Supplementary figure legends

Supplementary Fig. S1. Phylogenetic comparative analysis for position-specific weight matrix and secondary structure-based search for additional early branching chordate and echinoderm TRs. Multiple sequence alignment of 42 vertebrate (Chen et al. 2000) and 13 echinoderm (Li et al. 2013; Podlevsky et al. 2016b) TR pseudoknot (A) and box H/ACA (B) domains. Residues that are conserved 80% or more are shaded with white text on colored background. A dashed line separates vertebrates and echinoderms. Conserved primary sequence and secondary structural motifs are labeled below the alignment.

Supplementary Fig. S2. Phylogenetic comparative analysis based secondary structure determination of deuterostome TR domains. Multiple sequence alignment of 23 novel deuterostome TR pseudoknot domains (A) and box H/ACA domains (B). Residues conserved 80% or more are shaded with white text on colored background. Conserved primary sequence and secondary structural motifs are labeled below the alignment. (C) Secondary structure models of select novel deuterostome TR, pseudoknot and box H/ACA domains. Universal co-variations (green lines), invariant residues (red) and residues with >80% conservation (orange) are based on the sequence alignment of 55 previously identified animal TRs and 82 novel metazoan TRs identified in this study. Group-specific covariations (blue lines) are indicated and based on the sequence alignment of TRs from individual groups including 50 chordate TRs (42 previously identified and 8 novel), 23 echinoderm (13 previously identified and 10 novel) and 4 acorn worm TRs.

Supplementary Fig. S3. Phylogenetic comparative analysis based secondary structure determination of protostome TR domains. Multiple sequence alignment of 29 novel protostome TR pseudoknot (A) and box H/ACA domains (B). Residues conserved 80% or more are shaded with white text on colored background. Conserved primary sequence and secondary structural motifs are labeled below the alignment. (C) Secondary structure models of select novel protostome TR, pseudoknot and box H/ACA domains. Universal co-variations (green lines), invariant residues (red) and residues with >80% conservation(orange) are based on the sequence alignment of 55 previously identified animal TRs and 82 novel metazoan TRs identified in this study. Group-specific covariations (blue lines) are indicated and based on the sequence alignment of TRs from individual groups including 21 mollusca, 7 annelida, 1 brachiopda and 1 phoronida TRs, respectively.

Supplementary Fig. S4. Identification of metazoan TRs. A simplified phylogenetic tree representing major metazoan clades. Lineages are colored; Deuterostomia (blue),

Protostomia (orange) and basal metazoan (green). Branch lengths do not represent evolutionary distances. The number of previously identified TRs are shown in black to the right of respective classes and number of TRs identified in this study are shown in red. A total of 82 novel TRs were identified in this study spanning 10 metazoan phyla and 18 classes.

Supplementary Fig. S5. Phylogenetic comparative analysis based secondary structure determination of basal metazoan TR domains. Multiple sequence alignment of 29 novel basal metazoan TR pseudoknot (A) and box H/ACA domains (B). Residues conserved 80% or more are shaded with white text on colored background. Conserved primary sequence and secondary structural motifs are labeled below the alignment. (C) Secondary structure models of select novel basal metazoan TR, pseudoknot and box H/ACA domains. Universal co-variations (green lines), invariant residues (red) and residues with >80% conservation (orange) are based on the sequence alignment of 55 previously identified animal TRs and 82 novel metazoan TRs identified in this study. Group-specific covariations (blue lines) are indicated and based on the sequence alignment of TRs from individual groups including 26 cnidaria, 1 placozoa and 3 porifera species.

Supplementary Fig. S6. *In vitro* synthesis of sponge TERT proteins. The sponge TERT proteins were synthesized in RRL in the presence of ^{35}S methionine and analyzed on SDS-PAGE gel, exposed to phosphorstorage screen, and imaged with a Typhoon gel imager (GE healthcare). (A) FLAG tagged *Amphimedon queenslandica* TERT, FLAG-AquTERT (lane 2) and FLAG tagged human TERT, FLAG-hTERT (lane 1) expressed as control and a size marker. (B) FLAG tagged *Oscarella carmela* TERT (OcaTERT).

Supplementary Fig. S7. Conservation of CAB box in metazoan TRs. Multiple sequence alignments of the distal P8 stem-loop of TRs from select representative species of each clade are shown. RNA secondary structure is indicated using dot-bracket notations above each alignment and the RNA structural element is labeled above base-pairing notations. Residues conserved 80% or more are shaded with white text on colored background. CAB box conservation is shown within red box and labeled above the box in red.

Table S1. Species with TR identified in this study

Clade	Phylum	Class	Species	Accession	Start Coordinates[a]	End Coordinates[b]	Strand	Source
Deuterostomia	Chordata	Cyclostomata	*Petromyzon marinus*	PIZI01000001.1	7,205,787	7,206,194	+	NCBI
		Cyclostomata	*Lethenteron camtschaticum*	APJL01104664.1	144	547	-	NCBI
		Cyclostomata	*Lampetra planeri*	SRR5230929	n/a	n/a	n/a	NCBI-SRA
		Cyclostomata	*Eptatretus burgeri*	FYBX02010827.1	605,218	605,647	-	NCBI
		Cyclostomata	*Eptatretus atami*	DRR062483	n/a	n/a	n/a	NCBI-SRA
		Cephalochordata	*Asymmeteron lucayanum*	LZCU01053182.1	582	974	+	NCBI
		Cephalochordata	*Branchiostoma belcheri*	AYSS01005866.1	20,970	21,389	-	NCBI
		Cephalochordata	*Branchiostoma floridae*	ABEP02000017.1	29,201	29,618	-	NCBI
	Echinodermata	Asteroidea	*Asterias amurensis*	GAVL01049438.1	1	372	-	NCBI-TSA
		Asteroidea	*Asterias rubens*	GAUU01010066.1	1	370	+	NCBI-TSA
		Asteroidea	*Asterias forbesi*	SRR1138708	n/a	n/a	n/a	NCBI-SRA
		Asteroidea	*Pisaster giganteus*	SRR11293188	n/a	n/a	n/a	NCBI-SRA
		Asteroidea	*Pisaster ochraceus*	SRR11293187, SRR10982254, SRR10982249	n/a	n/a	n/a	NCBI-SRA
		Asteroidea	*Patiria miniata*	AKZP01087997.1	1879	2303	+	NCBI
		Asteroidea	*Patiriella regularis*	CYSQ010112690.1	45	475	-	NCBI
		Asteroidea	*Acanthaster planci*	BDGF01006146.1	22,662	23,102	+	NCBI
		Asteroidea	*Echinaster spinulosus*	GAVE01110158.1	49	503	-	NCBI-TSA
		Ophiuroidea	*Ophionereis fasciata*	CZLG010265874.1	525	944	+	NCBI
	Hemichordata	Enteropneusta	*Saccoglossus bromophenolosus**	No source data	n/a	n/a	n/a	n/a
		Enteropneusta	*Saccoglossus kowalevskii**	NW_003116016.1	11,881	12,316	-	NCBI
		Enteropneusta	*Harrimaniidae sp. N. clade 3 JTC-2014*	SRR1695462	n/a	n/a	n/a	NCBI-SRA
		Enteropneusta	*Ptychodera flava*	BCFJ01016025.1	62,725	63,164	+	NCBI
Protostomia	Brachiopoda	Lingulata	*Lingula anatina*	NW_013580819.1	173,755	174,151	+	NCBI
	Phoronida		*Phoronis australis*	NMRA01000149.1	753,300	753,694	+	NCBI
	Annelida	Polychaeta	*Capitella teleta*	AMQN01027543.1	126	490	+	NCBI
		Polychaeta	*Arenicola marina*	SRR2005653	n/a	n/a	n/a	NCBI-SRA
		Polychaeta	*Hydroides elegans*	LQRL01164149.1	3,818	4,257	-	NCBI
		Clitellata	*Eisenia fetida*	CYRZ010654929.1	282	695	+	NCBI
		Clitellata	*Lumbricus rubellus*	scaffold682|size41541	33,676	34,086	-	DataSTORRE
		Clitellata	*Olavius algarvensis*	SRR5421607	n/a	n/a	n/a	NCBI-SRA
		Clitellata	*Amynthas corticis*	FXXH010317067.1	93	484	-	NCBI
	Mollusca	Gastropoda	*Pomacea diffusa**	No source data	n/a	n/a	n/a	n/a
		Gastropoda	*Pomacea canaliculata*	PZQS01000002.1	30,270,917	30,271,327	+	NCBI

Protostomia	Mollusca	Gastropoda	*Conus tribblei*	LFLW010801771.1	6,342	6,711	-	NCBI
		Gastropoda	*Rapana venosa*	GDIA01241434.1	1	338	-	NCBI-TSA
		Gastropoda	*Littorina saxatilis*	Contig56765	8,751	9,136	+	DRYAD
		Gastropoda	*Haliotis discus hannai*	GIGJ01057632.1	465	866	-	NCBI-TSA
		Gastropoda	*Haliotis laevigata*	VKKT01005222.1	43,381	43,787	-	NCBI
		Gastropoda	*Haliotis tuberculata*	GEAU01287953.1	1	369	+	NCBI-TSA
		Gastropoda	*Elysia timida*	GBRM01107526.1	147	495	+	NCBI-TSA
		Gastropoda	*Elysia cornigera*	GBRW01213247.1, SRR1583663	n/a	n/a	n/a	NCBI-TSA/SRA
		Gastropoda	*Bathyberthella antarctica*	GFXA01051430.1	1	247	-	NCBI-TSA
		Bivalvia	*Mytilus galloprovincialis*	LNJA010333678.1	187	535	+	NCBI
		Bivalvia	*Bathymodiolus platifrons*	MJUT01034425.1	591,096	591,451	+	NCBI
		Bivalvia	*Modiolus philippinarum*	MJUU01052557.1	27,325	27,680	-	NCBI
		Bivalvia	*Pinctada fucata*	DRR050170	n/a	n/a	n/a	NCBI-SRA
		Bivalvia	*Pinctada martensii*	NIJJ01006429.1	9,898	10,264	-	NCBI
		Bivalvia	*Crassostrea gigas*	SZQM02000004.1	154,764	155,125	+	NCBI
		Bivalvia	*Crassostrea virginica**	KV918323.1	2,331,345	2,331,700	-	NCBI
		Bivalvia	*Nodipecten subnodosus*	GFNL01243408.1	1	252	-	NCBI
		Bivalvia	*Mizuhopecten yessoensis*	NEDP01005424.1	678,838	679,170	+	NCBI
		Aplacophora	*Alexandromenia crassa*	comp40353_c0_seq1	15	384	+	DRYAD
Basal metazoa	Cnidaria	Anthozoa	*Acropora digitifera*	BACK02028654.1	1,815	2,284	-	NCBI
		Anthozoa	*Acropora cervicornis*	GASU01042147.1	1	361	-	NCBI-TSA
		Anthozoa	*Acropora hyacinthus*	GDIF01035769.1	16	409	+	NCBI-TSA
		Anthozoa	*Stylophora pistillata*	LSMT01000472.1	108,604	109,085	+	NCBI
		Anthozoa	*Seriatopora hystrix*	SRR2300678, SRR3144612, SRR3144610	n/a	n/a	n/a	NCBI-SRA
		Anthozoa	*Madracis auretenra*	comp285766_c0_seq1	1	424	+	
		Anthozoa	*Pseudodiploria strigosa*	HACD01239751.1	1	266	+	NCBI-TSA
		Anthozoa	*Favia lizardensis*	GDZU01016292.1	24	495	-	NCBI-TSA
		Anthozoa	*Orbicella faveolata*	MZGG01000725.1	580,730	581,215	-	NCBI
		Anthozoa	*Platygyra daedalea*	c38845_g1_i1	1	505	+	PDTD
		Anthozoa	*Montipora capitata*	RDEB01000170.1	373,653	374,130	-	NCBI
		Anthozoa	*Galaxea fascicularis*	GFAZ01037277.1	7	471	-	NCBI-TSA
		Anthozoa	*Montastraea cavernosa*	comp748712_c0_seq1	1	216	+	DRYAD
		Anthozoa	*Porites astreoides*	GEHP01404864.1	216	699	-	NCBI-TSA
		Anthozoa	*Ctenactis echinata*	GDZV01002545.1	1	285	-	NCBI-TSA
		Anthozoa	*Anthopleura elegantissima*	GBXJ01057767.1	34	437	+	NCBI-TSA
		Anthozoa	*Actinia tenebrosa*	SRR3207346	n/a	n/a	n/a	NCBI-SRA

Basal metazoa	Cnidaria	Anthozoa	*Corynactis australis*	GELM01043505.1	5	483	+	NCBI-TSA
		Anthozoa	*Ricordea yuma*	SRR3201251, SRR3201252, SRR3201253	n/a	n/a	n/a	NCBI-SRA
		Scyphozoa	*Aurelia aurita*	GBRG01038552.1	181	625	-	NCBI-TSA
		Scyphozoa	*Chrysaora fuscescens*	SRR3180892	n/a	n/a	n/a	NCBI-SRA
		Scyphozoa	*Rhopilema esculentum*	GEMS01118701.1	1	382	-	NCBI-TSA
		Scyphozoa	*Periphylla periphylla*	SRR1915828	n/a	n/a	n/a	NCBI-SRA
		Staurozoa	*Calvadosia cruxmelitensis*	HAHC01109237.1	3	350	+	NCBI-TSA
		Staurozoa	*Lucernaria quadricornis*	HAHD01078658.1	1	405	+	NCBI-TSA
		Hydrozoa	*Craspedacusta sowerbyi*	SRR923472	n/a	n/a	n/a	NCBI-SRA
	Placozoa		*Trichoplax adhaerens*	ABGP01000509.1	21,534	21,930	+	NCBI
	Porifera	Demospongiae	*Amphimedon queenslandica*	ACUQ01004887.1	26,508	26,870	+	NCBI
		Calcarea	*Sycon ciliatum*	ERR592860, ERR592861, ERR592862, ERR592868	n/a	n/a	n/a	NCBI-SRA
		Homoscleromorpha	*Oscarella carmela*	scaffold411	49,661	50,069	-	Compagen

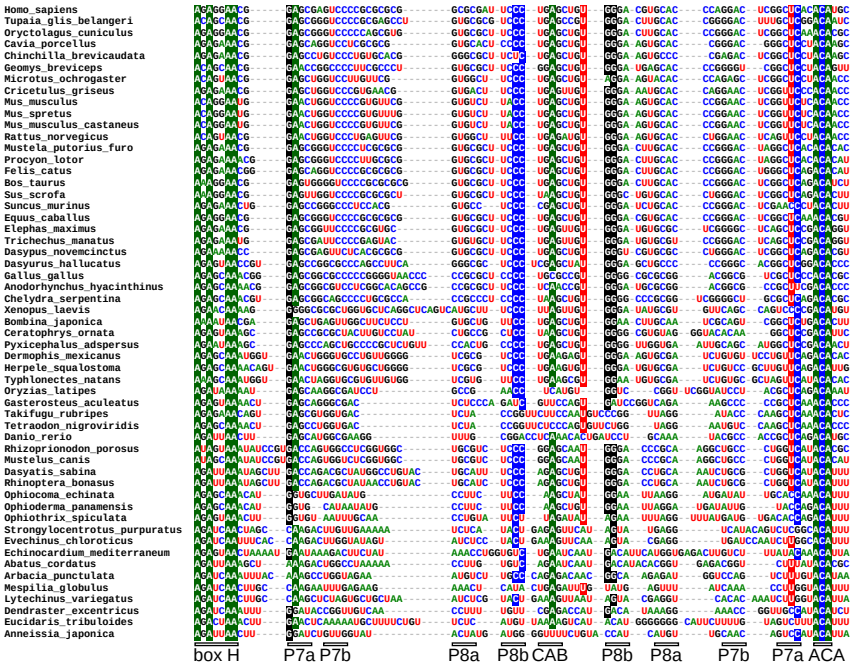
[a]: 5’ end predicted based on multiple sequence alignment
[b]: 3’ end of TR inferred as three nucleotides downstream of box ACA
[*]: TRs cloned in this study
NCBI: National center for Biotechnology Information – Genome Database, URL: www.ncbi.nlm.nih.gov/genome/
NCBI-TSA: National center for Biotechnology Information – Transcriptome Shotgun Assembly Sequence Database, URL : www.ncbi.nlm.nih.gov/genbank/tsa/
NCBI-SRA: National center for Biotechnology Information – Sequence Read Archive Database, URL : www.ncbi.nlm.nih.gov/sra/ (TRs identified using INFERNAL from assembled SRA reads or BLAST against raw reads)
DataSTORRE: Stirling Online Repository for Research Data – University of Stirling, URL: datastore.stir.ac.uk
DRYAD: Dryad digital repository, URL: datadryad.org
PDTD: *Platygyra daedalea* transcriptome database, URL: http://ib.oregonstate.edu/~meyere/DB/Pdae/search.php
Compagen: A comparative genomics platform for early branching metazoan, URL: http://www.compagen.org/index.html

A

Supplemental Fig. S1

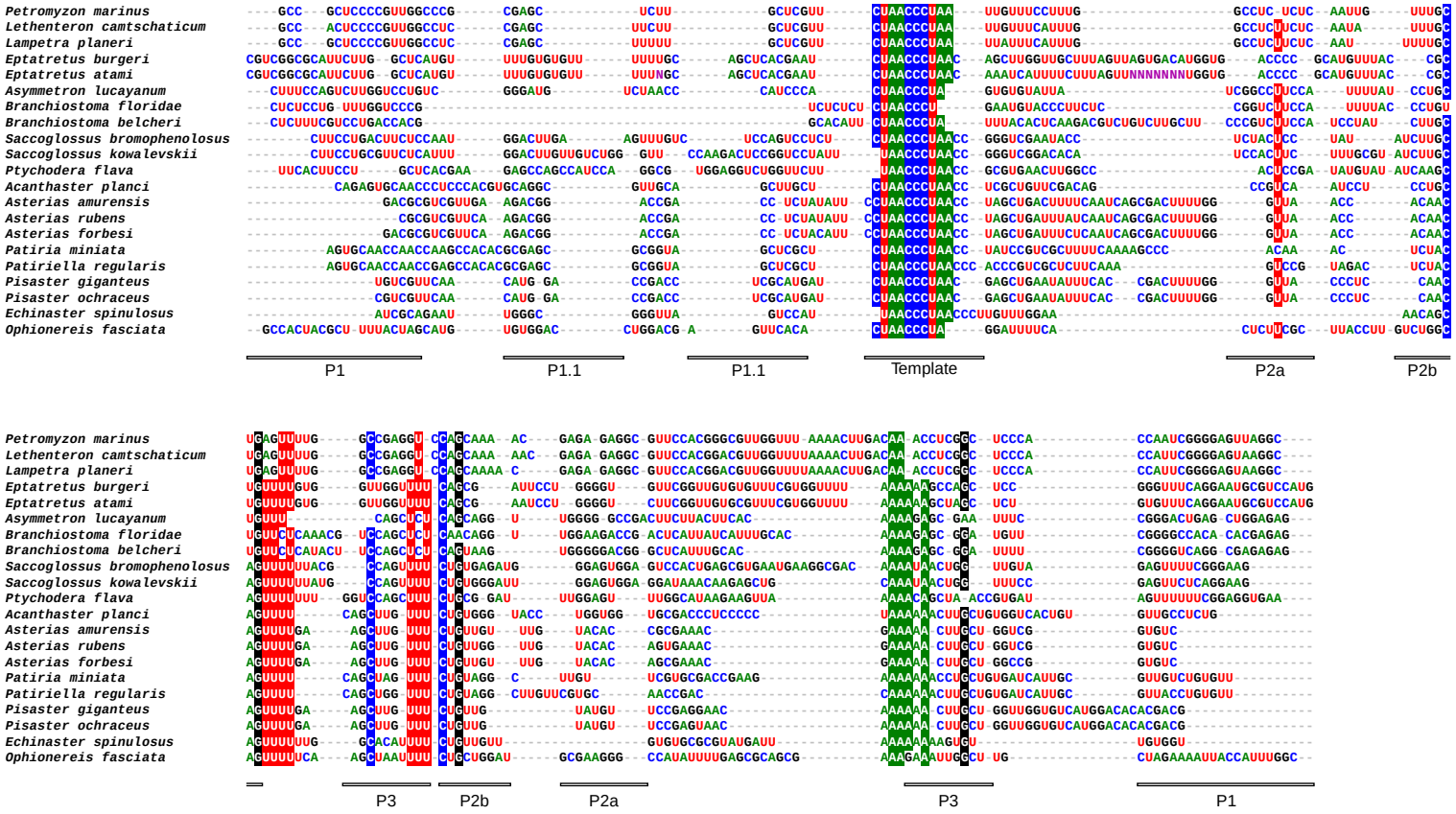
B

Supplemental Fig. S1

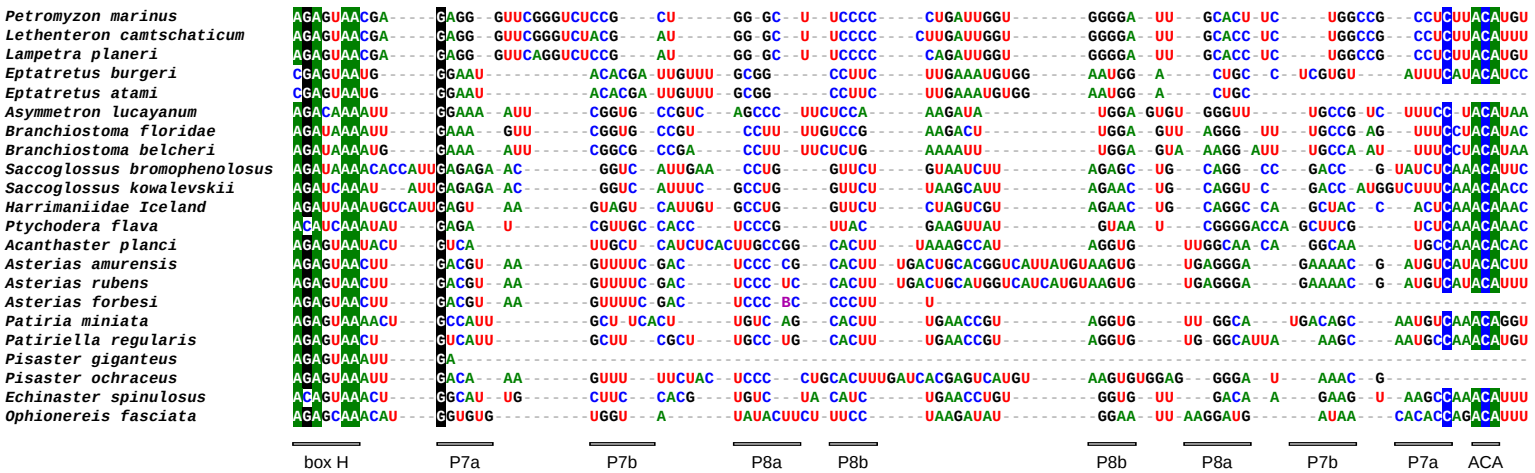


Supplemental Fig. S2

A



B

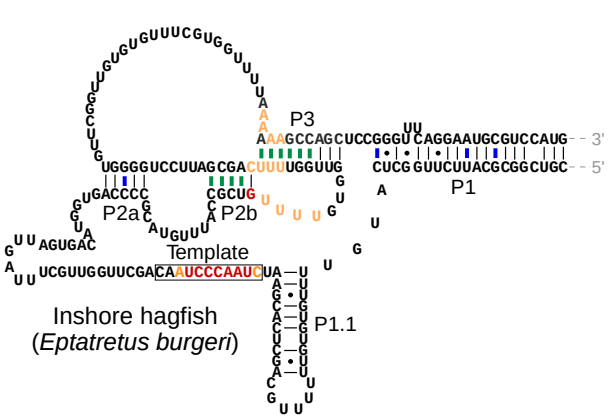


C

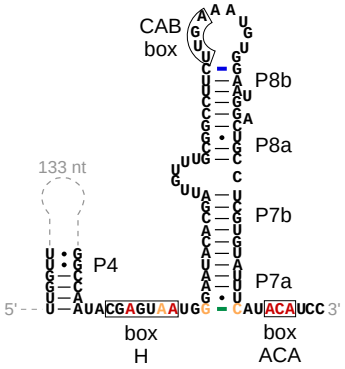
Pseudoknot

boxH/ACA

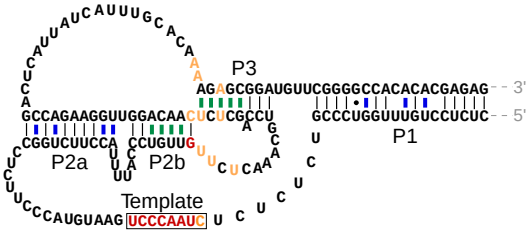
Chordata
(notochords)
Cyclostomata



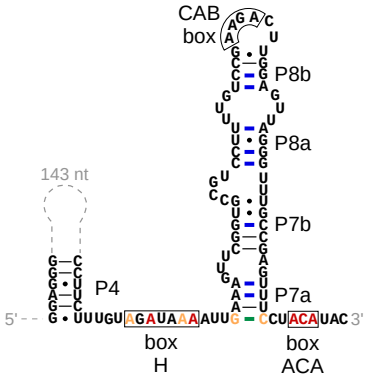
Inshore hagfish
(*Eptatretus burgeri*)



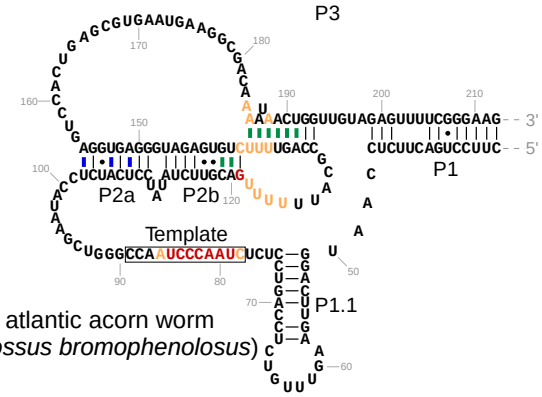
Chordata
(notochords)
Cephalochordata



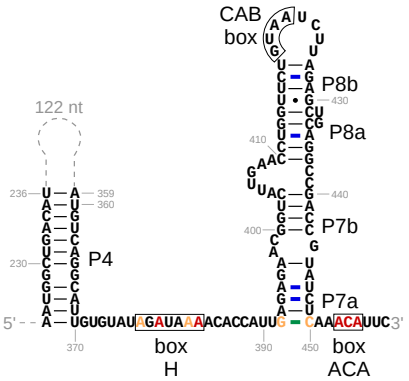
Florida lancelet
(*Branchiostoma floridae*)



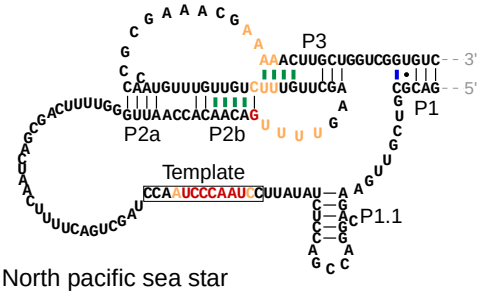
Hemichordata
(stomochords)
Enteropneusta



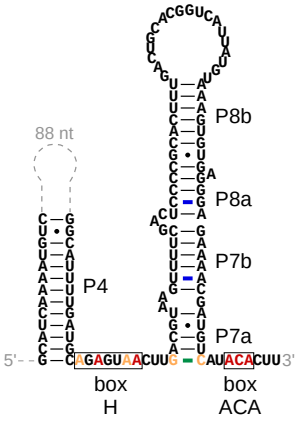
North atlantic acorn worm
(*Saccoglossus bromophenolosus*)



Echinodermata
(spiny skinned)
Asteroidea

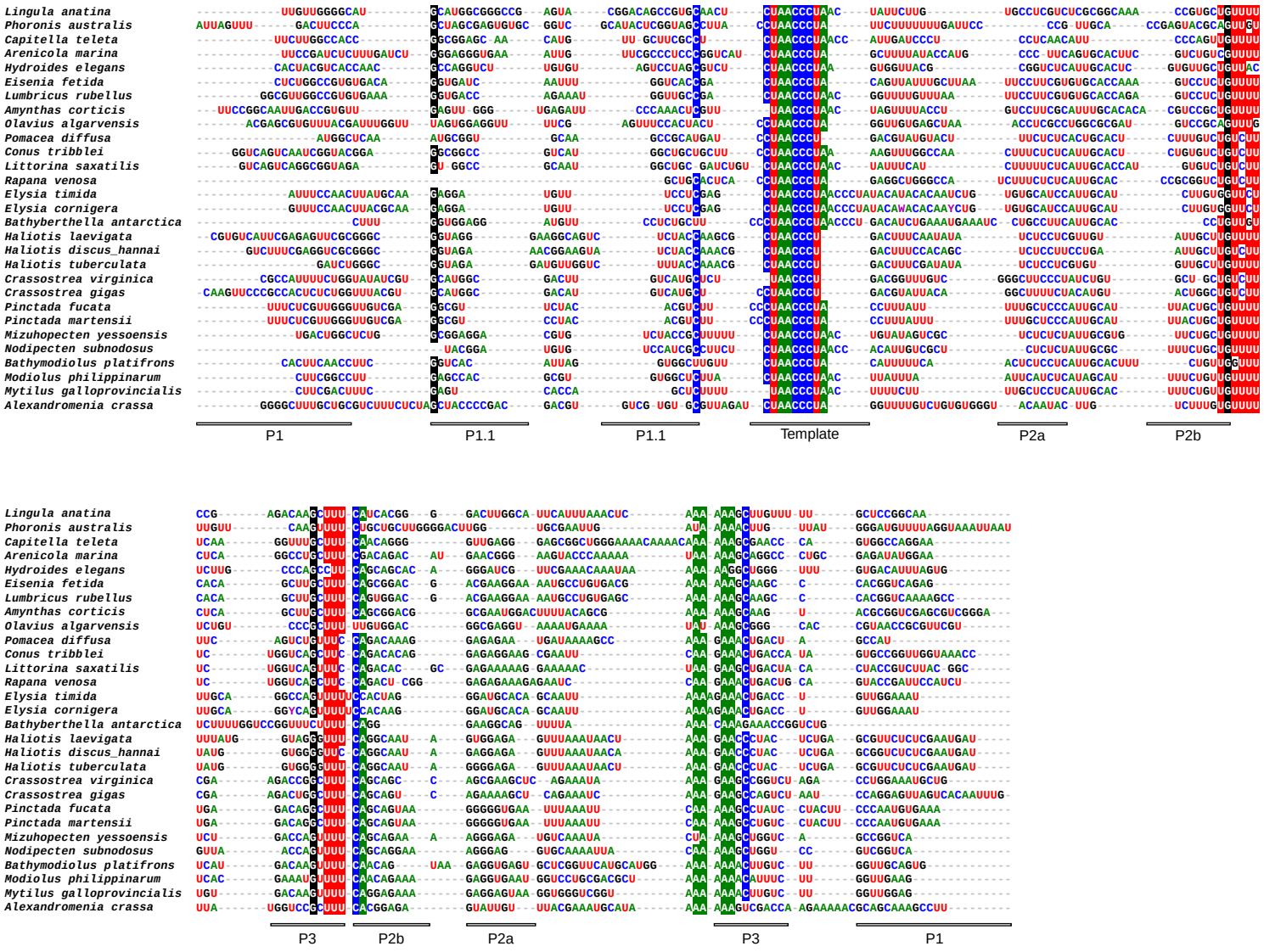


North pacific sea star
(*Asterias amurensis*)



Supplemental Fig. S3

A



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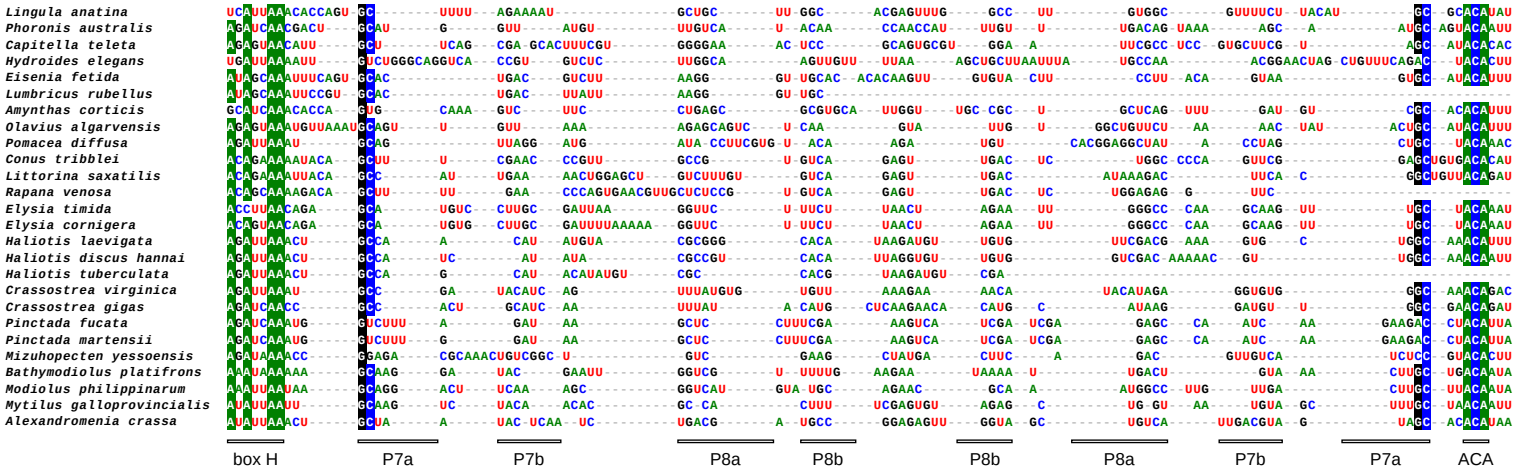
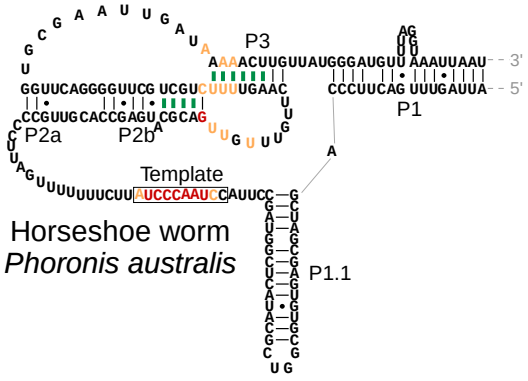


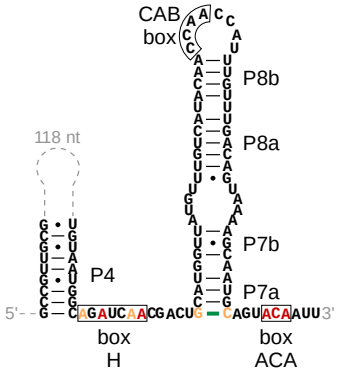
C

Pseudoknot

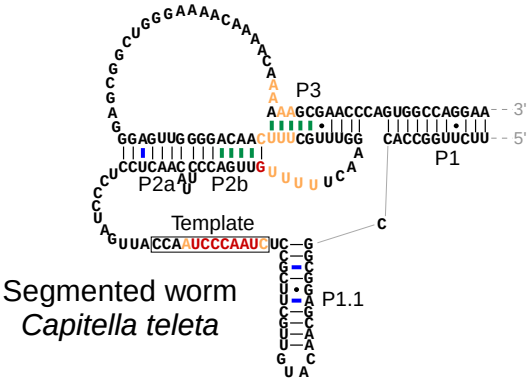
boxH/ACA

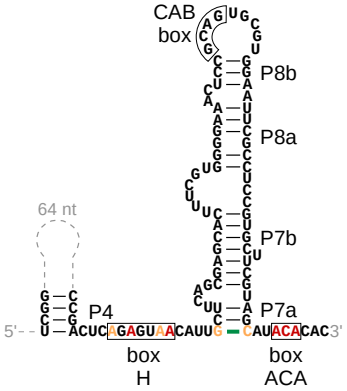
Phoronida
(horseshoe worms)



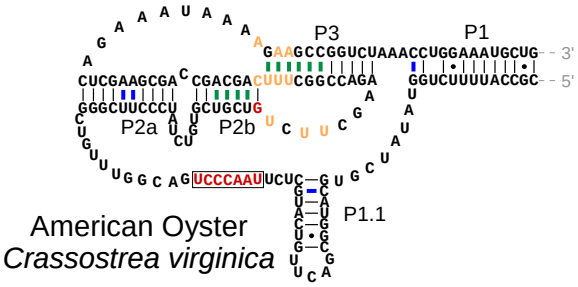


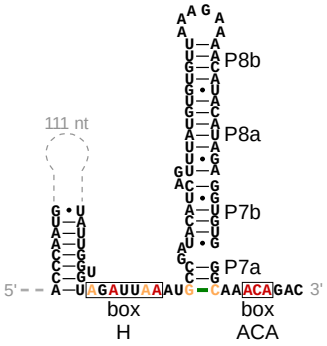
Annelida
(segmented worms)
Polychaeta



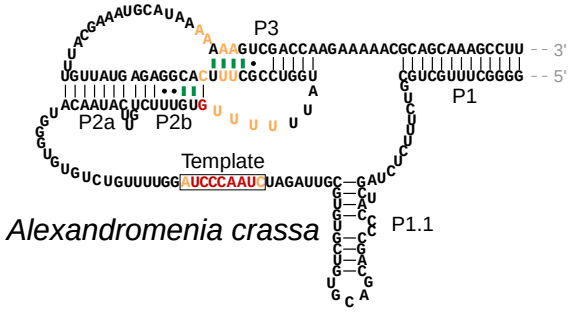


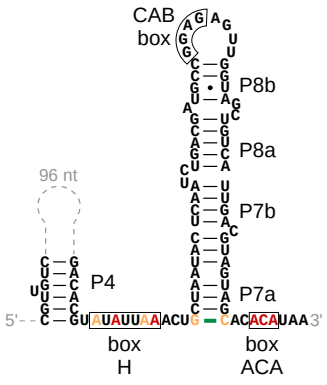
Mollusca
(soft-bodied)
Bivalvia



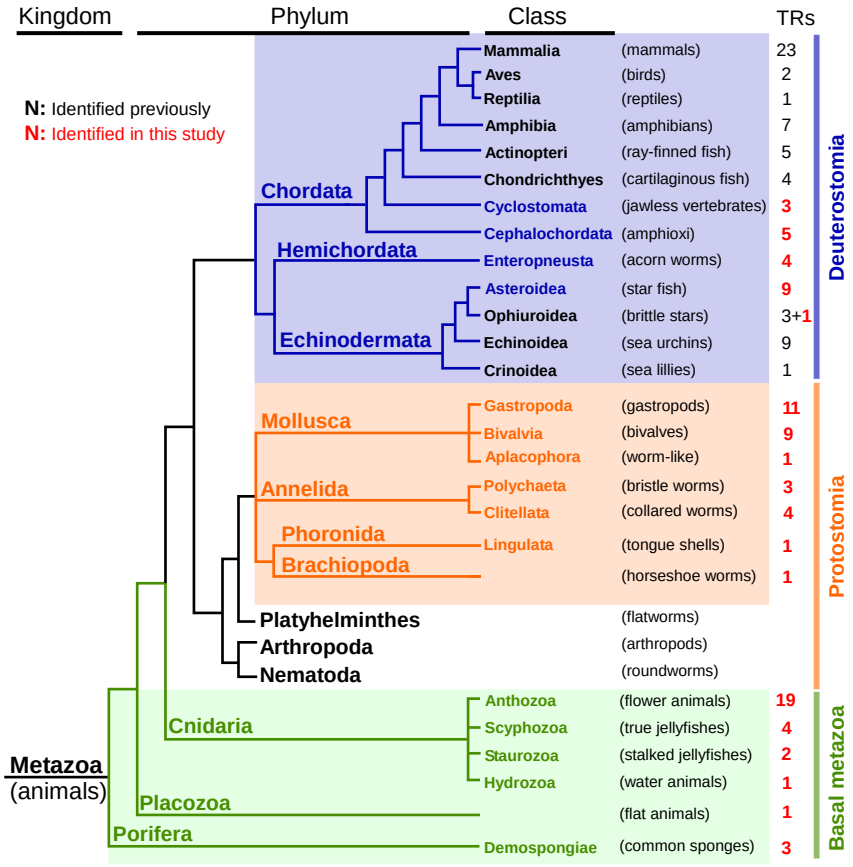


Mollusca
(soft-bodied)
Aplacophora

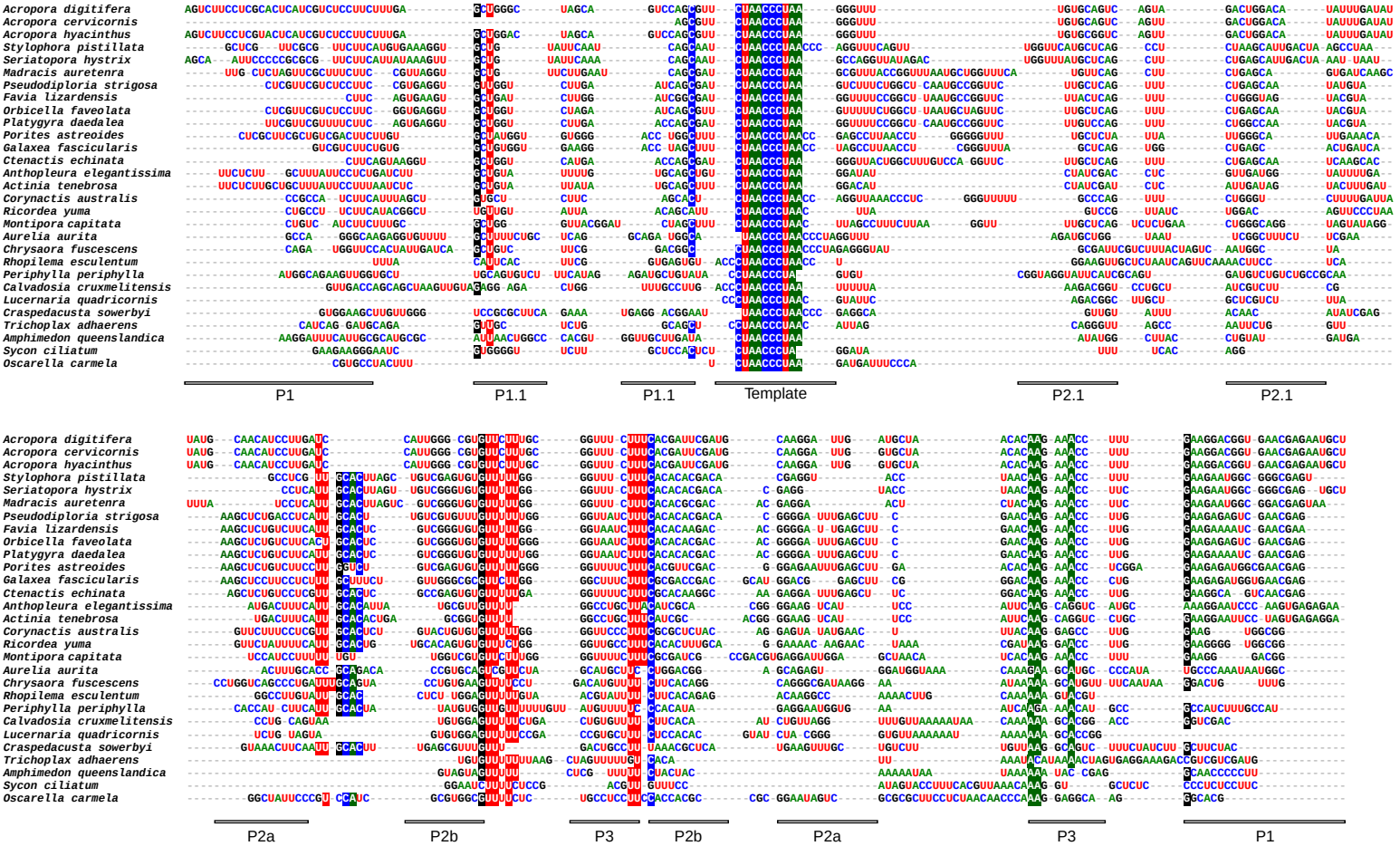




Supplemental Fig. S4



A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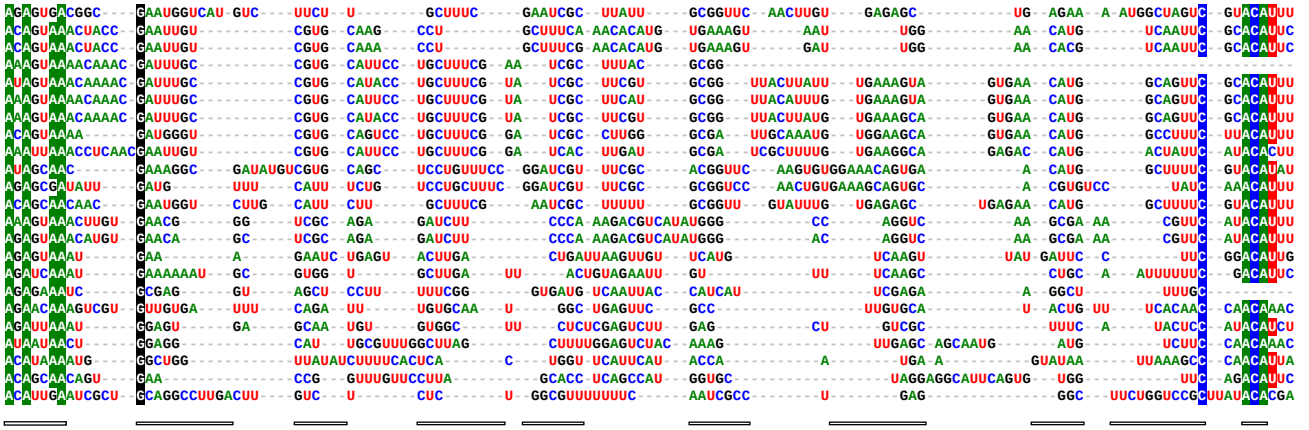
Acropora cervicornis
Acropora hyacinthus
Stylophora pistillata
Seriatopora hystrix
Madracis auretenra
Pseudodiploria strigosa
Favia lizardensis
Orbicella faveolata
Platygyra daedalea
Porites astreoides
Galaxea fascicularis
Ctenactis echinata
Anthopleura elegantissima
Actinia tenebrosa
Corynactis australis
Ricordea yuma
Montipora capitata
Aurelia aurita
Chrysaora fuscescens
Rhopilema esculentum
Periphylla periphylla
Calvadosia cruxmelitensis
Lucernaria quadricornis
Craspedacusta sowerbyi
Trichoplax adhaerens
Amphimedon queenslandica
Sycon ciliatum
Oscarella carmela
P1
P1.1
P1.1
Template
P2.1
P2.1
P2a
P2b
P3
P2b
P2a
P3
P1

B

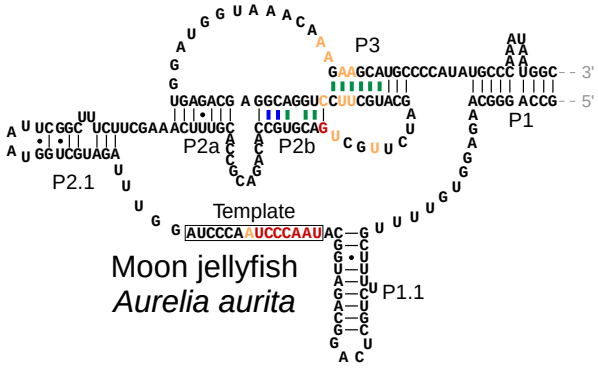

Acropora digitifera
Stylophora pistillata
Seriatopora hystrix
Pseudodiploria strigosa
Favia lizardensis
Orbicella faveolata
Platygyra daedalea
Porites astreoides
Galaxea fascicularis
Corynactis australis
Ricordea yuma
Montipora capitata
Anthopleura elegantissima
Actinia tenebrosa
Aurelia aurita
Chrysaora fuscescens
Rhopilema esculentum
Lucernaria quadricornis
Craspedacusta sowerbyi
Trichoplax adhaerens
Amphimedon queenslandica
Sycon ciliatum
Oscarella carmela
box H
P7a
P7b
P8a
P8b
P8b
P8a
P7b
P7a
ACA

C

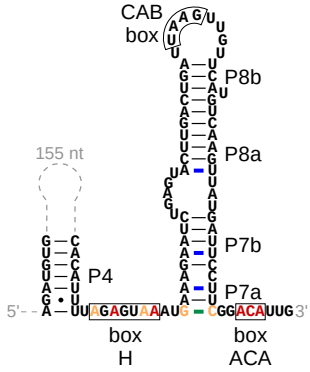
Pseudoknot

boxH/ACA

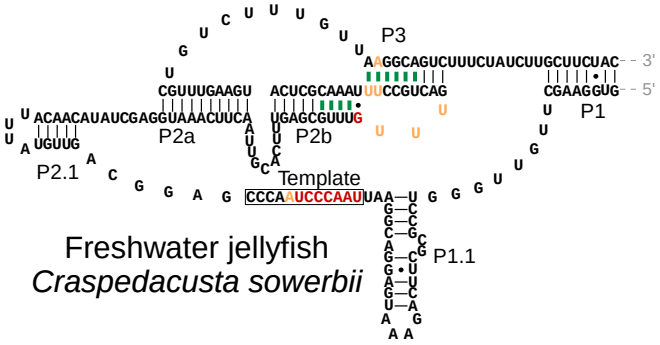
Cnidaria
(nettles)
Scyphozoa



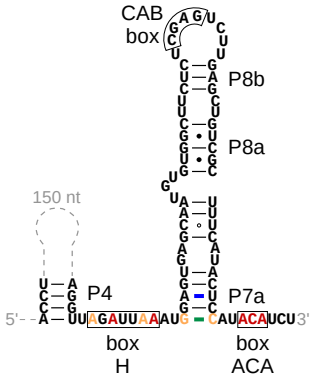
Moon jellyfish
Aurelia aurita



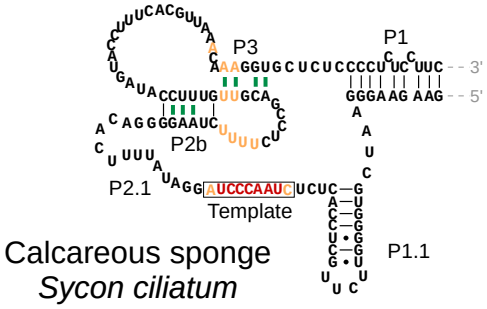
Cnidaria
(nettles)
Hydrozoa



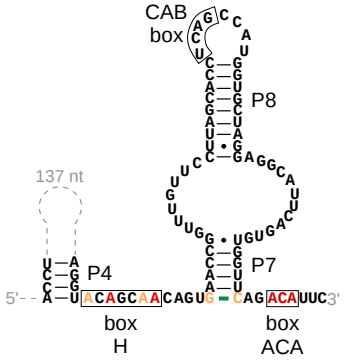
Freshwater jellyfish
Craspedacusta sowerbii



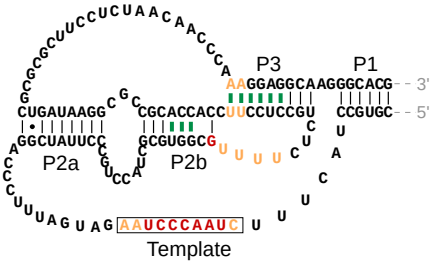
Porifera
(sponges)
Calcarea



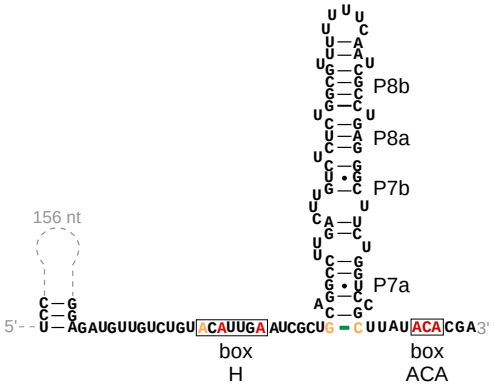
Calcareous sponge
Sycon ciliatum



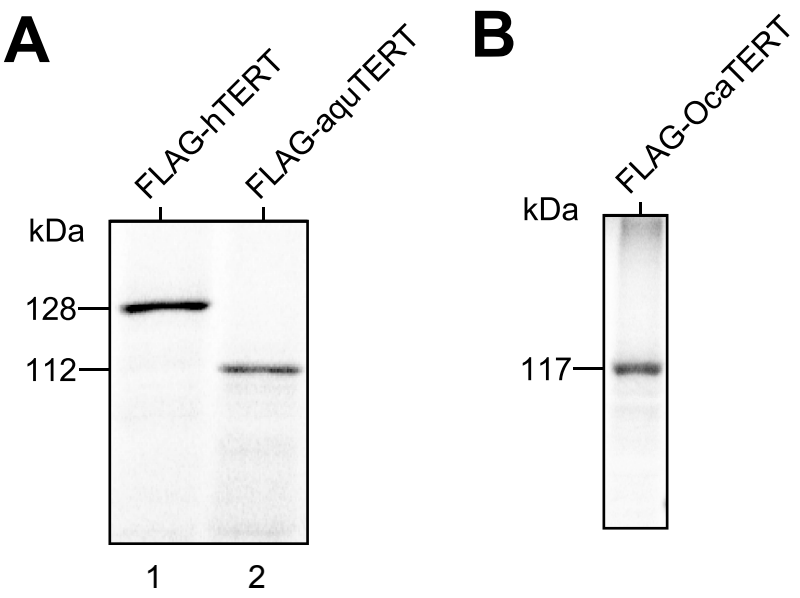
Porifera
(sponges)
Homoscleromorpha



Slime sponge
Oscarella carmela



Supplemental Fig. S6



Supplemental Fig. S7