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Towards the identification of Tudor SN interactome: cloning of putative interactors

- Mot identifieringen av Tudor SN interaktomet: kloning av förmodade interagerande proteiner

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Abstract

Tudor staphylococcal nuclease (TSN) is a protein found associated with stress granules (SG) and processing bodies (PB) where it acts as a part of the plant stress response. In plants not much is known for TSN. This project serves as a starting point in our attempt to elucidate the physically interacting proteins of TSN. Previously, our group identified proteins that can be physical interactors of TSN. Herein, we focused on TCTP, MAPK, Armadillo, FY, DHH1 and Kin11. By using overlay PCR, I fused the cDNAs of these genes with their corresponding promoters, and by using the Gateway technology I finally introduced these fusions to a binary vector which contains the protein fluorescent marker GFP. These binary vectors were transformed into *Agrobacterium tumefaciens*. In parallel, the promoters of these genes were introduced into a binary vector upstream of a uidA gene (encoding β-glucoronidase; GUS). In the future the aforementioned transformed Agrobacterium cells will be used to transform Arabidopsis plants to confirm the interactions of the above mentioned proteins with TSN and its association with SG and PB.

Introduction

Rapidly increasing world population makes us stand in front of a bigger demand on crops, especially in the developing countries of the world. At the same time our harvests stands in front of bigger challenges due to global warming and a more rapid change in climate with flooding, storms, an increased selection of pathogens and sudden heat peaks. These environmental challenges cause stress reactions in plants.

Plants have evolved through centuries to withstand changing climate and stress and can adapt to slow changes, for example changes in season. But our crops are seldom selected for stress tolerance but for yield which makes them vulnerable when changes in weather are too fast for them to adapt, leaving reduced harvests and starving people. Luckily there are breeding methods in today´s science that can improve plants tolerance to stress situations but for research to accomplish this there is a demand of knowledge of how the stress reactions affect plants and what the plants evolved response looks like.

Sudden heat peaks are perhaps the stress factor of most importance in plants. Plants responses to heat stress vary with duration, temperature and plant type. The plant pathways and metabolism are immediately affected by a moderate temperature increase since enzymes have temperature sensitive reaction optima and tend to unfold and aggregate. Heat will also affect the fatty acids in membranes of cells and make them more fluid. [2]. These events eventually lead to cell starvation, decreased ion influx, growth inhibition, production of toxic compounds and reactive oxygen species

(ROS). [2,3]. Plants can to some extent tolerate heat stress by several mechanisms. At an early stage the photosynthetic activity is reduced by several coordinated factors such as stomatal closure and decreased activity of photosynthetic enzymes. The metabolism also receives signals to act upon the osmotic imbalance that lack of water results with. It does this by producing osmoprotectants that works as osmotic imbalance agents in the cell by increasing the solute concentration in the cytoplasm to the surrounding environment outside the cell in this way protecting the sensitive proteins thus the normal metabolism can continue [4].

If the plants´ survival mechanisms are not sufficient the stress reaction culminates in a response known as cell death as the plant attempts to isolate the infected area or withdraw resources from the growing parts in order to survive. The mechanisms in plants which lead to cell death during stress are collectively known as programmed cell death (PCD). These mechanisms are finely tuned and occur in eukaryotes not only due to stress but as a part of the development and cell differentiation. PCD involves an activation of a specific set of genes that orchestrate the disassembly of cellular components which ultimately leads to cell death. PCD in plants is of major importance for the plant survival and development. It can be found during many growth stages starting in early embryogenesis and further on for example in leaf remodelling and senescence. The PCD machinery includes a number of different agents either working together or individually. These can be nucleases, caspase-like molecules, reactive oxygen species (ROS) and the mitochondrial released protein Cytochrome *c.* PCD in plants have similarities to animal PCD, possibly because of a common ancestor or even a symbiosis between proto-mitochondria and archaebacteria in ancient time [5]. Upon PCD-signals the plant cell protoplast will retract from the cell wall [6] caspase-like molecules cleave caspases which lead to activation of enzymes cleaving the DNA.[7,8] and enzymes will start degrade the rest of the organelles. However some of the organelles are needed to produce the destructive enzymes thus the mitochondria are functioning during the early stages of the PCD [9].

In stress situations the survival of the plant depends highly on faster responses than the previously described PCD -which takes around 6 hours- here post –transcriptional amendments are of great importance. One example is by preventing mRNA to reach the ribosomes and get translated into a protein. By stalling the protein translation in the cell the cell holds back anabolic processes that are related to growth and development and re-orientates its energy to reactions are needed to endure the stress situation, slightly like pulling the handgear. So how do the cells achieve this? Two types of cellular aggregates called Stress granules (SG) and Processing bodies (PG) which were earlier dismissed as cell artefacts in the cytoplasm has now been shown to be responsible for this function. These large cellular agglomerates are clustering with mRNA, proteins, eukaryotic initiation factors

(eIFs) and various other molecules [11-14]. Studies has also found that they carry RISC complexes that would indicate that they are integrated with miRNA-induced silencing pathways which would imply that they have a role in cellular pathways and cell fate decisions [15-17]. Even though PBs and SGs seem to perform similar tasks in silencing they are distinct from each other in several ways. PBs are present in the cytoplasm in unstressed cells but increase in number upon stress whereas SGs are only present under stress conditions. SGs seem to have a somewhat fixed position in the cytoplasm but changes appearance by fusions and divisions, PBs on the other hand move around rapidly without changing their shape. Occasionally these two aggregates associate and there, in this case there is a possibility of exchange of messenger ribonucleoproteins (mRNPs) and mRNA. [18]

The evolutionary conserved Tudor Staphylococcal nuclease (TSN) proteins have been identified as PB and SG components. The domain architecture of TSN consist of two subunits, one being a tandem repeat of four Staphylococcal Nuclease (SN) domains linked with a Tudor and one additional SN at the C-terminal [19]. The two domains both interact physically with various proteins [20-22] and the SN domains can bind to RNA due to its nucelolytic activity [23, 24]. TSN has been identified as components of the machinery that is involved in the regulation of mRNA levels [27-30]

The goal of this project was to transform translational fusions of proteins with GFP that have been previously identified by our group as TSN interacting proteins by TAP tag purification using *Arabidopsis* plants expressing Tap-TSN1, Tap-TSN 2 and Tag-GFP [29]. To achieve this, I established a protocol for ligating promoters with the corresponding cDNAs, using fusion PCR. The promoter-cDNA fusions were subcloned into destination vectors using Gateway technology. Furthermore, I constructed vectors in which I introduced the promoter of these genes upstream of GUS.

Materials and methods

Gene accessions used in this study

TCTP, MAPK, Armadillo, FY, DHH1 and Kin11.

Oligonucleotide sequences

All oligonucleotides used in this study are presented in Appendix 1.

Extraction of DNA from Arabidopsis plants

Leaf discs of ~0.5 cm^2 of *Arabidopsis columbia* were grinded and mixed with 30 µl 0.5 NaOH. Next 370 µl 100 mM Tris buffer pH~7 were added and the solution vortexed and stored at -20 ˚C. Two to 10 µl of DNA were used in the PCR-reactions.

Polymerase chain reaction

All PCR reactions in this study have used the following protocol unless otherwise stated. A 2x phusion master mix (Thermo scientific) of was used. Oligonucleotides concentrations was 10 µM reaction volumes was 50 uL. PCR was conducted on thermal cycler (Bio Rad) under the following conditions: 98˚C for 1 min, 98 ˚C for 30 s, 60 ˚C for 45 s and 72 ˚C for 2 min for 35 cycles. Products were resolved on a 0.5 µg/ml Ethidium Bromide (EtBr) containing 1% (w/v) agarose gel, ran at 80V. Molecular sizes were estimated by using a DNA ladder [1 kb plus ladder (Generuler)].

Purification of PCR-product

Buffers and columns from the GeneJET gel extraction kit (Thermo scientific) was used. Binding buffer was added 1:1 with PCR-product and added to column. The rest of the procedure was made according to manufacturer´s instructions with minor modifications. The product was eluted with 15µl mQ-water instead of elution buffer. The concentrations of the DNA were estimated using NanoDrop (Fisher scientific).

Purification of PCR-products from gel

Buffers and columns from the GeneJET gel extraction kit (Thermo scientific) were used. Binding buffer was added in equal volume to the PCR-product volume and the mixture was applied to the column. The rest of the procedure was made according to the manufacturer´s instructions with minor modifications. The product was eluted with 15µl mQ-water instead of elution buffer. The concentrations of the DNA were estimated using NanoDrop (Fisher scientific).

Fusion PCR

The concentration of DNA was diluted till 10-20 ng/µl for the fusion PCR. The cDNA was mixed at almost equal amount with the corresponding promoter-DNA. Fusion PCR was done without primers for one cycle with an annealing temperature of 65 ˚C and a final elongation time of 4 min. The amplification was conducted with attB1 and attB 2 primers (see appendix 3 for sequences) or gene specific primers for the individual constructs. The annealing temperature was 65 ˚C, and cycles 20-25.

BP cloning

The fusions were subcloned into pDONR/Zeo using the BP recombination (Invitrogen). Equimolar amount of the vector and the construct were added to the reaction in a final volume of 5 µl,

containing 1 µl of the BP Clonase. The reactions were incubated for 1.5 hours at RT. Reactions were terminated by the addition of 1 uL proteinase K, and incubation at 37 ˚C for 10 min. Subsequently, 2.5 uL of each reaction were transformed in E. coli NEB10 beta cells (New England Biolabs), according to the manufacturer's instructions. The cells were spread on solidified LB agar-containing petri dishes supplemented with100 µg mL^{-1} Zeocin. Dishes were incubated at 37 ˚C overnight.

Colony PCR

Individual colonies from the plates, 2 colonies per plate were sampled and diluted in 50 µl of H_2O. The tubes were placed at 95 ˚C for 10 min. The samples were then centrifuged for ~2 min and 1-2 µl supernatant was used as the template for the PCR-reaction. The attB1 and attB2 or gene specific primers were used. The annealing temperature was 65 ˚C, 30 cycles.

Plasmid extraction

The positive colonies from the colony PCR were sampled for overnight culturing in liquid LB-media (25%) with 100 µg mL^{-1} Zeocin. The colonies were incubated at 28˚C with shaking overnight. The overnight cultures were centrifuged for 1 min and supernatant was discarded. The purification was made using the Miniprep kit and procedure according to the manufacturer´s manual. The concentration of the product was measured using NanoDrop (Fisher scientific).

LR cloning

The confirmed pDONR vectors from the BP reactions were used for LR reactions, either withpGWB3(promoters)or with pGWB4 (promoter-cDNA). The procedure was made in the same way as the previously mentioned BP-cloning. E. coli transformations were done as described for BP reactions.

Digestions

Digestions to confirm the inserts of pDONR and the destination vectors were conducted with various restriction enzymes (see appendix 2). Three µl of the purified DNA from the BP cultures and 6 µl of the LR cultures were mixed with 10X Fast Digestion buffer (Thermo Scientific) and 14 µl mQ water and incubated for 30 min at 37˚C. Samples were analysed by agarose gel electrophoresis as described above.

Agrobacterium transformation

Transformation of *Agrobacterium tumefaciens* GV3101 electrocompetent cells was performed by using a Gene pulser Xcell (BioRad). Twenty µl of Agrobacterium cells were mixed with 1,5 µl of each plasmid purified from the LR cloning in a precooled manufacture cuvette. The settings were: 1500 V,

25 µF, 200 Ω cuvette width of 1 mm. After the pulse, 400 µl of YEP-media was added to the sample and Incubated at 28 ˚C for at least 1.5 h. The cells were spread on selective YEP agar plates with 100 µg mL^{-1} Kanamycin and Rifampicin and incubated for approximate 48 hours.

Results and discussion

Optimizing the fusion PCR reactions

The cDNA and the corresponding promoters (ca. 2kb upstream of ATG start codon) were amplified by individual PCR reactions and visualized using gel electrophoresis. The bands corresponding to the expected size were cut out and purified before Fusion PCR (Fig. 1 and 2). The result of the fusion PCR reactions are shown in figure 3. I was confronted with problems in the fusion PCR, since no sufficient yield or purity was achieved. I troubleshooted the Fusion PCR protocol by making gel purifications of the cDNA and the corresponding promoter prior to the fusion PCR. The conclusion from all the different trials is that the Fusion PCR protocol was probably of less importance whereas the purity and sufficient concentration of the gene PCR product and promoter PCR product was of main importance since I observed less cloning efficiency when purity was low even though concentration was similar.

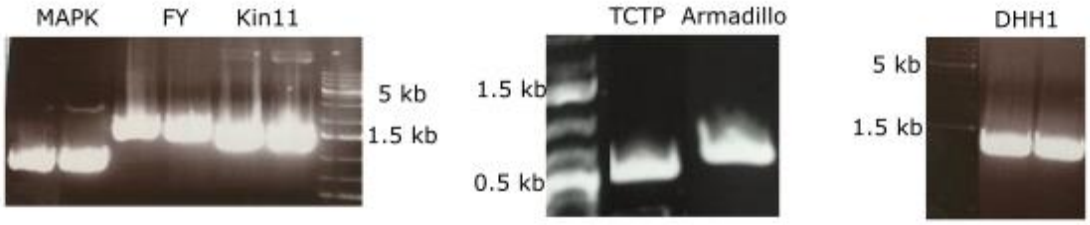

Figure 1. Agarose gel electrophoresis analyses of the cDNAs (MAPK: 1119 bp, FY: 1975 bp, Kin11: 1532 bp, TCTP 570 bp, Armadillo 732 bp and DHH1: 1559 bp).

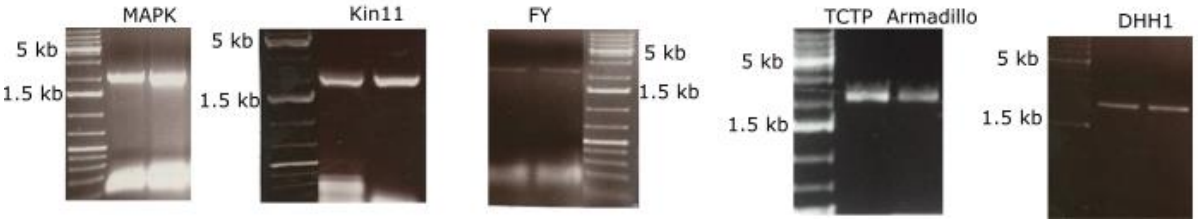

Figure 2. Agarose gel electrophoresis analyses of the promoters (TCTP, MAPK, Armadillo, Kin 11, FY and DHH1). All gene promoters have the approximate size of 2 kb.

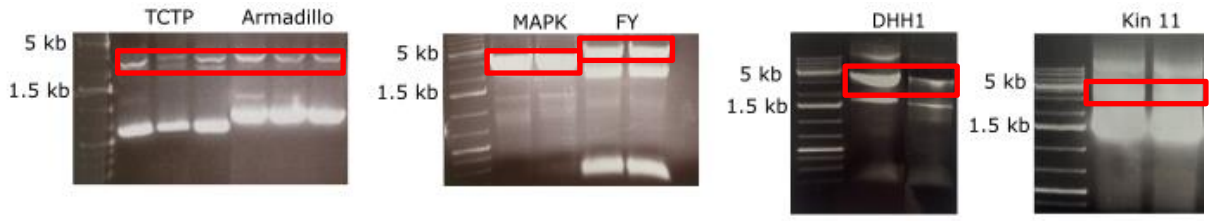

Figure 3. Agarose gel electrophoresis analyses of the of promoter-cDNA fusions. Three replicas were made for TCTP (2650 bp), and Armadillo (2804 bp), two of MAPK (3261 bp), FY (4140 bp), DHH1 (3625 bp) and Kin 11 (3702 bp). The bands corresponding to the size (marked in red) of the fused constructs were cut out and purified.

Cloning into pDONR vector

I extracted the DNA (fusion PCR and promoters) from the gels. The data on these purification concentrations and their purity are shown in table 1.

Next, I introduced the products of fusion PCR and promoters into pDONR/Zeo vector by using Gateway technology and in particular BP clonase.

Fused construct	Concentration (ng/µl)	260/230	Promoter construct	Concentration (ng/µl)	260/230
TCTP	34,1	0,56	TCTP	14,7	0,77
MAPK	136,8	1,81	MAPK	32,9	1,71
Armadillo	19,8	0,43	Armadillo	4,8	0,19
FY	44,4	1,73	FY	44,7	1,21
DHH1	23,5	0,35	DHH1	138,2	1,39
Kin11	25,7	0,84	Kin11	10,1	0,28

Table 1. The received concentrations and purity data of fused constructs and promoters. The 260/230 value is indicating the ratio of absorption at 260 (DNA) and 230 (contaminants).

The pDONR vectors were confirmed by restriction analysis using endonucleases. The gel pictures of the fusions from BP cloning are shown in fig 6, where TCTP, MAPK, DHH1 and Armadillo showed the expected pattern of digestion, whereas no positive clones could be obtained for FY and Kin11. The digestions of the pDONR vectors containing the promoters of DHH1, TCTP, MAPK, Armadillo and

Kin11 are shown in fig. 7, with two replicas for TCTP, MAPK, Armadillo and Kin11. I could not get positive clones for the FY promoter. It is unclear why FY could not be cloned into pDONR. The concentration and 260/230 ratio are not poorer than some of the other constructs which had positive colonies. The FY fusion is ~4000 bp, definitely in the range of the minimum and maximum size for inserts that can be introduced into pDONR/Zeo vector (70-12 000bp) according to the manufacturer, while the GC-content is not extraordinary. None of the ~10 screened colonies of the BP cloning of FY showed presence of the construct so of some reason the construct was not taken up by the vector or in such a small scale that I haven´t come across the positive clones

To ensure that the pDONR vectors contained correct inserts with no point mutations, the plasmids were confirmed by sequencing (GATC, Germany).

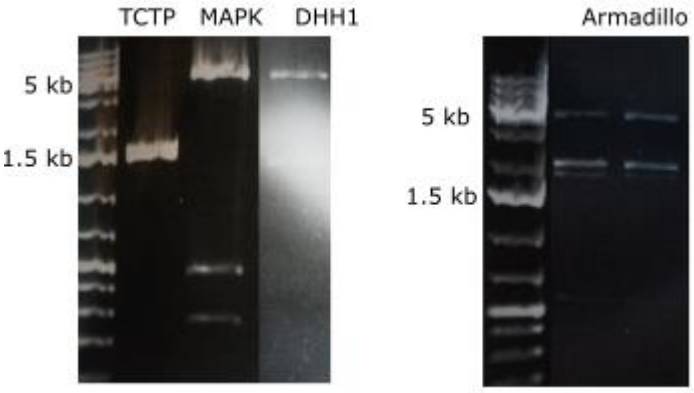

Fig 6. Digestions of fusions from BP cloning in pDONR/Zeo vector and NEB10 cells; TCTP (NCOI enzyme), MAPK (HindIII enzyme), DHH1 (HindIII) and Armadillo (XHOI and ECORI enzymes).

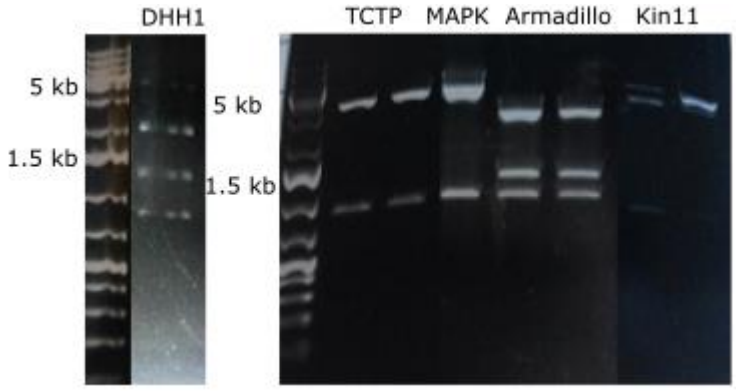

Fig 7. Digestions of promoters from BP cloning in pDONR/Zeo vector and NEB10 cells, all digested with the restriction enzyme ApaLI.

Sublconing into the destination vectors

To proceed with LR-cloning into destination vectors expressing GFP or GUS, colonies from the BP-cloning were incubated overnight and purified. The received concentrations in ng/µl are presented in table 2. The concentrations were used to calculate similar proportions of vector and plasmid in the LR cloning. The vectors were transformed into E.coli (NEB10) cells expressing kanamycin resistance.

Fused construct	Concentration (ng/µl)	260/230	Promoters	Concentration (ng/µl)	260/230
TCTP	67,3	2,19	TCTP	45,9	2.07
MAPK	70,9	2,28	MAPK	66,66	2.11
Armadillo	46,1	2,13	Armadillo	77,59	2.16
FY	-	-	FY	-	-
DHH1	12,5	1,65	DHH1	30,64	1,68
Kin11	-	-	Kin11	47,37	1.87

Table 2. Concentrations of BP-clones, FY promoter and fusion as well as Kin11 constructs were not verified in the digestions thus not continued with.

The colonies including pGWB3 and pGwB4 vectors individually were confirmed by restriction analysis using endonucleases (see appendix 2). The gel picture of the digested promoter-cDNA construct in the pGWB4 vector is shown in fig 8.

To investigate the concentration and the purity of the DNA of clones derived from the LR-cloning to have a successful cloning into Agrobacterium cells the concentration and purity was measured using NanoDrop, see table 3.

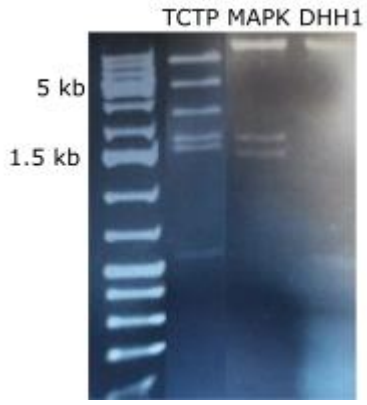

Fig 8. Digestions of fusions from LR cloning in PGWB4 promoter in NEB10 cells; TCTP (NCOI), MAPK (HindIII), DHH1 (HindIII).

Fusions	Concentration (ng/µl)	260/230
TCTP	16.2	2.17
MAPK	13.9	1.98
DHH1	13.87	1.96
Armadillo	-	-
FY	-	-
Kin11	-	-

Table 3. The received concentrations and purity of the DNA purified from the colonies of the LR cloning.

Agrobacterium transformation

The destination vectors were transformed into A. tumefaciens strain GV3101. Previously, this strain had been successfully used for A. thaliana transformation. Colonies of transformed A. tumefaciens were grown in YEP medium and stored as glycerol stocks (50% culture volume mixed with 50% sterile glycerol).

Future perspectives and remarks

The most troublesome and time consuming part in this project was to achieve the fused gene and promoter construct by fusion PCR and gel purification. To avoid this problem, especially for problematic genes such as FY, one can use Multi site Gateway cloning (Invitrogen) technology which allows the subcloning of several constructs at the same time into Expression clones in the LR reaction during a 16 h long incubation time. For this the gene-promoter and gene would have to be cloned into individual pDONR vectors by a BP reaction. The 16 h incubation time is of course a disadvantage compared to 1.5 h for a normal LR-cloning but there are still plenty with time to gain if one is confronted with problems with sufficient concentration of fusion PCR product and their purity.

In the short-term, the transformed *A. tumefaciens* produced by my project will be used to transform *A. thaliana* Col-0 plants using the floral dipping method. I anticipate that the constructed lines will be used to evaluate the localization of the proteins to SGs and PBs and to confirm the interactions with TSN. The promoters linked with GUS will give a good appreciation of the expression of the genes during and after a stress situation by visualization of plant leafs and roots in a confocal microscope. The promoter-cDNA fusions expressing GFP will be used to investigate the localisation of the proteins during and after a stress situation by visualization of plant material in a confocal microscope. Immunostaining of TSN with anti-TSN to have a red marker for the TSN in the same plants as the GFP-expressing proteins will visualize both of the proteins in different colours and one would be able to perfectly observe whether the proteins possibly interact by their signal position.

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29. **Emilio Gutierrez** unpublished data.

Appendix 1 Oligonucleotide sequences and primers

TCTP	Cloning promoter primers egb155 F GGGGACAAGTTTGTACAAAAAAGCAGGCTTGcctgtaattcttccatggtgca egb 156 R *GGGGACCACTTTGTACAAGAAAGCTGGGTAcaccaacatggtcgcttattga* Gene promoter primers egb155 F GGGGACAAGTTTGTACAAAAAAGCAGGCTTGcctgtaattcttccatggtgca egb 158 R ACCGGTGAGAAGATCTTGGTACACCAACATGGTCGCTTATTGA Gene primers egb 159 F TCAATAAGCGACCATGTTGGTGTACCAAGATCTTCTCACCGGT egb 160 R *GGGGACCACTTTGTACAAGAAAGCTGGGTA*gcacttgacctccttcaaac (no STOP) Full oligonucleotide sequence CCTGTAATTCTTCCATGGTGCATGCCACTCTGGACGGGGCCATCTACTAGGCATTCTCTCCATTAAAGCAGCAGTTGACAGATTTCTGTAACACATAG AGGGGAAATGATGGACAAATAATGAATAACTGAAGCATTGATACAAGTTAGATGCATTTCAGGTTAAATAAATATGGCCAAATTTAAATTTCATGAC TCTGAACCACCAATTCACCTTAGTGCGGCTTTATGTACCACCTAGGTATCGCAATCTAGATGTAGTGTCGTTATAGAGAGTCCATAAAAGTAGATAAG GATGCGACACAAACCTTTCGGAAAATGATCCAGGTGCAGGAAGAATGGCCGTGCTTTTGCCTGGATTACCAGTATTGTTCCTTCAATTCAAGAATCA ATAATTATCAGTATCAATCAGCACGATAAGTAGAGCATCAATCACAAAGACAAAACAGGAATAACATTCTTGAGAATGTCGTAAATTATTTATACTAA CTATATGGACTAGTCACATTCCAAATGAAAATGTATACTCACAAGTCTCTTGGCGGTAAAGTTGGACCAACAACTAAAGCTTTCTCTGTTGCGCTTTTT CGAAGTTCCTTGGACCCTTTTGGACCTGCGGAGAAAATAATTTGGTCTTTCAGAACGAGAAACAATAGTCATGAAAAAGGCTAAAAGATTACACTAA CAGCGTTTTTAGTGCAGCAGAAGTTTGTTTGAAAAGATACTCTTAAGAGGATACATTACTGAAACTTGTTTTCCAATCCTAATTATAGACATATATGAA TGAAACTCACAACTAATATAAATAGATATCAGGGAAACTAGAAAATAGGAAACTAGTTTTGTCTCACAACAGGAAAAACAGATAAAAGAACTACCTG GAAGCGCAAGGGCTTTCAATGGAGCCGTCTTTTCACTGTTATGGTCAGCAATACGTGTAGGCTTACTTGCAGGTTCTACGCCTCCAAATGCAACTTGA ATCTATACAAGTAACGTCCAAGTCATTAACTTTTATATTCTACTTGATGTTCACTAAAACGCTTGATGCAAACTAAACATCTTACTATAAAGATGAGGG TAATATGCTAGAAAGCAAACTCAAACAATAGTAGCTGATGAGAAGAATCTAACTCCAGATAAAAGGTAAACTATAAAAGTGATAAGCCATTGACTGT TCGATTTACACCTTATAAAATTTTCTCAGGTTCAACGTCTCTGAAGCTTGCTATAAAGCTATCCATTATCAAACTCAAAACAAAGAAGAAGCGAACAA AATTTTCAACACGATCAAAGATAAAATTTAAAAAATATTCGAAAACCCCCTTACCTTATGGCAGAGCCGAATTCTTTTGCTGCGAGACAAGAACAAAA ACAGACAAGAAGAAGAAATCGTCAATACACATAAAAATTCTGAGAAATTTCGAGAAAAAAAAGTGGACATTGCAAGCGAAACTGTTGATTAACCTT

	TCAGGATCAGGCTGAGGAAATTGGCCATGGACGGGGGAGAAAATTTCACGAGCTCGTCTAACAGATTTTAGACTGAGTTTTTTCAGTGACTGAGTTT CGACCTCCCTGTTCAAAGCTATCATCGTCATTCTAGGGTTTTACACGAACAATCAGAAACAGAACGAGAAAGACGGAAGAGAAGAGAGGGAAATAG GATTTTATCCGACCCGAACACTATTTATACGTAGCCCAACACTCGAATCCCCACCCGTTGGACCAAACCCGGCTCATTAAGCGTCGGTTCAGATTTATT TCCTTTATTTAAAAAAAAGGAAAGGGTAAAAAATAGAAAATTGGAAACAGTTAAAGCCCAAAATTGTAATTTACCGAGAATTGTAAATTTACCTGAA AACCCTACGCTATAGTTTCGACTATAAATACCAAACTTAGGACCTCACTTCAGAATCCCCTCGTCGCTGCGTCTCTCTCCCGCAACCTTCGATTTTCGTT TATTCGCATCCATCGGAGAGAGAAAACAATCAATAAGCGACCATGTTGGTGTACCAAGATCTTCTCACCGGTGATGAGCTTCTGTCTGACTCTTTCCC TTACAAGGAGATTGAGAATGGAATCCTCTGGGAAGTAGAAGGAAAGTGGGTTACTGTGGGAGCTGTAGATGTTAACATTGGTGCCAATCCATCTGC TGAAGAAGGTGGTGAGGATGAAGGTGTTGATGACTCTACTCAAAAGGTTGTTGACATTGTCGACACCTTCAGACTTCAGGAGCAACCAACTTATGAC AAGAAGGGATTCATCGCTTACATTAAGAAATACATTAAGCTTTTGACACCCAAGCTCAGCGAAGAAGATCAAGCTGTCTTCAAGAAGGGTATTGAGG GAGCTACCAAGTTTTTGCTCCCCAGGCTCAGTGACTTCCAATTCTTTGTTGGGGAGGGTATGCATGATGACAGCACTTTGGTCTTTGCTTACTACAAG GAGGGTTCAACTAACCCAACATTTTTGTACTTCGCTCATGGTTTGAAGGAGGTCAAGTGCTGA
MAPK	Cloning promoter primers egb 161 F GGGGACAAGTTTGTACAAAAAAGCAGGCTTGgatggttgatttatacccgct egb 162 R *GGGGACCACTTTGTACAAGAAAGCTGGGTAtttcttcatcgtcttcgtctacttcg* CGAAGTAGACGAAGACGATGAAGAAA Gene promoter primers egb 161 F GGGGACAAGTTTGTACAAAAAAGCAGGCTTGgatggttgatttatacccgct egb 163 R GAGATTATTGCTGAATCCACCTTTCTTCATCGTCTTCGTCTAC Gene primers egb 164 F GTAGACGAAGACGATGAAGAAAGGTGGATTCAGCAATAATCTC egb 165 R *GGGGACCACTTTGTACAAGAAAGCTGGGTA*cacggagaacgtaccagacag (no STOP) Full oligonucleotide sequence GATGGTTGATTTATACCCGCTTAGCAGCTACTACTTCGGTTCAAAAGAAGCTCTTCGCGTCAAGGACGAGATTATCTCTGATCGAGTCATCAGATTGA AATCCAAGTAAATCGAGTTTTGATGTGATTTAGATCATCTGGTTTTTTTCTTATTGTTTTAATCTATAGCTAAAGTTTCTTTTTTGTTTCTTTTGAAGTTA TGCTGCTCATGGATTGAGAACTTGCGTTGAAGCTGTTCTTCTGGTAATTTTATAGTTGGTAGCCAAATTAGGGATTTTGAAAAGTTTTACAGGGAAAA TTGGTTTGAAAGGATTGATTAAAATCTAATGTTGGGACACATCAGGTTGAGTTGTTCAAGCATCCTCATGTTTTGCTTCTTCAATATAGAAACTCCATT TTCAAGCTTCCTGGTGGTCGTTTGAGACCTGGAGAATCAGGTTTAGTATGCTGTTTCCTTGCATCTTTATGCATAAATATCGCTGTCTCAATTCTAAAC GCTAAAGTCATGTTTCTCCTTAATCTTCTAGATATTGAAGGTCTAAAACGGAAACTAGCATCAAAGCTATCGGTTAACGAAAATGTGGGTGTTTCGGG TTATGAGGTAGGAACTATGGCTTCTTGTTAAAGTGCTTTTGTATCTGATTGTTTCTGGCTTAAGAATGGTCTCTCTAATTACAACATTGTTGTAGGTAG GAGAATGTATTGGGATGTGGTGGAGACCCAACTTTGAGACATTGATGTATCCTTTCTTGCCACCTAATATTAAACACCCAAAGGTTGGTTAGTTAGTT TCACTTTCATTGTAAGAAAAAGTTGAGACTTTTGAAGATTTGCGCTTATACTAAAAGCTCTGCTCTATCCTCATCTTTTGTGTAGGAATGCACAAAGCT TTTTCTTGTGAGATTACCGGTACATCAACAGTTTGTTGTGCCTAAGAACTTCAAACTCCTCGCTGTTCCATTGTGTCAACTCCATGAAAATGAAAAGGT ATGTACTATCTTACTTGCATTGCCCTTAACTCATCGTGAACTTTTAATCGATGTTCCTGGTAAAACTAACAGGGTCAGTCAATCAAGCTGTATTTAATTA TGTGTTGTGATGTTTCATTTATGCGTTGCAGACTTATGGGCCGATCATGTCGCAAATTCCTAAGCTTCTTTCGAAATTCTCCTTCAACATGATGGAGAT ATGAACGAACCTCATGATCATTGCCTCTAGTAGCTCTTGCCCCCCGCAATTTGTGATAGGATTATGTTTGGGGAAATCGTTTGTGGAGCCTCTCTGAG TCTGTGACTATATAATGTATATAGGGCTTATAATATATAGACAATGTTGAAGAATAGCATGTTCTTGTTGGTTATAGAGACTAGACATGAAGTATACT GATAGATAGATTAATCAATTCGAAACAATTTTTCTTTGTGTGATAATTTCGATGTGTAATTGAATGTATACATGAGTAAAACAAATATAGGGGTGTAT GCTTATTTGTATATTCCTTGTTGAAGAAAACCTTGTTATCTTCAAACATGAAACCGAGAAACATAAGTAAAGATTTTACTGTTTGTTATATGTGTTTATT GTATTTGATCAAACAATATTTGAAATATTGAAAACTTTCTATAAATATCAAACGTTAGTTTGGCATTCTGCGTTAGCGTTTTGTTGTAGTGGTGATTTT GGGAGTGTTTAAAAACCAAAAGCTAAAGAACGAAGAATCACTGAATTAACCGGTTTGTTTCCGGTTAAAATGTGGATTAAAACTTTTCGGTCAAAGA AAACCGGGAAAAAGAAGTAGTAAATGGGAAAGTCTCTAAGTCGTTGGTCAACGTCGTGAACATGTGCTTGACTAATATTAATTAATGGCTGTTTTAT TTCCAAATTCCAATTTCTGGCTGAAAAGTACATGAATTATGCTTAAAACTGTGTTTTACTTAATATATAATGACGAGGAAACAACAAAAAGGAAGAAG CGAAGAACGAAGTAGACGAAGACGATGAAGAAAGGTGGATTCAGCAATAATCTCAAGCTCGCAATTCCTGTTGCTGGCGAGCAATCCATCACCAAA TTCCTGAGGAAAGGTTTTGGTTCTCTCTGTAGGACTCAAAGCGGTACGTTTAAGGATGGAGATCTACGTGTTAACAAGGATGGAGTTCGAATCATTT CTCAATTGGAGCCTGAAGTCCTGTCTCCAATTAAGCCAGCTGATGATCAGCTGAGCTTGTCGGATTTGGATATGGTTAAAGTCATTGGCAAAGGAAG TAGTGGTGTTGTTCAGCTGGTTCAACACAAATGGACTGGCCAATTTTTCGCCTTGAAGGTCATTCAACTAAATATTGATGAAGCAATTCGCAAGGCAA TTGCACAAGAGCTCAAAATAAATCAATCGTCACAGTGTCCAAATCTTGTTACCTCGTACCAGTCATTTTATGACAATGGCGCAATCTCACTAATCTTGG AGTACATGGACGGAGGATCTCTAGCAGACTTTCTCAAGTCAGTTAAAGCCATCCCTGACTCCTATCTTTCTGCCATCTTTAGACAAGTGCTTCAAGGA TTAATCTATCTTCATCACGATAGGCATATCATCCATCGTGACTTGAAACCATCCAATCTGTTGATCAACCACAGAGGAGAAGTCAAAATAACTGACTTT GGTGTGAGTACCGTTATGACAAACACCGCAGGTTTAGCAAACACATTTGTGGGGACTTACAATTATATGTCTCCAGAGAGAATCGTTGGAAACAAGT ACGGAAATAAAAGTGATATATGGAGCTTGGGTTTAGTAGTACTCGAATGTGCAACAGGAAAGTTCCCTTATGCACCTCCGAATCAAGAGGAAACAT GGACCAGTGTTTTCGAGTTGATGGAAGCCATTGTTGACCAACCGCCACCCGCTCTTCCTTCAGGAAATTTCTCCCCTGAGTTATCTTCATTCATCTCCA CATGTTTGCAGAAGGATCCAAACAGTCGAAGCTCTGCAAAGGAACTGATGGAACATCCTTTCTTGAACAAATACGACTACTCGGGGATCAATCTCGC GTCCTACTTCACAGATGCAGGATCGCCACTTGCAACACTTGGGAACCTGTCTGGTACGTTCTCCGTGTAA
Armadill	Cloning promoter primers egb 166 F GGGGACAAGTTTGTACAAAAAAGCAGGCTTGgaatttgtaattaagtaccgtac egb 167 R *GGGGACCACTTTGTACAAGAAAGCTGGGTAtagaaacatggttttgacggag* CTCCGTCAAAACCATGTTTCTA Gene promoter primers egb 166 F GGGGACAAGTTTGTACAAAAAAGCAGGCTTGgaatttgtaattaagtaccgtac egb 168 R TTTACAGCTCCGGAACTGACTTAGAAACATGGTTTTGACGGAG Gene primers egb 169 F CTCCGTCAAAACCATGTTTCTAAGTCAGTTCCGGAGCTGTAAA egb 170 R *GGGGACCACTTTGTACAAGAAAGCTGGGTAtatgagagggtcatatttttttg* (no STOP)

o	Full oligonucleotide sequence GAAAGAGAGAATATATTTTTTTTTTCTTTTTGAATTTGTAATTAAGTACCGTACTTATTGAAGATTTA TTTTTATTAATTTTGCAGAGGGTGCACGGCGTATATGTTATATGCAATGTATATTTTTTTTTTATGAAGGATGTATAGATGTATAAATAGGGTGTGAAT GTGTCTCATGCATAAATCCATAATAGATGTATGATAGGGTGTGAATGTGTTCATACATGGCACGAACGTGTCTCATATGCATAATAGATTGTTGATTG GGTGTGAATGTGTCTCATGCATGGCACGAATGTCATACTCATATGCATAATAGATGTACGTATAGGGTGTGAATGTGTGTGCATAGATTACGTGTGA CCGAGCGTAGGGTAGTAGCAAAAACATAAAACACAAAATACCAAGCGCTCGCTAGTTGAAACACTCAAAAATTCAAAAAATGCGGTTTTTATAGCTA GACAAAAAAACACAAAAATATCAAACTCAAAATAAAAATCACTAGACACAAACCAAACACTCGTACTTGAAGCTGAGAAAGACCCAAATACGTCGCTT GAGACGTCTTTATCTGAGGAGGAGGCTTCGTCGTCCGCTCCTTTACCAATCACCAAAGAGCGAGATGCGCAAGAGTGACCACTAATCGTTGTTTCCA GATACAGTTGCCGGTTGACAGACTTGTATTGTAGTCGGCCCGGAATGCATCGGTAGATTCTATGGGCCTATGGCGAGGCTTTAAGCACAGAAACTGC TTGGAGAAGGACCATCCTACACTATTCGATTCGCTGGCATGTGTGGAAACACCCAAAACACATAAAATACGATTGCTCGAAACACTCAAGTCTCAAG TTATATCGCTAGAAAAAACATACAAAAAAACAAGCTAAAAACTGCGGTTTTTAATCGCTGGACAAAACACACAAAAATACCAAACGATCGCTAGTCG AAACACCAAAAACACAACAAAAATGATAACTTAAAAAACAAAAAACAAAAAAAAAAAACCACACACCAAACAACTTTATGCGTTTGCGCTTTAATGC TCTTGGGCGAACCAATGGTTACATGGGTAAAATGTAACCATATTAATGGACCTTTAATGGGTCAAAAAGTTTCTTATCTAACTAAGGCCCAAATACGG GAAGAGATCGATGACTAATGCTCCTGGTGGACCCAATAGTTAAATGGAGAACATGTATCTCGAATAATGGGCCTTTAATGGGTCAAACTTATTATCA ATCAAACTTCTATTCAAACAAAGGCCCAAATATTCTTGACTAATTTTGCTCCGCCGTTAATCAGTCCCTTTTCACCGACTTCGCCATTGCTAATCGCCGT CGAGCTTCCATGGCTGCTGCTCCATGGCTCTATCGCTCTTTGAGAAGGAGCTCCATTAGAGTTACTCACAGCTTTCAATGGCTTCATTGCTTCCTCATT TGCCTGATGCTGCGGTTTACACGTGTCTGATCACGGGGTTTGGGACTCAGAAGAAGCTGGTTACTGTTTACGAACTGCTCAAGGAGATGCAAGAAA AGGGACATCCTCCGGATGGCAAAACGTATAATGCGCTGATCAAACTGATGGCGAACCAGAAAATTTCGGAACATGCCACAAGGATCTACAACAAGC TGATCCAGAACAATATTGAGCCATCGATACACACATTCAACATGATAATGAAGTCATACTTCATGGCGAGAAACTATGAGATGGGGAGAGCAGTTTG GGACGATATGAACAATTGAATTAATATTGAAAACTAAGAGAAATTTGGTTTTGGGTTATTACAAAAAAACAATTACAGTATTAGCCAAGAAAAGAGA GAGTTTGCTCTTATAGGGTCTAAAAGAAGGCCCATTTACAAATGGGCTTGTTAGTTTAAAGGACTGGTGCTTCTCTTCGTCAATGGAGGTTGGTATAA TAATAGGTACGAAAGAAAACCCTATTTGCGCCGCATCTCTCTCGTCTTCCTTTACTCCGTCAAAACCATGTTTCTAAGTCAGTTCCGGAGCTGTAAACC TTTGCTCGAGTCTCGTCTACCTTTGCTCCGCCTCTCTCTCTCTAAATGTCTCGATCAGTCCTTCAAAACCACGGCTAGTAGCGAATACGGTAGTAAGAT CAACAAAGTCACAAAGCATGTCAATGAGGAGGATGGTGCAACTTTCTATGTTACTCGAAGGGGGGCAATCGATAGTTCTGCCCCTGCTGAATCAAA AGCATATCCAAAAGCAGCTAATATCAGTTCAATCCATAGTATGAGGGAATCTCTTCTGGAGGAAACAGAGGAAGACTACTACAGACGCTTGGCACTT TTTGCCCTGAGGAATCATGGTGGAGAGGATGCCATAAATGTTATTATAGAATCTCTAGGTGTTGAGAGTTCTATGATTCGCATTGAGGCTGCATTTGT GTTGGGACAATTGGAATCCAAAACAGCTATAGCTTCTCTAAGCAAGATCTTGAGAGATGTGAAGGAGCACCCCATGGTTCGAGTAGAGGCTGCAAA AGCCCTTGGTTTCATTGCAGATGAGAAGAGCAGAGAAGTACTCCAAGAACTTTCAGGCGACCTCGATCCCATTATCGCTAAAGGTTGTGATTCTTCAT TGAGTATCTTGGAATTTAAGAATTCAAAAAAATATGACCCTCTCATATAA
F Y	Cloning promoter primers egb 171 F GGGGACAAGTTTGTACAAAAAAGCAGGCTTGccacaggtacttttttctga egb 172 R *GGGGACCACTTTGTACAAGAAAGCTGGGTAccgcatcatcggtggttgtgg* CCACAACCACCGATGATGCGG Gene promoter primers egb 171 F GGGGACAAGTTTGTACAAAAAAGCAGGCTTGccacaggtacttttttctga egb 173 R AGTGGAAGAAGCTGACGACTGCCGCATCATCGGTGGTTGTGGC GCCACAACCACCGATGATGCGGCAGTCGTCAGCTTCTTCCACT Gene primers egb 174 F GCCACAACCACCGATGATGCGGCAGTCGTCAGCTTCTTCCACT egb 175 R *GGGGACCACTTTGTACAAGAAAGCTGGGTActgatgttgctgattgttg* (no STOP) Full oligonucleotide sequence CCACAGGTACTTTTTCTGAAACATTCTCTTCGAAAGGAACTTGGATGCAAATTAGACAAATTTCTCCTCATGTGGCTTGGCATAAAGCTGTCTGGTTTA AGGAATCTGTCCCAAGGTTTTCATTTATCCTTTGGATGGCTTCCCATGAAAGGTTGACAACGAGAGATAGGCTTTTACAGTGGGGTATGAATGTACA TGGTTCTTGTGTTCTGTGTGATTCTGGCCTTGATTCCCATGATCACTTGTTCTTCAAATGTGAGTTTTCTAGATCACTTTGGATTTCGCTCGCTGGAAGG ATTTCGCCGAATCCTCCACTCTCTTTACCCTTGGCTTCTATGTGGGTTCTTGGTGTACATTCTTCTCCGCCGCACTATGCAAGTAAGATCTTAAAACTTC TTCTTCAAACCGCTGTTTATATGATTTGGAAAGAGCGCAACCGCAGAGATTTCTCAGCAGATGCTACCTCTCTCTCCGCTACAAAAATTCTCGTTGATC GCACAATGCGTAACCGTCTCATCTCCTTCTCGCCGGAGGAATCAGTTACCACTATCTCTCTCCTTGGATTCTACTTTGGTTGTATTAGTACCCCACTATA GGTGTGGCAAAGCTCATATTTATGTATGTATGGTGTTCCATTAGTCTCTCTTCACAAGAGCTCGGTGTGTTTTTGGGGTTTCATTGTACTATGTAAACC TTTTTTCATTTGGTATAAATCTTAACATTAAACCAAAAAAAAAAAAAATGTTGAAACCTGTTAACATAGTCTATTTATACTCTATAAGTGCAAAACAGGG TTTTAACATGATTAAGTTTCGTAGTGGAATATAATAGCGGATTGGGATTGTGTTTCATATAGCATTGCCATTGGGATGTAGTGAATCTTGTTTGGAAT AACGTATATCGATTTTTTTTTTTTTTTGTTATTTGGGAGATTTTAAGTTCTAATATGACAAGATTATAACATTTAATTTAACATTAAACCAAAAAAAAAA AGATTGTAACATTTTCTGAATTTTTGTAATTCGTTTTACTAGTGTCAGTTTTTTTTTCTTTTACTTTTAACTCTTTCAAATATTTAGTTATTTTAATGTTTTA AAAAAAAAGACTAATTTGCATCACCACTATCTTTGGGTTGAATATTTATTCAATATGTTCCACTATGAATACGTGAATTATGTGTGTGTTTTCATCTACA ATTCATACACACACTCTGTCATGATTGTGAAATAAGCAGTAAAACAACAACAATACTAGAAAAAAACCACACTTTGGGACATTAGAGTTCGATGTGAA TACAATACTAGGATTTTCTTAATGTTAATGGCGATGCCTCTATTCATTCTTCTTCCTCTTTCTTTTATAGTCGTCCTTCACGTGTCCTGCCCGATCCGCAG TATCCACCCTAGAATCTCGTTGATCTCAACAGTTCTTCAGATCTTTTTCATGCCATCTCGCGGTTAGTTGCTTAAGATACACTCCTCGAGCTTGTTGATC AACCGGAAGGCCGACGGATGGGTTTACTAAACAAACTCATCCGTTATTTCCTACTCGCAATACATAGTTCAGCCTGAACATATGTGTCTAATTGATAG GGTAGGCCAAGTAACAACATACACCCAAAAAAAAAAAAAATAAAAAAAAAAAATAAAAAAATCGTGGAGATTAAAAAAGCTAAACCAATCGACTTCTT GATTAGTTTGATGATTTTATTAATCTTATCGGTTAAAACCGACCGGCGATCGAGGGTTTGGCTTGCCGAGAAGGAAGAAAAAAAAAACCCTAAGATTT ATTTCTACTCTTCACTCCATTATCGACTTCTCATCATTTCTCTACCCATTGTACTTCTCTGCTCTTGGGTTGTCATCAATTCGCGAATTTGATACACGCAC ACCCTAAAGAGAGATCCACGCTCGTTTCAAGGTAACCTAAATTCGAACACTTTCGCAGTTTCCCTTCGATTCGAATAAATCACTGTTCTCCGTGGGCA ACAATGTACGCCGGCGGCGATATGCACAGGGGTTCACAGATGCCACAACCACCGATGATGCGGCAGTCGTCAGCTTCTTCCACTAACATCAATCCCG ACTACCACCACCCATCTGGCCCCTTCGACCCCAATGTAGACAGTTTTGGGGCGAAACGAATGAGAAAGCATACACAGAGAAGAGCTGTTGATTACAC CAGCACCGTTGTTAGATATATTCAGTTCAACGAACTCATACTTTATATGGAACTAAAAGTAGAGAGTGTTTACTCCATATATGTTTTTGTTCAGGCTCG AACATGGCAGCGAGACTCAAGGGATAGGACCACTCTGCAACCAACCCCAGCTGCAGCAGTTGATATGCTTCCCACAGTTGCCTATTCGGATAATCCT

	TCAACAAGCTTTGCTGCAAAGTTTGTTCACGCATCTCTAAACAAGAACCGTTGTTCAATCAACCGTGTATTGTGGACACCTTCAGGAAGACGTCTTAT CACGGGCTCCCAAAGTGGGGAGTTTACTCTTTGGAATGGGCAATCTTTTAATTTTGAAATGATTCTTCAGGCACATGATCAACCTATTAGGTCGATGG TATGGAGCCACAATGAAAATTATATGGTTTCTGGTGATGATGGAGGCACATTAAAGTATTGGCAGAACAATATGAACAATGTGAAAGCCAATAAAA CTGCTCACAAGGAATCAATTCGCGATTTAAGTTTTTGTAAAACAGATTTGAAGTTCTGTTCGTGTTCTGATGATACGACTGTTAAAGTCTGGGATTTTA CCAAGTGCGTAGATGAAAGCTCATTAACCGGCCATGGTTGGGATGTCAAGAGCGTTGACTGGCACCCCACAAAGTCCCTACTAGTTTCTGGTGGAAA AGACCAACTTGTCAAACTCTGGGATACTAGATCTGGGAGAGAGCTTTGCTCACTCCATGGTCACAAAAACATAGTACTTAGCGTGAAGTGGAACCAA AATGGCAATTGGCTTTTAACGGCCTCGAAAGATCAGATAATTAAGCTGTATGATATAAGGACTATGAAGGAGCTTCAATCCTTTCGTGGGCACACGA AAGATGTAACATCTTTGGCGTGGCATCCCTGCCATGAAGAATATTTTGTCAGTGGGAGCTCTGACGGCTCCATTTGTCATTGGATTGTGGGGCATGA AAACCCGCAGATTGAAATCCCAAATGCTCATGATAACAGTGTTTGGGATCTTGCATGGCATCCTATTGGATATCTTCTTTGCAGTGGTAGCAATGATC ACACAACCAAGTTTTGGTGCAGAAACAGGCCTGCAGATAATCCCCGAGATGTTCTTATGCAGAACCAAGGCTATAATGAACAAGGTTTTGGTCGCCA ACCTGATAATTTCCAACCATCTGAGGCATCGCCAATCCCTGGAGCATTTGTGCCTGGGTTGACACGGAACGAGGGGACTATCCCAGGAATAGGAATA GCAATGCCATTTGATGCATCCTCTCAAGGGGATCATAAACAGCCTCTTCCAGGTTCCATGGCACTGGGGGCTCCTCCTTTGCCACCTGGTCCCCACCC ATCGCTTCTTGGAAGTGGCCAGCAGCAAGGGTATCAGCAACAACAACAACACCAAGGTCATCCCCAGCAAATGCTTCCAATGCCCAATATGCCTCAC CATCAGCTACCACCATCATCTCATATGCCATTGCACCCTCATCATCTTCCCCGGCCTATGCAAATGCCTCCTCACGGTCACATGCCACCTCCCTCAATGC CTATGTCTCATCAGATGCCTGGATCAATGGGAATGCAAGGTGGCATGAATCCTCAGATGTCGCAAAGTCATTTTATGGGTGCTCCTTCAGGAGTATTT CAAGGACAACCAAACAGTGGCGGACCCCAAATGTATCCCCAAGGACGTGGTGGTTTCAACCGTCCACAGATGATTCCAGGCTACAACAACCCTTTCC AACAGCAGCAGCAGCCACCTTTACCTCCTGGCCCTCCACCAAACAACAATCAGCAACATCAGTAG
DHH1	Cloning promoter primers egb 176 F GGGGACAAGTTTGTACAAAAAAGCAGGCTTGgattcatgttcttcagct egb 177 R *GGGGACCACTTTGTACAAGAAAGCTGGGTAgtattcatcacaaatctctctc* Gene promoter primers egb 176 F GGGGACAAGTTTGTACAAAAAAGCAGGCTTGgattcatgttcttcagct egb 178 R CGGTGGATATCTTCCTCTGTTAGTATTCATCACAAATCTCTCT AGAGAGATTTGTGATGAATACTAACAGAGGAAGATATCCACCG Gene primers egb 179 F AGAGAGATTTGTGATGAATACTAACAGAGGAAGATATCCACCG egb 180 R *GGGGACCACTTTGTACAAGAAAGCTGGGTActgacagtagattgcttga* (no STOP) Full oligonucleotide sequence GGATTCATGTTCTTCAGCTGCTGCATCAGAAGTTTCTTCTTCTGTAGGGTTTGCTTCATCTTCTTTGGGTTCGGTTTTGTCTTCCTCAGCCGTCGAGCGA CGGTGGTTAGCCGCGGCTTGGAGGACATGATTGTAATTACCTTTCTCAAGGAAAAGCTGGCTGAAGTGGACCTTACAGTAAAGGACGCCATCGAGA GCAGCATATGAAGAATGTGTTAATGGACAACCACTATGTGCACATCTGAAGCAAGTCTTGTGGTAAGATTCTCCTTCCATTGTCATCTTCTCTAGAGG ATAAACTGTTTTCTTACAAGCTGCACATTTGTCTTGTGTGCCGCTAAAGAAGGATGATAACCTGTTTGGAGCCTTCGTCTATGCAAATGATCACACCA AGAAATCAGATTAGAAAACAAAAAGAGAAAACAGAGGAATGTTGATGAATTATGTGCTTACCGCATCATTCGATTTCTCGGTCTTTCCAGCTGTAAA AGACCAAGAAGAGAAAGTAGCAAAGATTGTTAGCTTTTCAAGAGAGGTTAATAAAAAACAGAGCAAAGAAAACAGAGGATTTGTTAGCAACCTGTT TGGAAATTCTTGCTGAAATTTCCAGATTCTTTGAAGAGCTGTTCAAAATGGGTCTTGCAATACAAAACTCCATCCATGGATGAGTAGTTACATATCTG AAAAGGAGAAGAAAGATGTTCTCTTTGTGTTTTTTTTTTCATGAGGGAACAATGGAGGAAAAGGAAGATAGATAGATACCACGAGAGTGCCATTGC AATGGCTGCACCTGAAGCATGACTTGTGATAAGGCATTCCTTCCAAAGTCATCAAGTCCATAACGTATACCGTCTTGTCACACGCCTTGCATTTGTCTG TTGTCCCTGTAAACGCCGCCATTGGTATCTCTTCTTCTTCTTTCAAATTACGAAAGAGTTGGAAGATGGATCAAAAGTTTTCTTCTTCTTTTTGTTG TTCGTTTAGACACGAATCAAGGTTTGTTTTGCCATCATCTATGAGCTCTCTGCCTTTTTATTATATCAAAATCATGGATTGAGATTTTTAGGGGTAAAA TTTTCTTCTGATGGTTGAGATTCAAATGAGGAGAGCCCTTAAAATGTTCTTCTACATTCTAAATCTATTTCTGGCTATATTTTATTTTATTTAACACTTAT TTTTGTTTTGCTTTCTAAATTATATCGTGTAAACCAAGGGATTAGGTGCAAAGTTTATATATAGTTAGCCTCCGATTTTAAGATGTTATTAATTCATATA CACAATTATAAGGATCTTTATACAAAATAAGTAACACCTAATCTAACATCACAAGACTGCCTTTTAAGCATAGCTTTTAATAATCATAAGCATAATTTG TTTTGTTAGATATCATTAATCAAAGGGCAAAGAATCTGGGGGTCTAAAATGACAAAAACAATACAACATGGGGTGAATACCTGAAAGAAAAGTAAA TCCGAGTTATACTGTAAATAATATAAAGCTTATGGGTCATGTATTTATTTAACGTAAACATAAAAGGCCATTTTGATAAATAGTGAGAGTCTGAAAAA AAGAAAGAGGAGAAAATCCATTGACGCCCTAACAAAAGACGCCGGCGAGTGTATTCGTCACTTTCTCTAGTTTCCTGTAACCCAACCCACTCAAACG AAATTTTTTCTTAATCTCTCTTTCGTTCTTCTTCTTCTTCTTCTTTAGATTGTTGTAATTCCAATCGGAACTCTCGATTCGCTTTCTTCTCCGTTCTTCT GTGGCGGACCGGTTTTGTGGTTTTTTATTTGAACATTCCCGATCTTGCCCTGAGTTCCACGATCAGTTTCTTTAAACCCTAGGTTTTACACAAGCATTCT GTTTCTCCAGATTATCGCAGACTCTTTCGTTCTATTCTTTGTAGTTTTGTAAAAGGTATTTCGGTAGATTTAGGGTTTGTCTGTGTCTAGTTTTCGAGAT CGAGAGAGATTTGTGATGAATACTAACAGAGGAAGATATCCACCGGGTGTTGGAACGGGTCGTGGTGCGCCTCCGAATCCAGATTATCATCAGTCG TATCGGCAGCAACAACCACCTCAAGATCAGCAGTATGTTCAACGCGGTTATTCTCAGAACCCTCAGCAGATGCAACTCCAGCAACAACATCAACAAC AACAGCAGCAGCAACAGTGGTCAAGACGCCCTCAGCTTCCTGGAAACGCTAGTAACGCTAATGAGGTGGTTCAGCAGACGACCCAGCCCGAAGCTA GCAGCGATGCTAATGGTCAAGACTGGAAGGCTACGTTAAGGCTACCACCTCCTGATACTCGTTATCAGACAGCGGATGTGACAGCTACAAAGGGAA ATGAATTCGAAGATTACTTTCTGAAAAGAGATCTGTTAAAGGGAATATATGAGAAGGGTTTTGAGAAGCCATCTCCAATTCAAGAAGAGAGCATTCC AATTGCTTTAACTGGTAGTGATATTCTTGCTAGAGCTAAAAACGGTACAGGAAAGACTGGTGCCTTCTGCATTCCAGTCCTCGAGAAAATTGACCCAA ATAACAATGTTATTCAAGCCATGATTCTAGTTCCAACGCGAGAGCTGGCCCTTCAGACATCACAAGTTTGCAAGGAGCTTTCCAAATATTTGAATATC CAGGTTATGGTCACCACTGGCGGTACCAGTCTGAGAGATGATATTATGCGATTACATCAACCTGTGCATCTGCTGGTTGGAACTCCTGGAAGAATAT TGGATCTTACAAAAAAGGGTGTCTGTGTTTTGAAAGACTGTGCGATGCTTGTAATGGATGAGGCCGACAAGCTTTTGTCTGCAGAATTCCAACCTTCT CTAGAGGAATTGATACAGTTTCTACCCCAAAATCGTCAGTTTTTGATGTTTTCCGCCACATTCCCTGTCACTGTTAAGGCTTTTAAGGATCGACATCTC CGGAAGCCCTATGTTATCAATCTCATGGATCAACTCACGCTTATGGGTGTCACGCAATATTATGCTTTTGTCGAAGAAAGACAGAAGGTTCACTGCCT CAACACACTTTTCTCTAAGCTGCAAATAAATCAATCGATAATCTTTTGCAACTCTGTCAATCGCGTGGAGCTGTTGGCTAAGAAAATCACAGAACTTG GTTATTCATGCTTCTACATTCATGCAAAGATGGTTCAAGACCACAGGAACAGAGTATTCCACGAGTTCCGCAATGGTGCTTGCAGGAATCTCGTTTGC ACTGATCTGTTTACTCGAGGAATTGACATTCAAGCTGTGAATGTCGTGATCAACTTTGATTTTCCTAGGACTTCTGAGTCATATCTACACAGGGTGGG TCGATCAGGACGGTTTGGACACCTTGGATTGGCTGTGAATTTGGTAACTTATGAGGACCGTTTCAAAATGTATCAGACTGAGCAAGAACTTGGGACC GAAATCAAACCAATTCCTTCTAATATCGATCAAGCAATCTACTGTCAGTAA

Kin11	Cloning promoter primers egb 181 F GGGGACAAGTTTGTACAAAAAAGCAGGCTTGcgatatagaaatataaattggtatatgtat egb 182 R *GGGGACCACTTTGTACAAGAAAGCTGGGTAatgatccatttcctgtgtccca* Gene promoter primers egb 181 F GGGGACAAGTTTGTACAAAAAAGCAGGCTTGcgatatagaaatataaattggtatatgtat egb 183 R ATTGCCAAATCTATTTGATGAATGATCCATTTCCTGTGTCCCA Gene primers egb 184 F TGGGACACAGGAAATGGATCATTCATCAAATAGATTTGGCAAT egb 185 R *GGGGACCACTTTGTACAAGAAAGCTGGGTAgatcacacgaagctctgta* (no STOP) TAAGAAAATTTTAAGTTAAATTTTTCGATATAGAAATATAAATTGGTATATGTATTTGTTTTTGTTAAATTTCCTATTTCTTTTAGAAAAAATATTTAAA AAATCTCGATTTTGCAATACAATTTAAATATCTATTTTATTATATAAAAATACAATTTAATTCGATTCCATACAATTCATATACAATTTGATTTTGCAATA CAAATATAATTTTATTCTATACACTCCATCTTATTGTGTTAGTTTTCCTTCTTAGTTAAGAAAATTTTAAGTTAAATTTTTTAGATATAGAAATATAAATT GGTGTATGTATTTTTATTGGTTAAATTTCCTATTTCTTTTAGAAAAAATATTTAAAAAATCTCGATTATGCAATACAATTTAAATATCTATTTTATTATAA TACAAATACAATTAAATTTGATTGTATACATTTCAAATACAATTCGATTTTGCAATACAAATACAATTTAATTCTATACACTCCATCTTATTGTTAGTTTC CCTTCTTAGTTAAGTAAACTATAACTTATTTTTTTTAGATATAGACATATAAATTAGTATATGTAAATATTTGTTTTTGTTAAATTGCCTATTTCTTTTAG GAAAAAAGTAAAAAAAAAATCCGACACAATATCAACATATGAACGATTGACTTGTTACGTGTCACGATCAGTTGAGTTATTAACTTTAAAAACTCTACT TTATATAATAAAATGCGTCTGAAACTTCTTAATTTATCCGCTTTATCATTAGCACGCTTTTATATATAGAAAACTAGATTTTAACCCGCGGTATACCGCG GAGACAATTTATTTTTTAAAGTTACTATATATAAAAATTTGCAAATTATATTTATCTATAAAATATTTTTATTTTATAGTTTACGATTGTTATTAAGTAAC GTCCTGTCAAACCCGTCCCGCCAATCCGTCCCGTAAAAAAAATTCACTGATTTTATTTATGATATGTTTATGAATCATTGTCTAAATATTTTTTAATTTT GTAGTAATTAAATGCTATCTAATATCTCTGCGGTTTGATGATTATAATCGAATTATTATAGTCATGTAGAAAAACAATATCAAAAATGATAATTTTGTA GTAATTAAATGATATTAAATATTTCGGAAGTTTTATTTTAGTAACTAATCGTGTAGTTAATGTGGAAAGATTTTGGGAAGATTGTCAAATATTTAATTT AAAGATTTTCTTTCTAATTATCAAAAACAATTATTAAATGCGAGTGGCAGCCACTGTAAATAAACTCCAACTCTAGGATTTATTTCACAAAGTGGCTGC AAAAATGTATATATAGATCATAAAAACAGTAATATAAAAATTTTAAACCTTAAAAAACTAGTTTTTCAAACAAAAACAGAAAATGGGCAAAAAGGCC CTAATTTAAGGGCTTGCAAATGGCAATAAATGTTTATCTGGGTTTTGTGGTTTCTTGATTCGTCATTACCAATTTTCACCATGAAGACTTAAAAAAACC TGACTCAGCTCTGCGTCACCTACGAGTTGGAAATCTGACTTCCCCAAGAATTTCTTCAGATTACATCGTAATTTACAATTCTGCCCTATTTATACCCATC AATCTGATTCTCCTTGTTTCTGTATATTTCTTCGCTCTTTGTTTGAACAATTTATCTGGGTTTTTGATTCTCGGATCTACTCCAAGACGGTTTTCGAAATT AACAAGCTTTTCAGGTAAATTGACTTTTTTTTCTTTGTAAAGGTTTATCAATTTTGTCTTCTAAACTCAAACTTGTGAGTCTCTTGTTTGGTTTCAGACCA AAACCACCATTACAAAGTAGTTATGTCGGTGGTTCTTGTCAGTTTTCTTACAAGCTTATGGTTGGTTTAGATTAGATTTGATGAAATGAAAATTGAGTT GAAGAATGTTTTTTTTTATTTGAGCAAGTTAATATGTTTACTGATGTTTTTCTATAGAGAAGTTGCTAAGAGAGCTTACTTATGAGTTTGATGTTGAAT TGGGACACAGGAAATGGATCATTCATCAAATAGATTTGGCAATAATGGAGTGGAATCGATTTTACCGAATTACAAGCTTGGTAAAACTCTTGGAATT GGGTCTTTTGGGAAGGTGAAAATAGCAGAGCATGTTGTCACAGGGCATAAGGTTGCTATCAAAATCCTTAATCGTCGTAAGATCAAGAACATGGAG ATGGAAGAGAAAGTGAGGAGGGAGATTAAGATTCTACGGTTGTTTATGCATCCTCATATTATTCGGCAGTATGAGGTAATAGAGACCACGAGTGAC ATTTATGTTGTGATGGAGTATGTCAAGTCTGGAGAGCTCTTTGATTATATTGTTGAGAAAGGCAGATTACAAGAAGATGAGGCTCGTAACTTTTTCCA GCAGATAATATCTGGTGTAGAGTACTGCCATCGTAATATGGTTGTCCATAGAGACCTGAAGCCTGAGAATTTACTATTGGATTCGAGGTGTAATATT AAGATTGCAGACTTTGGGTTGAGTAATGTTATGCGGGATGGTCATTTTCTAAAGACGAGTTGTGGAAGCCCCAACTACGCTGCTCCCGAGGTTATAT CAGGTAAATTATATGCTGGACCTGAAGTAGATGTATGGAGTTGCGGAGTTATATTGTACGCTCTATTATGCGGTACTCTTCCTTTTGATGATGAAAAC ATTCCCAACCTTTTCAAGAAAATTAAGGGTGGGATTTACACTCTTCCAAGTCATTTATCATCTGAGGCTAGAGACCTGATCCCAAGGATGCTTATAGTT GACCCGGTGAAACGAATCACCATTCCTGAGATCCGTCAACACCGTTGGTTCCAGACTCATCTCCCTCGTTATCTTGCTGTCTCTCCACCGGATACAGTA GAGCAGGCTAAAAAGATCAATGAGGAGATAGTTCAAGAAGTGGTTAACATGGGATTTGATAGAAACCAGGTTTTGGAATCTCTACGCAACAGAACA CAAAACGATGCTACTGTTACATACTACCTGTTATTGGATAACCGGTTCCGTGTTCCAAGTGGCTATCTAGAATCCGAGTTTCAGGAGACAACAGACAG TGGTTCCAATCCTATGCGCACACCTGAAGCGGGCGCTTCACCTGTAGGCCACTGGATTCCTGCACATGTGGATCACTACGGGTTGGGAGCAAGATCA CAAGTCCCTGTTGATCGAAAATGGGCTCTTGGACTTCAGTCTCATGCGCATCCTCGTGAAATCATGAATGAAGTTTTGAAAGCTCTTCAAGAACTCAA TGTGTGTTGGAAGAAGATTGGTCACTACAACATGAAATGTCGATGGGTTCCTGGTTTAGCTGATGGTCAGAATACTATGGTCAACAATCAGCTGCAC TTCAGAGATGAATCCAGCATCATTGAGGATGACTGTGCCATGACTTCACCCACTGTCATCAAATTTGAACTTCAGCTATACAAAGCCCGGGAAGAGA AGTACTTGCTGGATATACAGAGAGTTAACGGTCCGCAGTTTCTCTTCTTGGATCTATGCGCCGCCTTTCTTACAGAGCTTCGTGTGATCTGA

Appendix 2 Endonucleases

Fused construct	Endonucleases	Promoters	Endonucleases
TCTP	NCOI	TCTP	ApaLI /NCOI+SacI
MAPK	HindIII	MAPK	ApaLI /NCOI+SacI
DHH1	HindIII	DHH1	ApaLI /NCOI+SacI
FY	NCOI	FY	ApaLI /NCOI+SacI
Armadillo	EcoRI+ApaLI/XHOI+ECORI	Armadillo	ApaLI /NCOI+SacI
Kin11	HaeIII/XbaI+SacI	Kin11	ApaLI /NCOI+SacI

Appendix 3 attB primers

attB1: 5´-GGGG ACA AGT TTG TAC AAA AAA GCA GGC
attB2: 5´-GGGG AC CAC TTT GTA CAA GAA AGC TGG G