



# Protocol for Testing for the Presence of Genetically Modified Plant Material

31 October 2017

## **TITLE**

Operational Code: Protocol for Testing for the Presence of Genetically Modified Plant Material

## **COMMENCEMENT**

This Operational Code is effective from the date of issue.

## **REVOCATION**

This Operational Code replaces the *Protocol for Testing for the Presence of Genetically Modified Plant Material*, dated 9<sup>th</sup> June 2017.

## **ISSUING BODY**

This Operational Code is issued by the Ministry for Primary Industries.

Dated at Wellington this 31<sup>st</sup> day of October 2017.

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## Introduction

This introduction is not part of the Operational Code, but is intended to indicate its general effect.

## Purpose

The purpose of this Operational Code is to describe the approved method for sampling seed and nursery stock and the DNA testing method for the presence of genetically modified (GM) plant material for imported consignments of seeds for sowing and nursery stock for planting in New Zealand.

Adherence to the *Protocol for Testing for the Presence of Genetically Modified Plant Material* (the Protocol) reduces the likelihood that unapproved GM plants are imported and grown in the New Zealand environment.

## Background

It is illegal to import GM seeds for sowing and nursery stock into New Zealand without approval under the Hazardous Substances and New Organisms (HSNO) Act 1996.

GM crops and their seeds are defined as new organisms under HSNO. This Act deems unlawful the import, development, field-testing and release of any organism without approval from the Environmental Protection Authority (EPA). The HSNO Act is enforced at the New Zealand border under section 28 of the Biosecurity Act 1993 (hereafter referred to as the Act).

Seeds and plants imported for planting into New Zealand must meet the phytosanitary requirements detailed in the MPI Import Health Standard (IHS): 155.02.05: *Seeds for Sowing* – <http://www.mpi.govt.nz/document-vault/1151> and 155.02.06: *Importation of Nursery Stock* - <https://www.mpi.govt.nz/document-vault/1152>

Seed and nursery stock importers must take appropriate precautions to ensure consignments of seed for sowing and nursery stock do not contain unapproved GM plant material. Such precautions might include purchasing seed or plants which have been produced under a quality assurance system, such as field isolation and other production management practices, and testing to exclude GM presence through the production chain.

For seed, this Protocol ensures a 95% level of confidence that the inadvertent presence of 1 GM seed in 1000 seeds (0.1%) will be detected in the final sample tested. For nursery stock, this protocol ensures that the presence of a genetic modified organism can be accurately detected. An accurate test method minimizes the likelihood of unapproved GM plants being released into the environment.

## Who should read this Operational Code?

The Protocol applies to New Zealand importers of seed for sowing of the following crops: maize, sweetcorn, soybean, oilseed rape, lucerne/alfalfa, squash, cotton, petunia and flaxseed/linseed. It also applies to importers of specific species listed in the nursery stock standard.

## Why is this important?

Importers must take all reasonable steps to ensure that the goods comply with the HSNO Act and the requirements of the IHS (section 16B of the Act).

Non-compliance with the IHS may result in clearance not being given and the goods may be reshipped or destroyed.

## **Document History**

Refer to Appendix 1 for the amendment record for this Protocol.

## **Other information**

The information contained within a border throughout this document is for guidance and is not part of the requirements.

## Part 1: Requirements

### 1.1 Application

- (1) The Protocol applies to the following seeds imported under IHS 155.02.05: *Seeds for Sowing*:
  - a) Maize - *Zea mays* var. *indentata*
  - b) Sweetcorn - *Zea mays* var. *saccharata*
  - c) Soybean - *Glycine max*
  - d) Oilseed rape – *Brassica napus* var. *oleifera*
  - e) Lucerne/alfafa – *Medicago sativa*
  - f) Squash - *Cucurbita pepo*
  - g) Cotton - *Gossypium hirsutum*
  - h) Flaxseed/linseed – *Linum usitatissimum*
  - i) Petunia – *Petunia* spp.
- (2) The Protocol applies to the following nursery stock material imported under IHS 155.02.06: *Importation of Nursery Stock*:
  - a) *Petunia* spp.

### 1.2 Incorporation by reference

- (1) The Protocol is incorporated by reference under MPI 155.02.05: *Seeds for sowing* and 155.02.06: *Importation of Nursery Stock* and is a mandatory requirement under these IHSs (section 28).

### 1.3 Definitions

- (1) **Composite sample** means a sample is formed by combining and mixing all the primary samples taken from the seed lot/line.
- (2) **Consignment** means goods listed on, or covered by, a phytosanitary certificate, a single bill of lading, airway bill, declaration, invoice or goods in a mail package.

Notes:

  - a) commercial unaccompanied consignments are consignments covered by an airway bill/bill of lading intended for resale or manufacture.
  - b) commercial accompanied consignments are single consignments, imported by one passenger, at one time intended for re-sale, as samples and/or for analysis.
  - c) private unaccompanied consignments are consignments covered by an airway bill/bill of lading, or items via the mail pathway, imported as personal property not intended for resale.
  - d) private accompanied consignments are consignments imported as personal property not intended for resale.
- (3) **Genetically Modified** Section 2 of the HSNO Act defines genetically modified organisms (GMOs) as any organism in which any of the genes or other genetic material:
  - a) have been modified by *in vitro* techniques, or
  - b) are inherited or otherwise derived from any other genes or genetic material that has been modified by *in vitro* techniques.

*In vitro* techniques refers to using test tubes, cell culture plate or other methods outside a living organism.
- (4) **Primary sample** means a portion taken from the seed lot/line during one single sampling action.

- (5) **Representative/submitted sample** means a sample that is to be submitted to the testing laboratory and may comprise either the whole of the composite sample or a subsample thereof.
- (6) **Sealed** means that a container in which seed is held is closed in such a way, that it cannot be opened to gain access to the seed and closed again, without either destroying the seal or leaving evidence of tampering. This definition refers to the sealing of seed lots, as well as of seed samples.
- (7) **Seed lot/line** means a specified quantity of seed that is physically and uniquely identifiable and nominated on an import permit (s) and/or phytosanitary certificate and GM certificate.
- (8) **Subsample** means a portion of a sample obtained by reducing a sample.
- (9) **Official seed sampler** means a person officially recognised and authorized by their local NPPO and/or ISTA and/or AOSA as competent to operate as per ISTA and/or AOSA seed sampling methodology.
- (10) **Permit to import** means an official document authorising importation of a commodity in accordance with specified phytosanitary requirements. This may be a requirement of an import health standard (IHS) pursuant to section 22(2) of the Biosecurity Act 1993, and it may also include certain conditions which must be met in order for the commodity to receive biosecurity clearance.
- (11) **Transitional facility** means a place approved or part of a port declared, in accordance with section 39 of the Biosecurity Act 1993 for the purpose of inspection, storage, treatment, quarantine, holding or destruction of uncleared goods.

## Part 2: General Requirements (Seeds)

- (1) All individual seed lots, as specified in Part 1.1: *Application*, must be tested for the presence of unapproved GM seeds.
- (2) Importers can either have seed consignments sampled and tested at the New Zealand border or offshore prior to importation.
- (3) A testing certificate for each seed lot in the consignment is required by MPI at the border;
  - a) all certificates must be presented to MPI if more than one certificate is produced for the same seed lot,
  - b) alternative options for importers of small volumes of seed for research, breeding and multiplication are provided in Part 2.2: *Seed sampling - small lots*,
  - c) testing must be done by an MPI-approved laboratory.
- (4) Seed lots in a consignment will only be given biosecurity clearance if no unapproved GM seeds are detected (testing result is negative) and all requirements of the IHS have been met.
- (5) All seed sampling and testing costs are the responsibility of the importer, as per the Biosecurity (costs) Regulations 2015.
- (6) Seeds must be sampled as per:
  - a) Part 2.1: *Seed sampling - large lots*; or
  - b) Part 2.2: *Seed sampling - small lots*.
- (7) The sample must be tested using a DNA-based technique (end-point or real-time PCR). The required DNA tests are described in Part 2.3: *Seed Testing Methods*.

### Guidance

- A list of [MPI - approved and recognised laboratories](#) can be found on the MPI website.

## 2.1 Seed sampling – large lots

- (1) The sampling procedure is designed to collect a representative sample based on the following assumptions:
  - a) individual seeds are either GM or not GM:
    - i) if seeds are present which are heterozygous for the GM trait (for example, due to cross-pollination) the confidence of detection might be reduced.
  - b) any GM seeds present are randomly dispersed throughout the seed lot/line;
  - c) the sample will be ground and analysed as a whole and not individually;
  - d) laboratory testing will accurately identify the presence of 1 or more GM seeds in a 3200 seed sample.
- (2) A representative sample must contain a minimum of 3200 seeds drawn from each seed lot. To obtain a seed sample of at least 3200 seeds by weight;
  - a) count and weigh 100 seeds,
  - b) multiply this weight by 32, and
  - c) round up to the nearest 50 grams; or in the case of small seeded species such as *Brassica* and *Medicago sativa*, round to the nearest 5g.
- (3) Sub-samples are combined to form one uniform bulk sample, and then divided to obtain a final representative sample containing no fewer than 3200 seeds.
- (4) The sampling method used for each seed lot (for example, automatic in-line machine) must be stated on the GM testing certificate.



- (5) Sampling must be drawn by an automatic in-line machine or by an official seed sampler following the International Seed Testing Association (ISTA) or Association of Official Seed Analysts (AOSA) methodology.
- (6) The ISTA sampling methodology for medium-sized and bulk-sized containers is summarised in the following tables:

<b>Sacks: Containers up to and including 100 kg capacity</b>						
No. containers per seed lot	1-4	5-8	9-15	16-30	31-59	> 60
No. sub-samples to be taken	3 primary samples per container	2 primary samples per container	1 primary sample per container	15 primary samples – one each from 15 different containers	20 primary samples – one each from 20 different containers	30 primary samples – one each from 30 different containers

<b>Bulk bins: Containers greater than 100 kg capacity</b>				
Weight of seed lot (kg)	100- 500	501- 3,000	3001- 20,000	> 20,000
No. sub-samples to be taken	5	1 per 300 kg, not fewer than 5	1 per 500 kg, not fewer than 10	1 per 700 kg, not fewer than 40

- (7) Seed samples collected prior to shipping to New Zealand must comply with the ISTA and/or AOSA seed sampling methodology.

<b>Guidance</b>
<ul style="list-style-type: none"> <li>• Samples taken at the border on arrival in New Zealand will be collected under controlled conditions by MPI staff, or organisations approved by MPI.</li> <li>• The sample will be held under MPI supervision until it can be sent to the MPI-approved testing facility of the importer's choice. The rest of the consignment will be held in a MPI-approved transitional facility until a negative result is received and biosecurity clearance is given.</li> <li>• If a positive result is found, positive lots will not be given clearance, but either destroyed or re-shipped back to its destination.</li> <li>• Records of sampling and testing done at the border will be kept by MPI. This information is subject to the Official Information Act 1982.</li> </ul>

## 2.2 Seed sampling – small lots

- (1) Importers of small quantities of seed for sowing for research, education, cultivar trials and/or multiplication for re-export may not be able to meet the 3200 seed sampling requirements of the Protocol.
- (2) MPI defines small quantities as follows:
- for large seeded species (e.g. *Zea mays*, *Glycine max*, *Cucurbita pepo*, *Petunia (if pelleted)* and *Gossypium*), less than 5 kg total volume per seed lot/line;
  - for small seeded species (e.g. *Brassica napus*, *Petunia (not pelleted)*, *Linum* and *Medicago sativa*), less than 100g total volume per seed lot/line.
- (3) Importers of small volumes can apply for three alternative options:
- proportionate sampling;
  - leaf disc sampling; and
  - exporter/ importer non-GM assurance declaration.

- (4) A permit to import is required for options b) leaf disc sampling, and c) exporter/ importer non-GM assurance declaration.
- (5) If any of the lots which make up the consignment weigh 5kg or more (large seeded species) or 100g or more (small seeded species) they are not considered small volume and must comply with Part 2.1: Seed Sampling- large lots and Part 2.4: Test Certification.

**Guidance**

- An import permit application form can be found on the MPI website or by contacting MPI [plantimports@mpi.govt.nz](mailto:plantimports@mpi.govt.nz)

**2.2.1 Proportionate sampling**

- (1) This option may only be applied to seed lots from the same species.
- (2) A proportionate sub-sample of each lot from the same species, up to a maximum of 50 seed lots, drawn by an official seed sampler can be pooled together to make up a 3200 seed sample to obtain the final representative sample:
  - a) these sub-samples will be tested as one sample.
- (3) For clarity and border clearance purposes, GM testing certificates must contain:
  - a) the lot/line number or unique identifier of each individual seed lot/line that formed part of the representative sample.
- (4) If a positive result is found, none of the lots that formed part of the representative sample can be given clearance and they will be destroyed or re-shipped.

**Guidance**

- Guidance for proportionate sampling can be found in [Schedule 2](#) and [Schedule 3](#) of this Protocol.

**2.2.2 Leaf disc sampling**

- (1) Living material (such as leaf discs) can also be tested for GM DNA sequences.
- (2) Untested seed may be imported into New Zealand under an import permit, and grown in an appropriate Transitional Facility approved to [Facility Standard: Post Entry Quarantine for Plants \(MPI.STD.PEQ\)](#):
  - a) this may be Level 1, though there may be additional phytosanitary requirements that require the seed to be grown in a Level 2 facility.
- (3) During growth and before pollen is produced, leaf samples must be taken and sent for testing at a MPI-approved testing laboratory (under importer's choice);
  - a) the sampling and testing plan must be approved by MPI.
- (4) Biosecurity clearance will be given to all rows (or blocks) of plants testing negative for the presence of unapproved GM material.
- (5) If unapproved GM material is detected in a plant row, this row must be destroyed, and any remaining unplanted seed of this seed lot will not receive biosecurity clearance.

**2.2.3 Exporter/ importer non-GM assurance declaration**

- (1) Seed for sowing imported under this option is not required to be tested for GM presence but must be imported into New Zealand under an import permit, and grown in an appropriate Transitional Facility approved to [Facility Standard: Post Entry Quarantine for Plants \(MPI.STD.PEQ\)](#).

- a) The seed for sowing must be accompanied by a non-GM assurance declaration, signed by both the exporter and importer, stating that the seed was produced neither from nor by genetically modified crops. The declaration form is provided in Schedule 1 and must be completed.
  - b) The declaration form must be presented at the border with the consignment and other documentation required by this Protocol and the MPI 155.02.05: Seeds for sowing.
- (2) Plants grown from this seed will not be tested and will not receive biosecurity clearance.
  - (3) All harvested seed must be exported out of New Zealand and the remaining vegetative material, including any subsequent emerging volunteer plants, destroyed under supervision from MPI.

## Part 3: General Requirements (Nursery Stock)

- (1) All individual varieties, as specified in Part 1.1: Application, must be tested for the presence of unapproved GM material.
- (2) Importers must sample and test their nursery stock varieties offshore prior to importation.
- (3) All sampling and testing costs are the responsibility of the importer, as per the Biosecurity (costs) Regulations 2015.
- (4) The sample must be tested using a DNA-based technique (end-point or real-time PCR). The required DNA tests are described in Part 4: Testing Methods.
- (5) A testing certificate for each variety in the consignment is required by MPI at the border;
  - a) all certificates must be presented to MPI if more than one certificate is produced for the same variety,
  - b) testing must be done by an MPI-approved or recognised laboratory.
- (6) A permit to import will only be issued for varieties which have been tested and certified as not containing unapproved GM plant material (testing result is negative)

### Guidance

- A list of [MPI - approved and recognised laboratories](#) can be found on the MPI website.

## 3.1 Sampling

### Guidance

- A sample must contain sufficient leaf material, either fresh or dried, for each variety.
- Leaf material shall be collected as leaf punches (at least 2 x 10 punches per sample) or as whole leaves (at least 10 per sample).
- If sending tissue culture plantlets, 5 per variety should produce enough and they should be at least 5 cm (2 inches) in height.
- Fresh material must be kept cool during transport to an MPI-approved or recognised laboratory

## Part 4: Testing Methods

- (1) Testing laboratories must have validated methods capable of detecting haploid GM DNA sequences present in the sample at the lowest reliable limit of detection:
  - a) this limit shall not exceed 0.03% at the 95% confidence level; and
  - b) validated methods must be made available to MPI auditors.
- (2) Both qualitative and quantitative PCR tests can be used to test for the presence of GM sequences;
  - a) qualitative PCR tests are used to screen for the presence of specific gene sequences, either commonly incorporated into GM plants (e.g. CaMV 35S promoter, NOS terminator and nptII) or event specific (e.g. DAS-40278-9):
    - i) qualitative PCR test is the recommended method of choice for the Protocol.
  - b) quantitative PCR tests that estimate the likely concentration of GM plant material present in the consignment may also be used, but are only acceptable if there is a negative result at the limit of detection which must be clearly reported on the testing certificate.
- (3) The GM screening tests required for specific species of seeds and nursery stock are shown in the following table.

Species	Type of Screen
Maize – <i>Zea mays</i> var. <i>indentata</i> AND Sweetcorn – <i>Zea mays</i> var. <i>sacharata</i>	CaMV 35S promoter; AND NOS terminator; AND DAS-40278-9 event specific.
Soybean – <i>Glycine max</i>	CaMV 35S promoter; AND FMV 34S promoter or EPSPS or equivalent assay (MON 89788 event specific); AND DP-3Ø5423-1 event specific or equivalent assay; AND MON 87701 event specific or equivalent assay.
Oilseed rape – <i>Brassica napus</i> var. <i>oleifera</i>	Bar gene or equivalent assay (Seedlink/Restorer); AND Pat (syn) gene or equivalent assay (Liberty Link T45/TOPAS19/2); AND RT73/GT73 event-specific or FMV-34S or EPSPS or equivalent assay (Roundup Ready); AND OXY235 event-specific or NOS terminator or equivalent assay; AND Laurical (thioesterase) gene or equivalent assay (nptII).
Lucerne/alfafa – <i>Medicago sativa</i>	FMV-34S promoter or EPSPS.
Squash – <i>Cucurbita pepo</i>	CaMV 35S promoter.
Cotton – <i>Gossypium hirsutum</i>	CaMV 35S promoter; AND NOS terminator; AND GHB614 event specific or EPSPS or an equivalent assay; AND 281-24-236 event specific or Pat (syn) gene or an equivalent assay (3006-210-23 event specific); AND MON88913 event specific.

Species	Type of Screen
Flaxseed – <i>Linum usitatissimum</i>	NOS terminator (construct specific).
Petunia – <i>Petunia</i> spp.(seed and nursery stock)	CaMV 35S promoter; AND NOS promoter AND NPTII gene;

## 4.1 Test certification

- (1) In the case of seeds the sampling method used for each seed lot (for example, automatic in-line machine) must be stated in the GM certificate.
- (2) GM certificates must contain the name of the species tested and the same lot/line number or unique identifier as stated on all other import documentation for consignments arriving in New Zealand.
- (3) When testing plant material, the exporter's name, the species and each variety tested must be specified on the testing certificate.

## Schedule 1: Exporter/importer non-GM assurance declaration

### To be completed and signed by the exporter and importer.

As defined by the New Zealand HSNO Act 1996, Genetically modified organism means, unless expressly provided otherwise by regulations, any organism in which any of the genes or any other genetic material (a) have been modified by in vitro techniques; or (b) are inherited or otherwise derived, through any number of replications, from any genes or other genetic material which has been modified by in vitro techniques.

Note that under the Hazardous Substances and New Organisms (HSNO) Act 1996. The import and release of any genetically modified crop without approval from the Environmental Protection Authority (EPA) it is unlawful.

I,..... (Exporter's name and address)...

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declare that according to the requirements set out in the Seed for Sowing Import Health Standard (MPI IHS: 155.02.05: *Seeds for Sowing* - <https://www.mpi.govt.nz/dmsdocument/1151-seeds-for-sowing-import-health-standard>

Insert species name and lot/line number or unique identifier as stated on all the other import documentation
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was produced neither "from" nor "by" genetically modified crops.

I undertake to inform immediately the importer and the Ministry for Primary Industries, MPI, New Zealand of any information that can undermine the accuracy of this declaration.

Note that MPI may request evidence as to how production, handling and transport of these seeds is performed in the field, or require and audit as a way to provide quality to the production system.

I,..... (Importer's name and address)...

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declare to the best of my knowledge that according to the requirements set out in the Seed for Sowing Import Health Standard (MPI IHS: 155.02.05: *Seeds for Sowing* - <https://www.mpi.govt.nz/dmsdocument/1151-seeds-for-sowing-import-health-standard>

Insert species name and lot/line number or unique identifier as stated on all the other import documentation
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was produced neither "from" nor "by" genetically modified crops.

Signed by <b>Exporter</b> and Company Name (details) and date	Signed by <b>Importer</b> and Company Name (details) and date
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Warning: Any person who knowingly makes a statement of information or a declaration that is false or misleading in a material particular may on summary conviction, be sentenced to a term of imprisonment and/or fined not exceeding \$500,000.00

## Schedule 2: Minimum consignment size for proportionate sampling

### Guidance

- For the proportionate sample regime to be cost-effective for small seed consignments a minimum total consignment size which is 3-4 times as large as the sample is required.
- This means that between 9,600 and 12,800 seeds in total is required within a consignment regardless of the number of lots/lines within the consignment



## Schedule 3: Stratified proportionate sampling and examples

Proportionate sampling is carried out across all lots imported to achieve a composite sample. A total sample of 3,200 seeds is achieved by ensuring a sample is taken from each imported lot within a consignment. The size of the sample from each lot must be proportionate to the size of the lot as part of the consignment.

The sample size from each lot should be calculated as follows:

- (1) Work out the proportion of each lot in the total consignment (weight or seed number) using the following equation:

$$\text{Proportion of total consignment size} = \frac{\text{Weight of each line (g)}}{\text{Total weight in consignment(g)}}$$

- (2) Calculate the sample size for each lot (weight or seed number) using a total composite sample size of 3,200 seeds or weight equivalent (1.1kg for *Zea mays*):

$$\begin{aligned} \text{Sample size of each line} \\ = \text{weight of total composite sample} \times \text{proportion of total consignment size} \end{aligned}$$

- (3) Take the sum of the sample size for each lot to check the total composite sample for the consignment is at least 3,200 seeds or rounded up to the nearest whole seed.

### Guidance

- Tables 1-4 illustrate the sample size required from each lot for a consignment of 10 or 50 lots for both *Zea mays* and *Medicago sativa*.
- Examples are given using the number of seeds and weight of sample as the unit of measurement for guidance only.

**Table 1:**

The proportionate sampling regime required to ensure a composite sample size of at least 3,200 seeds is taken. The consignment consists of 10 lots of *Zea mays* and has a total consignment weight of 11.72 kg. This weight equates to 34,090 seeds of *Zea mays*.

Sample sizes are rounded up to the nearest whole seed.

Line/Lot Number	No. of seeds in each lot	Weight of each lot / g	Percentage of total consignment size / %	Sample size from each lot / no. of seeds	Weight of sample from each lot / g
1	2910	1000	8.54	274	95
2	30	10	0.09	3	2
3	1450	500	4.25	136	47
4	5820	2000	17.07	547	189
5	1450	500	4.25	136	47
6	30	10	0.09	3	2
7	11640	4000	34.14	1093	376
8	580	200	1.7	55	19
9	8730	3000	25.61	820	282
10	1450	500	4.25	136	47

**Table 2:**

The proportionate sampling regime required to ensure a composite sample size of at least 3,200 seeds is taken. The consignment consists of 50 lots of *Zea mays* and has a total consignment weight of 60.50kg. This weight equates to 175,970 seeds of *Zea mays*.

Sample sizes are rounded up to the nearest whole seed.

Line/Lot Number	No. of seeds in each lot	Weight of each lot / g	Percentage of total consignment size /%	Sample size from each lot /no. of seeds	Weight of sample from each lot /g
1	2,910	1,000	1.65	53	19
2	30	10	0.02	1	1
3	1,450	500	0.82	27	10
4	5,820	2,000	3.31	106	37
5	1,450	500	0.82	27	10
6	30	10	0.02	1	1
7	11,640	4,000	6.61	212	73
8	580	200	0.33	11	4
9	8,730	3,000	4.96	159	55
10	1,450	500	0.82	27	10
11	1,450	500	0.82	27	10
12	1,450	500	0.82	27	10
13	8,730	3,000	4.96	159	55
14	2,910	1,000	1.65	53	19
15	7,270	2,500	4.13	133	46
16	7,270	2,500	4.13	133	46
17	30	10	0.02	1	1
18	1,450	500	0.82	27	10
19	1,450	500	0.82	27	10
20	1,160	400	0.66	22	8
21	1,020	350	0.58	19	7
22	290	100	0.16	6	3
23	870	300	0.49	16	6
24	13,090	4,500	7.44	239	83
25	580	200	0.33	11	4
26	2,910	1,000	1.65	53	19
27	11,640	4,000	6.61	212	73
28	8,730	3,000	4.96	159	55
29	1,450	500	0.82	27	10
30	1,450	500	0.82	27	10

Line/Lot Number	No. of seeds in each lot	Weight of each lot / g	Percentage of total consignment size /%	Sample size from each lot /no. of seeds	Weight of sample from each lot /g
31	2,620	900	1.49	48	17
32	1,750	600	0.99	32	11
33	290	100	0.16	6	3
34	2,330	800	1.32	43	15
35	30	10	0.02	1	1
36	2,620	900	1.49	48	17
37	2,910	1,000	1.65	53	19
38	5,820	2,000	3.31	106	37
39	1,450	500	0.82	27	10
40	1,450	500	0.82	27	10
41	290	100	0.16	6	3
42	8,730	3,000	4.96	159	55
43	30	10	0.02	1	1
44	8,730	3,000	4.96	159	55
45	11,640	4,000	6.61	212	73
46	1,450	500	0.82	27	10
47	8,730	3,000	4.96	159	55
48	1,450	500	0.82	27	10
49	2,910	1,000	1.65	53	19
50	1,450	500	0.82	27	10

**Table 3.**

The proportionate sampling regime required to ensure a composite sample size of at least 3,200 seeds is taken. The consignment consists of 10 lots of *Medicago sativa* and has a total consignment weight of 143 g. This weight equates to 65,000 seeds of *Medicago sativa*.

Due to the weight of *Medicago sativa* seeds, weights have been round to the nearest 2 decimal places.

Sample sizes are rounded up to the nearest whole seed.

Line/Lot Number	No. of seeds in each lot	Weight of each lot / g	Percentage of total consignment size /%	Sample size from each lot /no. of seeds	Weight of sample from each lot /g
1	22,860	50	35.17	1,126	2.47
2	9,140	20	14.06	450	0.99
3	30	0.07	0.05	2	0.01
4	6,860	15	10.56	338	0.74
5	18,290	40	28.14	901	1.98
6	4,570	10	7.03	225	0.5
7	460	1	0.71	23	0.06
8	30	0.07	0.05	2	0.01
9	460	1	0.71	23	0.06
10	2,290	5	3.52	113	0.25

**Table 4.**

The proportionate sampling regime required to ensure a composite sample size of at least 3,200 seeds is taken. The consignment consists of 50 lots of *Medicago sativa* and has a total consignment weight of 1.67 kg. This weight equates to 722,000 seeds of *Medicago sativa*.

Due to the weight of *Medicago sativa* seeds, weights have been round to the nearest 2 decimal places.

Sample sizes are rounded up to the nearest whole seed.

Line/Lot Number	No. of seeds in each lot	Weight of each lot / g	Percentage of total consignment size /%	Sample size from each lot /no. of seeds	Weight of sample from each lot /g
1	30	0.07	0.01	1	0.01
2	22,860	50	3.17	102	0.23
3	4,570	10	0.63	21	0.05
4	9,140	20	1.27	41	0.09
5	6,860	15	0.95	31	0.07
6	4,570	10	0.63	21	0.05
7	30	0.07	0	0	0
8	2,290	5	0.32	11	0.03
9	460	1	0.06	2	0.01
10	11,430	25	1.58	51	0.12
11	36,570	80	5.07	163	0.36
12	460	1	0.06	2	0.01
13	9,140	20	1.27	41	0.09
14	30	0.07	0	0	0
15	4,570	10	0.63	21	0.05
16	27,430	60	3.8	122	0.27
17	30	90	0	0	0
18	41,140	90	5.7	183	0.41
19	22,860	50	3.17	102	0.23
20	2,290	5	0.32	11	0.03
21	4,570	10	0.63	21	0.05
22	13,710	30	1.9	61	0.14
23	30	0.07	0	0	0
24	22,860	50	3.17	102	0.23
25	27,430	60	3.8	122	0.27
26	4,570	10	0.63	21	0.05
27	2,290	5	0.32	11	0.03
28	41,140	90	5.7	183	0.41

Line/Lot Number	No. of seeds in each lot	Weight of each lot / g	Percentage of total consignment size /%	Sample size from each lot /no. of seeds	Weight of sample from each lot /g
29	18,290	40	2.53	81	0.18
30	27,430	60	3.8	122	0.27
31	41,140	90	5.7	183	0.41
32	41,140	90	5.7	183	0.41
33	22,860	50	3.17	102	0.23
34	2,290	5	0.32	11	0.03
35	4,570	10	0.63	21	0.05
36	13,710	30	1.9	61	0.14
37	11,430	25	1.58	51	0.12
38	18,290	40	2.53	81	0.18
39	2,290	5	0.32	11	0.03
40	460	1	0.06	2	0.01
41	4,570	10	0.63	21	0.05
42	27,430	60	3.8	122	0.27
43	4,1140	90	5.7	183	0.41
44	4,1140	90	5.7	183	0.41
45	22,860	50	3.17	102	0.23
46	2,290	5	0.32	11	0.03
47	4,570	10	0.63	21	0.05
48	13,710	30	1.9	61	0.14
49	36,570	80	5.07	163	0.36
50	460	1	0.06	2	0.01

## Appendix 1: Amendment Record – Protocol for testing for the presence of Genetically Modified Plant Material

Amendments to this protocol will be given a consecutive number and will be dated.

Number	Details	Date
1	Protocol on genetically modified contamination in sweet corn seed imports	23 May 2001
2	Protocol for testing imports of <i>Zea mays</i> seed for sowing for the presence of genetically modified seed.	1 August 2002
3	Protocol for testing imports of <i>Glycine max</i> seed for sowing for the presence of genetically modified seed.	22 September 2002
4	Protocol for testing imports of <i>Brassica napus var. oleifera</i> seed for sowing for the presence of genetically modified seed.	1 October 2002
5	Protocol for testing imports of <i>Zea mays</i> seed for sowing for the presence of genetically modified seed.	1 June 2004
6	Protocol for testing imports of <i>Zea mays</i> seed for sowing for the presence of genetically modified seed.	21 March 2005
7	Protocol for testing imports of <i>Brassica napus var. oleifera</i> seed for sowing for the presence of genetically modified seed.	21 March 2005
8	Protocol for testing imports of <i>Glycine max</i> seed for sowing for the presence of genetically modified seed.	21 March 2005
9	Protocol for testing imports of <i>Brassica napus var. oleifera</i> seed for sowing for the presence of genetically modified seed.	1 March 2006
10	Protocol for testing imports of <i>Medicago sativa</i> seed for sowing for the presence of genetically modified seed.	1 August 2006
11	Protocol for testing imports of <i>Medicago sativa</i> seed for sowing for the presence of genetically modified seed.	30 November 2006
12	Protocol for testing seed imports for the presence of genetically modified seed (combined protocol).	1 March 2010
13	Protocol for testing seed imports for the presence of genetically modified seed (combined protocol).	27 July 2012
14	Protocol for testing for the presence of genetically modified plant material developed in new template (review and update).	12 November 2015
15	Protocol for testing for the presence of genetically modified plant material for Petunia seeds (review and update).	9 June 2017
16	Protocol for testing for the presence of genetically modified plant material for Petunia nursery stock (review and update).	31 October 2017