Bayer CropScience

Application to FSANZ to Vary Food Standard 1.5.2 to Include the Glufosinate Ammonium-Tolerant Canola (*B. napus*) Event MS11

Prepared by [redacted]

Submitted December 2016

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Executive Summary

Bayer Crop Science Pty Ltd seeks to vary FSANZ Standard 1.5.2 to allow the use of genetically modified canola (*Brassica napus*) derived from transformation event MS11 *B. napus* in the Australian and New Zealand food industries. We seek specifically to allow the use of genetically modified *Brassica napus* (canola) oil derived from transformation event MS11 *B. napus* in the Australian and New Zealand food industries. Canola seed contains 44% oil which is extracted and used as a cooking oil. The remainder of the seed (meal) is used as livestock feed.

Bayer's Crop Science Division (Bayer CS) has developed a highly successful breeding tool that is used to produce *Brassica napus (B. napus)* glufosinate-ammonium tolerant hybrids that are sold in Canada and the USA. Currently, BCS hybrids are based on events MS8 *B. napus* and RF3 *B. napus* MS8 *B. napus* will be phased out of use by the mid-2020's and MS11 *B. napus* will be the replacement event.

The hybrid technology comprises three components: a dominant gene for male sterility – the *barnase* gene (event MS11), a dominant gene for fertility restoration – the *barstar* gene (event RF3) and a selectable marker gene to make the system more convenient for breeding and seed production – the *bar* gene (found in both MS11 and RF3) conferring tolerance to glufosinate-ammonium. MS11 *B. napus* is a male sterile line that segregates 1:1 for sterility and fertility and is only used for the production of the MS11xRF3 *B. napus* hybrid seed. It will never be commercialized as a standalone product.

MS11 *B. napus* (male sterile line) was produced by means of *Agrobacterium* mediated transformation using the vector pTCO113. MS11 *B. napus* contains the *barnase* gene (origin *Bacillus amyloliquefaciens*) coding for a ribonuclease, Barnase. The *barnase* gene is driven by the Pta29 promoter that restricts gene expression to the tapetum cells during anther development. Expression of Barnase in the tapetum cells of MS11 *B. napus* results in lack of viable pollen and male sterility. MS11 *B. napus* contains the *barstar* gene (origin *Bacillus amyloliquefaciens*) coding for the Barstar protein, which is an inhibitor of the Barnase protein. This prophylactic *barstar* gene, driven by the Pnos promoter, is included to enhance transformation frequency. MS11 *B. napus* also contains the *bar* gene (origin *Streptomyces hygroscopicus*) coding for phosphinothricin acetyl transferase (PAT/*bar*) confering tolerance to glufosinate-ammonium. The *bar* gene is driven by the PssuAt plant promoter that is active in all green tissues of the plant. The OECD identifier of MS11 *B. napus* is BCS-BNØ12-7.

The incorporation and expression of the MS11 transgenic locus in the *B. napus* genome has been characterized according to international standards for the safety assessment of biotechnology products. This information is included with this application to support the food safety of the PAT, Barnase and Barstar proteins. Hybrid *B. napus* varieties containing MS11 *B. napus* will be grown commercially in the *B. napus* producing areas of Canada, USA and Australia.

The *bar, barnase and barstar* genes were introduced into the *B. napus* genome in a single gene construct via direct-gene transfer. The regulatory sequences used in this construct are derived from common plants or plant pathogens that are routinely used in plant biotechnology and have a history of safe use.

In the molecular characterisation of the MS11 *B. napus* transgenic locus, bioinformatics analysis of the full DNA sequence revealed no evidence supporting cryptic gene expression or unintended effects resulting from the genetic modification. The transgenic locus also shows structural stability over different generations and growing environments, and in different genetic backgrounds.

Food safety evaluation of the PAT/*bar*, Barnase and Barstar proteins was undertaken utilising guidance provided by Codex (2003). No health-related adverse effects have been associated with the proteins.

The source organism for the Barnase and Barstar proteins, *Bacillus amyloliquefaciens,* is ubiquitous in nature and found throughout the world as common soil bacteria. The Barnase and Barstar proteins

have no amino acid sequence homology to know allergens and both are rapidly degraded in simulated gastric fluid and simulated intestinal fluid assays. The Barnase and Barstar proteins have no amino acid sequence similarity to know toxins and exhibited no effects in acute oral mouse toxicity tests. Both proteins have a good history of safe use.

The source organism for the phosphinothricin acetyltransferase (PAT/*bar*) protein, *Streptomyces hygroscopicus*, is a common saprophytic bacterial species that is found worldwide, predominately in soil. The PAT/*bar* protein does not possess structural or functional similarity with known toxic proteins or allergens; it shares no sequence homology with known allergens and toxins, no N-glycosylation sites, and rapidly degrades in simulated digestive environments. The Pat/*bar* protein exhibited no effects in an acute oral mouse toxicity test. The PAT/*bar* protein has a good history of safe use.
Therefore, it is concluded that MS11 *B. napus* has negligible impact on canola nutritional value.

Part 1 General Information on the Application

1.1 Applicant Details

(a) Applicant (individual organisation's) name

Bayer Crop Science Pty Ltd

(b) Name of contact person

[redacted]

(c) Address (street and postal)

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Level 1, 8 Redfern Road
Hawthorn East
Victoria 3123
Australia

(d) Telephone numbers

Tel: [redacted]

(e) Email address

[redacted]

(f) Nature of applicant's business

Seeds and traits, biotechnology.

(g) Details of other individuals, companies or organisations associated with the application.

Not applicable.

1.2 Purpose of the Application

This application, on behalf of Bayer Crop Science Pty Ltd, seeks to vary FSANZ Standard 1.5.2 to allow the use of genetically modified canola (*B. napus*) derived from transformation event MS11 *B. napus* in the Australian and New Zealand food industries.

The food products derived from canola are oil and meal. The primary food product consumed by humans in Australia is refined oil. Canola meal is used as a component of animal feed, and has industrial uses (Bonnardeaux, 2007).

Canola varieties containing MS11 *B. napus* will be commercially cultivated in the major canola producing countries of the world, including Australia. It is anticipated that food products derived from canola containing this event will enter the Australian and New Zealand food supply via local production and imports from major canola producing countries such as Canada.

1.3 Justification for the Application

MS11 *B. napus*t introduced three genes to the *B.napus* genome. These genes confer two novel traits: tolerance to broad spectrum herbicides with glufosinate ammonium as the active ingredient, and through the dominant gene *barnase* the trait for male sterility within the MS11 x RF3 hybrid breeding system. Canola varieties containing MS11 *B. napus* will be produced commercially in Australia and the major canola producing countries of the world.

(a) Need for the proposed change

The proposed change is required as a key advancement in *B. napus* hybrid breeding systems for Bayer, both internationally and locally in the future. The advantages that MS11 *B. napus* supplies over currently approved male-sterile components of the Bayer SeedLink® hybrid breeding system (e.g. MS8) are outlined under Section 1.3 (b) below.

(b) The advantage of the proposed change over the status quo, taking into account any disadvantages

Advantages of MS11 *B. napus*, taking into account any disadvantages of the proposed change
The novel traits expressed by canola varieties containing MS11 *B. napus* provide several agronomic benefits over conventional canola varieties and other transgenic canola currently under commercial cultivation in Australia. These include:

- The use of MS11 *B. napus* in the MS11 x RF3 hybrid breeding system confers increased vigour to the crop and hence increased yields of canola seeds and oil yield.
- Glufosinate ammonium is a broad spectrum, post-emergence weed control system that provides an alternative to pre-emergent and residually active compounds, and encourages herbicide use on an as-needed basis.
- Glufosinate ammonium provides an alternative broad-spectrum herbicidal mode of action for canola farming systems facilitating the use of an alternative mode of herbicide action within the crop rotation system of a farm.
- Broad spectrum weed control reduces cultivation needs, reducing on-farm fuel consumption, decreasing CO_2 emissions and also importantly improving soil health (Brookes, G. and Barfoot, P.; 2016; http://www.tandfonline.com/doi/full/10.1080/21645698.2016.1192754).

Note:

(a) Any public health and safety issues related to the proposed change including details of target groups and population groups that may be adversely affected
(b) Any consumer choice issues related to the proposed change
(c) Any evidence tht the food industry generally or other specific companies have an interest in, or support, the proposed change.

In relation to points (a), (b) and (c) above, the data contained within this submission indicates the general safety of MS11 *B. napus*-derived foods and their close similarity to non-GM comparators that have been used in studies. From the work conducted there is no indication that there are

public health or safety issues related to the proposed change to Standard 1.5.2 of the Food Standards Code. The section below discussing food safety of MS11 *B. napus* goes into further detail in this respect.

Consumer choice with respect to the proposed change is anticipated to be dealt with by FSANZ via their assessment of the data included in this package. It should be noted that MS11 *B. napus* when used in hybrid breeding systems to deliver *B. napus*-derived food products will result in the primary food product – canola oil – which contains novel proteins which are below the limit of quantification. This food item therefore does not result in the need for labelling to differentiate it from canola oil derived from non-GM *B. napus* varieties.

As MS11 *B. napus* is still in the developmental stage with Bayer, there is no specific information available to indicate that the food industry have interest in, or support, the proposed change to the Standard 1.5.2. However, due to reasonably rapid uptake by the farming community of GM *B. napus* in past years and the impact that this has had on the price per tonne of seed for crushing to oil it may be anticipated that the food industry generally support technology that leads to lower commodity prices for the canola oil that they wish to purchase, process into foods and on-sell.

Food safety

The PAT/*bar* protein from *Streptomyces hygroscopicus*, and the barnase and barstar proteins from *Bacillus amyloliquefaciens*, each have a long history of safe use in agriculture. These proteins have been successfully used for herbicide tolerance and in hybrid breeding systems (barnase and barstar) for more than 20 years. They are expressed by a number of transgenic crops that have been in commercial production for more than 20 years in many parts of the world, most notably in canola (*B. napus*) in Canada. FSANZ has assessed these proteins previously (see Table 2, Section A.2(a)(i)), and have not identified public health or safety concerns.

Information is provided in this application to support the safety of the novel proteins expressed by MS11 *B. napus*. The PAT/*bar*, Barnase and Barstar proteins have no characteristics consistent with known toxins or allergens, and have limited potential for mammalian toxicity. Compositional and nutritional analyses demonstrate that food derived from canola containing MS11 *B. napus* is equivalent to food derived from conventional canola varieties.

The PAT protein has been assessed by FSANZ previously for several transgenic crops, including seven previous Bayer CropScience applications. No public health or safety concerns were identified associated with the PAT protein expressed by other Bayer CropScience events in Liberty Link® cotton (A533; A1028; A1040), Liberty Link® canola (A372), LibertyLink® corn (A375), LibertyLink® soy (A481), or rice event LLRICE62 (A589). In the review of LLCotton25, FSANZ stated: "*The safety of PAT has been assessed on numerous previous occasions by FSANZ. In all instances, it has been concluded that PAT is non-toxic to humans and has limited potential as a food allergen*" (FSANZ, 2005).

Status of similar applications made in other countries by the applicant, if applicable

COUNTRY	AGENCY	FOOD/FEED/CULT/IMPORT	SUBMISSION DATE
USA	United States Department of Agriculture (USDA)	Feed/Cultivation	16/8/2016
	Food and Drug Administration (FDA)	Food	26/8/2016
Korea	Ministry of Food and Drug Safety (MFDS)	Food/Import	8/9/2016
	Rural Development Administration (RDA)	Feed/Import	26/10/2016

1.4 Regulatory impact information

Costs and benefits, and impacts on trade

Varying FSANZ Standard 1.5.2 to include commercial canola (*B. napus*) varieties containing MS11 *B. napus* is unlikely to have a detrimental impact on the Australian canola or food industries. Despite being a small canola producer, Australian canola is sourced for many food products on the domestic market. These ingredients are also obtained from imported canola products, with Canada a major source of imports. Once canola varieties containing MS11 event *B. napus* are launched for commercial production in Australia, Canada, as well as in other parts of the world, food products derived from canola containing this event are likely to enter the domestic food supply.

If MS11 *B. napus* is not incorporated into the FSANZ Standards, this could have wide ranging impacts on the price of food products containing ingredients derived from canola. These would arise from the need to source other canola varieties that do not contain MS11 *B. napus*. These products may attract a premium price that must be met by the manufacturer, with those costs eventually passed on to the consumer. This would be compounded by the costs of segregating MS11 *B. napus* products from other canola products, where trading partners are willing to comply with this requirement. Other factors to consider include disruptions to the food supply, and the significant costs of recalling food products if MS11 *B. napus* were to be distributed in the local food supply.

Varying the FSANZ Standards to include MS11 *B. napus* will contribute to maintaining stable food prices, consumer choice in the marketplace, and decreased production costs for transgenic canola varieties in the longer term. Further, including MS11 *B. napus* in the FSANZ Standards will also enable Australia to compete internationally in the production of canola, and provide Australian canola producers with more environmentally sustainable alternatives.

The potential trade implications of not including MS11 *B. napus* in the FSANZ Standards are significant. Segregating MS11 *B. napus* products from other canola products has compliance and identification requirements that are difficult and costly to meet. Canada is a major trading partner of Australia, and approved transgenic crops are considered to be substantially equivalent to conventional crops. Therefore, in Canada where more than 95% of the canola (*B. napus*) crop is trangenic, there are no intentions of segregating or labelling transgenic crops or their products. Products containing MS11 *B. napus* imported into Australia from Canada, or other trading partners with similar treatments of transgenic crops, may need to be removed from sale. This could expose Australia to disputes with trading partners at the World Trade Organisation.

1.5 Information to support the application

All of the relevant information to support the application is supplied within this summary and the associated electronic dossier that has been supplied to FSANZ. The relevant studies are listed in the "List of Appended Electronic Documents" above, and suitable literature references are provided in a reference list at the end of this document. To navigate the electronic dossier a direction to which "Node" of the dossier the document may be found under is supplied.

1.6 Assessment Procedure

We consider that the appropriate assessment for this application is the General Procedure since the PAT, Barnase and Barstar proteins have been evaluated by FSANZ previously.

1.7 Confidential Commercial Information

Information in the Bayer CropScience reports provided in Nodes A.3 (c), (i) (CCI) (Document M-547543-01-1), A.3 (c), (iii) (CCI) (Documents M-304805-01-1, M-545355-01-1)A.3, (c), (v) (CCI) (Document no. M-552421-01-1), A.3 (e), (i) (CCI) (Document M-547544-01-1), B.1 (a) (CCI) (Documents, M-232685-01-1, M-232692-01-1) contain confidential commercial information. A formal request for this information to be treated as such has been submitted to FSANZ.

1.8 Other Confidential Information

There are no documents regarded as "Other Confidential Information" associated with this submission.

1.9 Exclusive Capturable Commercial Benefit (ECCB)

The application is expected to confer an ECCB upon Bayer's Crop Science Division since it will contribute to facilitating commercial activities with MS11 *B. napus* in Australia.

1.10 International and Other Standards

The Bayer CropScience reports and studies included in the information supporting this application have been conducted according to international standards. In the safety assessment of biotechnology products, Bayer's Crop Science Division refers primarily to the *Codex Alimentarius* Commission weight-of-evidence approach (CAC, 2009), and the relevant Codex Standard is:

Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants. CAC/GL 45-2003. Adopted in 2003, Annexes II and III adopted in 2008. (CAC, 2009).

Other guidelines and recommendations are also considered including those of the World Health Organisation (WHO), the United Nations Food and Agriculture Organisation (FAO), the United States Food and Drug Administration (US-FDA), the United States Environment Protection Agency (US-EPA), and the European Food Safety Agency (EFSA) (see CAC, 2009 above; EFSA, 2011; FAO/WHO, 2001; US-FDA, 2012).

1.11 Statutory Declaration

Included in the application cover letter to FSANZ, which is appended as an electronic document to the DVD which contains the submission.

1.12 Checklist for Standards Related to New Foods

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Part A Technical Information on the Food Produced Using Gene Technology

A.1 Nature and Identity of the Genetically Modified Food

(a) A description of the GM organism from which the new GM food is derived. The description must include the nature and purpose of the genetic modification.

The GM organism is cultivated canola (*Brassica napus*) transformed with MS11 *B. napus*. Transformation of the *B. napus* variety N90-740 was achieved using standard *Agrobacterium*-mediated transformation methodology. The transformation methodology is described in [redacted] (2008; Dossier Node A.3 (a), M-307476-01-1).

The MS11 event introduced three genes to the *B. napus* genome:

(i) The *barnase* gene encodes for a ribonuclease Barnase. This gene is derived from the common soil bacterium, *Bacillus amyloliquefaciens*. The *barnase* gene is driven by the Pta29 promoter that restricts gene expression to the tapetum cells during anther development. Expression of Barnase in the tapetum cells of MS11 *B. napus* results in lack of viable pollen and male sterility.

(ii) The *barstar* gene (origin *Bacillus amyloliquefaciens*) coding for the Barstar protein, which is an inhibitor of the Barnase protein. This prophylactic *barstar* gene, driven by the Pnos promoter, is included to enhance transformation frequency.

(iii) The *bar* gene that encodes for the phosphinothricin acetyl transferase protein (PAT). This gene is derived from a common soil bacterium, *Streptomyces hygroscopicus*.

The PAT protein exhibit highly specific activity. The PAT protein promotes the detoxification of glufosinate ammonium, therefore MS11 canola shows tolerance to field applications of herbicides with glufosinate ammonium as the active ingredient. This trait offers canola farmers an alternative broad spectrum, post-emergent weed control system.

The Barnase ribonuclease confers male sterility in MS11 *B. napus* breeding lines that form part of the overall MS11 x RF3 hybrid breeding system. RF3 *B. napus* within this hybrid breeding system is the fertility restorer line that renders the conventionally bred hybrid fertile once again. RF3 *B. napus* contains the *barstar* gene (origin *Bacillus amyloliquefaciens*), coding for the Barstar protein, which is an inhibitor of the Barnase protein. The barstar gene is driven by the Pta29 promoter that restricts gene expression to the tapetum cells during anther development. Expression of the Barstar protein in the tapetum cells leads to restoration of fertility after crossing to a male sterile (MS) *B. napus* line. RF3 *B. napus* also contains the bar gene (origin *Streptomyces hygroscopicus*) coding for phosphinothricin acetyltransferase (PAT/*bar*) confering tolerance to glufosinate-ammonium. The *bar* gene is driven by the PssuAt plant promoter that is active in all green tissues of the plant.

FSANZ has previously assessed the PAT protein, as expressed by the *bar* or *pat* gene, in other Bayer CropScience food crops including Liberty Link® cotton (A533), the elements of TwinLink® cotton (A1028 and A1040), corn (A375) and soybean (A481), InVigor® Hybrid canola (A372), and the LLRICE62 rice event (A589). Both the MS11 and RF3 events will be present in canola varieties to be commercialised in Australia.

(b) The name, line number and OECD Unique identifier of each of the new lines or strains of GM organism from which the food is derived.

The transformation event is named “MS11”, and *B. napus* transformed with this event will be referred to as MS11 *B. napus*. The OECD Unique identifier of MS11 *B. napus* is BCS-BNØ12-7.

(c) The name the food will be marketed under (if known).

This is unknown as this application is related to a commodity crop rather than a specific food or additive.

A.2 History and Use of the Host and Donor Organisms

The common and scientific names of the host and donor organisms must be stated.

The taxonomic classifications of the organisms from which the genetic elements of MS11 *B. napus*are derived are presented below in Table 1.

Table 1 Taxonomy of the donor organisms from which the genetic elements of MS11 *B.napus*are derived

GENETIC ELEMENT	DONOR ORGANISM TAXONOMY							
	Kingdom	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Scientific Name**	**Common Name**
Plant Genome								
Genomic DNA	Plantae	Streptophyta	Magnoliopsida	Brassicales	Brassicaceae	*Brassica*	*Brassica napus* (2n=38)	Canola, Oilseed rape
Gene Construct								
3'g7	Eubacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Agrobacterium*	*Agrobacterium tumefaciens*	
bar	Eubacteria	Actinobacteria	Actinobacteridae	Actinomycetales	Streptomycetaceae	*Streptomyces*	*Streptomyces hygroscopicus* strain ATCC21705	
PssuAt	Plantae	Streptophyta	Magnoliopsida	Brassicales	Brassicaceae	*Arabidopsis*	*Arabidopsis thaliana*	Mouse-ear cress, Thale-cress
3'nos								
3'barnase	Eubacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	*Bacillus amyloliquefaciens*	
barnase	Eubacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	*Bacillus amyloliquefaciens*	
Pta29	Plantae	Streptophyta	Magnoliopsida	Solanales	Solonaceae	*Nicotiana*	*Nicotiana tabacum* L.	Tobacco
Pnos	Eubacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Agrobacterium*	*Agrobacterium tumefaciens*	
barstar	Eubacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	*Bacillus amyloliquefaciens*	
3'g7	Eubacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Agrobacterium*	*Agrobacterium tumefaciens*	
aadA	Eubacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Escherichia*	*Escherichia coli*	*E.coli*
barstar	Eubacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	*Bacillus amyloliquefaciens*	
aadA	Eubacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Escherichia*	*Escherichia coli*	*E.coli*
ORI pVS1	Eubacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*Pseudomonas aeruginosa*	

Where information relating to an organism has been included in previous safety assessments prepared by FSANZ, it is not necessary to provide any further information. Where an organism has not been considered previously by FSANZ, the following information must be provided. A complete package of data has been provided to FSANZ for MS11 *B. napus* in order to provide the agency with as up to date data and information as possible according to the data requirements of the March 2016 Application Handbook.

(a) For the donor organism(s) from which the genetic elements are derived:

(i) Any known pathogenicity, toxicity or allergenicity of relevance to the food;

Brassica napus

The host organism, cultivated canola (*B. napus*), is an established agricultural field crop that is grown as a source of food and feed. Rape seed oil is used widely as an edible oil in Asia. Only through breeding for improved oil quality, and the development of improved processing techniques, has rapeseed oil become important in western countries. Since the Second World War, rapeseed production in Europe and Canada has increased dramatically as a result of improved oil and meal quality. Modern techniques of plant transformation and genotype identification using isozymes, restriction fragment length polymorphism (RFLP) markers, or random amplified polymorphic DNA (RAPDs) markers will complement classical breeding for the production of other improved lines (Buzza, 1995). China, India, Europe and Canada are now the top producers, although this crop can be successfully grown in the United States, South America and Australia, where annual production has increased sharply over the last few years (OECD, 1997). Low erucic acid, low glucosinolate canola is not known to be capable of causing disease or other ill health in people, plants or animals. Plants commonly produce toxins and allergens that serve as a natural defence against pests and pathogens. Seeds and other canola plant tissues contain toxic and anti-nutritional secondary defence chemicals including erucic acid and glucosinolates (e.g. 3-Butenyl- , 4-Pentenyl-, 2- Hydroxy-3-butenyl-, 4-Hydroxy-3-indoylmethyl-glucosinolate amongst others).

Canola oil is valued for its high mono-unsaturated oleic acid level. It also contains a good proportion of polyunsaturates including 10-12% omega-3 linolenic acid. Early rapeseed cultivars contained high levels of erucic acid, C22:1, although current cultivars have only trace quantities (< 0.1%) of this undesirable product (Mailer, 2007). Variation in fatty acids still occurs with the main variation obvious in oleic acid, which ranges from approximately 59-62%. Linoleic and linolenic acids also vary from 18-22% and 10-12% respectively.

Glucosinolates are considered anti-nutritional factors in low erucic acid rapeseed meal. On their own they are innocuous, but when cells of the seed are ruptured glucosinolates come in contact with myrosinase. The myrosinase enzyme hydrolyzes the glucosinolates releasing sulphur, glucose and isothiocyanates. The isothiocyanates are goitrogenic, reducing the ability of the thyroid to absorb iodine (Downey, 2007). These metabolites of glucosinolates can affect animal performance and can be toxic to the liver and kidneys (Tripathi and Mishra, 2007). Heating during processing of the meal eliminates most of the myrosinase, but is not completely effective in eliminating the effects of glucosinolates because some intestinal microflora also produces myrosinase (Tripathi and Mishra, 2007).

Early rapeseed varieties introduced into Australia and some subsequent Australian varieties had high glucosinolate concentrations, in excess of canola standards. Through plant breeding and selection, varieties today have only very low glucosinolate levels, generally less than 7 µmoles of total glucosinolate /g of whole seed. This is equivalent to approximately 11 µmoles/g of oil-free meal and well less than the canola standard of 30 µmoles/g of meal of only four specified glucosinolates. Mailer and Cornish (1987) found that environment has a major influence on glucosinolate content with sulphur availability and water stress contributing to an increase. Despite some year to year variation, maximum levels never approach the canola limit in the Australian crop. In 1994, under water stress conditions, glucosinolate concentration reached 9µmoles per gram of seed, or 15 µmoles per gram of meal, at some sites. Despite dry conditions in 1997, as indicated by low oil and high protein concentrations, glucosinolates remained low, perhaps indicating more stability in more recent varieties (Mailer, 2007).

Despite the natural presence of these compounds, canola has a long history of safe use.

Canola oil is the only product of *B. napus* that represents a major component of human food, and it is an important vegetable oil source. Canola oil intended for human consumption is highly processed to reduce its toxicological properties (OECD, 2011; Bonnardeaux, 2007).

Several studies have investigated the potential for *B. napus* to cause food allergy. In general, sensitisation in children was associated with multiple allergies to other foods and pollen (HealthCanada, 2010, Monsalve et al, 2001 and Poikonen et al, 2009). There were a small number of DBPCFC (double-blind placebo controlled food challenges) with adults or children (Figueroa et al, 2005 and Morisset et al, 2003) and SBPCFC (single-blind placebo controlled food challenge) studies with children (Rancé, 2003), due mainly to the difficulty of masking the mustard taste and the unethical health risk to conduct clinical studies with highly sensitive patients.

Because the protein was either at very low levels or absent in canola oil, the significance of the results of these allergenicity studies in determining the safety of consumption of canola oil by the general population was considered to be low (Gylling, 2006). In addition, the food allergy to canola oil in adults has not been reported in the scientific literature (Verhoeckx et al., 2015).

Although *B. napus* contains a 2S-albumin, which is known to be strongly allergenic, the lack of reported allergenicity records is very likely due to the harsh nature of the combined heat and mechanical processing for producing oil. According to the OECD (2011), canola seed is traditionally crushed and solvent extracted in order to separate the oil from the meal. The process usually includes seed cleaning, seed pre-conditioning and flaking (i.e. preheating of the seeds to approx. 35 °C), seed cooking/conditioning (including a steam-heating with a temperature, which is rapidly increased and which ranges between 80 and 105 °C, for 15–20 min), pressing the flake to mechanically remove a portion of the oil, solvent extraction of the press-cake to remove the remainder of the oil, oil and meal desolventising (with final stripping and drying at a temperature of 103–107 °C), degumming and refining of the oil. The most probable hypothesis is that the proteins are removed from the oil by extrusion. All these steps extract the potential allergens from oil (Verhoeckx et al., 2015).

A combination of physical- and thermal treatment (e.g. extrusion) can suppress allergenicity of mustard/canola seed allergens by extracting the potential allergens from oil. Edible oils that are bleached and deodorised are devoid of allergenicity (Verhoeckx et al., 2015).

The absence of proteins in processed canola oil also suggests that canola oil from any transgenic variety should be as safe for human consumption as canola oil from conventional *B. napus* varieties.

bar gene

The *bar* gene in MS11 *B. napus* was isolated from strain ATCC21705 of *Streptomyces hygroscopicus* (Murakami *et al*., 1986). *Streptomyces hygroscopicus* is a common saprophytic bacterial species that is found worldwide (Kützner, 1981). The *Streptomycetae* bacteria were first described in 1916 (Waksman and Curtis, 1916; see also Kützner, 1981; Bradbury, 1986). Soil is the predominant habitat but these organisms may also be isolated from water.

Many *Streptomyces* species biosynthesise antimicrobial compounds, and this is thought to aid in competition between microbial populations for nutrients. *Streptomyces hygroscopicus* produces a variety of useful antimicrobial and herbicidal compounds (Dunne *et al*., 1998), of which the PAT enzyme confers phosphinothricin tripeptide (phosphinothricin or bialaphos) tolerance. This tolerance is conferred through inactivation by transfer of an acetyl group. Acetyltransferase activity has been identified in six other bacterial species from five different genera of common soil bacteria. This is thought to have evolved as a protective mechanism to protect these microorganisms from antimicrobials produced by both themselves and other competing microorganisms. Consequently, natural resistance to phosphinothricin and N-acetyltransferase has also been reported in various genera of soil bacteria (Bartsch and Tebbe, 1989). It is expected that humans would be exposed to

these microorganisms and anti-microbial compounds directly through the consumption of roots and other vegetables that are eaten fresh.

Streptomyces species very rarely cause human disease, most often manifesting as a localized, chronic suppurative infection of the skin and underlying soft tissue (Dunne *et al*., 1998). The PAT protein is expressed by a number of transgenic crops that have been in commercial production for many years. Therefore this protein has been well characterised and demonstrated to be non-toxic to humans and animals. FSANZ did not identify any public health or safety concerns associated with the expression of PAT, as encoded by the *pat* or *bar* gene, in Liberty Link® cotton (LLCotton25; A533), Liberty Link® canola (A372), LibertyLink® soy (A481), LLRICE62 (A589), T304-40 cotton (A1028; combined with *cry1Ab* gene) or GHB119 cotton (A1040; combined with *cry2Ae* gene). In the review of LLCotton25, FSANZ stated: "*The safety of PAT has been assessed on numerous previous occasions by FSANZ. In all instances, it has been concluded that PAT is non-toxic to humans and has limited potential as a food allergen*" (FSANZ, 2005). Similarly, the PAT protein, as expressed by the *pat* gene, has been approved in LibertyLink® corn (A375), and DBT418 corn (Monsanto; A380). The *pat* gene encodes for a PAT protein with 87% homology to the PAT protein encoded by the *bar* gene (Wohlleben *et al*., 1988). The *pat* gene has been combined with *cry* genes previously also, and approved by FSANZ for cotton (A518) and corn (A446; A543).

Table 2 Gazetted FSANZ Standards for events encoding for the expression of PAT/*bar* and PAT/*pat* proteins

CROP	APP	EVENTS/LINES EVALUATED	OTHER TRANSGENIC TRAITS
Canola	A372	Event MS8 and Event RF3	Glufosinate ammonium tolerance, barnase and barstar hybrid breeding system
Corn	A375	Event T25 (ACS-MS003-2)	Glufosinate ammonium tolerance
	A380	Line DBT418	Glufosinate ammonium tolerance, Lepidoptera protection
	A385	Line BT-176	Glufosinate ammonium tolerance, Lepidoptera protection
	A386	Line BT-11	Glufosinate ammonium tolerance, Lepidoptera protection
	A446	Line 1507	Glufosinate ammonium tolerance, Lepidoptera protection
	A543	Line DAS-59122-7	Glufosinate ammonium tolerance, Coleoptera protection
Corn	A1106	Line 4114	Glufosinate ammonium tolerance, Coleoptera protection, Lepidoptera protection
	A1112	Line MZHG0JG	Glyphosate and glufosinate ammonium tolerance
Cotton	A518	Line MXB-13	Glufosinate ammonium tolerance, Lepidoptera protection
	A533	Line LL25	Glufosinate ammonium protection
	A1028	Event T304-40	Glufosinate ammonium tolerance, Lepidoptera protection
	A1040	Event GHB119	Glufosinate ammonium tolerance, Lepidoptera protection

CROP	APP	EVENTS/LINES EVALUATED	OTHER TRANSGENIC TRAITS
	A1080	Event MON88701	Glufosinate ammonium tolerance, dicamba tolerance
	A1094	Line DAS-81910-7	2,4-D and glufosinate ammonium tolerance
Soybean	A481	Lines A2704-12 and A5547-127	Glufosinate ammonium tolerance
	A592	Event MON-89788-1	Glufosinate ammonium tolerance
	A1073	Event DAS-44406-6	Glufosinate ammonium, 2,4-D and glyphosate tolerance
	A1081	Event SYNHT0H2	Glufosinate ammonium and mesotrione tolerance

Regulatory sequences

The promoter and terminator sequences used in MS11 *B. napus* are derived from common plants or plant pathogens. These genetic elements constitute a minute component of their respective genomes, no genes that may be implicated in human disease, allergies or toxic effects have been transferred. Many of the organisms from which these elements are derived are model species in plant science with a history of safe use. These elements are described in Table 4, Section A.3(c)(i).

The *bar* gene is driven by the PssuAt plant promoter that is active in all green tissues of the plant. The PssuAt plant promoter is derived from *Arabidopsis thaliana* (mouse-ear cress), a model plant used extensively in plant biotechnology with a long history of safe use in plant genetics. The *bar* gene terminator sequence consists of the 3' untranslated region of the TL-DNA gene 7 (3'g7) of the *Agrobacterium tumefaciens* octopine Ti plasmid (Dhaese *et al*., 1983). *Agrobacterium tumefaciens* is a soil born, gram-negative bacterium that has been extensively studied since it was identified as the causative agent of crown gall disease in plants. *Agrobacterium tumefaciens* is a well-known prokaryotic organism capable of transferring DNA to the eukaryotic cell (Bundock and Hooykaas, 1998). This gene transfer ability may have evolved from bacterial conjugal transfer systems which mobilise plasmids for transfer between bacterial cells (Stachel and Zambryski, 1986) and is exploited in biotechnology. Consequently, *A. tumefaciens* is widely used transformation system in plant biotechnology.

Barnase and *Barstar* genes

MS11 *B. napus* contains the *barnase* gene (origin *Bacillus amyloliquefaciens*) coding for a ribonuclease, Barnase and the *barstar* gene (origin *Bacillus amyloliquefaciens*) coding for the Barstar protein, which is an inhibitor of the Barnase protein (Hartley, 1988). The source organism for the Barnase and Barstar proteins, *Bacillus amyloliquefaciens,* is ubiquitous in nature and found throughout the world as common soil bacteria. It has an excellent safety profile and has been characterised and utilised extensively in the development of GM crops over the last 20 years or more, particularly within GM *B. napus* in Canada and Australia (FSANZ application A372).

Regulatory sequences

The *barnase* gene is driven by the Pta29 promoter that restricts gene expression to the tapetum cells during anther development. This promoter is derived from *Nicotiana tabacum* (tobacco) (Suerinck *et al*., 1990), and is extensively used as a model plant species in biotechnology developments in plants. Although tobacco produces toxins and carcinogens, the regulatory sequence Pta29 comprises a small part of its total genome, and in itself has no pathogenic, toxic or carcinogenic properties. The terminator sequence for the *barnase* gene is the 3'' untranslated region of the nopaline synthase gene from the T-DNA of pTiT37 from *A. tumefaciens* (3'nos) (Depicker *et al*., 1982).

As mentioned previously, *A. tumefaciens* is a well-known prokaryotic organism capable of transferring DNA to the eukaryotic cell (Bundock and Hooykaas, 1998). This gene transfer ability may have evolved from bacterial conjugal transfer systems which mobilise plasmids for transfer between bacterial cells (Stachel and Zambryski, 1986) and is exploited in biotechnology. Consequently, *A. tumefaciens* is widely used transformation system in plant biotechnology.

The *barstar* gene is driven by the Pnos promoter region of the nopaline synthase gene of *A. tumefaciens*. The terminator for the *barstar* gene, the 3' untranslated region of the TL-DNA gene 7 of the *A. tumefaciens* octopine Ti plasmid is also derived from *A. tumefaciens* (Dhaese et al., 1983).

(ii) history of use of the organism in the food supply or history of human exposure to the organism through other than intended food use (e.g. as a normal contaminant).

<u>*Brassica napus*</u>
The host organism, cultivated *Brassica napus*, is an established agricultural field crop that has been grown for millennia as a source of food and feed, and has a long history of safe use.
B. napus is a member of the subtribe Brassicinae of the tribe Brassiceae of the Cruciferous (Brassicaceae) family, sometimes referred to as the mustard family (OECD, 1997).
There are two types of *B. napus*: 1) oil-yielding oleiferous rape, of which one subset with specific quality characteristics is often referred to as "canola" (vernacular name), and 2) the tuber-bearing swede or rutabaga. Canola, the oleiferous type can also be subdivided into spring and winter forms. Sanskrit writings of 2000 to 1500 BC directly refer to oleiferous *B. napus* forms (sarson types) and mustard. In Europe, domestication is believed to have occurred in the early Middle Ages. Commercial plantings of rapeseed are recorded in the Netherlands as early as the 16th century. At that time rapeseed oil was used primarily as an oil for lamps. Later it came to be used as a lubricant in steam engines (OECD, 1997).
Although used widely as an edible oil in Asia, only through breeding for improved oil quality, and the development of improved processing techniques, has rapeseed oil become important in western countries. Since the Second World War, rapeseed production in Europe and Canada has increased dramatically as a result of improved oil and meal quality. Modern techniques of plant transformation and genotype identification using isozymes, restriction fragment length polymorphism (RFLP) markers, or random amplified polymorphic DNA (RAPDs) markers will complement classical breeding for the production of other improved lines (Buzza, 1995). China, India, Europe and Canada are now the top producers, although this crop can be successfully grown in the United States, South America and Australia, where annual production has increased sharply over the last few years. Today, two species of Brassica have commercialized varieties with "double low" characteristics, i.e. low erucic acid content in the fatty acid profile and very low glucosinolate content in the meal, characteristics desirable for high-quality vegetable oil and high-quality animal feed. In North America these species (*B. napus* and *B. rapa*) are considered to be of "canola" quality (OECD, 1997), although *B. rapa* is no longer grown commercially.

B. napus is grown as a winter annual in regions where winter conditions do not result in very low temperatures, which would kill the plants. These biotypes typically require vernalisation before the onset of stem elongation, raceme development, flowering and seed set. In North America and northern parts of Europe, a spring biotype of *B. napus* that requires no vernalisation prior to flowering is grown. These biotypes are typically lower yielding than the winter annual types, but require considerably less time to complete their life cycle (OECD, 1997).

<u>*bar* gene</u>
The *bar* gene (coding for the PAT protein) was isolated from strain ATCC21705 of *Streptomyces hygroscopicus* (Murakami *et al*., 1986). *Streptomyces hygroscopicus* is a common soil bacterium that produces a variety of useful compounds including antimicrobials (such as rapamycin and hygromycin B) and herbicides (such as Bialaphos, a derivative of phosphinothricin). The *bar* gene has been released in transgenic commercial food crops in Australia and overseas and is considered to pose no risks to human health or safety. Extensive animal testing has shown that the PAT protein is non-toxic to humans and animals. In Australia, FSANZ did not identify any public health or safety concerns associated with the PAT protein in cotton (A533; A518; A1028; A1040), canola (A372), soybeans (A481), rice (A589), or corn (A375; A380; A446; A543). The history of safe use of *S. hygroscopicus*, and safety data for the PAT protein are also provided in Herouet *et al.* (2005).

<u>*barnase* and *barstar* genes</u>
The barnase and barstar genes were isolated from *Bacillus amyloliquefaciens* (Hartley, 1988). The source organism for the Barnase and Barstar proteins, *Bacillus amyloliquefaciens,* is ubiquitous in nature and found throughout the world as common soil bacteria. It has an excellent safety profile and

has been characterised and utilised extensively in the development of GM crops over the last 20 years or more, particularly within GM *B. napus* in Canada and Australia (FSANZ application A372).

Regulatory sequences

The genetic elements used as promoters and terminators of transgene expression in MS11 *B. napus* are derived from common plants or plant pathogens with specific host ranges. These genetic elements constitute a minute component of their respective genomes, no genes that may be implicated in human disease, allergies or toxic effects have been transferred. Many of the organisms from which these elements are derived are model species in plant science and are widely used for various research applications and have a history of safe use.

(b) For the host organism into which the genes were transferred:

(i) Its history of safe use for food

Canola is the name used for rapeseed (*B. napus*, *Brassica rapa*, or *Brassica juncea*) crops that have less than 2% erucic acid (a fatty acid)[1] and less than 30 micromoles of glucosinolates per gram of seed solids (OECD 2011). Canola varieties were first developed in Canada in the 1970s, using traditional breeding techniques, in response to a demand for food-grade rapeseed products and animal feed with improved palatability. Rapeseed-derived products that do not meet the compositional standard cannot use the trademarked term, canola.
Since the release of canola in Australia in 1980, it has become an important oilseed crop in most grain growing regions of Australia (FSANZ, A1089).

Rapeseed is the second largest oilseed crop in the world behind soybean. In 2012/13, the major oilseed rape producers globally were European Union (18.8m mt), Canada (13.3m mt) and China (12.6m mt) (USDA Foreign Agricultural Service; www.fas.usda.gov). While Canada is the largest exporter of canola, Australia regularly exports over one million tonnes of canola seed to Japan, Europe, China, Pakistan and other markets. This represents 15-20% of the world's trade in canola (AOF, 2007) (FSANZ, A1089).

(ii) The part of the organism typically used as food

Canola seeds are processed into two major products, oil and meal. The oil is the major product for human consumption, being used in a variety of manufactured food products including salad and cooking oil, margarine, shortening and a range of prepared foods such as mayonnaise, sandwich spreads, creamers and coffee whiteners. The meal provides a good protein source in stockfeed for a variety of animals, primarily pigs, poultry and dairy cattle. Whole canola seeds are being used increasingly in products such as breads (FSANZ, A1089).

(iii) The types of products likely to include the food or food ingredient

See the information under Section A.2 (b)(iii) above.

(iv) Whether special processing is required to render food derived from the organism safe to eat.

Canola seed is traditionally crushed and solvent extracted in order to separate the oil from the meal. The process usually includes seed cleaning, seed pre-conditioning and flaking, seed cooking/ conditioning, pressing the flake to mechanically remove a portion of the oil, solvent extraction of the press-cake to remove the remainder of the oil, oil and meal desolventizing, degumming and refining of the oil, and toasting of the meal. Canola seed can also be subject to cold-press extraction (*i.e.* no heat or solvent). The main steps of the solvent extraction process are schematised in Figure 1 (OECD, 2011).

Seed cleaning

The seed is cleaned to remove plant stalks, grains from other plant species and other materials from the bulk of the seed. Aspiration, indent cleaning, sieving, or some combination of these is used in the cleaning process. Dehulling of the seed is, at present, not a commercial process.

Seed pre-conditioning and flaking
Many crushing plants in colder climates preheat the seed to approximately 35°C through grain dryers in order to prevent shattering which may occur when cold seed from storage enters the flaking unit (Unger, 1990). The cleaned seed is first flaked by roller mills set for a narrow clearance to physically rupture the seed coat. The objective here is to rupture as many cell walls as possible without damaging the quality of the oil. The thickness of the flake is important, with an optimum of between 0.3 and 0.4 mm. Flakes thinner than 0.2 mm are very fragile while flakes thicker than 0.4 mm result in lower oil yield.

Seed cooking/conditioning
Flakes are cooked/conditioned by passing them through a series of steam-heated drum or stack-type cookers. Cooking serves to thermally rupture oil cells which have survived flaking, reduce oil viscosity and thereby promote coalescing of oil droplets, increase the diffusion rate of prepared oil cake, and denature hydrolytic enzymes. Cooking also adjusts the moisture of the flakes, which is important in the success of subsequent pre-pressing operations. At the start of cooking, the temperature is rapidly increased to 80–90°C. The rapid heating serves to inactivate the myrosinase enzyme present in canola. This enzyme can hydrolyze the small amounts of glucosinolates present in canola and will produce undesirable breakdown products which affect both oil and meal quality.
The cooking cycle usually lasts 15 to 20 minutes and the temperatures usually range between 80 and 105°C, with an optimum of about 88°C. In some countries, especially China, cooking temperatures of up to 120°C have been traditionally used when processing high glucosinolate rapeseed to volatize some of the sulphur compounds which can cause odours in the oil. However, these high temperatures can negatively affect meal protein quality.

Pressing
The cooked canola seed flakes are then pressed in a series of low pressure continuous screw presses or expellers. This action removes most of the oil while avoiding excessive pressure and temperature. The objective of pressing is to reduce the oil content of the seed from about 42% to 14–20%, making the solvent extraction process more economical and efficient, while producing acceptable quality presscake.

Solvent extraction
Since the pressing is not able to remove all of the oil from the canola seed, the presscake is solvent extracted to remove the remaining oil. The cake from the expellers, containing between 14 and 20% oil, is sometimes broken into uniform pieces prior to solvent extraction. In solvent extraction, hexane specially refined for use in the vegetable oil industry is used. After a series of extractions, the marc (hexane saturated meal) that leaves the solvent extractor contains less than 1% oil.

Desolventizing of oil and meal
The micella and meal are “stripped” of solvent, to recover solvent-free oil and meal. The micella containing the oil is desolventized using evaporator equipment. The solvent is removed from the marc in a desolventizer-toaster. This is done in a series of compartments or kettles within the desolventizer, often by injection of live steam, followed by final stripping and drying at a temperature of 103–107°C. The final, solvent-free meal contains about 1% oil and 8 to 10% moisture.

Degumming of oil
The “crude” oil from the two extraction stages (physical and chemical) is usually blended and then degummed before being stored for sale or further processing. Degumming removes phosphatides co-extracted with the oil, which tend to separate from the oil as sludge during storage. The phosphatide content of crude oil varies, but is usually in the order of 1.25% (or 500 ppm if measured as phosphorus). Two degumming methods are in use: (a) using water to precipitate phosphatides and; (b) using an acid such as citric, malic, or phosphoric and water (super-degumming).

Alkali and physical refining of oil
Degummed oil is further purified in a process of refining. One of two methods are used, namely, alkali refining, especially with water degummed oil, and physical refining with acid-water degummed oil. Alkali refining is the most common process used, even with acid-water degummed oil. Physical refining is a relatively new development. While it is very economical, physical refining requires well-degummed oil of moderate chlorophyll and free fatty acid content. Alkali refining reduces soap, free fatty acid, and phosphorus levels. The further removal of free fatty acids is done by steam distillation in a deodorizer. This simultaneously deodorizes the oil. Because deodorization is the last process normally carried out on edible oils, this step may be delayed until other processes, such as hydrogenation of the oil, have been done. Alkali-refined oil contains chlorophylloid compounds which give the oil a green colour, and catalyze oil oxidation. These compounds are removed by adsorptive bleaching with acid-activated clays.

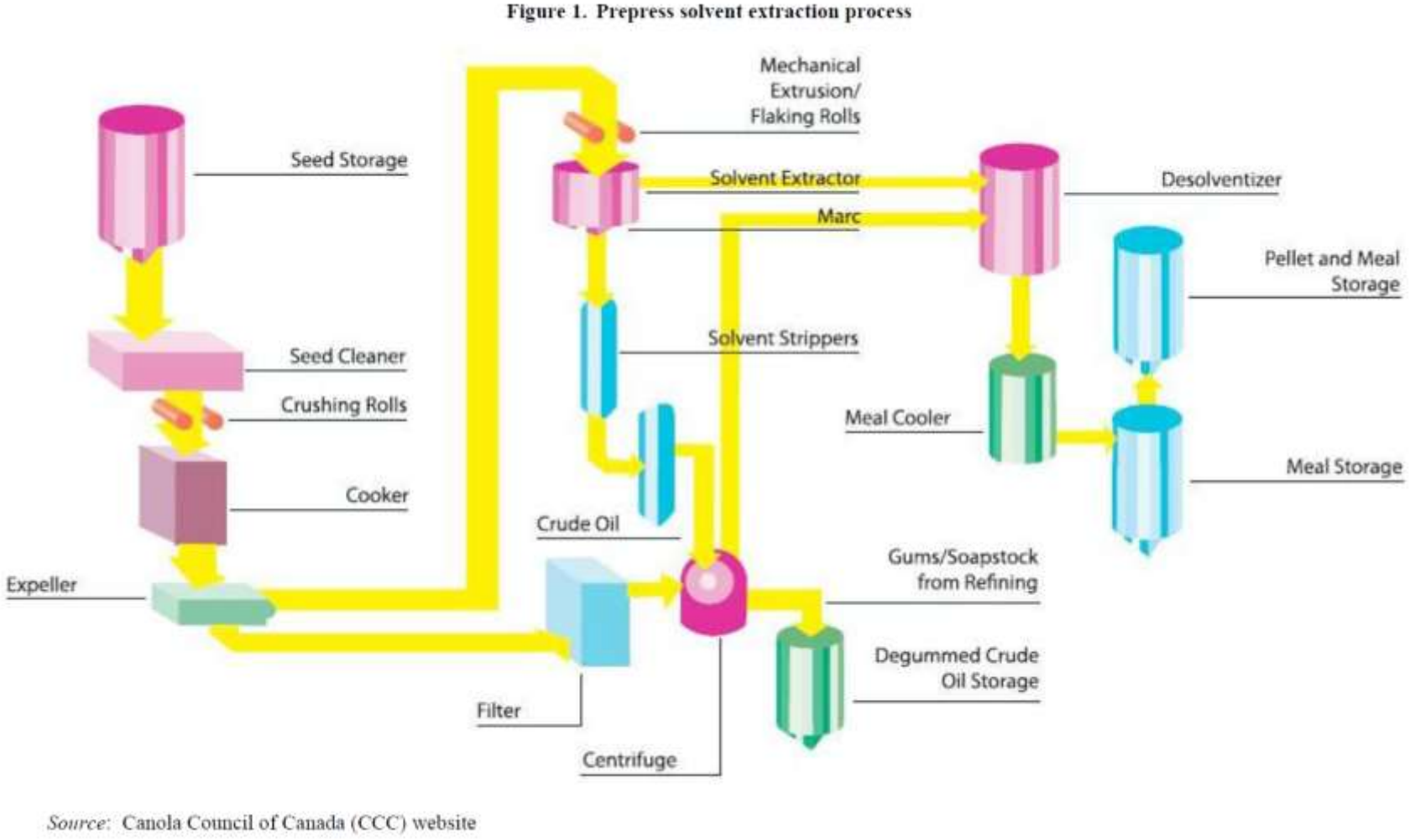


Figure 1. Schematic of canola oil and meal processing

A.3 The Nature of the Genetic Modification

(a) A description of the method used to transform the host organism.

Brassica napus seeds of variety N90-740 were germinated on solid germination medium. Hypocotyl segments were dissected from the *Brassica napus* seedlings and incubated on solid modified Murashige & Skoog (MS) medium for callus induction. Callus was isolated from the wounded sites of the hypocotyls and transferred to the same medium for embryogenic callus (EC) development.
Small clumps of EC were transformed with the vector pTCO113 using the vector system as described by Deblaere *et al*. (1987). This vector system consists of an *Agrobacterium* strain, C58C1Rlf (Van Larebeke *et al*., 1974), and two plasmid components namely a non-oncogenic helper Ti-plasmid pGV4000 and a T-DNA cloning vector pTCO113, essentially derived from pGSC1700 (Cornelissen and Vandewiele, 1989). The non-oncogenic helper Ti-plasmid pGV4000 from which the T-region has been deleted carries the *vir* genes required for transfer of an artificial T-DNA cloned on the second plasmid to the plant genome.
After this cocultivation, the transformed calli were selected on medium supplemented with glufosinate ammonium. After induction of somatic embryogenesis and regeneration into plantlets, the plantlets were transferred to the greenhouse for flowering, seed setting and further characterization. (see Table 3 below for a description of the vector) [redacted] (2008; M-307476-01-1; Node A.3 (a)).

(b) A description of the construct and the transformation vectors used, including:

(i) The size, source and function of all the genetic components including marker genes, regulatory and other elements; and

The genetic components comprising MS11 *B. napus* are detailed in Table 3 below, and in [redacted] (2015; M-182728-04-1; Node A.3 (b) (i), (ii)). The sequence between nucleotide positions 1 – 5865 bp represents the intended transgenic locus, with the remaining sequence comprising the vector backbone. These components are shown in Figure 2 in Section A.3(b)(ii) below.

Table 3 Genetic elements comprising the pTCO113 vector used in MS11 *B. napus*

GENETIC ELEMENT	NT POSITION	SIZE (KB)	ORIENTATION	DESCRIPTION AND FUNCTION	REFERENCE
RB	1 – 25			Right border region of the T-DNA of *Agrobacterium tumefaciens.*	Zambryski, 1988
	26 - 97			Polylinker sequences: sequence used in cloning	
3'g7	98-309		Counter clockwise	3' untranslated region of the TL-DNA gene 7 of the *Agrobacterium tumefaciens* octopine Ti plasmid.	Dhaese *et al*., 1983
	310-331			Polylinker sequences: sequence used in cloning	
bar gene	332 – 883		Counter clockwise	Coding sequence of the phosphinothricin acetyltransferase gene of *Streptomyces hygroscopicus*.	Thompson *et al*., 1987
PssuAt	884 – 2613		Counter clockwise	Promoter region of the ribulose-1,5-biphosphate carboxylase small subunit gene of *Arabidopsis thaliana*	Krebbers *et al*., 1988
	2614-2658			Polylinker sequences: sequence used in cloning	
3'nos	2659 – 2919		Counter clockwise	3'' untranslated region of the nopaline synthase gene from the T-DNA of pTiT37	Depicker *et al.*, 1982
	2920-2935			Polylinker sequences: sequence used in cloning	
3'barnase	2936 – 3033		Counter clockwise	3' untranslated region of the barnase gene from *Bacillus amyloliquefaciens*.	Hartley, 1988
barnase gene	3034 – 3369		Counter clockwise	Coding sequence of the *barnase* gene of *Bacillus amyloliquefaciens*.	Hartley, 1988
	3370-3371			Polylinker sequences: sequence used in cloning	
Pta29	3372 – 4879		Counter clockwise	Promoter of the anther-specific gene TA29 of *Nicotiana tabacum* (tobacco)..	Seurinck *et al*., 1990
	4880-4920			Polylinker sequences: sequence used in cloning	
Pnos	4921 – 5214		Clockwise	Promoter region of the nopaline synthase gene of *Agrobacterium tumefaciens*.	Depicker *et al.*,1982
	5215-5216			Polylinker sequences: sequence used in cloning	
barstar gene	5217 – 5489		Clockwise	Coding sequence of the *barstar* gene of *Bacillus amyloliquefaciens*.	Hartley, 1988
	5490-5554			Polylinker sequences: sequence used in cloning	
3'g7	5555 – 5766		Clockwise	3' untranslated region of the TL-DNA gene 7 of the *Agrobacterium tumefaciens* octopine Ti plasmid.	Dhaese *et al*., 1983
	5767-5840			Polylinker sequences: sequence used in cloning	
LB	5841 – 5865			Left border rion of the T-DNA of *Agrobacterium tumefaciens*.	Zambryski, 1988
aadA	5866 – 7745		Counter clockwise	Fragment including the aminoglycoside adenyltransferase gene of *Escherichia coli*.	Fling *et al*., 1985
barstar	7746 – 8181		Counter clockwise	Fragment including the barstar gene of *Bacillus amyloliquefaciens*.	Hartley, 1988
aadA	8182 – 8405		Counter clockwise	Fragment including the residual upstream sequences of the aminoglycoside adenyltransferase gene of *Escherichia coli*.	Fling *et al*., 1985

GENETIC ELEMENT	NT POSITION	SIZE (KB)	ORIENTATION	DESCRIPTION AND FUNCTION	REFERENCE
ORI pVS1	8406-12177			Fragment including the origin of replication of the plasmid pVS1 of *Pseudomonas aeruginosa*.	Heeb *et al*., 2000
ORI ColE1	12178 – 13540			Fragment including the origin of replication from the plasmid pBR322 for replication in *Escherichia coli*.	Bolivar *et al*., 1977

(ii) A detailed map of the location and orientation of all the genetic components contained within the construct and vector, including the location of relevant restriction sites.

A vector map of pTCO113, containing the genetic elements described above in Table 3, is presented below in Figure 2, and in [redacted] (2015; M-182728-04; Node A.3 (b) (i), (ii)). The locations of restriction sites within the transgenic locus are shown below in Figure 4 in Section A.3 (c)(i).

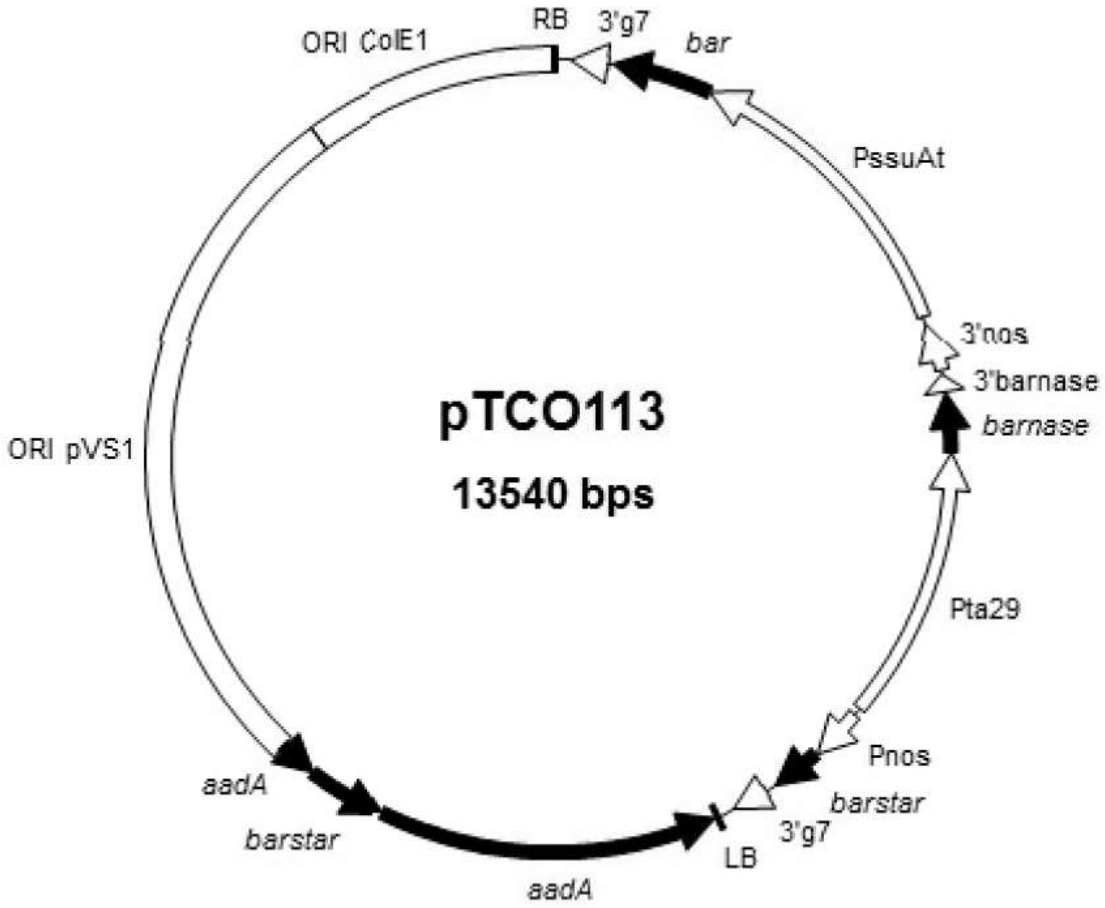


Figure 2 Map of plasmid vector pTCO113 used in MS11 *B. napus*

(c) A full molecular characterisation of the genetic modification in the new organism, including:

(i) Identification of all transferred genetic material and whether it has undergone any rearrangements;

The transgenic locus of MS11 *B. napus* was characterized by means of Southern blot analysis ([redacted]; 2016; M-547543-01, Node A.3 (c), (i) (CCI)).

Seeds from the T2 generation were used to produce MS11 *B. napus* leaf material. The identity of the leaf material was confirmed. Non-genetically modified (non-GM) *B. napus* variety N90-740 (non-GM counterpart) was used as a negative control. The positive control was the transforming plasmid of MS11 *B. napus* (pTCO113).

To characterize the transgenic locus of MS11, pooled gDNA from MS11 *B. napus* samples were digested with the restriction enzymes *Afl*III, *Bcl*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Hpa*I, *Kpn*I, *Mfe*I, *Nco*I, *Nde*I and *Sty*I. Pooled gDNA from plants of the non-GM counterpart was digested with the restriction enzyme *Eco*RI. Plasmid DNA of pTCO113 was digested with the *Eco*RI restriction enzyme.

The resulting DNA fragments were separated by agarose gel-electrophoresis. Transfer of the separated DNA fragments from the agarose gel to a positively charged nylon membrane was performed by a neutral Southern blotting procedure. The resulting membranes were hybridized with DIG-labeled probes covering the different components of the transgenic cassettes as well as the full T-DNA (P014, P016 to P023 and P028) (Figure 3). Table 4 provides details of the probes used in the Southern blot analysis. A schematic overview of the MS11 transgenic locus, with indication of the restriction enzymes, the probes used and the expected fragments is presented in Figure 4.

Each membrane contained one negative control, in which the template DNA was digested gDNA prepared from the non-GM counterpart. This negative control showed no hybridization with any of the probes used, confirming the absence of any background hybridization. Similarly, each membrane contained a positive control of digested gDNA prepared from non-GM plant material, supplemented with an equimolar amount of digested transforming plasmid pTCO113. For each of the probes used, the expected fragments were detected for the positive control, confirming that the applied experimental conditions allowed specific hybridization of the probes used with the target sequences. Hybridization of the positive control with the Pta29 probe showed a second band of >10 kb which is the result of incomplete digestion of the plasmid (Figure 11, lane 15).

The banding pattern expected for a single insertion was observed for MS11 *B. napus* samples with all restriction digests and probe combinations tested. (Table 5, Figure 5 to Figure 17).

Membranes containing gDNA digested with *Hpa*I and hybridized with the *barstar*, 3'barnase-*barnase*, Pta29, Pnos and the T-DNA probes

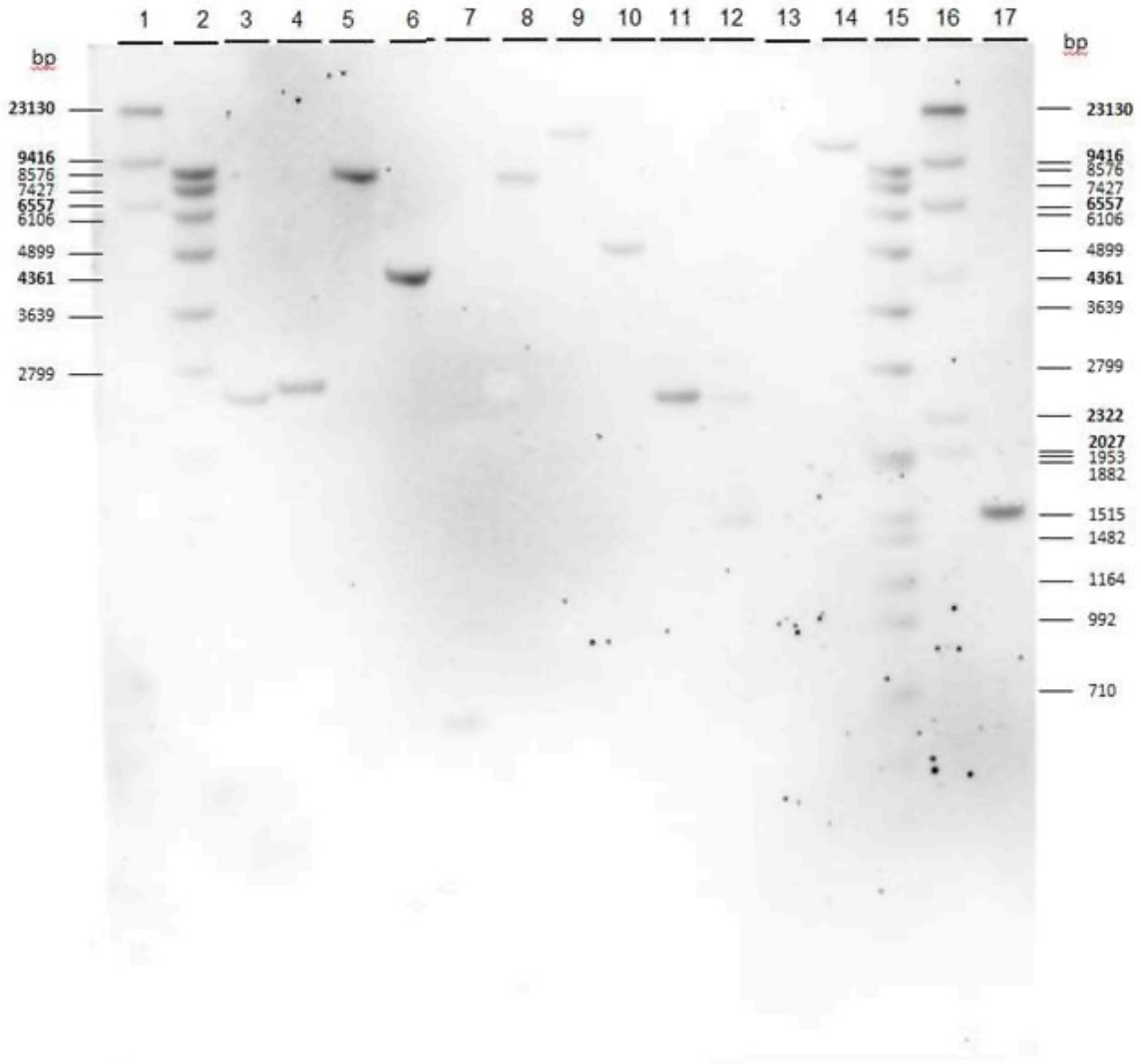


Figure **6**, Figure 10, Figure 11, Figure 12 and Figure 15, lane 8) showed an additional weak fragment of approx. 10 kb. The probes with which this fragment is visualized and the fact that the size of this fragment (approx. 10 kb) is an approximate summation of the 2296 bp internal fragment and a 8200 bp 3' integration fragment demonstrates that this fragment is the result of an incomplete digestion of the *Hpa*I restriction site within the Pta29 promoter. Hybridization of a freshly-prepared *Hpa*I digested gDNA sample with the T-DNA probe confirmed the presence of this incomplete digested fragment (Figure 16, lane 3).

Additionally, membranes containing gDNA digested with *Sty*I and hybridized with any of the probes (Figure 6, Figure 7 and Figure 12, lane 12; Figure 5, Figure 8 to Figure 11, Figure **14** and Figure 15, lane 13) resulted in a smear of fragments as a consequence of partially digested gDNA. To confirm the single copy model as present in the pTCO113 plasmid for MS11, the *Sty*I restriction digestion and Southern blot analysis was repeated. Hybridization of this freshly-prepared *Sty*I restriction digestion with the T-DNA probe (Figure 17, lane 3) resulted in all expected fragments and confirmed the single copy model as present in the pTCO113 plasmid for MS11.

Finally, the membrane containing gDNA digested with *Nco*I and hybridized with the Pnos probe (Figure 12, lane 11) showed two bands corresponding to both the integration fragments, whereas only hybridization with the 2500 bp fragment was expected. Since the hybridization signal with the 5300 bp

fragment was assumed to be the result of a not fully stripped membrane, the experiment was repeated. This hybridization (Figure 13, lane 3) confirmed the absence of a signal of the Pnos probe with the 5300 bp fragment.

In conclusion, the Southern blot results demonstrated the presence of one complete T-DNA insert containing the *bar*, the *barnase* and the *barstar* gene cassettes in MS11 *B. napus*.

Table 4: Information on the probes used

Probe ID	Probe template ID	Description	Primer pair/ Restr. digest	Primer sequence (5' → 3')	Primer position on pTCO113 (bp)	Size probe template (bp)
P014	PT023	*bar*	GLPA343	[redacted]	395 → 417	425
			GLPA344	[redacted]	819 → 796	
P016	PT035	*barstar*	GLPA345°	[redacted]	5226 → 5249 and 8049 → 8026	262°
			GLPA346°	[redacted]	5487 → 5465 and 7788 → 7810	
P017	PT073	RB - 3'g7	GLPA174	[redacted]	1 → 25	317 8311**
			GLPA048	[redacted]	317 → 293 and 5547 → 5571	
P018	PT092	3'nos	GLPA348	[redacted]	2666 → 2686	217
			GLPA349	[redacted]	2882 → 2861	
P019	PT108	PssuAt	GLPA001	[redacted]	855 → 876	1870
			GLPA005	[redacted]	2724 → 2702	
P020	PT109	3'barnase - *barnase*	GLPA006	[redacted]	2860 → 2879	573
			GLPA009	[redacted]	3432 → 3405	
P021	PT110	Pta29	GLPA012	[redacted]	3290 → 3324	1660
			GLPA013	[redacted]	4949 → 4928	
P022	PT111	Pnos	GLPA015	[redacted]	4873 → 4891	374 10758***
			GLPA017	[redacted]	5246 → 5225 and 8029 → 8050	
P023	PT116	3'g7 - LB	GLPA020	[redacted]	5516 → 5535 and 7759 → 7740	350 2244****
			GLPA359	[redacted]	5865 → 5841	
P028	PT108	T-DNA	GLPA174	[redacted]	1 → 25	5865
			GLPA359	[redacted]	5865 → 5841	

NA means not applicable
** An additional PCR product of 8311 bp can be produced
*** An additional PCR product of 10758 bp can be produced
**** An additional PCR product of 2244 bp can be produced
° These primers amplify two identical regions

Table 5: Expected and obtained hybridization fragments determined for the insert characterization of MS11 *B. napus*

Part 1:

Enzyme	Expected fragment size (bp)	Fragment description	Obtained fragment size (bp)	H4/LJS018/08-F5		H1/LJS018/06-F9		H4/LJS018/06-F4		H8/LJS018/09-F2		H5/LJS018/09-F3		H1/LJS018/10-F4	
				P014-2		P016-1		P017-3		P018-4		P019-2		P020-2	
				bar		*Barstar*		RB-3'g7		3'nos		PssuAt		3'barnase-*barnase*	
				Figure 5		Figure 6		Figure 7		Figure 8		Figure 9		Figure 10	
				Expected	Obtained	Expected	Obtained	Expected	Obtained	Expected	Obtained	Expected	Obtained	Expected	Obtained
***Afl*III**	>305	5' integration fr.	4300	No	No	No	No	Yes	Yes	No	No	No	No	No	No
	2476	internal fr.	2476	Yes	Yes	No	No	No	No	Yes ** (132)	Yes	Yes	Yes	No	No
	550	internal fr.	550	No	No	No	No	No	No	Yes ** (84)	No	No	No	Yes	Yes
	>2467	3' integration fr	2500	No	No	Yes	Yes	Yes	Yes	No	No	No	No	Yes ** (84)	No
***Bcl*I**	>1637	5' integration fr.	1850	Yes	Yes	No	No	Yes	Yes	No	No	Yes	Yes	No	No
	2761	internal fr.	2761	No	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes
	509	internal fr.	509	No	No	No	No	No	No	No	No	No	No	No	No
	>891	3' integration fr.	2650	No	No	Yes	Yes	Yes	Yes	No	No	No	No	No	No
***Eco*RI**	>2614	5' integration fr.	> 10 kb	Yes	Yes	No	No	Yes	Yes	No	No	Yes	Yes	No	No
	2260	internal fr.	2260	No	No	No	No	No	No	Yes	Yes	Yes ** (93)	No	Yes	Yes
	>924	3' integration fr.	8400	No	No	Yes	Yes	Yes	Yes	No	No	No	No	No	No
***Eco*RV**	>3895	5' integration fr.	4900	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	>1903	3' integration fr.	4400	No	No	Yes	Yes	Yes	Yes	No	No	No	No	No	No
***Hin*dIII**	>948	5' integration fr.	2100	Yes	Yes	No	No	Yes	Yes	No	No	Yes ** (110)	No	No	No
	3938	internal fr.	3938	No	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes
***Hin*dIII**	629	internal fr.	629	No	No	Yes	Yes	No	No	No	No	No	No	No	No
	>283	3' integration fr.	1450	No	No	No	No	Yes	Yes	No	No	No	No	No	No
***Hpa*I**	>1867	5' integration fr.	3200	Yes	Yes	No	No	Yes	Yes	No	No	Yes	Yes	No	No

Enzyme	Expected fragment size (bp)	Fragment description	Obtained fragment size (bp)	H4/LJS018/08-F5		H1/LJS018/06-F9		H4/LJS018/06-F4		H8/LJS018/09-F2		H5/LJS018/09-F3		H1/LJS018/10-F4	
				P014-2		P016-1		P017-3		P018-4		P019-2		P020-2	
				bar		*Barstar*		RB-3'g7		3'nos		PssuAt		3'barnase-*barnase*	
				Figure 5		Figure 6		Figure 7		Figure 8		Figure 9		Figure 10	
				Expected	Obtained	Expected	Obtained	Expected	Obtained	Expected	Obtained	Expected	Obtained	Expected	Obtained
	2296	internal fr.	2296	No	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes
	>1635	3' integration fr.	8200	No	No	Yes	Yes	Yes	Yes	No	No	No	No	No	No
		Additional fr.	>10 kb	No	No	No	Yes	No	No	No	No	No	No	No	Yes
***Kpn*I**	>349	5' integration fr.	7000	No	No	No	No	Yes	Yes	No	No	No	No	No	No
	2256	internal fr.	2256	Yes	Yes	No	No	No	No	No	No	Yes	Yes	No	No
	21*	internal fr.	NA	No	No	No	No	No	No	No	No	Yes ** (21)	No	No	No
	719	internal fr.	719	No	No	No	No	No	No	Yes	Yes	Yes ** (81)	No	Yes	Yes
	>2453	3' integration fr.	>10 kb	No	No	Yes	Yes	Yes	Yes	No	No	No	No	Yes ** (70)	No
***Mfe*I**	>3827	5' integration fr.	4100	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	>1971	3' integration fr.	5000	No	No	Yes	Yes	Yes	Yes	No	No	No	No	No	No
***Nco*I**	>3349	5' integration fr.	5300	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	>2449	3' integration fr.	2500	No	No	Yes	Yes	Yes	Yes	No	No	No	No	Yes ** (66)	No
		Additional fr.	5300	No	No	No	No	No	No	No	No	No	No	No	No
***Nde*I**	>4557	5' integration fr.	6900	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	64*	internal fr.	NA	No	No	No	No	No	No	No	No	No	No	No	No
	>1177	3' integration fr.	1600	No	No	Yes	Yes	Yes	Yes	No	No	No	No	No	No
***Sty*I**	>1279	5' integration fr.	3000	Yes	Unknown	No	Unknown	Yes	Unknown	No	Unknown	Yes	Unknown	No	No
	2070	internal fr.	2070	No		No		No		Yes		Yes		Yes	No
	982	internal fr.	982	No		No		No		No		No		Yes ** (66)	No
	>1467	3' integration fr.	1500	No		Yes		Yes		No		No		No	No
		Additional fr.		No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	No

Enzyme	Expected fragment size (bp)	Fragment description	Obtained fragment size (bp)	H4/LJS018/08-F5		H1/LJS018/06-F9		H4/LJS018/06-F4		H8/LJS018/09-F2		H5/LJS018/09-F3		H1/LJS018/10-F4	
				P014-2		P016-1		P017-3		P018-4		P019-2		P020-2	
				bar		*Barstar*		RB-3'g7		3'nos		PssuAt		3'barnase-*barnase*	
				Figure 5		Figure 6		Figure 7		Figure 8		Figure 9		Figure 10	
				Expected	Obtained	Expected	Obtained	Expected	Obtained	Expected	Obtained	Expected	Obtained	Expected	Obtained
Non-GM counterpart genomic DNA *Eco*RI digested	/	negative control	NA	No	No	No	No	No	No	No	No	No	No	No	No
Non-GM counterpart genomic DNA *Eco*RI digested + 1 equimolar amount pTCO113 *Eco*RI digested	2260	positive control	2260	No	No	No	No	No	No	Yes	Yes	No	No	Yes	Yes
	11280		11280	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	No	No

Table 5: Expected and obtained hybridization fragments determined for the insert characterization of MS11 *B. napus*

Part 2:

Enzyme	Expected fragment size (bp)	Fragment description	Obtained fragment size (bp)	H3/LJS018/09-F2		H6/LJS018/06-F2		H1/LJS018/18-F2	H5/LJS018/08-F4		H6/LJS018/08-F4		H1/LJS018/16-F5	H1/LJS018/15-F3
				P021-2		P022-2		P022-3	P023-2		P028-12		P028-8	P028-12
				Pta29		Pnos			3'g7-LB		T-DNA probe			
				Figure 11			Figure 12	Figure 13	Figure 14			Figure 14	Figure 16	Figure 17
				Expected	Obtained	Expected	Obtained		Expected	Obtained	Expected	Obtained		
***Afl*III**	>305	5' integration fr.	4300	No	No	No	No	N.A.	Yes	Yes	Yes ** (119)	No	N.A.	N.A.
	2476	internal fr.	2476	No	No	No	No	N.A.	No	No	Yes	Yes$	N.A.	N.A.
	550	internal fr.	550	Yes ** (58)	No	No	No	N.A.	No	No	Yes	No	N.A.	N.A.
	>2467	3' integration fr	2500	Yes	Yes	Yes	Yes	N.A.	Yes	Yes	Yes	Yes$	N.A.	N.A.
***Bcl*I**	>1637	5' integration fr.	1850	No	No	No	No	N.A.	Yes	Yes	Yes	Yes	N.A.	N.A.
	2761	internal fr.	2761	Yes	Yes	No	No	N.A.	No	No	Yes	Yes$	N.A.	N.A.
	509	internal fr.	509	Yes	Yes	Yes ** (52)	No	N.A.	No	No	Yes	No °	N.A.	N.A.
	>891	3' integration fr.	2650	Yes ** (25)	No	Yes	Yes	N.A.	Yes	Yes	Yes	Yes$	N.A.	N.A.
***Eco*RI**	>2614	5' integration fr.	> 10 kb	No	No	No	No	N.A.	Yes	Yes	Yes	Yes	N.A.	N.A.
	2260	internal fr.	2260	Yes	Yes	Yes ** (19)	No	N.A.	No	No	Yes	Yes	N.A.	N.A.
	>924	3' integration fr.	8400	Yes ** (58)	Yes	Yes	Yes	N.A.	Yes	Yes	Yes	Yes	N.A.	N.A.
***Eco*RV**	>3895	5' integration fr.	4900	Yes	Yes	No	No	N.A.	Yes	Yes	Yes	Yes	N.A.	N.A.
	>1903	3' integration fr.	4400	Yes	Yes	Yes	Yes	N.A.	Yes	Yes	Yes	Yes	N.A.	N.A.
***Hind*III**	>948	5' integration fr.	2100	No	No	No	No	N.A.	Yes	Yes	Yes	Yes	N.A.	N.A.
	3938	internal fr.	3938	Yes	Yes	Yes ** (31)	No	N.A.	No	No	Yes	Yes	N.A.	N.A.
	629	internal fr.	629	Yes ** (46)	Yes	Yes	Yes	N.A.	Yes ** (16)	No	Yes	No	N.A.	N.A.
***Hind*III**	>283	3' integration fr.	1450	No	No	No	No	N.A.	Yes	Yes	Yes ** (128)	No	N.A.	N.A.
***Hpa*I**	>1867	5' integration fr.	3200	No	No	No	No	N.A.	Yes	Yes	Yes	Yes	Yes	N.A.
	2296	internal fr.	2296	Yes	Yes	No	No	N.A.	No	No	Yes	Yes	Yes	N.A.

Enzyme	Expected fragment size (bp)	Fragment description	Obtained fragment size (bp)	H3/LJS018/09-F2		H6/LJS018/06-F2		H1/LJS018/18-F2	H5/LJS018/08-F4		H6/LJS018/08-F4		H1/LJS018/16-F5	H1/LJS018/15-F3
				P021-2		P022-2		P022-3	P023-2		P028-12		P028-8	P028-12
				Pta29		Pnos			3'g7-LB		T-DNA probe			
				Figure 11			Figure 12	Figure 13	Figure 14			Figure 14	Figure 16	Figure 17
				Expected	Obtained	Expected	Obtained		Expected	Obtained	Expected	Obtained		
	>1635	3' integration fr.	8200	Yes	Yes	Yes	Yes	N.A.	Yes	Yes	Yes	Yes	Yes	N.A.
		Additional fr.	>10 kb	No	Yes	No	Yes	N.A.	No	No	No	Yes	Yes	N.A.
***Kpn*I**	>349	5' integration fr.	7000	No	No	No	No	N.A.	Yes	Yes	Yes ** [163]	No	N.A.	N.A.
	2256	internal fr.	2256	No	No	No	No	N.A.	No	No	Yes	Yes	N.A.	N.A.
	21*	internal fr.	NA	No	No	No	No	N.A.	No	No	Yes ** [21]	No	N.A.	N.A.
	719	internal fr.	719	Yes ** [72]	Yes	No	No	N.A.	No	No	Yes	No	N.A.	N.A.
	>2453	3' integration fr.	>10 kb	Yes	Yes	Yes	Yes	N.A.	Yes	Yes	Yes	Yes	N.A.	N.A.
***Mfe*I**	>3827	5' integration fr.	4100	Yes	Yes	No	No	N.A.	Yes	Yes	Yes	Yes	N.A.	N.A.
	>1971	3' integration fr.	5000	Yes	Yes	Yes	Yes	N.A.	Yes	Yes	Yes	Yes	N.A.	N.A.
***Nco*I**	>3349	5' integration fr.	5300	Yes ** [76]	Yes	No	No	No	Yes	Yes	Yes	Yes	N.A.	N.A.
	>2449	3' integration fr.	2500	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	N.A.	N.A.
		Additional fr.	5300	No	No	No	Yes	No	No	No	No	No	N.A.	N.A.
***Nde*I**	>4557	5' integration fr.	6900	Yes	Yes	No	No	N.A.	Yes	Yes	Yes	Yes	N.A.	N.A.
	64*	internal fr.	NA	Yes ** [64]	No	No	No	N.A.	No	No	Yes ** [64]	No	N.A.	N.A.
	>1177	3' integration fr.	1600	Yes	Yes	Yes	Yes	N.A.	Yes	Yes	Yes	Yes	N.A.	N.A.
***Sty*I**	>1279	5' integration fr.	3000 [b]	No	Unknown	No	Unknown	N.A.	Yes	Unknown	Yes	Unknown	N.A.	Yes [b]
	2070	internal fr.	2070	Yes ** [76]		No		N.A.	No		Yes		N.A.	Yes
	982	internal fr.	982	Yes		No		N.A.	No		Yes		N.A.	Yes
	>1467	3' integration fr.	1500 [b]	Yes		Yes		N.A.	Yes		Yes		N.A.	Yes [b]
		Additional fr.		No	Yes	No	Yes	N.A.	No	Yes	No	Yes	N.A.	No

Enzyme	Expected fragment size (bp)	Fragment description	Obtained fragment size (bp)	H3/LJS018/09-F2		H6/LJS018/06-F2		H1/LJS018/18-F2	H5/LJS018/08-F4		H6/LJS018/08-F4		H1/LJS018/16-F5	H1/LJS018/15-F3
				P021-2		P022-2		P022-3	P023-2		P028-12		P028-8	P028-12
				Pta29		Pnos			3'g7-LB		T-DNA probe			
				Figure 11			Figure 12	Figure 13	Figure 14			Figure 14	Figure 16	Figure 17
				Expected	Obtained	Expected	Obtained		Expected	Obtained	Expected	Obtained		
Non-GM counterpart genomic DNA *Eco*RI digested	/	negative control	NA	No	No	No	No	No	No	No	No	No	No	No
Non-GM counterpart genomic DNA *Eco*RI digested + 1 equimolar amount pTCO113 *Eco*RI digested	2260	positive control	2260	Yes	Yes	No	No	No	No	No	Yes	Yes	Yes	Yes
	11280		11280	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

* Based on the technical limits of Southern Blotting, this fragment might be too small to be visualized.

** Due to a small overlap with the probe, these framgents may not be visible. The size of the overlap is indicated between brackets.

$: These bands have a comparable size and cannot be distinguished using this experimental setup

° This band is not observed because of the small size of the fragment in comparison with the large probe

[a] this band overlaps with the 2761 bp internal fragment

[b] With this experimental setup, it is not possible to determine if this fragment represents the 5' or 3' integration fragment

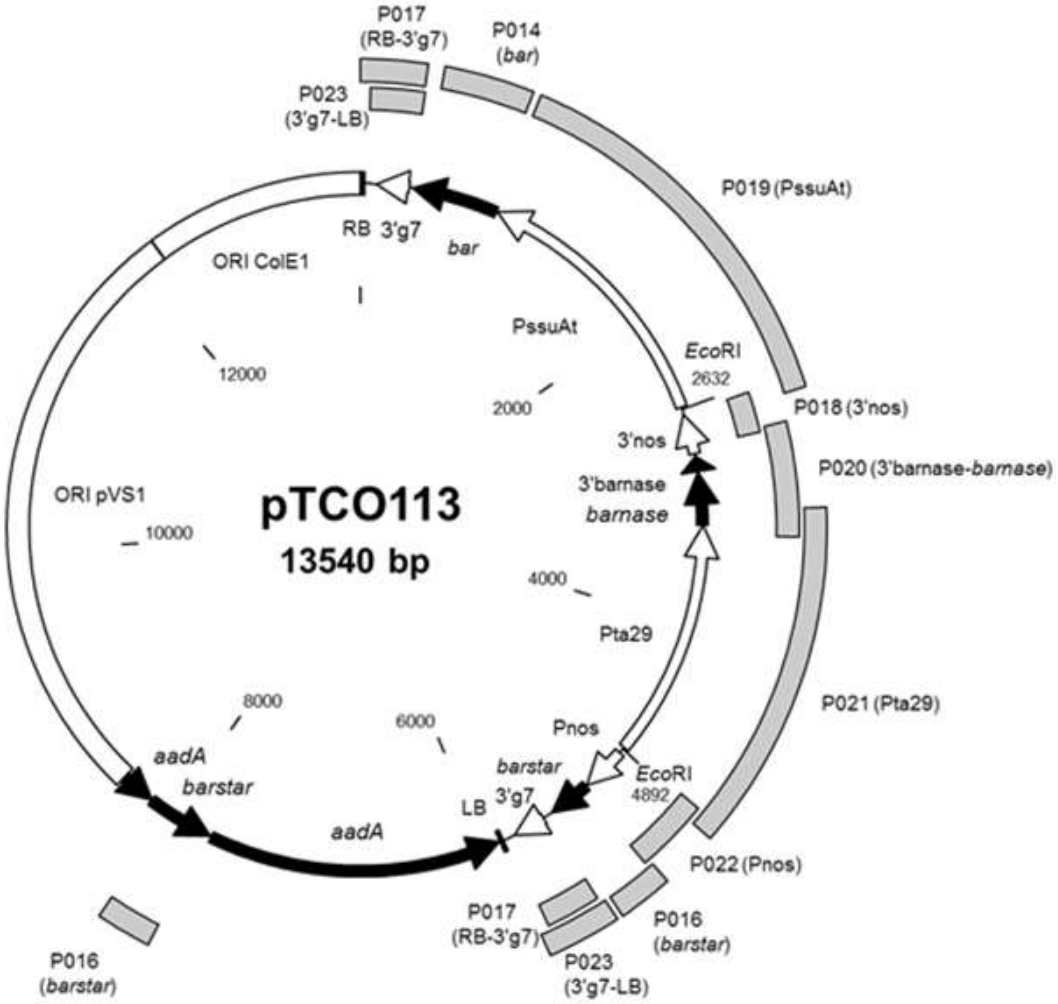


Figure 3: Map of transformation vector pTCO113 with indication of the position of enzymes used for plasmid digestion and the probes covering the different individual features of the T-DNA region.

The indicated restriction enzyme positions between brackets refer to the first base after the cleavage site of the restriction enzyme.

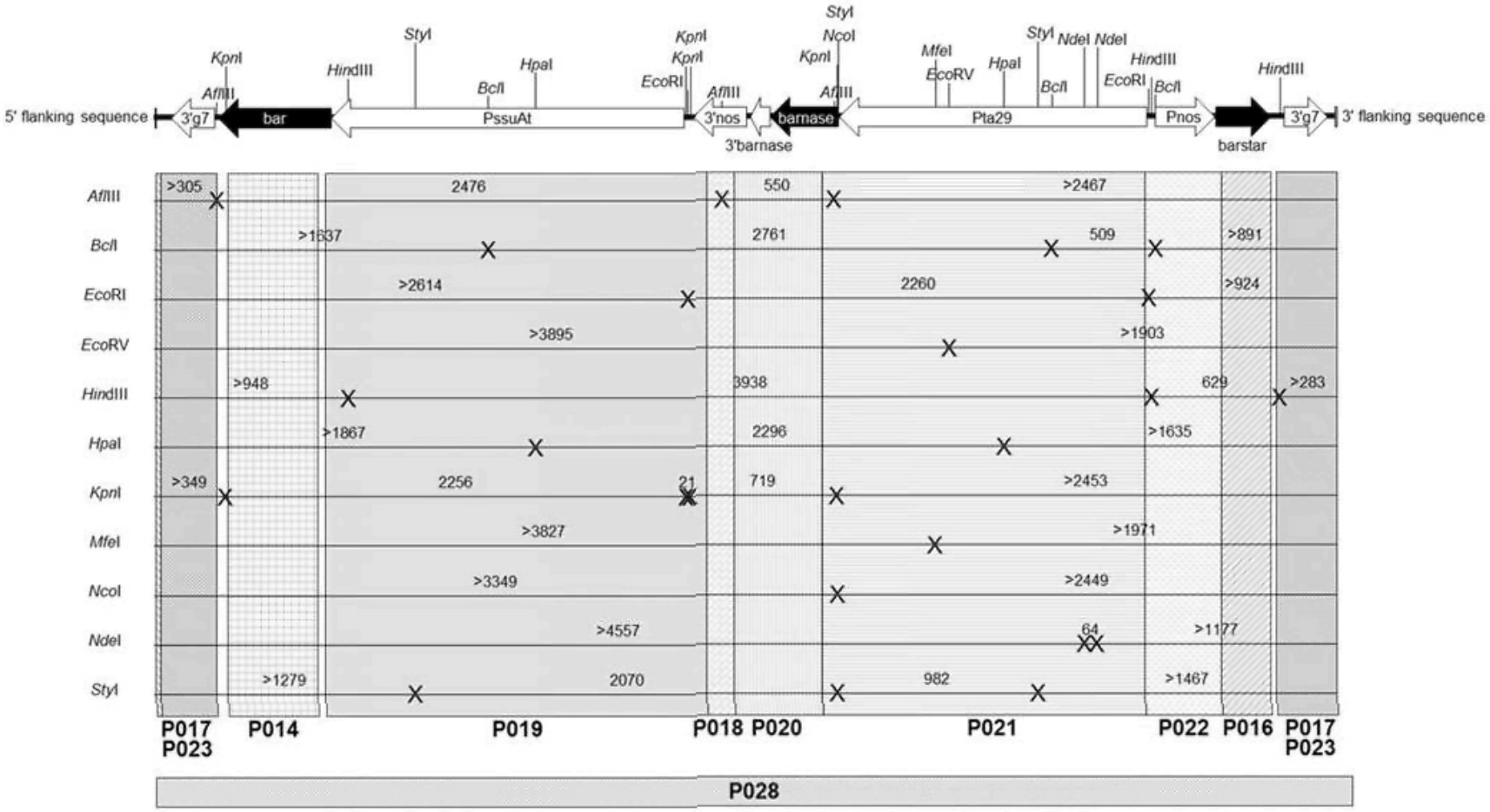


Figure 4: Schematic overview of the MS11 *B. napus* transgenic locus with indication of the different restriction enzymes and probes used in this study to assess the insert organization, and expected fragment sizes in bp

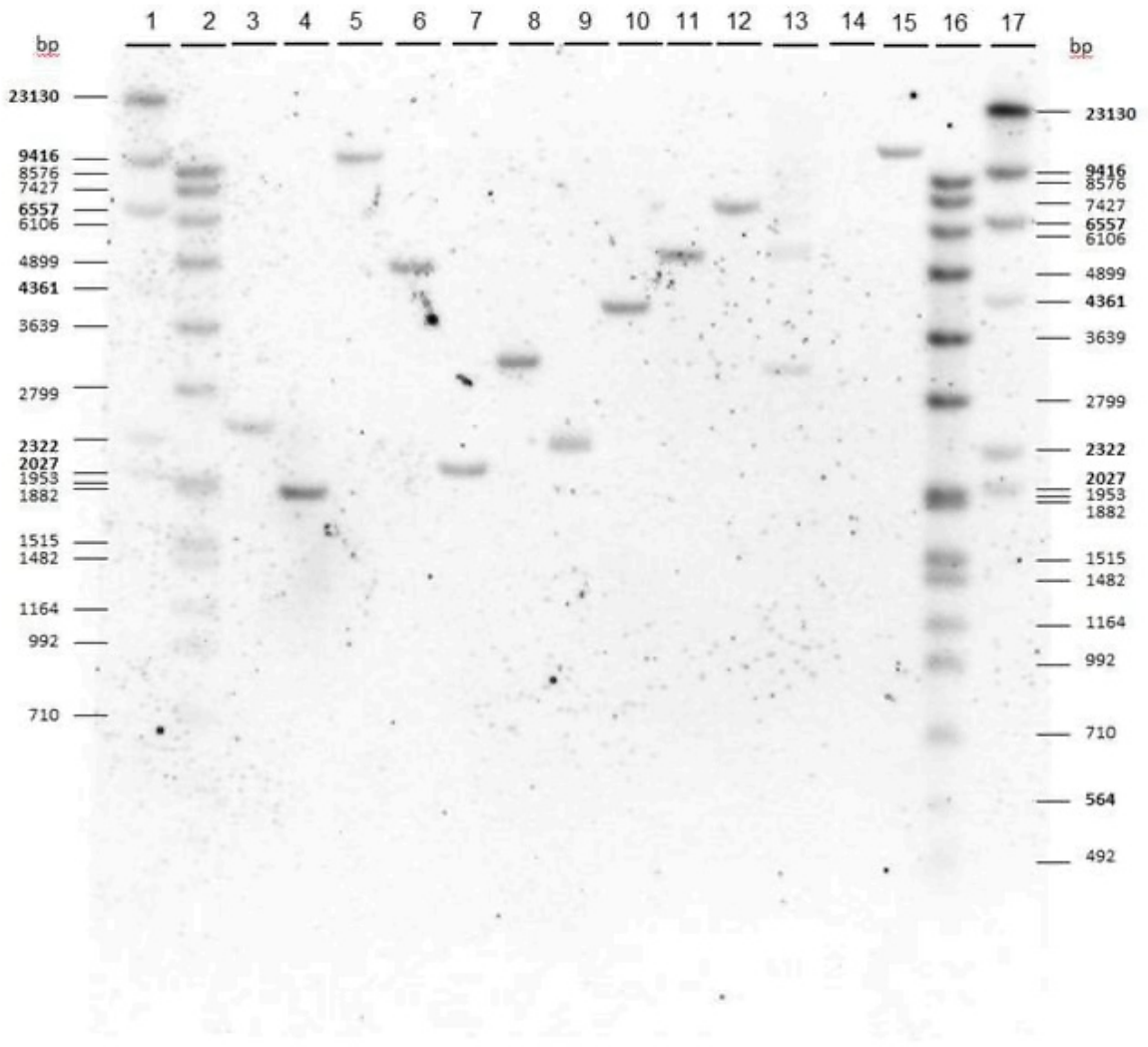


Figure 5: Hybridization performed with a *bar* probe (P014) to determine the insert organization of MS11 *B. napus*.

gDNA was isolated from MS11 *B. napus* plants and from the non-GM counterpart. The gDNA samples were digested with different restriction enzymes and hybridized with a probe corresponding to the MS11 *bar* sequence (P014-2, PCR labeling).

Lane 1: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)
Lane 2: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 3: 5 µg gDNA from MS11 *B. napus* - *Afl*III digested
Lane 4: 5 µg gDNA from MS11 *B. napus* - *Bcl*I digested
Lane 5: 5 µg gDNA from MS11 *B. napus* - *Eco*RI digested
Lane 6: 5 µg gDNA from MS11 *B. napus* - *Eco*RV digested
Lane 7: 5 µg gDNA from MS11 *B. napus* - *Hin*dIII digested
Lane 8: 5 µg gDNA from MS11 *B. napus* - *Hpa*I digested
Lane 9: 5 µg gDNA from MS11 *B. napus* - *Kpn*I digested
Lane 10: 5 µg gDNA from MS11 *B. napus* - *Mfe*I digested
Lane 11: 5 µg gDNA from MS11 *B. napus* - *Nco*I digested
Lane 12: 5 µg gDNA from MS11 *B. napus* - *Nde*I digested
Lane 13: 5 µg gDNA from MS11 *B. napus* - *Sty*I digested
Lane 14: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested
Lane 15: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested + an equimolar amount of pTCO113 - *Eco*RI digested
Lane 16: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 17: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)

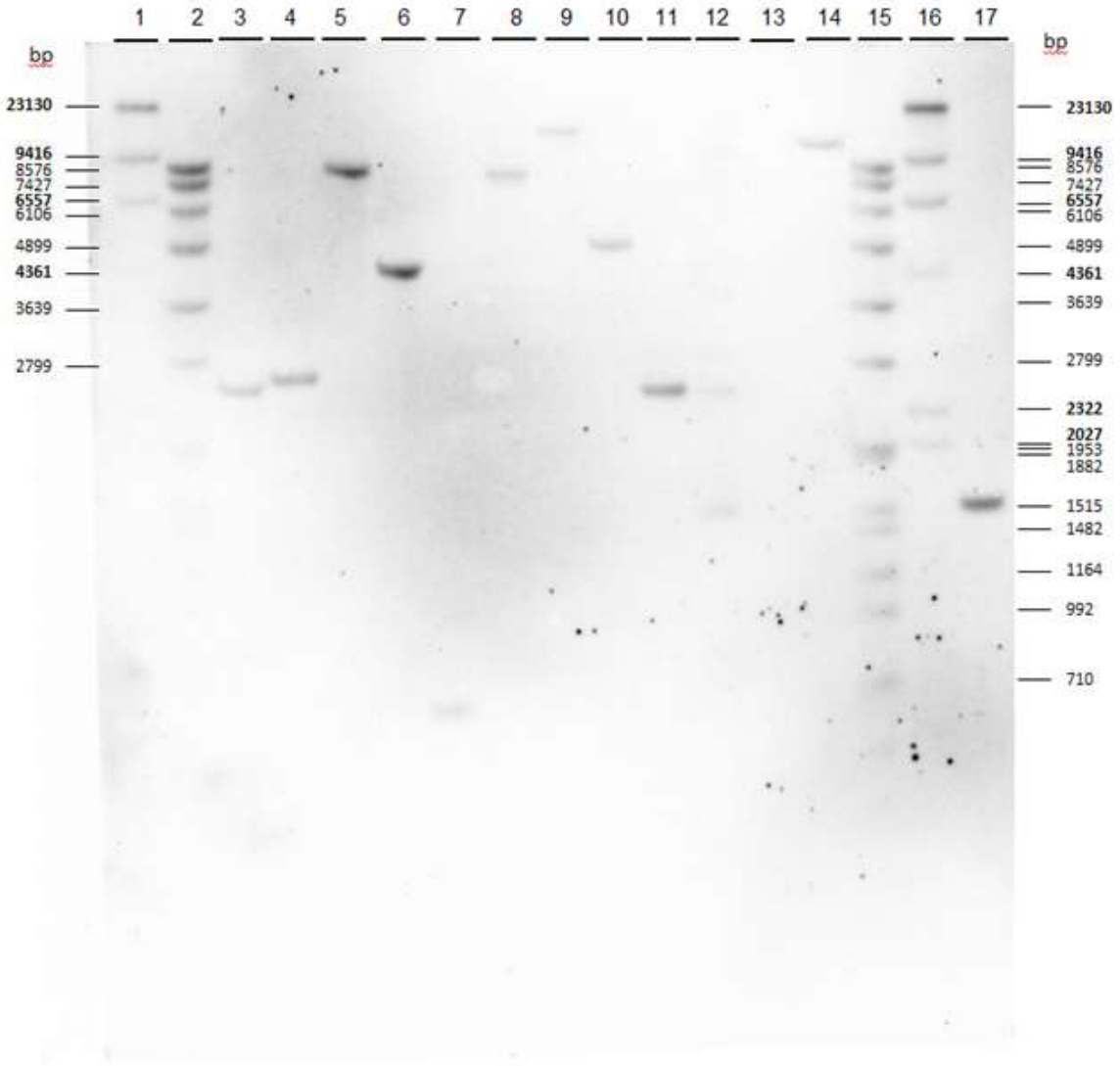


Figure 6: Hybridization performed with a barstar probe (P016) to determine the insert organization of MS11 *B. napus*.

gDNA was isolated from MS11 *B. napus* plants and from the non-GM counterpart. The gDNA samples were digested with different restriction enzymes and hybridized with a probe corresponding to the MS11 *barstar* sequence (P016-1, random primed labeling).The size of the low molecular weight band in lane 7 is determined using another exposure of this membrane (data not shown).

Lane 1: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)
Lane 2: 5 µg gDNA from the non-GM counterpart- *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 3: 5 µg gDNA from MS11 *B. napus* - *Afl*III digested
Lane 4: 5 µg gDNA from MS11 *B. napus* - *Bcl*I digested
Lane 5: 5 µg gDNA from MS11 *B. napus* - *Eco*RI digested
Lane 6: 5 µg gDNA from MS11 *B. napus* - *Eco*RV digested
Lane 7: 5 µg gDNA from MS11 *B. napus* - *Hin*dIII digested
Lane 8: 5 µg gDNA from MS11 *B. napus* - *Hpa*I digested
Lane 9: 5 µg gDNA from MS11 *B. napus* - *Kpn*I digested
Lane 10: 5 µg gDNA from MS11 *B. napus* - *Mfe*I digested
Lane 11: 5 µg gDNA from MS11 *B. napus* - *Nco*I digested
Lane 12: 5 µg gDNA from MS11 *B. napus* - *Sty*I digested
Lane 13: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested
Lane 14: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested + an equimolar amount of pTCO113 - *Eco*RI digested
Lane 15: 5 µg gDNA from the non-GM counterpart- *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 16: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)
Lane 17: 5 µg gDNA from MS11 *B. napus* - *Nde*I digested

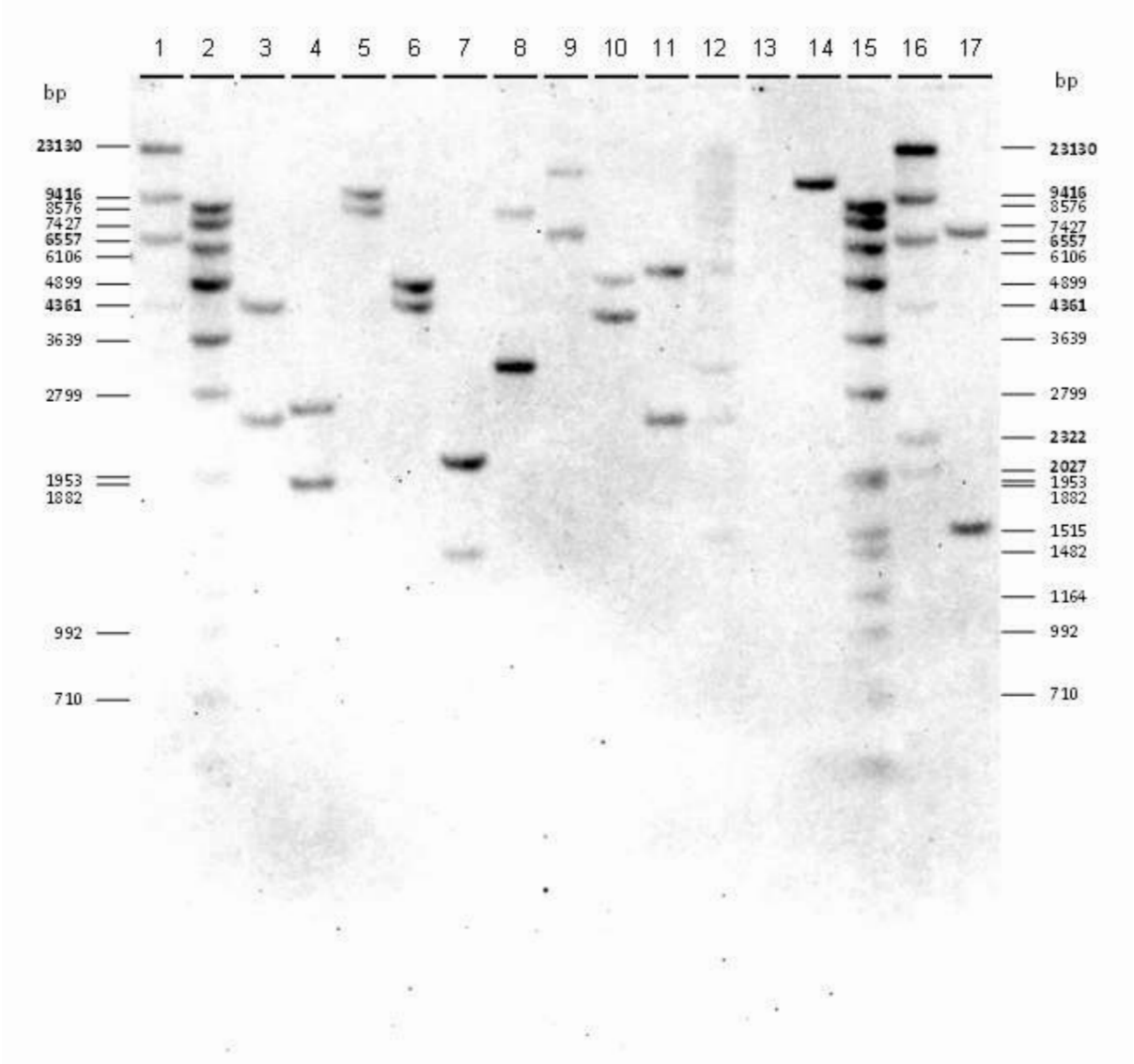


Figure 7: Hybridization performed with a RB-3'g7 probe (P017) to determine the insert organization of MS11 *B. napus*.

gDNA was isolated from MS11 *B. napus* plants and from the non-GM counterpart. The gDNA samples were digested with different restriction enzymes and hybridized with a probe corresponding to the MS11 RB-3'g7 sequence (P017-3, PCR labeling).

Lane 1: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)
Lane 2: 5 µg gDNA from the non-GM counterpart- *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 3: 5 µg gDNA from MS11 *B. napus* - *Afl*III digested
Lane 4: 5 µg gDNA from MS11 *B. napus* - *Bcl*I digested
Lane 5: 5 µg gDNA from MS11 *B. napus* - *Eco*RI digested
Lane 6: 5 µg gDNA from MS11 *B. napus* - *Eco*RV digested
Lane 7: 5 µg gDNA from MS11 *B. napus* - *Hin*dIII digested
Lane 8: 5 µg gDNA from MS11 *B. napus* - *Hpa*I digested
Lane 9: 5 µg gDNA from MS11 *B. napus* - *Kpn*I digested
Lane 10: 5 µg gDNA from MS11 *B. napus* - *Mfe*I digested
Lane 11: 5 µg gDNA from MS11 *B. napus* - *Nco*I digested
Lane 12: 5 µg gDNA from MS11 *B. napus* - *Sty*I digested
Lane 13: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested
Lane 14: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested + an equimolar amount of pTCO113 - *Eco*RI digested
Lane 15: 5 µg gDNA from the non-GM counterpart- *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 16: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)
Lane 17: 5 µg gDNA from MS11 *B. napus* - *Nde*I digested

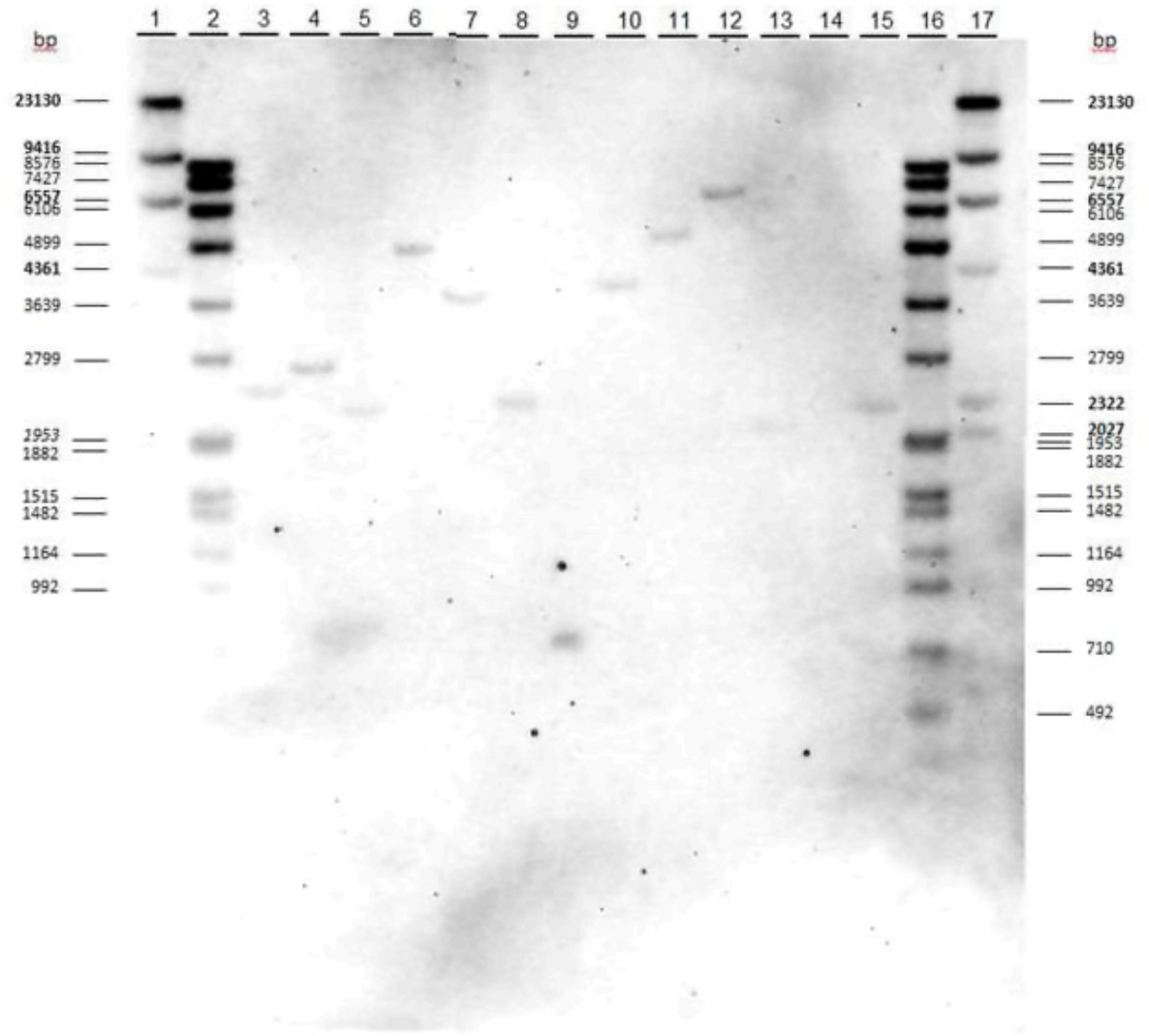


Figure 8: Hybridization performed with a 3'nos probe (P018) to determine the insert organization of MS11 *B. napus*.

gDNA was isolated from MS11 *B. napus* plants and from the non-GM counterpart. The gDNA samples were digested with different restriction enzymes and hybridized with a probe corresponding to the MS11 3'nos sequence (P018-4, PCR labeling).
Lane 1: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)
Lane 2: 5 µg gDNA from the non-GM counterpart- *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 3: 5 µg gDNA from MS11 *B. napus* - *Afl*III digested
Lane 4: 5 µg gDNA from MS11 *B. napus* - *Bcl*I digested
Lane 5: 5 µg gDNA from MS11 *B. napus* - *Eco*RI digested
Lane 6: 5 µg gDNA from MS11 *B. napus* - *Eco*RV digested
Lane 7: 5 µg gDNA from MS11 *B. napus* - *Hin*dIII digested
Lane 8: 5 µg gDNA from MS11 *B. napus* - *Hpa*I digested
Lane 9: 5 µg gDNA from MS11 *B. napus* - *Kpn*I digested
Lane 10: 5 µg gDNA from MS11 *B. napus* - *Mfe*I digested
Lane 11: 5 µg gDNA from MS11 *B. napus* - *Nco*I digested
Lane 12: 5 µg gDNA from MS11 *B. napus* - *Nde*I digested
Lane 13: 5 µg gDNA from MS11 *B. napus* - *Sty*I digested
Lane 14: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested
Lane 15: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested + an equimolar amount of pTCO113 - *Eco*RI digested
Lane 16: 5 µg gDNA from the non-GM counterpart- *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)

Lane 17: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)

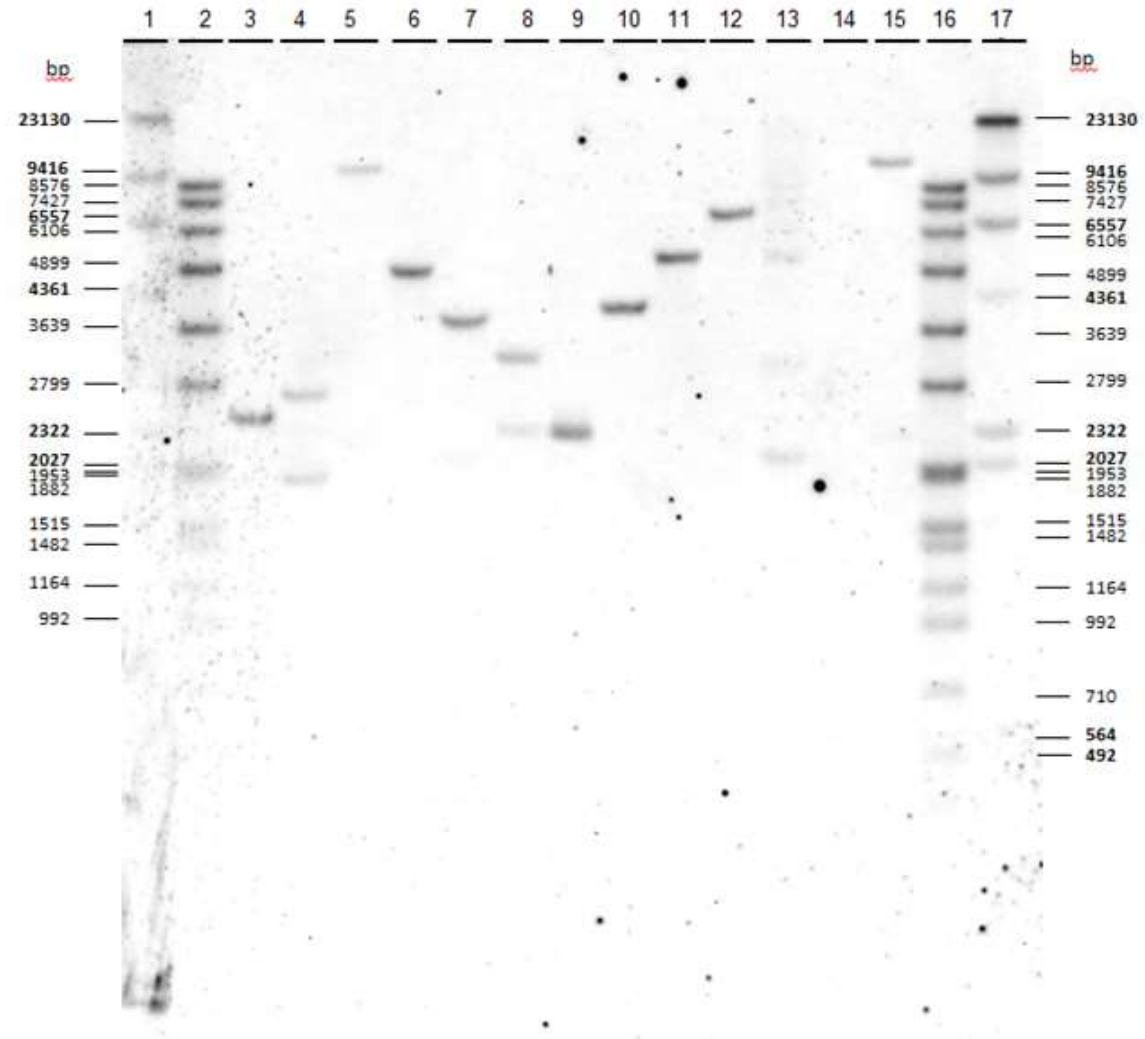


Figure 9: Hybridization performed with a PssuAt probe (P019) to determine the insert organization of MS11 *B. napus*.

gDNA was isolated from MS11 *B. napus* plants and from the non-GM counterpart. The gDNA samples were digested with different restriction enzymes and hybridized with a probe corresponding to the MS11 PssuAt sequence (P019-2, PCR labeling).

Lane 1: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)
Lane 2: 5 µg gDNA from the non-GM counterpart- *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 3: 5 µg gDNA from MS11 *B. napus* - *Afl*III digested
Lane 4: 5 µg gDNA from MS11 *B. napus* - *Bcl*I digested
Lane 5: 5 µg gDNA from MS11 *B. napus* - *Eco*RI digested
Lane 6: 5 µg gDNA from MS11 *B. napus* - *Eco*RV digested
Lane 7: 5 µg gDNA from MS11 *B. napus* - *Hin*dIII digested
Lane 8: 5 µg gDNA from MS11 *B. napus* - *Hpa*I digested
Lane 9: 5 µg gDNA from MS11 *B. napus* - *Kpn*I digested
Lane 10: 5 µg gDNA from MS11 *B. napus* - *Mfe*I digested
Lane 11: 5 µg gDNA from MS11 *B. napus* - *Nco*I digested
Lane 12: 5 µg gDNA from MS11 *B. napus* - *Nde*I digested
Lane 13: 5 µg gDNA from MS11 *B. napus* - *Sty*I digested
Lane 14: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested
Lane 15: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested + an equimolar amount of pTCO113 - *Eco*RI digested
Lane 16: 5 µg gDNA from the non-GM counterpart- *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 17: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)

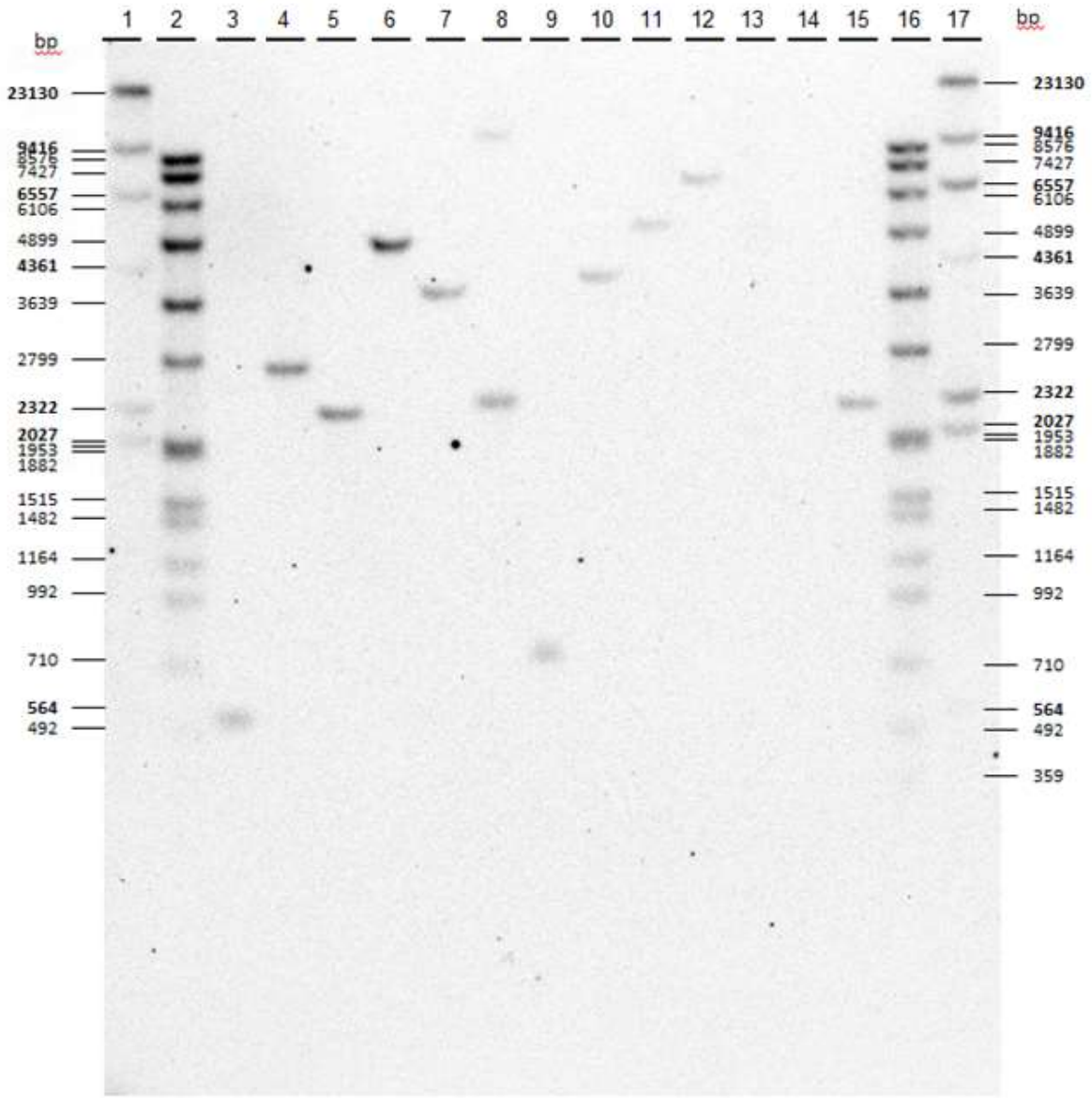


Figure 10: Hybridization performed with a 3'barnase-*barnase* probe (P020) to determine the insert organization of MS11 *B. napus*.

gDNA was isolated from MS11 *B. napus* plants and from the non-GM counterpart. The gDNA samples were digested with different restriction enzymes and hybridized with a probe corresponding to the MS11 3'barnase-*barnase* sequence (P020-2, PCR labeling).

Lane 1: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)
Lane 2: 5 µg gDNA from the non-GM counterpart- *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 3: 5 µg gDNA from MS11 *B. napus* - *Afl*III digested
Lane 4: 5 µg gDNA from MS11 *B. napus* - *Bcl*I digested
Lane 5: 5 µg gDNA from MS11 *B. napus* - *Eco*RI digested
Lane 6: 5 µg gDNA from MS11 *B. napus* - *Eco*RV digested
Lane 7: 5 µg gDNA from MS11 *B. napus* - *Hin*dIII digested
Lane 8: 5 µg gDNA from MS11 *B. napus* - *Hpa*I digested
Lane 9: 5 µg gDNA from MS11 *B. napus* - *Kpn*I digested
Lane 10: 5 µg gDNA from MS11 *B. napus* - *Mfe*I digested
Lane 11: 5 µg gDNA from MS11 *B. napus* - *Nco*I digested
Lane 12: 5 µg gDNA from MS11 *B. napus* - *Nde*I digested
Lane 13: 5 µg gDNA from MS11 *B. napus* - *Sty*I digested
Lane 14: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested
Lane 15: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested + an equimolar amount of pTCO113 - *Eco*RI digested

Lane 16: 5 µg gDNA from the non-GM counterpart- *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 17: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)

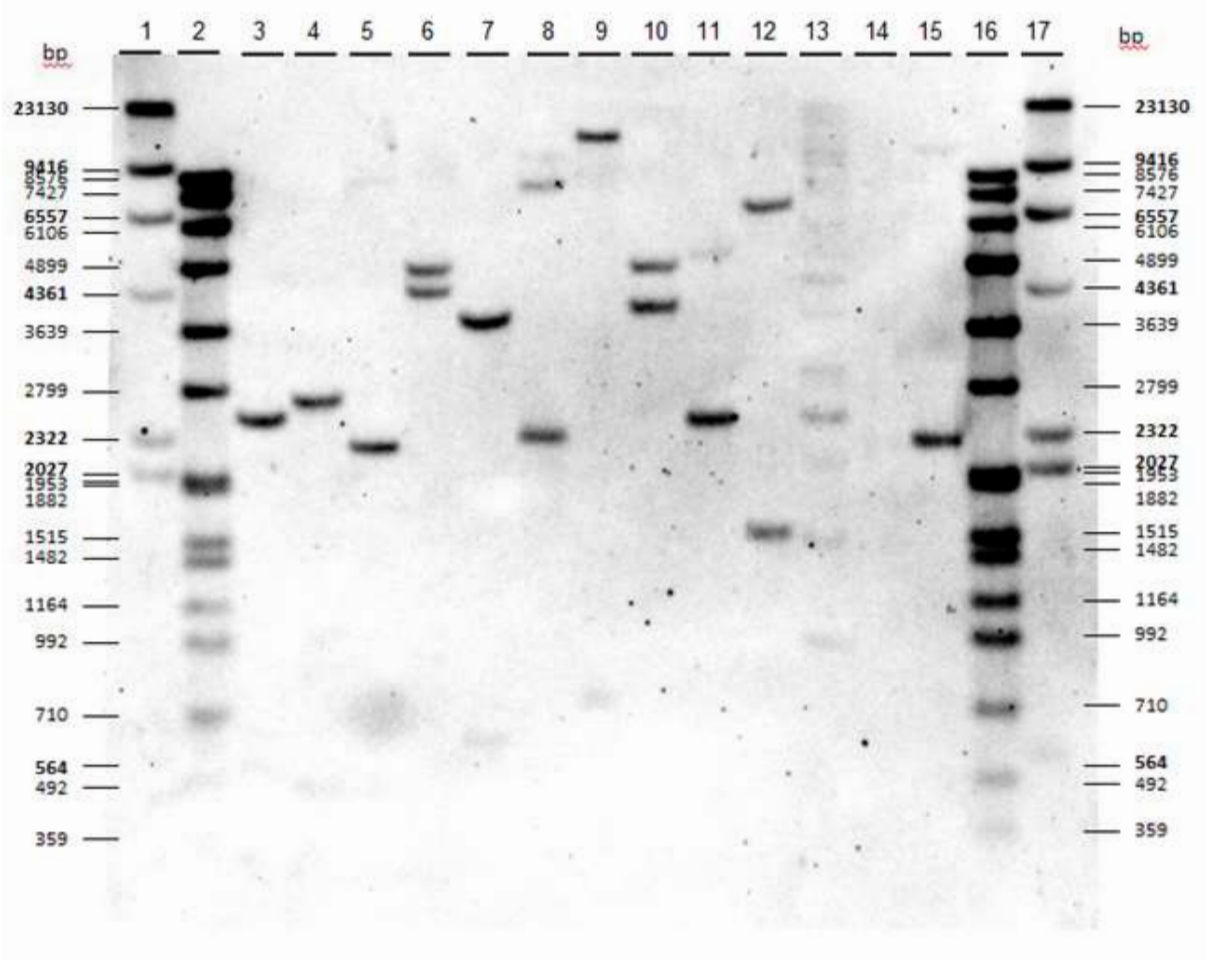


Figure 11: Hybridization performed with a Pta29 probe (P021) to determine the insert organization of MS11 *B. napus*.

gDNA was isolated from MS11 *B. napus* plants and from the non-GM counterpart. The gDNA samples were digested with different restriction enzymes and hybridized with a probe corresponding to the MS11 Pta29 sequence (P021-2, PCR labeling).

Lane 1: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)
Lane 2: 5 µg gDNA from the non-GM counterpart- *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 3: 5 µg gDNA from MS11 *B. napus* - *Afl*III digested
Lane 4: 5 µg gDNA from MS11 *B. napus* - *Bcl*I digested
Lane 5: 5 µg gDNA from MS11 *B. napus* - *Eco*RI digested
Lane 6: 5 µg gDNA from MS11 *B. napus* - *Eco*RV digested
Lane 7: 5 µg gDNA from MS11 *B. napus* - *Hin*dIII digested
Lane 8: 5 µg gDNA from MS11 *B. napus* - *Hpa*I digested
Lane 9: 5 µg gDNA from MS11 *B. napus* - *Kpn*I digested
Lane 10: 5 µg gDNA from MS11 *B. napus* - *Mfe*I digested
Lane 11: 5 µg gDNA from MS11 *B. napus* - *Nco*I digested
Lane 12: 5 µg gDNA from MS11 *B. napus* - *Nde*I digested
Lane 13: 5 µg gDNA from MS11 *B. napus* - *Sty*I digested
Lane 14: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested
Lane 15: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested + an equimolar amount of pTCO113 - *Eco*RI digested
Lane 16: 5 µg gDNA from the non-GM counterpart- *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 17: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)

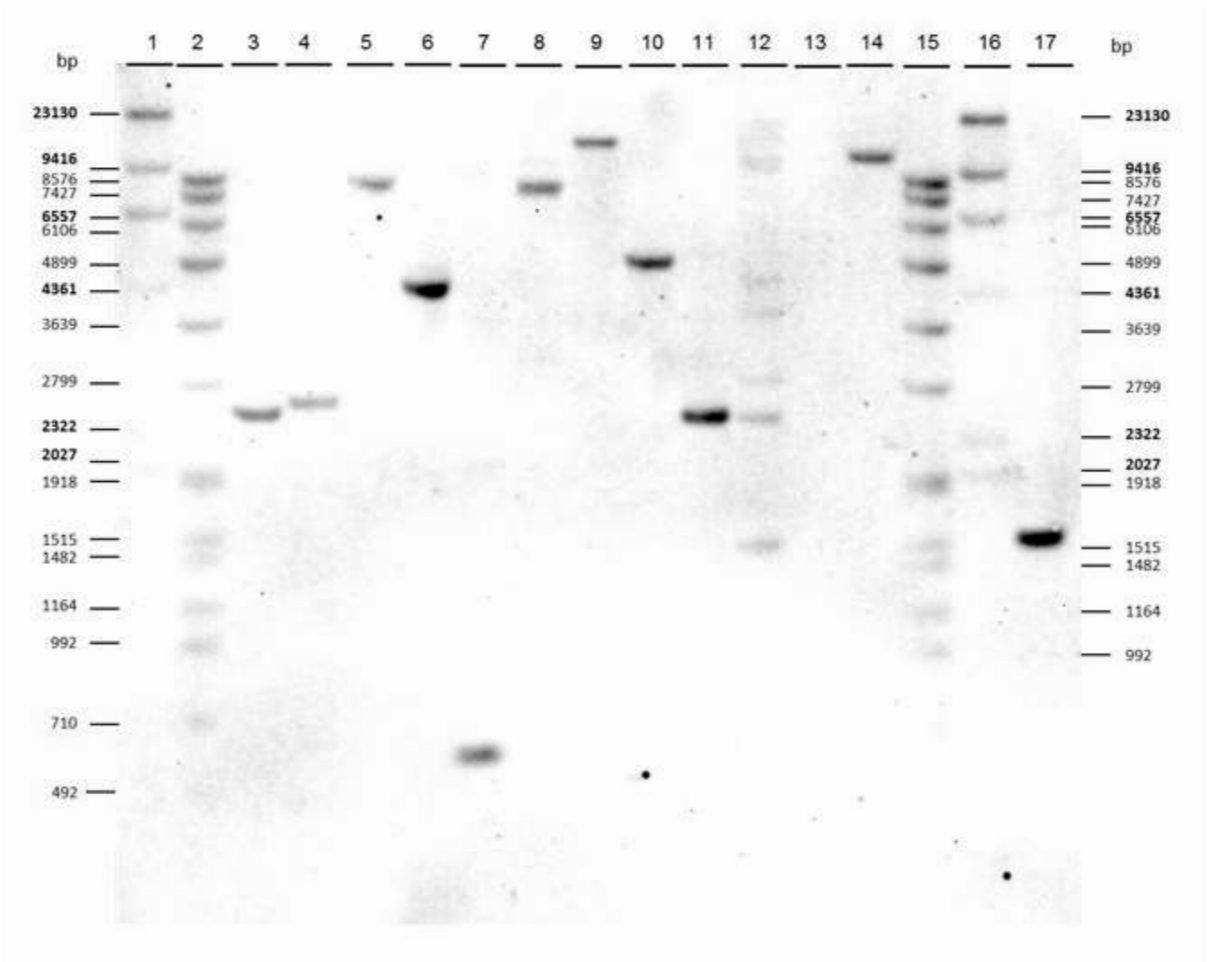


Figure 12: Hybridization performed with a Pnos probe (P022) to determine the insert organization of MS11 *B. napus*.

gDNA was isolated from MS11 *B. napus* plants and from the non-GM counterpart. The gDNA samples were digested with different restriction enzymes and hybridized with a probe corresponding to the MS11 Pnos sequence (P022-2, PCR labeling).

Lane 1: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)
Lane 2: 5 µg gDNA from the non-GM counterpart- *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 3: 5 µg gDNA from MS11 *B. napus* - *Afl*III digested
Lane 4: 5 µg gDNA from MS11 *B. napus* - *Bcl*I digested
Lane 5: 5 µg gDNA from MS11 *B. napus* - *Eco*RI digested
Lane 6: 5 µg gDNA from MS11 *B. napus* - *Eco*RV digested
Lane 7: 5 µg gDNA from MS11 *B. napus* - *Hin*dIII digested
Lane 8: 5 µg gDNA from MS11 *B. napus* - *Hpa*I digested
Lane 9: 5 µg gDNA from MS11 *B. napus* - *Kpn*I digested
Lane 10: 5 µg gDNA from MS11 *B. napus* - *Mfe*I digested
Lane 11: 5 µg gDNA from MS11 *B. napus* - *Nco*I digested
Lane 12: 5 µg gDNA from MS11 *B. napus* - *Sty*I digested
Lane 13: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested
Lane 14: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested + an equimolar amount of pTCO113 - *Eco*RI digested
Lane 15: 5 µg gDNA from the non-GM counterpart- *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 16: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)
Lane 17: 5 µg gDNA from MS11 *B. napus* - *Nde*I digested

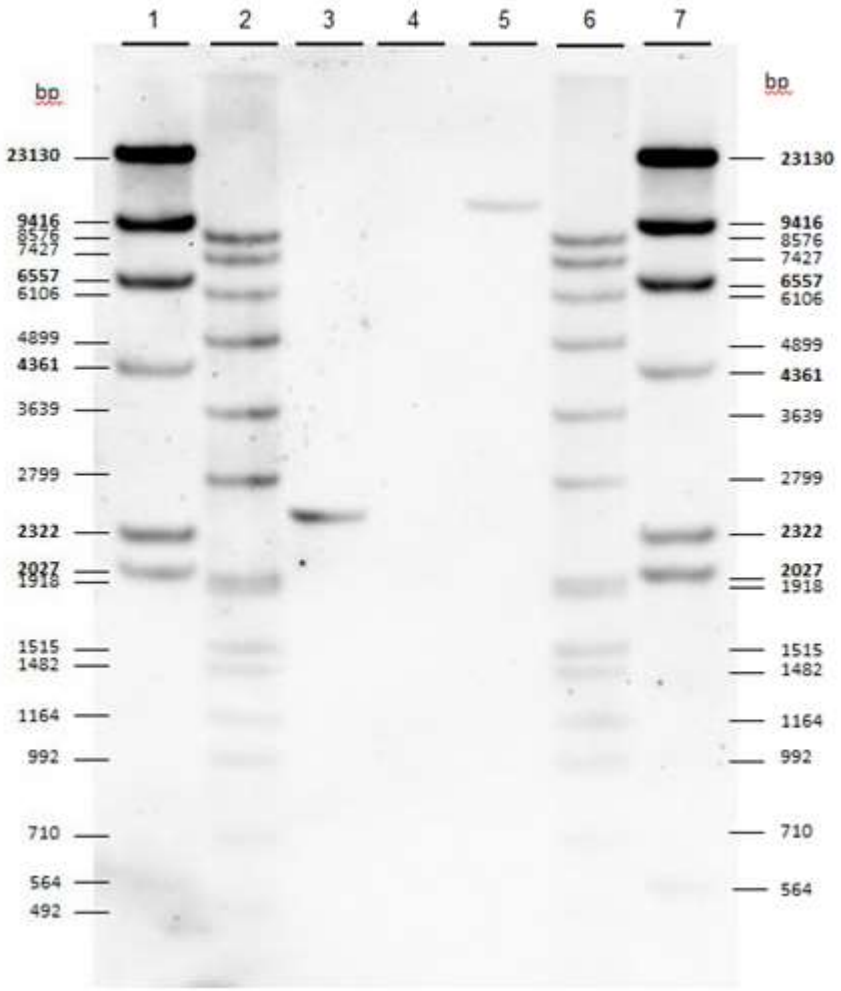


Figure 13: Hybridization performed with a Pnos probe (P022) to determine the insert organization of MS11 *B. napus*.

gDNA was isolated from MS11 *B. napus* plants and from the non-GM counterpart. The gDNA samples were digested with different restriction enzymes and hybridized with a probe corresponding to the MS11 Pnos sequence (P022-3, PCR labeling).

Lane 1: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)
Lane 2: 5 µg gDNA from the non-GM counterpart- *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 3: 5 µg gDNA from MS11 *B. napus* - *Nco*I digested
Lane 4: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested
Lane 5: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested + an equimolar amount of pTCO113 - *Eco*RI digested
Lane 6: 5 µg gDNA from the non-GM counterpart- *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 7: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)

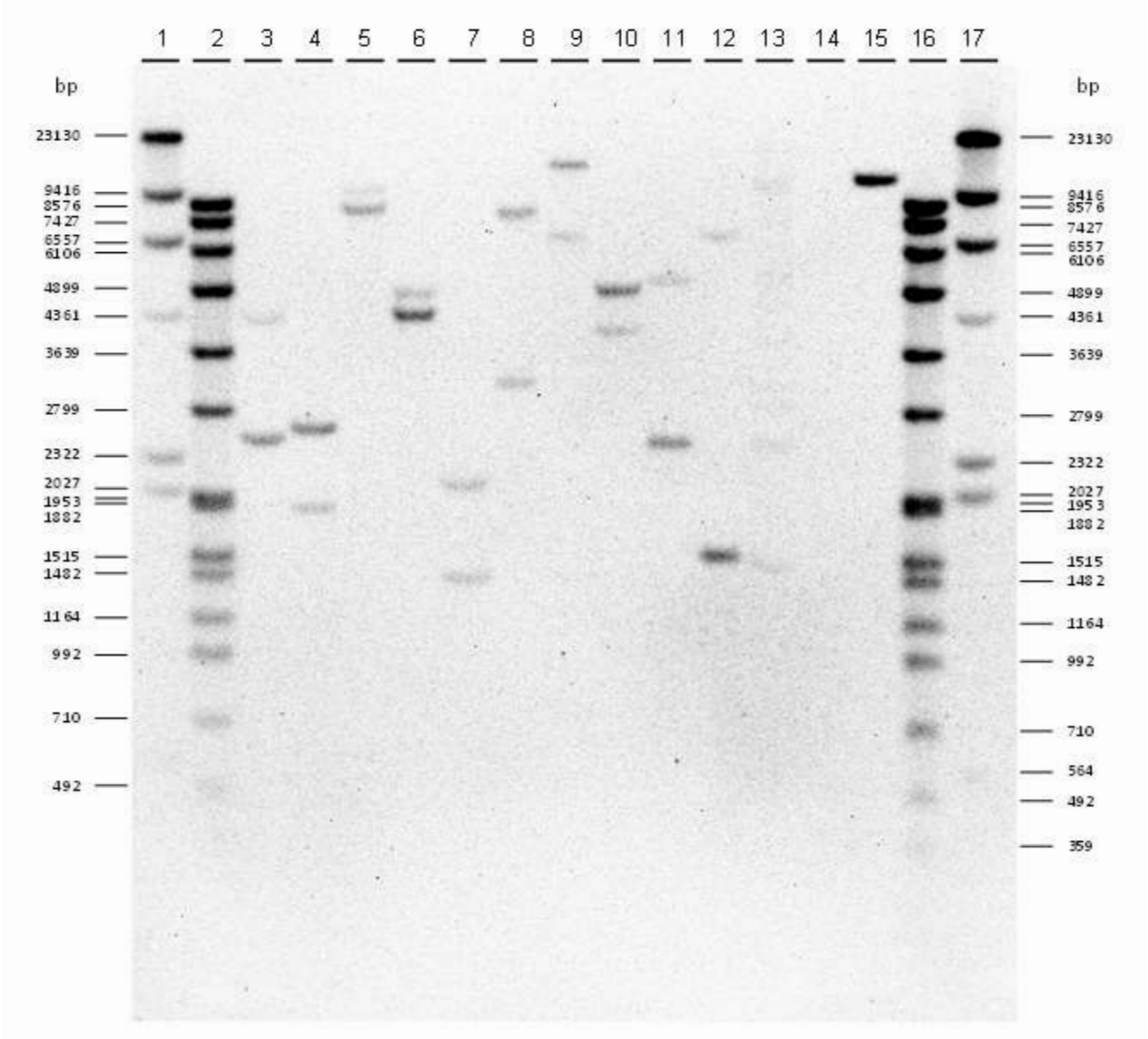


Figure 14: Hybridization performed with a 3'g7-LB probe (P023) to determine the insert organization of MS11 *B. napus*.

gDNA was isolated from MS11 *B. napus* plants and from the non-GM counterpart. The gDNA samples were digested with different restriction enzymes and hybridized with a probe corresponding to the MS11 3'g7-LB sequence (P023-2, PCR labeling).

Lane 1: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)
Lane 2: 5 µg gDNA from the non-GM counterpart- *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 3: 5 µg gDNA from MS11 *B. napus* - *Afl*III digested
Lane 4: 5 µg gDNA from MS11 *B. napus* - *Bcl*I digested
Lane 5: 5 µg gDNA from MS11 *B. napus* - *Eco*RI digested
Lane 6: 5 µg gDNA from MS11 *B. napus* - *Eco*RV digested
Lane 7: 5 µg gDNA from MS11 *B. napus* - *Hind*III digested
Lane 8: 5 µg gDNA from MS11 *B. napus* - *Hpa*I digested
Lane 9: 5 µg gDNA from MS11 *B. napus* - *Kpn*I digested
Lane 10: 5 µg gDNA from MS11 *B. napus* - *Mfe*I digested
Lane 11: 5 µg gDNA from MS11 *B. napus* - *Nco*I digested
Lane 12: 5 µg gDNA from MS11 *B. napus* - *Nde*I digested
Lane 13: 5 µg gDNA from MS11 *B. napus* - *Sty*I digested
Lane 14: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested
Lane 15: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested + an equimolar amount of pTCO113 - *Eco*RI digested
Lane 16: 5 µg gDNA from the non-GM counterpart- *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 17: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)

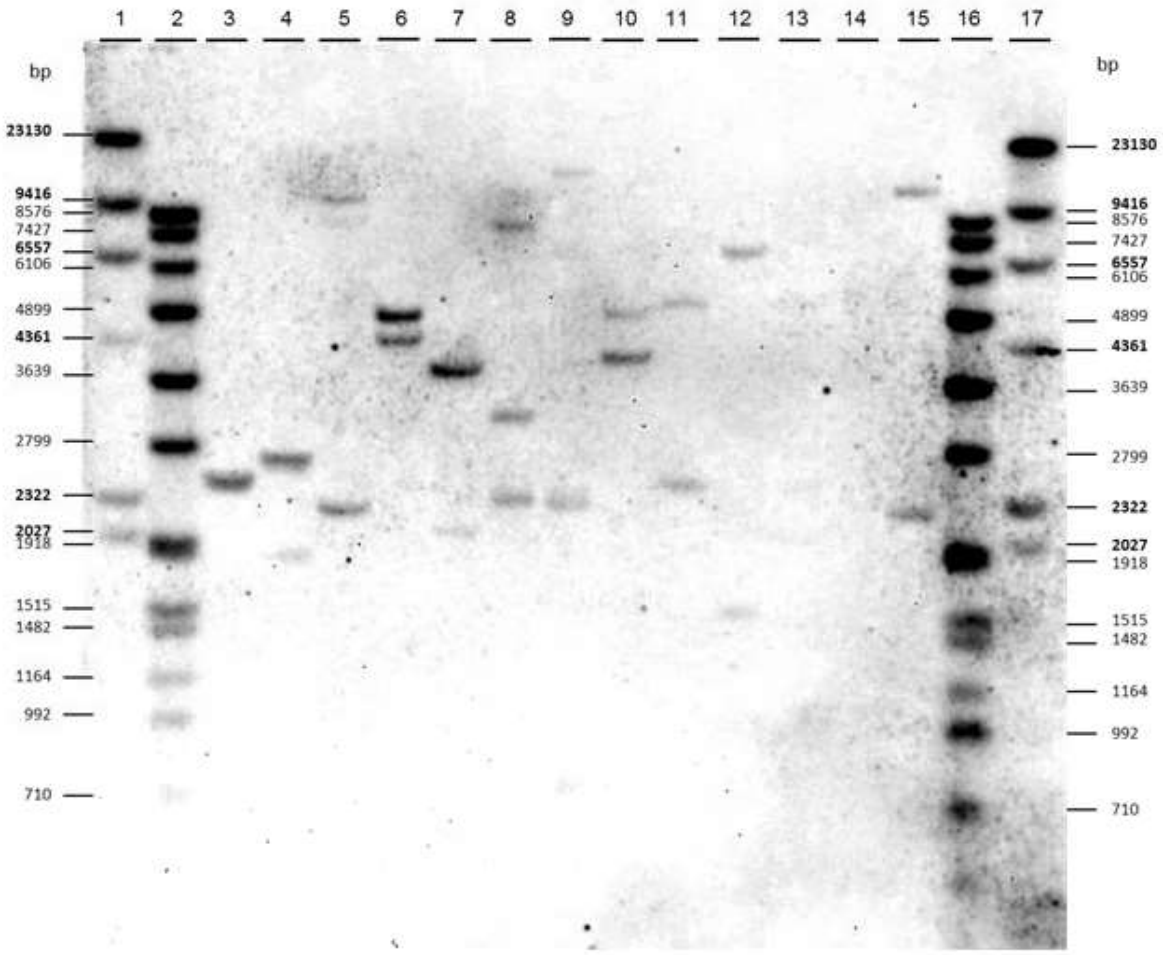


Figure 15: Hybridization performed with a T-DNA probe (P028) to determine the insert organization of MS11 *B. napus*.

gDNA was isolated from MS11 *B. napus* plants and from the non-GM counterpart. The gDNA samples were digested with different restriction enzymes and hybridized with a probe corresponding to the MS11 T-DNA sequence (P028-12, PCR labeling).

Lane 1: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)
Lane 2: 5 µg gDNA from the non-GM counterpart- *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 3: 5 µg gDNA from MS11 *B. napus* - *Afl*III digested
Lane 4: 5 µg gDNA from MS11 *B. napus* - *Bcl*I digested
Lane 5: 5 µg gDNA from MS11 *B. napus* - *Eco*RI digested
Lane 6: 5 µg gDNA from MS11 *B. napus* - *Eco*RV digested
Lane 7: 5 µg gDNA from MS11 *B. napus* - *Hind*III digested
Lane 8: 5 µg gDNA from MS11 *B. napus* - *Hpa*I digested
Lane 9: 5 µg gDNA from MS11 *B. napus* - *Kpn*I digested
Lane 10: 5 µg gDNA from MS11 *B. napus* - *Mfe*I digested
Lane 11: 5 µg gDNA from MS11 *B. napus* - *Nco*I digested
Lane 12: 5 µg gDNA from MS11 *B. napus* - *Nde*I digested
Lane 13: 5 µg gDNA from MS11 *B. napus* - *Sty*I digested
Lane 14: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested
Lane 15: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested + an equimolar amount of pTCO113 - *Eco*RI digested
Lane 16: 5 µg gDNA from the non-GM counterpart- *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 17: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)

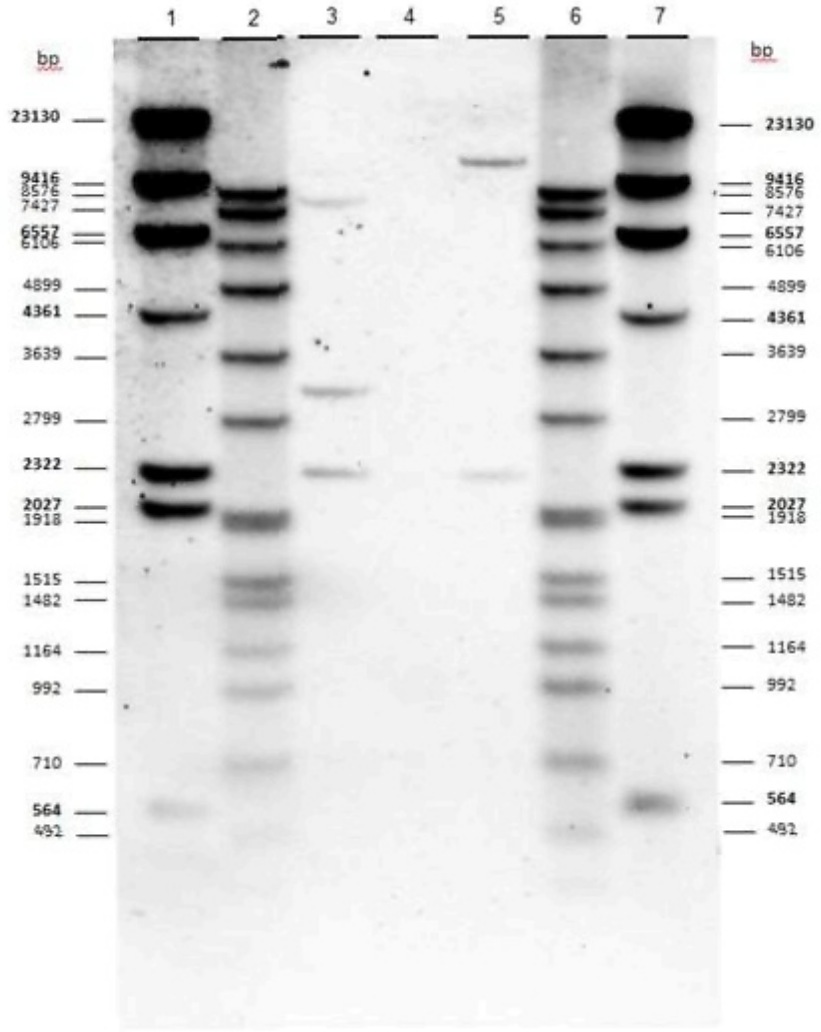


Figure 16: Hybridization performed with a T-DNA probe (P028) to determine the insert organization of MS11 *B. napus*.

gDNA was isolated from MS11 *B. napus* plants and from the non-GM counterpart. The gDNA samples were digested with different restriction enzymes and hybridized with a probe corresponding to the MS11 T-DNA sequence (P028-8, PCR labeling).

Lane 1: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)
Lane 2: 5 µg gDNA from the non-GM counterpart- *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 3: 5 µg gDNA from MS11 *B. napus* - *Hpa*I digested
Lane 4: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested
Lane 5: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested + an equimolar amount of pTCO113 - *Eco*RI digested
Lane 6: 5 µg gDNA from the non-GM counterpart- *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 7: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)

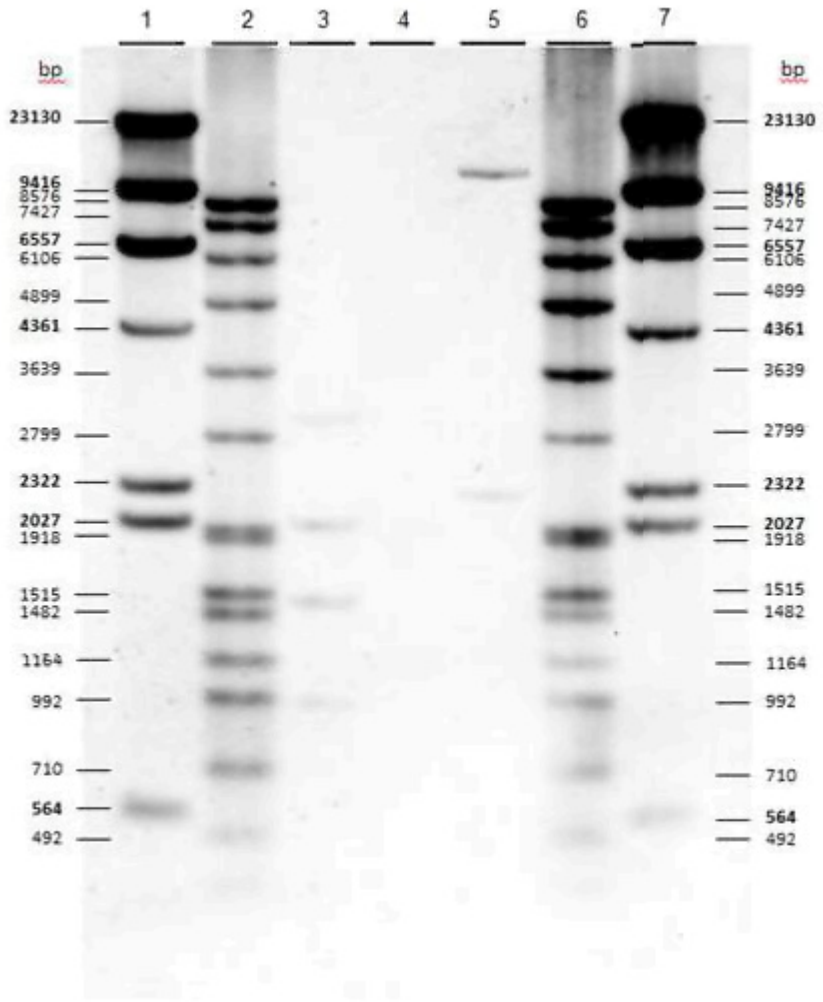


Figure 17: Hybridization performed with a T-DNA probe (P028) to determine the insert organization of MS11 *B. napus*.

gDNA was isolated from MS11 *B. napus* plants and from the non-GM counterpart. The gDNA samples were digested with different restriction enzymes and hybridized with a probe corresponding to the MS11 T-DNA sequence (P028-12, PCR labeling).

Lane 1: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)
Lane 2: 5 µg gDNA from the non-GM counterpart- *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 3: 5 µg gDNA from MS11 *B. napus* - *Sty*I digested
Lane 4: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested
Lane 5: 5 µg gDNA from the non-GM counterpart - *Eco*RI digested + an equimolar amount of pTCO113 - *Eco*RI digested
Lane 6: 5 µg gDNA from the non-GM counterpart- *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 7: 5 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)

In an earlier study ([redacted], 2008, M-304805-01-1, Node A.3 (c), (iii) (CCI)), the DNA sequence of the transgenic locus including the inserted transgenic sequences and both flanking sequences, and the pre-insertion locus of MS11 *B. napus* were determined.
Genomic DNA was isolated from transgenic plants. To determine the sequence of the elite event locus, six overlapping fragments were amplified. The pre-insertion locus could be amplified in one fragment. After sequencing of all the PCR fragments, one consensus sequence was obtained from the fragments deriving from the event locus, and one consensus sequence was obtained for the wild type locus at the integration site.
The determined transgenic locus sequence contains 419 bp 5-prime flanking sequences, 5778 bp inserted transgenic sequences and 555 bp 3-prime flanking sequences. 1014 bp were determined at the pre integration locus, including 419 bp 5-prime flanking sequences, 555 bp 3-prime flanking sequences and a target site deletion of 40 bp.
The 5-prime and 3-prime flanking sequences determined at the pre-insertion locus are completely identical to the 5-prime and 3-prime flanking sequences determined at the transgenic locus. This demonstrates that the flanking sequences of MS11 *B. napus* correspond exactly to the *Brassica napus* genome in its original organization (refer Figure 18, below).

MS11 – transgenic locus

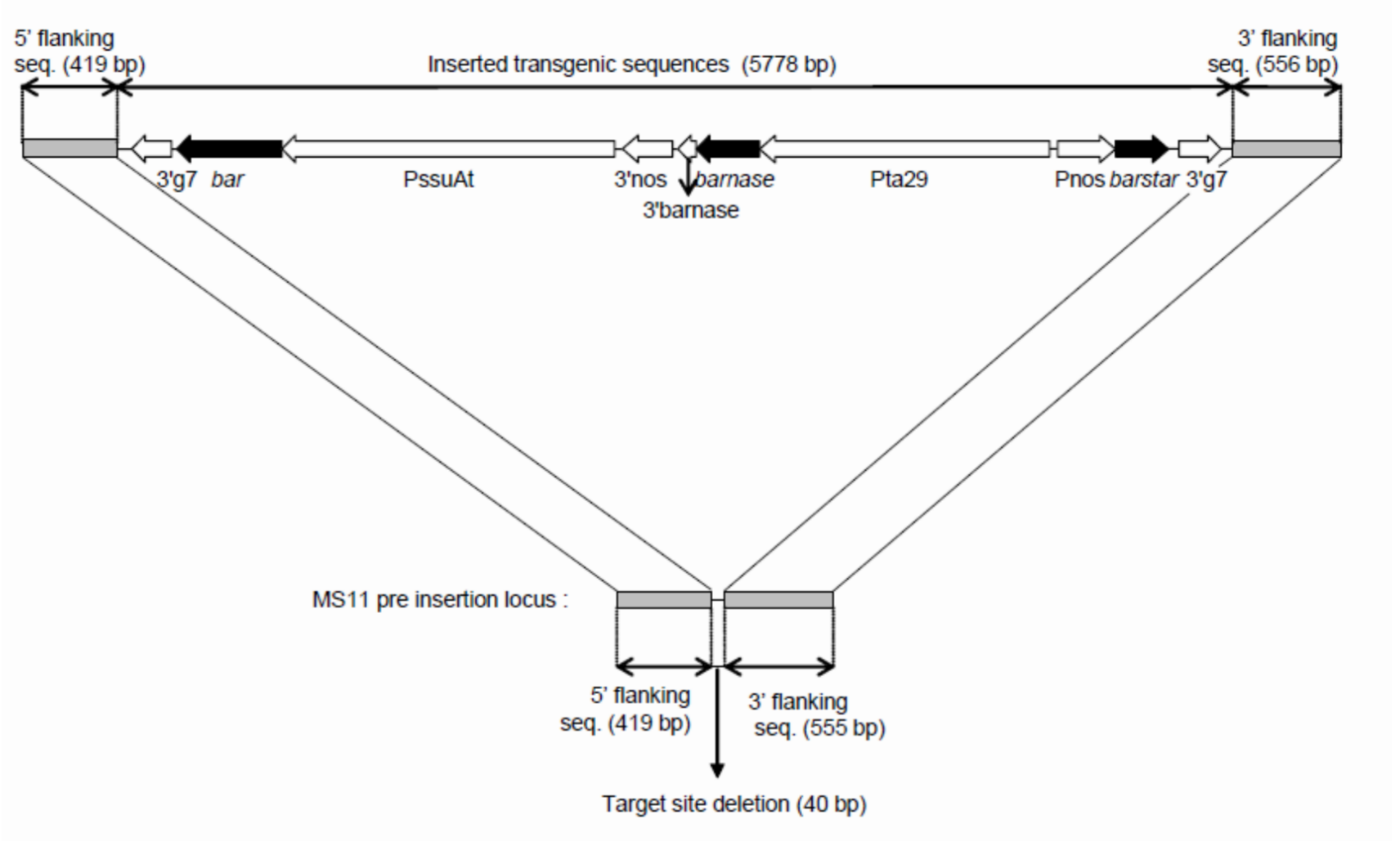


Figure 18 Organisation of the MS11 *B. napus* transgenic and pre-insertion loci in the *Brassica napus* genome

The potential presence of vector backbone sequences in MS11 *B. napus* was assessed by means of Southern blot and PCR analysis ([redacted] 2016; M-547543-01, Node A.3 (c), (i), (CCI)).

Seeds from the T2 generation were used to produce MS11 *B. napus* leaf material. The identity of the leaf material was confirmed. Non-genetically modified (non-GM) *B. napus* variety N90-740 (non-GM counterpart) was used as a negative control. The positive control was the transforming plasmid of MS11 *B. napus* (pTCO113).

To assess the presence of vector backbone sequences in MS11 *B. napus*, the gDNA from individual MS11 *B. napus* plants were digested with the restriction enzymes *Afl*III and *Nde*I. Equal amounts of digested gDNA of five different MS11 *B. napus* plants were pooled for each restriction digestion and further analysed. Pooled gDNA from plants of the non-GM counterpart was digested with the restriction enzyme *Nde*I. Plasmid DNA of pTCO113 was digested with the *Eco*RI restriction enzyme.

The resulting DNA fragments were separated by agarose gel-electrophoresis. Transfer of the separated DNA fragments from the agarose gel to a positively charged nylon membrane was performed by a neutral Southern blotting procedure. The resulting membranes were hybridized with four overlapping, DIG-labeled vector backbone probes (P024 to P027) that cover every bp of the vector backbone except the *barstar* sequences. The absence of the *barstar* gene contained within the vector backbone could not be confirmed by Southern blot analysis since the gene is also part of the MS11 insert sequence. Therefore, the absence of the *barstar* gene as part of the vector backbone was confirmed by means of PCR analysis.

Table 6 provides details of the probes used in the Southern blot analysis. A schematic overview of the plasmid pTCO113 with indication of the restriction enzymes and probes used to assess the presence of vector backbone sequences in MS11 *B. napus* is presented in Figure 19.

Each membrane contained one negative control, in which the template DNA was digested gDNA prepared from the non-GM counterpart. This negative control showed no hybridization with any of the probes used, confirming the absence of any background hybridization with all the probes used. Similarly, each membrane contained two positive controls, one consisting of digested gDNA prepared from non-GM plant material and supplemented with an equimolar amount of digested transforming plasmid pTCO113, and a second positive control consisting of digested gDNA prepared from non-GM plant material that was supplemented with 0.1 equimolar amount of pTCO113 digested plasmid DNA. Both positive controls showed the expected hybridization fragments after hybridization with the vector backbone probes (Figure 20 to Figure 23, lanes 6 and 7). This demonstrated that the hybridizations were performed under conditions allowing detection of the possible presence of vector backbone sequences in one of the five pooled MS11 *B. napus* plants tested.

Hybridization of the digested MS11 *B. napus* gDNA samples with the vector backbone probes resulted in no hybridization fragments, as expected (Table 8, Figure 20 to Figure 23, lanes 3 and 4). This demonstrated the absence of vector backbone sequences in MS11 *B. napus* gDNA samples. When hybridizing the same membranes with the T-DNA probe, all expected fragments were obtained. This demonstrated that an adequate amount of a sufficient quality of digested MS11 *B. napus* gDNA was loaded on the gels to be able to detect vector backbone sequences in MS11 *B. napus*, if present.
The absence of *barstar* sequence originating from the vector backbone was verified by PCR analysis. Five primer combinations were used to perform the PCR analysis. Primers targeting T-DNA sequences at the RB were included to serve as an internal control. Primer sequences and the positions of the primers in plasmid pTCO113 are presented in Table 7 and Figure 19. No amplicons were obtained using MS11 *B. napus* gDNA as template in PCR analysis to test for the presence of *barstar* sequence originating from the vector backbone (Table 9, Figure 24, panel A: lane 2, 7 and 12; panel B: lane 2 and 7). As a result, the absence of *barstar* originating from the vector backbone sequence was demonstrated.

In conclusion, the Southern blot and PCR results demonstrated the absence of vector backbone sequences in MS11 *B. napus*.

Table 6: Information on the probes used

Probe ID	Probe template ID	Description	Primer pair/ Restr. digest	Primer sequence (5' → 3')	Primer position on pTCO113 (bp)	Size probe template (bp)	Overlap between probe
P024	PT112	Vector backbone - *aadA*	GLPA019	[redacted]	5891 → 5912	1840	No overlap with PT113 → *barstar* gene
			GLPA361	[redacted]	7730 → 7711		
P025	PT113	Vector backbone - 5'ORI pVS1, version 1	GLPA380	[redacted]	8214 → 8233	2382	
			GLPA396	[redacted]	10595 → 10576		611 bp (version1) or 282 bp (version 2)
		Vector backbone - 5'ORI pVS1, version 2	GLPA378	[redacted]	8217 → 8236	2050	
			GLPA151	[redacted]	10266 → 10247		
P026	PT114	Vector backbone - 3' ORI pVS1	GLPA148	[redacted]	9984 → 10003	2246	
			GLPA156	[redacted]	12229 → 12210		39 bp
P027	PT115	Vector backbone - ORI ColE1	GLPA160	[redacted]	12191 → 12212	1389	
			GLPA162	[redacted]	39 → 20		
P028	PT108	T-DNA	GLPA174	[redacted]	1 → 25	5865	NA
			GLPA359	[redacted]	5865 → 5841		

NA means not applicable
** An additional PCR product of 8311 bp can be produced
*** An additional PCR product of 10758 bp can be produced
**** An additional PCR product of 2244 bp can be produced
° These primers amplify two identical regions

Table 7: Information on the primers used in the PCR analysis to test for presence of the barstar sequence originating from the vector backbone

Description amplicon	Primer pair	Primer sequence (5' → 3') *	Primer position in pTCO113 (bp)	PCR amplicon position in pTCO113 (bp)	Amplicon size (bp)
T-DNA (part of 3'g7)	GLPA047	[redacted]	143 → 163 5721 → 5701**	143 → 317 5547 → 5721 143 → 5521*** 5547 → 317***	175 5579*** 8311***
	GLPA048	[redacted]	317 → 293 5547 → 5571**		
complete *barstar* in vector backbone	GLPA049	[redacted]	7663 → 7682	7663 → 8218	556
	GLPA050	[redacted]	8218 → 8198		
barstar + downstream sequences in vector backbone	GLPA181	[redacted]	7478 → 7497	7478 → 8027 5248 → 8027 ***	550 2780***
	GLPA045	[redacted]	8027 → 8004 5248 → 5271 **		
	GLPA181	[redacted]	7478 → 7497	7478 → 8049 5226 → 8049***	572 2824***
	GLPA345	[redacted]	8049 → 8026 5226 → 5249**		
	GLPA180	[redacted]	7457 →7476	7457 → 8049 5226 → 8049***	593 2824***
	GLPA345	[redacted]	8049 → 8026 5226 → 5249**		
barstar + upstream sequences in vector backbone	GLPA046	[redacted]	7792 → 7815 5483 → 5460**	7792 → 8218 7792 → 5483 ***	427 11232 ***
	GLPA050	[redacted]	8218 → 8198		

* A lowercase 'g' is used to avoid confusion between 'G' and 'C'
** An additional binding site is present for this primer
*** Additional PCR products can be produced

Table 8: Expected and obtained hybridization fragments determined for the vector backbone assessment of MS11 *B. napus*
Part 1:

Sample	T-DNA or plasmid fragment sizes (bp)	Fragment description	Obtained fragment size (bp)	H1/LJS018/11-F4		H2/LJS018/11-F2		H1/LJS018/12-F6		H3/LJS018/12-F3	
				P024-2		P028-1		P025-2		P028-2	
				Vector backbone probe (*aad*A)		T-DNA probe		Vector backbone probe (5' ORI pVS1)		T-DNA probe	
				Figure 20				Figure 21			
				Exp.	Obt.	Exp.	Obt.	Exp.	Obt.	Exp.	Obt.
Ms11 - *Afl*III	>305	5' integration fr.	NA	No	No	Yes ** (119)	No	No	No	Yes ** (119)	No
	2476	internal fr.	2476	No	No	Yes	Yes$	No	No	Yes	Yes$
	550	internal fr.	550	No	No	Yes	No °	No	No	Yes	No °
	>2467	3' integration fr	2500	No	No	Yes	Yes$	No	No	Yes	Yes$
Ms11 - *Nde*I	>4557	5' integration fr.	6900	No	No	Yes	Yes	No	No	Yes	Yes
	64*	internal fr.	NA	No	No	Yes	No	No	No	Yes	No
	>1177	3' integration fr.	1600	No	No	Yes	Yes	No	No	Yes	Yes
Non-GM counterpart - *Nde*I	/	Negative control	NA	No	No	No	No	No	No	No	No
Non-GM counterpart - *Nde*I + 0.1 equimolar amount pTCO113 - *Eco*RI	2260	Positive control	2260	No	No	Yes	No	No	No	Yes	No
	11280	Positive control	11280	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Non-GM counterpart - *Nde*I + 1 equimolar amount pTCO113 - *Eco*RI	2260	Positive control	2260	No	No	Yes	Yes	No	No	Yes	Yes
	11280	Positive control	11280	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Table 8: Expected and obtained hybridization fragments determined for the vector backbone assessment of MS11 *B. napus*

Part 2:

Sample	T-DNA or plasmid fragment sizes (bp)	Fragment description	Obtained fragment size (bp)	H1/LJS018/13-F2		H3/LJS018/13-F1		H1/LJS018/14-F5		H3/LJS018/14-F1	
				P026-2		P028-3		P027-2		P028-10	
				Vector backbone probe (3' ORI pVS1)		T-DNA probe		Vector backbone probe (ORI Col E1)		T-DNA probe	
				Figure 22				Figure 23			
				Exp.	Obt.	Exp.	Obt.	Exp.	Obt.	Exp.	Obt.
Ms11 - *Afl*III	>305	5' integration fr.	NA	No	No	Yes ** (119)	No	No	No	Yes ** (119)	No
	2476	internal fr.	2476	No	No	Yes	Yes$	No	No	Yes	Yes$
	550	internal fr.	550	No	No	Yes	No °	No	No	Yes	No °
	>2467	3' integration fr	2500	No	No	Yes	Yes$	No	No	Yes	Yes$
Ms11 - *Nde*I	>4557	5' integration fr.	6900	No	No	Yes	Yes	No	No	Yes	Yes
	64*	internal fr.	NA	No	No	Yes	No	No	No	Yes	No
	>1177	3' integration fr.	1600	No	No	Yes	Yes	No	No	Yes	Yes
Non-GM counterpart - *Nde*I	/	Negative control	NA	No	No	No	No	No	No	No	No
Non-GM counterpart - *Nde*I + 0.1 equimolar amount pTCO113 - *Eco*RI	2260	Positive control	2260	No	No	Yes	No	No	No	Yes	No
	11280	Positive control	11280	Yes	Yes	Yes	No	Yes	Yes	Yes	No
Non-GM counterpart - *Nde*I + 1 equimolar amount pTCO113 - *Eco*RI	2260	Positive control	2260	No	No	Yes	Yes	No	No	Yes	Yes
	11280	Positive control	11280	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

$ These bands have a comparable size and cannot be distinguished using this experimental setup
* Based on the technical limits of Southern Blotting, this fragment might be too small to be visualized
** Due to a small overlap with the probe, these framgents may not be visible. The size of the overlap is indicated between brackets
° This band is probably not observed because of the small size of the fragment in comparison with the large probe

Table 9: Expected and obtained PCR results to investigate the potential presence of *barstar* sequences as part of the vector backbone

Primer combinations	Target	MS11	WT (negative control)	WT + equimolar amount of pTCO113 (positive control)
GLPA049 - GLPA050 GLPA047 - GLPA048*	complete *barstar* in vector backbone	/ 175 bp°	/ /	556 bp 175 bp°
GLPA181 - GLPA045 GLPA047 - GLPA048*	*barstar* + downstream sequences in vector backbone	/ 175 bp° 474 bp[a]	/ /	550 bp 2780 bp** 175 bp° 474 bp[a]
GLPA181 - GLPA345 GLPA047 - GLPA048*		/ 175 bp° 496 bp[b]	/ /	572 bp 2824 bp** 175 bp° 496 bp[b]
GLPA180 - GLPA345 GLPA047 - GLPA048*		/ 175 bp° 496 bp[b]	/ /	593 bp 2824 bp** 175 bp° 496 bp[b]
GLPA046 - GLPA050 GLPA047 - GLPA048*	*barstar* + upstream sequences in vector backbone	/ 175 bp°	/ /	427 bp 11232 bp** 175 bp°

* Primers targeting T-DNA sequences at the RB are included to serve as an internal control
** An additional PCR product can be produced, see also Table 5
° Additional PCR products might be obtained also for the positive control, see Table 5 for the expected amplicon sizes of these additional fragments
[a] Additional PCR product of 474 bp may be expected as a result of the combination of primers GLPA045 and GLPA047.
[b] Additional PCR product of 496 bp may be expected as a result of the combination of primers GLPA047 and GLPA345

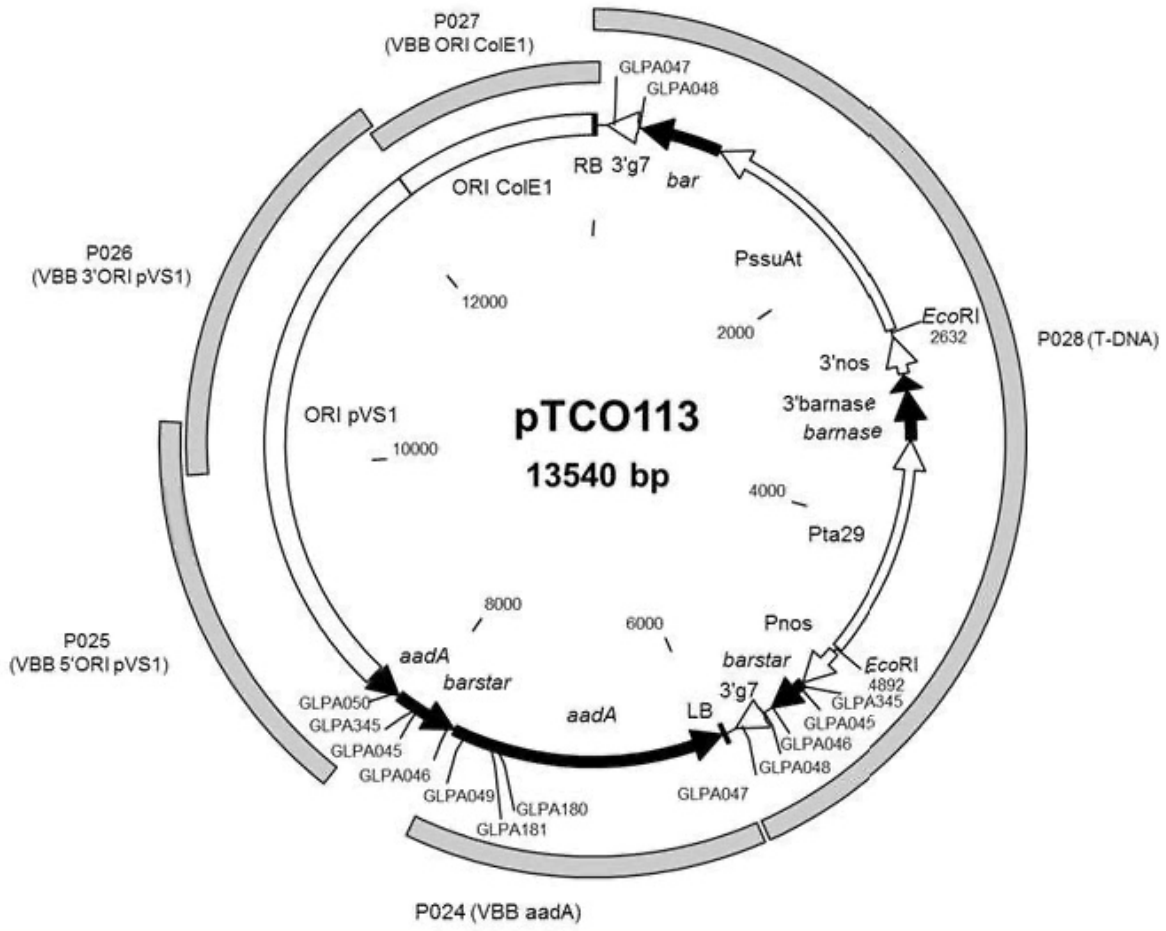


Figure 19: Map of transformation vector pTCO113 with indication of the primers used for the investigation of the presence of *barstar* sequences as part of the vector backbone, the position of enzymes used for plasmid digestion in this study and the vector backbone probes and T-DNA probe (P028) indicated.

The indicated restriction enzyme positions between brackets refer to the first base after the cleavage site of the restriction enzyme

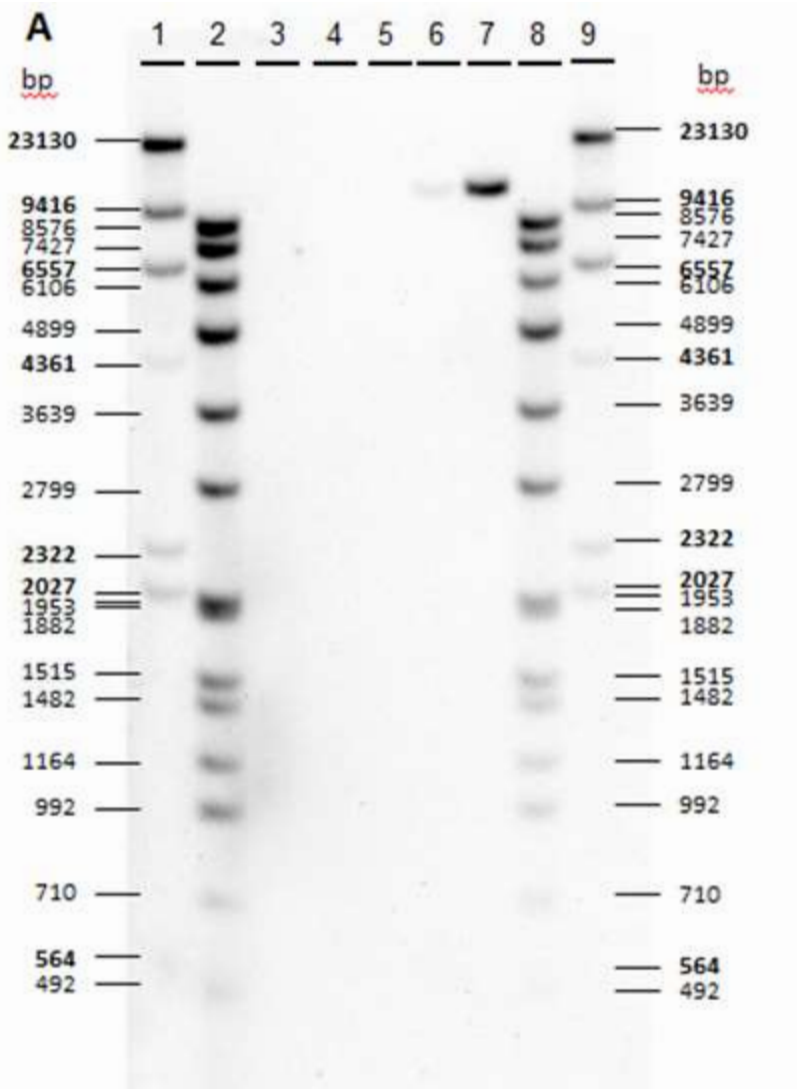


Figure 20: Hybridization performed with a vector backbone probe covering the *aadA* sequence (P024) to assess the vector backbone presence in the T2 generation of MS11 *B. napus*.

gDNA was isolated from MS11 *B. napus* plants and from the non-GM counterpart. The gDNA samples were digested with restriction enzymes *Afl*III and *Nde*I and hybridized with a vector backbone probe (P024-2, PCR labeling) and with the T-DNA probe (P028-01, PCR labeling) (data not shown).

Lane 1: 3 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)
Lane 2: 3 µg gDNA from the non-GM counterpart - *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 3: 3 µg gDNA from MS11 *B. napus* - *Afl*III digested
Lane 4: 3 µg gDNA from MS11 *B. napus* - *Nde*I digested
Lane 5: 3 µg gDNA from the non-GM counterpart - *Nde*I digested
Lane 6: 3 µg gDNA from the non-GM counterpart - *Nde*I digested + 1/10th of an equimolar amount of pTCO113 - *Eco*RI digested
Lane 7: 3 µg gDNA from the non-GM counterpart - *Nde*I digested + an equimolar amount of pTCO113 - *Eco*RI digested
Lane 8: 3 µg gDNA from the non-GM counterpart - *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 9: 3 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)

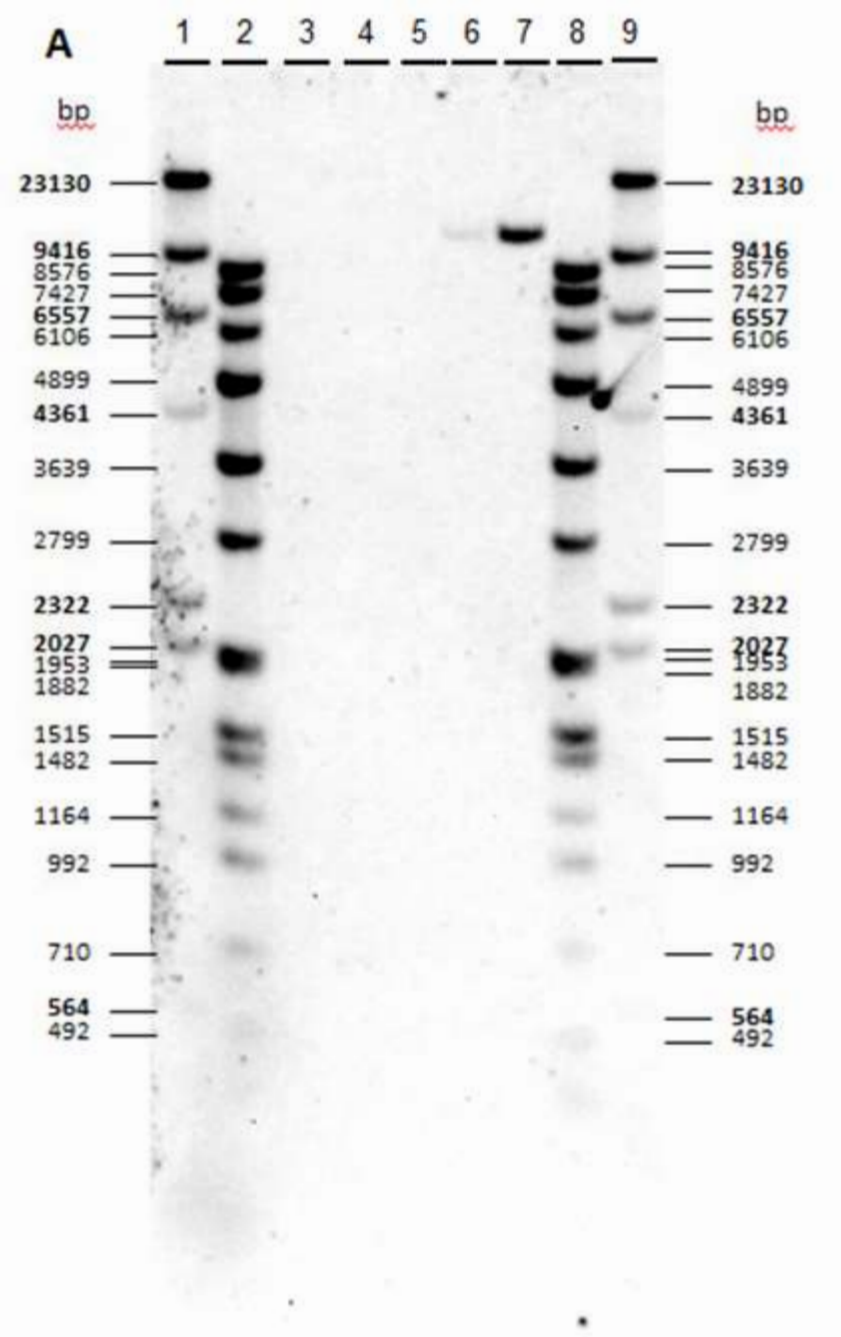


Figure 21: Hybridization performed with a vector backbone probe covering the 5'ORI pVS1 sequence (P025) to assess the vector backbone presence in the T2 generation of MS11 *B. napus*.

gDNA was isolated from MS11 *B. napus* plants and from the non-GM counterpart. The gDNA samples were digested with restriction enzymes *Afl*III and *Nde*I and hybridized with a vector backbone probe (P025-2, PCR labeling) and with the T-DNA probe (P028-02, PCR labeling) (data not shown).

Lane 1: 3 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)
Lane 2: 3 µg gDNA from the non-GM counterpart - *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 3: 3 µg gDNA from MS11 *B. napus* - *Afl*III digested
Lane 4: 3 µg gDNA from MS11 *B. napus* - *Nde*I digested
Lane 5: 3 µg gDNA from the non-GM counterpart - *Nde*I digested
Lane 6: 3 µg gDNA from the non-GM counterpart - *Nde*I digested + 1/10th of an equimolar amount of pTCO113 - *Eco*RI digested
Lane 7: 3 µg gDNA from the non-GM counterpart - *Nde*I digested + an equimolar amount of pTCO113 - *Eco*RI digested

Lane 8: 3 µg gDNA from the non-GM counterpart - *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 9: 3 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)

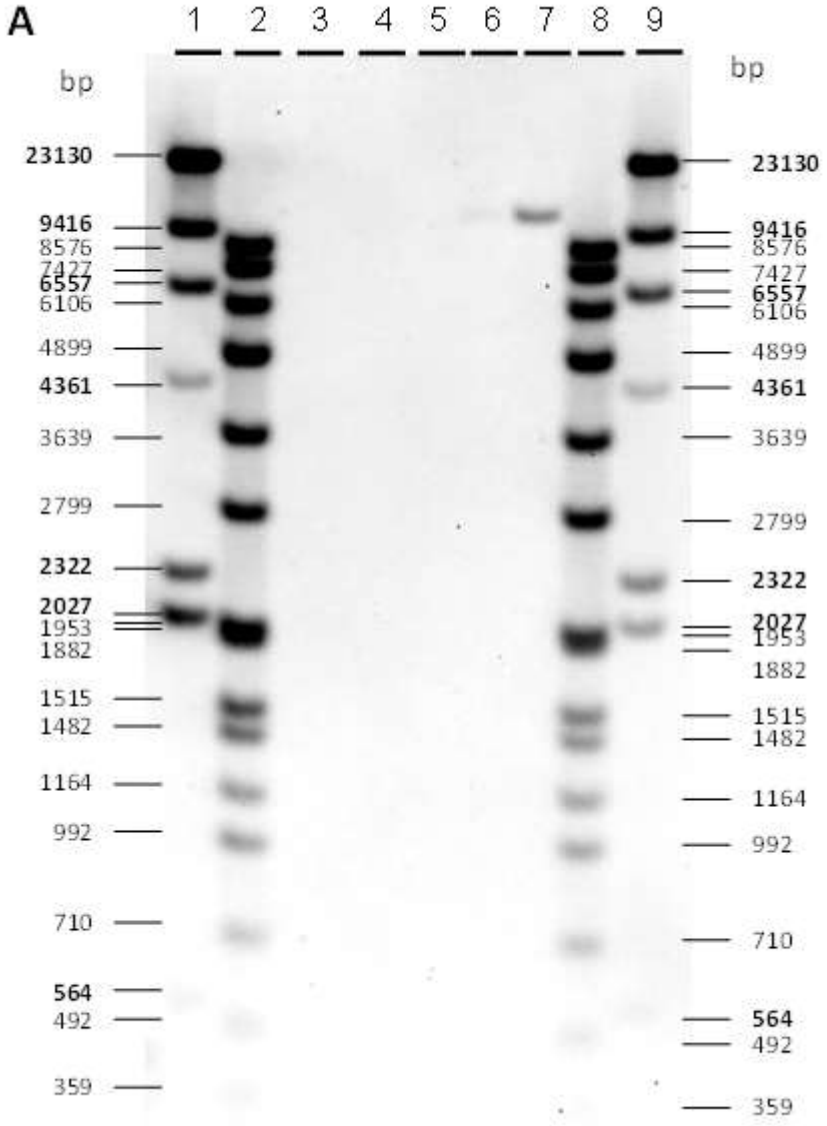


Figure 22: Hybridization performed with a vector backbone probe covering the 3'ORI pVS1 sequence (P026) to assess the vector backbone presence in the T2 generation of MS11 *B. napus*.

gDNA was isolated from MS11 *B. napus* plants and from the non-GM counterpart. The gDNA samples were digested with restriction enzymes *Afl*III and *Nde*I and hybridized with a vector backbone probe (P026-2, PCR labeling) and with the T-DNA probe (P028-03, PCR labeling) (data not shown).

Lane 1: 3 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)
Lane 2: 3 µg gDNA from the non-GM counterpart - *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 3: 3 µg gDNA from MS11 *B. napus* - *Afl*III digested
Lane 4: 3 µg gDNA from MS11 *B. napus* - *Nde*I digested
Lane 5: 3 µg gDNA from the non-GM counterpart - *Nde*I digested
Lane 6: 3 µg gDNA from the non-GM counterpart - *Nde*I digested + 1/10th of an equimolar amount of pTCO113 - *Eco*RI digested
Lane 7: 3 µg gDNA from the non-GM counterpart - *Nde*I digested + an equimolar amount of pTCO113 - *Eco*RI digested
Lane 8: 3 µg gDNA from the non-GM counterpart - *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 9: 3 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)

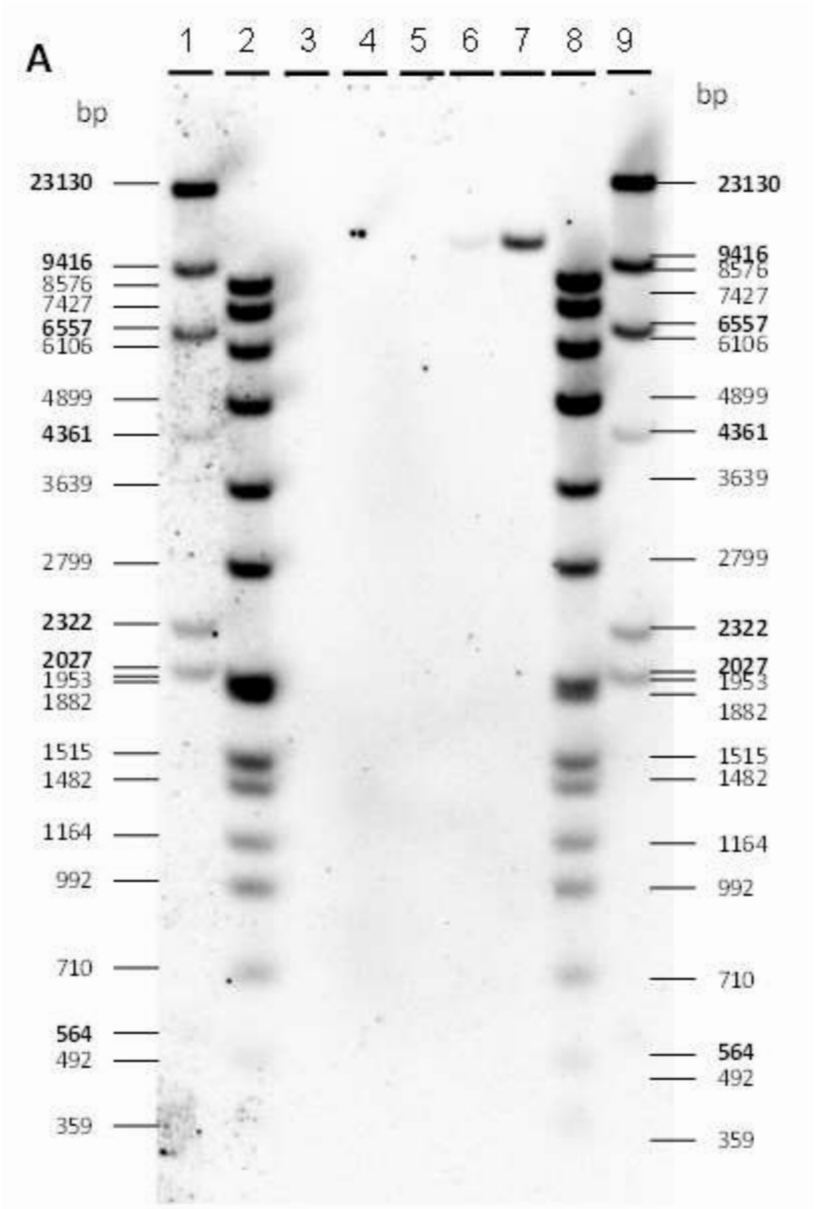


Figure 23: Hybridization performed with a vector backbone probe covering the 3'ORI colE1 sequence (P027) to assess the vector backbone presence in the T2 generation of MS11 *B. napus*.

gDNA was isolated from MS11 *B. napus* plants and from the non-GM counterpart. The gDNA samples were digested with restriction enzymes *Afl*III and *Nde*I and hybridized with a vector backbone probe (P027-2, PCR labeling) and with the T-DNA probe (P028-10, PCR labeling) (data not shown).

Lane 1: 3 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)
Lane 2: 3 µg gDNA from the non-GM counterpart - *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 3: 3 µg gDNA from MS11 *B. napus* - *Afl*III digested
Lane 4: 3 µg gDNA from MS11 *B. napus* - *Nde*I digested
Lane 5: 3 µg gDNA from the non-GM counterpart - *Nde*I digested
Lane 6: 3 µg gDNA from the non-GM counterpart - *Nde*I digested + 1/10th of an equimolar amount of pTCO113 - *Eco*RI digested
Lane 7: 3 µg gDNA from the non-GM counterpart - *Nde*I digested + an equimolar amount of pTCO113 - *Eco*RI digested
Lane 8: 3 µg gDNA from the non-GM counterpart - *Eco*RI digested + 10 ng DNA Molecular Weight Marker VII, DIG-labeled (Roche)
Lane 9: 3 µg gDNA from the non-GM counterpart - *Hin*dIII digested + 10 ng DNA Molecular Weight Marker II, DIG-labeled (Roche)

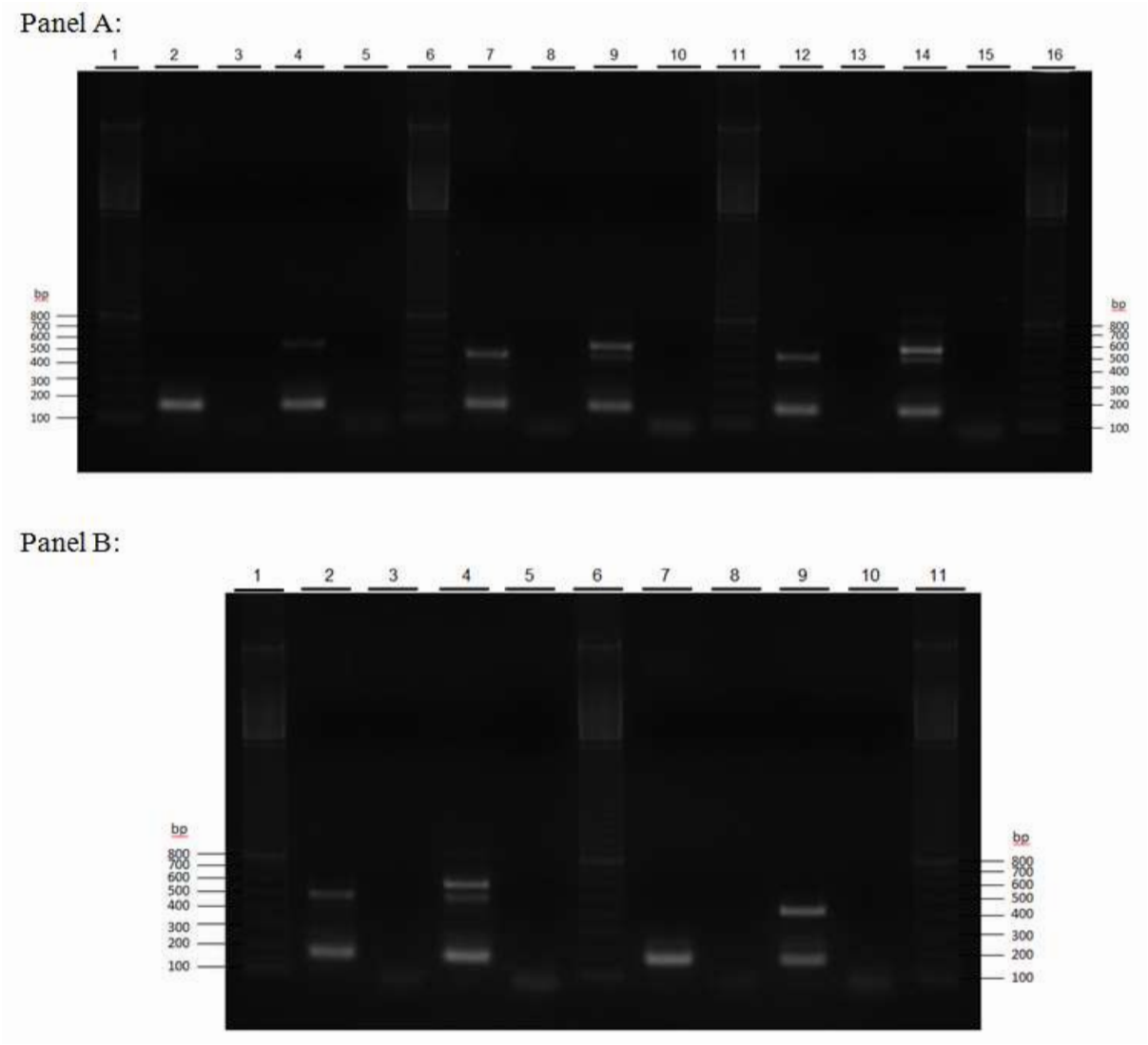


Figure 24: PCR analysis to assess absence of barstar originating from vector backbone sequence in MS11 *B. napus.*

Panel A:

Lane 1, 6, 11 and 16: 100 bp molecular weight marker

PCR template:
Lane 2, 7 and 12: gDNA from *B. napus* MS11
Lane 3, 8 and 13: gDNA from *B. napus* N90-740 (negative control)
Lane 4, 9 and 14: gDNA from *B. napus* N90-740 + equimolar amount of pTCO113 (positive control)
Lane 5, 10 and 15: water sample (no template control)

Primer combinations used:
Lane 2 to 5: GLPA049-GLPA050 (complete *barstar* in vector backbone; 556 bp)
GLPA047-GLPA048 (part of 3'g7 in T-DNA; 175 bp)

Lane 7 to 10: GLPA181-GLPA045 (*barstar* + downstream sequences in vector backbone; 550 bp)
GLPA047-GLPA048 (part of 3'g7 in T-DNA; 175 bp)

Lane 12 to 15: GLPA345-GLPA181 (*barstar* + downstream sequences in vector backbone; 572 bp)
GLPA047-GLPA048 (part of 3'g7 in T-DNA; 175 bp)

Panel B:

Lane 1, 6 and 11: 100 bp molecular weight marker

PCR template:

Lane 2 and 7: gDNA from *B. napus* MS11
Lane 3 and 8: gDNA from *B. napus* N90-740 (negative control)
Lane 4 and 9: gDNA from *B. napus* N90-740 + equimolar amount of pTCO113 (positive control)
Lane 5 and 10: water sample (no template control)

Primer combinations used:
Lane 2 to 5: GLPA345-GLPA180 (*barstar* + downstream sequences in vector backbone; 593 bp)
GLPA047-GLPA048 (part of 3'g7 in T-DNA; 175 bp)

Lane 7 to 10: GLPA050-GLPA046 (*barstar* + upstream sequences in vector backbone; 427 bp)
GLPA047-GLPA048 (part of 3'g7 in T-DNA; 175 bp)

(ii) A determination of the number of insertion sites, and the number of copies at each insertion site;

As detailed above in Section A.3 (d), Southern blot analysis and full DNA sequencing of the MS11 *B. napus* transgenic locus revealed that the inserted genetic material consists of one complete copy of the T-DNA that corresponds to the transforming plasmid. The arrangement of the MS11 *B. napus* transgenic locus is shown in Figure 27 in Section A.3(d)(iv) below (also in Figure 18 above). The Southern blot analysis is detailed in [redacted] (2016; M-547543-01; Node A.3 (c), (i), (CCI)), and sequencing of the transgenic locus is detailed in [redacted] (2008, M-304805-01-1, Node A.3 (c), (iii) (CCI)).

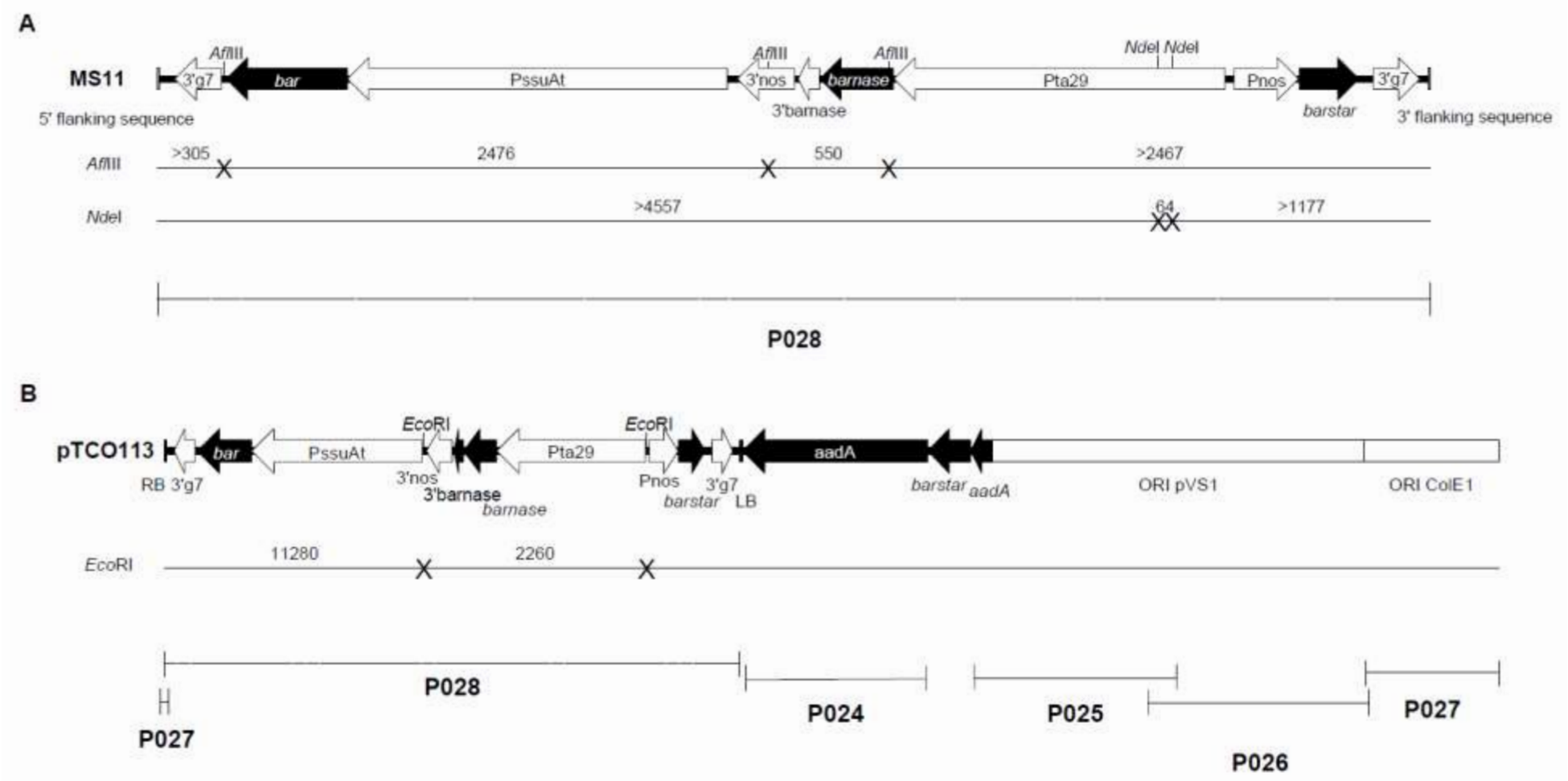


Figure 25 Schematic drawing of MS11 *B. napus* and pTCO113 with indication of the relevant restriction sites and position of the used probes to assess the potential vector backbone presence. Panel A: transgenic locus MS11 *B. napus* – Panel B: pTCO113

(iii) Full DNA sequence of each insertion site, including junction regions with the host DNA;

The DNA sequence of the MS11 *B. napus* transgenic locus and the corresponding insertion locus was determined ([redacted] 2016; M-545355-01; Node A.3 (c), (iii) (CCI); [redacted] 2008; M-304805-01; Node A.3 (c), (iii) (CCI)).

In initial experiments, six overlapping fragments were prepared to determine the sequence of the MS11 *B. napus* transgenic locus ([redacted] 2008; M-304805-01; Node A.3 (c), (iii) (CCI)). The insertion locus was amplified in one fragment. As the MS11 *B. napus* plants used in these experiments were hemizygous, containing one copy of the MS11 *B. napus* transgenic locus and one copy of the insertion locus, gDNA extracted from leaf material of MS11 *B. napus* plants was used as template for all amplifications. For each PCR fragment, multiple identical PCR reactions were performed. After amplification all identical PCR reactions were pooled for sequencing. Sanger sequencing was performed.

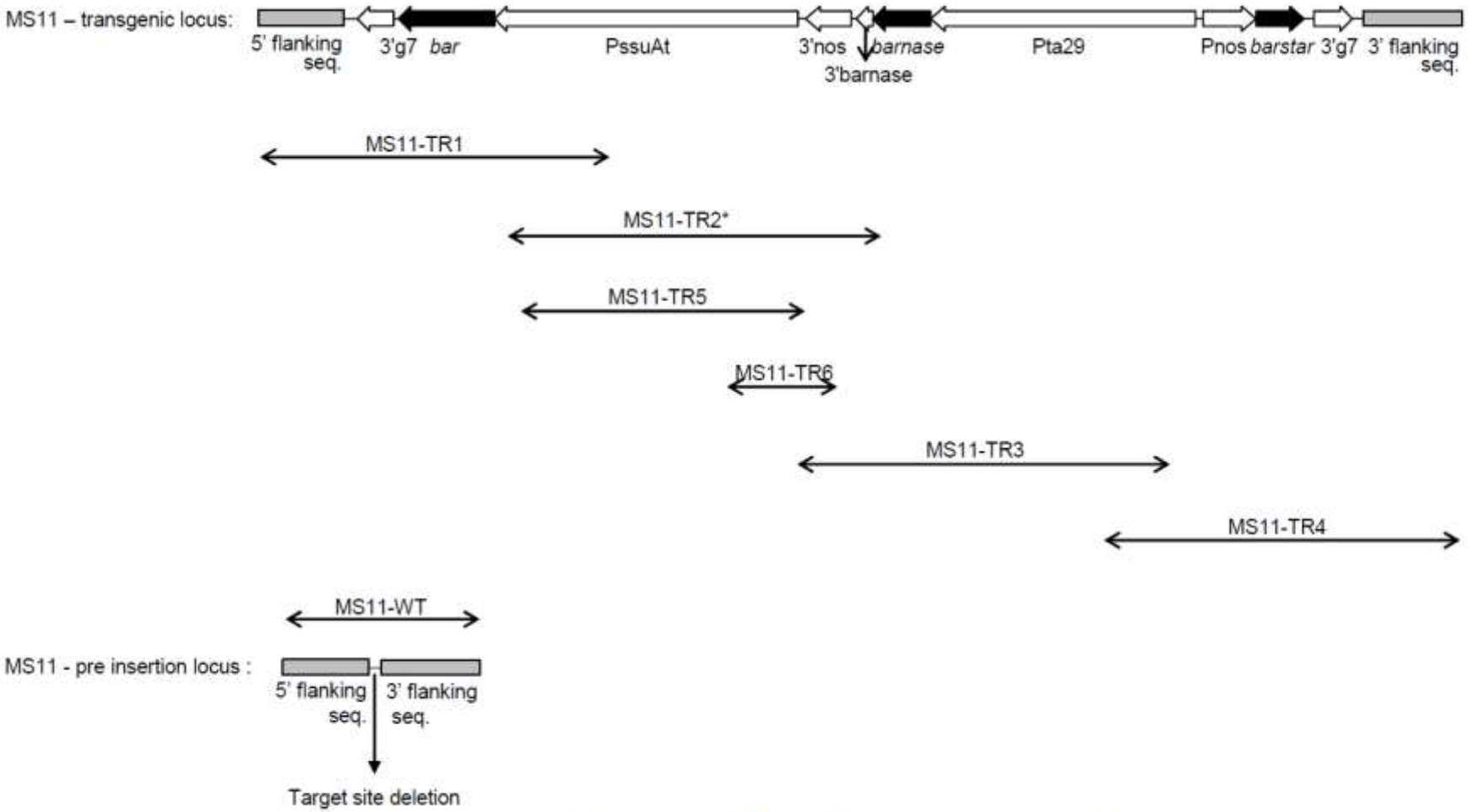
The obtained consensus sequences of the transgenic and insertion loci were annotated by pairwise alignments using the Clone Manager software. The consensus sequence of the MS11 *B. napus* transgenic locus was compared with the pTCO113 plasmid sequence to identify the T-DNA region. The consensus sequence of the MS11 *B. napus* transgenic locus was also compared to the MS11 *B. napus* insertion locus sequence to identify sequence regions of *B. napus* origin within the MS11 *B. napus* transgenic locus as well as the target site deletion (TSD) within the MS11 *B. napus* insertion locus.

To determine additional 5' and 3' flanking sequences of MS11 *B. napus* to obtain at least 1 Kb of both flanking regions, and the corresponding insertion locus sequence of MS11 *B. napus*, three additional fragments were prepared to generate additional sequence ([redacted] 2016; M-545355-01; Node A.3 (c), (iii) (CCI)). To determine additional MS11 *B. napus* flanking sequences, gDNA extracted from leaf material of MS11 *B. napus* plants was used as a template. To extend the MS11 *B. napus* insertion locus sequence, gDNA extracted from leaf material of non-GM *B. napus* variety N90-740 was used as a template. For each PCR fragment, multiple identical PCR reactions were performed. After amplification all identical PCR reactions were pooled for sequencing. Sanger sequencing was performed using the ABI PRISM® BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). A consensus sequence of all sequencing reads was generated. Each bp of the consensus sequence had 4-fold coverage, 4 independent sequencing reads.

The extended MS11 *B. napus* transgenic locus sequence was validated by comparing with the sequences of the MS11 *B. napus* trasngenic locus obtained from previous experiments. A pairwise alignment demonstrated 100% identity in the overlapping regions of the sequenced fragments of the MS11 *B. napus* transgenic locus with the previously determined MS11 *B. napus* transgenic locus sequence of [redacted] 2008 (M-304805-01-1, Node A.3 (c), (iii) (CCI)).

The extended MS11 *B. napus* insertion locus sequence was validated by comparing with the sequences of the MS11 *B. napus* insertion locus obtained from previous experiments. A pairwise alignment demonstrated 100% identity in the overlapping regions of the sequences determined for the amplified MS11 *B. napus* insertion locus fragment with the previously determined MS11 *B. napus* insertion locus sequence of [redacted] 2008 (M-304805-01-1, Node A.3 (c), (iii) (CCI)).

The final MS11 *B. napus* transgenic locus with extended flanking sequences consisted of 8209 bp, which included 1129 bp of 5' flanking sequence and 1302 bp of 3' flanking sequence. The corresponding MS11 *B. napus* insertion locus consisted of 2471 bp, which included 1129 bp of sequence 100% identical to the 5' flanking sequence, 1302 bp of sequence 100% identical to the 3' flanking sequence, and a target site deletion (TSD) of 40 bp. The results demonstrated that upon transformation, 40 bp from the MS11 *B. napus* insertion locus were replaced by 5778 bp of T-DNA from plasmid pTCO113. The flanking sequences obtained at the MS11 *B.* napus transgenic locus were identical to the corresponding sequences obtained from the insertion locus. This demonstrates that the MS11 *B. napus* flanking sequences are of *B. napus* origin within its original genomic organization.



* Fragment MS11-TR2 was not used to determine the sequence. The sequence of this region was determined using the fragments MS11-TR5 and MS11-TR6.

Figure 26 Schematic drawing of the MS11 *B. napus* elite event and the pre-insertion locus with indication of the fragments amplified for sequence determination

Table 10 Details of the primers used to determine the sequence of the MS11 *B. napus* transgenic and pre-insertion loci

FRAGMENT ID	PRIMER PAIR	POSITION IN VECTOR pTCO113 OR FLANKING REGIONS	LENGTH OF AMPLICON (BP)	OVERLAP BETWEEN FRAGMENTS (BP)
MS11-TR1	EM036	5' flanking region	ca. 1990 bp	ca. 514 bp
	MDB318	1597 → 1577		
MS11-TR5	KVM043	1084 → 1104	ca. 1563 bp	
	DPA449	2646 → 2628		ca. 413 bp
MS11-TR6	MDB750	2234 → 2253	ca. 630 bp	
	NEL040	2863 → 2845		ca. 233 bp
MS11-TR3	MAE044	2631 → 2650	ca. 2088 bp	
	MLD008	4718 → 4698		ca. 377 bp
MS11-TR4	MDB380	4342 → 4368	ca. 2019 bp	
	TVS005	3'flanking region		
MS11-WT	EM036	5' flanking region	ca. 1015 bp	
	TVS005	3' flanking region		
MS11-TR2	MLD005	1010 → 1031	ca. 2117 bp	
	MAE024	3126 → 3107		

(iv) A map depicting the organisation of the inserted genetic material at each insertion site; and

The organisation of the MS11 transgenic locus within the *B. napus* genome, as confirmed by Southern blot ([redacted], 2016; M-547543-01-1; Node A.3 (c), (i), (CCI)) and DNA sequence analyses ([redacted], 2008; M-304805-01-1; Node A.3 (c), (iii) (CCI)), and described above in Sections A.3(d)(i-iii), is shown in Figure 27 below.

Figure 27 Organisation of the MS11 *B. napus* transformation event in the *Brassica napus* genome demonstrated by Southern blot and DNA sequence analyses

(v) Details of an analysis of insert and junction regions for the occurrence of any open reading frames (ORFs).

MS11 *B. napus* expresses the Barnase protein in the tapetal cells of the anthers during pollen development. This expression leads to the abortion of pollen grains and thus leads to male sterility. Crossing of female MS11 *B. napus* plants with the pollen of RF3 *B. napus*, which expresses the Barstar protein, results in generation of hybrid seeds. In these seeds, the Barstar protein inhibits the activity of the Barnase protein, and therefore fully restores the pollen development and thus the fertility.

A bioinformatics analysis was performed on the transgenic locus sequence of the MS11 *Brassica*

napus to identify potential open reading frames (ORF). In the next step, the predicted ORF sequences of at least 30 amino acids were used as query sequences in homology searches to known allergens and toxins ([redacted] 2016; M-552421-01, Node A.3 (c), (v) (CCI)).

A bioinformatics analysis was first performed on the transgenic locus sequence of the MS11 *B napus* and identified 554 ORF crossing a junction or overlapping the inserted DNA, between two translation stop codons, with a minimum size coding for 3 amino acids. From them, 107 unique ORF sequences were of ≥30 amino acids length.

These ORF sequences of ≥30 amino acids length showed no biologically relevant sequence identities with known allergens and known toxins.

Therefore, there are neither allergenic nor toxicological *in silico* findings associated with the presence of the potential ORF polypeptides.

Bioinformatic analysis of the MS11 *B. napus* insertion locus

Bioinformatics analysis on the MS11 *B. napus* insertion locus sequence was performed to identify the insertion locus and to determine whether regulatory sequences or endogenous *B. napus* genes were interrupted upon the insertion of T-DNA sequences. ([redacted] 2016; M-307568-02; Node A.3 (c), (v)).

BLAST (Basic Local Alignment Search Tool) searches were performed in order to search for identity with known genes and proteins. The BLAST tool available on the National Center for Biotechnology Information (NCBI) website (http://blast.ncbi.nlm.nih.gov) was used with default parameters. Database definitions are provided in Table 11.

BLASTn, which compares a nucleotide query sequence against a nucleic sequence database, was used to identify similarities between the MS11 *B. napus* insertion locus and sequences within the nucleotide collection and the Expressed Sequence Tag (EST) databases available on the NCBI website.

BLASTx, which compares the six-frame theoretical translation products of the nucleotide query sequence (both strands) against a protein sequence database, was used to compare the MS11 *B. napus* insertion locus sequence to the NCBI non-redundant protein database.

Similarities between the MS11 *B. napus* insertion locus and *B. napus* genome were identified using the BLAT tool (BLAST-like alignment tool) and a *B. napus* reference genome (Chalhoub, B.; *et al.*; 2014; M-541668-01; published) available on the Genoscope website (http://www.genoscope.cns.fr/brassicanapus/). Default parameters were used.

Based on the bioinformatics analysis performed, the MS11 *B. napus* insertion locus originates from *B. napus* chromosome A03. Similarity searches indicated the presence of an endogenous gene in the 3' flanking sequence region of the MS11 *B. napus* insertion locus. The coding sequence of this gene is not interrupted upon insertion of T-DNA sequences. Therefore, the insertion of T-DNA sequences in the MS11 *B. napus* insertion locus is unlikely to interrupt or alter transcriptional or translational activity of known endogenous *B. napus* genes.

Table 11: Database definitions used for BLAST analysis

Database	Algorithm	Posted Date	Analysis date	Number of sequences	Number of letters
NCBI Nucleotide collection (nr/nt)	BLASTN 2.3.1+	Feb 29, 2016 8:13 AM	Mar 02, 2016	34,921,546	113,216,291,083
NCBI Expressed Sequence Tags (EST)	BLASTN 2.3.1+	Feb 28, 2016 6:19 AM	Mar 02, 2016	76,144,851	42,428,242,625
NCBI Non-redundant protein sequences (nr)	BLASTX 2.3.1+	Feb 29, 2016 8 :12 AM	Mar 02, 2016	82,777,350	30,298,809,097
Genoscope Brassica napus L Reference Genome	BLAT	N.A.	Mar 02, 2016	N.A.	N.A.

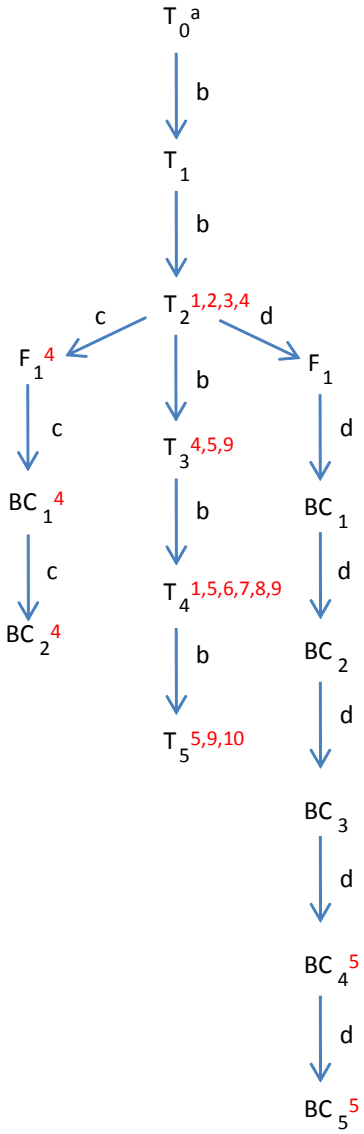
(d) A description of how the line or strain from which food is derived was obtained from the original transformant (i.e. provide a family tree or describe the breeding process) including which generations have been used for each study.

Following *Agrobacterium*-mediated transformation of the conventional breeding line, N90-740 resulting in MS11 *B. napus*, T_0 plants were treated with glufosinate-ammonium to select for the expression of the *bar* gene. T_0 hemizygous MS11 *B. napus* plants were cross-pollinated with non-genetically modified (non-GM) plants (N90-740 variety) to produce the T_1 generation. MS11 *B. napus* hemizygous plants from the T_1 generation were cross-pollinated with non-GM plants (N90-740 variety) to produce the T_2 generation. The process of crossing MS11 *B. napus* hemizygous plants with non-GM plants (N90-740 variety) was repeated to produce the T_3, T_4, and T_5 generations.

MS11 *B. napus* hemizygous plants from the T_2 generation were also cross-pollinated with non-GM plants (B144 variety) creating a F_1 generation. MS11 *B. napus* hemizygous plants from the F_1 generation were backcrossed to non-GM plants (B144 variety) to produce a BC_1 generation. The process of backcrossing MS11 *B. napus* hemizygous plants with non-GM B144 plants was repeated to produce the BC_2, BC_3, BC_4, and BC_5 generations.

MS11 *B. napus* hemizygous plants from the T_2 generation were also cross-pollinated with non-GM plants (Ebony variety) creating a F_1 generation. MS11 *B. napus* hemizygous plants from the F_1 generation were backcrossed to non-GM plants (Ebony variety) to produce a BC_1 generation. The process of backcrossing MS11 *B. napus* hemizygous plants with non-GM plants (Ebony variety) was repeated to produce a BC_2 generation.

The breeding program for the development of event MS11 *B. napus* and its introgression into *Brassica napus* germplasm is demonstrated in Figure 28 below. Table 12 describes the MS11 *B. napus* generations used for analysis and the associated reports describing these studies.



a: N90-740 variety was used for transformation
b: crossing with N90-740 variety
c: crossing with Ebony variety
d: crossing with B144 variety

Figure 28. Pedigree of MS11 *B. napus*

Table 12. Generations used for analysis of MS11 *Brassica napus*

No. in Tree	Experiment	Generation(s)	Comparator
1	DNA sequencing of insert and flanking region	T2 T4	N90-740 None
2	Insert Characterization by Southern Analysis	T2	N90-740
3	Absence of Vector Backbone by Southern Analysis	T2	N90-740
4	Structural Stability by Southern Analysis	T2 T3 F1 (Ebony) BC1 (Ebony) BC2 (Ebony)	N90-740
5	Inheritance of the Insert	T3 T4 T5 BC4 (B144) BC5 (B144)	None
6	Agronomic and phenotypic Analysis	T4	N90-740
7	Composition Analysis	T4	N90-740
8	Protein Expression Analysis	T4	N90-740
9	Protein Expression over generations	T3 T4 T5	N90-740
10	Toxicity feeding study (90-day rat) – to be provided to FSANZ when study is complete	T5	N90-740

(e) Evidence of the stability of the genetic changes, including:

(i) The pattern of inheritance of the transferred gene(s) and the number of generations over which this has been monitored; and

The structural stability of the MS11 *B. napus* was demonstrated by assessing individual MS11 *B. napus* plants from five generations (T2, T3, F1, BC1, and BC2) by means of Southern blot analysis ([redacted] 2016; M-547544-01, Node A.3 (e), (i) (CCI)).

Seeds from five different seed lots were used to produce MS11 *B. napus* leaf material. The identity and zygosity of the individual plants were confirmed. Non-genetically modified (non-GM) *B. napus* variety N90-740 (non-GM counterpart) was used as a negative control. The positive control was the transforming plasmid of MS11 *B. napus* (pTCO113).

The MS11 *B. napus* and non-GM counterpart gDNA samples were digested with the *Eco*RV restriction enzyme. Plasmid DNA of pTCO113 was digested with the *Eco*RI restriction enzyme. The resulting DNA fragments were separated by agarose gel-electrophoresis. Transfer of the separated DNA fragments from the agarose gel to a positively charged nylon membrane was performed by a neutral Southern blotting

procedure. The resulting membranes were hybridized with a DIG-labeled T-DNA probe P028 (Table 13). A schematic overview of the MS11 *B. napus* transgenic locus, with indication of the restriction sites, the T-DNA probe used, and the expected fragments is presented in Figure 29.

Each membrane used for the analysis contained one negative control. For all hybridizations, this negative control showed no hybridization with the T-DNA probe, confirming the absence of any background hybridization with the probe used. Similarly, each membrane contained one positive control. For all hybridizations, the expected fragments were detected for the positive control indicating that the conditions of Southern blot experiments allowed specific hybridization of the T-DNA probe with the target sequences. A number of small, weak additional fragments were obtained for the positive control on the membrane containing the T3 generation (Figure 31). These additional fragments are artifacts, which are most likely due to star activity since these fragments were no longer observed after hybridization of a freshly digested transforming plasmid pTCO113 with the same T-DNA probe -
Figure 30 Figure 32; Figure 33; and Figure 34).

Genomic DNA from individual MS11 *B. napus* plants was digested with *Eco*RV. For all individual plants confirmed as positive for the presence of MS11 *B. napus* from the T2, T3, F1, BC1 and BC2 generations, both expected fragments (4400 bp and 4900 bp) were obtained -
Figure 30 to Figure 34 and Table 14).

These results demonstrate the structural stability of MS11 *B. napus* in the T2, T3, F1, BC1 and BC2 generations.

Table 13: Information on the probe used

Probe ID	Description	Primer pair	Primer sequence (5' → 3')	Primer position in pTCO113 (bp)	Probe size (bp)
P028	T-DNA	GLPA174	[redacted]	1 → 25	5865
		GLPA359	[redacted]	5865 → 5841	

Table 14: Stability of MS11 *B. napus* in the individual plants - Expected and obtained hybridization fragments

Sample	Reference to figure[$]	Fragment size (bp)	Fragment description	Probe P028 T-DNA	
				Exp.	Obt.
10 samples scoring positive for MS11 - T2 generation – EcoRV	Figure 2	approx. 4900 *	5' integration fragment	Yes	Yes
		approx. 4400 *	3' integration fragment	Yes	Yes
10 samples scoring positive for MS11 – T3 generation – *Eco*RV	Figure 3	approx. 4900 *	5' integration fragment	Yes	Yes
		approx. 4400 *	3' integration fragment	Yes	Yes
9 samples scoring positive for MS11 – F1 generation – *Eco*RV	Figure 4	approx. 4900 *	5' integration fragment	Yes	Yes
		approx. 4400 *	3' integration fragment	Yes	Yes
10 samples scoring positive for MS11 – BC1 generation – *Eco*RV	Figure 5	approx. 4900 *	5' integration fragment	Yes	Yes
		approx. 4400 *	3' integration fragment	Yes	Yes
10 samples scoring positive for MS11 – BC2 generation – *Eco*RV	Figure 6	approx. 4900 *	5' integration fragment	Yes	Yes
		approx. 4400 *	3' integration fragment	Yes	Yes
non-GM counterpart – *Eco*RV	Figure 2 to Figure 6	/	Negative control	/	/
non-GM counterpart – *Eco*RI digested + an equimolar amount of pTCO113 – *Eco*RI digested	Figure 2 to Figure 6	2260	Positive control°	Yes	Yes
		11280		Yes	Yes

[$] lane numbers see legend of figures
* Fragment sizes as determined in the "Detailed insert characterization and confirmation of the absence of vector backbone sequences in MS11 *B. napus"* study
°For the membrane containing the T3 generation samples, additional weak fragments were obtained in the positive control, see Figure 3

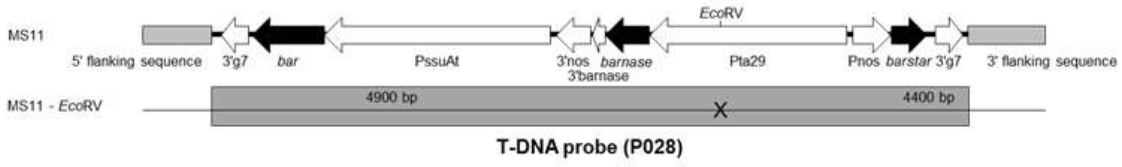


Figure 29: Schematic overview of the MS11 *B. napus* transgenic locus with indication of the restriction sites, the probe used and expected fragment sizes in bp

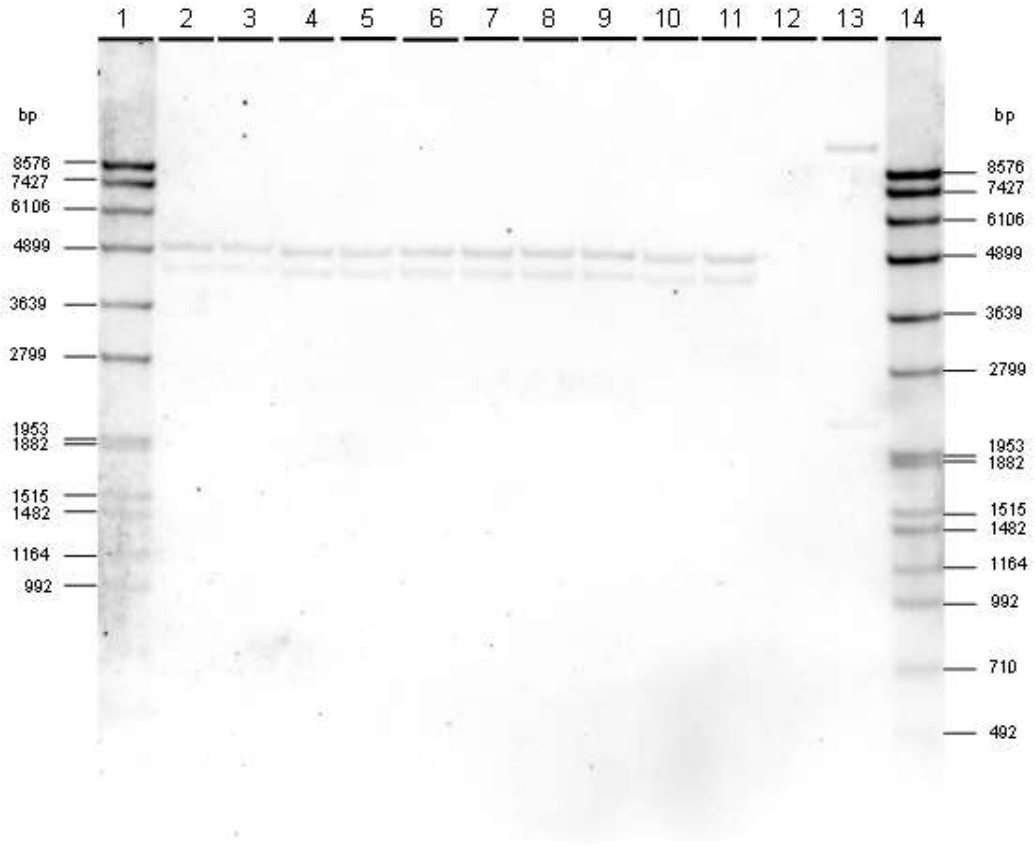


Figure 30: Southern blot analysis of MS11 *B. napus* – Hybridization performed with a MS11 *B. napus* T-DNA probe to assess structural stability of the individual plants of the T2 generation

Genomic DNA was isolated from individual MS11 *B. napus* plants of the T2 generation confirmed as positive for the presence of MS11 *B. napus* and from the non-GM counterpart. The gDNA samples were digested with the restriction enzyme *Eco*RV and hybridized with a probe corresponding to the MS11 *B. napus* T-DNA region (P028-13).

Lane 1: 10 ng DNA Molecular Weight marker VII, DIG-labeled + 3 µg of gDNA of the non-GM counterpart – *Eco*RI digested
Lane 2 to 11: 3 µg gDNA of individual samples of MS11 *B. napus* of the T2 generation scoring positive for the presence of MS11 *B. napus* – *Eco*RV digested
Lane 12: 3 µg gDNA of the non-GM counterpart – *Eco*RV digested (negative control)
Lane 13: 3 µg gDNA of the non-GM counterpart – *Eco*RI digested + an equimolar amount of plasmid pTCO113 – *Eco*RI digested (positive control)
Lane 14: 10 ng DNA Molecular Weight marker VII, DIG-labeled + 3 µg of gDNA of the non-GM counterpart – *Eco*RI digested

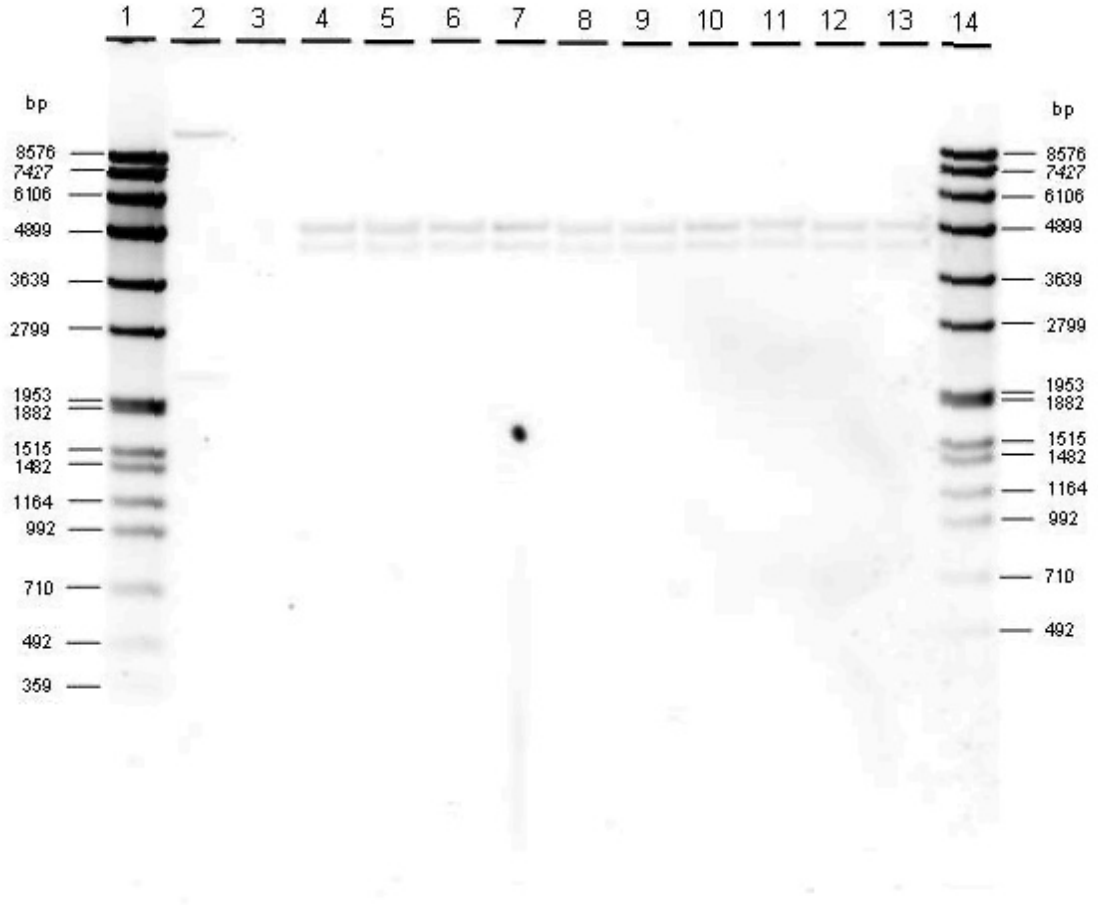


Figure 31: Southern blot analysis of MS11 *B. napus* – Hybridization performed with a MS11 *B. napus* T-DNA probe to assess structural stability of the individual plants of the T3 generation

Genomic DNA was isolated from individual MS11 *B. napus* plants of the T3 generation confirmed as positive for the presence of MS11 *B. napus* and from the non-GM counterpart. The gDNA samples were digested with the restriction enzyme *Eco*RV and hybridized with a probe corresponding to the MS11 *B. napus* T-DNA region (P028-02).

Lane 1: 10 ng DNA Molecular Weight marker VII, DIG-labeled + 3 µg of gDNA of the non-GM counterpart – *Eco*RI digested
Lane 2: 3 µg gDNA of the non-GM counterpart – *Eco*RI digested + an equimolar amount of plasmid pTCO113 – *Eco*RI digested (positive control)
Lane 3: 3 µg gDNA of the non-GM counterpart – *Eco*RV digested (negative control)
Lane 4 to 13: 3 µg gDNA of individual samples of MS11 *B. napus* of the T3 generation scoring positive for the presence of MS11 *B. napus* – *Eco*RV digested
Lane 14: 10 ng DNA Molecular Weight marker VII, DIG-labeled + 3 µg of gDNA of the non-GM counterpart – *Eco*RI digested

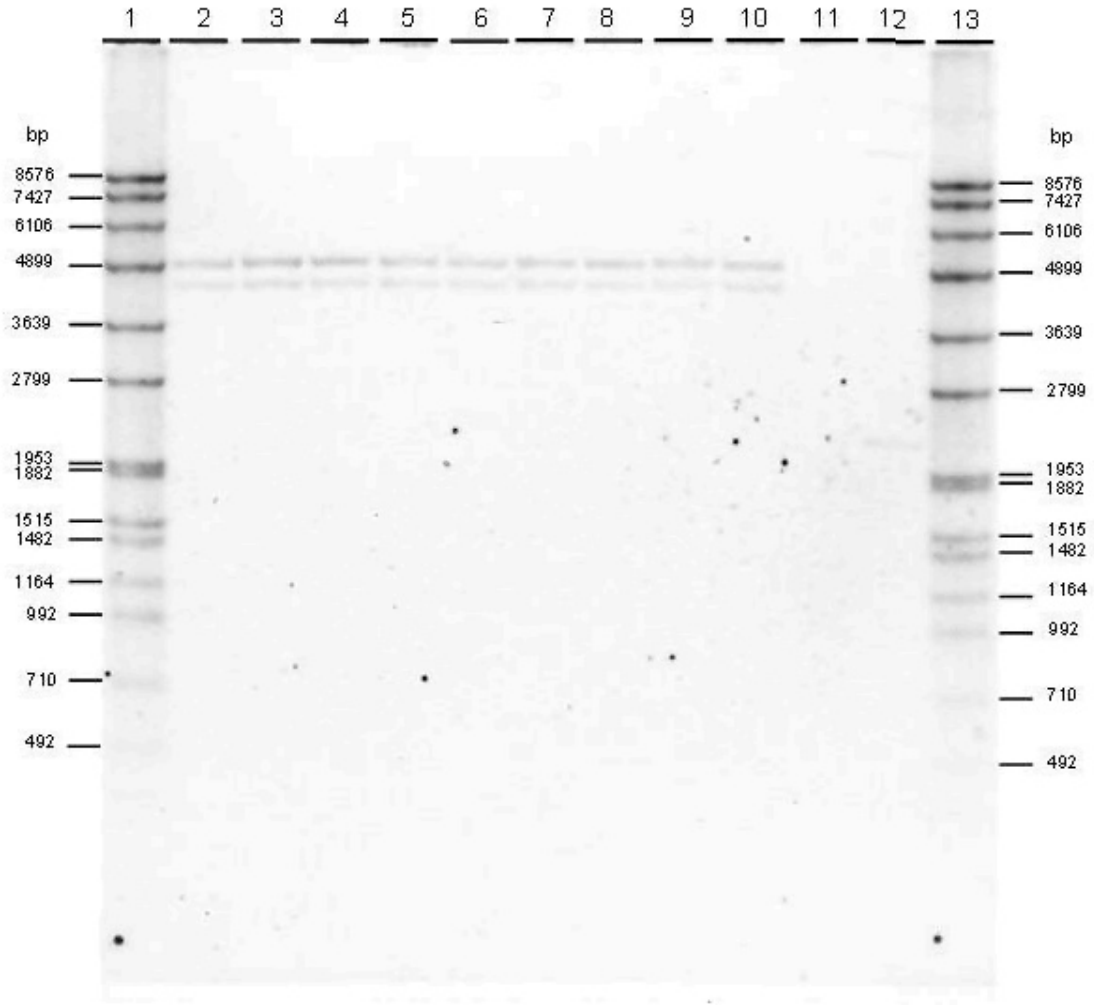


Figure 32: Southern blot analysis of MS11 *B. napus* – Hybridization performed with a MS11 *B. napus* T-DNA probe to assess structural stability of the individual plants of the F1 generation

Genomic DNA was isolated from individual MS11 *B. napus* plants of the F1 generation confirmed as positive for the presence of MS11 *B. napus* and from the non-GM counterpart. The gDNA samples were digested with the restriction enzyme *Eco*RV and hybridized with a probe corresponding to the MS11 *B. napus* T-DNA region (P028-05).

Lane 1: 10 ng DNA Molecular Weight marker VII, DIG-labeled + 3 µg of gDNA of the non-GM counterpart – *Eco*RI digested
Lane 2 to 10: 3 µg gDNA of individual samples of MS11 *B. napus* of the F1 generation scoring positive for the presence of MS11 *B. napus* – *Eco*RV digested
Lane 11: 3 µg gDNA of the non-GM counterpart – *Eco*RV digested (negative control)
Lane 12: 3 µg gDNA of the non-GM counterpart – *Eco*RI digested + an equimolar amount of plasmid pTCO113 – *Eco*RI digested (positive control)
Lane 13: 10 ng DNA Molecular Weight marker VII, DIG-labeled + 3 µg of gDNA of the non-GM counterpart – *Eco*RI digested

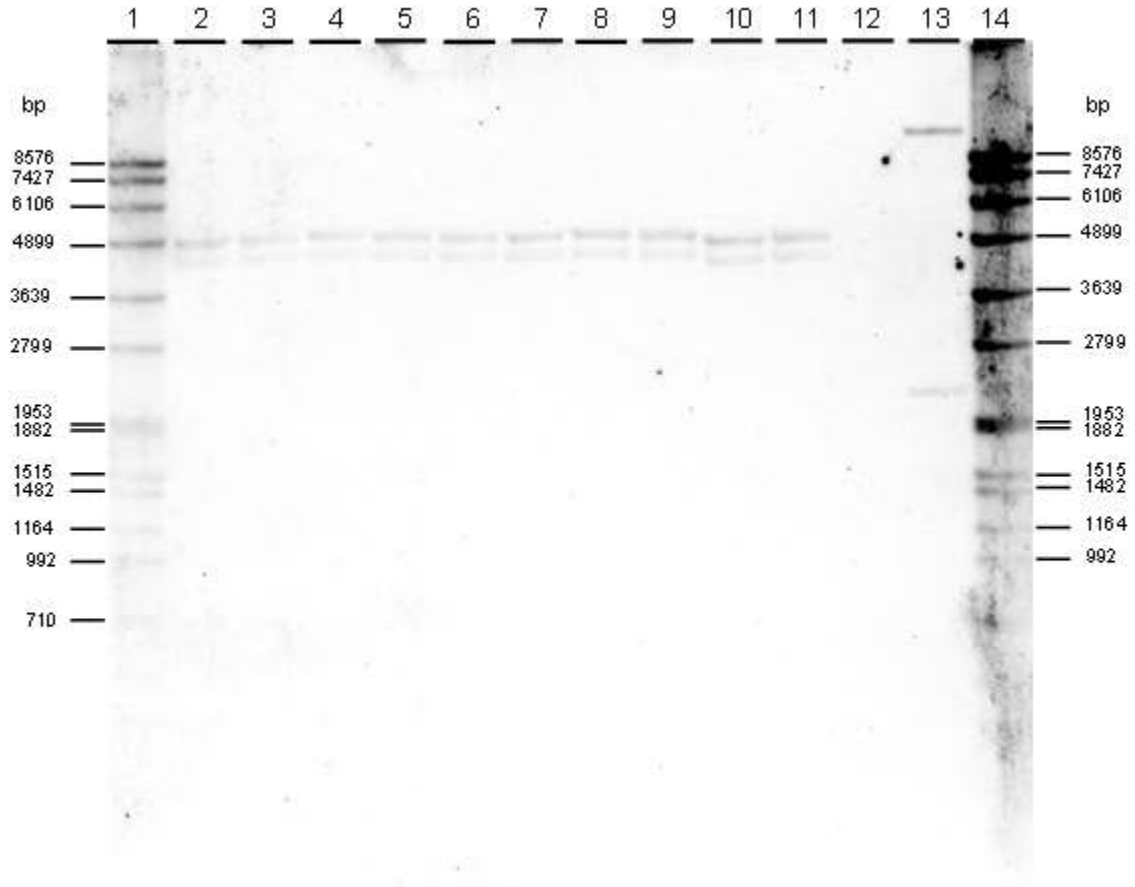


Figure 33: Southern blot analysis of MS11 *B. napus* – Hybridization performed with a MS11 *B. napus* T-DNA probe to assess structural stability of the individual plants of the BC1 generation

Genomic DNA was isolated from individual MS11 *B. napus* plants of the BC1 generation confirmed as positive for the presence of MS11 *B. napus* and from the non-GM counterpart. The gDNA samples were digested with the restriction enzyme *Eco*RV and hybridized with a probe corresponding to the MS11 *B. napus* T-DNA region (P028-05).

Lane 1: 10 ng DNA Molecular Weight marker VII, DIG-labeled + 3 µg of gDNA of the non-GM counterpart – *Eco*RI digested
Lane 2 to 11: 3 µg gDNA of individual samples of MS11 *B. napus* of the BC1 generation scoring positive for the presence of MS11 *B. napus* – *Eco*RV digested
Lane 12: 3 µg gDNA of the non-GM counterpart – *Eco*RV digested (negative control)
Lane 13: 3 µg gDNA of the non-GM counterpart – *Eco*RI digested + an equimolar amount of plasmid pTCO113 – *Eco*RI digested (positive control)
Lane 14: 10 ng DNA Molecular Weight marker VII, DIG-labeled + 3 µg of gDNA of the non-GM counterpart – *Eco*RI digested

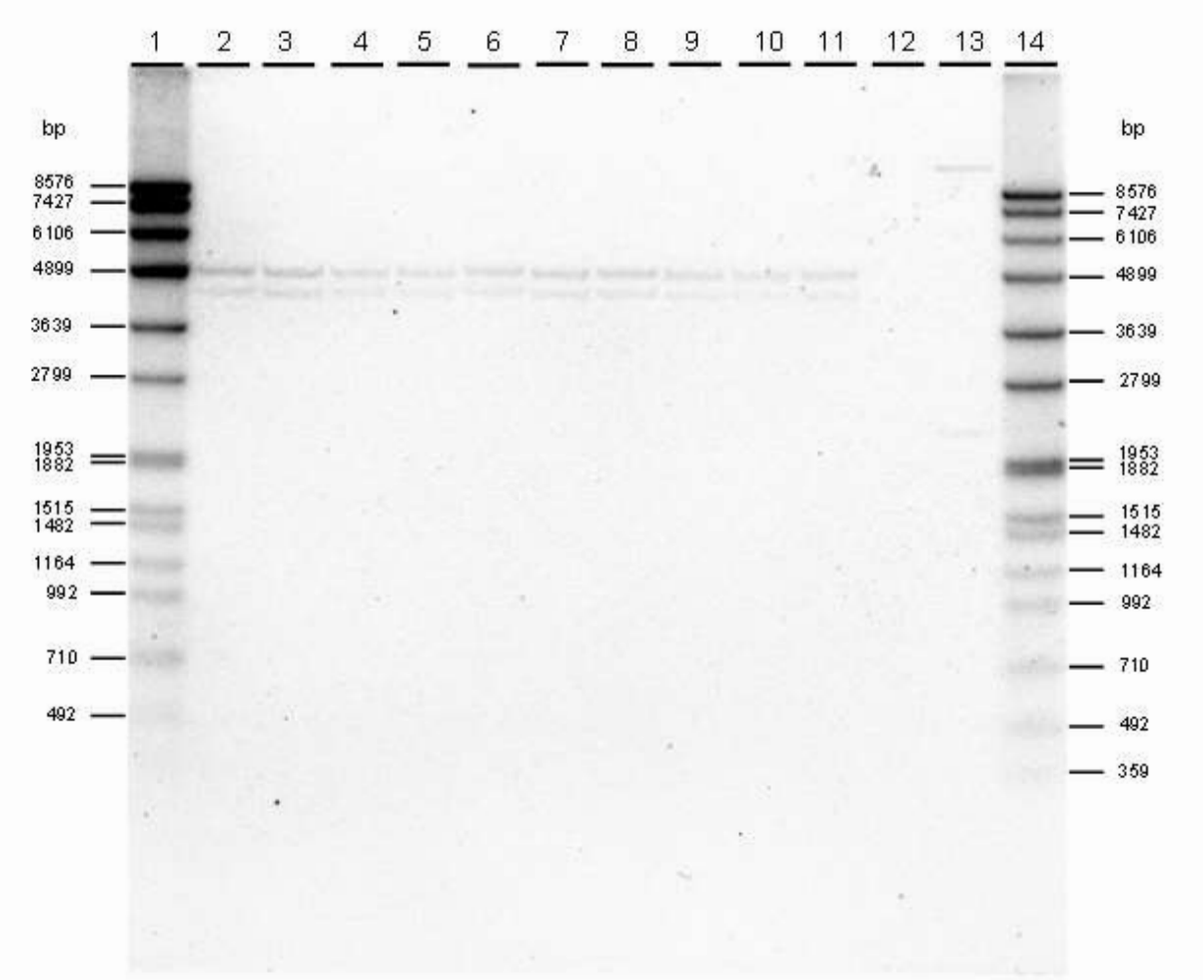


Figure 34: Southern blot analysis of MS11 *B. napus* – Hybridization performed with a MS11 *B. napus* T-DNA probe to assess structural stability of the individual plants of the BC2 generation

Genomic DNA was isolated from individual MS11 *B. napus* plants of the BC2 generation confirmed as positive for the presence of MS11 *B. napus* and from the non-GM counterpart. The gDNA samples were digested with the restriction enzyme *Eco*RV and hybridized with a probe corresponding to the MS11 *B. napus* T-DNA region (P028-05).

Lane 1: 10 ng DNA Molecular Weight marker VII, DIG-labeled + 3 µg of gDNA of the non-GM counterpart – *Eco*RI digested
Lane 2 to 11: 3 µg gDNA of individual samples of MS11 *B. napus* of the BC2 generation scoring positive for the presence of MS11 *B. napus* – *Eco*RV digested
Lane 12: 3 µg gDNA of the non-GM counterpart – *Eco*RV digested (negative control)
Lane 13: 3 µg gDNA of the non-GM counterpart – *Eco*RI digested + an equimolar amount of plasmid pTCO113 – *Eco*RI digested (positive control)
Lane 14: 10 ng DNA Molecular Weight marker VII, DIG-labeled + 3 µg of gDNA of the non-GM counterpart – *Eco*RI digested

(ii) The pattern of inheritance and expression of the phenotype over several generations and, where appropriate, across different environments.

Section A.3(e) above details the MS11 *B. napus* breeding program, and section A.3(f)(i) (directly above) details experiments undertaken to test the structural stability of the MS11 *B. napus* transgenic locus over different generations and in different environments. The expression of the PAT protein was tested at every step of the breeding program through the application of glufosinate ammonium to select for the MS11 *B. napus* phenotype.

Genomic DNA from individual plants of five MS11 *B. napus* generations (T3, T4, T5, BC4, and BC5) was tested for the absence or presence of MS11 *B. napus* by polymerase chain reaction (PCR) analysis ([redacted]; 2016; M-545765-01, Node A.3 (e), (ii)). The results from event-specific PCR analysis were used to calculate the segregation ratios of the MS11 *B. napus* insert.

Chi-square analysis of the segregation data for each of the five generations was performed to test the hypothesis that the MS11 *B. napus* insert is inherited in a manner that is predictable according to Mendelian principles and is consistent with insertion into a single chromosomal locus within the *B. napus* nuclear genome.

Plant samples were analyzed using event-specific PCR to determine the presence or absence of the MS11 *B.napus* insert. PCR analysis included the amplification of the MS11 *B. napus* event-specific sequence and the amplification of an endogenous gene sequence. Samples with signal corresponding to the MS11 *B. napus* event-specific sequence and the endogenous sequence were recorded as positive for the MS11 *B. napus* insert. Samples with signal corresponding to the endogenous sequence only were recorded as negative.

The Chi-square analysis is based on testing the observed segregation ratio relative to the segregation ratio expected from Mendelian inheritance principles. For the T3, T4, T5, BC4, and BC5 generations of MS11 *B. napus*, the expected segregation ratio of positive and negative was 1:1. The $\chi 2$ values were calculated using the following equation.

$$\chi 2 = \sum \frac{|(\text{Observed} - \text{Expected})|2}{\text{Expected}}$$

The results for MS11 *B. napus* event-specific PCR are summarized in Table 15. In addition, the absence or presence of the *bar*, *barstar*, and *barnase* genes was determined using gene-specific PCR analysis. The results from the gene-specific PCR analysis confirmed that the *bar*, *barstar*, and *barnase* genes are present for samples positive for MS11 *B. napus* and are absent for samples negative for MS11 *B. napus*.

Segregation ratios determined for five generations of MS11 *B. napus* confirmed that the MS11 *B. napus* insert is inherited in a predictable manner and as expected for a single insertion. These data are consistent with Mendelian principles and support the conclusion that MS11 *B. napus* consists of a single insert integrated at a single chromosomal locus within the *B. napus* nuclear genome.

Table 15: Observed Versus Expected Identity for MS11 in T3, T4, T5, BC4, and BC5 as Determined by PCR Analysis

MS11 Insert	T3		T4		T5		BC4		BC5	
	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected
Positive	42	42	48	46	39	47.5	43	44.5	51	49
Negative	42	42	44	46	56	47.5	46	44.5	47	49
χ^2 Value *	0		0.174		3.042		0.101		0.163	

* The critical value to reject the null hypothesis at the 5% confidence level is < 3.84 with one degree of freedom.

(g) an analysis of the expressed RNA transcripts, where RNA interference has been used.

RNA interference has not been used to develop this food product.

B.1 Characterisation and safety assessment of new substances

(a) A full description of the biochemical function and phenotypic effects of all new substances (e.g. a protein or an untranslated RNA) that are expressed in the new GM organism, including their levels and site of accumulation, particularly in edible portions

PAT/*bar* protein

The phosphinothricin acetyltransferase (PAT) protein is encoded by the *bar* gene that was isolated from *Streptomyces hygroscopicus* in the mid-1980s (Murakami, T.; et al.; 1986). *Streptomyces hygroscopicus* is a common saprophytic bacterial species that is found worldwide (Kutzner, H. J.; 1981; M-204308-01). Soil is the predominant habitat of these organisms but they may also be isolated from water. It is expected that humans would be exposed to these microorganisms and compounds directly through the consumption of roots and other vegetables that are eaten fresh. These organisms are not known to be pathogens of plants, humans or other animals (OECD; 1999; M-204493-01).

The *Streptomycetae* bacteria were first described in 1916 (Waksman, S. A.; Curtis, R. E.; 1916). Species of these genera are Gram-positive, sporulating soil micro-organisms that are commonly referred to as actinomycetes (OECD; 1999; M-204493-01). *Streptomyces hygroscopicus* produces a variety of useful antimicrobial (e.g. rapamycin and hygromycin B) and herbicidal compounds (L-PPT and Bialaphos, a derivative of phosphinothricin; Dunne *et al.*, 1998). *Streptomyces* is one of only two genera that are reported to synthesise L-phosphinothricin (L-PPT; the other is *Kitasatosporia*), the L-isomer of the amino acid phosphinothricin (OECD; 1999; M-204493-01).

Acetyltransferase activity and natural resistance to phosphinothricin have been reported for several genera of soil bacteria (Bartsch *et al.*, 1989). This resistance is thought to have evolved as a competitive mechanism to protect these microorganisms from antimicrobials produced by both themselves and other competing microorganisms (Bartsch and Tebbe, 1989). Tolerance to phosphinothricin is conferred through acetylation of L-phosphinothricin (L-PPT), the active isomer of glufosinate ammonium (Bartsch *et al.*, 1989).

Through the use of recombinant DNA technologies to introduce the *bar* gene, a range of commercial transgenic crops have been developed that express the PAT enzyme (OECD; 1999; M-204493-01). The crops include cotton, maize, oilseed rape, rice and soybean (Herouet, C.; et al.; 2005; M-247779-01-2). Through the expression of PAT, these crops are able to detoxify L-phosphinothricin (L-PPT), and exhibit tolerance to post-emergent application of herbicides containing glufosinate ammonium as the active ingredient (International Life Sciences Institute (ILSI); 2011; M-411628-01).

The safety evaluation of transgenic crops expressing novel proteins considers the source of the protein, the activity and intrinsic properties of the protein, and the potential allergenicity and toxicity of the protein (Herouet, C.; et al.; 2005; M-247779-01-2). *Streptomyces hygroscopicus* is not known to be a pathogen of plants, humans or other animals (OECD; 1999; M-204493-01), and the PAT protein, like other acetyletransfereases, is not known to have any allergenic or toxic properties, and has a well-characterised activity and substrate specificity. A battery of tests performed according to internationally accepted methods and standards have established that the PAT protein does not possess structural or functional similarity with known toxic proteins or allergens; it shares no sequence homology with known allergens and toxins, no N-glycosylation sites, and rapidly degrades in simulated digestive environments (Herouet, C.; et al.; 2005; M-247779-01-2).

Transgenic crops expressing the PAT protein have been grown for more than two decades in the USA and Canada. In the 2011 review by ILSI, it was estimated that regulatory authorities in seven countries had issued approvals for the environmental release of six transgenic crop species that express PAT proteins (encoded by the *bar* or *pat* gene), either alone or in combination with genes for other traits (e.g. insect resistance) (International Life Sciences Institute (ILSI); 2011; M-411628-01).

Expression of PAT/*bar* in Plant Tissues

In the protein expression study that is provided, not only was MS11 B. napus analysed, but also the other component line RF3 B. napus and the resultant MS11x RF3 B. napus stack, as this is the intended commercial product.

The untreated MS11 *B. napus* entry contained positive plants as well as negative segregants, as expected. All samples with PAT/*bar* expression levels <LLOQ except for root samples were excluded from mean and standard deviation calculations and ranges as they were determined to be negative segregants. Protein expression levels in positive roots samples were close to the LLOQ, and it was not possible to distinguish between root samples with low expression levels and root samples obtained from negative segregants.
As a result, root samples with PAT/*bar* protein expression levels <LLOQ were excluded from mean and standard deviation calculations but included in the ranges. The level of PAT/*bar* expression in untreated and treated (sprayed) MS11 *B. napus* matrices ranged from <LLOQ (observed in root at stem elongation and first flowering growth stages) to 74.44 µg/g DW (observed in whole plant at 3-5 leaf growth stage). The level of PAT/*bar* expression in untreated and treated RF3 *B. napus* matrices ranged from 0.27 µg/g DW (observed in root at stem elongation growth stage) to 181.94 µg/g DW (observed in whole plant at 3-5 leaf stage growth stage). The level of PAT/*bar* expression in untreated and treated MS11 x RF3 *B. napus* matrices ranged from 0.25 µg/g DW (observed in grain at maturity) to 108.12 µg/g DW (observed in raceme) (

Table 16) ([redacted], 2015; M-549123-01; Node B.1 (a)).
Root (stem elongation and first flowering growth stages) and grain matrices all exhibited lower mean PAT/*bar* DW expression levels relative to mean DW values for other matrices of MS11 , RF3, and MS11 x RF3 *B. napus*. Mean (±SD) PAT/*bar* expression levels in untreated and treated root and grain matrices of MS11, RF3, and MS11 x RF3 varied within the range of 0.17 ± 0.03 µg/g DW and 2.56 ± 2.33 µg/g DW.
Mean PAT/*bar* DW expression levels were highest in whole plant (3-5 leaf growth stage) for RF3 and MS11 x RF3 *B. napus*. Mean (±SD) PAT/*bar* expression levels in whole plant samples (3-5 leaf growth stage) of untreated and treated RF3 were 62.70 ± 21.39 and 63.71 ± 37.54 µg/g DW, respectively. Mean (±SD) PAT/*bar* expression levels in whole plant samples (3-5 leaf growth stage) of untreated and treated MS11 x RF3 were 41.16 ± 15.35 and 52.09 ± 18.17 µg/g DW, respectively.
Mean (±SD) PAT/*bar* DW expression levels were highest in whole plant samples of untreated and treated MS11 *B. napus* at stem elongation and 3-5 leaf growth stages, respectively. Mean PAT/*bar* expression levels in whole plant samples at stem elongation growth stage of untreated MS11 was 24.68 ± 12.02 µg/g DW. Mean PAT/*bar* expression levels in whole plant samples at the 3-5 leaf growth stage of treated MS11 was 35.40 ± 16.22 µg/g DW.

Table 16: PAT/*bar* (µg/g Fresh Weight and Dry Weight). Mean Fresh Weight and Dry Weight Concentrations and Expression Ranges of PAT/*bar* in Plant Matrices Harvested from Untreated and Treated MS11, RF3 and MS11 x RF3 *B. napus* Entries Grown at Three Sites

Growth Stage	Sample Matrix	Entry ID	PAT/*bar* (µg/g FW)		PAT/*bar* (µg/g DW)	
			Mean ± SD	Range	Mean ± SD	Range
3-5 Leaf	Whole Plant	B (n=4)	2.34 ± 0.13	2.20 – 2.52	22.02 ± 7.09	14.93 – 30.88
		C (n=15)	2.95 ± 0.64	2.25 – 4.01	35.40 ± 16.22	7.32 – 74.44
		D (n=15)	5.88 ± 1.07	4.35 – 7.67	62.70 ± 21.39	22.10 – 92.77
		E (n=15)	6.14 ± 1.18	4.73 – 9.33	63.71 ± 37.54	23.91 – 181.94
		F (n=15)	3.75± 1.51	2.30 – 7.76	41.16 ± 15.35	16.03 – 75.90
		G (n=15)	5.06 ± 1.59	2.49– 7.88	52.09 ± 18.17	20.42 – 83.02
Stem Elongation	Whole Plant	B (n=6)	3.29 ± 1.75	0.70 – 4.83	24.68 ± 12.02	9.53 – 40.73
		C (n=14)	2.72 ± 1.82	0.54 – 5.14	21.89 ± 9.59	7.35 – 40.66
		D (n=15)	6.21 ± 3.76	1.18 – 12.24	49.11 ± 19.58	13.77 – 85.09
		E (n=14)	6.04 ± 3.49	1.72 – 12.44	56.84 ± 22.66	28.11 – 107.38
		F (n=15)	2.82 ± 1.78	0.94 – 7.56	22.70 ± 13.63	8.22 – 61.83
		G (n=15)	3.02 ± 1.44	0.90 – 6.52	25.52 ± 7.87	12.98 – 37.60
	Root	B (n=3)	0.04 ± 0.01	0.03 – 0.05	0.17 ± 0.03	0.15 – 0.20
		C (n=6)	0.09 ± 0.07	0.03 – 0.22	0.39 ± 0.19	0.18 – 0.64
		D (n=13)	0.26 ± 0.10	0.07 – 0.42	1.61 ± 0.79	0.27 – 2.94
		E (n=15)	0.31 ± 0.13	0.16 – 0.63	2.56 ± 2.33	0.95 – 10.57
		F (n=15)	0.17 ± 0.07	0.09 – 0.31	0.97 ± 0.55	0.46 – 2.30
		G (n=15)	0.20 ± 0.10	0.09 – 0.40	2.35 ± 4.80	0.56 – 19.50
First Flowering	Whole Plant	B (n=3)	2.12 ± 1.11	0.84 – 2.85	18.93 ± 9.55	7.91 – 24.54
		C (n=14)	1.85 ± 0.83	0.97 – 3.47	14.82 ± 5.01	6.13 – 27.52
		D (n=15)	3.33 ± 0.97	2.22 – 5.44	33.06 ± 10.98	14.12 – 52.95
		E (n=15)	4.21 ± 1.18	2.68 – 6.2	43.20 ± 20.16	6.49 – 89.33
		F (n=15)	2.28 ± 1.25	0.80 – 5.51	22.50 ± 11.56	8.58 – 42.13
		G (n=14)	2.61 ± 1.41	1.05 – 5.61	28.07 ± 13.10	12.30 –59.38
	Root	B (n=1)	0.03± ND	<LLOQ – 0.03	0.17± ND	<LLOQ – 0.17
		C (n=6)	0.09 ± 0.06	0.03 – 0.16	0.37 ± 0.25	0.15 – 0.76
		D (n=15)	0.27 ± 0.12	0.15 – 0.59	2.03 ± 2.97	0.59 – 12.56
		E (n=15)	0.28 ± 0.14	0.07 – 0.54	1.62 ± 0.85	0.36 – 3.53
		F (n=14)	0.14 ± 0.06	0.07 – 0.25	0.74 ± 0.34	0.27 – 1.31
		G (n=15)	0.13 ± 0.06	0.06 – 0.29	0.75 ± 0.36	0.30 – 1.49
	Raceme	B (n=4)	2.32 ± 0.31	2.03 – 2.68	13.95 ± 1.50	12.54 – 16.06
		C (n=14)	2.99 ± 0.78	1.91 – 4.78	23.89 ± 10.73	9.37 – 55.29
		D (n=15)	5.82 ± 0.87	4.13 – 7.70	43.27 ± 8.26	24.44 – 55.38
		E (n=15)	5.32 ± 1.59	1.51 – 7.67	40.59 ± 13.29	12.54 – 62.63
		F (n=15)	3.91 ± 1.12	2.21 – 5.99	30.77 ± 13.62	18.17 – 75.69
		G (n=15)	4.83 ± 1.17	2.88 – 6.46	41.94 ± 20.83	17.04 – 108.12
Maturity	Grain	B (n=9)	0.30 ± 0.17	0.06 – 0.56	0.34 ± 0.18	0.06 – 0.59
		C (n=15)	0.44 ± 0.18	0.27 – 0.77	0.49 ± 0.18	0.31 – 0.84
		D (n=15)	0.66 ± 0.08	0.48 – 0.79	0.74 ± 0.09	0.51 – 0.92
		E (n=15)	0.76 ± 0.24	0.51 – 1.30	0.83 ± 0.25	0.57 – 1.39
		F (n=15)	0.53 ± 0.24	0.24 – 0.90	0.59 ± 0.26	0.28 – 1.01
		G (n=15)	0.55 ± 0.28	0.22 – 1.01	0.60 ± 0.30	0.25 – 1.12

Mean, standard deviation, and range for each entry matrix was based on the total matrix sample population (n=15) (three trial sites x five independent matrix samples analyzed per trial site) except as otherwise noted. Where n<15, the sample values excluded from calculations were below LLOQ or not available for analysis.

Barnase protein

Barnase protein is a ribonuclease which, when expressed in the tapetal cells of the anthers during pollen development, leads to cell death and consequently, to male sterility. The Barnase protein encoded by the *barnase* gene has 111 amino acids ([redacted]; 2003; M-232685-01, Node B.1 (a) (CCI)) with a theoretical molecular weight of 12.5 kDa. The amino acid sequence of this plant-produced protein differs from the native barnase protein isolated from *Bacillus amyloliquefaciens* (as described by Hartley, R. W.; 1988; M-180195-01; published), by the addition of a methionine from the start codon followed by the substitution of valine and proline for alanine and glutamine, respectively.

Barnase protein is derived from a well-known source organism, *Bacillus amyloliquefaciens*, which is ubiquitous in nature and has an excellent safety profile. Because of its relatively small and simple structure, Barnase has been extensively studied in terms of structure, function, enzymatic activity, and molecular interactions for several years.

Barnase occurs frequently in nature for four reasons:

1. There are many similar species of *B. amyloliquefaciens* in nature
2. *B. amyloliquefaciens* is used in detergent and food industries.
3. Barnase is also present in other bacteria such as other *Bacilli* species, *Clostridium acetobutylicum* and the Gram-negative *Yersinia pestis*.
4. The RNase family and RNase inhibitors play a central role in every aspect of cellular RNA metabolism, not only in prokaryotes but also in eukaryotes

Therefore, exposure to Barnase protein is not new.

Barnase (and its inhibitor Barstar) have been utilized to develop plant phenotypes with direct agronomic application. Male sterility and restoration of fertility was one of the first to be reported, and hybrid canola varieties engineered with this technology have been commercialized since 1996 ([redacted] 2009; M-355152-01; Node B.1 (a)).

In MS11 *B. napus*, the *barnase* gene expression is under the control of a tapetum-specific promoter**,** Pta29. (Mariani, C.; et al.; 1990; M-147935-01; published , and [redacted]; 2008; M-304805-01; Node A.3 (c), (iii) (CCI)). Therefore, the Barnase protein is expected to be specifically expressed in flower buds during anther development. Barnase exhibits RNAse activity; hence, presence of Barnase protein leads to RNA degradation, cell disruption, and cell death (Mariani, C.; et al.; 1992; M-147936-01; published and Hartley, R. W.; 1989; M-147934-01; published). Since cells expressing Barnase protein are quickly disrupted, the levels of Barnase protein in MS11 *B. napus* tissues would be expected to be low. This was substantiated in protein expression studies where expression levels of Barnase protein determined in different matrices of MS11 *B. napus,* including flower buds, were below the lower limit of quantification for the ELISA method in all matrices analyzed ([redacted].; 2015; M-542702-01; Node B.1 (a)). Furthermore, Barnase was not detected by western blot analysis in crude extracts or upon immuno-affinity purification attempts.

Due to the low levels of Barnase in MS11 *B. napus* tissues, protein of sufficient quantity and quality could not be extracted from the MS11 *B. napus* plant to experimentally confirm the equivalence of the microbially produced Barnase protein with the MS11 *B. napus* plant-produced protein. As such, the Barnase protein in MS11 *B. napus* would be classified as an intractable protein as described in Bushey, D. F.; et al.; 2014; M-549822-01; published . Therefore, a weight of evidence approach was used to assess the equivalence of the intractable protein with the microbially-produced protein.

Sequence analysis of the MS11 *B. napus* insert confirmed the sequence of the *barnase* gene was as expected ([redacted] 2008; M-304805-01; Node A.3 (c), (iii) (CCI)). Peptide mapping of the microbially-produced protein demonstrated 100% coverage against the theoretical amino acid sequence of the Barnase protein ([redacted] 2013; M-467079-01; Node B.1 (a), [redacted] 2016; M-551100-01; Node

B.1 (a), and [redacted] 2003; M-232685-01; Node B.1 (a) (CCI)) and was 100% identical to the amino acid sequence predicted from the nucleotide sequence of the MS11 *B.napus* insert. MS11 *B. napus* plants exhibited the male sterile phenotype, demonstrating that an active Barnase was expressed and was efficacious. Likewise, the microbially-produced protein had confirmed enzymatic activity.

This information cumulatively provides evidence that the Barnase in MS11 *B. napus* was produced as intended.

Barstar protein

The *barstar* gene, an intracellular inhibitor of the Barnase ribonuclease, was isolated from *Bacillus amyloliquefaciens* (Hartley, R. W.; 1988; M-180195-01; published). The Barstar protein encoded by the *barstar* gene has 90 amino acids ([redacted] 2003; M-232692-01; Node B.1 (a) (CCI)), and a theoretical molecular weight of 10.3 kDa.

The Barstar protein is an inhibitor of the Barnase protein. The prophylactic *barstar* gene in MS11 *B. napus*, driven by the Pnos promoter, was included to enhance transformation frequency.

Barstar protein is derived from a well-known source organism, *Bacillus amyloliquefaciens*, which is ubiquitous in nature and has an excellent safety profile. Because of its relatively small and simple structure, Barstar has been extensively studied in terms of structure, function, enzymatic activity, and molecular interactions for several years.

Barstar occurs frequently in nature for four reasons:

1. There are many similar species of *B. amyloliquefaciens* in nature and
2. *B. amyloliquefaciens* is used in detergent and food industries.
3. Barstar is also present in other bacteria such as other *Bacilli* species, *Clostridium acetobutylicum* and the Gram-negative *Yersinia pestis*.
4. The RNase family and RNase inhibitors play a central role in every aspect of cellular RNA metabolism, not only in prokaryotes but also in eukaryotes

Therefore, exposure to Barstar protein is not new.

Barnase and its inhibitor Barstar have been utilized to develop plant phenotypes with direct agronomic application. Male sterility and restoration of fertility was one of the first to be reported, and hybrid canola varieties engineered with this technology have been commercialized since 1996 ([redacted] 2009; M-355152-01; Node B.1 (a)).

The only known function of the Barstar protein is to protect the bacteria from the lethal effect of the Barnase activity (Hartley, R. W.; 1988; M-180195-01; published ; Smeaton, J. R.; et al.; 1964; M-228147-01; published). The inhibition of Barnase by Barstar is highly specific and non-covalent. The Barstar protein sterically blocks the active site of the Barnase protein with an alpha-helix and adjacent loop (Hartley, R. W.; 1989; M-147934-01; published).

Barstar protein was only consistently expressed in roots from field grown samples treated with glufosinate herbicide. The protein expression levels of Barstar were consistently below LLOQ in all grain samples and most raceme and whole plant samples ([redacted] 2015; M-542702-01; Node B.1 (a)). Western blot analysis of crude root extracts only very faintly detected Barstar protein, but a band of the anticipated size was detected in concentrated preparations. However, repeated attempts to purify or further enrich the Barstar protein using immuno-affinity chromatography were unsuccessful, as contaminants were also co-purified during this process.

As such, the Barstar protein in MS11 *B. napus* would be classified as an intractable protein as described in Bushey, D. F.; et al.; 2014; M-549822-01; published . Therefore, a weight of evidence approach was used to assess the equivalence of the intractable protein with the microbially-produced protein.

Sequence analysis of the MS11 *B. napus* insert confirmed that the sequence of the *barstar* gene was as expected (2008; M-304805-01; Node A.3 (c), (iii) (CCI)). Peptide mapping of the microbially-produced protein demonstrated 100% coverage against the theoretical amino acid sequence of the Barstar protein (2003; M-232692-01; Node B.1 (a) (CCI); 2009; M-433234-01; Node B.1 (a); 2012; M-433174-01; Node B.1 (a) and 2014; M-495269-01; Node B.1 (a); 2012; M-433233-01; Node B.1 (a); and 2016; M-548907-01; Node B.1 (a)) and was 100% identical to the amino acid sequence predicted from the nucleotide sequence of the MS11 *B. napus* insert. Since no additional start codon is present in the MS11 *B. napus* insert sequence that could result in a slightly different protein, it was concluded that the amino acid sequence of the Barstar protein expressed in MS11 *B. napus* is identical to the amino acid sequence confirmed for the microbially-produced protein (2003; M-232692-01; Node B.1 (a) (CCI), and 2014; M-495269-01; Node B.1 (a)).

Additionally, concentrated crude protein extract was used to confirm the molecular weight and immuno-reactivity of the Barstar protein expressed in MS11 *B. napus* (2016; M-548891-01; Node B.1 (a)). Enough protein of sufficient quantity and quality could not be extracted from the MS11 *B. napus* plants to perform additional experiments typically conducted to demonstrate equivalency to the microbially-produced protein.

This information cumulatively provides evidence that the Barstar in MS11 *B. napus* was produced as intended.

Barnase-barstar protein complex

Barnase protein is a ribonuclease which, when expressed in the tapetal cells of the anthers during pollen development, leads to cell death and consequently, to male sterility. Barstar is a specific inhibitor for the Barnase activity. When a plant expressing Barstar is crossed with a male sterile plant expressing Barnase, the hybrid obtained has restored fertility.

Barnase and Barstar proteins are derived from a well-known source organism, *Bacillus amyloliquefaciens*, which is ubiquitous in nature and has an excellent safety profile. Barnase is an extracellular endoribonuclease (RNase) encoded by the *barnase* gene. It has 110 amino acids with an apparent molecular weight of 12.5 kDa. The Barstar protein, encoded by the *barstar* gene, has 90 amino acids, with an apparent molecular weight of 10.3 kDa. Their linear amino acid sequences are known.

Barnase, Barstar and their homologous proteins occur frequently in nature for four reasons:

1. There are many similar species of *B. amyloliquefaciens* in nature
2. *B. amyloliquefaciens* is used in detergent and food industries.
3. Barnase and Barstar are also present in other bacteria such as other *Bacilli* species, *Clostridium acetobutylicum* and the Gram-negative *Yersinia pestis*.
4. The RNase family and RNase inhibitors play a central role in every aspect of cellular RNA metabolism, not only in prokaryotes but also in eukaryotes

Therefore, exposure to the Barnase and Barstar proteins, including to the Barnase/Barstar complex, is not new.

Although Barnase is known to be cytotoxic by nature, due to its ribonuclease activity, it is expressed selectively in the tapetum during anther development. The co-expression of Barstar in hybrid plants neutralizes the enzymatic activity of Barnase by forming an inert stable complex. Because of its

cytotoxicity, the Barnase protein presence in the plant is only possible when it is complexed with Barstar. Therefore, no exposure is expected to the Barnase protein by animals or humans.

Barnase and its inhibitor, Barstar, have been utilized to develop plant phenotypes with direct agronomic application. Male sterility and restoration of fertility was one of the first to be reported. Hybrid canola varieties engineered with this technology have previously been evaluated by regulators and were released onto the market in 1996. GM crops expressing Barnase and Barstar have subsequently been consumed by humans and animals, and there have been no adverse effects reported in their 20 year history. Therefore, GM canola expressing Barnase and Barstar proteins have good safety profiles, and there is a reasonable certainty of no harm after their consumption.

The mode of action of Barnase is well known. The Barnase protein is an endoribonuclease, i.e., it cleaves RNA at internal sites. Barnase catalyzes the cleavage of phosphodiester linkages in RNA oligo- and polynucleotides. This reaction leads to the formation of intermediate nucleoside-2'3'-cyclophosphates and mono- and small oligonucleotides as final products. Barnase shows preference towards phosphodiester bonds with guanosine at their 3' end when cleaving RNA.

The only known function of the Barstar protein is to protect the bacteria from the lethal effect of the Barnase activity. Inhibition of the Barnase protein by the Barstar protein involves the formation of a stable non-covalent one-to-one complex of the two proteins. The Barstar protein sterically blocks the active site of the Barnase protein with an alpha-helix and adjacent loop. This inhibition of Barnase by Barstar is highly specific and of high affinity. This complex is reversible through manipulation of pH, salt concentration, and/or temperature, and active Barnase and Barstar proteins can be recovered after dissociation. The relative molecular weight of the Barnase/Barstar complex protein is 22-23 kDa.

The mode of inhibition of Barnase by Barstar is simple. Barstar binds to the active site of Barnase and sterically blocks this site. More precisely, some segments of Barstar (α-helix 2 and loop connecting α-helices 2 to 3) enter into the binding pocket of Barnase, in the same manner as a substrate. Asp39 of Barstar binds to the phosphate-binding site of Barnase, mimicking enzyme-substrate interaction. The active site of the enzyme is then covered and access to substrate effectively denied. This Barnase/Barstar complex protein is very tight and stable.

(b) Information about prior history of human consumption of the new substances, if any, or their similarity to substances previously consumed in food.

See the relevant parts of Section B.1(a) above on history of safe use and refer to the relevant studies.

PAT/*bar* protein

PAT/*bar* protein is derived from a well-known source organism, *S. hygroscopicus*, which is a common saprophytic bacterial species that is found worldwide (Kutzner, H. J.; 1981; M-204308-01; published) and has an excellent safety profile. *S. hygroscopicus* is not known to be a pathogen of plants, humans or other animals (OECD; 1999; M-204493-01), and the PAT protein, like other acetyletransfereases, is not known to have any allergenic or toxic properties, and has a well-characterised activity and substrate specificity. A battery of tests performed according to internationally accepted methods and standards have established that the PAT protein does not possess structural or functional similarity with known toxic proteins or allergens; it shares no sequence homology with known allergens and toxins, no N-glycosylation sites, and rapidly degrades in simulated digestive environments (Herouet, C.; et al.; 2005; M-247779-01; published).

Transgenic crops expressing the PAT protein have been grown for more than a decade in the USA and Canada. In the 2011 review by ILSI, it was estimated that regulatory authorities in seven countries had issued approvals for the environmental release of six transgenic crop species that express PAT proteins

(encoded by the *bar* gene or the *pat* gene), either alone or in combination with genes for other traits (e.g. insect resistance) (CERA/ILSI.; 2011; M-411628-01; published).

Barnase and Barstar proteins

Barnase and Barstar proteins are derived from a well-known source organism, *Bacillus amyloliquefaciens*, which is ubiquitous in nature and has an excellent safety profile. Barnase is an extracellular endoribonuclease (RNase) encoded by the *barnase* gene. It has 110 amino acids with an apparent molecular weight of 12.5 kDa. The Barstar protein, encoded by the *barstar* gene, has 90 amino acids, with an apparent molecular weight of 10.3 kDa. Their linear amino acid sequences are known.

Barnase, Barstar and their homologous proteins occur frequently in nature for four reasons:

1. There are many similar species of *B. amyloliquefaciens* in nature
2. *B. amyloliquefaciens* is used in detergent and food industries.
3. Barnase and Barstar are also present in other bacteria such as other *Bacilli* species, *Clostridium acetobutylicum* and the Gram-negative *Yersinia pestis*.
4. The RNase family and RNase inhibitors play a central role in every aspect of cellular RNA metabolism, not only in prokaryotes but also in eukaryotes

Therefore, exposure to the Barnase and Barstar proteins, including to the Barnase/Barstar complex, is not new.

Although Barnase is known to be cytotoxic by nature, due to its ribonuclease activity, it is expressed selectively in the tapetum during anther development. The co-expression of Barstar in hybrid plants neutralizes the enzymatic activity of Barnase by forming an inert stable complex. Because of its cytotoxicity, the Barnase protein presence in the plant is only possible when it is complexed with Barstar. Therefore, no exposure is expected to the Barnase protein by animals or humans.

Barnase and its inhibitor, Barstar, have been utilized to develop plant phenotypes with direct agronomic application. Male sterility and restoration of fertility was one of the first to be reported. Hybrid canola varieties engineered with this technology have previously been evaluated by regulators and were released onto the market in 1996. GM crops expressing Barnase and Barstar have subsequently been consumed by humans and animals, and there have been no adverse effects reported in their 20 year history. Therefore, GM canola expressing Barnase and Barstar proteins have good safety profiles, and there is a reasonable certainty of no harm after their consumption ([redacted] 2009; M-355152-01; Node B.1 (a)).

(c) Information on whether any new protein has undergone any unexpected post-translational modification in the new host

Post-translational modification is determined by glycosylation analysis. For Barnase and Barstar, glycosylation testing was performed on the microbially-produced proteins, as sufficient protein for testing could not be extracted from *B. napus* plants. No glycosylation was determined in the microbially-produced proteins. For PAT/*bar*, neither the plant-purified nor the microbially-produced protein was glycosylated. Therefore, it is deduced that there is no unexpected post-translational modification via glycosylation for PAT/*bar*, Barnase, or Barstar.

(d) Where any ORFs have been identified (in subparagraph A.3 (c)(v) of this Guideline (3.5.1)), bioinformatics analyses to indicate the potential for allergenicity and toxicity of the ORFs.

Bioinformatic analysis of the MS11 *B. napus* transgenic locus

A bioinformatics analysis was performed on the transgenic locus sequence of MS11 *B. napus* to identify potential open reading frames (ORF) ([redacted].; 2016; M-552421-01; Node A.3 (c), (v) (CCI)).

The ORF search was performed using the GetORF search program from the European Molecular Biology Open Software Suite (EMBOSS) tools (version 6.3.1, July 2010). An ORF was defined as the region between two translation stop codons (TAA, TAG, or TGA) with a minimum size coding for 3 amino acids. All ORF crossing a junction or overlapping the inserted DNA were reported.

Translated amino acid sequences from all identified ORF with a minimum size of 30 amino acids were used as query sequences for homology search with known allergens and known toxins.

The 8-mer homology search was carried out to identify any short sequences of 8 amino acids or longer that share 100% identity to an allergenic protein. Additionally, each complete query sequence was compared with all the sequences available in the allergen database (FARRP; www.allergenonline.org) (Table 17).

The overall homology search used the FASTA program (version 35.04, Jan. 15, 2009). Only the matches of ≥35% identity over at least 80 amino acids were considered potentially relevant. For all ORF shorter than 80 amino acids, the percentage of identity was calculated over a hypothetical 80 amino acid window, with gaps treated as mismatches:

$$\text{Calculated \% identity} = \frac{\text{\% identity} \times \text{length of ORF coverage}}{80}$$

Each complete query sequence was also compared with all the sequences available in the NCBI non-redundant protein database database (Table 17) using the FASTA program. For each ORF, only the best scoring 1000 matches were reported when more than 1000 matches were found.

The biological relevance of the matches was further assessed by examining the alignments (e.g., identity, length of alignment, presence of gaps, E-value), as well as the published information on toxicity of the matching proteins. The biologically relevant matches provided insight on the familiarity and potential toxic properties of the potential polypeptide.

In the MS11 *B. napus* transgenic locus, GetORF identified 554 ORF (corresponding to 526 unique sequences) defined between two stop codons and with a minimum size of 3 amino acids. After elimination of duplicates, translated amino acid sequences of at least 30 amino acids length represented 107 unique sequences.

No 100% identities were found between the 8 or longer linearly contiguous amino acid blocks that compose the query sequences and known allergens. Additionally, no biologically relevant identities were found between the query sequences and known allergens. For all ORFs of 80 amino acids or more, there was no match of ≥ 35% identity over at least 80 amino acids. For all ORFs shorter than 80 amino acids, there was no match of ≥ 35% identity recalculated over 80 amino acids.

None of the matches obtained from the NCBI non-redundant database were toxicologically relevant (i.e., indicative of a potential identity with a toxin), for one of the following reasons:

- the match was not biologically relevant (e.g., short alignment, low % identity, presence of gaps, high E-value) or
- the matching protein was not a known toxin.

Results indicate that these ORF sequences of ≥30 amino acids length showed no biologically relevant sequence identities with known allergens and known toxins. Therefore, there are neither allergenic nor toxicological *in silico* findings associated with the presence of the potential ORF polypeptides.

Table 17: Summary of the database releases and date of search

Name	Database type	Number of sequences	Version	Date of release (Year-Month-Day)
AOL	Allergen database	1 956	16	2016-01-26
NCBI non-redundant protein database	General database	81 622 391	2016.0206	2016-02-19

B.2 New proteins

If it can be shown the new protein(s) is identical to one previously assessed by FSANZ, the only other safety information that must be provided is an updated bioinformatics comparison of the amino acid sequence to known protein toxins, anti-nutrients and allergens.

Where the new protein is not identical to one previously assessed by FSANZ, the following must be provided:

(a) Information on the potential toxicity of any new proteins, including:

(i) A bioinformatics comparison of the amino acid sequence of each of the new proteins to known protein toxins and anti-nutrients (e.g. protease inhibitors, lectins)

Although barnase, barstar and the PAT/*bar* proteins are not new, updated bioinformatics analysis for known proteins and anti-nutrients have been provided for each of the amino acid sequences associated with the proteins.

PAT/*bar* protein

As described in the document number M-084188-01-2 (Node B.1 (a) (CCI)), the query sequence corresponding to the PAT/*bar* protein is as follows:

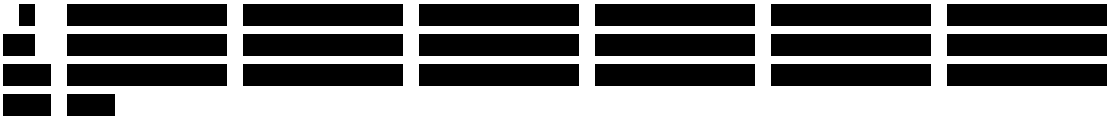


Figure 1. Amino Acid Sequence of the PAT Protein

Two *in silico* approaches based on the FASTA algorithm associated with the BLOSUM50 scoring matrix were used to evaluate the potential amino acid sequence identity of the query protein with known toxins (2016; M-084359-10; Node B.2 (a) (i)):

An overall identity search with all protein sequences present in the NCBI non-redundant database. An E-value threshold of 0.1 was used for pre-selecting the most similar proteins. The biological relevance of the matches was further assessed by the author. Biologically relevant matches provide insight on the familiarity and potential toxic properties of the query protein.

An overall identity search with all protein sequences present in the in-house Bayer toxin database. An E-value threshold of 10 was used for pre-selecting the most identical toxins. The biological relevance of the

matches was further assessed by the author. Biologically relevant matches provide insight on the potential toxic properties of the query protein.

Table 18. Summary of the database releases and date of search

Name	Database type	Number of sequences	Version	Date of release (Year-Month-Day)
NCBI non-redundant protein database	General database	81 622 391	2016.0206	2016-02-19
BCS 2015 toxin database	Toxin database	24 496	16.1	2016-03-10

Date of search: March 21, 2016

As expected, the results of the overall homology search with general protein database showed only main identities with the ribonuclease family from various origins. Furthermore, no biologically relevant identities were found with any toxic proteins from the Bayer toxin database.

Overall, all matches were considered not toxicologically relevant.

Barnase protein

Described in document number M-232685-01-1 (Node B.1 (a) (CCI)), the query sequence corresponding to the Barnase protein (111 amino acids) is as follows:

Figure. Amino Acid Sequence of the Barnase Protein

Two *in silico* approaches based on the FASTA algorithm associated with the BLOSUM50 scoring matrix were used to evaluate the potential amino acid sequence identity of the query protein with known toxins (; 2016; M-552256-01; Node B.2 (a) (i)):

An overall identity search with all protein sequences present in the NCBI non-redundant database. An E-value threshold of 0.1 was used for pre-selecting the most similar proteins. The biological relevance of the matches was further assessed by the author. Biologically relevant matches provide insight on the familiarity and potential toxic properties of the query protein.

An overall identity search with all protein sequences present in the in-house Bayer toxin database. An E-value threshold of 10 was used for pre-selecting the most identical toxins. The biological relevance of the matches was further assessed by the author. Biologically relevant matches provide insight on the potential toxic properties of the query protein.

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Table 19. Summary of the database releases and date of search

Name	Database type	Number of sequences	Version	Date of release (Year-Month-Day)
NCBI non-redundant protein database	General database	81 622 391	2016.0206	2016-02-19
BCS 2016 toxin database	Toxin database	24 496	16_1	2016-03-10

Date of search: March 17, 2016

The overall homology search with general protein databases showed that most of the matches corresponded to sequences from the ribonuclease family from various origins. Furthermore, no biologically relevant identities were found with any toxic protein from the Bayer toxin database.

In conclusion, there are neither allergenic nor toxicological *in silico* findings associated with the Barnase protein.

Barstar protein

Described in document number M-232692-01-1 (Node B.1 (a) (CCI)), the query sequence corresponding to the Barstar protein (90 amino acids) is as follows:

[redacted]

Figure. Amino Acid Sequence of the Barstar Protein

Two *in silico* approaches based on the FASTA algorithm associated with the BLOSUM50 scoring matrix were used to evaluate the potential amino acid sequence identity of the query protein with known toxins ([redacted] 2016; M-552416-01; Node B.2 (a) (i)):

- An overall identity search with all protein sequences present in the NCBI non-redundant database. An E-value threshold of 0.1 was used for pre-selecting the most similar proteins. The biological relevance of the matches was further assessed. Biologically relevant matches provide insight on the familiarity and potential toxic properties of the query protein.
- An overall identity search with all protein sequences present in the in-house Bayer toxin database. An E-value threshold of 10 was used for pre-selecting the most identical toxins. The biological relevance of the matches was further assessed. Biologically relevant matches provide insight on the potential toxic properties of the query protein.

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Table 20. Summary of the database releases and date of search

Name	Database type	Number of sequences	Version	Date of release (Year-Month-Day)
NCBI non-redundant protein database	General database	81 622 391	2016.0206	2016-02-19
BCS 2016 toxin database	Toxin database	24 496	16.1	2016-03-10

Date of search: March 17, 2016

The overall homology search with general protein databases showed that most of the matches corresponded to sequences from the ribonuclease family from various origins. Furthermore, no biologically relevant identities were found with any toxic protein from the Bayer toxin database.

In conclusion, there were no toxicological *in silico* findings associated with the Barstar protein.

(ii) Information on the stability of the protein to proteolysis in appropriate gastrointestinal model systems

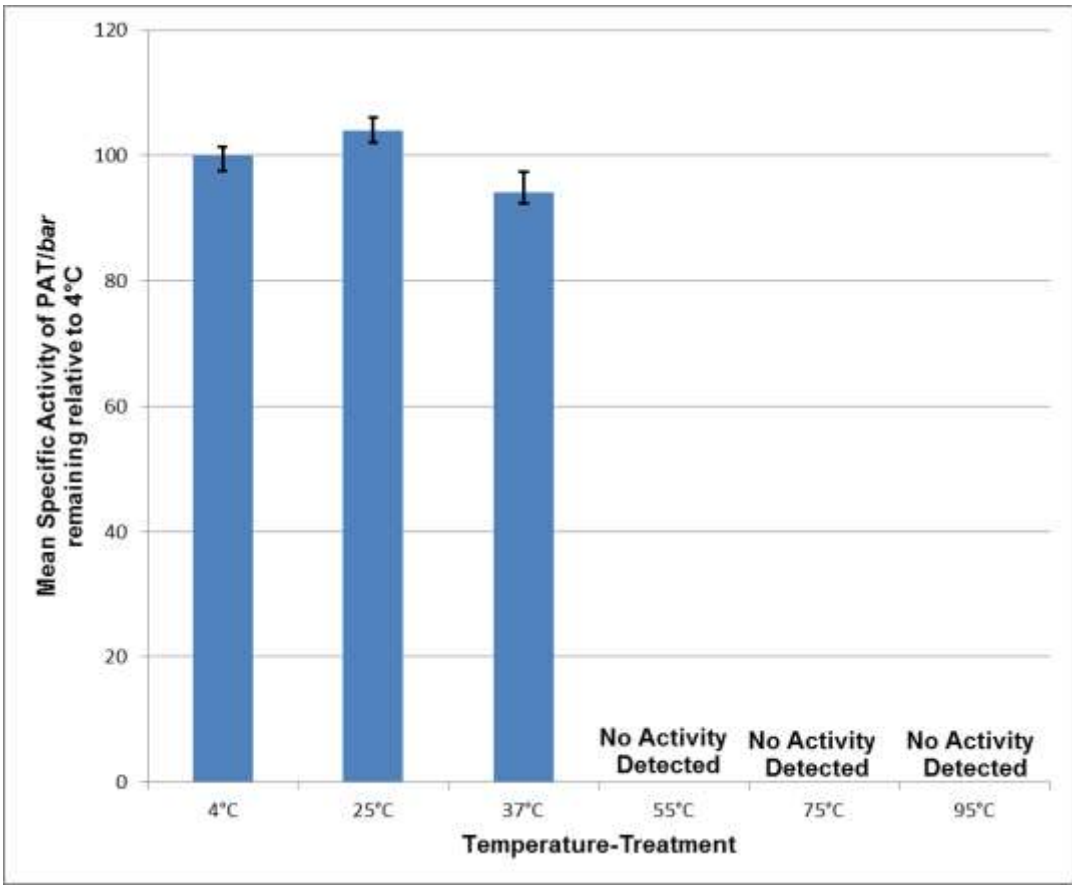
PAT/*bar* protein

The effect of temperature on the microbially-produced PAT/*bar* protein was assessed using the PAT quantitative activity assay ([redacted] 2016; M-554703-01; Node B.2 (b) (iii)). Samples of PAT/*bar* were incubated for 30 minutes at 4°C, 25°C, 37°C, 55°C, 75°C, and 95°C. The specific activity of each temperature-treated sample was then measured by the PAT quantitative activity assay. The specific activity for the sample treated at 4°C was used for comparison with the other temperature-treated samples. There was no decrease of the mean specific activity of PAT/*bar* after treatment at 25°C and 37°C and there was no residual mean specific activity detected for PAT/*bar* after treatment at 55°C and above (Table 21 and Figure 35).

Table 21. Mean specific Activity of PAT/*bar* after Temperature Treatment

Temperature Treatment (°C)	Mean Specific Activity of PAT/*bar* (µmol/min*mg protein)	% Activity remaining relative to 4°C
4	19.6	100
25	20.4	104
37	18.5	94
55	Not Active	Not Active
75	Not Active	Not Active
95	Not Active	Not Active

Mean specific activity was annotated as “Not Active” if the mean specific activity was equal to, or less than, zero.



Error Bars are displayed as a range of PAT/*bar* activity relative to 4°C

Figure 35 Effect of Temperature on the Mean Specific Activity of PAT/*bar*

SGF/SIF stability

The microbially-produced PAT/*bar* protein was tested for stability in human simulated gastric fluid (SGF) ([redacted], 2009; M-217195-04; Node B.2 (a) (ii)) and human simulated intestinal fluid (SIF) ([redacted], 2016; M-208793-04; Node B.2 (a) (ii)). Test proteins were incubated with human SGF (pepsin solution at pH 1.2) and human SIF (pancreatin solution at pH 7.5) at approximately 37°C. Samples were taken for analysis at incubation times ranging from 0 to 60 minutes. The resultant protein solutions were analysed for presence of the test protein and potential stable protein fragments by SDS-PAGE and western blot analyses. The PAT/*bar* protein was degraded very rapidly (within 30 seconds) in simulated gastric fluid, as well as in simulated intestinal fluid.

Barnase protein

The heat stability of the microbially produced Barnase protein was assessed using SDS-PAGE, western blot analyses, ELISA, and a quantitative activity assay ([redacted], 2012; M-440532-01; Node B.2 (b) (iii); [redacted], 2013; M-475710-01; Node B.2 (b) (iii); [redacted], 2014; M-490632-01; Node B.2 (b) (iii)). Samples of Barnase were incubated for 30 minutes at 4°C, 25°C, 37°C, 55°C, 75°C, and 95°C. The sample treated at 4°C was used for comparison to the other temperature treated samples.

For the SDS-PAGE and western blot analyses, two samples for each temperature treatment (except 4°C) were incubated. One of the two samples was centrifuged and the supernatant (S) containing the soluble protein and pellet (P) containing the insoluble protein were separated. The pellet was suspended in buffer prior to analysis. The other sample for each temperature was not centrifuged and labeled uncentrifuged (UC). The SDS-PAGE and western blot analyses produced similar results. After heat treatment at 25°C, 37°C, and 55°C, the majority of Barnase remained in the supernatant. Treatments at 55°C and above resulted in smaller bands under the Barnase band, suggesting the protein was degrading. The smaller bands were more pronounced in the 75°C and 95°C treated samples.

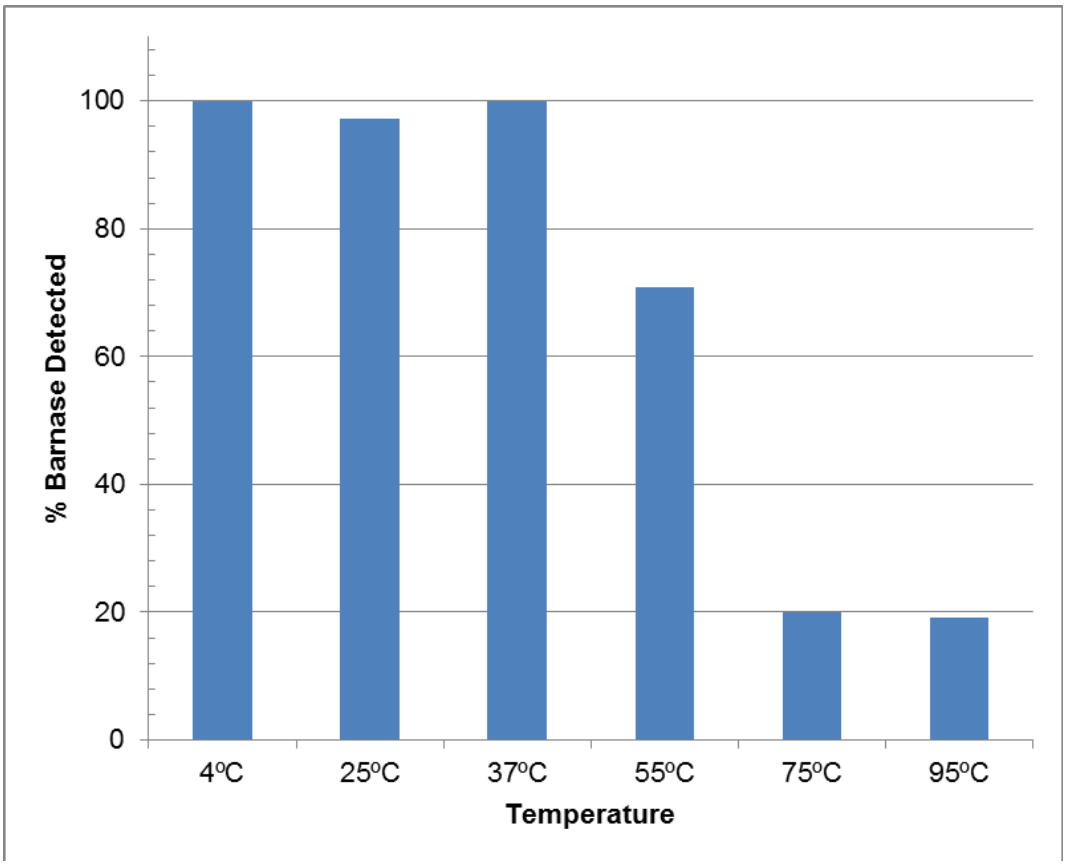
Barnase exhibited decreased detection by ELISA with increasing temperature treatments (Table 22 and Figure 36). There was no decrease of the amount of Barnase detected after treatment at 25°C and 37°C. After treatment at 55°C, there was a small decrease in detection of Barnase, and after treatment at 75°C and 95°C there was a large decrease in the amount of Barnase detected.

For the Barnase quantitative activity assay, the specific activity of each temperature-treated sample was measured. Barnase exhibited decreased specific activity with increasing temperature treatments (Table 23 and Figure 37). There was no decrease of the specific activity of Barnase after treatment at 25°C and 37°C. After treatment at 55°C and 75°C, there was a decrease in specific activity of Barnase. There was no residual specific activity of Barnase detected after treatment at 95°C.

Taken together, these results suggest Barnase loses stability upon heating at 55°C or above.

Table 22. Amount of Barnase Detected by ELISA after Heat Treatment

Temperature Treatment (°C)	Amount of Barnase (mg/ml)	% Detected
4	1.10	100
25	1.07	97.3
37	1.10	100.0
55	0.78	70.9
75	0.22	20.0
95	0.21	19.1



Note: The sample treated at 4°C was used for comparison to the other temperature treated samples.

Figure 36 Effect of Temperature on Amount of Barnase Detected using ELISA

Table 23. Specific Activity of Barnase after Temperature-Treatment

Temperature Treatment (°C)	Specific Activity of Barnase (µmoles product*min^{-1}*mg of enzyme^{-1}) [1]	% Specific Activity Remaining
4	1089.24	100.0
25	1052.68	96.6
37	1162.64	106.7
55	908.37	83.4
75	356.31	32.7
95	< LOD [2]	

[1] Specific activity calculated using 62.7 pg/mL as the amount of Barnase used in the assay.
[2] The amount of Fluorescent product measure was below the limit of detection of the method. The limit of detection was 0.309 µM.

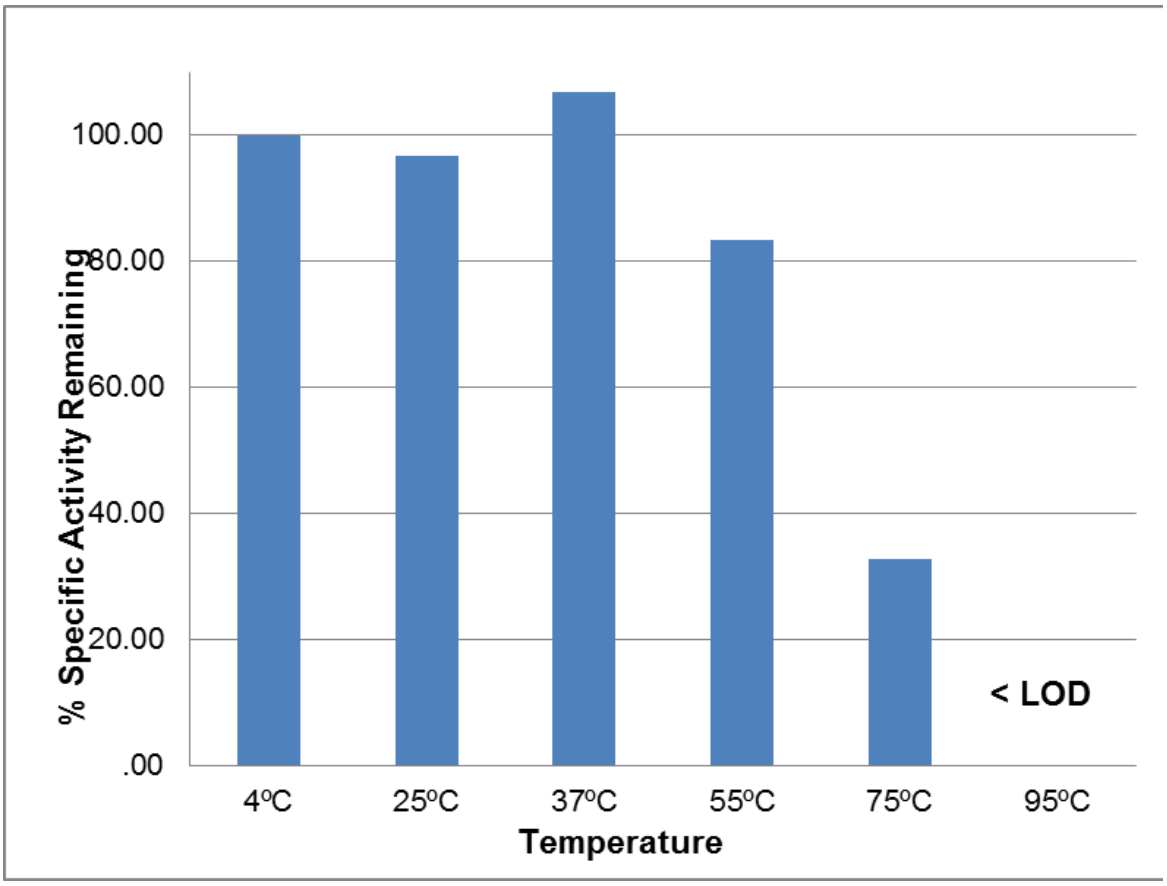


Figure 37 Effect of Temperature on the Specific Activity of Barnase

The microbially produced Barnase protein was tested for stability in human simulated gastric fluid (SGF) ([redacted], 2012; M-430109-01; Node B.2 (a) (ii)) and human simulated intestinal fluid (SIF) [redacted] 2016; M-430112-02; Node B.2 (a) (ii)). Test proteins were incubated with human SGF (pepsin solution at pH 1.2) and human SIF (pancreatin solution at pH 7.5) at approximately 37°C. Samples were taken for analysis at incubation times ranging from 0 to 60 minutes. The resultant protein solutions were analysed for presence of the test protein and potential stable protein fragments by SDS-PAGE and western blot analyses. The Barnase protein was degraded very rapidly (within 30 seconds) in simulated gastric fluid, but was still visible at 60 minutes when incubated in the presence of pancreatin, indicating a slow degradation rate in simulated intestinal fluid.

Barstar protein

The heat stability of the microbially produced Barstar protein was assessed using SDS-PAGE, western blot analyses, ELISA, and a quantitative activity assay ([redacted], 2012; M-433396-01; Node B.2 (b) (iii); [redacted], 2014; M-479248-01; Node B.2 (b) (iii); [redacted], 2014; M-490635-01; Node B.2 (a) (ii)). Samples of Barstar were incubated for 30 minutes at 4°C, 25°C, 37°C, 55°C, 75°C, and 95°C. The sample treated at 4°C was used for comparison to the other temperature treated samples.

For the SDS-PAGE and western blot analyses, two samples for each temperature treatment (except 4°C) were incubated. One of the two samples was centrifuged and the supernatant (S) and pellet (P) were separated. The pellet was suspended in buffer prior to analysis. The other sample for each temperature was not centrifuged and labeled uncentrifuged (UC). The SDS-PAGE and western blot analyses produced similar results. After heat treatment at 25°C, 37°C, and 55°C, the majority of Barstar remained

in the supernatant. After heat treatment at 75°C and 95°C, there was a small amount of Barstar in the pellet, but the majority still remained in the supernatant. In addition, the heat treatment at 95°C produced some multimers of protein indicated by the higher molecular weight bands in the supernatant. This suggests that Barstar forms soluble oligomers upon heating at 95°C, and the native form of Barstar is not stable upon heating at 95°C.

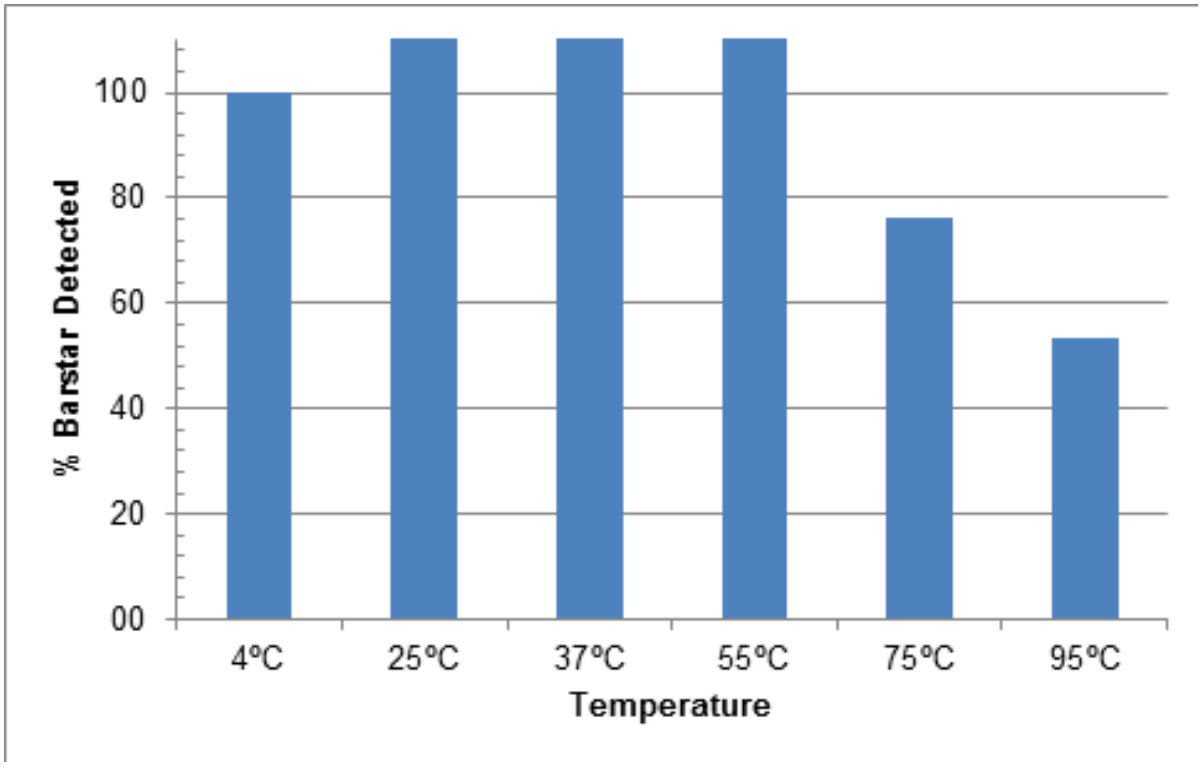
Barstar exhibited decreased detection by ELISA with increasing temperature treatments (Table 24. and Figure 38). There was no decrease in the amount of Barstar detected by ELISA after heat treatment at 25°C, 37°C, and 55°C. After treatment at 75°C and 95°C, there was a decrease in the amount of Barstar detected.

For the Barstar quantitative activity assay, the half maximal inhibitory concentration (IC_{50}), a measure that indicates how much of an inhibitor (Barstar) is needed to inhibit a given biological process by half (Barnase activity), of each temperature-treated sample was determined. Barstar exhibited a decrease in inhibition (increase of IC_{50}) of Barnase with increasing temperature-treatments (Table 25 and Figure 39). There was no increase in the IC_{50} for Barstar after treatment at 25°C, 37°C, and 55°C. After treatment at 75°C, there was an increase in the IC_{50} of Barstar. The IC_{50} could not be calculated for Barstar after treatment at 95°C, since the temperature-treated Barstar was unable to inhibit Barnase. This indicated that Barstar sample treated at 95°C had no activity as an inhibitor of Barnase.

Taken together, these results suggest Barstar loses stability upon heating at 75°C or above.

Table 24. Amount of Barstar Detected by ELISA after Heat Treatment

Temperature Treatment (°C)	Amount of Barstar (mg/ml)	% Detected
4	0.63	100.0
25	0.77	122.2
37	0.78	123.8
55	0.77	122.2
75	0.48	76.2
95	0.34	54.0



Note: The sample treated at 4°C was used for comparison to the other temperature treated samples.

Figure 38 Effect of Temperature on Amount of Barstar Detected using ELISA

Table 25. IC_{50} of Barstar after Temperature-Treatment

Temperature Treatment (°C)	IC_{50} (µM) [1]	Fold Difference in IC_{50} from 4°C control
4ºC	1.04	1.00
25ºC	1.11	1.07
37ºC	1.02	0.98
55ºC	1.04	1.00
75ºC	1.73	1.66
95ºC	Not Determined [2]	

[1] The concentration of Barnase used was 1.57 µM.
[2] The IC_{50} for 95°C was not determined because there was no difference in the observed rates of Barnase at any concentration of Barstar assayed.

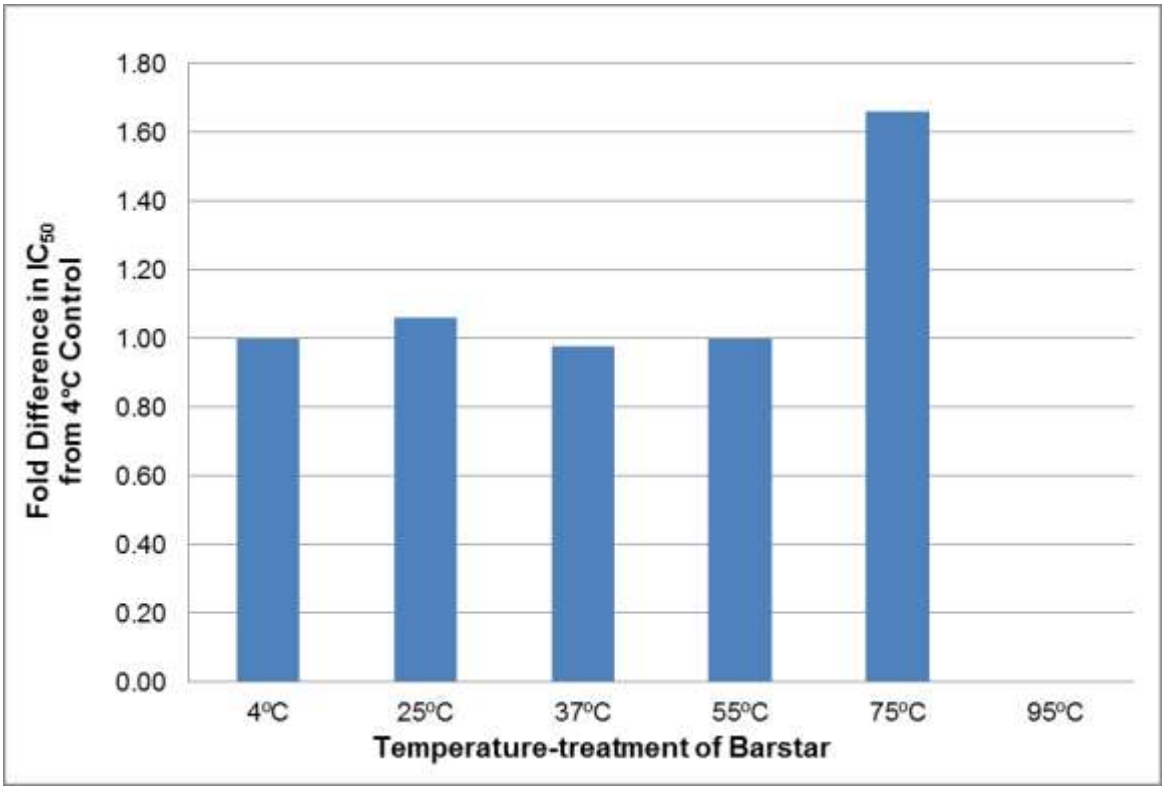


Figure 39. Effect of Temperature on the IC_{50} of Barstar

The microbially produced Barstar protein was tested for stability in human simulated gastric fluid (SGF) ([redacted], 2012; M-429793-01; Node B.2 (a) (ii)) and human simulated intestinal fluid (SIF) ([redacted], 2012; M-429800-02; Node B.2 (a) (ii)). Test proteins were incubated with human SGF (pepsin solution at pH 1.2) and human SIF (pancreatin solution at pH 7.5) at approximately 37°C. Samples were taken for analysis at incubation times ranging from 0 to 60 minutes. The resultant protein solutions were analysed for presence of the test protein and potential stable protein fragments by SDS-PAGE and western blot analyses. The Barstar protein was degraded very rapidly (within 30 seconds) in simulated gastric fluid, and (>90% of the Barstar protein) was degraded within 10 minutes in simulated intestinal fluid.

Barnase-barstar complex

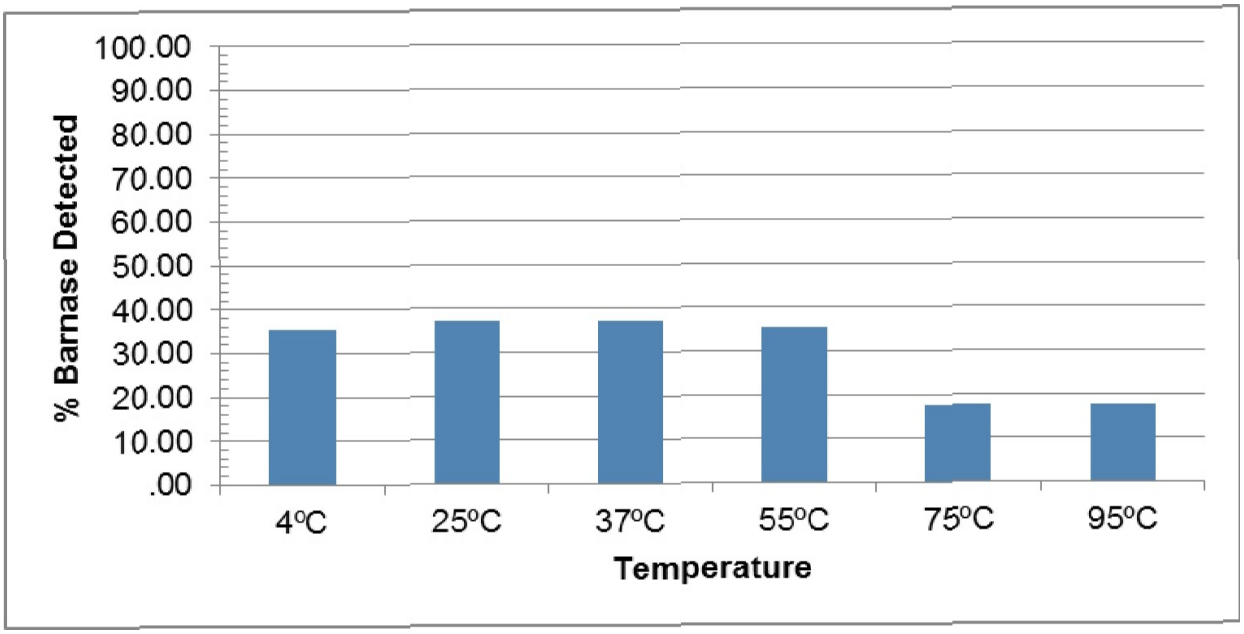
The heat stability of the microbially-produced Barnase/Barstar protein complex was assessed using SDS-PAGE, western blot analyses, ELISA, and quantitative activity assays ([redacted], 2013; M-549535-01; Node B.2 (b) (iii); [redacted], 2014; M-477906-01; Node B.2 (b) (iii); [redacted], 2014; M-492536-01; Node B.2 (b) (iii)). Samples of Barnase/Barstar protein complex were incubated for 30 minutes at 4°C, 25°C, 37°C, 55°C, 75°C, and 95°C. The sample treated at 4°C was used for comparison to the other temperature treated samples.

For the SDS-PAGE and Western Blot analyses, two samples for each temperature treatment (except 4°C) were incubated. One of the two samples was centrifuged and the supernatant (S) containing the soluble protein and pellet (P) containing the insoluble protein were separated. The pellet was suspended in buffer prior to analysis. The other sample for each temperature was not centrifuged and labeled uncentrifuged (UC). The SDS-PAGE and western blot analyses produced similar results. After heat treatment at 25°C, 37°C, and, 55°C, the majority of the Barnase/Barstar protein complex remained in the supernatant. After heat treatment at 75°C and 95°C, the Barnase/Barstar protein complex produced multimers that are mostly in the supernatant. The results suggest that the Barnase/Barstar protein complex is degrading and forms oligomers upon heating at 75°C; therefore, the native form of the Barnase/Barstar protein complex is not stable upon heating at 75°C.

After incubation, the samples of microbially produced Barnase/Barstar complex were analyzed by two ELISA methods, one to detect the Barnase protein and the other to detect the Barstar protein**.** The amount of Barnase or Barstar initially used for each heat treatment was referenced for the amount of each protein detected after heat treatments. The effect of temperature on the complex was determined by the ability of the ELISA analysis to resolve the individual subunits. The Barnase ELISA was able to resolve the complex at all temperature treatments (Table 26 and Figure 40). There was no decrease in the percent (%) protein detected for the Barnase protein after treatments up to 55°C. After treatment at 75°C and 95°C, there was a decrease in the amount of Barnase detected. This suggests that the complex degrades above 75°C. The results of the Barstar ELISA at 4°C, 25°C and 37°C were below the LLOQ, while at 55°C, below LOD, suggesting that the protein, while in the complex, does not have a recognized epitope by the Barstar ELISA method (Table 27 and Figure 41). After treatment at 75°C and 95°C, Barstar was detected, suggesting the Barnase/Barstar protein complex had degraded, allowing the free Barstar protein to bind to antibodies on the ELISA plate. In summary, both ELISA analyses produced similar results; the Barnase/Barstar protein complex is stable to 55°C, but begins to degrade at 75°C and 95°C.

Table 26. Amount of Barnase Detected by ELISA after Heat Treatment

Temperature Treatment (°C)	Amount of Barnase (mg/ml)	% Detected
4	0.22	35.5
25	0.23	37.1
37	0.23	37.1
55	0.22	35.5
75	0.11	17.7
95	0.11	17.7

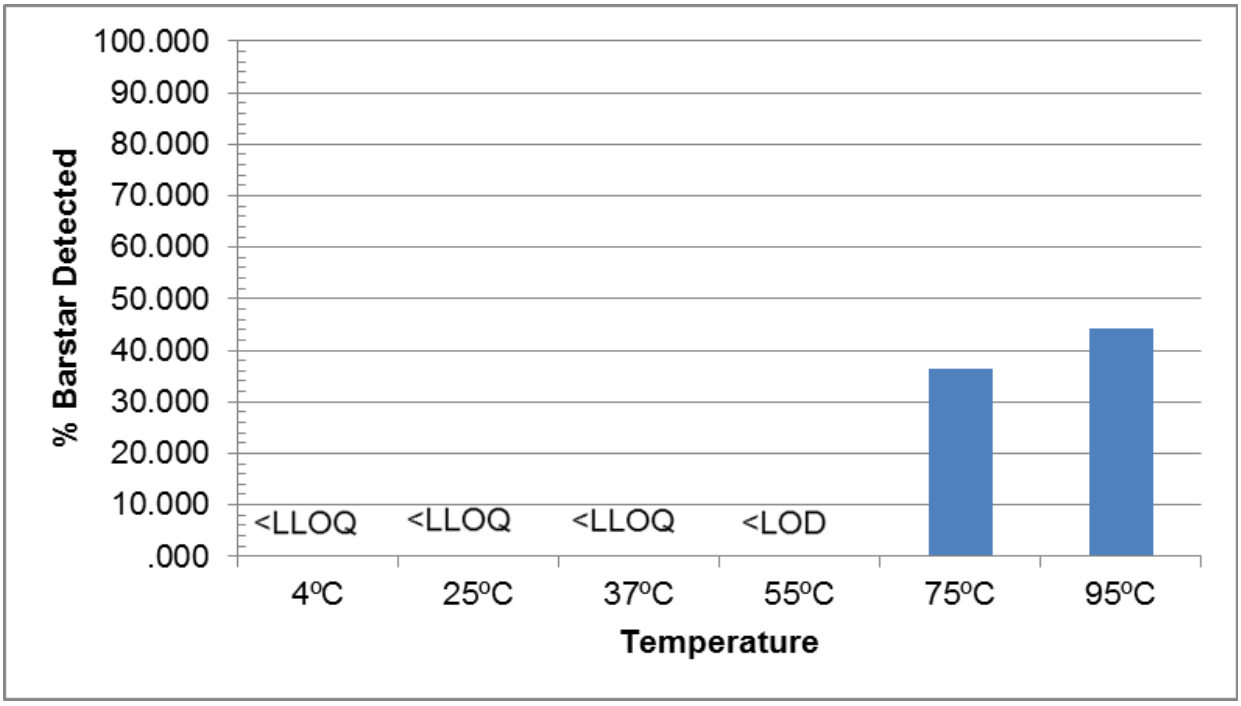


Note: The sample treated at 4°C was used for comparison to the other temperature treated samples.

Figure 40. Effect of Temperature on Amount of Barnase Detected using ELISA

Table 27. Amount of Barstar Detected by ELISA after Heat Treatment

Temperature Treatment (°C)	Amount of Barstar (mg/ml)	% Detected
4	<LLOQ	<LLOQ
25	<LLOQ	<LLOQ
37	<LLOQ	<LLOQ
55	<LOD	<LOD
75	0.19	36.5
95	0.23	44.2



Note: The sample treated at 4°C was used for comparison to the other temperature treated samples.

Figure 41. Effect of Temperature on Amount of Barstar Detected using ELISA

The residual Barnase specific activity of each temperature-treated sample was measured by the Barnase quantitative activity assay which measures the specific activity of each temperature-treated sample. Additionally, the residual Barstar IC_{50} of each temperature-treated sample was measured by the Barstar quantitative activity assay. The specific activity and IC_{50} for each temperature-treated sample was compared to the previously measured specific activity and IC_{50} of the individual proteins [redacted], 2014; M-490632-01; Node B.2 (b) (iii); [redacted], 2014; M-490635-01; Node B.2 (a) (ii)). The Barnase/Barstar complex protein exhibited no residual Barnase or Barstar activity at any of the incubation temperatures (Table 28 and Table 29). The observation of no residual Barnase activity for the Barnase/Barstar complex protein treated at 4°C suggests that the Barstar protein binds to Barnase within the Barnase/Barstar complex, resulting in inhibition of the Barnase activity.

Table 28. Specific Activity of Barnase after Temperature-Treatment

Temperature Treatment (°C)	Specific Activity of Barnase (µmoles product*min^{-1}*mg of enzyme^{-1}) [1]	% Specific Activity Remaining
4	1089.24	< LOD [2]
25	1052.68	< LOD [2]
37	1162.64	< LOD [2]
55	908.37	< LOD [2]
75	356.31	< LOD [2]
95	< LOD [2]	< LOD [2]

[1] Determined in M-490632-01.
[2] The amount of Fluorescent product measure was below the limit of detection of the method. The limit of detection is 0.309 µM.

Table 29. IC_{50} of Barstar and Barnase/Barstar Complex Protein after Temperature Treatment

Temperature Treatment (°C)	IC_{50} (µM) for Barstar [3]	IC_{50} (µM) for Barnase/Barstar Complex Protein
4	1.04	Not Determined [4]
25	1.11	Not Determined [4]
37	1.02	Not Determined [4]
55	1.04	Not Determined [4]
75	1.73	Not Determined [4]
95	Not Determined [5]	Not Determined [4]

[3] Determined in M-490635-01.
[4] The IC_{50} was not determined because there was no difference in the observed rates of Barnase at any concentration of Barnase/Barstar complex protein assayed.
[5] The IC_{50} for 95 °C was not determined because there was no difference in the observed rates of Barnase at any concentration of Barstar complex protein assayed.

The microbially produced Barnase/Barstar protein complex was tested for stability in human simulated gastric fluid (SGF) ([redacted], 2014; M-476903-01; Node B.2 (a) (ii)) and human simulated intestinal fluid (SIF) ([redacted], 2014; M-476904-01; Node B.2 (a) (ii)). Test proteins were incubated with human SGF (pepsin solution at pH 1.2) and human SIF (pancreatin solution at pH 7.5) at approximately 37°C. Samples were taken for analysis at incubation times ranging from 0.5 to 60 minutes. The resultant protein solutions were analysed for presence of the test protein and potential stable protein fragments by SDS-PAGE and western blot. The Barnase/Barstar protein complex was degraded very rapidly (within 30 seconds) when incubated with SGF. Small molecular weight residual fragments (2.5 to 3.5 kDa) were fully degraded within 5 minutes. The Barnase/Barstar protein complex was not degraded within 60 minutes when incubated with SIF; the Barnase protein band showed a partial digestion in that there was a decrease in intensity of the bands with time, while the Barstar protein bands showed no decrease in intensity.

(iii) An animal toxicity study if the bioinformatics comparison and biochemical studies indicate either a relationship with known protein toxins/anti-nutrients or resistance to proteolysis.

PAT/*bar* protein

The PAT protein has no amino acid sequence similarity to other known toxins, as demonstrated by overall amino acid homology searches ([redacted] 2016; M-084359-10; Node B.2 (a) (i)). As expected, the PAT protein only has high structural similarity to the non-toxic proteins of the same functional family, in particular with the PAT protein encoded by the *pat* gene. None of the proteins belonging to the class of acetyltransferase enzymes have been described as toxic.

The mode of action and biochemical properties of the PAT enzyme are well known. This is a highly substrate specific enzyme which follows a Michaelis-Menten kinetic that is pH- and heat-dependant. Its substrate is L-phosphinothricin. The metabolic effect of its expression in plants thus confers tolerance to glufosinate ammonium herbicides.

The PAT protein is rapidly and completely degraded in human simulated gastric ([redacted], 2009; M-217195-04; Node B.2 (a) (ii)) and intestinal ([redacted], 2016; M-208793-04; Node B.2 (a) (ii)) fluids. This minimizes the likelihood that this protein could survive in the human digestive tract and be absorbed. In conclusion, it is considered that the *Streptomyces hygroscopicus* (source of gene) is non-pathogenic and the *bar* gene as well as its encoded PAT protein is not toxic to mammals. PAT also does not possess characteristics associated with food allergens ([redacted] 2016; M-084359-10; Node B.2 (a) (i)). Based on this information, there is a reasonable certainty of no harm resulting from the inclusion of the PAT protein (encoded by the *bar* gene) in human food and in animal feed.

Barnase protein

Described in document number M-232685-01-1 (Node B.1 (a) (CCI)), the query sequence corresponding to the Barnase protein (111 amino acids) is as follows:

Figure 1. Amino Acid Sequence of the Barnase Protein

As mentioned previously, two *in silico* approaches based on the FASTA algorithm associated with the BLOSUM50 scoring matrix were used to evaluate the potential amino acid sequence identity of the query protein with known toxins ([redacted] 2015; M-552256-01; Node B.2 (a) (i)):

An overall identity search with all protein sequences present in the NCBI non-redundant database. An E-value threshold of 0.1 was used for pre-selecting the most similar proteins. The biological relevance of the matches was further assessed by the author. Biologically relevant matches provide insight on the familiarity and potential toxic properties of the query protein.

An overall identity search with all protein sequences present in the in-house Bayer toxin database. An E-value threshold of 10 was used for pre-selecting the most identical toxins. The biological relevance of the matches was further assessed by the author. Biologically relevant matches provide insight on the potential toxic properties of the query protein.

Database used are provided in Table 30.

Table 30. Summary of the database releases and date of search

Name	Database type	Number of sequences	Version	Date of release (Year-Month-Day)
NCBI non-redundant protein database	General database	81 622 391	2016.0206	2016-02-19
BCS 2016 toxin database	Toxin database	24 496	16_1	2016-03-10

Date of search: March 17, 2016

The overall homology search with general protein databases showed that most of the matches corresponded to sequences from the ribonuclease family from various origins. Furthermore, no biologically relevant identities were found with any toxic protein from the Bayer toxin database.

In conclusion, there are neither allergenic nor toxicological *in silico* findings associated with the Barnase protein.

It cannot be ruled out that Bamase as well as all known ribonucleases have toxic properties. Two experimental models have shown that Barnase or derived Bamase-constructs could present some level of toxicity (Prior et al. 1996; Ilinskaya et al., 1997 – refer [redacted], 2004; M-227262-01, Node B.2 (a) (iii)).
In one model (Ilinskaya, 1997), the Bamase produced by *Bacillus amyloliquefaciens* was dissolved in the perfusion media (15 ug/ml and 150 ug/ml) and applied to the kidney after removal from the animal for the *in vitro* approach. Alternatively, the Bamase was administered to rats *in vivo.* The renal effects were assessed in the isolated perfused rat kidney 1 and 6 h after treatment by a biochemical analysis of the urine samples.
The results showed slight alterations of the renal function in the isolated male rat kidney system after application of the Barnase protein at very high concentrations. However, only catalytically active Barnase induced time- and concentration-dependent nephrotoxicity. No adverse effects were observed upon perfusion of the catalytically inactive Barnase.
The other model (Prior et al., 1996) investigated the cytotoxic activity of the chimeric *Pseudomonas exotoxin* >A-Barnase (PE-Bar) toxins as a novel cytotoxic agents for the treatment of human disease on a variety of cell lines or on BALB/c mice for 48h (10 and 25 mg/kg).
The results showed that PE-Bar was cytotoxic to a variety of human cell lines due to its ribonuclease activity which had been delivered to the cytosol. However, the PE-Bar protein was not lethal when injected into mice, either intravenously, up to 10 mg/kg, or intraperitoneally, at 25 mg/kg which was at least 2200 fold more that the amount required for killing by PE. Moreover, Bamase alone was not toxic to mice up to 10 mg/kg. Despite high concentrations, the very low toxicity of PE-Bar or Bamase compared to PE may reflect the mechanism by which the molecules kill cells: irreversible inactivation of protein synthesis for PE and reversible destruction of RNA by PE-Bar or Bamase.
In conclusion, a relatively low reversible toxic effect is expected in the presence of Bamase alone at concentrations much higher than any possible levels related to the exposure to plant products. Nevertheless, this toxicity appears to be mediated by the catalytic activity of the Barnase. Therefore, this adverse effect is prevented by the presence of the Barstar protein that clearly protects the organism from the toxic effects of intracellular Bamase activity (Smeaton et al., 1965; [redacted], 2004; M-227262-01; Node B.2 (a) (iii)) by forming a one-to-one Barstar-Barnase complex (Hartley, 1973; [redacted], 2004; M-227262-01; Node B.2 (a) (iii)) that inactivates the Barnase protein.
In addition, the presence of the Barnase protein cannot be detected *in plants* in other tissues than the flower buds during pollen development. Based on Northern and Western results, neither the gene expression or the protein is detectable in either the leaves or seeds ([redacted], 2004; M-227262-01; Node B.2 (a) (iii)). Thus, there is a remote risk of any potential toxicity in mammals or humans by the consumption of product derived from varieties containing the Bamase protein.

Barstar protein

Described in document number M-232692-01-1 (Node B.1 (a) (CCI)), the query sequence corresponding to the Barstar protein (90 amino acids) is as follows:

Figure . Amino Acid Sequence of the Barstar Protein

[redacted]

As mentioned previously, two *in silico* approaches based on the FASTA algorithm associated with the BLOSUM50 scoring matrix were used to evaluate the potential amino acid sequence identity of the query protein with known toxins ([redacted].; 2016; M-552416-01; Node B.2 (a) (i)):

- An overall identity search with all protein sequences present in the NCBI non-redundant database. An E-value threshold of 0.1 was used for pre-selecting the most similar proteins. The biological relevance of the matches was further assessed. Biologically relevant matches provide insight on the familiarity and potential toxic properties of the query protein.
- An overall identity search with all protein sequences present in the in-house Bayer toxin database. An E-value threshold of 10 was used for pre-selecting the most identical toxins. The biological relevance of the matches was further assessed. Biologically relevant matches provide insight on the potential toxic properties of the query protein.

Database used are provided in Table 31.

Table 31. Summary of the database releases and date of search

Name	Database type	Number of sequences	Version	Date of release (Year-Month-Day)
NCBI non-redundant protein database	General database	81 622 391	2016.0206	2016-02-19
BCS 2016 toxin database	Toxin database	24 496	16.1	2016-03-10

Date of search: March 17, 2016

The overall homology search with general protein databases showed that most of the matches corresponded to sequences from the ribonuclease family from various origins. Furthermore, no biologically relevant identities were found with any toxic protein from the Bayer toxin database.

In conclusion, there were no toxicological *in silico* findings associated with the Barstar protein.

No toxicity of Barstar or Barnase-Barstar complex
No adverse effects have been reported for the Barstar protein or for the Bamase-Barstar complex ([redacted], 2004; M-227262-01; Node B.2 (a) (iii)). This indirectly suggests a potential for toxicity in the absence of the Barstar protein, however as mentioned previously, MS11 *B. napus*, the source of Barnase will not be released as a commercial product for supply in the food chain. It is Bayer's intention to market MS11 *B. napus* as a breeding stack – MS11 x RF3 – in future.

The Barstar protein has no homology with known toxins. However, as expected, the Bamase protein has a high similarity with other ribonuclease proteins from different species of origin. Nevertheless, the co-expression of the Barstar protein neutralizes the enzymatic activity of the Bamase protein by forming an inert stable Barstar-Barnase complex. In addition, the detection of Barnase is only possible in plants when it is complexed with Barstar and the complex or protein alone can only be detected in the tapetal cells of the anthers during pollen formation. Therefore, there is no exposure of the Barnase protein to animals or humans ([redacted], 2004; M-227262-01; Node B.2 (a) (iii)).

(b) Information on the potential allergenicity of any new proteins, including:

(i) Source of the new protein

PAT/*bar* protein is derived from the *bar* gene which in turn is derived from *Streptomyces hygroscopicus* (Murakami *et al*., 1986). Barnase protein is derived from the *barnase* gene and Barstar protein is derived from the *barstar* gene, both of which are derived from *Bacillus amyloliquefaciens* (Hartley, 1988).

None of these proteins are new, however up to date information on the potential allergenicity of these proteins is provided for assessment purposes.

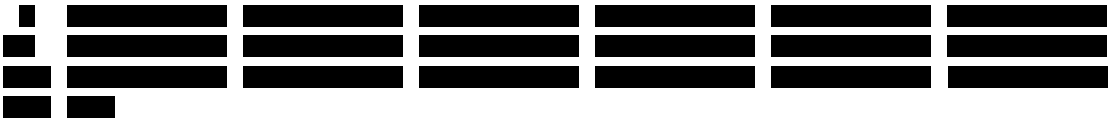
(ii) A bioinformatics comparison of the amino acid sequence of the novel protein to known allergens

PAT/*bar* protein

An integral component of protein safety assessment is whether the protein is known to be allergenic, or the protein is sufficiently similar to a known allergen to indicate potential cross-reactivity in individuals sensitised to the known allergen (Metcalfe *et al*. 1996). Cross-reactivity of immunoglobin E (IgE) between a novel protein and a known allergen should be considered a possibility where a segment of 80 or more amino acids show more than 35% identity (Thomas *et al*. 2005).

As described in the document number M-084188-01-2 (Node B.1 (a) (CCI)), the query sequence corresponding to the PAT/*bar* protein is as follows:

Figure. Amino Acid Sequence of the PAT Protein



This search evaluated the potential amino acid sequence identity of the query protein with known allergens by using three *in silico* approaches (2016; M-084359-10; Node B.2 (a) (i)). Database used are provided in

Table 32.

An 80-mer sliding window search was carried out to compare the query sequence, subdivided into 80 amino acid blocks, with all known allergens present in the public allergen database AllergenOnline (www.allergenonline.org). The FASTA algorithm was used, with the BLOSUM50 scoring matrix, and an E-value threshold of 10. The criterion indicating potential allergenicity was a 35 % identity with an allergenic protein. For alignment lengths shorter than 80 amino acids, the percentage of identity was recalculated over a hypothetical 80 amino acid window, with gaps treated as mismatch. From this calculation, only the matches of >35% identity were considered potentially relevant.

An 8-mer search was carried out to identify any short sequences of 8 amino acids or longer that share 100% identity to an allergenic protein. This search was performed using SeqMatchAll from the EMBOSS suite, which compared the query sequence with all known allergens present in the allergen database.

An overall identity search was carried out to compare the complete query sequence with all protein sequences present in the AllergenOnline database. The FASTA algorithm was used, with the BLOSUM50 scoring matrix and an E-value threshold of 10. The criterion indicating potential allergenicity was ≥35% identity over at least 80 consecutive amino acids with an allergenic protein.

Table 32. Summary of the database releases and date of search

Name	Database type	Number of sequences	Version	Date of release (Year-Month-Day)
AOL	Allergen database	1 956	16	2016-01-26
NCBI non-redundant protein database	General database	81 622 391	2016.0206	2016-02-19

Date of search: March 21,2016

Overall homology search
No biologically relevant identities were found between the query protein and known allergens from the AOL database, based on a '35% identity over an 80 amino acid segment' matching criterion.

8-mer search
No identities were found between the 8 linearly contiguous amino acid blocks that compose the query protein and known allergens from the AOL database.

N-glycosylation site search
Glycosylation is one of the principal co-translational and post-translational modifications of various membrane-bound and secreted proteins, and many food allergens are N-glycosylated (Huby *et al*., 1995; Jenkins *et al*., 1996).
No potential N glycosylation sites were identified on the query sequence by using the N - X~(P) - [S,T] consensus sequence or the N - X - C consensus sequence.

The overall identity search showed no biologically relevant identity between the query protein and any known allergenic proteins. In addition, the 8-mer search showed no 100% identity with known allergenic proteins.
No potential N-glycosylation sites were identified on the amino acid sequence of the query protein.

There were no allergenic *in silico* findings associated with the PAT/*bar* protein

Barnase protein

An integral component of protein safety assessment is whether the protein is known to be allergenic, or the protein is sufficiently similar to a known allergen to indicate potential cross-reactivity in individuals sensitised to the known allergen (Metcalfe *et al*. 1996). Cross-reactivity of immunoglobin E (IgE) between a novel protein and a known allergen should be considered a possibility where a segment of 80 or more amino acids show more than 35% identity (Thomas *et al*. 2005).

Described in document number M-232685-01-1 (Node B.1 (a) (CCI)), the query sequence corresponding to the Barnase protein (111 amino acids) is as follows:

Figure . Amino Acid Sequence of the Barnase Protein

This search evaluated the potential amino acid sequence identity of the query protein with known allergens by using two *in silico* approaches (2016; M-552256-01; Node B.2 (a) (i)). Database used are provided in Table 33.

- An overall identity search was carried out to compare the complete query sequence with all protein sequences present in the public allergen database AllergenOnline (www.allergenonline.org). The FASTA algorithm was used, with the BLOSUM50 scoring matrix and an E-value threshold of 1. The criterion indicating potential allergenicity was ≥35% identity over at least 80 consecutive amino acids with an allergenic protein.

- An 8-mer search was carried out to identify any short sequences of 8 amino acids or longer that share 100% identity to an allergenic protein. This search was performed using SeqMatchAll from the EMBOSS suite, which compared the query sequence with all known allergens present in the allergen database.

Furthermore, the study considered the potential N-glycosylation sites by searching their known consensus sequences, potentially found in allergenic proteins.

Table 33. Summary of the database releases and date of search

Name	Database type	Number of sequences	Version	Date of release (Year-Month-Day)
AOL	Allergen database	1 956	16	2016-01-26
NCBI non-redundant protein database	General database	81 622 391	2016.0206	2016-02-19

Date of search: March 17, 2016

No biologically relevant identities were found between the query protein and known allergens from the AOL database, based on a '35% identity over an 80 amino acid segment' matching criterion.

No identities were found between the 8 linearly contiguous amino acid blocks that compose the query protein and known allergens from the AOL database.

N-glycosylation site search

Glycosylation is one of the principal co-translational and post-translational modifications of various membrane-bound and secreted proteins. The attachment of saccharides to target proteins is thought to enhance protein folding and stability. Some food allergens are N-glycosylated, therefore it is possible that glycosyl groups may contribute to allergenicity (Huby *et al*., 1995; Jenkins *et al*., 1996). However, many allergens are not glycosylated, and a large number of non-allergens are glycoproteins, indicating the glycosylation is neither necessary nor sufficient for allergenicity. It is therefore important to carefully interpret and confirm the results of glycosylation analyses in the safety assessment of a novel protein.
The best studied mode of glycosylation is the formation of an N-glycosidic linkage to Asparagin in the polypeptide chain. One criterion for protein N-glycosylation is the presence of the sequence N-X~(P)-S/T, where N = Asparagin, X~(P) = any amino acid except Proline (P), S = Serin and T = Threonine, in the query sequence. Another potential receptor site is N-X-C, where N = Asparagin, X = any amino acid and C = Cysteine. Therefore, the consensus sequences searched for in this analysis were N-X~(P)-[S/T] or N-X-C.
No potential N-glycosylation site was identified on the Barnase protein amino acid sequence by using the N - X~(P) - [S,T] and N - X - C consensus sequences.

Barstar protein

Described in document number M-232692-01-1 (Node B.1 (a) (CCI)), the query sequence corresponding to the Barstar protein (90 amino acids) is as follows:

Figure . Amino Acid Sequence of the Barstar Protein

This search evaluated the potential amino acid sequence identity of the query protein with known allergens by using two *in silico* approaches (2016; M-552416-01; Node B.2 (a) (i)). Database used are provided in Table 34.

- An overall identity search was carried out to compare the complete query sequence with all protein sequences present in the public allergen database AllergenOnline (www.allergenonline.org). The FASTA algorithm was used, with the BLOSUM50 scoring matrix and an E-value threshold of 1. The criterion indicating potential allergenicity was ≥35% identity over at least 80 consecutive amino acids with an allergenic protein.

- An 8-mer search was carried out to identify any short sequences of 8 amino acids or longer that share 100% identity to an allergenic protein. This search was performed using SeqMatchAll from the EMBOSS suite, which compared the query sequence with all known allergens present in the allergen database.

Furthermore, this study considered the potential N-glycosylation sites by searching their known consensus sequences, potentially found in allergenic proteins.

Table 34. Summary of the database releases and date of search (Barstar)

Name	Database type	Number of sequences	Version	Date of release (Year-Month-Day)
AOL	Allergen database	1 956	16	2016-01-26
NCBI non-redundant protein database	General database	81 622 391	2016.0206	2016-02-19

Date of search: March 17, 2016

Overall homology search
No biologically relevant identities were found between the query protein and known allergens from the AOL database, based on a '35% identity over an 80 amino acid segment' matching criterion.

8-mer search
No identities were found between the 8 linearly contiguous amino acid blocks that compose the query protein and known allergens from the AOL database.

N-glycosylation site search
No potential N-glycosylation site was identified on the Barstar protein amino acid sequence by using the N - X~(P) - [S,T] and N - X - C consensus sequences.

(iii) The new protein's structural properties, including, but not limited to, its susceptibility to enzymatic degradation (e.g. proteolysis), heat and/or acid stability

Please refer to Section B.1 (d) (ii) above for details on enzymatic degradation, heat and acid stability for the PAT/*bar*, Barnase and Barstar proteins.

(iv) Specific serum screening where a new protein is derived from a source known to be allergenic or has sequence homology with a known allergen

Not applicable. The proteins are not from a source known to be allergenic nor do they display sequence homology with known allergens.

(v) Information on whether the new protein(s) have a role in the elicitation of gluten-sensitive enteropathy, in cases where the introduced genetic material is obtained from wheat, rye, barley, oats, or related cereal grains

Not applicable. The introduced genetic materail is not obtained from wheat, rye, barley, oats or related cereal grains.

Where the new protein has been produced from an alternative source (e.g. microbial expression system) in order to obtain sufficient quantities for analysis, information must be provided to demonstrate that the protein tested is biochemically, structurally and functionally equivalent to that expressed in the food produced using gene technology.

PAT/*bar* protein

PAT/*bar* protein was extracted and purified from MS11 *B. napus* leaves to determine the structural and functional characteristics of the plant-purified protein. The structural and functional comparability with the microbially-produced PAT/*bar* protein batch 1215_PATbar was assessed by several complementary methods: molecular weight and purity determination by SDS-PAGE or UPLC-UV-MS, immuno-reactivity by western blot towards an anti- Pat/*bar* antibody, peptide mapping by mass spectrometry, N-terminal sequencing by Edman Degradation, glycosylation by glycostaining, and enzymatic activity assay using a qualitative and quantitative spectrophotometric method ([redacted], 2015; M-544805-01-1; Node B.2 (b) (v)).

For the plant-purified PAT/*bar* protein, SDS-PAGE analysis demonstrated a purity of 74%. Peptide mapping against the theoretical amino acid sequence of the PAT/*bar* protein resulted in coverage of 100%. The N-terminal sequence (MDPER) was consistent with the expected theoretical sequence although acetylation of the N-terminus was observed, and the intact molecular mass confirmed the theoretical molecular mass of the acetylated PAT/*bar* protein (21 kDa).

Comparisons of the plant-purified and the microbially-produced PAT/*bar* proteins by SDS-PAGE demonstrated comparable molecular masses, and the immuno-reactivity of the plant-purified PAT/*bar* protein was confirmed. Neither the plant-purified nor the microbially-produced PAT/*bar* proteins were glycosylated. The activity of the plant-purified and microbially-produced PAT/*bar* proteins were functionally equivalent.

The comparison of the structural and functional characteristics of the plant-purified PAT/*bar* protein with the microbially-produced PAT/*bar* protein batch 1215_PATbar demonstrated that both PAT/*bar* proteins have similar protein-specific characteristics. The identity of the plant-purified PAT/*bar* protein was confirmed and both plant-purified and the microbially-produced PAT/*bar* proteins are structurally and functionally equivalent.

Barnase protein

For the safety assessment of GM crops, toxicology studies are typically performed using a microbially-produced protein as a surrogate, which is equivalent to the plant protein, (Raybould, A.; et al.; 2013; M-549824-01; published). Structural and functional equivalence between the microbially-produced protein and the plant protein are demonstrated by comparing molecular weight, immuno-reactivity, glycosylation status, N-terminal sequence, and biological activity.

In MS11 *B. napus*, the *barnase* gene expression is under the control of a tapetum-specific promoter, Pta29. (Mariani, C.; et al.; 1990; M-147935-01; published , and [redacted].; 2008; M-304805-01; Node A.3 (c), (iii) (CCI)). Therefore, the Barnase protein is expected to be specifically expressed in flower buds during anther development. Barnase exhibits RNAse activity; hence, presence of Barnase protein leads to RNA degradation, cell disruption, and cell death (Mariani, C.; et al.; 1992; M-147936-01; published and Hartley, R. W.; 1989; M-147934-01; published). Since cells expressing Barnase protein are quickly disrupted, the levels of Barnase protein in MS11 *B. napus* tissues would be expected to be low. This was substantiated in protein expression studies where expression levels of Barnase protein determined in different matrices of MS11 *B. napus,* including flower buds, were below the lower limit of quantification for the ELISA method in all matrices analyzed ([redacted]; 2015; M-542702-01; Node B.1 (a)). Furthermore, Barnase was not detected by western blot analysis in crude extracts or upon immuno-affinity purification attempts.

Due to the low levels of Barnase in MS11 *B. napus* tissues, protein of sufficient quantity and quality could not be extracted from the MS11 *B. napus* plant to experimentally confirm the equivalence of the microbially produced Barnase protein with the MS11 *B. napus* plant-produced protein. As such, the Barnase protein in MS11 *B. napus* would be classified as an intractable protein as described in Bushey, D. F.; et al.; 2014; M-549822-01; published . Therefore, a weight of evidence approach was used to assess the equivalence of the intractable protein with the microbially-produced protein.

Sequence analysis of the MS11 *B. napus* insert confirmed the sequence of the *barnase* gene was as expected ([redacted] 2008; M-304805-01; Node A.3 (c), (iii) (CCI)). Peptide mapping of the microbially-produced protein demonstrated 100% coverage against the theoretical amino acid sequence of the Barnase protein ([redacted] 2013; M-467079-01, Node B.1 (a), [redacted] 2016; M-551100-01, Node B.1 (a), and [redacted] 2003; M-232685-01, Node B.1 (a) (CCI)) and was 100% identical to the amino acid sequence predicted from the nucleotide sequence of the MS11 *B.napus* insert. MS11 *B. napus* plants exhibited the male sterile phenotype, demonstrating that an active Barnase was expressed and was efficacious. Likewise, the microbially-produced protein had confirmed enzymatic activity.

This information cumulatively provides evidence that the Barnase in MS11 *B. napus* was produced as intended and that the microbially-produced Barnase protein can be considered as a surrogate for the Barnase expressed in MS11 *B. napus.*

Barstar protein

The Barstar protein in MS11 *B. napus* total protein extract was observed at a comparable migration distance to the recombinant 1340_Barstar protein (*E. coli* expressed) spiked into the non-GM counterpart within the SDS-PAGE and Western blot ([redacted], 2016; M-548891-01; Node B.1 (a)). This confirmed that the immuno-reactivity and the apparent molecular weight of Barstar protein in MS11 *B. napus* total protein extract and the recombinant Barstar protein spiked into non-GM counterpart are comparable. No Barstar protein specific signal was detected in the non-GM counterpart total protein extract.
In conclusion, the Barstar protein in MS11 *B. napus* total protein extract and the recombinant Barstar protein batch 1340_Barstar are comparable.

Information on the potential toxicity and potential allergenicity of a newly expressed protein is also not required if:

(a) The protein is expressed from a transferred gene that is derived from the same species as the host or a species that is cross-compatible with the host, provided evidence is provided to demonstrate the following:

(i) The gene donor belongs to a species that is commonly used as food and has a history of safe use

Not applicable.

(ii) The protein is expressed at levels in the new food produced using gene technology that are consistent with the levels in the gene donor.

Not applicable.

(b) Evidence is provided to demonstrate the absence of the newly expressed protein from the parts of the host organism consumed as food.

Grain and processed fractions were analyzed by Enzyme-Linked ImmunoSorbent Assay (ELISA), to determine the protein expression levels of Barnase, Barstar, and PAT/*bar* in MS11 *B. napus* grain and processed fractions ([redacted], 2016; M-552078-01-1; Node B.2 (b) (v)). The quantitation of Barnase protein was conducted with a validated Barnase-specific ELISA method using the EnviroLogix Barnase Plate Kit (Catalog Number: AP 127). The quantitation of Barstar protein was conducted with a validated Barstar-specific ELISA method using the EnviroLogix Barstar Plate Kit (Catalog Number: AP 125). The quantitation of PAT/*bar* protein was conducted with a validated PAT/*bar* -specific ELISA method using the Envirologix QualiPlate™ Kit for LibertyLink® PAT/*bar* (Catalog number: AP 013).

The expression levels of Barnase and Barstar were <LLOQ for MS11 *B. napus* grain and all processed fractions. The mean expression levels of PAT/*bar* ranged from 0.25 to 0.31 µg/g DW in MS11 *B. napus* grain, press cake, and solvent extracted meal (Table 35). The expression levels of PAT/*bar* protein were <LLOQ in MS11 *B. napus* toasted meal, crude oil, and refined, bleached, deodorized (RBD) oil (Table 35). Levels of PAT/*bar* were similar between grain, press cake and solvent extracted meal fractions and were <LLOQ in toasted meal, crude oil and RBD oil fractions.

Table 35. Amounts of PAT/*bar* Detected in MS11 *B. napus* Matrices

Matrix	Fresh Weight (µg/g)	Dry Weight (µg/g)
Grain	0.23	0.25
Press cake[1]	0.23 ± 0.02	0.26 ± 0.02
Solvent extracted meal[1]	0.27 ± 0.03	0.31 ± 0.03
Toasted meal	<LLOQ	<LLOQ
Crude oil[2]	<LLOQ	
RBD oil[2]	<LLOQ	

[1] Results reported as mean ± standard deviation with a sample size of 2

[2] Results of the analyses for the oil samples are reported “as is”

B.3 Other (non-protein) new substances

If other (non-protein) substances are produced as a result of the introduced DNA, information must be provided on the following:

(a) The identity and biological function of the substance

Non-protein substances cannot be created from DNA. The central maxim of molecular biology is that DNA makes RNA and RNA makes protein. Therefore, no non-protein substances could be created from the introduction of the DNA insert.

(b) Whether the substance has previously been safely consumed in food

Not relevant.

(c) Potential dietary exposure to the substance

Not relevant.

(d) Where RNA interference has been used:

(i) The role of any endogenous target gene and any changes to the food as a result of silencing that gene

Not applicable. RNA interference has not been used.

(ii) The expression levels of the RNA transcript

Not applicable. RNA interference has not been used.

(iii) The specificity of the RNA interference

Not applicable. RNA interference has not been used.

Information on the Labelling of the GM Food

Although there has been no requirement in the Application Handbook for quite some time to supply FSANZ with information related to labelling of the GM food, the following details are provided to assist in the review of whether MS11 *B. napus* derived foods will require labelling.

(a) Information on whether novel DNA or protein is likely to be present in final food.

To assess the potential exposure of humans and animals to the PAT/*bar*, Barnase and Barstar proteins in food and feed products derived from MS11 *B. napus*, *B. Napus* seed and processed commodities were analysed for novel protein content.

The *B. napus* plants that produced the grain were grown in Saskatchewan, Canada in 2014. MS11 *B. napus* plants were treated with the trait-specific herbicide glufosinate-ammonium at the 1-2 and the 4-5 leaf stages. Both applications were at the rate of 370 gram a.i per ha. After harvest, the grain was packaged and stored at ambient temperature.
Grain was shipped to GLP Technologies in Navasota, TX where each grain sample was processed to generate press cake, solvent extracted meal, toasted meal, crude oil, and refined, bleached, deodorized oil (RBD oil). Subsamples of the grain and processed fractions were shipped frozen on dry ice to Bayer CropScience LP in Morrisville, NC ([redacted]; 2016; M-552078-01; Node B.2 (b) (v)).

Grain and processed fractions were analyzed by Enzyme-Linked ImmunoSorbent Assay (ELISA), at Bayer CropScience to determine the protein expression levels of Barnase, Barstar, and PAT/*bar* in MS11 *B. napus* grain and processed fractions. Expression levels

were less than the lower limit of quantitation (<LLOQ) for Barnase and Barstar in the grain and all processed fractions. Levels of PAT/*bar* were similar between grain, press cake and solvent extracted meal fractions and were <LLOQ in toasted meal, crude oil and RBD oil fractions.

(b) Detection methodology for the GM food suitable for analytical purposes.

MS11 *B. napus* can be detected using molecular genetic (DNA) or biochemical (protein) techniques. For the detection of event-specific DNA, a real-time polymerase chain reaction (RT-PCR) technique has been developed and can be provided to FSANZ on request. These methods enable the detection and quantification of minute quantities of DNA derived from MS11 *B. napus* and are specific enough to detect event-specific DNA within complex DNA pools.

Detection of the proteins expressed by the MS11 event can be achieved using standard immunoassay methodology such as the enzyme-linked inmmunosorbent assay (ELISA). The use of this methodology to detect the PAT/*bar* and Barstar proteins in various plant tissues is described in [redacted]., 2015; M-549123-01; Node B.1 (a); and [redacted] (2016; M-552078-01-1; Node B.2 (b) (v)). Note that due to the intractable nature of the Barnase protein *in planta*, it could not be detected in relevant plant matrices using ELISA methodology.

B.4 Novel herbicide metabolites in GM herbicide-tolerant plants

Data must be provided on the identity and levels of herbicide and any novel metabolites that may be present in the food produced using gene technology.

If novel metabolites are present then the application should address the following, where appropriate:

(a) Toxicokinetics and metabolism

(b) Acute toxicity

(c) Short-term toxicity

(d) Long-term toxicity and carcinogenicity

(e) Reproductive and developmental toxicity

(f) Genotoxicity

The PAT enzyme is not anticipated to function within MS11 *B. napus* differently to the way that it functions within MS8 *B. napus* (A372), therefore glufosinate-ammonium metabolism is not anticipated to differ in MS11 B. napus compared to the previously approved MS8 event, i.e. no novel metabolites would be expected to be formed. Hence glufosinate-ammonium metabolism studies submitted to FSANZ previously in association with LibertyLink canola (A372) are expected to sufficiently describe the metabolism of glufosinate-ammonium in MS11 *B. napus*.

B.5 Compositional Analyses of the Food Produced Using Gene Technology

(a) The levels of key nutrients, toxicants and anti-nutrients in the food produced using gene technology compared with the levels in appropriate comparator (usually the non-GM counterpart). A statistical analysis of the data must be provided.

Field Production

MS11 *B. napus* along with the non-genetically modified (non-GM) conventional counterpart (N90-740) and six non-GM commercial reference varieties were grown in 2014 in 10 field trials in the *B. napus* growing regions of Canada and the USA. The field trial design for MS11 *B. napus* assessment consisted of six entries replicated four times (24 plots total) in a randomized complete block design at each site. For detailed information on the field trials and production, refer to report [redacted]; 2016; M-549076-01; Node B.5 (a). The field trial site locations were selected based on representative agro-climatological conditions and *B. napus* production history to be representative of the range of likely environments where the *B. napus* will be commercially grown. Geographical distribution of the sites was determined by representativeness of the commercial *B. napus* production regions of Canada and the USA, based mainly on the agricultural census data (Canola Council of Canada and United States Department of Agriculture). Composition analysis was conducted on samples collected from nine of the ten field trials. The field trial locations selected for composition analysis are presented in the table below.

Table 36 Test Site Locations

Site	County/Rural Municipality	Nearest Town	State or Province/Country	Principal Field Investigator
01	Rural Municipality of Hoodoo	Wakaw	Saskatchewan, Canada	Dean Ngombe
02	Sturgeon	Gibbons	Alberta, Canada	Jennifer Patterson
03	North Norfolk	MacGregor	Manitoba, Canada	Lynn Grant
04	Rural Municipality of MacDonald	Starbuck	Manitoba, Canada	Melissa Fuchs
05	Rural Municipality of Whitewater	Minto	Manitoba, Canada	Angela De Koninck
06	Rural Municipality of Corman Park	Saskatoon	Saskatchewan, Canada	Sonya Fieldmeier
07	Grand Forks	Northwood	North Dakota, USA	Ted Lloyd
09	Jerome	Jerome	Idaho, USA	Tim Vargas
10	Grant	Ephrata	Washington, USA	Mark Qualls

The entries included for the composition assessment of MS11 *B. napus* are presented in Table 37. In addition to the MS11 *B. napus* and conventional counterpart, six different non-GM commercial reference varieties were included to provide reference ranges for the composition assessment. Each field trial location planted only three of the six commercial reference varieties. Each entry in each field trial was replicated four times in a randomized complete block design (RCBD).

Table 37 Description of Entries

Entry ID	Description	Background	Trait-Specific Herbicide Treatment	Seed Lot Number	Locations
A	Conventional Counterpart (N90-740)	N90-740	Not Treated	14CHBN000009	All
B	MS11	N90-740	Not Treated	14CHBN000006	All
C	MS11	N90-740	Treated	14CHBN000006	All
F	46A65	Non-GM	Not Treated	11CNBN000019	1, 2, 3, 7 and 9
G	AC Elect	Non-GM	Not Treated	11CNBN000023	1, 2, 3, 7 and 9
H	AC Excel	Non-GM	Not Treated	11CNBN000027	1, 2, 3, 7 and 9
I	Peace	Non-GM	Not Treated	04CEBN000077	4, 5, 6 and 10
J	Spectrum	Non-GM	Not Treated	11CNBN000039	4, 5, 6 and 10
K	Westar	Non-GM	Not Treated	11CNBN000068	4, 5, 6 and 10

The selected *B. napus* reference varieties were registered commercial varieties that were listed in either the OECD list of non-GM commercial varieties (www.oecd.org/tad/seed, accessed on July 15, 2015) (46A65, AC Elect, and Westar) or the Canadian Food Inspection Agency database (http://www.inspection.gc.ca/active/netapp/regvar/regvare.aspx?id=3179, id=5220, and id=4659, accessed on July 17, 2015) (AC Excel, Peace, and Spectrum).

The MS11 *B. napus* plots treated with trait-specific herbicide (Entry C) received one spray application of Liberty 280 SL (nominally containing 280 g glufosinate-ammonium per liter) at a target rate of 500 g ai/ha at BBCH Growth Stage 12 to 14 (2 to 4 leaves unfolded). Ammonium Sulfate (AMS) was added at a rate of approximately 10 g/L in the tank mix as an additive to the Liberty application. The trait-specific herbicide treatment removed the fertile segregants from the treated plots; therefore the remaining plants in these plots depended on pollen from neighboring plots for fertilization. Details on the field production of the MS11 *B. napus* are provided in the Field Production Report ([redacted] 2016; M-549076-01; Node B.5 (a)).

Composition Analysis

The composition parameters, units and Covance method mnemonics are presented in Table 38. The analytical methods employed and the reference standards used are detailed in the appendix of the composition analytical report ([redacted] M-549080-01-1; Node B.5 (a)).

Table 38 Composition Parameters

Parameter	Units	Covance Method Mnemonic
Proximates		
Moisture	% FW	M100_T100
Crude Protein	% FW, DW	PGEN
Crude Fat	% FW, DW	FSOX
Ash	% FW, DW	ASHM
Carbohydrate (Calculated)	% FW, DW	CHO
Acid Detergent Fiber	% FW, DW	ACID_DF
Neutral Detergent Fiber	% FW, DW	NEUT_DF
Amino Acids		
Alanine, Arginine, Aspartic Acid, Cystine, Glutamic Acid, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tyrosine, and Valine.	% FW, DW	TAALC
Tryptophan	% FW, DW	TRPLC
Vitamins and Minerals		
Minerals: Calcium, Copper, Iron, Magnesium, Manganese, Phosphorus, Potassium, Sodium, and Zinc	mg/kg FW, DW	ICP
Vitamin E: Tocopherols (α, β, γ)	mg/kg FW, DW	TTLC
Vitamin K1	mg/kg FW, DW	VKGS
Anti-Nutrients		
Phytic Acid	% FW, DW	PHYT
Tannins (Insoluble, Soluble, Total)	% FW, DW	TANNINS
Sinapine	% FW, DW	SINAPINE
Glucosinolates:		
4-Hydroxyglucobrassicin, 4-Methoxyglucobrassicin Epi-progoitrin, Glucoalyssin, Glucobrassicanapin Glucobrassicin, Glucoiberin, Gluconapin Gluconapoleiferin, Gluconasturtiin, Glucoraphanin Glucotropaeolin, Neoglucobrassicin, Progoitrin Total Glucosinolates	µmol/g FW, DW	GLLC
Fatty Acids (% Total Fatty Acids)		
Caproic (C6:0), Caprylic (C8:0), Capric (C10:0), Lauric (C12:0), Myristic (C14:0), Myristoleic (C14:1), Pentadecanoic (C15:0), Pentadecenoic (C15:1), Palmitic (C16:0), Palmitoleic (C16:1), Heptadecanoic (C17:0), Heptadecenoic (C17:1), Stearic (C18:0), Oleic (C18:1), Linoleic (C18:2), Linolenic (C18:3), Gamma Linolenic (18:3), Octadecatetraenoic (C18:4), Arachidic (C20:0), Eicosenoic (C20:1), Eicosadienoic (C20:2), Eicosatrienoic (C20:3), Homogamma Linolenic (20:3), Arachidonic (C20:4 N3), Arachidonic (C20:4 N6), Eicosapentaenoic (C20:5), Behenic (C22:0), Erucic (C22:1), Docosadienoic (C22:2), Docosapentaenoic (C22:5 N6), Docosapentaenoic (C22:5 N3), Docosahexaenoic (C22:6), Lignoceric (C24:0) and Nervonic (C24:1)	% FW, DW, % Total Fatty Acids	FALC

Statistical analysis

B. napus grain samples from nine sites generated a total of 215 observations for each analyte. There were 35, 32 and 40 observations each for entries A, B, and C, respectively, and 108 observations for the reference variety entries (Entries F – K), which were analyzed together as 'Reference varieties' for descriptive statistics.

Analytes where more than one third of the values were less than the limit of quantitation (< LOQ) were excluded from the statistical analysis (Table 39). For several analytes, glucobrassicin, insoluble tannins, soluble tannins and total condensed tannins, there were sufficient sample values above LOQ to be statistically evaluated. In these cases a value equal to half the LOQ (dry weight basis or % total fatty acids) was substituted. The statistical analyses were performed using SAS version 9.3 (SAS, 2011).

Combined sites analysis
Descriptive statistics (mean, standard deviation, minimum, and maximum) were summarized by Entry ID for each composition analyte for overall combined site analysis. Descriptive statistics (mean and standard deviation) were determined for entries A, B, and C, and ranges and tolerance intervals (specified to contain 99% of the population with 95% confidence) were determined across all commercial varieties.

The composition data for entries A, B and C were analyzed for each analyte combined over all sites with a mixed model analysis of variance with the fixed entry effect and the random site, interaction of entry by site, and block nested within site effects. Based on the mixed model, entry differences (A vs B and A vs C) were estimated and presented with 95% confidence intervals, along with the p-values (t-test) for the entry differences. Statistical significance was evaluated at $p<0.05$ level.

By site analysis
For each composition analyte, the by-site analysis was performed using a mixed model analysis of variance with the fixed entry effect and the random block effect, followed by pairwise t-tests comparing entries A vs B and A vs C. A summary of the analytes, by analyte category, indicating the number of sites with significant differences for each analyte is presented. By-site descriptive statistics; mean, standard deviation, minimum and maximum value are also presented together with tolerance intervals across all sites.

Table 39. Parameters with Values Below the Limit of Quantitation (LOQ)

	Number of Values		Excluded from analysis
Parameter	**≥ LOQ**	**< LOQ**	
6:0 Caproic	0	215	Yes
8:0 Caprylic	0	215	Yes
10:0 Capric	0	215	Yes
12:0 Lauric	0	215	Yes
14:0 Myristic	0	215	Yes
14:1 Myristoleic	0	215	Yes
15:0 Pentadecanoic	0	215	Yes
15:1 Pentadecenoic	0	215	Yes
17:0 Heptadecanoic	0	215	Yes
17:1 Heptadecenoic	0	215	Yes
18:3 Gamma Linolenic	0	215	Yes
18:4 Octadecatetraenoic	0	215	Yes
20:2 Eicosadienoic	45	170	Yes
20:3 Eicosatrienoic	0	215	Yes
20:3 Homogamma Linolenic	0	215	Yes
20:4 Arachidonic (n3)	0	215	Yes
20:4 Arachidonic (n6)	0	215	Yes
20:5 Eicosapentaenoic	0	215	Yes
22:1 Erucic	26	189	Yes
22:2 Docosadienoic	0	215	Yes
22:5 Docosapentaenoic (n3)	0	215	Yes
22:5 Docosapentaenoic (n6)	0	215	Yes
22:6 Docosahexaenoic	0	215	Yes
Sodium	67	148	Yes
Beta Tocopherol	1	214	Yes
4-Methoxyglucobrassicin	0	215	Yes
Epi-progoitrin	16	199	Yes
Glucoalyssin	26	189	Yes
Glucobrassicanapin	135	80	Yes
Glucobrassicin	200	15	No
Glucoiberin	0	215	Yes
Gluconapoleiferin	0	215	Yes
Gluconasturtiin	54	161	Yes
Glucoraphanin	95	120	Yes
Glucotropaeolin	0	215	Yes
Neoglucobrassicin	13	202	Yes
Insoluble Tannins	206	9	No
Soluble Tannins	211	4	No
Total Condensed Tannins	214	1	No

Results and Discussion

Proximates and Fiber in *B. napus* Grain (Table 40**).** No significant differences were observed between the non-GM conventional counterpart (Entry A) and MS11 *B. napus* not treated with trait-specific herbicides (Entry B) for any of the proximates or fibers.

No significant differences were observed between the non-GM conventional counterpart (Entry A) and MS11 *B. napus* treated with trait-specific herbicides (Entry C) for total carbohydrates.

Statistically significant differences ($p < 0.05$) were observed between the non-GM conventional counterpart (Entry A) and MS11 *B. napus* treated with trait-specific herbicides (Entry C) for moisture, ash, fat, protein, acid detergent fiber and neutral detergent fiber. However, the means for all entries, for all proximates and fiber, were within the range of the reference varieties and the tolerance intervals.

Amino Acids in *B. napus* Grain (Table 41**).** No significant differences were observed between the non-GM conventional counterpart (Entry A) and MS11 *B. napus* not treated with trait-specific herbicides (Entry B) for any of the amino acids.

No significant differences were observed between the non-GM conventional counterpart (Entry A) and MS11 *B. napus* treated with trait-specific herbicides (Entry C) for arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, threonine, tryptophan, tyrosine and valine.

Statistically significant differences ($p < 0.05$) were observed between the non-GM conventional counterpart (Entry A) and MS11 *B. napus* treated with trait-specific herbicides (Entry C) for alanine, cystine, lysine, methionine, phenylalanine, proline and serine. However, the means for all entries, for all the amino acids, were within the range of the reference varieties and the tolerance intervals.

Fatty Acids in *B. napus* Grain (Table 42**).** No significant differences were observed between the non-GM conventional counterpart (Entry A) and MS11 *B. napus* not treated with trait-specific herbicides (Entry B) for any of the fatty acids.

No significant differences were observed between the non-GM conventional counterpart (Entry A) and MS11 *B. napus* treated with trait-specific herbicides (Entry C) for 16:1 palmitoleic acid, 18:2 linoleic acid, 18:3 linolenic acid, and 20:1 eicosenoic acid.

Statistically significant differences ($p < 0.05$) were observed between the non-GM conventional counterpart (Entry A) and MS11 *B. napus* treated with trait-specific herbicides (Entry C) for 16:0 palmitic acid, 18:0 stearic acid, 18:1 oleic acid, 20:0 arachidic acid, 22:0 behenic acid, 24:0 lignoceric acid and 24:1 nervonic acid. However, the means for all entries, for all the fatty acids, were within the range of the reference varieties and the tolerance intervals.

Minerals in *B. napus* Grain (Table 43**).** No significant differences were observed between the non-GM conventional counterpart (Entry A) and MS11 *B. napus* not treated with trait-specific herbicides (Entry B) for any of the minerals.

No significant differences were observed between the non-GM conventional counterpart (Entry A) and MS11 treated with trait-specific herbicides (Entry C) for calcium, iron and manganese.

Statistically significant differences ($p < 0.05$) were observed between the non-GM conventional counterpart (Entry A) and MS11 *B. napus* treated with trait-specific herbicides (Entry C) for copper, magnesium, phosphorus, potassium and zinc. However, the means for all entries, for all minerals, were within the range of the reference varieties and the tolerance intervals.

Vitamins in *B. napus* Grain (Table 44**).** No significant differences were observed between the non-GM conventional counterpart (Entry A) and MS11 *B. napus* not treated with trait-specific herbicides (Entry B) for any of the vitamins.

No significant differences were observed between the non-GM conventional counterpart (Entry A) and MS11 *B. napus* treated with trait-specific herbicides (Entry C) for alpha tocopherol.
Statistically significant differences ($p < 0.05$) were observed between the non-GM conventional counterpart (Entry A) and MS11 *B. napus* treated with trait-specific herbicides (Entry C) for gamma

tocopherol and vitamin K. However, the means for all entries, for all the vitamins were within the range of the reference varieties and the tolerance intervals.

Anti-nutrients in *B. napus* Grain (Table 45**).** No significant differences were observed between the non-GM conventional counterpart (Entry A) and MS11 *B. napus* not treated with trait-specific herbicides (Entry B) or the non-GM conventional counterpart (Entry A) and MS11 *B. napus* treated with trait-specific herbicides (Entry C) for 4-Hydroxyglucobrassicin, glucobrassicin, phytic acid, sinapine, soluble tannins or total condensed tannins.

Statistically significant differences ($p < 0.05$) were observed between both the non-GM conventional counterpart (Entry A) and MS11 *B. napus* not treated with trait-specific herbicides (Entry B) and the non-GM conventional counterpart (Entry A) and MS11 *B. napus* treated with trait-specific herbicides (Entry C) for gluconapin. Statistically significant differences ($p < 0.05$) were observed between the non-GM conventional counterpart (Entry A) and MS11 *B. napus* not treated with trait-specific herbicides (Entry B) for insoluble tannins. Statistically significant differences ($p < 0.05$) were observed between the non-GM conventional counterpart (Entry A) and MS11 *B. napus* treated with trait-specific herbicides (Entry C) for progoitrin and total glucosinolates. However, the means for all entries, for all the anti-nutrients were within the range of the reference varieties and the tolerance intervals; therefore the differences are not considered biologically relevant.

Of the 67 composition analytes, 57 had sufficient levels above LOQ for statistical analysis. Of the 57 analytes that were statistically analyzed, statistically significant differences ($p < 0.05$) between the non-GM conventional counterpart (Entry A) and the MS11 *B. napus* not treated with trait-specific herbicide (Entry B) were observed for two analytes (gluconapine and insoluble tannins). Statistically significant differences ($p < 0.05$) between the non-GM conventional counterpart (Entry A) and the MS11 *B. napus* treated with trait-specific herbicide (Entry C) were observed for 30 analytes. The large number of differences observed in the treated entry is a result of the cross pollination necessary to generate seed for the male sterile MS11 *B. napus*. However the means of all analytes were within the range of the reference varieties and the tolerance intervals; reflecting that the insertion that resulted in this event did not result in any unintended impact on the composition of the *B. napus* grain produced.

Table 40 Comparison of Proximates and Fiber in Grain of MS11 *B. napus* with its Non-GM Conventional Counterpart[a]

Parameter	Non-GM Conventional Counterpart (Entry A) Mean ± SD	MS11 Not Treated (Entry B) Mean ± SD	MS11 Treated (Entry C) Mean ± SD	Non-GM Reference Varieties Range (Entries F-K) [b] (Min-Max)	Tolerance Interval Non-GM Reference Varieties (Entries F-K) [c] (Lower-Upper)	Comparison t-test A vs B [d] p-value	Comparison t-test A vs C [d] p-value
Moisture (% FW)	9.73 ± 2.78	9.49 ± 2.28	11.54 ± 4.20	4.16 - 14.50	1.32 - 17.25	0.922	0.010
Ash (% DW)	4.87 ± 0.86	5.00 ± 0.84	5.47 ± 0.91	0.77 - 10.60	1.36 - 7.99	0.316	0.001
Total Carbohydrates (% DW)	29.7 ± 2.3	29.4 ± 1.8	31.2 ± 3.3	22.7 - 43.2	20.7 - 37.3	0.994	0.078
Fat (% DW)	37.1 ± 4.6	36.5 ± 4.3	33.8 ± 4.7	26.3 - 46.7	26.4 - 49.7	0.261	<.001
Protein (% DW)	28.4 ± 2.3	29.1 ± 2.5	29.6 ± 1.3	23.2 - 33.4	21.0 - 35.6	0.213	0.006
Acid Detergent Fiber (% DW)	21.3 ± 1.9	21.5 ± 1.7	20.1 ± 2.7	15.2 - 24.6	14.0 - 25.4	0.979	0.023
Neutral Detergent Fiber (% DW)	25.0 ± 1.9	24.6 ± 1.9	23.4 ± 2.5	18.9 - 29.1	17.1 - 29.4	0.413	0.011

[a] Composition samples were derived from nine field trials conducted in Canada and the USA in 2014.
[b] Range of results from six reference lines (Entries F-K).
[c] 99% Tolerance Interval: Range of reference lines based on tolerance intervals specified to contain 99% of the population with 95% confidence.
[d] t-Test p-value: Pairwise comparison to the non-GM conventional counterpart (Entry A).

Table 41 Comparison of Amino Acids in Grain of MS11 *B. napus* with its Non-GM Conventional Counterpart[a] (% DW)

Parameter	Non-GM Conventional Counterpart (Entry A) Mean ± SD	MS11 Not Treated (Entry B) Mean ± SD	MS11 Treated (Entry C) Mean ± SD	Non-GM Reference Varieties Range (Entries F-K) [b] (Min-Max)	Tolerance Interval Non-GM Reference Varieties (Entries F-K) [c] (Lower-Upper)	Comparison t-test A vs B [d] p-value	Comparison t-test A vs C [d] p-value
Alanine	1.27 ± 0.10	1.28 ± 0.09	1.32 ± 0.06	0.98 - 1.44	0.96 - 1.53	0.984	0.005
Arginine	1.96 ± 0.17	1.98 ± 0.17	2.01 ± 0.11	1.51 - 2.34	1.38 - 2.43	0.745	0.064
Aspartic Acid	2.25 ± 0.23	2.25 ± 0.22	2.29 ± 0.15	1.63 - 2.49	1.46 - 2.67	0.822	0.227
Cystine	0.627 ± 0.064	0.639 ± 0.059	0.644 ± 0.056	0.539 - 0.849	0.474 - 0.910	0.428	0.039
Glutamic Acid	4.91 ± 0.46	4.99 ± 0.41	5.03 ± 0.30	4.09 - 5.94	3.57 - 6.28	0.567	0.057
Glycine	1.43 ± 0.12	1.44 ± 0.10	1.45 ± 0.08	1.16 - 1.68	1.06 - 1.75	0.774	0.115
Histidine	0.697 ± 0.058	0.721 ± 0.064	0.720 ± 0.048	0.585 - 0.880	0.522 - 0.910	0.237	0.061
Isoleucine	1.19 ± 0.10	1.20 ± 0.09	1.21 ± 0.06	0.914 - 1.39	0.84 - 1.46	0.729	0.239
Leucine	2.06 ± 0.17	2.08 ± 0.16	2.10 ± 0.10	1.64 - 2.36	1.51 - 2.49	0.770	0.107
Lysine	1.52 ± 0.11	1.57 ± 0.11	1.59 ± 0.09	1.38 - 1.90	1.20 - 1.98	0.120	0.002
Methionine	0.505 ± 0.037	0.511 ± 0.040	0.522 ± 0.035	0.399 - 0.644	0.379 - 0.657	0.757	0.021
Phenylalanine	1.19 ± 0.10	1.20 ± 0.10	1.22 ± 0.06	0.931 - 1.37	0.87 - 1.44	0.704	0.044
Proline	1.73 ± 0.21	1.77 ± 0.21	1.83 ± 0.26	1.46 - 2.28	1.22 - 2.32	0.362	<.001
Serine	1.22 ± 0.10	1.23 ± 0.09	1.25 ± 0.06	0.99 - 1.42	0.92 - 1.47	0.685	0.043
Threonine	1.22 ± 0.09	1.23 ± 0.08	1.24 ± 0.06	0.99 - 1.39	0.93 - 1.47	0.946	0.205
Tryptophan	0.416 ± 0.033	0.420 ± 0.036	0.428 ± 0.028	0.320 - 0.499	0.283 - 0.534	0.718	0.050
Tyrosine	0.899 ± 0.067	0.901 ± 0.064	0.911 ± 0.040	0.715 - 1.020	0.669 - 1.072	0.933	0.187
Valine	1.45 ± 0.12	1.46 ± 0.11	1.47 ± 0.07	1.15 - 1.67	1.06 - 1.77	0.996	0.126

[a] Composition samples were derived from nine field trials conducted in Canada and the USA in 2014.
[b] Range of results from six reference lines (Entries F-K).
[c] 99% Tolerance Interval: Range of reference lines based on tolerance intervals specified to contain 99% of the population with 95% confidence.
[d] t-Test p-value: Pairwise comparison to the non-GM conventional counterpart (Entry A).

Table 42 Comparison of Fatty Acids in Grain of MS11 *B. napus* with its Non-GM Conventional Counterpart[a] (% Total Fatty Acids)

Parameter	Non-GM Conventional Counterpart (Entry A) Mean ± SD	MS11 Not Treated (Entry B) Mean ± SD	MS11 Treated (Entry C) Mean ± SD	Non-GM Reference Varieties Range (Entries F-K) [a] (Min-Max)	Tolerance Interval Non-GM Reference Varieties (Entries F-K) [b] (Lower-Upper)	Comparison t-test A vs B [c] p-value	Comparison t-test A vs C [c] p-value
16:0 Palmitic	4.17 ± 0.18	4.21 ± 0.20	4.34 ± 0.20	3.53 - 5.03	3.34 - 5.22	0.357	0.013
16:1 Palmitoleic	0.226 ± 0.018	0.215 ± 0.018	0.235 ± 0.021	0.194 - 0.295	0.179 - 0.302	0.067	0.175
18:0 Stearic	2.16 ± 0.25	2.22 ± 0.28	2.27 ± 0.34	1.62 - 2.66	1.40 - 2.72	0.263	0.009
18:1 Oleic	63.1 ± 2.0	63.5 ± 2.0	61.6 ± 2.5	54.3 - 66.2	52.2 - 69.3	0.433	<.001
18:2 Linoleic	18.4 ± 1.0	18.2 ± 1.0	18.8 ± 1.3	16.0 - 25.2	13.9 - 26.6	0.543	0.207
18:3 Linolenic	9.05 ± 1.40	8.70 ± 1.41	9.55 ± 1.49	6.82 - 13.10	4.06 - 14.37	0.085	0.053
20:0 Arachidic	0.731 ± 0.068	0.757 ± 0.080	0.782 ± 0.103	0.530 - 0.909	0.436 - 0.936	0.097	<.001
20:1 Eicosenoic	1.34 ± 0.10	1.38 ± 0.11	1.42 ± 0.12	0.933 - 3.33	0.11 - 2.95	0.486	0.071
20:2 Eicosadienoic	<LOQ - 0.0861	<LOQ	<LOQ - 0.0897	<LOQ - 0.124	NA	NA	NA
22:0 Behenic	0.408 ± 0.042	0.425 ± 0.049	0.452 ± 0.060	0.215 - 0.487	0.183 - 0.547	0.076	<.001
22:1 Erucic	<LOQ	<LOQ	<LOQ - 0.166	<LOQ - 1.96	NA	NA	NA
24:0 Lignoceric	0.198 ± 0.039	0.209 ± 0.043	0.234 ± 0.055	0.114 - 0.319	0.075 - 0.314	0.132	<.001
24:1 Nervonic	0.195 ± 0.043	0.191 ± 0.050	0.221 ± 0.052	0.121 - 0.337	0.057 - 0.338	0.896	0.018

[a] Composition samples were derived from nine field trials conducted in Canada and the USA in 2014.
[b] Range of results from six reference lines (Entries F-K).
[c] 99% Tolerance Interval: Range of reference lines based on tolerance intervals specified to contain 99% of the population with 95% confidence.
[d] t-Test p-value: Pairwise comparison to the non-GM conventional counterpart (Entry A).
NA=Not Applicable because more than 1/3 of the values are <LOQ. Minimum and maximum are reported instead of mean and standard deviation.

Table 43 Comparison of Minerals in Grain of MS11 *B. napus* with its Non-GM Conventional Counterpart[a] (mg/kg DW)

Parameter	Non-GM Conventional Counterpart (Entry A) Mean ± SD	MS11 Not Treated (Entry B) Mean ± SD	MS11 Treated (Entry C) Mean ± SD	Non-GM Reference Varieties Range (Entries F-K) [b] (Min-Max)	Tolerance Interval Non-GM Reference Varieties (Entries F-K) [c] (Lower-Upper)	Comparison t-test A vs B [d] p-value	Comparison t-test A vs C [d] p-value
Calcium	4885 ± 1102	4651 ± 718	5277 ± 1361	3540 - 7200	2359 - 7211	0.360	0.078
Copper	4.33 ± 0.79	4.29 ± 0.94	4.68 ± 0.91	2.91 - 7.25	1.57 - 6.27	0.966	<.001
Iron	131.6 ± 85.1	119.6 ± 75.8	158.5 ± 95.6	46.4 - 844.0	0 - 461.9	0.731	0.264
Magnesium	3659 ± 438	3654 ± 413	3938 ± 560	2210 - 4610	2126 - 5060	0.126	<.001
Manganese	39.4 ± 7.5	38.1 ± 6.7	38.4 ± 5.4	27.7 - 55.9	20.3 - 60.5	0.675	0.148
Phosphorus	7947 ± 1450	8219 ± 1540	8674 ± 1253	4870 - 12100	2316 - 13044	0.450	0.003
Potassium	8577 ± 1374	8865 ± 1424	9516 ± 1667	4770 - 11400	3672 - 12636	0.144	<.001
Sodium	<LOQ - 416	<LOQ - 786	<LOQ - 590	<LOQ - 955	NA	NA	NA
Zinc	48.4 ± 8.8	50.2 ± 9.6	54.1 ± 9.3	29.5 - 68.1	21.8 - 70.3	0.211	<.001

[a] Composition samples were derived from nine field trials conducted in Canada and the USA in 2014.
[b] Range of results from six reference lines (Entries F-K).
[c] 99% Tolerance Interval: Range of reference lines based on tolerance intervals specified to contain 99% of the population with 95% confidence.
[d] t-Test p-value: Pairwise comparison to the non-GM conventional counterpart (Entry A).
NA=Not Applicable because more than 1/3 of the values are <LOQ. Minimum and maximum are reported instead of mean and standard deviation.

Table 44 Comparison of Vitamins in Grain of MS11 *B. napus* with its Non-GM Conventional Counterpart[a] (mg/kg DW)

Parameter	Non-GM Conventional Counterpart (Entry A) Mean ± SD	MS11 Not Treated (Entry B) Mean ± SD	MS11 Treated (Entry C) Mean ± SD	Non-GM Reference Varieties Range (Entries F-K) [b] (Min-Max)	Tolerance Interval Non-GM Reference Varieties (Entries F-K) [c] (Lower-Upper)	Comparison t-test A vs B [d] p-value	Comparison t-test A vs C [d] p-value
Tocopherols							
Alpha Tocopherol	94.8 ± 11.6	93.5 ± 7.4	91.2 ± 10.9	47.8 - 151.0	48.0 - 154.9	0.356	0.436
Beta Tocopherol	<LOQ	<LOQ	<LOQ	<LOQ - 9.03	NA	NA	NA
Gamma Tocopherol	171 ± 29	165 ± 24	153 ± 29	96 - 381	44 - 326	0.316	0.028
Vitamin K	1.297 ± 0.410	1.286 ± 0.359	1.702 ± 0.604	0.668 - 2.050	0.168 - 2.140	0.891	0.002

[a] Composition samples were derived from nine field trials conducted in Canada and the USA in 2014.
[b] Range of results from six reference lines (Entries F-K).
[c] 99% Tolerance Interval: Range of reference lines based on tolerance intervals specified to contain 99% of the population with 95% confidence.
[d] t-Test p-value: Pairwise comparison to the non-GM conventional counterpart (Entry A).
NA=Not Applicable because more than 1/3 of the values are <LOQ. Minimum and maximum are reported instead of mean and standard deviation.

Table 45 Comparison of Anti-nutrients in Grain of MS11 *B. napus* with its Non-GM Conventional Counterpart[a].

Parameter	Non-GM Conventional Counterpart (Entry A) Mean ± SD	MS11 Not Treated (Entry B) Mean ± SD	MS11 Treated (Entry C) Mean ± SD	Non-GM Reference Varieties Range (Entries F-K) [a] (Min-Max)	Tolerance Interval Non-GM Reference Varieties (Entries F-K) [b] (Lower-Upper)	Comparison t-test A vs B [c] p-value	Comparison t-test A vs C [c] p-value
Glucosinolates (umol/g DW)							
4-Hydroxyglucobrassicin	3.93 ± 0.92	4.08 ± 1.03	4.19 ± 1.07	2.58 - 7.00	1.96 - 7.28	0.520	0.284
Epi-progoitrin	<LOQ - 0.238	<LOQ - 0.244	<LOQ - 0.294	<LOQ - 0.283	NA	NA	NA
Glucoalyssin	<LOQ - 0.337	<LOQ - 0.381	<LOQ - 0.315	<LOQ - 0.631	NA	NA	NA
Glucobrassicanapin	<LOQ - 1.09	<LOQ - 1.15	<LOQ - 0.643	<LOQ - 1.04	NA	NA	NA
Glucobrassicin	0.391 ± 0.222	0.425 ± 0.204	0.479 ± 0.219	0.106 - 1.230	0 - 1.364	0.516	0.177
Gluconapin	2.09 ± 0.85	2.69 ± 1.11	2.99 ± 1.16	0.723 - 4.83	0 - 5.25	0.013	<.001
Gluconasturtiin	<LOQ - 0.293	<LOQ - 0.39	<LOQ - 0.342	<LOQ - 0.391	NA	NA	NA
Glucoraphanin	<LOQ - 0.579	<LOQ - 0.81	<LOQ - 0.706	<LOQ - 0.668	NA	NA	NA
Neoglucobrassicin	<LOQ - 0.525	<LOQ - 0.351	<LOQ - 0.426	<LOQ - 0.574	NA	NA	NA
Progoitrin (umol/g DW)	5.48 ± 2.75	5.88 ± 2.86	7.38 ± 3.51	1.49 - 14.10	0 - 13.67	0.601	0.001
Total Glucosinolates	12.3 ± 4.1	13.7 ± 4.2	15.8 ± 4.8	6.25 - 24.9	0.673 - 26.6	0.179	<.001
Phytic Acid (% DW)	2.11 ± 0.43	2.18 ± 0.46	2.29 ± 0.30	1.04 - 3.48	0.31 - 3.78	0.698	0.051
Sinapine (% DW)	0.717 ± 0.060	0.738 ± 0.052	0.701 ± 0.087	0.351 - 0.894	0.337 - 1.022	0.526	0.739
Insoluble Tannins (% DW)	0.403 ± 0.095	0.455 ± 0.110	0.458 ± 0.125	0.043 - 0.604	0 - 0.749	0.027	0.109
Soluble Tannins (% DW)	0.099 ± 0.038	0.100 ± 0.047	0.134 ± 0.081	0.0204 - 0.2500	0 - 0.194	0.778	0.051
Total Condensed Tannins (% DW)	0.503 ± 0.121	0.554 ± 0.147	0.591 ± 0.189	0.021 - 0.773	0 - 0.923	0.097	0.060

[a] Composition samples were derived from nine field trials conducted in Canada and the USA in 2014.
[b] Range of results from six reference lines (Entries F-K).
[c] 99% Tolerance Interval: Range of reference lines based on tolerance intervals specified to contain 99% of the population with 95% confidence.
[d] t-Test p-value: Pairwise comparison to the non-GM conventional counterpart (Entry A).
NA=Not Applicable because more than 1/3 of the values are <LOQ. Minimum and maximum are reported instead of mean and standard deviation.

By-Site Analysis

By-site results were examined for those analytes, which showed significant combined-site differences between the non-GM conventional counterpart (Entry A) and MS11 *B. napus* not treated with trait-specific herbicides (Entry B) (two analytes) or the non-GM conventional counterpart (Entry A) and MS11 *B. napus* treated with trait-specific herbicides (Entry C) (30 analytes).

There were significant differences between the non-GM conventional counterpart (Entry A) and MS11*B. napus* treated with trait-specific herbicides (Entry C) at over half the sites for moisture, fat, neutral detergent fiber, lysine, 16:0 palmitic acid, 18:0 stearic acid, 18:1 oleic acid, 20:0 arachidic acid, 22:0 behenic acid 24:0 lignoceric acid, phosphorus, zinc, and vitamin K (Table 46). This is consistent with the observations in the combined-site analysis as a result of the cross pollination necessary to generate seed for the male-sterile MS11 *B. napus*.

For the remaining analytes where there were significant differences between the non-GM conventional counterpart (Entry A) and MS11 *B. napus* not treated with trait-specific herbicides (Entry B) or the non-GM conventional counterpart (Entry A) and MS11*B. napus* treated with trait-specific herbicides (Entry C), less than half of the sites showed significant differences.

Table 46 Summary of the By-Site Analyses of Composition Analytes by Composition Group for Canola Grain

Analyte Category	Parameter	N of sites	Entry effect not estimable #	N of sites without significant (p>=0.05) entry effect in the ANOVA	N of sites with a significant (p<0.05) entry effect in the ANOVA	N of sites with significant entry differences *	
						A vs B	A vs C
Proximates and Fiber	Moisture	9	-	2	7	4	5
	Ash	9	-	3	6	1	3
	Total Carbohydrates	9	-	1	8	1	4
	Fat	9	-	1	8	-	8
	Protein	9	-	1	8	-	4
	Acid Detergent Fiber	9	-	3	6	1	3
	Neutral Detergent Fiber	9	-	-	9	1	5
Amino Acids	Alanine	9	-	3	6	-	3
	Arginine	9	-	2	7	1	2
	Aspartic Acid	9	-	-	9	-	5
	Cystine	9	-	4	5	-	1
	Glutamic Acid	9	-	4	5	-	2
	Glycine	9	-	1	8	-	3
	Histidine	9	-	6	3	-	2
	Isoleucine	9	-	1	8	1	2
	Leucine	9	-	1	8	-	4
	Lysine	9	-	3	6	1	5
	Methionine	9	-	6	3	-	2
	Phenylalanine	9	-	1	8	-	3
	Proline	9	-	4	5	-	4
	Serine	9	-	1	8	-	3
	Threonine	9	-	-	9	-	3
	Tryptophan	9	-	4	5	-	1
	Tyrosine	9	-	-	9	-	4
	Valine	9	-	1	8	-	3
Fatty Acids	16:0 Palmitic	9	-	-	9	1	7
	16:1 Palmitoleic	9	-	-	9	1	4
	18:0 Stearic	9	-	-	9	1	7
	18:1 Oleic	9	-	-	9	1	5
	18:2 Linoleic	9	-	-	9	1	4
	18:3 Linolenic	9	-	1	8	3	5
	20:0 Arachidic	9	-	-	9	2	6
	20:1 Eicosenoic	9	-	-	9	1	3
	22:0 Behenic	9	-	1	8	2	7
	24:0 Lignoceric	9	-	1	8	1	6
	24:1 Nervonic	9	-	3	6	-	3
Minerals	Calcium	9	-	3	6	1	4
	Copper	9	-	-	9	-	4
	Iron	9	-	8	1	-	-
	Magnesium	9	-	5	4	1	4
	Manganese	9	-	5	4	-	1
	Phosphorus	9	-	3	6	1	5
	Potassium	9	-	-	9	1	4
	Zinc	9	-	2	7	-	5
Vitamins	Alpha Tocopherol	9	-	3	6	-	1
	Gamma Tocopherol	9	-	4	5	-	1
	Vitamin K	9	-	-	9	1	6
Anti-nutrients	4-Hydroxyglucobrassicin	9	-	6	3	-	-
	Glucobrassicin	9	-	7	2	-	1
	Gluconapin	9	-	7	2	1	2
	Progoitrin	9	-	8	1	1	1
	Total Glucosinolates	9	-	6	3	1	1
	Phytic Acid	9	-	3	6	-	4
	Sinapine	9	-	2	7	1	4
	Insoluble Tannins	9	-	1	8	1	1
	Soluble Tannins	9	-	3	6	-	3
	Total Condensed Tannins	9	-	1	8	1	3

***** Only if the ANOVA for a respective site resulted in a significant overall entry effect, the individual t-tests were taken into account and the 'significant' entry differences were counted

Conclusions

Of the 57 analytes evaluated, statistically significant differences were observed for only two analytes (gluconapin and insoluble tannins) between the non-GM conventional counterpart (Entry A) and the MS11 *B. napus* not treated line (Entry B). When comparing the non-GM conventional counterpart and MS11 *B. napus* treated with trait-specific herbicide (Entry C), 30 analytes were statistically different, reflecting the contribution of pollen from other plots within the field to the male sterile flowers of MS11 *B napus.* However, in all cases, the means for all analytes were within the range of the reference varieties and within the tolerance intervals for the reference varieties. Therefore, the statistically significant differences are not considered biologically relevant.

Based on the composition analysis, nutrient and anti-nutrient levels in MS11 *B. napus* grain are equivalent to that of *B. napus* reference varieties.

Composition analysis of processed products

MS11 *B. napus* and its conventional counterpart, N90-740, were grown in Saskatchewan, Canada, in 2014 to produce bulk grain for processing. The MS11 *B. napus* plants were treated with the trait-specific herbicide glufosinate-ammonium at BBCH 11-12 and BBCH 14-15 (1-2 leaf and 4-5 leaf) stages. Both applications were at the rate of 370 gram a.i per ha. After harvest, the bulk grain was packaged and stored at ambient temperature until shipment to the processing facility. Each bulk grain sample was processed in a manner that simulated commercial practice to generate press cake, solvent extracted meal, toasted meal, crude oil, and refined, bleached, deodorized oil (RBD oil) ([redacted], 2016; M-552078-01; Node B.2 (b) (v)).

Composition Analysis

Grain and processed fraction samples were analyzed to determine their composition. Grain was analyzed for proximates and fiber, amino acids, fatty acids, minerals, vitamins and anti-nutrients. Press cake, solvent extracted meal and toasted meal were analyzed for proximates and fiber, amino acids, minerals, and anti-nutrients. Crude oil and RBD oil were analyzed for fatty acids and vitamins. The analytical methods employed and the reference standards used are detailed in the appendix of the composition analytical report ([redacted]., M-552078-01-1; Node B.2 (b) (v)).Analytes and matrices where all values were <LOQ are presented in Table 47.

Means and standard deviations for proximates and fiber levels in grain, press cake, solvent extracted meal and toasted meal are provided in Table 48 - Table 51. Similar levels of proximates and fiber were observed between the two samples; MS11 *B. napus* and the non-GM conventional counterpart. For both samples, crude fat levels decreased upon processing to press cake and then to solvent extracted meal.

Means and standard deviations for amino acid levels in grain, press cake, solvent extracted meal and toasted meal are provided in Table 52 - Table 55. Similar levels of amino acids were observed between the two samples; MS11 *B. napus* and the non-GM conventional counterpart. For both samples, amino acid levels increased when press cake was processed to solvent extracted meal.

Means and standard deviations for fatty acid levels in grain, crude oil and RBD oil are provided in Table 56 - Table 58. Similar levels of fatty acids were observed between the two samples; MS11 *B. napus* and the non-GM conventional counterpart. For both samples, fatty acid profiles were similar.

Means and standard deviations for mineral levels in grain, press cake, solvent extracted meal, and toasted meal are provided in Table 59 - Table 62. Similar levels of minerals were observed between the two samples; MS11*B. napus* and the non-GM conventional counterpart. For both samples, mineral levels increased upon processing grain to press cake and then to solvent extracted meal.

Means and standard deviations for vitamin levels in grain, crude oil and RBD oil are provided in Table 63 - Table 65. Similar levels of vitamins were observed between the two samples; MS11 *B. napus* and the non-GM conventional counterpart. For both samples, vitamin levels decreased when crude oil was processed to RBD oil.

Means and standard deviations for anti-nutrient levels, in grain, press cake, solvent extracted meal and toasted meal are provided in Table 66 - Table 69. Similar levels of phytic acid, sinapine and tannins were observed between the two samples; MS11 *B. napus* and the non-GM conventional counterpart. Glucosinolate levels tended to be higher in MS11 than in the non-GM conventional counterpart. However for all samples, total glucosinolate levels were <30 µmol/g and erucic acid (an anti-nutritive fatty acid) levels were <2% total fatty acids, and therefore below the thresholds for these anti-nutrients required for Canola quality *B. napus* as specified in the OECD Low Erucic Acid Rapeseed (Canola) document (Anon.; 2011; M-223000-02; published).

Table 47. Analytes and Matrices Where All Values were <LOQ

Analyte	Grain	Press Cake	Solvent Extracted Meal	Toasted Meal	Crude Oil	RBD Oil
C8:0 Caprylic Acid	X	NA	NA	NA	X	X
C10:0 Capric Acid	X	NA	NA	NA	X	X
C12:0 Lauric Acid	X	NA	NA	NA	X	X
C14:0 Myristic Acid	–	NA	NA	NA	X	X
C14:1 Myristoleic Acid	X	NA	NA	NA	X	X
C15:0 Pentadecanoic Acid	X	NA	NA	NA	X	X
C15:1 Pentadecenoic Acid	X	NA	NA	NA	X	X
C17:0 Heptadecanoic Acid	–	NA	NA	NA	X	X
C18:4 Stearidonic Acid	X	NA	NA	NA	X	X
C20:3 Eicosatrienoic Acid	X	NA	NA	NA	X	X
C20:4 Arachidonic Acid	X	NA	NA	NA	X	X
C20:5 Eicosapentaenoic Acid	X	NA	NA	NA	X	X
C22:1 Erucic Acid	–	NA	NA	NA	X	X
C22:5 N3 Docosapentaenoic Acid	–	NA	NA	NA	X	X
C22:5 N6 Docosapentaenoic Acid	X	NA	NA	NA	X	–
Beta Tocopherol	X	NA	NA	NA	X	X
4-methoxyglucobrassicin	X	X	X	X	NA	NA
Epi-progoitrin	X	X	X	X	NA	NA
Glucoalyssin	X	X	X	X	NA	NA
Glucobrassicanapin	X	X	–	X	NA	NA
Glucoiberin	X	X	X	X	NA	NA
Gluconapoleiferin	X	X	X	X	NA	NA
Gluconasturtiin	–	X	X	X	NA	NA
Glucoraphanin	X	X	X	X	NA	NA

X = the results of the analyte were <LOQ for all of the samples of the specified matrix
NA = the analyte was not measured in the specified matrix
– = the results of the analyte had at least one result >LOQ for the specified matrix

Table 48. Proximates and Fiber in Grain (%)

Bayer Sample ID	15-RSLJS026-001B		15-RSLJS026-002B	
Description	Non-GM Conventional Counterpart		MS11 *B. napus* Treated	
	FW	DW	FW	DW
	Mean ± SD			
Moisture	7.31 ± 0.25	–	6.46 ± 0.26	–
Ash	4.14 ± 1.17	4.47 ± 1.25	4.32 ± 1.46	4.63 ± 1.58
Carbohydrates	23.1 ± 0.1	24.9 ± 0.1	21.5 ± 2.8	23.0 ± 3.0
Crude Fat	38.7 ± 1.0	41.8 ± 1.2	40.1 ± 1.7	42.9 ± 1.9
Crude Protein	26.7 ± 0.0	28.8 ± 0.1	27.6 ± 0.1	29.5 ± 0.1
Acid Detergent Fiber	34.4 ± 2.3	37.1 ± 2.6	35.7 ± 4.1	38.2 ± 4.5
Neutral Detergent Fiber	32.3 ± 2.1	34.8 ± 2.2	33.5 ± 3.9	35.8 ± 4.1

Table 49. Proximates and Fiber in Press Cake (%)

Bayer Sample ID	15-RSLJS026-003B		15-RSLJS026-004B	
Description	Non-GM Conventional Counterpart		MS11 *B. napus* Treated	
	FW	DW	FW	DW
	Mean ± SD			
Moisture	11.67 ± 0.12	–	11.27 ± 0.32	–
Ash	4.33 ± 0.01	4.90 ± 0.02	4.34 ± 0.03	4.89 ± 0.02
Carbohydrates	30.6 ± 1.8	34.7 ± 2.0	30.3 ± 0.8	34.1 ± 0.8
Crude Fat	20.7 ± 0.7	23.4 ± 0.7	20.3 ± 0.6	22.9 ± 0.6
Crude Protein	32.7 ± 1.1	37.0 ± 1.3	33.8 ± 0.1	38.1 ± 0.2
Acid Detergent Fiber	15.3 ± 2.3	17.3 ± 2.6	11.7 ± 0.3	13.2 ± 0.3
Neutral Detergent Fiber	16.3 ± 2.3	18.4 ± 2.6	16.2 ± 3.1	18.2 ± 3.5

Table 50. Proximates and Fiber in Solvent Extracted Meal (%)

Bayer Sample ID	15-RSLJS026-005B		15-RSLJS026-006B	
Description	Non-GM Conventional Counterpart		MS11 *B. napus* Treated	
	FW	DW	FW	DW
	Mean ± SD			
Moisture	14.07 ± 0.31	–	13.30 ± 0.10	–
Ash	5.24 ± 0.02	6.09 ± 0.04	5.35 ± 0.06	6.17 ± 0.06
Carbohydrates	36.8 ± 0.7	42.8 ± 0.9	36.8 ± 0.8	42.5 ± 0.9
Crude Fat	2.7 ± 0.9	3.2 ± 1.0	1.7 ± 0.4	2.0 ± 0.4
Crude Protein	41.2 ± 0.4	47.9 ± 0.5	42.8 ± 0.4	49.4 ± 0.4
Acid Detergent Fiber	19.2 ± 3.8	22.3 ± 4.5	16.2 ± 2.2	18.7 ± 2.6
Neutral Detergent Fiber	18.8 ± 0.7	21.9 ± 0.8	18.2 ± 0.6	21.0 ± 0.7

Table 51. Proximates and Fiber in Toasted Meal (%)

Bayer Sample ID	15-RSLJS026-007B		15-RSLJS026-008B	
Description	Non-GM Conventional Counterpart		MS11 *B. napus* Treated	
	FW	DW	FW	DW
	Mean ± SD			
Moisture	9.99 ± 0.53	–	9.61 ± 0.23	–
Ash	5.49 ± 0.02	6.11 ± 0.05	5.60 ± 0.01	6.19 ± 0.02
Carbohydrates	39.0 ± 1.5	43.3 ± 1.6	37.4 ± 0.9	41.4 ± 0.9
Crude Fat	2.9 ± 1.1	3.3 ± 1.2	3.0 ± 0.5	3.3 ± 0.5
Crude Protein	42.6 ± 0.3	47.3 ± 0.4	44.4 ± 0.2	49.1 ± 0.4
Acid Detergent Fiber	17.0 ± 0.3	18.9 ± 0.4	16.7 ± 1.6	18.5 ± 1.7
Neutral Detergent Fiber	21.0 ± 0.1	23.3 ± 0.2	19.5 ± 0.2	21.6 ± 0.2

Table 52. Amino Acids in Grain (%)

Bayer Sample ID	15-RSLJS026-001B		15-RSLJS026-002B	
Description	Non-GM Conventional Counterpart		MS11 *B. napus* Treated	
	FW	DW	FW	DW
	Mean ± SD			
Alanine	1.14 ± 0.05	1.23 ± 0.06	1.17 ± 0.01	1.24 ± 0.01
Arginine	1.55 ± 0.10	1.67 ± 0.11	1.65 ± 0.06	1.76 ± 0.06
Aspartic acid	2.00 ± 0.09	2.16 ± 0.09	2.06 ± 0.03	2.20 ± 0.03
Cystine	0.69 ± 0.11	0.75 ± 0.12	0.80 ± 0.06	0.85 ± 0.06
Glutamic acid	5.02 ± 0.24	5.42 ± 0.27	5.39 ± 0.03	5.77 ± 0.03
Glycine	1.28 ± 0.07	1.38 ± 0.08	1.34 ± 0.03	1.43 ± 0.04
Histidine	0.70 ± 0.04	0.75 ± 0.05	0.75 ± 0.03	0.80 ± 0.03
Isoleucine	1.06 ± 0.06	1.14 ± 0.06	1.11 ± 0.01	1.18 ± 0.02
Leucine	1.85 ± 0.11	2.00 ± 0.12	1.95 ± 0.02	2.08 ± 0.03
Lysine	1.53 ± 0.06	1.65 ± 0.07	1.59 ± 0.03	1.70 ± 0.04
Methionine	0.45 ± 0.03	0.48 ± 0.03	0.50 ± 0.03	0.53 ± 0.03
Phenylalanine	1.06 ± 0.07	1.14 ± 0.08	1.10 ± 0.06	1.18 ± 0.06
Proline	1.56 ± 0.08	1.68 ± 0.09	1.66 ± 0.02	1.77 ± 0.03
Serine	1.09 ± 0.04	1.18 ± 0.04	1.15 ± 0.03	1.23 ± 0.03
Threonine	1.07 ± 0.06	1.16 ± 0.07	1.12 ± 0.03	1.20 ± 0.04
Tryptophan	0.31 ± 0.01	0.33 ± 0.01	0.31 ± 0.02	0.33 ± 0.02
Tyrosine	0.62 ± 0.04	0.67 ± 0.05	0.63 ± 0.02	0.68 ± 0.03
Valine	1.34 ± 0.07	1.45 ± 0.08	1.39 ± 0.02	1.49 ± 0.03

Table 53. Amino Acids in Press Cake (%)

Bayer Sample ID	15-RSLJS026-003B		15-RSLJS026-004B	
Description	Non-GM Conventional Counterpart		MS11 *B. napus* Treated	
	FW	DW	FW	DW
	Mean ± SD			
Alanine	1.46 ± 0.01	1.65 ± 0.01	1.44 ± 0.02	1.62 ± 0.02
Arginine	1.94 ± 0.04	2.20 ± 0.05	1.96 ± 0.06	2.21 ± 0.07
Aspartic acid	2.60 ± 0.01	2.94 ± 0.01	2.55 ± 0.01	2.88 ± 0.02
Cystine	0.99 ± 0.08	1.12 ± 0.08	0.96 ± 0.10	1.08 ± 0.11
Glutamic acid	6.38 ± 0.01	7.22 ± 0.02	6.54 ± 0.06	7.37 ± 0.08
Glycine	1.61 ± 0.03	1.82 ± 0.04	1.62 ± 0.04	1.82 ± 0.05
Histidine	0.87 ± 0.02	0.99 ± 0.03	0.88 ± 0.03	1.00 ± 0.03
Isoleucine	1.34 ± 0.01	1.52 ± 0.02	1.34 ± 0.03	1.51 ± 0.03
Leucine	2.36 ± 0.03	2.67 ± 0.03	2.36 ± 0.05	2.66 ± 0.05
Lysine	1.97 ± 0.01	2.22 ± 0.01	1.97 ± 0.01	2.22 ± 0.03
Methionine	0.59 ± 0.04	0.67 ± 0.05	0.59 ± 0.02	0.66 ± 0.02
Phenylalanine	1.32 ± 0.03	1.50 ± 0.04	1.33 ± 0.05	1.50 ± 0.05
Proline	1.98 ± 0.02	2.24 ± 0.03	2.03 ± 0.04	2.28 ± 0.05
Serine	1.37 ± 0.03	1.55 ± 0.04	1.37 ± 0.03	1.54 ± 0.03
Threonine	1.35 ± 0.02	1.52 ± 0.03	1.33 ± 0.03	1.50 ± 0.03
Tryptophan	0.41 ± 0.00	0.46 ± 0.01	0.42 ± 0.01	0.47 ± 0.01
Tyrosine	0.77 ± 0.01	0.87 ± 0.02	0.75 ± 0.03	0.85 ± 0.04
Valine	1.69 ± 0.02	1.91 ± 0.03	1.69 ± 0.03	1.90 ± 0.04

Table 54. Amino Acids in Solvent Extracted Meal (%)

Bayer Sample ID	15-RSLJS026-005B		15-RSLJS026-006B	
Description	Non-GM Conventional Counterpart		MS11 *B. napus* Treated	
	FW	DW	FW	DW
	Mean ± SD			
Alanine	1.75 ± 0.01	2.04 ± 0.01	1.77 ± 0.02	2.04 ± 0.03
Arginine	2.43 ± 0.07	2.82 ± 0.08	2.42 ± 0.05	2.79 ± 0.06
Aspartic acid	3.06 ± 0.02	3.56 ± 0.03	3.13 ± 0.04	3.61 ± 0.04
Cystine	1.22 ± 0.05	1.42 ± 0.06	1.20 ± 0.03	1.38 ± 0.04
Glutamic acid	7.64 ± 0.02	8.90 ± 0.04	8.07 ± 0.11	9.31 ± 0.12
Glycine	2.02 ± 0.07	2.35 ± 0.08	2.00 ± 0.03	2.31 ± 0.03
Histidine	1.11 ± 0.04	1.29 ± 0.05	1.11 ± 0.02	1.28 ± 0.02
Isoleucine	1.65 ± 0.02	1.93 ± 0.02	1.65 ± 0.05	1.90 ± 0.05
Leucine	2.90 ± 0.03	3.38 ± 0.04	2.94 ± 0.04	3.38 ± 0.04
Lysine	2.33 ± 0.02	2.72 ± 0.02	2.43 ± 0.03	2.80 ± 0.03
Methionine	0.74 ± 0.01	0.86 ± 0.01	0.74 ± 0.01	0.86 ± 0.01
Phenylalanine	1.67 ± 0.07	1.94 ± 0.08	1.65 ± 0.04	1.90 ± 0.05
Proline	2.43 ± 0.03	2.83 ± 0.04	2.50 ± 0.02	2.89 ± 0.02
Serine	1.72 ± 0.03	2.00 ± 0.04	1.71 ± 0.02	1.97 ± 0.02
Threonine	1.70 ± 0.03	1.98 ± 0.04	1.68 ± 0.02	1.94 ± 0.02
Tryptophan	0.50 ± 0.03	0.58 ± 0.03	0.52 ± 0.02	0.60 ± 0.02
Tyrosine	0.98 ± 0.03	1.14 ± 0.03	0.93 ± 0.04	1.08 ± 0.05
Valine	2.09 ± 0.02	2.43 ± 0.02	2.06 ± 0.06	2.38 ± 0.06

Table 55. Amino Acids in Toasted Meal (%)

Bayer Sample ID	15-RSLJS026-007B		15-RSLJS026-008B	
Description	Non-GM Conventional Counterpart		MS11 *B. napus* Treated	
	FW	DW	FW	DW
	Mean ± SD			
Alanine	1.83 ± 0.02	2.04 ± 0.01	1.87 ± 0.04	2.06 ± 0.05
Arginine	2.46 ± 0.05	2.74 ± 0.06	2.60 ± 0.02	2.87 ± 0.01
Aspartic acid	3.19 ± 0.04	3.55 ± 0.04	3.26 ± 0.11	3.61 ± 0.12
Cystine	1.22 ± 0.09	1.35 ± 0.10	1.26 ± 0.08	1.39 ± 0.08
Glutamic acid	7.96 ± 0.05	8.84 ± 0.01	8.42 ± 0.17	9.31 ± 0.22
Glycine	2.06 ± 0.03	2.30 ± 0.04	2.16 ± 0.01	2.39 ± 0.01
Histidine	1.12 ± 0.03	1.25 ± 0.03	1.18 ± 0.01	1.31 ± 0.01
Isoleucine	1.72 ± 0.02	1.92 ± 0.02	1.78 ± 0.01	1.97 ± 0.02
Leucine	3.05 ± 0.03	3.39 ± 0.03	3.18 ± 0.02	3.51 ± 0.03
Lysine	2.26 ± 0.04	2.51 ± 0.04	2.27 ± 0.07	2.51 ± 0.08
Methionine	0.73 ± 0.05	0.81 ± 0.06	0.79 ± 0.04	0.87 ± 0.04
Phenylalanine	1.71 ± 0.04	1.90 ± 0.05	1.80 ± 0.02	1.99 ± 0.02
Proline	2.53 ± 0.02	2.80 ± 0.03	2.66 ± 0.02	2.95 ± 0.03
Serine	1.77 ± 0.02	1.97 ± 0.02	1.84 ± 0.01	2.04 ± 0.02
Threonine	1.76 ± 0.02	1.95 ± 0.03	1.81 ± 0.01	2.00 ± 0.01
Tryptophan	0.54 ± 0.02	0.59 ± 0.02	0.52 ± 0.05	0.57 ± 0.06
Tyrosine	0.98 ± 0.05	1.09 ± 0.06	1.02 ± 0.01	1.13 ± 0.01
Valine	2.18 ± 0.02	2.42 ± 0.03	2.23 ± 0.02	2.47 ± 0.02

Table 56. Fatty Acids in Grain

Bayer Sample ID	15-RSLJS026-001B			15-RSLJS026-002B		
Description	Non-GM Conventional Counterpart			MS11 *B. napus* Treated		
	% FW	% DW	% Total Fatty Acids	% FW	% DW	% Total Fatty Acids
	Mean ± SD					
C14:0 Myristic	0.0156 ± 0.0013	0.0169 ± 0.0015	0.0546 ± 0.0021	0.0158 ± 0.0017	0.0169 ± 0.0019	0.0534 ± 0.0012
C16:0 Palmitic	1.13 ± 0.07	1.22 ± 0.07	3.93 ± 0.03	1.14 ± 0.11	1.23 ± 0.12	3.86 ± 0.04
C16:1 Palmitoleic	0.0583 ± 0.0032	0.0630 ± 0.0036	0.2027 ± 0.0012	0.0565 ± 0.0047	0.0605 ± 0.0051	0.1907 ± 0.0042
C17:0 Heptadecanoic	0.0132 ± 0.0010	0.0143 ± 0.0012	0.0458 ± 0.0018	0.0138 ± 0.0017	0.0148 ± 0.0018	0.0463 ± 0.0017
C17:1 Heptadecenoic	0.0329 ± 0.0116	0.0355 ± 0.0127	0.1129 ± 0.0366	0.0325 ± 0.0102	0.0347 ± 0.0110	0.1078 ± 0.0263
C18:0 Stearic	0.599 ± 0.035	0.646 ± 0.040	2.07 ± 0.02	0.554 ± 0.059	0.592 ± 0.064	1.85 ± 0.04
C18:1 Oleic	18.4 ± 0.9	19.8 ± 1.0	63.5 ± 0.2	18.7 ± 1.7	20.0 ± 1.8	62.8 ± 0.1
C18:2 Linoleic	4.97 ± 0.31	5.36 ± 0.34	17.17 ± 0.23	5.11 ± 0.51	5.47 ± 0.56	17.13 ± 0.21
C18:3 Linolenic	2.88 ± 0.15	3.11 ± 0.17	9.95 ± 0.01	3.26 ± 0.27	3.48 ± 0.30	10.93 ± 0.06
C20:0 Arachidic	0.200 ± 0.009	0.216 ± 0.011	0.688 ± 0.013	0.189 ± 0.016	0.202 ± 0.018	0.631 ± 0.004
C20:1 Eicosenoic	0.431 ± 0.020	0.464 ± 0.023	1.48 ± 0.01	0.474 ± 0.037	0.507 ± 0.041	1.58 ± 0.02
C20:2 Eicosadienoic	0.0225 ± 0.0024	0.0243 ± 0.0027	0.0773 ± 0.0046	0.0248 ± 0.0032	0.0266 ± 0.0035	0.0829 ± 0.0036
C22:0 Behenic	0.118 ± 0.007	0.127 ± 0.007	0.403 ± 0.002	0.113 ± 0.011	0.121 ± 0.012	0.377 ± 0.007
C22:1 Erucic	< 0.0207	<LOQ	<LOQ	0.0217 ± 0.0104	0.0232 ± 0.0111	0.0740 ± 0.0404
C22:5 N3 Docosapentaenoic	0.0160 ± 0.0020	0.0173 ± 0.0022	0.0549 ± 0.0047	0.0169 ± 0.0028	0.0181 ± 0.0030	0.0560 ± 0.0046
C22:6 Docosahexaenoic	0.0219 ± 0.0334	0.0235 ± 0.0359	0.0778 ± 0.1196	0.0211 ± 0.0320	0.0225 ± 0.0341	0.0772 ± 0.1194
C24:0 Lignoceric	0.0563 ± 0.0046	0.0608 ± 0.0052	0.192 ± 0.006	0.0514 ± 0.0077	0.0550 ± 0.0084	0.170 ± 0.013

Table 57. Fatty Acids in Crude Oil

Bayer Sample ID	15-RSLJS026-009B		15-RSLJS026-010B	
Description	Non-GM Conventional Counterpart		MS11 *B. napus* Treated	
	% FW	% Total Fatty Acids	% FW	% Total Fatty Acids
	Mean ± SD			
C16:0 Palmitic	2.67 ± 0.09	4.71 ± 0.04	3.19 ± 0.13	3.90 ± 0.01
C16:1 Palmitoleic	0.121 ± 0.004	0.214 ± 0.004	0.151 ± 0.004	0.1850 ± 0.0036
C17:1 Heptadecenoic	0.0881 ± 0.0014	0.155 ± 0.004	0.105 ± 0.006	0.128 ± 0.002
C18:0 Stearic	1.40 ± 0.05	2.45 ± 0.04	1.58 ± 0.08	1.93 ± 0.02
C18:1 Oleic	35.1 ± 1.7	61.5 ± 0.3	51.5 ± 2.0	62.7 ± 0.1
C18:2 Linoleic	10.5 ± 0.4	18.5 ± 0.2	14.03 ± 0.61	17.1 ± 0.1
C18:3 Linolenic	5.27 ± 0.21	9.25 ± 0.06	9.05 ± 0.35	11.00 ± 0.00
C20:0 Arachidic	0.384 ± 0.021	0.671 ± 0.010	0.525 ± 0.032	0.637 ± 0.013
C20:1 Eicosenoic	0.709 ± 0.035	1.24 ± 0.02	1.33 ± 0.06	1.61 ± 0.01
C20:2 Eicosadienoic	0.0868 ± 0.0016	0.152 ± 0.009	0.0783 ± 0.0052	0.0949 ± 0.0025
C22:0 Behenic	0.283 ± 0.005	0.492 ± 0.016	0.351 ± 0.018	0.424 ± 0.005
C22:6 Docosahexaenoic	0.145 ± 0.006	0.253 ± 0.007	< 0.0548	<LOQ
C24:0 Lignoceric	0.233 ± 0.007	0.405 ± 0.019	0.225 ± 0.008	0.271 ± 0.003

Table 58. Fatty Acids in RBD Oil

Bayer Sample ID	15-RSLJS026-011B		15-RSLJS026-012B	
Description	Non-GM Conventional Counterpart		MS11 *B. napus* Treated	
	% FW	% Total Fatty Acids	% FW	% Total Fatty Acids
	Mean ± SD			
C16:0 Palmitic	2.83 ± 0.09	4.69 ± 0.08	3.40 ± 0.09	3.84 ± 0.01
C16:1 Palmitoleic	0.127 ± 0.002	0.210 ± 0.003	0.155 ± 0.009	0.175 ± 0.005
C17:0 Heptadecanoic	0.0367 ± 0.0165	0.0610 ± 0.0291	< 0.0543	<LOQ
C17:1 Heptadecenoic	0.0836 ± 0.0014	0.138 ± 0.002	0.098 ± 0.004	0.111 ± 0.003
C18:0 Stearic	1.50 ± 0.05	2.47 ± 0.02	1.73 ± 0.04	1.95 ± 0.01
C18:1 Oleic	37.8 ± 1.0	62.3 ± 0.4	56.4 ± 1.6	63.4 ± 0.1
C18:2 Linoleic	11.1 ± 0.3	18.3 ± 0.3	15.17 ± 0.42	17.1 ± 0.1
C18:3 Linolenic	5.17 ± 0.13	8.54 ± 0.09	9.25 ± 0.29	10.43 ± 0.06
C20:0 Arachidic	0.423 ± 0.025	0.694 ± 0.029	0.57 ± 0.01	0.640 ± 0.006
C20:1 Eicosenoic	0.773 ± 0.026	1.27 ± 0.02	1.50 ± 0.04	1.68 ± 0.01
C20:2 Eicosadienoic	0.0900 ± 0.0028	0.148 ± 0.006	0.0830 ± 0.0024	0.0929 ± 0.0021
C22:0 Behenic	0.308 ± 0.003	0.504 ± 0.007	0.379 ± 0.008	0.423 ± 0.004
C22:5 N6 Docosapentaenoic	< 0.0548	<LOQ	0.039 ± 0.021	0.0441 ± 0.0227
C22:6 Docosahexaenoic	0.154 ± 0.011	0.252 ± 0.014	< 0.0548	<LOQ
C24:0 Lignoceric	0.253 ± 0.007	0.412 ± 0.004	0.224 ± 0.017	0.250 ± 0.024

Table 59. Minerals in Grain (mg/kg)

Bayer Sample ID	15-RSLJS026-001B		15-RSLJS026-002B	
Description	Non-GM Conventional Counterpart		MS11 *B. napus* Treated	
	FW	DW	FW	DW
	Mean ± SD			
Calcium	3,679 ± 134	3,969 ± 155	3,255 ± 117	3,480 ± 124
Copper	2.70 ± 0.04	2.92 ± 0.06	2.78 ± 0.10	2.98 ± 0.11
Iron	68.5 ± 1.8	73.8 ± 2.1	76.3 ± 2.2	81.6 ± 2.5
Magnesium	3,610 ± 79	3,895 ± 92	3,747 ± 53	4,006 ± 54
Manganese	41.9 ± 0.7	45.3 ± 0.8	34.1 ± 0.6	36.4 ± 0.7
Phosphorus	6,728 ± 193	7,259 ± 215	7,317 ± 189	7,822 ± 187
Potassium	6,721 ± 122	7,251 ± 143	6,906 ± 88	7,384 ± 98
Sodium	15.7 ± 1.7	16.9 ± 1.7	13.3 ± 3.6	14.2 ± 3.9
Zinc	51.5 ± 1.1	55.5 ± 1.2	54.3 ± 2.2	58.0 ± 2.5

Table 60. Minerals in Press Cake (mg/kg)

Bayer Sample ID	15-RSLJS026-003B		15-RSLJS026-004B	
Description	Non-GM Conventional Counterpart		MS11 *B. napus* Treated	
	FW	DW	FW	DW
	Mean ± SD			
Calcium	4,896 ± 176	5,541 ± 201	4,313 ± 26	4,860 ± 43
Copper	3.88 ± 0.02	4.40 ± 0.01	3.88 ± 0.04	4.37 ± 0.04
Iron	87.6 ± 0.4	99.2 ± 0.5	94.7 ± 0.4	107 ± 1
Magnesium	4,682 ± 84	5,298 ± 102	4,612 ± 11	5,196 ± 25
Manganese	53.0 ± 0.4	60.0 ± 0.5	42.8 ± 0.1	48.3 ± 0.2
Phosphorus	8,695 ± 185	9,840 ± 216	8,955 ± 192	10,089 ± 184
Potassium	8,809 ± 201	9,969 ± 241	8,562 ± 62	9,648 ± 91
Sodium	20.5 ± 0.7	23.3 ± 0.9	15.2 ± 0.4	17.2 ± 0.5
Zinc	64.6 ± 2.3	73.1 ± 2.6	65.2 ± 0.5	73.4 ± 0.5

Table 61. Minerals in Solvent Extracted Meal (mg/kg)

Bayer Sample ID	15-RSLJS026-005B		15-RSLJS026-006B	
Description	Non-GM Conventional Counterpart		MS11 *B. napus* Treated	
	FW	DW	FW	DW
	Mean ± SD			
Calcium	5,761 ± 95	6,704 ± 120	4,995 ± 71	5,761 ± 73
Copper	4.66 ± 0.49	5.43 ± 0.59	4.90 ± 0.05	5.65 ± 0.05
Iron	127 ± 6	148 ± 7	121 ± 2	139 ± 2
Magnesium	5,546 ± 102	6,454 ± 127	5,672 ± 49	6,543 ± 47
Manganese	66.1 ± 4.6	76.9 ± 5.5	53.0 ± 0.8	61.1 ± 0.9
Phosphorus	10,210 ± 274	11,882 ± 324	10,881 ± 214	12,551 ± 228
Potassium	10,509 ± 124	12,230 ± 161	10,557 ± 51	12,178 ± 55
Sodium	34.6 ± 11.8	40.3 ± 13.8	18.9 ± 0.4	21.8 ± 0.5
Zinc	83.1 ± 3.6	96.6 ± 4.1	79.3 ± 0.6	91.5 ± 0.9

Table 62. Minerals in Toasted Meal (mg/kg)

Bayer Sample ID	15-RSLJS026-007B		15-RSLJS026-008B	
Description	Non-GM Conventional Counterpart		MS11 *B. napus* Treated	
	FW	DW	FW	DW
	Mean ± SD			
Calcium	6,123 ± 227	6,802 ± 242	5,524 ± 221	6,112 ± 250
Copper	5.35 ± 0.11	5.94 ± 0.13	5.54 ± 0.17	6.13 ± 0.20
Iron	126 ± 3	141 ± 3	136 ± 6	151 ± 6
Magnesium	5,708 ± 161	6,341 ± 157	6,002 ± 154	6,640 ± 180
Manganese	66.3 ± 0.8	73.7 ± 1.1	56.9 ± 1.4	62.9 ± 1.6
Phosphorus	10,579 ± 294	11,753 ± 265	11,453 ± 330	12,671 ± 388
Potassium	10,776 ± 265	11,971 ± 263	11,293 ± 295	12,494 ± 342
Sodium	35.5 ± 10.2	39.5 ± 11.6	23.4 ± 3.3	25.9 ± 3.7
Zinc	79.8 ± 2.2	88.6 ± 2.9	86.0 ± 2.2	95.2 ± 2.7

Table 63. Vitamins in Grain (mg/kg)

Bayer Sample ID	15-RSLJS026-001B		15-RSLJS026-002B	
Description	Non-GM Conventional Counterpart		MS11 *B. napus* Treated	
	FW	DW	FW	DW
Tocopherols				
Alpha Tocopherol (Vitamin E)	80.1 ± 4.3	86.4 ± 4.3	75.2 ± 1.2	80.4 ± 1.0
Delta Tocopherol	3.5 ± 0.4	3.8 ± 0.4	2.6 ± 0.0	2.8 ± 0.1
Gamma Tocopherol	164 ± 10	176 ± 10	146 ± 3	155 ± 3
Total Tocopherols	247 ± 15	266 ± 15	223 ± 4	238 ± 4
Vitamin K1	0.712 ± 0.082	0.767 ± 0.086	0.805 ± 0.043	0.861 ± 0.048

Table 64. Vitamins in Crude Oil (mg/kg)

Bayer Sample ID	15-RSLJS026-009B	15-RSLJS026-010B
Description	Non-GM Conventional Counterpart	MS11 *B. napus* Treated
	FW	
	Mean ± SD	
Tocopherols		
Alpha Tocopherol (Vitamin E)	208 ± 4	202 ± 5
Delta Tocopherol	10.7 ± 1.6	8.2 ± 1.0
Gamma Tocopherol	453 ± 7	426 ± 13
Total Tocopherols	672 ± 12	636 ± 19
Vitamin K1	2.08 ± 0.08	1.92 ± 0.02

Table 65. Vitamins in RBD Oil (mg/kg)

Bayer Sample ID	15-RSLJS026-011B	15-RSLJS026-012B
Description	Non-GM Conventional Counterpart	MS11 *B. napus* Treated
	FW	
	Mean ± SD	
Tocopherols		
Alpha Tocopherol (Vitamin E)	191 ± 6	183 ± 4
Delta Tocopherol	8.0 ± 0.5	6.6 ± 0.8
Gamma Tocopherol	375 ± 10	366 ± 8
Total Tocopherols	575 ± 15	556 ± 13
Vitamin K1	1.59 ± 0.04	1.65 ± 0.12

Table 66. Anti-Nutrients in Grain

Bayer Sample ID	15-RSLJS026-001B		15-RSLJS026-002B	
Description	Non-GM Conventional Counterpart		MS11 *B. napus* Treated	
	FW	DW	FW	DW
	Mean ± SD			
Glucosinolates (µmol/g)				
4-hydroxyglucobrassicin	4.67 ± 0.92	5.04 ± 0.99	5.73 ± 0.64	6.12 ± 0.69
Glucobrassicin	0.630 ± 0.048	0.680 ± 0.054	0.946 ± 0.099	1.010 ± 0.104
Gluconapin	2.73 ± 0.17	2.94 ± 0.19	5.72 ± 0.13	6.12 ± 0.12
Gluconasturtiin	0.195 ± 0.031	0.211 ± 0.034	0.185 ± 0.015	0.198 ± 0.017
Neoglucobrassicin	0.039 ± 0.002	0.042 ± 0.002	0.051 ± 0.001	0.055 ± 0.001
Progoitrin	2.75 ± 0.10	2.97 ± 0.11	5.37 ± 0.08	5.74 ± 0.07
Total Glucosinolates	11.0 ± 1.0	11.9 ± 1.1	18.0 ± 0.8	19.4 ± 0.9
Phytic Acid (%)	1.51 ± 0.09	1.63 ± 0.10	1.56 ± 0.19	1.67 ± 0.21
Sinapine (%)	0.774 ± 0.028	0.835 ± 0.030	0.863 ± 0.036	0.923 ± 0.041
Tannins (%)				
Insoluble Condensed Tannins	0.133 ± 0.024	0.144 ± 0.026	0.133 ± 0.021	0.142 ± 0.023
Soluble Condensed Tannins	0.0930 ± 0.0043	0.100 ± 0.005	0.1089 ± 0.0140	0.116 ± 0.014

Table 67. Anti-Nutrients in Press Cake

Bayer Sample ID	15-RSLJS026-003B		15-RSLJS026-004B	
Description	Non-GM Conventional Counterpart		MS11 *B. napus* Treated	
	FW	DW	FW	DW
	Mean ± SD			
Glucosinolates (µmol/g)				
4-hydroxyglucobrassicin	4.16 ± 0.71	4.70 ± 0.80	5.10 ± 0.55	5.74 ± 0.61
Glucobrassicin	0.463 ± 0.089	0.524 ± 0.100	0.695 ± 0.042	0.783 ± 0.049
Gluconapin	1.60 ± 0.30	1.80 ± 0.34	2.99 ± 0.21	3.37 ± 0.24
Neoglucobrassicin	0.056 ± 0.004	0.063 ± 0.005	0.061 ± 0.004	0.068 ± 0.005
Progoitrin	1.66 ± 0.26	1.88 ± 0.30	2.55 ± 0.22	2.87 ± 0.25
Total Glucosinolates	7.9 ± 0.9	9.0 ± 1.0	11.4 ± 1.0	12.8 ± 1.1
Phytic Acid (%)	1.88 ± 0.14	2.12 ± 0.16	1.85 ± 0.18	2.08 ± 0.21
Sinapine (%)	0.960 ± 0.044	1.09 ± 0.05	0.985 ± 0.031	1.11 ± 0.03
Tannins (%)				
Insoluble Condensed Tannins	0.197 ± 0.018	0.223 ± 0.020	0.203 ± 0.035	0.229 ± 0.039
Soluble Condensed Tannins	0.117 ± 0.004	0.133 ± 0.005	0.125 ± 0.023	0.141 ± 0.027

Table 68. Anti-Nutrients in Solvent Extracted Meal

Bayer Sample ID	15-RSLJS026-005B		15-RSLJS026-006B	
Description	Non-GM Conventional Counterpart		MS11 *B. napus* Treated	
	FW	DW	FW	DW
	Mean ± SD			
Glucosinolates (µmol/g)				
4-hydroxyglucobrassicin	2.86 ± 0.24	3.33 ± 0.27	4.82 ± 0.92	5.56 ± 1.05
Glucobrassicanapin	<0.166	<LOQ	0.150 ± 0.059	0.173 ± 0.068
Glucobrassicin	0.274 ± 0.021	0.319 ± 0.024	0.626 ± 0.136	0.722 ± 0.155
Gluconapin	1.04 ± 0.17	1.20 ± 0.19	2.93 ± 0.58	3.38 ± 0.67
Neoglucobrassicin	0.0343 ± 0.0167	0.0399 ± 0.0193	0.070 ± 0.007	0.080 ± 0.008
Progoitrin	1.04 ± 0.18	1.21 ± 0.21	2.19 ± 0.31	2.53 ± 0.36
Total Glucosinolates	5.2 ± 0.6	6.1 ± 0.7	10.8 ± 1.6	12.5 ± 1.7
Phytic Acid (%)	2.84 ± 0.16	3.30 ± 0.17	3.07 ± 0.13	3.54 ± 0.15
Sinapine (%)	1.24 ± 0.07	1.45 ± 0.08	1.29 ± 0.09	1.48 ± 0.11
Tannins (%)				
Insoluble Condensed Tannins	0.178 ± 0.041	0.207 ± 0.048	0.225 ± 0.017	0.259 ± 0.020
Soluble Condensed Tannins	0.178 ± 0.002	0.207 ± 0.002	0.213 ± 0.004	0.246 ± 0.004

Table 69. Anti-Nutrients in Toasted Meal

Bayer Sample ID	15-RSLJS026-007B		15-RSLJS026-008B	
Description	Non-GM Conventional Counterpart		MS11 *B. napus* Treated	
	FW	DW	FW	DW
	Mean ± SD			
Glucosinolates (µmol/g)				
4-hydroxyglucobrassicin	1.47 ± 0.24	1.63 ± 0.27	1.57 ± 0.11	1.73 ± 0.12
Glucobrassicin	0.219 ± 0.033	0.243 ± 0.036	0.313 ± 0.040	0.346 ± 0.044
Gluconapin	0.95 ± 0.11	1.06 ± 0.12	1.78 ± 0.20	1.97 ± 0.22
Neoglucobrassicin	0.0339 ± 0.0160	0.0377 ± 0.0176	0.0465 ± 0.0189	0.0514 ± 0.0209
Progoitrin	1.01 ± 0.21	1.12 ± 0.23	1.43 ± 0.13	1.59 ± 0.14
Total Glucosinolates	3.7 ± 0.6	4.1 ± 0.7	5.1 ± 0.5	5.7 ± 0.5
Phytic Acid (%)	2.97 ± 0.09	3.30 ± 0.11	3.14 ± 0.08	3.47 ± 0.09
Sinapine (%)	1.20 ± 0.01	1.33 ± 0.02	1.28 ± 0.04	1.42 ± 0.05
Tannins (%)				
Insoluble Condensed Tannins	0.260 ± 0.056	0.290 ± 0.065	0.291 ± 0.096	0.322 ± 0.107
Soluble Condensed Tannins	0.141 ± 0.007	0.156 ± 0.009	0.171 ± 0.004	0.190 ± 0.004

Conclusion

Based on the composition analysis, nutrient and anti-nutrient levels were similar between the non-GM conventional counterpart and the MS11 *B. napus* grain and processed fraction samples.

(b) Information on the range of natural variation for each constituent measured to allow for assessment of biological significance should any statistically significant differences be identified

The OECD "Revised Consensus Document on Compositional Considerations for New Varities of Low Erucic Acid Rapeseed (Canola): Key Food and Feed Nutrients, Anti-nutrients and Toxicants" (M-223000-02) provides the required information on natural variation for each constituent measured within the compositional studies to allow assessment of biological significance should any statistically significant differences be identified in the above studies by FSANZ.

(c) The levels of any other constituents that may potentially be influenced by the genetic modification, as a result, for example, of downstream metabolic effects, compared with the levels in an appropriate comparator as well as the range of natural variation.

Other than the intended presence of the PAT/*bar*, Barnase and Barstar proteins in *B. napus* containing MS11 *B. napus*, food products derived from MS11 *B. napus* have been shown to be compositionally and nutritionally similar to products derived from commercial varieties of non-transgenic *B. napus* (see Section B.5(a) directly above).

(d) The levels of any naturally occurring allergenic proteins in the GM food compared with the levels in an appropriate comparator. Particular attention must be paid to those foods that are required to be declared when present as an ingredient, and where significant alterations to protein content could be reasonably anticipated.

B. napus is not considered to produce allergenic proteins.

Part C Information Related to the Nutritional Impact of the Food Produced Using Gene Technology

The application must contain the following information if the compositional analysis indicates biologically significant changes to the levels of certain nutrients in the food produced using gene technology compared to the non-GM counterpart food:

(a) Data are required on the anticipated dietary intake of the GM food in relation to the overall diet, together with any information which may indicate a change to the bioavailability of the nutrients from the GM food

Based on the composition analysis, where nutrient and anti-nutrient levels were found to be similar between the non-GM conventional counterpart and the MS11 *B. napus* grain and processed fraction samples, no analysis of dietary intake in relation to the overall diet is required as bioavailability of the nutrients from MS11 *B. napus* derived foods is expected to be similar to bioavailability of nutrients from non-GM *B. napus* derived foods.

(b) Where the GM food contains an intended nutritional change, information, such as clinical trial data, must be provided to determine the nutritional impact of the GM food.

Not applicable.

Part D Other Information

There is no requirement to conduct animal feeding or whole food toxicity studies on the food produced using gene technology. However, if a 90-day (or longer) whole food toxicity study in rodents has been provided to satisfy the data and information requirements of another jurisdiction, this should also be provided to FSANZ as additional supporting information.

A 90-day whole food toxicity study has been conducted and is presented in this submission ([redacted], Document no. M-569171-01-1, Node D).
The objective of this study was to determine the potential toxic effects of toasted MS11 *Brassica napus* (*B. napus*) meal incorporated at 15% (w/w) in the diet when administered continuously for at least 90 days to Sprague Dawley rats.
Toasted MS11 *B. napus* meal was compared to its conventional counterpart. In addition, another toasted meal from one non-Genetically Modified (GM) commercial reference variety was included in the study.
The study included three groups of Crl:CD(SD) rats with each group consisting of 16 males and 16 females (approximately 51 days old at study diet administration), all pair-housed by sex and assigned to treatment using a randomized complete block design. The test substance-treated group (Test) was offered a diet formulated to contain 15% GM *B. napus*. A concurrent control group (Control) was offered a control diet containing a conventional counterpart at 15%. In addition, a reference group (Reference) was offered a diet containing 15% non-GM *B. napus.* Diets were provided *ad libitum* to the rats for a minimum of 90 consecutive days.
All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily, and detailed physical examinations were performed weekly (± two days). Individual body weights were recorded weekly (± two days). Cage food weights were recorded once weekly (± two days) beginning following randomization. Functional observational battery (FOB) and motor activity data were recorded for all animals prior to the initiation of dose administration and near the end of treatment (study week 13). Ophthalmic examinations were performed during study weeks -2 and 12. Clinical pathology parameters (hematology, coagulation, serum chemistry, and urinalysis) were analyzed for all animals at the scheduled necropsy (study week 13). Complete necropsies were conducted on all animals, and selected organs were weighed at the scheduled necropsy. Selected tissues were examined microscopically from all animals.
There were no effects on survival, clinical observations, body weights, food consumption, functional observational battery, motor activity, ophthalmic examinations, hematology, coagulation, serum chemistry, urinalysis, macroscopic, organ weights or microscopic parameters that were attributed to test diet administration.
Based on the results of this study, dietary administration of MS11 *B. napus* meal for at least 90 consecutive days at a concentration of 15% in the diet had no adverse effects on the growth or health of Sprague Dawley rats.

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