Teagasc response to EPA queries of April 4th and May 23rd on Notification B/IE/12/01 **June 13th, 2012**

Query No.	Comment	EPA query and Teagasc response
1.	No. 11, EPA request of 4th April, 2012	11. Please provide molecular data (gel data) supporting the data given in Table 1 and Figure 4 on pages 16 and 17 of the application, respectively. Response: Molecular data has been provided in Appendix 1.
2.	No. 1, EPA request of 23rd May, 2012	1. How will the comparator plants, grown in parallel to the GM lines within each site be treated / managed, following completion of the trial? Response: Harvested tubers from the comparator plants will be removed off the field and stored in a secure location within the biotechnology building. All plots on the site will then undergo repeat harvesting to minimise tuber loss post-harvest and the site will then be surveyed by project personnel who will collect mini tubers and tuber pieces (irrespective of being GM or comparator) in sealed bags for disposal by steam sterilisation or burial. It is intended that the comparator tubers collected from the 2012 harvest will be used to supplement the number of tubers required for the following 2013 season and the same will occur from 2013-2014 and 2014-2015. Although they are non-GM in origin, comparator tubers will be stored within the confines of the EPA licensed facility in Oak Park between growing seasons. This will ensure their complete separation from any non-GM commercial potato lots. No comparator tuber material will be supplied to animals for feed purposes during the course of the study.
3.	No. 2, EPA request of 23rd May, 2012	2. Please provide a definition for 'agri-environment' and 'cisgenesis'? Response: Within the confines of the proposed notification, the term 'agri-environment' refers to the farmland-related biodiversity and associated ecological networks within the confines of a managed agricultural land

		system. Cisgenesis is the modification of a recipient organism with a gene (cisgene) from a crossable - sexually compatible – organism (same species or closely related species). The gene includes its introns (if present) and its flanking native promoter and terminator in the normal sense orientation.
4.	No. 3, EPA request of 23rd May, 2012	3. Please provide evidence to support the following statements made in the notification. **Pages 27/28, Section H2**: • "The cisgenic line A15-031 only possesses a significant selective advantage over its non-cisgenic comparator within the managed environment of a cropping system and only in the presence of *P.infestans*" Response: A15-031 is genetically equivalent to its comparator Desiree, with the exception of the presence of the Rpi-vnt1.1 cisgene from the wild potato species *Solanum venturii*. The Rpi-vnt1.1 cisgene is disease specific and only confers durable resistance to *P. infestans*. Therefore in comparison to the non-cisgenic comparator (which does not contain Rpi-vnt1.1) A15-031 is equipped with a selective ability to resist *P. infestans* disease. • "In the absence of *P. infestans*, A15-031 is substantially equivalent to its non-cisgenic comparator, with the exception that A15-031 contains the Rpi-vnt1.1 gene, from the wild potato species *S. venturii*". Response: The cisgenic line A15-031 has only been modified with the Rpi-vnt1.1 gene, which remains under the control of the native promoter and terminator sequence. As such, the expression of the insert is dependent on whether particular tissues of the plant are exposed to the pathogenic organism *P. infestans*. Cultivation in the Netherlands indicates the phenotypic equivalence of A15-031 and the comparator Desiree in the absence of *P.*

		infestans as evident in images in Appendix 2 • "…Coupled with the fact that *P. infestans* resistance is not a key determinant for inducting potential invasiveness. It can be concluded …" Response: The primary criterion for an invasive plant species is an ability to out-compete neighbouring plants outside the confines of a managed environment (e.g rhododendron). Potato is incapable of establishing feral populations outside of the managed field environment (e.g. hedgerows), due to its inability to compete with weed species. As *P. infestans* does not infect common weed species it can be concluded that *P. infestans* resistance would not infer a fitness advantage that would permit A15-031 to become invasive. **Page 19, Section D5**: • "The cisgenic A15-031 line to be studied at Oak Park is phenotypically stable as per previous testing conducted in the Netherlands". Response: See images provided in Appendix 2 • **Section D5**: "As the desired trait of increased resistance to *P. infestans* has been consistently expressed in successive generations it has been concluded that the inserts are genetically stable" Response: In the event that the Rpi-vnt1.1 gene was genetically unstable it would not confer the desired phenotype. As a result, significant degrees of *P. infestans* infection and disease would be expected, equivalent to

		what is recorded on the comparator Desiree. In such a scenario A15-031 would be of no use to the proposed study and would not be included in the notification.
5.	No. 4, EPA request of 23rd May, 2012	**5. Finally please provide evidence that the Rpi-vnt1.1 gene gives rise to a single trait i.e. blight resistance and that alternative splicing does not occur.** Response: The genetic sequence that has been inserted into A15-031 is the Rpi-vnt1.1 cisgene, which includes the native promoter and terminator. Rpi-vnt1.1 confers resistance to *P. infestans* only (Vleeshouwers et al. 2011; Zhu et al, 2011). The phenomenon of alternative splicing refers to the ability of a cell to produce multiple proteins from a single RNA transcript, which is translated from a specific DNA sequence, containing exons (coding regions) interspersed with introns (non-coding regions). In alternative splicing, the introns play a key role in deciding the prevalence of alternative splicing. The Rpi-vnt1.1 gene belongs to the NBS-LRR type of resistance genes and containing a single exon does not contain any introns. Indeed, to date no alternative splicing has been detected among intronless CC-NBS-LRR resistance genes in the model research plant, Arabidopsis (Lozano et al. 2012). Separately, it is important to note that alternative splicing is a natural phenomenon in eukaryotic organisms and provides increased protein diversity and functionality. Indeed, recent literature (Lozano et al. 2012) discusses the subject of alternative splicing in potato and the suggested role that alternative splicing may play a crucial role in preserving the stability of the hyper-resistance resistance response. Therefore, while the absence of introns in Rpi-vnt1.1 negates the potential for alternative splicing to occur; if it were to occur the consequence of such an event is likely to extend the durability of the cisgene's activity against *P. infestans*. This is due to the fact that resistance against *P. infestans* is only possible due to the precise recognition of specific *P. infestans* effector proteins by the resistant proteins of the GM potato. So if alternative splicing occurred it would lead to the production by the GM plant of variant resistant proteins against *P. infestans*, hence extending the durability and efficacy of A15-031's blight resistance phenotype.

6		Comment on the proximity of the nearest river to Oak Park Response: The Barrow runs through Carlow town and at its nearest point is positioned 1.47km from the proposed trial site across farmland, the Waterford – Dublin railway line and a section of Oak Park forest.

References used above and attached:

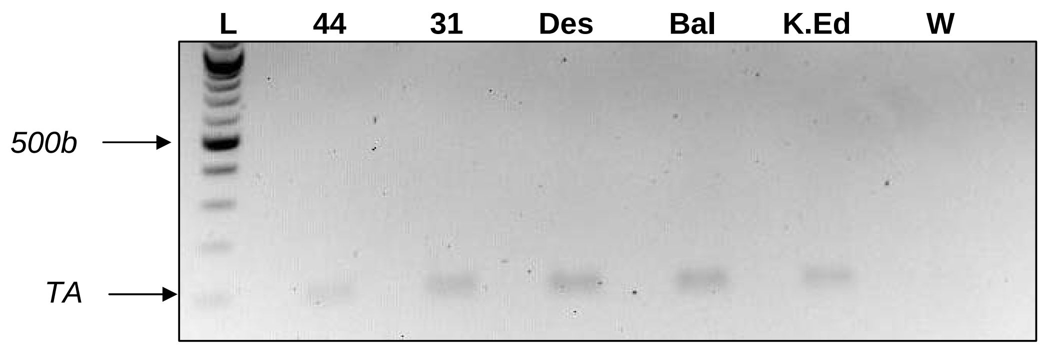
Vleeshouwers et al. (2011). Understanding and Exploiting Late Blight Resistance in the Age of Effectors. Annu. Rev. Phytopathol. 2011.49:507-531.

Zhu et al. (2011). Functional stacking of three resistance genes against Phytophthora infestans in potato. Transgenic Research, DOI 10.1007/s11248-011-9510-1

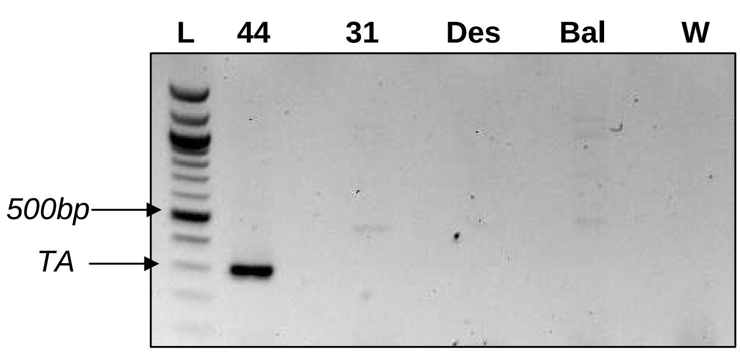
Lozano R, Ponce O, Ramirez M, Mostajo N, Orjeda G (2012) Genome-Wide Identification and Mapping of NBS-Encoding Resistance Genes in *Solanum tuberosum* Group Phureja. PLoS ONE 7(4): e34775. doi:10.1371/journal.pone.0034775,

Appendix 1: Molecular characterisation of A15-031. DNA was extracted from A15-031, A15-044 and Desiree by CTAB based methodology in the Netherlands and provided to Teagasc. PCR cycling conditions were completed as detailed on p. 34 of the Teagasc Notification of February 27th.

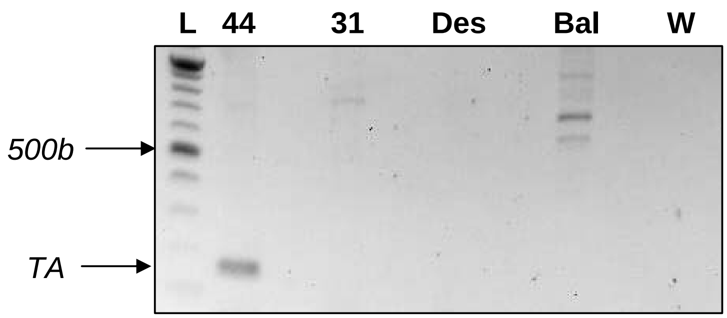
A. Verifying the presence of the target amplicon (TA, size = 110bp) elongation factor 1 alpha (EF1-alpha) sequence in the GM line A15-031 (31), the GM line A15-044 (44), Desiree (Des) and two additional conventional varieties, Balfour (Bal) and King Edward (K.Ed). The non-template control was water (W) and L = 100bp molecular ladder. EF1-alpha is a conserved sequence required for cell division. Present in all potato varieties, irrespective of it being GM or non-GM, EF1-alpha is employed as a control for diagnostic PCR methodologies. (Note; a review of the expected target size of the TA on NCBI, confirmed a size of 110bp, and not the listed size of 200bp in Table 2, p.35 of the Notification of February 27th).



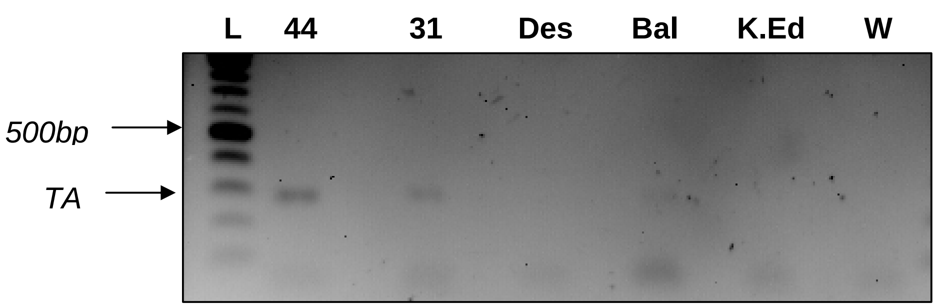
B. Verifying the absence of the tetracycline antibiotic marker gene (*tetA*) in A15-031 (31) and the presence of the target amplicon (TA) of 296bp in the GM line A15-044 (positive control). Desiree (Des) was the non-GM control, from which A15-031 and A15-044 were derived. Balfour (Bal) was included as an additional control for conventional potato. The non-template control was water (W) and L = 100bp molecular ladder.



C. Verifying the absence of the kanamycin antibiotic marker gene (*nptIII*) in A15-031 and the presence of the target amplicon (TA) of 162bp in the GM line A15-044 (positive control). Desiree (Des) was the non-GM control, from which A15-031 and A15-044 were derived. Balfour (Bal) was included as an additional control for conventional potato. The non-template control was water (W).



D. Verifying the presence of the Rpi-vnt1.1 cisgene in the GM lines A15-031 and A15-044 with a target amplicon (TA) ~300bp. Desiree (Des) was the non-GM comparator control and Balfour (Bal) and King Edward (K. Ed) were included as additional conventional potato controls. The non-template control was water (W) and L = 100bp molecular ladder.



Appendix 2:
Images illustrating true-to-type phenotype of A15-031 relative to its non-cisgenic comparator, Desiree, while under cultivation in the Netherlands. **(A)** Large scale image of plots currently being cultivated at Wageningen University, with A15-031 and Desiree as labelled. **(B.)** Images detailing equivalent phenotypes of both A15-031 and the non-GM comparator, Desiree.

A.

B.

Desiree	A15-031