IDENTIFICATION AND DEVELOPMENTAL EXPRESSION OF THE ZEBRAFISH ZGC:154061 GENE, A CONSERVED YET UNCHARACTERIZED MATERNALLY EXPRESSED *MEIS2* LINKED GENE

A Thesis by BRANDON SCOTT CARPENTER

Submitted to the Graduate School Appalachian State University In partial fulfillment of the requirements for the degree of MASTER OF SCIENCE

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FOREWORD

The organization and formatting of this thesis strictly follows the instruction to the author for manuscript submission to *Gene Expression Patterns*, the official journal of The International Society of Developmental Biologists. The general organization of the text is similar to that of a *Nature* letter, with the whole text in a single main section headed "Results and Discussion."

ABSTRACT

IDENTIFICATION AND DEVELOPMENTAL EXPRESSION OF THE ZEBRAFISH ZGC:154061 GENE, A CONSERVED YET UNCHARACTERIZED MATERNALLY EXPRESSED*MEIS2* LINKED GENE (May 2010)

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We have identified a novel and previously undescribed gene, zgc:154061, located directly downstream of the zebrafish *meis2.2* gene. We have identified putative orthologs of this gene in all animals that we have been able to examine. The zgc:154061 gene and its vertebrate orthologs are organized in a convergently transcribed manner with respect to the *Meis2* gene in all species we have examined (*meis2.2* in teleosts). It appears that the homologs of *Meis* and zgc:154061 are also linked in amphioxus and sea urchins but that this linkage is not present in urochordates, nor in protostomes. During zebrafish development, transcripts of zgc:154061 are observed in every cell of the embryo from the earliest stage through the shield stage indicating this gene is a maternal transcript since its expression of zgc:154061 gradually decreases from its peak value at 0 hpf until 8 hpf and then is observed to be activated again at 12 hpf throughout the neural tube before becoming restricted to the retina and tectum opticum by 48 hpf.

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The vertebrate *Meis* homeobox-containing gene family consists of at least three members in vertebrates. The products of the *Meis* genes appear to function as cofactors, directly interacting with other homeodomain proteins as well as DNA to facilitate transcriptional regulation. During the process of searching for *cis*-regulatory elements associated with *Meis2* using phylogenetic footprinting, we identified a novel conserved gene sequence located directly downstream of *Meis2* in every vertebrate examined. While this novel gene appears to be present in every animal we have been able to examine, nothing is known concerning its function or expression. Here we report the first spatial and temporal expression pattern to date for any ortholog of this gene, in zebrafish.

1. RESULTS and DISCUSSION

1.1 Identification of zgc:154061

In zebrafish, the *zgc:154061* gene is found directly downstream of the *meis2.2* gene on chromosome 17 (Fig. 1A), the two genes separated by approximately 10.9-Kb. The *zgc:154061* gene codes for the production of a transcript 1914-nt in length (Fig. 1B) that is convergently transcribed with respect to *meis2.2* and is predicted to be encoded by 9 exons using the NCBI Model Maker tool for genomic sequence analysis. It does not appear that there is a second paralog of *zgc:154061*, associated with the second zebrafish *Meis2* homolog *meis2.1* or elsewhere in the zebrafish genome for that matter, based on BLAST analysis

against the available zebrafish genome. The gene encodes an open reading frame that would be predicted to produce a protein 300 amino acids in length (Fig. 1B). This protein has been previously reported as hypothetical protein LOC767755 as part of a large scale gene identification effort (Strausberg et al., 2002), and a cDNA clone of the gene made available through Open Biosystems, from whom we obtained it. Nothing else has been reported concerning this gene or its product in any species.

We have identified predicted orthologs of zgc:154061 in all vertebrates with publicly available genome data that we have been able to examine. These vertebrate orthologs are present in the same convergently transcribed orientation directly adjacent to that species' Meis2 ortholog, although the spacing separating the two genes varies between species. An amino acid alignment of representative vertebrate species is shown in Fig. 2 and summarized also in Table 1. As shown in Fig. 3, phylogenetic analysis revealed that the translated zebrafish zgc:154061 protein is most closely related to the Takifugu ortholog amongst all species examined. The zebrafish predicted protein represents the longest protein from the orthologs examined. Furthermore there seems to be some variability in the amino terminus of the orthologous proteins with several different translation start points. The mouse and chicken proteins have similar start and stop sites as do the human and *Takifugu* proteins. The zebrafish protein includes an 18-aa amino terminal sequence not found in tetrapods. In addition the chicken protein appears to have an 11-aa deletion compared to human, mouse and zebrafish. There does not appear to be any similarity between zgc:154061, nor any of its orthologs, to any previously described gene to provide any clues to functional domains coded for by this gene or about the function of this gene in general.

Putative homologs of zgc:154061 have also been identified in the urochordate *Ciona* floridae cephalochordate Branchiostoma intestinalis. the and the echinoderm Strongylocentrotus purpuratus. A comparison of representative homologs of the translated product of these genes is included in Table 1. In C. intestinalis, based on the available genome data, it appears that the zgc:154061 ortholog and the Meis ortholog are on different chromosomes. However, in the amphioxus genome, the zgc:154061 ortholog and the Meis ortholog are found adjacently on scaffold 120 and in the sea urchin the two genes are found adjacent to one another but it appears that they are transcribed from the same strand of DNA as opposed to the convergently transcribed orientation observed in vertebrates. Putative homologs of zgc:15406 have also been identified in a number of invertebrate species, based on sequence similarities, including fruit fly (Drosophila melanogaster) as indicated in Table 1 and Fig. 3. It appears that all nonvertebrate putative orthologs of the product of zgc:154061 share several short conserved domains in the C-terminal region as indicated in Fig 2 but none of the protostome orthologs appear to be adjacent to the invertebrate Meis orthologue homothorax in any of these species. Together this suggests that the genomic organization and linkage of *Meis2* and orthologs of zgc:154061 is ancient and arose early in the deuterostome lineage, although this organization seems to have been lost in urochordates.

1.2 Spatial and temporal expression of zgc:154061

As a first step in characterizing zgc:154061, we examined the spatial and temporal expression of this gene using whole mount *in situ* hybridization (Fig. 4 and Fig. 5) and quantitative real-time PCR (Fig. 6) between the zygote and early larva periods. Strong

expression is observed at the one cell stage and expression persists ubiquitously in every cell beyond the onset of transcription of the zygotic genome, at the midblastula transition, to the shield stage (Fig. 4A-C). By 9 hpf, expression of zgc:154061 is observed to be very low and almost undetectable by whole mount *in situ* hybridization (Fig. 4D). These observations are supported by quantitative real-time PCR where we see the quantity of transcripts of zgc:154061 steadily decrease from their maximum level at the earliest stage of development to barely detectable levels at 8 hpf (Fig. 6). These observations would be consistent with that of a maternal transcript that is present in the zygote and that gradually degrades over time while not being replenished by new transcriptional activity. This is not unexpected since at least the first 3 h of zebrafish development are not accompanied by transcription of the embryo's genome. Transcripts of the zebrafish β -actin gene, used as endogenous control for the quantitative real-time PCR experiments, have been reported to be present at nearly even levels throughout most of development, although with an inexplicable slight increase in expression observed prior to the onset of embryonic transcription (McCurley and Callard, 2008). The decrease in expression of zgc:154061 relative to the steady expression β -actin prior to activation of transcription of the embryo's genome suggests varying half lives of these two transcripts, or perhaps the existence of a mechanism by which zgc:154061 is actively reduced. Following epiboly, low expression of zgc:154061 is again observed, this later expression peaking at 12 hpf based on quantitative real-time PCR (Fig. 6). Whole mount in situ hybridization reveals that this expression is largely restricted to the developing neural tube. Expression is observed throughout the neural tube and the optic vesicle until 24 hpf (Fig. 4G and Fig. 5C,D) becoming more anteriorly restricted by 48 hpf of development where expression is observed throughout the forebrain, the tectum of the midbrain and very

faintly in the anterior hindbrain (Fig. 5G,H). The strongest expression at 48 hpf is observed in the retina and tectum opticum (Fig. 5H,J,K). Following 48 hpf, *zgc:154061* expression gradually decreases in these areas to below observable levels for the remainder of development.

A reasonable explanation for the conservation of the genomic linkage of *Meis2* and orthologs of zgc:154061 would be that the two genes are sharing cis-regulatory elements. This logic has been used to help explain the clustered organization of Hox genes (Harding et al., 1985; Gould et al., 1997; Mann, 1997; Sharpe et al., 1998; Prince, 2002) as well as the clustered organization of the *Dlx* gene family, for example, which are found as convergently transcribed gene pairs in vertebrates (Ellies et al., 1997; Zerucha et al., 2000). If Meis2 and zgc:154061 orthologs are sharing cis-regulatory elements, one would predict that they should exhibit overlapping patterns of expression. Comparing the expression of zgc:154061 described here to that of meis2.2 shown here (Fig. 5) and reported previously (Waskiewicz et al., 2001; Thisse, 2005; Bessa et al., 2008; Santos et al., 2010), it appears that there is some overlap between these two genes in the developing eye as well as anterior neural tube and brain leading up to and at 24 hpf and particularly in the retina at 48 hpf. Specifically, meis2.2 is expressed throughout the optic vesicle from 15 to 18 hpf (Bessa et al., 2008) but is completely lost from the eye by 24 hpf as shown in Fig 5A and as reported previously (Bessa et al., 2008). We see zgc:154061 expressed throughout the optic vesicle during this same time frame (Fig. 4G and Fig. 5C,D), however its expression persists to 24 hpf whereas meis2.2 expression has disappeared by this point. Expression of meis2.2 is also observed in the forebrain at 24 hpf (Fig. 5A,B) and zgc:154061 is expressed throughout the neural tube at this same time, including the forebrain (Fig. 5C,D). By 48 hpf, meis2.2 expression reappears

in the retina and tectum of the midbrain and exhibits overlapping expression with *zgc:154061* in these regions (Fig.5E-H). The highly conserved nature of *meis2.2* and *zgc:154061* expression, particularly at 48 hpf in the retina suggests the possibility that these genes are sharing *cis*-regulatory elements, at least for one directing expression in the developing eye. Examples consistent with our observations of expression of *meis2.2* and *zgc:154061* earlier in development, such as at 24 hpf, also exist however, where tandem genes are thought to share *cis*-regulatory elements but do not share perfectly overlapping patterns of expression, likely because of interactions between the shared enhancers and the promoters as well as developmental stage-specific repressors associated with each gene (Irvine et al., 2007). It is also possible that the organization of these genes has been preserved because of the presence of *cis*-regulatory elements within introns of one of the genes but directing expression of the other gene, such as that which is seen for the limb-specific long range enhancer controlling sonic hedgehog expression and that is found in an intron of the *Lmbr1* locus (Lettice et al., 2002).

Recently, conserved syntenic regions containing long-range *cis*-regulatory elements distributed over long distances and encompassing conserved developmental regulatory genes as well as phylogenetically and functionally unrelated "bystander" genes have been termed genomic regulatory blocks, or GRBs (Kikuta et al., 2007). It has been proposed that following the whole genome duplication in teleosts, certain GRBs that contain conserved developmental genes have been maintained in only one of the duplicated syntenic regions, suggesting that evolutionary pressure acts to maintain only a single-copy of the GRB. For example *Pax6*, a highly conserved gene involved in vertebrate retinal and central nervous system development, has been duplicated in teleosts and as a result zebrafish contain two

copies, pax6.1 and pax6.2. Using enhancer detection, Kikuta et al. (2007) located a region ~68 kb downstream of pax6.2 within an intron of a neighboring gene, elp4, that was able to recapitulate the expression pattern of pax6.2. This suggests that the *cis*-regulatory sequence driving pax6.2 expression exists within the elp4 gene despite elp4 expression being more wide spread and consequently not appearing to be regulated by the regulatory elements within its own introns. Interestingly, elp4 was only maintained downstream of pax6.2 similar to what we report here in terms of zgc:154061 being maintained downstream of meis2.2 but there being no paralog associated with meis2.1. Thus, the presence of any putative *cis*-regulatory element within an intron of zgc:154061 may explain why this gene is maintained downstream of meis2.2. It is possible that the single-copy GRB containing zgc:154061 and meis2.2 is protecting this locus from chromosomal breakage while any paralogous regulatory region associated with meis2.1 has been lost by neutral evolution; a phenomenon predicted by the duplication degeneration complementation model (Force et al., 1999).

We have described here a novel zebrafish gene zgc:154061 that is highly conserved amongst vertebrates in terms of sequence identity and linkage to the *Meis2 (meis2.2* in zebrafish) homeobox gene. It appears that the genomic organization of the zgc:154061 and *Meis2* genes arose early in the deuterostome lineage as it is also observed in cephalochordates and echinoderms. The amino acid alignment of the vertebrate orthologs of zgc:154061 indicates a very well conserved C-terminal region, which may very well represent a functional domain. This gene is maternally expressed and also expressed in the developing neural tube and eye in a manner at least partially overlapping with its genomic neighbor *meis2.2*. The role of zgc:154061 and its orthologs remain to be investigated and we are currently exploring RNA over-expression and morpholino knockdown experiments in zebrafish to pursue this.

2. EXPERIMENTAL PROCEDURES

2.1 Zebrafish Husbandry

Zebrafish embryos were obtained by pair-wise breeding of adult AB line fish housed in an enclosed Z-Mod system (Aquatic Habitats) and maintained at 28 °C on a 16 h light / 8 h dark cycle. Embryos were staged according to (Kimmel et al., 1995). All experimental procedures involving zebrafish were approved by the Appalachian State University IACUC.

2.2 In situ hybridization

The full length 1914-bp cDNA clone of *zgc:154061* was isolated by the Zebrafish Gene Collection (ZGC), an NIH initiative, in conjunction with the NIH Mammalian Gene Collection (MGC) project (Strausberg et al., 2002) and made available through Open Biosystems (Clone Id: 8334609, Accession: BC124527) from whom we purchased it. So as to utilize a smaller probe size for our *in situ* hybridizations, a partial 393-bp fragment of the zebrafish *zgc:154061* gene (underlined in Fig. 1B) corresponding to positions 608-1000 of the full length cDNA clone was isolated by PCR using the primer sequences GGTCTGGAACATGAAGAC and CCTCATGCCATCAGAAAC (locations indicated in Fig. 1B). The PCR amplification was performed with Phusion High-Fidelity DNA Polymerase (New England BioLabs), the product subcloned into the pGEM-T Vector System (Promega, Madison WI) and confirmed by sequencing. An antisense DIG-labelled riboprobe

was synthesized directly from this subclone using T7 RNA Polymerase after linearizing the plasmid with *Not*I. A sense DIG-labelled riboprobe, used as a negative control, was synthesized from the same construct using SP6 RNA Polymerase after linearizing the plasmid with *Sac*II. Whole mount *in situ* hybridization was performed as described by Thisse and Thisse (2008) (Thisse and Thisse, 2008). Embryos were photographed using a Leica MZ6 dissecting microscope, Leica DFC320 digital camera and the Leica Application Suite Version 3.3.1. Images were compiled for Figures 4 and 5 using Adobe Photoshop 7.0. For sectioning, 48 hpf embryos were positioned in 1.5% agarose melted in 5% sucrose in PBS following *in situ* hybridization. Agarose blocks were incubated in 30% sucrose in PBS at 4°C overnight. Agar blocks were frozen in optical cutting temperature (O.C.T.) media and 20µm sections were cut using a Leica CM 1100 cryostat. Images of sections were obtained using an Olympus IX81 inverted microscope and processed with MicroSuite Biological Suite software. Images were compiled for Fig.5J,K using Adobe Photoshop 7.0.

2.3 RNA Extraction And Quantitative real-time PCR

Total RNA was extracted from 30-100 staged embryos by homogenizing with RNase, DNase-, pyrogen-free disposable pestles (Kontes) in TRIzol (Invitrogen) and following the protocol described by (Chomczynski and Mackey, 1995). RNA quality and quantity was determined by NanoDrop and denaturing gel electrophoresis. Reverse transcription of RNA samples into cDNA were performed using the Applied Biosystems High Capacity RNA-tocDNA Master Mix and following the manufacturer's instructions.

Relative Quantitative real-time PCR was performed to analyze temporal expression of zgc:154061 during zebrafish development using the Applied Biosystems 7300 real-time PCR System. For each stage examined, 200 ng of cDNA was used as template together with TaqMan Universal PCR Master Mix (Applied Biosystems) and gene specific primers and probe: zgc:154061 forward 5'-GCAGACGCACTTCACACATCTC-3'; zgc:154061 reverse 5'-TGCGCTTCATTCTCTTGGTA-3'; zgc:154061 probe 5'-FAM-CGCGTCCACTCTGCTGAGCATCTTC-TAMRA-3' (see Fig. 1B for locations of primers and probe). The zebrafish β -actin gene was used as an internal control with gene and specific primers and probe: ß -actin forward 5'-GCTGTTTTCCCCTCCATTGTTG-3'; ß-actin 5'-TTTCTGTCCCATGCCAACCAT-3'; ß-actin probe 5'-FAMreverse CCCAGACATCAGGGAGTG-TAMRA-3'. Primers and probes were designed using Primer Express (Applied Biosystems) and purchased from Operon. The following amplification protocol was used: 50°C for 2 m; 95°C for 10 m; 95°C for 15 s, 60°C for 1 m repeated for 40 cycles. All reactions were performed in triplicate twice and two separate RNA extractions from each stage of development examined. Results were interpreted and are shown as level of relative expression calibrated to expression in an adult zebrafish using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

FIGURE LEGENDS

Fig. 1 Genomic organization and sequence of zgc:154061 (A) Position of zgc:154061 with respect to *meis2.2* in zebrafish. Vertical lines/boxes represent exons. Arrows indicate the direction of transcription. The predicted exons of the Zgc:154061 gene and its location with respect to *meis2.2* were identified using the publicly available genome sequence and the Model Maker tool in the NCBI Database. (B) Nucleotide and translated sequence of the zgc:154061 gene. The sequence used as a probe for *in situ* hybridization is underlined and the primer sites used to amplify this sequence are double underlined. The primer sites used for quantitative real-time PCR are indicated by red double underlines and the site of the probe by a red single underline. Amino acid sequence is shown above each codon. Start and stop codons are indicated by boxes.

Table 1. Amino acid sequence identity percentages based on pairwise comparisons between putative, representative homologues of zgc:154061 that we have been able to identify based on searches of that organisms's corresponding publicly available genome data. Species examined are: human (Homo sapien - Hs); mouse (Mus musculus - Mm); chicken (Gallus gallus - Gg); African clawed frog (Xenopus laevis - Xl); zebrafish (Danio rerio - Dr); pufferfish (Takifugu rubripes - Tr); tunicate (Ciona intestinalis - Ci); lancelet (Branchiostoma floridae - Bf); sea urchin (Strongylocentrotus purpuratus - Sp); fruit fly (Drosophila melanogaster - Dm). Amino acid sequences were obtained from publicly available genome sequences through the NCBI database (accession numbers: Hs -NP_115888.1; Mm - XP_001480310.1; Gg - NP_001026371.1; XI - NP_001090210.1; Dr - NP 001070190.1; Ci - XP 002128870.1; Bf - XP 002221176.1; Sp - XP 780639.1; Dm - NP 648806.1) except that of *Takifugu rubripes* which was obtained from the IMCB Project Fugu Genome database (gene:SINFRUG00000137928 transcript:SINFRUT00000146276).

Fig. 2. Amino acid sequence alignments of products of zgc:154061 orthologues for: human (*Homo sapiens* - hypothetical protein LOC84529); mouse (*Mus musculus* - hypothetical protein LOC399568); chicken (*Gallus gallus* - hypothetical protein LOC423293); African clawed frog (*Xenopus laevis* - hypothetical protein LOC779112); pufferfish (*Takifugu rubripes* – translation of SINFRUT00000146276) and; zebrafish (*Danio rerio* - hypothetical protein LOC767755). Yellow shaded regions represent complete identity amongst all six species, blue shading indicates identity between at least three of the species shown. Amino acid sequences are based on publicly available genome sequences though the NCBI database except that of *Takifugu rubripes* which was obtained from the IMCB Fugu Genome Project database. Amino acid alignments were generated using Vector NTI Advance Version 11.0 Align X (Invitrogen) with the following pairwise settings: K-tuple size-1, number of best diagonals-5, gap penalty-3 and multiple alignment settings: gap opening penalty-10, gap extension penalty-0.05, Gap separation penalty range-40, % identity for alignment delay-40. Underlined regions indicate domains that are also found in putative invertebrate orthologs of zgc:154061.

Fig. 3. Phylogenetic tree of zgc:154061 orthologous amino acid sequences. The phylogenetic tree was constructed using Phylogeny.fr (http://www.phylogeny.fr/) using MUSCLE 3.7 for multiple sequence alignment, GBlocks 0.91b for alignment refinement, PhyML 3.0 aLRT for phylogeny, and TreeDyn 198.3 for tree rendering (Dereeper et al., 2008). Numbers on branches represent the percentage of how many times clades grouped following 500 replications. Species examined are: human (Homo sapien); chimp (Pan troglodytes); mouse (Mus musculus); dog (Canis familiaris); cow (Bos taurus) chicken (Gallus gallus); African clawed frog (Xenopus laevis); zebrafish (Danio rerio); pufferfish (Takifugu rubripes); lancelet (Branchiostoma floridae); sea urchin (*Ciona intestinalis*); tunicate (Strongylocentrotus purpuratus); fruit fly (Drosophila melanogaster). Amino acid sequences were obtained from publicly available genome sequences through the NCBI database (accession numbers: Homo sapien - NP_115888.1; Pan troglodytes - XP_510289.2; Mus musculus - XP 001480310.1; Canis familiaris - XP 849922.1; Bos taurus NP_001015668.1; Gallus gallus - NP_001026371.1; Xenopus laevis - NP_001090210.1; Danio rerio - NP 001070190.1; Ciona intestinalis - XP 002128870.1; Branchiostoma floridae - XP_002221176.1; Strongylocentrotus purpuratus - XP_780639.1; Drosophila *melanogaster* - **NP 648806.1**) except that of *Takifugu rubripes* which was obtained from the Fugu Project database (gene:SINFRUG00000137928 IMCB Genome transcript:SINFRUT00000146276).

Fig. 4. Whole-mount *in situ* hybridization on analysis of early zebrafish zgc:154061 expression. Embryos are shown as whole mounts with the anterior to the left. (A) 16-cell (1.5 hpf), (B) 4 hpf, (C) 6 hpf, (D) 9 hpf (90% epiboly), (E) 14 hpf, (F) 20 hpf, and (G) 20 hpf dorsal view with yolk removed. e, eye.

Fig. 5. Whole-mount *in situ* hybridization on analysis of late zebrafish *zgc:154061* and *meis2.2* expression. Embryos are shown as whole mounts the anterior to the left. (A) 24 hpf *meis2.2*, (B) 24 hpf *meis2.2* dorsal view with yolk removed, (C) 24 hpf *zgc:154061*, (D) 24 hpf *zgc:154061* dorsal view with yolk removed, (E) 48 hpf *meis2.2*, (F) 48 hpf *meis2.2* dorsal view with yolk removed, (G) 48 hpf *zgc:154061*, (H) 48 hpf *zgc:154061* dorsal view with yolk removed, (I) 48 hpf *zgc:154061* sense RNA probe negative control. (J) and (K) represent 48 hpf *zgc:154061* transverse sections through the brain and retina following *in situ* hybridization. e, eye; fb, forebrain; r, retina; teo, tectum opticum.

Fig. 6. Quantitative real-time PCR analysis of *zgc:154061* expression. Total zebrafish mRNA was isolated from 30-100, 0 hpf to 120 hpf embryos. Expression of *zgc:154061* relative to that of the β -actin endogenous control at each developmental stage indicated is shown, calibrated to expression in an adult zebrafish using the 2^{- $\Delta\Delta$ Ct} method. Error bars reflect standard error of the mean for each sample. Each value on the graph is significantly different from the neighboring values as determined by t-test (p 0.05).

FIGURES

Fig. 1A



Fig. 1B

	gtco	gogto	ytgct	rgcag	gaact	ggag	gette	gtaca	agtgt	tatt	iggto	yctgi	ttgt	gtto	gcaga
60	gtt	gtgaa	aat												
	М	D	А	V	S	G	G	S	A	Т	G	Т	G	Ε	Q
71	ATG	GAC	GCA	GTG	TCA	GGC	GGC	AGC	GCC	ACA	GGA	ACA	GGT	GAA	CAG
	V	Ν	Ν	L	R	I	С	R	А	Ε	Y	R	۰s	I	S
116	GTG	AAT	AAT	CTG	AGG	ATC	TGC	AGG	GCA	GAA	TAC	AGG	AGC	ATC	AGC
	R	ਜ	V	E	0	T,	R	P	т	R	0	С	М	ĸ	• T
161	AGG	- 	GTG	GAG	CÂG	CTG	CGT	CCC	ACA	CGG	CÂG	TGC	ATG	AAG	ACC
	т.	0	Τ	Н	F	Ψ	H	Τ.	P	A	S	T	Т.	Τ.	S
206	CTC	CAC	ACG	CAC	- 		САТ	CTTC	CCC	CCC	TCC		СТС	СТС	ACC
200	T	F	000	0	F	v	0	N N	R R	м	K ICC	R	910	M	<u>2001</u>
251		TTC T	TCC	CAC	GAG	TAC	CAC	AAC			AAC	CCC	АСТ	ATC	CCC
201	D		ц Ц	cAG	DAU	F	V	T	P	W	V	v	0	D	v
296		CAT	CNC		CCT	CAC	CTTC			CTTC	т тлт	 	CNC	ACC	 האה
200	R	D	F	Z	F	UAU T	P	A	ЛОЛ	F 010	D	T.	T.	T.	F
2/1		CAT	C 7 7	CCT	CAC	ACC				C 7 7					CAC
JHI	T	5AI 7	M	GCI	UAB	D	T	GCC	D	JAD	T	T	N CIG	D	T
206	СШС	C C M	IN N N C	Q C A C	V CTTC			ы ПСП	E CCA	CCTT			CCTT		
200	CIC	GCI	AAC	CAG	GIG	GAI	CIA	TCT	CCA	GCI		TIG	GCI	CGI	CIG
101		Ц					E C A C		R	IN 7 7 C	A	D D D D	V CTTC	r	5
431	ATG	CTG	GAG	TGT	TTC	CTA	GAG	GAG	CGC	AAC	GCT	TCA	GTC	CCT	TCC
170	R	Q	V	L	N	N	M	L	R	E	P	Y	L	1	P
4/6	AGA	CAA	GTC	CTC	AAC	AAC	ATG	CTG	CGT	GAG	CCG	TAT	TTA	A'I''I'	CCA
501	D	L	V	ட — — – –	A	K	Н	1	E	Q	C	T	V	N	D
521	GA'I'	CTG	GTG	'I''I'A	GCC	AAG	CAC	ATC	GAG	CAG	TGC	ACA	GTA	AA'I'	GAC
	C	C	Y	G	Р	Ц 	V	D	C	1	K	H	·A	1	G
566	TGC	TGT	TAT	GGA	CCG	CTG	GTC	GAC	TGC	ATC	AAA	CAT	GCC	ATC	<u>GGT</u>
	L	E	Н	E	D	T	L	R	D	K	L	R	E	R	• N
611	CTG	GAA	CAT	GAA	GAC	ACT	CTG	AGA	GAC	AAA	CTC	AGA	GAG	AGG	AAC
	-	~	_					~	-				~		
65.6	L	S	F	L	D	E	N	Q	L	R	V	K	G	Y	D
656	L CTG	S TCG	F TTT	L TTA	D GAT	E GAG	N AAT	Q CAG	L CTG	R CGG	V GTC	K AAA	G GGA	Y TAC	D GAC
656	L CTG K	S TCG T	F TTT P	L TTA D	D GAT I	E GAG I	N AAT L	Q CAG E	L CTG V	R CGG P	V GTC I	K AAA A	G GGA V	Y TAC D	D GAC G
656 701	L CTG K AAA	S TCG T ACC	F TTT P CCG	L TTA D GAC	D GAT I ATC	E GAG I ATC	N AAT L CTG	Q CAG E GAG	L CTG V GTG	R CGG P CCG	V GTC I ATC	K AAA A GCT	G GGA V GTT	Y TAC D GAT	D GAC G GGC
656 701	L CTG K AAA H	S TCG T ACC I	F TTT P CCG · V	L TTA D GAC H	D GAT I ATC W	E GAG I ATC I	N AAT L CTG E	Q CAG E GAG S	L CTG V GTG K	R CGG P CCG A	V GTC I ATC S	K AAA A GCT F	G GGA V GTT G	Y TAC D GAT D	D GAC G GGC D
656 701 746	L CTG K AAA H CAC	S TCG T ACC I ATC	F TTT P CCG · V GTT	L TTA D GAC H CAC	D GAT I ATC W TGG	E GAG I ATC I ATC	N AAT L CTG E GAG	Q CAG E GAG S AGT	L CTG V GTG K AAA	R CGG P CCG A GCT	V GTC I ATC S TCA	K AAA A GCT F TTT	G GGA V GTT G GGA	Y TAC D GAT D GAT	D GAC GGC D GAT
656 701 746	L CTG K AAA H CAC H	S TCG T ACC I ATC S	F TTT P CCG · V GTT H	L TTA D GAC H CAC N	D GAT I ATC W TGG TGG	E GAG I ATC I ATC Y	N AAT L CTG E GAG L	Q CAG E GAG S AGT N	L CTG V GTG K AAA E	R CGG P CCG A GCT Q	V GTC ATC S TCA F	K AAA A GCT F TTT W	G GGA V GTT G GGA S	Y TAC D GAT GAT Y	D GAC GGC D GAT C
656 701 746 791	L CTG K AAA H CAC H CAC	S TCG T ACC I ATC S AGT	F TTT P CCG V GTT H CAC	L TTA D GAC H CAC N AAC	D GAT I ATC W TGG · T ACA	E GAG I ATC I ATC Y TAC	N AAT L CTG E GAG L CTG	Q CAG E GAG S AGT N AAC	L CTG V GTG K AAA E GAG	R CGG P CCG A GCT Q CAG	V GTC ATC S TCA F TTC	K AAA GCT F TTT W TGG	G GGA GTT G GGA S AGC	Y TAC D GAT GAT Y TAC	D GAC GGC D GAT C TGC
656 701 746 791	L CTG K AAA H CAC H CAC N	S TCG T ACC I ATC S AGT R	F TTT P CCG · V GTT H CAC F	L TTA D GAC H CAC N AAC G	GAT I ATC W TGG · T ACA P	E GAG I ATC J ATC Y TAC G	N AAT L CTG E GAG L CTG L	Q CAG E GAG S AGT N AAC V	L CTG V GTG K AAA E GAG I	R CGG P CCG A GCT Q CAG Y	V GTC I ATC S TCA F TTC W	K AAA GCT F TTT W TGG F	G GGA GTT G GGA S AGC G	Y TAC D GAT Q GAT Y TAC F	D GAC GGC D GAT C TGC I
656 701 746 791 836	L CTG K AAA H CAC H CAC N AAC	S TCG T ACC I ATC S AGT R AGG	F TTT P CCG GTT H CAC F TTT	L TTA D GAC H CAC N AAC G GGT	D GAT I ATC W TGG CCG	E GAG I ATC J ATC Y TAC G GGT	N AAT L CTG E GAG L CTG L CTG	Q CAG E GAG S AGT N AAC V GTC	L CTG V GTG K AAA E GAG I ATC	R CGG P CCG A GCT Q CAG Y TAC	V GTC ATC S TCA F TTC W TGG	K AAA GCT F TTT W TGG F TTC	G GGA GTT G GGA S AGC G GGC	Y TAC D GAT QAT Y TAC F TTC	D GAC GGC D GAT C TGC I ATC
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656 701 746 791 836 881 926 971 1031 1091 1151 1211 1271 1331 1391 1451 1511 1571 1631 1691 1751	L CTG K AAA H CAC N AAC S TCA G GGC TGA Caga gtgt tggt tggt tggt taaa ctggt taaa ctggt taaa ctggt	S TCG T ACC I ACC S AGT R AGG E GAG F TTC Gagact S S C C S C C S C C S C C C C C C C C	F TTT P CCG V GTT H CAC F TTT L CTG P CCC GGGG CCC GGGGG CCC GGGGG CCC GGGGG CCC CCC GGGGG CCC GCC GCC GCC CCC GCC CCC CCC CCC CCC CCC CCC F TTT L CCC CCC F TTT L CCC CCC CCC F TTT L CCC CCC CCC CCC CCC CCC CCC CCC C	L TTA D GAC H CAC G GGT D GGT D GAC T C GGT T C GAC T C GAC C G G C C C C C C C C C C C C C C	D GAT I ATC W TGG C C C C C C C C C C C C C C C C C C	E GAG I ATC Y TAC G G G G G CAG CAG CAG CAG CAG CAG CAG	N AAT L CTG E GAG L CTG CTG R CTG CTG R CTG S CTG S CTG S CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG	Q CAG E GAG S AGT N AAC V GTC C GTC C GTC S AGC GTC S AGC GTC S C AGC C GTC S C C GTC S C C C C C C C C C C C C C C C C C C	L CTG V GTG K AAA E GAG I ATC R CGA L CTG CCAC CCAC CCAC CCAC CCAC CCAC CCAC	R CGG P CCG A GCT Q CAG Y TAC G GGG C TGT G GGG C TGT G G G G C C TGT G C C TGT G G G G	V GTC I ATC S TCA F TTC W TGG I ATC A GCG GCG GCG GCG GCG GCG GCG GCG GCG	K AAA A GCT F TTT W TGG F TTC L CTG G GGA CTG GGA CTG CTG G GGA CTG CTG CTG CTG CTG CTG CTG CTG CTG CTG	GGA V GTT G GGA S AGC G GGC CTG P CCC GCC GCC GCC GCC GCC GCC	Y TAC D GAT D GAT Y TAC F TTC K AAG Q CAG CAG CCCC G G G G G G G G G G G G G	D GAC G G G D C TGC I C C C C C C C C C C C C C C C C C C
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Table 1	ole 1
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	Hs	Mm	Gg	Xl	Dr	Tr	Ci	Bf	Sp	Dm
Hs		94	82	76	70	77	42	52	51	38
Mm			82	72	61	77	34	44	50	27
Gg				68	59	74	32	42	58	24
Xl					64	70	38	44	52	31
Dr						76	34	41	50	26
Tr							42	49	50	38
Ci								36	41	28
Bf									54	33
Sp										36
Dm										

Fig. 2

Mus musculus (1)MILTKAQYDEIAQCLSVPPTRQSLRKLKQRFPSQSQATLLS Gallus gallus (1)	Homo sapien	(1)	
Gallus gallus (1)	Mus musculus	(1)	MILTKAOYEEIAOCLVSVPPTROSLRKLKORFPSOSOATLLS
Xenopus laevis (1) Takifugu repribes (1) Danio rerio (1) MDAVSGGSATGTGEQVNNLRICRAEYRSISRFVEQLRPTRQCMKTLQTHFTHLPASTLS Homo sapien (1) Mus musculus (43) IFSQEVQKJIKRTHAKHHTPAEALSINGRVINGAARVILELANEVTYAPSIMAR Gallus gallus (43) IFSQEVQKJIKRTHAKHHTPAEALSINGRVINGARAAVILELANEVTYAPSIMAR Takifugu repribes (1) Takifugu repribes (1) Mus musculus (43) IFSQEVQKIKRTHAKHHTPAEALSIN ORVINGAR DEAETRATE LILEANEVTSES MART Takifugu repribes (1) Takifugu repribes (1) Mus musculus (13) Gallus gallus (103) Gallus gallus (103) Mus Rusculus (103) Mus Rusculus (103) Gallus gallus (103) Mart Deelog AN DERL DEO CAN DEV CURNOCY CLUND CYGELVD CIKHATG Kenopus laevis (37) VLREFL DED CLEAR KYDNEW MART MARTHESEN THE LANGE CONNOCY CELVD CIKHATG Mus musculus (163) Mus musculus (163) Mus musculus (161) Mus P	Gallus gallus	(1)	RKLTKAOYDEIAOFLGHVOPTRÖSLRKLKEKFPSÖSÖSTLLS
Takifugu repribes (1) Danio rerio (1) MDAVSGGSATGTGEQVNNLRICRAEYRSISRFVEQLRPTRQCMKTLQTHFTHLPASTLLS Homo sapien (1) Mus musculus (3) Gallus qallus (43) IFSQEYQKHIKRTHAKHHTPEAIESTYORYLNEVGRAARVLDELANEWDYARSIMARI Xenopus laevis (1) Takifugu repribes (1) Danio rerio (61) IFSQEYQKQIKRTHAKHHTPEAIESTYORYLNEVARAADVLDELANEWDYSERAMART Takifugu repribes (1) Danio rerio (61) IFSQEYQKHKKRSMARHHSPEVLKVYQOR NDELETARTE LUBELANEVDYSERAMART Mus musculus (103) ILBERICHENTERSISTINGULAR SQLEADAVYC INNOVYC INNOCYGELUDCIKHAIG Mus musculus (103) (103) ILBERICHENTERSISTINGULAR SQLEADAVYC INNOCYGELUDCIKHAIG Salus galus (103) (103) VLREFICDBOGPTREWIS HITEPYLANOVYC INNOCYGELUDCIKHAIG Mus musculus (163) Mus CFFEERNASV SRQUINNMLREPYLIPLUAAVYC INNOCYGELUNCYGELUNCYGELUNCKIKANG Mus musculus (163) Mus CFFEERNASV SRQUINNMLREPYLIPLUAAVYC INNOCYGELUNCYGELUKAAYE Mus musculus (163) Mus musculus (163)	Xenopus laevis	(1)	
Danio rerio (1) MDAVSGGSATGTGEQVNNLRICRAEYRSISRFVEQLRPTRQCMKTLQTHFTHLPASTLLS Homo sapien (1)	Takifugu repribes	(1)	
 Homo sapien Homosocintorsey WMERCONDUCTION OF QUERTINGTIAL VEGENTING THE TREMEMENT AND TREMEMENT AND TREASES TO TREMEMENT AND TREASES TO TREMEMENT AND TREASES TO TREMEMENT AND TREASES TO TREMEMENT AND TREASES TO TREASES T	Danio rerio	(1)	MDAVSCCSATCTCFOVNNLRTCRAFYRSTSRFVFOLRDTROCMKTLOTHFTHLDASTLLS
Homo sapien (1)	Danie Terro	(1)	
Mus musculus (43) IFSQEYQKHIKRTHAKHHTPEAIES WQRYLNGVGKNGAA PVLEELANEVDYAFSLMAFI Gallus gallus (43) IFSQEYQKQIKRTHAKHHTPEAIES WQRYLNGVMKNAAA PVLEELANEMDFASSLMAFI Xenopus laevis (1)	Homo sapien	(1)	MARL
Gallus gallus (43) IFSQEYQKQIKRTHAKHHTAEAVETYYQKYLNGVMKNAAARVLLELANENTFAESIAARI Xenopus laevis (1) MYXAYLNEVARDPKVEILELANENTFAESIAARI Takifugu repribes (1) MYXAYLNEVARDPKVEILELANENTFAESIAARI Takifugu repribes (1) ISQEYQKRMKRSMARHHSPEVLRVYYQKYRDEAETRATEFLELELANEVTSSEALAARI Mus musculus (103) ILERFLOEHETTPESKSIINSULROSQIEDGYLANGVYGCIVNDCOYCELUDCIKHAIG Gallus gallus (103) ILERFLOEHETTPESKSIINSULROSQIEDGYLANGVYGCIVNDCOYCELUDCIKHAIG Gallus gallus (103) VLERFLOEDGARDSKINNURDSCIEDGYLANGVYGCIVNDCOYCELUDCIKHAIG Mus musculus (103) VLERFLOEDGARDSKINNURDSCIEDGYLANGVYGCIVNDCOYCELUDCIKHAIG Takifugu repribes (5) ILDRFLOEDGEARSKINNURDSCIEDGYLANGVYGCIVNDCOYCELUDCIKHAIG Mus musculus (163) YLERPLOEDGEARSKINNURDSCIEDGYLANGVYGCIVNDCOYCELUDCIKHAIG Gallus gallus (163) YHEVULROLLIKKNISFLDEDCULARKGYDKTPDFILOVPAVEGHI HWIESKASFGDE Gallus gallus (163) YHEVULROLLIKKNISFLDEDCULARKGYDKTPDFILOVPAVEGHI HWIESKASFGDE Takifugu repribes (5) OHENVLLEOKKKENISFLDETCULARKGYDKTPDFILOVPAVEGHI HWIESKASFGDE Gallus gallus (123) YHEVULROKKKENISFLDETCULARKGYDKTPDFILOVPAVEGHI HWIESKASFGDE Danio rerio (181) <td< td=""><td>Mus musculus</td><td>(43)</td><td>IFSQEYQKHIKRTHAKHHTPEAIES<mark>YYQRY</mark>LNGVGKNGAA<mark>PVLLELAN</mark>EV<mark>D</mark>YA<mark>P</mark>SLMARI</td></td<>	Mus musculus	(43)	IFSQEYQKHIKRTHAKHHTPEAIES <mark>YYQRY</mark> LNGVGKNGAA <mark>PVLLELAN</mark> EV <mark>D</mark> YA <mark>P</mark> SLMARI
Xenopus laevis (1)	Gallus gallus	(43)	IFSOEYOKOIKRTHAKHHTAEAVET <mark>YYORY</mark> LNGVMKNAAA <mark>PVLLELAN</mark> EMDFA <mark>PSLMAR</mark> I
Takifugu repribes Danio rerio (1)	Xenopus laevis	(1)	
 Danio rerio (1) IFSQEYQKRMKRSMARHHSPEVLRVYY ORYRDEAETRATE LLELLAN QVDLS ALLARL Homo sapien (5) ILER FLOCH ETP SKSIINSMIRDES OIPD GVLANQVYC GIVNDC OY GPLVDCIKHAIG Mus musculus (103) ILER FLOCH OTP SKSUINSMIRDES OIPD GVLANQVYC GIVNDC OY GPLVDCIKHAIG Gallus gallus (103) VLER FLOCH OTP SKSUINSMIRDES OIPD GVLANQVYC GIVNDC OY GPLVDCIKHAIG Gallus gallus (103) VLER FLOCH OTP SKSUINSMIRDES OIPD GVLANQVYC GIVNDC OY GPLVDCIKHAIG Gallus gallus (103) VLER FLOCH OPP SKSUINSMIRDES OIPD GVLANQVYC GIVNDC OY GPLVDCIKHAIG Takifugu repribes (5) ILD ETI ODLOOP TKPTLS MIR DESI IPP VLANQVHLCINDC OY GPLVDCIKHAIG Danio rerio (121) MLEC FLOCH ODLOOP TKPTLS MIR DESI IPP VLANQVHCI GIVNDC OY GPLVDCIKHAIG Mus musculus (163) VEHEVULROLLEKNIS FLOED OLRAK GYDKTPD FILOV VAVE GHIH HWIESKASFGDE Gallus gallus (163) -NRSCSLCVAE OLRAK GYDKTPD FILOV VAVE GHIH HWIESKASFGDE Mas musculus (163) VEHEVULROLLEKNIS FLDED OLRAK GYDKTPD FILOV VAVE GHIHWIESKASFGDE Takifugu repribes (65) OPH VLLCOKIKERNIS FLDED OLRAK GYDKTPD FILOV VAVE GHIHWIESKASFGDD Danio rerio (181) LEHED TRDKIRERNIS FLDED OLRAK GYDKTPD FILOV VAVE GHIHWIESKASFGDD Danio rerio (181) LEHED TRDKIRERNIS FLDED OLRAK GYDKTPD FILOV VAVE GHIHWIESKASFGDD Danio rerio (181) LEHED TRDKIRERNIS FLDEN OLRAK GYDKTPD IIE VFVAVE GHIHWIESKASFGDD Mus musculus (223) CSHHAVIH DOWSYNNEGOGLVIYW GFIOL DLONK FRGILLKAC FFTNIVTICH STA-Gallus gallus (212) SHQAYLQ OPWSYNNEGOGLVIYW GFIED LOCNERGILLKAC FFTNIVTICH STA-Gallus gallus (212) SHQAYLQ DEWSYNNEGOGLVIYW GFIED LOCNERGILLK GFFTDIVTICH STA-Danio rerio (241) HSHNVINE GFWSYNNEGOGLVIYW GFIED LOCNERGILLK GFFTDIVTICH A Danio rerio (241) HSHNVINE GFWSYNNEGOGLVIYW GFIED LOCNERGILLK GFFTDIVTICH A Danio rerio (301)	Takifugu repribes	(1)	MARV
Homo sapien (5) I REFIGEN ETPESKSIINSMIRDESO IPOGVLANOVYO IVNDOVYGEUVDO IKHAIG Mus musculus (103) ULERFIGEN OTPESKSVINSMIRDESO IPOGVLANOVYO IVNDOVYGEUVDO IKHAIG Gallus gallus (103) (103) VLERFIOCH OTPESKSVINSMIRDESO IPOGVLANOVYO IVNDOVYGEUVDO IKHAIG Gallus gallus (103) Takifugu repribes (37) VLERFIODHDGOPTKPUSSMIRDESI IPDVLANOVHO IVNDOVYGEUVDOVYGEUVDO IKHAIG Takifugu repribes (5) ILDEFIODL GEM SKTVINSMIREESI PDLILAONOVO TVNDOVYGEUVDIKHAIG Danio rerio (121) MLECFIEERNASVPSROVLNNMIREFYI IPDLILAONOVO TVNDOVYGEUVDIKHAIG Mus musculus (163) (163) Y HEVULRDLILEKNISELDED QLRAKGYDKTPDFILOVYAVEGHIHWIESKASFGDE Gallus gallus (163) Y HEVULRDLILKKNISELDED QLRAKGYDKTPDFILOVYAVEGHIHWIESKASFGDE Takifugu repribes (65) PHEVULROVIKEHNIAFDED QLRAKGYDKTPDFILOVYAVEGHIHWIESKASFGDE Danio rerio Mus musculus (223) CSHHAYLHODILKKENNISELDET QLRAKGYDKTPDFILEVPYAVEGHIHWIESKASFGDE Danio rerio Mus musculus (223) CSHHAYLHODILEKKENNISELDET QLRAKGYDKTPDIILEVPYAVEGHIHWIESKASFGDE Danio rerio Mus musculus (223) CSHHAYLHODILEKKENNISELDET QLRAKGYDKTPDIILEVPYAVEGHIHWIESKASFGDE Danio rerio Mus musculus (223) CSHHAYLHODILEKKENNISELDET QLRAKGYDKTPDIILEVPYAVEGHIHWIESKASFGDE Danio rerio Mus musculus (223) CSHHAYLHODILEKKENNISELDET QLRAKGYDK	Danio rerio	(61)	TESOFYOKRMKRSMARHHSPEVI.RVYYORYRDEAETRATEPI.LLELANOVDI.SPATIARI.
Homo sapien (5) ILER FLOEHEETP SKSIINSMIRDESOIPDGULANOVYOIVNDOY GPUVDIKHAIG Mus musculus (103) ILER FLOEHEETP SKSIINSMIRDESOIPDGULANOVYOIVNDOY GPUVDIKHAIG Gallus gallus (103) VLER FLOEGEQAT SKKIINSMIRDESOIPDGULANOVYOIVNDOY GPUVDIKHAIG Sandous gallus (103) VLER FLOEGEQAT SKKIINSMIRDESOIPDGULANOVYOIVNDOY GPUVDIKHAIG Takifugu repribes (37) VLER FLOEDEGEP TKPUSSMIRDESI PPDULANOVHIINDECNOPUVDIKHAVG Danio rerio (121) MECFLEERNASVESROVINNMIREPYL PPDULANOVACIVNDOY GPUVDIKHAVG Mus musculus (163) YEHEVLLRDILLEKNISPILEDQLRAKGYDKTPDFILOVPVAVEGHI HWIESKASFGDE Gallus gallus (163) YEHEVLLRDILLEKNISPILEDQLRAKGYDKTPDFILOVPVAVEGHI HWIESKASFGDE Mus musculus (163) YEHEVLLRDILLEKNISPILETQLRAKGYDKTPDFILOVPVAVEGHI HWIESKASFGDE Gallus gallus (163) YEHEVLLROKKERNISPILETQLRAKGYDKTPDFILOVPVAVEGHI HWIESKASFGDE Takifugu repribes (65) QEHEVLLCOKKERNISPILETQLRAKGYDKTPDFILE VPVAVEGHI HWIESKASFGDE Takifugu repribes (15) QEHEVLLCOKKERNISPILETQLRAKGYDKTPDFILE VPVAVEGHI HWIESKASFGDE Mus musculus (223) CSHHAYLHOGFWSYNNRFGFGLVIYWGFIQELDONRERGILLKACFFENTYTINGKASSASFGDE Homo sapien (125) SHAYLHOGFWSYNNRFGFGLVIYWGFIQELDONRERGILLKACFFENTYTINGKASAFGDE	201110 10110	(01)	
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Gallus gallus (103) VLERFLOEQQAIPSKTLINSMLRDSOIPDGVLANOTYQCTVNDCCYCPLVDCIKHFIN Xenopus laevis (37) VLERFLOPLOGQPTKPVLSSMLAPSLIPDVLANOVHLCIINDCFNSPLVDGIKHAIG Takifugu repribes (5) ILDRFLODLEGEMESKTVLNSMLKEPSLIPDLLAONTOOCYCPLVDCYCPLVDCIKHAYG Danio rerio (121) MLECFLEERNASVPSRQVLNNMLREPYLIPDLVLAKHIEQCTVNDCCYCPLVDCIKHAYG Mus musculus (163) YEHEVLLRDLLEKNLSFLDEDQLRAKGYDKTPDFILQVPVAVEGHIHWIESKASFGDE Gallus gallus (163) YEHEVLLRDLLEKNLSFLDEDQLRAKGYDKTPDFILQVPVAVEGHIHWIESKASFGDE Gallus gallus (163) YEHEVLLRDLLEKNLSFLDEDQLRAKGYDKTPDFILQVPVAVEGHIHWIESKASFGDE Gallus gallus (163) YEHEVLLRDLEKKNISFLDEDQLRAKGYDKTPDFILQVPVAVEGHIHWIESKASFGDE Takifugu repribes (65) QEHEVLLCOKIKERNISFLDEDQLRAKGYDKTPDFILQVPVAVEGHIHWIESKASFGDE Danio rerio (181) LEHEDTERDKIRERNISFLDEDQLRAKGYDKTPDIILEVPVAVEGHIHWIESKASFGDE Mus musculus (223) CSHHAYLHOOFWSYNRFGPGLVIYWGFIQELDONRERGILLKACFPTNIVTLCHSIA- Mus musculus (125) CSHHAYLHOOFWSYNRFGPGLVIYWGFIQELDONRERGILLKACFPTNIVTLCHSIA- Gallus gallus (125) SHQAYLQDOFWSYNRFGPGLVIYWGFIGELDONRERGILLKACFPTNIVTLCHSIA- Danio rerio (125) HAYLHOOFWSYNRFGPGLVIYWGFIGELDONRERGILLKACFPTNIVTLCHSIA- Danio rerio	Mus musculus	(103)	I <mark>LERFLQ</mark> GHEQTP <mark>PSK</mark> S <mark>VINSMLRDPSQIPD</mark> GVLANQVYQCIVNDCCYGPLVDCIKHAIG
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Danio rerio (301)	Takifugu repribes	(182)	
	Danio rerio	(301)	

Fig. 3











Fig. 6



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BIOGRAPHICAL SKETCH

Brandon Scott Carpenter was born in Gastonia, North Carolina on June 18, 1984. He attended elementary schools in Cherryville, North Carolina and graduated as valedictorian of his senior class from Cherryville High School in 2002. The following autumn he entered Appalachian State University to study Biology, and in May of 2007 he graduated magna cum laude as the Outstanding Biology Senior with a B.S. in Biology and a minor in Chemistry. In the fall of 2007, he remained at Appalachian State University and began study towards a M.S. degree with a concentration in Cell and Molecular Biology. After receiving his M.S. in Biology from Appalachian State University in May of 2010, Brandon commenced work toward his Ph.D in Cell and Developmental Biology at the University of Michigan Ann Arbor.

Brandon is the son of Gary Carpenter of Belmont, North Carolina and Donna Thomas of Cherryville, North Carolina and is married to Heather Atwell Carpenter. Since entering Appalachian State University to study Biology in 2002, Brandon has been determined to research cancer as a result of his childhood battle with leukemia. In the future, he plans to attain his goal of becoming an independent research scientist and apply his knowledge and skills towards saving the lives of cancer patients.