APPLICATION FOR CONSENT TO RELEASE A GMO – HIGHER PLANTS

PART A1: INFORMATION REQUIRED UNDER SCHEDULE 1 OF THE GENETICALY MODIFIED ORGANISMS (DELIBERATE RELEASE) REGULATIONS 2002

PART 1

General information

1. The name and address of the applicant and the name, qualifications and experience of the scientist and of every other person who will be responsible for planning and carrying out the release of the organisms and for the supervision, monitoring and safety of the release.

Applicant:

The Sainsbury Laboratory

John Innes Centre

Colney Lane

Norwich NR4 7UH

2. The title of the project.

Improving late blight (*Phytophthora infestans*) resistance in potato using resistance genes from South American potato relatives.

PART II

Information relating to the parental or recipient plant

3. The full name of the plant -

(a) family name, Solanaceae

(b) genus, *Solanum*

(c) species, *tuberosum*

(d) subspecies, *tuberosum*

(e) cultivar/breeding line, Desiree

(f) common name. Potato

4. Information concerning -

(a) the reproduction of the plant:

(i) the mode or modes of reproduction,
For agricultural purposes, vegetative reproduction via tubers is the primary mode of reproduction. Sexual reproduction resulting in seed production is also possible. Selfing is more likely than cross-pollination; estimates of the rates of cross-pollination under field conditions range from 0 to about 20% (Plaisted, 1980). Other studies having shown that the cross-pollination rates are 2% at a distance of 3 metres from the crop, reducing to 0.017% at a distance of 10 metres (McPartlan and Dale, 1994).

(ii) any specific factors affecting reproduction,
Tubers are frost-sensitive and are rendered non-viable if exposed to temperatures of -3 °C or lower. During the winter period, wet soils also reduce tuber viability.

(iii) generation time; and
Tuber to tuber or seed to tuber generation time is one year or growing season under European conditions.

(b) the sexual compatibility of the plant with other cultivated or wild plant species, including the distribution in Europe of the compatible species.
Solanum tuberosum cv. Desiree is sexually compatible with other cultivated genotype of the same species. It is not sexually compatible with other UK crops or with either of the only two known wild *Solanum* species that grow in the UK, *Solanum dulcamara* (woody nightshade) and *Solanum nigrum* (black nightshade) (Eijlander and Stiekema, 1994; Raybould and Gray, 1993; McPartlan and Dale, 1994).

5. Information concerning the survivability of the plant:

(a) its ability to form structures for survival or dormancy,
Potatoes can survive as tubers or seed.

(b) any specific factors affecting survivability.

Potato tubers are sensitive to frosts and generally cannot survive temperatures of -3 °C and below. Generally, temperatures below zero impact on survivability with tubers being rendered non-viable after 2 hours of exposure to temperatures of -1.9 °C (Boydston et al, 2006). Tubers rarely survive winters in European soils due to the cool, wet conditions and the use of agricultural practices such as ploughing and the application of herbicides to clear land following potatoes. Potatoes are more often than not rotated and crops grown on land previously sown to potatoes often out-compete any surviving ground keepers. Tubers that are not harvested (ground keepers) and survive periods of ground frost may persist and produce plants in subsequent growing seasons. Careful management of the site will minimise such occurrences. Any volunteer plants that do form will be removed to prevent further survival through tuber production.

Although potato seed can survive winter temperatures, berries do not typically mature under UK field conditions and thus seed is rare. The heterozygous tetraploid genetic nature of cultivated potatoes means that seeds arising from sexual reproduction are often weak, have much lower agronomic performance than the parent plants and suffer competitively. Although potato seeds may survive in the soil for up to 8 years (Bock et al, 2002), plants arising from any seed that does germinate in the ground are unlikely to survive the winter conditions in the UK. In any case, any such volunteers that grow on the trial site will be identified and destroyed.

6. Information concerning the dissemination of the plant:

(a) the means and extent (such as an estimation of how viable pollen and/or seeds decline with distance where applicable) of dissemination; and

Potato can be spread as tubers, botanical seeds and pollen. Dissemination of tubers and botanical seed is normally limited to the area of cultivation. Dissemination of tubers and botanical seed is mainly caused by man while carrying out transports, handling and cultural practices. Animals, especially large birds, may also cause a limited amount of dissemination. Such dissemination of botanical seed, however, is practically excluded, as the seeds are contained in very poisonous berries.

Dissemination of pollen is facilitated almost exclusively by insects. Pollen is produced in low quantities and can be disseminated either by wind or insects. However, dissemination by wind is considered to be very limited (Eastham & Sweet, 2002). Dissemination usually less than 10 metres so the transgenic trial crop can be easily isolated reproductively from other potato crops. Selfing is the most likely and most frequently observed form of reproduction; cross-pollination rates have been shown to be just 2% at a distance of 3 metres from the crop, reducing to 0.017% at a distance of 10 metres (McPartlan and Dale, 1994).

(b) any specific factors affecting dissemination.

Tubers are dispersed by activities of man in crop husbandry and transport. Fruits are not often consumed by animals as they are highly poisonous and hence seed is not dispersed by this means. Pollen is produced by some cultivars. It is consumed by some insects such as bumble bees although, as potato flowers lack nectar, pollen dissemination by bees is unlikely and they tend not to forage in potato crops (Sanford & Hanneman, 1981). Hive bees also do not commonly forage in potato crops as its flowers lack nectar. Wind dissemination is considered to be marginal (Eastham and Sweet, 2002). Overall, pollen dissemination is minimal at distances of 5 to 10 metres from a potato crop (Bock et al., 2002).

7. The geographical distribution of the plant.

The potato originates from South America (the Andes). Potatoes are widely cultivated throughout the world and rank as the 4th most important food crop. In the UK potatoes are grown solely as agricultural produce, there are no ornamental or wild potato varieties.

8. Where the application relates to a plant species which is not normally grown in the United Kingdom, a description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts.
Not applicable.

9. Any other potential interactions, relevant to the genetically modified organism, of the plant with organisms in the ecosystem where it is usually grown, or elsewhere, including information on toxic effects on humans, animals and other organisms.
Potatoes in the UK are hosts to a number of pests and disease-causing organisms, including slugs, insects, nematodes, viruses, bacteria and fungi. A number of beneficial organisms, such as bees, parasitoids and insects that feed upon aphids for example, also associate with potato crops.

Above ground parts of potato plants, including berries, contain signifcant levels of glycoalkaloids which are toxic to mammals and birds and nitrates which are anti-nutritional. Glycoalkaloid levels in tubers of cultivated potatoes are generally less than 100 mg/kg fresh weight which is below the maximum acceptable level of 200 mg/kg fresh weight established by OECD. The modifications made to the transgenic potatoes referred to in this application do not affect these characteristics.

PART III

Information relating to the genetic modification

10. A description of the methods used for the genetic modification.
Transgenic potato plants were generated using *Agrobacterium tumefaciens* strain AGL1. For transformation, a standard protocol similar to that of Kumar et al (1996) was used. Stem internode sections of the potato cultivar Desiree were co-cultivated with AGL1 and encouraged to develop callous tissue. Shoots which regenerated from callous tissue were excised and encouraged to develop roots. All transgenic plants were treated with the antibiotic Claforan to kill any remaining *Agrobacterium*.

11. The nature and source of the vector used.
The transformation vector pBIN19 (Frisch et al, 1995) carries on its backbone the *nptIII* gene (for bacterial selection only) and a fragment of ColE1 from pBR3222 which includes an *E. coli* origin of replication and the *bom* site which allows mobilization from *E. coli* to *A. tumefaciens*. Located between the two border sequences of the T-DNA region of the Ti plasmid are the kanamycin-resistance gene (*nptII*) and the *lacZ'* gene, which contains a multiple cloning site to enable insertion of the genes to be transferred to plant hosts. The orientation of the boundary sequences in pBIN19 means that the lacZ′ and *nptII* genes, as well as any new DNA ligated into the restriction sites within lacZ′, are transferred to the plant DNA. This vector was used to create the transgenic plant PL3056 containing *Rpi-vnt1*.

The transformation vector pCLD04541 is a binary cosmid/TAC vector and is derived from SLJ1711 (1) which is in turn derived from pRK290. The backbone of pCLD04541 contains a gene for tetracycline resistance in bacterial hosts, Sequences between the left and right border sequences include *nptII* for kanamycin selection of transformed plants, driven by a

CaMV35S promoter. The octopine synthase gene terminator (*ocs 3'*) from *Agrobacterium tumefaciens* terminates transcription of the *nptII* gene. This sequence is not related to any technology that prevents seed propagation of plants. This vector was used to create the transgenic plant PL2808 which contains *Rpi-mcq1.1*.

12. The size, intended function and name of the donor organism or organisms of each constituent fragment of the region intended for insertion.

All plants will contain the selectable marker *nptII* for kanamycin resistance. This is required only for selection of transgenic lines. Additionally, each transgenic line will contain a gene conferring resistance to *Phytophthora infestans* as described below.

Plasmid pSLJ21152 contains *Rpi-vnt1.1* which originates from the wild South American potato relative *Solanum venturii* (Foster et al, 2009). *Rpi-vnt1.1* is a plant resistance (*R*) gene of the CC-NB-LRR class which confers resistance to some isolates of the late blight pathogen *P. infestans*. The expression of *Rpi-vnt1.1* will be under the control of endogenous promoter and terminator regions.

Plasmid pSLJ21153 contains *Rpi-mcq1.1*. This resistance gene originates from the wild South American potato relative *Solanum mochiquense*. *Rpi-mcq1.1* is a plant resistance (*R*) gene of the CC-NB-LRR class and confers resistance to a different spectrum of isolates of *P. infestans* to that conferred by *Rpi-vnt1.1*. Expression of *Rpi-mcq1.1* will be under the control of endogenous promoter and terminator regions. Plasmid pSLJ21153 also contains a truncated copy of a CC-NB-LRR gene. This truncated copy lacks a start codon and the CC-domain and thus consists of a NB-LRR region with high (77%) identity to the *Rpi-mcq1.1* gene. Due to the lack of a start codon we predict that this sequence is non-functional.

Table of genetic elements in T-DNA

Abbreviation	Name & Function	Size (bp)	Origin
pSLJ21152			
T-DNA		7709	
p-*nos*	Promoter of nopaline synthase gene	307	*A. tumefaciens*
nptII	Neomycin phosphotransferase gene	792	*E. coli*
t-*nos*	Terminator of nopaline synthase gene	256	*A. tumefaciens*
p-*Rpi-vnt1.1*	Promoter region of *Rpi-vnt1.1* gene	708	*Solanum venturii*
Rpi-vnt1.1	Coding region of *Rpi-vnt1.1 R* gene	2677	*Solanum venturii*
t-*Rpi-vnt1.1*	Terminator region of *Rpi-vnt1.1* gene	926	*Solanum venturii*
pSLJ21153			
T-DNA		21,000	
CaMV35S	Promoter controlling expression of *nptII*	618	Cauliflower mosaic virus
nptII	Neomycin phosphotransferase gene	795	*E. coli*
ocs 3'	Octopine synthase terminator	560	*A. tumefaciens*
p-*Rpi-mcq1.1*	Promoter region of *Rpi-mcq1.1* gene	9124	*Solanum mochiquense*
Rpi-mcq1.1	Coding region of *Rpi-mcq1.1 R* gene	2589	*Solanum mochiquense*
t-*Rpi-mcq1.1*	Terminator region of *Rpi-mcq1.1* gene	3998	*Solanum mochiquense*
Trunc-*Rpi-mcq*	Truncated NB-LRR gene with homology to *Rpi-mcq1.1*	2183	*Solanum mochiquense*
t-Trunc-*Rpi-mcq*	Sequence 3' to truncated NB-LRR gene with homology to *Rpi-mcq1.1*	1169	*Solanum mochiquense*

PART IV

Information relating to the genetically modified plant

13. A description of the trait or traits and characteristics of the genetically modified plant which have been introduced or modified.

The modified plants contain introduced plant *R* genes, which were isolated from wild South American potato species. The introduced genes confer useful resistance against different isolates of the late blight pathogen. Resistance genes enable plant to recognise certain isolates of the pathogen which possess a specific corresponding avirulence gene or effector. The recognition event triggers a pre-existing signal cascade culminating in expression of the plant defence response which acts to prevent further pathogen growth within the host plant. Other than increasing the range of *P. infestans* genotypes to which the plants are resistant, no other traits have been altered.

14. The following information on the sequences actually inserted or deleted:

(a) the size and structure of the insert and methods used for its characterisation, including information on any parts of the vector introduced into the genetically modified plant or any carrier or foreign DNA remaining in the genetically modified plant,

The sizes of the T-DNA sequences to be inserted are approximately 7.7 kb for pSLJ21152 and 21.0 kb for pSLJ21153. The respective plasmid maps can be found in Annex 1 to this application.

PCR experiments have been done to show that no sequences outside of the T-DNA borders are present in the plant line to be released that contains pSLJ21153 (*Rpi-mcq1.1*). To determine this, PCR primers designed to amplify regions from the vector backbone close to the left and right borders were used. PCR products were not obtained from the plant line PL2808 to be released (Annex 1, Figure 3). We also tested this plant line using primers designed to amplify the tetracycline resistance bacterial selection marker present in the vector backbone. No PCR products were obtained from the plants (Annex 1, Figure 3).

PCR experiments were also done to show that no sequences outside of the T-DNA borders are present in the plant line to be released that contains pSLJ21152 (*Rpi-vnt1.1*). To determine this, PCR primers designed to amplify regions from the vector backbone close to the left and right borders were used. PCR products were not obtained from the plant line PL3056 to be released (Annex 1, Figure 4) We also tested this plant line using primers designed to amplify the *nptIII* bacterial selection marker present in the vector backbone. No PCR products were obtained from the plants (Annex 1, Figure 4).

In summary, PCR-based characterisation of the plants intended for release provide no evidence for integration of vector backbone into the GM plants.

(b) the size and function of the deleted region or regions,

Not applicable.

(c) the copy number of the insert, and

Given the low levels of expression observed, we expect that the inserted genes are present as 1-2 copies.

(d) the location or locations of the insert or inserts in the plant cells (whether it is integrated in the chromosome, chloroplasts, mitochondria, or maintained in a non-integrated form) and the methods for its determination.
As plants were transformed using *A. tumefaciens,* all transformation events will result in a nuclear location for the transgenes. All lines produced have shown stability of the inserted sequence during propagation associated with nuclear integration.

15. The following information on the expression of the insert -

(a) information on the developmental expression of the insert during the lifecycle of the plant and methods used for its characterisation,

The expression of the resistance genes *Rpi-vnt1.1* and *Rpi-mcq1.1* in the transgenic plants to be released is governed by their respective native promoters and terminators. *R* genes of the same class (NB-LRR) have previously been shown to exhibit very weak expression in vegetative parts of the plant (Michelmore et al., 2001).

To examine expression of the transgenes *Rpi-mcq1.1* and *Rpi-vnt1.1* in the transgenics plants nominated for release, plants of the transgenic lines carrying *Rpi-mcq1.1* and *Rpi-vnt1.1* were inoculated with either water (as a negative control) or spores of *Phytophthora infestans*. 18 hours after inoculation, RNA was extracted and RT-PCRs done for 22, 26 and 30 PCR cycles (see Annex 1 for further details and results). Expression of *Rpi-mcq1.1* was undetectable until 30 PCR cycles. Expression of *Rpi-vnt1.1* was undetectable even at 30 cycles. In contrast, expression of the constitutively expressed reference gene *PHA1* (potato plasma membrane ATPase) was detectable from 22 PCR cycles.

These expression analyses confirm that the expression levels of the transgenes under the control of their native regulatory sequences are very low. Despite these very low expression levels, the transgenic plants are resistant to strains of *P. infestans* which are able to cause disease on control, non-transgenic plants, demonstrating that the resistance genes are functional within the plants intended for release.

(b) the parts of the plant where the insert is expressed, such as roots, stem or pollen.
R genes are known to be expressed, albeit at very low levels, in the vegetative aerial parts of plants (Michelmore et al., 2001).

16. Information on how the genetically modified plant differs from the parental or recipient plant in the following respects -

(a) mode or modes and/or the rate of reproduction,
The inserted genes are not expected to alter either the mode or rate of reproduction of the genetically modified plants. The only known function of the plant resistance genes inserted is to confer resistance against specific genotypes of the late blight pathogen *P. infestans*.

(b) dissemination,
The dissemination capacity of the genetically modified plants is not expected to differ from the parental lines.

(c) survivability.

The genetically modified plants will have enhanced survivability in the field due to an increase in the range of *P. infestans* isolates to which they are resistant. This increased survivability will only be apparent in the event that the local *P. infestans* population is comprised of isolates against which the plants are resistant. Should the local population comprise of genotypes which are not recognised either by the introduced *R* genes, or by *R* genes already present in the genome of the potato plant, no increase in survivability will be apparent.

17. The genetic stability of the insert and phenotypic stability of the genetically modified plant.

No genetic or phenotypic instability of the genetically modified plants has been observed during the time that the plants have been cultivated. The plants have all been regenerated from tubers obtained from the original transformed lines and no changes in phenotype are evident.

18. Any change to the ability of the genetically modified plant to transfer genetic material to other organisms.

The only mechanism by which potatoes could conceivably transfer genetic material to other organisms would be via uptake of potato DNA from dead plant material by soil living bacteria, by transfer of DNA to bacteria in the stomachs of animals that consume potatoes or by cross-pollination of compatible wild species. The transfer of genetic material from the potato plants to soil microorganisms, and their successful expression and long-term establishment is very improbable under field conditions (Schlüter et al., 1995). The transfer and subsequent establishment and expression of genetic material in bacteria or in cells of the gastrointestinal tract in man or animals after unintended consumption of plant parts derived from the potato plants to be released is very improbable under natural conditions (van den Eede, 2004). In any case, due to the toxicity of the above ground plant parts, animals do not feed on this material. The tubers produced by the transgenic plants released will not be used for animal feed and will be destroyed following harvest. There are no wild Solanaceous species in the UK with which the potatoes could outcross. The modifications made to the transgenic plants will not alter the ability to transfer genetic material by any of these routes.

19. Information on any toxic, allergenic or other harmful effects on human health arising from the genetic modification.

The potato plants intended for release contain genes conferring increased resistance to potato late blight and are not expected to exert any toxic, allergenic or other harmful effects on human health.

The introduced genes, *Rpi-vnt1.1* and *Rpi-mcq1.1* are members of a class of resistance genes (NB-LRR; nucleotide binding site-leucine rich repeat) that are already known to be abundant within potato and other plant genomes. This particular class of *R* genes contains the majority of plant *R* genes identified thus far and each possesses the same protein structure. Many of the European cultivated potato varieties already contain *R* genes of the NB-LRR class that were derived from the wild potato species *Solanum demissum* (Wastie, 1991). Thus far, no member of the NB-LRR class of *R* genes has been shown to confer toxic or allergenic properties. The abundance of this class of genes in plants, many of which are food crops, suggests that there is no particular hazard associated with their presence in the genome. In the genomes of plants for which a complete genome sequence is available, Arabidopsis is known to possess approximately 200 *R* genes and *R* gene homologues, rice possesses approximately 500. Recent estimates from the draft potato sequence suggest that the potato contains at least 180 *R* genes and *R* gene homologues (Dan MacLean, Sainsbury Laboratory, unpublished data). The expression of the introduced genes in the transgenic potatoes to which this application for release applies is under the control of native

promoters and thus they are expressed at very low levels, comparable with what is known for other native resistance genes.

The truncated NB-LRR located downstream from the *Rpi-mcq1.1* coding sequence lacks a recognised transcription start site and therefore will not be expressed in the recipient plants. Therefore there are no potential toxic, allergenic or other harmful effects on human health predicted.

The marker gene *nptII* (or *aph*(3')-IIa) is expressed as an enzyme (aminoglycoside 3-phosphotransferase II or neomycin phosphotransferase II) that inactivates the antibiotics neomycin, kanamycin, geneticin (G418), and paromomycin by phosphorylation. The protein encoded by the gene has been shown to be bio-safe, non-toxic and poses no risk to human or animal health. The following passage is taken from Appendix A of the Statement of EFSA on the "Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants" (The EFSA Journal, 2009, 1034: 66-82):

"The safety of the *aph*(3')-IIa gene and its protein product APH(3')-IIa has been verified by a number of studies. The exposure of humans and animals to the gene and protein via food and feed is very low due to the initially low levels in plants and further losses during processing. The protein is readily digested in the gastrointestinal tract. Bioinformatic analyses indicate no concerns as regards toxicity or allergenicity. Lack of toxicity has been verified by acute oral toxicity in mice. The *aph*(3')-IIa gene has been used in human gene therapy studies with no clinical signs of toxicity. Subchronic toxicity study on rats and nutritional studies on broilers and heifers with plant material containing APH(3')-IIa provide further assurance of safety."

In summary no toxicity of the NPTII protein has been observed and in simulated digestive fluids this protein is rapidly degraded. The safety of the transgenic protein NPTII involves no outstanding safety issues and derived products are no more likely to cause adverse effects on human and animal health than conventional potato (EFSA Journal (2006) 323, 1-20).

In addition to the absence of known toxic or allergenic properties of any of the genetic elements present in the modified potatoes, tubers will be destroyed at harvest and thus there will be no risk of the genetically modified material entering the food chain.

20. Information on the safety of the genetically modified plant to animal health, particularly regarding any toxic, allergenic or other harmful effects arising from the genetic modification, where the genetically modified plant is intended to be used in animal feeding stuffs.

The modified plants are not intended to be used as animal feed.

21. The mechanism of interaction between the genetically modified plant and target organisms, if applicable.

The target organism of the enhance resistance conferred by the introduced *R* genes is *Phytophthora infestans*, the cause of potato late blight. The interaction will be manifested by a reduction in the ability of the late blight pathogen to infect the genetically modified potatoes. *R* genes encode molecules with both recognition and signal transduction properties. The external part of the protein (the leucine rich repeat, LRR) recognises specific molecules (effectors or avirulence factors) secreted by the pathogen which are intended to help the pathogen cause disease on the host. Recognition by the LRR region results in a signal transduction event culminating in the triggering of plant defence responses which result in localised host plant cell death and preventing spread of the pathogen through host tissues (Jones & Dangl, 2006). The recognition and triggering of defence responses may also induce expression of defence-related genes in distant parts of the plant to the original infection site (Heil & Bostock, 2002).

22. The potential changes in the interactions of the genetically modified plant with non-target organisms resulting from the genetic modification.
Resistance genes of the NB-LRR class are very specific, limited to species or race, and cause initiation of a resistance response (Hammond-Kosack and Parker, 2003; Jones and Dangl, 2006). For recognition of the target organism a very specific avirulence factor has to be injected by the pathogen. The specific avirulence factors for *Rpi-vnt1.1* and *Rpi-mcq1.1* are, based on current knowledge, expected to be produced only by *P. infestans*. Due to the specificity of the response reaction no effects on other organisms than *P. infestans* are expected other than those that also apply to the interaction of non-genetically modified potatoes with non-target organisms under conventional agricultural practice. Due to a reduced need for fungal treatments an increase in the populations of those non-target organisms that respond to the fungal treatments might be expected. No other changes in interactions are anticipated. Further the trial will provide an opportunity to investigate any potential changes in the interactions with non-target organisms via observations on disease and pest susceptibility.

23. The potential interactions with the abiotic environment.
None of the introduced genes will have any effect on frost, drought or salt tolerance and therefore we do not expect the genetically modified potatoes to differ in any of these respects to other potato varieties or crops. With the exception of a reduced fungicide input, the field trial will be treated no differently to a standard potato crop. We do not expect the modified plants to respond any differently to any standard agricultural practices such as herbicide treatment or fertiliser application.

24. A description of detection and identification techniques for the genetically modified plant.
PCR primers for specific detection of the introduced *R* genes and for the *nptII* kanamycin resistance gene are available and details are given in Annex 1.

25. Information about previous releases of the genetically modified plant, if applicable.
These plants have not been previously released.

PART V

Information relating to the site of release

(Applications for consent to release only)

26. The location and size of the release site or sites.
The plants will be released on an area of arable land no larger than 1000 metres squared located at the John Innes Centre (JIC, Ordnance Survey map grid reference TG 1707). Each year the area planted with the genetically modified plants will be approximately 200 metres squared. In accordance with potato planting practice, the plot will rotate within the release site each year of the trial.

27. A description of the release site ecosystem, including climate, flora and fauna.
The release site (Ordnance Survey map grid reference TG 1707) is arable land located at the John Innes Centre; some areas are bordered by deciduous hedges or trees. Flora in the immediate vicinity will be unknown until decisions on other local (non GM) field trials are made each year but will likely be limited to cereals (wheat/barley and peas). With the exception of a surrounding guard crop of Maris Piper no potatoes will be grown within the

accepted distance of 20 metres from the release site. The guard crop is in place to protect the release plants against edge effects such as wind and rain. As the tubers are white skinned (Desiree is red skinned) these guard potatoes will also serve a useful visual marker during harvesting of the trial. With respect to planting and waste management these potatoes will be treated as part of the trial and will be disposed of as described for the transgenic potatoes.

28. Details of any sexually compatible wild relatives or cultivated plant species present at the release sites.

There are no sexually compatible wild Solanaceous relatives present on either release site. If present at all nearby the trial site, other related Solanaceous wild species will be limited to the boundary hedge/field margins of the trial site and thus will be separated by a distance of >20m from the genetically modified crop.

29. The proximity of the release sites to officially recognised biotopes or protected areas which may be affected.

There are no officially recognised biotopes, protected areas or SSSIs within 4km of the release site. Given that potato pollen is not normally disseminated more than 10m from the parent plants, this distance equates to 400x the normal dissemination distance. The closest SSSI to the release sites is Sweet Briar Road Meadows which is ~4 km away and is a series of unimproved wet meadows with permanent water-logging and thus very unlikely to host any potato plants. Potato does not hybridise with any British native plants. This combined with the fact that potato is not a wind-pollinated plant and is not visited frequently by pollinators such as bees (due to lack of nectar production) mean that there is no risk to any officially recognised biotopes or protected areas listed by Natural England.

PART VI

Information relating to the release

30. The purpose of the release of the genetically modified plant, including its initial use and any intention to use it as or in a product in the future.

This release is part of a programme of publically funded research and is not linked to commercial interests.

Since 2001, we have been working towards identifying, mapping and isolating resistance genes from potato that confer resistance against potato late blight (*Phytophthora infestans*). Recently, two such genes (*Rpi-vnt1.1* and *Rpi-mcq1.1*) were successfully isolated from wild South American relatives of the potato and these have been transformed into the potato cultivar Desiree. The genes identified are potentially valuable weapons in the fight against potato blight as between them they confer resistance against many different isolates of late blight, including the very aggressive Blue 13 strain which is currently responsible for major potato losses in the UK and Europe. Thus there is a need to test these genes in a 'real' environment.

The aims of the trial are:

1) to demonstrate that the transferred resistance genes offer a valuable method for controlling late blight of potatoes which does not rely on agricultural inputs (pesticides).
2) to confirm that the transferred resistance genes still function in a 'real life' situation (i.e. in a field as opposed to a lab/greenhouse).

3) to expose plants containing the newly identified genes to the local populations of late blight to confirm that they are indeed useful.
4) if infection does result in disease, to isolate the corresponding pathogen race.

31. The foreseen date or dates and duration of the release.

The field trial start date will be on 1st May during the year that consent is granted and will continue until 30 November 2010. If consent is granted, the trial will continue for 3 years and will subsequently be from 1st May until 30th November in 2011 and from 1st May until 30th November in 2012. The exact timing of sowing of the trial will depend upon weather conditions at the time. Harvesting of tubers will take place during September or October of each year of the field trials.

32. The method by which the genetically modified plants will be released.

Tubers or small glasshouse-grown plants will be planted in the field by hand according to the randomised block trial design shown in Annex 2.

33. The method for preparing and managing the release site, prior to, during and after the release, including cultivation practices and harvesting methods.

The ground will be prepared by staff from The Operations Centre (JIC) who look after field trials on the NRP site according to normal agricultural practices for potato. Ground preparations will consist of existing grass being sprayed with herbicide to clear the ground. Manure will be applied if necessary and the ground will be prepared for planting using a power harrow. Harvest will occur late September/October depending on weather conditions at the time (if the plants senesce prior to this then harvesting will be brought forward). Plants will be lifted by haulms and harvesting of tubers will be by fork and hand to ensure removal of all GM material. The plot will be then sown to grass, monitored for ground-keepers during the remainder of the year and sprayed with a broadleaf herbicide. Any ground keepers identified will be removed by hand and destroyed as detailed above. The monitoring of the plot for ground-keepers will be continued for a period of 2 years following the 3 year experiment in accordance with Defra guidance. During this time the plot will be sown to grass to enable easy identification and removal of ground keepers.

34. The approximate number of genetically modified plants (or plants per square metre) to be released.

For each year of the field trial we will release not more than 200 plants of each of the transgenic lines containing *Rpi-vnt1.1* and *Rpi-mcq1.1*.

PART VII

Information on control, monitoring, post-release and waste treatment plans

35. A description of any precautions to -

i. maintain the genetically modified plant at a distance from sexually compatible plant species, both wild relatives and crops.

Although there are no sexually compatible wild relatives capable of hybridising with potato present in the UK, transgenic plants will be isolated from any other Solanaceous relatives, including other potato crops, by a distance of at least 20 metres. The release site will be routinely monitored for volunteers and any discovered will be destroyed. Post-harvest, the plot will be sown to grass to allow identification of volunteers. For a two-year period following the trial the only crops grown on the release site will be those that allow easy identification and destruction of volunteers.

(b) any measures to minimise or prevent dispersal of any reproductive organ of the genetically modified plant (such as pollen, seeds, tuber).

Pollen will be allowed to be produced and disperse but it's low viability and distance (at least 20 metres) from other potato crops will ensure that the only recipients will be local potatoes within the trial. Prior to planting, plant and/or tubers will be transported to the release site in a vehicle not used for general transport purposes and the plants will not be mixed with either other plants or with equipment used for working on other agricultural land. Any equipment used for the planting (and harvesting) of transgenic material will be thoroughly cleaned after use. Harvesting of tubers will be by fork and hand which will minimise dispersal. The use of a guard crop with a different tuber skin colouration will help identify that all transgenic tubers have been harvested.

36. A description of the methods for post-release treatment of the site or sites.
Harvest will occur late September/October depending on weather conditions at the time (if the plants senesce prior to this then harvesting will be brought forward). Harvesting will be by fork and hand to ensure removal of all GM material. The plot will be then sown to grass, monitored for ground-keepers during the remainder of the year and sprayed with a broadleaf herbicide. Any ground keepers identified will be destroyed by herbicide treatment (e.g. glyphosate) or removed by hand and destroyed by autoclaving. The monitoring of the plot for ground-keepers will be continued at monthly intervals by walking the trial site for a period of 2 years following the 3-year experiment in accordance with Defra guidance. During this time the plot will be sown to grass or another crop that will enable easy identification and removal of ground keepers.

37. A description of the post-release treatment methods for the genetically modified plant material including wastes.
All harvested material (plant tops and tubers) will be placed in a container at the trial site and disposed of directly from the site by a specialist company licensed for such work.

38. A description of monitoring plans and techniques.
The purpose of the monitoring plan is to enable early detection any unintended effects related to the release of the transgenic potato plants.

The release site will be visited by trained laboratory personnel who are working on the project at no less than weekly intervals. Visits will usually occur more frequently. Any unexpected occurrences that could potentially result in adverse environmental effects or the possibility of adverse effects on human health will be notified to the national inspectorate immediately. Should the need arise to terminate the release at any point the emergency plans detailed below will be followed.

Post trial the release site will remain fallow or will be planted with grass to enable easy identification of volunteers. The site will be inspected monthly between April and October (the growing season of potato) and any volunteers identified will be immediately destroyed either by application of a systematic broad leaf herbicide or by hand pulling plants and digging out tubers/root systems, followed by autoclaving. If volunteers are found at the end of the 2 year period, monthly inspections will continue during April-October of the subsequent season until 3 successive months have passed in which no volunteers have been found.

39. A description of any emergency plans.

At any time point post planting, should the release need to be terminated, any plant material will be sprayed with an appropriate broad leaf systemic herbicide and tubers dug up by fork and hand and destroyed by a specialist waste disposal company licensed for such work.

Should the release site be subject to vandalism, care will be taken to ensure that all uprooted plant material within and outside of the trial site is identified and destroyed accordingly as described above.

40. Methods and procedures to protect the site.

Potatoes are not grazed on by animals due to the toxic nature of the above ground plant parts. The release site will be fenced to protect against animal damage and entry by unauthorised persons. The site will also be monitored by remote security cameras visible from the John Innes Centre reception which is manned through out the day by JIC reception staff and by security guards out of normal working hours.

PART VIII

Information on methodology

41. A description of the methods used or a reference to standardised or internationally recognised methods used to compile the information required by this Schedule, and the name of the body or bodies responsible for carrying out the studies.

Methods are detailed in appropriate references listed at the end of this application or are included in Annex 1 where results are also detailed.

PART A2: DATA OR RESULTS FROM ANY PREVIOUS RELEASES OF THE GMO

Give information on data or results from any previous releases of this GMO by you either inside or outside the European Community, [especially the results of monitoring and the effectiveness of any risk management procedures].

No previous releases

PART A3: DETAILS OF PREVIOUS APPLICATIONS FOR RELEASE

Give details of any previous applications to release the GMO made to the Secretary of State under the 2002 Regulations or to another Member State under the Deliberate Release Directive 2001/18/EC.

No previous release applications have been made

PART A4: RISK ASSESSMENT AND A STATEMENT ON RISK EVALUATION

Summary

Environmental risks

Four hundred years of cultivation of the potato has established that the potato has limited ability to survive in UK environments except when cultivated. Plants generated from tubers are readily identifiable and easily eliminated either by hand pulling or use of herbicides. Potato plants are not invasive of natural habitats. The pollen of potato normally disperses less than 10m, is often infertile and potatoes cannot cross with other crop plants to produce hybrids. A major factor contributing to the lack of pollen dispersal is the fact that flowers of *Solanum* spp produce no nectar, so pollen is the only food reward offered. Consequently, they are not frequently visited by Honey bees seeking nectar. In addition, the anthers of these plants require sonication by insects to release pollen, and thus the spectrum of pollinating insects is restricted. Bumble bees typically forage over 70–631m (Osborne et al., 1999), but pollen from one flower is usually deposited only across a limited number that are subsequently visited. This and factors such as residence time in one crop favours highly localized cross-pollination of plants near the pollen source (Cresswell et al., 2002). Estimates of the rates of cross-pollination under field conditions range from 0 to about 20% (Plaisted, 1980). Other studies have shown that the rate of cross-pollination rates are 2% at a distance of 3 metres from the crop, reducing to 0.017% at a distance of 10 metres McPartlan and Dale, 1994).

The overall risk to the environment from transgenic potatoes sited at least 20 m from other plants with which it is cross-fertile is low to effectively zero. The resistance traits to be expressed only affect the target pathogen, *Phytophthora infestans*. The expected environmental impact is negligible to effectively zero and will reduce the level of other agricultural inputs such as use of fungicides to control late blight in potato crops.

Any evaluation of biosafety of transgenic potato crops to animals must be set in the context that these plants are a natural hazard to a range of animals. Their tissues naturally contain steroidal glycoalkaloids such as α-chaconine and α-solanine that are potent neurotoxins,

particularly if administered by an intraperitoneal route. Their levels in leaves are normally higher than safe levels accepted in tubers for food.

Human health risks

R genes of the CC-NB-LRR class are not new to the human diet, being present in all plants consumed by both humans and animals. The model plant species Arabidopsis is known to possess approximately 200 *R* genes and *R* gene homologues, rice possesses approximately 500. Recent estimates from analysis of the draft potato sequence suggest that the potato contains at least 180 *R* genes and *R* gene homologues (Dan MacLean, Sainsbury Laboratory, unpublished data). *R* genes themselves are not toxic even to crop pathogens. They simply serve a recognition function, enabling plants to recognise specific molecules produced by the pathogens, resulting in the triggering of plant defence responses. These plant defence responses are not specific to late blight resistance. They are triggered upon recognition of any plant pathogen.

Any evaluation of biosafety of transgenic potato crops to humans must be set in the context that these plants are a natural hazard as they naturally contain steroidal glycoalkaloids (see above). The total content of such glycoalkaloids in tubers of varieties to be used for food should not exceed 20 mg/100 g fresh weight (Krits et al., 2007).

RISK ASSESSMENT

Conclusions on the Potential Environmental Impact from the Release or the Placing on the Market of GMOs

i. Likelihood of the genetically modified higher plant (GMHP) becoming more persistent than the recipient or parental plants in agricultural habitats or more invasive in natural habitats.

Neither the *R* genes *Rpi-vnt1.1* or *Rpi-mcq1.1* nor the kanamycn resistance gene confer characteristics to the GM potato that would increase the competitiveness of plants containing the genes in unmanaged ecosystems. Neither would the genes enable plants carrying them to out-compete plants of similar type for space. None of the transferred genes are anticipated to affect pollen production and fertility, seed dispersal or frost tolerance. Seeds and tubers, which might be spread outside cultivated fields, would have no competitive advantage in this environment. Potatoes are not persistent outside the agricultural environment and feral potato plants do not generally occur in the UK. The introduced *R* genes and the kanamycin resistance gene are thus not anticipated to confer any advantage compared to conventional potato varieties with respect to persistence in agricultural habitats or invasiveness in natural habitats.

To further minimise any risk, the following risk management measures will be applied: crop rotation as per conventional agricultural practise, implementation of isolation distances of a minimum of 20 metres from any other potato plants not included in the trial and volunteer management to ensure effective control of volunteers emerging on the field and the immediate surroundings. The overall impact is therefore considered negligible.

ii. Any selective advantage or disadvantage conferred to the GMHP

The intended effect of the genetic modification described here is to improve the resistance of recipient plants to *P. infestans*. Under *P. infestans* pressure resistant potatoes are therefore intended to have a selective advantage in comparison to untreated non-resistant conventional potatoes included in the trial. This advantage is only applicable in the agricultural environment and only in those cases where no other plant protection measures against *P. infestans* (such as fungicide treatments) are applied. Conventional agricultural practices as well as volunteer management will ensure effective control of volunteers emerging on the field and the immediate surroundings. Potato plants are never seen established outside the agricultural environment and resistance to *P. infestans* is not a characteristic that would enhance the invasiveness of potatoes.

The introduced kanamycin resistance trait used is used for selection of transgenic plants during tissue culture and confers improved tolerance to the antibiotics neomycin, kanamycin, geneticin (G418), and paromomycin. These antibiotics are not used in agriculture and hence will not confer any selective advantage to the transgenic plants. The nptII gene responsible for the resistance has been approved as safe for use by the European Food Safety Authority.

iii. Potential for gene transfer to the same or other sexually compatible plant species under conditions of planting the GMHP and any selective advantage or disadvantage conferred to those plant species.

Genetic material can be transferred from conventional potatoes as well as genetically modified potatoes to sexually compatible plants via pollen. Transfer via pollen to other species or wild relatives at or near the release site is very unlikely due to the absence of sexually compatible species. Therefore out-crossing to those species can be excluded. Transfer of genetic material via pollen to conventional potato varieties is possible, however the proposed risk management measures (e.g. isolation distance, monitoring and volunteer management) will prevent any unintended pollination. In the unlikely case that pollen is transferred to non-genetically modified potatoes, the consequences are negligible. No selective advantage or disadvantage is being transferred to those potatoes (see point ii). There is no risk of introduction of the GM traits into conventional potato material as potato is propagated vegetatively.

iv. Potential immediate and/or delayed environmental impact resulting from direct and indirect interactions between the GMHP and target organisms, such as predators, parasitoids and pathogens (if applicable).

The target organism of the introduced *R* genes is *Phytophthora infestans*. The intended effect of the genetic modification is to confer tolerance to *P. infestans*, thereby reducing the population in the trial plants. Under conventional agricultural practice *P. infestans* is also controlled by fungicide-treatment of potato fields and thus the outcome of the interaction (i.e. a reduction in the population of *P. infestans*) is a desirable one and does not differ from the outcome of these other pratices. The overall impact of *P. infestans* tolerant potatoes on target organisms is therefore considered comparable to the impact of fungicide applications on non-genetically modified potatoes conducted according to conventional agricultural practice.

v. Possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of the GMHP with non-target organisms, (also taking into account organisms which interact with target organisms), including impact on population levels of competitors, herbivores, symbionts (where applicable), parasites and pathogens.

The resistance genes introduced into the genetically modified potatoes are of the NB-LRR class. Genes of this class recognise specific molecules produced by some plant pathogens (in this case *P. infestans*) and trigger a hypersensitive response, leading to plant cell necrosis, which limits the spread of the pathogen. Due to the specificity of the recognition no effects on other organisms than *P. infestans* are expected other than those that also apply to the interaction with non-genetically modified potatoes under conventional agricultural practice. Pathogens other than the particular races of *P. infestans* to which the introduced genes confer resistance, that are able to infect the non-transgenic plants grown as part of the trial will also be able to infect the transgenic plants. Due to a reduced need for fungal treatments, an increase in the populations of those non-target organisms that respond to fungal treatments might be expected. Any effects on disease and susceptibility to pests other than *P. infestans* will be monitored during the release. The overall impact on non-target organisms is considered negligible.

vi. Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMHP and persons working with, coming into direct contact with, or in the vicinity of the GMHP release(s).

The genetically modified potatoes differ from conventional potato varieties in their tolerance to *P. infestans* conferred by the introduced *R* genes. Potato already contains a large number of resistance genes conferring tolerance against other plant diseases. Recent estimates based on our analysis of the potato genome sequence indicate that there are 189 genes of the NB-LRR class within potato (Dan MacLean, The Sainsbury Laboratory, unpublished data). Included in this number are NB-LRR *R* genes that were originally introgressed from other wild potato species, namely *Solanum demissum*, during breeding efforts made during the 20th Century. None of the genes are known to exert any toxic or allergenic effects to human health. The *R* genes themselves are not toxic even to *P. infestans.* The mode of action is due to a hypersensitive response triggered upon recognition of the late blight pathogen by the *R* genes, leading to plant cell necrosis. The introduced genes are expressed by their endogenous promoters at extremely low levels that are comparable to those from other endogenous resistance genes. Due to the lack of any identified toxic effects of the NB-LRR class of *R* genes we do not expect there to be any immediate or delayed effects on human health resulting from direct or indirect human interactions with the modified plants.

The introduced selection marker gene is expressed as the enzyme neomycin phosphotransferase. This selectable marker has been considered safe for use in this context by The European Food Safety Authority (see “Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants”, The EFSA Journal, 2009, 1034: 66-82).

In summary, none of the introduced genes encode for products that are known to be toxic to humans either by ingestion or by contact. In any case, the potato plants are not for human consumption and measures taken with regard to planting, harvest, storage and transportation of the plant material will minimize any contact to humans. Therefore the overall impact on human health is negligible.

vii. Possible immediate and/or delayed effects on animal health and consequences for the food/feed chain resulting from consumption of the GMO and any products derived from it if it is intended to be used as animal feed.

The GM potatoes will not be used for animal feed. Potatoes are not grazed on by animals due to the toxic nature of alkaloids in the green parts of the plant and which are features on non-transgenic potato plants. Measures to be taken during the proposed trial will in any case protect the trial against damage by wild animals (e.g. fences) and also ensure that seed stock and plant material are harvested, stored, transported or disposed of (e.g. cleaning of machinery, packaging) in such a way to prevent contact with animals. Therefore the overall impact on animal health is negligible.

viii. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release(s).

The R-genes introduced into the genetically modified potatoes confer resistance to *Phytophthora infestans*, which is the target organism. The *R* genes encode receptors that will recognize specific elicitors injected by the pathogen into the plant cell. This recognition will, through a signalling network, trigger both local and systemic defence responses. The local response aims at trapping the pathogen in the cells by localized cell death thus stopping further penetration and spread. Based on this mechanism of response none of the newly expressed proteins are expected to be exuded from the plants to the soil. Thus no effects on biogeochemical processes are anticipated other than those which also apply to non-modified potato varieties under conventional agricultural practise. Due to a reduced need for fungal treatments an increase in the populations of other foliar pathogens and soil organisms might be expected. The overall impact on biogeochemical processes is negligible.

ix. Possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the GMHP where these are different from those used for non-GMHPs.

The small scale trial will be conducted according to conventional agricultural practice except for a reduction in fungicide treatments in order to evaluate the efficacy of the introduced R-genes against *Phytophthora infestans*. Differences in the scale of fungicide treatments are also standard practice either in conventional or organic agriculture or in plant protection trials conducted according to applicable agricultural practice. Alterations in fungicide use are likely to have implications on organisms associated with the plants, either present in the soil or on the plant leaves, possibly increasing the populations of both foliar pathogens, other than *P. infestans,* and soil organisms. Therefore overall impact on the environment is negligible and is comparable to the effect of the cultivation of non-genetically modified potatoes with a potentially positive impact on soil and plant-associated microflora.

	Step1: *Potential hazards which may be caused by the characteristics of the novel plant*	**Step 2: *Evaluation of how above hazards could be realised in the receiving environments***	**Step 3: *Evaluation the magnitude of harm caused by each hazard if realised***	**Step 4: *Estimation of how likely/often each hazard will be realised as harm***	**Step 5: *Modification of management strategies to obtain lowest possible risks from the deliberate release***	**Step 6: *Overall risk of the estimate of each hazard the risk of harm caused by the release***
a	**Increased invasiveness in natural habitats or persistence in agricultural habitats.**	*Negligible.* The introduced traits do not confer competitive abilities in natural or agricultural habitats. Conventional practice and volunteer management are applied.	*Very unlikely.* Neither R-genes nor the *nptII* gene confer characteristics to the GM potato that add competitive abilities in unmanaged ecosystems or allow the plants to compete against plants of similar type for space. None of the characteristics transferred to the potato plants are anticipated to affect pollen production/fertility, seed dispersal or frost tolerance.	*Negligible*. Surviving, reproductive potato plants are rarely seen outside the field.	Conventional agricultural practice and volunteer management (monitoring for volunteers and removal/destruction of volunteers in the field, isolation distance, crop rotation).	Overall impact is negligible.
b	**Selective advantage; improved resistance to *P. infestans***	*Moderate*. The intended effect of the genetic modification is to improve the	*Likely.* The intended effect of the genetic modification is to improve the	The advantage is applicable only in the agricultural environment and only in those cases where no other plant	Conventional agricultural practice and volunteer management	Overall impact is negligible.

		resistance to P. infestans, therefore a selective advantage is conferred in comparison to untreated non-resistant conventional potatoes.	resistance to P. infestans. Thus under *P. infestans* pressure resistant potatoes are intended to have a selective advantage in comparison to untreated non-resistant conventional potatoes in the agricultural environment.	protection measures against *P. infestans* are applied. Potato plants are rarely seen outside the field. Resistance to P. infestans is not the key determinant for potential invasiveness of potatoes.	(monitoring for volunteers and removal/destruction of volunteers).	
c	**Selective advantage-resistance to certain antibiotics provided by the antibiotic selectable marker gene (*nptII*)**	*Negligible*. The potato plant will not benefit from expression of this selectable marker as it is not used in agricultural environments Acquisition of resistance by certain other microorganisms.	*Very unlikely* in all aspects. Resistance genes to this antibiotic are already widely distributed among soil bacteria.	The chance of a microbe acquiring the gene is negligible given a) the small number of plants in the trial and b) no ecological advantage would be conferred to soil microorganisms. In the very highly unlikely situation that such transfer occurs to microbes occurring in mammals there would be little harm. The antibiotics have only minor therapeutic relevance in human medicine and restricted use in veterinary medicine.	*None.* This marker has a 14-year history of safe use in food crops.	Overall impact is negligible.
d	**Selective advantage or disadvantage conferred to**	*Negligible*. Potato is a vegetatively propagated crop and	*Very unlikely*. Neither of the traits confers a selective advantage in	In the unlikely case that pollen is transferred to non-genetically modified potatoes,	Conventional agricultural practice and volunteer	Overall impact is negligible.

	sexually compatible plant species	none of the traits confer a selective advantage in the agricultural environment under conventional agricultural practice.	the agricultural environment under conventional agricultural practice. Pollen transfer to other cultivated potatoes is possible, but likely due to short distance of pollen flow. The are two wild Solanum species in the UK but their cross fertilisation with potato crops has not been recorded.	the consequences are negligible since potato is a vegetatively propagated crop. True potato seed is not saved by growers.	management. Isolation distance to other potato crops.	
e	**Potential environmental impact due to interactions between the novel plant and target organism (*P. infestans*)**	*Low*. The intended effect of the transferred resistance genes is to reduce the infection and reproductive success of *P. infestans*, thereby reducing the local population of *P. infestans*. As *P. infestans* is a damaging crop disease, this effect is beneficial.	*Very likely*. The intended effect of the genetic modification is to confer tolerance against the target organism *P. infestans*.	The intended effect is a reduced population of *P. infestans* in the potato field. However, this is acceptable and desired also under conventional agricultural practice and is usually achieved by fungicide-treatment of potato fields.	*None* but impact on *P. infestans* populations will be monitored as the main aim of the field trial.	Overall impact is negligible.
f	**Potential environmental impact due to interactions**	*Negligible.* Other than carrying an extra resistance genes, the plants do not differ	*Very unlikely* due to the inherent specificity and mode of action of *R* genes.	Any effect on non-target organism due to the introduced trait of *P. infestans* tolerance is anticipated to be	Monitoring plan including observations on disease and pest	Overall impact is negligible.

	between the novel plant and non-target organisms	from non-genetically modified potatoes. Any effect is anticipated to be comparable to that of non-genetically modified potatoes under conventional agricultural practice.		comparable to that of non-genetically modified potatoes under conventional agricultural practice. Due to a reduced need for fungal treatments an increase in the populations of non-target organisms might be expected.	susceptibility, including any unintended or unexpected effects.	
g	**Potential effect on human or animal health due to introduced *R* genes**	*Negligible*. NBS-LRR genes are not known to confer toxic or allergenic properties.	*Very unlikely*. NBS-LRR genes are not known to confer toxic or allergenic properties. The endogenous promoters used are known to drive expression of the introduced *R* genes at a very low level, no higher than that at which other *R* genes present in non-transgenic potatoes are expressed.	Material from the field trial is not intended for human/animal consumption.	Measures with regard to planting, harvest, storage and transportation minimize the contact to humans and animals.	Overall impact is negligible.
h	**Potential effect on human or animal health due to introduced *nptII* gene**	*Negligible*. *nptII* gene is not known to confer toxic or allergenic properties. Antibiotics to which the gene confers resistance are not routinely used on humans.	*Very unlikely*. *nptII* gene is not known to confer toxic or allergenic properties. Antibiotics to which the gene confers resistance are not routinely used on	Material from the field trial is not intended for human/animal consumption.	Measures with regard to planting, harvest, storage and transportation minimize the contact to humans and animals.	Overall impact is negligible.

			humans.			
i	**Potential effects on biogeochemical processes (changes in soil decomposition of organic material)**	*Negligible.* None of the newly expressed proteins is expected to be exuded from the plants to the soil.	*Very unlikely*. Soil fertility is not expected to be affected any differently due to the cultivation of the genetically modified potato plants as compared to conventional potatoes. None of the newly expressed proteins is expected to be exuded from the plants to the soil.	*Negligible*. Any effect is expected to be comparable to that of non-genetically modified potatoes under conventional agricultural practice. Due to a reduced need for fungicide treatments, an increase in the populations of soil organisms might be expected.	*None.*	Overall impact is negligible.
j	**Possible environmental impact due to changes in cultivation practice**	*Low*. Potential positive effects on the population of other foliar pathogens and soil organisms, due to a reduction in fungicide treatments.	*Likely*. Application of conventional agricultural practice will be as for a convention, non-transgenic crop, other than a reduction in fungal treatments against *P. infestans*.	Potential positive effects on the populations of foliar pathogens other than *P. infestans* also possible on soil organisms.	*None*.	Overall impact is negligible. Potentially there may be a positive impact on foliar and soil microflora.

PART A5: ASSESSMENT OF COMMERCIAL OR CONFIDENTIALITY OF INFORMATION CONTAINED IN THIS APPLICATION.
Identify clearly any information that is considered to be commercially confidential. A clear justification for keeping information confidential must be given.

This is publically funded research and has no associated commercial confidentiality considerations.

PART A6: STATEMENT ON WHETHER DETAILED INFORMATION ON THE DESCRIPTION OF THE GMO AND THE PURPOSE OF RELEASE HAS BEEN PUBLISHED
Make a clear statement on whether a detailed description of the GMO and the purpose of the release have been published, and the bibliographic reference for any information so published.
This is intended to assist with the protection of the applicant's intellectual property rights, which may be affected by the prior publication of certain detailed information, e.g. by its inclusion on the public register.

Research detailing the production of the plants containing the *R* gene *Rpi-vnt1.1* has been published (Foster et al, 2009; Pel et al 2009). It has also been discussed at scientific conferences and lectures to members of the public.

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Improving late blight (*Phytophthora infestans*) resistance in potato using resistance genes from South American potato relatives.

Annex 1.

This annex contains details of the experiments done to characterise the genetically modified plants referred to in the associated release application by The Sainsbury Laboratory.

Included here are details of a number of independently generated transgenic lines. However, only two plant lines, PL2808 (*Rpi-mcq1.1*) and PL3056 (*Rpi-vnt1.1*) are nominated for release.

Section 1: Sequences present in insert from pSLJ21152 (*Rpi-vnt1.1)*
Section 2: Sequences present in insert from pSLJ21153 (*Rpi-mcq1.1)*
Section 3: Results from characterisation of transgenic plants
Section 4: Methods used for characterisation of transgenic plants
Section 5: Vector maps of pSL21152 (*Rpi-vnt1.1*) and pSL21153 (*Rpi-mcq1.1*)

SECTION 2: SEQUENCES PRESENT IN INSERT FROM pSLJ21152 (*Rpi-vnt1.1)*

Sequence of *nos* promoter present in pSLJ21152

```
  1 gatcatgagc ggagaattaa gggagtcacg ttatgacccc cgccgatgac gcgggacaag
 61 ccgttttacg tttggaactg acagaaccgc aacgttgaag gagccactca gccgcgggtt
121 tctggagttt aatgagctaa gcacatacgt cagaaaccat tattgcgcgt tcaaaagtcg
181 cctaaggtca ctatcagcta gcaaatattt cttgtcaaaa atgctccact gacgttccat
241 aaattcccct cggtatccaa ttagagtctc atattcactc tcaatccaaa taatctgcac
301 cggatct
```

Sequence of neomycin phosphotransferase (*nptII*) present in pSLJ21152

```
  1 atgattgaac aagatggatt gcacgcaggt tctccggccg cttgggtgga gaggctattc
 61 ggctatgact gggcacaaca gacaatcggc tgctctgatg ccgccgtgtt ccggctgtca
121 gcgcaggggc gcccggttct ttttgtcaag accgacctgt ccggtgccct gaatgaactg
181 caggacgagg cagcgcggct atcgtggctg gccacgacgg gcgttccttg cgcagctgtg
241 ctcgacgttg tcactgaagc gggaagggac tggctgctat tgggcgaagt gccggggcag
301 gatctcctgt catctcacct tgctcctgcc gagaaagtat ccatcatggc tgatgcaatg
361 cggcggctgc atacgcttga tccggctacc tgcccattcg accaccaagc gaaacatcgc
421 atcgagcgag cacgtactcg gatggaagcc ggtcttgtcg atcaggatga tctggacgaa
481 gagcatcagg ggctcgcgcc agccgaactg ttcgccaggc tcaaggcgcg catgcccgac
541 ggcgatgatc tcgtcgtgac ccatggcgat gcctgcttgc cgaatatcat ggtggaaaat
601 ggccgctttt ctggattcat cgactgtggc cggctgggtg tggcggaccg ctatcaggac
661 atagcgttgg ctacccgtga tattgctgaa gagcttggcg gcgaatgggc tgaccgcttc
721 ctcgtgcttt acggtatcgc cgctcccgat tcgcagcgca tcgccttcta tcgccttctt
781 gacgagttct tc
```

Sequence of *nos* terminator present in pSLJ21152

```
  1 gatcgttcaa acatttggca ataaagtttc ttaagattga atcctgttgc cggtcttgcg
 61 atgattatca tataatttct gttgaattac gttaagcatg taataattaa catgtaatgc
121 atgacgttat ttatgagatg ggtttttatg attagagtcc cgcaattata catttaatac
181 gcgatagaaa acaaaatata gcgcgcaaac taggataaat tatcgcgcgc ggtgtcatct
241 atgttactag atcggg
```

Sequence of *Rpi-vnt1.1* promoter present in pSLJ21152

```
   1 agttatacac cctacattct actcgagtca ttatgatgat gtctcacgac caaatcaaat
  61 caaagttaaa taaatatcga accgaacgcc cactctgtat gagtatggca aaagattttg
 121 agagaatcaa gttgcataaa agcctaattt tcatggaaca tacaaattga gtctcataat
 181 agcccaaact cacagccatg aacccaaatt gggtaaagtt ttgcaagacg ttcatcaaac
 241 agttaggaaa cataaaatgg cgctagatat ataataaatt tttttaacat atggtgtgat
 301 tgatagttat atactaaaga tgtttgctta gttacgtaat ttttttcaaaa aaaaaaggta
 361 cattatcaat catcagtcac aaaatattaa aagttactgt ttgtttttta aattccatgt
 421 cgaatttaat tgaatgacac ttaaattggg acgaacggtg taatttcttt tgactattct
 481 actagtatct atccacagca cgtgttgttc ctttcttctt tcgtttttca tttacttgac
 541 attattagga gacttggccc tgaactccaa ctattctaag ctgacctttc ttttccttta
 601 ccaattatct tcttctttct aatttcgttt tacgcgtagt actgcctgaa ttttctgact
 661 ttcaacgttt gttattcatg cttgaaaacg aaataccagc taacaaaag
```

Sequence of *Rpi-vnt1.1* present in pSLJ21152

```
   1 atgaattatt gtgtttacaa gacttgggcc gttgactctt actttccctt cctcatcctc
  61 acatttagaa aaaagaaatt taacgaaaaa ttaaaggaga tggctgaaat tcttctcaca
 121 gcagtcatca ataaatcaat agaaatagct ggaaatgtac tctttcaaga aggtacgcgt
 181 ttatattggt tgaaagagga catcgattgg ctccagagag aaatgagaca cattcgatca
 241 tatgtagaca atgcaaaggc aaaggaagtt ggaggcgatt caagggtgaa aaacttatta
 301 aaagatattc aacaactggc aggtgatgtg gaggatctat tagatgagtt tcttccaaaa
 361 attcaacaat ccaataagtt catttgttgc cttaagacgg tttcttttgc cgatgagttt
 421 gctatggaga ttgagaagat aaaaagaaga gttgctgata ttgaccgtgt aaggacaact
 481 tacagcatca cagatacaag taacaataat gatgattgca ttccattgga ccggagaaga
 541 ttgttccttc atgctgatga aacagaggtc atcggtctgg aagatgactt caatacacta
 601 caagccaaat tacttgatca tgatttgcct tatggagttg tttcaatagt tggcatgccc
 661 ggtttgggaa aaacaactct tgccaagaaa ctttataggc atgtctgtca tcaatttgag
 721 tgttcgggac tggtctatgt ttcacaacag ccaagggcgg gagaaatctt acatgacata
 781 gccaaacaag ttggactgac ggaagaggaa aggaaagaaa acttggagaa caacctacga
 841 tcactcttga aaataaaaag gtatgttatt ctcttagatg acatttggga tgttgaaatt
 901 tgggatgatc taaaacttgt ccttcctgaa tgtgattcaa aaattggcag taggataatt
 961 ataacctctc gaaatagtaa tgtaggcaga tacataggag gggatttctc aatccacgtg
1021 ttgcaacccc tagattcaga gaaaagcttt gaactcttta ccaagaaaat ctttaatttt
1081 gttaatgata attgggccaa tgcttcacca gacttggtaa atattggtag atgtatagtt
1141 gagagatgtg gaggtatacc gctagcaatt gtggtgactg caggcatgtt aagggcaaga
1201 ggaagaacag aacatgcatg gaacagagta cttgagagta tggctcataa aattcaagat
1261 ggatgtggta aggtattggc tctgagttac aatgatttgc ccattgcatt aaggccatgt
1321 ttcttgtact ttggtcttta ccccgaggac catgaaattc gtgcttttga tttgacaaat
1381 atgtggattg ctgagaagct gatagttgta aatactggca atgggcgaga ggctgaaagt
1441 ttggcggatg atgtcctaaa tgatttggtt tcaagaaact tgattcaagt tgccaaaagg
1501 acatatgatg gaagaatttc aagttgtcgc atacatgact tgttacatag tttgtgtgtg
1561 gacttggcta aggaaagtaa cttctttcac acggagcaca atgcatttgg tgatcctagc
1621 aatgttgcta gggtgcgaag gattacattc tactctgatg ataatgccat gaatgagttc
1681 ttccatttaa atcctaagcc tatgaagctt cgttcacttt tctgtttcac aaaagaccgt
1741 tgcatatttt ctcaaatggc tcatcttaac ttcaaattat tgcaagtgtt ggttgtagtc
1801 atgtctcaaa agggttatca gcatgttact ttccccaaaa aaattgggaa catgagttgc
1861 ctacgttatg tgcgattgga gggggcaatt agagtaaaat tgccaaatag tattgtcaag
1921 ctcaaatgtc tagagaccct ggatatattt catagctcta gtaaacttcc ttttggtgtt
1981 tgggagtcta aaatattgag acatctttgt tacacagaag aatgttactg tgtctctttt
2041 gcaagtccat tttgccgaat catgcctcct aataatctac aaactttgat gtgggtggat
2101 gataaatttt gtgaaccaag attgttgcac cgattgataa atttaagaac attgtgtata
2161 atggatgtat ccggttctac cattaagata ttatcagcat tgagccctgt gcctagagcg
2221 ttggaggttc tgaagctcag atttttcaag aacacgagtg agcaaataaa cttgtcgtcc
2281 catccaaata ttgtcgagtt gggtttggtt ggtttctcag caatgctctt gaacattgaa
2341 gcattccctc caaatcttgt caagcttaat cttgtcggct tgatggtaga cggtcatcta
```

```
2401 ttggcagtgc ttaagaaatt gcccaaatta aggatactta tattgctttg gtgcagacat
2461 gatgcagaaa aaatggatct ctctggtgat agctttccgc aacttgaagt tttgtatatt
2521 gaggatgcac aagggttgtc tgaagtaacg tgcatggatg atatgagtat gcctaaattg
2581 aaaaagctat ttcttgtaca aggcccaaac atttccccaa ttagtctcag ggtctcggaa
2641 cggcttgcaa agttgagaat atcacaggta ctataa
```

Sequence of *Rpi-vnt1.1* terminator present in pSLJ21152

```
   1 ataattattt acgtttaata tccatgattt ttttaaattt gtatttagtt catcaactaa
  61 atattccatg tctaataaat tgcagggatg cctttgaaaa tgattctgtg ttggagagaa
 121 tcttctgatg cctgttggta ttataatact aataataaga gaaaaagttt gattactgtt
 181 tcaagttaat tgcttgtgat ttgtaaaaac aaattacttt tatatttctc tttgttttat
 241 tttatgttta tttatcttta attaatggag taataaaata aaaatcttat tttcaataga
 301 aaaaagtaga ccttatttgt ggtgcatgta tggtatcttt ttgaaatttt tgatatattt
 361 gctctttgat tcgaatttct tgcttatatg atgatttgca taaatataaa atattataca
 421 aatacctatg ggttggaaaa tatagaaata tgccaatcaa atgtatacaa aaatcattaa
 481 tagatagaat cgtaaaagat atacaaatga gaaatgcttg actaagaagc ttcgtgcaac
 541 ctctcacact gagcacaatg catttggtga tctcggcact attgctgtta cttgtaagac
 601 tacgttcccc aataagtctt tccaaacggc ttgcaaagct gagaatatga aaatctcata
 661 ggttagtttg ctgcgttaat tatttacatt taatatgctc gataaggtga ttttaaaaaa
 721 atttgtacta gttaattcat gaactaaata tttcatttaa tactccataa ttctgaatat
 781 ggaaaataaa taatatttaa taacaagaat aaaatgataa attattcatt gattttataa
 841 attggataaa tattattaaa tattcttaaa taatataatg aacaagtgaa gatgaacgga
 901 gggagtatga agcctctttt caaag
```

SECTION 2: SEQUENCES PRESENT IN INSERT FROM pSLJ21153 (*Rpi-mcq1.1)*

Sequence of CaMV35S promoter present in pSLJ21153

```
   1 catggagtct aagattcaaa tcgaggatct aacagaactc gccgtgaaga ctggcgaaca
  61 gttcatacag agtcttttac gactcaatga caagaagaaa atcttcgtca acatggtgga
 121 gcacgacact ctggtctact ccaaaaatgt caaagataca gtctcagaag accaaagggc
 181 tattgagact tttcaacaaa ggataatttc gggaaacctc ctcggattcc attgcccagc
 241 tatctgtcac ttcatcgaaa ggacagtaga aaaggaaggt ggctcctaca aatgccatca
 301 ttgcgataaa ggaaaggcta tcattcaaga tctctctgcc gacagtggtc ccaaagatgg
 361 acccccaccc acgaggagca tcgtggaaaa agaagacgtt ccaaccacgt cttcaaagca
 421 agtggattga tgtgacatct ccactgacgt aagggatgac gcacaatccc actatccttc
 481 gcaagaccct tcctctatat aaggaagttc atttcatttg gagaggacac gctcgatcga
 541 gtataagagt ctatttttac aacaattacc aacaacaaca aacaacaaac aacattacaa
 601 ttactattta caattacc
```

Sequence of *nptII* gene present in pSLJ21153

```
   1 atggttgaac aagatggatt gcacgcaggt tctccggccg cttgggtgga gaggctattc
  61 ggctatgact gggcacaaca gacaatcggc tgctctgatg ccgccgtgtt ccggctgtca
 121 gcgcaggggc gcccggttct ttttgtcaag accgacctgt ccggtgccct gaatgaactg
 181 caggacgagg cagcgcggct atcgtggctg gccacgacgg gcgttccttg cgcagctgtg
 241 ctcgacgttg tcactgaagc gggaagggac tggctgctat tgggcgaagt gccggggcag
 301 gatctcctgt catctcacct tgctcctgcc gagaaagtat ccatcatggc tgatgcaatg
 361 cggcggctgc atacgcttga tccggctacc tgcccattcg accaccaagc gaaacatcgc
 421 atcgagcgag cacgtactcg gatggaagcc ggtcttgtcg atcaggatga tctggacgaa
 481 gagcatcagg ggctcgcgcc agccgaactg ttcgccaggc tcaaggcgcg catgcccgac
 541 ggcgaggatc tcgtcgtgac tcatggcgat gcctgcttgc cgaatatcat ggtggaaaat
 601 ggccgctttt ctggattcat cgactgtggc cggctgggtg tggcggaccg ctatcaggac
 661 atagcgttgg ctacccgtga tattgctgaa gagcttggcg gcgaatgggc tgaccgcttc
```

```
 721 ctcgtgcttt acggtatcgc cgctcccgat tcgcagcgca tcgccttcta tcgccttctt
 781 gacgagttct tctga
```

Sequence of octopine synthase terminator (*ocs 3'*) present in pSLJ21153

```
   1 tgctttaatg agatatgcga gacgcctatg atcgcatgat atttgctttc aattctgttg
  61 tgcacgttgt aaaaaacctg agcatgtgta gctcagatcc ttaccgccgg tttcggttca
 121 ttctaatgaa tatatcaccc gttactatcg tatttttatg aataatattc tccgttcaat
 181 ttactgattg taccctacta cttatatgta caatattaaa atgaaaacaa tatattgtgc
 241 tgaataggtt tatagcgaca tctatgatag agcgccacaa taacaaacaa ttgcgtttta
 301 ttattacaaa tccaatttta aaaaaagcgg cagaaccggt caaacctaaa agactgatta
 361 cataaatctt attcaaattt caaaagtgcc ccaggggcta gtatctacga cacaccgagc
 421 ggcgaactaa taacgctcac tgaagggaac tccggttccc cgccggcgcg catgggtgag
 481 attccttgaa gttgagtatt ggccgtccgc tctaccgaaa gttacgggca ccattcaacc
 541 cggtccagca cggcggccgg
```

Sequence of *Rpi-mcq1.1* promoter present in pSLJ21153

```
   1 cattatcata tttcttttct agctcttcaa taacttcttt atcagcttca gcacttccaa
  61 aaattatgtc atctatctct ccacctgaaa tttggccttc aagattatct tcttcatcta
 121 tttgatcttt atcttctttc tctgtttcca atgttcgatt taagccaatg attgaccttg
 181 ttatagctaa cgattgatca gagacttcaa cttctgatac atctccctca ttgctttctc
 241 taaaatgtac attgttggca tatatagctt tagatactgt ttgttcaaaa tctacttctt
 301 tgatttcttc gataaaagct accatatcac tagtatgagc atcttcgaga atttcttgct
 361 tcatatcgat gacatcagtg tcttgcttct ccacgtgatc gactacatta tattctgaat
 421 cctcgatcaa ctctttttca agattgatgg aaactatagc atctattgct caagccttag
 481 ctactaaaca tttttcccac tcttcttctt ctttagcaac cttttgagct atattacttt
 541 ccttggctgg acaatatctt tttatgtggc ctacttcgcc acaacgataa catctgagag
 601 gcttcttacc ataatagtta gaaggctctt ccttctctct aggtgagtgt gaaccacctg
 661 aggatcgaga tgtgacatat ctcttgtttt tcctatagag ttcctcttgt tagctacaag
 721 agcatttttt tctccttctt tgacaaatac accagccaat tgcttggcta gtagttcctg
 781 tgacgacaac aaatttttcaa actccctcca agaatggtta atgagcccat cccttgaatt
 841 gacgtcacaa atgaaatata ttctggcttc aaaccacgaa tgactattct tctcattcgt
 901 gcttccgaga tagcctcttt cgggttcaac aaagatatct cagaacataa gttcttaata
 961 ttcaaaaaat actcgaagat agaaagatta ccttgagtga tgttagccaa tttattctcc
1021 aatatctgca gccgagcttc attcttgttg ttgaacaact gatcgagggt cttccatatt
1081 tcatgagcta atttacactt tataatgtga tcaaataaat cggaggagat tgtcctcttt
1141 agaatgaact ccgccttcgc attaacctgc ttctacttct tgtgtatgct actatttttc
1201 ggtccgtcag cagaaggact tatgttactc ctattcacaa catcccacaa atcctcgccc
1261 tcaaggtatg attccataca tgtcctccat accttgtaat tggattggtt caacaactcc
1321 atccccaatc cgttaacacg acctcttaaa tccatttaca caaactaatt gaatctacca
1381 ccaacccaat cttgaaataa aacccagaaa ccaatgtatg gttatggctc tgataccatg
1441 tagagaattg cagaagaaaa gtataatcgg agaacccttc tgctagccca agatagttaa
1501 caaagatcgg aagactcaac taaagaagag gaagctagaa aggagaactt gtttatggaa
1561 gaagactctt ttatttgagt tgttcttatg ttgtcttggg ttttggttgt gtacaaatga
1621 ctaatgccac ctctatttat agttgtgtag ggatgattct agatactaat aatctagact
1681 attctaacct cttctacaaa tatcttcctc aaatatctaa aactctagac tatctagatt
1741 tttcctaact acaaatatca aagatattac tctacaatat tctagaaagt tctacaaaat
1801 atctaagata ttttttgtac ctccaaaaaa tcaactttaa ttttttacag tatgaactcg
1861 acgtcagatt aatcatgata ttttcattca tagaagttga tattccaatg ggtcaattgg
1921 tttagtactc gtattttata aatttatttt tagttttata tatttttat aaaaaaatct
1981 tcattttcac acataatatt gtaagttgct gtatattctt caccctaaat aaccctaaag
2041 tctttaactc aagattcctt cactatttcc tctcatccta ttttctttat accaaactct
2101 tcgagtgttt ttagcatgtt tgagcataga caataacttt gctttgtaat tagtaacgtc
2161 acactcttaa ttataacgac aataataaca tatttaatcc gttatagttt tacaaaaaaa
2221 aataaaaagt tatttttgat gagctccttc attcaagtaa atgtcacact cttaattgat
2281 ttgtaaatat caatttattt ggaccatagg cacatgttaa taactcaatg atgaaaagga
2341 catttgagaa aattaaagtg tcggcaaggc ttaatttgtt gcacaaaaag ccaattagca
```

```
2401 ataagattat taaacattga aaagacttaa ctctaaaatt tgtatgtaaa tagtgggaca
2461 acattcttat tattataata ttaatttatt aattagggac tatgatttca tgtcaattga
2521 aatttagact ttagaaaaac aataaaagtt ctttgttaac aagaaagtga ctaattggtg
2581 ctacacatga agtatgaaaa aacacttttt taggaaaata agttcgttaa agaataaaaa
2641 aagggactta tctaatagaa gtaggaaaaa taagttatac aaatgacatt ttacattgat
2701 tatgttctcc gcatcattca acacacctca tctccaccct acctccgtat aggtatagcc
2761 tccatcatca ccactcacca ccctatcacc cctaccccta tctcgactac atcccacaat
2821 atttatctaa attatataca aatatttgta acatcttttc tttatacctt gggcttaaga
2881 cttgtttcct gggctttaga gaacagaaca attagtcttg gcccaaatta atttttccaa
2941 ttgtacaatc actcatacaa ggcaaagtgc atggatatcc aattttgacg tcgccaaagt
3001 tgtcatttaa cttgtgttgc ttacatttca gataatacgg tgtctgaact taatctttaa
3061 cgaagtccaa ctgcacatag ccgacaagta catatgcctg aagttcaatt atttttttgtt
3121 gaactttagc acataaaaac tcaatgaata ataaatactc tctactaatt tgaatcgact
3181 tgcccactta gaccacagtt agggcaaggg ctgccatgaa gttgtgaata actagaagac
3241 agaagcagtc aagtgatcat atgataaaac cttaatatag aagaatacat taaaagctaa
3301 ggaaaatccg ttcaacacaa catcgcgttt aaatcctatc tcctacctac gaaatttgcc
3361 atgcttgtat agacaagtga gcaaagatta caagttttac ttagtatgca tcattttgtc
3421 tagatccacc atagccacca ccaaatccac caccaccata tccaccacca ccagaacgaa
3481 aaccactgct gcgtggagct ctctcttggg ctagagagac gcggatgttc cttccgttca
3541 gatcctgcag gtaacataat gtaatatcag ccatgattca tgaataatca gcctctctac
3601 ctctgagagg taggtctgag aggtaggagt aaaatctgca tacattctac cctcctcaga
3661 ccccactttg taagactaca ctaagtatgt tgttgttggg tttcaactaa tatccacaac
3721 cgaaaatgat cgaaaattta atccaaatcc ttgttaccaa acagtccctt aaactataca
3781 aaggcataaa ccaaaagtgg gcattccact tgcatattca atggtttcat gtattactaa
3841 caaaatttca cctgtccatc cattgctgac atagcctctt gagcagattc gccatctgtg
3901 aagttcacga atccaaatcc ctttgatttc ccagaatctc tatccatgat gaccttagct
3961 gtgccacaat caaagtacac aagtatttaa cacaagagca aagttcaaac agcagaaagt
4021 aagagctagc aagcgagatg aaagggagga gcatgtcaaa aaagccagaa atattggttc
4081 aactccaatg ttaactaatc ctagcaccca caacctaaac gagggagatg aagatagtaa
4141 ctgataatgc cttaaccgat agaacaaaat aagaaactac cagaaaaatg ccactatccc
4201 agcccaatca ctagagtatg caaccaaata tgcaattaat gactaaatcg tgtggagcat
4261 tcgttatata aaaacttaag gtcatgaaca caaatcacga gctgcagaaa atccatcaag
4321 tttcatagcc tagttagcta gaggaacaca agacaacagg taaatttatg catctaccat
4381 gccatatata ggcttaaatt atagagatcg caagctatta tcagatacat atcgtactat
4441 gtattatcct tcataaaaga tatagtatat ttcatctaac ttgattttca ttgtgttgtg
4501 cattcaaata ttcatcatca gagaataaaa acaagatgga aactaaagca agccagactc
4561 aagatagttg ggagattacc ctcgacgaca tcaccaaagc tgttgaaggc ttccttcagg
4621 gactgatcat cagttccaaa tgaaagacct attaaattga ggaataaata agcaaaactt
4681 tacatttcgc agagtattca ctaagaaaca atacttgtga tatttttttc agacagcaag
4741 tcacatacct ccaataaaaa gctttgttga catgcaacgg atggcgttaa gcattgaagg
4801 tgctgatgat tgtgcattca atgcattgcc gcttgttaaa atgctctgct tcaaaagacc
4861 accaagtttg ttgtagaaag ccatagatat ctataaaaag aaggttatac aaatttagct
4921 tttaagtatg aaaaccttta atctcacagg caacatcaat aagaagattg acaaaacata
4981 aaacttatac aaagggtaga cgaatgacac gactaaatga agcaaaactc attcatttaa
5041 atgcatactc gaacaaaaaa aacatagcag cacacatcca caaacaagat ttcagaaagc
5101 acatatgagg tgaaatctat aagctctcta ggtcctcctt tccttcactc cttatccccc
5161 tgggagttgt tactagatat tagaactcat gttcagatac actacacaaa aattaaattt
5221 ctagcctaat attatatctt aggttcacaa ccatcttcac aaatcacaga ggcagagcta
5281 cgattttgag cttatgagtt ctagattatt ttttttaaaa aagtagttta ttgggttctg
5341 gatagactat ttatacatct tgagtgaaat tttcaacacg aatacagagt cagttttccc
5401 aaacccatga gatgcattgc ggctttgccc tgccaactag gtaatcattc aacagcagtc
5461 agttaagttt ctgaagcaaa taacaagtga gagcaacatt caagtatctt tatttcaaaa
5521 cagaacaatc atcaaactcg tttaactcaa ttacacaaat tagagaaata cacttcatac
5581 atacaagaaa aagaaacacc actaattaca aaagatatca aactaataag aaggccaaag
5641 catcatctca ctttgaacta gacacacaac aatattttcc accaaacata aaaaagcaat
5701 gagtaaaagt tctacaagga aaacattatc agacttgaac agcgcaaaga gtgatttttt
5761 tttaaccaaa catgaaaaac gagtaaaagt tctacaatat gaaaccaaac ataaaaccaa
5821 gatacaccaa agacttacca aaactccata ttaaccaaaa aataaagaaa cataacataa
5881 caattaggta aagaaccatg atataaccaa ataagcataa atccatctca aaaaactcat
5941 ttttttttcac caaacatcaa aaacaatgag taaaagctct acaatataaa ccaaacataa
6001 aaccaatata ccccaaaaac ttaacaaagc tccatattaa ctaaaagagg aaatacataa
```

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6061 ttaccccatc aaacttagac ccctttttg aagtagacac ttaaagtttg cgcaagtcct
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6181 catcagctgt gatatcacgc ttaagaagcg catgaatgag gaaaatttag cttcaaccag
6241 tggaaatatg actcatatca tgtcaatttg atctcccaat tactgcatct acttcactta
6301 acgaataggg caacaaaatt gaagaaaacc cgagttctga gcaaaacctt tagcggagta
6361 aaaataactg ttgaatgctc catcccaccc ccaccgacga agaagggaga gaaaaaaaca
6421 gagaatgaaa caacaaaatc tgtctccgtt gccaaggaaa tcagctagcc gtggttgaaa
6481 ttcttcgccg gcaacgaccc taaaaggtgt tttggttgat ggactgagat tgtgttattc
6541 agttacaact caatcctttc atgaaaattc catccgaaac aataaaaaaa atacccataa
6601 tttaaaattg aaattattag agagtggtca attagttgca gtatctgata aagatacaaa
6661 atgtgcagag ttagtttttt ggaacacaca ttctaggaaa aattgataat ctaaacatca
6721 aatttattgt ttgattcgct taacttgtct agttcatggt tgcatttgaa aaaaaaaaca
6781 aagcaatagt ttaagcggaa tgaaggagaa aggagagagt gaaatgatat atccagttgg
6841 gtttagaggt tgaaatgtga acggatctgg gttttacccg gtcttttatt aaatgggtgg
6901 tagaaaataa attatatata tatatttttt ggagtgaaca cacgcgcagg tgctgagatt
6961 accattgttg tccaaatggt gtatttataa tggttgaaaa ttgtttcgtg gtgtaatagg
7021 actcccacaa actttaagtg tctgcttcaa aaaatggttt aagtttaatg gggtaactat
7081 gtatttcctc taactaaaaa tcaaaaacca tagcaaaaaa ataaggtaaa gaaccataat
7141 ataatcaaat aagcataaac ccatctcaaa aaactcattt tttttaacaa taaaccaaac
7201 ataaaaccaa tataccccaa agacttaaca aagtttcata ttaactaaaa atcaaaaacc
7261 atagtaaagc aataacgtaa agaaccataa tataatcaaa taagcataaa cccatctcaa
7321 aaactcattt ttttcatcaa acatcaaaaa acaatgagta aaagttctac aacaagaacc
7381 aaacataaaa ccaagagacc ccaaagactt aacaaagttc catattaaca aaaaatcaac
7441 aaccataaca aaacaataag gcaaagaaca atagcataac caaataagca taaacccatc
7501 ttaaaaaact catttttatc accaaacatt aaaaaactca ttttttcac caaacatcaa
7561 aaaacaatga gtaaaagttc tacaacatga accaaacata aaaccaacat accccaaaca
7621 cttaacaaag ctccatataa acaacaaaac aacaaggcaa agaagcataa tatagcaaaa
7681 taagcataaa tccatctcaa acaaattata aaaaaactaa cctaatgaag acaagttttc
7741 agggtttaag aggcaagaaa atgagaagcg gctaggtctt actgtgaact gtggggttta
7801 agaaagggta tatataagta cactgccttt cgacttttc agagtgaaaa aaatactcat
7861 atatctgcgg cgttttaaaa ggagctcgag ggtaatttta ctgcttagag gtgttgtacc
7921 ttgatttta aagagagtat ttttggaatt aatgtacaac atgcattatg cgaactcata
7981 atagtttgta aatgagcaat tgtcgagatt atgaaagcta ttttaggatg ttatgtgaat
8041 tatttgtatt tatttcgaaa tagtttttca ctttatttca aaagcagttt gattgtaaaa
8101 atcgtcaatt tttagttgtt ttattctttc atttgcaaga aaaaaaaatt aagcataaat
8161 ctattttcaa tttcaattct ataaatatta cgaaaaatat ttgaatttca caatcaaatg
8221 cccatttagt ttttttttt ttaaacttta atacgagact tttttcatat tttatatttt
8281 cctcaaatta gatccttttt tttcctttcc ttgttgtaag tccttgtgaa aaaacctcca
8341 aatcctaact tgtgttgtga taccacaagg atttaaagat tacacataat gaaacaaaaa
8401 aaaaaaaaaa tcaattcgag cttcgaaaat gaaaaaaatt gataaatttt ttttttcttt
8461 aatcactatt acgtgataca aatttgaatt agtcgaatta atatatttaa aacaaaacac
8521 tccttatcag aaaagtgaag aaattctgac cattccacta gagtcattat ggtgatggaa
8581 gtttaataaa atagaaccga agaatcgaat gcccactcaa atttttttga gagcccaaac
8641 tcacagccat gaacccaaat tgggtaaagt tttgcaagac gttcatctaa cagttaggaa
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8761 actaaagatg tttgcttagt tacgtgattt ttttaaaaaa aaagagagta cattatcaat
8821 catcagccac aaaatattaa aagtcacagt ttgtttctta aattccatat cgaattaaat
8881 tgaatgacag ttaaattgga atgaatggtg taatttcctt tgactattgt actagtatct
8941 tatccacagc atgtgttgtt ccttccttct ttcgtttttc atttacttga cattagtagg
9001 agacttggca gtggactcca actattctaa gctgaccttt cttttccttt accaattatc
9061 ttctcttttc taatttctca ttctgatcgg tttttgtagc tactgaaaaa gaaagagtga
9121 agaa
```

Sequence of *Rpi-mcq1.1* present in pSLJ21153

```
   1 atggctgaaa ttcttcttac agcagtcatc aataaatctg tagaaatagc tggaaatgta
  61 ctctttcaag aaggtacgcg tttatattgg ttgaaggagg atatagattg gctccaaaga
 121 gaaatgagac acattcgatc atatgtagac aatgcaaagg ccaaggaagt tggaggtgat
 181 tcaagggtga aaaacttatt aaaagatatt caacaactcg caggtgatgt ggaggatctc
 241 ctagatgagt ttcttccaaa aattcaacaa tccagtaagt tcaaaggcgc aatttgttgc
```

```
 301 cttaagaccg tttcttttgc ggatgagttt gctatggaga ttgagaagat aaaaagaagg
 361 gttgtggaca ttgatcgtgt aaggacaact tacaacatca tggatacaaa taacaacaat
 421 gattgcattc cattggacca gagaagattg ttccttcatg ttgatgaaac agaggtcatc
 481 ggtttggatg atgacttcaa tacactacaa gccaaattac ttgaccaaga tttgccttat
 541 ggagttgttt caatagttgg catgcccggt ctaggaaaaa caactcttgc caagaaactt
 601 tataggcatg tccgtcataa atttgagtgt tcgggactgg tctatgtttc acaacagcca
 661 agggcgggag aaatcttaat cgacatagcc aaacaagttg gactgacgga agacgaaagg
 721 aaagaaaact tggagaacaa cctacggtca ctcttgaaaa gaaaaaggta tgttattctc
 781 ttagatgaca tttgggatgt tgaaatttgg gatgatctaa aacttgtcct tcctgaatgt
 841 gattcaaaaa ttggcagtag gataattata acctctcgaa atagtaatgt aggcagatac
 901 ataggagggg atttctcaat tcacgtgttg caacctctaa attcggagaa cagttttgaa
 961 ctctttacca agaaaatctt tatttttgat aacaataata attggaccaa tgcttcacca
1021 aacttggtag atattggtag aagtatagtt ggtagatgtg gtggtatacc actagccatt
1081 gtggtgactg caggcatgtt aagggcaaga gaaagaacag aacgtgcatg gaacaggtta
1141 cttgagagta tgagccataa agttcaagat ggatgtgcta aggtattggc tctgagttac
1201 aatgatttgc caattgcatt aaggccatgt ttcttgtatt ttggccttta ccccgaggat
1261 catgaaattc gtgcttttga tttgacaaat atgtggattg ctgagaagtt gatagttgta
1321 aatagtggca atgggcgaga ggctgaaagt ttggcggatg atgtcctaaa tgatttggtt
1381 tcaagaaaca tgattcaagt tgccaaaagg acatatgatg gaagaatttc aagttgtcgc
1441 atacatgact tgttacatag tttgtgtgtt gacttggcta aggaaagcaa cttctttcac
1501 accgagcaca atgcattggg tgatcccgga aatgttgcta ggctgcgaag gattacattc
1561 tactctgata ataatgccat gaatgagttc ttccgttcaa atcctaagct tgagaagctt
1621 cgtgcacttt tctgttttac agaagaccct tgcatatttt ctcaactggc tcatcttgat
1681 ttcaaattat tgcaagtgtt ggttgtagtc atctttgttg atgatatttg tggtgtcagt
1741 atcccaaaca catttgggaa catgaggtgc ttacgttatc tgcgattcca ggggcatttt
1801 tatgggaaac tgccaaattg tatggtgaag ctcaaacgtc tagagaccct cgatattggt
1861 tatagcttaa ttaaatttcc tactggtgtt tggaagtcta cacaattgaa acatcttcgt
1921 tatggaggtt ttaatcaagc atctaacagt tgcttttcta taagcccatt tttcccaaac
1981 ttgtactcat tgcctcataa taatgtacaa actttgatgt ggctggatga taaatttttt
2041 gaggcgggat tgttgcaccg attgatcaat ttaagaaaac tgggtatagc aggagtatct
2101 gattctacag ttaagatatt atcagcattg agccctgtgc caacggcgct ggaggttctg
2161 aagctcaaaa tttacaggga catgagtgag caaataaact tgtcgtccta tccaaatatt
2221 gttaagttgc gtttgaatgt ttgcggaaga atgcgcttga actgtgaagc atttcctcca
2281 aatcttgtca agcttactct tgtcggcgat gaggtagacg gtcatgtagt ggcagagctt
2341 aagaaattgc ccaaattaag gatacttaaa atgtttgggt gcagtcataa tgaagaaaag
2401 atggatctct ctggtgatgg tgatagcttt ccgcaacttg aagttctgca tattgatgaa
2461 ccagatgggt tgtctgaagt aacgtgtagg gatgatgtca gtatgcctaa attgaaaaag
2521 ttgttacttg tacaacgccg cccttctcca attagtctct cagaacgtct tgcaaagctc
2581 agaatatga
```

Sequence of *Rpi-mcq1.1* terminator present in pSLJ21153

```
   1 aattcacaat gtgtcaatat ataggttagt ttgctacgtt aatctcccat tatgtctaat
  61 gaattgcgcg cagatgcatt tgagaatgat tgattgtaaa ttgtaattgt aataaataaa
 121 taaatgtttg attgctttct gaagttgatg tatttgtggc ttgtgatttg taaaacatat
 181 ttatttattg tcttatcact tatgtttatt tacctttgga attagcagta gctttcgttt
 241 cttctcttct tcaataatca atgctcgcaa atataaatta ggggcgtatt ttattggttt
 301 ggtttatcgg tttataaatt cgtttaatta ataaccaatt caattaaata ttttttatc
 361 ggttttgggt ccttagcggt tcgatatttg atttaaccaa taagaaaata cttataaaac
 421 aaatatatga cttctcaaac aatttagcgt ggcaagataa taccgtaact ttacaaatac
 481 tcataaaata gaaacaacaa taactaacat gaaaagaatt atacaagtgt aacacaaaga
 541 aaaactaaga ggaatatgct tcttacttta cattttgacg ttttgtataa tgtgaatttt
 601 tgaacttaaa gtcactgtga agtgtgatgt gaaggtgaaa ggacaaatgc actaactagt
 661 aaggtattgc gattaatatt taatgtttat gtatgagtaa aatagtaaat tattatagtt
 721 ttattgggtt atcagtatac ccaataactc aatattaaaa atcaaaatcg aaccggtaac
 781 ccaatatttt tttctttcta taaaaccatt aaaacctcat tgacccaata acccaataac
 841 aataaatcaa tagcactttt ttcattttaa tttatcgatc gattagattt ttgcaaccca
 901 ctaatataaa ttactacctg ttatagcaag tgcaagtaga gaattgatat atagctcaca
 961 ttttacaaat tctttctagt gttaatcgtc aaaaacatta gcttctcaat aatatatggc
1021 taataattat tttaagtaat catatttgtg acaattaata ggtttaagat gagaaataaa
```

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1081 tcaaagtatt ttatttaata aagggaatgt atataatttt cttcactatc acgcgaagtg
1141 caagttgttt tattatgtac tctcccgatc tcagttgatc attttccttt ttacaagcac
1201 actaagtatc atagataaga agtaaatttt attaattcat tccttaaaaa aattcttaaa
1261 atttaataaa caaatgtgaa cactataaaa aagaataaat tgcatggata atatagaaaa
1321 aagaaattaa ttaatggttt cttcatttag aaagatgaac aactatatat ttttaataaa
1381 ataatcaact aatatggaac aaaactacag attgaatagt tacaactaaa gtgaaaaaaa
1441 gagttcttca cgagaaatac ttctgaaata ttcatttatt tcttttgtaa catttacatc
1501 cagacaaaag tgaaaatatg attatctttt tttaattctc caacattata ctttccttct
1561 acttttttcat gagaaaaaaa ggtaagatgc ctatatatgg tttaagtaaa gaataaaatg
1621 gaaattatgg cccatcttac cctcacattc aagtcaaata ttgaactaat ttatttattt
1681 tttataaaaa atatacttat aaatagaatt gaagtgcttg ggttaatttg tcaatttcca
1741 tcttttgaaa aaaacactta taattaatat ttactccaat caaggaactg catcacctaa
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1861 agttagaact agtagtaaat aaattgtata attactatgc tcctatatgt atgaccaata
1921 ttatcaatta aaaagatacg tacaattatt ctattttaat tgataaaacg gaaaagggtc
1981 aaaattgctc taaactatgt aaatagatta tttatacccct ccattatatt ttgggatcaa
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2221 attaccacta aaggtatgta atgaagggta taagcggtct atttcacata gttcaggggc
2281 aattttgacc ctttttttgtt gataaaatga tattaagaag aaaaaaaaat tctttctact
2341 tatttattaa ttaaagttat tagagaaaaa aaaataagaa tatgattctt taagaaaata
2401 agaatacggt taaatattga aacgtatacc ataactcaac cacaaaatta acttattgat
2461 attgttatca gattaacact cggtgaagtt aataagactt gggtattatg aagaattgac
2521 atggtttatt gtcagatgct ttgttcacta taaataatat tttgttaggt ttttaaaata
2581 ggaataactt atcaaaagtt gtactttaaa atttgtagtt ccttcttgca ttatttttta
2641 ctaagaaatt aatctaaagt cttttgagat aatatctcaa atggtcactc aactatgaat
2701 atctttctca gaaagtcact caactattga tagttttctc agaaagttac ttaactttat
2761 tttgtaaatc aaaagacact caactatgtg tagttatctt agaaaatcat tcaactagga
2821 ataattatct tagacagtca ctcagatatt gatatttcac atagactgtc accaaaccaa
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2941 aagaaataaa aaagttcatt ttaatctttt tattgacgta tcccactaaa attaagtaat
3001 atatataaaa caacaaaact taatagtata gttagtaaaa caaaattata tctaacgtac
3061 caatataaat tataaataca aatataaaca actcagttac ttattttata taagaaaatt
3121 aacttataag tggataatca gttaattatg tcaacgtttg aacaatagtt agacaataac
3181 gaagactcca atgtttctaa ttatgtgaga aaaattagag tagcaaatta tgtagtcata
3241 gttgagtgat tgctttaaat attattttaa acgcatccaa aatgaagtct tgataaatat
3301 atatttctct ataattttag tggggtgaat caataaaatg attaaaattg acacttttt
3361 atttttaaa atttagtata aaataaaaat ctcaagtcaa caaaattaat gaagaaatta
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3601 atctcaagcc ttttactttc ttcaccccaa tgatggaagg caggaaatta gattacatgc
3661 atgttggtac actttgtgct aatcgtaata attgttaact tggtgttaaa tcaatcgata
3721 ttttattgat aattagctag catattatag tacatttaaa ttcattaact gatatagtac
3781 attttattga taattcatta actgatatag tacatttaaa ttcattaact gatagctagc
3841 atattatagt acattttatt gataattagc tagcatatta tagtacattt aaagccgtta
3901 gtaatgtttc ttctgccaac aagtttgtcg cagagattga aaaaataaaa agaagagttg
3961 ctgagattga tcgtttgaga acaacttatg gtatcact
```

Sequence of truncated NB-LRR gene present in pSLJ21153

```
   1 gatacaagta acaacaacaa tcaacatgac tacattccgt tggaccagag aggattattc
  61 cttcatgctg atgaaacaga ggtcatcggt ttggatgatg acttcaataa gctacaagcc
 121 aaattacttg atcatgattt gccttatgga gttgtttcaa tagttggcat gcccggtttg
 181 ggaaaaacaa ctcttgccaa gaaactctat aggcatgttc gtgatcaatt tgagtgttct
 241 ggactgatct atgtttcaca acagccaagg gtgggaggaa tcttacatga catagccaaa
 301 caagttggac tgacggaaga ggaaaggaaa gaaaacttgg agaacaacct acgatcactc
 361 ttgaaaataa aaaggtatgt tatcctctta gatgacattt gggatgttga aatttgggat
 421 gatctaaaac ttgtccttcc tgaatgtgat tcaaaaattg gcagtaggat aattataacc
```

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 481 tctcgaaata gtaacgttgg aagatatata ggaggagatt cctcactctg tgtgttgcaa
 541 cctctagatt tagataatag ttttgaactc tttagcaaaa aaatctttaa ttttgataac
 601 aataataatt gggccaatgc ttcaccagag ttggtagata ttggtagaag tatagttggg
 661 agatgtggag gtataccact agccattgtg gtgacggcag gcatgttaag ggcaagagaa
 721 agaacagaac gtgcatggaa cagagtactt gagagtatga gccataaagt tcaagatgga
 781 tgtgctaagg tattggttct gagttactat gatttgccca ttgcattaag gccatgtttc
 841 ttgtactttg gccttttccc cgaggaccat gaaattcgtg cttttgattt gacaaatatg
 901 tggattgctg agaagctgat agttgtaaat agtggtaata tgcgagaggc tgaagatttg
 961 gcggaggatg tcctaaatga tttggtttca agaaacttga ttcaagttgc caaaaggaca
1021 tatgatggaa gaatttcaag ttgtcgcata catgacttgt tacatagttt gtgtgtggat
1081 ttggcaaagg aaagtaactt ctttcacacc gagcacaatg catttggtga tcccagcaat
1141 gttgctaggg tgcgaaggat tacattctac tctaataata atgccatgaa tgagttcttc
1201 tgttcaaatc ctaaacctac gaagcttcgt gcacttttct gtttcaacaa taacagttgc
1261 ctattttctc atatggctca ccttaatttc aaattattgc aagtgttggt tgtagtcaca
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1681 gatgtatccg gttctaccat taagatatta tcagcattga gccctgtgcc taaatcgttg
1741 gaggttctga agctcagatt tttcaagaac acgagtgatc aaataaactt gtcgtcccat
1801 ccaaatattg tcgagttggg tttgtttggt ttctcagcaa tgctcttgaa cattgaagca
1861 ttccctccaa atcttgtcaa gcttaatctt gtcggcttga tggtagacgg tcatctattg
1921 gcagtgctta agaaattgcc caaattaagg atacttacat tgcttaggtg cagccattat
1981 gcagaaaaaa tggatctctc tggtgatagc tttccgcaac ttgaagtttt acatattgag
2041 gatgcacaag ggttgtctga agtaacgtgc atggatgata tgagtatgcc taaattgaaa
2101 aagctattaa ttgtacaagg cccaatcatt tattccccaa ttagtctcag ggtctcggaa
2161 cggcttgcaa tgttgagaat ata
```

Sequence 3’ of truncated NB-LRR gene present in pSLJ21153

```
   1 aaaatctcac aggtactata aataattatt tacgtttaat atccatgatt ttttgaaatt
  61 tgtatttagt tcatcaacta aatattccat ctctaataaa ttgcagggat gcctttgaaa
 121 atgattaagt ctgtgttgga gagaatcttc tgattcctgt tggtattaaa tactaataat
 181 aagagaaaaa gtttgattgc tgtttcaagt taattgcttg tcatttgtaa aaacaaatta
 241 atattgtatt tctctttgtt ttgttttatg tatggtatct ttttgaaaat tttgatatat
 301 ttactctttg attcgaattt cttgcttata tgatgatttg cataaatata aaatattata
 361 catttaccta tgggatggaa aaaattagaa atatgtcaat caaatgtata caaaaatcat
 421 taatagatag aatcgtaaaa gatctacaaa tgagaaatgc tagactaaga agcttcgtgc
 481 aacctctcac accgaccaca atgcatttgg tgatctcggc aatattgcta ttacttgtaa
 541 gactccgttc cccaataagt ctttccaaaa ggcttgcaaa gctgagaata tgaaaatctc
 601 accggttagt ttgctgcgtt aattatttac gtttaatatg ctggataagg tgatttttt
 661 aaaaatttgt actagttagt tcatgaacta aatatttgat ttaatactcc ataattctga
 721 atatgcgtgg aaggatttgg atgtgagtac cgggtggaaa accaagaaaa tggatttact
 781 tgtcatgttt cgtgagagtt aatttgatta actttcagag tcaaattgaa taaaattcat
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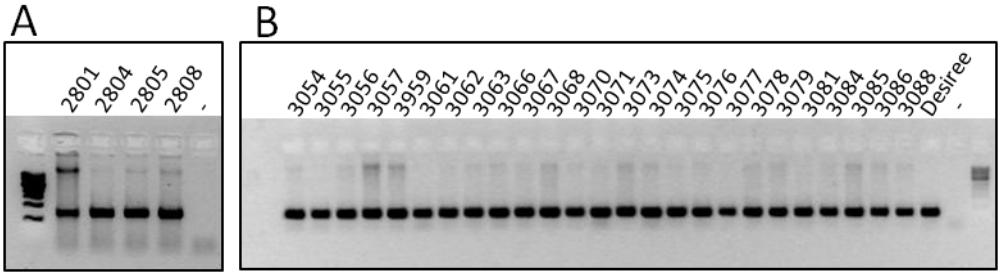
 901 aataatatga tgaaaaaata catctcaaag ttcatatata tatataattt aactctcaaa
 961 aaacgaaacg taacaagtta aaataaaagg atagaataat taattgcaaa aatattcatc
1021 ttaaaatgtt aatcaaagtt catatagttt gactctcaaa aaatgaaacg taataagttg
1081 aaataaacgg atgtaataat ttattgcaaa attattcatc ttaaaatatt gatcccccgg
1141 gctgcaggaa ttcgatatca agcttatcg
```

Section 3: Results from characterisation of transgenic plants

Genomic DNA quality determination

All genomic DNAs analysed for the presence of insert and vector backbone sequences were tested for their quality. We used primers designed to amplify the potato plasma membrane ATPase (primers #3 and #4, designed on HPA1 from *Solanum tuberosum*). The desired product was amplified from all genomic DNAs indicating that they are suitable templates of sufficient quality for use in characterisation of the transgenic plants by PCR (Figure 1).

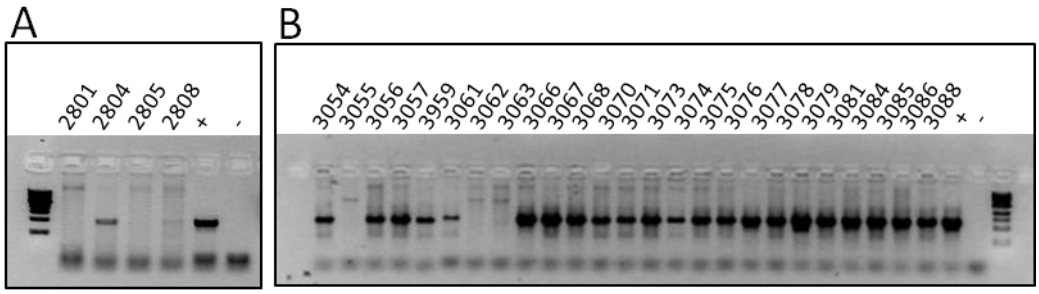
***Figure 1**: Amplification of HPA1 from genomic DNA extracted from Desiree and Rpi-mcq1.1 (A) and Rpi-vnt1.1 (B) transgenic plants.*



Analysis for the presence of transgene in potato lines (Desiree) transformed with pSLJ21152 and pSLJ21153

PCRs using primers against *Rpi-mcq1.1* with DNA from the non-transformed Desiree control produced amplification products, indicating that there are sequences with high homology to *Rpi-mcq1.1* present within the Desiree genome. Consequently, the presence of the insert in the *Rpi-mcq1.1* transgenic lines was confirmed using primers against the *nptII* gene (primer #30 and #31, Annex 1 Section 5). According to this analysis two plants transformed with pSLJ21153 were shown to contain the inserted sequence. The presence of insert in *Rpi-vnt1.1* transgenic plants was confirmed using primers against *Rpi-vnt1* (primer #14 and #15, Annex 1 Section 5). According to this analysis 22 plants transformed with pSLJ21152 were shown to contain the inserted sequence. The results are shown in Figure 2A and 2B and Table 1.

***Figure 2**: Presence of insert in Desiree + Rpi-mcq1.1 and Rpi-vnt1.1 transgenics.*



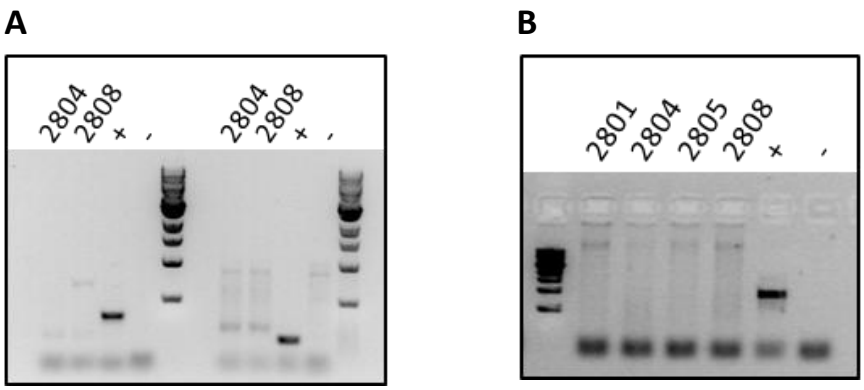
A) Amplification of *nptII* gene in *Rpi-mcq1.1* transgenics (+ve control is pSLJ21153)

B) Amplification of *Rpi-vnt1* gene in *Rpi-vnt1.1* transgenics (+ve control is pSLJ21152)

Analysis of potato lines (Desiree) transformed with pSLJ21153 for vector backbone sequences

PCR experiments were done to show that sequences outside of the T-DNA borders are absent from the plant line to be released that contains pSLJ21153 (*Rpi-mcq1.1*). To determine this, PCR primers pairs designed to amplify regions from the vector backbone close to the left and right borders were used (primers #118/#119 and #120/#121, Annex 1 Section 5). PCR products were not obtained from either of the plant lines tested (PL2804 and PL2808) (Figure 3A). We also tested these plant lines using primers #20/#21 (Annex 1 Section 5) designed to amplify the tetracycline resistance bacterial selection marker gene present in the vector backbone. No PCR products were obtained from the plants (Figure 3B). Plant line PL2808 is the line containing *Rpi-mcq1.1* chosen for release.

***Figure 3:** Testing for absence of vector backbone in transgenics plants containing Rpi-mcq1.1*



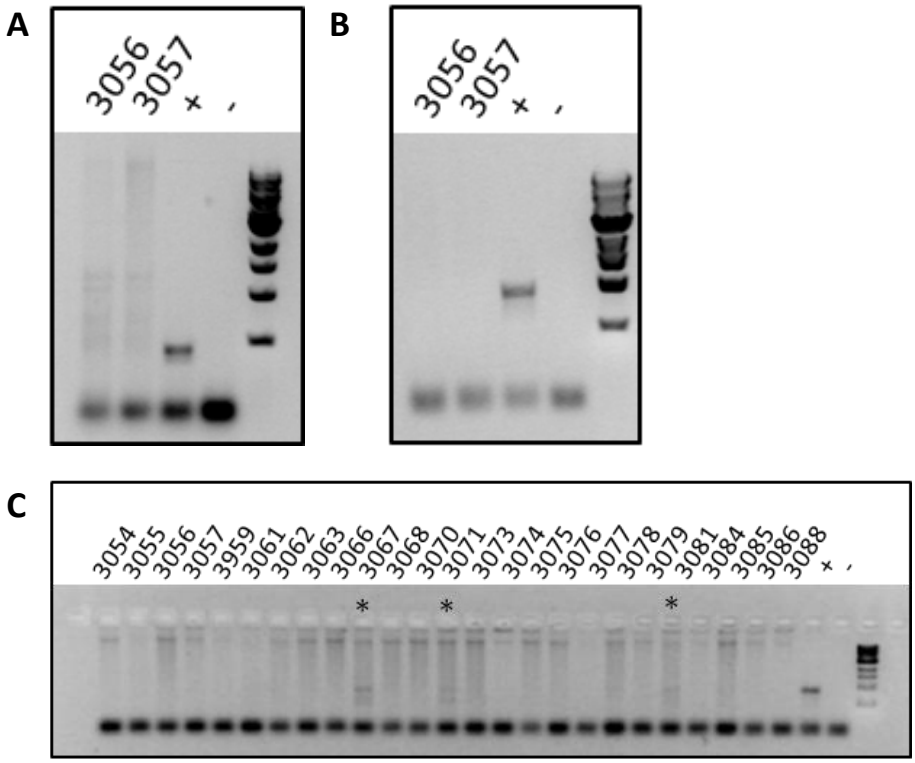
A) Amplification of sequences close to Right and Left borders from lines containing *Rpi-mcq1.1.* +ve control is pSLJ21153.

B) Amplification of tetracycline resistance gene in lines containing *Rpi-mcq1.1.* +ve control is pSLJ21153.

Analysis of potato lines (Desiree) transformed with pSLJ21152 for vector backbone sequences

PCR experiments were done to show that sequences outside of the T-DNA borders are absent from the plant line to be released that contains pSLJ21152 (*Rpi-vnt1.1*). To determine this, PCR primers pairs designed to amplify regions from the vector backbone close to the left and right borders were used (primers #117/#122 and #114/#115, Annex 1 Section 5). PCR products were not obtained from either of the plant lines tested (PL3056 and PL3057) (Figure 4A, 4B). We also tested plant lines using primers #22/23 (Annex 1 Section 5) designed to amplify the *nptIII* bacterial selection marker present in the vector backbone. No PCR products were obtained from the majority of plants (the only exception being lines PL3067, PL3071 and PL3081) (Figure 4C). Plant line PL3056 is the line containing *Rpi-vnt1.1* chosen for release.

***Figure 4**: Testing for absence of vector backbone in transgenic plants containing Rpi-vnt1.1*



A) Amplification of sequences close to Right borders from lines containing *Rpi-vnt1.1.* +ve control is pSLJ21152.
B) Amplification of sequences close to Left borders from lines containing *Rpi-vnt1.1.* +ve control is pSLJ21152.
C) Amplification of *nptIII* gene in *Rpi-vnt1.1* transgenics (+ve control is pSLJ21152)

Table 1. Data for plants transformed with construct pSLJ21152 and pSLJ21153

Plant number	Line	Construct	*Rpi-vnt1* PCR result	Backbone *nptIII* PCR result
3054	Desiree	pSLJ21152	pos	neg
3055	Desiree	pSLJ21152	neg	neg
3056	Desiree	pSLJ21152	pos	neg
3057	Desiree	pSLJ21152	pos	neg
3059	Desiree	pSLJ21152	pos	neg
3061	Desiree	pSLJ21152	pos	neg
3062	Desiree	pSLJ21152	neg	neg
3063	Desiree	pSLJ21152	neg	neg
3066	Desiree	pSLJ21152	pos	neg
3067	Desiree	pSLJ21152	pos	pos
3068	Desiree	pSLJ21152	pos	neg
3070	Desiree	pSLJ21152	pos	neg
3071	Desiree	pSLJ21152	pos	pos
3073	Desiree	pSLJ21152	pos	neg
3074	Desiree	pSLJ21152	pos	neg
3075	Desiree	pSLJ21152	Pos	neg
3076	Desiree	pSLJ21152	pos	neg
3077	Desiree	pSLJ21152	pos	neg
3078	Desiree	pSLJ21152	pos	neg
3079	Desiree	pSLJ21152	pos	neg
3081	Desiree	pSLJ21152	pos	pos
3084	Desiree	pSLJ21152	pos	neg
3085	Desiree	pSLJ21152	pos	neg
3086	Desiree	pSLJ21152	pos	neg
3088	Desiree	pSLJ21152	pos	neg

Plant number	Line	Construct	Insert *nptII* PCR result	Backbone *tet* PCR result
2801	Desiree	pSLJ21153	neg	neg
2804	Desiree	pSLJ21153	pos	neg
2805	Desiree	pSLJ21153	neg	neg
2808	Desiree	pSLJ21153	pos	neg

Table 2. List of primers used to characterise transgenic plants

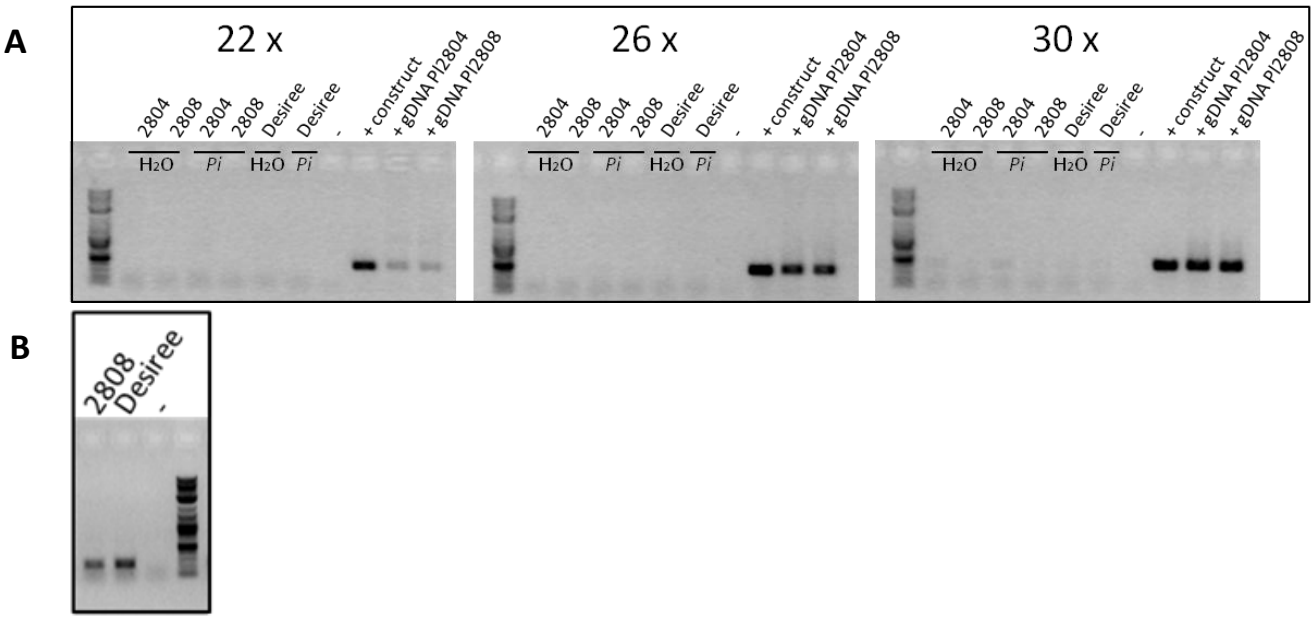
Construct	Gene	Primer number	Sequence 5'-3'
Desiree	*PHA1*	3	TGCTGCAATCGAAGGAATTGGC
Desiree	*PHA1*	4	CTTCACCACTGATTCCACGTGAC
pSLJ21152/pSLJ21153	*nptII*	30	ATGGCTGAAATTCTTCTTAC
pSLJ21152/pSLJ21153	*nptII*	31	TGTCCTTACACGATCAATGTC
pSLJ21152	*nptIII*	22	CGTCGATACTATGTTATACGCC
pSLJ21152	*nptIII*	23	ATATCCTCCCTGATCGACCGG
pSLJ21153	*tet*	20	CTCGTAGGAGAACTTGACCTTC
pSLJ21153	*tet*	21	GCGATGACAACGCAAGCAGCAC
pSLJ21153	*Rpi-mcq1.1*	12	ATGGCTGAAATTCTTCTTAC
pSLJ21153	*Rpi-mcq1.1*	13	TGTCCTTACACGATCAATGTC
pSLJ21152	*Rpi-vnt1*	14	TTCAACGTTTGTTATTCATGC
pSLJ21152	*Rpi-vnt1*	15	ATACTCTCAAGTACTCTGTTC
pSLJ21152	Outside LB	117	TAAGCTGCCGGGTTTGAAACAC
pSLJ21152	Outside LB	122	TGCGCATCTTCATCCTCGGC
pSLJ21152	Outside RB	114	ACGATCCGACAGCGCGCCCAGC
pSLJ21152	Outside RB	115	CCTGAAGTGCCAGTAAAGCGC
pSLJ21153	Outside LB	120	CACCGGGCAGGCGCGCAACAC
pSLJ21153	Outside LB	121	GTGATCGCCGCCGAGAATGC
pSLJ21153	Outside RB	118	ATGGCATTACGTCATTCCTCG
pSLJ21153	Outside RB	119	ATGACGCTGATGCTTCATCGC

Expression analysis of *Rpimcq1.1* and *Rpi-vnt1.1*

To analyse expression levels of the transgenic lines, primers against *Rpi-mcq1.1* (primer #12 and # 13) and *Rpi-vnt1* (primer #14 and # 15, Table 1) were used in semi-quantitative RT-PCR.

Plants of the transgenic lines PL2804 and PL2808 carrying *Rpi-mcq1.1* were inoculated with either water (as a negative control) or spores of *Phytophthora infestans*. 18 hours after inoculation, RNA was extracted and RT-PCRs done for 22, 26 and 30 cycles. As shown in Figure 5A, expression of *Rpi-mcq1.1* was undetectable until 30 cycles. In contrast, expression of the constitutively expressed reference gene *PHA1* was detectable from 22 cycles (Figure 5B). Plant lines PL2808 is the line carrying *Rpi-mcq1.1* chosen for release.

***Figure 5**: expression analysis of Rpi-mcq1.1 transgene.*

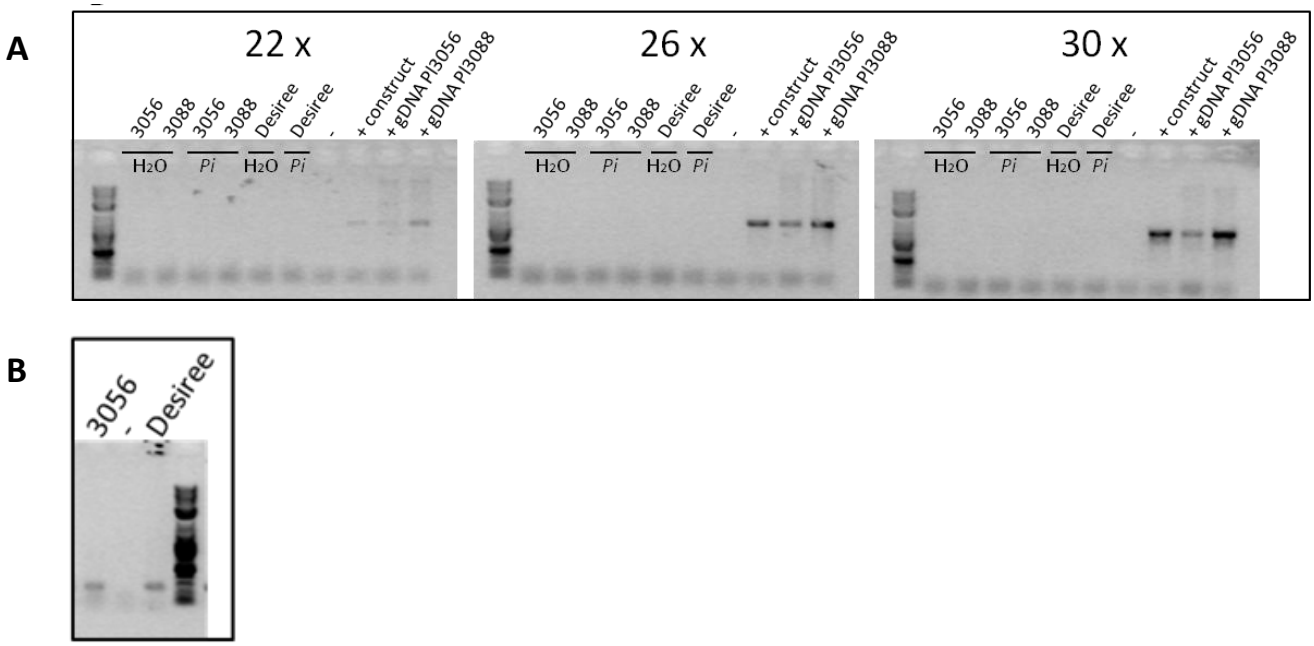


A) RT-PCR of *Rpi-mcq1.1* from plants of lines PL2804, PL2808 and non-transformed Desiree inoculated with either water (H20) or *P. infestans* (*Pi*). Positive controls include pSLJ21153 plasmid DNA and genomic DNA from the transgenic plants PL2804 and PL2808.

B) RT-PCR of the reference gene *PHA1* from PL2808 and non-transformed Desiree (results from 22 cycles shown).

Plants of the transgenic lines PL3056 and PL3058 carrying *Rpi-vnt1.1* were inoculated with either water (as a negative control) or spores of *Phytophthora infestans*. 18 hours after inoculation, RNA was extracted and RT-PCRs done for 22, 26 and 30 cycles. As shown in Figure 6A, expression of *Rpi-vnt1.1* was undetectable even after 30 cycles. In contrast, expression of the constitutively expressed reference gene *PHA1* was detectable from 22 cycles (Figure 6B). Plant lines PL3056 is the line carrying *Rpi-vnt1.1* chosen for release.

***Figure 5**: expression analysis of Rpi-vnt1.1 transgene.*



A) RT-PCR of *Rpi-vnt1.1* from plants of lines PL3056, PL3058 and non-transformed Desiree inoculated with either water (H20) or *P. infestans* (*Pi*). Positive controls include pSLJ21152 plasmid DNA and genomic DNA from the transgenic plants PL3056 and PL3058.

B) RT-PCR of the reference gene *PHA1* from PL3056 and non-transformed Desiree (results from 22 cycles shown).

Section 4: Methods used for characterisation of transgenic plants

DNA isolation

Genomic DNA was isolated by using the Retsch DNA isolation protocol detailed in Park et al (2005). DNA was eluted in 75 µl DNAse free water.

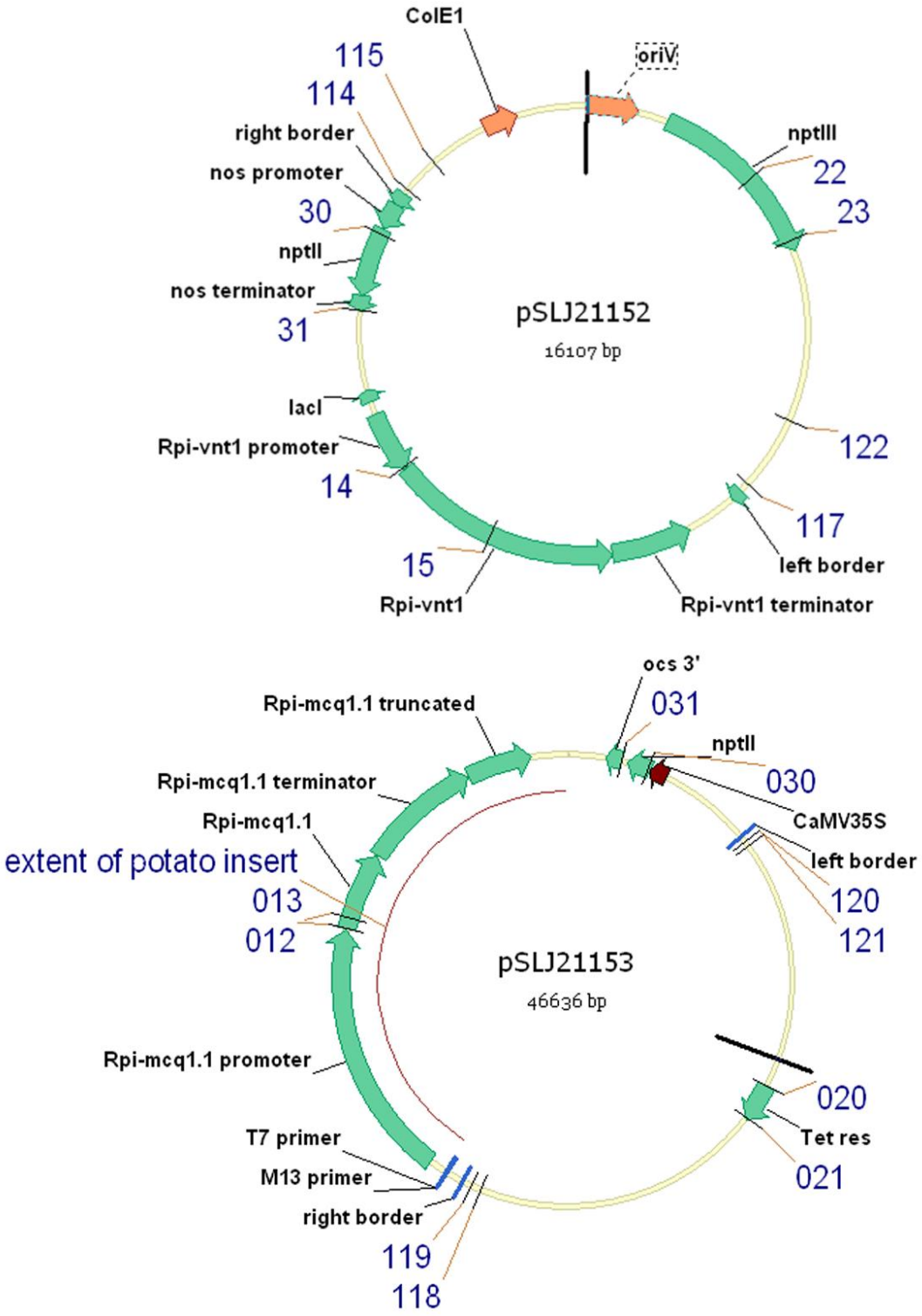
Amplification

All PCR reactions were performed in 30 ul with (3µl 10x PCR buffer, 3 µl 0.8 mM dNTPs, 0.25 µl Taq polymerase (homemade) and 2 µl of genomic DNA or 5 µl cDNA per PCR reaction). The following PCR program was used for all PCR reactions: 94 ^{o}C, 30'' - 55 ^{o}C, 30'' – 72 ^{o}C, 2'30'' – 26 - 33x. 7,5 µl of this reaction was loaded and run on a 1.2 % TAE agarose gel.

RNA isolation

RNA was isolated from young fresh leaves using the Tri reagent (Sigma, T9424). After DNAse treatment, the pellet was disolved in 100 µl RNA free water. Subsequently, 2.5 µg of total RNA was used to make first strand cDNA using Superscript III Reverse Transcriptase (Invitrogen, 18080-444). 100 µl water was added after cDNA synthesis. 5 µl was used as template per RT-PCR reaction.

Section 5: Vector maps of pSL21152 (*Rpi-vnt1.1*) and pSL21153 (*Rpi-mcq1.1*)



Improving late blight (*Phytophthora infestans*) resistance in potato using resistance genes from South American potato relatives.

Annex 2.

Proposed Plot Layout