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

Norwich Research Park

Norwich NR4 7UH

2. The title of the project.

Improving late blight (*Phytophthora infestans*) resistance in potato using resistance genes from wild potato relatives and from the Sarpo Mira potato variety.

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* L.

(d) subspecies, *tuberosum*

(e) cultivar/breeding line, Maris Piper and 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 have 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 subsp. *tuberosum* is sexually compatible with other cultivated genotypes 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 groundkeepers. Tubers that are not harvested (groundkeepers) 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.

Pollen is produced in low quantities and can be disseminated either by wind or insects. In the case of potatoes, dissemination of pollen is almost exclusively by

insects, with the contribution of wind being very limited (Eastham and Sweet, 2002; White, 1983). Dissemination is usually restricted to less than 10 metres (Conner and Dale, 1996; McPartlan and Dale, 1994; Tynan et al, 1990) so the transgenic trial crop can be easily isolated reproductively from other potato crops. Selfing is the most frequently observed form of reproduction (Plaisted, 1980); 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 can be dispersed by insects such as bumblebees although, as potato flowers lack nectar, pollen dissemination by honeybees is unlikely and they tend not to forage in potato crops (Sanford and Hanneman, 1981). Wind dissemination is considered to be marginal (Eastham and Sweet, 2002; White, 1983). 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, oomycetes 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 significant 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 cultivars Maris Piper and Desiree were co-cultivated with AGL1 and incubated in conditions that favour the development of callous tissue. Shoots that regenerated from callous tissue were excised and incubated in conditions that promote root development. All transgenic plants were treated with the antibiotic Claforan to kill any remaining *Agrobacterium*.

11. The nature and source of the vector used.

For *Rpi-vnt1.1* (plasmid pSLJ21152):

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 *Escherichia coli* origin of replication and the *bom* site which allows mobilization from *E. coli* to *Agrobacterium tumefaciens*. Located between the two border sequences of the T-DNA region of the Ti plasmid are the kanamycin-resistance gene for plant selection (*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 plasmid pSLJ21152 containing *Rpi-vnt1.1*.

For *Rpi-amr3* (plasmid pSLJ24119), *Rpi-amr1e* (plasmid pSLJ24156) *and Rpi-am1k* (plasmid pSLJ24478):

The transformation vector pICSLUS0001 derives from the Golden Gate Level 2 Acceptor pAGM4723 (Engler et al, 2014). It carries on its backbone the *nptIII* gene (for bacterial selection only) and the pMB1 replicon which functions as a bacterial origin of replication. Located between the two border sequences of the T-DNA region of the Ti plasmid are the glufosinate/phosphinothricin-resistance gene for plant selection (*bar*) and the USER cassette sequence which enables the insertion of the genes to be transferred to plant hosts by the USER cloning technique (Nour-Eldin et al, 2010). The orientation of the boundary sequences in pICSLUS0001 means that the *bar* gene as well as any new DNA integrated in the USER cassette site are transferred to the plant DNA. This vector was used to create the plasmids pSLJ24119, pSLJ24156 and pSLJ24478 containing *Rpi-amr3, Rpi-amr1e* and *Rpi-amr1k* respectively.

For *Rpi-Smira1* (plasmid pSJL24466) *and Rpi-Smira3* (plasmid pSJL24468):

The transformation vector pICSL86900 derives from the Golden Gate Level 2 Acceptor pAGM4723 (Engler et al, 2014). It carries on its backbone the *nptIII* gene (for bacterial

selection only) and the pMB1 replicon which functions as a bacterial origin of replication. Located between the two border sequences of the T-DNA region of the Ti plasmid are the kanamycin-resistance gene for plant selection (*nptII*) and a Golden Gate cassette sequence which enables the insertion of the genes to be transferred to plant hosts by the Golden Gate cloning technique (Engler et al, 2008). The Golden Gate cassette includes the *lacZ'* gene which is replaced by the gene of interest upon cloning. The orientation of the boundary sequences in pICSL86900 means that the *nptII* gene as well as any new DNA integrated in the Golden Gate cassette site are transferred to the plant DNA. As mentioned before, the *lacZ'* gene is removed during the cloning procedure. This vector was used to create the plasmids pSJL24466 and pSJL24468 containing *Rpi-Smira1 and Rpi-Smira3* respectively.

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

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 *Phytophthora infestans*. The expression of *Rpi-vnt1.1* will be under the control of endogenous promoter and terminator regions. In addition, this plasmid carries (within the T-DNA) the selectable marker *nptII* from *Escherichia coli*, which confers kanamycin resistance. This gene is required only for the *in vitro*-selection of transgenic lines and is under the control of the promoter and terminator sequences of the nopaline synthase gene from *Agrobacterium tumefaciens*.

Plasmids **pSLJ24119**, **pSLJ24156** and **pSLJ24478** contain *Rpi-amr3, Rpi-amr1e* and *Rpi-amr1k* respectively. These genes were isolated from the wild potato relative *Solanum americanum*. They are plant resistance (*R*) genes of the CC-NB-LRR class which confer resistance to some isolates of the late blight pathogen *Phytophthora infestans*. The expression of these genes will be under the control of endogenous promoter and terminator regions. In addition, these plasmids carry (within the T-DNA) the selectable marker *bar* from *Streptomyces hygroscopicus*, which confers resistance to glufosinate-containing herbicides. This gene is required only for the *in vitro*-selection of transgenic lines and is under the control of the promoter and terminator sequences of the nopaline synthase gene from *Agrobacterium tumefaciens*.

Plasmids **pSJL24466** and **pSJL24468** contain *Rpi-Smira1* and *Rpi-Smira3* respectively. These genes were isolated from the potato (*Solanum tuberosum*) variety Sarpo Mira. They are plant resistance (*R*) genes of the CC-NB-LRR class which confer resistance to some isolates of the late blight pathogen *Phytophthora infestans*. The expression of these genes will be under the control of endogenous promoter and terminator regions. In addition, these plasmids carry (within the T-DNA) the selectable marker *nptII* from *Escherichia coli*, which confers kanamycin resistance. This gene is required only for the *in vitro*-selection of transgenic lines and is under the control of the promoter sequence of the nopaline synthase gene and the terminator sequence of the octopine synthase gene, both from *Agrobacterium tumefaciens*. In addition, the tobacco mosaic virus 5'-unstranlated region known as 'omega

leader' is present in between the nopaline synthase promoter and the *nptII* coding region. This sequence acts solely as an enhancer of translation and is not translated into protein.

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* gene	2677	*Solanum venturii*
t-*Rpi-vnt1.1*	Terminator region of *Rpi-vnt1.1* gene	926	*Solanum venturii*
pSLJ24119			
T-DNA		6915	
p-*nos*	Promoter of nopaline synthase gene	299	*A. tumefaciens*
bar	Bialaphos *N*-acetyltransferase gene	549	*Streptomyces hygroscopicus*
t-*nos*	Terminator of nopaline synthase gene	268	*A. tumefaciens*
p-*Rpi-amr3*	Promoter region of *Rpi-amr3* gene	1918	*Solanum americanum*
Rpi-amr3	Coding region of *Rpi-amr3* gene	2664	*Solanum americanum*
t-*Rpi-amr3*	Terminator region of *Rpi-amr3* gene	770	*Solanum americanum*
pSLJ24156			
T-DNA		7223	
p-*nos*	Promoter of nopaline synthase gene	299	*A. tumefaciens*
bar	Bialaphos *N*-acetyltransferase gene	549	*Streptomyces hygroscopicus*

t-*nos*	Terminator of nopaline synthase gene	268	*A. tumefaciens*
p-*Rpi-amr1e*	Promoter region of *Rpi-amr1e* gene	1632	*Solanum americanum*
Rpi-amr1e	Coding region of *Rpi-amr1e* gene	2763	*Solanum americanum*
t-*Rpi-amr1e*	Terminator region of *Rpi-amr1e* gene	1265	*Solanum americanum*
pSLJ24478			
T-DNA		6774	
p-*nos*	Promoter of nopaline synthase gene	299	*A. tumefaciens*
bar	Bialaphos *N*-acetyltransferase gene	549	*Streptomyces hygroscopicus*
t-*nos*	Terminator of nopaline synthase gene	268	*A. tumefaciens*
p-*Rpi-amr1k*	Promoter region of *Rpi-amr1k* gene	1620	*Solanum americanum*
Rpi-amr1k	Coding region of *Rpi-amr1k* gene	2580	*Solanum americanum*
t-*Rpi-amr1k*	Terminator region of *Rpi-amr1k* gene	1011	*Solanum americanum*
pSJL24466			
T-DNA		8546	
p-*nos*	Promoter of nopaline synthase gene	270	*A. tumefaciens*
TMV Omega	5'-Untranslated Sequence (Omega Leader)	62	Tobacco Mosaic Virus
nptII	Neomycin phosphotransferase gene	795	*E. coli*
t-*ocs*	Terminator of octopine synthase gene	732	*A. tumefaciens*
p-*Rpi-Smira1*	Promoter region of *Rpi-Smira1* gene	1835	*Solanum tuberosum var. Sarpo Mira*
Rpi-Smira1	Coding region of *Rpi-Smira1* gene	3786	*Solanum tuberosum var. Sarpo Mira*
t-*Rpi-Smira1*	Terminator region of *Rpi-Smira1* gene	684	*Solanum tuberosum var. Sarpo Mira*
pSJL24468			
T-DNA		8799	

p-*nos*	Promoter of nopaline synthase gene	270	*A. tumefaciens*
TMV Omega	5'-Untranslated Sequence (Omega Leader)	62	Tobacco Mosaic Virus
nptII	Neomycin phosphotransferase gene	795	*E. coli*
t-*ocs*	Terminator of octopine synthase gene	732	*A. tumefaciens*
p-*Rpi-Smira3*	Promoter region of *Rpi-Smira3* gene	1732	*Solanum tuberosum var. Sarpo Mira*
Rpi-Smira3	Coding region of *Rpi-Smira3* gene	4023	*Solanum tuberosum var. Sarpo Mira*
t-*Rpi-Smira3*	Terminator region of *Rpi-Smira3* gene	803	*Solanum tuberosum var. Sarpo Mira*

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 the wild potato relatives *Solanum venturii* and *Solanum americanum* and from the *Solanum tuberosum* Sarpo Mira variety. 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 avirulent effector gene. The recognition event triggers a 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 (*Rpi-vnt1.1*), 6.9 kb for pSLJ24119 (*Rpi-amr3*), 7.2 kb for pSLJ24156 (*Rpi-amr1e*), 6.8 kb for pSLJ24478 (*Rpi-amr1k*), 8.5 kb for pSJL24466 (*Rpi-Smira1*) and 8.8 kb for pSJL24468 (*Rpi-Smira3*).

The plasmid map corresponding to pSLJ21152 (*Rpi-vnt1.1*) can be found in **Annex 1** to this application (Section 3).

Rpi-amr3, *Rpi-amr1e* and *Rpi-amr1k* have been (or will be) cloned in the USER cassette site of vector pICSLUS0001 to generate plasmids pSLJ24119 (*Rpi-amr3*), pSLJ24156 (*Rpi-amr1e*) and pSLJ24478 (*Rpi-amr1k*), with the native *R* gene terminator sequence located next to (and upstream of) the right border (RB). The vector map corresponding to pICSLUS0001 is included in **Annex 1** to this application (Section 4).

Rpi-Smira1 and Rpi-Smira3 have been cloned in the Golden Gate cassette site of vector pICSL86900 to generate plasmids pSJL24466 (*Rpi-Smira1*) and pSJL24468 (*Rpi-Smira3*), with the native *R* gene terminator sequence located next to (and upstream of) the right border (RB). The Golden Gate cloning site includes 4-nucleotide-overhang sequences to determine the polarity of the insert and a copy of the *lacZ* gene which is removed upon cloning. The vector map corresponding to pICSL86900 is included in **Annex 1** to this application (Section 5).

A. Molecular characterization of the insertion in plants carrying Rpi-vnt1.1

Details on the molecular characterization of the plants carrying *Rpi-vnt1.1* are included in **Annex 1** to this application (Section 1). Briefly, genomic DNA was extracted from plants transformed with pSLJ21152 (*Rpi-vnt1.1*) and the quality of the genomic DNA was assessed by PCR, using control primers. The presence of the transgene was confirmed in the line nominated for release (PL3056) by PCR, using primers against *Rpi-vnt1.1*.

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*). 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 using primers located near to the right border. PCR primers designed against sequence close to the left border did not yield a PCR product even in the positive control. However, 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 demonstrating the absence of this antibiotic marker.

B. Molecular characterization of the insertion in plants carrying Rpi-amr3

Details on the molecular characterization of the plants carrying *Rpi-amr3* are included in **Annex 1** to this application (Section 1). Briefly, genomic DNA was extracted from plants transformed with pSLJ24119 (*Rpi-amr3*) and the quality of the genomic DNA was assessed by PCR, using control primers. The presence of the transgene was confirmed in the six lines tested by PCR, using primers against *Rpi-amr3*. Primers against the T-DNA selectable marker were also used, and confirmed the presence of the pSLJ24119 T-DNA in all the lines analysed.

In addition, PCR experiments were done to test whether sequences outside of the T-DNA borders are present in the *Rpi-amr3* transgenic plants (Annex 1, Section 1). To determine this, PCR primer pairs designed to amplify regions from the vector backbone close to the left and right borders were used.

When using the primers binding close to the right border, only the positive control yielded a product of the expected size. When using the primers binding close to the left border, the positive control and three of the transgenic lines yielded a product of the expected size. In addition, two lines (as well as the Maris Piper WT control) show extremely faint bands (which might be a consequence of cross-contamination) an a sixth line shows no band.

To further investigate this, additional tests are in progress. We will extract new genomic DNA samples from these lines and we will test them for the presence of the *nptIII* selectable marker gene located in the pICSLUS0001 vector backbone. For this, we will use primers that amplify the *nptIII* full-length sequence. Lines giving a positive result in that test will be **excluded** from the proposed trial.

C. Molecular characterization of the insertion in plants carrying Rpi-amr1e, Rpi-amr1k, Rpi-Smira1 and Rpi-Smira3

Constructs carrying *Rpi-amr1e, Rpi-amr1k, Rpi-Smira1* and *Rpi-Smira3* are in the pipeline for plant transformation at the moment. The plants obtained after the transformation will be characterized in the same way as the plants carrying *Rpi-amr3*, analysing the presence of the T-DNA insert and the integration of backbone sequences as described above.

Details on the molecular characterization of these plants are included in **Annex 1** to this application (Section 1).

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

Not applicable.

(c) the copy number of the insert, and

A. Plants carrying Rpi-vnt1.1

Given the low levels of *Rpi-vnt1.1* expression observed in the plants transformed with pSLJ21152 (Annex 1, Section 1), we expect that the inserted gene is present as 1-2 copies.

B. Plants carrying Rpi-amr3

A preliminary analysis of insert copy number in the *Rpi-amr3* lines was performed by Droplet Digital PCR (ddPCR). The details of the protocol followed are indicated in **Annex 1** (Section 2). Primers MP013/MP014 that amplify a potato vacuolar invertase gene were used as control. This gene has 4 copies in the potato tetraploid genome.

To assess the copy number of the insert, primers binding to the 3' region of the *Rpi-amr3* gene (primers MP015/MP016; Annex 1, Section 2) as well as primers binding to the *bar* selectable marker gene (MP017/MP018; Annex 1, Section 2) were used.

The 3' region of the *Rpi-amr3* gene is adjacent to the right border (RB) of the T-DNA. When using primers MP015/MP016 which bind to this region, three of the six *Rpi-amr3* lines tested showed around 4 to 6 insertions while the other three lines showed a higher number of insertions (over 15).

The selectable marker gene is adjacent to the left border (LB) region of the T-DNA. When using primers MP017/MP018 that bind to the *nos* promoter and *bar* coding region respectively, the *Rpi-amr3* lines tested showed only 1 to 3 insertions.

Transference of the T-DNA to the plant starts in the RB region and finishes in the LB region. The results described above suggest that in most of the cases, only a fragment of the T-DNA was transferred and inserted, with just 1 to 3 full T-DNA copies present per transgenic line. This is in agreement with the expression data described below.

The multiple insertions observed have no obvious deleterious effect since none of the six *Rpi-amr3* lines under study shows an abnormal phenotype when grown in the glasshouse.

C. Plants carrying Rpi-amr1e, Rpi-amr1k, Rpi-Smira1 and Rpi-Smira3

Rpi-amr1e and *Rpi-amr1k* have been (or will be) cloned in the same transformation vector as *Rpi-amr3* (i.e., vector pICSLUS0001). *Rpi-Smira1* and *Rpi-Smira3* have been cloned in transformation vector pICSL86900. Both pICSLUS0001 and pICSL86900 derive from the same Golden Gate Level 2 Acceptor plasmid (pAGM4723; Engler et al, 2014). Therefore we expect that plants carrying *Rpi-amr1e, Rpi-amr1k, Rpi-Smira1* or *Rpi-Smira3* will have similar insert copy numbers as plants carrying *Rpi-amr3* (described above).

(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, which is 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 in the transgenic plants to be released is governed by their respective native promoters and terminators. Resistance genes of the same class (NB-LRR; nucleotide binding site-leucine rich repeat) have previously been shown to exhibit very low expression levels in vegetative parts of the plant (Tan et al, 2007).

A. Plants carrying Rpi-vnt1.1

To examine expression of the transgene *Rpi-vnt1.1* in the transgenic plants nominated for release, plants of the transgenic lines 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 PCR cycles (see **Annex 1** for further details and results). 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 level of the *Rpi-vnt1.1* transgene under the control of its native regulatory sequences is very low.

B. Plants carrying Rpi-amr3

Details on the expression analysis of *Rpi-amr3* lines are included in **Annex 1** to this application (Section 1).

Expression of the *Rpi-amr3* transgene was assessed by quantitative PCR (qPCR) using primers MP015/MP016 (Annex 1, Section 2). *Elongation factor 1* gene (*EF1*) was used as control for the normalization of the qPCR data. The expression of *EF1* was assessed with primers EF1_F/EF1_R (Annex 1, Section 2) and the result was expressed as [Number of mRNA molecules of *Rpi-amr3* per 1 Million mRNA molecules of *EF1* control].

RNA of suitable quality for cDNA synthesis and qPCR was only obtained from the leaves of three out of six *Rpi-amr3* lines (A1, A2 and A3). The other three lines under study were senescent at the moment of collecting the samples, so the expression of the transgene in those plants could not be determined with confidence. In addition to RNA from the *Rpi-amr3* lines, an RNA sample from the *Solanum americanum* accession from which *Rpi-amr3* was isolated was included as control (SP1102). Accession SP1102 is diploid and contains 2 copies of *Rpi-amr3* per diploid genome.

The level of expression of the *Rpi-amr3* transgene in lines A1, A2 and A3 is not higher than the level of expression of *Rpi-amr3* in the resistant accession from which this gene has been isolated. In addition, and in agreement with the results previously obtained with *Rpi-vnt1.1* plants, more than 30 qPCR cycles were required to detect the expression of this transgene. This is consistent with the preliminary results of the insert copy number determination which suggest that only 1 to 3 full T-DNA copies are present per transgenic line.

C. Plants carrying Rpi-amr1e, Rpi-amr1k, Rpi-Smira1 and Rpi-Smira3

Rpi-amr1e and *Rpi-amr1k* have been (or will be) cloned in the same transformation vector as *Rpi-amr3* (i.e., vector pICSLUS0001). *Rpi-Smira1* and *Rpi-Smira3* have been cloned in transformation vector pICSL86900. Both pICSLUS0001 and pICSL86900 derive from the same Golden Gate Level 2 Acceptor plasmid (pAGM4723; Engler et al, 2014) and all the genes will be controlled by their native regulatory elements. Therefore we expect that plants carrying *Rpi-amr1e, Rpi-amr1k, Rpi-Smira1* or *Rpi-Smira3* will show similar characteristics to that of plants carrying *Rpi-amr3* (described above) regarding insert copy number and level of expression of the transgene (which is predicted to be low).

(b) the parts of the plant where the insert is expressed, such as roots, stem or pollen.

Resistance genes are known to usually be expressed, albeit at very low levels, in all vegetative parts of plants (Tan et al, 2007).

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 resistance genes as well as the selectable marker genes included in the T-DNAs 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.

The plants carrying *Rpi-amr3*, *Rpi-amr1e* and *Rpi-amr1k* will also contain the *bar* gene which confers resistance to herbicides that have glufosinate as active

ingredient. However, these plants can be readily eliminated with other herbicides such as glyphosate.

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

No genetic or phenotypic instability has been observed in the lines that are already growing in the glasshouse. The plants that will be taken to the field if the permit is granted, will be regenerated from tubers obtained from the original transformed lines. Any plant showing genetic or phenotypic instability when grown in the glasshouse will be excluded from the trial. Only lines for which no changes in phenotype are evident will be selected for field testing.

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 are not predicted to 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 are members of a class of resistance (*R*) 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 they all possess highly similar protein structures. Many of the European cultivated potato varieties already contain additional *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. Considering plants for which a complete genome sequence is available, *Arabidopsis thaliana* is known to possess approximately 200 *R* genes and *R* gene homologues (Meyers et al, 2003), while rice possesses approximately 500 (Zhou et al, 2004). Within the potato genome, a set of 438 NB-LRR-type genes has been predicted (Jupe et al, 2012), and further analysis showed that the doubled monohaploid reference potato genome encodes ~ 750 NB-LRR proteins (Jupe et al, 2013). The expression of the introduced genes in the transgenic potatoes to which this application for release applies is under the control of native promoters. This correlates with very low levels of expression, comparable with what is known for other native resistance genes.

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 characteristics of the transgenic protein NPTII involve no outstanding safety issues and derived products are no more likely to cause adverse effects on human and animal health than conventional potato (The EFSA Journal, 2006, 323: 1-20).

The marker gene *bar* is expressed as an enzyme (*N*-acetyltransferase) that acetylates the herbicide glufosinate (also known as phosphinothricin), causing its inactivation and detoxification (Thompson et al, 1987; De Block et al, 1987). No toxic or harmful effects on human or animal health have been described for the *bar* gene. The protein encoded by this gene is highly specific and does not posses any of the properties of food allergens or toxins (e.g. it has no sequence homology with known toxins or allergens, it is heat labile and it is not stable in simulated human and animal gastric fluids) (Herouet et al, 2005; Wehrman et al, 1996). Studies carried out in mice also support this conclusions (Herouet et al, 2005; Wang et al, 2000). This gene has previously been used as plant selectable marker in transgenic lines that were released for field trials. This includes wheat and barley field trials in the UK (wheat released at John Innes Centre, Consent Date April 1997, and barley released at John Innes Centre in 1998-2000 and 2001-2003). Furthermore, transgenic events including the *bar* gene have been assessed by the EFSA GMO panel and no

concerns were identified (The EFSA Journal, 2013, 11(6): 3251). Finally, crop cultivars that carry this gene are commercially available (Green and Owen, 2011).

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 enhanced resistance conferred by the introduced resistance (*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 (Takken and Goverse, 2012). Current models indicate that the LRR (leucine-rich repeat) region recognises specific molecules secreted by the pathogen (effectors or avirulence factors) which are intended to help the pathogen cause disease on the host. Recognition by the LRR region results in a signal transduction event that culminates in the triggering of plant defence responses. These result in localised host plant cell death and prevent spread of the pathogen through host tissues (Jones and 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 and 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 initiate a resistance response upon recognition of the target organism (Jones and Dangl, 2006). For recognition to take place, a specific avirulence factor has to be injected into the plant by the pathogen. Based on current knowledge about the resistance genes that target *P. infestans* and on the information available on *P. infestans* avirulence factors (Vleeshouwers et al, 2011; Rodewald and Trognitz, 2013), it is not expected that any of the introduced genes will affect other non-target organisms that could be in contact with these plants during the trial.

Overall, 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 anti-fungal treatments, an increase in the populations of those non-target organisms that respond to the anti-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 is predicted to 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 T-DNAs are available and details are given in **Annex 1** (Section 2).

25. Information about previous releases of the genetically modified plant, if applicable.

One of the genotypes included in this application (i.e., Desiree potato plants carrying the *Rpi-vnt1.1* transgene) has been previously released in the UK as part of a successful field trial carried out between 2010 and 2012 (Consent number 10/R29/01). No unexpected effects were seen during this release and the trial is currently in the post-trial monitoring phase.

The results of the previous *Rpi-vnt1.1* field trial have been reported in Jones et al (2014) and showed that this gene could confer resistance to the races of the late blight pathogen that circulated in the UK at the time of the trial. We aim at using these *Rpi-vnt1.1* transgenic Desiree plants as positive control in the field trial of the *Rpi-amr3, Rpi-amr1e, Rpi-amr1k, Rpi-Smira1 and Rpi-Smira3* genes, if permission is granted.

None of the other transgenic lines included in this application (i.e., Maris Piper potatoes carrying *Rpi-amr3, Rpi-amr1e, Rpi-amr1k, Rpi-Smira1 or Rpi-Smira3*) has 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 100 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 (JIC); 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 Desiree 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 Desiree tubers are red skinned (Maris Piper is white 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 the 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 more than 20 metres 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 Sites of Special Scientific Interest (SSSIs) within 4km of the release site. Given that potato pollen is not normally disseminated more than 10 metres from the parent plants, this distance equates to 400 times the normal dissemination distance. The closest SSSI to the release site 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 honeybees (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.

Since 2001, we have been working towards identifying, mapping and isolating resistance genes from potato that confer resistance against potato late blight (*Phytophthora infestans*). This research has been publicly funded.

Recently, five such genes were successfully isolated from the potato relative *Solanum americanum* (*Rpi-amr3, Rpi-amr1e, Rpi-amr1k)* and from the *Solanum tuberosum* Sarpo Mira variety (*Rpi-smira1 and Rpi-smira3)*. These have been (or will be) transformed into the potato cultivar Maris Piper. Previously, the gene *Rpi-vnt1.1* was isolated from the wild South American potato relative *Solanum venturii* (Foster et al, 2009). This gene has been transformed into the potato cultivar Desiree and successfully tested in the field in recent years (Jones et al, 2014; Consent 10/R29/01). The line carrying *Rpi-vnt1.1* will be used as positive control in the proposed trial.

The genes identified are potentially valuable weapons in the fight against potato late blight as they confer resistance against many different isolates of this pathogen, including the strains which are 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.

There are no plans to use these particular plants as or in a product in the future.

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

If consent is granted, this year's field trial will start in May 2016 and will continue until 30th November 2016. The trial will continue for 3 years and will subsequently be from 1st May until 30th November in 2017 and from 1st May until 30th November in 2018. 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 following a randomised block trial design.

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 (John Innes Centre, JIC) who look after field trials on the Norwich Research Park (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 monitored for groundkeepers during the remainder of the year and will be shallow tilled to remove weeds and encourage germination of any shed true potato seed. Any groundkeepers identified will be removed by hand and destroyed by autoclaving within the Sainsbury Laboratory. The monitoring of the plot for groundkeepers 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 left fallow to enable easy identification and removal of groundkeepers.

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 no more than 200 transgenic plants. These will include different lines of the different genotypes described in this application.

PART VII

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

35. A description of any precautions to -

(a) 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 left fallow 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 to disperse but it's low viability and the distance of the transgenic plants from other potato crops (at least 20 metres) 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 left fallow, monitored for groundkeepers during the remainder of the year and sprayed with a systemic broadleaf herbicide. Any groundkeepers identified will be destroyed by herbicide treatment (e.g. glyphosate) or removed by hand and destroyed by autoclaving as described below. The monitoring of the plot for groundkeepers 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 left fallow to enable easy identification and removal of groundkeepers.

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 sealed bags or containers and removed from site to an authorised waste disposal facility. Disposal will be carried out by incineration through our contractor SRCL.

38. A description of monitoring plans and techniques.

The purpose of the monitoring plan is to enable early detection of 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 to enable easy identification of volunteers. The site will be inspected monthly between April and November (the growing season of potato) and any volunteers identified will be immediately destroyed either by application of a systemic broadleaf herbicide or by hand pulling plants and digging out tubers/root systems. These will then be autoclaved within the Sainsbury Laboratory. If volunteers are found at the end of the 2-year period, DEFRA recommendations will be followed for the management of the release site.

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 systemic broadleaf herbicide and tubers dug up by fork and hand and transferred to an authorised waste facility for disposal by deep burying or incineration.
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 (JIC) reception which is manned throughout 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** (Sections 1 and 2) where results are also detailed. Results included in Annex 1 where obtained at The Sainsbury Laboratory (Norwich, UK).

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