Part B: Information about the release application to be included on the public register

B1. The name and address of the applicant

The Sainsbury Laboratory - Norwich Research Park, Norwich, NR4 7UH

B2. A general description of the genetically modified organisms in relation to which the application is being made

The organisms to be released are genetically . potato plants that have been modified to improve different traits: resistance to *Phytophthora infestans* (the organism responsible for the devastating late blight of potatoes), resistance to potato cyst nematodes (PCN), resistance to Potato Virus Y (PVY) and improved tuber quality. To generate the genetically modified plants, *Agrobacterium*-mediated transformation was used, which utilises the natural ability of the soil bacterium *A. tumefaciens* to stably incorporate foreign DNA into the genotype of plants.

All of the transgenic lines contain a stack of three *P. infestans* resistance (*R*) genes (*Rpi-amr3*, *Rpi-amr1* and *Rpi-vnt1.1*). Deployment of *R*-gene stacks has the potential to confer an efficient and relatively durable resistance by combining different recognition specificities. These genes were isolated from the wild potato relatives *Solanum americanum* and *S. venturii* and they confer useful resistance against different isolates of the late blight pathogen. All the lines also contain another *R* gene, a PVY resistance gene Ry_{sto}, sourced from *Solanum stoloniferum*, a wild potato species. *R* genes enable plants to recognise certain isolates of the pathogen, which possess a specific corresponding avirulent effector gene. The recognition event triggers a signalling cascade culminating in expression of the plant defense response, which acts to prevent further pathogen growth within the host plant. All of these *R* genes are regulated by their endogenous promoters and terminators. These terminator sequences regulate the expression of the genes and are not related to any technology that prevents seed propagation of plants.

In some lines, the *P. infestans* and PVY resistance genes have been introduced in combination with a gene-silencing module that improves tuber quality. This silencing module is designed to silence the polyphenol oxidase gene *PPO* and the vacuolar acid invertase gene *VInv* in a tuber-specific manner. Silencing is triggered by the expression of sense and antisense sequences of the above-mentioned genes from convergent tuber-specific promoters and occurs via the endogenous gene-silencing mechanisms of plants.

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. This phenomenon has a negative impact on tuber quality. The gene-silencing module contains sense and antisense sequences derived from the gene encoding the predominant PPO variant in tubers and its silencing significantly decreases enzymatic browning upon bruising.

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. To silence this gene in tubers, the gene-silencing module contains sense and antisense sequences derived from the potato *Vlnv* gene.

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. The Food Standards Agency (FSA) has recently released a report on foods with high potential for acrylamide formation, advising on actions to reduce dietary intake of this compound. Silencing of the *Vlnv* gene in tubers contributes to a reduction in the acrylamide-forming potential, since it decreases the availability of reducing sugars.

Some of the transgenic lines included in this application contain a stack of another two R genes (*Hero* and *NRC6* from tomato) that confer resistance against PCN, an important quarantine pathogen. Hero on its own does not confer resistance to PCN in potato, therefore a tomato helper gene, *NRC6*, is delivered together with Hero to enable PCN resistance. Both these *R* genes are regulated by their endogenous promoters and terminators. These terminator sequences regulate the expression of the genes and are not related to any technology that prevents seed propagation of plants.

In addition to the previous traits, all the transgenic plants proposed for release in this application also contain the *CSR* 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.

B3. The location at which the genetically modified organisms are proposed to be released

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)

B4. The purpose for which the genetically modified organisms are proposed to be released (including any future use to which they are intended to be put)

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 pathogen, *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 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. The stack of three R genes providing resistance to *P. infestans* proved highly effective against late blight in the field conditions across several years. Similarly, the gene silencing module performed as expected in the tubers of the transgenic lines. To increase the range of the pathogens targeted, Ry_{sto} was added to all newly created transgenic lines. Some of the new lines also received the stack of PCN resistance genes, *Hero* and *NRC6*.
Four distinct groups of transgenic potatoes are proposed to be tested, as below:

Name	*P. infestans* resistance	PVY resistance	Gene silencing module	PCN resistance
SLJ25057	YES	NO	YES	NO
SLJ25566	YES	YES	YES	YES
SLJ25586	YES	YES	YES	NO
SLJ25587	YES	YES	NO	NO

Lines transformed with SLJ25057 have been tested in the field in Norwich and Cambridge under consents 17/R29/01 and 19/R29/01 in the years 2017-2021.

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.

B5. The intended dates 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.

B6. The environmental risk assessment

Four hundred years of cultivation have established that the potato has limited ability to survive in UK environments except when cultivated. Plants generated from tubers are readily eliminated and potato plants are not invasive of natural habitats. We expect no difference with respect to persistence in agricultural habitats or invasiveness into natural habitats as compared to conventional potato varieties under normal agricultural practice. The pollen of potato normally disperses less than 10 metres and cannot cross with other crop plants to produce hybrids. Through the precautionary measures undertaken for the duration of the release and the maintained distance from, or absence of, conventionally cultivated potatoes or wild

species, the possibility of any gene transfer is effectively zero. Even in the very improbable event that pollen was to be transferred to non-genetically modified potato plants, no consequences are to be expected, since potato propagation conventionally takes place via tubers and not via seeds.

Most of the lines included in this application carry resistance (*R*) genes. Non-transgenic potato plants also contain many *R* genes, which are active against a wide range of potential pathogens. Many conventional potato varieties also contain additional *R* genes against *P. infestans* and PVY that have been introgressed from wild *Solanum* species. An intended effect of the introduced trait is increased survivability of the genetically modified potatoes exposed to *P. infestans*, PVY and PCN. These possible selective advantages, however, are of importance only in the agricultural field and will not intrinsically improve the survivability in the surrounding environment.

The resistance traits to be expressed are predicted to affect only the target pathogens, *P. infestans*, PVY and PCN (if present). The expected environmental impact is negligible and will reduce the level of other agricultural inputs such as use of fungicides to control late blight in potato crops.

The gene-silencing module linked to the stack of late blight resistance genes is designed to modify tuber quality traits that are important in post-harvest management and processing of the potato tubers. They are not expected to affect the fitness of the plants in field conditions.

All the plasmids used to generate the plants included in this application carry an allele of the tomato acetolactate synthase (*ALS*) gene encoding a variant of the ALS enzyme that is resistant to inhibition by sulfonylureas and imidazolinones. Resistance to ALS-inhibiting herbicides is present in several commercially-available crops. This trait will be only used for the *in vitro* selection of transgenic lines during tissue culture. The plants remain sensitive to other herbicides such as glyphosate or glufosinate, which could readily be used to eliminate them in the field. In addition, sulfonylureas and imidazolinones will not be used in the context of this trial, so no selective advantage will be conferred to these plants.

Finally, in the unlikely event of backbone integration, no detrimental effect is expected from any of the elements in the vector backbone and no other emergent advantages or disadvantages are expected from the proposed combinations of genes and traits.

The interactions of the genetically modified potato lines with non-target organisms and the effects resulting from this will be comparable to those of conventional potato varieties. Due to a reduced need for fungal treatments, an increase in the

populations of those non-target organisms that respond to fungal treatments might be expected. Similarly, the nematode resistance trait has been previously tested in the field. Works performed with similar transgenic lines established considerable advantages of this approach to soil micro-organisms relative to nematicide use, with no detrimental effects on non-target organisms and soil health.

No toxic or allergenic effects are expected from any of the additional proteins expressed in the transgenic lines proposed for release. Measures which are taken under current release practice will both protect the trial against damage by wild animals, and also ensure that seed stock and plant material are harvested, transported and disposed in a way that minimises or prevents contact with people or animals. No effects on biogeochemical processes are expected, other than those that apply also to non-genetically modified potatoes.

B7. The methods and plans for monitoring the genetically modified organisms and for responding to an emergency

The release site will be visited by trained personnel who are working on the project at approximately weekly 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. The monitoring of the plot for groundkeepers will be continued at monthly intervals for the duration of the three-year trial by walking the trial site.

Following completion of the trial, the plot will be 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. Both raw data and reports of inspections of groundkeepers and volunteers will be maintained and provided to DEFRA. The

cultivation of the release site after the monitoring programme has concluded will be according to local crop rotation practice for potatoes.

Emergency procedures: 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 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.