BASF Plant Science

Notification for the release into the environment of genetically modified potatoes with improved resistance to *Phytophthora infestans* (2007 - 2011)

PART A1

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PART A1

INFORMATION REQUIRED UNDER SCHEDULE 1 OF THE 2002 REGULATIONS

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 organism and for the supervision, monitoring and safety of the release.

Applicant:
BASF Plant Science GmbH
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Germany

Regulatory Contact:
Dr. Andreas Heise
BASF Plant Science Holding GmbH
Manager Regulatory Compliance Europe
Agricultural Center, Li444
D-67117 Limburgerhof
Germany

Andreas Heise (PhD) has experience in Molecular Biology, Phytopathologie and Quality Management and acts as Biological Safety Officer.

Responsible Scientist:
Dr. Robert Storer
BASF PLC
Technical Services Advisor, Compliance Officer
Earl Road
SK8 6QG Cheadle

Robert Storer (MSc, PhD, FRES) has experience in developmental field trials, involving a range of GM field crops and will act as Compliance Liaison for BASF Plant Science.

2. The title of the project.
Notification for the release into the environment of genetically modified potatoes with improved resistance to *Phytophthora infestans*.

PART II

INFORMATION RELATING TO THE PARENTAL OR RECIPIENT PLANT

3. The full name of the plant

Family: *Solanaceae*
Genus: *Solanum*
Species: *tuberosum*
Subspecies: *tuberosum*
Cultivar: P698, P835, P880
Trivial name: potato

Properties of varieties	P698	P835	P880
Ripening	Relatively late	Medium	Medium
Growth habit	Intermediate type	Intermediate type	Stem type
Flower colour	White	White	White
Flowering	Middle	Abundant	Abundant
Berry formation	Rarely	Few	Frequently
Tuber shape	Oval-long	Oval	Oval
Tuber skin	Yellow	Yellow	Yellow

4. Information concerning -

(a) the reproduction of the plant:

(i) the mode or modes of reproduction
Reproduction of potato is mainly vegetatively via tubers, though sexual reproduction via botanical seeds is possible. Under field conditions selfing is most likely, with 80-100 % of seeds formed due to selfing.

(ii) any specific factors affecting reproduction
Potatoes survive as tubers or as seed. As the tubers are generally frost sensitive their survivability and reproduction is dependent on temperature. The tuber cannot survive a temperature of –3 °C and lower. It is reported that potato tubers are destroyed by a frost period of 25 hours at –2 °C or a frost period of five hours at –10 °C (OECD 1997).

(iii) generation time
The generation time of potato as it is cultivated in Europe is one year.

(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 is compatible with other cultivated genotypes of the same species in Europe. *Solanum tuberosum* is not compatible with the wild related

species *Solanum dulcamara* (woody nightshade) and *Solanum nigrum* (black nightshade) in Europe (Eijlander and Stiekema, 1994; Raybould and Gray, 1993; McPartlan and Dale, 1994). No viable seeds or plants can be formed (OECD, 1997).

5. Information concerning the survivability of the plant:

(a) its ability to form structures for survival or dormancy

In its original habitat (South America) potato is a perennial plant, but in Europe it is grown as an annual crop. Potatoes survive as tubers or as seed.

(b) any specific factors affecting survivability

As the tubers are generally frost sensitive their survivability and reproduction is dependent on temperature. The tuber cannot survive a temperature of – 3 °C and lower. It is reported that potato tubers are destroyed by a frost period of 25 hours at – 2 °C or a frost period of five hours at – 10 °C (OECD, 1997). Under European conditions the tubers persist poorly in cold wet soils and plants rapidly become infected with a range of fungal and viral diseases (Eastham and Sweet, 2002). The survivability is also limited by cultivation practices such as ploughing, harrowing and application of herbicides and by competition from other crops in the crop rotation. Botanical seed over-winter regardless of temperature. Under field conditions the berries normally do not mature. As European cultivated potatoes are of a heterozygous tetraploid genetic status there is a high level of genetic segregation while forming seeds. Plants potentially arising out of those seeds are usually weak, with poor agronomic performance and low competitiveness. Their survival depends on cultivation practices and crop rotation. Volunteer plants are eliminated by ploughing, harrowing, herbicide treatment and competition in crop rotation.

6. Information concerning the dissemination of the plant:

(a) the means and extent (such as an estimation of how viable pollen and/or seed declines with distance where applicable) of 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.

Dissemination of pollen - if available as many varieties are sterile - is executed almost exclusively by insects. Pollen dissemination is limited with a maximum distance of 5 to 10 m (Bock et al., 2002). Wind dissemination is considered marginal (OECD, 1997; Eastham and Sweet, 2002). Under field conditions selfing is most likely, with 80-100 % of seeds formed due to selfing.

(b) any specific factors affecting dissemination
See above.

7. The geographical distribution of the plant
Potato originates from the Andean region of South America, where it has been cultivated for several thousand years. It is one of the most important crop plants throughout the world and is cultivated in the whole of Europe. Potato is only found in the agricultural ecosystem.

8. Where the application relates to a plant species that is not normally grown in the United Kingdom, a description of the natural habitat of the plant, including information on natural predators, parasites, competitors and symbionts.
Not applicable.

9. Any other potential interactions, relevant to the genetically modified organism, of the plant with organism in the ecosystem where it is usually grown, or elsewhere, including information on toxic effects on humans, animals and other organisms.
Insects like aphids (*Myzus persicea*, *Aphis nasturtii*, *A. frangulae* and others), leaf hoppers (*Empoasca* spp) and the Colorado beetle (*Leptinotarsa decemlineata*) are well known parasites in European potato cultivation, as are nematodes (*Globodera* spp, *Ditylencus* spp, *Paraditylencus* spp, *Tricodorus* spp and *Paratricodorus* spp).
Just like other plants there are many microorganisms, viruses and viroids interacting with the potato plant. Well known pathogenic fungi are for example potato late blight (*Phytophthora infestans*), black scurf (*Rhizoctonia solanii*), potato wart disease (*Synchythrium endobioticum*), early blight (*Alternaria solani*), powdery scab (*Spongospora subterranea*), skin spot (*Polyscytalum pustulans*), silver scurf (*Helminthosporium solani*), grey mold (*Botrytis cinerea*), watering wound rot (*Pythium ultimum*), wilt (*Verticillium* spp) and storage rots (*Phoma foveata* and *Fusarium* spp).
Among pathogenic bacteria, the most common ones are black leg (*Erwinia carotovora* ssp *carotovora*, *Erwinia carotovora* ssp *atroseptica*, and *Erwinia chrysanthemi*) and common scab (*Streptomyces scabies*), while in Europe brown rot (*Pseudomonas solanaceareum*) and ring rot (*Corynebacterium sepedonicum*) are quarantine diseases.
There are many viruses that attack the potato plant. Economically most important are *Potato leaf roll virus* (PLRV), *Potato virus Y* (PVY), *Potato virus A* (PVA), *Potato virus X* (PVX), *Potato virus S* (PVS), *Potato virus M* (PVM), *Tobacco rattle virus* (TRV) and *Potato mop-top virus* (PMTV). Among viroids the *Potato spindle tuber viroid* (PSTVd) is the most important one.
Potatoes are a significant part of the diet in large parts of the world. The only part of the plant which is consumed is the tubers. The main toxic or anti-nutritional substances in potatoes are glycoalkaloids and nitrates. Glycoalkaloids which in high concentrations are toxic, are found in harmful amounts mainly in the above ground parts of the plant stems, leaves and fruits. In the tubers of cultivated potato varieties, the content is usually low, below

100 mg per kilogram fresh weight. A maximum glycoalkaloid content of 200 mg per kilogram fresh weight in table potatoes has been established (OECD, 1997). The highest content can be found in inflorescence and in sprouts, in tubers generally in skin and upper layers of flesh.
Nitrates are found in the entire plant and are considered anti-nutritional, especially for babies. Therefore plant breeders aim at maintaining very low contents in new potato varieties.
Potatoes are also commonly used as feed throughout the world. Wild animals, mammals and birds, occasionally feed on potatoes exposed in the field or in potato clamps. As is the case for humans, a high content of glycoalkaloids is toxic and poisoning may occur.

PART III
INFORMATION RELATING TO THE GENETIC MODIFICATION

10. A description of the methods used for the genetic modification

Transformation of potato with recombinant DNA was performed using *Agrobacterium tumefaciens* strain AGL0, AGL1 or LBA4404. A binary vector system was used where the T-DNA, containing the genes that are to be transferred, is found on one plasmid while the DNA mobilizing functions are found on a modified Ti-plasmid (Hoekema et al., 1983). Transformation was carried out by cutting leaf or stem tissue followed by *A. tumefaciens* inoculation. After a certain period of time in which explants and bacteria were subcultured together, *A. tumefaciens* was killed with Claforan (Visser et al., 1991). Shoots were regenerated under Imazamox selection (Andersson et al., 2003).

11. The nature and source of the vector used

The binary vectors, VCPMA16 and VCPMA19, are both based on pPZP200 (Hajdukiewicz et al., 1994), which can be propagated in *E. coli* as well as *A. tumefaciens*. The backbone contains a ColE fragment (o-ColE1) from pBR322 including origin of replication in *E. coli* as well as a *bom* site for mobilization from *E. coli* to *A. tumefaciens*. A fragment derived from plasmid pVS1 contains broad host range replication functions (o-VS1-repA), including origin of replication and the *repA* gene, as well as a *sta* gene (c-sta) encoding stabilizing functions. Additionally, the backbone contain a gene coding for spectinomycin resistance (c-aadA) making bacterial selection possible but which is not intended to be transferred to the plant.

The T-DNA in VCPMA16 and VCPMA19 is delimited by pTiT37 right and left T-DNA border regions (b-RB and b-LB) originating from *A. tumefaciens*. The sequences within the T-DNA are described below.

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

T-DNAs in the plasmids VCPMA16 and VCPMA19 contain an acetohydroxyacid synthase gene for selection of transformed plant tissue as well as genes for improving resistance to *Phytophthora infestans*.

The acetohydroxyacid synthase gene (*ahas*) originates from *Arabidopsis thaliana* and has a point mutation corresponding to S653N in the expressed AHAS protein (Chang and Duggleby, 1998). The point mutation results in expressed protein, which confers resistance to herbicides from the Imidazolinone family during tissue culture and is used to select transformed tissue. The promoter and terminator sequences originate from the nopaline synthase gene (*nos*) with origin from *A. tumefaciens*.

The genes for improving resistance to *P. infestans* are R-genes from *Solanum bulbocastanum.* Plasmids VCPMA16 and VCPMA19 both contain a genomic

fragment from *S. bulbocastanum* containing the *Rpi-blb2* gene with endogenous promoter and terminator regions. Both constructs also contain genomic fragments from *S. bulbocastanum* containing the *Rpi-blb1* gene with endogenous promoter and terminator regions. The bulbocastanum *Rpi-blb1* fragment of VCPMA19 is longer that in VCPMA16 containing both longer promoter as well as terminator regions.

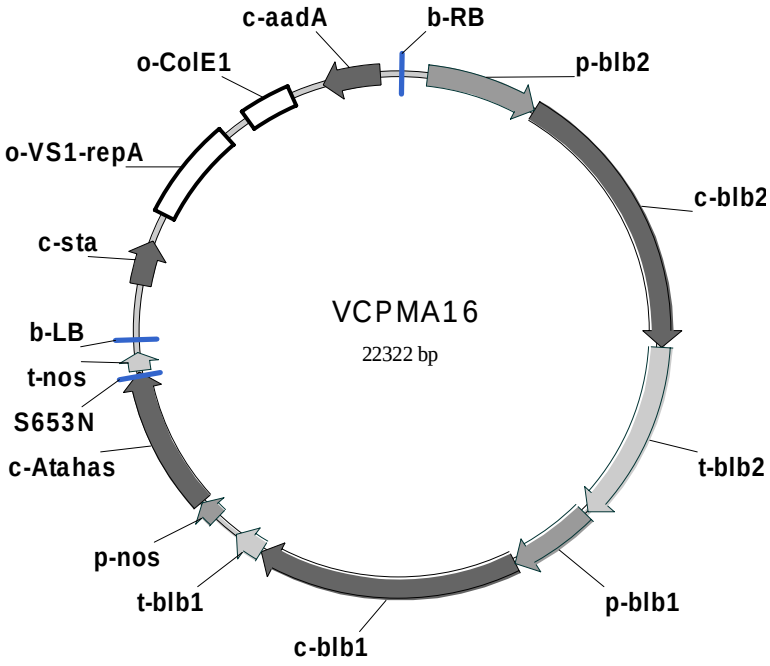
R-genes can be divided into different classes, where the majority of genes belong to the nucleotide binding site (NBS)-leucine rich repeat (LRR) class (Young, 2000). The *Rpi-blb1* (van der Vossen et al., 2003) and *Rpi-blb2* (van der Vossen et al., 2005) both belong to the NBS-LRR class of R-genes.

There are no components of the vectors known to code for harmful substances.

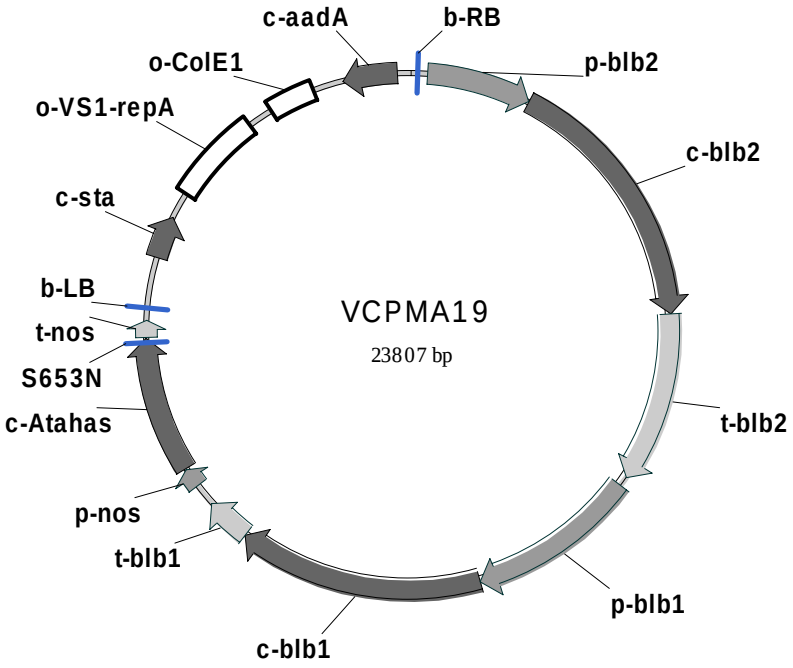
Table of genetic elements in T-DNA

Abbreviation	Name and function	Size (bp)	Origin
VCPMA16			
T-DNA		16700	
p-blb2	Promoter region of gene *Rpi-blb2* (including intron)	1530	*S. bulbocastanum*
c-blb2	Coding region of gene *Rpi-blb2* (including intron)	3890	*S. bulbocastanum*
t-blb2	Terminator region of gene *Rpi-blb2*	2530	*S. bulbocastanum*
p-blb1	Promoter region of gene *Rpi-blb1*	1173	*S. bulbocastanum*
c-blb1	Coding region of gene *Rpi-blb1* (including intron)	3592	*S. bulbocastanum*
t-blb1	Terminator region of gene *Rpi-blb1*	406	*S. bulbocastanum*
p-nos	Promoter of nopaline synthase gene	288	*A. tumefaciens*
c-At*ahas*	Coding region of acetohydroxyacid synthase gene containing mutation S653N	2013	*A. thaliana*
t-nos	Terminator of nopaline synthase gene	253	*A. tumefaciens*
VCPMA19			
T-DNA		18100	
p-blb2	Promoter region of gene *Rpi-blb2* (including intron)	1530	*S. bulbocastanum*
c-blb2	Coding region of gene *Rpi-blb2* (including intron)	3890	*S. bulbocastanum*
t-blb2	Terminator region of gene *Rpi-blb2*	2530	*S. bulbocastanum*
p-blb1	Promoter region of gene *Rpi-blb1*	2516	*S. bulbocastanum*
c-blb1	Coding region of gene *Rpi-blb1* (including intron)	3592	*S. bulbocastanum*
t-blb1	Terminator region of gene *Rpi-blb1*	669	*S. bulbocastanum*
p-nos	Promoter of nopaline synthase gene	288	*A. tumefaciens*
c-At*ahas*	Coding region of acetohydroxyacid synthase gene containing mutation S653N	2013	*A. thaliana*
t-nos	Terminator of nopaline synthase gene	253	*A. tumefaciens*

Map of plasmid VCPMA16



Map of plasmid VCPMA19



PART IV

INFORMATION RELATING TO THE GENETICALLY MODIFIED PLANT

13. A description of the trait or traits and characteristics of the genetically modified plant which have been introduced or modified.

The inserted resistance genes, *Rpi-blb1* and *Rpi-blb2*, confer improved resistance against *Phytophthora infestans*.
The introduced *ahas* gene confers resistance to imidazolinones used for selection during transformation.

14. The following information on the sequences actually inserted or deleted:

(a) The size and structure of the insert and methods used for its characterisation, including information on any parts of the vector introduced into the genetically modified plant or any carrier or foreign DNA remaining in the genetically modified plant

The sizes of the T-DNA sequences intended to be inserted are approximately 17 kb and 18 kb respectively for VCPMA16 and VCPMA19. According to analysis with real-time PCR (Ingham et al., 2001) all genes of the T-DNA; the *Rpi-blb1, Rpi-blb2* as well as *ahas* gene are confirmed to be present in all of the transgenic lines intended for the field trial.
Additionally, analysis by real-time PCR for vector sequences outside the T-DNA has been performed, but no such sequences were detected in any of the genetically modified potato lines intended for field trial. For this analysis two different primer-probe sets within the backbone of the vector were used, one close to left border and one close to right border. The primer and probe set at the right border is directed to a sequence within the *aadA* gene (spectinomycin resistance) and thus the analysis also shows the absence of this gene. For details of the PCR analysis for the *ahas* gene and right border – aadA sequence refer to *Annex 1*.

(b) the size and function of the deleted regions

No regions have been deleted.

(c) the copy number of the insert

According to real-time PCR analysis (Ingham et al., 2001) with primers and probe directed against the *ahas* gene all transgenic lines intended for field trial contain 1 or 2 copies.

(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 used for its determination.

The transgenic plants are produced by *Agrobacterium*-mediated transformation thus resulting in nuclear localization of the insert (Zambryski, 1980; Hohn et al., 1991). The inserts have been found stable when shoots are propagated via cuttings. Therefore the inserts are considered to be stably integrated into the nuclear plant genome.

15. The following information on the expression of the insert:

(a) information on the developmental expression of the insert during the lifecycle of the plant and methods used for its characterisation.

The expression of *Rpi-blb1* and *Rpi-blb2* genes is regulated by their respective native promoters. Other R-genes of the NBS-LRR class have been shown to have very weak expression in vegetative tissue (Michelmore et al., 2001). In the genetically modified lines a low level of expression of both *Rpi-blb1* and *Rpi-blb2* has been demonstrated by real-time PCR analysis in leaves, stems, tubers and roots. In flowers a low expression of *Rpi-blb2* can be detected, while *Rpi-blb1* is expressed at even lower levels or not at all.

The *ahas* gene is controlled by the *nos* promoter, which is known to result in low expression levels in all parts of the plant. Expression of the *ahas* gene in the genetically modified potato material has been demonstrated in tissue culture by survival on media containing Imazamox.

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

See above.

16. Information on how the genetically modified plant differs from the parental or recipient plant in the following respects:

(a) mode or modes and/or the rate of reproduction

Neither the resistance genes nor the *ahas* gene are expected to affect tuber formation, seed setting, flower development or pollen formation. When the genetically modified potato lines covered in this application were grown under greenhouse conditions, no changes in these properties were noted. Three genetically modified potato lines carrying the *Rpi-blb2* gene were released in the field in Sweden in 2005. Throughout the trial the performance of the GM lines in comparison to the non-GM parental potato variety and the variety Bintje with regard to resistance against *Phytophthora infestans* was followed. Growth and development of the GM lines was recorded as normal and comparable to the non-GM comparator lines and no significant differences were observed till the early flowering stage. Upon early flowering the plants became infected with *Phytophthora infestans.* While the non-GM comparator plants suffered substantial defoliation by the disease, the three GM potato lines tested showed the intended phenotype of late blight resistance. Genetically modified potato plants expressing the AHAS protein have been studied in multiple field trials in the Netherlands, Sweden, Germany and the Czech Republic and no changes in the properties mentioned have been observed.

(b) dissemination

Neither resistance genes nor the *ahas* gene are expected to affect pollen or seed dispersal. There is thus no reason to assume that the modified potato clones differ from the parental varieties in this respect.

(c) survivability

Survival of potato tubers is depending on temperature. The introduction of additional R-genes or the *ahas* gene is not expected to alter the frost sensitivity. Potatoes expressing the AHAS protein have been evaluated for frost tolerance in field, but no alterations compared to the parental varieties were observed.

Potato already contains many other R-genes of the NBS-LRR class (Wouters et al., 2004; see section 19). Some NBS-LRR genes are introgressed from *Solanum demissum* and confer resistance to some races of *P. infestans* (Ballvora et al., 2002). It is therefore not expected that the genetically modified potato lines with an improved resistance to *Phytophthora infestans* differ in their mode of reproduction, in dissemination or survivability from non-genetically modified potato plants.

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

Agrobacterium-mediated transformation generally results in stable insertions. The inserts have been found to be stably present when material propagated via several rounds of cuttings and grown in the greenhouse has been analysed.

18. Any change to the ability of the genetically modified plant to transfer genetic material to other organisms.

No change is expected with regard to the ability of the genetically modified potatoes with improved resistance to *Phytophthora infestans* to transfer genetic material to other organisms in comparison to conventional potatoes. Transfer of genetic material might either occur via pollen or via uptake of decomposing material in the soil. Outcrossing to related European wild species is not possible for potato and there is no reason to assume that the inserted R-genes or the *ahas* gene could influence this property. The possibility of outcrossing to conventional potato varieties is expected to be the same as for the respective parental varieties. None of the plant characteristics observed in the greenhouse and in the field (for genetically modified potato lines similar to those intended for the release) indicate any significant difference to the parental varieties. 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). A transfer to viruses can also be ruled out, since viruses, which have both plants and also bacteria as host are as yet unknown. Further 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).

19. Information on any toxic, allergenic or harmful effects on human health and the environment arising from the genetic modification.

The genetically modified potatoes for improved resistance to *Phytophthora infestans* are not expected to exert any toxic, allergenic or harmful effects on human health or the environment.

The introduced genes *Rpi-blb1* and *Rpi-blb2* (R-genes) are added to the potato genome that already harbours resistance genes belonging to the NBS-LRR class (nucleotide binding site – leucine rich repeat). The NBS-LRR gene family contains the majority of plant disease resistance genes (R-genes) known to date and the R-gene derived proteins feature the same protein structure. In addition the potato genome carries R-genes derived from the wild potato species *Solanum demissum* (Wastie, 1991). No member of the NBS-LRR protein class so far has been identified to confer toxic or allergenic properties. R-gene homologues are very abundant in all plant species (Dangl and Jones, 2001) and for those where genome DNA sequence information is available, the number of genes could be determined (rice with approx. 500 genes and *Arabidopsis thaliana* with approx. 200 genes). The expression of the introduced R-genes (*Rpi-blb1* and *Rpi-blb2*) in the genetically modified potatoes, under the control of the respective endogenous promoters is at very low levels and comparable to those from other resistance genes.

The introduced selection marker gene is expressed as the enzyme AHAS. AHAS (EC 4.1.3.18, also known as acetolactate synthase ALS) is found in bacteria, other micro-organisms and plants. It catalyses the first step in the biosynthesis of the essential branched chain amino acids isoleucine and valine. A single amino acid substitution in the AHAS enzyme alters the binding site for imidazolinone herbicides and thus confers a tolerant phenotype. Many studies have demonstrated that except their tolerance to imidazolinone herbicides, the imidazolinone tolerant AHAS enzymes are catalytically identical to the native, imidazolinone sensitive AHAS enzymes, including feedback inhibition by isoleucine and valine. Induced as well as acquired mutations are known to confer tolerance to a particular group of herbicides in crop plants (Chang et al., 1998). The food, feed, and environmental safety of plants tolerant to imidazolinone has been assessed by Health Canada and the Canadian Food Inspection Agency for imidazolinone tolerant maize, rice, canola, sunflower, lentils and wheat. Imidazolinone herbicide tolerant maize or CLEARFIELD maize has been cultivated in the US since 1992, CLEARFIELD canola since 1996 and CLEARFIELD wheat since 2001.

20. Information on the safety of the genetically modified plant to animal health, particularly regarding any toxic, allergenic or other harmful effects arising from the genetic modification, where the genetically modified plant is intended to be used in animal feedstuffs.

The genetically modified potatoes intended for the field trial will not be used as animal feedstuff.

21. The mechanism of interaction between the genetically modified plants and target organisms.

The target organism of the introduced resistance is *Phytophthora infestans*. A reduction in the ability of *P. infestans* to infect the genetically modified potatoes is expected due to the inserted R-genes. The R-genes encode receptors that will recognize specific avirulence factors injected by the pathogen. This recognition will, through a signalling network, trigger both local and systemic defense responses. The local response aims at trapping the pathogen, in those cells first penetrated, by localized cell death thus stopping further penetration and spreading. The systemic response induces expression of defense related genes also in remote parts of the plant (Heil and Bostock, 2002).

22. The potential changes in the interactions of the genetically modified plant with non-target organisms resulting from the genetic modification.

Resistance genes of the NBS-LRR class are very specific, limited to species or race, and cause initiation of a resistance response (Hammond-Kosack and Parker, 2003). For recognition of the target organism a very specific avirulence factor has to be injected by the pathogen. The specific avirulence factors for *Rpi-blb1* and *Rpi-blb2* are, based on current knowledge, expected to be produced only by *P. infestans*. Due to the specificity of the response reaction no effects on other organisms than *P. infestans* are expected other than those that also apply to the interaction of non-genetically modified potatoes with non-target organisms under conventional agricultural practice. Due to a reduced need for fungal treatments an increase in the populations of those non-target organisms that respond to the fungal treatments might be expected. No other changes in interactions are anticipated. Further the trial will provide an opportunity to investigate any potential changes in the interactions with non-target organisms via observations on disease and pest susceptibility.

23. The potential interactions with the abiotic environment.

The genetically modified potatoes with increased resistance to *Phytophthora infestans* are unlikely to have any change in their interaction with the abiotic environment compared to non-genetically modified potatoes. None of the introduced genes are related to frost, drought tolerance or salt tolerance. The hypersensitive response by the potato plants induced upon infection by the fungus is not expected to alter any interactions with the abiotic environment. Conventional agricultural practice will be applied to the field trial except for a reduction in fungal treatment in order to analyse the efficacy of the introduced trait. Further the release will provide an opportunity to investigate any potential interactions with the abiotic environment via recording and

comparing potato tuber yield data. *Phytophthora infestans* is already present in the soil and relies on favourable climatic conditions (e.g. humidity) for potato plant infection. Conventional agricultural practice is applied (e.g. crop rotation, herbicide and insecticide applications, reduced fungicide applications). The hypersensitive response by the genetically modified potato plants induced upon infection by the fungus is also not expected to alter any interactions of the fungus with the abiotic environment.

24. A description of detection and identification techniques for the genetically modified plant.

Assays based on real-time PCR have been developed for the *Rpi-blb1*, the *Rpi-blb2* as well as the *ahas* gene and can all be used to distinguish the genetically modified potato lines from their parental varieties.

25. Information about previous releases of the genetically modified plants.

Genetically modified potato lines carrying the *Rpi-blb2* gene were released in the field in Sweden in 2005 and 2006 (consent no. B/SE/05/450, B/SE/05/8615), in Netherlands in 2006 (consent no. B/NL/05/03) as well as in Germany (consent no. B/DE/05/174).

Field trials with potatoes modified for starch composition also carrying the *ahas* gene as selection marker have taken place at several locations in Sweden (since 2002), Germany (since 2004), the Netherlands (since 2004) and the Czech Republic (since 2005). During these trials no unforeseen effects as compared to conventional potato varieties have been observed.

PART V

INFORMATION RELATING TO THE SITE OF RELEASE

26. The location and size of the release site or sites.

The location of the release site is as follows:

District East Yorkshire, community Preston/Hedon,

- one field with 9.5 ha on a farm at ordnance survey map grid reference TA 1729

The genetically modified plants will occupy no more than 1 ha in an experimental plot of less than 2 ha. The approximate dimensions of the release plot are outlined in the proposed trial plan in *Annex 2*. The plot will rotate in any given year on the available release site in order to allow for monitoring of volunteers and to follow crop rotation requirements for potato planting. Likewise the plot will rotate in any given year on the available release site if a field will be planted in consecutive years. A maximum of 45,000 potato plants per year will be planted per release site on the experimental plot. A total maximum of 225,000 potato plants will be planted over the 5 years of the release.

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

The release site is arable land. Some of this is bordered by deciduous trees and hedgerows.

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

The fields surrounding the release site will be cultivated commercially according to conventional agricultural practice including crop rotation. No potatoes will be cultivated in close vicinity (< 20 m) to trial. There are no sexually compatible wild relatives present at the release site.

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

There are no officially recognized biotopes or protected areas such as SSSIs, National Parks or Nature Reserves within a given distance of 1000 m of the site.

The nearest protected area to the release site is the SSSI Humber Estuary at a distance of 3.0 km of the site.

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.

The trials will be conducted for development purposes. In the first three to four years the purpose of the small-scale experimental trial will be the screening of events for improved resistance to *Phytophthora infestans* (proof of concept under UK field conditions with UK specific *Phytophthora infestans* strains). In addition during the course of the trial the following will be observed and recorded: agronomic performance (e.g. plant vigour and yield), and selected plant characteristics (e.g. emergence, flowering, maturation), as well as stability of the trait. Further, the last one to two years of trial the release is aiming at, in the context of risk assessment research, collecting data for selected *Phytophthora* resistant potato lines in comparison to unmodified recipient varieties or to conventional potato varieties. This relates to the management of volunteers, to the stability of expression (e.g. sampling of plant tissue at various developmental stages in to order to conduct gene expression studies), to potential effects on potato-associated insects, and to altered qualitative properties (e.g. tuber quality). The harvested plant material is to be used for various analyses (including molecular biological and biochemical tests). If appropriate, the tuber material is also to be used as seed stock for the following season.

The long-term aim is to develop potatoes with improved resistance to *P. infestans.*

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

The genetically modified potato lines are planned to be released from April to October in the years 2007 to 2011 with planting taking place earliest in April and latest in June and harvesting in September/October of each year.

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

The genetically modified potato lines will be planted as tubers or mini-tubers according to conventional field testing practice by hand or machine. Tubers will be placed in rows and plots according to appropriate 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.

Preparation and management of the release site will be according to conventional agricultural practice. This includes but is not limited to fertilisation, use of fungicides or insecticides. Weeds will be removed manually or chemically. In addition *Phytophthora infestans* inoculation with UK borne pathotypes will be carried out for efficacy studies. Inoculation with UK borne pathotypes of *Phytophthora infestans* is standard practice and will be performed according to conventional field testing practice by hand or machine. Seed potatoes will be planted directly into the field. The potato lines will be

planted in a randomised trial design. An example of the trial design is given in *Annex 2*. Infestation strips consisting of conventional potato varieties will be placed between and outside the plot blocks. Harvesting will be preceded by defoliation (using an approved defoliant) and after the tops have been removed the tubers will be harvested by hand or machine.

34. The approximate number of genetically modified plants (or plants per m^2) to be released.

Planting density will be according to conventional agricultural practice with a total maximum of 45,000 potato tubers to be planted per location per year. Therefore the number of plants per genetically modified potato line (see Annex 1 for the list of available lines) will not exceed 500 per location in the first year. In later years fewer selected GM potato lines will be planted with the number of GM plants per selected potato line and per year not exceeding 2000.

PART VII

INFORMATION ON CONTROL, MONITORING, POST-RELEASE PLANS AND WASTE TREATMENT PLANS

35. A description of any precautions to -

(a) maintain the genetically modified plant at a distance from sexually compatible plant species, both wild relatives and crops.

There are no sexually compatible wild relatives to potato in the UK. The only sexually compatible species will be commercially cultivated potatoes. An isolation distance of 20 m between the GM potato lines and commercial potato cultivation will be observed throughout the testing period. The release site will be monitored for volunteers, any emerging volunteer potato plants will be destroyed. After harvest only those crops will be cultivated on the release site that allow monitoring for volunteers. The year directly following the release the field plot will either remain fallow or will be cultivated with a species that facilitates weed management of the area.

(b) any measures to minimise or prevent dispersal of any reproductive organ of the genetically modified plant (such as pollen, seeds, tuber).

During transport and handling the potatoes will be clearly labelled, separated from conventional potatoes and packaged in closed, double layer packaging. Any equipment or machinery used for planting and harvesting will be cleaned on site. Any excess potato material (tubers after planting, after harvest) will be inactivated (e.g. via heat or via chopping). An isolation distance of at least 20 m to commercially cultivated non-genetically modified potatoes will be observed.

36. A description of the methods for post-release treatment of the site or sites.

The release site will be managed according to conventional agricultural practice. The first year following the release the volunteer monitoring programme starts and the field plot will either remain fallow or will be cultivated with a species that facilitates weed management of the area that year. For the duration of the volunteer monitoring programme no potatoes will be planted on the field plot, however other crops can be planted as long as they allow volunteer monitoring. Emerging volunteers will be destroyed by herbicide treatment (systemic herbicide e.g. glyphosate) prior to flower setting. The monitoring for volunteers will continue till no volunteers emerge in two successive years. The cultivation of the release site in the years after the monitoring programme has concluded will be according to local crop rotation practice for potatoes.

37. A description of post-release treatment methods for the genetically modified plant material including wastes.

Harvesting will be performed according to conventional agricultural practice either manually or mechanically. Harvested tubers will be transported from the release site either for interim storage (e.g. quality assessment) and disposal (inactivation e.g. via heat or buried in a landfill site) to National Institute of Agricultural Botany, Huntington Road, Cambridge, UK or for analyses to BASF Plant Science GmbH, Limburgerhof, Germany; biovativ GmbH, Groß Lüsewitz, Germany, and Plant Science Sweden, Svalöv, Sweden. Any left-over tubers identified on the release area after harvest, will be collected and transported off site for inactivation. Above ground green parts will be inactivated prior to harvest either chemically or mechanically and be left at the release site for decomposition. The genetically modified potato tubers originating from the trial will not be used for human food or animal feed.

38. A description of monitoring plans and techniques.

The following monitoring plan is based on the conclusions of the environmental risk assessment and aims at early observation and identification of intended and unintended effects related to the release of the GM potato plants.

Assumptions of risk assessment	Observations performed by notifier
Case specific monitoring	
No selective advantage due to improved resistance to *P. infestans*	Monitoring for volunteers
No selective advantage or disadvantage conferred to sexually compatible plant species	Monitoring for volunteers
Intended effects on target organism *P. infestans*	Observations on changes in tolerance to *P. infestans*
No impact on the environment due to interactions with non-target organisms	Observations on changes in susceptibility to potato-associated insects and pests under conventional agricultural practice
General Surveillance	
No differences in general characteristics of the plant: size, shape, flowering, development	Observations on general plant characteristics and agronomic performance
No differences in disease and pest susceptibility	Observations on changes in susceptibility to potato-associated insects and pests under conventional agricultural practice
No difference in competitive behaviour	Monitoring for volunteers
Limitations of the potato to the release site	Control (notebook, restricted access) over implementation of risk management measures

Baselines

The performance of the genetically modified potato lines will be compared to the performance of the recipient varieties grown in parallel at the same release site.

Time period

During the course of the entire vegetation period (from about April to October) of the potato lines the area of release will be visited by the compliance liaison and trained personnel to observe the release at defined intervals (at least once a month). The compliance liaison or trained personnel will observe the area post-release at defined intervals for the duration of the volunteer monitoring program.

Responsibilities
The notifier is responsible for the monitoring plan. Case-specific and general surveillance will be carried out by BASF Plant Science and contracted individuals including compliance liaison and trained personnel.

Area
It is the site of release and the individual release plots that will be monitored.

Inspections
The area of release will be visited by the compliance liaison and trained personnel. Inspections may also be performed by the responsible authority.

Data collection and evaluation
BASF Plant Science will be responsible for all records of observations and analyses performed in accordance with the monitoring plan. Data will be collected and analysed according to specifications by BASF Plant Science. Field notebooks are kept during the period of release.

Reporting
Information regarding any unexpected occurrences of relevance regarding potential adverse effects on the environment and human health directly related to the genetically modified potato lines will be communicated to the appropriate Authority and required measures will be implemented accordingly. A report summarising the observations during the field trial will be submitted annually.

39. A description of any emergency plans.

Should the trial become a target of vandalism, measures to prevent an unintended release of the genetically modified potatoes will be taken, e.g. removal and destruction of uprooted plants or premature termination of the trial according to the harvesting measures described earlier. Alternatively, depending on the development stage of the potato plants, the area could be sprayed with an approved herbicide and the material ploughed under.

40. Methods and procedures to protect the site.

The release site will be fenced to protect against damage by animals. A sign will be posted at the release site in order to prohibit the entrance by unauthorised persons.

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 and their description have been included with the appropriate references in the respective sections of this Schedule.

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ANNEX 1

This annex contains a list of currently available lines that will be considered for the proposed release.

1. Analysis for presence of the insert in potato lines transformed with VCPMA16 or VCPMA19

The presence of the insert in lines transformed with VCPMA16 or VCPMA19 was confirmed by real-time PCR. For this analysis primers and probe directed against the *ahas* gene were used. Primers and probe to an endogenous control were also included in the PCR reactions as reference. According to this analysis all lines transformed with construct VCPMA16 as well as VCPMA19 listed in this Annex were shown to contain the targeted insert sequence. Data are shown in *Tables 1* and *2* (section 'insert analysis'). The data are given as Ct values, which is the number of PCR-cycles required to reach a threshold value of PCR products. The maximum number of cycles was 40. If the difference in Ct values between the *ahas* gene and the endogenous control (dCt) is lower than 4, the sample is judged as positive.

Additionally, for all of the VCPMA16 and VCPMA19 lines intended for the field trial, presence of the *Rpi-blb1* and *Rpi-blb2* genes in the insert is confirmed by real-time PCR analysis (data not shown).

2. Analysis of potato lines transformed with VCPMA16 or VCPMA19 for detection of vector backbone sequences

In the vectors VCPMA16 and VCPMA19 the backbone is derived from pPZP200. This backbone contains the *aadA* gene encoding resistance to spectinomycin.

Presence of backbone for lines transformed with VCPMA16 or VCPMA19 is analysed by real-time PCR with two different primer-probe sets within the backbone, one close to left border (LB) and one close to right border (RB) (*Figure 1*). The primer and probe set at the right border is directed to a sequence within the *aadA* gene (spectinomycin resistance). A negative analysis result with this primer-probe set will thus show the absence of the *aadA* gene.

The analyses with the RB-aadA primer-probe set also included primers and probe for an endogenous control as reference. If the difference in Ct values between the RB-aadA sequence and the endogenous control (dCt) is higher than 4, the sample is judged as negative (a difference of more than 4 in the analysed sample is equivalent to less than 0.125 (0.5^3) copies of the target; such values likely arise due to very small levels of contamination during sample preparation or assay set-up or when disproportionately low Ct values have been obtained without any true amplification). DNA extracts from potato plants known to contain backbone

sequences were used as positive controls to confirm the assay. All the lines transformed with construct VCPMA16 as well as VCPMA19 presented in this Annex were shown not to contain the right border *aadA*-sequence. For data see *Tables 1* and *2* (section 'backbone analysis').

Similarly, all presented VCPMA16 and VCPMA19 lines were shown to be negative also when analysed with the LB primer-probe set (data not shown).

Table 1. Data for lines transformed with construct VCPMA16

			Insert analysis				Backbone analysis			
	Line no	Parental variety	Ct AHAS	Ct end ctrl	dCt	Result	Ct RB-aadA	Ct end ctrl	dCt	Result
1	TS-PH05-001-0098	P880	30,74	29,37	1,37	positive	40,00*	29,07	10,93	negative
2	TS-PH05-001-0100	P880	29,90	28,56	1,34	positive	40,00*	28,37	11,63	negative
3	TS-PH05-002-0003	P698	27,81	27,30	0,50	positive	38,52	27,02	11,51	negative
4	TS-PH05-002-0018	P698	27,25	27,00	0,26	positive	40,00*	27,49	12,51	negative
5	TS-PH05-002-0055	P698	26,58	26,57	0,01	positive	40,00*	25,51	14,49	negative
6	TS-PH05-002-0082	P698	26,99	27,10	-0,11	positive	40,00*	25,53	14,47	negative
7	TS-PH05-004-0012	P880	30,30	28,84	1,45	positive	40,00*	29,32	10,68	negative
8	TS-PH05-004-0014	P880	26,77	26,19	0,58	positive	40,00*	26,96	13,04	negative
9	TS-PH05-004-0018	P880	28,94	27,37	1,57	positive	40,00*	27,66	12,34	negative
10	TS-PH05-004-0019	P880	28,90	27,70	1,20	positive	40,00*	27,40	12,60	negative
11	TS-PH05-004-0044	P880	29,90	28,22	1,68	positive	40,00*	28,28	11,72	negative
12	TS-PH05-007-0188	P880	27,23	27,63	-0,40	positive	40,00*	28,13	11,87	negative
13	TS-PH05-008-0004	P880	27,28	26,64	0,64	positive	40,00*	26,65	13,35	negative
14	TS-PH05-009-0001	P835	26,71	25,26	1,45	positive	40,00*	25,61	14,39	negative
15	TS-PH05-009-0003	P835	28,60	28,05	0,55	positive	39,04	27,71	11,33	negative
16	TS-PH05-009-0051	P835	32,41	30,73	1,67	positive	40,00*	30,65	9,35	negative
17	TS-PH05-009-0057	P835	33,68	31,67	2,01	positive	40,00*	31,96	8,04	negative
18	TS-PH05-009-0060	P835	28,32	28,27	0,05	positive	36,80	27,37	9,43	negative
19	TS-PH05-009-0061	P835	26,99	25,74	1,25	positive	40,00*	25,62	14,38	negative
20	TS-PH05-009-0070	P835	28,28	28,01	0,27	positive	34,36	27,53	6,83	negative
21	TS-PH05-009-0073	P835	27,50	26,81	0,69	positive	40,00*	27,73	12,27	negative
22	TS-PH05-009-0078	P835	27,78	27,60	0,18	positive	36,36	26,75	9,60	negative
23	TS-PH05-009-0079	P835	28,44	28,11	0,33	positive	38,11	27,00	11,11	negative
24	TS-PH05-009-0110	P835	28,60	27,92	0,69	positive	39,07	27,74	11,33	negative
25	TS-PH05-009-0131	P835	26,50	25,09	1,41	positive	40,00*	25,39	14,61	negative
26	TS-PH05-009-0133	P835	27,67	26,07	1,60	positive	40,00*	25,84	14,16	negative
27	TS-PH05-009-0163	P835	26,88	25,40	1,48	positive	40,00*	25,76	14,24	negative
28	TS-PH05-009-0168	P835	28,09	27,16	0,93	positive	40,00*	27,01	12,99	negative
29	TS-PH05-009-0174	P835	27,76	25,94	1,82	positive	40,00*	26,14	13,86	negative
30	TS-PH05-009-0186	P835	26,73	25,21	1,51	positive	40,00*	25,55	14,45	negative
31	TS-PH05-009-0200	P835	30,33	29,15	1,18	positive	40,00*	29,46	10,54	negative
32	TS-PH05-009-0207	P835	29,94	28,99	0,95	positive	40,00*	29,73	10,27	negative
33	TS-PH05-009-0209	P835	27,24	25,81	1,43	positive	40,00*	25,62	14,38	negative
34	TS-PH05-009-0216	P835	26,94	27,23	-0,28	positive	38,72	25,77	12,95	negative
35	TS-PH05-010-0002	P835	30,66	29,28	1,38	positive	40,00*	29,54	10,46	negative
36	TS-PH05-010-0023	P835	32,18	30,19	1,99	positive	40,00*	30,75	9,25	negative
37	TS-PH05-010-0032	P835	29,04	28,65	0,38	positive	38,68	28,48	10,20	negative
38	TS-PH05-010-0041	P835	27,22	25,60	1,62	positive	40,00*	25,98	14,02	negative
39	TS-PH05-010-0043	P835	32,07	29,66	2,40	positive	40,00*	30,64	9,36	negative
40	TS-PH05-010-0049	P835	32,34	30,71	1,62	positive	40,00*	30,99	9,01	negative
41	TS-PH05-010-0061	P835	31,53	30,05	1,48	positive	40,00*	30,17	9,83	negative
42	TS-PH05-010-0070	P835	30,82	29,43	1,39	positive	40,00*	29,49	10,51	negative
43	TS-PH05-010-0079	P835	28,75	27,28	1,47	positive	40,00*	27,66	12,34	negative
44	TS-PH05-010-0085	P835	29,14	28,95	0,19	positive	40,00*	28,09	11,91	negative
45	TS-PH05-010-0091	P835	26,80	25,34	1,46	positive	40,00*	25,70	14,30	negative
46	TS-PH05-010-0099	P835	29,52	29,05	0,46	positive	36,68	29,00	7,68	negative
47	TS-PH05-010-0102	P835	31,71	30,16	1,54	positive	40,00*	30,74	9,26	negative
48	TS-PH05-010-0105	P835	30,43	29,59	0,84	positive	40,00*	29,23	10,77	negative
49	TS-PH05-010-0112	P835	29,79	29,45	0,34	positive	37,33	28,78	8,55	negative
50	TS-PH05-010-0118	P835	27,04	25,40	1,64	positive	40,00*	26,02	13,98	negative
51	TS-PH05-010-0119	P835	30,68	28,97	1,71	positive	40,00*	29,07	10,93	negative

52	TS-PH05-010-0125	P835	29,57	29,40	0,17	positive	37,29	28,93	8,36	negative
53	TS-PH05-010-0137	P835	31,13	31,07	0,06	positive	40,00*	30,36	9,64	negative
54	TS-PH05-010-0142	P835	32,57	30,99	1,58	positive	40,00*	31,13	8,87	negative
55	TS-PH05-010-0150	P835	31,54	30,21	1,33	positive	40,00*	30,54	9,46	negative
56	TS-PH05-010-0152	P835	31,54	29,92	1,62	positive	38,40	30,35	8,05	negative
57	TS-PH05-010-0156	P835	27,01	25,48	1,52	positive	40,00*	25,03	14,97	negative
58	TS-PH05-010-0163	P835	27,74	26,88	0,86	positive	40,00*	26,32	13,68	negative
59	TS-PH05-010-0173	P835	26,25	24,78	1,47	positive	40,00*	25,22	14,78	negative
60	TS-PH05-010-0178	P835	26,41	24,78	1,62	positive	40,00*	25,20	14,80	negative
61	TS-PH05-010-0186	P835	26,23	24,74	1,48	positive	40,00*	25,15	14,85	negative
62	TS-PH05-010-0199	P835	26,45	24,98	1,47	positive	40,00*	24,80	15,20	negative
63	TS-PH05-010-0204	P835	26,98	24,96	2,01	positive	40,00*	26,21	13,79	negative
64	TS-PH05-010-0210	P835	26,63	25,07	1,56	positive	40,00*	25,23	14,77	negative
65	TS-PH05-010-0233	P835	29,42	28,94	0,48	positive	34,38	28,92	5,46	negative
66	TS-PH05-010-0244	P835	28,94	28,51	0,43	positive	40,00*	27,86	12,14	negative
67	TS-PH05-010-0246	P835	28,32	27,61	0,71	positive	36,89	26,41	10,48	negative
68	TS-PH05-010-0248	P835	27,09	25,55	1,54	positive	40,00*	25,54	14,46	negative
69	TS-PH05-010-0299	P835	26,99	25,55	1,43	positive	40,00*	25,96	14,04	negative
70	TS-PH05-010-0300	P835	28,74	28,46	0,28	positive	40,00*	28,11	11,89	negative
71	TS-PH05-010-0303	P835	26,62	25,11	1,51	positive	40,00*	25,65	14,35	negative
72	TS-PH05-010-0316	P835	30,53	30,53	0,00	positive	38,97	30,12	8,85	negative
73	TS-PH05-010-0327	P835	30,27	29,39	0,88	positive	40,00*	29,18	10,82	negative
74	TS-PH05-010-0329	P835	29,27	28,95	0,32	positive	40,00*	28,66	11,34	negative
75	TS-PH05-010-0332	P835	28,46	27,99	0,47	positive	36,78	27,54	9,24	negative
76	TS-PH05-010-0350	P835	28,87	28,63	0,24	positive	37,87	28,20	9,67	negative
77	TS-PH05-010-0352	P835	28,26	26,42	1,83	positive	40,00*	27,01	12,99	negative
78	TS-PH05-010-0419	P835	27,77	26,13	1,65	positive	40,00*	25,38	14,62	negative
79	TS-PH05-010-0421	P835	28,39	26,83	1,56	positive	40,00*	26,96	13,04	negative
80	TS-PH05-010-0466	P835	26,73	25,20	1,53	positive	40,00*	25,07	14,93	negative
81	TS-PH05-010-0471	P835	27,27	25,51	1,75	positive	40,00*	25,80	14,20	negative
82	TS-PH05-010-0472	P835	27,29	25,67	1,62	positive	40,00*	25,85	14,15	negative
83	TS-PH05-010-0475	P835	27,78	25,78	2,01	positive	40,00*	25,83	14,17	negative
84	TS-PH05-010-0483	P835	26,93	25,41	1,51	positive	39,87	25,29	14,59	negative
85	TS-PH05-010-0485	P835	29,00	28,29	0,71	positive	40,00*	28,92	11,08	negative
86	TS-PH05-010-0486	P835	27,95	26,79	1,16	positive	40,00*	26,63	13,37	negative
87	TS-PH05-011-0001	P835	28,80	28,67	0,13	positive	37,77	27,88	9,90	negative
88	TS-PH05-011-0002	P835	32,09	30,55	1,55	positive	40,00*	30,91	9,09	negative
89	TS-PH05-011-0005	P835	27,50	26,06	1,44	positive	40,00*	26,88	13,12	negative
90	TS-PH05-011-0007	P835	26,88	25,44	1,44	positive	40,00*	25,28	14,72	negative
91	TS-PH05-011-0022	P835	28,29	28,22	0,07	positive	38,38	27,50	10,88	negative
92	TS-PH05-011-0023	P835	28,17	27,91	0,26	positive	38,78	27,08	11,70	negative
93	TS-PH05-011-0026	P835	28,50	28,46	0,04	positive	40,00*	27,80	12,20	negative
94	TS-PH05-011-0027	P835	27,07	25,09	1,97	positive	40,00*	25,46	14,54	negative
95	TS-PH05-011-0029	P835	26,45	25,04	1,42	positive	40,00*	25,46	14,54	negative
96	TS-PH05-011-0048	P835	26,37	24,96	1,41	positive	40,00*	24,99	15,01	negative
97	TS-PH05-011-0049	P835	27,64	26,77	0,87	positive	40,00*	26,48	13,52	negative
98	TS-PH05-011-0083	P835	29,32	29,09	0,23	positive	40,00*	27,96	12,04	negative
99	TS-PH05-011-0086	P835	27,83	27,50	0,32	positive	40,00*	27,15	12,85	negative
100	TS-PH05-011-0088	P835	26,60	25,10	1,50	positive	40,00*	25,31	14,69	negative
101	TS-PH05-011-0089	P835	26,26	24,88	1,39	positive	38,52	25,24	13,27	negative
102	TS-PH05-011-0093	P835	27,01	25,38	1,64	positive	40,00*	25,59	14,41	negative
103	TS-PH05-011-0098	P835	27,20	25,86	1,34	positive	40,00*	25,94	14,06	negative
104	TS-PH05-011-0110	P835	28,41	27,59	0,82	positive	40,00*	28,36	11,64	negative
105	TS-PH05-011-0119	P835	27,22	25,90	1,32	positive	40,00*	25,53	14,47	negative
106	TS-PH05-011-0122	P835	27,87	27,46	0,41	positive	40,00*	25,28	14,72	negative
107	TS-PH05-011-0124	P835	28,14	26,51	1,64	positive	40,00*	26,42	13,58	negative
108	TS-PH05-011-0144	P835	27,43	26,68	0,75	positive	40,00*	26,81	13,19	negative
109	TS-PH05-011-0148	P835	27,31	25,92	1,38	positive	40,00*	25,52	14,48	negative
110	TS-PH05-011-0149	P835	26,96	25,29	1,67	positive	40,00*	25,51	14,49	negative

111	TS-PH05-011-0170	P835	27,93	27,12	0,81	positive	40,00*	27,73	12,27	negative
112	TS-PH05-012-0008	P880	29,97	29,52	0,45	positive	40,00*	28,91	11,09	negative
113	TS-PH05-012-0020	P880	29,98	29,02	0,97	positive	40,00*	29,03	10,97	negative
114	TS-PH05-012-0040	P880	26,16	26,49	-0,33	positive	37,16	26,61	10,55	negative
115	TS-PH05-012-0104	P880	31,14	29,88	1,26	positive	40,00*	29,93	10,07	negative
116	TS-PH05-013-0018	P698	29,46	28,56	0,90	positive	40,00*	28,57	11,43	negative
117	TS-PH05-014-0007	P880	28,50	27,08	1,42	positive	40,00*	26,98	13,02	negative
118	TS-PH05-014-0008	P880	29,12	28,53	0,59	positive	40,00*	28,90	11,10	negative
119	TS-PH05-014-0009	P880	27,96	26,35	1,61	positive	40,00*	26,08	13,92	negative
120	TS-PH05-014-0010	P880	27,72	27,38	0,34	positive	40,00*	25,92	14,08	negative
121	TS-PH05-014-0011	P880	27,47	27,62	-0,15	positive	40,00*	27,09	12,91	negative
122	TS-PH05-014-0043	P880	27,47	27,77	-0,30	positive	40,00*	27,90	12,10	negative
123	TS-PH05-014-0044	P880	27,16	26,93	0,23	positive	38,26	25,32	12,94	negative
124	TS-PH05-014-0059	P880	32,96	30,76	2,20	positive	40,00*	31,06	8,94	negative
125	TS-PH05-015-0001	P698	31,59	29,88	1,71	positive	40,00*	30,12	9,88	negative
126	TS-PH05-015-0003	P698	32,96	31,03	1,93	positive	40,00*	31,07	8,93	negative
127	TS-PH05-015-0007	P698	27,95	27,29	0,65	positive	40,00*	27,30	12,70	negative
128	TS-PH05-015-0016	P698	32,49	30,49	2,00	positive	40,00*	30,41	9,59	negative
129	TS-PH05-015-0018	P698	31,57	29,21	2,36	positive	40,00*	29,70	10,30	negative
130	TS-PH05-015-0019	P698	27,93	26,50	1,43	positive	40,00*	26,76	13,24	negative
131	TS-PH05-015-0021	P698	26,59	25,47	1,12	positive	40,00*	25,50	14,50	negative
132	TS-PH05-015-0024	P698	26,38	26,22	0,16	positive	40,00*	24,82	15,18	negative
133	TS-PH05-015-0025	P698	26,92	25,59	1,34	positive	40,00*	25,97	14,03	negative
134	TS-PH05-015-0059	P698	28,38	28,10	0,27	positive	40,00*	27,72	12,28	negative
135	TS-PH05-015-0061	P698	26,33	24,87	1,46	positive	40,00*	24,85	15,15	negative
136	TS-PH05-015-0086	P698	26,93	25,09	1,84	positive	40,00*	25,81	14,19	negative
137	TS-PH05-015-0105	P698	27,19	27,28	-0,09	positive	40,00*	25,04	14,96	negative
138	TS-PH05-015-0123	P698	28,46	27,39	1,07	positive	40,00*	27,20	12,80	negative
139	TS-PH05-015-0128	P698	28,19	28,17	0,02	positive	37,34	26,18	11,16	negative
140	TS-PH05-016-0006	P835	27,76	26,38	1,38	positive	40,00*	26,67	13,33	negative
141	TS-PH05-016-0019	P835	27,44	25,96	1,48	positive	40,00*	26,38	13,62	negative
142	TS-PH05-016-0022	P835	26,97	25,55	1,42	positive	40,00*	25,15	14,85	negative
143	TS-PH05-016-0023	P835	26,77	25,19	1,58	positive	40,00*	25,82	14,18	negative
144	TS-PH05-016-0032	P835	27,66	26,13	1,53	positive	40,00*	26,97	13,03	negative
145	TS-PH05-016-0034	P835	28,80	28,48	0,32	positive	40,00*	27,99	12,01	negative
146	TS-PH05-016-0037	P835	29,96	29,21	0,75	positive	37,00	28,66	8,34	negative
147	TS-PH05-016-0038	P835	27,48	25,90	1,59	positive	40,00*	26,33	13,67	negative
148	TS-PH05-016-0044	P835	27,78	26,15	1,63	positive	40,00*	26,69	13,31	negative
149	TS-PH05-016-0047	P835	27,10	25,70	1,40	positive	40,00*	26,06	13,94	negative
150	TS-PH05-016-0049	P835	28,56	28,00	0,56	positive	36,98	27,80	9,18	negative
151	TS-PH05-016-0050	P835	28,68	28,23	0,45	positive	39,83	27,63	12,20	negative
152	TS-PH05-016-0056	P835	27,62	27,23	0,39	positive	34,36	26,84	7,52	negative
153	TS-PH05-016-0057	P835	28,54	28,43	0,11	positive	37,27	28,17	9,10	negative
154	TS-PH05-016-0059	P835	28,66	28,11	0,55	positive	35,54	27,61	7,93	negative
155	TS-PH05-016-0078	P835	28,44	27,99	0,45	positive	39,52	27,49	12,03	negative
156	TS-PH05-016-0099	P835	27,28	26,89	0,39	positive	36,52	26,25	10,27	negative
157	TS-PH05-016-0104	P835	27,39	26,87	0,52	positive	40,00*	27,27	12,73	negative
158	TS-PH05-016-0105	P835	27,17	26,56	0,60	positive	40,00*	27,58	12,42	negative
159	TS-PH05-016-0110	P835	28,99	27,90	1,10	positive	40,00*	28,40	11,60	negative
160	TS-PH05-016-0117	P835	27,61	27,32	0,28	positive	40,00*	25,05	14,95	negative
161	TS-PH05-016-0123	P835	27,75	27,02	0,74	positive	40,00*	27,60	12,40	negative
162	TS-PH05-016-0127	P835	28,08	27,58	0,50	positive	40,00*	28,02	11,98	negative
163	TS-PH05-016-0166	P835	29,05	28,22	0,82	positive	40,00*	28,50	11,50	negative
164	TS-PH05-016-0168	P835	27,62	26,85	0,76	positive	40,00*	26,94	13,06	negative
165	TS-PH05-017-0002	P880	26,84	27,05	-0,21	positive	40,00*	27,13	12,87	negative
166	TS-PH05-017-0004	P880	30,46	29,83	0,63	positive	36,81	29,19	7,62	negative
167	TS-PH05-017-0005	P880	28,22	27,76	0,46	positive	35,17	27,09	8,08	negative
168	TS-PH05-017-0012	P880	27,03	25,80	1,23	positive	40,00*	25,57	14,43	negative
169	TS-PH05-017-0016	P880	29,52	28,02	1,50	positive	40,00*	27,78	12,22	negative

170	TS-PH05-017-0026	P880	27,21	27,04	0,17	positive	40,00*	25,69	14,31	negative
171	TS-PH05-017-0043	P880	27,50	27,43	0,07	positive	40,00*	26,10	13,90	negative
172	TS-PH05-017-0044	P880	31,09	29,61	1,48	positive	40,00*	30,00	10,00	negative
173	TS-PH05-017-0056	P880	29,29	27,87	1,42	positive	40,00*	27,73	12,27	negative
174	TS-PH05-017-0058	P880	26,87	26,64	0,23	positive	40,00*	25,53	14,47	negative
175	TS-PH05-017-0093	P880	27,65	26,68	0,97	positive	40,00*	27,27	12,73	negative
176	TS-PH05-017-0096	P880	30,83	29,91	0,91	positive	40,00*	29,80	10,20	negative
177	TS-PH05-018-0011	P698	27,97	26,47	1,50	positive	40,00*	26,94	13,06	negative
178	TS-PH05-018-0027	P698	26,39	25,03	1,37	positive	40,00*	24,74	15,26	negative
179	TS-PH05-018-0028	P698	26,95	25,74	1,22	positive	38,57	25,71	12,85	negative
180	TS-PH05-018-0031	P698	26,71	24,95	1,76	positive	40,00*	25,46	14,54	negative
181	TS-PH05-018-0039	P698	27,92	27,86	0,06	positive	38,58	25,91	12,68	negative
182	TS-PH05-018-0046	P698	27,42	25,95	1,47	positive	40,00*	26,10	13,90	negative
183	TS-PH05-018-0059	P698	27,24	25,88	1,35	positive	40,00*	26,35	13,65	negative
184	TS-PH05-018-0062	P698	29,09	28,33	0,75	positive	38,14	28,09	10,05	negative
185	TS-PH05-018-0082	P698	26,95	25,38	1,57	positive	40,00*	25,70	14,30	negative
186	TS-PH05-018-0092	P698	27,31	26,04	1,27	positive	40,00*	25,76	14,24	negative
187	TS-PH05-018-0093	P698	26,80	26,87	-0,07	positive	40,00*	25,22	14,78	negative
188	TS-PH05-018-0096	P698	26,61	26,59	0,02	positive	40,00*	24,70	15,30	negative
189	TS-PH05-018-0100	P698	29,94	28,99	0,96	positive	40,00*	29,13	10,87	negative
190	TS-PH05-018-0104	P698	28,67	28,07	0,59	positive	40,00*	27,94	12,06	negative
191	TS-PH05-018-0117	P698	28,54	26,88	1,66	positive	40,00*	27,14	12,86	negative
192	TS-PH05-018-0118	P698	28,12	26,36	1,76	positive	40,00*	26,49	13,51	negative
193	TS-PH05-018-0120	P698	27,75	26,07	1,68	positive	40,00*	25,93	14,07	negative
194	TS-PH05-018-0125	P698	27,59	27,49	0,10	positive	40,00*	25,81	14,19	negative
195	TS-PH05-018-0133	P698	28,74	28,07	0,66	positive	40,00*	27,98	12,02	negative
196	TS-PH05-018-0134	P698	27,55	26,11	1,43	positive	40,00*	25,90	14,10	negative
197	TS-PH05-018-0137	P698	27,78	26,08	1,70	positive	40,00*	26,28	13,72	negative
198	TS-PH05-018-0147	P698	29,40	28,60	0,80	positive	40,00*	28,61	11,39	negative
199	TS-PH05-018-0149	P698	29,08	28,28	0,80	positive	40,00*	28,46	11,54	negative
200	TS-PH05-018-0152	P698	29,76	28,75	1,01	positive	40,00*	28,27	11,73	negative
201	TS-PH05-018-0180	P698	28,45	27,49	0,96	positive	40,00*	27,42	12,58	negative
202	TS-PH05-018-0192	P698	28,74	27,85	0,89	positive	40,00*	27,83	12,17	negative
203	TS-PH05-018-0239	P698	29,21	27,94	1,27	positive	40,00*	28,38	11,62	negative
204	TS-PH05-018-0253	P698	29,15	28,38	0,78	positive	40,00*	27,71	12,29	negative
205	TS-PH05-018-0260	P698	28,58	27,72	0,86	positive	40,00*	27,78	12,22	negative
206	TS-PH05-019-0006	P698	29,15	29,01	0,14	positive	38,27	28,31	9,96	negative
207	TS-PH05-019-0023	P698	27,60	26,05	1,55	positive	40,00*	26,35	13,65	negative
208	TS-PH05-019-0025	P698	27,19	25,78	1,41	positive	40,00*	25,41	14,59	negative
209	TS-PH05-019-0046	P698	27,51	25,87	1,64	positive	40,00*	25,70	14,30	negative
210	TS-PH05-019-0047	P698	27,48	26,14	1,34	positive	38,80	25,84	12,95	negative
211	TS-PH05-019-0051	P698	28,41	27,24	1,17	positive	40,00*	26,86	13,14	negative
212	TS-PH05-020-0002	P698	27,67	26,69	0,99	positive	40,00*	26,41	13,59	negative
213	TS-PH05-020-0018	P698	27,87	27,13	0,74	positive	36,21	27,21	8,99	negative
214	TS-PH05-020-0019	P698	28,69	28,23	0,47	positive	36,98	27,91	9,07	negative
215	TS-PH05-020-0020	P698	28,80	28,39	0,41	positive	38,05	27,67	10,38	negative
216	TS-PH05-020-0030	P698	28,13	27,72	0,41	positive	33,91	27,25	6,66	negative
217	TS-PH05-020-0031	P698	27,94	26,25	1,69	positive	40,00*	26,06	13,94	negative
218	TS-PH05-020-0032	P698	26,55	25,09	1,46	positive	40,00*	25,34	14,66	negative
219	TS-PH05-020-0033	P698	27,43	26,59	0,84	positive	40,00*	26,85	13,15	negative
220	TS-PH05-020-0034	P698	27,05	25,52	1,53	positive	40,00*	25,47	14,53	negative
221	TS-PH05-020-0040	P698	27,98	26,56	1,42	positive	40,00*	26,93	13,07	negative
222	TS-PH05-020-0043	P698	27,44	27,29	0,16	positive	40,00*	26,02	13,98	negative
223	TS-PH05-020-0075	P698	27,37	27,01	0,36	positive	40,00*	24,96	15,04	negative
224	TS-PH05-020-0084	P698	27,37	26,52	0,84	positive	40,00*	26,64	13,36	negative
225	TS-PH05-022-0007	P698	29,40	29,10	0,30	positive	36,40	28,26	8,14	negative
226	TS-PH05-022-0013	P698	27,80	26,28	1,52	positive	40,00*	26,25	13,75	negative
227	TS-PH05-022-0015	P698	26,56	24,80	1,76	positive	40,00*	25,32	14,68	negative
228	TS-PH05-022-0019	P698	28,21	27,57	0,64	positive	40,00*	25,72	14,28	negative

229	TS-PH05-022-0020	P698	27,97	27,74	0,23	positive	40,00*	25,63	14,37	negative
230	TS-PH05-023-0003	P880	27,44	25,91	1,53	positive	40,00*	25,88	14,12	negative
231	TS-PH05-023-0005	P880	28,01	26,17	1,84	positive	40,00*	25,99	14,01	negative
232	TS-PH05-025-0014	P698	27,77	26,55	1,21	positive	40,00*	27,14	12,86	negative
233	TS-PH05-025-0015	P698	27,61	27,62	-0,01	positive	40,00*	25,93	14,07	negative
234	TS-PH05-025-0019	P698	27,51	26,71	0,79	positive	40,00*	26,53	13,47	negative
235	TS-PH05-025-0037	P698	26,99	25,77	1,22	positive	40,00*	25,25	14,75	negative
236	TS-PH05-025-0038	P698	28,56	26,96	1,60	positive	40,00*	26,81	13,19	negative
237	TS-PH05-025-0046	P698	27,61	28,00	-0,39	positive	40,00*	26,22	13,78	negative
238	TS-PH05-025-0048	P698	27,13	27,17	-0,04	positive	40,00*	25,48	14,52	negative
239	TS-PH05-025-0064	P698	27,15	25,74	1,40	positive	40,00*	25,62	14,38	negative
240	TS-PH05-025-0067	P698	27,22	25,38	1,83	positive	40,00*	25,74	14,26	negative
241	TS-PH05-025-0090	P698	27,45	26,90	0,54	positive	40,00*	27,04	12,96	negative
242	TS-PH05-025-0092	P698	28,10	27,04	1,06	positive	40,00*	26,97	13,03	negative
243	TS-PH05-025-0093	P698	26,80	26,59	0,21	positive	39,14	25,27	13,87	negative
244	TS-PH05-025-0096	P698	27,63	26,82	0,81	positive	40,00*	26,92	13,08	negative
245	TS-PH05-025-0134	P698	27,56	27,21	0,35	positive	40,00*	25,26	14,74	negative
246	TS-PH05-025-0174	P698	26,96	26,11	0,85	positive	40,00*	26,12	13,88	negative
247	TS-PH05-025-0193	P698	26,91	26,72	0,19	positive	40,00*	25,02	14,98	negative
248	TS-PH05-025-0194	P698	27,79	26,96	0,83	positive	40,00*	26,72	13,28	negative
249	TS-PH05-025-0210	P698	28,00	27,99	0,01	positive	38,36	25,39	12,97	negative
250	TS-PH05-026-0002	P880	27,42	27,28	0,15	positive	40,00*	25,08	14,92	negative
251	TS-PH05-026-0025	P880	27,30	27,42	-0,12	positive	40,00*	25,66	14,34	negative
252	TS-PH05-026-0026	P880	31,04	29,64	1,40	positive	40,00*	29,70	10,30	negative
253	TS-PH05-026-0048	P880	32,20	31,75	0,45	positive	40,00*	30,02	9,98	negative
254	TS-PH05-026-0066	P880	29,06	27,60	1,47	positive	40,00*	28,05	11,95	negative
255	TS-PH05-026-0069	P880	30,60	28,89	1,71	positive	40,00*	29,35	10,65	negative
256	TS-PH05-026-0091	P880	27,53	27,77	-0,24	positive	40,00*	27,73	12,27	negative
257	TS-PH05-027-0062	P880	29,39	29,70	-0,31	positive	40,00*	27,83	12,17	negative
258	TS-PH05-027-0085	P880	28,67	27,20	1,47	positive	40,00*	26,81	13,19	negative
259	TS-PH05-028-0011	P880	27,46	27,34	0,12	positive	40,00*	25,39	14,61	negative
260	TS-PH05-028-0030	P880	27,69	27,57	0,12	positive	40,00*	27,48	12,52	negative
261	TS-PH05-030-0009	P698	26,82	25,86	0,96	positive	40,00*	26,12	13,88	negative
262	TS-PH05-030-0015	P698	27,97	27,63	0,34	positive	40,00*	25,49	14,51	negative
263	TS-PH05-030-0020	P698	27,34	27,09	0,25	positive	40,00*	25,64	14,36	negative
264	TS-PH05-030-0023	P698	26,50	26,67	-0,17	positive	40,00*	24,64	15,36	negative
265	TS-PH05-030-0030	P698	28,06	27,06	0,99	positive	40,00*	27,10	12,90	negative
266	TS-PH05-030-0034	P698	26,49	25,97	0,52	positive	40,00*	25,76	14,24	negative
267	TS-PH05-030-0042	P698	28,89	27,96	0,92	positive	40,00*	28,68	11,32	negative
268	TS-PH05-030-0075	P698	28,65	28,23	0,42	positive	40,00*	26,00	14,00	negative
269	TS-PH05-030-0085	P698	27,89	27,04	0,86	positive	40,00*	26,70	13,30	negative
270	TS-PH05-033-0006	P880	27,54	27,25	0,28	positive	40,00*	27,76	12,24	negative
271	TS-PH05-033-0009	P880	28,98	27,63	1,35	positive	40,00*	26,99	13,01	negative
272	TS-PH05-034-0004	P698	26,77	25,36	1,41	positive	40,00*	24,92	15,08	negative
273	TS-PH05-034-0026	P698	27,30	26,41	0,89	positive	40,00*	25,91	14,09	negative
274	TS-PH05-035-0006	P698	27,60	25,95	1,65	positive	40,00*	25,85	14,15	negative
275	TS-PH05-035-0015	P698	28,55	27,47	1,08	positive	40,00*	27,14	12,86	negative
276	TS-PH05-035-0018	P698	26,91	26,72	0,19	positive	40,00*	25,46	14,54	negative
277	TS-PH05-035-0019	P698	27,16	27,04	0,12	positive	37,21	25,92	11,30	negative
278	TS-PH05-036-0003	P835	32,79	31,18	1,61	positive	40,00*	31,24	8,76	negative
279	TS-PH05-036-0007	P835	28,18	28,02	0,16	positive	35,46	26,89	8,57	negative
280	TS-PH05-036-0011	P835	28,19	27,73	0,47	positive	40,00*	26,79	13,21	negative
281	TS-PH05-036-0012	P835	29,10	28,37	0,74	positive	38,40	28,00	10,40	negative
282	TS-PH05-036-0015	P835	28,23	27,63	0,60	positive	36,88	27,29	9,59	negative
283	TS-PH05-036-0016	P835	31,19	29,30	1,89	positive	40,00*	29,96	10,04	negative
284	TS-PH05-036-0017	P835	26,75	25,13	1,62	positive	40,00*	25,35	14,65	negative
285	TS-PH05-036-0018	P835	27,20	26,99	0,21	positive	40,00*	26,84	13,16	negative
286	TS-PH05-036-0020	P835	28,59	28,00	0,59	positive	34,59	28,02	6,57	negative
287	TS-PH05-036-0026	P835	28,95	28,38	0,57	positive	40,00*	28,07	11,93	negative

288	TS-PH05-036-0031	P835	31,01	30,50	0,51	positive	39,02	30,09	8,93	negative
289	TS-PH05-036-0034	P835	27,51	26,19	1,32	positive	40,00*	26,39	13,61	negative
290	TS-PH05-036-0044	P835	27,23	25,43	1,80	positive	40,00*	25,73	14,27	negative
291	TS-PH05-036-0051	P835	27,76	26,09	1,66	positive	40,00*	26,07	13,93	negative
292	TS-PH05-036-0074	P835	28,09	27,97	0,12	positive	40,00*	26,99	13,01	negative
293	TS-PH05-036-0075	P835	27,84	27,61	0,23	positive	34,01	27,03	6,98	negative
294	TS-PH05-036-0081	P835	28,80	28,01	0,79	positive	40,00*	28,25	11,75	negative
295	TS-PH05-036-0082	P835	26,79	24,90	1,89	positive	40,00*	25,32	14,68	negative
296	TS-PH05-036-0089	P835	26,79	25,43	1,36	positive	40,00*	25,60	14,40	negative
297	TS-PH05-036-0118	P835	27,58	25,65	1,93	positive	40,00*	26,02	13,98	negative
298	TS-PH05-036-0148	P835	27,44	26,04	1,40	positive	40,00*	26,37	13,63	negative
299	TS-PH05-037-0002	P880	29,25	28,67	0,58	positive	40,00*	28,06	11,94	negative
300	TS-PH05-037-0003	P880	27,10	25,88	1,22	positive	40,00*	25,75	14,25	negative
301	TS-PH05-037-0005	P880	26,99	26,28	0,71	positive	40,00*	26,28	13,72	negative
302	TS-PH05-037-0007	P880	27,52	25,83	1,69	positive	40,00*	26,44	13,56	negative
303	TS-PH05-037-0011	P880	28,94	26,55	2,39	positive	40,00*	26,95	13,05	negative
304	TS-PH05-037-0020	P880	27,58	25,86	1,72	positive	40,00*	25,25	14,75	negative
305	TS-PH05-037-0022	P880	28,22	26,99	1,23	positive	40,00*	27,60	12,40	negative
306	TS-PH05-037-0026	P880	27,25	27,54	-0,29	positive	40,00*	28,15	11,85	negative
307	TS-PH05-037-0033	P880	28,24	27,06	1,17	positive	40,00*	26,89	13,11	negative
308	TS-PH05-037-0039	P880	27,44	27,67	-0,23	positive	40,00*	25,65	14,35	negative
309	TS-PH05-037-0045	P880	26,60	26,46	0,14	positive	40,00*	24,75	15,25	negative
310	TS-PH05-037-0054	P880	32,05	30,43	1,62	positive	40,00*	30,14	9,86	negative
311	TS-PH05-037-0068	P880	29,48	28,00	1,48	positive	40,00*	27,63	12,37	negative
312	TS-PH05-037-0079	P880	27,88	26,48	1,40	positive	40,00*	26,60	13,40	negative
313	TS-PH05-037-0096	P880	29,42	28,83	0,60	positive	40,00*	29,81	10,19	negative
314	TS-PH05-037-0097	P880	32,47	32,02	0,46	positive	40,00*	30,68	9,32	negative
315	TS-PH05-037-0116	P880	30,28	28,81	1,47	positive	40,00*	28,93	11,07	negative
316	TS-PH05-037-0118	P880	31,00	29,59	1,41	positive	40,00*	29,55	10,45	negative
317	TS-PH05-037-0135	P880	27,74	26,54	1,20	positive	40,00*	26,41	13,59	negative
318	TS-PH05-037-0138	P880	27,63	27,29	0,35	positive	40,00*	25,48	14,52	negative
319	TS-PH05-037-0147	P880	28,49	28,19	0,30	positive	40,00*	25,49	14,51	negative
320	TS-PH05-037-0149	P880	26,68	26,67	0,01	positive	38,14	25,38	12,76	negative
321	TS-PH05-037-0153	P880	27,83	27,74	0,09	positive	40,00*	26,39	13,61	negative
322	TS-PH05-037-0157	P880	26,68	26,45	0,23	positive	40,00*	27,30	12,70	negative
323	TS-PH05-037-0199	P880	27,99	27,46	0,53	positive	40,00*	27,58	12,42	negative
324	TS-PH05-037-0207	P880	26,79	26,59	0,19	positive	40,00*	26,83	13,17	negative
325	TS-PH05-037-0215	P880	27,26	27,20	0,07	positive	40,00*	27,73	12,27	negative
326	TS-PH05-037-0244	P880	28,66	28,35	0,31	positive	40,00*	28,40	11,60	negative
327	TS-PH05-037-0281	P880	30,15	29,00	1,16	positive	40,00*	28,10	11,90	negative
328	TS-PH05-037-0303	P880	30,40	28,81	1,59	positive	40,00*	28,83	11,17	negative
329	TS-PH05-037-0432	P880	30,72	29,39	1,33	positive	40,00*	29,39	10,61	negative
330	TS-PH05-037-0436	P880	30,07	29,75	0,32	positive	40,00*	29,15	10,85	negative
331	TS-PH05-037-0441	P880	31,44	30,28	1,15	positive	40,00*	30,44	9,56	negative
332	TS-PH05-037-0448	P880	28,16	27,93	0,24	positive	40,00*	28,41	11,59	negative
333	TS-PH05-037-0449	P880	30,65	30,45	0,20	positive	40,00*	29,95	10,05	negative
334	TS-PH05-037-0457	P880	29,75	30,44	0,34	positive	40,00*	29,72	10,28	negative
	neg ctrl, P698		40,00*	27,79	12,21	negative	35,23	25,26	9,97	negative
	neg ctrl, P698		36,65	25,17	11,48	negative	40,00*	27,71	12,29	negative
	neg ctrl, P698		38,24	31,14	7,10	negative	36,75	27,73	9,02	negative
	neg ctrl, P698		37,40	29,12	8,28	negative	37,30	28,87	8,43	negative
	neg ctrl, P698		40,00*	30,02	9,98	negative	38,98	27,99	11,00	negative
	neg ctrl, P835		40,00*	25,46	14,54	negative	40,00*	27,13	12,87	negative
	neg ctrl, P835		40,00*	26,43	13,57	negative	36,17	29,55	6,62	negative
	neg ctrl, P835		39,27	28,93	10,34	negative	40,00*	28,76	11,24	negative
	neg ctrl, P835		37,44	27,87	9,57	negative	36,23	28,50	7,73	negative
	neg ctrl, P835		35,69	26,69	9,00	negative	38,88	30,65	8,23	negative
	neg ctrl, P880		34,86	27,83	7,03	negative	40,00*	27,94	12,06	negative
	neg ctrl, P880		40,00*	27,36	12,64	negative	40,00*	26,25	13,75	negative

	neg ctrl, P880		40,00*	25,71	14,29	negative	35,15	27,11	8,03	negative
	neg ctrl, P880		37,98	29,60	8,37	negative	38,20	28,59	9,60	negative
	ctrl with backbone		-	-	-	-	27,32	25,98	1,35	positive
	ctrl with backbone		-	-	-	-	27,63	26,08	1,55	positive
	ctrl with backbone		-	-	-	-	27,11	26,90	0,21	positive
	ctrl with backbone		-	-	-	-	27,15	26,07	1,08	positive
	ctrl with backbone		-	-	-	-	28,61	28,52	0,09	positive
	ctrl with backbone		-	-	-	-	28,69	28,22	0,47	positive
	ctrl with backbone		-	-	-	-	27,59	26,31	1,28	positive
	ctrl with backbone		-	-	-	-	26,67	27,00	-0,33	positive
	ctrl with backbone		-	-	-	-	26,62	25,71	0,91	positive
	ctrl with backbone		-	-	-	-	26,61	25,81	0,81	positive
	ctrl with backbone		-	-	-	-	26,36	25,69	0,67	positive
	ctrl with backbone		-	-	-	-	26,99	25,60	1,39	positive
	ctrl with backbone		-	-	-	-	26,47	25,93	0,54	positive
	ctrl with backbone		-	-	-	-	28,25	29,09	-0,85	positive
	ctrl with backbone		-	-	-	-	28,07	28,28	-0,21	positive

* no signal detected after 40 cycles

Table 2. Data for lines transformed with construct VCPMA19

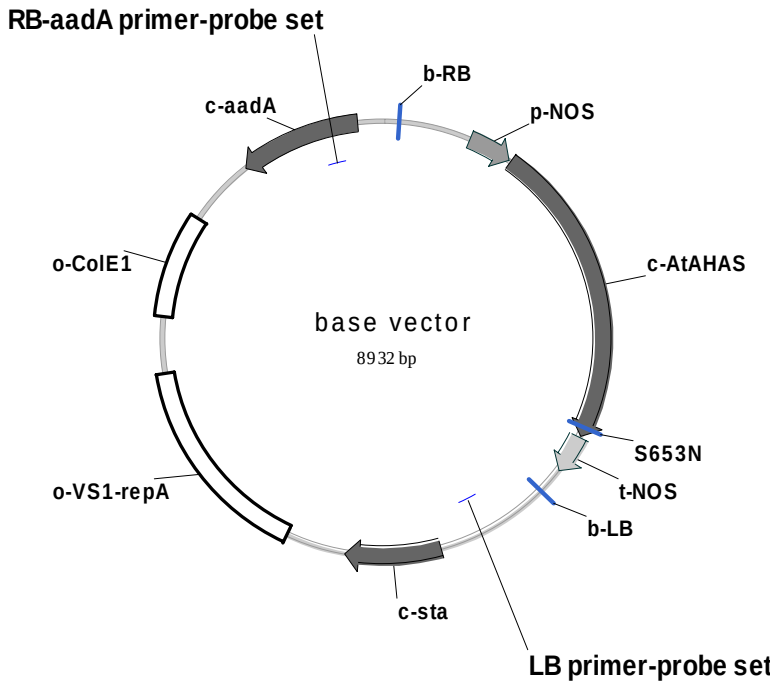
			Insert analysis				Backbone analysis			
	Line no	Parental variety	Ct AHAS	Ct end ctrl	dCt	Result	Ct RB-aadA	Ct end ctrl	dCt	Result
335	TS-PH05-038-0029	P698	28,91	28,01	0,90	positive	40,00*	28,21	11,79	negative
336	TS-PH05-038-0035	P698	27,22	26,96	0,26	positive	40,00*	25,07	14,93	negative
337	TS-PH05-038-0043	P698	28,18	27,70	0,47	positive	40,00*	27,40	12,60	negative
338	TS-PH05-038-0067	P698	32,71	31,85	0,86	positive	40,00*	31,96	8,04	negative
339	TS-PH05-038-0071	P698	30,43	28,94	1,50	positive	40,00*	28,63	11,37	negative
340	TS-PH05-039-0004	P698	26,99	26,83	0,16	positive	38,30	25,68	12,62	negative
341	TS-PH05-039-0013	P698	27,00	26,35	0,65	positive	40,00*	27,12	12,88	negative
342	TS-PH05-039-0029	P698	27,42	26,58	0,84	positive	40,00*	26,43	13,57	negative
343	TS-PH05-039-0038	P698	27,52	27,10	0,42	positive	40,00*	25,62	14,38	negative
344	TS-PH05-039-0039	P698	26,89	26,78	0,10	positive	40,00*	24,97	15,03	negative
345	TS-PH05-040-0005	P698	30,01	29,01	1,00	positive	40,00*	28,43	11,57	negative
346	TS-PH05-040-0010	P698	29,21	29,23	-0,02	positive	40,00*	27,23	12,77	negative
347	TS-PH05-040-0022	P698	29,80	29,06	0,73	positive	40,00*	28,61	11,39	negative
348	TS-PH05-040-0023	P698	30,81	29,21	1,60	positive	40,00*	29,77	10,23	negative
349	TS-PH05-040-0025	P698	29,39	27,86	1,53	positive	40,00*	27,83	12,17	negative
350	TS-PH05-040-0044	P698	28,95	27,54	1,41	positive	40,00*	27,50	12,50	negative
351	TS-PH05-040-0051	P698	29,70	28,45	1,25	positive	40,00*	28,15	11,85	negative
352	TS-PH05-040-0055	P698	29,75	28,58	1,17	positive	40,00*	28,53	11,47	negative
353	TS-PH05-041-0002	P698	27,48	26,82	0,66	positive	40,00*	26,85	13,15	negative
354	TS-PH05-041-0004	P698	27,27	26,49	0,78	positive	40,00*	26,62	13,38	negative
355	TS-PH05-041-0008	P698	29,69	28,80	0,89	positive	40,00*	28,93	11,07	negative
356	TS-PH05-041-0012	P698	30,00	28,86	1,14	positive	40,00*	28,75	11,25	negative
357	TS-PH05-042-0001	P835	27,29	26,40	0,90	positive	40,00*	26,60	13,40	negative
358	TS-PH05-042-0002	P835	26,84	26,29	0,54	positive	40,00*	26,56	13,44	negative
359	TS-PH05-042-0003	P835	28,34	27,13	1,21	positive	40,00*	27,07	12,93	negative
360	TS-PH05-042-0007	P835	28,37	27,28	1,08	positive	40,00*	27,26	12,74	negative
361	TS-PH05-042-0035	P835	29,24	28,10	1,14	positive	40,00*	28,01	11,99	negative
362	TS-PH05-042-0036	P835	29,38	27,83	1,55	positive	40,00*	28,08	11,92	negative
363	TS-PH05-042-0053	P835	29,06	27,56	1,50	positive	40,00*	27,75	12,25	negative
364	TS-PH05-042-0054	P835	28,29	26,97	1,32	positive	40,00*	27,09	12,91	negative
365	TS-PH05-042-0061	P835	30,40	29,36	1,04	positive	40,00*	29,25	10,75	negative
366	TS-PH05-042-0064	P835	28,18	26,58	1,60	positive	40,00*	26,42	13,58	negative
367	TS-PH05-042-0070	P835	28,44	27,27	1,17	positive	40,00*	27,18	12,82	negative
368	TS-PH05-042-0081	P835	27,74	26,61	1,13	positive	40,00*	26,43	13,57	negative
369	TS-PH05-042-0086	P835	28,67	27,26	1,41	positive	40,00*	27,09	12,91	negative
370	TS-PH05-042-0092	P835	29,83	28,56	1,27	positive	40,00*	28,62	11,38	negative
371	TS-PH05-043-0001	P835	27,64	26,22	1,42	positive	40,00*	26,29	13,71	negative
372	TS-PH05-043-0008	P835	29,38	28,32	1,06	positive	40,00*	27,76	12,24	negative
373	TS-PH05-043-0021	P835	28,20	27,04	1,16	positive	40,00*	26,71	13,29	negative
374	TS-PH05-043-0034	P835	29,84	28,74	1,10	positive	40,00*	28,36	11,64	negative
375	TS-PH05-043-0067	P835	29,86	28,56	1,30	positive	40,00*	28,24	11,76	negative
376	TS-PH05-044-0009	P880	27,58	27,20	0,38	positive	40,00*	25,39	14,61	negative
377	TS-PH05-044-0020	P880	28,20	27,01	1,18	positive	40,00*	26,88	13,12	negative
378	TS-PH05-046-0001	P880	26,95	25,43	1,51	positive	40,00*	25,47	14,53	negative
379	TS-PH05-046-0002	P880	27,07	26,84	0,23	positive	39,97	25,96	14,00	negative
380	TS-PH05-046-0019	P880	30,80	29,14	1,66	positive	40,00*	29,06	10,94	negative
	neg ctrl, P698		36,65	25,17	11,48	negative	40,00*	27,71	12,29	negative
	neg ctrl, P698		38,24	31,14	7,10	negative	36,75	27,73	9,02	negative
	neg ctrl, P698		37,40	29,12	8,28	negative	37,30	28,87	8,43	negative

	neg ctrl, P698		40,00*	30,02	9,98	negative	38,98	27,99	11,00	negative
	neg ctrl, P835		40,00*	25,46	14,54	negative	40,00*	27,13	12,87	negative
	neg ctrl, P835		39,27	28,93	10,34	negative	40,00*	28,76	11,24	negative
	neg ctrl, P835		37,44	27,87	9,57	negative	36,23	28,50	7,73	negative
	neg ctrl, P835		35,69	26,69	9,00	negative	38,88	30,65	8,23	negative
	neg ctrl, P880		34,86	27,83	7,03	negative	40,00*	27,94	12,06	negative
	neg ctrl, P880		40,00*	27,36	12,64	negative	40,00*	26,25	13,75	negative
	ctrl with backbone		-	-	-	-	27,32	25,98	1,35	positive
	ctrl with backbone		-	-	-	-	27,63	26,08	1,55	positive
	ctrl with backbone		-	-	-	-	27,11	26,90	0,21	positive
	ctrl with backbone		-	-	-	-	27,15	26,07	1,08	positive
	ctrl with backbone		-	-	-	-	28,61	28,52	0,09	positive
	ctrl with backbone		-	-	-	-	26,61	25,81	0,81	positive
	ctrl with backbone		-	-	-	-	26,36	25,69	0,67	positive
	ctrl with backbone		-	-	-	-	26,99	25,60	1,39	positive
	ctrl with backbone		-	-	-	-	26,47	25,93	0,54	positive
	ctrl with backbone		-	-	-	-	28,25	29,09	-0,85	positive
	ctrl with backbone		-	-	-	-	28,07	28,28	-0,21	positive

* no signal detected after 40 cycles

The following plasmid map (*Figure 1*) shows the location of the RB-aadA and LB primer-probe sets on the backbone of the base vector that was used for preparing constructs VCPMA16 and VCPMA19.
The RB-aadA primer probe set is located about 300 base pairs from the RB and lies completely within the *aadA* gene. The amplicon size is 137 bp.
The LB primer-probe set is located about 400 base pairs from the LB and has an amplicon size of 103 bp.
The endogenous control primer-probe set is located on the potato genome within an endogenous gene of low copy number. The amplicon size is 94 bp.

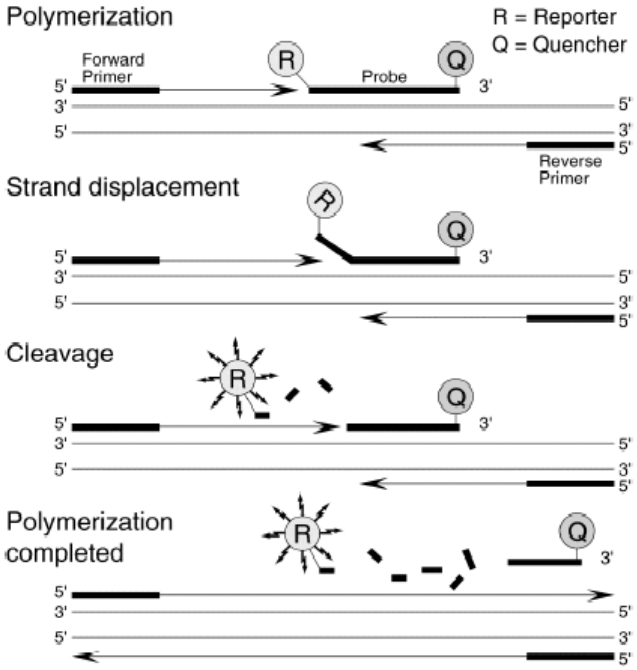
Figure 1.



The spectinomycin resistance gene is homologous in sequence to accession numbers AAX97761 (protein) and AY995143 (nucleotide, first CDS).

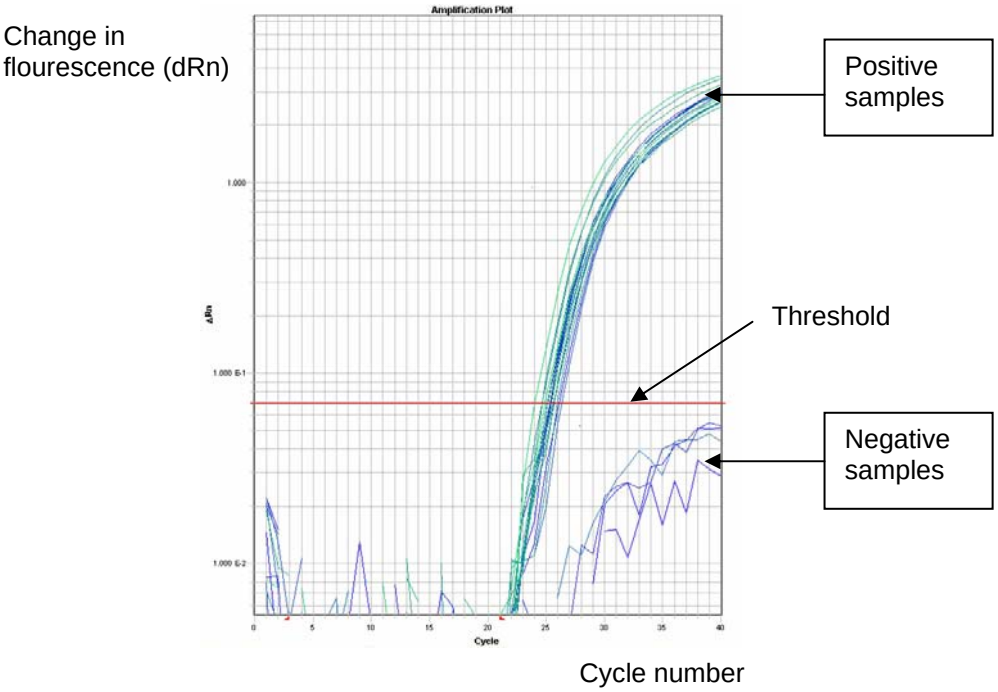
Real-time PCR is a very sensitive, quantitative PCR method. In addition to PCR-primers a probe directed to a sequence between the two primers is included. This probe is labelled with a fluorescent reporter and a quencher (see Figure below). During strand displacement the probe is broken down and the reporter and quencher are separated allowing the reporter to fluoresce. The production of PCR-products is measured quantitatively as fluorescence during the PCR process. Real-time PCR data are given as Ct values, which is the number of PCR-cycles required to reach a threshold value of PCR products. The maximum number of PCR-cycles used in our analysis is 40.

Figure 2.



In real-time PCR the amplification can be followed for each cycle. In the analyses the fluorogenic 5´ nuclease (TaqMan) assay has been applied, where the probe emits fluorescence and the fluorescence detected by the instrument is proportional to the amplification product. This change in fluorescence is shown in a graph on the instrument (ABI Prism 7900HT). To transfer these results into figures a threshold line is set and the Ct determined as “the fractional cycle number at which the amount of amplified target reaches a fixed threshold” (“User Bulletin #2:ABI PRISM 7700 sequence Detection System”, Applied Biosystems).

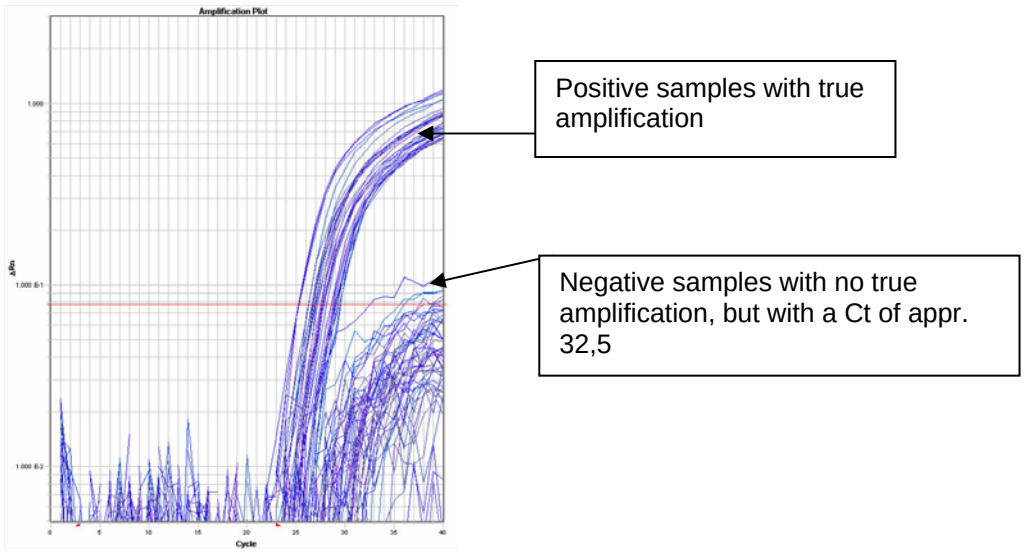
Figure 3. Amplification plot



In the analysis of presence/absence of the aadA gene, all samples are analysed in duplex reactions that in addition to the RB-aadA primer-probe set also contain primers and probe for the endogenous control. The endogenous control is used to confirm that the quality and amount of DNA as well as reaction conditions are satisfactory. Comparing the Ct value of the RB-aadA primer-probe set to the Ct value of the endogenous control gives a dCt value (dCT = CT,X – CT,R, the difference in threshold cycles for target and reference). This value is used for the copy number calculation but also for the RB-aadA assay and can be considered to distinguish true insertions from signals arisen due to e.g. potential contaminations or similar reasons. All plates analysed contain both positive (potato samples known to contain backbone sequences) as well as negative controls. As negative controls non-transformed potato as well as NTC (non-template controls) are used.
(In addition to the data presented in the tables, two additional samples have been analysed for each line with the RB-aadA primer probe set confirming the negative results.)

In the amplification plot, the amplification curve can, in some cases, come just above the threshold, generating a disproportionately low Ct value, without that any real amplification has taken place. This can be seen when studying the original graph of the amplification (see Figure below). The tendency of this phenomenon varies between different assays (primer/probe combinations), oligonucleotide batches and extract qualities.

Figure 4. Amplification plot



3. Detection of genetically modified potato lines (*ahas*-gene)

DNA extraction
Genomic DNA is isolated from fresh leaf tissue using Nucleon PhytoPure (Amersham Biosciences).

PCR method
Setup of PCR reaction
4 µl DNA
2 µl Red Taq PCR reaction buffer 10X (Sigma)
1 µl Red Taq DNA polymerase (Sigma)
0.4 µl forward primer (25 µM)
0.4 µl reverse primer (25 µM)
0.4 µl dNTP (10 µM) (Sigma)
11.8 µl H2O

Primer pair for AHAS detection
AHAS1 frw 5´- AAC AAC AAC ATC TTC TTC GAT C- 3´
AHAS1 rev 5´TAA CGA GAT TG TAG CTC CG- 3´

PCR program for AHAS detection

1. 94 °C 1.5 min

2. 94 °C 30 s
3. 55 °C 30 s
4. 72 °C 1 min

5. 72 °C 7 min

Steps 2-4 are repeated for 32 cycles.
Size of amplicon is 509 bp.

ANNEX 2

Proposed plot design map