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 modified potato plants that have been modified for improved resistance to *Phytophthora infestans*, the organism responsible for the devastating late blight of potatoes. To generate the genetically modified plants, *Agrobacterium*-mediated transformation was used, which utilises the natural ability of the soil bacterium *Agrobacterium tumefaciens* to stably incorporate foreign DNA into the genotype of plants.

For the production of potato lines with improved resistance to *P. infestans* six additional resistance genes (*R* genes) were (or will be) introduced independently generating six distinct transgenic lines. *R* genes specifically recognize their target pathogen and induce a defense response in the plant, thus protecting the plant from infection by the pathogen. Six *R* genes, one from *Solanum venturii* (a wild potato species from South America), three from *Solanum americanum* (a wild potato relative commonly known as American black nightshade) and two from the potato variety Sarpo Mira were used. All of these *R* genes are regulated by their endogenous promoters and terminators.

Plants carrying the *R* genes *Rpi-vnt1.1, Rpi-Smira1* and *Rpi-Smira3* will also contain the selectable marker gene *nptII*, required only for the pre-selection of transgenic lines. Expression of *nptII* produces the enzyme neomycin phosphotransferase, which confers on the plants resistance to the antibiotic kanamycin. This antibiotic is not used routinely for medical treatment of humans or animals and is known to be biosafe as used.

Plants carrying the *R* genes *Rpi-amr3*, *Rpi-amr1e* and *Rpi-amr1k* will also contain the selectable marker gene *bar*, used only for the pre-selection of transgenic lines. Expression of *bar* produces an *N*-acetyltransferase enzyme which catalyses the acetylation of the herbicide glufosinate. Therefore, plants carrying this gene will be resistant to herbicides that have glufosinate as active ingredient. No toxic or harmful effects on human health and the environment have been described for the *bar* gene. Plants expressing this gene will still be susceptible to other commonly used broadleaf herbicides and glufosinate-containing herbicides will not be applied in the context of this trial.

In plants carrying *Rpi-vnt1.1,* the *nptII* selectable marker is flanked by promoter and terminator sequences from the nopaline synthase gene of *A. tumefaciens*.

In plants carrying *Rpi-Smira1* or *Rpi-Smira3,* the *nptII* selectable marker is flanked by the promoter sequence of the nopaline synthase gene of *A. tumefaciens,* a short regulatory sequence from the tobacco mosaic virus (TMV omega leader) and the terminator sequence from the octopine synthase gene of *A. tumefaciens.* The TMV regulatory sequence modulates the expression of the transgene and is not translated into protein. Finally, in plants carrying *Rpi-amr3*, *Rpi-amr1e* or *Rpi-amr1k,* the *bar* selectable marker is flanked by promoter and terminator sequences from the nopaline synthase gene of *A. tumefaciens*. All the terminator sequences mentioned above regulate the expression of the genes and are not related to any technology that prevents seed propagation of plants.

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

The plants will be released on an area of 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 on the release site each year of the trial. For each year of the field trial we will release no more than 200 transgenic plants.

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, 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*. This gene has been transformed into the potato cultivar Desiree and successfully tested in the field in recent years. The line carrying *Rpi-vnt1.1* will be used as 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.

B5 The intended dates of the release.

If consent is granted, the 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.

B6 The environmental risk assessment.

The genetically modified potato lines each contain one of six *R* genes: *Rpi-vnt1.1* from *Solanum venturii, Rpi-amr3, Rpi-amr1e or Rpi-amr1k* from *Solanum americanum* and *Rpi-smira1 or Rpi-smira3* from the potato variety Sarpo Mira. All of these genes confer improved resistance to *Phytophthora infestans*. 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* 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*. This possible selective advantage, however, is of importance only in the agricultural field, and will not otherwise improve the survivability in the surrounding environment.

Four hundred years of cultivation of the potato 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 were 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.

The interactions of the genetically modified potato line with non-target

organisms and the effects resulting from this will be comparable to those of conventional potato varieties. No toxic or allergenic effects are expected which could be attributed to the improved resistance to *P. infestans*, the neomycin phosphotransferase enzyme or the glufosinate *N*-acetyltransferase enzyme. 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 such a way in order to minimise or prevent contact with people or animals. No effects on biogeochemical processes are expected, other than those that apply also to non-genetically modified potatoes.

The *nptII* gene expressed in some of the potato plants imparts resistance to certain antibiotics only used during the selection process in tissue culture. This confers no selective advantage in the field. It has been considered safe for such use by the European Food Safety Authority and it has an over 20-year history of use with transgenic crops for this purpose.

The *bar* gene present in some of the potato plants confers resistance to glufosinate-containing herbicides and will only be used during the selection process in tissue culture. No adverse effects on human health and the environment have been described for this gene. Transgenic events including the *bar* gene have been previously assessed by the EFSA GMO panel and no concerns were identified. Glufosinate will not be used in the context of the proposed trial and plants expressing the *bar* gene are still susceptible to other commonly used herbicides.

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 laboratory personnel who are working on the project at no less than weekly intervals during May-November (the potato growing season) of each year of the trial. 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.

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 heat treatment. 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 three-year 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 systematic 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 two-year 'fallow' 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. 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 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.