DNA conserved across animals that controls genes for embryonic development

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Summary

How does one fertilized cell develop into an exquisitely complex animal? Complex development evolved via simple development, and retains ancient components: genes that control development in similar ways in distantly-related animals, like humans and flies. Expression of these genes is controlled by DNA segments near the genes, but these segments seem less widely conserved. Arguably, just four have been found conserved between vertebrates and non-deuterostome animals.

Here are reported twenty-five regulatory DNA segments conserved across bilaterian animals, of which seven are conserved in cnidaria (coral and sea anemone). They control developmental genes (e.g. *Nr2f*, *Ptch*, *Rfx1/3*, *Sall*, *Smad6*, *Sp5*, *Tbx2/3*), including six homeobox genes: *Gsx*, *Hmx*, *Meis*, *Msx*, *Six1/2*, and *Zfhx3/4*. The segments contain perfectly or near-perfectly conserved CCAAT boxes, E-boxes, and other sequences recognized by regulatory proteins. More such DNA conservation will surely be found soon, as more genomes are published and sequence comparison is optimized. This reveals a control system for animal development conserved since the Precambrian.

Introduction

Genes often remain similar across vast gulfs of evolution. For example, the genes that encode ribosomal RNA (rRNA) in humans and bacteria have recognizably-similar DNA sequences. Gene expression is controlled by DNA segments near the genes, which curiously lack such long-term conservation. Some are conserved across vertebrate animals, but few have been found conserved between vertebrates and invertebrates [1–3].

Early animal evolution produced bilaterian animals, which then split into two superphyla: protostomes and deuterostomes (fig. 1). A pioneering study by Royo et al. found several regulatory DNA segments conserved across deuterostomes, but found none of them in any non-deuterostome, except, amazingly, two in sea anemone, which is not even bilaterian [4]. These two are enhancers of the *Sox21* and *Hmx* genes. Soon after, Clarke et al. found two regulatory elements conserved between deuterostomes and protostomes [5]: an enhancer of the *Id* gene in gastropods (but no other protostomes), and an enhancer of *Znf503* in tick (but no other protostomes).

	Gsx enhancer	Hmx enhancer	Meis promoter	Msx enhancer	Six1 enhancer	Six2 promoter	Zfhx3/4 promoter	Coe intron element	Nr2f promoter	Rfx1/3 promoter	Sall promoter	Smad6 promoter	Sox21 enhancer	Sp5 promoter	Tbx2/3 promoter	Tbx3 enhancer	Znf207 3′-UTR	Cpeb2/3/4 3′-UTR	Id enhancer	Lin28b promoter	Mex3 3′-UTR	Ptch promoter	Ssbp2 promoter	Znf503 enhancer	Znf503/703 enhancer	
chordate	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	human
chordate	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	chimaera
chordate	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	lancelet
hemichordate	○	○	○	○		○	○		○	○	○	○	○	○	○	○	○		○		○	○	○	○	○	acorn worm
echinoderm				○			○		○	○	○	○	○	○		○			○				○	○		sea urchin
arthropod																	○									lobster
arthropod									○	○		○											○			centipede
arthropod									○	○		○											○	○		millipede
arthropod									○		○	○										○	○	○		scorpion
arthropod										○		○					○						○	○		tick
arthropod									○	○	○	○		○								○	○	○		horseshoe crab
arthropod									○																	sea spider
onychophora	○				○	○		○	○	○		○		○	○	○			○	○	○		○	○	○	velvet worm
priapulid	○								○	○		○		○			○	○	○		○			○		cactus worm
mollusc	○							○	○	○				○					○		○	○	○			chiton
mollusc			○							○				○					○			○	○			sea hare
mollusc	○	○	○			○	○		○	○		○		○	○				○		○	○	○	○		abalone
mollusc						○			○	○									○		○		○			owl limpet
mollusc			○			○			○	○				○					○			○	○			oyster
mollusc	○								○	○		○		○					○		○		○			nautilus
mollusc	○									○		○									○		○			octopus
mollusc										○																solenogaster
annelid	○					○			○	○		○		○					○				○			peanut worm
annelid						○			○	○		○							○				○			sandworm
annelid				○		○			○	○		○							○		○		○			Satsuma tubeworm
annelid									○	○				○					○				○			shingle tubeworm
brachiopod	○	○	○	○	○	○			○	○		○		○		○		○	○		○	○	○	○	○	shamisen shell
phoronid	○									○				○					○				○			horseshoe worm
nemertea	○								○	○				○	○	○			○				○			bootlace worm
cnidaria			○																							carnation coral
cnidaria		○	○						○			○	○	○												stony coral
cnidaria		○	○						○			○	○													lace coral
cnidaria		○							○																	starlet sea anemone
cnidaria		○	○						○				○								○					pale anemone

Figure 1: Animals in which each regulatory DNA element was detected. Regulatory segments are colored according to their nearby gene: homeobox genes red, other transcription factors blue. Each animal's phylum is written on the left, deuterostomes in purple and protostomes in green.

Regulatory DNA conserved across vertebrates or beyond tends to regulate genes that control embryonic development [2]. Many of these developmental genes encode transcription factors: proteins that control other genes by interacting with their regulatory DNA. One family of transcription factor genes contain homeobox sequences, which encode a DNA-binding structure called the homeodomain: these are especially used in development. Development involves the appearance of different territories in the embryo that express different combinations of transcription factors, and then develop into different body parts. These territories form in response to varying concentrations of signalling molecules, such as BMP, Wnt, and hedgehog proteins, that are secreted from cells. Often, one developmental gene contributes to many parts of development, presumably because development evolved by redeploying these genes.

It is curious that developmental genes are widely conserved across animals, but their regulatory DNA rarely is. One theory is that conserved regulatory segments correspond to conserved body plans such as vertebrate [1]. This does not explain, however, why the expression patterns of developmental genes are often deeply conserved across animals [2]. One possible answer is that regulatory function can be conserved even as the DNA evolves so much that sequence similarity is lost [6].

Here, many more DNA segments are found that control developmental gene expression and are conserved across bilaterian animals, and even in non-bilaterian corals and sea anemone. They were found by exploiting the wealth of recently-published animal genomes, and by optimizing DNA homology detection [7]. This reveals a system of DNA sequences conserved since the Precambrian that control animal development.

Results

The first step was to find conserved DNA segments between the genomes of human and chimaera, a cartilaginous fish. These genomes are related closely enough that many conserved segments can be found, but distantly enough that most of their DNA lacks similarity. Another reason for using chimaera is that its genome has evolved slowly [8]. Next, conserved regions were removed if they encode protein, rRNA, tRNA, snRNA, snoRNA, or miRNA. Pseudogenes were also removed, i.e. DNA that used to encode protein, rRNA, tRNA, etc. (Such DNA might also regulate gene expression, but its conservation can be explained by what it does or did encode.) The remaining conserved regions cover 5.7 million base-pairs in chimaera: 1/174-th of the genome.

The next step was to seek conserved segments between these chimaera regions and various invertebrate genomes. By not using the whole chimaera genome, we get 174-fold fewer false matches, so we can find 174-fold weaker similarities. Each similarity was given an E-value, which means the number of times such a similarity would be expected between random sequences of shuffled bases. More specifically, between random sequences with 40% `g+c` and the same lengths as: the one invertebrate genome, and all the chimaera regions. Similarities were accepted with either E-value ≤ 0.0001, or E-value ≤ 10 and near homologous genes. For example, a chimaera segment near the *smad6* gene matched octopus DNA with E-value 0.46, and the nearest gene in octopus is the homolog of *smad6*.

As a negative control, each search was repeated after reversing (but not complementing) the invertebrate genome. DNA doesn't evolve by reversal, because that would require flipping the $3'$-to-$5'$ bonds between all the nucleotides. In most cases a reversed genome had about 10 matches with E-value ≤ 10, and the lowest E-value ever seen was 0.0009.

Further sensitivity was gained by using conserved parts of invertebrate genomes. For example, by using oyster DNA segments that are conserved in scallop (1/59-th of the oyster genome), sensitivity

was boosted a further 59-fold, revealing matches near the *Ptch* and *Meis* genes.

These methods found 25 DNA segments with similarity between vertebrates and non-deuterostome animals (fig. 1). They can be explained by either common ancestry or convergent evolution. It seems implausible that most are due to convergent evolution, because they are in a wide range of animals (fig. 1, 2) and they preserve position and orientation relative to the genes (fig. 3). The segments were not found in some invertebrates (fig. 1): either they were lost during evolution of those animals, or they are present but undetected. The segments are labelled "promoter" if they occur at a gene's transcription start site in human (the best-annotated genome), else "enhancer". One segment lies in an intron, and three lie in 3′-UTRs (UnTranslated Regions of exons, downstream of the translated protein-coding region).

Interestingly, all of these genes, except *Znf207*, have multiple (2–4) copies in genomes of jawed vertebrates. For example, jawed vertebrates have three similar *Meis* genes (*Meis1*, *Meis2*, and *Meis3*), two *SoxB2* genes (called *Sox14* and *Sox21*), two *sine oculis* genes (*Six1* and *Six2*), and two *NET* genes (*Znf503* and *Znf703*). *Sp5* is a semi-exception: vertebrates have two copies, named *Sp5* and *Sp5-like*, but *Sp5-like* was lost in mammals and birds [9]. In early vertebrate evolution, there were two rounds of whole-genome duplication. Most gene duplicates were rapidly lost, so that vertebrates do not have many more genes than invertebrates, but some genes remain in 2–4 copies, including nearly all the ones here.

The conserved DNA segments were detected at all, some, or just one of these multi-copy genes. For example, the conserved *Gsx* enhancer was found near both *Gsx* genes in human and chimaera (fig. 2, 3), but the *SoxB2* enhancer was only found at *Sox21*. The *sine oculis* enhancer was only found at *Six1*, whereas the *sine oculis* promoter was only found at *Six2*. One *NET* enhancer was found at both *Znf503* and *Znf703*, the other only at *Znf503*. While "not found" does not necessarily mean "absent", it seems that duplication of these genes enabled divergence of their regulation and developmental roles.

The functions of these genes and conserved DNA segments are partially known. *Gsx* and *Msx* encode transcription factors that define territories in early development of the CNS (central nervous system), in a manner conserved across bilaterian animals [10–12]. They produce an ancient sensory/inter/motor neuron system. *Msx* is expressed at the lateral edges of the CNS, and produces Rohon–Beard mechanosensory neurons. *Gsx* is expressed more medially and produces interneurons, and the most medial territory produces motor neurons. The *Gsx* enhancer seems to contribute by preventing lateral expression of *Gsx*, thus defining the brain's pallium-subpallium boundary [13, 14]. *Msx* plays many other roles in development: the enhancer is the previously-identified *Msx1* "proximal element" that produces expression in mouse embryo nasal epithelium, myotome, limb mesenchyme, eye, ear, roof plate, second arch, genital ridge and epiphysis [15].

The *Six1/2* genes control development of eyes and other cranial sensory organs (e.g. olfactory epithelium, inner ear, taste papillae), and also contribute to development of kidney, thymus, parathyroid gland, and more [16]. The *Six1* enhancer is the previously-described Six1-13 element, which produced variable expression patterns of unclear importance [16]: it would be interesting to re-examine this element.

Tbx2/3 control formation of the brain's neurohypophysis [17], and contribute to development of vertebrate limbs, heart, liver, and more [18]. The *Tbx3* enhancer was shown to affect expression of *Tbx3* and limb length in mice and horses [19].

The *Coe* genes encode transcription factors affecting subpallium development, expressed in *Gsx*+ brain regions of mice and annelids [10]. They also define subtypes of body-wall motor neuron in mice and roundworms [20], and promote development of fat cells [21]. The conserved DNA segment is in

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tagcaacagttgccccggtgagggaaacggaggcgccatagccacggtagtc  human RFX1
tagcaaccgttgccttgtgaat-------caagcgccatagtcaccgtagtc  human RFX3
tagcaactgttgccttgaggac--------gagcgccatagccaccgtagtc  chimaera rfx1
tagcaactgttgccttgtgact--------cagcgccatagtcaccgtagtc  chimaera rfx3
tggcaacagttgccttggttgt--------gtgcgccatagccaccgtagtc  lancelet
tggcaactgttgccttgagaat--------aaacgccatagccaccgtagtc  acorn worm
tagcaacagttgccttgagaat--------aaacgccatagtcaccgtagtc  sea urchin
tggcaactgttgccttaggaat--------ccgacgccatagacacggtagct  centipede
tggcaactgttgccctaggaat--------aagcgccatagacacggtaact  millipede
tggcaactgttgcccttggagt--------caacgccataactacggaagct  tick
tggcaactgttgcccttggaat--------caacgccataccgacggtaacc  horseshoe crab
tggcaacagttgccttaggaat--------aaacgccatagccaccgtagct  velvet worm
tggcaactgttgcccaagg-----------aaacgccatagacacggtagct  cactus worm
tggcaacagttgccgtaggaat--------aaacgccatagccacggtagct  chiton
tggcaactgttgccgtaggaat--------aaacgccatagacacggtagct  sea hare
tggcaacagttgccgtaggaat--------aaacgccatagccaccgtagct  abalone
tggcaacagttgccgtaggaat--------aaacgccatagccaccgtagct  owl limpet
tggcaactgttgccgtaggaat--------aaacgccatagccaccgtagct  oyster
tggcaactgttgccgtaggaat--------aaacgccatagccacggtagtt  nautilus
tggcaactgttgccctaggaat--------aaacgccatagccacggtatct  octopus
tggcaactgttgccctaggaat--------aaacgccatagacacggtagct  solenogaster
tggcaacagttgccgtaggaat--------aaacgccatagccaccgtagct  peanut worm
tggcaacagttgccgtaggaat--------aaacgccatagccacggtagct  sandworm
tggcaacagttgccgtaggaat--------aaacgccatagccacggtagct  Satsuma tubeworm
tggcaacagttgccgtaggaat--------aaacgccatagccaccgtagct  shingle tubeworm
tggcaacagttgccgtaggaat--------aaacgccatagccaccgtagct  shamisen shell
tggcaacagttgccgtaggaat--------aaacgccatagccaccgtagct  horseshoe worm
tggcaacagttgccgtaggaat--------aaacgccatagacaccgtagct  bootlace worm
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gtcttttcaca----aaccagcagctggt  human GSX1
ttctattcagg---agttctgcagctggt  human GSX2
gtcttttcaca----aaccagcagctggt  chimaera gsx1
gtcttttcacg----agctagcagctggt  chimaera gsx2
ggtgggtttcc------acggcagatggt  lancelet
gtttttcacc----aaacggcagatggt  acorn worm
gtttttctacc----aaacggcagatggt  velvet worm
gtttttccacc----aaacgccagctggt  cactus worm
gtttttctacc----aaccggcagatggt  chiton
gtt--------------------------  abalone
gtttttctacc----aaacggcagatggc  nautilus
gtttttctacc----aaacggcagatggc  octopus
ggtttttgccttcaatacggcagatgtt  peanut worm
tttttccgca----aaacggcagatgtt  shamisen shell
gttctttcgtc----atacggcagatggt  horseshoe worm
gcttttctggc----aaacggcagatgtc  bootlace worm
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                                          -----
cccgcccccggagacccacaattggtcc  human TBX2
cacgcctatgcagcaacacaattggtcc  human TBX3
cacgcctatacgggcacacaattggtcc  chimaera tbx2
cacgcctatggaggcacacaattggtcc  chimaera tbx3
cacgcctctctggcgaggggattggctg  lancelet
cacgcctatactcaacgctgattggtcg  acorn worm
cccgcctatttagcgcccggattggtgg  velvet worm
cccgccccgctcgttaaatgattggttg  abalone
cacgcctatttctcaccctgattggccc  bootlace worm
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aaccaatggcg...tgacacg----agctccatataaggca-gcgatctccatag  human NR2F1
-----------...caaccccggctgctgccttataaggcgcgcgtcgccatgg  human NR2F2
aaccaatggtg...tgacatg----agctccatataaggca-gcgatctccatag  chimaera nr2f1
atccaatgcgg...tcgggtg---tagcgccatataaggaa-ggcatctccatag  chimaera nr2f2
agccaatggga...tgacatg---tcactccatatatggta-gtaattgtcatag  lancelet
agccaatcagc...tgacatt---tctttccatataaggca-ataattgtcatag  acorn worm
-----------...tgacacc---gcctgccatataaggca-gtgggtgtcatgg  sea urchin
agccaatgaga...tgacacg--ctcgcgccatatatggaa-gcagttgccatgg  centipede
ggccaatggca...tgacacg---------------------------------  millipede
agccaatgaga...tgacacg---ttttgccatatatggaa-gtggttaccatgc  scorpion
agccaatgaga...tgacacg---ttttaccatatatggaa-tacgttgccatgc  horseshoe crab
gcccactgtg-...tgacatg---tctatccttataaggaa---tatggtcggtg  sea spider
agccaatgaga...tgacatg---ttttaccatatatggag-ttagtatccatgg  velvet worm
gaccaatagga...tgacaca---gtactccatatatggaa-gtagtcgccatag  chiton
gaccaatagga...tgacacc---gctctccatataagga--gaggtaaccatag  abalone
ggccaatcaga...tgactcc---cagcgccatataagga--ggtgtaaccatag  owl limpet
--ccaataggga...tgacatt---agactccttatatggaa---aactgccatag  oyster
gaccaatggga...tggcagt---gtcatccatatatggg--cttgtatccatag  nautilus
agccaatgagc...tgacatc---acctgccttatatggca-gtggttgctatag  peanut worm
gaccaatagga...tgacatg---ccctgccatataaggaa-gtggtttccatgg  sandworm
-----------...tgacacg---gcgcgccatatatggaa-gacgtcaccatag  Satsuma tubeworm
agccaatgaga...tgacttg---tcctcccaaatatggaa-gaggtaaccatgg  shingle tubeworm
agccaatagga...tgacacg---ttgtgccttataaggta-gatgttgtcatag  shamisen shell
agccaatagga...tgacatg---cggagccttatatggca-cgagttactatag  bootlace worm
-----------...tgacagg----cttaccatatatagaa-tcagttgctaggg  stony coral
-----------...tgacagc----cggaccatatatagca-gtggtttccacgg  lace coral
-----------...tgacagt---tattgccatatatggtg-cacgcttccatgg  starlet sea anemone
-----------...tgactct---tgctcccttatatggca-tg-gctgctatgg  pale anemone
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cctttgat---caggaaaatccaat  human SP5
gctttgat---cagacaaatccaat  chimaera sp5
cctttgat---gaagtcaacccaat  chimaera sp5-like
cctttgatcaagagttggcccctat  lancelet
tctttgat---ggcacgaatccaat  acorn worm
cctttgat---aactgaaatccaat  sea urchin
ccctcaaa---ggtcggaatccaat  horseshoe crab
gctttgat---ctcctcaatccaat  oyster
cctttgat---ctcccgaatccaat  nautilus
tctttgat---gtgccaaatccaat  peanut worm
gctttgaa---gttgaaaatccaat  shingle tubeworm
cctttgat---ctcgtaaatccaat  shamisen shell
gctttgat---catagaaatccaat  stony coral
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                                          -----
ttctgccct-ccgcgagccaatcg  human PTCH1
ttctgcct--tcctcggccaatga  chimaera ptch1
ttgcttcttccctttagccaatgg  chimaera ptch2
ttgtggag--cccgcggccaatca  lancelet
ttggccg---tgagtgaccaatca  acorn worm
tagaaag---ccacgagccaatcg  horseshoe crab
tagaaag---ccacgagccaatcg  horseshoe crab
ctgcgcta--taattgaccaatca  chiton
cctactga--taattgaccaatca  sea hare
cctactga--taattgaccaatca  abalone
ctgtgggc--ttaagaaccaatca  shamisen shell
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Figure 2: Parts of alignments between regulatory DNA sequences. The full alignments are in the Appendix. The over-bars show: CCAAT boxes (in the *Nr2f*, *Sp5*, and *Ptch* promoters), reverse-strand CCAAT box ATTGG (*Tbx2/3* promoter), E-box CAnnTG (*Gsx* enhancer), Wnt response element CTTTG (*Sp5* promoter), and CArG box CC(A/T)$_6$GG (*Nr2f* promoter). The *Ptch* promoter matches two places in the horseshoe crab genome, both near genes annotated as "patched-like".

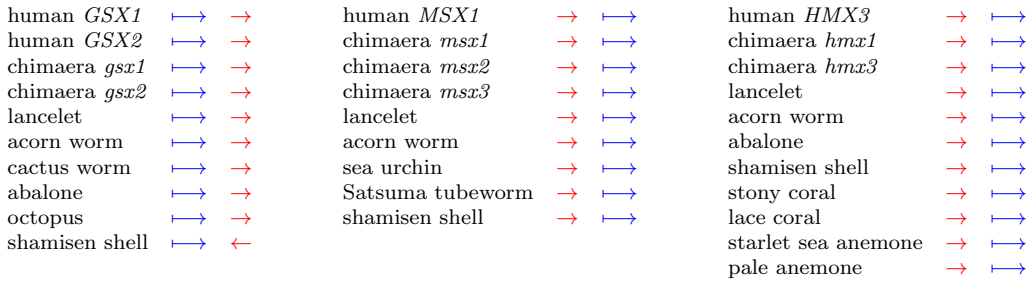

Figure 3: Position and orientation of conserved DNA segments (red arrows) relative to nearby genes (blue arrows). Within each column, all the genes are homologous to each other. Animals without gene annotations are not shown. A regulatory segment doesn't really have an orientation, but once an orientation is arbitrarily chosen for any one segment, that defines the orientations of all its homologs.

an intron, so is transcribed into RNA: it might function in the DNA or RNA or both. This RNA region, of the mouse *Coe* gene *EBF1*, interacts with PSPC1 protein during fat cell development, promoting export of the RNA from the nucleus and expression of EBF1 protein [21].

The *Znf503/703* genes encode transcriptional repressors that are related to Sp transcription factors, but are thought not to bind directly to DNA. They contribute to brain and eye development in vertebrates and flies. In vertebrates they define hindbrain territories and motor neuron subtypes, and contribute to development of the brain's striatum, and limbs [22]. The *Znf503* enhancer was previously found in tick [5]: here it is found in diverse protostomes (fig. 1). The *Znf503/703* enhancer is 2.5 kb further upstream: it directs expression, in mouse embryos, in branchial arches and their derivatives, apical ectodermal ridge of limb buds, and urogenital tissues [23].

The other genes also regulate development [24–27]. *Hmx* interacts with *Gsx* in development of the brain's hypothalamus [28]. *Ptch* genes encode the main receptors that recognize hedgehog signalling molecules, and Smad6 proteins inhibit response to BMP signalling molecules [29]. *Sp5* is a target of Wnt signalling in bilaterians, and also in cnidaria where it prevents development of multiple heads [30]. It has a promoter region conserved from human to coral, which includes an especially conserved sequence resembling a Wnt response element (fig. 2). The *Rfx1/3* genes control formation of cilia on cells, which are used for hedgehog signalling, establish left-right body asymmetry, and more [31]. The promoter is spectacularly conserved across bilateria (fig. 2). Ssbp2 protein partners with LIM homeodomain proteins, which specify motor neurons in the same way in vertebrates and flies, among other roles [32]. *Nr2f* participates in neural development of bilaterians and cnidaria [33]: its promoter has been conserved since early neural evolution (fig. 1, 2).

Interestingly, the three genes with conserved 3′-UTR segments encode proteins that bind to RNA. The Cpeb proteins control mRNA localization and translation, in neural development and memory formation [34]. They are regulated by miRNAs binding to their 3′-UTRs [35–37]. *Mex3* contributes to defining anterior territories in bilaterian embryos, by destabilizing mRNAs [38, 39]. Its 3′-UTR contains elements for translational enhancement and destabilization, including by its own protein or miRNA [38, 40]. The UTR was previously found conserved between vertebrates and lancelet [38]: here it is found in other phyla and even a sea anemone (fig. 1). The Znf207 protein is a bit of an outlier: it contributes to chromosome alignment during cell division, a function not specific to animal development. It also, however, contributes to early neural development by binding to DNA or RNA [41].

Do these particular developmental genes have a common theme? It is hard to say, but perhaps there is a tendency for roles in ventral CNS (e.g. subpallium, striatum, hypothalamus, neurohypophysis, motor neurons).

In the conserved DNA segments, conserved transcription factor binding sites were identified (see the Appendix), using the JASPAR database of DNA-binding tendencies [42]. The results are uncertain, because transcription factors have weak sequence preferences, often similar to those of other transcription factors. Perhaps different factors bind to overlapping sites at different times depending on molecule concentrations.

On the other hand, there are clearly-conserved "boxes", including E-boxes and many CCAAT boxes (fig. 2). The E-box, `CAnnTG`, binds to various transcription factors that have a bHLH (basic-helix-loop-helix) structure. bHLH factors initiate neural development in bilateria and cnidaria [11], perhaps by binding to these conserved E-boxes. CCAAT boxes bind to the transcription factor NF-Y, which controls many developmental genes [43]. One CArG box was found, conserved from human to sea anemone (though imperfect in coral), in the *Nr2f* promoter (fig. 2). In general, CArG boxes bind to the Srf transcription factor, which controls cell migration and neural outgrowth in development [44].

Another interesting result is that the conserved DNA segments rarely change position (upstream/downstream) or orientation relative to the nearby genes. Figure 3 shows just one change, for *Gsx* in shamisen shell. There are a few others. In chimaera, the *sox21* gene has been inverted relative to its neighboring genes, but the inverted region doesn't include the enhancer. So, the enhancer is upstream of *sox21* in chimaera but downstream in the other animals.

Some protostome animals have more detected DNA elements than others (fig. 1). This is partly artifactual, e.g. some genomes lacked gene annotations so had the stricter 0.0001 E-value threshold. With that said, these seem to have many conserved elements: abalone, shamisen shell, and velvet worm (which lacks gene annotations). Perhaps these animals have undergone less evolution of their development and body plan. All three have sometimes been regarded as "primitive" or "living fossils": the shamisen shell (*Lingula*) was noted in Darwin's Origin of Species as little changed from ancient fossils.

On the other hand, no conserved segments were found in fly (*Drosophila melanogaster*), roundworm (*Caenorhabditis elegans*), or the leech *Helobdella robusta*. Perhaps the developmental systems of these animals have evolved more drastically [45]. Beyond bilateria, no conserved segments were found in ctenophores (comb jellies), sponges, or *Trichoplax* (table 2). A recent study found three regulatory DNA elements conserved between mammals and flies [46]: the present study confirmed they are conserved between human and chimaera, but didn't find them in invertebrates. Even when comparing each pair of human and fly sequences, boosting sensitivity 10^{12}-fold over comparing the whole genomes, the present methods found tiny similar fragments in just one out of three pairs. Another study found identical DNA segments ≥ 50 bp between human and fly, and even human and sponge [47]. A replication attempt here also found such matches, but they overlap regions that encode protein, rRNA, tRNA, or snRNA, or pseudogenes. A third study found 6 533 non-protein-coding segments conserved across deuterostomes [48], vastly more than found here or elsewhere [4, 5]. Some possible reasons are that they used E-value ≤ 0.05 per 5 kb (which is 1 per 100 kb), and didn't exclude pseudogenes or RNA genes.

In summary, we begin to see a system of DNA elements controlling gene expression for animal development, conserved since ancestral bilaterians and beyond in the Precambrian. This conservation helps us understand how early animals developed, and how modern development is built on these ancient components.

Why have these DNA sequences been so conserved? Judging by present-day bilaterians and cnidaria, the common ancestor of these animals already had an exquisitely integrated multicellular body, with a nervous system, that developed from a single fertilized cell [11, 12]. This complex development must have evolved by accretion of developmental processes, often by redeploying existing pathways [28]. So, one regulatory DNA element could become used in many parts of development, or its outcomes might get built upon by many processes. This brings us to Hyrum's Law of software development: "With a sufficient number of users... all observable behaviors of your system will be depended on by somebody" [49]. Thus, DNA changes with even subtle effects on transcription factor interactions, e.g. though DNA shape [50], would likely be harmful. This resulted in strongly-conserved DNA controlling core animal development, upon which evolved arthropods, molluscs, chordates, and so on.

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Appendix

Data availability

The alignments between pairs of whole genomes (e.g. chimaera-human, oyster-scallop) are in the 2023 section of `https://github.com/mcfrith/last-genome-alignments`. Other files used in this study are at `https://github.com/mcfrith/conserved-animal-dna`.

Methods

Finding conserved segments between the human and chimaera genomes

The genomes were compared using LAST version 1453 (`https://gitlab.com/mcfrith/last`). First, `chrEBV` (Epstein-Barr virus) was removed from the human genome. An index called (say) `cdb` was made for the chimaera genome:

```
lastdb -P8 -uMAM8 -c cdb chimaera.fa
```

`-P8` makes it faster by using 8 processors, `-uMAM8` increases sensitivity and memory use [51], and `-c` suppresses simple sequences like `atatatatatatatatat`. Next, rates of insertion, deletion, and substitutions between the genomes were found [52]:

```
last-train -P8 --revsym -D1e9 --sample-number=5000 cdb human.fa > hc.train
```

Then, many-to-one human-to-chimaera alignments were found (based on these rates) [53]:

```
lastal -P8 -D1e9 -m100 --split-f=MAF+ -p hc.train cdb human.fa > many-to-one.maf
```

`-m100` makes it more slow and sensitive, and `-D1e9` gets similarities that would be expected between shuffled-base sequences at most once per 10^9 human bp. The alignments were cut down to one-to-one alignments [53]:

```
last-split -r many-to-one.maf > one-to-one.maf
```

Finally, isolated alignments (not near other alignments) were discarded, using `maf-linked` from `https://gitlab.com/mcfrith/protein-fossils/`:

```
maf-linked one-to-one.maf > linked.maf
```

This final step removes non-homologous insertions of homologous transposons [54].

Human genes and pseudogenes

In order to find conserved regulatory DNA, we need to exclude conserved DNA in protein-coding regions, RNA genes, and pseudogenes. The following genome coordinates were used, where `.txt` and `.bigPsl` refer to files in the UCSC genome database:

- Protein-coding regions in `ncbiRefSeq.txt`, `wgEncodeGencodeCompV43.txt`, `xenoRefGene.txt`, and `hg38.refseq.transMapV5.bigPsl`.
- rRNA, tRNA, and snRNA genes and pseudogenes in `rmskOutCurrent.txt`.
- Exons of RNA genes in `ncbiRefSeq.txt`, extracted like this:

  ```
  awk '$13 ~ /^RNU|^MIR|^SNOR|^SCARNA|^RNA5-8/' ncbiRefSeq.txt
  ```

- Protein fossils derived from host genes, in `https://github.com/mcfrith/protein-fossils`.

Chimaera genes and pseudogenes

First, repeats were found with RepeatMasker 4.1.5 (using RMBlast 2.13.0 and TRF 4.09):

```
RepeatMasker -xsmall -engine rmblast -s -species "Callorhinchus milii" chimaera.fa
```

Then, protein fossils were found as described previously [54], except that `fasta-nr` option `-s` was used this time. Finally, these genome coordinates were obtained:

- Protein-coding regions and RNA genes in the genome's `genomic.gff` file, extracted like this:

  ```
  awk -F"\t" '$3 ~ /CDS|rRNA|tRNA|snRNA|snoRNA|guide_RNA/' genomic.gff
  ```

  Here, `guide_RNA` means scaRNA (small Cajal body-specific RNA), which is a type of snoRNA.
- rRNA, tRNA, and snRNA genes and pseudogenes from RepeatMasker.
- Protein fossils derived from host genes.

Non-genic chimaera DNA conserved in human

First, each human (pseudo)gene segment was extended by 50 base-pairs in both directions. This is because the edges of the human-to-chimaera alignments cannot be perfectly reliable: they may overshoot slightly beyond conserved regions into non-conserved DNA, or undershoot [55]. Then, chimaera bases were retained if they align to human bases not in these segments. These chimaera regions were extended by 50 bases in both directions. Finally, bases in chimaera (pseudo)genes were removed from these regions.

Comparing invertebrate genomes to these chimaera regions

An index called (say) `sdb` was made for the chimaera DNA regions:

```
lastdb -c -uMAM8 -S2 sdb chimaera-segments.fa
```

Then, similar segments were sought between each genome and the chimaera regions:

```
lastal -P8 -J1 -D$D -m100 -p my.train sdb genome.fa > out.maf
```

Here, `$D` was set to (genome size) / 10: this gets similarities that would be expected at most once per `$D` genome base-pairs if we compared a shuffled genome to shuffled DNA with the same length as the whole of `sdb`. In other words, it gets similarities with E-value ≤ 10. This step requires a file `my.train`, which is explained later.

E-value adjustment

For each pair of similar segments, `lastal` outputs a per-sequence E-value. This is the number of times such a similarity would be expected between random DNA sequences with the same lengths as: the one sequence from `genome.fa` (chromosome or fragment), and the whole of `sdb`. These E-values were converted to per-genome E-values:

genome E-value = (sequence E-value) $\times$ (genome length) / (sequence length) .

Simple sequences and reversed genomes

Simple sequences, like `atatatatatatatatat`, evolve frequently and independently. This leads to similar segments that are not conserved homologs. There are several methods to suppress simple sequences from homology search, but these methods do not work equally well. LAST uses the `tantan` method, which seems to suppress them effectively [56].

One way to test this is by comparing two DNA sequences after reversing (but not complementing) one of them. Then there are no true homologs, but simple sequences produce many strong similarities [56]. In the present study, reversed genomes did not have much stronger matches than would be expected between random sequences of shuffled bases, indicating that simple sequences were suppressed effectively.

Finding conserved regions between invertebrate genomes

This step found, for example, oyster DNA segments that are conserved in scallop. The genomes were compared in the same way as human-to-chimaera, without the final `maf-linked` step. Oyster protein-coding regions and RNA genes were extracted like this:

```
awk -F"\t" '$3 ~ /CDS|rRNA|tRNA|snRNA|snoRNA|guide_RNA/' genomic.gff
```

Each of these regions was extended by 50 base-pairs in both directions. Then, these regions were cut from the oyster segments that align to scallop. The remaining oyster segments were then extended by 50 bases in both directions.

This was repeated for the genome pairs in table 4, with oyster replaced by the left genome and scallop by the right genome. Some of these genomes did not have a `genomic.gff` file, so an empty file was used instead.

The `my.train` file

This file has rates of insertion, deletion and substitutions. It defines "similar": for example, it might specify that `a`↔`g` and `c`↔`t` substitutions are more likely than other substitutions.

Typically, `last-train` finds these rates from the sequences we wish to compare [52]. But that doesn't work here, because the invertebrate DNA is too distantly related to the chimaera regions. Therefore, a more closely-related animal, lamprey (a jawless vertebrate), was used instead. Using

the whole lamprey genome doesn't work well either, so lamprey DNA segments were used that are conserved in hagfish (another jawless vertebrate). These conserved lamprey segments were found in the same way as oyster-versus-scallop, removing protein-coding regions and RNA genes in the same way too. These lamprey segments were compared to chimaera in two ways:

```
last-train -s1 --revsym --matsym --gapsym -D1e7 --sample-number=5000
                                                        sdb lampreySegs.fa > try1.train

last-train -s1 --revsym --matsym --gapsym -D1e7 --sample-number=5000 --pid=65 --scale=6
                                                        sdb lampreySegs.fa > try2.train
```

`--pid=65` makes `last-train` ignore similarities with > 65% identity, so the rates will be tuned for finding homologies with low percent-identity, but will be worse for finding short homologies with high percent-identity. (`--scale=6` may be unnecessary: it makes the output have units of 1/6 bits). Both of these `train` files were used for homology searches.

Finding matches to conserved deuterostome DNA

After comparing invertebrate DNA to the chimaera regions, there were 45 chunks of the chimaera regions conserved in at least one invertebrate. Most of these chunks are conserved in lancelet or acorn worm.

To boost sensitivity further, the searches were repeated against these chunks of chimaera, lancelet, and acorn worm. The chunks were first extended by 50 bases in both directions. Then, protein-coding regions and RNA genes of the three animals were extracted like this:

```
awk -F"\t" '$3 ~ /CDS|rRNA|tRNA|snRNA|snoRNA|guide_RNA/' genomic.gff
```

and cut from the conserved chunks. The chunks were indexed:

```
lastdb -c -uMAM8 -S2 ddb chimaera-lancelet-acornWorm-chunks.fa
```

Then similarities were found using `lastal` as above.

Thus, the searches were against deuterostome DNA (from chimaera, lancelet, and acorn worm). In future, more DNA conservation can probably be found by using non-deuterostomes too. The present study stopped here, to avoid risk of "transitive catastrophe": see for example stony coral and bootlace worm in the *Sp5* promoter alignment.

Multiple sequence alignment

In the end, after finding the conserved segments, the DNA sequences were aligned with the `linsi` method in MAFFT v7.520 [57]:

```
linsi sequences.fa > alignment.fa
```

Transcription factor binding signals

The DNA sequences were scanned with transcription factor binding site profiles from the JASPAR database (non-redundant vertebrate profiles in JASPAR CORE 2022) [42]. The scanning was done by FIMO version 5.5.2 [58].

miRNA target signals

Potential miRNA binding sites were found with TargetScan Release 8.0 [59].

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Tables

Table 1: Genomes with detected regulatory elements (size means number of a/c/g/t bases in the file)

Animal	Phylum	Scientific name	Genome	Size (bp)	Has gene annotation
human	chordate	*Homo sapiens*	`hg38_no_alt_analysis_set`	2934876451	yes
chimaera	chordate	*Callorhinchus milii*	`GCF_018977255.1`	991467956	yes
lancelet	chordate	*Branchiostoma floridae*	`GCF_000003815.2`	487128766	yes
acorn worm	hemichordate	*Saccoglossus kowalevskii*	`GCF_000003605.2`	642223636	yes
sea urchin	echinoderm	*Strongylocentrotus purpuratus*	`GCF_000002235.5`	921518293	yes
lobster	arthropod	*Homarus americanus*	`GCF_018991925.1`	2291242282	yes
centipede	arthropod	*Strigamia maritima*	`GCA_000239455.1`	173599458	
millipede	arthropod	*Glomeris maerens*	`GCA_023279145.1`	149766888	
scorpion	arthropod	*Centruroides sculpturatus*	`GCF_000671375.1`	863041242	yes
tick	arthropod	*Ixodes scapularis*	`GCF_016920785.2`	2226616818	yes
horseshoe crab	arthropod	*Limulus polyphemus*	`GCF_000517525.1`	1705786612	yes
sea spider	arthropod	*Nymphon striatum*	`GCA_016618385.1`	744500873	yes
velvet worm	onychophora	*Euperipatoides rowelli*	`GCA_003024985.2`	1744674727	
cactus worm	priapulid	*Priapulus caudatus*	`GCF_000485595.1`	436582939	yes
chiton	mollusc	*Acanthopleura granulata*	`GCA_016165875.1`	544712841	
sea hare	mollusc	*Aplysia californica*	`GCF_000002075.1`	737797481	yes
abalone	mollusc	*Haliotis rufescens*	`GCF_023055435.1`	1334438814	yes
owl limpet	mollusc	*Lottia gigantea*	`GCF_000327385.1`	298894987	yes
oyster	mollusc	*Crassostrea gigas*	`GCF_902806645.1`	647867327	yes
nautilus	mollusc	*Nautilus pompilius*	`GCA_018389105.1`	729019367	
octopus	mollusc	*Octopus bimaculoides*	`GCF_001194135.2`	1986279820	yes
solenogaster	mollusc	*Wirenia argentea*	`GCA_025802215.1`	528706996	
peanut worm	annelid	*Sipunculus nudus*	`GCA_026874595.1`	1426680931	
sandworm	annelid	*Alitta virens*	`GCA_932294295.1`	671131125	
Satsuma tubeworm	annelid	*Lamellibrachia satsuma*	`GCA_022478865.1`	664980950	yes
shingle tubeworm	annelid	*Owenia fusiformis*	`GCA_903813345.2`	499118861	yes
shamisen shell	brachiopod	*Lingula anatina*	`GCF_001039355.2`	389003419	yes
horseshoe worm	phoronid	*Phoronis ovalis*	`GCA_028565635.1`	325077420	
bootlace worm	nemertea	*Lineus longissimus*	`GCA_910592395.2`	391166316	
carnation coral	cnidaria	*Dendronephthya gigantea*	`GCF_004324835.1`	286150628	yes
stony coral	cnidaria	*Acropora millepora*	`GCF_013753865.1`	475342477	yes
lace coral	cnidaria	*Pocillopora damicornis*	`GCF_003704095.1`	225744106	yes
starlet sea anemone	cnidaria	*Nematostella vectensis*	`GCF_932526225.1`	269383238	yes
pale anemone	cnidaria	*Exaiptasia diaphana*	`GCF_001417965.1`	210157303	yes

Table 2: Genomes analyzed but no regulatory elements detected

Animal	Phylum	Scientific name	Genome	Size (bp)	Has gene annotation
fruit fly	arthropod	*Drosophila melanogaster*	GCF_000001215.4	142573024	yes
bee	arthropod	*Apis mellifera*	GCF_003254395.2	223937270	yes
bristletail	arthropod	*Machilis hrabei*	GCA_003456935.1	1320889507	
roundworm	nematode	*Caenorhabditis elegans*	GCF_000002985.6	100286401	yes
jawless leech	annelid	*Helobdella robusta*	GCF_000326865.1	215435648	yes
planarian	platyhelminth	*Schmidtea mediterranea*	GCA_022537955.1	773867583	
flatworm	platyhelminth	*Macrostomum lignano*	GCA_002269645.1	762829307	yes
brown bryozoan	bryozoa	*Bugula neritina*	GCA_010799875.2	214708255	yes
hydra	cnidaria	*Hydra vulgaris*	GCF_022113875.1	817163865	yes
trichoplax	placozoa	*Trichoplax adhaerens*	GCF_000150275.1	94748975	yes
sea walnut	ctenophore	*Mnemiopsis leidyi*	GCA_000226015.1	150338246	
comb jelly	ctenophore	*Bolinopsis microptera*	GCA_026151205.1	265424760	
sea gooseberry	ctenophore	*Hormiphora californensis*	GCA_020137815.1	110660632	
cigar comb jelly	ctenophore	*Beroe ovata*	GCA_946803715.1	84911038	
demosponge	porifera	*Amphimedon queenslandica*	GCF_000090795.2	143136305	yes
glass sponge	porifera	*Oopsacas minuta*	GCA_024704765.1	60959430	yes

Table 3: Other genomes used in this study

Animal	Phylum	Scientific name	Genome	Size (bp)	Has gene annotation
lamprey	chordate	*Petromyzon marinus*	GCF_010993605.1	1074036726	yes
hagfish	chordate	*Eptatretus burgeri*	GCA_024346535.1	1507324335	
Bahama lancelet	chordate	*Asymmetron lucayanum*	GCA_001663935.1	452764958	
Hawaiian acorn worm	hemichordate	*Ptychodera flava*	GCA_001465055.1	1126321642	
sea cucumber	echinoderm	*Apostichopus japonicus*	GCA_002754855.1	798993713	yes
robber fly	arthropod	*Machimus atricapillus*	GCA_933228815.1	268640068	
woodlouse	arthropod	*Armadillidium nasatum*	GCA_009176605.1	1223004717	yes
sea butterfly	mollusc	*Limacina bulimoides*	GCA_009866985.1	2900980226	
top snail	mollusc	*Phorcus lineatus*	GCA_921293015.1	957783961	
scallop	mollusc	*Mizuhopecten yessoensis*	GCF_002113885.1	907590157	yes
firefly squid	mollusc	*Watasenia scintillans*	GCA_015471945.1	649114004	
medicinal leech	annelid	*Hirudo medicinalis*	GCA_011800805.1	156118814	
waratah anemone	cnidaria	*Actinia tenebrosa*	GCF_009602425.1	214400470	yes
zoanthid	cnidaria	*Epizoanthus planus*	GCA_025388665.1	215910112	

Table 4: Invertebrate genomes that were compared to each other

Given to `lastdb`	Given to `lastal`
stony coral	waratah anemone
sea hare	sea butterfly
lancelet	Bahama lancelet
lancelet	acorn worm
oyster	scallop
fruit fly	robber fly
millipede	centipede
abalone	top snail
jawless leech	medicinal leech
lobster	woodlouse
Satsuma tubeworm	shingle tubeworm
horseshoe crab	scorpion
shamisen shell	horseshoe worm
sea walnut	sea gooseberry
starlet sea anemone	zoanthid
octopus	firefly squid
acorn worm	Hawaiian acorn worm
sea urchin	sea cucumber

Alignments

Above each alignment are shown transcription factor binding motifs that are conserved in most of the sequences. Some are labeled by name of the (family of) transcription factor. Others are labeled by "box" or "element":

- E-box: `canntg`, bound by many bHLH (basic helix-loop-helix) transcription factors and some zinc finger transcription factors.
- CCAAT: CCAAT-box, bound by NF-Y.
- WRE: Wnt response element, bound by TCF/LEF proteins, the consensus is said to be `ctttg` or `cctttgww`.
- NRRE: nuclear receptor response element, the consensus is said to be `aggtca` or `(a/g)g(g/t)tca`.
- GC-box: bound by Sp transcription factors, the consensus is `gggcggg` or similar.
- CArG-box: `ccwwwwwwgg`, bound by transcription factor Srf.

```
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
human GSX1    --gggtctgtta-------gacggtgcggtgcctgcagctgatttgatgggttggaaatgcgcacgaaacaaaatacttgaat-cctgaaaaagacccttttgtaagtttctt-tagaaatcaagcaattctgcatgacaa
human GSX2    --gggtctggca----ggcgcagaggtgccgtatgcaaatggttc------actattaggcgagaaaga-----------aatacttcaaatggccctttgtaaccttctat-agaaaatcgaggtactctgcatgacaa
chimaera gsx1 -----tctggta-------gacggtgcgatgcacgcagctgatttgatgagttggaaatacgcacgaa--aaaatacttgaat-cctgaaaaagatcctttgtaagtttctt-tagaaatcaagcaattctgcatgacaa
chimaera gsx2 --gggtctggta-------ggcgaggtgggatatgcaaatga-----------caggatattcgcaaggaaaaatactcgaatcctccaaatggctcttttgtgagtttctt-tagaaatcaagcaattctgcatgacaa
lancelet      cccaacctgctgtcgggggcagagataattttccaaaatggagt-----ggaaaatagacc--------aaaatatatagca-gcgcgccgggctttgtgctgcttgtatataagaaaacaacatattctacatgacaa
acorn worm    cgcaatctgctg--gccgcagccagataatttatgcaaatga-----------ggcgcggcaacgccag-aaaatacttgaaaccccaaaagcctctttgtttcttctgta-aagaaaaccaacaattgtgcataacaa
velvet worm   -gcaatatgcta-gacggagggggataatttatgcaaatga-----------agtaggcgctctgga-aaaatactttaaa-cctgaaaagcctctttgtgccttatgta-aagaaaaccaacaattgtgaataacaa
cactus worm   ----------tg--gctagagcccgataatgtatgcaaaata---------ttcgagacactctctaga-aaaatagtttaat-cgtgaaaagctcttttgtgcaaagcgtc-aaagcagccctcagtgttgaacaacaa
chiton        -------------------------ataatgtatgcgaatgtt--------ctcggcacacttatagca-ctgatacatggatccccaaaaggtctttttctccgttccata-tagaaagttaagaaacgtcgacaacaa
abalone       ----------------------aggcaatgtatgcaaatga-------------gtgtgcacccttagaacactgcccaaga-tttccacagattctttgt-atttctagc-aaagaaaccaacaatttgcagcgacaa
nautilus      -------------------------------atgcaaatga-----------gaaaaaacgcgcttcg-caactactctaaa-gctgaaaagattctttgtacggcctgca-aagaaaaccaacagtcgtgaataacaa
octopus       ------------------------------tcatgcaaatga-----------gaaaatgctggcttga-aaactacttttgg-cctgaaaagcttcattgtacacggtgta-aagaaaaccaacaatcgaaaataacaa
peanut worm   ------------------------------------------------------------------------------------------agaaagcctttgtgcctcccca-aagaaactcaataaatgcgaataacaa
shamisen shell ----------------------------------------------------------------------------------------aaagaacctttgtatgttagta-aggaacaccagcctcgttgaatgacaa
horseshoe worm ----------------------------------------------------------------------------------------aaagaagtctttgtgtcctatgta-aagaaaaccaacaattgaaaataacaa
bootlace worm --caacctgctc--tctctgcccagataatttatgtaaatga------------acggggcgcgctgga-aatatactttaaa-catgaaaagctgcattgtgttttgtgta-aagaaaaccaacaattgtgaataacaa

                  Sox_____
                Hmx_________________
                ------                                E-box_
human GSX1    ag--aacaattaat-aagccgtcttttcaca----aaccagcagctggtttccccgtcaaggaaagttggaaaaaattcaggctgaatgcgcgcagaagctcttt------------
human GSX2    ag--cacaatt----aagctttctattcagg---agttctgcagctggtagcccat--tgggaaagtgggaggaaa-----------------------------------------
chimaera gsx1 ag--aacaattaat-aagccgtcttttcaca----aaccagcagctggtttgccgt--caggaaagttggaaaaaattcaggctgaatgcgcgcaaagctcttt------------
chimaera gsx2 ag--aacaattaat-aagccgtcttttcacg----agctagcagctggtaacccgt--ggggaaagtaggggaaaatcagagatgaatgcgcgcaaaagtt---------------
lancelet      aaggggcgattgat-tacttggtgggtttcc------acggcagatggttaagg-----gggagaggtggcgggaggg-----------------------------------------
acorn worm    ag--aacaattaat-aagttgtttttcacc----aaacggcagatggttcgaacg--agaagaagggagcgaaaagttgaaatgcctcgctggaaacaaaatgcgcgctttttaca
velvet worm   ag--aacaattaat-aagttgtttttctacc----aaacggcagatggttcgagcg--actgtaacatactcaaaagttgatatgctttgcgaaactttt-tgcgctccttttaca
cactus worm   ag--aacaattcac-aagttgtttttccacc----aaacgccagctggttaggccg--ctcgtaacatattcaaacgtcgagaggcgctgacgtaacccgcgatgc------------
chiton        ag--actaattaataaaattgtttttctacc----aaccggcagatggtttggacg--acggga-----------------------------------------------------
abalone       ag-aaacaattgcc-atgttgtt-------------------------------------------------------------------------------------------
nautilus      ag--aacaattaat-aagttgtttttctacc----aaacggcagatggctgg-------------------------------------------------------------
octopus       ag--aacaattaat-aagttgtttttctacc----aaacggcagatggctgg-------------------------------------------------------------
peanut worm   ag--acctgttaat-aagttggtttttgccttcaatacggcagatgttt---------------------------------------------------------------
shamisen shell ag--aacaattaat-aacctttttttccgca----aaacggcagatgttttctcg-------------------------------------------------------------
horseshoe worm ag--aacaattaatcagaaagttctttcgtc----atacggcagatggt-------------------------------------------------------------
bootlace worm ag--aacaattaat-accttgcttttctggc----aaacggcagatgtccaacccg--atgggaacatatttaaaacttg-----------------------------------
```

Figure 4: *Gsx* enhancer

```
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
                  CCAAT
human HMX3    ----------------------------------------------------------------------------------------------------------------ttcgcctgcctaatcccatttgccattgtacgcgcccaatcgccgt-ata
chimaera hmx1 ----------------------------------------------------------------------------------------------------------------ttttcttgagtaatcctatttgccattgtgcccgtccaatcgtgga-ata
chimaera hmx3 ----------------------------------------------------------------------------------------------------------------ctctcccccccttcagtaatcccatttgccattgtgcgtacccaatcgccgt-ata
lancelet      aaccggttgttgggatcgcccaataagagggggttttccaccactcgtccccattgttcaagatttgaga-ggtgtggtcacttgagcttttcgttcttcgtaatccaattacccattgtgtgtgtccaatcaccga-gtc
acorn worm    ----------------tcgcccaataagggtatgttttcaccactctgt-gcattgttatccatataagaaggcgtggttacagtggagtcgctttcactgtaatcctattgcgtcgtccccgtggccaatcacaga-gct
abalone       ---------------------------------------------------cgatcgtcgatgattggagagggcgtggtgtactggaggattcctctttcattaatccgattagcttttgtgtttggccaatcagtgatttt
shamisen shell ----------------------------------------------------------------------------------------------------------------tctaatcctattatgtattgtcctcgaccaatcgc-ga-atg
stony coral   ----------------------------------------------------------------------------------------------------------------tttctcatagttccattagccgtcattttagaccaatcacagc-agc
lace coral    ----------------------------------------------------------------------------------------------------------tgattattcgagctttctcgtggttccattagtcgtcaccctggaccaatcacgga-cgc
starlet sea anemone ---------------------ccaatcacagtggg-ttttatcagttt---tcccggttgtttgcaaagaagggggcgggaagtgcattttagctttctcacggtcccattagatgtattgctcgtccaatcagcgc-tct
pale anemone  ----------------------------------------------------------------------------------------------------------tgcattttctctttctcacggttccattagacacaaaggtcgaccaatcatagt-aca

                                                                EVX_______
                                BARHL_____     NR4A______     POU6______
human HMX3    ctcccc---------gcatttaacttggatgacattttgatt--tcatcattagcatc----------------cggcgccggattgacgc---
chimaera hmx1 ctctttgcttgttaagcatttaacttgcatgacattttttatt--tcatcattatcatttttcccccttct-----------------------
chimaera hmx3 ctttct---------gcattgaacttgtgtgacatttttatt--tcatcgttatcacc----------------cggcgccggattgacgc---
lancelet      gttttcagttgtcaagcatttaacttgtatgacacgtttatt--tagacgttattattgttatctctgctggacggcgccatttcgactcagc
acorn worm    tcattagcttgtcacccatttaacttgcacgccacttgtatt--tatatgttattagcgtg---ttagatgagcggcgccaatacgactc---
abalone       gcttgaagttgtcactcgtttaa-------------------------------------------------------------------
shamisen shell cagtccatttgtcatccatttaacttctctgctactctttctcttgtagttattatcgct---ccctgtgatcggcgccatcacggttaagc
stony coral   gcatttgcatgtcagacgtttaacaagtttgacatttttattcgtgatcattatcaccggc------ttaaattgtcgcctcgcatgc-----
lace coral    gcatttacatgtcagacgtttaacaagtttgacatttttattcttgatcattatcact-----------------------------------
starlet sea anemone ccgtttgcttgtcaaccgtttaacaagtttgacgtttttattcgcgatcattatcacaggc------ttaaaacgtcgccacttaagctca--
pale anemone  gttcttccttgtcaaacgcttaacaagtttgacattttttattcgcgatcattatcatcgac------ttaaaacgtcgccactt---------
```

Figure 5: *Hmx* enhancer

```
                                                                                MEIS___    NFI_______________                                                                                FOXO___
human MEIS1   -cagagcggatgatcattcattcaccacgttgacaacctcgcctgtgattgacagctggagtggcagaaagccatgagatttg-----gtagttgg---gtctgaggggcgc---tctttttttccttt---tctttct
human MEIS2   tcatcatcaaccatcattcattcactaccttgacattccgggctttgattgacagctggagtggcaaaaagccatgaaacacg-----acagttcg---gttacatgtgggc---tgctgacgggccgct---cgtaacc
chimaera meis2 --acggcagaacatcattcattcaatacctgacaaccc-tgctctgattgacagctggagtggcaaaaagccatgagagacg-----acagtttt---gttacaagcgggc---tgttgacgggccgat---cgtaacc
lancelet      tcatgacagatcatcagtcattcagtactatgacaaccg-cgctttgattgacagcttgagtggcaaaaagccacgagacacg-----acagtttt---gttaca-ggcgct---tgttgacgggccgacattcatttcc
acorn worm    ---tgacatctcatcactcacgtccttccatgacaacct-cactttgattgacagcttgaatggcaaaaagccacgagacacg-----acagtttt---gttacagggctgt---tgttgatgggccgacataggtttcc
sea hare      ---tgacagctcatttattatccgggccctcgcggacggctcgtttgattgacagatttagtatca-gcatccacgagtcgcg-----acaggccctgttgttcgagtgtgctcgtgttgacgggc--------------
abalone       ---tgacagctcatttgttatcccgttctatagtaaccc-tggcttgattgacagatttagtggta-------------------------------------------------------------------------
oyster        ---tgacagctcgtacattatttcttgctagagtaacgt-ggttttgattgacatatt--gcgagaaatagccatgagttaag-----acaggtt-----------------------------------------
shamisen shell --atgacagctcatttgttactcgtcactatggcaacgg--cggatgattgacagcctcgctgg-aagaggccacgagcatcg-----acagttta---gtt-------------------------------------
carnation coral -----------------------cctctatggaaactt-tagtttgattgacaggagatatggcaggcagccatgatacaaaccttgacaattc----gttacgact-----tgtgatgacgggggaatatgctatggg
stony coral   ----------------------------------------------tgattgacagacttgt-ggccaaaagccacgagacagg-cttgacaattc----gttactagt-----tgtgttgacggccgtacacatcttttc
lace coral    ----------------------------------------------tgattgacagacttgt-ggccaaaagccacgagacagg-cttgacaattc----gttactagt-----tgtgttgacggccgtacacatcttttc
pale anemone  ----------------------------------------------tgattgacagatcagtggggcaagagccacgagacaag-cttgacaatcc----gttacgggt-----tgtgttgacgggaatacacatctttct

human MEIS1   ttc------------------tttctttttttttttttaaactgattttt-------------------
human MEIS2   ttcagttcgggggcttgacaattttttcttcttttcttcttttcttttcttt--------------
chimaera meis2 ttcagt-------------aattttttttttttttttggcttgacatttttttt--------------
lancelet      ttctgt-------------tgcctgacattcttgtgtaagcttagaaagtccccggtacgttcaaactg
acorn worm    ttctgt-------------tagctgacacatttctgtaagcttagaaagtccccggcctgttcaaattg
sea hare      ------------------------------------------------------------------
abalone       ------------------------------------------------------------------
oyster        ------------------------------------------------------------------
shamisen shell ------------------------------------------------------------------
carnation coral ttctcatt-----------tggttgacgggt---tgtaagcttagagagcc------------------
stony coral   cttacatt-----------gagttgacgggt---tgtaagtttagaaagctatccaactgt--------
lace coral    cttacata-----------cagttgacgggt---tgtaagtttagaaagctatccaactgt--------
pale anemone  tctgca--------------acttgacgtgt---tgtaagtttagaaag--------------------
```

Figure 6: *Meis* promoter

```
                                                WRE__                    E2F________              Smad_____
human MSX1    ----------------cccgtgcgccgggtgcagcctttgat-ccggccg-----------ccgctggcgccttaaaa--caacat-cctagtctgcctatt-----aggcgcgctgaggaatcgtgacacattgtttat
chimaera msx1 ----------------ctctccctgaagtcgcctcctttgat-cgcgccg-----------ctgctggcgcctaaaaa--caacataacttgtctgcctatt-----aaaggcactgactaatcgtgacagattgcttat
chimaera msx2 ----------------ccccgggggcgggggggggctttgat-caggccg-----------atcctggcgcctatgaa--caaccccacttgtctgcctctt-----aacttgtctgactaatggtgacagactgtttat
chimaera msx3 ---------------tctccctgaggtagcgtctcggtttgat-cccaccg-----------aagctggcgccttccaa---aacataacttgtctgcctatt-----aaatgccttgacttatcttgaagaattgc----
lancelet      gggtaagtgttacatattcggagtgattgtggtccctttgat-cccgccg-----------ttcgtggcgcctagaaa--caacacaacttgtctgcgcatt-----gaaatatctgacaaatcaggtcagattgtttat
acorn worm    gggtaactgttccatatgctaagcaatgcatttctctttgat-cgggccgttaacgctctcttagtggcgcctgtacactcactaaaggatgtctgctttttttaaaaaaaggatggcaagtcgtgatggattatttgt
sea urchin    ------------------------------------------------------------ttgtggcgccgactgc--cgtcctaagatgtctactcagt-----gaataaggtgacaaatggtgacggagtgtttgt
Satsuma tubeworm ----------------------------tggtctcctttgaa-gcgtccg-----------acgttggcgcctagcgc--gaacataacttgtctgtctatt-----cttcggcctgacaaatcgtgacagattgtttag
shamisen shell ----------------ctatgcgcggggctgttttcttgatgtacccg-----------accctggcgccttcttg--aaacttaacttgtctccctata-----gaaaagagtgacaagacttgactgcctgcttgc

                  MSX_____
human MSX1    ccccgcaattagcgatg-------------------------------------------------------------------------
chimaera msx1 agtcgcaattagcaatgcagacgctc-ggcgg-------------------------------------------------------
chimaera msx2 ccgcgcggttacccagcaggtgccgc-gggg---------------------------------------------------------
chimaera msx3 -----------------------------------------------------------------------------------------
lancelet      cctcacaattagtactggctaggtca-caaggtaattgatcactc-------------------------------------------
acorn worm    cgagccaattagctctggcttggtcc-gagggtaattgatcacct----aaaggggtgtgtactcattgcaaataactcctttatcttc
sea urchin    acttgcaattagcctggctatgcctcctgaggtaattgatcgcccgaatagaaagagccgttcccactgaaaaaccctctccctcctc
Satsuma tubeworm acgcacaattagcgacgccctgacgc-gg-----------------------------------------------------
shamisen shell tcaagcaattag---------------------------------------------------------------------------
```

Figure 7: *Msx* enhancer

```
                                                                                                                E-box_ FOX_______                        TEAD____
human SIX1    gtgcgcacttgctccagactttagggtattaactgatttttagagccgggtttaagcctccgttc---cggtcctcacacagctgccatgtcaata--agga-ctgtttcaagca---gaggcattccttctgggagggc
chimaera six1 gtgcgcgttcgtttggtgatttacggtacaatctgactccgatag--agggttaaggatcagttc---cggtccccaaacagctgccttgtcaata--aggatctgccacaagtg---gagacatccctgctgtcaggac
lancelet      -------------------taccttatacttactaatactgagtc--cgatccacgtattggttc---cggtatcgaaacagctgttttgtcaata--agaa-ctgtcaaacgaa---aaagcattccaacgatgccggt
velvet worm   ------------------------------------------------------------------------------------------------------------------------------ggg---gaagcattccgctaataccagt
shamisen shell -------------------taccttacactgatcaatttagagcaaaacgttagagcattggtccaagtagcactggaacagctgttttgtcaatattagcg-cagtctggggagcataaagcataccactaataccggc

                      MEIS___
human SIX1    ---------------------------------------
chimaera six1 ---------------------------------------
lancelet      gatgtactctacctgtcaaacattatctctaataacat
velvet worm   gatgtact-tacctgtcaaatattatctttacaaacat
shamisen shell -----------cctgtcaaatattatct----------
```

Figure 8: *Six1* enhancer

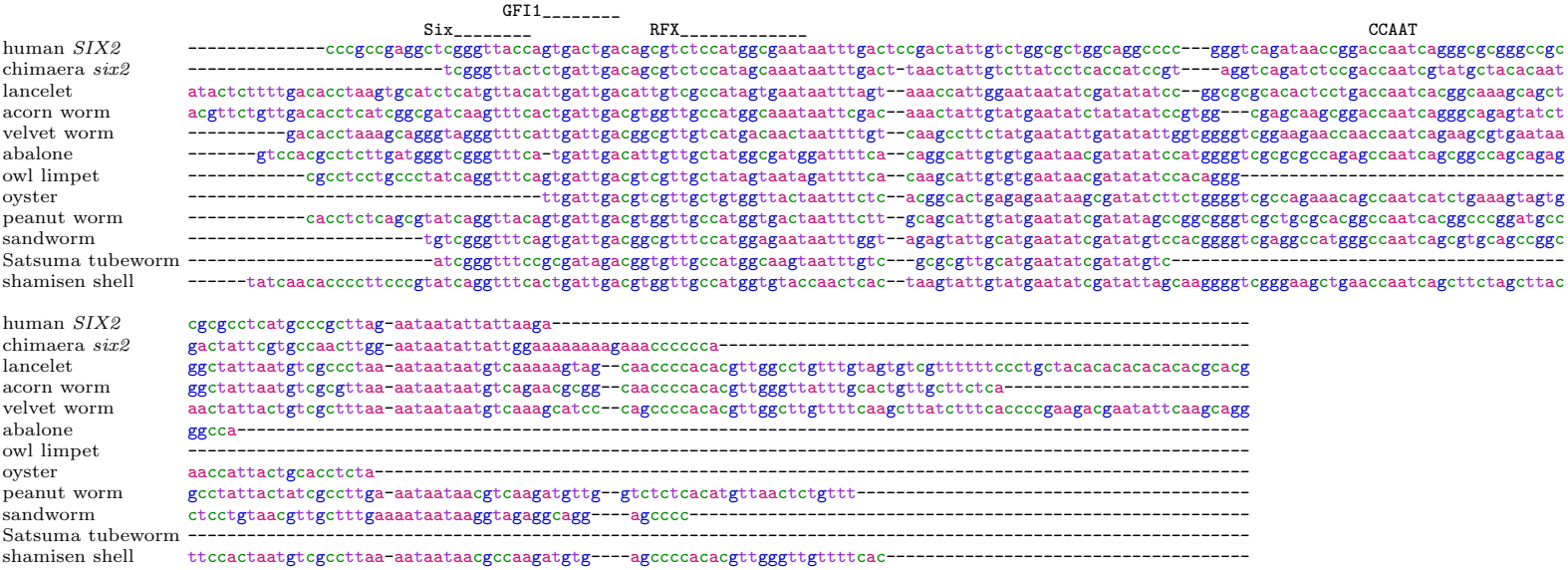

Figure 9: *Six2* promoter

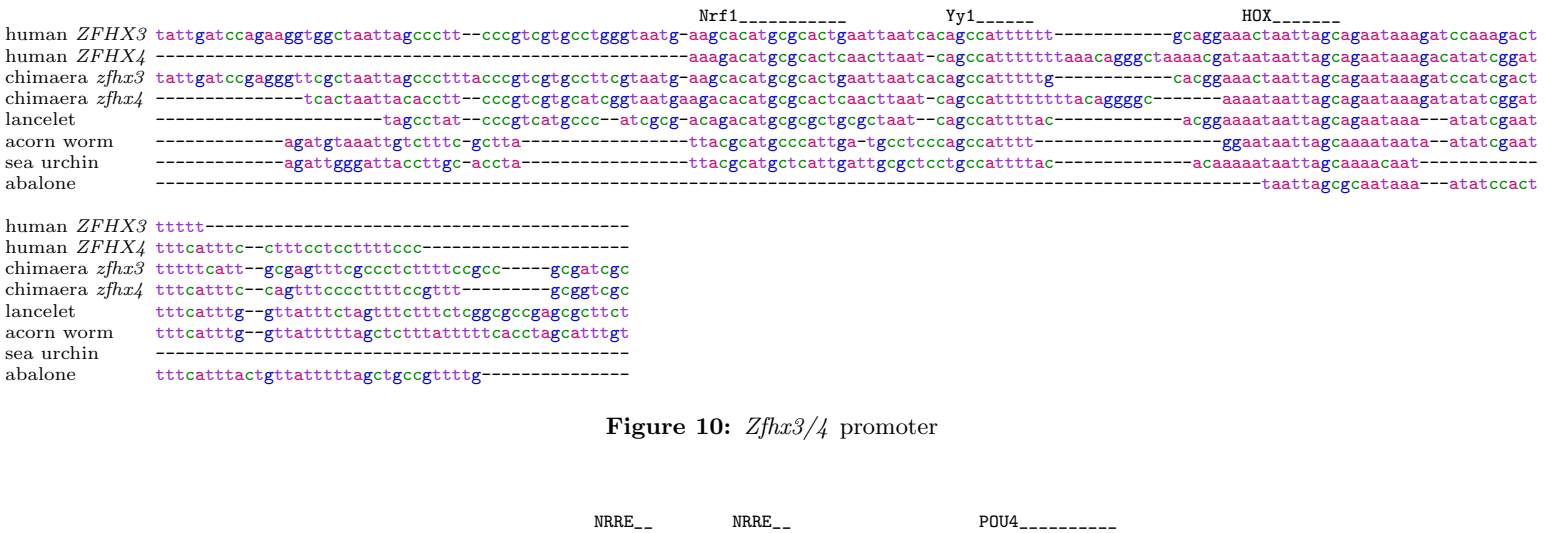

Figure 10: *Zfhx3/4* promoter

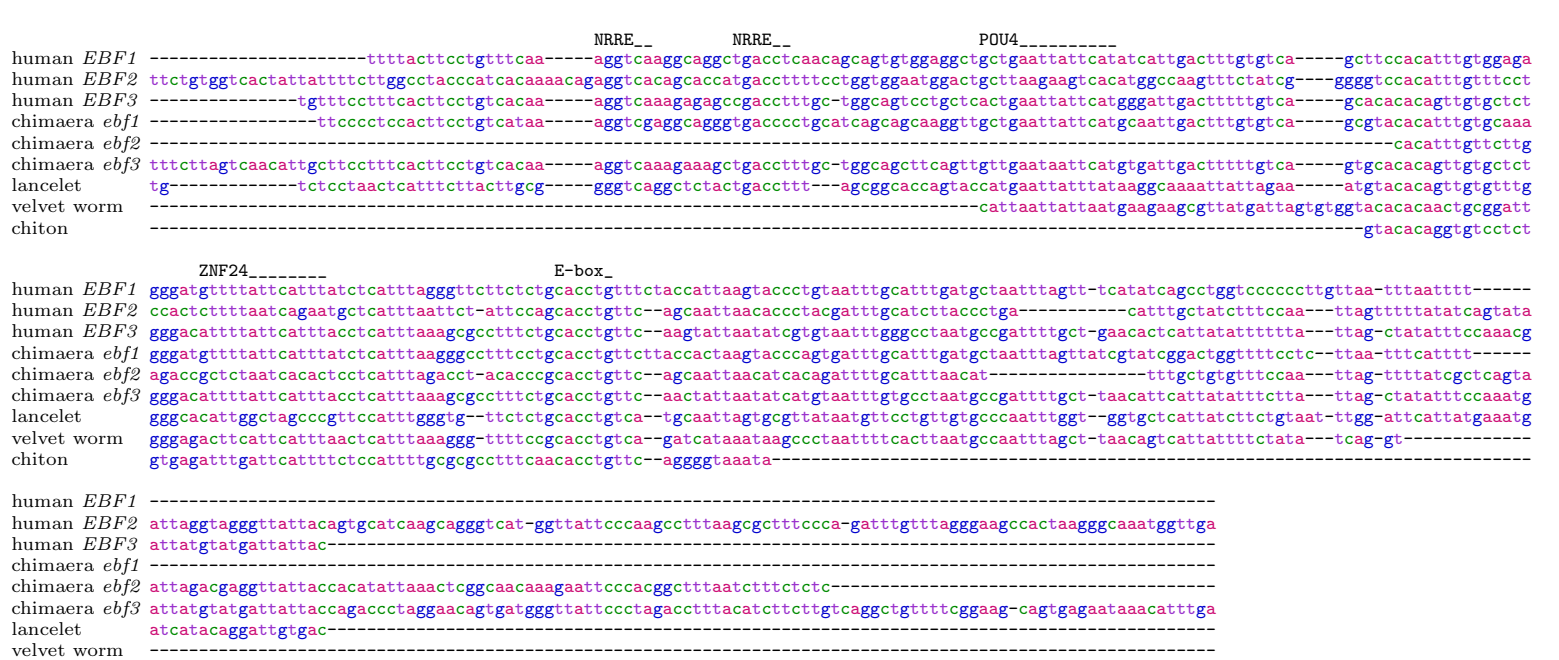

Figure 11: *Coe* intron element

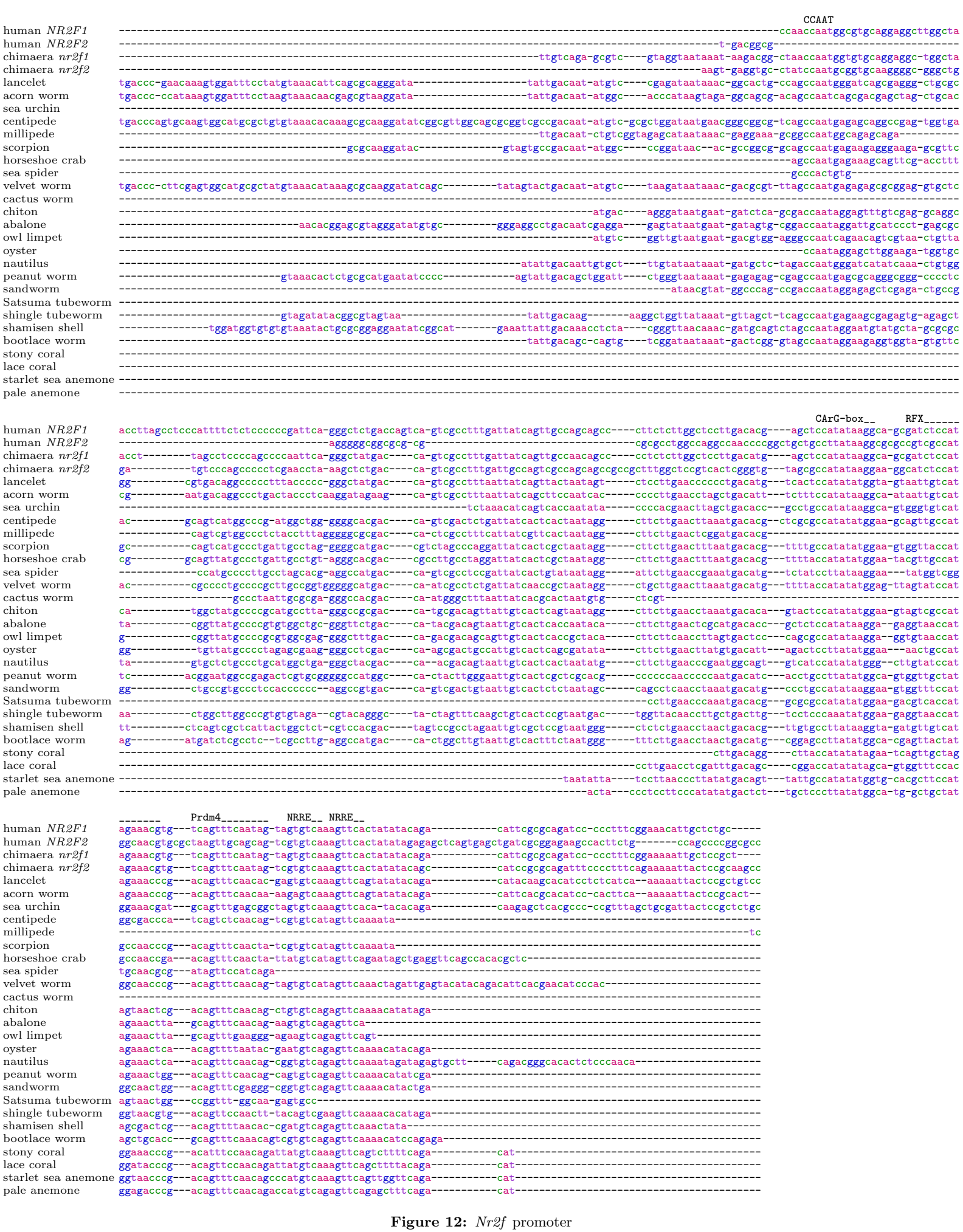

Figure 12: *Nr2f* promoter

```
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
human *RFX1*  ------------------cgcccccgcgcgtgacgtaactagggtgggtagcaacagttgccccggtgagggaaacggaggcgccatagccacggtagtcg-t----ggcgaccaagca-accc-----ggcaacgcg--
human *RFX3*  ----------------------------------------------tatagcaaccgttgccttgtgaat-------caagcgccatagtcaccgtagtcc-t----ggcgact--gtt-accc-----atcaacaacaa
chimaera *rfx1*  ------------------cgccgcgttgcctgggttgattccggaggctagcaactgttgccttgaggac--------gagcgccatagccaccgtagtcc-t----ggcgact--gtt-accccgtcaaacaacaactt
chimaera *rfx3*  -acgg-----------------------------ttgctgggctcttatagcaactgttgccttgtgact--------cagcgccatagtcaccgtagtcc-t----ggcgact--gtt-accc-----accaacaacag
lancelet  ggcgggtaggcggagt-gatccgtgttgtgtgtgcgtgtgtcgccgtgtggcaacagttgccttggttgt--------gtgcgccatagccaccgtagtca-c----ggcgact--gtt-tccc-----gccaacaacac
acorn worm  ---------------------------gtgtactgtggttaggttatttggcaactgttgccttgagaat--------aaacgccatagccaccgtagtcc-t----ggcgact--gtt-accc-----accaacacggc
sea urchin  --------------------------------tacagtggtgaggtttagcaacagttgccttgagaat--------aaacgccatagtcaccgtagtcc-t----ggcgact--gtt-accc-----gccaacga---
centipede  ----------------------------------------------tggcaactgttgccttaggaat-------ccgacgccatagacacggtagctg-t----gacgactgcgta-acccc----atcaacaggac
millipede  ----------------------------------------------tggcaactgttgccctaggaat--------aagcgccatagacacggtaactg-t----gacgact--ggt-agcc-----atcaacagtaa
tick  ------------------------------------------gttgcggcagttggcaactgttgccctggagt--------caacgccataactacggaagctg-c----aacggcttggta-acct-----gtcaaca----
horseshoe crab  ---------------------------------------------ttggcaactgttgcccttggaat--------caacgccataccgacggtaaccc-g----gacaacc--aaa-atct-----gtcaaca----
velvet worm  ----------------------------------tgtacagaggtttggcaacagttgccttaggaat--------aaacgccatagccaccgtagctg-t----gacgact--gtt-accc-----gccaacga---
cactus worm  ----ggtcggcgcaataatctattgccctgcatcgatgtgatccggtttggcaactgttgcccaagg----------aaacgccatagacacggtagctg-t----ggcgact--gtt-accccg---aacaacaaagc
chiton  ggcggttatgcggaataat----cgttgatttgctgacgcgctgagtctggcaacagttgccgtaggaat--------aaacgccatagccacggtagctg-t----gacgact--gtt-accc-----gccaacaagac
sea hare  ------------------------------------------gagtctggcaactgttgccgtaggaat--------aaacgccatagacacggtagctg-t----gacgact--gtt-tccc-----atcaacaacta
abalone  ------------------------------------------gaggctggcaacagttgccgtaggaat--------aaacgccatagccaccgtagctg-t----gacgact--gtt-accc-----accaacacgac
owl limpet  ------------------------------------------gagtctggcaacagttgccgtaggaat--------aaacgccatagccaccgtagctg-t----gacgact--gtt-accc-----accaacagaac
oyster  -----------------------------------------ggagactggcaactgttgccgtaggaat--------aaacgccatagccaccgtagctgtt----gacgact--gtt-accc-----accaacacgta
nautilus  ----------------------------------------------tctggcaactgttgccgtaggaat--------aaacgccatagccacggtagttg-ttgacgacgagt--gtt-accc-----gccaacaaga-
octopus  ------------------------------------------gagtgtggcaactgttgccctaggaat--------aaacgccatagccacggtatctact----gacgact--gca-gccc-----gccaacaacac
solenogaster  ------------------------------------------agtctggcaactgttgccctaggaat--------aaacgccatagacacggtagctg-t----gacgact--gtt-accc-----accaacaactc
peanut worm  ------------------------------------------gagtctggcaacagttgccgtaggaat--------aaacgccatagccaccgtagctg-t----gacgact--gtt-accc-----gccaacac---
sandworm  ------------------------------------------cgtctggcaacagttgccgtaggaat--------aaacgccatagccacggtagctg-t----gacaact--gta-tccc-----gccaacaagac
Satsuma tubeworm  ------------------------------------------cttctggcaacagttgccgtaggaat--------aaacgccatagccacggtagctg-t----gacgact--gtt-accc-----gccaacaagac
shingle tubeworm  ------------------------------------------cgtctggcaacagttgccgtaggaat--------aaacgccatagccaccgtagctg-t----gacgact--gtt-accc-----gccaacacgac
shamisen shell  ------------------------------------------cgtctggcaacagttgccgtaggaat--------aaacgccatagccaccgtagctg-t----gacgact--gtt-accc-----gccaacaagtc
horseshoe worm  ------------------------------------------cgtctggcaacagttgccgtaggaat--------aaacgccatagccaccgtagctg-t----gacgact--gtt-accc-----gccaacaagaa
bootlace worm  ------------------------------------------cggagtctggcaacagttgccgtaggaat--------aaacgccatagacaccgtagctg-t----gacgact--gttgaccc-----gccaacaagac

human *RFX1*  ------------------------------------------------------------
human *RFX3*  ctac---tcctctcctcctcctcctcttcctcctcctcct------ccccaccacc
chimaera *rfx1*  acgccacaaaggtgattcttttctctctctctcttctctc----------------
chimaera *rfx3*  gagc---gcaggtgatctcctcctcactct----------------------------
lancelet  caacc-acaaggtgattttcctccatcctctactcccactaccgcaccgcgccactacc
acorn worm  a------taaggtgatttttttcctttcagttcctagtcttcc----------------
sea urchin  -------caaggtaatttttttcctttta-----------------------------
centipede  ------agaaggtga--------------------------------------------
millipede  -------cgaggtgattctctc-------------------------------------
tick  ------------------------------------------------------------
horseshoe crab  ------------------------------------------------------------
velvet worm  ------------------------------------------------------------
cactus worm  cagc---tgaggtaatttttccttc---------------------------------
chiton  -------taaggtgattttcctt----------------------------------
sea hare  -------taaggtgaattt-------------------------------------
abalone  caac---taaggtgattttctttcctt-----------------------------
owl limpet  -------taaggtgattttctttta----------------------------------
oyster  -------aaaggtgattttc-------------------------------------
nautilus  -------gaaggtgatttttctttccatttcggttcgtag------------------
octopus  acacc-aaaaggtgatttcctttccc-----------------------------
solenogaster  -------cgaggtgatatacttattattc-----------------------------
peanut worm  --tc---taaggtgattttc-------------------------------------
sandworm  -------taaggtgattttt------------------------------------
Satsuma tubeworm  t--------aggtgattttcctttac-----------------------------
shingle tubeworm  ------------------------------------------------------------
shamisen shell  -------taaggtgattttcct----------------------------------
horseshoe worm  c------taaggtgattttt------------------------------------
bootlace worm  -------taaggtgattttacttcctattttgctctaactta----cacgcc------
```

Figure 13: *Rfx1/3* promoter

```
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
human *SALL1* ---gctggcccggtgccaatcgcctttcaaagagcccctctatgattaatcgcaatgcattattg-at-aatcataattatag----------
human *SALL4* ----------gccaatcagctgtcagggc------tcatgataaatcgcaatgcattattg-at-aataataattactg----------
chimaera *sall1* ---gctggagagttgccaatcggctttcaagaagc----ggatgattaatcgcaatgcattattg-at-aatcataattataccccccaccccacccaccccccaaaacccccccccccccaccaccccaccaccaccaccacatgc
chimaera *sall3* ----ctggagagcagccaatccgctgtcaggag---cggcgatgattaatcgcaatgcattattg-at-aatcataattatat----------
chimaera *sall4* ----------gggggggg----cgatgataaatcgcaatgcattattg-at-aatcataattacca----------
lancelet ----------tcagcgcgctcctcgctgattaatcacaccccactattg-at-aatcataattatac----------
acorn worm taatgagtagagcggccaatca------cggcaatgcattcgaaacataacgcaatgcattattg-ataaatcataaatttct----------
sea urchin taattagtatatcgaccaatca------gaaaggaccattcatagcggagcaacaagctctattgcataaatcataaattggt----------
scorpion ----------taatcgcaatgcattattg-at-aatcataatagtg----------
horseshoe crab ----------taatcttaatgcattattg-at-aatcataattgtg----------

human *SALL1* ----------caggacacatgcgcgctgcgccc----------
human *SALL4* ----------ggacatgcgcgttccggccgaagggggggta--------aatttccc-
chimaera *sall1* acacacacacacacacacactcactcactcactcacacacatacacacacactcactcactcactcacacacatatatgcacaacgcacacacatgcgcactggaggcgccagg-gtcaatttccgtaattttgc-
chimaera *sall3* ----------ggtgacatgcgcactccacggccccgcagctaatttccgtaattttgc-
chimaera *sall4* ----------tcacacatgcgcactccactccccgca----------gtaatttggc-
lancelet ----------agacatgcgcaatgaccaccccttc--ttaatttccgtaattttcg-
acorn worm ----------gtacatgcgcactggggcccccact--gtaatttccgtaattttgcc
sea urchin ----------gtacatgcgcac----------
scorpion ----------acatgcgca----------
horseshoe crab ----------acatgcgcagt----------

human *SALL1* ----------
human *SALL4* -aactcca--ggaatttgt----------g
chimaera *sall1* -aactcca---gaattagt---aattttttattcgc----------ttgctgatgtatcaac----------cacc-------a
chimaera *sall3* -aactcgg--ggtatttgt--gaaattttttttt-------ttttatttgttgctgatgtatctgc----------cacc-------a
chimaera *sall4* -aactccaccagaatttgtttgaaatttttttttttgggggttttatttgttactgatgcatctgc--catgtcgcgtcgcaagcagccgaacccccagc-------a
lancelet -aactcga---gaatttca---aaatttttaatct-------ttttcattgttgctgctgctgtgggaggatgtcgcggcgcaagcaggcccggccgcaacatttggag
acorn worm aaactcggc----------gaatttctaatca--------tttccccgctgctgttgtttccag-ccatgtcgcgccgaaagcaggccaaaccgcagcacctaggg
sea urchin ----------
scorpion ----------
horseshoe crab ----------
```

Figure 14: *Sall* promoter

```
                                                                                                                CEBP__________                        E2F____________ Smad____    E-box_
human            ----------------------------aagagtt-----------aagaaacttgaa-accttgtttgcgcaacaatcag-------c----gcc---gcgg-agccgccaaagtgtctagac-tggcatatgatggga
chimaera         ----------------------------aagggtt-----------aagaaacttgag-accttgtttgcgcaaccatcgc-------tgcagatg---agag-ccgcgccaaaatgtctagaccggccacatgattgga
lancelet         ------------------------------------------------cggtaatgtgat-ctgttatttgcgcacccatcgg------------------cgga-gcgcgccaaaacgtctagac-gcgctgccgccgcac
acorn worm       actgtttttcggtgagatattc---cgggggagaagtaacagccaagtgtaatttgag-ctgttatttgcgcaacaatcat-------tttagact---cggg-gcgcgccaaaatgtctagac-gaccacgtgataatt
sea urchin       ------------------------------------------------------------gttatttgctcaacaatcat-------ttcagact---cgag-ccgcgccaaaatgtctagac-atccacatgacccaa
sea urchin       ------------------------------------------------------------gttatttgctcaacaatcat-------ttcagact---cgag-ccgcgccaaaatgtctagac-atccacatgacccaa
centipede        ------------------------------------------------cggtaattcgat-ttgttatttgcgcaacaatcac-------tgaagaca---tggg-gcgcgccaaaaagtctagacgaatcacgtgtccgct
millipede        attctttttcgcgaaaatggcgagaggagaagcatataatcagtagccgtaatttgat-attttatttgcgctacaatcgt-------ccgagacag-tgtgc-gcgcgccattgagtctagac-gatcacgtgat----
scorpion         ------------------------------------------------agtaatttgat-ctcttatttgcgcaacaatcgt-------ttcgg-----------gcgcgccgaaaagtctagac-gtccacgtgatcggt
tick             ------------------------------------------------cgaaggtaatttgat-cggttatttgcgcaactatctcgcgcagacagaagga---ggag-gcgcgccgtcctgtctagac-gggcacgtg------
horseshoe crab   ------------------------------------------------------------------ttatttgcgcaatgatcac-------cttaaaca---ccga-acgcgccaaaatgtctagac-actcgtgtgagcata
horseshoe crab   --------------------------gggaggaacgtagtctcagaaagtaacttgat-cacttatttgcgcaacaatcgc-------atcagaca---cta--ccgcgtcaaaatgtctagac-acgcacgtgaacata
horseshoe crab   ------------------------------------------------taatttgat-ctattatttgcgtaacaatcgt-------atctgtgc---ttag-ccgcgccaaaatgtctagac----------------
horseshoe crab   ------------------------------------------------agtaatttgag-ctcttatttgcgcaacaatcgc-------ttcagaca---cca--gcgcgccaaaatgtctagac-gtgtacgtaata---
velvet worm      --------------------------gggaggaatgtaatcattggaagtaatttgat-atgttatttgcgcaacaatcac-------tttagaca---tggg-gcgcgccaaaaagtctagac-aagcacgtgactaac
cactus worm      ------------------------------------------------------------------------------cagtcac-------tatagatg---tgtg-gcgcgccagcgagtctagac-ggccacgtga-----
abalone          ------------------------------------------------cagtaatttgat-atgttatttgcgcaacaatcat-------agtcgaca---tcag-ccgcgccaaaaagtctagac-acccacgtgctacat
nautilus         ------------------------------------------------gtaatttgat-atgttatttgcgcaacaatcac-------tttaggca---tcagtccgcgccaaaaagtctagac-tgccacgtgaccgtc
octopus          ------------------------------------------------tgaaatttgatcccgatatttgtgcaacaatcact------gaaagacagagtgag-ccgcgccaaagtgtctagac-tagcacgtgacccgg
peanut worm      ------------------------------------------------agaaacttgat-atgttatttgtgcaacaatcac-------tgtagaca---gtgg-ccgcgccatggagtctagacgagccatgtgaccatt
sandworm         ------------------------------------------------gaaacttgat-acgatatttgcgcaacaatcac-------tttagaca---gcga-ccgcgccaaaaagtctagacaaggcatgtgactgaa
Satsuma tubeworm ------------------------------------------------aactcgat-gccttattggcgcaacaataac-------tttagaca---gcta-ccgcgccaaaatgtctagacctcgcacgtgacccgc
shamisen shell   ------------------------------------------------agtaattcgat-aggttatttgcgcaacaatcac-------tttagaca---tcag-ccgcgccaaaaagtctagacaaaccatgtgatcaag
stony coral      ------------------------------------------------attgaaa-ttggtatttacgtaacaccctc-------atcagacaactatag-acgcgcctggctgtctagac-aagcatgtgacagga
lace coral       ------------------------------------------------attgaaa-ttgttatttgcgtaaca-ccac-------atcagacaactatag-acgcgccaatgcgtctagac-atgcacgtgacaata

                     CCAAT
human            ggcag-ccaatgactccgcggcgctcc---------------------------
chimaera         gccaa-ccaatgactcaaaagtgtccc---------------------------
lancelet         gga---ccaatcagcgcgcagct-------------------------------
acorn worm       ttgaa-ccaatcaagtgttagcaatgg-tgtgg-cagtaagtgttgtaaatttc
sea urchin       gtcaa-ccaatcagcgtagaagtaatcgtgtggtcagagagagtagtaaatttc
sea urchin       gtcaa-ccaatcagcgtagaagtaatcgtgtggtcagagagagtagtaaatttc
centipede        cccgaaccaatggcggcgc-----------------------------------
millipede        --------------------------------------------------
scorpion         tatag-ccaacc------------------------------------------
tick             --------------------------------------------------
horseshoe crab   ctcag-ccaatggtt----------------------------------------
horseshoe crab   ttcaa-ccaatagc------------------------------------------
horseshoe crab   --------------------------------------------------
horseshoe crab   --------------------------------------------------
velvet worm      ataaa-ccaatcagatct------------------------------------
cactus worm      --------------------------------------------------
abalone          cttgg-ccaatcagcgtgcagct-------------------------------
nautilus         gtcgg-ccaatca-----------------------------------------
octopus          ccgcc-tcaaccaatcaga-----------------------------------
peanut worm      tccga-ccaatca-----------------------------------------
sandworm         attgg-ccaatggc----------------------------------------
Satsuma tubeworm cccag-ccaat-------------------------------------------
shamisen shell   ataag-ccaatca-------------------------------------------
stony coral      atcaa-ccaatcaa----------------------------------------
lace coral       tttca-gccaat------------------------------------------
```

Figure 15: *Smad6* promoter. This matches four places in the horseshoe crab genome: three upstream of genes annotated as "smad6-like", and one upstream of the edge of a genome fragment.

```
                                                                                                                
```

```
                                                                WRE__              CCAAT                                                                                GC-box_
human *SP5*          -----------------gggtc-tccaggcggcaa--ggcccccttgat---caggaaaatccaattatttgtgtatat--acatttcccacatagtgattacttcattaattgtcccgccttta-tctcctcccttcc
chimaera *sp5*       -----------------gggtc-tcctggcggcagc-tgaaggctttgat---cagacaaatccaattatttgtgtatat--acatttcccacatagtgattacttcattaattgtcccgccttta-tctcctcctttgc
chimaera *sp5-like*  --------------------tc-ctgtgggagtggccggggccctttgat---gaagtcaacccaattatttctgtacatggagatctcccagatggtgataacttcttcagt-------------------------
lancelet             ----------------------------------------cctttgatcaagagttggcccctatttagcatgtagat--agattacgtggctagtgattaggtcgttcttttacccgccttt--gtcccgcccaaga
acorn worm           agtggcgtccaatcagtggaca---gtttggtcggtgacacgtctttgat---ggcacgaatccaatttgcagtgtgtct--aaaagtggcacatagtgattacttcatttacttgcccgccttttt-gtcccgccttgcc
sea urchin           -----------------------cttggcggtccgt-cggcacctttgat---aactgaaatccaattatttgtttatac--aaaagtggcagtgggctattacttcattaagttgcccgccccattgtaccgcctatcc
horseshoe crab       ------------------------------------tggcacccccctcaaa---ggtcggaatccaattatttgtgtg-gc--taatgctagaagtagtgattac---------tttcccctcccct---acctgccctact
velvet worm          ------------------------------------------------------------------------------------------------------------cccctcccaa---tccggcctccct
cactus worm          ------------------------------------------------------------------------------------------------------------ccgccccat---gcctgcccccct
chiton               ------------------------------------------------------------------------------------------------------------ccccgcctcg---ttcggcccatct
sea hare             ------------------------------------------------------------------------------------------------------ttgc---------ttccccgccctc---tccgtcccctcg
abalone              ------------------------------------------------------------------------------------------------------------gtcccgccttc---gcctgcccctca
oyster               ----------------tgaatg---ggttccttggtattgaagctttgat---ctcctcaatccaattatttatgtgtgt--aaacgtcctgcgagaagattac---------ttgcccgccctc---gacaaccccgtt
nautilus             ----gcagccaatcgccgcacg---ccgccctcggcgcttctcctttgat---ctcccgaatccaattactgcgctgtat--acgttctctacgaggtgattgc---------ttgcccgccttc---gccaaccccctct
peanut worm          ------------------------------------ggcctgtctttgat---gtgccaaatccaattatttgtgtatat--aaattgcacatgggacgattag---------ttccccacccct---gccggcccctcc
shingle tubeworm     ----gccctcaatagtcaaacaacccccctcagcggcacttttgctttgaa---gttgaaaatccaattatttgtgtgtgt--aaattccctgcctagtgattag---------ttgccctccctt---gccagcccctgt
shamisen shell       ----------------------------ggtcggcgtcggtcctttgat---ctcgtaaatccaattatttgtgtatat--aaattccctacgaggtgattag---------ttgcccaccctt---gccagcccctct
horseshoe worm       ------------------------------------------------------------------------------------------------------------------------------------------
bootlace worm        ------------------------------------------------------------------------------------------------------------------------------------------
stony coral          agcgatggccaatcaatgagca---ctttgctcggc-ggcttgctttgat---catagaaatccaat-------------------------------------------------------------------

                                                                                HOX_____          Fox______
human *SP5*          catcccccct-------------------------------------------------aataatcag-ttcttttatccagaccaaca-aacacaccataggagctttgtggatt---caaa-ggatttgctttcgc
chimaera *sp5*       catccccatccccatcccccatccatccccaccccccaccccatcccccccctataataataatcagcccctttatacataccaaca-aacacaccacaggagctttgtgtatt---caaaggatttggttcccc
chimaera *sp5-like*  ------------------------------------------------------------------------------------------------------------------------------------------
lancelet             caacccctct-------------------------------------------------aataatcgc-tagtattctacatgtcaaca-aacaagccgcgggagccctgtgtata---gaag-ggattgaatttc-c
acorn worm           ctgcccacgt-------------------------------------------------aataatcgc-ttctattatatgtgtcaaca-aacacacagcaggccctttgtcagca---caaa-agattcgatttc-c
sea urchin           cggcccctct-------------------------------------------------tataatgaa-cgctatttgcgagtcaaca-aaccctcttgcgggtctttgtggtgaactaat-ggacggggcccc-c
horseshoe crab       aataacctcg-------------------------------------------------aataatccg-caccattataagtgtcaaca-a-------------------------------------------
velvet worm          aataactg---------------------------------------------------cataatcag-cgccattataagtgtcaaca-aacactttcggagacgcgaggggata---taaa-agatttgatttc-c
cactus worm          aataatggcg-------------------------------------------------cataatcaa-cgctattatgattgtcaaca-aacactcgccagcgagcatatggaca---taaa-agattgaatttc-c
chiton               aatatctccg-------------------------------------------------cataatcaa-acctattatatgagtcaaca-aacagcctctaagaagcttgtagaca---gaaa-ggatttaatttc-c
sea hare             gacaacagcg-------------------------------------------------tcttaatcag-ctctatttttcgtgtcaacacagtccgctctaagaaacatatggaca---aaaa-ggatttcatttc-c
abalone              aataacaaac-------------------------------------------------cataatcag-ccctattatgcgtgtcaaca-cactcgctctaagaagcatatggact---gaaa-ggatttaatttc-c
oyster               aataacaagt-------------------------------------------------tataatcgc-ttctattatgactgtaaaca-atctctctcttagaacttgtatttt---gaaa-ggatttaatttc-c
nautilus             aataacaccg-------------------------------------------------tataatcag-cgctattataagtgtcaaca-aacactctccgaccgccatatggaca--taaaa-aggttgaatttc-c
peanut worm          aataacgccc-------------------------------------------------cataatcag-ggctattatgtctgtcaaca-aacacgctcaaggaagcctatgtgct---cgag-ggatttaatttc-c
shingle tubeworm     aataacaagg-------------------------------------------------aataatcac-ttctattatgactgtcaaca-aacacagccttacttctatatggaca---caat-ggattaaatttc-c
shamisen shell       aataacattg-----------------------------------------------gcataatcag-ctctattataaacggcaaca-acccctcgccaggaagcatatggaca---taaa-ggatttaattct-c
horseshoe worm       ------------------------------------------------------------ataatcag-tgccattatcaacgctaaca-aacactctcttagactcctatggaca---gaaa-agatttaatttc-c
bootlace worm        -----cgcgg-------------------------------------------------cataatgga-cgctattattcccgtcaaca-aacacacactgcgaccactgccaggc---ccac--tgttcaatatc-c
stony coral          ------------------------------------------------------------------------------------------------------------------------------------------

                                                  CCAAT                WRE__                  CCAAT
human *SP5*          ttctgaaag-agccgctattctttgatgattgggtagc--ggcaaacttcaaagcca-taaatcttccctctgactggctggcggcccagcaaagtcctt---------------------------------------
chimaera *sp5*       ctctgaaag-agccgctggtctttgatgattgggtggtgggggaacatcaaagtggctgtggctctctgctcactggctggtgggctgg-------------------------------------------------
chimaera *sp5-like*  ------------------------------------------------------------------------------------------------------------------------------------------
lancelet             ttttaaatc-gcccgctgatcacatatgaatggcgggc--ggcacacatcacaggaa-ttttctcgcgtgccgattggctggcggtgagatcagaggcagttgc----------aagtctggtctgtgt-taccgcctttt
acorn worm           ttttaaatc-acccgctagttggcgctaattggacagc--ggtaaagatgacacgaa-gtgccctccaatgcgattggttggcggtgtttcaaagctcctcgtt----------cgcgtctgatgtgc-gaccgcctat
sea urchin           tttcaaatc-caccg-caccgcttcctcattggtgggc--ggcatctctcaaaggac-ttagaccgtcagatcattgggtagcggcgagataaaagactagacg----------taatgcggctttccctcactgactat
horseshoe crab       ------------------------------------------------------------------------------------------------------------------------------------------
velvet worm          tttacattc-acccgctttgctaacggattggcggtc--ggcgaacttcaaaaatc-cgcgagctatttacaattggctcgcggtctgtgaaagcaatagctc------------gttcacatagtgt-taccgcctct
cactus worm          ttttaaata-acccgccagcccactctcattggtctgc--ggcactatcaaacaggc-atctctcgt-tcgatattggctggcggcacgtcaaagagagcgaagcgccgcatatttatgtatgaagtgtgcaccgcctat
chiton               ttttaaacc-acccgcctattcaatgtaattggctggc--ggtagacctcaaagggc-tggaggcgcgctccgattggctggcaccacatcaaagc-------------------------------------------
sea hare             tt----------------------------------------------------------------------------------------------------------------------------------------
abalone              ttttaaacc-accccgctattgatcgtaattggctggc--ggcgaacatcaaaggaa-aaatatcgcgttttgattggagggcggtagatcagagccgatactc-gca----------gcactaagtgt-gaccgcctat
oyster               ttttaaactaacccgcctatagatttcaattggctcgc--ggcaaacttcaaagaca-ttgtcaagtattttgattggctcgcggaaacatcagag-----------------------------------------
nautilus             ctttaaacc-acccgcgtgccggctgtaattggctgtc--ggcgcacttcaaaggag-cgctcctgcgccccgattggcgggcggtggatctgaggcgatggc-----------------------------------
peanut worm          tttcatttc-tgccgactattgattgtaattggtccgc--ggtggggatcaaagctg-gtgcctcgcgcgctgattggctagcggcacttctaagacccaggtt-----------------------------------
shingle tubeworm     ttttaaatc-gcccgcctattgattttgattggatagc--ggcagacatcaaaggaa-aaccccaac-ataggattggatatcggtacttcaaaggggatcaac-----------tgttcgtaaagcat-tacggcctat
shamisen shell       ctttagtgc-acccgacagcgtgagactattggttggc--ggtatacatcaaaataa--ctcctagagctccgattggctggcggcatcttcagatctccgtga----------taacgcttgaagtgt-tactgcttat
horseshoe worm       tttcaaatg-acccgcttctaacattttattggatagc--ggtacgattaaaggcaa---acattcactcgtgattggttagcgg-------------------------------------------------
bootlace worm        gcc--aacc-aaaccaccattgatcctaattggcccgc--ggcggacatcaaagaca-ccgcattctcctctgattggtcagcggcacatcaaaggccattg-----------------------------------
stony coral          ------------------------------------------------------------------------------------------------------------------------------------------

human *SP5*          -------
chimaera *sp5*       -------
chimaera *sp5-like*  -------
lancelet             tcacac-
acorn worm           tcacact
sea urchin           acacac-
horseshoe crab       -------
velvet worm          tcgc---
cactus worm          tgaca--
chiton               -------
sea hare             -------
abalone              tcacagt
oyster               -------
nautilus             -------
peanut worm          -------
shingle tubeworm     tgatgtt
shamisen shell       tga----
horseshoe worm       -------
bootlace worm        -------
stony coral          -------
```

Figure 17: *Sp5* promoter

```
                                                
```

```
                                                                                                                                                                                                                                                miR-92_
                                miR-130                                                                                                                                                                                                      Let-7__
human CPEB2  ccattttgacatttgttatgcactatttttatatctctgtgagagatttttccaacagtcagc--tatttta-------------------tggcacactttttttgactgatgacatctc-ctttgctatacctcaa--
human CPEB3  -----------tttgttatgcactactactttttgtat-tctagacagttttcaaaagttggcagatttttttttttgtttttaaggaaaaaggcatgc--tttctgactgacatgatc---ttttgcaatacctcaa--
human CPEB4  --------atatttgttatgcactactttttgtatatct------tgtttttccaacagt-gaa--catttttt-------------------aggcacact-tttcactgacgggatatctctttatgcaatacctcaa--
chimaera cpeb2 ccatattggcatttgttgtgcactactttttgtatcttggtgagagtttttccaacagtcagc--tgtttta-------------------gggcacac--tttttgactgatggcatctc-ctttgcaatacctcaa--
chimaera cpeb3 --------acatttgttatgcactactttttgta-----tctggacggttttcaacagtcagctgatttttt-------------------aagcgtgc--tttctgactgacatgatc---ttttgcaatacctcaa--
chimaera cpeb4 --------acatttgttatgcactactttttgtatatct------tgtttttccaacagt-gga--tatttttt-------------------cagatcact-tttttcacagatggcatctcttttgcaatacctcgatt
lancelet     ------------------------------------------------------------------actttttt-------------------aggcaggc--tttccaccta--tgtacc---atttgcaatacctcaagg
cactus worm  ------------------------------------------------------------------------------------------------------------------------------------------------
shamisen shell ---------------------------------------------------------------------------------------------------------------------------------------------

human CPEB2  -tttttggaatttagagaagaaatcagtagttttgc--aatgttaattatttagatattt-----------------------------------------------------------------------------
human CPEB3  --ttttgaaatataggaaagaaaatgatattttctaagtaagtttattc---------agaaaaatatatattaatttaattctgca-agaaaatgattta------acagatgggta------actttattttttcat
human CPEB4  -tttttcatattgcaaagagtagc------tttttg--tactttttattactgagagatcttcatatac-------ttcatttttaa-tataaataatttt-----aataaattttat------tttcttatattctgct
chimaera cpeb2 -ttttctgaatttagaagagaaatcgatagttttgc--tatgtttattattgaaagatctgcatatata-attaatttaattcagca-aataaatgattta-----acaaaattgata------tttttattttcatac
chimaera cpeb3 --ttttgaaatataggaaagaaattgatattttctaagtaagtttattc----------taaaaatatatattaatttaattctgca-aggaaatgattta------acagatgggta------atttttattttttcat
chimaera cpeb4 -tttttcatattctaaagagagatgaatagtttttc--tatgcttattgttcaaagatctgcaaatatg-gttaacttaattgtgca-aagaaatgagttg-----aagcaatcagtt------ttgctttctttcgttt
lancelet     ttttccagaattt---gagcaaatagatatttt-----gaagtttattcttgaaagttaggaatgagagtattaatttaattctgca-----gatgatttaaagagaagatgtgggta------attttattttcttgt
cactus worm  ------------------------------------------------------------ggagtgacattattatttaattctgca-atcaaacagttgaaaaataaaatatgggtaagattgattttattttctcgt
shamisen shell ---------------------------------------------------------aagatgtatgttaatttaattcagcacacaagataatttatggtaggaaaatgggta------attttattttt----

human CPEB2  ---------------------------------------------------------------------------------------------
human CPEB3  gct------------------tatttgtgtttt-----ttgaaaa-------tgaagaagttagagctttataactataatattaaagatcatatctaac
human CPEB4  ttt------------------tatacattt-------------------------------------------------------------
chimaera cpeb2 atc------------------ttgctttct----------aaatt-------tggaagggtctaaacatttgta--------------------------
chimaera cpeb3 gct------------------tatttgctttt-----tggaaat-------tgaaggagctagagctttataactttaatatggagaattgtagttagc
chimaera cpeb4 tat------------------tattttttt-------------------------------------------------------------
lancelet     att------------------gttctgcttttgcatatgaaaac-------tggaaagctttaaactttttattata---------------------
cactus worm  aatattattatgtatgattccttttcagcctttgcctgtaaggatgccagtgcaggagattttaaactttttattata---------------------
shamisen shell ---------------------------------------------------------------------------------------------
```

Figure 21: *Cpeb2/3/4* 3′-UTR. Previous studies showed regulation by miR-92 [35, 36] and miR-130 [37], and predicted these Let-7 and miR-92 binding sites [35].

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human *ID1*
human *ID2*
human *ID4*
chimaera *id1*
chimaera *id2*
chimaera *id3*
chimaera *id4*
lancelet
acorn worm
sea urchin *emc*
sea urchin *emc-like*
velvet worm
cactus worm
chiton
sea hare
abalone
owl limpet
oyster
nautilus
peanut worm
sandworm
Satsuma tubeworm
shingle tubeworm
shamisen shell
horseshoe worm
bootlace worm

                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
human *ID1*
human *ID2*
human *ID4*
chimaera *id1*
chimaera *id2*
chimaera *id3*
chimaera *id4*
lancelet
acorn worm
sea urchin *emc*
sea urchin *emc-like*
velvet worm
cactus worm
chiton
sea hare
abalone
owl limpet
oyster
nautilus
peanut worm
sandworm
Satsuma tubeworm
shingle tubeworm
shamisen shell
horseshoe worm
bootlace worm

                                                                                                                                                                                                                                                                                                                                                
human *ID1*
human *ID2*
human *ID4*
chimaera *id1*
chimaera *id2*
chimaera *id3*
chimaera *id4*
lancelet
acorn worm
sea urchin *emc*
sea urchin *emc-like*
velvet worm
cactus worm
chiton
sea hare
abalone
owl limpet
oyster
nautilus
peanut worm
sandworm
Satsuma tubeworm
shingle tubeworm
shamisen shell
horseshoe worm
bootlace worm
```

Figure 22: *Id* enhancer

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                                                                                                                                                                                              
human *LIN28B*      ----------------------------------------------------------cccttaa-ggatacgaggtgaaattagtagcaagaaagcatgtaattgacaaagtcacgtgtgctcagggggccagaaactggagagagg
chimaera *lin28b*   ttatgtgcggtgacaggttggagcagagtgggattggctcggcgctgcctctccgaa-ggctgcgatgtgtaattagcggcaagaaagcatgtaattgacggagtcacgtgcgccag---ggccaggaa----aggagga
chimaera *lin28b-like* ttatgtgcggtgacaggttggagcagagtgggattggctcggcgctgcctctccgaa-ggctgcgatgtgtaattagcggcaagaaagcatgtaattgacggagtcacgtgcgccag-----------------------
lancelet            ttattctcaatgacaggtagcagagccacgctattggctgttgccagggcggccgct-gattggtatgtgtaattagtggcaaagagccatgtaattg--tacgtcacgtgtagcag-----------------------
velvet worm         ---------------------------------attggttgttgctaagataataattccactagtatttgtaattagtgccaaagaagcatgtaattg--tacgtcacgtg---------------------------

human *LIN28B*      agagaaaaaaatcaaaagaaggaaagcacattag
chimaera *lin28b*   aaaaaaaaggggctaaagaggaaaagcacgttag
chimaera *lin28b-like* ----------------------------------
lancelet            ----------------------------------
velvet worm         ----------------------------------
```

Figure 23: *Lin28b* promoter

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human *MEX3B*       ------------tgctttctaac-----------atttgtataaatag-aat--tcagtgcaagttacaaaaattctgttgcaccac-tctagtttt-agtatttcta-ttttaata--catttgtttaccacttgttta
human *MEX3C*       ------------------------------------------------------------------------------------------atcaa-tatagttta-gtatttgctaattttacta---cactct-------tttgtta
human *MEX3D*       ------------------------------------------------------------------------------------------------------ttta-attttttttt-ttttttac---ttttactttta-cctcttg
chimaera *mex3b*    aaggaacagttaagctttctaac-----------atttgtacaaatagaaaa--ccagtggaaattac--aaattctgttgcaccac-tatagttta--gtatttcta-ttttaata--catttgtttaccacttgttta
chimaera *mex3c*    ccg-------------------------------------------------------------------------------------------------------------------------------------------tttgtta
chimaera *mex3d*    ------------------------------------------------------------------------------------gcaccag-tacagattactaaatattca-agtttatc---atttgtttacct-cttactg
lancelet            ------------tgctttctaac-----------atttgtataaaata-gct--acacatgccgatacgataatttcgttgcac----catagtctaggcatgtacta-ttttaata---tattatttacaa-cagttta
acorn worm          ----------------ttctaacatat-tatattatttgtataaatag------caactgactatatggtaaaatccgttgcact---catagctag-aatttacttattttaata---tattattta----cagttta
velvet worm         ------------------------------------------------------------------------------------------------------atagttta-gtattttcta-ttttaata---cattatttacca-cagttac
cactus worm         ----------------------------------atttgtata-----------gtagcgaccgacttgtatgatttgttgcacat--gttagttta--gtatttcta-ttttaata---tattatttacta-ccgtttg
chiton              ----------------------------------atttgtataaatag-ca----tttcaaaatgaccatttttgttgtttcacact-catagttta-gtatttcta-ttttaatatgatattatttaataccagccga
abalone             ---gaagatttaagctttctaacatacatatattatttgtataaatag----------attctcaaagacttaatttgttgcacatc-catagttta-gtatttcta-ttttaata-----ttatttacta-ctctgtg
owl limpet          ------------------------------------------------------------------------------------------gttgcactta-aatagttta-gattttcta-ttttaata--ttattatttactatttgactg
nautilus            aaagcaagattatgctttctaac------attatatttgtataaatag-------------tatctgaaaaaaatttgttgcacaac-catagacta-gtatttcta-ttttaata-----ttatttacgaccagtctg
octopus             ------------------------------------------------------------------------------tttgttgcacaacttagagacta-gtatttcta-ttttaata------tatttacgagctatctg
Satsuma tubeworm    ------------------------------------------------------------------------------------------------------------------------------------------------
shamisen shell      ---------ttatgctttctaatg----catattatttgtatgaatag-------caaaactgacaacaattaatttgttgcacaac-catagttta-gtatttcta-ttttaata--ttattatttatcacaattctg
pale anemone        a--------------------------------tatttgtacaagata-tgtagtcataatttggatacgatattccgttgcactac-tatagttgg-------acta-ttttaata---t-------------------

                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
human *MEX3B*       tgtatatgtaggtgatgttacttgagcttaaatgtacttta--------ctgagcaaagtttaaaaaaca---aagta-----tattttatttt----atgataaag-ggcctttaacct--catggtcaaat--actaa
human *MEX3C*       tgtatatgtagggaagtcatagggattataaattcaattt-----------gagtaaaatttaaaa-----------------ccatatatttt----atgataaag-ggcctttaacttaagatggccaaag-cactga
human *MEX3D*       tgtatatgtagggaatttata---gggaaatatgtacttta--------tggaataaattttaagaacta---aaata-----tattttattttaaataaagtaatg-gacctttaatcttacacagctaaat-tactga
chimaera *mex3b*    tgtatatgtaggtaaagttacttgagcgtaaatgtacttta--------ttgagcaaagttt-aaaaacg---aaata-----tattttatttt----atgataaag-ggcctttaacct--catggtcaaat--actaa
chimaera *mex3c*    tgtatgtgtagggaagtgataaggattataaattcaacctattgagtcgaaacaaaaagtttaaaa-----------------ccatatatttt----atgataaaatggcctttaacttaacgtggccaaag-cactga
chimaera *mex3d*    tgtatatgtagggaatttata-ggactgaaaatgtacttta-------ttgaataaattttaaaaaaaac-taaata-----tattttatttt----aagataatg-gacctttaaccttacatggctaaag-tactga
lancelet            tgtatatctaggtgtgaaaca-tgaccgcaaatatactatg-------gagaaaaagtttactaaataca---aaata-----ttttttatttt----gggcctggt-aaccatg-------tttggccagatatactaa
acorn worm          tgtatatgtaggtgtgagata-tgaccaaaaatg---------------ttgaacaaagtttaatacaaatgcaaatat----ttttttattttgtttggccagct---------tactgaaatggccagatatactaa
velvet worm         tgtgtatattagtatagaact-ttatcaccagca---------------aaaaaatgaatactaaaga---------------tattttatttt-ataaggtccatt-----------tcgttgtggccat---tactaa
cactus worm         tgtatatgtaggtgtga-------------------------------------------------------------------------------------------------------------------------------
chiton              tgtgtatatgtagagaaactc---aatacagatgttgtaaa--------caagtgaaaagtctaaaaaaa---agata-----tttttatttt---caaggccaat-gcaaatg----t--tgtggccagat--actaa
abalone             tatat-tgtagatgttccaca--gattaccaataactcgtc-----------gacaaagtacaagaa------agata------tttttatttt----gaggccaag-caaaata---------tggccgtt---actaa
owl limpet          tatatgattgtagacgtttat---------------------------aaagacaaaataccatataag---atgaaaagatattttatttaataaagaccact-aaataaa---------tggtcgat---actaa
nautilus            tgtatatgtaga------------------------------------------------------------------------------------------------------------------------------acaag
octopus             tgtatatgtaga------------------------------------------------------------------------------------------------------------------------------acaag
Satsuma tubeworm    ------------------------------------------------------------------------------------ttttatttt----atggcaata-tgcagtg---------tggccaaa---actaa
shamisen shell      tgtatgtaggtgtgtgactatggaacccaaagtaaagatta--------agatgcagagtttaaagttaa---atacaaaga-tattttatttt-gttaggctcagc-taataag---------agcctgta--actaa
pale anemone        -------------------------------------------------------------------------------------------------------------------------------------------------

                    miR-374
human *MEX3B*       ---tattatatttgctgagaca---agatttgaaa------------ttgtatcaagagttttatttttctgacattta---------------aagttctacataataaaggtaaaact
human *MEX3C*       ta-ttatatatttgctgtaaag-------------------------agaattataagagtttattttctgatattaa-aagttacttaataaagacttgtttccattaacttgaa---
human *MEX3D*       ---ttatatatttgctgagctgatttaagggttaa-------aaaaattgtatcaagagttttatttttgacttcaaa--gccttcttaataaagcctcttttctacatgt--------
chimaera *mex3b*    ---tattatatttgctgagaca---agaattgaaa------------ttgtatcaagagttttatttttctgacattta---------------aagttctacataataaaggtaaaact
chimaera *mex3c*    ta-ttatatatttgctgtaaag-------------------------agaattataagagtttattttctgatattaaaaagttacttaataaagac-cgtttccattaaagtgaa---
chimaera *mex3d*    ta----tatatttgctgaactgacttgatggtatg--------aaattgtattaagagtttattttttgacttaaaaaaaccttcttaataaagactttttacagtatgt--------
lancelet            tattattatatttgttgagact--tgaggattata------------aaattgtaagagtttattttctgccattaa---------------agttgtattacaataaatgacaagct
acorn worm          ---tattatatttgccgagatt---tgagattatt----atggaattgttattaaaaagtttattttctgtcattaa---------------agtaaaagttct--------------
velvet worm         ---tattatataaactaagaga-----------------------tatacaatttaaagtttattttctgccagta-----------------------------------------
cactus worm         ------------------------------------------------------------------------------------------------------------------------
chiton              ---tattatatttgctgaactc---aaatattata-------------aatgtaaaaggttttattttctggcattaa---------------aa----------------------
abalone             ---tattatatttgctgaaact---cagaagtata-------------agatgtaagagtttattttctgccattaa---------------agtcaagttccaa-------------
owl limpet          tattattatatttgccggaact---caattataag-------------agatgtaaga-ttttattttctgccattaa---------------agtaaa--------------------
nautilus            t-----------------------------------------------------------------------------------------------------------------------
octopus             t-----------------------------------------------------------------------------------------------------------------------
Satsuma tubeworm    tgttattatatttgctggaccc---agaattttaa-------------gattgtaagagtttattttctgccattaa---------------aaat----------------------
shamisen shell      ---tattatatttgtggagagt---aagaaaaaaagtataaaaaaaagaattgtaagagtttattttctggcattaata-----------aaagttgtatttcaa-------------
pale anemone        ------------------------------------------------------------------------------------------------------------------------
```

Figure 24: *Mex3* 3′-UTR. MRDE: previously predicted Mex3b-responsive destabilizing element [38]. MRE: resembles a MEX-3–recognition element [39]. miR-374: previously predicted miRNA binding site [40].

```
                                                   GLI___________                 RFX______________                                                  CC
human PTCH1     gaggctgccc-----cgaagccgaggatgcacacactgggttgcctacctgggtggtctc-tctac-tttggtgagctgttgctaagcagctaataatag---tcatgtttgctgagtaatttctgccct-ccgcgagcc
chimaera ptch1  --------------------------------tggatgcactcacagcctgggtggtctc-tctac-tttggtgagctgttgctaagcagctaataatag---tcgtgtttgctgagtaatttctgcct--tcctcggcc
chimaera ptch2  --------------------cccacccccccattgatgctctggcagtctgggtggtcctggccac-tttggtgagctgttgcttagcagctaataatagt--agatgtttgctaagtaatttgcttcttccctttagcc
lancelet        gaaggcgcctggcggtggaggatggatgtgtcagggtacccctacgacttgtgtggtctg-tcttc-tttggcgagccgtttcctagcagcaaatgacggg--ccgtgttactcgagcaaattgtggag--cccgcggcc
acorn worm      ----------------------tcctctttttatcctatcccaacttcttgtgtggtcta-cctctttttctggtgacgttactaagcaactaatgagaac--tcgtgttacatgagtcatttggccg---tgagtgacc
scorpion        ---------------------------cctctttggtggctaggcaacgtgtgtggtccggacgac-gtcggtgatccgttgcctagcaactaat-------------------------------------------
horseshoe crab  ----------------------------gccacagcgtccccgcgacgtgtgtggtcttctccac-tttagcgaaccgttgcttggcaactaatgcaaggttcgctgttacgagaggagctagaaag---ccacgagcc
horseshoe crab  --------------------cccgcattctcaacagcgtccccgcgatgtgtgtggtcttctatac-tttagtaagccgttgcttggcaactaatacaaggctcgctattacgagaggagctagaaag---ccacgagcc
chiton          -------------------------ttccctcttcgacctaccgcaatctgggtggtctt-tctac-tttggtgagccgttgcctggcaactaatgtgcagc-tgatgttacttgagcaatctgcgcta--taattgacc
sea hare        ----------------------------------------tcacttcttgggtggtcta-tctac-ttgggtgagctgttgctaagcaactaatgttgggc-tggtgttacacgagcaatcctactga--taattgacc
abalone         --------------------------------tgggttcccttccttcttgggtggtctg--ctac-ctgggggagctgttgccaggcaactaatgttgggc-tggtgttacacgagcaatcctactga--taattgacc
oyster          --------------------cttatcgcactgttggcggtcccgcaacttgggtggccgagcttctatgtggtgaacggttgcttagcaactaatg-----------------------------------------
shamisen shell  ----------------------------ccgtttgttcctggccagcttgggtggtctt-tctac-tttggtgagctgttgctaggcaactaatgtcgggc-tgcagttacatgagctgcctgtgggc--ttaagaacc

                AAT
human PTCH1     aatcgcgtcgcagaaacccaagccatctgaca------------gccccgggggcggga----g
chimaera ptch1  aatgagatcccagcaatacaagccttctcccagccatctgacg-gccctggaggcgggtttt-g
chimaera ptch2  aatgggaatccggtcgagaggactttccatcaactctatctgatgggctggaggcgggtt----
lancelet        aatcagagcgcgggaaggtgacccctcgatcacgggagctcga-ggcggagaggcgggtttgt
acorn worm      aatcagattgaagaaaattgacccttctgtggggcc-gtttga-caggaggggcgtggtt---
scorpion        ------------------------------------------------------------
horseshoe crab  aatcgaagcg-----------------------------------------------------
horseshoe crab  aatcgcagcg-----------------------------------------------------
chiton          aatcagagcgctgatctgtttcccatcaatcatg------------------------------
sea hare        aatcag--------------------------------------------------------
abalone         aatcag--------------------------------------------------------
oyster          ------------------------------------------------------------
shamisen shell  aatcatcgccgcgctctgcgcgccatcaatcacgtc-accgca-ttcccgggggcgggccttgt
```

Figure 25: *Ptch* promoter

```
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                
human ----------aaggggcggagctttggcg----------tgggcggccaatagt----------gggggt--ggctgcgtgggtcgccatggggacgggg---ctgttcccggggaggctgtg-
chimaera ----------aacggggcggcgccggctgc----------ggcgcgggccaatgag----------ggcggc--ccgtcgtttggtcgccatggtgacgggg---ttgttcccggga----cctg-
lancelet ----gtgggttttgaaaattatagcttcaagtatgggctgtctcccgccaaacaaatggaccaatcagcgaagaccttttgacaag--tcatcatcgtgtttccatggttaccggg---tcgggctgggg-----cttg-
acorn worm tcacgtgatgcaatgctaagttagtttctagatc--tccataccaattactgcgaactagccaatcaggatacactcttcgacgggtcatcaactgcagtttctatggtaacgggg---tcggactcggc-----cttg-
sea urchin ----------agtttctagaat--ccctgaattaat-cagtgcttcgaccaatcaacgtcgcgtactgggcagt--tcacgatgtagtcgtcatagctacgggg---tcagtctctgc-----cttg-
centipede ----------tc--cccttagcaacc-acaccgaacgaccaatgagcgtgcgggctttgacagg---tccattgacgtttctatggtaaccggg---tcagtctcaac-----cttg-
millipede ----------caccacaatgtttctatagtaacggagtttttggtgtcgtc-----cttg-
scorpion ----------atcaattaatgtttctatggtaacaggg---tcggtgtcgtc-----ctga-
scorpion ----------ccaatcacatagccgcatttgacaga-tcccaattgatgttactatggcaacaggg---tcggtgtcgtc-----ctga-
tick ----------ccaatcacaaggcagtctttgacag---atcaattgatgtttctatggtaacatgg---tcggtgtcgtc-----ctga-
horseshoe crab ----------ccaatcacgttactgcctctgacag---atcaattgacgtttctatggtaacaagg---tcggtatcgtc-----gtat-
horseshoe crab ----------atgaactgatgtttctatggtaacaagg---acaaaatcatc-----gtga-
horseshoe crab ----------atgaactaatgtttctatggtaacaagg---agaaaatcatc-----gtga-
horseshoe crab ----------ccaatcacacagctgcttcccacag---atcaattgatgtttctatggtaacgagg---tcggtgtcgtc-----gtgt-
velvet worm ----------atgggccaatcacatttgagcttttgacag---gtcgattgatgttgtcatggaaacagg---tcggtgtcgtc-----cttg-
chiton ----------c--cccacaactactataattaaacatccaatcgcaatgtgccatttgacagg--tctacttgacgttgtcacggtaacgagg---tcagtgtcgtc-----cttg-
sea hare ----------ccaatcaacgcgaagcgactgacagc--cgcagttgatgttggcaagacaacgggg---tcagtgtcgtc-----cttg-
abalone ----------aactatccaatagagacttgccatttgacagg--tctacttgatgttggcaaggcaacgggg---tcagtgtcgtc-----cttg-
owl limpet ----------aaccgaccaatcgagactagctatttgacagg--tctacttgatgttggcaaggcaacgggg---tcagtgtcgtc-----cctg-
oyster ----------agttggtttctagatc--gcagattctgctataattaacgaactaatcgtagaacgcctttcttcagt--tctacttgtggttgctatagtgacgggg---tcagtgtcgtc-----cttg-
nautilus -cacatgatgttagaccggaccgttttaaggcac--tccaaacttactataattaactacccaatcgtaagttgcattttgacagg--tctacttgatgttggcaaggcaacgggg---tcagtgtcgtc-----cttg-
octopus ----------aactacccaatcgtaacttgccttttgacagc--tctccttgacgttggcaaggcaacgggg---tcagtgtcgtc-----cttg-
peanut worm ----------acccagtgacgtacccatggaaacgagg---tcagtgtcgtc-----cttg-
sandworm ----------cctactcagcggctcagccaatcggagcgcttctcatgacag---ttctcttgatgtcgtcatggtgacgagg---tcagtgtcgtc-----cttg-
Satsuma tubeworm tcatgtgatattttgactcatgagtttctagtctc-cccacatctactaaaattactccaccaatcgtattgctccgtttgacaag-tctcattagatgtcgccatggtgatgagg---tcagtgtcatc-----cttg-
shingle tubeworm ----------ttcgacagg--tctttctgacgtcgctatggaaacaagg---tcagtgtcgtc-----cgtg-
shamisen shell ----gtgatgtttgaacacgacagtttcaaggct--caccaatcctccgtaataactcgcccaatccaagattgctatttgacagg--tccttgtgacgtctctatgacgacgagg---tcagtgtcgtc-----cttga
horseshoe worm ----------catg--tggtgatgacgtcactatagcaacaagg---tcagtgtcatc-----ctta-
bootlace worm ----------tacccaatcgaaaggcgctatttgacagg--tcttcttgacgtcaccatagagacgggg---tcagtgtcgtc---ctcttg-

human --at-gggttgacaggtg-cgtgacagt----------
chimaera --at-ggcttgacaggtg-cgtgacaatccagctcttca--
lancelet --at-gggttgacaggtg-cgtgacagtccagctatccagc
acorn worm --at-ggcttgacaggtg-cgtgacagttcagctacccagc
sea urchin --at-tgcttgacaggtg-cgtgtcagtatagctacccagc
centipede --atggcttgacaggttgtctgacatttc----------
millipede --at-ggcttgacaggtg-cgtgacagttc----------
scorpion --at-gtcttgacaggtg-cgtgacagtttaacttgacagc
scorpion --at-gtcttgacaggtg-cgtgacagtttaacttcacagc
tick --at-gtcttgacaggtg-tgtgactgttcaaatccacag-
horseshoe crab --at-gtcttgacaggtg-cgtgacagttcagcctcacagc
horseshoe crab --at-gtcttgacaggtg-tgtgacagtttaac--------
horseshoe crab --at-gtcttgacaggtg-tgtgacagttcaac--------
horseshoe crab --at-gtcttgtcaggtg-cgtgacacttcggccacacag-
velvet worm --at-ggcttgacaggtg-cgtgacagttcagctccacagc
chiton --at-ggcttgacaggtg-catgacagttcagct-------
sea hare --at-ggcttgacaggtg-cgtgacagttcagctct-----
abalone --at-ggcttgacaggtg-cgtgacagttcagctctc----
owl limpet --at-gtcttgacaggtg-cctgcgagt----------
oyster --at-atgttgacaggtg-cgtgacagttcagctccc----
nautilus --at-ggcttgacaggtg-cgtgacagttcagct-------
octopus --at-ggcttgacaggtgccgtgacacttcagctac-----
peanut worm --at-ggcttgacaggtg-cgtgacaatccaact-------
sandworm --at-ggcttgacaggtg-cgtgacagtttcagctc-----
Satsuma tubeworm --at-ggcttgacaggtg-cgtgacagttcagc--------
shingle tubeworm --aa-gcattgacaggtg-catgacagttcagctgc-----
shamisen shell tcat-ggtttgacaggtg-cgtgacagtcagctccc-----
horseshoe worm --at-gggttgacaggtg-cgtgacagttcggc--------
bootlace worm --at-ggcttgacaggtg-cgtgacagttcagct-------
```

Figure 26: *Ssbp2* promoter. This matches two places in the scorpion genome and four places in horseshoe crab, all upstream of genes annotated as "Ssbp3-like".

```
human ZNF503        ----------------------------------------------------------------------------------------------------------------ctcttttccctcaagtcgca
chimaera znf503     ---------------------------------------cgtccgccttttgttcgcatacaaattgagtttactctgagaacgatttatttccctatattgaatgcctcttttcaaa-agccgttctttttcaagtcaca
lancelet            aaaggggcacaaattgccgcctacttacag----tgtttcggcgttcttttgttcgc-cgccaaacgggtttattt--ccaacgattta-tatcaccaattcaatgacgcttttcaaagcctttctttttttcaaatcaca
acorn worm          ----------------------------------------------gactccctttgttgtcggacaaattgagctcacac--agaccgatttactttcggcaattcactggcctttcagaa------aactttttcaaattacc
sea urchin znf503   ---------------------------------------cggtctctttgttgtcacacattaattgcgattatt---gggccgatttatctcggggaatcataccccctttcaaaag------ccgtttttcaaattact
sea urchin znf503-like ------------------------------------cggtctctttgttgtcacacattaattgcgattatt---gggccgatttatctcggggaatcataccccctttcaaaag------ccgtttttcaaattact
millipede           ----------------------------------------------------------------------------------------------------------------------------------------
scorpion            ----------------------------------------------------------------------------------------------------------------------------------------
scorpion            ----------------------------------------------------------------------------------------------------------------------------------------
scorpion            ----------------------------------------------------------------------------------------------------------------------------------------
tick                ----------------------------------------------------------------------------------------------------------------------------------------
horseshoe crab      ----------------------------------------------------------------------------------------------------------------------------------------cg
horseshoe crab      ----------------------------------------------------------------------------------------------------------------------------------------
velvet worm         ------------------------------------------------------------cgcaaattgcgtttattt--tgaacgattag-tgtgaccaattcaatgtgtttt------------attctttatttgctcgg
cactus worm         ----------------------------------------------------------------------------------------------------------------------------------------
abalone             ----------------------------------------------------------------------------------------------------------------------------------------
shamisen shell      agacggatacaaacttccatct-cctacaggatcggcttcaaattctttgttcacgcacaaattgcgtatactt--tcagcgattag-aattaccaattccgtgtggttt------------atcctttatttgcctct

                                                MEIS_________
                                     MafF_____________        PKNOX___________        MEIS_________                NFI______________
human ZNF503        cccgc-cttg---taatcagcaacaaaa-gctgacataaatcacgggaggattgacaagagctgaca-aataactgattgattg-cggtcgaggaacattgacaattgatggaggggtggagatgcccaaagaaggggggg
chimaera znf503     ccagg-cttg---taatcagcaacaaaa-gctgacataaatca-acgcagattgaca-gggctgaca-aataactgattgattg-cggtcggggaa-attgacagtctggggaggggaagg-------aggggaggggag
lancelet            ccggaccatg---taatcaacaacaaaa-ggtgacatgaatca-gccaggattgaca-gccttgaca-ggagattgattgatgg-gggtctggat--atcgacggatggcgacatgccagg--tgattaa----------
acorn worm          ctagc-catg---taatcgcctacaaag-ggtgacataaatca-gacgcgattgaca-gccatcacg-ttggaatgattgatta-cgct-------------------------------------------------
sea urchin znf503   cgagc-catg---ttatcaacaacaaaacgctgacataaatca-gacggaattgaca-aggctgacaccgcatttgattgatcg-cag-------------------------------------------------
sea urchin znf503-like cgagc-catg---ttatcaacaacaaaacgctgacataaatca-gacggaattgaca-aggctgacaccgcatttgattgatcg-cag-------------------------------------------------
millipede           -----------------cgacaacaaaa-ggtgacataaatca-agtatgattgacgtgccgtgaca-ggcgcttgatggatga-ggtcct-------------------------------------------------
scorpion            -------------------acaacaaaa-gctgacataaatca-accttgattggca-cttgtgaca-ggtgtgtgatagatgg-cgctgtaaac--catgacggctggcggcttgccaag--tcattag----------
scorpion            -------------------acaacaaaa-gctgacataaatca-accttgattggca-cttgtgaca-ggtgtgtgatagatgg-cgctgtaaac--catgacggctggcggcttgccaag--tcattag----------
scorpion            ----------------ttgacaacaaaa-gctgacataaatca-actgtgattggca-cttgtgaca-gccgcttgatggatcg-ccttgtagcc--tgcgacgcctggcatcttgccaag--tcatta----------
tick                -------------------acaacaaaa-gctgacataaatca-tgcctgattggca-gctctgaca-acgggctgatggatag-cgttgtaaaa--gttgacagctggccccacgccaga--tcatta----------
horseshoe crab      tcgcactgta---ggcccgacaacacaa-cctgacataaatca-actgtgattgaca-cttctgaca-gatccttgatggatgg-tgttgcaaag--actgacaactggcggcttgccaaa--tcatta----------
horseshoe crab      ----------------tcaagcactaag-cctaacataaatca-gttcctactgaca-ctcttgact-ctcgactgatggatga-cgttgtaaac--cttgacagctggcactttgccaag------------------
velvet worm         ctatataatgtatacattaacaacaaaa-ggtgacataaatca-acgttgattgaca-gagctgaca-ggcgattgattgatcg-tagtctgaag--cgtgacggctggcggcttgccaag--ctatta----------
cactus worm         ----------------attactaacaaag-ggtgacataaatca-gttgtgattgaca-gccgacgca-ttgaagtgattgatgg-cg-------------------------------------------------
abalone             ----------------attaacagcgcgc-ggtgacataaatca-agcttgattgacg-gacaggcca-tcaaaatgattgattgttggggcatga--ggtgacgtctggctagctgccaag--taatta----------
shamisen shell      cttca-agtgtatacacgaagaactaaa-gctgacataaatca-agcatgattgacg-tgcggctca-tcaacttgattgatca-cagtcggtgc--aa-------------------------------------
```

```
human ZNF503        gagggaggaaa
chimaera znf503     ggcagtggaaa
lancelet            -----aatgtc
acorn worm          -----------
sea urchin znf503   -----------
sea urchin znf503-like -------
millipede           -----------
scorpion            -----tacatc
scorpion            -----tacatc
scorpion            -----------
tick                -----------
horseshoe crab      -----------
horseshoe crab      -----------
velvet worm         -----------
cactus worm         -----------
abalone             -----------
shamisen shell      -----------
```

Figure 27: *Znf503* enhancer. This matches three places in the scorpion genome, two upstream of genes annotated as "Noc-like", and two places in horseshoe crab upstream of Noc-like annotations. (*noc* is an insect homolog of *Znf503/703*.)

```
                                                                                GC-box_
                                                                     EGR_____________                                                  DUX__________          ALX____
human ZNF503        gg---cctgacctgtcaggcggatta-----tcttcgagggagattaataggggaggcgggctgcaataataatcgttttgtttgatgtgacaactctgatagg-cgttgatttacttacaaactgataggcttttaatt
human ZNF703        tga-ccctgagccgtcaagaag-----------gaacagccagattaatggggtgggcgggctgggtaataatcg-tttgtttgatgtgacaagcctgatagg-cgttgatttacttacagactgatgggcttttaatt
chimaera znf503     ga---cctgacctgtcaagcggatta-----tcatacagagagattaatgggtgggcgggttgcaataataatcgttttgtttgatgtgacaagtctgatagg-cgttgatttacttacaaactgataggcttttaatt
chimaera znf703     tgaccccgagactgtcaagcga-----------agtcccggagattaatggggtgggcgggttaaagcaataatta-tttgtttgatgtgacaggcttgatggg-cattgatttagttgcggactgataggcttttaatt
lancelet            ta----taaacgtgtcagtaggattaccgaatgatggagagtgataaatggggtgggcggtctatgataataatcattttgtttgatctgacacttttgatagg-cgttgatttactttaaaactgataggcttttaatt
acorn worm          ------------------------------------------ataaatagtatgggcgtacatgggtaataatcttattgtccacgttgacggtttgattga-cgttagtttact---------acgtcgttttcata
velvet worm         ta----taaacgcgtcagccacttcactgattgatgcaagcagataaataggataggcggggcagccaaacaatcattctgtttgatttgacacgtctgatagaccgttaatttact----------------------
shamisen shell      ------------------------------------caagtagattgatggagaaggcggggtgtctaaacagttcccttgtaccacttgacacgtctgatagg-gcttaatttacttaaagcct--ctgcgttccgatt

                    ---
human ZNF503        gagcgcctccg-ccgagcccgagatgaaagggaagcggcg-----------------------------------------gtg
human ZNF703        gagcacgccat-cctagtc--acttcaaag-------------------------------------------------------
chimaera znf503     gagcgcctacatccaagttttagatgaaaggaaagcggcg-----------------------------------------gcg
chimaera znf703     gagaacctccaattccgaggcacatcaaag-------------------------------------------------------
lancelet            gagcgcctaag-gagcggc-gacttcaaacaagtcgaggacg--taagctgtctctttggcggggcaacaaaacaatttccc
acorn worm          gcacaccgccg--agcggc-gacttcaaagaaatactccccactatagctgtct---------gagcaacaaaacaatttgcc
velvet worm         ------------------------------------------------------------------------------------
shamisen shell      actatcaaaacacggcggccaacatcaaaggcgtcctgcgtagcctaccgtctcgtttgggcaagggaacgtaacaatttgcc
```

Figure 28: *Znf503/703* enhancer

```
human       caggttggcagatctggagggctaatccaaacaa----ggcgcttgacttgtgaggtc-gcgagtctagacagagctggtggctcggcggctccttatctttccctggaatcagccgaagag
chimaera    caggttggtgtatctctgagcctaatcaaaacaa-------gtttgacttgtgaggtcctcacgtctagacagagctggactctcggcggctccttatctgagaacggaatcagcagagagg
acorn worm  ------------------------aatcaaaacagttttgtttttgacttgtgaggtctgtacgtctagacagaaagcgtcgcaaggcggcggcttatctcccttcggcatcaaactaa---
```

Figure 29: *Isl1* enhancer

```
human       gtctttaatgtaccattaactattgtctttacacaatatgggaactgt---aaagcatgacatgtgttataataaaacaca-ttttcaatg-atacacttggacttgagg-ctggggtgc-agcaaacaaaca
chimaera    gtctttaatgtaccattaactattgtctttgcacaatatgggaactgt---aaagcatgacatgtgttataataaaacaca-ttttcaatt-atacacttgtactggaag-ctctgatgcaagcaaacaaaca
acorn worm  gcctttaatgttctctgggcctttgtacgttcacaatgtgggcacact---agagaatgacgtgtctcggataaaaacacacttttcaatt-atctagttatagtgtagg-ctgtgaagcaaacaaacaatca
sea urchin  --------------------ccattgtcccttgacaatgtatgcacagctcgtgatgatgacgtctgccccataaaaacacacttttccattggtctaggtttcctgtaagcctttgaagtaaaca--------
```

Figure 30: *Ptf1a* enhancer

```
human *SIX1*   -agacttgtccccctggcatctcaggttaa--agaaggaggtccaggaaaatgt-ggtagagccattacaagaaacccgagccgcagtcatca--------
chimaera *six1* tgaggtcggccctccagcttctcgggttaa--aggaggcgggcctgcaaaatgt-ggtagagccattacaagaaacccgagatccattcatcatgcactca
chimaera *six2* --ggtcgaccccaccagcctctcgggttaggtagtgggagg-ttgtcaaaatacaagagcagccattagtagaaacgtgatatctgttcatca--------
lancelet       -agggtcggccctctgccggctcgggttgt--agggggagg-tttagaaaatat-ggtatagtcattaagcgaaatttgataccccattcatcatgcgc---
acorn worm     tcaggtcagccctctcgcgtctcgggttga--agtaggagg-tttaagaaatat-ggcatagtcattaagggtaattcgataccccattcacgatctagtca
```

Figure 31: *Six1/2* enhancer

```
human *SP8*   gaggccgcagattggctggcc--gcgcctccgcggcacttcaaagccggagagggagctacaattg--tggtggaat-gggcggaactgatcatt-gtattgcacacct
chimaera *sp8* -----ccctgattggctgccg--gggtctgagcggtacttcaaaggcgagagaggcgctacaattg--cggttgaatggggcggaactgatcatt-gtgttgcacacct
chimaera *sp9* -------ctgattggccgagggctcggcacagcggcacttcaaagcccaca--cccgctacaatta--gggtcgagt------------------------------cg
lancelet      gagctcgctgattggtcgagag-gggcctgagcgggccttcaaagggaagc--cacaccacaattatatggtcaaat-gggcggaaccgaccatttgtattgtacacct
acorn worm    --------tcattggtggatca-gccgataggcggctcttcaaagcccttt-gcaagcggcaattatcgggccaaat-gggcggacctggtcatttgcat---------
```

Figure 32: *Sp8/9* promoter

```
human *ZFP36L1* gcttgt---------------------------cactgcacat-caatataaaaaa---gcttatttaacttatcaaaacgtattt-attgccaaactatgcttt-----tttttgttaattttgt------tcatattt
human *ZFP36L2* tttttagctgttttgagttgattcgcaccactgcaccacaact-caatatgaa------aactatttaactta------tttatta---tcttgtgaaaagtata------caatgaaaattttgt-tcatactgtattt
chimaera *zfp36l1* gcttct---------------------------cactgcacacacaacacaaaaaaattgcttatttaacttatc-gaacatattt-attgccaaac--------------atatgcatttttttgt------ttgtattt
chimaera *zfp36l2* tttttagctgttgagtttttgatggcccactggtggcccagct-cgctataa-------aactatttaactta------tttatta---tctcg-agaaagtata------cattggtaa-tttgt-tcatactgtattt
lancelet       tattta-------------------------------------------------------acttatgtgactta------tttatttgaaccttatacgtaacgttatgcatttttggtaaatttattatgctcgttatgt
acorn worm     ------------------------------------------------------------tatttaagttaacaggccttattt---atttgaagtaagcatt------ttttggtaaatttataatgcctcctattt

human *ZFP36L1* atcgggatgacaaat---ccataga-atatatt---cttttatg-ttaaattatgatcttca-tattaatcttaaaattttgtgacgtgtctttttcct----------------------------------------
human *ZFP36L2* atcaagtatgatgaaaagcaatagatatatattcttttattatg-ttaaattatgattgccattattaatcggcaaaatgtggagtgtatgttcttttcacagtaatatatgcc---------------------ttttgt
chimaera *zfp36l1* atcaaaaatgatgaa---ccataga-gtatatt-ttcttttatgtttaaattatgatcttc----ttaatcttaagattatgaggtcctttttttggtc-----------------------------------------
chimaera *zfp36l2* atcgcgtatgatgaaaa-caatagatatatattcttttattatgtttaaattatgattgccattattaatctgcagaatgtggagtgtatgttgttttcacagtaatatatgcctttttttgttgggtttttttgttttgt
lancelet       acgaga--cgatgagag-tgatagatatttatt---atattatgtttaatttatgattgtt--tgtgaatcacaggattggagtgtgtgtactatgtgtatggtaatatgtg----------------------------
acorn worm     atcgggttccatgtggg-acatagatatatatt-ataaactttg-ttaaattatgattgtgaattgaaatggacagagactggagtgt-------------------------------------------------

human *ZFP36L1* ----------------tttttt
human *ZFP36L2* aacttcac-ttggttattttat
chimaera *zfp36l1* ----------------tttttt
chimaera *zfp36l2* aacttcactttggttattttat
lancelet       ----------------------
acorn worm     ----------------------
```

Figure 33: *Zfp36l* 3′-UTR

```
human EBF1 gggcccggtatggatgattaggtt------tcgggctttgaattgttgacaaggcaggggtaatgaattgcgggacactggctcctgcatttccccctcattagtcagtattagctacattaatagtaataatt------
human EBF3 ------------------------------------------------------------------------------------cacatgtgcccctcattaagctgcattacctgcattaaaactaataattgccact
chimaera ebf1 ggcccagccgaggatgattagctttgtggatttgactgtgatttgttgacaaggcagagg-gatgaattgtgggac--ttgagcaagtgtgtgactctcattattcagtattagccgcattaatagtaataatt------
chimaera ebf3 ------------------------------------------------------------------------------------ttgcacacatgtgcccctctcattaagctgcattacctgcattaaaactaataattgccact
lancelet ------------------------------------------------------------------------------------ttgcaggcatgcctctcattaagttacatcacatgcattagaagtaatcattcctgct

human EBF1 ---------ggatatattcatcatttaaaatgttttaataaatgtttggacagtacccgatcagagctgtcaaa-------
human EBF3 cagagctgtgcgcccattaatcatttataatgttttaataagtttttaatcaggacctaa--aaggctagagagccgcata
chimaera ebf1 ---------acatgaattaatcatttataatgttttaataaatgtttggtcagcacctgatctacactgtcaaa-------
chimaera ebf3 cagctctgtgctcacattaatcatttataatgttttaataagtttttaatcaggatttga--aaggctagaaagct-----
lancelet cacctccaagcccccattaatgatttaccaggtcctaatcaggttttgatgggagtgtac--ggggctagcgagctgtgca
```

Figure 34: *Coe* intron element 2

```
human IRX1 ---ttttctacttgaacatgaaataatgatttttgctcatataattacaaagctaacatctgatagagtcagcatccagggggattatcgcatgcatgagtattagacctcattaccagcttgactgcaaaattatta
human IRX3 tcattttctatttgaacatgaaataatgattttctgcccgtctaattacaaagccaacatctgatcaagccagcatccagaggggattatcgcatgcacgagtattagacctcattaccagcttgagtgcaaaattatta
human IRX4 tcatttttctgctgaacatgaaataatgattttctgccagtataattacaaagctaacatctgatagagtcagcatccagggggattatcacaggcatgagtattagacctcattaccagcttgactgcaaaattatta
human IRX6 ----ttttttcctgaacatgaaataatgattttctgcccgtctaattacaaagccaacatctgatcaagccagcatccagaggggattatcgcatgcacgagtattagacctcattaccagcttgagtgcaaaattatta
chimaera irx1 ----tttctacttgaacatgaaataatgatttttgctcatataattacaaagctaacatctgatagagtcagcatccagggggattatcacatgcatgagtattagacctcattaccagctcgactgcaaaattatta
chimaera irx3 tcattttctatttgaacatgaaataatgattttctgccagtctaattacaaagccaacatctgatcaagccggcatccagaggggattatcacatgcacgagtattagacctcattaccagctcgagtgcaaaattatta
chimaera irx4 ---ttttttgctgaacatgaaataatgattttctgccagtataattacaaagctaacatctgatagagtcagcatccagggggattatcacatgcatgagtattagacctcattaccagcttgactgcaaaattatta
chimaera irx6 ----ttttttcctgaacatgaaataatgattttctgccagtctaattacaaagccaacatctgatcaagccggcatccagaggggattatcacatgcacgagtattagacctcattaccagcttgagtgcaaaattatta
lancelet IrxA ------------------------------------------------------------------------------------agaattataactaattataagcatgactgccacaatatta
lancelet IrxC ------------------------------------------------------------------------------------agaattataactaattgtaagcatgactgccacaatatta

human IRX1 catcttgtaattggatggtgag--------atttatatacagattgg----ccgggtt-tctgtaagattgtaattacaagcttcattggtttgctacttatcgga-acttcacagaga----aaacaactggtaaaatg
human IRX3 catcttgtaattggatggtgag--------atttatatacagatcgg----cccggtt-tctgtaagattgtaattacaagcttcgttggttagctacttatcagaactttgcaggaaaaca-aaacaaatgactgagga
human IRX4 catcttgtaattggagggtgag--------atttatatacagattgg----ccgggtt-tctgtaagattgtaattacaagcttcgttggtttgctacttatcaca-cggtgcaggaggcggc--aaaaacaagc-agag
human IRX6 catcttgtaattggatggtgag--------atttatatacagatcgg----cccggtt-tctgtaagattgtaattacaagcttcgttggttagctacttatcact-cagcacagaagag---aggaaaataagc-aaca
chimaera irx1 catcttgtaattggatggcgag--------atttatatacagattgg----ccaggtt-tctgtaagattgtaattacaagcttcattggtttgctacttatcgga-acttcacagaga----aaacaactggtaaaatg
chimaera irx3 catcttgtaattggatggtgag--------atttatatacagatcgg----cctggtt-tctgtaagattgtaattacaagcttcgttggttagctacttatcagaactttgcaggaaaaca-aaacaaatgactgagca
chimaera irx4 catcttgtaattggatggcgag--------atttatatacagattgg----cccggttctctgtaagattgtaattacaagcttcgttggtttgctacttatcaca-cagtgcaggaggaggcaaaaaaacaagc-agag
chimaera irx6 catcttgtaattggatggtgag--------atttatatacagatcgg----cctggtt-tctgtaagattgtaattacaagcttcgttggttagctacttatcaca-cagcacaggaga----aaaaaaataagc-aaca
lancelet IrxA caactgataattggaaggttggatggcttcatttgcatgtagagctgagacctgggtt-cccaccaga---caattatagctttggttagagggagcctcttggct-ccatgcaggatgctgctaca---------cacc
lancelet IrxC catctgataattggaaggttggatggcttcatttgcatgtagagctgagacctgggtt-cccaccaga---caattatagctttggttagagggagcctcttggct-caatgcaggatgctgctaca---------cacc

human IRX1 aaatgagcatttcattaacctattgccttatcatgggaaaacctgtgtaacagccgtgtatgcattaacataaatag---------
human IRX3 aaacgagcatttcattaacctgtaacccgagcgcgaggg-----------------------------------------------
human IRX4 ggaaggccatttcattaagctgtaaccctatcaagggaggagcgctgtaacgtccctgtatttattaggccaaacag---------
human IRX6 gaaaaggcatttcattaacccataacccctatcaagggaaagccactgtaacacccatgtctgcattagggagaacagctaaattca
chimaera irx1 aaatgagcatttcattaacctatcgccttatcatgggaaaacctgtgtaacgttcatgtatgcattaacataaatag---------
chimaera irx3 aaatgagcatttcattaacctgtaaccctatcatgggggaacctgtgtaacattcatgtatgcattaaggaaaatgggttcatcca
chimaera irx4 gaaagaccatttcattaacctgtaaccctatcaagggaggagcactgtaacatccctgtatttattaggccaaacag---------
chimaera irx6 gaaagagcatttcattaacccgtaaccctatcaagggagaactgctgtaacatccatgtttgcattagggagaacag---------
lancelet IrxA acaagaccactttattaacc----------------------------------------------------------------
lancelet IrxC acaagaccactttattaacc----------------------------------------------------------------
```

Figure 35: *Irx* enhancer

```
human NR2F1 ----------------------aaacaagaattaggggaaaataacattttccaaataattataaaaaattgtcctgtgtctatgtatctatatctg---ttttgta-tttttttctggttccaaaccagatt-tcctgt
human NR2F2 ------------------------------------------------ttttccaaattatt------aattgtcctgtgtctatgtacctctagctgttctttttg-tactttctggttccaaaccagtttattctgt
chimaera nr2f1 gcgcatttaaaagacaga---atcaagagattaggggaaaataacattttccaaataattat---aaattgtcctgtgtctatgtatctatatctg---ttttgtattttttttctggttcaaaaccaggtt-tcgtgt
chimaera nr2f2 gtgcatttaaacaagaatttcaaaattaggaggaaagaaaagtaacattttccaaattattat---aaattgtcctgtgtctatgtatctatagctg---ttttgta-tttttcctggttccaaaccagtctcctctgt
lancelet ------------------------------------------------------------------------tgtgtctatgtatctt--gctg---ttttata-----tacatggttccaaaccaaacg-ttaagt

human NR2F1 gatt-ctatactaataatttttgatataaccctttgcttcttataatgagtgcgatatatgttgtcgaggc--tgttcttcaagaattaaaattgaagtga----aaatttaaacaaaaa
human NR2F2 ggtt-ctataata--ag-ttttgatataatc-ttggcttctt-----aaaaactgtgtatcattaaaatat-atgttctgcaagaattaaaactgagtccatgaaaataccataggaaga
chimaera nr2f1 gattcctatactaataatttttgatataaccttttgcttcttataatgagtgagatttatgtcgtcaaagctatgttctccaagaattaaaattgaaatca--------------------
chimaera nr2f2 gatt-ctctactaataa-ttttgatataacc-attgcttcttat--cagatacggtgtatcacgtaaat---atgttctgcaagaattaaaactgaattcactaaaatgttagataaaaa
lancelet gatt-tactaataataa-ttttgatataacc-------------------------------------------------------------------------------------------
```

Figure 36: *Nr2f* 3′-UTR