Department for Environment, Food and Rural Affairs

Application for consent to release a GMO – Higher plants

Part A1: Information required under Schedule 1 of the Genetically Modified Organisms (Deliberate Release) Regulations 2002 (as amended)

Part I 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

   Responsible Scientist:

   Lead Scientist

   The Sainsbury Laboratory

   Norwich Research Park

   Colney Lane

   Norwich NR4 7U

   The lead Scientist has almost 40 years of experience in molecular biology, plant pathology and working with transgenic plants.

2. **The title of the project**

   Development of new potato varieties with various combinations of late blight resistance, PVY resistance, PCN resistance, reduced bruising and improved

processing quality.

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	*Solanum tuberosum* L.
***(d)* subspecies**	*tuberosum*
(e) cultivar/breeding line	Maris Piper, Charlotte
(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 3 metres from the crop, reducing to 0.017% at 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^{o}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 usually 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 transport, 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 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 3 metres from the crop, reducing to 0.017% at 10 metres (McPartlan and Dale, 1994).

(b) any specific factors affecting dissemination:

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 3 metres from the crop, reducing to 0.017% at 10 metres (McPartlan and Dale, 1994).

7. The geographical distribution of the plant in Europe

The potato originates from South America (the Andes). Potatoes are widely cultivated throughout the world and rank as the 4th most important food crop (FAO 2021). In Europe, including 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 Europe, 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 several pests and disease-causing organisms, including slugs, insects, nematodes, viruses, bacteria, oomycetes and fungi. Several 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 are not expected to affect any of these characteristics (as initially confirmed in trials 17/R29/01 and 19/R29/01; Witek AI, unpublished).

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 (Hellens et al, 2000) For transformation, a standard protocol like that of Kumar et al (1996) was used. Stem internode sections of the potato cultivar Maris Piper or Charlotte 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. Internode sections and shoots were exposed to the selectable agent chlorsulfuron during tissue culture to favour regeneration of lines carrying the T-DNA of interest. In addition, all transgenic plants were treated with the antibiotics timentin and cefotaxime to kill any remaining Agrobacterium.

11. The nature and source of the vector used

To assemble plasmids SLJ25586 and SLJ25587, vector pICH32281_LacZ was used. It belongs to the Level 2 class of Golden Gate vectors (Werner et al, 2012; Engler et al, 2014). pICH32281_LacZ carries on its backbone the *nptII* gene (for bacterial selection only) and the *ipt* gene (for counter-selection of plants where the backbone has been integrated) (Richael et al, 2008). It also carries sequences that function as bacterial origins of replication: pBR322 and RiA4. The left and right border were adjusted to minimise the amount of bacterial DNA involved (TSL SynBio).

Located between the two border sequences of the T-DNA region of the Level 2 transformation vector is the Golden Gate cassette sequence. This enables the insertion of the genes to be transferred to plant hosts by the Golden Gate cloning technique (Engler et al, 2008). This cloning cassette includes recognition sites for Type IIS restriction endonucleases and 4-nucleotide-overhang sequences to determine the polarity of the insert. The Golden Gate cassette includes the LacZ gene which is replaced by the genes of interest upon cloning. The orientation of the T-DNA boundary sequences in these vectors means that any new DNA integrated in the Golden Gate cassette site is transferred to the plant DNA.

Details of the vectors' constituent fragments that remain in the plasmids after cloning the genes of interest are listed in the following table. As mentioned above, the LacZ gene is removed during the cloning procedure. In addition, 'silent' nucleotide changes (i.e., changes that do not affect the protein sequence) have been introduced into the *ipt* gene to make it amenable to cloning.

To assemble the plasmids SLJ25606 and SLJ25057, vector pAGM32305 was used. It carries the same genetic elements as the pICSL 32281_LacZ, the only differences being the different recognition sites for Type IIS restriction endonucleases, and non-trimmed borders.

Table 1. Genetic elements in the backbone of the vectors pICSL32281_LacZ* and pAGM32305**

Abbreviation	Name & Function	Size (bp)	Origin
LB	Left border of T-DNA from a nopaline-type Ti plasmid.	29* 151**	*Agrobacterium tumefaciens*
RBO	Right border of T-DNA from a nopaline-type Ti plasmid (with overdrive sequence).	139* 155**	*Agrobacterium tumefaciens*
ipt	*Isopentenyl transferase (ipt)* gene.	1640	*Agrobacterium tumefaciens*
p-*bla*	Promoter region of the *beta-lactamase (bla)* gene. Drives the expression of the bacterial	108	*Escherichia coli*

	selectable marker gene (*nptII)*. Cloned from the pUC19 vector.		
nptII	Coding region of the *neomycin phosphotransferase II* (*nptII*) gene.	795	*Escherichia coli*
pBR322 Replicon	Fragment of the pBR325 cloning vector containing the moderate-copy-number replicon pBR322. Functions as origin of replication in *Escherichia coli*.	1863	*Escherichia coli*
RiA4 Replicon	Plasmid pRiA4 replicator region. Functions as origin of replication in *Agrobacterium tumefaciens*.	4604	*Agrobacterium rhizogenes*

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

The T-DNAs of all plasmids (SLJ25057, SLJ25606, SLJ25586 and SLJ25587) contain as below:

- a plant selectable marker, an allele of the tomato acetolactate synthase (*ALS/CSR*) that is resistant to inhibition by the herbicide chlorsulfuron (CS). This resistance is due to 2 mutations that have been described in *Nicotiana tabacum* (Lee et al, 1988). The *ALS/CSR* gene is required only for the *in vitro* selection of transgenic lines. It is under the control of its endogenous regulatory sequences and is located next to the vector's left border.

- an extra T-DNA left-border sequence in between the *ALS/CSR* gene and the vector's left border. This sequence derives from an *A. tumefaciens* octopine-type *Ti* plasmid. It was added with the aim of decreasing the chances of backbone integration due to left-border 'read-through' during T-DNA transfer.

- a stack of the three late blight resistance genes (*Rpi*), *Rpi-vnt1.1, Rpi-amr3 and Rpi-amr1*, combined with a gene-silencing module. *Rpi-vnt1.1* originates from the wild potato relative *Solanum venturii* (Foster et al, 2009). Both *Rpi-amr3* and *Rpi-amr1* were isolated from the wild potato relative *Solanum americanum* (Witek et al, 2016; Witek et al, 2021). They are plant resistance (*R*) genes of the CC-NB-LRR class which confer resistance to a large range of isolates of the late blight pathogen *Phytophthora infestans* (Witek et al, 2021). The expression of these genes is under the control of endogenous regulatory sequences.

In addition to the previous traits, the transgenic lines transformed with plasmids SLJ25606, SLJ25586 and SLJ25587 carry a Potato Virus Y (PVY) resistance gene, Ry-f_{sto} (Grech-Baran et al, 2020) from the wild relative *S. stoloniferum*. *Ry_{sto}* confers extreme resistance (ER) to PVY and related viruses. It encodes a nucleotide-binding leucine-rich repeat (NLR) protein with an N-terminal TIR domain and was proven to

drive PVY perception and ER in transgenic potato plants (Grech-Baran et al, 2020). The expression of this gene is under control of its own regulatory elements.

The transgenic lines created using plasmid SLJ25606 also contain a pair of tomato genes, *Hero* and *NRC6*, that contribute to potato cyst nematode (PCN) resistance in tomato (Ernst et al, 2002). *Hero* gene encodes a NLR protein with an N-terminal CC domain and was shown to confer resistance to *Globodera pallida* and *Globodera rostochiensis* in transgenic tomato plants. *Hero* on its own does not confer resistance to PCN in potato (Sobczak et al, 2005), as potato lacks functional counterpart of NLR helper protein NRC6 that is required to initiate immune response (WO2019108619A1). To overcome that, *Hero* and *NRC6* are delivered together to confer nematode resistance in potato. Both genes are expressed under the control of their own regularory elements.

All transgenic lines except SLJ25587 contain the gene-silencing module designed to silence the polyphenol oxidase gene *Ppo* (Rommens et al, 2006) and the vacuolar acid invertase gene *Vlnv* (Ye et al, 2010; Bhaskar et al, 2010) in a tuber-specific manner. Silencing of these genes in potato tubers aims to prevent browning upon bruising and cold-induced potato sweetening, lowering the potential for blackening and acrylamide formation upon cooking.

The gene-silencing module contains two tuber-specific promoters in convergent orientation: one from the ADP-glucose pyrophosphorylase (*Agp*) gene (Müller-Röber and Kossman, 1994) and the other from the granule-bound starch synthase (*Gbss*) gene (Visser et al, 1991). Sense and antisense sequences from the *Ppo* and the *Vlnv* genes are located in between the convergent promoters and they are separated by a ‘spacer’ sequence. The structure of this module is such that it directs the transcription of RNA molecules with complementary sequences. The consequent formation of double-stranded RNA triggers the post-transcriptional silencing of the above-mentioned genes via the endogenous silencing machinery of plants.

All the plasmids described above contain ‘end-linker’ sequences in their T-DNAs. These elements are part of the Golden Gate cloning toolbox (Engler et al 2014) and their sole function is to link the genes of interest to the vector backbone. Also, the plasmid SLJ25057 contains a ‘dummy-linker’ in its T-DNA. This linker is required to connect genetic elements when assembling the T-DNA by the Golden Gate cloning technique (Engler et al 2014). The linker was specially designed for this project and contains stop codons in the six possible reading frames; like end-linkers, it does not encode any proteins.

Finally, ‘silent’ nucleotide changes (i.e., changes that do not affect the protein sequence) have been introduced into some of the genes mentioned above, to make them amenable to cloning (domesticate). For technical reasons, plasmids SLJ25057 and SLJ25606 feature non-domesticated *Rpi-amr3* and *Rpi-amr1*, which doesn’t change the nature or function of either of these genes.

Table 2. Genetic elements in T-DNAs

Elements in T-DNA	Name & Function	Size (bp)	Origin	Included in
Extra LB	Extra left border sequence derived from an octopine-type Ti plasmid	25	*Agrobacterium tumefaciens*	SLJ25057, SLJ25606, SLJ25586, SLJ25587
p-*CSR*	*Acetolactate synthase / chlorsulfuron resistance* gene (*ALS/CS*^{R})	3751	*Solanum lycopersicum*	SLJ25057, SLJ25606, SLJ25586, SLJ25587
Rpi-vnt1.1	*Rpi-vnt1.1* gene	3999	*Solanum venturii*	SLJ25057, SLJ25606, SLJ25586, SLJ25587
Rpi-amr3 (nd)	Non-domesticated *Rpi-amr3* gene	5209	*Solanum americanum*	SLJ25057, SLJ25606
Rpi-amr3	Domesticated *Rpi-amr3* gene	5352	*Solanum americanum*	SLJ25586, SLJ25587
Rpi-amr1 (nd)	Non-domesticated *Rpi-amr1* gene	7349	*Solanum americanum*	SLJ25057, SLJ25606
Rpi-amr1	Domesticated *Rpi-amr1* gene	7201	*Solanum americanum*	SLJ25586, SLJ25587
Ry$_{sto}$	*Ry*$_{sto}$ gene	6790	*Solanum stoloniferum*	SLJ25606, SLJ25586, SLJ25587
Dummy-Linker	Linker sequence required for Golden Gate cloning	28	Synthetic	SLJ25057
p-*Agp*	Promoter of the ADP-glucose pyrophosphorylase gene (*Agp*)	2259	*Solanum tuberosum*	SLJ25057, SLJ25606, SLJ25586
Sense-*Vlnv*	Fragment of the vacuolar acid invertase gene (*Vlnv*) in sense orientation	504	*Solanum tuberosum*	SLJ25057, SLJ25606, SLJ25586
Antisense-*Ppo*	Fragment of the polyphenol oxidase gene (*Ppo*) gene in antisense orientation	144	*Solanum tuberosum*	SLJ25057, SLJ25606, SLJ25586
Spacer	Spacer sequence that creates a loop in between the sense and antisense fragments of the hairpin structure	162	*Solanum tuberosum*	SLJ25057, SLJ25606, SLJ25586

Sense-*Ppo*	Fragment of the polyphenol oxidase gene (*Ppo*) gene in sense orientation	144	*Solanum tuberosum*	SLJ25057, SLJ25606, SLJ25586
Antisense-*Vlnv*	Fragment of the vacuolar acid invertase gene (*Vlnv*) in antisense orientation	504	*Solanum tuberosum*	SLJ25057, SLJ25606, SLJ25586
p-*Gbss*	Promoter of the granule-bound starch synthase gene (*Gbss*)	686	*Solanum tuberosum*	SLJ25057, SLJ25606, SLJ25586
NRC6	*NRC6* gene	6250	*Solanum lyopersicum*	SLJ25606
Hero	*Hero* gene	6363	*Solanum lycopersicum*	SLJ25606
End-Linker	Linker sequence required for Golden Gate cloning	36	Synthetic	SLJ25057, SLJ25606, SLJ25586, SLJ25587

Part IV Information relating 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.

Plants transformed with plasmids SLJ25057, SLJ25606, SLJ25586 and 25587 contain a stack of the three *Rpi* genes *Rpi-amr3, Rpi-amr1* and *Rpi-vnt1.1*. The introduced genes confer useful resistance against different isolates of the late blight pathogen, *Phytophthora infestans*. *R* genes enable plants to recognise isolates of the pathogen that possess a specific corresponding avirulent effector gene. The recognition event triggers a signalling cascade culminating in expression of the plant defence response, which acts to prevent further pathogen growth within the host plant. Deployment of *R*-gene stacks has the potential to confer a more efficient and durable resistance by combining different recognition specificities (Haverkort et al, 2016; Jo et al, 2016). The effectiveness of this stack of genes in the field was repeatedly confirmed in the past field trials under consents 17/R29/01 and 19/R29/01.

Plants transformed with plasmids SLJ25057, SLJ25606 and SLJ25586 also contain a gene-silencing module designed to silence the polyphenol oxidase gene *Ppo* (Rommens et al, 2006) and the vacuolar acid invertase gene *Vlnv* (Ye et al, 2010; Bhaskar et al, 2010) in a tuber-specific manner, by using convergent tuber-specific promoters.

The enzyme PPO plays a major role in tuber discolouration after impact-induced bruising. Upon mechanical damage of the tuber, PPO-mediated oxidation of polyphenols leads to the precipitation of black or brown pigment deposits that affect tuber quality. The gene-silencing module in plasmid SLJ25057 contains sense and antisense sequences derived from the 3'UTR of *POT32*, the predominant PPO variant in tubers (Thygesen et al, 1995). Its silencing significantly decreases enzymatic browning upon bruising (Rommens et al, 2006).

Cold storage of tubers triggers the accumulation of reducing sugars (i.e., glucose and fructose). This process, known as cold-induced potato sweetening, is responsible for the potato blackening upon cooking at temperatures above 120 ºC in low-moisture environments. Blackening is the result of the accumulation of dark (and bitter-tasting) compounds, which are products of the non-enzymatic Maillard reaction between reducing sugars and amino acids. The enzyme VINV hydrolyses sucrose to glucose and fructose in the vacuole and its activity correlates with potato sweetening during cold storage. Silencing of the *Vlnv* gene decreases potato blackening upon cooking and may be correlated with increased sucrose levels (Ye et al, 2010; Bhaskar et al, 2010).

The Maillard reaction also leads to the formation of acrylamide from reducing sugars and asparagine, which is the predominant free amino acid in potato tubers. Acrylamide is a neurotoxic compound and potential carcinogen (Friedman, 2003). The Food Standards Agency (FSA) advises on actions to reduce dietary intake of this compound (FSA, Chief Scientific Advisor Science Reports, 2015; https://www.food.gov.uk/safety-hygiene/acrylamide). Silencing of the *Vlnv* gene in tubers contributes to a reduction in the acrylamide-forming potential, since it decreases the availability of reducing sugars (Ye et al, 2010).

The results so far repeatedly confirmed the expected effect of the silencing module on the tubers, which have significantly reduced both discolouration upon bruising and accumulation of reducing sugars (Witek AI, unpublished).

In addition to the previous traits, the transgenic lines transformed with plasmids SLJ25606, SLJ25586 and SLJ25587 carry a Potato Virus Y (PVY) resistance gene, Ry-f_{sto} (Grech-Baran et al, 2020). PVY is an aphid-transmitted pathogen of a major economic significance, affecting both agri-food sector and seed potato production. Ry_{sto}, from the wild relative *S. stoloniferum*, confers extreme resistance (ER) to PVY and related viruses, and was previously proven to drive PVY perception and ER in transgenic potato plants (Grech-Baran et al, 2020).

The transgenic lines created using plasmid SLJ25606 also contain a pair of tomato genes, *Hero* and *NRC6*, that contribute to potato cyst nematode (PCN) resistance in tomato (Ernst et al, 2002). *Globodera pallida* and *Globodera rostochiensis* are quarantine pathogens, and PCN infestation is a major problem in potato production, causing crop losses up to and above 80%. *Hero* gene was shown to confer

resistance to *G. pallida* and *G. rostochiensis* in transgenic tomato plants. *Hero* on its own does not confer resistance to PCN in potato (Sobczak et al, 2005), as potato lacks functional counterpart of NLR helper protein NRC6 that is required to initiate immune response (Derevnina et al, 2018). To overcome that, *Hero* and *NRC6* are delivered together to confer nematode resistance in potatoes.

All the transgenic plants proposed for release in this application also contain the *CS*^{R} gene which confers resistance to some herbicides (sulfonylureas and imidazolinones). This trait will be used **only** for the *in vitro* selection of transgenic lines during tissue culture and these plants remain sensitive to other herbicides.

The goal of the proposed trial is to evaluate the initial set of transgenic lines for their field resistance to P. infestans and PVY, to select lines that are true to type for both varieties, to assess the yield and tuber quality. Plants transformed with plasmid SLJ25057 will be used as positive control for *P. infestans* resistance and field performance.

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:

Table 3. T-DNA sizes

T-DNA	Size (bp)
SLJ25057	24,800
SLJ25606	44,175
SLJ25586	27,154
SLJ25587	31,557

Plasmids SLJ25057 and SLJ25606 were assembled in vector pAGM32305. Plasmids SLJ25586 and SLJ25587 were assembled in vector pICSL32281_LacZ. Maps of both vectors can be found in Annex 1 to this application (Section 1) and a detailed description of vector elements has been presented in Section 11 of this document.

In both cases, the selectable marker gene *CS*^{R} is located next to the LB of the T-DNA. Schematic representations of T-DNA structures of all four plasmids are

presented in Annex 1 to this application (Section 2) and a detailed description of T-DNA elements has been presented in Section 12 of this document.

The initial molecular characterization of plants carrying the above-mentioned plasmids is done by polymerase chain reactions (PCR). Genomic DNA samples are extracted from several independent lines of each genotype. The quality of the genomic DNA is assessed by PCR with primers designed to bind to the potato elongation factor 1 alpha (*EF1*α) gene. The presence of the transgenes is assessed by PCR with primers specific to the sequences of the selectable marker gene and genes of interest. Amplicons have been designed within the transgenes and spanning the junctions between the different genes in the T-DNAs.

Sequences outside the T-DNA are not expected to integrate; however, this may still happen in some instances. For that reason, we have optimised the design of the transformation vector to reduce the chances of unintended backbone integration and to be able to counter-select plants in which this has happened.

Transference of the T-DNA to the plant normally starts in the RB region and finishes in the LB region. We have included an overdrive sequence in the RB region of both vectors (RBO) to ensure an efficient transfer of T-DNAs in the correct orientation (Peralta et al, 1986; Shurvinton and Ream, 1991). In addition, all plasmids carry an extra T-DNA LB sequence in between the selectable marker gene and the vector's LB. This sequence was added with the aim of decreasing the chances of backbone integration due to LB 'read-through' during T-DNA transfer. Finally, both vectors carry the *A. tumefaciens* isopentenyl transferase (*ipt*) gene in the backbone region just outside the LB. This gene codes for an enzyme that drives the synthesis of the natural cytokinin isopentenyl adenosine. Production of this cytokinin during tissue culture induces a characteristic bushy shooting/non rooting phenotype that allows to easily counter-select plants where the backbone has been integrated (Richael et al, 2008).

In addition to the *ipt* gene, the backbone also contains the neomycin phosphotransferase II (*nptII*) gene for bacterial selection only. This gene is expressed as an enzyme that inactivates the antibiotics neomycin, kanamycin, geneticin (G418), and paromomycin by phosphorylation. The expression of this gene is driven by a bacterial promoter and the protein encoded has been shown to be bio-safe, non-toxic and poses no risk to human or animal health (The EFSA Journal, 2009, 1034: 66-82).

The rest of genetic elements in the vectors' backbone are regulatory sequences that do not encode proteins. Border sequences (LB and RBO) derive from *A. tumefaciens*. Replicon pBR322 derives from *Escherichia coli* while replicon RiA4 derives from *A. rhizogenes*. The function of these replicons is to serve as origins of replication in bacterial cells. Finally, the promoter region of the beta-lactamase (*bla*)

gene derives from *E. coli* and drives the expression of *nptII* in bacterial cells. All these elements have been detailed in Section 11 of this document.

In summary, in the unlikely event of backbone integration, none of the elements in the vectors' backbone poses a significant risk. Both the *ipt* and the *nptII* genes are already widely present in the environment and the use of the *nptII* gene has been deemed safe by EFSA (The EFSA Journal, 2009, 1034: 66-82). The rest of the elements are also present in the environment and do not code for proteins.

After analysing the presence of the T-DNAs, transgenic plants are also assessed for the presence of backbone sequences by PCR. Amplicons span the *ipt* coding sequence next to the LB region, the *nptII* coding sequence and the RiA4 replicon next to the RBO region. Details of the methods and primers used in PCR tests can be found in Annex 1 to this application (Section 3).

It is worth noting that lines transformed with SLJ25057 have already been tested in the field in Norwich and Cambridge under consents 17/R29/01 and 19/R29/01. Field trials took place in the summers of 2017-2021 and further details on the characterisation of such lines have been submitted to APHA as part of the corresponding release reports.

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

Not applicable.

(c) the copy number of the insert, and

Since both plasmids carry the *Agrobacterium* low-copy-number RiA4 replicon, insert copy numbers in all the transgenic lines is expected to be low. In line with this, plants transformed with constructs based on the pAGM32305 or pICSL32281_LacZ vectors normally show between 1 and 2 inserts. The copy number and integration site will be further assessed by local capture on a preselected set of lines.

(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 localisation of the transgenes.

15. The following information on the expression of the insert

(a) The genetic stability of the insert and phenotypic stability of the genetically modified plant:

Agrobacterium-mediated transformation generates stable insertions. So far, no genetic or phenotypic instability has been observed in the lines that have been tested in the glasshouse and in field trials in Norwich and Cambridge. The plants that will be taken to the field if the permit is granted, will be obtained from the original transformed lines generated at The Sainsbury Laboratory, Norwich. Lines showing genetic or phenotypic instability when characterised 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.

(b) Conclusions on the molecular characterisation of the genetically modified plant:

The molecular characterisation of the set of transgenic lines described in this proposal is aimed at establishing the presence of the full sequences of the T-DNAs as well as absence of any vector elements beyond left and right borders. This is established by PCR amplifying sequences at the junctions between all the genes in the T-DNAs, and by PCR amplifying the selected sequences in the vector backbones (in *nptII* gene, *ipt* gene and RiA4 sequence). Only the lines that carry full T-DNA as expected and do not contain parts of the vectors are considered as suitable for release. The primer sequences and the PCR amplification details can be found in Part A1 Annex, Section 3 and Table 1.

Part IVA Information on specific areas of risk

16. Any change to the persistence or invasiveness of the genetically modified plant and its ability to transfer genetic material to sexually compatible relatives and the adverse environmental effects arising:

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). Potato plants are not invasive of natural habitats. The pollen of potato normally disperses less than 10 metres, is often infertile and potatoes cannot cross with other crop plants to produce hybrids. Estimates of the rates of cross-pollination under field conditions range from 0 to about 20% (Plaisted, 1980). Other studies have shown that the rates of cross-pollination are 2% at 3 metres from the crop, reducing to 0.017% at 10 metres (McPartlan and Dale, 1994).

Neither the genes or the gene-silencing module introduced into the potato plants proposed for release confer characteristics that would increase the competitiveness of plants 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.

Based on current knowledge, the overall risk to the environment from transgenic potatoes sited at least 20 metres from other plants with which it is cross-fertile is effectively zero. The resistance traits to be expressed are predicted to affect only the target pathogens, *P. infestans*, PVY and potato cyst nematodes (if present). The expected environmental impact is negligible and will most probably reduce the level of other agricultural inputs such as use of fungicides or nematicides to control late blight or potato cyst nematodes in potato crops.

17. Any change in the ability of the genetically modified plant to transfer genetic material to microorganisms and the adverse environmental effects arising:

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, following harvest, will be destroyed, or kept under contained conditions for experimental purposes. 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.

18. The mechanism of interaction between the genetically modified plant and target organisms, if applicable, and the adverse environmental effects arising:

The target organism of the plants carrying *Rpi-vnt1.1, Rpi-amr3* and *Rpi-amr1* is *P. infestans*, the cause of potato late blight. The goal of the proposed trial is to assess the level of resistance of such plants to circulating strains of the late blight pathogen. It is expected, as seen before, that this interaction will be manifested by a reduction in the ability of *P. infestans* to infect the genetically modified potatoes. The benefits of this approach have been extensively reported in the scientific literature, including

examples of potato plants carrying *R*-gene stacks that have been field-trialled within the European Union (Haverkort et al, 2016; Jo et al, 2016). The same *Rpi* gene stack has been extensively trialled in releases under consents 17/R29/01 and 19/R29/01, and it's expected to perform equally well in the new release.

The effects of Ry_{sto} and *Hero-NRC6* will be observed if the relevant pathogens (PVY and *G. pallida*) are present at the release sites, which is more likely for aphid-transmitted PVY. An enhanced resistance against PVY should be observed, which should manifest in the absence of detectable PVY material in haulm samples and tubers, as Ry_{sto} provides extreme resistance to PVY.

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).

The gene-silencing module incorporated in some of the plants proposed for release does not target organisms and is expected to improve tuber quality only. It is worth noting that the enzyme PPO has been linked to plant defence responses; however, silencing of *Ppo* in tubers does not enhance susceptibility to the late blight pathogen (Rommens et al, 2006). Also, commercial potatoes were the *Ppo* gene has been silenced by the same mechanism don't show increased disease susceptibility. This is probably due to an incomplete suppression of the browning process and to other plant defence mechanisms.

19. Potential changes in the interactions of the genetically modified plant with no-target organisms resulting from the genetic modification and the adverse environmental effects arising:

Resistance proteins 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 late blight *R* genes will affect other non-target organisms that could be in contact with these plants during the trial.

Further, as mentioned above, the gene-silencing module incorporated in some of the plants proposed for release do not target organisms and are expected to improve

tuber quality only. In a similar way, the selectable marker gene is not expected to affect the interaction of the plants with target or non-target organisms.

Overall, no effects on non-target organisms 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 use of anti-fungal chemicals in the experimental field, an increase in the populations of non-target organisms that are normally affected by 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.

20. Potential changes in agricultural practices and management of the genetically modified plant resulting from the genetic modification, if applicable, and the adverse environmental effects arising,

The only significant difference between the genetically modified potatoes in this application and regular cultivated potatoes is the reduced need for use of conventional disease controls (mainly fungicides, but also nematicides and insecticides), which is a desirable effect, reducing the cost and environmental impact of the potato cultivation. This is especially relevant in case of larger scale cultivation, out of the scope of the proposed trials.

Within the trial, the alterations in fungicide use are likely to have some 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. Overall impact of the trial 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.

21. Potential interactions with the abiotic environment and the adverse environmental effects arising:

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. Except for 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 fertiliser application.

22. Any toxic, allergenic or other harmful effects on human health arising from the genetic modification:

Potato plants transformed with all four plasmids contain genes conferring increased resistance to potato late blight (*Rpi-vnt1.1, Rpi-amr3* and *Rpi-amr1*). The proteins

encoded by these genes are not expected to exert any toxic, allergenic, or other harmful effects on human health.

Rpi-vnt1.1, Rpi-amr3, *Rpi-amr1*, Ry_{sto}, *Hero* and *NRC6* 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 class of *R* genes contains most 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 *S. 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 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 around 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 *R* 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.

Potato plants transformed with all plasmids except SLJ25587 also contain a gene-silencing module. As described previously, this module only includes potato sequences and their structure is such that they do not code for proteins. Instead, they direct the transcription of RNA molecules with complementary sequences and their mode of action is based on using the endogenous post-transcriptional silencing machinery of plants to reduce the expression of the *Ppo* and *VInv* genes in tubers. No toxic or allergenic potential is therefore expected, and nucleic acids (such as the endogenous RNA and DNA molecules of plants) are readily degraded by human digestive fluids (Liu et al, 2015). It is also worth noting that transgenic potatoes developed with an equivalent technology have been approved for commercialization in the US, and several varieties are available on the market.

All the plasmids used to generate the plants included in this application carry the selectable marker gene CS^R. CS^R is an allele of the tomato acetolactate synthase (*ALS*) gene that has been cloned under the control of its native regulatory elements. It codes for a variant of the ALS enzyme that is resistant to inhibition by some herbicides (sulfonylureas and imidazolinones). Resistance to ALS-inhibiting herbicides is present in several commercially-available crops, including wheat, soybean, rice, canola and sunflower (Green and Owen, 2011; Hanson et al, 2014). In all of them, resistance is due to mutations in the *ALS* gene. This is also the case for the tomato *ALS* allele introduced in the plants proposed for release. Resistance

to these herbicides has been typically achieved by traditional breeding methods but at least one transgenic event that includes a resistant *ALS* allele has been deregulated in the US (Green and Owen, 2011). Therefore, no harmful effects are predicted to arise from the use of this marker gene.

Furthermore, linker sequences used to assemble the plasmids included in this application do not code for proteins so no toxic or allergenic potential is predicted.

Finally, as mentioned above, several measures have been taken to avoid backbone integration in the transgenic plants to which this application refers. In the unlikely event of backbone sequences being inserted, the only two protein-coding genes present in the vectors' backbones are the marker gene *nptII* and the *ipt* gene.

The marker gene *nptII* (or *aph(3')-IIa*) is under the control of a bacterial promoter and is used for bacterial selection only. It 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 isopentenyl transferase (*ipt*) gene derives from the soil bacteria *A. tumefaciens*. This gene codes for an enzyme that catalyses the synthesis of the cytokinin isopentenyl adenosine, which naturally occurs in plants (Sakakibara et al, 2005). Plants have their own isopentenyl transferase genes for cytokinin production, some

of which are expressed in edible parts of crops like maize kernels (Brugiere et al, 2008).

In this case, the presence of the *ipt* gene in the vector backbone of pAGM32305 allows the counter-selection of plants where the backbone has been integrated. The enzyme IPT encoded by the backbone *ipt* gene stimulates the production of natural cytokinins during tissue culture of transformed plants. This induces a characteristic shooting phenotype that allows the easy identification of such plants (Richael et al, 2008).

If the backbone *ipt* gene is not significantly expressed due to positional effects or has been only partially inserted, it is possible that plants where parts of the backbone have been integrated 'scape' the counter-selection step. Therefore, plants selected for release will be screened for the presence of backbone sequences in any case. However, in line with the above discussion on the *nptII* backbone gene, no harmful effects are expected in relation to this gene either. If the gene is normally expressed and the IPT enzyme produced, plants will display an abnormal development and will be discarded. In addition, the IPT enzyme sequence is not flagged as a potential allergen by Allergenonline (www.allergenonline.com). An '80mer Sliding Window Search' was carried out and it yielded no matches of significant identity. Such search is described as 'a precautionary search using a sliding window of 80 amino acid segments of each protein to find identities greater than 35% (according to CODEX Alimentarius guidelines, 2003)'.

Furthermore, integration of coding sequences from *Agrobacterium spp.* into plant genomes is a phenomenon that occurs in nature. For example, it has been described that the cultivated sweet potato's genome contains *Agrobacterium* T-DNA sequences with expressed genes (Kyndt et al, 2015).

In addition to the absence of known harmful properties of any of the genetic elements present in the modified potatoes, no harmful properties are expected to emerge when the above-mentioned genes and traits are combined. Finally, tubers will be destroyed or kept under contained experimental conditions after harvest and thus there will be no risk of the genetically modified material entering the food chain.

23. Conclusions on the specific areas of risk

The genetically modified plants proposed for release in this application, are unlikely to pose any risk on the areas of potential influence as described above. The long-term possibility of reduced use of chemical pest control and associated putative positive impact on soil and plant-associated microflora, can only be considered a beneficial and desired effect.

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 3, Table 1).

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

A set of lines transformed with SLJ25057 has been tested in the field by The Sainsbury Laboratory in Norwich under consent 17/R29/01 in the years 2017-2021, and by NIAB Cambridge under consent 19/R29/01 in 2020 and 2021.

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 at two locations:

1. (2022-2025) The Sainsbury Laboratory, Dorothea de Winton field station, JIC (Ordnance Survey map grid reference TG 1525)
2. (2023-2025) NIAB trial site Cambridge (Ordnance Survey map grid reference TL 4362)

27. A description of the release site ecosystem, including climate, flora and fauna

The release site 1 is in arable agricultural areas of the Church Farm, Bawburg. The flora and fauna are typical of agricultural land in the East of England.

The release site 2 (NIAB) 1 is in arable agricultural areas of the NIAB trial site in Cambridge Park Farm, Histon. The flora and fauna are again typical of agricultural land in the East of England.

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

No sexually compatible wild relatives of potato are present in the UK. The only compatible cultivated species is potato, and the already minimal risk of sexual reproduction (see section 6 above), will be further minimised by observing a 20 m distance between the experimental plots and any other potato plots.

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

For site 1, there are no officially recognised biotopes, protected areas or Sites of Special Scientific Interest (SSSIs) within approx. 3.5 km of the release site. The closest SSSI to the release site is River Wensum SAC which is ~3.5 km away across A47 motorway and is comprised of inland running water body, bogs, marshes, water fringed vegetation, fens, humid grassland, mesophile grassland, and broad-leaved deciduous woodland and thus unlikely to be in any way affected.

For site 2, the two closest SSSIs are Traveller's Rest Pit at ~1 km away and Histon Road at ~1.5 km away, but both of those are of geological significance only. The closest SSSI of relevance is Madingley Wood, ~2.5 km away from site 2, which is a protected woodland area unlikely to host any potato plants.

Given that potato pollen is not normally disseminated more than 10 metres from the parent plants, the distance from both sites to the nearest relevant SSSIs equates to 250-350 times the normal dissemination distance.

Potato does not hybridise with any British native plants. This combined with the fact that potato is not a wind-pollinated plant and is rarely visited 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, The Sainsbury Laboratory in Norwich has been working towards identifying, mapping and isolating resistance (*R*) genes from potato that confer resistance against potato late blight (*P. infestans*). This research has been publicly funded. In addition to that, other valuable R genes have been identified at the TSL, including a PVY resistance gene, Ry_{sto}, that targets the most economically important viral pathogen of potato.

The genes identified are 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.

The *Rpi* genes *Rpi-vnt1.1, Rpi-amr3* and *Rpi-amr1* have been transformed into Maris Piper or Hermes potato as a three-gene stack in combination with a gene-silencing

module conferring increased tuber quality (SLJ25057) and tested extensively in the field trials in Norwich and Cambridge, in the years 2017-2021. Two such lines in the Maris Piper background will be used as positive controls for the *Rpi* gene stack and tuber quality module.

The setup of SLJ25057 was enhanced with PVY resistance gene, Ry_{sto}, and Hero-NRC6 set (SLJ25606) or Ry_{sto} only (SLJ25586) for transformation of Maris Piper. This next generation of *P. infestans* resistant lines is designed to also benefit from PVY/PCN or PVY resistance.

Finally, the same stack of *Rpi* genes was combined with Ry_{sto} only to form plasmid SLJ25587 for transformation of potato cultivar Charlotte. Charlotte, as non-industrial potato, mostly grown for individual consumption, would benefit less from enhancing tuber quality, thus the silencing module was omitted from the line-up.

Robust assessment of performance in the field normally requires testing the plants in different locations. The main goals of the proposed release are:

1) to expose plants containing the *Rpi* stack to the current local populations of late blight to reconfirm that they are indeed useful and capable of conferring resistance in different geographical locations with changing *P. infestans* populations;

2) to assess the field performance of Ry_{sto} against PVY in the trial conditions;

3) to assess the agronomic performance and yield of the modified plants in comparison to wild-type Maris Piper and Charlotte plants under standard fungicide sprays;

4) to harvest tubers for detailed assessment of potential for browning and cold-induced sweetening, as well as other relevant characteristics such us dry-matter content

5) to select the best lines of each type for further development towards a GM variety (varieties).

The transgenic plants included in this application have been generated with funding from the Horticulture and Potato Initiative (HAPI) and Follow-on Fund. These programs stemmed from British Biotechnology and Biological Sciences Research Council's (BBSRC) strategy to support innovative developments in bioscience. The goal of the HAPI was to address challenges faced by the horticulture and potato industries in the UK, and funding has been granted for collaborative works between research institutions and industrial partners. Follow-on Fund was granted to complete the work mainly funded by HAPI.

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

For location 1, the releases will be conducted between 1 April and 30 November in the years 2022-2025. For location 2, the releases will be conducted between 1 April and 30 November in the years 2023-2025.

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

Plants or tubers will be planted in the field by hand following a non-randomised or 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 release sites will be subjected to shallow tillage, herbicide application, irrigation, fungicide spraying, following best agricultural practice and the requirements of the trials. Planting and harvesting will be done by hand and using handheld tools (forks, hand trowels), not machinery. Post-trial removal of volunteers will be done by hand and fork or by herbicide spraying.

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

The total number of genetically modified plants to be released across all years and both locations won't exceed 10,000.

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

35. (1) A description of any precautions to maintain spatial and, as the case may be, temporal separation of the genetically modified plant from sexually compatible plant species

(2) In sub-paragraph (1) "plant species" means:

(a) Wild and weedy relatives, or

There are no sexually compatible wild relatives capable of hybridising with potato present in the UK.

(b) Crops

Transgenic plants will be isolated from 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 two years of left

fallow, the only crops potentially grown on the release site will be those that allow easy identification and destruction of volunteers.

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 volunteers during the remainder of the year and sprayed with a systemic broadleaf herbicide. Any volunteeers 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 every season of the release in accordance with DEFRA guidance. During this time the plot will be left fallow to enable easy identification and removal of groundkeepers. Monitoring will continue for another two years after every season of the release, but crops easy to distinguish from potato may be grown.

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, removed from site and destroyed by deep burial, incineration or autoclaving.

38. A description of monitoring plans and techniques

The release site will be visited by trained personnel who are working on the project at minimum monthly intervals from planting to harvest in each year of the trial. 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.

At the end of each season, the plot will be left fallow and monitored for groundkeepers during the remainder of the year. Any groundkeepers identified will be destroyed by herbicide treatment (e.g. glyphosate) or removed by hand and destroyed by deep burying or incineration/autoclaving. Following the completion of each year of the trial, the individual release plots will remain fallow for two years to enable easy identification of volunteers. The sites will be inspected monthly between March and November and any volunteers identified will be immediately destroyed

either by application of a systematic broadleaf herbicide or by hand pulling plants and digging out tubers/root systems. These will then be destroyed by deep burying or incineration/autoclaving. For another two years, monitoring will continue, but crops easily distinguishable from potato may be grown. After the end of the monitoring period, DEFRA recommendations will be followed for the management of the release site. Both raw data and reports of inspections of groundkeepers and volunteers will be maintained and provided to DEFRA.

39. A description of any emergency plans

Emergency procedures: At any time 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 destroyed by deep burying or incineration/autoclaving. 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

Both release sites are contained within the field experimentation stations, with access limited to trained personnel. Standard Operating procedures will further regulate the access to the sites, sites maintenance and monitoring before, during and post release, planting and harvesting practices and emergency situations.

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 (Section 3). The transgenic plants were generated and initially characterised in the laboratory, glasshouses and field trials at The Sainsbury Laboratory (Norwich, UK).

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