## Characterization of actin genes in Bonamia ostreae and their application to phylogeny of the Haplosporidia

I. López-Flores<sup>1,\*</sup>, V.N. Suárez-Santiago<sup>2</sup>, D. Longet<sup>3</sup>, D. Saulnier<sup>1</sup>, B. Chollet<sup>1</sup>, I. Arzul<sup>1</sup>

<sup>1</sup>Laboratoire de Génétique et Pathologie. Station La Tremblade, IFREMER. Avenue Mus de Loup, 17390 La Tremblade, France

<sup>2</sup>Department of Botany, Faculty of Sciences, University of Granada, Avenida Severo Ochoa s/n, 18071 Granada,

Spain <sup>3</sup>Department of Zoology and Animal Biology, University of Geneva, Sciences III, 30 Quai Ernest Ansermet, CH 1211 Geneva 4, Switzerland

\*: Corresponding author : present address at Laboratoire de Génétique et Pathologie, IFREMER. Avenue Mus de Loup, 17390 La Tremblade, France. Tel.: +33 546762647; Fax: +33 546762611. e-mail address : ilopez@ugr.es

#### Abstract:

Bonamia ostreae is a protozoan parasite which infects the European flat oyster Ostrea edulis, causing systemic infections and resulting in massive mortalities in populations of this valuable bivalve species. In this work, we have characterized B. ostreae actin genes and used their sequences for a phylogenetic analysis. Design of different primer sets was necessary to amplify the central coding region of actin genes of B. ostreae. Characterisation of the sequences and their amplification in different samples demonstrated the presence of two intragenomic actin genes in B. ostreae, without any intron. The phylogenetic analysis placed B. ostreae in a clade with Minchinia tapetis, Minchinia teredinis and Haplosporidium costale as its closest relatives, and demonstrated that the paralogous actin genes found in Bonamia resulted from a duplication of the original actin gene after the Bonamia origin.

Keywords: Bonamia ostreae, Ostrea edulis parasite, actin gene, molecular phylogeny.

## INTRODUCTION

*Bonamia ostreae* is a small-size (2-3 μm) protozoan parasite, responsible for a bonamiosis, an haemocytic disease affecting European flat oyster *Ostrea edulis*. In Europe, it was first described in France (Pichot *et al.*, 1980) as the causative agent of serious mortalities in this native species. Since then, it has been responsible for a drastic decrease in *O. edulis* production in different farming areas along the European Atlantic coast (see review in Carnegie and Cochennec-Laureau 2004). It has also been detected in *O. edulis* populations on the Pacific and Atlantic coasts of North America (Carnegie and Cochennec-Laureau 2004). It has also been detected species of the genus are *B. exitiosa* from *O. chilensis* in New Zealand and Chile, *O. angasi* in Australia, *O. puelchana* in Argentina, and *Crassostrea ariakensis* in North Carolina, USA (Campalans *et al.*, 2006); *B. roughleyi* (previously known as *Mikrocytos roughleyi*; Carnegie and Cochennec-Laureau, 2004) in *Saccostrea glomerata* in Australia (Farley *et al.*, 1988); and *B. perspora* in *Ostreola equestris* in North Carolina, USA (Carnegie *et al.*, 2006).

Recent isolation of the gene coding for the small subunit of the ribosomal RNA in Bonamia ostreae (18S or SSU rDNA) has clarified phylogenetic affinities of Bonamia species. Thus, after different taxonomic affiliations, analysis of SSU rRNA placed Bonamia within the Haplosporidia (Carnegie et al., 2000; Reece et al., 2004). Genes coding for proteins provide the possibility of performing phylogenetic analyses under a different evolutionary rate compared to those governing ribosomal genes. In this respect, actin has the advantage of being an ubiquitous protein in eukaryotic cells and one of the most conserved throughout evolution, from yeast to human (Sheterline and Sparrow, 1994). Actin is a cytoskeletal protein involved in cellular functions like maintaining cell morphology, cell motility and division, and intracellular transport (Sheterline and Sparrow, 1994). Actin proteins are encoded by a multigene family in all organisms examined so far, except in yeast and in some alveolata where they are encoded by one single gene (Hightower and Meagher, 1986; Reece et al., 1997; Zhou et al., 2006). In other protozoans, like Plasmodium falciparum, Entamoeba histolytica, several species of foraminifera and the haplosporidians Haplosporidium louisiana, Minchinia chitonis, Minchinia teredinis and Minchinia tapetis, two to four actin genes have been described (Wesseling et al., 1988; Huber et al., 1987; Pawlowski et al., 1999;

Reece *et al.*, 2004). The gene family differs in size among these organisms, and the number and location of introns within actin genes are variable (Hightower and Meagher, 1986; Reece *et al.*, 2004; Flakowski *et al.*, 2006).

This study reports, through the use of nucleotide and amino acid sequence analyses, the identification of at least two actin genes within *Bonamia ostreae*. Phylogenetic analyses were carried out to assess orthologous/paralogous relationships of the sequences and to infer the phylogenetic position of *B. ostreae* within the Haplosporidia based on the actin gene sequences.

## MATERIALS AND METHODS

#### Samples and DNA extraction

Infected oysters (*Ostrea edulis*) were obtained by experimental infections. Healthy animals were injected with *Bonamia ostreae* cells previously purified (see below) from naturally infected oysters from Quiberon bay, Brittany (France) and maintained in a laboratory tank. This procedure allowed us to obtain highly infected oysters which were used for parasite purification. In parallel, non injected oysters were used as negative control. Infections were confirmed by microscopic examination of tissue imprints and by PCR assay based on the 18S rDNA gene of the parasite, using primers Bo and BoAs (Cochennec *et al.*, 2000).

Parasites were purified as described previously (Cochennec *et al.*, 2000). About 50 x  $10^6$  cells could be obtained from each highly infected oyster. Cells were centrifuged at 12000 g and pellets from several purifications were pooled and conserved at  $-20^{\circ}$ C.

In addition, two samples (gill tissues fixed in 95% ethanol) of *O. chilensis* from New Zealand infected with *B. exitiosa* were used as controls.

Genomic DNA from purified parasites and oyster gills was extracted according to Winnepenninckx *et al.* (1993) using  $2 \times 10^8$  parasite cells or 100 mg of oyster tissue. Integrity and quantity of DNA was measured by electrophoresis on agarose gel stained with ethidium bromide and spectrophotometry respectively.

#### Amplification of actin genes by PCR

Different samples and sets of degenerate primers (four forward primers were combined with five reverse ones) were used to amplify *Bonamia ostreae* actin gene fragments. Two of these primer pairs were used previously for the amplification of actin genes in dinoflagellata and haplosporidia species (Reece *et al.*, 1997; 2004), and in a rhizopod species (Longet *et al.*, 2004). The remaining primers tested have been designed for this work based on alignment of conserved regions of actin protein (GenBank accession numbers: AY450412, AY450407, AY450414, AY450416, AJ132374, AJ32375, J01016, D50839, AF057161, M86241, U84287, TGU10429, M19871, M19871). An available oyster actin sequence (AAB81845) was also included to prevent amplification of the host gene. DNA from a non-infected *Ostrea edulis* was used as control.

Amplification products were separated on 1% agarose gels stained with ethidium bromide. Testing of all primers combinations showed that one forward primer (F) and two reverse primers (R1 and R2) allowed amplification of two putative *B. ostreae* sequences (Fig. 1, panel A). The reaction was carried out in a volume of 50  $\mu$ l with 2 mM Mg<sub>2</sub>SO<sub>4</sub>, 0.2 mM of each dNTP, 1  $\mu$ M of each primer, 1.5 U of *Taq* polymerase (New England Biolabs) and 5-10 ng of DNA. Thermal cycling was 95 °C for 1 min, 40 cycles of 95 °C for 1 min, 50 °C for 1 min and 65 °C for 1 min, followed by 65 °C for 10 min.

Amplified products were cloned using TOPO vector system (Invitrogen) and the nucleotide sequences were determined using an ABI Prism Dye Terminator Cycle Sequencing kit (Applied Biosystems) following the manufacturer's recommendations. Sequences were identified by comparison with those included in the GenBank and EMBL databases using BLAST algorithm (Altschul *et al.*, 1997).

Primer pairs BostAct1F/BostAct1R and BostAct2F/BostAct2R were designed for independent amplification of Actin1 and Actin2 genes of *B. ostreae* based on the cloned sequences (Fig. 1, panel A). Actin genes sequences from *Ostrea edulis* and *Ostrea chilensis* obtained during this work (recorded in GenBank with accession numbers AM410916, AM410917 and AM410918) were aligned with *B. ostreae* sequences to ensure specificity of the primers for *B. ostreae* actins. DNA from a non-infected *Ostrea edulis* and DNA from an *O. chilensis* highly infected with *Bonamia exitiosa* were used as controls. Amplification reactions were performed as described previously but increasing annealing and extension temperatures from 50 to 55 °C and 65 to 72 °C respectively. Identification of each sequence type, Actin1 and Actin2, was done by digestion of PCR product (PCR-RFLP) with the restriction enzyme *Bst*UI (Promega).

The reactions consisted in 16  $\mu$ l of PCR product, 2  $\mu$ l 10x Buffer and 5 U of enzyme in a final volume of 20  $\mu$ l.

## Sequence and phylogenetic analyses

Phylogenetic analyses of the actin genes were performed using both nucleotide and amino acid sequences. Sequences used in the phylogenetic analyses from haplosporidian species were downloaded from GenBank (Haplosporidium costale, AY450407; H. louisiana, AY450411; H. nelsoni, AY450412; Minchinia chitonis, AY450414; M. tapetis, AY450417; M. terenidis, AY450420; Urosporidium crescens, AY450422). Sequences from foraminiferan species Reticulomyxa filosa (AJ132374), Ammonia sp. (AJ132372) and Allogromia sp. (AJ132370) and the Cercozoa Cercomonas sp. (AF363534) were chosen as outgroup according to the results obtained by Reece et al. (2004). Multiple sequence alignment was done using the program MEGALIN of the DNAstar package (LASERGEN) by Clustal method and revised by eye. Phylogenetic analyses were performed with the program PAUP\* version 4.0b10 (Swofford, 2003) using the maximum parsimony criterion. Parsimony analysis involved heuristic searches. Data matrices were subjected to 1000 replicates of random sequence additions using tree bisection-reconnection branch-swapping. In the protein analysis the characters were considered as unordered states with equal weight, while in the nucleotide analysis the third nucleotide of each codon was downweighted. Gaps were treated as missing data and the ambiguous regions in the alignment were deleted from the analysis. The starting tree was obtained by stepwise addition. The characters were optimized by accelerated transformation. Finally, 1,000 bootstrap replicates (Felsenstein, 1985) with 10 heuristic searches were performed to assess internal support for nodes.

## RESULTS

## Sequence characterisation

Two major actin gene fragments, different in size, could be obtained by combining one forward with two reverse primers in different PCR reactions using DNA of purified *Bonamia ostreae*. They were 869 bp and 784 bp in length. The shorter one was similar

to the larger one except on the 3' end 85 nucleotides (Fig. 1, panel B). Comparison with sequences included in GenBank and EMBL data bases by BLAST allowed to identify partial actin DNA fragments, corresponding to central region of the gene. These sequences were named Actin1 (BoAct1, 869 bp) and Actin2 (BoAct2, 784 bp).

Three cloned sequences per actin sequence type were obtained (Fig. 2A). The GenBank Accession numbers are AM410919-AM410921 and AM410922-AM410924. Excluding primer sites, the sequence to be compared was 745 bp in length. Intra-type variability between clones was low, and estimated at five nucleotides between Actin1 sequences and one between Actin2 sequences (Fig. 2A). Three of the five sites corresponded to non-synonymous changes with respect to deduced Actin1 amino acid sequence (TCT coding S to CCT coding P, GAT coding D to GAA coding E, and ATG coding M to TTG coding L at positions 451, 474 and 700 respectively). The variable site of Actin2 partial sequences corresponded also to a non-synonymous change (ATC coding I to GTC coding V at position 403) (Fig. 2B). Nucleotide sequence type 1 and type 2 (Actin1 and Actin2) demonstrated 83.7% of identity. Variable positions were mainly located in the second half of the sequences (Fig. 2A). These variable positions (108 in total) usually corresponded to the third position of the codons (58) while 33 and 17 variable sites corresponded to the second and the first codon positions respectively. No gene intron was identified when sequences were aligned with homologous actin genes.

Encoded amino acid sequences of these two actin gene fragments (289 and 261 amino acid residues for Actin1 and Actin2 respectively) showed a full length open reading frame and demonstrated 87.2% of sequence identity. The first 121 amino acid residues were identical between the sequences and corresponded to identical nucleotide sequences (Fig. 2B).

In order to determine if sequence types, Actin1 and Actin2, corresponding to actin genes belong to *Bonamia ostreae* genome, a variable region of nucleotide sequences was used to design primers for amplification of Actin1 and Actin2 sequences in different isolates (BostAct1F/BostAct1R and BostAct2F/BostAct2R respectively, see Fig. 2A). Both primer pairs amplified the expected 264 bp product when DNA of the parasite purified from four different naturally infected oysters (*O. edulis*) was used as template. The different sequence types were determined by digestion of PCR products with the restriction enzyme *Bst*UI (PCR-RFLP). Actin2 sequences had no recognition site for this enzyme while Actin1 sequences contained two of them, yielding, after

digestion of the 264 bp amplicon, three fragments of 13, 83 and 168 bp. Figure 3 shows differential band patterns obtained by PCR-RFLP. Both types of sequences could be easily identified in the four different DNAs from *B. ostreae* purified cells, while no amplification was obtained for oyster DNA either healthy *Ostrea edulis* or *O. chilensis* infected with *B. exitiosa*.

#### Phylogeny based on actin genes

Alignment of all 17 actin gene nucleotide sequences (all Bonamia ostreae actin gene sequences, seven of haplosporidian, and the four outgroup sequences) resulted in a 1113 bp matrix (after excluding ambiguous regions). Of the 1113 characters 416 were parsimony-informative (285 in the ingroup). Phylogenetic analysis based on nucleotide sequences produced three most-parsimonious trees of 3377 steps in length (CI: 0.585; RI: 0.595; HI: 0.415), which differ only in the relationships between the Actin2 sequences of Bonamia ostreae. Figure 4A shows the strict consensus tree which coincides with one of the most-parsimonious trees. The tree shows that Bonamia ostreae Actin1 and Actin2 were paralogous sequences to each other, and orthologous to the remaining haplosporidian actin gene sequences (Fig. 4A). B. ostreae actin genes clustered together with 100% bootstrap support (Fig. 4A), and this grouping remained when the multiple haplosporidian paralogous sequences described by Reece and coworkers (Reece et al., 2004) were included in an overall analysis (data not shown). B. ostreae resulted a sister clade to that formed by Minchinia tapetis and M. teredinis and Haplosporidium costale. Both Haplosporidium and Minchinia genera resulted paraphyletic. Urosporidum crecens was the basal species of the haplosporidians.

Alignment of all 17 protein sequences resulted in a 370 amino acid matrix (excluding an ambiguous region), from which 84 were parsimony-informative (65 in the ingroup). Phylogenetic analysis using the protein sequences yielded four most-parsimonious trees of 329 steps in length (CI: 0.796; RI: 0.765; HI: 0.204), which differ only in the relationships between the Actin2 sequences of *B. ostreae*, and also in the relationships between *H. costale*, *M. tapetis* and *M. teredinis*. Strict consensus tree is shown in Figure 4B. The main difference of this tree with respect to the nucleotide tree (Fig. 4A) was the closer relationships of *B. ostreae* with *H. nelsoni* instead of the clade consisting of *M. tapetis*, *M. teredinis* and *H. costale*.

## DISCUSSION

#### Two actin genes in the genome of Bonamia ostreae

Amplification by PCR using "universal" primers of unknown genes from non-cultivable eukaryotic parasites like Bonamia ostreae is a difficult aim. Conserved or "universal" primers are mostly designed from higher organism sequences and frequently amplified host DNA (always present in the samples) instead of parasite DNA. Combination of conserved primers and primers designed using sequences from related organisms, and the use of DNA from Ostrea edulis and from O. chilensis infected with B. exitiosa as separate controls, allowed us to amplify actin genes in B. ostreae. Characterization of amplified products allowed the identification of two sequence types encoding two proteins. Both types were identical at the amino terminal end. Variable positions were mainly located at third codon positions with decreasing proportions at second and first codon positions. Deduced amino acid sequences of Actin1 and Actin2 were about 13% divergent, a relatively high value for actin proteins encoded by genes from the same species (Hightower and Meagher, 1986). Nevertheless, higher divergence values could be observed between both actin paralogs described in species of the related foraminiferan phylum, 16% in Reticulomyxa filosa, 17% in Ammonia sp and 18% in Allogromia sp (personal obs. based on sequences analysed by Pawlowski et al., 1999). In the apicomplexa Plasmodium falciparum, actin protein sequences showed 21% sequence divergence and were considered extremely divergent (Wesseling et al., 1988).

All together these data suggested that two paralogous actin genes are present in the genome of *Bonamia ostreae*. The absence of amplification from non-infected oyster and a sister species (*B. exitiosa*) DNAs, the comparison with *O. edulis* sequences and the amplification by PCR of both sequence types in different *B. ostreae* samples from different origins demonstrated that both types of sequences corresponded to *B. ostreae* genes and not to actin genes from different species (the host, other parasites, algae, etc.), coding for one actin protein each. The two actin genes fragments of *Bonamia ostreae* reported in this study represent the first protein-encoding sequences obtained for any *Bonamia* species. Isolation and characterization of new genes or loci of its genome is of great interest in order to create a molecular data base for this parasite as exist for other protozoans useful for further research.

## Actin Phylogeny

PCR amplification and sequencing analysis suggested that there were at least two actin genes in the genome of Bonamia ostreae and phylogenetic analysis supported this assumption. Both types of actin genes sequences obtained in this study (Actin1 and Actin2) grouped together on the basis of nucleotide and amino acid sequences when trees were inferred using orthologous sequences from species belonging to the genera Haplosporidium, Minchinia and Urosporidium, as well as when their paralogous sequences were included in the analysis. This result suggests that the two Actin1 and Actin2 nucleotide sequences are the result of a gene duplication event (resulting in two paralogous loci) in B. ostreae after differentiation from the common ancestor. Within haplosporidians, multiple paralogs are present in species of the genus Minchinia, while Haplosporidium genus includes species, like H. nelsoni and H. costale in which only a single type of actin gene has been amplified by PCR until now. Many of these sequences were found to contain introns, and genes with introns as well as single intronless actin genes were establish to be orthologous (Reece et al., 2004). In Bonamia ostreae at least two paralogous actin genes without introns exist. Gain of introns in actin sequences throughout the evolution has been described in the group of foraminifera (Flakowski et al., 2006), and this could be applied to the haplosporidian sequences to explain the existence of paralogous genes in some species containing different number of introns.

Actin phylogeny based on amino acid sequences placed *Haplosporidium nelsoni* as the closest species to *Bonamia ostreae*, in a sister clade to that formed by *Haplosporidium costale*, *Minchinia tapetis* and *Minchinia teredinis*. In the nucleotide based tree, this topology is reversed since *B. ostreae* is more closely related to the clade of *H. costale-M. tapetis* and *M. teredinis* than to *H. nelsoni*, although the bootstrap support was low. *Minchinia* and *Haplosporidium* resulted as paraphyletic genera in both analysis. Our results are similar to those previously obtained using actin gene sequences (Reece *et al.*, 2004). Phylogeny based on small subunit of ribosomal gene performed by these authors allowed to include more haplosporidian species in the analysis and therefore they described more precisely the ingroup relationships. The genus *Bonamia* showed closest relationship to the genus *Minchinia*, which resulted monophyletic on the basis of ribosomal gene sequence. Characterisation of actin genes in the remaining haplosporidian species and subsequent analysis may clarify the discrepancies between actin and 18S phylogenies concerning the relationships between *Bonamia, Minchinia and Haplosporidium* genera, and also provide a wider knowledge of relationships within the group.

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Fig. 1. Primer description (A) and schematic representation of the genes that encode for actin in *Bonamia ostreae* (B). Arrows indicate approximate locations of the primers used for PCR amplifications. Primers BostAct1F/R and BostAct2F/R, designed after amplification of Actin1 with primers F/R1 and Actin 2 with primers F/R2, amplify a 264 bp fragment of Actin1 and Actin2 genes.

Fig. 2. Sequences alignment of *Bonamia ostreae* actin genes 1 and 2. (A) Nucleotide sequences corresponding to cloned actin sequences Type 1 (BostAct1) and Type 2 (BostAct2). Last numeric values indicate the recombinant *E. coli* clones. Dots (.) indicate identical bases. Locations of degenerate forward primer (position 1-17) and reverse ones (763-784 and 850-869) are underlined. Locations of specific primers for each actin gene type (BostAct1F and 2F, position 454-472, and BostAct1R and 2R, position 697-717) are highlighted in grey. Recognition sites for restriction enzyme *Bst*UI (CG<sup>A</sup>CG) in Type 1 sequences are showed in bold and underlined (cut sites: positions 537 and 705). (B) Inferred amino acid sequences corresponding to protein Actin 1 and Actin 2. Dots (.) indicate identical residue.

Fig. 3. Detection of a polymorphism involving *Bst*UI restriction sites in *Bonamia ostreae* actin genes sequences. DNA from four different *B. ostreae* samples (purified parasite) independently amplified with primers BostAct1F/R (Actin Type 1) and BostAct2F/R (Actin Type 2) yielded amplicons 264 bp in length. After digestion with *Bst*UI, two RFLP profiles were observed: a no-digested 264 bp band in Actin2 sequences (lanes 6 to 9) and bands of 168 + 83 bp for Actin1 sequences (lanes 2 to 5; the third expected band of 13 bp can not be observed in the gel). Lanes 1 and 10

correspond to the molecular weigth marker (Smart Ladder SF, Eurogentec). Negative controls were non-infected *Ostrea edulis* DNA (lane 11), DNA from *O. chilensis* highly infected with *B. exitiosa* (lane 12) and no-DNA water control (lane 13).

Fig. 4. Parsimony analysis based on actin gene sequences showing the paralogy of *Bonamia ostreae* sequences obtained in this study. Numbers at nodes indicate percentage support of 1000 bootstrap replicates (bootstrap support values below 50 are not presented). Phylogeny was performed using (A) nucleotide sequences and (B) translated amino acid sequences.

## Bonamia ostreae actin gene

# Fig. 1

Primer	Sequence (5'-3')	Reference
F	AACTGGGAYGAYATGGA	Longet et al., 2004
R1	GGWCCDGATTCATCRTAYTC	Longet et al., 2004
R2	CNSWRTAYTTYCTYTCNGGN	Designed for this study
BostAct1F	GCTTCGACCGAAAGTTCCG	Designed for this study
BostAct1R	TTCAGACTGCACGCGCATATC	Designed for this study
BostAct2F	TCTTCCCAATCTTGTTCGA	Designed for this study
BostAct2R	CTCCTTCCGTAGACGTTCATT	Designed for this study

**(B)** 



(A) and I. Arzul; *Bonamia ostreae* actin gene; Fig. 2A

BoAct1 1	AAC	TGG	GAT	GAC	ATG	GAG	AAG	ATC	TGG	CAC	CAC	AGC	TTC	TTC	AAC	GAA	CTG	AGA	GTC	GCC	60
BoAct1_2				T																	
BoAct1_14	• • •	• • •	• • •	•••		• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	
BoAct2 34			C																		
BoAct2_45																					
Dolat1 1	000	<b>C A A</b>	a.a	a.a	000	ana	arra	ama	200	a.a.	<i>ca</i> <sub>2</sub>	000	ama	7 A TT	000		caa	አአጥ	000	C . C	1 2 0
BoAct1 2			GAG			GIC				GAG										GAG	120
BoAct1_14																					
BoAct2_26		• • •				• • •	• • •		• • •	• • •		• • •		• • •	• • •		• • •	• • •		• • •	
BOACL2_34 BoAct2 45		• • •					• • •		• • •	• • •	• • •	• • •					• • •	• • •	• • •	• • •	
BoAct1_1	AAG	ATG	ACT	CAG	ATC	ATG	TTC	GAG	ACC	TTC	AAC	ACC	CCC	GCG	ATG	TAC	GTC	GCC	ATC	CAA	180
BoAct1_2 BoAct1_14	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •		• • •	• • •	• • •	• • •	• • •	• • •	
BoAct2_26																					
BoAct2_34																					
BoAct2_45	• • •	• • •	• • •	• • •		• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	
BoActl 1	GCG	GTC	CTC	TCC	CTC	TAC	GCG	TCC	GGA	AGA	ACC	ACC	GGA	ATC	GTC	TTG	GAC	TCC	GGA	GAT	240
BoAct1_2																					
BoAct1_14		• • •					• • •		• • •	• • •		• • •		• • •	• • •		• • •	• • •		• • •	
BoAct2_26 BoAct2_34	• • •	• • •				• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •		• • •	• • •	• • •	• • •	
BoAct2_45																					
										~	~ ~ -			~~~~	~ ~ ~						
BOACT1_1 BOACt1 2	GGC	GI,G	AGC	CAC	ACC	GIC	CCG	A'I'C	TAC	GAA	GGA	TAT	GCC	CTT	CCT	CAC	GCC	A'I'T	C'1'G	AGA	300
BoAct1_14																					
BoAct2_26																					
BoAct2_34 BoAct2_45		• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	•••	• • •	• • •	• • •	• • •	• • •	• • •	
DUNCL2_40	• • •	• • •	• • •	• • •	• • •	• • •	• • •	•••	• • •	• • •	• • •	• • •	•••	•••	•••	• • •	•••	• • •	• • •	• • •	
BoAct1_1	TTG	GAC	ATC	GCC	GGA	AGA	GAT	CTC	ACC	GAC	TAC	TTG	ATC	AAA	ATC	CTC	ACC	GAG	CGC	GGC	360
BoAct1_2 BoAct1_14	• • •	• • •				• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	
BoAct2 26																		· · · ·			
BoAct2_34							•••			•••		•••									
BoAct2_45	• • •	• • •	• • •	• • •	• • •	• • •	• • •		• • •	• • •	• • •	• • •					• • •	• • •		• • •	
BoAct1 1	CAC	AGC	TTC	ACC	ACC	ACC	GCC	GAA	CGC	GAA	ATC	GTC	CGC	GAC	ATC	ААА	GAA	ACG	CTT	TGC	420
BoAct1_2																					
BoAct1_14	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •	• • •		• • •	• • •	• • •	• • •	• • •	
BOACL2_20 BOACL2_34		.A.					• • •		•••	• • •	• • •	• • •			G		• • •	•••	• • •	• • •	
BoAct2_45		.A.																			
		-			~~~		~		~		mam		-	1.07	<i>a</i>	1.07	-			~ ~ ~	400
BOACt1_1 BOAct1_2	TAC	IGC	GCA	AAG	GAI		GAA	GCA	GAA	AIG		GCI	TCG	ACT	GAA	AGI	100	GAA	AIC	GAC	480
BoAct1_14		T									с			c				T			
BoAct2_26	• • •	• • •		.CT							G.C	т	C	CAA	TCT	т	G	A.G		G	
											~ ~					_					
BoAct2_34 BoAct2_45	• • •	• • •		.CT	• • •	• • •	• • •	• • •	• • •	• • •	G.C	Т	c	CAA	TCT	т	G	A.G	• • •	G	
BoAct2_34 BoAct2_45			· · · · · · ·	.CT .CT	 	· · · · · ·	· · · · · ·	 	 	· · · · · · ·	G.C G.C	Т Т	c c	CAA CAA	TCT TCT	т т	G G	A.G A.G	 	G G	
BoAct2_34 BoAct2_45 BoAct1_1	  AAG	  TCG	  TAC	.CT .CT GAA	  стс	 	  GAT	  gga	  CAA	  act	G.C G.C TTG	т т асс	C C ATC	CAA CAA GGA	TCT TCT AAC	Т Т GAG	G G CGT	A.G A.G TTC	  CGC	G G <b>G</b> TC	540
BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_14	  AAG	 TCG 	 TAC	.CT .CT GAA 	 стс	 ccc	 GAT 	 GGA 	 CAA 	 ACT	G.C G.C TTG	T T ACC	C C ATC 	CAA CAA GGA	TCT TCT AAC	T T GAG	G G CGT 	A.G A.G TTC	 <u>CGC</u>		540
BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_14 BoAct2_26	 AAG 	 TCG  AAC	TAC	.CT .CT GAA 	 CTC  T	 CCC  A	 GAT 	 GGA 	CAA	 ACT  A	G.C G.C TTG  G	T T ACC  G	C C ATC 	CAA CAA GGA  G	TCT TCT AAC  	T T GAG  	G CGT  C	A.G A.G TTC  T	 CGC  A	G G <u>G</u> TC  	540
BOACt2_34 BOACt2_45 BOACt1_1 BOACt1_2 BOACt1_14 BOACt2_26 BOACt2_34	 AAG  	TCG  AAC AAC	TAC	.CT .CT GAA  	 CTC  T T	 CCC  A A	GAT  	GGA 	CAA 	 ACT  A A	G.C G.C TTG  G G	T T ACC  G G	C C ATC 	CAA CAA GGA  G G	TCT TCT AAC  T T	T T GAG  A A	G CGT  C	A.G A.G TTC  T T	CGC 	G G <b>G</b> TC  T T	540
BOACt2_34 BOACt2_45 BOACt1_1 BOACt1_2 BOACt2_26 BOACt2_26 BOACt2_34 BOACt2_45	AAG	TCG  AAC AAC AAC	TAC	.CT .CT GAA  	 CTC  T T	 CCC  A A	GAT 	GGA 	CAA 	ACT       	G.C G.C TTG  G G G	T T ACC     G G	C C ATC 	CAA CAA GGA  G G	TCT TCT AAC  T T	T T GAG  A A A	G G CGT  C C	A.G A.G TTC  T T T	CGC	G G GTC  T T	540
BOACT2_34 BOACT2_45 BOACT1_1 BOACT1_2 BOACT2_26 BOACT2_26 BOACT2_34 BOACT2_45 BOACT2_1	AAG     CCC	TCG TCG AAC AAC AAC AAC	TAC	.CT .CT GAA    CTG	 CTC  T T T TTC	 CCC  A A A A	GAT    CCT	GGA    TCT	CAA    GCT	ACT     ACT  ACT	G.C G.C TTG  G G GGA	T T ACC  G G AGC	C C ATC   GAG	CAA CAA GGA  G G TCA	TCT TCT AAC  T T TCG	T T GAG  A A A GGA	G G CGT  C C C ATC	A.G A.G TTC  T T CAC	CGC   A A A A	G G GTC  T T T TTG	540
BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_24 BoAct2_26 BoAct2_24 BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2	AAG    CCC	TCG  AAC AAC AAC GAA	TAC    GTA	.CT .CT GAA    CTG	 CTC  T T T TTC	 CCC  A A A AAT 	GAT    CCT	GGA    TCT	CAA   GCT	ACT     A  A ATC	G.C G.C TTG  G G G GGA	T T ACC  G G AGC	C C ATC   GAG	CAA CAA GGA  G G TCA	TCT TCT AAC  T T TCG 	T T GAG  A A A GGA	G G CGT  C C ATC	A.G A.G TTC  T T CAC	CGC        	G G  T T T TTG 	540
BOACT2_34 BOACT2_45 BOACT1_1 BOACT1_2 BOACT1_14 BOACT2_26 BOACT2_34 BOACT2_45 BOACT1_14 BOACT1_2 BOACT1_14 BOACT1_26	AAG    CCC 	TCG TCG AAC AAC AAC GAA	TAC   GTA 	.CT .CT GAA   CTG 	 CTC  T T TTC 	 CCC  A A A AAT 	GAT    CCT 	GGA   TCT  AG.	CAA CAA   GCT  TTG	ACT  A A A ATC 	G.C G.C TTG  G G GGA 	T T ACC   G G AGC 	C C ATC   GAG	CAA CAA GGA  G G TCA 	TCT TCT AAC  T T TCG  GAA	T T GAG  A A A GGA 	G G CGT  C C C ATC 	A.G A.G TTC  T T CAC	CGC  A A A AAA 	G G GTC  T T TTG  C	540
BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_14 BoAct2_26 BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct1_24 BoAct2_26 BoAct2_34	AAG    CCC  A.A A.A	TCG TCG  AAC AAC AAC GAA 	TAC    GTA   	.CT .CT GAA   CTG  T	 CTC  T T TTC 	 CCCC  A A A AAT  C.G C.G	GAT   CCT 	GGA    TCT  AG. AG.	CAA   GCT  TTG TTG	ACT A A A A ATC 	G.C G.C TTG G G G GGA 	T T ACC G G AGC  AGC T	C C ATC  GAG 	CAA CAA GGA  GGA G G TCA  T	TCT TCT AAC  T T TCG GAA GAA	T T GAG  A A GGA  G G	G G CGT  C C C ATC  G.G G.G	A.G A.G TTC  T T CAC 	CGC  A A A A AAA 	G G  T T T TTG  C	540 600
BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct2_26 BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_24 BoAct2_26 BoAct2_34 BoAct2_34	AAG    CCC  A.A A.A	TCG  AAC AAC AAC GAA  	TAC    GTA     	.CT .CT GAA   CTG  T T T	 CTC  T T TTC  	 CCC  A A A AAT  C.G C.G C.G	GAT    CCT  	GGA    TCT  AG. AG.	CAA   GCT  TTG TTG TTG	ACT    A  A ATC  	G.C G.C TTG  G G GGA  	T T ACC   G G AGC      T	C C ATC  GAG  GAG 	CAA CAA GGA  G G TCA  TCA  T T	TCT TCT AAC  T T TCG GAA GAA GAA	T GAG  A A GGA  G G G	G CGT  C C ATC  G.G G.G G.G	A.G A.G TTC  T CAC  	CGC  A A A AAA  	G G  T T T TTG  C C	540
BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct2_26 BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_24 BoAct2_26 BoAct2_34 BoAct2_34 BoAct2_45	AAG    CCC  A.A A.A A.A	TCG  AAC AAC AAC GAA 	TAC    GTA      	.CT .CT GAA   CTG  T T T	CTC  T T T TTC  	CCC  A A A A AAT  C.G C.G C.G C.G	GAT    CCT  	GGA    TCT  AG. AG.	CAA CAA CAA CAA CAA CAA CAA CAA CAA CAA	ACT  A A A ATC  	G.C G.C TTG  G G GGA  	T T ACC   G G AGC  AGC  T T	C C ATC   GAG 	CAA CAA GGA  G G TCA  T T	TCT TCT AAC  T T TCG GAA GAA GAA	T T GAG  A A A GGA  GGA G G	G G CGT  C C C ATC  G.G G.G G.G	A.G A.G TTC  T T CAC  CAC 	CGC CGC    AAA  AAA	G G GTC  T T TTG  C C	540 600
BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct2_26 BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_26 BoAct2_34 BoAct2_45 BoAct2_45	AAG   CCC  A.A A.A A.A GCC 	 TCG  AAC AAC AAC GAA  CAC	TAC TAC  GTA  GTA  T  ACC	.CT .CT GAA   CTG  T T T T	 CTC  T T TTC   ATT	 CCC  A A A AAT  C.G C.G C.G C.G C.G	GAT   CCT   AAG	GGA    TCT  AG. AG. AG.	CAA CAA CAA CAA CAA CAA CAA CAA CAA CAA	ACT    ATC  ATC  ATC 	G.C G.C TTG  G GGA  GGA  GAT 	T T ACC  G G AGC  T T T T T	C C ATC  GAG  GAG 	CAA CAA GGA  G G TCA  T T T T T	TCT TCT AAC  .T .T TCG GAA GAA GAA GAA	T T GAG  .A .A .A GGA  GGA  GCA  CTC 	G G CGT  C C C ATC  G.G G.G G.G TTC 	A.G A.G TTC  T T CAC  GCC	CGC  A A A AAA  AAA  AAA	G G GTC  T T TTG  C C	540 600 660
BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_26 BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_14 BoAct2_26 BoAct2_26 BoAct2_34 BoAct2_45 BoAct1_2 BoAct1_2 BoAct1_2	AAG   CCC  A.A A.A A.A GCC 	 TCG  AAC AAC GAA  CAC	TAC  GTA  GTA     ACC 	.CT .CT GAA  CTG CTG T T T T	 CTC  T T TTC  ATT	 CCCC  A A A AAT  C.G C.G C.G C.G C.G C.G XTG 	GAT   CCT  AAG	GGA   TCT  AG. AG. AG. 	CAA   GCT TTG TTG TTG GAC 	ACT A A A ATC  ATT 	G.C G.C TTG G G G G G G G G G	T T ACC   G G AGC   T T AGC  AGC   T	C C ATC  GAG GAG  AGA	CAA CAA GGA  G G TCA  TCA      	TCT TCT AAC  T TCG GAA GAA GAA GAA	T T GAG   A  A  A GGA  GGA  G CTC  	G G CGT  C C ATC G.G G.G G.G G.G TTC 	A.G A.G TTC   T CAC  GCC 	CGC  A A A AAAA A AAAA  AAAA	G G GTC  T T TTG  C C	540 600 660
BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_26 BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_26 BoAct2_45 BoAct2_45 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct2_26	AAG   CCC  A.A A.A A.A GCC  A.G	 TCG  AAC AAC GAA  CAC CAC  TT. TT	TAC   GTA     ACC     	.CT .CT GAA  CTG  T T T T	CTC  T T TTC  ATT 	CCCC  A A AAT C.G C.G C.G C.G C.G C.G	GAT   CCT  AAG  AAG	GGA   TCT  AG. AG. AG. TGC  T	CAA CAA  GCT TTG TTG TTG GAC 	 ACT  A A ATC  ATT  G.CC G.C	G.C G.C TTG G. G. G. G. G. G. G. G. G. C C C C C	T T ACC  G G G G G G	C C ATC  GAG  AGA  AGA	CAA CAA GGA  G G TCA  TCA  TCA  TCA  TCA	TCT TCT AAC  TCT TCG GAA GAA GAA GAA GAA C	T T GAG  A A A GGA  GGA  GGA CTC  CTC  T	G G CGT  C C ATC  G.G G.G G.G TTC   AT	A.G A.G TTC  TTC  T.T GAC  GCC  T.G	CGC  A A AAA AAA  AAAC  T	G G G T T T TTG  C C	540 600 660
BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_14 BoAct2_26 BoAct2_34 BoAct2_45 BoAct1_14 BoAct1_2 BoAct1_14 BoAct2_26 BoAct1_14 BoAct2_26 BoAct2_34 BoAct2_45	AAG   CCC  A.A A.A A.A A.A GCC  A.G A.G A.G A.G	 TCG AAC AAC AAC GAA  CAC CAC  TT. TT. TT.	TAC TAC  GTA  GTA   T ACC   ACC  	.CT .CT GAA  CTG  T T T T T	CTC CTC  TTC TTC  ATT  ATT  ATT  ATT  ATT  A A	 CCCC  A A AAT  C.G C.G C.G C.G C.G C.G C.G C.G C.	GAT GAT  CCT  AAG  AAG  AAG	GGA GGA  TCT  AG. AG. AG.  TGC  T T	CAA CAA CAA CAA CAA CAA CAA CAA CAA CAA	 ACT  A A ATC  ATT  G.CC G.C G.C	G.C G.C TTG G G G G G G G G G	T T ACC  G G G G G G	C C ATC  GAG GAG  AGA  G GG	CAA CAA GGA  G G G TCA T TT T T AAA 	TCT TCT AAC  T T TCG GAA GAA GAA GAA GAA  T T	T T GAG   A  GGA  GGA  GGA  GCTC   T T T	G G CGT  C C ATC  G.G G.G G.G TTC  T TAT	A.G A.G TTC  T T CAC  GCC  T.G T.G T.G	CGC  A A AAA  AAAA  AAAC  T T	G G GTC  T TTG C C C.	540 600 660
BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct2_26 BoAct2_34 BoAct2_45 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_1 BoAct1_2 BoAct2_26 BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_1 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_45	AAG  CCCC  A.A A.A A.A GCC  A.G A.G A.G A.G	 TCG  AAC AAC GAA  CAC CAC  TT. TT. TT.	TAC  GTA  GTA  	.CT .CT GAA   CTG  T T T T T	CTC CTC  TT TTC  ATT  ATT  ATT  ATT  ATT  ATT 	CCCC  A A AAT C.G C.G C.G C.G C.G C.G C.G C.G C.G C.G	GAT GAT CCT CCT AAG  AAG  AAG	GGA  TCT  AG. AG. AG.  TGC   TGC   TGC 	CAA  GCT  TTG TTG GAC 	ACT ACT  ACT  ATC  ATC  ATT G.C G.C G.C	G.C G.C TTG G G G GGA GGA   GAT  C C	T T ACC  G G G G G G	C C ATC  GAG  AGA  AGA  G G	CAA CAA GGA  G G TCA T TT AAAA  	TCT TCT AAC   TCG GAA GAA GAA GAA GAC  T.T T	T T GAG   A  A  A  A  GGA  GGA  G CTC CTC  T  T  T  T 	G G CGT  C C C ATC  G.G G.G G.G G.G TTC  .AT .AT	A.G A.G TTC  T T CAC  GCC  T.G T.G	CGC 	G G GTC  T TTG C C C.	540 600 660
BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_34 BoAct2_45 BoAct2_26 BoAct1_1 BoAct1_2 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_45	AAG   CCCC  A.A A.A A.A A.A A.A	 TCG  AAC GAA  CAC CAC  TT. TT. TT. TTG	TAC  GTA    ACC     	. CT . CT GAA   CTG  T T T T T	CTC CTC  T T TTC  ATT  ATT  ATT  ATT  A  A  A	 CCC  A A A AAT C.G C.G C.G C.G C.G C.G C.G C.G C.G C.G	GAT GAT CCT CCT AAG  AAG  AAG	GGA GGA  TCT TCT  AG. AG. AG.  TGC  TGC  TGC  TGC	CAA  GCT  TTG TTG GAC   TTC	ACT ACT A A ATC A ATC A ATC A ATC CC G.C G.C G.C CCCC	G.C G.C TTG G G GG. GG. GG. GGA  GGA  C C.C C.C AAT	T T ACC   G  G  G  G  G  T  T  T  T  T  T  T  C G  T T A  G  G  T T	C C ATC  GAG  AGA  AGA  GAG G GAT	CAA CAA GGA  G G TCA  TCA  TCA  TTA AAA   TTG A	TCT TCT AAC  T T TCG GAA GAA GAA GAA CAA CAA CAA CAA CAA C	T T GAG   GGA  GGA  GGA  CTC  T.T T T	G G CGT  C C C ATC  G.G G.G G.G TTC  .AT .AT .AT	A.G A.G TTC  T T CAC CAC   GCC T.G T.G T.G	CGC      AAAA  AAAA  AAAC   AAAC    AAAC   AAAC  AAAC  AAAC   AAAA  AAAA  AAAA 	G G GTC  T TTG C C C.	540 600 660 720
BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_14 BoAct2_26 BoAct2_34 BoAct2_45 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct1_1 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_34 BoAct2_45 BoAct2_45	AAG   CCCC  A.A A.A A.A A.A A.A	 TCG  AAC GAA  CAC CAC  TT. TT. TT. TT. TTG 	TAC  GTA    ACC     	.CT .CT GAA   CTG T T T T GGA	CTC CTC  T T TTC  ATT  ATT  ATT  ATT  A CGGA	 CCC  A A A AAT  C.G C.G C.G C.G C.G C.G C.G C.G C.	GAT GAT CCT CCT AAG  AAG  AAG  ACA	GGA GGA  TCT TCT  AG. AG. AG. TGC  TGC  TGC  TGC  TGC  TGC	CAA CAA CAA CAA CAA CAA CAA CAA CAA CAA	ACT ACT A A ATC A ATC A ATC A ATC C.CC G.C G.C CCCC	G.C G.C TTG G G GG. GG. GG. GGA  GGAT  C C.C C AAT	T T ACC   G  G  G  G  G  T  T  T  T  ACCC  ACC  ACC  ACC  ACC  ACCC ACCCC ACCCC ACCCC ACCCC ACCCC ACCCC ACCCC ACCCC ACCCCC ACCCCC ACCCCC ACCCCC ACCCCCC	C C ATC  GAG  AGA  AGA  GAT 	CAA CAA GGA   G G TCA T T.T AAA  TTG A A	TCT TCT AAC  T T TCG GAA GAA GAA GAA GAA CAA CAA CAA CAA C	T T GAG   A  GGA  GGA  GGA  GCTC  T  T  T	G G CGT  C C ATC  G.G G.G G.G G.G TTC  .AT .AT .AT	A.G A.G TTC   CAC  GCC  T.G T.G T.G T.G	CGC      AAA  AAA  AAA    AAA   	G G GTC  T T TTG  C C	<ul><li>540</li><li>600</li><li>660</li><li>720</li></ul>
BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_14 BoAct2_26 BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_34 BoAct2_245 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_45 BoAct2_45 BoAct1_1 BoAct2_26 BoAct1_1 BoAct2_26 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2	AAG  CCCC  A.A A.A A.A A.A A.A GCC C A.G GCC GCT  A.G GCT 	 TCG  AAC AAC AAC GAA  CAC  TT. TT. TT. TT. TT. TT. A	TAC  GTA   GTA  	.CT .CT GAA   CTG T T T T GGA  	CTC  T T TTC  ATT  ATT  GGA 	CCCC  A A AAAT A AAAT C.G C.G C.G C.G C.G C.G C.G C.G C.G C.G	GAT  CCT  AAG  AAG  ACA 	GGA GGA  TCT  AG. AG. AG. AG. T T.T T ATG  T	CAA CAA CAA CAA CAA CAA CAA CAA CAA CAA	ACT  A A ATC  ATC  ATC CCC G.C G.C G.C G.C G.C G.C CCC  GAA	G.C G.C TTG G G G G G G	T T ACC  G G G AGC  T T T T ATC  ATC  ATC	C C ATC  GAG  AGA G G GAT G A	CAA CAA GGA G G TCA T T T AAA  CAA TTG A A GAA	TCT TCT AAC  T TCG GAA GAA GAA GAA GAA C TT T	T T GAG  A A GGA  GGA  GGA  G CTC CTC C T T T T	G G CGT  C C ATC  G.G G.G G.G G.G TTC  .AT .AT .AT .AT .AT	A.G A.G TTC   CAC  GCC  T.G T.G T.G T.G	CGC      AAA  AAA  AAA    AAA   	G G GTC  T T TTG  C C	540 600 660 720
BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_26 BoAct2_34 BoAct2_45 BoAct1_1 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_245 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_245 BoAct2_34 BoAct2_34 BoAct2_34 BoAct2_34 BoAct2_26 BoAct1_14 BoAct2_26 BoAct2_26 BoAct2_34 BoAct2_27 BoAct1_245	AAG  CCCC  A.A A.A A.A A.A A.A GCC C A.G GCT C GTT  C.CC CC	 TCG  AAC AAC AAC GAA  CAC  TT. TT. TT. TT. TT. TT. A A	TAC  GTA  GTA     ACC   	.CT .CT GAA   CTG  T T T GGA  GGA 	CTCC   TTC  ATT  ATT  GGA  GGA	CCCC  A A AAAT A AAAT C.G C.G C.G C.G C.G C.G C.G C.G C.G C.G	GAT  CCT  AAG  AAG  ACA  ACA	GGA  TCT  AG. AG. AG. TGC  TGC  ATG  ATG	CAA  GCT  TTG TTG GAC  TTC  TTC 	ACT  A A ATC  ATC  ATC  G.C G.C G.C G.C C CCC GAA GAA	G.C G.C TTG G G G G G G	T T ACC  G G G AGC  T T T T ATC  ATC  ATC 	C C ATC  GAG  AGA G GAT G GAT G A A	CAA CAA GGA G G TCA T T T AAA  CAA CAA CAA	TCT TCT AAC  TC GAA GAA GAA GAA GAA C TT T T	T T GAG  A A GGA  GGA  GGA  G CTC CTC CTC CTC CTC C T T T T	G G CGT C C ATC C G.G G.G G.G G.G TTC AT AT AT AT AT AT	A.G A.G TTC  TTC T T CAC CAC  T.G CAC T.G T.G T.G T.G T.G T.G T.G AAG AAG	CGC     AAA  AAA  AAA    AAA  AAA  AAA  AAA  AAA  A	G G GTC  T T TTG  C C	540 600 660 720
BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_26 BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_26 BoAct2_245 BoAct1_14 BoAct2_26 BoAct2_34 BoAct2_34 BoAct2_34 BoAct2_34 BoAct2_26 BoAct1_14 BoAct2_26 BoAct1_245	AAG  CCC  A.A A.A A.A A.A A.A GCC  A.G A.G A.G GTT  C C	TCG  AAC AAC AAC GAA  CAC CAC TT. TT. TT. TT. TT. A. A. A.	TAC  GTA  	.CT .CT GAA   CTG  T T T T GGA  GGA 	CTC  TT T TTC  ATT  ATT  GGA 	CCCC  A A AAT C.G C.G C.G C.G C.G C.G C.G C.G C.G C.G	GAT  CCT  AAG  AAG  ACA  ACA  	GGA  TCT  AG. AG. AG. TGC  TGC  ATG  ATG	CAA  GCT  TTG TTG GAC  TTC  TTC 	ACT    ATC  ATC  ATC G.C G.C G.C C CCC C CCC G.C C CCC G.C C CCC G.C C C C	G.C G.C G.C TTG G G G G G G G	T T ACC  G G G G G G	C C ATC  GAG G GAT G GAT G GAT G A A	CAA CAA GGA G G G TCA T TT AAA T TTG A A GAA GAA GAA	TCT TCT AAC  T T T TCG GAA GAA GAA GAA GAA CAA  T T T	T T GAG  A A A GGA  GGA CTC CTC CTC CTC CTC CTC CTC CTC CTC CT	G G CGT C C ATC C G.G G.G G.G G.G TTC  .AT .AT .AT .AT .AT 	A.G A.G TTC  TTC  CAC CAC  GCC T.G T.G T.G T.G T.G T.G AAG AAG	CGC      AAAA  AAAA  AAAC     CGAA  GAAA  	G G G GTC  T T TTG T C C	540 600 660 720
BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_26 BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_26 BoAct2_245 BoAct1_14 BoAct2_26 BoAct2_34 BoAct2_34 BoAct2_34 BoAct2_34 BoAct2_34 BoAct2_34 BoAct2_26 BoAct1_14 BoAct2_26 BoAct1_245 BoAct1_245 BoAct1_245 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_45 BoAct2_45 BoAct2_26 BoAct2_45 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_27 BoAct2_26 BoAct2_27 BoAct2_26 BoAct2_27 BoAct2_2	AAG  CCCC  A.A A.A A.A A.A GCC  A.G A.G A.G GTT  C C.CC TCT	 TCG  AAC AAC AAC  CAC CAC  TT. TT. TT. TTG A A A TTG	TAC TAC  GTA  	.CT .CT GAA  CTG  T T T T T	CTC    TTC  ATT  ATT  AGGA  CCT	CCCC  A A AAT A AAAT C.GG C.GG C.GG C.GG C.GG C.GG C.GG C.G	GAT  CCT  AAG  AAG  ACA  ACA  GG G TCC	GGA  TCT  AG. AG. AG. TGC T T ATG T ATG  ATG	CAA  GCT  GCT TTG TTG GAC  TTC  CAA	ACT A A ATC A ATC G.C G.C G.C G.C C CCC G.C GAA GAA GAA GAA	G.C G.C TTG G G G G G G G G C C.	T T ACC  G G AGC  AGC  T T T ATC  ATC  ATC  GTG	C C ATC  GAG  AGA  AGA G GAT  A A	CAA CAA GGA G G TCA T TT AAA T TTG A A GAA GAA GAA TGT	TCT TCT AAC  T T T TCG GAA GAA GAA GAA GAA C TT T T T	T T GAG  A A A GGA  GGA  CTC CTC CTC C C C C C.	G G CGT  C C ATC  G.G G.G G.G G.G G.G TTC  	A.G A.G TTC T T CAC CAC  GCC T.G T.G T.G T.G T.G T.G AAG AAG AAG	CGC 	G G G T T TTG T TTG C C	<ul><li>540</li><li>600</li><li>660</li><li>720</li><li>780</li></ul>
BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_34 BoAct2_45 BoAct1_2 BoAct1_2 BoAct1_14 BoAct2_26 BoAct2_34 BoAct2_45 BoAct1_14 BoAct2_26 BoAct1_14 BoAct2_45 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct2_45 BoAct2_45 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct2_45 BoAct2_34 BoAct2_45 BoAct2_34 BoAct2_45 BoAct2_15 BoAct2_14 BoAct2_26 BoAct2_34 BoAct2_45 BoAct2_14 BoAct2_45 BoAct1_1 BoAct2_12 BoAct1_12 BoAct1_14 BoAct2_14 BoAc	AAG  CCCC  A.A A.A A.A A.A GCC  A.G GTT  CCC C TCT 	TCG  AAC AAC GAA  CAC  TT. TT. TT. TT. TT. TT. TT. TT. T	TAC  GTA  GTA    ACC  	.CT .CT GAA  CTG  T T T T T	CTC    TTC  ATT  ATT  ATT  ATT  A  CCT	CCCC  A A AAT  C.G C.G C.G C.G ATG  TCC  AG. AG. AG. TCT	GAT  CCT  AAG A A A A A G G G TCC	GGA  TCT  AG. AG. AG. TGC  TGC  AG. AG. AG. AG. AG. AG. AG. AG. AG	CAA  GCT TTG TTG GAC  TTC TTC  CAA	ACT A A A ATC  ATT G.C G.C G.C CCC  GAA GAA GAA GAC	G.C G.C TTG G G G G G GGA  C.C C.C C.A C C.A C C.A C C.A C C.A C C.A C C C C	T T ACC  G ACC ACC	C C ATC  GAG  AGA  AGA  GAT  A A	CAA CAA GGA G G G TCA T T.T AAA  TTG A GAA GAA GAA GAA GAA TGT 	TCT TCT AAC  T  TCG GAA GAA GAA GAA GAA C T T T  T T  T T  T  T  T  T  T  T  T  T T  T  T T  T T T  T  T  T  T T T  T T  T T  T T  T T  T T T  T T T T  T T  T T T  T T  T  T  T T  T T  T T   	T T GAG  A A A GGA  GGA  CTC CTC C C C C C C	G G CGT C C ATC C ATC C G.G G.G G.G G.G TTC  .AT AT AT AT G G 	A.G A.G TTC  TTC  CAC  GCC T.G T.G T.G T.G T.G T.G CAC CCC	CGC 	G G G T T TTG T TTG C C	<ul><li>540</li><li>600</li><li>660</li><li>720</li><li>780</li></ul>
BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_34 BoAct2_45 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_34 BoAct2_245 BoAct2_34 BoAct2_245 BoAct1_1 BoAct2_26 BoAct2_234 BoAct2_245	AAG AAG CCCC  A.A A.A A.A A.A A.A GCC  A.G A.G A.G A.G CCC  A.C TCT  	 TCG  AAC AAC GAA  CAC  TT. TT. TT. TT. TT. TT. TT. TT. T	TAC  GTA  GTA     ACC   	.CTT .CT GAA  CTG T T T GGGA  GGGA  CC C C C	CTC T T TTC T TTC  ATT A GGA A CCT 	CCCC  A A AAT A AAT  C.G C.G C.G C.G ATG  AG. AG. AG. AG. AG. AG. 	GAT  CCT  AAG  AAG  ACA  CC CC	GGA  TCT  AG. AG. AG. TGC T T ATG T ATG  ATG  ATG  ATG 	CAA  GCT  TTG TTG GAC  TTC GAA  GAA 	ACT A A A ATC  ATT  G.C G.C G.C G.C C CCC  GAA GAA GAA GAA GAA GAA	G.C G.C TTG G G G G G G G G C C.	T T ACC  G.G  AGC  AGC  T.T  T  T  T  T  T  T  T  T  G G G G	C C ATC  GAG  AGA  AGA  GAT  A A	CAA CAA CAA GGA G G G TCA T T.T AAA  A.A GAA GAA GAA GAA GAA CAA CAA CAA CAA CA	TCT TCT AAC  TCT TCG GAA GAA GAA GAA GAA GAA C T.T T T T CCC  CCT	T T GAG  A A A GGA  GGA  GCT C CA CA CA CA	G G CGT C C C ATC C ATC         	A.G A.G TTC T T T CAC  GCC  T.G T.G T.G T.G T.G T.G AAG AAG AAG AAG	CGC 	G G GTC  T TTG T TTG C C	<ul><li>540</li><li>600</li><li>660</li><li>720</li><li>780</li></ul>
BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_34 BoAct2_45 BoAct2_45 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_25 BoAct1_14 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_27 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct2_34 BoAct2_34	AAG AAG CCCC  A.A A.A A.A A.A GCC  A.G A.G A.G A.G C.C C.C  C.C  A.C C.C C.C	 TCG  AAC GAA  GAA  CAC C TT. TT. TT. TT. TT. TT. TT. TT. T	TAC  GTA  	.CT .CT GAA  CTG T T T T GGA  CC C GGA  C C C.	CTC  .T .T TTC  ATT  ATT  ATT  CCT   CCT    CCT 	CCCC A A A AAT C.G C.G C.G C.G C.G C.G C.G C.G C.G C.G	GAT GAT CCT CCT AAG AAG AAG ACA ACA CC CCT CC CCT CC CCT CC CCT CC CCT CC CC	GGA  TCT  AG. AG. AG.  TGC  TGC  AG. AG	CAA  GCT TTG TTG TTG GAC  TTC GAC   CTC  CTC  CTC  CTC  CAA	ACT A A A ATC A ATC  ATT G.CC G.CC G.CC G.CC GAA GAA GAA GAA GAA GAA	G.C G.C TTG G G G G G G G C C C.	T T ACC  G.G G.G AGC  T.T  T ATC ATC  ATC  GTG GTG	C C ATC  GAG GAG G GAG G GAT A A	CAA CAA GGA G GGA G TCA T TT AAA CT AAA CT GAA GAA GAA TGT C	TCT TCT TCT AAC  T  TCG GAA GAC GAA GAA GAA GAA C T T T T  T T   T T  T  T  T  T  T  T  T  T  T  T  T	T T GAG  A A A GGA  GGA  G CTC CTC CTC CTC CTC CTC CTC CTC CTC C	G G CGT C C C ATC C ATC C ATC  AT AT AT AT C G.G G.G G.G G.G G.G  AT C 	A.G A.G TTC T T T CAC  GCC GCC T.G T.G T.G T.G T.G T.G CAC AAG AAG AAG AAG AAG	CGC 	G G GTC T TTG T TTG C C	<ul><li>540</li><li>600</li><li>660</li><li>720</li><li>780</li></ul>
BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct2_26 BoAct2_34 BoAct2_45 BoAct2_45 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_45 BoAct2_45 BoAct1_2 BoAct1_2 BoAct2_45 BoAct2_45 BoAct2_45 BoAct1_2 BoAct1_2 BoAct1_2 BoAct2_45	AAG AAG CCCC  A.A A.A A.A A.A A.A GCC  A.G GTT      	 TCG  AAC GAA  GAA  CAC CAC CAC TT. TT. TT. TT. TT. TT. TT. TT. TT. TT	TAC  GTA  GTA      ACC   	.CT .CT GAA  CTG CTG T T T T GGGA  GGCT GCT 	CTC  TT T TTC T TTC T TTC T TTC T TTC T TTC T T TC TC T TC TC T TC TC T TC TC TC TC TC TC TC TC TC TC TC	CCCC A A A AAT C.G C.G C.G C.G C.G C.G C.G C.G C.G C.G	GAT GAT CCT CCT AAG AAG AAG AAG CCT CCT CCT CCT CCT CCT CCT CCT CCT CC	GGA  TCT  AG. AG. AG.  TGC  TGC  AG. AG	CAA  GCT TTG TTG GAC  TTG GAC  CTC C C C C C C C C C C C C C C C	ACT A A A ATC A ATC  ATT G.CC G.CC G.CC G.CC GCC GAA GAA GAA GAA GAA GAA G	G.C G.C TTG G G G G G GGA   C C C C C C C C	T T ACC  G G AGC  T T ATC  ATC  GTG GTG  GTG	C C ATC  GAG GAG G GAG G GAT A A	CAA CAA GGA G G G TCA T TT T AAA C CT GA A A GAA GAA GAA GAA GAA CAA	TCT TCT TCT AAC  T T TCG GAA GAA GAA GAA GAA CAA CAA CAA CAA C	T T GAG  A A GGA  GGA  GGA  CTC CTC CTC CTC CTC CTC CTC CTC CTC	G G CGT C C C ATC C ATC C ATC C ATC C ATC C G.G G.G G.G G.G G.G G.G G.G C ATC C C C C C ATC 	A.G A.G TTC   CAC CAC CAC   GCC T.G T.G T.G T.G T.G T.G CCC CCC CAC   AAG AAG A.A A.A	CGC 	G G GTC T T TTG C C C	<ul><li>540</li><li>600</li><li>660</li><li>720</li><li>780</li></ul>
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BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_34 BoAct2_45 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_245 BoAct1_1 BoAct2_26 BoAct2_245 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_245 BoAct2_245 BoAct2_245 BoAct2_26 BoAct2_245 BoAct2_26 BoAct2_245 BoAct1_14 BoAct2_26 BoAct2_25 BoAct1_14 BoAct2_26 BoAct2_245 BoAct1_14 BoAct2_26 BoAct2_245 BoAct1_14 BoAct2_26 BoAct2_245 BoAct1_14 BoAct2_26 BoAct2_245 BoAct2_45 BoAc	AAG AAG CCCC  A.A A.A A.A A.A A.A GCC  A.G A.G A.G A.G CTCT       	TCG AAC AAC AAC AAC CAC CAC CAC CAC CAC TT. TT. TT. TT. TT. TT. TT. TT. GTT A A	TACC GTA  GTA       	.CTT .CT GAAA  CTG  T T T T T	CTC   TTC  TTC  ATT  ATT  ATT  ATT  ATT  ATT  ATT  ATT  ATT  ATT  ATT  ATT  ATT  ATT  ATT  ATT  ATT  ATT  ATT   ATT   ATT  	CCCC 	GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT	GGA  TCT TCT  AG. AG. AG. AG. AG. AG. AG. AG. AG	CAA GCT GCT TTG TTG GAC C.C. C.C. C.C. C.C. C.C. C.C. C.C.	ACT  ACT  ATC  ATC  G.C G.C G.C G.C CCC  GAA GAA GAA GTC  A A CC  CC CC  A	G.C G.C G.C TTG G G GGA  GGA  GAT  C C C C C C C C C.	T T ACC  G.G AGC  AGC  ATC  ATC  ATC  CTG  CTG 	C C ATC  GAG  AGA  AGA  AGA  AGA  C 	CAA CAA CAA GGA G TCA G TCA T T.T AAA T T.T AAA T T.T AAA C.T C.T C.T C.T C.T C.T C.T C.T C.T C.T	TCT TCT AACC  TCG GAA GAA GAA GAA GAA GAA GAA C TTC CCC CCC TTC  TTC CCC TTC  TTC 	T T GAG GAG  A A GGA  G GGA  G CTC C.A C.A C.A C.A C.A C.A C.A C.A C.A C.	G G CGT C C ATC C ATC C ATC C 	A.G A.G TTC  TTC  CAC CAC CAC  T.G T.G T.G T.G T.G T.G T.G T.G T.G	CGC 	G G G GTC  T TTG C C	<ul> <li>540</li> <li>600</li> <li>660</li> <li>720</li> <li>780</li> <li>840</li> </ul>
BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_34 BoAct2_45 BoAct2_45 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_27 BoAct1_1 BoAct2_26 BoAct2_26 BoAct2_28 BoAct2_28 BoAct2_29 BoAct1_12 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_26 BoAct2_27 BoAct1_14 BoAct2_26 BoAct2_26 BoAct2_245 BoAct2_245 BoAct1_14 BoAct2_26 BoAct2_245 BoAct2_245 BoAct2_245 BoAct2_245 BoAct2_245 BoAct2_245 BoAct2_245 BoAct2_245 BoAct2_245 BoAct2_245 BoAct2_25 BoAct2_245 BoAct2_25 BoAct2_245 BoAct2_25 BoAct2_245 BoAct2_25 BoAct2_245 BoAct2_245 BoAct2_245 BoAct2_245 BoAct2_245 BoAct2_245 BoAct2_25 BoAct2_25 BoAct2_25 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_27 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_27 BoAct2_26 BoAct2_26 BoAct2_27 BoAct2_26 BoAct2_27 BoAct2_26 BoAct2_26 BoAct2_27 BoAct2_26 BoAct2_27 BoAct2_26 BoAct2_27 BoA	AAG AAG CCCC  A.A A.A A.A A.A A.A A.G A.G A.G A.G	 TCG AAC AAC AAC CAC  TT. TT. TT. TT. TT. TT. TT. TT. T	TACC  GTA  GTA      ACC  	.CTT .CT GAAA  CTG CTG T T T T T GGAA   GGA GCT  ATC GCT  C C C C C C C C C C C C C C C C C	CTC    TTC  ATT  ATT  ATT  ATT  ATT  ATT  ATT  ATT  ATT  ATT  ATT  ATT  ATT  ATT  ATT   ATT  ATT   ATT      ATT  	CCCC 	GAT GAT GAT GAT GAT GAA GAT GAA GAT GAA	GGA  TCT TCT  AG. AG. AG. AG. TGC  TT T ATG  AG  ATG  ATG  ATG  ATG  ATG  ATG  ATG  ATG  ATG  ATG  ATG  AG  ATG  AG 	CAA GCT GCT TTG TTG GAC C C GAA TTC GAA C C C C C C C	ACT  ACT  ATC  ATC  ATC  ATC G.C G.C G.C G.C G.C CCC  ATC  A	G.C G.C G.C TTG G G GGA G GGA G GGA C C C C C C C C C C.	T T ACC   G AGC  AGC  T T T ATC  ATC  CTG  CTG  CTG 	C C ATC  GAG G G G GAT G G GAT G G	CAA CAA CAA GGA G TCA G TCA T T.T AAA T T.T AAA  CAA CAA CAA CAA CAA CAA CAA CAA	TCT TCT AAC  T T TCG GAA GAA GAA GAA GAA GAA C TT T T T T T	T T GAG GAG  A A GGA  GGA  CTC C C C C C C	G G CGT C C ATC C ATC C ATC C ATC C ATC C G.G G.G G.G G.G G.G G.G G.G CAG C 	A.G A.G TTC  TTC  CAC CAC C T.G T.G T.G T.G T.G T.G T.G T.G T.G	CGC   	G G G GTC  T TTG C C	<ul> <li>540</li> <li>600</li> <li>660</li> <li>720</li> <li>780</li> <li>840</li> <li>869</li> </ul>
BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct2_26 BoAct2_34 BoAct2_45 BoAct2_45 BoAct2_45 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_245 BoAct1_1 BoAct2_26 BoAct2_245 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct2_34 BoAct2_245 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_34 BoAct2_45	AAG AAG CCCC  A.A A.A A.A A.A A.A A.A A.A A.	 TCG AAC AAC AAC AAC AAC CAC CAC C	TACC TACC  GTA   	.CTT .CT GAAA  CTG CTG T T T T T CTG  C C  C  C  C  C  C C  C  C  C  C C C C  C	CTC      ATT  ATT   ATT  	CCCC  A A A	GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT	GGA  TCT T AG. AG. AG. TGC  TGC  ATG ATG  ATG  ATG  AT	CAA  GCT  TTG TTG TTG GAC  TTG TTG TTG C C A A A A A C	ACT  ACT  ATC  ATC  ATC  ATC G.C G.C G.C G.C G.C CCC  A A A CC  CC 	G.C G.C G.C TTG G G G G G G G G C C.	T T ACC  G ACC  ACC  ACC  ACC  ACC  ACC  G G	C C ATC  GAG G GAG G GAT  AGA  AGA  AGA  TCT T TCT 	CAA CAA CAA GGA G TCA G TCA T TT AAA  GAA GAA GAA TGT G GC. GC. ACG 	TCT TCT AAC  T TCG GAA GAA GAA GAA GAA GAA GAA C TT T CCC T T T	T T GAG GAG  A A A A A A A A A A A A	G G CGT C C C C C	A.G A.G TTC   CAC CAC   GCC T.G T.G T.G T.G T.G T.G T.G AAG AAG A.A AAG A.A A.A A.A A.A	CGC 	G G G GTC T T TTG T TTG T CC T ATT T ATT T TTT TTT ATT T ATT T ATT T ATT T	<ul> <li>540</li> <li>600</li> <li>660</li> <li>720</li> <li>780</li> <li>840</li> <li>869</li> </ul>
BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_26 BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_26 BoAct2_34 BoAct2_26 BoAct1_14 BoAct2_26 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct2_34 BoAct2_34 BoAct2_45 BoAct1_14 BoAct2_26 BoAct2_34 BoAct2_45 BoAct1_12 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_45 Bo	AAG AAG CCC  A.A A.A A.A A.A A.A A.A	 TCG AAC AAC AAC AAC CAC CAC  TT TT TT TT TT TT AA. A A A.	TAC TAC GTA         	.CT .CT GAA   CTG  T T T T T	CTC   TTC  ATT  ATT  ATT  ATT  ATT  ATT  ATT  ATT  ATT  ATT   ATT  	CCCC 	GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT	GGA GGA GGA GGA G GGA AG AG AG AG AG AG	CAA  GGCT  TTG TTG GAC  C GAA  A A A	ACT  ACT  ATC  ATC  ATC G.C G.C G.C G.C CCC  A A A CCC  CC 	G.C G.C G.C TTG G G G G G G G C C C.	T T ACC  G.G  AGC  AGC  ATC  ATC  CTG CTG CTG CTG	C C ATC  GAG  AGA  AGA G GAT G GAT G A A	CAA CAA CAA GGA G G TCA T T T AAA  CAA CAA GAA GAA GAA GAA GAA GAA CGC. GC. GC. ACG C. 	TCT TCT TCT AACC   TCG GAA GAA GAA GAA GAA GAA GAA C  TC  TC  T TC GCT   TTCC  TTC  TTC  TTCC  TTCC  TTCC  TTCC 	T T GAG   A GGA G GGA G CTC C CTC C C C C C	G G CGT C C ATC C ATC C TTC C TTC  AT AT AT AT AT AT AT AT AT C C	A.G A.G TTC   CAC CAC  GCC T.G T.G T.G T.G T.G T.G AAG AAG AAG A.A A.A A.G	CGC 	G G G GTC T TTG T TTG T C C	<ul> <li>540</li> <li>600</li> <li>660</li> <li>720</li> <li>780</li> <li>840</li> <li>869</li> </ul>
BoAct2_34 BoAct2_45 BoAct1_1 BoAct1_2 BoAct1_2 BoAct2_26 BoAct2_34 BoAct2_45 BoAct2_45 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_26 BoAct2_245 BoAct1_14 BoAct2_26 BoAct2_34 BoAct2_45 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct2_34 BoAct2_45 BoAct2_45 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_45 BoAct1_1 BoAct2_26 BoAct2_34 BoAct2_45 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct1_2 BoAct2_34 BoAct2_45 BoAct2_45 BoAct2_45 BoAct2_34 BoAct2_45 BoAct2_34 BoAct2_45 BoAct2_34 BoAct2_45 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_26 BoAct2_34 BoAct2_45	AAG AAAG  CCCC  A.A A.A A.A A.A A.	TCG           AAC           AAC           AAC           AAC           AAC           CACC	TACC  GTA  GTA      ACC   	.CT .CT GAA  CTG  T T T T T	CTC    TTC   ATT  ATT  	CCCC 	GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT	GGA  TCT  AG. AG. AG. AG. TGC  TGC  ATG 	CAAA  GGCT  TTG TTG GAC  TTG GAC  C C C C C C	ACT A A ATC A ATC A ATC A ATC G.C G.C G.C G.C CCC  A A A A A CCC  	G.C G.C G.C TTG G G G GGA   GAT  C C C C C C C C C.	T T ACC  G.G  AGC  AGC  T T ATC  ATC  GTG CTG CTG CTG	C C ATC  GAG  AGA  AGA  AGA  A	CAA CAA CAA GGA G G TCA T T T AAA  CAA CAA GAA GAA GAA GAA GAA GAA CAA CAA	TCT TCT TCT TCT AACC   T TCG GAA GAA GAA GAA GAA GAA GAA C  T  T C C C C C C T C C C C	T T GAG   A GGA G GGA G CTC  CTC  CTC C C C.	G G CGT C C ATC C ATC C ATC  AT AT AT AT AT AT AT AT AT AT AT C CAG C CAG C CAG C C	A.G A.G TTC  .TT  CAC  T.G CAC  T.G T.G T.G T.G T.G T.G AAG AAG AAG A.A A.A A.A A.A A.A	CGCC  	G G G T T TTG T TTG  C C	<ul> <li>540</li> <li>600</li> <li>660</li> <li>720</li> <li>780</li> <li>840</li> <li>869</li> </ul>

## Bonamia ostreae actin gene

# Fig. 2B

## **(B)**

60	NWDDMEKIWHHSFFNELRVAPEEHPVLLTEAPLNPKANREKMTQIMFETFNTPAMYVAIQ	Bonamia ostreae Actinel Bonamia ostreae Actine2
120	AVLSLYASGRTTGIVLDSGDGVSHTVPIYEGYALPHAILRLDIAGRDLTDYLIKILTERG	Bonamia ostreae Actinel Bonamia ostreae Actine2
180	HSFTTTAEREIVRDIKETLCYCAKDFEAEMSASTESSEIDKSYELPDGQTLTIGNERFRV .NIAS.QSC.K.E.NV	Bonamia ostreae Actinel Bonamia ostreae Actine2
240	PEVLFNPSAIGSESSGIHKLAHTSIMKCDIDIRKDLFANIVLSGGSTMFPNIDLRVQSEI TQ.LE.VTFNVYSMEH.NE.LRK	Bonamia ostreae Actinel Bonamia ostreae Actine2
289	SLLAPSSIEVKVICPPERKYSVWIGGSILSSLSTFQQMWITKDEYDESG .MMKINAT	Bonamia ostreae Actinel Bonamia ostreae Actine2

## Bonamia ostreae actin gene

# 1 2 3 4 5 6 7 8 9 10 11 12 13 400 bp-200 bp 4 5 6 7 8 9 10 11 12 13 400 bp-200 bp 4 5 5 6 7 8 9 10 11 12 13 400 bp-200 bp 4 5 5 6 7 8 9 10 11 12 13 400 bp-200 bp 4 5

# Fig. 3

## Bonamia ostreae actin gene

Fig. 4

