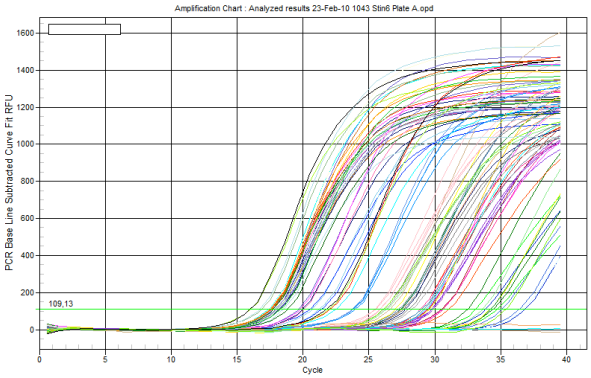
Gene expression profiles of invertase and sucrose synthase in potato leaves during drought stress in the CxE potato mapping population

Berend-Jan Dobma



Wageningen UR: Laboratory of Plant Breeding
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Preface

The report that you are reading at this moment is a report that concerns my MSc-thesis. With this thesis I will accomplish a big and important part of my MSc study Plant Sciences at the Wageningen University and Research center. I have chosen to come to Wageningen to study after finishing my Bachelors degree at Hogeschool Van Hall Larenstein in Leeuwarden. There I did the study Horti- and agriculture. During the time in Leeuwarden there was one aspect that attracted me. This was plant breeding. Therefore I wanted to go more into depth and I chose to go to Wageningen and study Plant Sciences with the specialization Plant Breeding and Genetic Resources.
I also have a big interest in the potato crop. The most important indication for this is that I grow my own potatoes as a hobby. So I tried to find a thesis which included doing research on potatoes. A research group that had potato as a crop of interest was the group that focused on abiotic stress. After having talked to mister Gerard van der Linden I decided to work on the project of Anitha Kumari. She already did research to drought tolerance in the CxE potato mapping population. Gerard van der Linden got me soon enthusiastic about the fact that Anitha found an interesting thing that directed in the way to invertases.
This resulted in the subject of my thesis. Gene expression profiles of invertases and sucrose synthases in the CxE potato mapping population during drought stress. In first instance I did not feel comfortable about it, because the focus of the work was a lot on molecular techniques. Something I was not familiar with until then. But I like practical work and I wanted to learn something about working in the lab, because my opinion is that molecular techniques are getting more important within breeding companies, I got to feel comfortable about working in a lab. However this was not possible by the support of several people, which I would like to thank. These are Hanneke van der Schoot, Johan Bucher, Linda Kodde and Doret Wouters for their technical support in the lab. I also would like to thank Gerard van der Linden and Anitha Kumari for their supervision and giving me the possibility to do my thesis within their research. I also would like to thank the rest of lab the personnel for the good atmosphere in the lab and the people in the student room, also known as the breeders' hole. Last but not least I would like to thank my girlfriend Noortje for her support and patience.

Berend-Jan Dobma
Wageningen, June, 2010

Summary

Potato is the fourth largest staple food crop and is therefore for a big part of the world population an important resource of energy and fibers. Potato produces also the most calories per liter water, compared to crops like wheat, maize and rice. However potato is also a drought sensitive crop. Abiotic stresses like drought cause more reduction in yield than pests and diseases.
Drought can cause stress to plants. This is expressed in several responses like inhibition of leaf expansion, loss of turgor, root extension, and stomatal closure. Stomatal closure causes a decrease in photosynthetic activity in the leaves. This results in a reduction of carbon assimilation and less sugars are produced. A plant that faces drought stress has to adjust its carbon partitioning. Two important enzymes that play role in carbon partitioning are invertase and sucrose synthase. There are apoplastic, cytosolic and vacuolar invertases. Sucrose synthase is located in the cytosol. In this report the expression of the apoplastic invertase and sucrose synthase are studied in the CxE potato mapping population by means of a qRT-PCR experiment.
In potato there are four apoplastic invertases, these are *InvCD141*, *InvCD111*, *InvGE* and *InvGF*. These have high similarities with *lin6*, *lin8*, *lin5* and *lin7* in tomato. There are 2 sucrose synthase genes, *sus3* and *sus4*, which are published. Searches in public nucleotide and protein databases resulted also in a third potato sucrose synthase gene, called *Susy2*. Unfortunately nothing was published about this gene. Further literature research indicated that *Susy2* is probably expressed in a later maturation stage of tissues.
Unfortunately the primers of *InvGE* and *InvGF* were not able to give amplification on cDNA template and *Susy2* gave several bands of different lengths. Because of time considerations it was decided to continue with *InvCD141*, *InvCD111*, *sus3* and *sus4*.
The qRT-PCR experiment was executed on two group of the same genotypes. One group was well watered and the other group with the same genotypes was induced with drought stress. The qRT-PCR experiment revealed that *InvCD141* and *InvCD111* were downregulated under drought stress and expression of sucrose synthase was upregulated under drought stress, but this was not significant. The decrease in apoplastic invertase expression in response to drought in our experiments may indicate that in the examined leaves the demand for energy-rich sugars decreases. This is in agreement with observations of stunted/decreased growth in conditions with low water supply. Sucrose synthase expression is negatively correlated under drought with invertase expression under drought. So it might be that sucrose synthase is taking over the function of apoplastic invertase. A down regulation of invertase and a trend of up regulation of sucrose synthase is seen. This means that hexoses are not entering the cell anymore through the apoplast, but that the sucrose in the cytosol is split in hexoses by the sucrose synthase. This might be done because of the initiation of starch synthesis.
In this experiment only the gene expression is measured. To have a better view on the effect of this expression it is recommended to measure the activity of the enzymes and the levels of hexoses and sucrose.
Mapping the data of gene expression experiments resulted in several eQTLs. Sometimes the positions were not the same as described in other gene studies. This means that other factors are regulating the expression of some of the genes studied in this report. These factors are interesting to study in further research.
The samples used in the qRT-PCR experiment of this report were taken from plants that were grown in a greenhouse pot experiment. The next step would be to do an experiment in the field.

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Introduction

1.1 Potato

For many people the potato tuber (*Solanum tuberosum* L.) is a big resource of energy. The tubers are produced by an annual herbaceous plant that can grow 100 cm tall and belongs to the Solanaceae. Potato is highly heterozygous and cross pollinating. It is tetraploid and has 48 chromosomes. The tubers are rich in starch and potato has the fourth position on the list of the world's largest staple food crops.
Originally the potato comes from the Andes region and nowadays it has spread and is grown on every continent. The Andes region is the centre of origin and wild potato species grow there, which can function as potential genetic resource.
Potatoes are mainly grown in Northern Europe and Northern America, former Soviet Union countries, China and India (www.potato2008.org, 2008). From the beginning of the 1990's developing countries have been increasing their potato production. Nowadays there are more potatoes produced in developing countries than in the developed countries. China and India are producing one third of the total. The total potato production in 2008 was 314,140,107 tonnes (FAOSTAT, 2009).
Because of the importance of this crop, the FAO declared 2008 as the year of the potato. On a potato fact sheet it is stated that potato produces the most calories per liter water used for growing compared to wheat, rice and maize (Lutaladio *et. al.*, 2009). All this above shows that potatoes have a good future, when it comes to becoming a more important staple food crop and the associated increase of growing area.

1.2 Drought stress in potato

Just like in every other crop, there are constraints during the cultivation of potatoes. These constraints exists of biotic stresses, such as *Phytophthora infestans*, bacteria, viruses, insects and nematodes, on one hand; on the other hand abiotic stresses such as drought and temperature are reducing tuber yield (Veerman, 2003).
In other words; despite the fact that potato has a good efficiency in the use of water and production of calories, it is a drought sensitive crop compared to other crops (Harris, 1978 in Van Loon, 1981).
According to Boyer, (1982) abiotic stress causes more crop loss than biotic stress, (see table 1.1.1)
Drought and salinity are the most severe abiotic stress factors accounting for yield loss world-wide.

Crop	Yield		Loss resulting from	
	highest	mean	Parasites and weeds	other
Maize	19.3	4.6	2.0	12.7
Wheat	14.5	1.9	0.7	11.9
Soy	7.4	1.6	0.7	5.1
Potato	94.1	28.3	14.9	50.9
Sugarbeet	121.0	42.6	17.1	61.3
average % of highest yield		24.8	10.9	64.3

Table 1.1.1: Crop loss over a number of years in the USA (based on Boyer et al,1982)

1.3 Drought response mechanisms

Drought can cause stress to plants. This is expressed in several responses like inhibition of leaf expansion, loss of turgor, root extension, and stomatal closure (Taiz and Zeiger, 2002). Photosynthesis itself is not directly affected by a lower water supply but indirectly by the closure of the stomata (Boyer 1970).

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For example roots are extended into deeper soil layers were water is still available. If the water supply is lowered, cells may stay turgid and leaves start to wilt. During the closure of the stomata less water is transpired, but also less carbon can be taken up. In the latter case the efficiency of photosynthesis is decreased, so indirectly by the closing of the stomata as a reaction on the lower water supply. A decrease in the efficiency of photosynthesis or the carbon assimilation itself has also consequences for the energy supply and metabolism in the plant. Enzymes like invertase and sucrose synthase play an important role in energy metabolism.

1.4 Invertase and sucrose synthase

In most plants sucrose is the form in which assimilated carbon is present and transported. Sucrose is a disaccharide of glucose and fructose. Sucrose can be cleaved into these two monosaccharides by two types of enzymes. These are invertase (EC 3.2.1.26) and sucrose synthase (EC 2.4.1.13), which initiate sucrose utilization.

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Invertase is a hydrolase and cleaves sucrose into glucose and fructose in an irreversible way. This is done within the source to sink relationship and the unloading of the phloem, (Sturm and Tang, 1999). So when sucrose is transported from the source tissue through the phloem to the sink tissue, the sucrose needs to be unloaded from the phloem. Once sucrose is unloaded into the sink tissue it can be hydrolyzed by invertase. By means of hexose transporters, glucose and fructose can enter the sink cell, for metabolic use. Besides that the hexoses can function as regulators of gene expression (Roitsch and González, 2004). See also picture 1.2.1.

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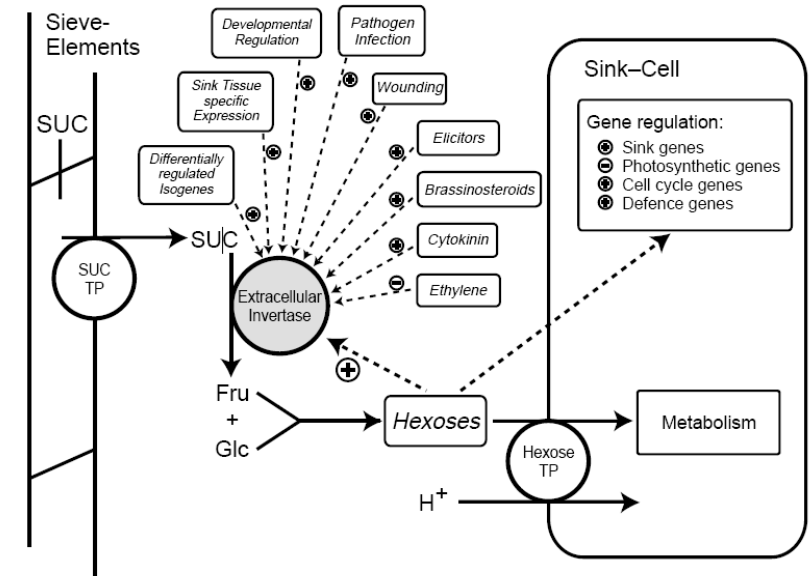
There are 3 kinds of invertases, these are vacuolar, neutral and cell wall bound invertases. Vacuolar and cell wall bound invertases have high similarities with respect to enzymatic and biochemical properties. They have both an acidic pH-optimum (Roitsch and González, 2004). Sucrose synthase catalyzes the reversible conversion of sucrose and uracil-diphosphate (UDP) into UDP-glucose and fructose. It is the predominant sucrose cleavage enzyme in cereal endosperm and potato tubers and provides substrates for starch synthesis in these and other storage organs. Sucrose synthase is also involved in meeting the increased glycolytic demand during anaerobic and cold stress as well as in supplying UDP-glucose for cell wall biosynthesis.

In addition, sucrose synthase appears to play a key role in supplying energy for loading and unloading in phloem by providing substrate for respiration (Fu and Park, 1995). Loading of sucrose into the phloem is done against the concentration gradient. This costs energy, but H^+-ATPase provides a proton gradient. The form of energy that is needed for H^+-ATPase is ATP. Sucrose synthase is therefore involved in the first step in metabolizing sucrose for respiration by supplying hexoses (Martin et. al., 1993).
Sucrose synthase is a cytosolic enzyme (Baroja-Fernández et. al., 2009).
Invertases and sucrose synthase enzymes are able to influence the expression of other genes and plant development in an indirect way, by means of changing the sugar concentrations as a result of their activity. E.g. when there is an abundance of sugars, genes that are active in the storage, utilization, sink activity and growth are upregulated. With a shortage of sugars, genes that are active in e.g. photosynthesis, source activity, export and remobilization are upregulated, where genes that are responsible for storage, sink activity and growth are downregulated (Koch, 1996). Also the glycolytic enzymes are affected by all kinds of factors. For example picture 1.2.1 shows the factors that influence the expression of apoplastic invertases. The picture is based on studies that focused on the regulation of cell wall invertase and its function in carbon partitioning, defence reactions and signalling of sugars in tomato, *Chenopodium rubrum*, and tobacco, (Roitsch, 2000).



Picture 1.2.1: Apoplasmic phloem unloading and regulation of extracellular invertase. Sucrose is unloaded from the sieve elements of the phloem into the apoplasm by a sucrose transporter, the disaccharide is cleaved by an extracellular invertase and the hexose monomers are taken up in the sink cell by monosaccharide transporters. Extracellular invertase expression is regulated by a range of signals and mechanisms including glucose, phytohormones and stress-related stimuli. Sugars are substrate for heterotrophic growth and function as signals for gene regulation. Solid lines indicate sugar and proton fluxes, dashed lines indicate signal transduction pathways. SUC, sucrose; Fru, fructose; Glc, glucose; H+, protons, TP transporter. (Picture taken from: Roitsch et. al., 2000)

1.5 Evaluation of drought tolerance in potato

Drought tolerance in potato was evaluated in a greenhouse experiment by growing potatoes in pots by Anitha Kumari. Details are described in section 2.1.
In this experiment the potato CxE mapping population was grown and at stolon initiation water supply was stopped. In order to measure Water Use Efficiency, $\Delta^{13}C$ was measured. These data were mapped, which made it possible to identify QTLs for this trait. On chromosome 10 a QTL for $\Delta^{13}C$ was mapped. On the location of this QTL a CAPS marker for an apoplastic invertase was present.
From literature it is known that the apoplastic invertase gene *InvCD141* is located on chromosome 10 in potato (Chen et al., 2001).

1.6 Aims of this research

From what is known in literature invertase and sucrose synthase are important enzymes with regard to sucrose metabolism, source to sink relationships, energy supply to developing tissues and the regulation of sugar signaling. The results from the greenhouse experiment, indicate the relevance of studying invertase and sucrose synthase expression in drought stressed potato. Therefore the aims of the study were:

- Identify invertase and sucrose synthase genes in potato
- Study expression profiles of invertase and sucrose synthase under drought

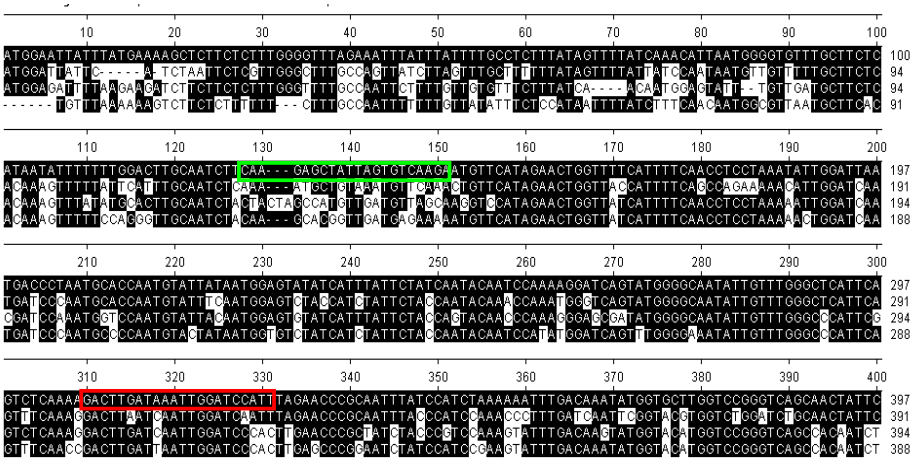
2. Material and methods

2.1 Plant material

Plants of the CxE potato population were grown in pots in a greenhouse. An overview of the used CxE genotypes is showed in Appendix A. During the experiment 94 progeny of CxE was grown together with the C and E parents, SH and RH parents and 10 tetraploid cultivars.
The CxE potato population is the result of a cross between parent C and parent E. Parent C is a crossing of *Solanum phureja* and *Solanum tuberosum*. Parent E is a crossing between parent C and a backcross clone of *Solanum vernei* and *Solanum tuberosum* (Jacobs *et. al*., 1995).
The experiment was done in two randomized blocks that were well watered throughout the growing period and six randomized blocks with drought stressed plants. In every block the 94 progeny were grown.
Stress was induced at the time of stolon initiation, by means of stopping the water supply. Leaf samples were taken from one replication of control and one replication of drought-stressed plants after four days of drought stress, when the first wilting symptoms were seen. The youngest fully expanded leaves were picked. By doing that it was assumed that these were source leaves (personal communication with A. Kumari, March 2010).
Leaf samples were picked, packed in tinfoil and frozen in liquid nitrogen. Until further use the samples were stored at -80 °C.

2.2 Search for sequences and primer design

The NCBI (nucleotide) database (http://www.ncbi.nlm.nih.gov/) and a potato EST database (http://compbio.dfci.harvard.edu/tgi/) were searched for sequences of invertase and sucrose synthase were performed by mean of keywords and BLAST searches. In the software program MegAlign of DNAStar, Lasergene, alignments of a shortlist with sequences were made with the ClustalW function.
PCR primers were designed manually, using the alignments in regions where discrimination between the different genes was possible, with the goal to have a primer with a high specificity. Some criteria were set for primer design. These were: a primer length of about 20 nucleotides, a melting temperature around 60 °C (4*(G+C) + 2*(A+T)= Tm, was the formula used), a GC content between the 40% and 60% ((G+C)/(total nt))*100%= GC%, was the formula used to calculate the GC content. Finally the PCR amplification product length had to be 150 till 250 nucleotides long. An example is depicted in picture 2.2.6.



Picture 2.2.6: Example of the alignment output and primer selection. The green square is the forward primer and the red square is the reverse primer. Also note that the 3'-ends are discriminating and therefore enhancing specificity.

2.3 Primer testing

Primers were tested to see whether they amplify the right product or not. This was done with a regular Polymerase Chain Reaction (PCR) on potato genomic DNA and at a later stage on cDNA samples.

2.4 RNA isolation cDNA synthesis

The total RNA isolation was done with a KingFisher Flex system and the MagMAX™-96 Total RNA Isolation Kit from Ambion cat#AM1830. To make it possible to isolate RNA from plant material a Plant Isolation Aid cat#AM9690 from Ambion was also used.
The protocol was executed according to the manufacturers' protocol except for filling plate A. Table 2.1.2 shows which reagent was pipetted in each plate.
For the homogenization the samples with the lysis/binding solution were spun down at 10000 G.

Plate	Volume per well in µl	Reagent(s)
A	100	Tissue homogenate in Lysis/binding Solution
	70	Isopropanol
	20	Bead mix
B	150	Wash solution 1
C	150	Wash solution 2
D	50	Diluted TURBO DNase
E	150	Wash solution 2
F	150	Wash solution 2
G	50	Elution buffer

Table 2.1.2: Filling of the different plates with the associated reagents

For convenience plates B,C,E,F and G were prepared first and covered with a seal and put aside until further use.
The RNA concentration and purity was measured with the Nanodrop ND1000 of Thermo Scientific. In appendix C the concentrations of each sample are given.
After RNA isolation it was needed to treat the RNA samples with an extra DNase treatment. This was done with the DNase I, Amplification Grade of Invitrogen, category number 18068-015.
cDNA was synthesized with iScript cDNA synthesis Kit made by Bio-Rad Laboratories inc, with the category number 170-8891.
Both treatments were executed according to the manufacturer's protocol.

2.5 qRT-PCR

To see how the genes of interest were expressed, qRT-PCR was used. EF 1α was used as a control RNA to compensate for the differences in the input of cDNA samples. Nicot et al., 2005 described that Elongation Factor 1α has a constant expression level under biotic and a-biotic stress conditions. With use of EF 1α as a reference the expression of the target gene could be normalized.
Table 2.1.3 shows the plate set-up used in the qRT-PCR experiment. Each cDNA sample was tested with primers of the target gene and primers of EF 1α. This was done in duplicate.
Each plate contained 12 samples of the control plants and 12 from the drought-stressed plants. During the greenhouse experiment 112 genotypes were in the control block and 112 in the drought stressed block. So 224 genotypes in total needed 9.33 plates per gene. 4 genes were screened so in total 37 plates were used in this experiment.

Gene	Gene	EF 1α	EF 1α	Gene	Gene	EF 1α	EF 1α	Gene	Gene	EF 1α	EF 1α
1 c	1 c	1 c	1 c	5 c	5 c	5 c	5 c	9 c	9 c	9 c	9 c
2 c	2 c	2 c	2 c	6 c	6 c	6 c	6 c	10 c	10 c	10 c	10 c
3 c	3 c	3 c	3 c	7 c	7 c	7 c	7 c	10 c	11 c	11 c	11 c
4 c	4 c	4 c	4 c	8 c	8 c	8 c	8 c	11 c	12 c	12 c	12 c
1 d	1 d	1 d	1 d	5 d	5 d	5 d	5 d	9 d	9 d	9 d	9 d
2 d	2 d	2 d	2 d	6 d	6 d	6 d	6 d	10 d	10 d	10 d	10 d
3 d	3 d	3 d	3 d	7 d	7 d	7 d	7 d	10 d	11 d	11 d	11 d
4 d	4 d	4 d	4 d	8 d	8 d	8 d	8 d	11 d	12 d	12 d	12 d

Table 2.1.3: Example of a plate set up during the qRT-PCR experiment. Numbers represent the way the duplo was positioned in the plate. C means control (well watered) and d means drought (no water). So the same genotypes from the control block as from the drought induced block were taken up in the same plate. The duplo's are technical repeats.

The qRT-PCR was run on a iCycler MyiQ™ Single Color Real Time PCR Detection system from Bio-Rad Laboratories. SYBR-green supermix of Bio-Rad Laboratories was used according to manufacturers protocol. The profile used for the qRT-PCR is explained in table 2.1.4.

Cycle nr.	Step nr.	Temperature in °C	Time
1	1	95	30 seconds
2	1	95	3 minutes
3	1	95	15 seconds
40x	2	60	1 minute
4	1	95	1 minute
5	1	65	1 minute
6	1	65	10 seconds
61x			

Table 2.1.4: Profile used during qRT-PCR

After running data files were stored on the personal computer for further processing and analysis.

2.6 Data analysis

Bio-Rad MyIQ software
Data of the qRT-PCR has been analyzed with the Bio-Rad iQ5 2.0 Standard Edition Optical System Software V2.0.148.060623.
In the Excel file a separation was made between the C and E parents, CxE progeny and the tetraploid cultivars.

Statistical analysis
Statistical analysis on the expression data of the CxE progeny was done with the GenStat 11th edition software.

3. Results

3.1 Invertase and sucrose synthase sequences in NCBI and EST databases and alignments

3.1.1 Invertases

Public nucleotide and protein databases were searched for invertases. The search was focused on sequences from Solanaceous species.
This resulted in mainly apoplastic invertases, but also vacuolar invertases. Sequences of the different genes were copied from the database and used for making alignments. A result of that is shown in table 3.1.1. The record of the sequence in the nucleotide database contained most of the times a link to the publication of the belonging gene.
Literature research based on these publications revealed that tomato apoplastic invertase genes *Lin5* and *Lin7* (tomato chromosome 9) are tandemly arranged; this is also true for tomato invertases *Lin6* and *Lin8* (tomato chromosome 10). Potato *InvGE* and *InvGF* are orthologues of *Lin5* and *Lin7* respectively. *InvCD111* and *InvCD141* are orthologues of *Lin8* and *Lin6* respectively, (Fridman and Zamir, 2003). In table 3.1.1 a similarity index is given for the homologues of potato and tomato. Similarities are at nucleotide level.

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gene	accession nr potato	putative function	Orthologue with	accession nr tomato	Similarity index
invGE	AJ133765	apoplastic invertase	lin5 tomato	AJ272306	90.80%
invGF	AJ133765	apoplastic invertase	lin7 tomato	AF506006	90.80%
InvCD141	Z22645	apoplastic invertase	lin6 tomato	AF506004	93.70%
InvCD111	Z21486	apoplastic invertase	lin8 tomato	AF506004	90.80%

Table 3.1.1: Similarity indices for the orthologues of potato and tomato apoplastic invertases

3.1.2 Sucrose synthase

Fu and Park published in 1995 that they found two differentially regulated classes of sucrose synthase genes, *Sus3* and *Sus4*, which were identified in potato. These genes were also found in nucleotide and protein databases. But on top of that a third potato sucrose synthase was found.
GenBank accession numbers: *Sus3* U24088
Sus4 U24087
Susy2 AY205302

Researchers that submitted the sequence did not publish about this gene. In the discussion, more is explained and reasoned about the putative function of Susy2.
Alignments resulted in similarity indices between the three sucrose synthase genes in potato. Those results show that there is a high similarity between *Sus3* and *Sus4*, but a lower similarity of these two genes when they are aligned with *Susy2* (see table 3.1.2 and picture 3.2.1).

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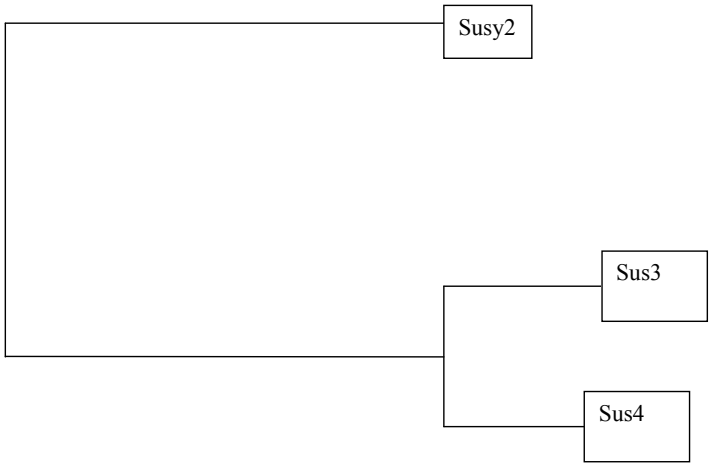
gene	accession nr potato	putative function	Similarity index	Similarity index
Susy2	AY205302	sucrose synthase		69,1%
Sus3	U24088	sucrose synthase	91,8%	
Sus4	U24087	sucrose synthase		

Table 3.1.2: Similarity indices between Sus3, Sus4 and Susy 2.

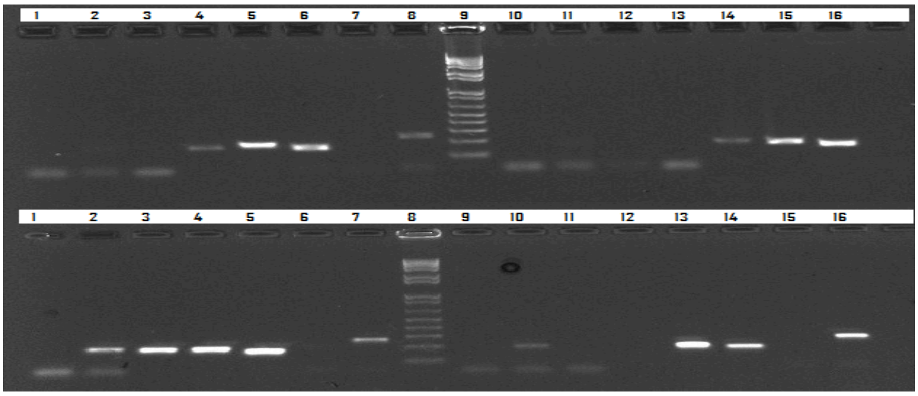


Picture 3.2.1.: Phylogenetic tree of the three sucrose synthase genes. *Sus3* and *Sus4* have a high similarity when compared to each other. *Susy2* is a potato sucrose synthase gene, but has less similarity compared to *Sus3* and *Sus4*.

3.2 Primer test

After making alignments, primers were designed (see materials and methods). An overview is given in appendix D.

The primers were tested on genomic DNA and later on cDNA. Pictures 3.2.2 and 3.2.3 show the amplification products on genomic DNA for invertase and sucrose synthase primers respectively. The primer pairs *InvGE*-F2/R2, *InvGF*-F1/R1, *InvGF*-F2/R2, *InvCD141*-F2/R2, *InvCD111*-F2/R2, *SuSy2*-F1/R1, *Sus3*-F1/R1 and *Sus4*-F1/R1 gave amplification on genomic DNA. Therefore it was decided to test these primers on cDNA in a regular PCR reaction.



Picture 3.2.2: Gel picture of invertase primers on genomic DNA. Primers are tested on parent C(lanes 1 till 8 in the upper row) and parent E (in the upper row, lanes 10, 11, 13, 14, 15, 16) and two randomly selected progeny, which are loaded in the lower row.

Upper row: 1: *InvGE*-F1/R1, 2: *InvGE*-F2/R2, 3: *InvCD141*-F1/R1, 4: *InvCD141*-F2/R2, 5: *InvGF*-F1/R1, 6: *InvGF*-F2/R2, 7: *InvCD111*-F1/R1, 8: *InvCD111*-F2/R2, 9: size marker, 10: *InvGE*-F1/R1, 11: *InvGE*-F2/R2, 12: *InvCD141*-F1/R1, 13: *InvCD141*-F1/R1, 14: *InvCD141*-F2/R2, 15: *InvGF*-F1/R1, 16: *InvGF*-F2/R2

Lower row: 1: *InvGE*-F1/R1, 2: *InvGE*-F2/R2, 3: *InvCD141*-F2/R2, 4: *InvGE*-F1/R1, 5: *InvGE*-F2/R2, 6: *InvCD111*-F1/R1, 7: *InvCD111*-F2/R2, 8: size marker, 9: *InvGE*-F1/R1, 10: *InvGE*-F2/R2, 11: *InvCD141*-F1/R1, 12: *InvCD141*-F2/R2, 13: *InvGF*-F1/R1, 14: *InvGF*-F2/R2, 15: *InvCD111*-F1/R1, 16: *InvCD111*-F2/R2.

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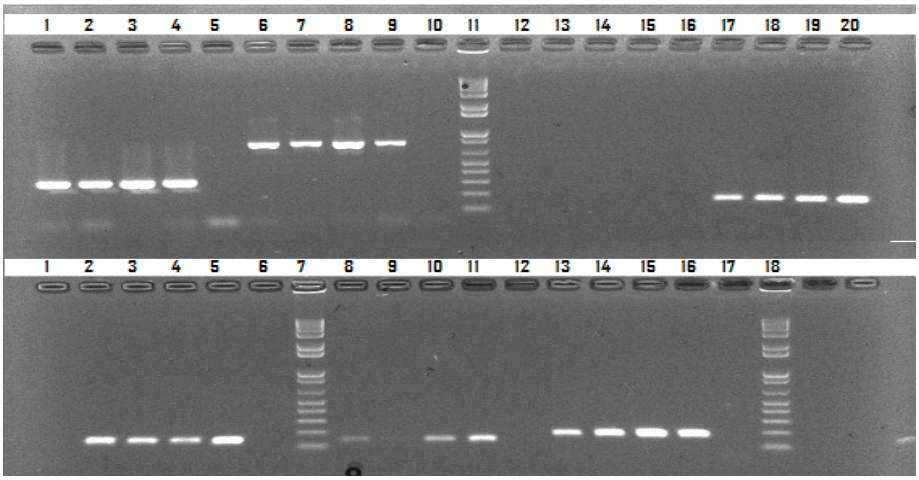
Verwijderd: InvGE-F2/R2 seems to work on the progeny. Inv CD111F1 doesnot work



Picture 3.2.3: Gel picture of sucrose synthase primers on genomic DNA. Primers that were tested on parent C can be seen in lanes: 1, 6, 12, 17 of the upper row, and lanes 2, 8 and 13 of the lower row. Results of the primer test on parent E can be seen in lanes: 2, 7, 13, 18 of the upper row, and lanes 3, 9 and 14 of the lower row. In the upper row lanes 3, 4, 8, 9, 14, 15, 19, 20 and lower row lanes 4, 5, 10, 11, 15, 16, two randomly picked samples of the CxE progeny is used.
Upper row: 1, 2, 3, 4: *SuSy2*-F1/R1. 6,7, 8, 9: *SuSy2*-F2/R2. 11: ladder. 12, 13, 14, 15: *Sus3*-F1/R1. 17, 18, 19, 20: *Sus3*-F2/R2. Lanes 5, 10 and 16 have no template
Lower row: 1(No template): *Sus3*-F2/R2. 2, 3, 4, 5: *Sus4*-F1/R1. 7: ladder. 8 , 9, 10, 11; *Sus4*-F2/R2. 13, 14, 15, 16: *InvGE*-F2/R2. 18: ladder. Lanes 6, 12, 17 have no template.

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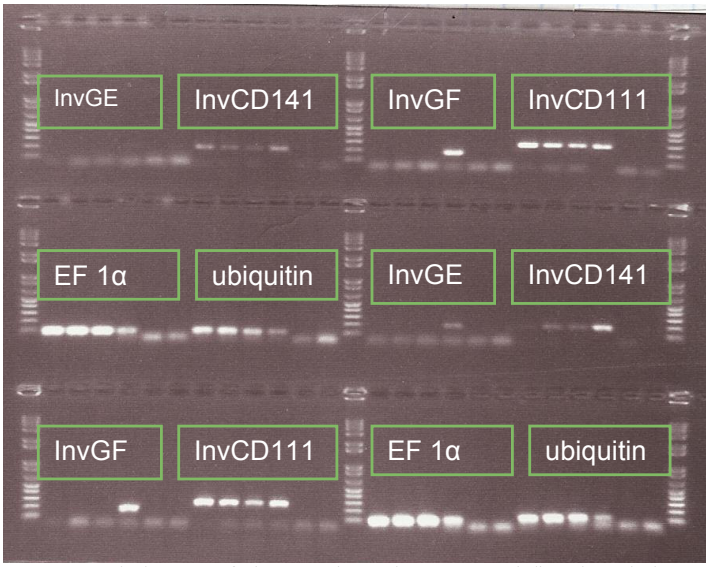
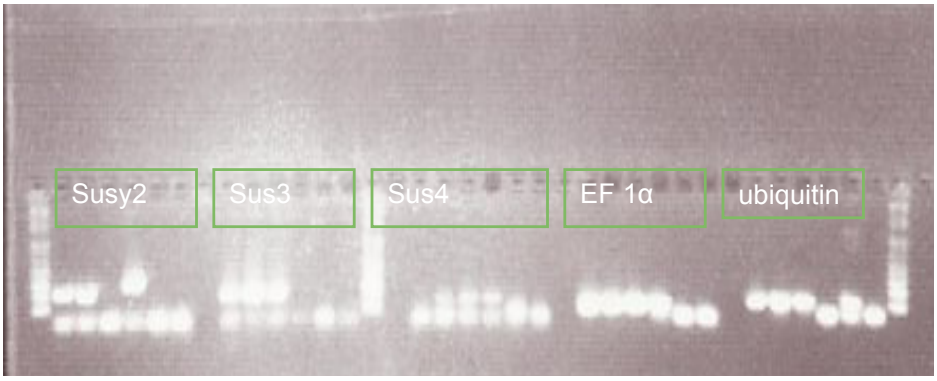


Picture 3.2.4: Check on cDNA for invertase primers. The green squares indicate the used primer set loaded per 6 lanes. Each 4th lane of a primer set has a template of parent C. Lanes 5 and 6 of each primer set are negative controls. The first 3 lanes of each primer set have template of randomly picked cDNA samples of the CxE progeny.
InvCD111-F2/R2 and InvCD141-F2/R2 gave good results. *EF 1α* was used as reference gene during qRT-PCR.



Picture 3.2.5: Check on cDNA of sucrose synthase primers. The green boxes indicate the tested primer set. Per box the first 3 lanes were used to test the primers on randomly picked cDNA samples of the CxE progeny. The fourth lane under each box has parent C as template. The last 2 lanes per box are negative controls.
Susy2-F1/R1 gave two different products, considering the product lengths. *Sus3*-F1/R1 and *Sus4*-F1/R1 gave good results, so those were accepted to use for qRT-PCR.

On cDNA of parent C and randomly picked CxE progeny only *InvCD141*-F2/R2, *InvCD111*-F2/R2, *Sus3*-F1/R1 and *Sus4*-F1/R1 gave a positive result (see pictures 3.2.4 for invertase and 3.2.5 for sucrose synthase). Because of time limits it was decided not to improve the primers of *InvGE*, *InvGF* and *Susy2*. *InvGE*-F2/R2 and *InvGF*-F2/R2 did not result in an amplification on the cDNA samples used. *Susy2*-F1/R1 gave amplifications with different product lengths. So hence with this it was decided to execute qRT-PCR only with these primer sets.

3.3 Gene expression

Performing qRT-PCR resulted in a dataset that contained the expression levels of each genotype within every treatment. The expression levels are calculated by the software that uses the ΔΔCt method. With this method a normalized expression of each sample is calculated.
As described in materials and methods the samples in the qRT-PCR plate had a technical duplicate. Good Ct values were values that differed less than 0.5 between the duplicates. This criterion was met throughout in the experiments with primers for *InvCD141*, *InvCD111* and *Sus3*. *Sus4* had more variation between the duplicates, sometimes 0.8 till 1.6. After running all the samples 56 samples were repeated, because these samples did not have amplification or a too big range between the duplicates. After repeating the 56 samples, still 27 samples did not result in data. Overall it can be said that in the control group 1 or 2 samples were missing for each primer pair. Each primer pair of all the four genes tested, were not able to amplify around 5 samples in the drought stressed group.
qRT-PCR measures fluorescence of a dye that is fixed between a double-stranded product. The qRT-PCR machine runs cycles, just as in a regular PCR machine. However in this case the fluorescence is measured. After every cycle the amplification of a region of interest increases quadratic. If the intensity of the fluorescence exceeds a threshold the number of cycles is counted. The cycle where it passes the threshold is numbered as the Ct-value. If there is a lot of template the amount of amplicon increases faster than when there is less input of template. So when a gene is less expressed, less mRNA synthesized to cDNA is present, more cycles are needed to exceed the threshold. A reference gene is used to correct for the input of cDNA and to calculate the expression of the target gene. In this experiment *EF 1α* was the used reference gene, which is normally a housekeeping gene in the plant.
Table 3.1.3 shows an impression of the Ct-values of the different primer pairs that were measured during the qRT-PCR experiment.

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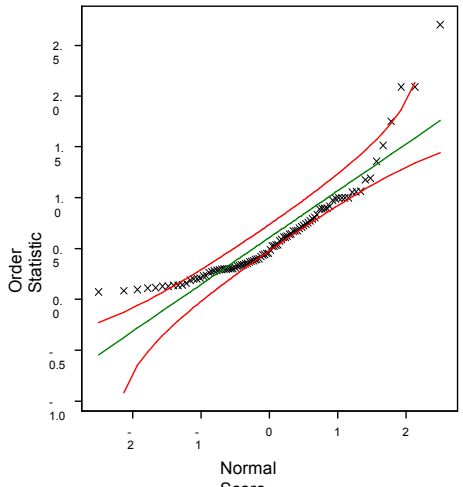
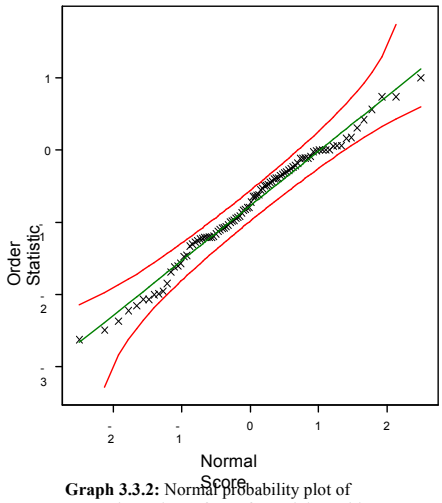
Gene (condition)	Average Ct-value of 8 mean Ct-values	Gene (condition)	Average Ct-value of 8 mean Ct-values
***InvCD141* (control)**	28	*EF 1α*	**18**
***InvCD141* (drought)**	32	*EF 1α*	**21**
***InvCD111* (control)**	24	*EF 1α*	**19**
***InvCD111* (drought)**	26	*EF 1α*	**19**
***Sus3* (control)**	25	*EF 1α*	**18**
***Sus3* (drought)**	26	*EF 1α*	**19**
***Sus4* (control)**	29	*EF 1α*	**18**
***Sus4* (drought)**	**31**	***EF 1α***	**19**

Table 3.1.3: General overview that resembles the Ct-values during the qRT-PCR. The higher the Ct-values, the lower the expression. Ct-values increase under drought *InvCD141* and *InvCD111*. Ct-values of the sucrose synthase do not change to much. Most important for a reference gene is that it has a stable level of expression under the different conditions. In this experiment the levels of *EF 1α* stayed fairly equal.

During the execution of statistical analysis it turned out that the raw expression values, directly received from the qRT-PCR software were not normally distributed. Therefore the raw data were transformed with a natural logarithm. This had a positive effect on the data being normally distributed. A graphical example is given in graphs 3.3.1 and 3.3.2.



Graph 3.3.1: Normal probability plot of expression values of non transformed data.



Graph 3.3.2: Normal probability plot of expression values of transformed data with a natural logarithm.

With the transformed data a two sample t-test and a correlation analysis were done. The results of these two forms of analyses are shown in tables 3.1.9, 3.1.10.

Gene	*Treatment*	*Mean (ln expression)*	*Regulation (control vs drought)*	**Significance**
InvCD141	Control	0.6067	Down	P=< 0.001
	Drought	0.3128		
InvCD111	Control	0.7572	Down	P=< 0.001
	Drought	0.2956		
Sus3	Control	0.7906	Up	P= 0.636
	Drought	1.1030		
Sus4	Control	0.6429	Up	P= 0.266
	Drought	0.9240		

Table 3.1.9: Means of expression values and significances based on a two sample t-test with an alpha-level of 0.05.

It can be concluded that *InvCD141* and *InvCD111* are both less expressed under drought conditions than under control conditions, see also table 3.1.9. Expression of *Sus3* and *Sus4* shows a trend of up-regulation during drought. This is not significant though.

Correlations

InvCD141 control								
InvCD141 drought	0.4131***							
InvCD111 control	0.0614	-0.0245						
InvCD111 drought	0.0885	0.2800*	0.3503**					
Sus3 control	0.1021	-0.0661	0.1943	0.0255				
Sus3 drought	-0.0286	-0.3448**	0.0880	0.0616	0.1936			
Sus4 control	0.0468	-0.0977	0.1398	0.1816	-0.0157	0.2473*		
Sus4 drought	0.0894	-0.1084	0.0221	0.1030	-0.1034	0.3487**	0.7713****	
	InvCD141 Control	InvCD141 drought	InvCD111 control	InvCD111 drought	Sus3 control	Sus3 drought	Sus4 control	Sus4 drougl

Number of observations: 82
*=P<0.05; **=P<0.01; ***=P<0.001; ****=P<0.0001

Table 3.1.10: Correlations between different genes and treatments

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Table 3.1.10 shows that there are significant correlations between *InvCD141* drought and *InvCD141* control, *InvCD111* drought and *InvCD111* drought, *Sus3* drought and *InvCD141* drought, *InvCD111* drought and *InvCD111* control, *Sus4* control and *Sus3* drought, Sus4 drought and *Sus3* drought, *Sus4* drought and *Sus4* control.
All the correlations are positive except for the correlation between *Sus3* drought and *InvCD141* drought. *Sus3* drought and *InvCD141* drought are negatively correlated.

3.4 Mapping of eQTLs

With the data from the 94 CxE progeny it was possible to do a QTL analysis, which in this case is done and kindly provided by Anitha Kumari.
Table 3.1.11 shows the outcome of the QTL analysis with the expression data. From the table it can be seen that the invertases locate on linkage group 10. *Sus3* control has a QTL on linkage group 2, where *Sus4* control and drought have an eQTL on linkage group 12.

QTL analysis: LOD threshold 4.0			
	Linkage group	LOD	% variation explained
InvCD111 control	10	7.61	31.4
InvCD141 stress	10	3.5	14.9
Sus3 control	2	4.21	19
Sus4 control	12	19.8	63
Sus4 stress	12	8.64	36

Table 3.1.11: Outcome of QTL analysis with the expression data derived from the qRT-PCR described in this report.

4. Discussion

Searching in the NCBI nucleotide and protein databases resulted in the invertase genes *InvGE*, *InvCD141*, *InvGF* and *InvCD111* of potato and respectively *lin5*, *lin6*, *lin7* and *lin8* as their orthologues in tomato. *Susy2*, *Sus3* and *Sus4* are the sucrose synthase genes in potato.

4.1 Invertases

Literature research revealed that the *InvGF* protein was only detected in mature flowers and flower buds and not in the source and sink leaves. This is different in comparison with *InvGE*, which was not only expressed floral tissue, but also in leaf tissue, (Maddison et. al., 1999). The tomato orthologs of these genes, *lin5* is primarily expressed in the ovary, developing fruits, stamen and pollen, *lin7* is expressed in the stamen and pollen (Fridman, et. al., 2003), (Godt and Roitsch, 1997).

Hedley et. al., reported in 1994 that *InvCD141* was expressed in source leaf, stem, and a little bit in tubers. *InvCD111* was expressed in the sink leaf and at a minor level in source leaf and stem. According to Hedley et. al., 1994, the potato genes for these apoplastic invertases may be developmental and tissue specific control with a switch in their expression during leaf maturation. In our experiment we do not see this switch during drought stress. Both genes are downregulated during drought stress and have positive correlations under control and as well under drought conditions.

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In tomato, *lin6* is expressed in sink tissues, like seedling roots, flower buds and tumors. Especially in situations that need a high carbohydrate supply. The function of lin8 was not so clear, because no amplification was found. However in the experiments described in this report, a product of the potato homologue was found. For tomato it was proven that apoplastic invertases play a very important role in the source-to-sink regulation within plants, (Godt and Roitsch, 1997).

4.2 Sucrose synthase

Fu and Park (1995) found two differentially regulated sucrose synthase genes, *Sus3* and *Sus4*, in potato.

The potato *Sus3* gene is expressed at the highest levels in stems and roots and appears to provide a vascular function of sucrose synthase. *Sus4* genes are expressed primarily in the storage and vascular tissue of tubers and appear to facilitate sink function.

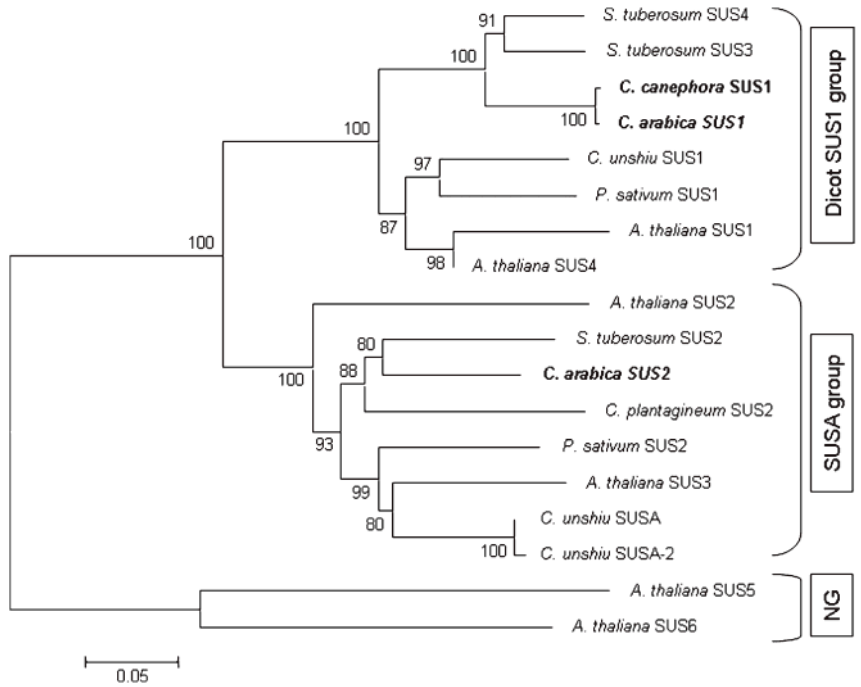
With BLAST analysis a third sucrose synthase (AY205302) was identified, submitted by Loureiro, et. al., 2002. No other literature reference was found related this gene.

A BLAST-search with this sequence resulted in hits with other sucrose synthases, indicating that *Susy2* is very likely a sucrose synthase.

Sus3 and *Sus4* are highly similar but *Susy2* was clearly different. It has 69,1% similarity index with *Sus3* and *Sus4* at the protein level, where the latter two have a 91,8% similarity.

To have a look at the putative similarities and differences between *Susy2* and *Sus3*/*Sus4*, another round of BLAST-searches was executed. Separate BLAST-searches with *Susy2*, *Sus3* and *Sus4* resulted in a few common hits that were listed after each search. These common hits were *Coffea canephora* sucrose synthase (*SS2*) (DQ834312), *Coffea arabica* sucrose synthase (*sus1* gene) (AM087674).

Geromel et. al., 2006 compared *sus1* and *sus2* genes in *Coffea arabica*, with other sucrose synthase genes in other crops. Baud et. al., 2004 stated that there are 6 sucrose synthase genes in Arabidopsis. It is also concluded that the isoforms are arranged in 3 pairs. These are *Sus1*/*Sus4*, *Sus2*/*Sus3* and *Sus5*/*Sus6*. Geromel, 2006 constructed a phylogenetic tree of the sucrose synthases. (picture 3.2.1). In this tree *Susy* 2 is called *Sus2*. The potato sucrose synthase gene (*Susy2*, AY205302) belongs to the SUSA group with the *Sus2* gene in *C. arabica* and *Sus2*/*Sus3* in *A. thaliana*.



Picture 3.2.1: Comparison of deduced amino acid sequences of plant sucrose synthase. The phylogenetic dendrogram was generated by multi alignment using CLUSTALW based on identity and the Neighbor–Joining method with Poisson correction. Bootstrap values are shown as percentages. EMBL database accession numbers of SUS proteins are as follows: Arabidopsis thaliana *SUS1* (NP197583), *SUS2* (NP199730), *SUS3* (NP192137), *SUS4* (AAK59464), *SUS5* (NP198534), *SUS6* (NP177480); Citrus unshiu *SUSA* (BAA88904), *SUSA-2* (BAA88981), *SUS1* (BAA88905); Coffea arabica *SUS1* (CAJ32596), *SUS2* (CAJ32597); Coffea canephora *SUS1* (CAI56037); Craterostigma plantagineum *SS2* (CAB38022); Pisum sativum *SUS1* (CAA09910), *SUS2* (CAA04512); Solanum tuberosum *SUS2* (AAO67719), *SUS3* (U24088), *SUS4* (U24087). (Picture taken from Geromel, et. al., 2006)

Komatsu et al., 2002 identified different groups of sucrose synthase. There is a group of monocot Sus genes. Another group are the Sus genes that are present in dicot plants. For example *Sus3* and *Sus4* in potato and Arabidopsis thaliana *Sus1* and *Sus4* belong to this group. The SUSA group contains the *Coffea arabica Sus2*, *Citrus unshiu Sus1* and *Sus2*, *Arabidopsis thaliana Sus2* and

Sus3, and the potato *Susy2* gene. The SUSA group is similar to the monocot Sus genes, when the exon structure is analyzed, (Komatsu et al., 2002).
So far nothing is published about the function and expression of *Susy2*. *Susy2* may be expressed at a later developmental stage of a sink organ. In *A. thaliana*, *Citrus unshiu* and *Coffea arabica* the sucrose synthase genes of the SUSA group are expressed at high levels during the later stages of seed and fruit development (Baud et. al., 2004, Komatsu et. al., 2002, Geromel et al., 2006). According to Komatsu et. al., 2002, *CitSUSA* had higher levels of mRNA transcripts in the mature source leaves and lower in the young sink leaves. In *A. thaliana* the *Atsus3* gene had a high expression during water stress (Baud et. al., 2004). As stated before *Susy2* of potato belongs to the same group as *CitSUSA* and *Atsus3*. This indicates the putative function and importance of the potato *Susy2* gene during plant development and response to drought stress.

4.3 Conclusions from the qRT-PCR experiment

Conclusions of the qRT-PCR results are that invertase genes *InvCD141* and *InvCD111* are down-regulated during drought in young leaves after 4 days (initial drought stage). *Sus3* and *Sus4* were not significantly changed in their expression by drought stress. However there is a trend of up-regulation of the latter two genes.
Invertases under control and drought conditions are positively correlated.
Sucrose synthase under control and drought are positively correlated.
But, *Sus3* is negatively correlated with *InvCD141* under drought.

4.4 Expression profiles of the screened genes

The qRT-PCR experiment revealed that over the whole population invertase genes *InvCD141* and *InvCD111* are down regulated during drought in young fully expanded leaves. In literature it is described that invertases are often upregulated due to biotic stress, increased sink strength and plant development (Sturm and Tang, 1999, Roitsch, 1999). It appears that when tissues are in high demand of energy, apoplastic invertase activity increases.

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The decrease in apoplastic invertase expression in response to drought in our experiments may indicate that in the examined leaves the demand for energy-rich sugars decreases. This is in agreement with observations of stunted/decreased growth in conditions with low water supply.
A reason for the results in this experiment could be that apoplastic invertases are down regulated to prevent negative feedback inhibition of the photosynthetic system. More hexoses in the cell means that photosynthesis is decreasing (Koch, 1996). On the other hand this is the opposite with what is seen with the expression of sucrose synthase. Sucrose synthase expression is negatively correlated under drought with invertase expression under drought. So it might be that sucrose synthase is taking over the function of apoplastic invertase. A down regulation of invertase and a trend of up regulation of sucrose synthase is seen. This means that hexoses are not entering the cell anymore through the apoplast, but that the sucrose in the cytosol is split in hexoses by the sucrose synthase. This might be done because of the initiation of starch synthesis.
In this experiment only the gene expression is measured. To have a better view on the effect of this expression it is recommended to measure the activity of the enzymes and the levels of hexoses and sucrose.

Sus3 and *Sus4* are not expressed in leaves according to Fu and Park, 1995. In this experiment expression of both genes is detected in leaves and therefore opposite to earlier publications. Different between the two experiments is that Fu and Park used just one cultivar. As explained in

the material and methods chapter the CxE population, used in this gene expression study, had a size of 94 genotypes added with the C and E parents and 12 tetraploid cultivars. Among the 94 progeny of CxE the expression levels of *Sus3* differed from 0.0220 to 2.789 under control conditions. *Sus4* expression levels differed from 0.001 to 3.116 under control conditions. Maybe the fact that the cultivar used by Fu and Park did not have any expression of the sucrose synthase in tissues they tested was a genotype-specific observation that should not be generalized.

4.5 Mapping the results of the expression profiles

The expression profiles of the genes does not always map to the same position as the actual genes in other gene studies. The expression profile of invertase genes map on linkage group 10, a location where the invertase genes are also mapped in other studies (Chen et. al., 2001) and (Li et. al., 2008). The expression profiles of *Sus3* mapped on linkage group 2 and *Sus4* on group 12. The *Sus4* gene was mapped on linkage group 12, colocalizing with the expression profiles. *Sus3* maps on linkage group 7 (Chen et. al., 2001) and (Li et. al., 2008), which is a different position than the eQTL calculated in this experiment.
This may indicate that a factor that regulates expression of *Sus3* is positioned on linkage group 2 and that it is interesting to study that factor in further research.

4.6 Change names of potato apoplastic invertase genes

Searches in the nucleotide and protein databases produced sequences of four apoplastic invertase genes and their tomato orthologues. The tomato genes are named *lin5*, *lin6*, *lin7* and *lin8*. The tomato genes are orthologues with *InvGE*, *InvCD141*, *InvGF* and *InvCD111* respectively. Because it concerns one group of genes it would be much clearer to use the same names as in tomato. In table 4.4.1 the current names of the potato and tomato genes are shown. The third column gives the proposed name for the potato apoplastic invertase genes.
The name is chosen for several reasons. “St” codes for *Solanum tuberosum* and “in” codes for invertase, the numbers indicate the orthologous relation with the tomato apoplastic invertase.

Gene name potato	Gene name tomato	Proposed gene name potato
InvGE	Lin5	Stin5
InvGF	Lin7	Stin7
InvCD141	Lin6	Stin6
InvCD111	Lin8	Stin8

Table 4.4.1: Proposed names of the potato apoplastic invertase genes.

4.7 Recommendations for further research

PCR reactions on genomic DNA resulted in amplification of all the genes discussed in this report. Only primers of *InvCD141*, *InvCD111*, *Sus3* and *Sus4* were able to amplify on cDNA and were subsequently used for qRT-PCR. This means that there are no expression profiles of *InvGE*, *InvGF* and *Susy2*.
During this experiment young source leaves were used. Literature studies indicate that *InvGF* may only be expressed in floral tissues. *InvGE* is primarily expressed in floral tissue, but also in leaves (Maddison et. al., 1999). Maybe *InvGE* was not expressed in the young source leaves used

in this study. This indicates that the choice of tissues, which are used in expression profile studies like this, is very important. Not only during primer testing, but also from a physiological point of view.
In the greenhouse experiment also samples of other tissues are taken, like shoots, roots, tubers and on different time points. It is recommended to do qRT-PCR experiments on the different tissues and samples picked at different time points. Expression of genes which are not detected in this experiment might be expressed in other tissues or at other stages of plant development. Doing a qRT-PCR experiment on for example tubers, may also give a better idea of the expression of invertase and sucrose synthase in sink tissues. Studying the samples of other time points might be especially interesting to see if there are any changes in expression. Samples used in this experiment were picked after 4 days of stress inducement and samples at a more severe level of stress need to be studied.
Also primer design is an important factor in not getting an expression profile. Especially *Susy2* was not examined, because in the primer check on cDNA this primer set gave different product lengths, see also picture 3.2.5. This resulted in skipping *Susy2* in the qRT-PCR experiment. So in future research special attention has to go to primer design of *Susy2*.

From this experiment it is concluded that invertase was down regulated under drought stress and sucrose synthase had a trend of up regulation. This is only the expression of the genes that code for the enzymes invertase and sucrose synthase. It is also interesting to measure the activity of these enzymes, because this gives insight in the actual effect of the up or down regulation of the gene.

Leaf samples used in this experiment were picked from plants that were grown under greenhouse conditions. Obviously in a greenhouse the environmental conditions are more constant and controllable compared to open field situations. Potatoes are mostly grown in the open field and therefore it is good to evaluate the gene expression profiles under these conditions.

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Appendices

Appendix A: list of used CxE genotypes

Appendix B: position of the isolated RNA in the different plates

Appendix C: concentrations of the isolated RNA

Appendix D: overview of designed primers

Appendix A: list of used (CxE) genotypes

017	632		
069	633	712	RH-parent
072	635	714	Bildtstar
084	636	715	Monalisa
110	639	717	Desiree
141	642	718	Biogold
145	648	721	Première
155	651	722	Bintje
164	653	723	
165	656	724	SH- parent
171	658	726	Mondial
195	659	732	Nicola
196	660	733	R. Burbank
202	663	736	Mozart
218	664	738	
222	666	740	E-parent
232	667	746	E-parent
233	668	747	E-parent
276	669	752	C-parent
277	673	755	C-parent
350	674	756	C-parent
447	675	761	
602	680	765	
603	681	769	
604	685	778	
605	688	782	
607	689	785	
608	694		
609	696		
615	697		
624	698		
628	701		
630	702		
631	709		

Appendix B: position of the isolated RNA in the different plates

4A control	1	2	3	4	5	6	7	8	9	10	11	12
A	680	697	664	RH-parent	E-parent	Bildtstar	171	702	714	084	155	746
B	632	722	718	756	607	Monalisa	715	Desiree	164	769	659	642
C	277	E-parent	782	724	624	648	785	673	447	738	604	663
D	110	608	195	635	688	202	Biogold	723	681	732	726	658
E	696	602	668	736	069	017	651	674	653	733	740	701
F	072	709	Première	628	Bintje	712	SH-parent	145	694	609	765	232
G	Mondial	Nicola	165	218	Russet Burbank	603	667	747	689	778	Mozart	639
H	605	717	E-parent	633	615	685	675	698	C-parent	721	C-parent	656

3A Drought	1	2	3	4	5	6	7	8	9	10	11	12
A	726	164	736	SH-parent	667	E-parent	712	Mozart	630	651	628	Bintje
B	Biogold	694	688	Première	609	709	155	675	698	724	642	Russet Burbank
C	084	738	712	656	Nicola	165	660	632	653	669	233	702
D	636	E-parent	232	714	778	765	732	069	721	718	Desiree	624
E	781	605	747	604	202	722	631	E-parent	723	701	145	195
F	746	C-parent	659	072	017	785	674	752	648	447	639	740
G	663	218	RH-parent	350	666	Bildtstar	635	608	733	C-parent	171	697
H	633	141	717	755	Monalisa	681	664	222	696	602	769	607

Left over samples

4A control									3A stress			
4A control	1	2	3	4	5	6	7	8	9	10	11	12
A	669	276	726 3A drought							680 4Acontr	680	C-parent
B	752	636									658	689
C	666	350									196	615
D	233	141									276	668
E	196	222									756	685
F	631	C-parent									110	Mondial
G	755	761									673	603
H	630	660									761	277

Appendix C: concentrations of the isolated RNA

4 A control Gemeten concentratie RNA (ng/µl)												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	620,09	364,59	493,53	827,29	654,02	818,28	526,18	434,95	777,46	519,15	844,2	864,78
B	390,44	269,42	1018,23	396,67	336,28	540,03	505,05	608,51	252,91	641,23	625,84	543,34
C	487,13	299,43	436,8	336,14	577,65	565,99	371,98	241,91	658,82	534,94	538,69	332,09
D	383,31	925,2	386,21	861,59	640,23	361,35	325,56	455,61	285,83	365,95	373,72	606,86
E	323,63	373,76	568,16	296,63	241,62	461,91	463,37	427,72	364,95	439,06	351,03	393,01
F	702,48	499,52	474,44	410,3	416,54	463,23	559,15	755,84	354,36	272,01	800,15	427,03
G	315,45	364,31	413,24	579,36	399,33	315,74	318,73	412,66	368,77	437,74	335,12	211,44
H	375,86	231,64	438,97	265,05	380,39	241,53	439,92	402,83	319,63	508,18	391,68	455,23

Toe te voegen RNA (µl)												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1,613	2,743	2,026	1,209	1,529	1,222	1,901	2,299	1,286	1,926	1,185	1,156
B	2,561	3,712	0,982	2,521	2,974	1,852	1,980	1,643	3,954	1,560	1,598	1,840
C	2,053	3,340	2,289	2,975	1,731	1,767	2,688	4,134	1,518	1,869	1,856	3,011
D	2,609	1,081	2,589	1,161	1,562	2,767	3,072	2,195	3,499	2,733	2,676	1,648
E	3,090	2,676	1,760	3,371	4,139	2,165	2,158	2,338	2,740	2,278	2,849	2,544
F	1,424	2,002	2,108	2,437	2,401	2,159	1,788	1,323	2,822	3,676	1,250	2,342
G	3,170	2,745	2,420	1,726	2,504	3,167	3,137	2,423	2,712	2,284	2,984	4,730
H	2,661	4,317	2,278	3,773	2,629	4,140	2,273	2,482	3,129	1,968	2,553	2,197

Toe te voegen 10x Dnase 1 reaction buffer (µl)												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
B	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
C	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
D	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
E	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
F	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
G	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
H	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000

Toe te voegen Dnase 1 (µl)												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
B	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
C	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
D	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
E	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
F	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
G	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
H	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000

Toe te voegen Elution buffer (µl)												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	6,387	5,257	5,974	6,791	6,471	6,778	6,099	5,701	6,714	6,074	6,815	6,844
B	5,439	4,288	7,018	5,479	5,026	6,148	6,020	6,357	4,046	6,440	6,402	6,160
C	5,947	4,660	5,711	5,025	6,269	6,233	5,312	3,866	6,482	6,131	6,144	4,989
D	5,391	6,919	5,411	6,839	6,438	5,233	4,928	5,805	4,501	5,267	5,324	6,352
E	4,910	5,324	6,240	4,629	3,861	5,835	5,842	5,662	5,260	5,722	5,151	5,456
F	6,576	5,998	5,892	5,563	5,599	5,841	6,212	6,677	5,178	4,324	6,750	5,658
G	4,830	5,255	5,580	6,274	5,496	4,833	4,863	5,577	5,288	5,716	5,016	3,270
H	5,339	3,683	5,722	4,227	5,371	3,860	5,727	5,518	4,871	6,032	5,447	5,803

3A drought Gemeten concentratie RNA (ng/µl)												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	334,15	240,34	440,47	356,05	372,80	673,03	276,79	449,49	115,29	467,24	302,10	438,34
B	367,80	167,85	271,86	356,22	220,87	302,81	319,82	291,08	217,08	412,03	153,56	254,94
C	218,55	383,10	225,40	473,55	255,40	286,49	447,47	225,29	233,15	366,84	228,88	364,36
D	200,41	212,74	154,84	217,67	222,41	234,77	273,37	474,23	179,69	474,46	556,56	234,07
E	262,24	232,93	374,10	256,37	199,82	124,63	176,74	232,45	196,46	280,33	398,47	349,40
F	354,67	288,06	337,31	203,94	370,63	219,33	137,25	164,15	228,21	181,31	189,42	244,91
G	211,82	312,31	420,52	517,48	394,14	426,58	229,06	345,16	221,82	358,02	206,40	184,72
H	346,30	564,16	367,16	313,29	213,78	177,59	326,84	187,66	194,38	297,02	216,13	236,04

Toe te voegen RNA (µl)												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	2,993	4,161	2,270	2,809	2,682	1,486	3,613	2,225	8,674	2,140	3,310	2,281
B	2,719	5,958	3,678	2,807	4,528	3,302	3,127	3,436	4,607	2,427	6,512	3,923
C	4,576	2,610	4,437	2,112	3,915	3,491	2,235	4,439	4,289	2,726	4,369	2,745
D	4,990	4,701	6,458	4,594	4,496	4,260	3,658	2,109	5,565	2,108	1,797	4,272
E	3,813	4,293	2,673	3,901	5,005	8,024	5,658	4,302	5,090	3,567	2,510	2,862
F	2,820	3,472	2,965	4,903	2,698	4,559	7,286	6,092	4,382	5,515	5,279	4,083
G	4,721	3,202	2,378	1,932	2,537	2,344	4,366	2,897	4,508	2,793	4,845	5,414
H	2,888	1,773	2,724	3,192	4,678	5,631	3,060	5,329	5,145	3,367	4,627	4,237

Toe te voegen 10x Dnase 1 reaction buffer (µl)												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
B	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
C	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
D	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
E	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
F	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
G	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
H	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000

Toe te voegen Dnase 1 (µl)												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
B	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
C	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
D	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
E	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
F	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
G	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
H	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000

Toe te voegen Elution buffer (µl)												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	5,007	3,839	5,730	5,191	5,318	6,514	4,387	5,775	-0,674	5,860	4,690	5,719
B	5,281	2,042	4,322	5,193	3,472	4,698	4,873	4,564	3,393	5,573	1,488	4,077
C	3,424	5,390	3,563	5,888	4,085	4,509	5,765	3,561	3,711	5,274	3,631	5,255
D	3,010	3,299	1,542	3,406	3,504	3,740	4,342	5,891	2,435	5,892	6,203	3,728
E	4,187	3,707	5,327	4,099	2,995	-0,024	2,342	3,698	2,910	4,433	5,490	5,138
F	5,180	4,528	5,035	3,097	5,302	3,441	0,714	1,908	3,618	2,485	2,721	3,917
G	3,279	4,798	5,622	6,068	5,463	5,656	3,634	5,103	3,492	5,207	3,155	2,586
H	5,112	6,227	5,276	4,808	3,322	2,369	4,940	2,671	2,855	4,633	3,373	3,763

Left over samples Gemeten concentratie RNA (ng/µl)												
	3 A drought		A1 drought	E1 without RT					F2 without RT	A1 control	4 A control	
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	587,71	421,33	334,15	488,85					170,12	620,09	198,65	119,52
B	362,68	739,49									462,79	122,52
C	546,24	409,83									344,88	525,23
D	358,15	328,24									382,78	296,10
E	488,85	496,99									523,32	287,23
F	456,97	253,31									200,95	170,12
G	285,96	254,10									574,93	567,78
H	537,89	421,12									293,78	300,01

Toe te voegen RNA (µl)												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1,702	2,373	2,993	2,046					5,878	1,613	5,034	8,367
B	2,757	1,352									2,161	8,162
C	1,831	2,440									2,900	1,904
D	2,792	3,047									2,612	3,377
E	2,046	2,012									1,911	3,482
F	2,188	3,948									4,976	5,878
G	3,497	3,935									1,739	1,761
H	1,859	2,375									3,404	3,333

Toe te voegen 10x Dnase 1 reaction buffer (µl)												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
B	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
C	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
D	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
E	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
F	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
G	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
H	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000

Toe te voegen Dnase 1 (µl)												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
B	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
C	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
D	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
E	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
F	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
G	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
H	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000

Toe te voegen Elution buffer (µl)												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	6,298	5,627	5,007	5,954					2,122	6,387	2,966	-0,367
B	5,243	6,648									5,839	-0,162
C	6,169	5,560									5,100	6,096
D	5,208	4,953									5,388	4,623
E	5,954	5,988									6,089	4,518
F	5,812	4,052									3,024	2,122
G	4,503	4,065									6,261	6,239
H	6,141	5,625									4,596	4,667

Appendix D: overview of designed primers

Primers for invertases in potato.

Gene name: InvGE

Primer name	**position**	**Sequence (5′ to 3′)**	**Length (nt)**	**Tm (C[1])**	**GC%[2]**	**Expected product length (nt)**
Stin5-F1	127-150	TCAAGAGCTATTAGTGTCAAG	21	58	38.09	
Stin5-R1	331-312	AATGGATCCAATTTATCAAGTCT	23	60	30.43	204
Stin5-F2	430-451	CGGAGTAGTAGATTCCCATG	20	60	50.00	
Stin5-R2	630-609	TCCTATTACAATTCTCCAAAGC	22	60	36.36	200

Gene name: InvGF

Primer name	**position**	**Sequence (5′ to 3′)**	**Length (nt)**	**Tm (C[1])**	**GC%[2]**	**Expected product length (nt)**
Stin7-F1	1038-1058	ATTCCCTGAGGATGATAATGC	21	60	42.86	
Stin7-R1	1241-1223	TGTGCAGGTGTGATTCCTG	19	58	50.00	203
Stin7-F2	531-551	TCCATTGATTGTAGCTGATGC	21	60	42.86	
Stin7-R2	717-697	AAGTGGGTGTTTAGCCTTAAC	21	60	42.86	186

Gene name: InvCD141

Primer name	**position**	**Sequence (5′ to 3′)**	**Length (nt)**	**Tm (C[1])**	**GC%[2]**	**Expected product length (nt)**
Stin6-F1	129-149	TACTAGCCATGTTGATGTTAG	21	58	38.10	
Stin6-R1	290-270	CAAACAATATTGCCCCATATC	21	58	38.10	161
Stin6-F2	795-814	CACGTCATACAATGGCAAAG	20	58	45.00	
Stin6-R2	989-969	CTATCAAAGAATGTCTTGGAC	21	58	38.10	174

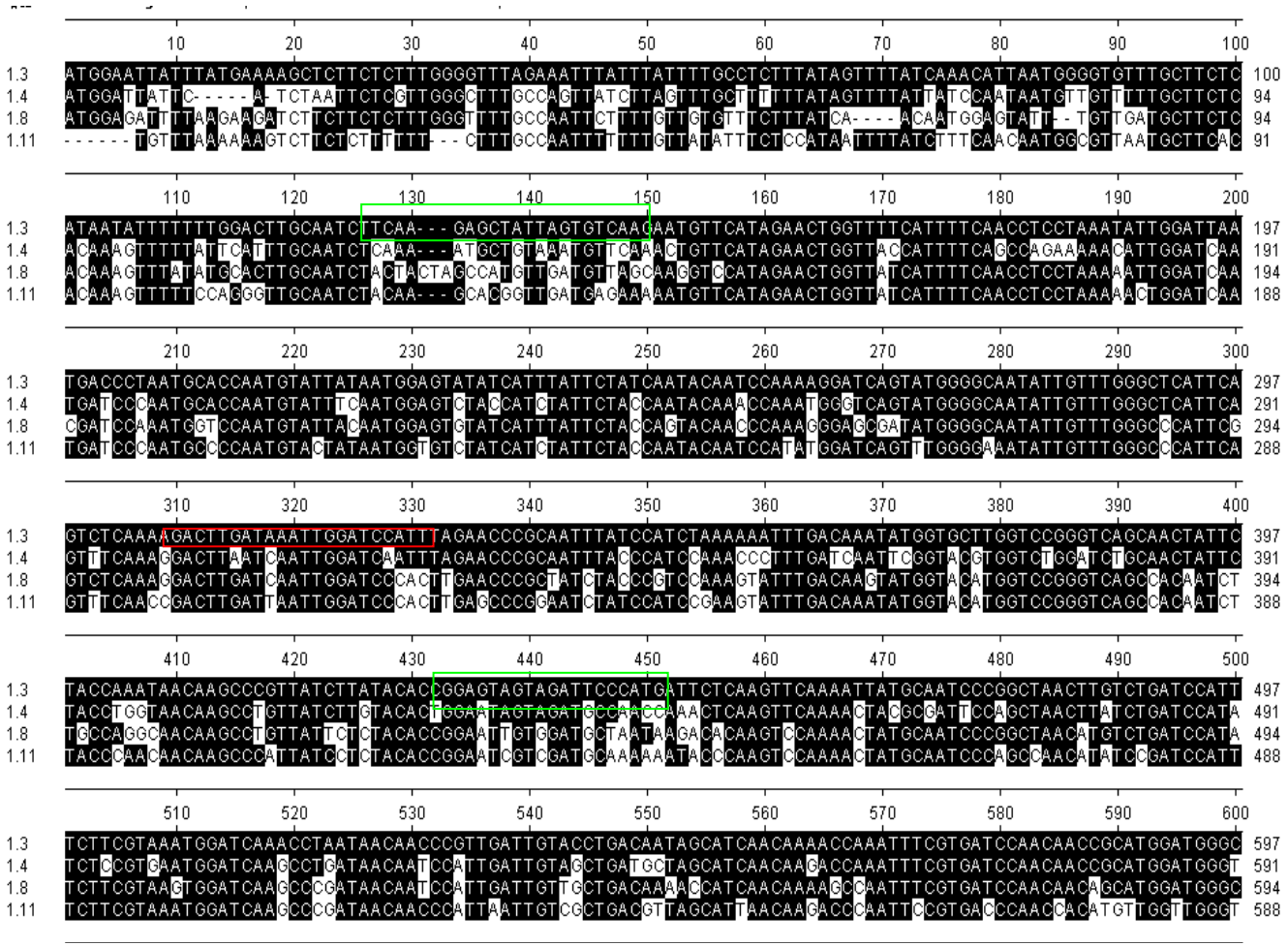
Gene name: InvCD111

Primer name	**position**	**Sequence (5′ to 3′)**	**Length (nt)**	**Tm (C[1])**	**GC%[2]**	**Expected product length (nt)**
Stin8-F1	95-115	CTTCACACAAAGTTTTTCCAG	21	58	38.10	
Stin8-R1	328-318	GGATCCAATTAATCAAGTCGG	21	60	42.86	233
Stin8-F2	790-810	TTGGATGCCTCATACAACAAG	21	60	42.86	
Stin8-R2	1064-1041	CCTTTCTTGACATCGTCATTG	21	60	42.86	271

[1]) 4*(G+C) + 2*(A+T)= Tm

[2]) ((G+C)/(total nt))*100%= GC%

1.3 = InvGE
1.4 = InvGF
1.8 = InvCD141
1.11 = InvCD111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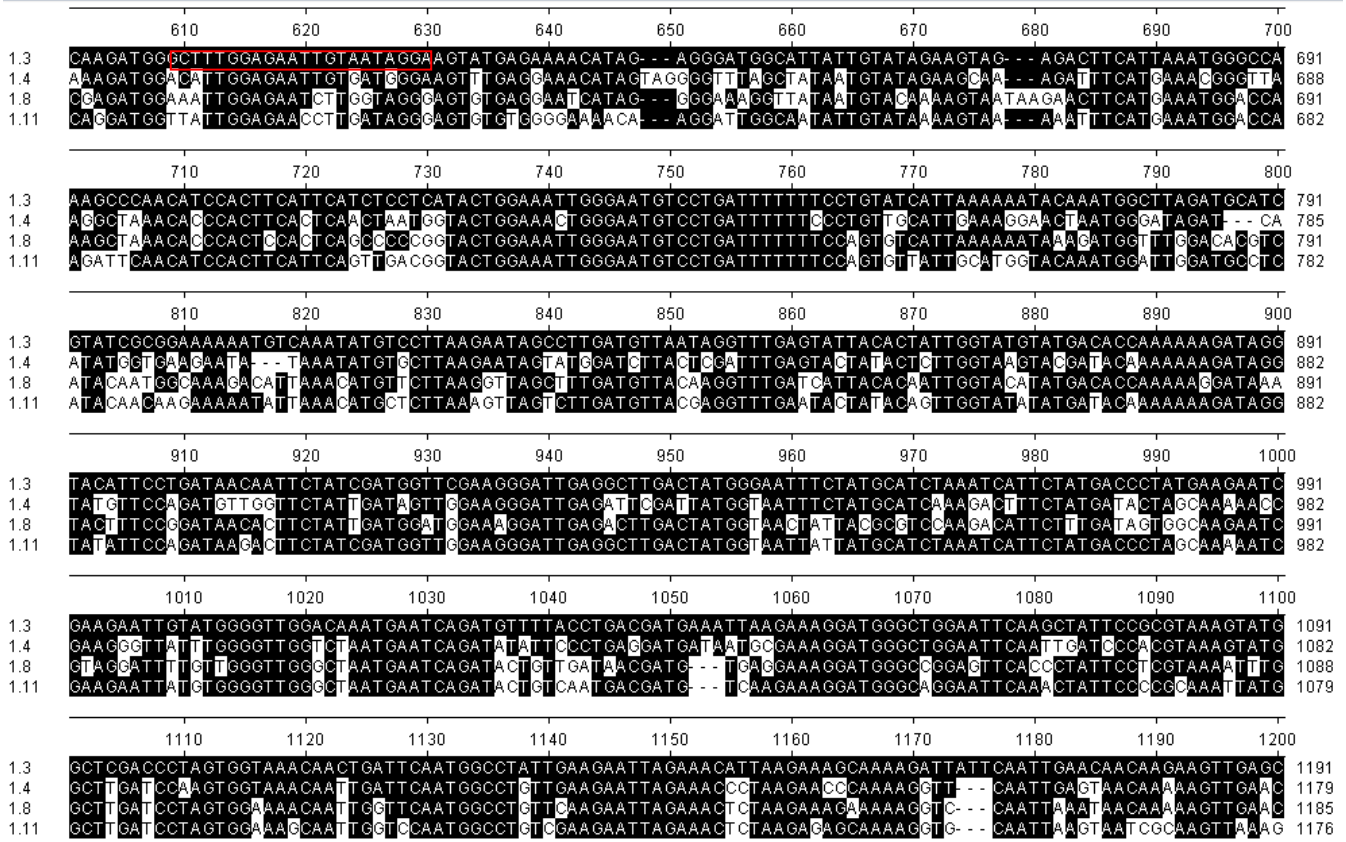
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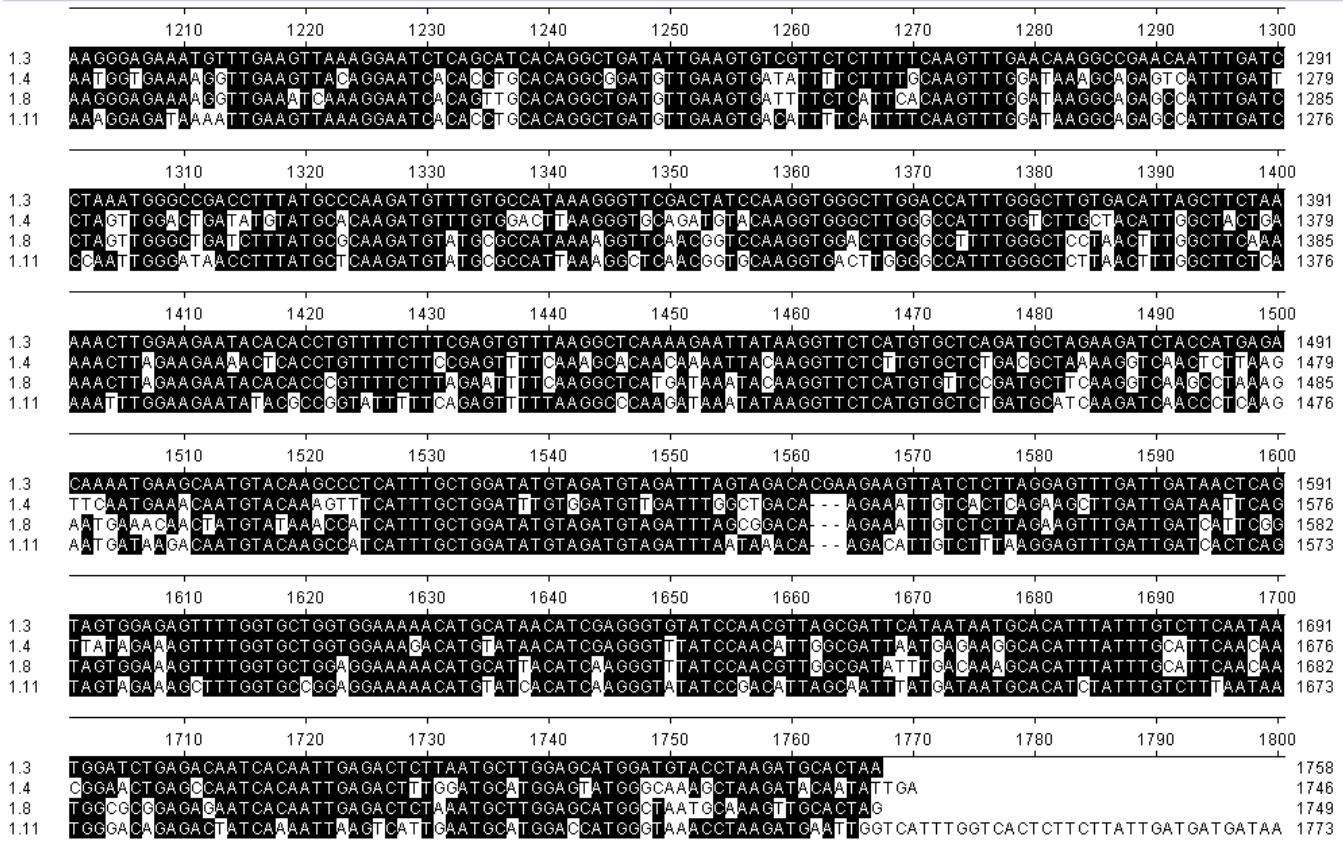
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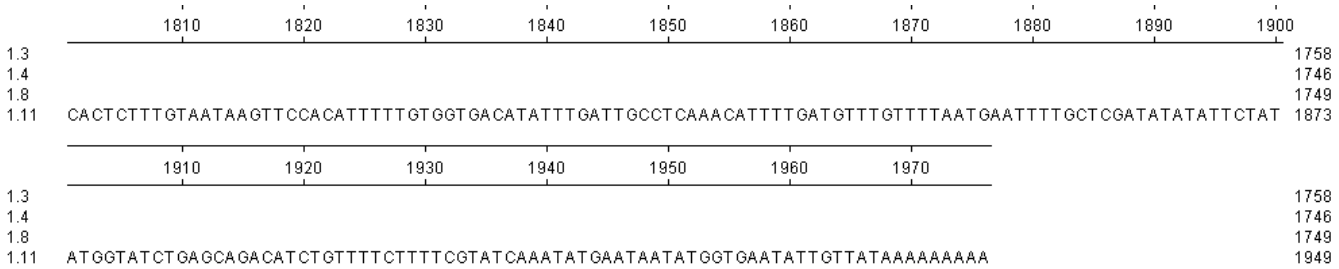

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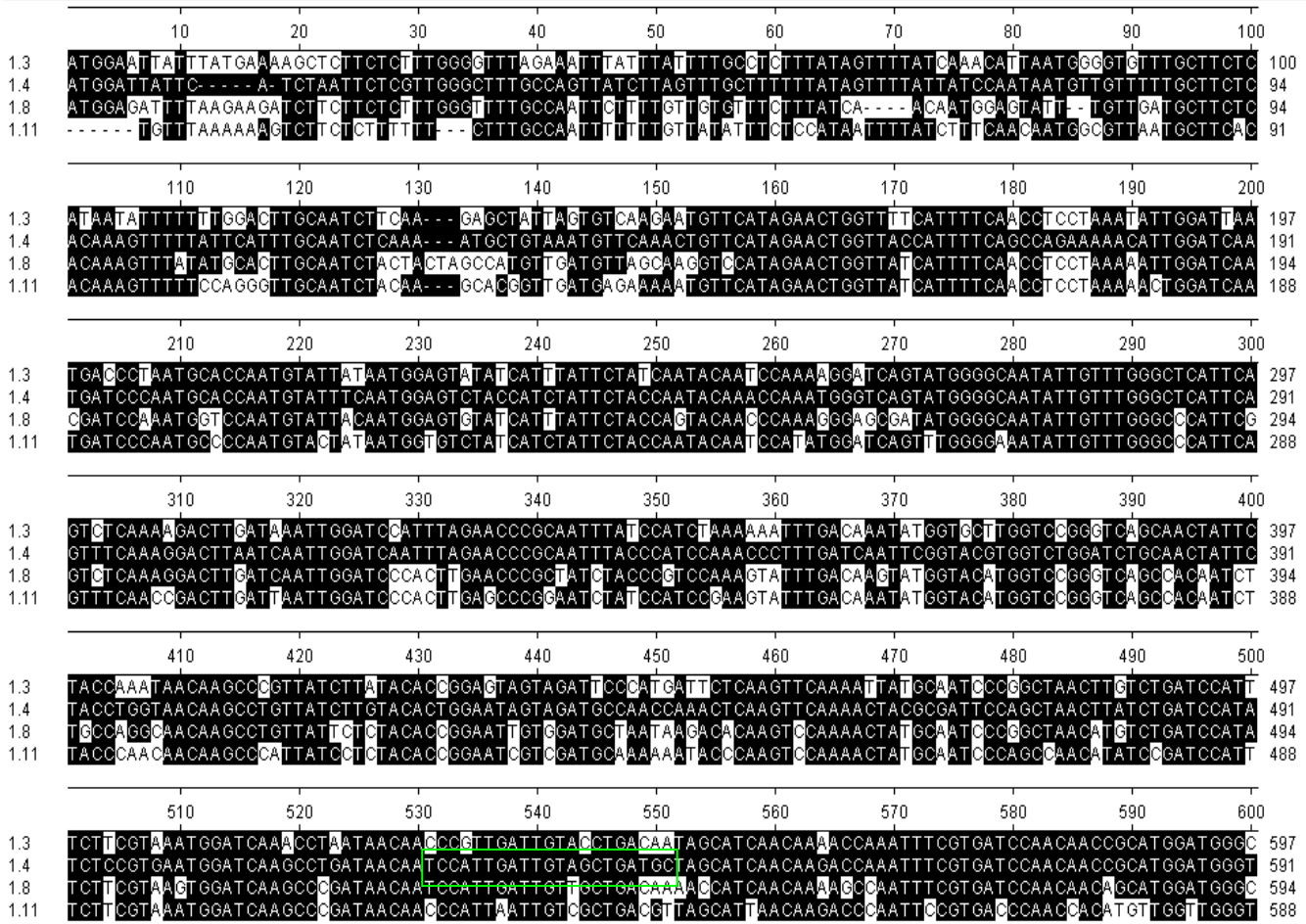
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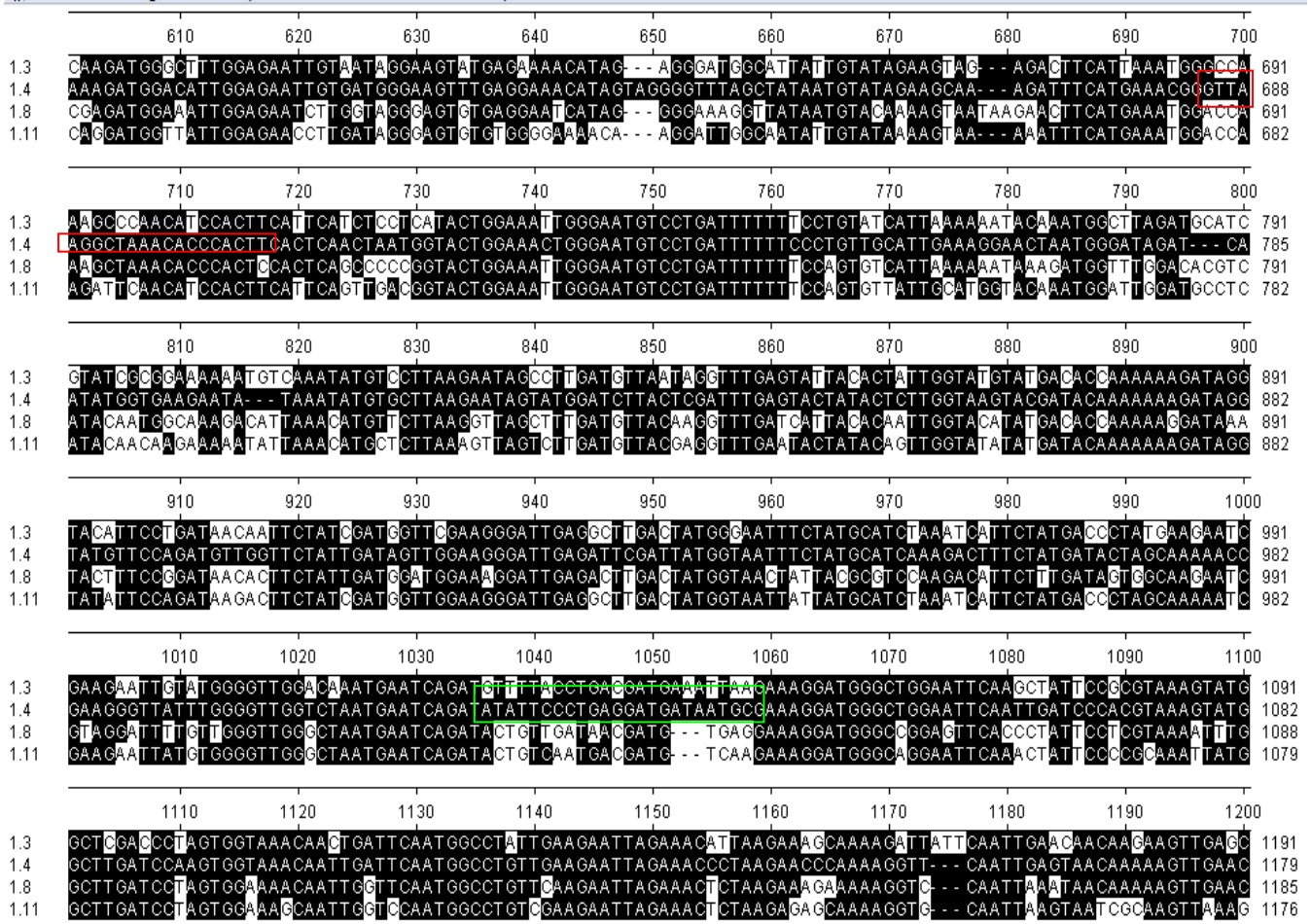
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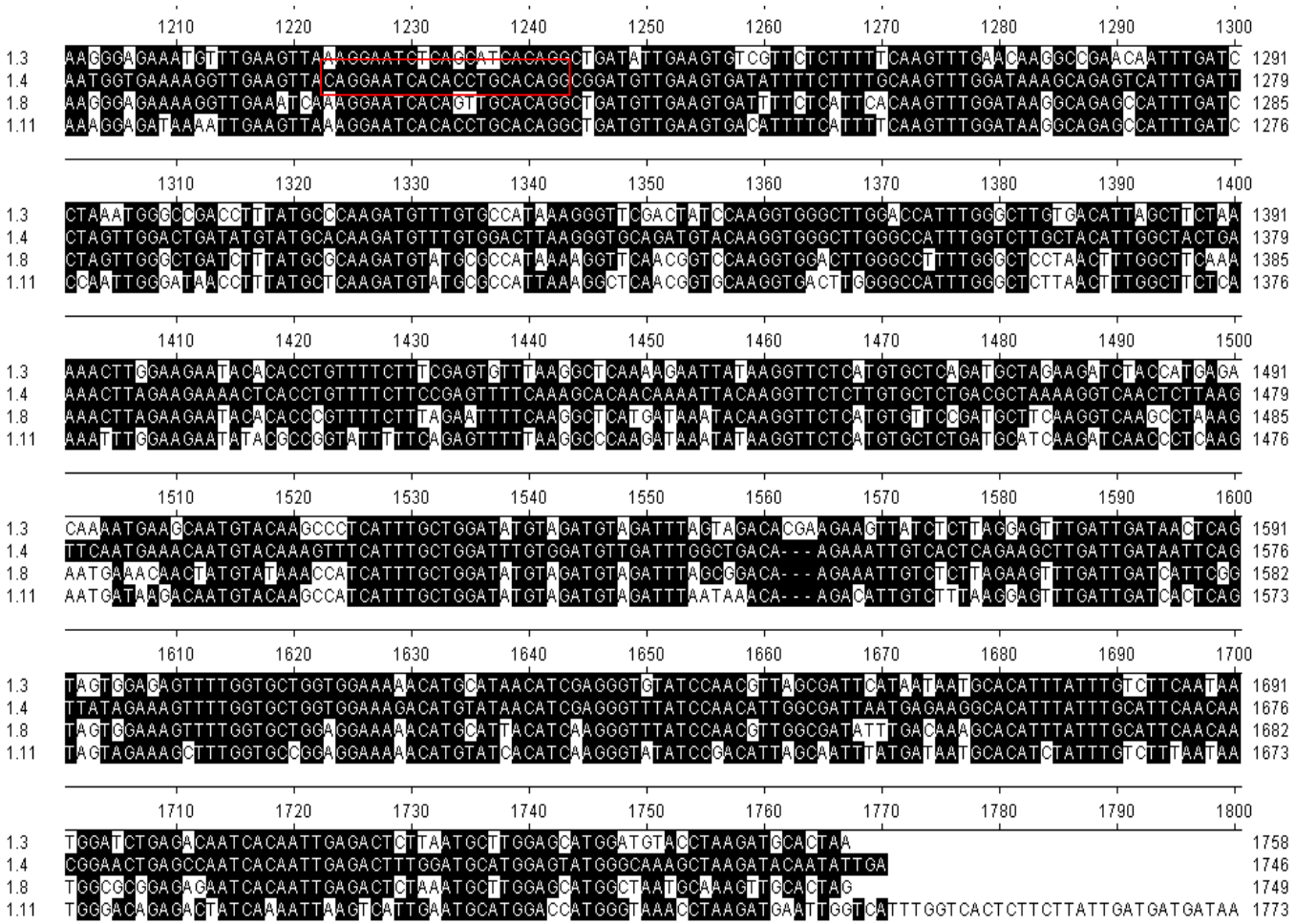
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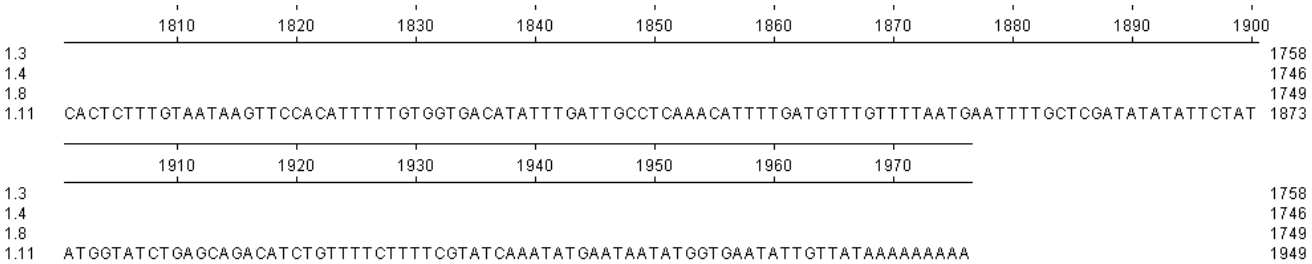
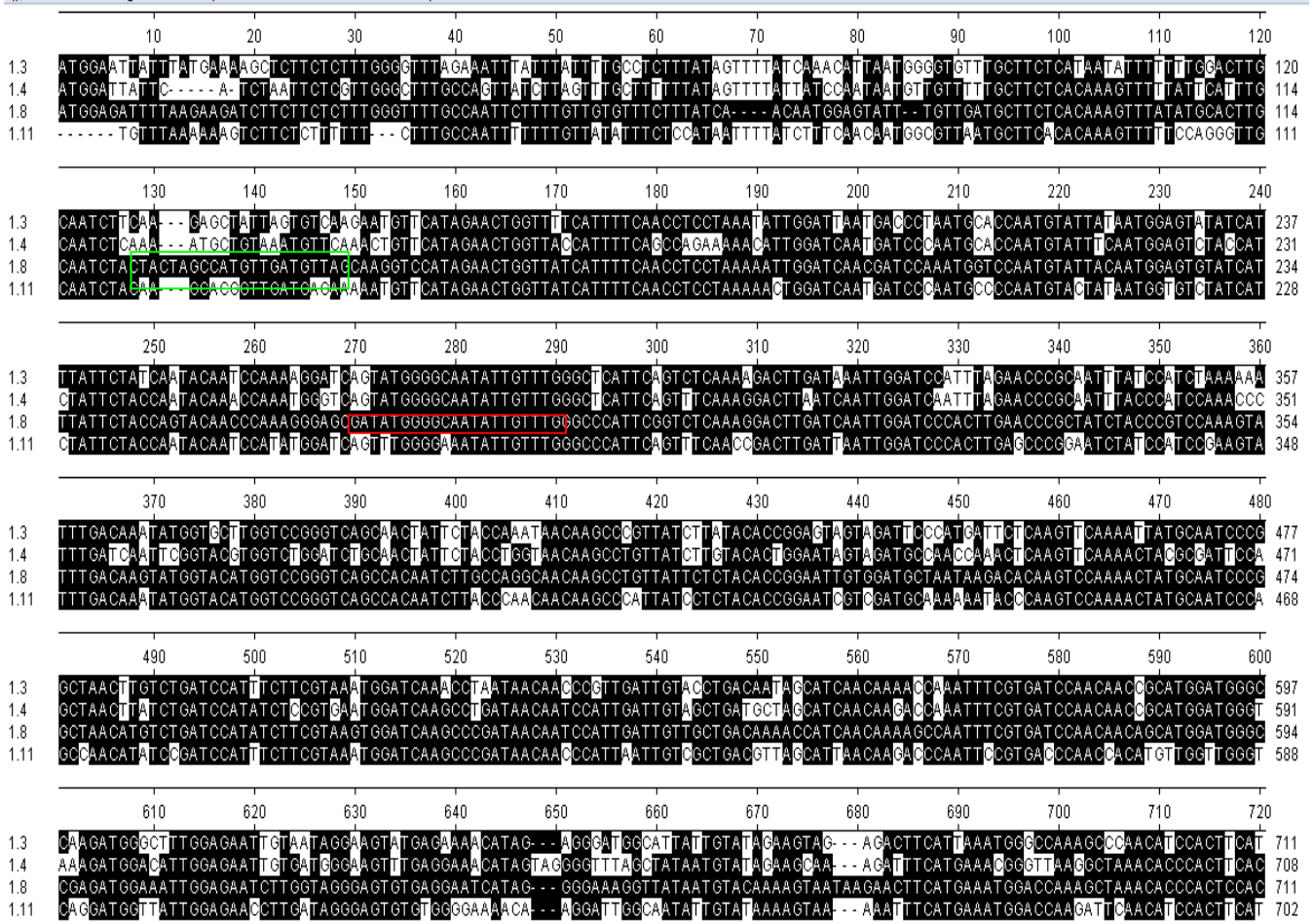


Decoration 'Decoration #1': Shade (with solid black) residues that match 1.4 exactly.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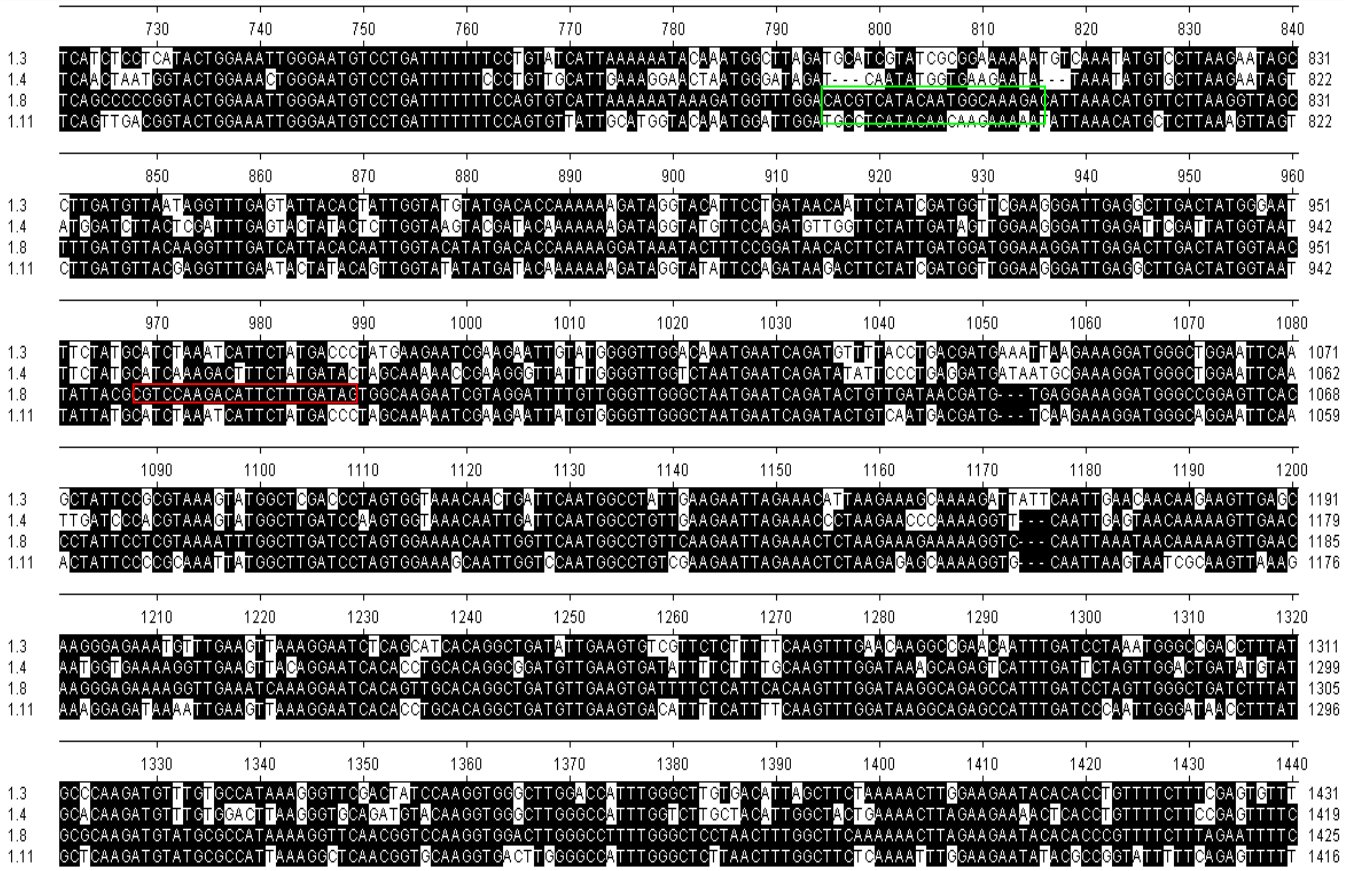
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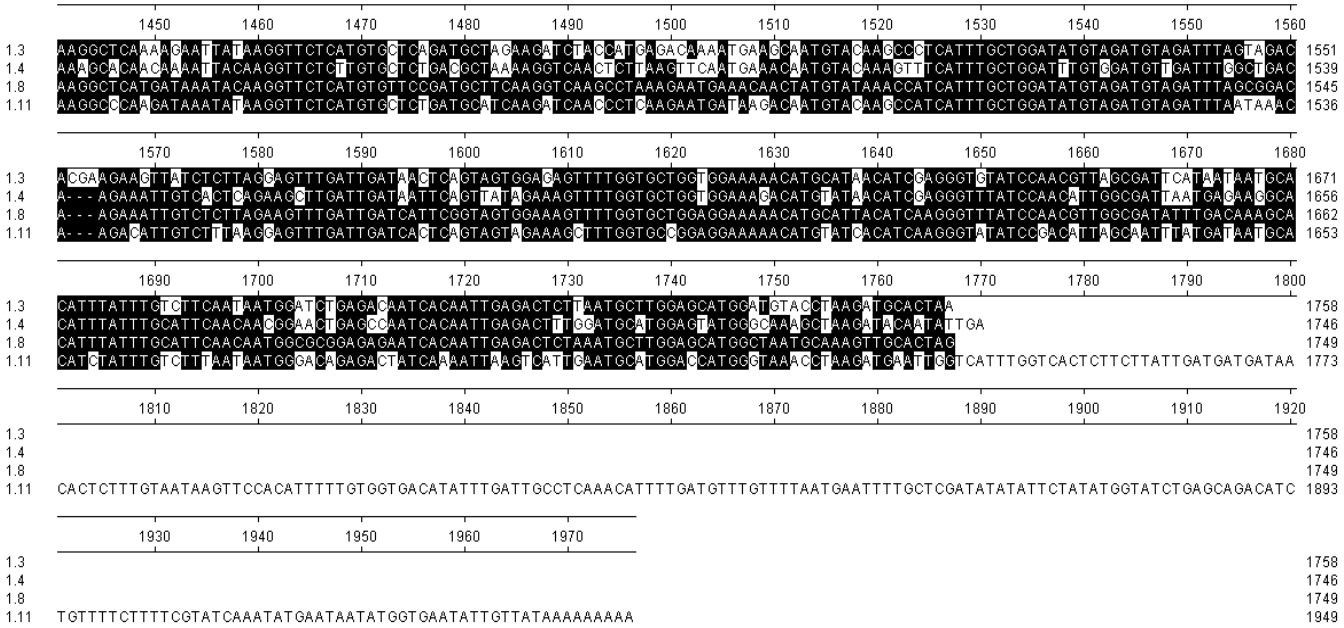
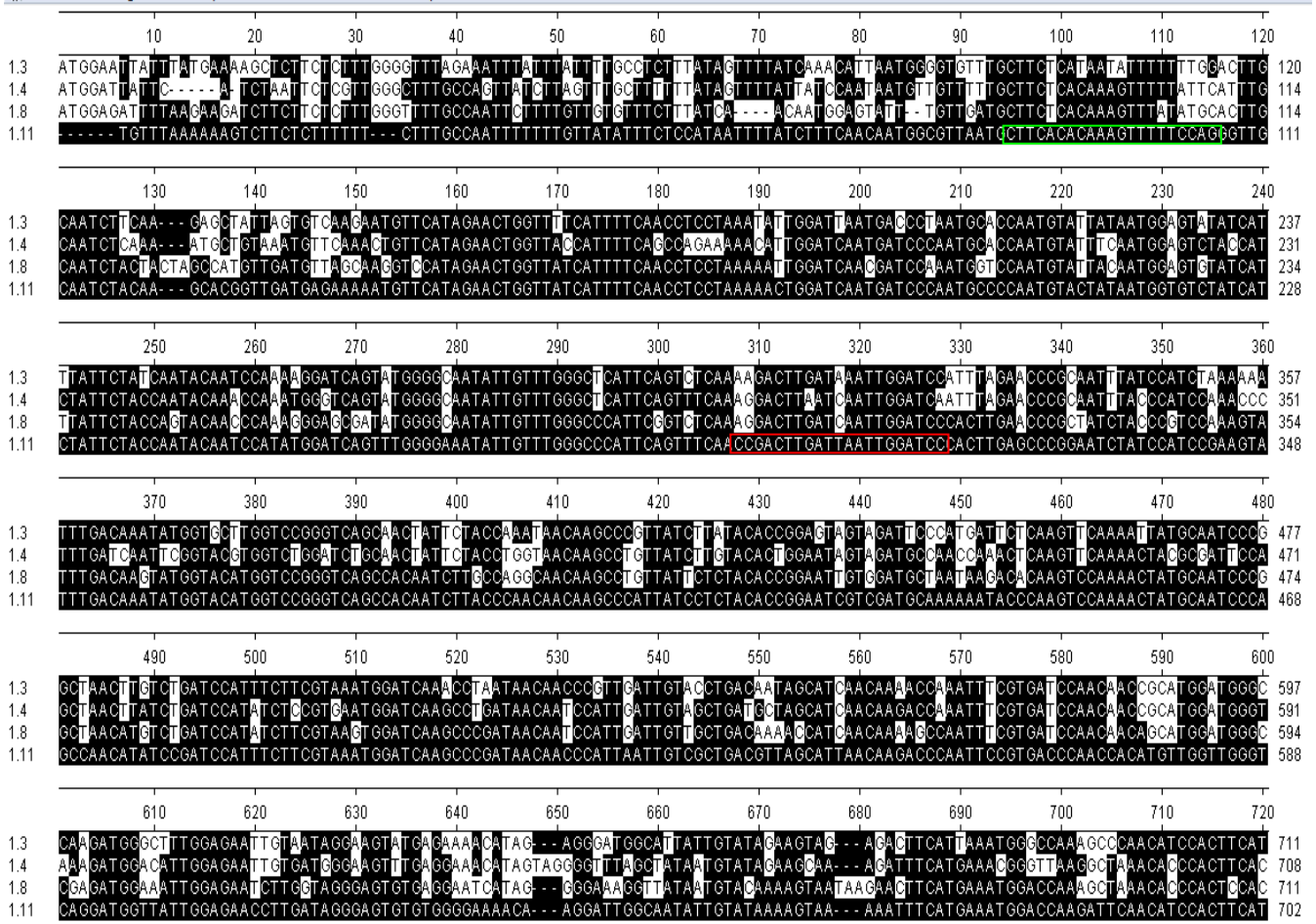


Decoration 'Decoration #1': Shade (with solid black) residues that match 1.8 exactly.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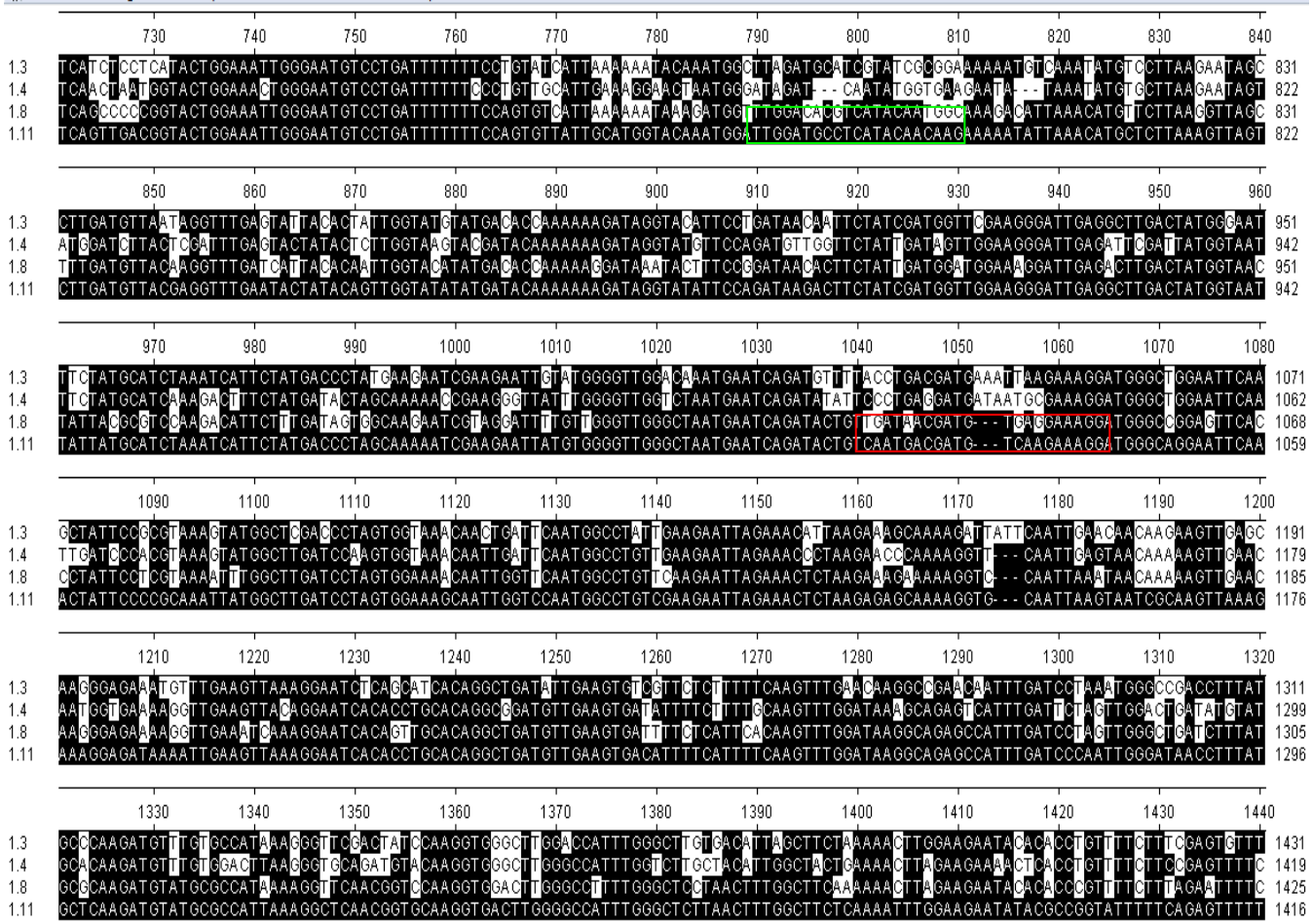
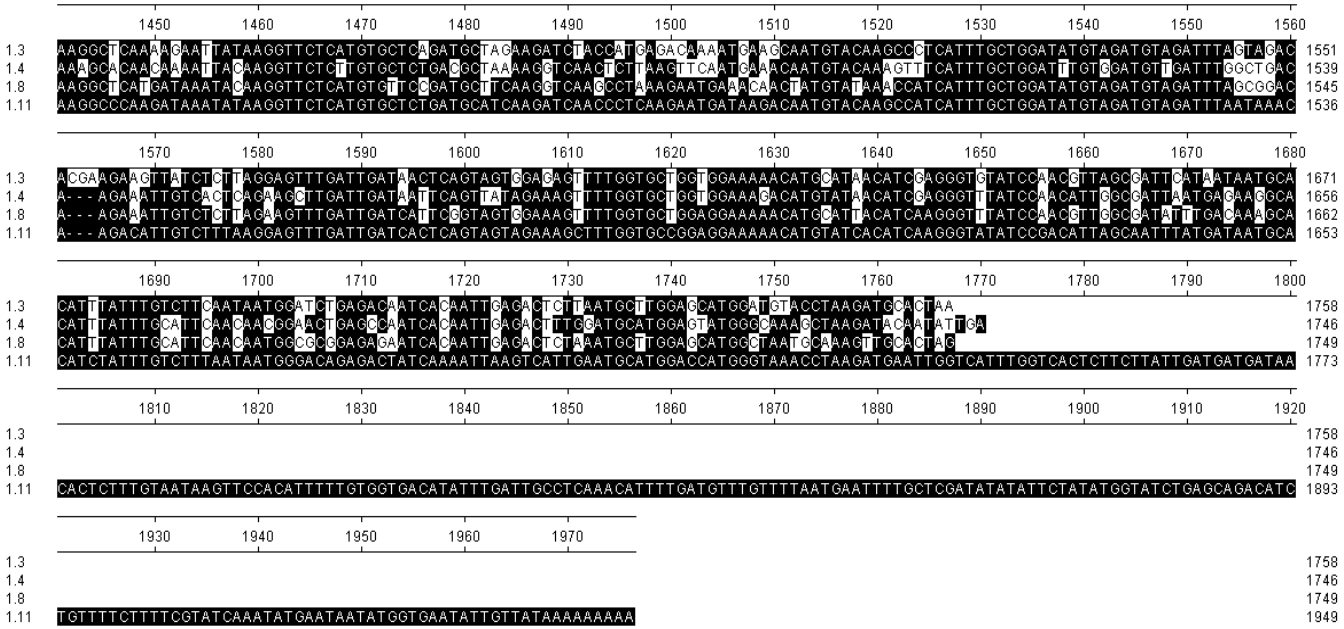
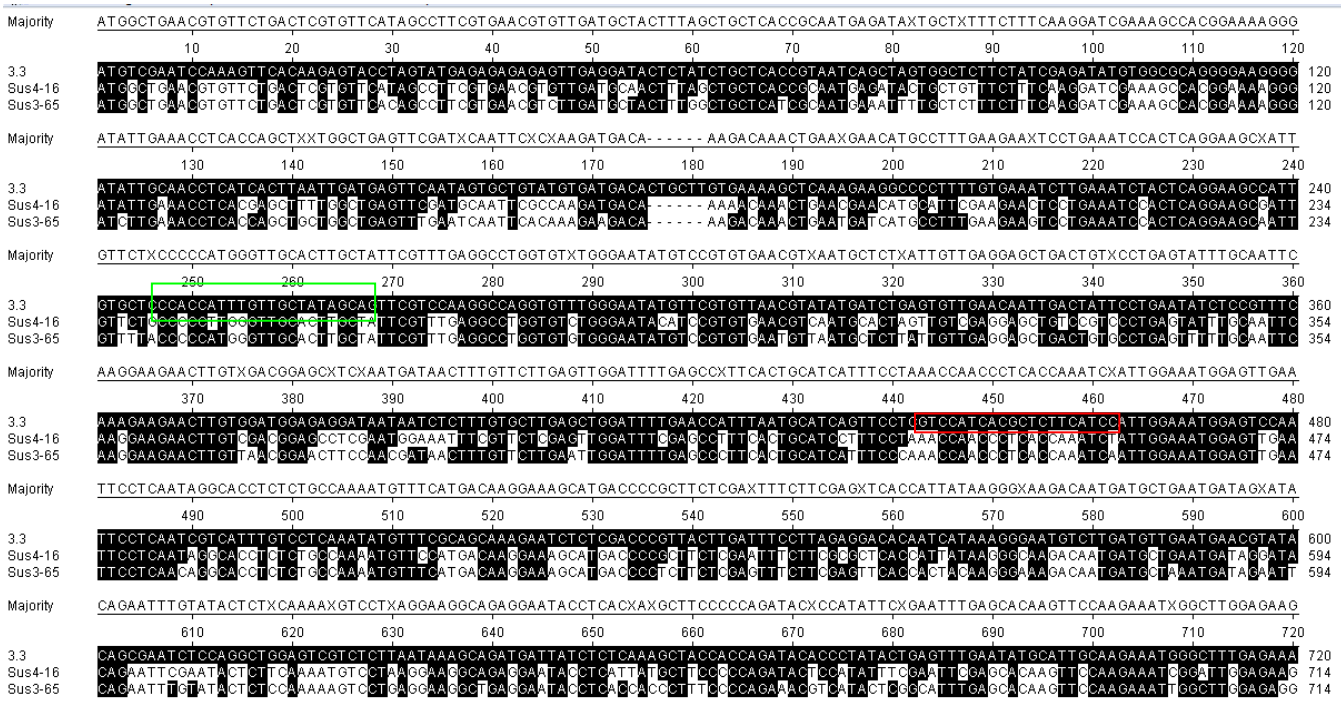
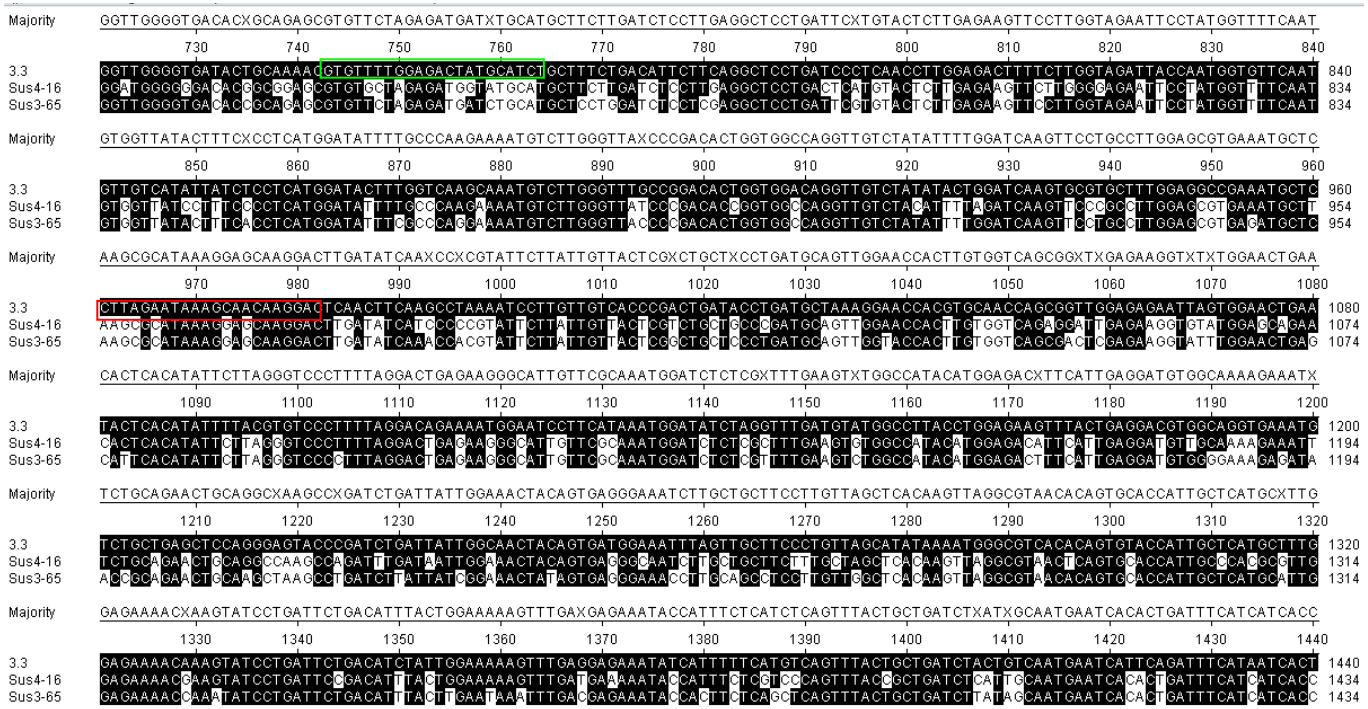




Decoration 'Decoration #1': Shade (with solid black) residues that match 1.11 exactly.


Majority
3.3
Sus4-16
Sus3-65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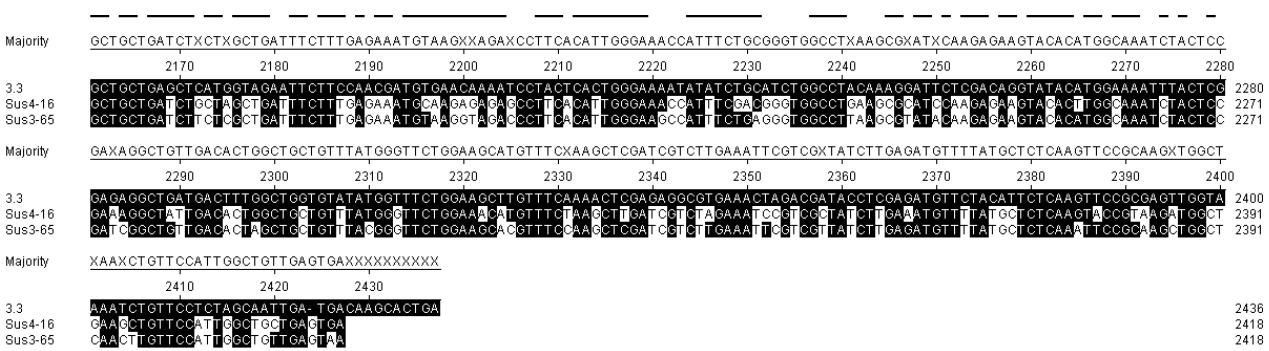
730 740 750 760 770 780 790 800 810 820 830 840
3.3 GGTTGGGGTGATACTGCAAAACGTGTTTTGGAGACTATGCATCTGCTTTCTGACATTCTTCAGGCTCCTGATCCCTCAACCTTGGAGACTTTTCTTGGTAGATTACCAATGGTGTTCAAT 840
Sus4-16 GGATGGGGGGACACGGCGGAGCGTGTGCTAGAGATGGTATGCATGCTTCTTGATCTCCTTGAGGCTCCTGACTCATGTACTCTTGAGAAGTTCTTGGGGAGAATTCCTATGGTTTTCAAT 834
Sus3-65 GGTTGGGGTGACACCGCAGAGCGTGTTCTAGAGATGATCTGCATGCTCCTGGATCTCCTCGAGGCTCCTGATTCGTGTACTCTTGAGAAGTTCCTTGGTAGAATTCCTATGGTTTTCAAT 834
Majority GTGGTTATACTTTCXCCTCATGGATATTTTGCCCAAGAAAATGTCTTGGGTTAXCCCGACACTGGTGGCCAGGTTGTCTATATTTTGGATCAAGTTCCTGCCTTGGAGCGTGAAATGCTC
850 860 870 880 890 900 910 920 930 940 950 960
3.3 GTTGTCATATTATCTCCTCATGGATACTTTGGTCAAGCAAATGTCTTGGGTTTGCCGGACACTGGTGGACAGGTTGTCTATATACTGGATCAAGTGCGTGCTTTGGAGGCCGAAATGCTC 960
Sus4-16 GTGGTTATCCTTTCCCCTCATGGATATTTTGCCCAAGAAAATGTCTTGGGTTATCCCGACACCGGTGGCCAGGTTGTCTACATTTTAGATCAAGTTCCCGCCTTGGAGCGTGAAATGCTT 954
Sus3-65 GTGGTTATACTTTCACCTCATGGATATTTCGCCCAGGAAAATGTCTTGGGTTACCCGGACACTGGTGGCCAGGTTGTCTATATTTTGGATCAAGTTCCTGCCTTGGAGCGTGAGATGCTC 954
Majority AAGCGCATAAAGGAGCAAGGACTTGATATCAAXCCXCGTATTCTTATTGTTACTCGXCTGCTXCCTGATGCAGTTGGAACCACTTGTGGTCAGCGGXTXGAGAAGGTXTXTGGAACTGAA
970 980 990 1000 1010 1020 1030 1040 1050 1060 1070 1080
3.3 CTTAGAATAAAGCAACAAGGACTCAACTTCAAGCCTAAAATCCTTGTTGTCACCCGACTGATACCTGATGCTAAAGGAACCACGTGCAACCAGCGGTTGGAGAGAATTAGTGGAACTGAA 1080
Sus4-16 AAGCGCATAAAGGAGCAAGGACTTGATATCATCCCCCGTATTCTTATTGTTACTCGTCTGCTGCCCGATGCAGTTGGAACCACTTGTGGTCAGAGGATTGAGAAGGTGTATGGAGCAGAA 1074
Sus3-65 AAGCGCATAAAGGAGCAAGGACTTGATATCAAACCACGTATTCTTATTGTTACTCGGCTGCTCCCTGATGCAGTTGGTACCACTTGTGGTCAGCGACTCGAGAAGGTATTTGGAACTGAG 1074
Majority CACTCACATATTCTTAGGGTCCCTTTTAGGACTGAGAAGGGCATTGTTCGCAAATGGATCTCTCGXTTTGAAGTXTGGCCATACATGGAGACXTTCATTGAGGATGTGGCAAAAGAAATX
1090 1100 1110 1120 1130 1140 1150 1160 1170 1180 1190 1200
3.3 TACTCACATATTTTACGTGTCCCTTTTAGGACAGAAAATGGAATCCTTCATAAATGGATATCTAGGTTTGATGTATGGCCTTACCTGGAGAAGTTTACTGAGGACGTGGCAGGTGAAATG 1200
Sus4-16 CACTCACATATTCTTAGGGTCCCTTTTAGGACTGAGAAGGGCATTGTTGCGCAAATGGATCTCTCGCTTTGAAGTGTGGCCATACATGGAGACATTCATTGAGGATGTTGCAAAAGAAATT 1194
Sus3-65 CATTCACATATTCTTAGGGTCCCCTTTAGGACTGAGAAGGGCATTGTTGCGCAAATGGATCTCTCGTTTTGAAGTCTGGCCATACATGGAGACTTTCATTGAGGATGTGGGGAAAGAGATA 1194
Majority TCTGCAGAACTGCAGGCXAAGCCXGATCTGATTATTGGAAACTACAGTGAGGGAAATCTTGCTGCTTCCTTGTTAGCTCACAAGTTAGGCGTAACACAGTGCACCATTGCTCATGCXTTG
1210 1220 1230 1240 1250 1260 1270 1280 1290 1300 1310 1320
3.3 TCTGCTGAGCTCCAGGGAGTACCCGATCTGATTATTGGCAACTACAGTGATGGAAATTTAGTTGCTTCCCTGTTAGCATATAAAATGGGCGTCACACAGTGTACCATTGCTCATGCTTTG 1320
Sus4-16 TCTGCAGAACTGCAGGCCAAGCCAGATTTGATAATTGGAAACTACAGTGAGGGCAATCTTGCTGCTTCTTTGCTAGCTCACAAGTTAGGCGTAACTCAGTGCACCATTGCCCAGCGCTTG 1314
Sus3-65 ACCGCAGAACTGCAAGCTAAGCCTGATCTTATTATCGGAAACTATAGTGAGGGAAACCTTGCAGCCTCCTTGTTGGCTCACAAGTTAGGCGTAACACAGTGCACCATTGCTCATGCATTG 1314
Majority GAGAAAACXAAGTATCCTGATTCTGACATTTACTGGAAAAAGTTTGAXGAGAAATACCATTTCTCATCTCAGTTTACTGCTGATCTXATXGCAATGAATCACACTGATTTCATCATCACC
1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430 1440
3.3 GAGAAAACAAAGTATCCTGATTCTGACATCTATTGGAAAAAGTTTGAGGAGAAATATCATTTTTCATGTCAGTTTACTGCTGATCTACTGTCAATGAATCATTCAGATTTCATAATCACT 1440
Sus4-16 GAGAAAACGAAGTATCCTGATTCCGACATTTACTGGAAAAAGTTTGATGAAAAATACCATTTCTCGTCCCAGTTTACCGCTGATCTCATTGCAATGAATCACACTGATTTCATCATCACC 1434
Sus3-65 GAGAAAACCAAATATCCTGATTCTGACATTTACTTGAATAAATTTGACGAGAAATACCACTTCTCAGCTCAGTTTACTGCTGATCTTATAGCAATGAATCACACTGATTTCATCATCACC 1434


Majority AGCACCTTCCAGGAGATAGCAGGAAGCAAGGACACTGTTGGACAGTATGAGAGCCATATGGCXTTCACAATGCCTGGATTGTATAGAGTTGTTCATGGCATTGATGTGTTCGATCCCAAA
1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
3.3 AGTACCTATCAAGAGATTGCAGGAACGAAGAATACTGTTGGTCAGTATGAGAGTCATACTGCTTTCACTCTCCCAGGCCTATATCGTGTTGTCCATGGCATTGATGTTTTCGATCCCAAA 1560
Sus4-16 AGCACCTTCCAGGAGATAGCAGGAAGCAAGGACACTGTGGGACAATATGAGAGCCATATGGCATTCACAATGCCTGGATTGTACAGAGTTGTTCATGGCATTAATGTGTTCGACCCAAA 1554
Sus3-65 AGCACCTTCCAGGAGATAGCAGGAAGCAAGGACACCGTTGGACAGTATGAGAGCCACATGGCCTTCACAATGCCTGGATTGTATAGAGTTGTTCATGGCATTGATGTGTTCGATCCCAAA 1554
Majority TTCAACATTGTGTCACCTGGAGCTGATATGAAXCTCTACTTCCCATACTCCGAAAAGGAAAAGAGACTGACAXCTTTTCACCCTGAAATTGAXGAGTTGCTGTTTAGTGATGTXGAGAAT
1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680
3.3 TTCAATATAGTGTCTCCTGGAGCTGACATGACAATTTACTTCCCATATTCTGACAAGGAAAAGAGACTGACATCTTTGCATCCCTCGATTGAGAAGTTGTTATTTGATCCTGAGCAGAAT 1680
Sus4-16 TTCAACATTGTCTCACCTGGAGCTGATATTAACCTCTACTTCTCCGTACTCCGAAACGGAAAAGAGACTTACAGCATTTCACCCTGAAATTGATGAGCTGCTGTTATAGTGACGTTCAGAAT 1674
Sus3-65 TTCAACATTGTGTCACCAGGAGCTGATGTGAATCTCTATTTTTCCATACTCCGAAAAGGAAAAGAGATTGACAACTTTTCACCCTGAAATCGAAGACTTGCTGTTTAGCGATGTCCAGAAT 1674
Majority GAAGAACATCTGTGTGTGCTGAAGGATAGGACTAAACCXATXATATTCACAATGGCAAGGTTGGACCGXGTGAAGAACTTAACTGGACTXGTTGAGTGGTATGCTAAGAATCCACGACTA
1690 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800
3.3 GAAGTGCATATAGGCAACCTGAATGATCAATCAAAACCGATAATTTTTTCAATGGCAAGGCTTGACCGGGTCAAGAACATTACGGGATTAGTTGAGTGCTATGCTAAAAATGCCACACTC 1800
Sus4-16 GACGAACATCTGTGTGTGCTCAAGGATAGGACTAAACCAATTTTATTCACAATGGCAAGGTTGGATCGTGTGAAGAATTTAACTGGACTTGTTGAGTGGTACGCCAAGAATCCACGACTA 1794
Sus3-65 GAAGAACACCTGTGTGTGTTGAAGGACAGGAATAAGCCCATCATATTCACCATGGCAAGATTGGACCGAGTGAAGAAGTTAACTGGACTCGTCGAGTGGTATGCTAAGAATCCACGACTA 1794
Majority AGGGAATTGGTTAACCTTGTTGTAGTTGGTGGAGAC--CGAAG-AAAGGAATCCAAAGATTTGGAAGAGCAGGCAGAGATGAAGAAGATGTATGAXCTTATAAAGACTCATAATTTGAAT
1810 1820 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920
3.3 AGGGAATTGGCAAACCTTGTTGTAGTAGCTGGATACAACGATGTAAAGAAATCCAATGATAGAGAAGAAATAGCAGAAATTGAGAAGATGCATGCTCTTATGAAGGAACATAACTTGGAT 1920
Sus4-16 AGGGGATTGGTTAACCTGGTTGTAGTTGGCGGAGAT--CGAAG-GAAGGAATCCAAAGATTTGGAAGAGCAGGCAGAGATGAAGAAGATGTATGAGCTAATAGAGACTCATAATTTGAAT 1911
Sus3-65 AGGGAGTTGGTTAACCTTGTTGTGGTTGGTGGAGAC--CGAAG-AAAGGAATCCAAAGACTTGGAAGAGCAGGCAGAGATGAAGAAGATGTATGAACTTATAAAGACTCACAATTTGAAT 1911
Majority GGCCAATTCAGATGGATTTCTTCCCAGATGAACCGXGTGAGGAATGGTGAGCTCTACCGXTACATTGCTGACACXAGGGGAGCTTTCGTTCAGCCTGCATTCTACGAGGCTTTTGGTCTG
1930 1940 1950 1960 1970 1980 1990 2000 2010 2020 2030 2040
3.3 GGTCAATTCAGATGGATATCAGCCCAAATGAACCGGGCACGTAATGGTGAGCTCTATCGCTATATAGCTGACAAGAGAGGTATATTTGTTCAGCCTGCATTTTATGAAGCGTTTGGACTC 2040
Sus4-16 GGCCAATTCAGATGGATTTCTTCCCAGATGAACCGAGTGAGGAATGGTGAGCTCTACCGATACATTGCTGACACTAAGGGAGCTTTCGTTCAGCCTGCATTCTACGAGGCTTTGGTCTG 2031
Sus3-65 GGCCAGTTGCGATGGATTTCTTCCCAGATGAACCGTGTGAGGAATGGGGAACTCTACAGGTACATTGCTGACACCAGGGGAGCTTTCGTGCAGCCTGCATTCTACGAGGCTTTCGGTCTG 2031
Majority ACTGTTGTTGAAGCXATGACTTGTGGTTTGCCTACATTTGCAACTAATCAXGGTGGTCCAGCTGAGATCATCGTTCACGGAAAGTCCGGXTTCCAXATTGATCCATATCATGGCGAGCAA
2050 2060 2070 2080 2090 2100 2110 2120 2130 2140 2150 2160
3.3 ACGGTGGTTGAAGCTATGACTTGTGGTCTTCCAACATTTGCAACTTGTCATGGTGGTCCTATGGAGATCATTCAGGACGGTGTATCCGGGTACCATATAGATCCTTATCATCCCAATAAA 2160
Sus4-16 ACTGTTGTTGAAGCAATGACTTGTGGTTTGCCTACATTTGCAACTAATCACGGTGGTCCAGCTGAGATCATCGTTCATGGAAAGTCCGGCTTCCACATTGATCCATATCACGGTGAGCAA 2151
Sus3-65 ACTGTTGTTGAGGCCATGAGCTGCGGTTTGCCTACATTTGCAACAAATCAAGGTGGTCCAGCTGAGATCATCGTTCACGGAAAGTCTGGTTTCCAAATTGATCCATACCATGGCGAGCAG 2151



Gene name: Susy2

Primer name	**position**	**Sequence (5′ to 3′)**	**Length (nt)**	**Tm (C[1])**	**GC%[2]**	**Expected product length (nt)**
Sus3.3-F1	248-268	CACCATTTGTTGCTATAGCAG	21	60	42,85	
Sus3.3-R1	462-443	GGATGAAGAGCGTGATGGAC	20	62	55	214
Sus3.3-F2	743-763	GTGTTTTGGAGACTATGCATC	21	60	42,86	
Sus3.3-R2	982-961	GTCCTTGTTGCTTTATTCTAAG	22	60	40,91	239


Majority ATGGCTGAACGTGTTCTGACTCGTGTTCATAGCCTTCGTGAACGTGTTGATGCTACTTTAGCTGCTCACCGCAATGAGATAXTGCTXTTTCTTTCAAGGATCGAAAGCCACGGAAAAGGG
10 20 30 40 50 60 70 80 90 100 110 120
3.3 ATGTCGAATCCAAAGTTCAGAAGAGTACCTAGTATTGAGAGAGAGTTGAGGATACTCTATCTGCTCACCGTAATCAGCTAGTGGCTCTTCTATCGAGATATGTGGCGCAGGGAAGGGG 120
Sus4-16 ATGGCTGAACGTGTTCTGACTCGTGTTCATAGCCTTCGTGAACGTGTTGATGCAACTTTAGCTGCTCACCGCAATGAGATACTGCTGTTTCTTTCAAGGATCGAAAGCCACGGAAAAGGG 120
Sus3-65 ATGGCTGAACGTGTTCTGACTCGTGTTCACAGCCTTCGTGAACGTCTTGATGCTACTTTCGCTGCTCATCGCAATGAAATTTTGCTCTTTCTTTCAAGGATCGAAAGCCACGGAAAAGGG 120

Majority ATATTGAAACCTCACCAGCTXXTGGCTGAGTTCGATXCAATTCXCXAAGATGACA------AAGACAAACTGAAXGAACATGCCTTTGAAGAAXTCCTGAAATCCACTCAGGAAGCXATT
130 140 150 160 170 180 190 200 210 220 230 240
3.3 ATATTGCAACCTCATCACTTAATTGATGAGTTCAATAGTGCTGTATGTGATGACACTGCTTGTGAAAAGCTCAAAGAAGGCGCCTTTTGTGAAATCTTGAAATCTACTCAGGAAGCCATT 240
Sus4-16 ATATTGAAACCTCACGAGCTTTTGGCTGAGTTCGATGCAATTCGCCAAGATGACA------AAAACAAACTGAACGAACATGCATTCGAAGAACTCCTGAAATCCACTCAGGAAGCGATT 234
Sus3-65 ATCTTGAAACCTCACCAGCTGCTGGCTGAGTTTGAATCAATTCAGAAAGAAGACA------AACACAAACTGAATGATCATGCCTTTGAAGAAGTCCTGAAATCCACTCAGGAAGCAATT 234

Majority GTTCTXCCCCCATGGGTTGCACTTGCTATTCGTTTGAGGCCTGGTGTXTGGGAATATGTCCGTGTGAACGTXAATGCTCTXATTGTTGAGGAGCTGACTGTXCCTGAGTATTTGCAATTC
250 260 270 280 290 300 310 320 330 340 350 360
3.3 GTGCTCCCACCATTTGTTGCTATAGCAGTTCGTCCAAGGCCAGGTGTTTGGGAATATGTTCGTGTTAACGTATATGATCTGAGTGTTGAACAATTGACTATTCCTGAATATCTCGGTTTC 360
Sus4-16 GTTCTGCCCCCTTGGGTTGCACTTGCTATTCGTTTGAGGCCTGGTGTCTGGGAATACATCCGTGTGAACGTCAATGCACTAGTTGTCGAGGAGCTGTCCGTCCCTGAGTATTTGCAATTC 354
Sus3-65 GTTTTACCCCCATGGGTTGCACTTGCTATTCGTTTGAGGCCTGGTGTGTGGGAATATGTCCGTGTGAATGTTAATGCTCTTATTGTTGAGGAGCTGACTGTGCCTGAGTTTTTGCAATTC 354

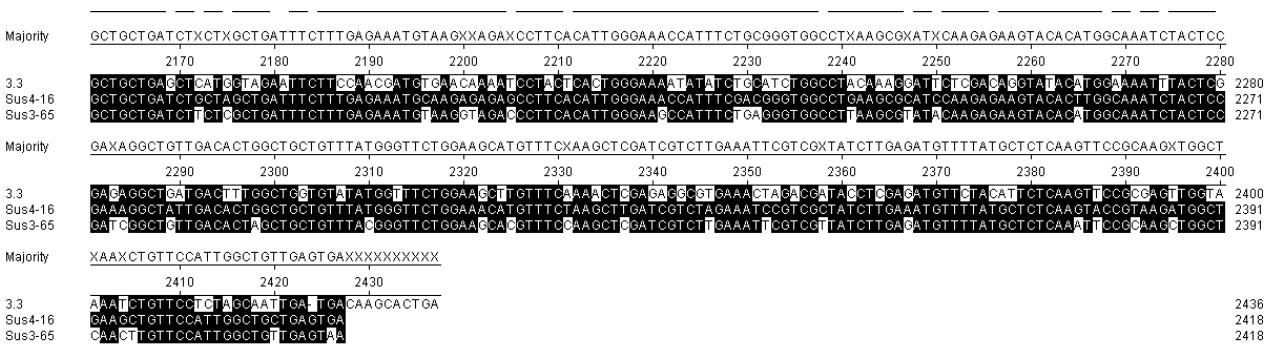
Majority AAGGAAGAACTTGTXGACGGAGCXTCXAATGATAACTTTGTTCTTGAGTTGGATTTTGAGCCXTTCACTGCATCATTTCCTAAACCAACCCTCACCAAATCXATTGGAAATGGAGTTGAA
370 380 390 400 410 420 430 440 450 460 470 480
3.3 AAAGAAGAACTTGTGGATGGAGAGGATAATAATCTCTTTGTGCTTGAGGTGGATTTTGAACCATTTAATGCATCAGTTCCTCGTCCATCACGCTCTTCATCCATTGGAAATGGAGTCGAA 480
Sus4-16 AAGGAAGAACTTGTCGACGGAGCCTCGAATGGAAATTTCGTTCTCGAGTTGGATTTCGAGCCTTTCACTGCATCCTTTCCTAAACCAACCCTCACCAAATCTATTGGAAATGGAGTTGAA 474
Sus3-65 AAGGAAGAACTTGTTAACGGAAGTTCGAACGATAACTTTGTTCTTGAATTGGATTTTGAGCCGTTCACTGCATCATTTCCGAAACCAACCCTCACCAAATCAATTGGAAATGGAGTTGAA 474

Majority TTCCTCAATAGGCACCTCTCTGCCAAAATGTTTCATGACAAGGAAAGCATGACCCCGCTTCTCGAXTTTCTTCGAGXTCACCATTATAAGGGXAAGACAATGATGCTGAATGATAGXATA
490 500 510 520 530 540 550 560 570 580 590 600
3.3 TTCCTCAATCGTCATTTGTCCTCAAATATGTTTCGCAGCAAAGAATCTCTCGACCCGTTACTTGATTTCCTTAGAGGACACAATCATAAAGGGAATGTCTTGATGTTGAATGAACGTATA 600
Sus4-16 TTCCTCAATAGGCACCTCTCTGCCAAAATGTTCCATGACAAGGAAAGCATGACCCCGCTTCTCGAATTTCTTCGCGCTCACCATTATAAGGGCAAGACAATGATGCTGAATGATAGGATA 594
Sus3-65 TTCCTCAACAGGCACCTCTCTGCCAAAATGTTTCATGACAAGGAAAGCATGACCCCTCTTCTCGAGTTTCTTCGAGTTCACCACTACAAGGGAAAGACAATGATGCTAAATGATAGAATT 594

Majority CAGAATTTGTATACTCTXCAAAAXGTCCTXAGGAAGGCAGAGGAATACCTCACXAXGCTTCCCCCAGATACXCCATATTCXGAATTTGAGCACAAGTTCCAAGAAATXGGCTTGGAGAAG
610 620 630 640 650 660 670 680 690 700 710 720
3.3 CAGCGAATCTCCAGGCTGGAGTCGTCTCTTAATAAAGCAGATGATTATCTCTCAAAGCTACCACCAGATACACGTATACTGAGTTTGAATATGCATTGCAAGAAATGGGCTTTGAGAAA 720
Sus4-16 CAGAATTCGAATACTCTTCAAAATGTCCTAAGGAAGGCAGAGGAATACCTCATTATGCTTCCCCCAGATACTCCATATTTCGAATTCGAGCACAAGTTCCAAGAAATCGGATTGGAGAAG 714
Sus3-65 CAGAATTTGTATACTCTCCAAAAAGTCCTGAGGAAGGCTGAGGAATACCTCACCACCCTTTCCCCAGAAACGTCATACTCGGCATTTGAGCACAAGTTCCAAGAAATTGGCTTGGAGAGG 714


Majority GGTTGGGGTGACACXGCAGAGCGTGTTCTAGAGATGATXTGCATGCTTCTTGATCTCCTTGAGGCTCCTGATTCXTGTACTCTTGAGAAGTTCCTTGGTAGAATTCCTATGGTTTTCAAT
730 740 750 760 770 780 790 800 810 820 830 840
3.3 GGTTGGGGTGATACTGCAAAACGTGTTTTGGAGACTATGCATCTGCTTTCTGACATTCTTCAGGCTCCTGATCCCTCAACCTTGGAGACTTTTCTTGGTAGATTACCAATGGTGTTCAAT 840
Sus4-16 GGATGGGGGGACACGGCGGAGCGTGTGCTAGAGATGGTATGCATGCTTCTTGATCTCCTTGAGGCTCCTGACTCATGTACTCTTGAGAAGTTCTTGGGGAGAATTCCTATGGTTTTCAAT 834
Sus3-65 GGTTGGGGTGACACGCAGAGCGTGTTCTAGAGATGATCTGCATGCTCCTGGATCTCCTCGAGGCTCCTGATTCGTGTACTCTTGAGAAGTTCCTTGGTAGAATTCCTATGGTTTTCAAT 834
Majority GTGGTTATACTTTCXCCTCATGGATATTTTGCCCAAGAAAATGTCTTGGGTTAXCCCGACACTGGTGGCCAGGTTGTCTATATTTTGGATCAAGTTCCTGCCTTGGAGCGTGAAATGCTC
850 860 870 880 890 900 910 920 930 940 950 960
3.3 GTTGTCATATTATCTCCTCATGGATACTTTGGTCAAGCAAATGTCTTGGGTTTCCCGGACACTGGTGCACAGGTTGTCTATATACTGGATCAAGTGCGTGCTTTGGAGGCCGAAATGCTC 960
Sus4-16 GTGGTTATCCTTTCCCCTCATGGATATTTTGCCCAAGAAAATGTCTTGGGTTATCCCGACACCGGTGGCCAGGTTGTCTACATTTTAGATCAAGTTCCCGCCTTGGAGCGTGAAATGCTT 954
Sus3-65 GTGGTTATACTTTCACCTCATGGATATTTCGCCCAGGAAAATGTCTTGGGTTACCCGACACTGGTGGCCAGGTTGTCTATATTTTGGATCAAGTTCCTGCCTTGGAGCGTGAGATGCTC 954
Majority AAGCGCATAAAGGAGCAAGGACTTGATATCAAXCCXCGTATTCTTATTGTTACTCGXCTGCTXCCTGATGCAGTTGGAACCACTTGTGGTCAGCGGXTXGAGAAGGTXTXTGGAACTGAA
970 980 990 1000 1010 1020 1030 1040 1050 1060 1070 1080
3.3 CTTAGAATAAAGCAACAAGGACTCAACTTCAAGCCTAAAATCCTTGTTGTCACCCGACTGATACCTGATGGTAAAGGAACCACGTGCAACCAGCGCTTGGAGAGAATTACTGGAACTGAA 1080
Sus4-16 AAGCGCATAAAGGAGCAAGGACTTGATATCATCCCCCGTATTCTTATTGTTACTCGTCTGCTGCCCGATGCAGTTGGAACCACTTGTGGTCAGAGGATTGAGAAGGTGTATGGAGCAGAA 1074
Sus3-65 AAGCGCATAAAGGAGCAAGGACTTGATATCAAACCACGTATTCTTATTGTTACTCGGCTGCTCCCTGATGCAGTTGGTACCACTTGTGGTCAGCGACTCGAGAAGGTATTTGGAACTGAG 1074
Majority CACTCACATATTCTTAGGGTCCCTTTTAGGACTGAGAAGGGCATTGTTCGCAAATGGATCTCTCGXTTTGAAGTXTGGCCATACATGGAGACXTTCATTGAGGATGTGGCAAAAGAAATX
1090 1100 1110 1120 1130 1140 1150 1160 1170 1180 1190 1200
3.3 TACTCACATATTTTACGTGTCCCTTTTAGGACAGAAAATGGAATCCTTCATAAATGGATATCTAGGTTTGATGTATGGCCTTACCTGGAGAAGTTTACTGAGGACGTGGCAGGTGAAATG 1200
Sus4-16 CACTCACATATTCTTAGGGTCCCTTTTAGGACTGAGAAGGGCATTGTTCGCAAATGGATCTCTCGCTTTGAAGTGTGGCCATACATGGAGACATTCATTGAGGATGTTGCAAAAGAAATT 1194
Sus3-65 CATTCACATATTCTTAGGGTCCCCTTTAGGACTGAGAAGGGCATTGTTCGCAAATGGATCTCTCGTTTTGAAGTCTGGCCATACATGGAGACTTTCATTGAGGATGTGGGGAAAGAGATA 1194
Majority TCTGCAGAACTGCAGGCXAAGCCXGATCTGATTATTGGAAACTACAGTGAGGGAAATCTTGCTGCTTCCTTGTTAGCTCACAAGTTAGGCGTAACACAGTGCACCATTGCTCATGCXTTG
1210 1220 1230 1240 1250 1260 1270 1280 1290 1300 1310 1320
3.3 TCTGCTGAGCTGCAGGGAGTACCCGATCTGATTATTGGCAACTACAGTGATGGAAATTTAGTTGCTTCCCTGTTAGCATATAAAATGGGCGTCACACAGTGTACCATTGCTCATGCTTTG 1320
Sus4-16 TCTGCAGAACTGCAGGCCAAGCCAGATTTGATAATTGGAAACTACAGTGAGGGCAATCTTGCTGCTTCTTTGCTAGCTCACAAGTTAGGCGTAACTCAGTGCACCATTGCCCACGCGTTG 1314
Sus3-65 ACCGCAGAACTGCAAGCTAAGCCTGATCTTATTATCGGAAACTATAGTGAGGGAAACCTTGCAGCTCCTTGTTCGCTCACAAGTTAGGCGTAACACAGTGCACCATTGCTCATGCATTG 1314
Majority GAGAAAACXAAGTATCCTGATTCTGACATTTACTGGAAAAAGTTTGAXGAGAAATACCATTTCTCATCTCAGTTTACTGCTGATCTXATXGCAATGAATCACACTGATTTCATCATCACC
1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430 1440
3.3 GAGAAAACAAAGTATCCTGATTCTGACATCTATTGGAAAAGTTTGAGGAGAAATATCATTTTTCATGTCAGTTTACTGCTGATCTACTGTCAATGAATCATTCAGATTTCATAATCACT 1440
Sus4-16 GAGAAAACGAAGTATCCTGATTCCGACATTTACTGGAAAAAGTTTGATGAAAAATACCATTTCTCGTCCCAGTTTACCGCTGATCTCATTGCAATGAATCACACTGATTTCATCATCACC 1434
Sus3-65 GAGAAAACCAAATATCCTGATTCTGACATTTACTTGAATAAATTTGACGAGAAATACCACTTCTCAGGTCAGTTTACTGCTGATCTTATAGCAATGAATCACACTGATTTCATCATCACC 1434

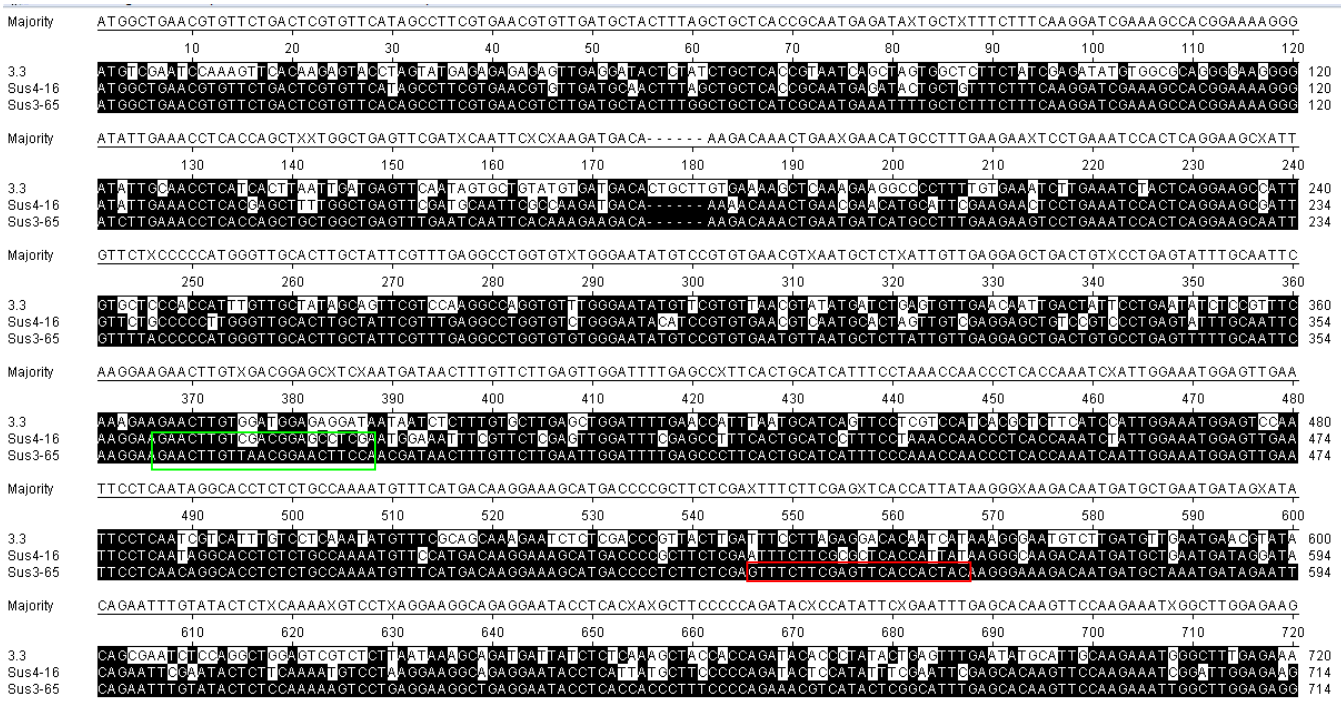

Majority AGCACCTTCCAGGAGATAGCAGGAAGCAAGGACACTGTTGGACAGTATGAGAGCCATATGGCXTTCACAATGCCTGGATTGTATAGAGTTGTTCATGGCATTGATGTGTTCGATCCCAAA
1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
3.3 AGTACCTATCAAGAGATTGCAGGAACGAAGAATACTGTTGGTCAGTATGAGAGTCATACTGCTTTCACTCTCCCAGGCCTATATCGTGTTGTCCATGGCATTGATGTTTCGATCCCAAA 1560
Sus4-16 AGCACCTTCCAGGAGATAGCAGGAAGCAAGGACACTGTGGGACAATATGAGAGCCATATGGCATTCACAATGCCTGGATTGTACAGAGTTGTTCATGGCATTAATGTGTTCGACCCCAAA 1554
Sus3-65 AGCACCTTCCAGGAGATAGCAGGAAGCAAGGACACCGTTGGACAGTATGAGAGCCACATGGCCTTCACAATGCCTGGATTGTATAGAGTTGTTCATGGCATTGATGTGTTCGATCCCAAA 1554
Majority TTCAACATTGTGTCACCTGGAGCTGATATGAAXCTCTACTTCCCATACTCCGAAAAGGAAAAGAGACTGACAXCTTTTCACCCTGAAATTGAXGAGTTGCTGTTTAGTGATGTXGAGAAT
1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680
3.3 TTCAATATAGTGTCTCCTGGAGCTGACATGACAATTTACTTCCGATATTGTGACAAGGAAAAGAGACTGACATCTTTGCATCCCTCGATTGAGAAGTTGTTATTTGATCCTGAGCAGAAT 1680
Sus4-16 TTCAACATTGTCTCACCTGGAGCTGATATTAACCTCTACTTCTCGTACTCCGAAACGGAAAAGAGACTTACAGCATTTCACCCTGAAATTGATGAGCTGCTGTATAGTGACGTTGAGAAT 1674
Sus3-65 TTCAACATTGTGTCACCAGGAGCTGATGTGAATCTCTATTTTCCATACTCCGAAAAGGAAAAGAGATTGACAACTTTTCACCCTGAAATCGAAGACTTGCTGTTTAGCGATGTCGAGAAT 1674
Majority GAAGAACATCTGTGTGTGCTGAAGGATAGGACTAAACCXATXATATTCACAATGGCAAGGTTGGACCGXGTGAAGAACTTAACTGGACTXGTTGAGTGGTATGCTAAGAATCCACGACTA
1690 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800
3.3 GAAGTGCATATAGGCAACCTGAATGATCAATCAAAACCGATAATTTTTTCAATGGCAAGGCTTGACCGGTCAAGAACATTACGGGATTAGTTGAGTGCTATGCTAAAAATGCCACACTC 1800
Sus4-16 GACGAACATCTGTGTGTGCTCAAGGATAGGACTAAACCAATTTTATTCACAATGGCAAGGTTGGATCGTGTGAAGAATTTAACTGGACTTGTTGAGTGGTACGCCAAGAATCCACGACTA 1794
Sus3-65 GAAGAACACCTGTGTGTGTTGAAGGACAGGAATAAGCCCATCATATTCACCATGGCAAGATTGGACCGAGTGAAGAACTTAACTGGACTCGTCGAGTGGTATGCTAAGAATCCACGACTA 1794
Majority AGGGAATTGGTTAACCTTGTTGTAGTTGGTGGAGAC- - CGAAG- AAAGGAATCCAAAGATTTGGAAGAGCAGGCAGAGATGAAGAAGATGTATGAXCTTATAAAGACTCATAATTTGAAT
1810 1820 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920
3.3 AGGGAATTGGCAAACCTTGTTGTAGTAGCTGGATACAACGATGTAAAGAAATCCAATGATAGAGAAGAAATAGCAGAAATTGAGAAGATGCATGCTCTTATGAAGGAACATAAGTTGGAT 1920
Sus4-16 AGGGGATTGGTTAACCTGGTTGTAGTTGGCGGAGAT- - CGAAG- GAAGGAATCCAAAGATTTGGAAGAGCAGGCAGAGATGAAGAAGATGTATGAGCTAATAGAGACTCATAATTTGAAT 1911
Sus3-65 AGGGAGTTGGTTAACCTTGTTGTGGTTGGTGGAGAC- - CGAAG- AAAGGAATCCAAAGACTTGGAAGAGCAGGCAGAGATGAAGAAGATGTATGAACTTATAAAGACTCACAATTTGAAT 1911
Majority GGCCAATTCAGATGGATTTCTTCCCAGATGAACCGXGTGAGGAATGGTGAGCTCTACCGXTACATTGCTGACACXAGGGGAGCTTTCGTTCAGCCTGCATTCTACGAGGCTTTTGGTCTG
1930 1940 1950 1960 1970 1980 1990 2000 2010 2020 2030 2040
3.3 GGTCAATTCAGATGGATATCAGCCCAAATGAACCGGGCACGTAATGGTGAGCTCTATCGCTATATAGCTGACAAGAGAGGTATATTTGTTCAGCCTGCATTTTATGAAGCGTTTGGACTC 2040
Sus4-16 GGCCAATTCAGATGGATTTCTTCCCAGATGAACCGAGTGAGGAATGGTGAGCTCTACCGATACATTGCTGACACTAAGGGAGCTTTCGTTCAGCCTGCATTCTACGAGGCTTTTGGTCTG 2031
Sus3-65 GGCCAGTTGCGATGGATTTCTTCCCAGATGAACCGTGTGAGGAATGGGGAACTCTACAGGTACATTGCTGACACCAGGGGAGCTTTCGTGCAGCCTGCATTCTACGAGGCTTTCGGTCTG 2031
Majority ACTGTTGTTGAAGCXATGACTTGTGGTTTGCCTACATTTGCAACTAATCAXGGTGGTCCAGCTGAGATCATCGTTCACGGAAAGTCCGGXTTCCAXATTGATCCATATCATGGCGAGCAA
2050 2060 2070 2080 2090 2100 2110 2120 2130 2140 2150 2160
3.3 ACGGTGGTTGAAGCTATGACTTGTGGTCTTCCAACATTTGCAACTTGTCATGGTGGTCGTATGAGATCATTCAGGAGGGTGTATCCGGGTACCATATAGATCCTTATCATCCCAATAAA 2160
Sus4-16 ACTGTTGTCGAAGCAATGACTTGTGGTTTGCCTACATTTGCAACTAATCACGGTGGTCCAGCTGAGATCATCGTTCATGGAAAGTCCGGCTTCCACATTGATCCATATCACGGTGAGCAA 2151
Sus3-65 ACTGTTGTTGAGGCCATGAGCTGCGGTTTGCCTACATTTGCAACAAATCAAGGTGGTCCAGCTGAGATCATCGTTCACGGAAAGTCTGGTTTCCAAATTGATCCATACCATGGCGAGCAG 2151

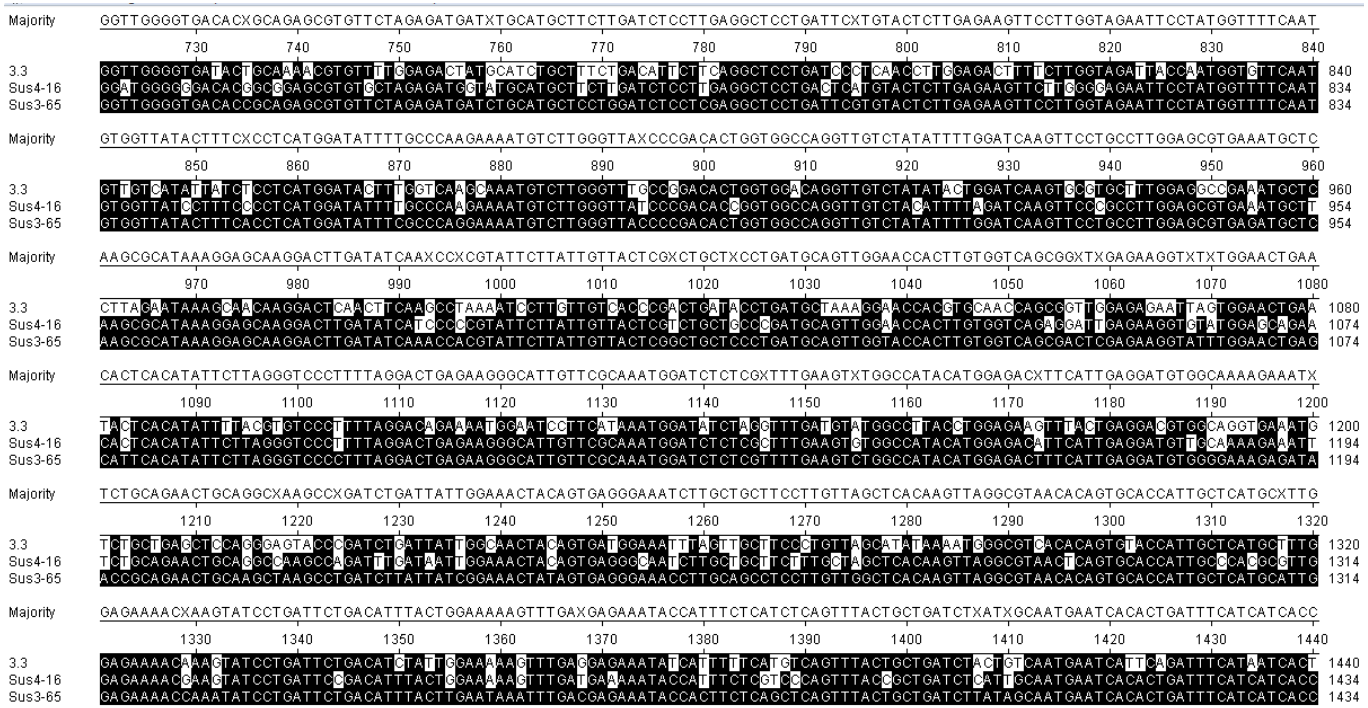


Decoration 'Decoration #1': Shade (with solid black) residues that match Sus4-16 exactly.

Gene name: sus4

Primer name	**position**	**Sequence (5′ to 3′)**	**Length (nt)**	**Tm (C[1])**	**GC%[2]**	**Expected product length (nt)**
Sus4-16-F1	661-682	CCCCCAGATACTCCATATTTC	21	58	42,86	151
Sus4-16-R1	812-792	AACTTCTCAAGAGTACATGAG	21	58	38,10	
Sus4-16-F2	1461-1479	AGGAAGCAAGGACACTGTG	19	58	36,00	152
Sus4-16-R2	1613-1593	GGAGTACGAGAAGTAGAGG	19	58	52,00	


Majority ATGGCTGAACGTGTTCTGACTCGTGTTCATAGCCTTCGTGAACGTGTTGATGCTACTTTAGCTGCTCACCGCAATGAGATAXTGCTXTTTCTTTCAAGGATCGAAAGCCACGGAAAAGGG
10 20 30 40 50 60 70 80 90 100 110 120
3.3 ATGTCGAATCCAAAGTTCACAAGAGTACCTAGTATGAGAGAGAGTTGAGGATACTCTATCTGCTCACCGTAATCAGCTAGTGGCTCTTCTATCGAGATATGTGGCGCAGGGAAGGGG 120
Sus4-16 ATGGCTGAACGTGTTCTGACTCGTGTTCATAGCCTTCGTGAACGTGTTGATGCAACTTTAGCTGCTCACCGCAATGAGATACTGCTGTTTCTTTCAAGGATCGAAAGCCACGGAAAAGGG 120
Sus3-65 ATGGCTGAACGTGTTCTGACTCGTGTTCACAGCCTTCGTGAACGTCTTGATGCTACTTTGGCTGCTCATCGCAATGAAATTTTGCTCTTTCTTTCAAGGATCGAAAGCCACGGAAAAGGG 120
Majority ATATTGAAACCTCACCAGCTXXTGGCTGAGTTCGATXCAATTCXCXAAGATGACA------AAGACAAACTGAAXGAACATGCCTTTGAAGAAXTCCTGAAATCCACTCAGGAAGCXATT
130 140 150 160 170 180 190 200 210 220 230 240
3.3 ATATTGCAACCTCATCACTTAATTGATGAGTTCAATAGTGCTGTATGTGATGACACTGCTTGTGAAAAGCTCAAAGAAGGCGCCTTTTGTGAAATCTTGAAATCTACTCAGGAAGCGATT 240
Sus4-16 ATATTGAAACCTCACGAGCTTTTGGCTGAGTTCGATGCAATTCGCCAAGATGACA------AAAACAAACTGAACGAACATGCATTCGAAGAACTCCTGAAATCCACTCAGGAAGCGATT 234
Sus3-65 ATCTTGAAACCTCACCAGCTGCTGGCTGAGTTTGAATCAATTCACAAAGAAGACA------AAGACAAACTGAATGATCATGCCTTTGAAGAAGTCCTGAAATCCACTCAGGAAGCAATT 234
Majority GTTCTXCCCCCATGGGTTGCACTTGCTATTCGTTTGAGGCCTGGTGTXTGGGAATATGTCCGTGTGAACGTXAATGCTCTXATTGTTGAGGAGCTGACTGTXCCTGAGTATTTGCAATTC
250 260 270 280 290 300 310 320 330 340 350 360
3.3 GTGCTCCCACCATTTGTTGCTATAGCAGTTCGTCCAAGGCCAGGTGTTTGGGAATATGTTCGTGTTAACGTATATGATCTGAGTGTTGAACAATTGACTATTCCTGAATATCTCCGTTTC 360
Sus4-16 GTTCTGCCCCCTTGGGTTGCACTTGCTATTCGTTTGAGGCCTGGTGTCTGGGAATACATCCGTGTGAACGTCAATGCACTAGTTGTCGAGGAGCTGTCCGTCCTGAGTATTTGCAATTC 354
Sus3-65 GTTTTACCCCCATGGGTTGCACTTGCTATTCGTTTGAGGCCTGGTGTGTGGGAATATGTCCGTGTGAATGTTAATGCTCTTATTGTTGAGGAGCTGACTGTGCCTGAGTTTTTGCAATTC 354
Majority AAGGAAGAACTTGTXGACGGAGCXTCXAATGATAACTTTGTTCTTGAGTTGGATTTTGAGCCXTTCACTGCATCATTTCCTAAACCAACCCTCACCAAATCXATTGGAAATGGAGTTGAA
370 380 390 400 410 420 430 440 450 460 470 480
3.3 AAAGAAGAACTTGTGGATGGAGAGGATAATAATCTCTTTGTGCTTGAGCTGGATTTTGAACCATTTAATGCATCAGTTCCTCGTCCATCACGCTCTTCATCCATTGGAAATGGAGTCCAA 480
Sus4-16 AAGGAAGAACTTGTCGACGGAGCCTCGAATGGAAATTTCGTTCTCGAGTTGGATTTCGAGCCTTTCACTGCATCGTTTCCTAAACCAACCCTCACCAAATCTATTGGAAATGGAGTTGAA 474
Sus3-65 AAGGAAGAACTTGTTAACGGAACTTCCAACGATAACTTTGTTCTTGAATTGGATTTTGAGCCCTTCACTGCATCATTTCCCAAACCAACCCTCACCAAATCAATTGGAAATGGAGTTGAA 474
Majority TTCCTCAATAGGCACCTCTCTGCCAAAATGTTTCATGACAAGGAAAGCATGACCCCGCTTCTCGAXTTTCTTCGAGXTCACCATTATAAGGGXAAGACAATGATGCTGAATGATAGXATA
490 500 510 520 530 540 550 560 570 580 590 600
3.3 TTCCTCAATCGTCATTTGTCCTCAAATATGTTTCGCAGCAAAGAATCTCTCGACCCGTTACTTGATTTCCTTAGAGGACACAATCATAAAGGGAATGTCTTGATGTTGAATGAACGTATA 600
Sus4-16 TTCCTCAATAGGCACCTCTCTGCCAAAATGTTCCATGACAAGGAAAGCATGACCCCGCTTCTCGAATTTCTTCGGCGCTCACCATTATAAGGGCAAGACAATGATGCTGAATGATAGGATA 594
Sus3-65 TTCCTCAACAGGCACCTCTCTGCCAAAATGTTTCATGACAAGGAAAGCATGACCCCTCTTCTCGAGTTTCTTCGAGTTCACCACTACAAGGGAAAGACAATGATGCTAAATGATAGAATT 594
Majority CAGAATTTGTATACTCTXCAAAAXGTCCTXAGGAAGGCAGAGGAATACCTCACXAXGCTTCCCCCAGATACXCCATATTCXGAATTTGAGCACAAGTTCCAAGAAATXGGCTTGGAGAAG
610 620 630 640 650 660 670 680 690 700 710 720
3.3 CAGCGAATTCTCCAGGCTGGAGTCGTCTCTTAATAAAGCAGATGATTATCTCTCAAAGCTACCACCAGATACACCCTATACTGAGTTTGAATATGCATTGCAAGAAATGGGCTTTGAGAAA 720
Sus4-16 CAGAATTCGAATACTCTTCAAAATGTCCTAAGGAAGGCAGAGGAATACCTCATTATGCTTGCCCCAGATACTCCATATTTCGAATTCGAGCACAAGTTCCAAGAAATCGGATTGGAGAAG 714
Sus3-65 CAGAATTTGTATACTCTCCAAAAAGTCCTGAGGAAGGCTGAGGAATACCTCACCACCCTTTCCCCAGAAACGTCATACTCGGCATTTGAGCACAAGTTCCAAGAAATTGGCTTGGAGAGG 714


Majority GGTTGGGGTGACACXGCAGAGCGTGTTCTAGAGATGATXTGCATGCTTCTTGATCTCCTTGAGGCTCCTGATTCXTGTACTCTTGAGAAGTTCCTTGGTAGAATTCCTATGGTTTTCAAT
730 740 750 760 770 780 790 800 810 820 830 840
3.3 GGTTGGGGTGATACTGCAAAACGTGTTTTGGAGACTATGCATCTGCTTTCTGACATTCTTCAGGCTCCTGATCCGTCAACCTTGGAGACTTTTCTTGGTAGATTACCAATGGTGTTCAAT 840
Sus4-16 GGATGGGGGGACACGGCGAGCGTGTGCTAGAGATGGTATGCATGCTTCTTGATCTCCTTGAGGCTCCTGACTCATGTACTCTTGAGAAGTTCTTGGGGAGAATTCCTATGGTTTTCAAT 834
Sus3-65 GGTTGGGGTGACACCGCAGAGCGTGTTCTAGAGATGATCTGCATGCTCCTGGATCTCCTCGAGGCTCCTGATTCGTGTACTCTTGAGAAGTTCCTTGGTAGAATTCCTATGGTTTTCAAT 834
Majority GTGGTTATACTTTCXCCTCATGGATATTTTGCCCAAGAAAATGTCTTGGGTTAXCCCGACACTGGTGGCCAGGTTGTCTATATTTTGGATCAAGTTCCTGCCTTGGAGCGTGAAATGCTC
850 860 870 880 890 900 910 920 930 940 950 960
3.3 GTTGTCATATTATCTCCTCATGGATAGTTTCGTCAAGCAAATGTCTTGGGTTTGCCGGACACTGGTGGACAGGTTGTCTATATACTGGATCAAGTGCGTGCTTTGGAGGCGAAATGCTC 960
Sus4-16 GTGGTTATCCTTTCCCCTCATGGATATTTTGCCCAAGAAAATGTCTTGGGTTATCCCGACACCGGTGGCCAGGTTGTCTACATTTTAGATCAAGTTCCCGCCTTGGAGCGTGAATGCTT 954
Sus3-65 GTGGTTATACTTTCACCTCATGGATATTTCGCCCAGGAAAATGTCTTGGGTTACCCCGACACTGGTGGCCAGGTTGTCTATATTTTGGATCAAGTTCCTGCCTTGGAGCGTGAGATGCTC 954
Majority AAGCGCATAAAGGAGCAAGGACTTGATATCAAXCCXCGTATTCTTATTGTTACTCGXCTGCTXCCTGATGCAGTTGGAACCACTTGTGGTCAGCGGXTXGAGAAGGTXTXTGGAACTGAA
970 980 990 1000 1010 1020 1030 1040 1050 1060 1070 1080
3.3 CTTAGAATAAAGCAACAAGGACTCAACTTCAAGCCTAAAATCCTTGTTGTCACCCGACTGATACCTGATGCTAAAGGAACCACGTGCAACCAGCGGTTGGAGAGAATTAGTGGAACTGAA 1080
Sus4-16 AAGCGCATAAAGGAGCAAGGACTTGATATCATCCCCCGTATTCTTATTGTTACTCGTCTGCTGCCCGATGCAGTTGGAACCACTTGTGGTCAGAGGATTGAGAAGGTGTATGGAGCAGAA 1074
Sus3-65 AAGCGCATAAAGGAGCAAGGACTTGATATCAAACCACGTATTCTTATTGTTACTCGGCTGCTCCCTGATGCAGTTGGTACCACTTGTGGTCAGCGACTCGAGAAGGTATTTGGAACTGAG 1074
Majority CACTCACATATTCTTAGGGTCCCTTTTAGGACTGAGAAGGGCATTGTTCGCAAATGGATCTCTCGXTTTGAAGTXTGGCCATACATGGAGACXTTCATTGAGGATGTGGCAAAAGAAATX
1090 1100 1110 1120 1130 1140 1150 1160 1170 1180 1190 1200
3.3 TACTCACATATTTTACGTGTCCGTTTAGGACAGAAATGGAATCCTTCATAAATGGATATCTAGGTTTGATGTATGGCCTTACGTGGAGAAGTTTACTGAGGACGTGCCAGGTGAAATG 1200
Sus4-16 CACTCACATATTCTTAGGGTCCCTTTAGGACTGAGAAGGGCATTGTTCGCAAATGGATCTCTCGCTTTGAAGTGTGGCCATACATGGAGACATTCATTGAGGATGTTGCAAAGAAATT 1194
Sus3-65 CATTCACATATTCTTAGGGTCCCCTTTAGGACTGAGAAGGGCATTGTTCGCAAATGGATCTCTCGTTTTGAAGTCTGGCCATACATGGAGACTTTCATTGAGGATGTGGGGAAAGAGATA 1194
Majority TCTGCAGAACTGCAGGCXAAGCCXGATCTGATTATTGGAAACTACAGTGAGGGAAATCTTGCTGCTTCCTTGTTAGCTCACAAGTTAGGCGTAACACAGTGCACCATTGCTCATGCXTTG
1210 1220 1230 1240 1250 1260 1270 1280 1290 1300 1310 1320
3.3 TCTGCTGAGCTCCAGGGAGTACCGATCTGATTATTGGCAACTACAGTGATGGAAATTTAGTTGCTTCCCTGTTAGCATATAAAATGGGCGTCACACAGTGTACCATTGCTCATGCTTTG 1320
Sus4-16 TCTGCAGAACTGCAGGCCAAGCCAGATTTGATAATTGGAAACTACAGTGAGGGCAATCTTGCTGCTTCTTGCTAGCTCACAAGTTAGGCGTAACTCAGTGCACCATTGCCCACGCGTTG 1314
Sus3-65 ACCGCAGAACTGCAAGCTAAGCCTGATCTTATTATCGGAAACTATAGTGAGGGAAACCTTGCAGCCTCCTTGTTGGCTCACAAGTTAGGCGTAACACAGTGCACCATTGCTCATGCATTG 1314
Majority GAGAAAACXAAGTATCCTGATTCTGACATTTACTGGAAAAAGTTTGAXGAGAAATACCATTTCTCATCTCAGTTTACTGCTGATCTXATXGCAATGAATCACACTGATTTCATCATCACC
1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430 1440
3.3 GAGAAAACAAAGTATCCTGATTCTGACATCTATTGGAAAAAGTTTGAGGAGAAATATCATTTTTCATGTCAGTTTACTGCTGATCTACTGTCAATGAATCATTCAGATTTCATAATCACT 1440
Sus4-16 GAGAAAACGAAGTATCCTGATTCCGACATTTACTGGAAAAAGTTTGATGAAAATACCATTTCTCGTCCCAGTTTACCGCTGATCTCATTGCAATGAATCACACTGATTTCATCATCACC 1434
Sus3-65 GAGAAAACCAAATATCCTGATTCTGACATTTACTTGAATAAATTTGACGAGAAATACCACTTCTCAGCTCAGTTTACTGCTGATCTTATAGCAATGAATCACACTGATTTCATCATCACC 1434


Majority AGCACCTTCCAGGAGATAGCAGGAAGCAAGGACACTGTTGGACAGTATGAGAGCCATATGGCXTTCACAATGCCTGGATTGTATAGAGTTGTTCATGGCATTGATGTGTTCGATCCCAAA
1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
3.3 AGTACCTATCAAGAGATTGCAGGAACGAAGAATACTGTTGGTCAGTATGAGAGTCATACTGCTTTCACTCTCCAGGCCTATATCGTGTTGTCCATGGCATTGATGTTTTCGATCCCAAA 1560
Sus4-16 AGCACCTTCCAGGAGATAGCAGGAAGCAAGGACACTGTGGGACAATATGAGAGCCATATGGCATTCACAATGCCTGGATTGTACAGAGTTGTTCATGGCATTAATGTGTTCGAGCCCAAA 1554
Sus3-65 AGCACCTTCCAGGAGATAGCAGGAAGCAAGGACACCGTTGGACAGTATGAGAGCCACATGGCCTTCACAATGCCTGGATTGTATAGAGTTGTTCATGGCATTGATGTGTTCGATCCCAAA 1554
Majority TTCAACATTGTGTCACCTGGAGCTGATATGAAXCTCTACTTCCCATACTCCGAAAAGGAAAAGAGACTGACAXCTTTTCACCCTGAAATTGAXGAGTTGCTGTTTAGTGATGTXGAGAAT
1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680
3.3 TTCAATATAGTGTCTCCTGGAGCTGACATGACAATTTACTTCCCATATTCTGACAAGGAAAAGAGACTGACATCTTTGCATCCCTCGATTGAGAAGTTGCTTATTTGATCCTGAGCAGAAT 1680
Sus4-16 TTCAACATTGTGTCACCTGGAGCTGATATTAACCTCTACTTCTCGTACTCCGAAACGGAAAAGAGACTTACAGCATTTCACCCTGAAATTGATGAGCTGCTGTATAGTGAGGTTGAGAAT 1674
Sus3-65 TTCAACATTGTGTCACCAGGAGCTGATGTGAATCTCTATTTTCCATACTCCGAAAAGGAAAAGAGATTGACAACTTTTCACCCTGAAATCGAAGACTTGCTGTTTAGCGATGTCGAGAAT 1674
Majority GAAGAACATCTGTGTGTGCTGAAGGATAGGACTAAACCXATXATATTCACAATGGCAAGGTTGGACCGXGTGAAGAACTTAACTGGACTXGTTGAGTGGTATGCTAAGAATCCACGACTA
1690 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800
3.3 GAAGTGCATATAGGCAACCTGAATTGATCAATCAAAACCGATAATTTTTTCAATGGCAAGGCTTGACCGGTCAAGAACATTACGGGATTAGTTGAGTGGTATGCTAAAAATGCCACACTC 1800
Sus4-16 GAGGAACATCTGTGTGTGCTCAAGGATAGGACTAAACCAATTTTATTCACAATGGCAAGGTTGGATCGTGTGAAGAATTTAACTGGACTTGTTGAGTGGTAGGCGAAGAATCCACGACTA 1794
Sus3-65 GAAGAACACCTGTGTGTGTTGAAGGACAGGAATAAGCCCATCATATTCACCATGGCAAGATTGGACCGAGTGAAGAACTTAACTGGACTCGTCGAGTGGTATGCTAAGAATCCACGACTA 1794
Majority AGGGAATTGGTTAACCTTGTTGTAGTTGGTGGAGAC- - CGAAG- AAAGGAATCCAAAGATTTGGAAGAGCAGGCAGAGATGAAGAAGATGTATGAXCTTATAAAGACTCATAATTTGAAT
1810 1820 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920
3.3 AGGGAATTGGCAAACCTTGTTGTAGTACCTGGATACAACGATGTAAAGAAATCCAATGATAGAGAAGAATAGCAGAAATTGAGAAGATGCATGCTCTTATGAAGGAACATAAGTTGGAT 1920
Sus4-16 AGGGGATTGGTTAACCTGGTTGTAGTTGGCGGAGAT- - CGAAG- GAAGGAATCCAAAGATTTGGAAGAGCAGGCAGAGATGAAGAAGATGTATGAGCTAATAGAGACTCATAATTTGAAT 1911
Sus3-65 AGGGAGTTGGTTAACCTTGTTGTGGTTGGTGGAGAC- - CGAAG- AAAGGAATCCAAAGACTTGGAAGAGCAGGCAGAGATGAAGAAGATGTATGAACTTATAAAGACTCACAATTTGAAT 1911
Majority GGCCAATTCAGATGGATTTCTTCCCAGATGAACCGXGTGAGGAATGGTGAGCTCTACCGXTACATTGCTGACACXAGGGGAGCTTTCGTTCAGCCTGCATTCTACGAGGCTTTTGGTCTG
1930 1940 1950 1960 1970 1980 1990 2000 2010 2020 2030 2040
3.3 GGTCAATTGAGATGGATATCAGCCCAATGAACCGGGCAGGTAATGGTGAGCTCTATCGCTATATAGCTGACAAGAGAGGTATATTTGTTCAGCCTGCATTTTATGAAGCGTTTGGACTC 2040
Sus4-16 GGCCAATTCAGATGGATTTCTTCCCAGATGAACCGAGTGAGGAATGGTGAGCTCTACCGATACATTGCTGACACTAAGGGAGCTTTCGTTCAGCCTGCATTCTACGAGGCTTTTGGTCTG 2031
Sus3-65 GGCCAGTTCCGATGGATTTCTTCCCAGATGAACCGTGTGAGGAATGGGGAACTCTACAGGTACATTGCTGACACCAGGGGAGCTTTCGTGCAGCCTGCATTCTACGAGGCTTTCGGTCTG 2031
Majority ACTGTTGTTGAAGCXATGACTTGTGGTTTGCCTACATTTGCAACTAATCAXGGTGGTCCAGCTGAGATCATCGTTCACGGAAAGTCCGGXTTCCAXATTGATCCATATCATGGCGAGCAA
2050 2060 2070 2080 2090 2100 2110 2120 2130 2140 2150 2160
3.3 ACGGTGGTTGAAGCTATGACTTGTGGTGTTCCAACATTTGCAACTTGTCATGGTGGTCCTATGGAGATCATTCAGGACGGTGTATCCGGGTACCATATAGATCCTTATCATCCCAATAAA 2160
Sus4-16 ACTGTTGTTGAAGCAATGACTTGTGGTTTGCCTACATTTGCAACTAATCACGGTGGTCCAGCTGAGATCATCGTTCATGGAAAGTCCGGCTTCCACATTGATCCATATCACGGTGAGCAA 2151
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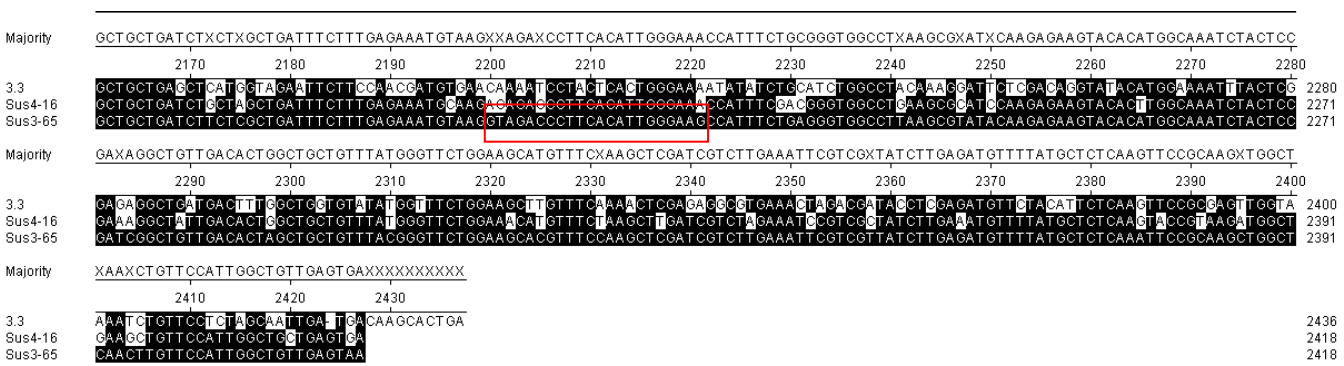

Decoration 'Decoration #1': Shade (with solid black) residues that match Sus3-65 exactly.

Gene name: sus3

Primer name	**position**	**Sequence (5′ to 3′)**	**Length (nt)**	**Tm (C[1])**	**GC%[2]**	**Expected product length (nt)**
Sus3-65-F1	367-388	GAACTTGTTAACGGAACTTCC	21	60	43	
Sus3-65-R1	566-546	TAGTGGTGAACTCGAAGAAAC	21	60	43	199
Sus3-65-F2	2043-2061	TGTTGTTGAGGCCATGAGC	19	58	52,60	
Sus3-65-R2	2221-2200	TCCCAATGTGAAGGGTCTAC	20	60	50,00	178