

Nitric Oxide regulates mouth development in amphioxus

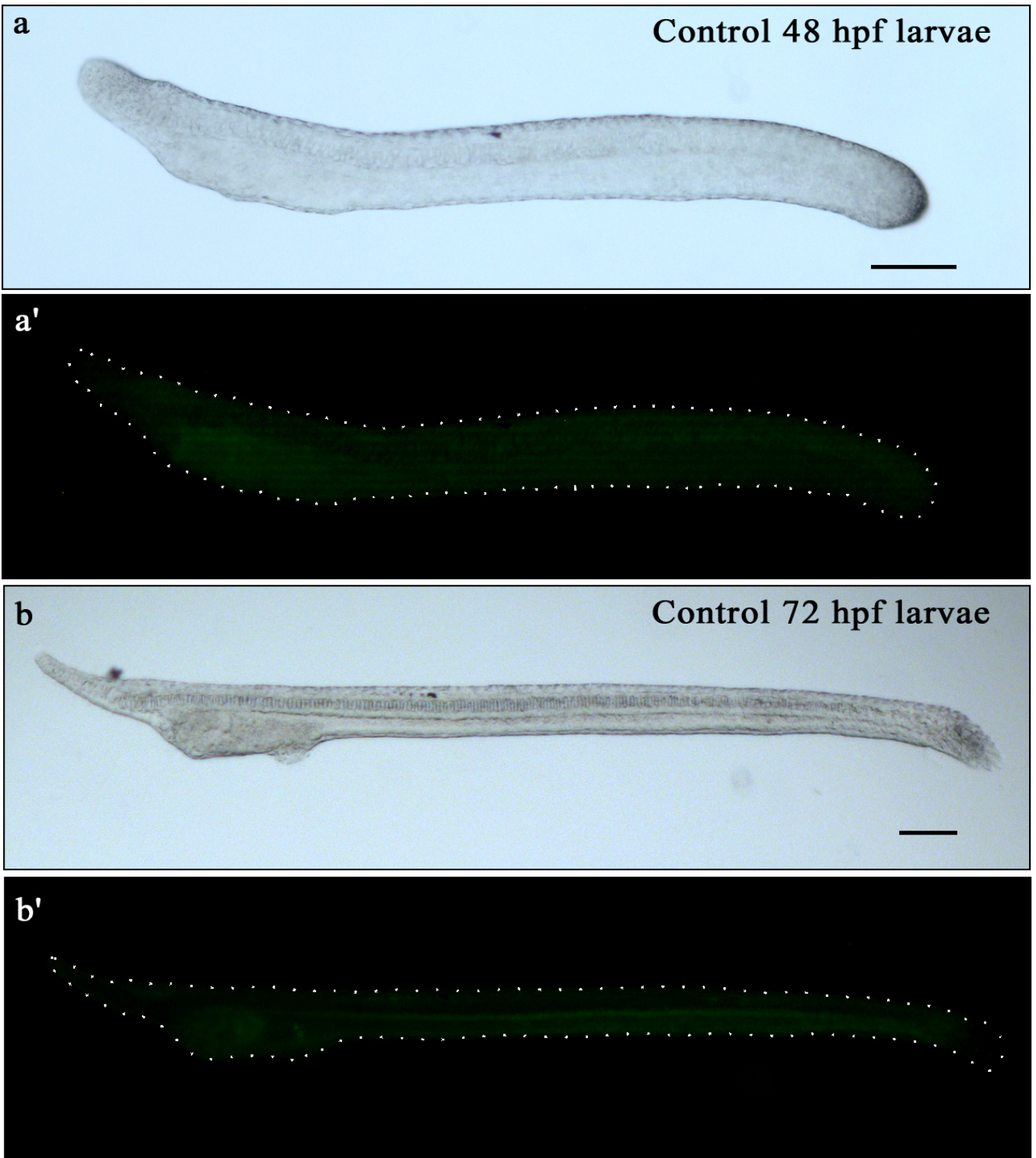
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Hyogo, Japan

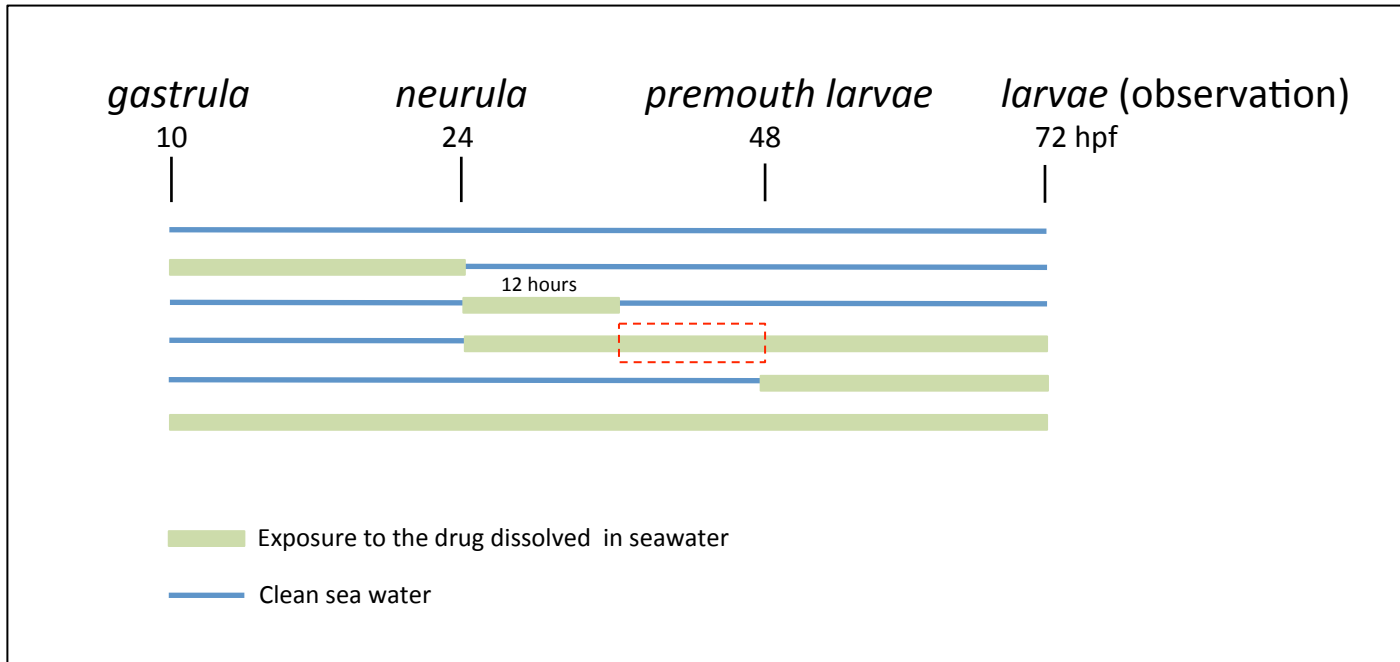
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Supplementary Figure S1:

Endogenous green fluorescence emission in not treated *B. lanceolatum* larvae (negative control), showing a negligible non-specific signal at the same laser intensity as used for DAF-FM-DA experiments. a) pre-mouth larvae (48 hpf) in bright field, a') the same specimen in fluorescence. b) 72 hpf larvae in bright field, b') the same specimen in fluorescence. Rostral part is on the left, dorsal part on the top. Scale bars: 50 μm .



Supplementary Figure S2: Scheme of drug treatments

The drug incubation period, from gastrula to larvae, is schematized by green bars, while blue thin bars indicate that the embryos are in FSW. Several washes with sea water were performed to end treatments. All samples were observed at 72 hpf for the general morphology (body plan shape, length) and in particular for the pharyngeal area development.

Supplementary Figure S3

Protein and coding sequences (CDS) of the *B. lanceolatum* Nos genes, extracted from the draft genome sequence. See Materials and Methods for details.

>*B. lanceolatum* NOSA, protein

MEGQQNIIVVGLVKREVDGLGVSAKRREVPGHSAVVIEEVIRGGPADHTGMVQPGDTIVAVN
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>*B. lanceolatum* NosA, CDS

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>*B. lanceolatum* NOSB, protein

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>B. lanceolatum NosB, CDS

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>B. lanceolatum NOSC, protein

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>B. lanceolatum NosC, CDS

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AGGTGCACCATAGAAACGTGGTACCCTGCAGACTCATCTCCAGAGAAAACCTGCAAGCACCC
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TGTGCCCCGGGACCATCTCGCCGTCTTCCC GGCCAACGAGGACCACCTTGTGCAGGCCGTGC
TGGACCGACTGGACAACGCTCCCGACCCGGACAGTATCGTCAACATGGAGGTTCTCCAAGAG
AAGCAGACGCCGCTAGGAGCCATACGAACATGGATGACGAGCGAGAGACTGCCCCCTGCTC
CCTCCGGACGGCCCTGTCTCGGTACTTTGACATCACGACGCCTCCATCGCCTCAGTTACTGC
AGCATCTGGCTACACAAGCTACAGATGAGGAGGAGAAGAAAGAACTGGAGGTTTTGGGAAAG
GGTGACGCGAGGTATGAAGACTGGAAGTTTGAGCGTACCCCGAACCTCGTAGAGGTCTTGA
GGACTACCCGTCCCTGAAGGTGGCTCCGACCCTCTGCTGAGTCAGCTACCCTTCTGCAGC
AGCGGTACTACTCCATCAGCTCATCACAGAGGATGTACCCGGGAGAGATCCACGCTACGGTC
GCAGTGGTCAGTTTTGCCACTCAGGGTGGCGTGGGTCTACCCACAACGGAGTCTGCTCATC
TTGGTTGAACCGAATCGAGAAGGATGATATCGTACCTTGTTCGTGCGGGGGGCACAAAAC
TCCATCTGCCCGAGGACCCACGGTTCGCTGATGATGGTAGGTCCGGGGACTGGCATCGCG
CCGTTCCGCAGCTTCTGGCAGCACAGGCAGATGGAGGTCACCTCTGGTGACCCACATCATCG
GCCAAAGTACGGCCAGATGACGTTAGTATTCGGCTGTGCGCAGTCGAAAATGGACGACATCT
ACAAGCACGAGACAGCACAGGCAAAGGAGGACGGCGCCTTGACTGAGGTCTACACGGCTCTG
TCCAGGGAGCCGGGCGTACCAAAGTCGTATGTCCAGAACGTCATCTTGGACCTGATTTCCGA
GAAGGTGTGCGACCTCCTGATGAAGCATAACGGCCATTTCTACGTCTGCGGGGACGTTTCCA
TGGCAGCAGACGTCTGCAACACGCTGGAGAAGGCCATGGAAAAACAGCAGGGCATGACCCCC
ACAAGGCCAAGGACTTCGTCGACAAGCTGAAGGACTGCAACCGATACCATGAGGACATCTT
TGGGGTAACCTTGAGGACCCAGGAGGTGACTGACCGTGTGCGCTCGGCCGCGCGTAAGAACT
GGGTGCGGGTGAAGAGACTGCGGCCAGCACGGTGGTCCC GCCACCAGAGGTCTCTCACCG
GTACGTGAGAGCTCGTCCTTATCGTCTACGCCCATGTCCACGCCGTCCGCCACTCCCGTCAA
CTCTCCCTACTCCTCACCGCTGCATCGACCCGATCCGACCGTGTGTGCCAACGGCGATTTAC
CAAGTCTACACATTGAGGAATAA

Supplementary Table S4

NOS sequences accession number used in the phylogenetic study.

species	NOSI	NOSII	NOSIII
<i>Homo sapiens</i>	AAB49040	AAB60366	AAA36365

species	NOSA	NOSB	NOSC
<i>Asymmetron lucayanum</i>	GESY01069733 GESY01069734 GESY01069736 GESY01069735 GESY01069737 GESY01069738	GETC01128572 GETC01128573	GETC01013682 GETC01013681 GESY01015049 GESY01007340 GESY01007341 GETC01000005 GESY01098362 GETC01143078
<i>Branchiostoma lanceolatum</i>	Suppl. Figure 2	Suppl. Figure 2	Suppl. Figure 2
<i>Branchiostoma floridae</i>	XP_002608547	XP_019636970	XP_002605826
<i>Branchiostoma belcheri</i>	XP_019623080	XP_019636970	XP_019631555
<i>Ciona intestinalis</i>	XP_009861972		
<i>Ciona savignyi</i>	ENSCSAVG00000009725		
<i>Strongylocentrotus purpuratus</i>	XP_003729305		

**A. lucayanum* *NosC* gene coding sequence was very fragmented in short contigs. Two of these, GESY01098362 and GETC01143078, overlapped and we reconstructed a longer sequence, which was used for the phylogenetic analysis.

Supplmentary File S5

1

Homo_sapiens_nNOS	ETGCTEYICM	GSIMHPQHAR	RPEDVRTKGO	LFPLAKEFID	QYYSSIKRFG
Homo_sapiens_iNOS	ILTCRSKSC	GSIMTPLTRG	PRDKPTTPDE	LLPQAIEFVN	QYYGSFKEAK
Homo_sapiens_eNOS	DGPCTPRRCL	GSLVFPLOGR	PSPGPPAPEQ	LLSQARDFIN	QYYSSIKRSG
Branchiostoma_floridae_NOSA	VNPCSDGKCL	GPLMRPRPFT	TPGYFRPKKEE	VLEQAQKFIF	EYYASLKRSD
Branchiostoma_floridae_NOSB	VSPCSSGKCM	GSIVRQREPF	PPYYKRPKEE	VLEQAQKFID	DYYASMKKEN
Branchiostoma_floridae_NOSC	VNPCSGTKCL	GSLMRPKSGR	PAGEVRPKKEE	VLEHAKEFLD	EFFASIKRAN
Branchiostoma_belcheri_NOSA	LNPCSDGRCL	GPLMRPRPYT	APGYFRPKKEE	VLEQAQKFIF	EYYASLKRSD
Branchiostoma_belcheri_NOSB	VSPCSSGKCM	GSVVQRQPF	PPDYKRPKEE	VLEQAQKFID	DYYASLKKAN
Branchiostoma_belcheri_NOSC	MNPCSGTKCL	GSLMRPKSTR	PAGEVRPKKEE	VLEHAKEFLD	EFFASIKRAN
Branchiostoma_lanceolatum_NOSA	LNPCNDGRCL	GPLMRPRPFT	APGYFRPKKEE	VLEQAQKFIF	EYYASLKSSD
Branchiostoma_lanceolatum_NOSB	VAPCSSGKCM	GSVVRQREPF	PPGYKRPKEE	VLEQAQKFID	DYYASMKKEN
Branchiostoma_lanceolatum_NOSC	INPCSGTKCL	GSLMRPKSAR	PAGEVRPKDE	VLEHAKEFLD	EFFASIKRAN
Asymmetron_lucayanum_NOSA	VTPCSHGRCV	GSLMRPRPYT	APGYFRPKDE	VLEQAQDFIF	DYYASLKKSS
Asymmetron_lucayanum_NOSB	LNPCSTSGKCM	GSTVVRQRYF	PPQYKRPNEE	ILEQAQKFID	DYYASMKKAN
Asymmetron_lucayanum_NOSC	-NPCSGTKCL	GSLMRPQVKR	PAGTARSKEE	VLEHAKEFMD	DFFASIKRAN
Ciona_intestinalis_NOS	INHCAPKKCN	GSLMQPMVCD	PRDGKPSVES	VRKLAKDFID	QYFASMKRND
Ciona_savignyi_NOS	-----	-----	-----	-----	----KHSRND
Strongylocentrotus_purpuratus_NOSa	ETSKRSGSAN	NDSVT-SPTR	QKPSMRSQDE	KIRLAREFID	QYYAHAQRSG

51

Homo_sapiens_nNOS	SKAHMERLEE	VNKEIDTTST	YQLKDTIELY	GAKHAWRNAS	RCVGRIQWSK
Homo_sapiens_iNOS	IEEHLARVEA	VTKEIETTGT	YQLTGDELIF	ATKQAWRNAP	RCIGRIQWSN
Homo_sapiens_eNOS	SQAHEQLQEF	VEAEVAATGT	YQLRESELVF	GAKQAWRNAP	RCVGRIQWGK
Branchiostoma_floridae_NOSA	AADRDRRWS	VQVQIEEKGV	YHLTYDELAF	AAKMSWRNSP	RCIGRIQWSK
Branchiostoma_floridae_NOSB	SEDHERRWRD	IQAOVAETGL	YDLEYDELLY	GAKMAWRNAP	RCIGRYQWNN
Branchiostoma_floridae_NOSC	TQAHKQRWAE	AKAQIEEKGW	YELTQMELTY	GAKLAWRNAP	RCVGRIQWSK
Branchiostoma_belcheri_NOSA	VADRDRRWRD	VQVQVEEKGV	YHLTYEELVY	GAKMSWRNAP	RCIGRIQWSK
Branchiostoma_belcheri_NOSB	SAEHEKRWQD	IQAOVSETGL	YDLEYDELLY	GAKMAWRNAP	RCIGRYQWNN
Branchiostoma_belcheri_NOSC	TQAHKQRWAE	AKSQIEEKGW	YELTQMELTY	GAKLGWRNAP	RCVGRIQWSK
Branchiostoma_lanceolatum_NOSA	AGERDRRWRD	VQVQVEEKGV	YHLTYDELLY	GAKMSWRNAP	RCIGRIQWSK
Branchiostoma_lanceolatum_NOSB	SAEHERRWID	IQAOVEEKGL	YDLEYDELLY	GAKMAWRNAS	RCIGRYQWNN
Branchiostoma_lanceolatum_NOSC	TQAHKQRWAE	AKVQIEEKGW	YELTQMELTY	GAKLAWRNAP	RCVGRIQWSK
Asymmetron_lucayanum_NOSA	ADDRDRRWAD	VQAQVEERGV	YQLTYDELVY	GAKMAWRNSP	RCIGRIQWSK
Asymmetron_lucayanum_NOSB	STEHDRRWRD	IQAOVEEKGV	YDLTYDELLY	GAKMAWRNAP	RCIGRYQWNN
Asymmetron_lucayanum_NOSC	TRAHKQRWAE	VEAQIEEKGL	YELTQMELTY	GAKLGWRNAP	RCVGRIQWSK
Ciona_intestinalis_NOS	SEAHGARLDE	VMNSIDSTGT	YELTKNELIF	GAKTAWRNAP	RCIGRIQWNN
Ciona_savignyi_NOS	SEAHSSRLAE	VLASISSTGT	YELTKNELIF	GAKTAWRNAP	RCVGRIQWNN
Strongylocentrotus_purpuratus_NOSa	TVSHERRLYE	VQMSLKESGN	YHLTEAELIF	GAKSAWRNLP	QSRCRIYWNK

101

Homo_sapiens_nNOS	LQVFDARDCT	TAHGMFNYIC	NHVKYATNKG	NLRSAITIFP	QRTDGKHDFFR
Homo_sapiens_iNOS	LQVFDARSCS	TAREMFEHIC	RHVRYSTNNG	NIRSAITVFP	QRSDBGKHDFFR
Homo_sapiens_eNOS	LQVFDARDCR	SAQEMFTYIC	NHIKYATNRG	NIRSAITVFP	QRCPGRGDFFR
Branchiostoma_floridae_NOSA	LQLFDAREVT	TARGMFEAIC	RHVKWCTNDG	NIRSAITVFP	PRTDGRHDYK
Branchiostoma_floridae_NOSB	LQVIDHRNVK	TAKEMFDAVC	DHVRYATKDG	QMKTAISIFP	MRTDGKHDYK
Branchiostoma_floridae_NOSC	LQVFDARYVT	TARGMYEAIC	NHIKYTTNKG	NLRSAITIFP	ARTDGKHDFFR
Branchiostoma_belcheri_NOSA	LQLFDARDVT	TARGMFEAIC	RHIKWCTNNG	NIRSAITVFP	PRTDGRHDYK
Branchiostoma_belcheri_NOSB	LQVIDYRNVK	TAKEMFDAVC	DHVRYATKDG	QMKTAISIFP	MRTDGKHDWK
Branchiostoma_belcheri_NOSC	LQVFDARYVT	TARGMYEAIC	NHIKYATNKG	NIRSAITIFP	ARTDGKHDFFR
Branchiostoma_lanceolatum_NOSA	LQLFDARDVT	TARGMFEAIC	RHIKWCTNNG	NIRSAITVFP	PRTDGRHDYK
Branchiostoma_lanceolatum_NOSB	LQVIDYRNVK	TAQEMFDAAC	DHIRYATKDG	QMKTAISIFP	MRTDGKHDYK
Branchiostoma_lanceolatum_NOSC	LQVFDARYVT	TARGMYEAIC	NHIKYATNKG	NLRSAITIFP	ARTDGKHDFFR
Asymmetron_lucayanum_NOSA	LQVFDARDVT	TARGMFEAIC	RHIKWATNNG	NLRSAITVFP	PRTDGRHDYK
Asymmetron_lucayanum_NOSB	LQVLDQRHVK	TAQEMFDAVC	EHVRYATKDG	QMKTAISIFP	VRTDGKHDYK
Asymmetron_lucayanum_NOSC	LQVFDARYVT	TARGMFEAIC	NHIKYATNKG	NIRSAITIFP	ARTDGKHDFFR
Ciona_intestinalis_NOS	LHVFDCKRVT	STYEMFEAIC	SHLKYATNKG	NIRSCITFFP	PRNDVKTEFR
Ciona_savignyi_NOS	LHVFDCKRVT	STREMFEAIC	VHLKYATNKG	NIRSCITFFP	PRNDVKTEFR
Strongylocentrotus_purpuratus_NOSa	LQVFDARKVT	NSRDMFEMLC	KHLLYATNKG	NVRPAITVFP	ARNDNQTDFFR

151

Homo_sapiens_nNOS	VWNSQLIRYA	GKQPDGSL	GDPAVQFTE	ICIQGWKPP	RGRFDVPLPL
Homo_sapiens_iNOS	VWNAQLIRYA	GYQMPDGSIR	GDPAVVEFTQ	LCIDLGWKPK	YGRFDVVPLV
Homo_sapiens_eNOS	IWNSQLVRYA	GYRQDGSVR	GDPAVVEITE	LCIQHGWTGP	NGRFDVPLPL
Branchiostoma_floridae_NOSA	VWNGQFLKYA	AYQLPDGSIL	GDPINLEFTE	VCQALGWKGE	GTRFDLLPLV
Branchiostoma_floridae_NOSB	FWNKYLFQYA	AYQQPDGSVI	GDPANLELTE	VCHSLGWKKG	GTPYDFLPAI
Branchiostoma_floridae_NOSC	VWNSQFVRYA	GKQPDGSIV	GDPASVEFTE	ICQSLGWKKG	NGPFDVPLV
Branchiostoma_belcheri_NOSA	VWNGQFMKYA	AYQLPDGSIL	GDPINLDFTE	VCQALGWKGA	GTRFDILPLV
Branchiostoma_belcheri_NOSB	FWNKLLFQYA	GYEQPDGSVI	GDPGSLELTK	VCQSLGWKKG	GTPYDFLPAI
Branchiostoma_belcheri_NOSC	VWNAQFIRYA	GKQPDGSVI	GDPASVEFTE	ICQSLGWKKG	NGPFDVPLV
Branchiostoma_lanceolatum_NOSA	VWNGQFLKYA	AYQLPDGSIL	GDPINLEFTE	VCQALGWKGE	GTRFDLLPMV
Branchiostoma_lanceolatum_NOSB	FWNKYLFQYA	GYQQPDGSVI	GDPANVELTE	VCQSLGWKKG	GTPYDFLPAI
Branchiostoma_lanceolatum_NOSC	VWNSQFVRYA	GKQPDGSVV	GDPASVEFTE	ICQSLGWKKG	NGPFDVPLV
Asymmetron_lucayanum_NOSA	VWNGQFMKYA	AYEMPDGSIL	GDPINLDFTE	VCQALGWKGA	GGYDILLPLV
Asymmetron_lucayanum_NOSB	FWNNFLFRYA	GYTQPDGSVI	GDPDMVEFTE	VCQSLGWRGK	GTPYDYLPAI
Asymmetron_lucayanum_NOSC	IWNAQFIRYA	GKXS-----	-----	-----WKGK	NGPFDVPLPL
Ciona_intestinalis_NOS	VWNSQLIRYA	CYREQDGSIV	GDPANLELTE	LCERLGWKKG	GGRFDLLPLL
Ciona_savignyi_NOS	VWNSQLIRYA	CYREQDGSIV	GDPANLELTE	LCMRLGWKKG	GGRFDLLPLL
Strongylocentrotus_purpuratus_NOSa	IWNSQLIGYA	GYRQPDGSVL	GDPSNLVFS	VCRTLGWRS	RTRFDVPLV

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Homo_sapiens_nNOS	LQANGDPEL	FQIPPELVLE	VPIRHPKFEW	FKDLGLKWYG	LPVSNMLLE
Homo_sapiens_iNOS	LQANGDPEL	FEIPPELVLE	VAMEHPKFEW	FRELELKWYA	LPVSNMLLE
Homo_sapiens_eNOS	LQAPDEPPEL	FILPPELVLE	VPLEHPTLEW	FAALGLRWYA	LPVSNMLLE
Branchiostoma_floridae_NOSA	LSANGEDPEW	FDIPRDIVME	VNITHPKYDW	FEELGIKWYC	VPVSNMLFD
Branchiostoma_floridae_NOSB	VSANGEDPVI	FDWPEVPLE	VELRHPKYDW	FEELAIKANC	IPMQDMLFD
Branchiostoma_floridae_NOSC	LSANGQDPEL	FELSKDLVLE	VELKHPKYGW	FKELGMKWYA	LPVSNMLFD
Branchiostoma_belcheri_NOSA	LSCNGDDPEW	FDIPKDIVME	VNITHPKYDW	FEELGIKWYC	LPVSNMLFD
Branchiostoma_belcheri_NOSB	VSANGEDPVI	FDWPEVPLE	LELRHPKHDW	FEELAIKVC	IPMQDMLFD
Branchiostoma_belcheri_NOSC	LSANGQDPEI	FELSKDLVLE	VEFKHPKYDW	FKELGIKWYA	LPVSNMLFD
Branchiostoma_lanceolatum_NOSA	LSANGEDPEW	FDLPKDIVME	VNITHPKYDW	FEELGIKWYC	VPVSNMLFD
Branchiostoma_lanceolatum_NOSB	VSANGEDPVI	FEWPEVPLE	LELRHPKHDW	FEELAIKVC	VPLODMLFD
Branchiostoma_lanceolatum_NOSC	LSANGQDPEL	FELSRDLVLE	VELKHPKYAW	FKELGMKWYA	LPVSNMLFD
Asymmetron_lucayanum_NOSA	LSANGEDPEW	FDIPKDIVME	VNITHPKYDW	FSELGIKWYC	VPVSNMLFD
Asymmetron_lucayanum_NOSB	VSANGEDPVI	FDWPEVPLE	LVLRHPKYDW	FEELNIKVC	IPMQDMLFD
Asymmetron_lucayanum_NOSC	LSANGQDPEL	FELSKDLVLE	VEIKHPKYEW	FKELGIWYA	VPVSNMLFD
Ciona_intestinalis_NOS	LQANGDPEL	YELPSNLVLE	VKLRHPDQEW	LEKMNWKWYA	VPVSNIMLN
Ciona_savignyi_NOS	LQANGDPEL	YELPANLVLE	VKLRHPDQDW	LEQMNWKWYA	VPVSNIMLD
Strongylocentrotus_purpuratus_NOSa	LQVNGEDPEV	FEIPPELIHE	VELAHPELRW	FMDLKLKWS	MPSYSNLLD

251

Homo_sapiens_nNOS	IGGLEFSACP	FSGWYMGTEI	GRDYCDNSRY	NILEEVAKKM	NLDMRKTSSL
Homo_sapiens_iNOS	VGGLEFPGCP	FNGWYMGTEI	GRDFCDVQRY	NILEEVGRM	GLETHKLASL
Homo_sapiens_eNOS	IGGLEFPAAP	FSGWYMSTEI	GRNLCDPHRY	NILEDVAVCM	DLDTTRTSSL
Branchiostoma_floridae_NOSA	CGGLEFTAAP	FSGWYMGTEV	GRDLCANRL	NITEAVGKRM	GLDVARNSL
Branchiostoma_floridae_NOSB	VGGMQFPACP	ITGWFOSTEP	VRDLDENRY	NMTEIIAKKL	GYDTSRYNSL
Branchiostoma_floridae_NOSC	VGGVEFPAAP	FSGWYMCTEI	GRDLCINRY	NFTEQIAKRM	GLDTGRASSL
Branchiostoma_belcheri_NOSA	CGGLEFTAAP	FSGWYMGTEV	GRDLCVNR	NITEAVGKRM	GLDIARNSSL
Branchiostoma_belcheri_NOSB	VGGMQFPACP	ITGWFOSTEP	VRDLDENRY	NMTEIIAKKL	GYDTSRYNSL
Branchiostoma_belcheri_NOSC	VGGVEFPAAP	FSGWYMCTEI	GRDLCVNR	NFTEQIAKRM	GLDTGRASSL
Branchiostoma_lanceolatum_NOSA	CGGLEFSAAP	FSGWYMGTEV	GRDLCENRL	NITEAVGKRM	GLDVARNSL
Branchiostoma_lanceolatum_NOSB	VGGMQFPACP	ITGWFOSTEP	VRDLDENRY	NMAEIIAKKL	GYDTSRYNSL
Branchiostoma_lanceolatum_NOSC	VGGVEFPAAP	FSGWYMCTEI	GRDLCINRY	NYTEQIAKRM	GLDTGRASSL
Asymmetron_lucayanum_NOSA	CGGVEFSAAP	FSGWYMGTEV	GRDLCVNR	NITETVGRM	GLDIARNSSL
Asymmetron_lucayanum_NOSB	CGGMHFPACP	ITGWFMSPE	VRDLDENRY	NFTEIIAKKL	GYDTSRYNSL
Asymmetron_lucayanum_NOSC	VGGIEX---	-----	-----	-----	-----
Ciona_intestinalis_NOS	IGGVEFPGVP	FNGWFMGTEI	GRDLLDTARY	NLILPVAEAM	GLNTKKTASL
Ciona_savignyi_NOS	IGGVEFPVP	FNGWFMGTEI	GRDLVDTARY	NMILPMAEAM	GLNTKKTSSL
Strongylocentrotus_purpuratus_NOSa	VGGIQFPACP	INERYVADI	ANAMCAPKRY	NMLTMIAERL	GLDISCTDSL

301

Homo_sapiens_nNOS	WKDQALVEIN	I AVLHSFQSD	KVTIVDHHS	TESFIKHMEN	EYRCRGGCPA
Homo_sapiens_iNOS	WKDQAVVEIN	I AVLHSFQKQ	NVTIMDHHS	AESFMKYMQN	EYRSRGGCPA
Homo_sapiens_eNOS	WKDKAAVEIN	I AVLHSYQLA	KVTIVDHHA	TASFMKHLEN	EQRKARGGCPA
Branchiostoma_floridae_NOSA	WKDAVFVEVN	I AVLHSFQRN	NVTIMDHHTA	SESFMKHLEN	EQRKRGCCPS
Branchiostoma_floridae_NOSB	WKDAAFLECH	I AVLHSFQMK	GVMTQDPYTL	TESFMKHMER	EHRVRGGCPS
Branchiostoma_floridae_NOSC	WRDLALIEAN	I AVLHSFQTC	NVTITDHHTA	CESEFMKHLEN	EQRKRGCCPA
Branchiostoma_belcheri_NOSA	WKDAVLVEVN	I AVLHSFQRS	NATIMDHHTA	SESEFMKHLEN	EQRKRGCCPS
Branchiostoma_belcheri_NOSB	WKDAAFLEVH	I AVLHFTQMN	GVMTQDPHTL	TESFMKHMER	EHRVRGGCPS
Branchiostoma_belcheri_NOSC	WRDLALIEAN	I AVLHSFQTC	NVTITDHHTA	CESEFMKHLEN	EQRKRGCCPA
Branchiostoma_lanceolatum_NOSA	WKDAVFVEVN	I AVLHSFQRN	NATIMDHHTA	SESEFMKHLEN	EQRKRGCCPS
Branchiostoma_lanceolatum_NOSB	WKDAAFLEVH	I AVIHSFQMK	GVMTQDPYTL	TESFMKHMER	EHRVRGGCPS
Branchiostoma_lanceolatum_NOSC	WRDLAMIEAN	I AVLHSFQTC	NVTITDHHTA	CESEFMKHLEN	EQRKRGCCPA
Asymmetron_lucayanum_NOSA	WKDAVLVEVN	I AVLHSFQSN	NVTISDHHTA	SETFIKHLEN	EQRKRGCCPS
Asymmetron_lucayanum_NOSB	WKDAAFLEVH	I AVLHSFQLE	GVMTQDPHTL	TESFMKHMER	EHRVRGGCPS
Asymmetron_lucayanum_NOSC	-----	-----	-----	-----	-----
Ciona_intestinalis_NOS	WKDKAILELN	I AVLHSFQDD	NVTIMDHHTA	TESFMKHFSN	EMKLRGGCPG
Ciona_savignyi_NOS	WKDKAILELN	I AVLHSFQSD	NVTIMDHHTA	TESFMKHFSN	EVKLRGGCPG
Strongylocentrotus_purpuratus_NOSa	WRDRTLVEMN	I AVLHSFQOY	GVTIMDHHTS	SAEFMKFFAS	EHEIROGCPT

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Homo_sapiens_nNOS	DVWVIVPPMS	GSITPVFHQE	MLNYRLTPSF	EYQDPWNTH	VWKGITNG-TK
Homo_sapiens_iNOS	DWIVLWPPMS	GSITPVFHQE	MLNYVLSPFY	YYQVEAWKTH	VWQDEKR--K
Homo_sapiens_eNOS	DWAWIVPPIS	GSLTPVFHQE	MVNYFLSPAF	RYQDPWPKGS	AAKG---TGI
Branchiostoma_floridae_NOSA	DWVWIVPPMS	ASLTFVFHQE	MANYHVRPSF	EYQEDAWLVH	LWRKKPKENP
Branchiostoma_floridae_NOSB	DWVWIVPPSC	SSLTPVFHQE	MLNYHLRPAL	EYQDAGWKIH	TWKTPPTIRT
Branchiostoma_floridae_NOSC	DWVWIVPPIS	GSLTPVFHQE	MISYYLRPSY	EYQEDAWMTH	VWKKKEDGKA
Branchiostoma_belcheri_NOSA	DWVWIVPPMS	ASLTFVFHQE	MANYHVRPSF	EYQEDAWLVH	LWRKKPKENP
Branchiostoma_belcheri_NOSB	DWVWIVPPSC	SSLTPVFHQE	MLNYHLRPAL	EYQAAGWTIH	TWKTPPTIRT
Branchiostoma_belcheri_NOSC	DWVWIVPPVS	ASLTPVFHQE	MISYYLRPSY	EYQEDAWKTH	VWKKKEDGKT
Branchiostoma_lanceolatum_NOSA	DWVWIVPPMS	ASLTFVFHQE	MANYHVRPSF	EYQEDAWLLH	LWRKKPEENP
Branchiostoma_lanceolatum_NOSB	DWVWIVPPSC	SSLTPVFHQE	MLNYHLRPAL	EYQEAGWKIH	TWKTPPTIRT
Branchiostoma_lanceolatum_NOSC	DWVWIVPPVS	ASLTPVFHQE	MISYYLRPSY	EYQEDAWKTH	VWKKKEDGKA
Asymmetron_lucayanum_NOSA	DWVWIVPPMS	ASLTFVFHQE	MAYYLRPSF	EYQDDAWLVH	LWRKKPEENP
Asymmetron_lucayanum_NOSB	DWVWIVPPSC	SSLTPVFHQE	MLNYHLRPAL	EYQEAGWKIH	EWKTLPTVRT
Asymmetron_lucayanum_NOSC	-----	-----	-----	-----DAWKTH	VWKKKEDGKA
Ciona_intestinalis_NOS	DWVWLVPPVS	GSATPIFHQE	ILNYTLNPAC	LYQDPWHYH	KWKDSVMKKR
Ciona_savignyi_NOS	DWVWLVPPVS	GSATPVFHQE	ILNYTLNPSC	LYQDPDWQYY	KWKDTVMVKKR
Strongylocentrotus_purpuratus_NOSa	DWRAVVSPLT	ASLTSLFHQE	TLNYQLMPTF	TVQDNPWEVP	EWKSKLSTDR

401

Homo_sapiens_nNOS	RRATGFKKLA	EAVKFSAKLM	QAMAKRVKA	TILYATETGK	SOYAKTLCCE
Homo_sapiens_iNOS	RREIPLKLV	KAVLFACMLM	RKTMASRVRV	TILFATETGK	SEALAWDLGA
Homo_sapiens_eNOS	TRKKTFKELV	NAVKISASLM	GTVMAKRVKA	TILYGSETGR	AQSYAQQLGR
Branchiostoma_floridae_NOSA	PRRCSLKEVA	LAVTFAAVLM	KKALDKRVKT	TILYATETGK	SESFASSTLG
Branchiostoma_floridae_NOSB	KKRCSFKAAA	MAVMFAAGLM	RKTLNKRKVS	TILFASQTGK	AETFSHSLCD
Branchiostoma_floridae_NOSC	KRKFGFKEVA	KAVKFSAKLM	GKALAKRVKA	TILYATETGK	SERYAKTICE
Branchiostoma_belcheri_NOSA	KRKYRLKEVA	LAVTFAAVLM	KKALHKRVKT	TILYATETGK	SESFATSTLG
Branchiostoma_belcheri_NOSB	KERC SFKAAA	MAVMFAAGLM	RKTLNRRVKS	TILFASQTGK	AETFSHSLCD
Branchiostoma_belcheri_NOSC	KRKFGFKEVA	KAVKFSAKLM	GKALAKRVKA	TILYATETGK	SERYAKTICE
Branchiostoma_lanceolatum_NOSA	KRKYRLKEVA	LAVTFAAVLM	KKALNKRKVT	TILYATETGK	SESFASSTLT
Branchiostoma_lanceolatum_NOSB	KKRCSFKAAA	MAVMFASGLM	RKTLNKRKVS	TILFASQTGK	AETFSNSLCD
Branchiostoma_lanceolatum_NOSC	KRKFGFKEVA	KAVKFSAKLM	GKALAKRVKA	TILYATETGK	SERYAKTICE
Asymmetron_lucayanum_NOSA	TRKYRFKEVA	LAVTFAAVLM	KKALDKRVKA	TILYATETGK	SESFASSTLG
Asymmetron_lucayanum_NOSB	KERC SFKAAA	LAVLFAAGSM	RKALDKRVKT	TILFASQTGK	AETFSHSVCD
Asymmetron_lucayanum_NOSC	KRKFGFKEVA	KAVKFSAKLM	GKALAKRVKA	TILFATETGK	SERYAKTICE
Ciona_intestinalis_NOS	KNKLHFKELA	RSVLMTSRMM	TKAMANRIKA	TILYASETGK	SYAYSKSLQE
Ciona_savignyi_NOS	KNKLHFKELA	KSMVMTSRMM	KKAMANRIKT	TILYATETGK	SYAYSLILQE
Strongylocentrotus_purpuratus_NOSa	LRSPG----A	DPSRFSSRIL	RRALSGTFKA	TVLYASEFGK	SEYYAKKVEE

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Homo_sapiens_nNOS	IFKHAFDAKV	MSMEEYDIVH	LEHETLVLVV	TSTFGNGDPP	ENGEKFGCAL
Homo_sapiens_iNOS	LFSCAFNPKV	VCMKYRLSC	LEERLLLIVV	TSTFGNGDCP	GNGEKLKKSLL
Homo_sapiens_eNOS	LFRKAFDPRV	LCMDEYDVVS	LEHETLVLVV	TSTFGNGDPP	ENGE SFAAAL
Branchiostoma_floridae_NOSA	IFKHAFDAKM	MCMDEYDITH	LEKEELVIVV	TSTFGNGDPP	DNGEAFGQAL
Branchiostoma_floridae_NOSB	LYKHAFDAKV	ICMDEYDVTO	MDKEQLVIVV	TSTFGNGDPP	DNGETFARAL
Branchiostoma_floridae_NOSC	IFKHAFDAKV	MCMDDYDIMH	LEHETLVIVV	TSTFGNGDPP	DNGESFGQAL
Branchiostoma_belcheri_NOSA	IFKHAFDAKI	MCMDEYDITN	LEKEELVIVV	TSTFGNGDPP	DNGEAFGQAL
Branchiostoma_belcheri_NOSB	LYKHAFHAKV	VCMDEYDMTO	LDKEQLVIVV	TSTFGNGDPP	DNGKTFARAL
Branchiostoma_belcheri_NOSC	IFKHAFDAKV	MCMDDYDIMH	LEHETLVIVV	TSTFGNGDPP	DNGESFGQAL
Branchiostoma_lanceolatum_NOSA	IFKHAFDAKM	MCMDEYDITN	LEKEELVIVV	TSTFGNGDPP	DNGEAFGQAL
Branchiostoma_lanceolatum_NOSB	LYKHAFDAKV	VCMDEYDMAQ	LDKEQLVIVV	TSTFGNGDPP	DNGETFARAL
Branchiostoma_lanceolatum_NOSC	IFKHAFDAKV	MCMDDYDIMH	LEHETLVIVV	TSTFGNGDPP	DNGESFGQAL
Asymmetron_lucayanum_NOSA	IFKHAFDAKM	MCDDYDVTE	LEKEDLVIIV	TSTFGNGDPP	DNGEAFGQAL
Asymmetron_lucayanum_NOSB	LYKHAFDAKV	VCMDEYDISR	LSKEQLVVVV	TSTFGNGDPP	DNGETFARAL
Asymmetron_lucayanum_NOSC	IFKHAFDAKV	MCMDDYDIMH	LEHETLVIVV	TSTFGNGDPP	DNGESFGQAL
Ciona_intestinalis_NOS	IFNHAFDAKV	VPMDQYDIGN	LEYEALVLIV	TSTFGNGDPP	ENGEYLAQAL
Ciona_savignyi_NOS	IFNHAFDAKV	VAMDQYDIGN	LEYESMLLVV	TSTFGNGDPP	ENGEDF--LL
Strongylocentrotus_purpuratus_NOSa	TFRKAFDIRL	LRMDEYDVMN	LEHENLLLIVV	TSTLGNGEPP	SNGQEFCRYL

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Homo_sapiens_nNOS	MEMRHPNSVQ	EERKSVRFVS	FGLGSRAYPH	FCAFGHAVDT	LLEELGGERI
Homo_sapiens_iNOS	FMLKELNNK-	-----FRYAV	FGLGSSMYPR	FCAFAHDIDQ	KLSHLGASQL
Homo_sapiens_eNOS	MEMSGPYNS	PRPEQLRFV	FGLGSRAYPH	FCAFAHAVIDT	RLEELGGERL
Branchiostoma_floridae_NOSA	LHMRHPPGSD	GKKRSVRYGV	FALGSRAYPH	FCAFGHAIDT	LFEELGAERI
Branchiostoma_floridae_NOSB	MQMKKQNGQT	SPGDSVRYAV	FALGSRAYPH	FCFGHSLNT	LLEQLGAQRV
Branchiostoma_floridae_NOSC	LEMRRHPPMDN	NDNRPVRFVS	FGLGSRAYPG	FCAFAHAVDT	LFGEELGGERI
Branchiostoma_belcheri_NOSA	LHMRHPPGND	GKKKSVRYCV	FALGSRAYPH	FCAFGHAIDT	LFEELGAERI
Branchiostoma_belcheri_NOSB	MQMKKQKQDT	SPDNNVRFV	FALGSRAYPH	FCFGHSSVDT	LLDQLGAQRI
Branchiostoma_belcheri_NOSC	LEMRRHPPMDN	NDNRPVRFSA	FGLGSRAYPG	FCAFAHAVIDT	LLGELGGERI
Branchiostoma_lanceolatum_NOSA	LHMRHPPGSD	KKRSVRYGV	FALGSRAYPH	FCAFGHAIDT	LFESLGAERI
Branchiostoma_lanceolatum_NOSB	MQMKKQNGQA	SPRDSVRFV	FALGSRAYPH	FCAFGHSSVDT	LLEQLGARRV
Branchiostoma_lanceolatum_NOSC	LEMRRHPPMDN	NDNRPVRFSA	FGLGSRAYPG	FCAFAHAVDT	LFGEELGGERI
Asymmetron_lucayanum_NOSA	LHMRHPPGSD	GKKKSVRYAV	FALGSRAYPH	FCAFGHAINT	LFEELGAERI
Asymmetron_lucayanum_NOSB	MQMKKNSGQ-	-----VRFV	FALGSRAYPH	FCFGHSSVDT	LFEQLGARRV
Asymmetron_lucayanum_NOSC	LEMRRHPPTDN	NDNKA	-----	-----	-----
Ciona_intestinalis_NOS	QKIVRDSKED	QKKAQVRFV	FGLGSRAYPH	FCAFAHAMDT	FLGELEGERL
Ciona_savignyi_NOS	FSFINIEHGI	HKIKSSRFV	FGLGSRAYPH	FCAFAHAVDT	LLGELEGERV
Strongylocentrotus_purpuratus_NOSa	MEMSDNNNR	SPVPQLSYGV	FGLGSSIYPY	YCAFARGVDQ	MLDSLGGDRL

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Homo_sapiens_nNOS	LKMREGDEL	GQEEAFRTWA	KKVFKAACDV	FCVGGDDVNI	KANNSLISNS
Homo_sapiens_iNOS	TPMGEDEL	GQEDAFRSWA	VQIFKAACET	FDVRGKQHIQ	IP--KLYTST
Homo_sapiens_eNOS	LQLGQDEL	GQEEAFRGWA	QAQFAACET	FCVGEDA--K	AAARDIFSPS
Branchiostoma_floridae_NOSA	HPVGEDEL	GQEESFRAWA	KGAFKSACER	YDVGHVNM	EANASLLGSS
Branchiostoma_floridae_NOSB	HAVGEGDEL	GQEESFRAWA	ESAFKSACLS	YDVGHGVDM	EATANLLGSS
Branchiostoma_floridae_NOSC	YKMGEDEL	GQEE SFRKWA	KGVFKAACDT	FCVGDDELNMS	EASATLLNST
Branchiostoma_belcheri_NOSA	HPVGEDEL	GQEE SFRAWV	KGAFKSACER	YDVGHVNM	EANASLLGSS
Branchiostoma_belcheri_NOSB	HAVGEGDEL	GQEE SFRTWA	ESAFKSACLS	YDVGHGVDM	EATANLLGSS
Branchiostoma_belcheri_NOSC	YKMGEDEL	GQEE SFRKWA	KGVFRAACDT	FCVGGDDLNMN	EASATLLNTT
Branchiostoma_lanceolatum_NOSA	HPVGEDEL	GQEE SFRVWV	KGAFKSACER	YDVGHVNM	EANASLLGSS
Branchiostoma_lanceolatum_NOSB	HAVGEGDEL	GQEE SFRAWA	ESAFKSACLS	YDVGHGVDM	EATANLLGSS
Branchiostoma_lanceolatum_NOSC	YKMGEDEL	GQEE SFRKWA	KGVFKAACDT	FCVGGDDLNMS	EASSTLLNST
Asymmetron_lucayanum_NOSA	HPVGEDEL	GQEE SFRAWV	KGAFKSACER	FDVGHVNM	EANASLLGNS
Asymmetron_lucayanum_NOSB	HTVGEDEL	GQEE SFRAWA	ESAFKSSCLS	FDVGHVDM	EATANLLGSS
Asymmetron_lucayanum_NOSC	-----P	GHTKRLSSIS	SSAT	-----	-----
Ciona_intestinalis_NOS	MEIGEGDEL	GQEE SFRVWA	QEVFTSACDT	FCLREQVNLE	DANASLSTSK
Ciona_savignyi_NOS	KEIGEGDEM	GQEECFRNWA	QQVFQGGNS	FDRLLI FNLE	DANASLKSTK
Strongylocentrotus_purpuratus_NOSA	VGMGWGDEM	GLDETDFDAWL	TKTFQKYAWL	FFIFDYTVLS	IFVCELILKG

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Homo_sapiens_nNOS	WKRNFRLTF	VAEAPELTQG	LSNVHKKRV	AARLLSRQNL	QSPKSSRSTI
Homo_sapiens_iNOS	WDPHRYRL--	VQDSQPLSKA	LSSMHAKNVF	TMRLKSRQNL	QSPTSSRATI
Homo_sapiens_eNOS	WKRQRYRLAQ	AEGQL--LLPG	LIHVHRRKMF	QATIRSVENL	QSSKSTRATI
Branchiostoma_floridae_NOSA	WAPGKFRIVQ	TKGLPEVLEG	LKSLHRRTL	SSTVISRQTL	QAPESGRQTC
Branchiostoma_floridae_NOSB	WAPDKFRLVQ	AKSNRDLLEG	LSTVHCKSVV	SCSFI SRQTL	QAPDSSRQTI
Branchiostoma_floridae_NOSC	WAADKFRLLP	AEGVKEIWE	LKSVH SRNVV	PCRLI SREN	QAPDSGRETI
Branchiostoma_belcheri_NOSA	WAPGKFRIVQ	TKGLPEILEG	LKSLHRRTV	SSTVISRQTL	QAPESRQTC
Branchiostoma_belcheri_NOSB	WAPDKFRLVQ	AKSNKDLLEG	LSTVHRKNV	PCTFISRQTL	QAPDSSRQTI
Branchiostoma_belcheri_NOSC	WSADKFRLLP	AEGVMEIWE	LKSVH SRTVV	PCRLI SREN	QAPDSGRETI
Branchiostoma_lanceolatum_NOSA	WAPGKFRVQ	TKGLPEILEG	LKSLHRRTV	SSTVISRQTL	QAPESRQTC
Branchiostoma_lanceolatum_NOSB	WAPDKFRLVQ	AKTKTDILQ	LSTVHRKNIV	PCTFISRQTL	QAPDSSRQTI
Branchiostoma_lanceolatum_NOSC	WSPDKFRLLP	AEGVKEIWE	LKSVH HRNVV	PCRLI SREN	QAPDSGRETI
Asymmetron_lucayanum_NOSA	WAPGKFHVQ	TKGLPEILEG	LKSLHRRTV	SSTVISRQTL	QAPESRQTC
Asymmetron_lucayanum_NOSB	WAPDKFRLVQ	AKGTRDLLEG	LSTLHRKVV	SCTFISRQTL	QAPDSSRQTI
Asymmetron_lucayanum_NOSC	-----	-----	-----ERRRK	SROXISRQTL	QAPESRQTC
Ciona_intestinalis_NOS	FERTDFRVVH	DKVQVPIKT	LGTMRKCLS	SCRLIRKKNL	QSEESGRSTI
Ciona_savignyi_NOS	FDKSNFRVVS	DVQVEPV--	-----	-----	---ESKRSTI
Strongylocentrotus_purpuratus_NOSA	WNILDFFIIV	LIILGPKFLG	SSRILRILRV	LRAFRSLR--	-----SRSTI

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Homo_sapiens_nNOS	FVRLHTNGSQ	ELQYQPGDHL	GVFPNGHEDL	VNALIERLED	APPVNMVKV
Homo_sapiens_iNOS	LVELSCEDGQ	GLNYLPGEHL	GVCPGNQPAL	VQGILERVVD	GTPHQTVRL
Homo_sapiens_eNOS	LVRLDITGGQE	GLQYQPGDHI	GVCPPNRPG	VEALLSRVED	PPAPTEPVAV
Branchiostoma_floridae_NOSA	LVELETHGAQ	ELKYVPGDHV	AIFPANEDRL	VQAILDRLEK	GTNPDAVILI
Branchiostoma_floridae_NOSB	LVQLDTKGNK	ELQFTPGDHL	GVFPANEDRL	VQEILDRIEG	GTNPDDVLEI
Branchiostoma_floridae_NOSC	LVRLDISQSD	DLNYVPGDHL	AVFPANEDHL	VQAVLDRLDN	APDPDSIVNM
Branchiostoma_belcheri_NOSA	LVQLETHGAH	ELKYVPGDHV	AVFPANEDRL	VQAILDRLEK	GTNPDAVIQI
Branchiostoma_belcheri_NOSB	LVQLDTKGRE	ELKFSPGDHL	GIFPANENHL	VQRILGRIDG	GTNPDDVLEI
Branchiostoma_belcheri_NOSC	LVRLDISQSD	DLNYVPGDHL	AVFPANEDHL	VQAVLDRLDN	APDPDSIVNM
Branchiostoma_lanceolatum_NOSA	LVQLETHGAQ	ELRYVPGDHV	AVFPANEDRL	VQAILDRVEK	GTNPDAVIQI
Branchiostoma_lanceolatum_NOSB	LVRLDITKNE	ELEFTPGDHL	GVFPANEDHL	VQAILDRIEG	GTNPDDVLEI
Branchiostoma_lanceolatum_NOSC	LVRLDISQSD	DLNYVPGDHL	AVFPANEDHL	VQAVLDRLDN	APDPDSIVNM
Asymmetron_lucayanum_NOSA	LVRLETHGAH	ELRYVPGDHV	GIFPANEERL	VREILDRLEQ	GTNPDCVIQI
Asymmetron_lucayanum_NOSB	LVRLDITKNE	ELKFTPGDHL	GVFPANEDHL	VQAILDRVEG	GTNPDEVLEV
Asymmetron_lucayanum_NOSC	LVRLGTHGDH	ELRYASGDH	GIFPANEDRL	VQEILDRMDK	GTNPDDVIQT
Ciona_intestinalis_NOS	LIQLETKNCE	NLNFQPGDHL	GIYPRNDPDL	VKELLSKIRD	ATDENTVVVL
Ciona_savignyi_NOS	LIQLEKKNSD	VLNFPQGDHL	GIYPRNDPAL	VKALISKIRD	ATDDSTVVRL
Strongylocentrotus_purpuratus_NOSA	LVRLLNTKER	GMAVQPGDHV	AFFPSNENAL	VARVLKRLDY	QKSPDAIFKL

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Homo_sapiens_nNOS	ELLERNNTWT	DELRLPPCTI	FQAFKYLDI	TTPPTPLQLQ	QFASLATSEK
Homo_sapiens_iNOS	EALESGSYVW	SDKRLPPCSL	SQALTYFLDI	TTPPTQLLLQ	KLAQVATEEP
Homo_sapiens_eNOS	EQLKSGSWV	RDRPLPPCSL	RQALTFFLDI	TSPSPQLLR	LLSTLAEPR
Branchiostoma_floridae_NOSA	EALKKSTWT	SHNRLPPCSL	RTALSRYLDV	TTPSPQLLL	YLAMHTSSR
Branchiostoma_floridae_NOSB	EALKKLTWV	PFERLPPCSM	RTALSRYLDV	TTPSPQLLG	SLAVHTGEPG
Branchiostoma_floridae_NOSC	EVLEKQTSWM	TSERLPPCSL	RTALSRYFDI	TTPSPQLLQ	HLATQATDEE
Branchiostoma_belcheri_NOSA	EALKKSTWT	PHDRLPCCSL	RTALSRYLDV	TTPSPQLLL	YLAMHATSNR
Branchiostoma_belcheri_NOSB	EALKKTVTW	PFERLPPCSL	RTALSRYLDI	TTPSPQLLG	SLAMHTNDIA
Branchiostoma_belcheri_NOSC	EVLEKQTTWM	TSERLPPCSL	RTALSRYFDI	TTPAPQLLQ	HLATQATDEE
Branchiostoma_lanceolatum_NOSA	EALKKISWT	PHDRLPTCSL	RTALSRYLDV	TTPSPQLLL	YLAMHATSSR
Branchiostoma_lanceolatum_NOSB	EALKKLTWV	PFERLPPCSL	RTALSRYLDI	TTPSPQLLG	SLSMHTSAPS
Branchiostoma_lanceolatum_NOSC	EVLEKQTTWM	TSERLPPCSL	RTALSRYFDI	TTPSPQLLQ	HLATQATDEE
Asymmetron_lucayanum_NOSA	EAQEKSTWT	THERLPPCSL	RTALSRYLDI	TTPSPQLLL	YLAMHATSSR
Asymmetron_lucayanum_NOSB	EALKKLTWV	PFERLPPCSL	RTALSRYLDI	TTPPTPQLLG	SLSMHAAPE
Asymmetron_lucayanum_NOSC	-----E	-----	-----	-----	-----
Ciona_intestinalis_NOS	EAKKRSYWE	EDTRLPPCTL	NMAFSRYLDI	TTPPTPQLLK	LFSECDVNEE
Ciona_savignyi_NOS	E-----YWD	ADTRLPSCTL	TMAFSRFLDI	TTPPTPQLLK	LFSECVNEE
Strongylocentrotus_purpuratus_NOSA	EKLPRETVWT	TVSRLPTCSL	RTALERYLDL	TTPPTPDFLR	LLATRADPR

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Homo_sapiens_nNOS	EKQRLLVLSK	GLOEYEEWKW	GKNPTIVEVL	EEFPSIQMPA	TLLLTQLSLL
Homo_sapiens_iNOS	ERQRLLEALCQ	-PSEYSKWK	TNSPTFLEVL	EEFPSLRVSA	GFLLSQLPIL
Homo_sapiens_eNOS	EQQLEALSQ	DPRRYEYWKW	FRCP TLLEVL	EQFPSVALPA	PLLLTQLPLL
Branchiostoma_floridae_NOSA	ERAELALGK	GGARYEDWK	EAAPTLPPEVL	QDYP SLQVPP	ALLLSQLPVL
Branchiostoma_floridae_NOSB	ERAELLELAK	GGTKYENWK	ERAPTLPPEVL	QDYP SLQVPA	ALLLSQLPVL
Branchiostoma_floridae_NOSC	EKKELEVLGK	GDSRYEDWK	ERTPNLPEVL	EDYPSLKVAP	TLLLSQLPFL
Branchiostoma_belcheri_NOSA	ERAELQALGK	GGLKYEDWK	EVAPTLPPEVL	QDYP SLQVPP	ALLLSQLPVL
Branchiostoma_belcheri_NOSB	ERAGLQELAK	GGSKYEDWK	EHAPTLPPEVL	QEFPSLTVPP	ALLLSQLPVL
Branchiostoma_belcheri_NOSC	EKKELEVLGK	GDSRYEDWK	ERTPNLPEVL	EDYPSLKVAP	TLLLSQLSFL
Branchiostoma_lanceolatum_NOSA	ERAELALGK	GGLRYEDWK	EAAPTLPPEVL	QHYP SLQVPP	ALLLSQLPVL
Branchiostoma_lanceolatum_NOSB	ERAELLELAK	GGTKYENWK	ERAPTLPPEVL	QEYPSLQVPP	ALLLSQLPVL
Branchiostoma_lanceolatum_NOSC	EKKELEVLGK	GDARYEDWK	ERTPNLPEVL	EDYPSLKVAP	TLLLSQLPFL
Asymmetron_lucayanum_NOSA	ERTELEALGK	GGPRYEDWK	EVAPTLPPEVL	QDFPSLQVPP	ALLLSQLPVL
Asymmetron_lucayanum_NOSB	EMAELLEELGK	GGKKYESWK	ERAPTLPPEVL	QNYPSLKVPP	ALLLSQLPVL
Asymmetron_lucayanum_NOSC	-----	-----	-----	-----	-----
Ciona_intestinalis_NOS	ESKRLEVLISQ	GQSEYEDWK	LNNPNLIEVL	NOFPSIQLEA	ALLLKQLPLL
Ciona_savignyi_NOS	QSTALRILGQ	GESEYEDWK	SNNPNLPEVL	NEFPSTQVDA	SLLLKQLPLL
Strongylocentrotus_purpuratus_NOSa	DKEKLSILGA	GCDEYEEWK	ENWPNMAEVL	EGYPSLKVPA	SLLLTQLPLL

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Homo_sapiens_nNOS	QPRYYSISS	PDMYPDEVHL	TVAIVSYRTR	DGEGPIHGV	CSSLNRIQA
Homo_sapiens_iNOS	KPRFYSISS	RDHTPTEIHL	TVAVVYHTG	DGQGPLHGV	CSTWLSLKP
Homo_sapiens_eNOS	QPRYYSVSSA	PSTHPGEIHL	TVAVLAYRTG	DGLGPLHYGV	CSTWLSLKP
Branchiostoma_floridae_NOSA	QQRYSISS	PHMYPGQIHA	TVDMVKYRTR	GGKGPEHSGV	CSNWLNNTINI
Branchiostoma_floridae_NOSB	QQRYSISS	PHMYRGEIHA	TLAVVSYRSQ	GGKGP IHNGV	CSSWFNRIE
Branchiostoma_floridae_NOSC	QQRYSISS	QLMYPGEIHA	TVAVVKFATQ	GGVGP IHNGV	CSSLNRIEK
Branchiostoma_belcheri_NOSA	QQRYSISS	PHMYPGQIHA	TVAVVKYRTR	GGKGP EHN	CSNWLNNTINI
Branchiostoma_belcheri_NOSB	QQALFHTAPP	PHMYPGQIHA	TLAVVSYRTR	GGKGP THNGV	CSSLNRIE
Branchiostoma_belcheri_NOSC	QQRYSISS	QRMFYPGEIHA	TVAVVRFATQ	GGVGP IHNGV	CSSLNRIEK
Branchiostoma_lanceolatum_NOSA	QQRYSISS	PHMYPGQIHA	TVAVVKYRTR	GGQGP EHS	CSNWLNNTINI
Branchiostoma_lanceolatum_NOSB	QQRYSISS	PHMYPGEIHA	TLAVVSYRSQ	GGKGP VHNGV	CSSWFNLEE
Branchiostoma_lanceolatum_NOSC	QQRYSISS	QRMYPGEIHA	TVAVVRFATQ	GGVGP THNGV	CSSLNRIEK
Asymmetron_lucayanum_NOSA	QQRYSISS	PHMHPGEIHA	TVAVVKYRTR	GGQGP EHR	CSNWLNNTINI
Asymmetron_lucayanum_NOSB	QQRYSISS	PHMYPGEIHA	TLAVVSFRAQ	GGKGP VHNGV	CSSWFNRIE
Asymmetron_lucayanum_NOSC	-----	-----	-----	-----	-----
Ciona_intestinalis_NOS	QARYYSISS	PEIFYPGEIHC	TVSVVYHTR	GGTGPLHGV	CSSYLNITD
Ciona_savignyi_NOS	QARYYSISS	PDVYPGEIHC	TVSVVYHTR	GGDGPLHGV	CSSYLNITD
Strongylocentrotus_purpuratus_NOSa	RPRYFSISS	PKQYPNEIHA	TVAVVSYHPN	GGRGPVHYGA	CSSWLYRAWK

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Homo_sapiens_nNOS	DELVPCFVRG	APSFHLP RNP	QVPCILVGGP	TGIAPFRSFW	QQRQFDIQHK
Homo_sapiens_iNOS	QDPVPCFVRN	ASAFHLPEDP	SHPCILIGPG	TGIAPFRSFW	QQR LHDSQHK
Homo_sapiens_eNOS	GDPVPCFIRG	APSFRLPPDP	SLPCILVGGP	TGIAPFRSFW	QER LHDIKSK
Branchiostoma_floridae_NOSA	NE SVPCFIRT	AKNFHLPEDP	SLPVL MVGGP	TGIAPLRSFW	QQRQVDIKTG
Branchiostoma_floridae_NOSB	GETVPCFVRP	AKNFHLPDDP	SMPVL MVGGP	TGIAPLRSFW	QQRQMEIKSG
Branchiostoma_floridae_NOSC	DDIVPCFVRA	AQNFHLPEDP	TVPLMMVGGP	TGIAPFRSFW	QHRQMEVTSG
Branchiostoma_belcheri_NOSA	NE SVPCFIRT	AKNFHLPEDS	SLPVL MVGGP	TGIAPLRSFW	QQRQVDIKAG
Branchiostoma_belcheri_NOSB	GETVPCFVRT	AKNFHLPDDP	SVPVL MVGGP	TGIAPLRSFW	QQRQADIKSG
Branchiostoma_belcheri_NOSC	DDIVPCFVRA	AQNFHLPEDP	TVPLMMVGGP	TGIAPFRSFW	QQRQMEVGS
Branchiostoma_lanceolatum_NOSA	NE SVPCFIRT	AKNFHLPENS	SLPVL MVGGP	TGIAPLRSFW	QQRQVDIKAG
Branchiostoma_lanceolatum_NOSB	GETVPCFVRA	AKNFHLPADP	SMPVL MVGGP	TGIAPLRSFW	QQRQMDIKSG
Branchiostoma_lanceolatum_NOSC	DDIVPCFVRG	AQNFHLPEDP	TVPLMMVGGP	TGIAPFRSFW	QHRQMEVTSG
Asymmetron_lucayanum_NOSA	GDSVPSFIRT	AKNFHLPENP	SLPVL MVGGP	TGIAPLRSFW	QQRQVDIKSG
Asymmetron_lucayanum_NOSB	GETVPCFVRP	AKNFHLPDDP	IMPVL MVGGP	TGIAPLRSFW	QQRQMDISSG
Asymmetron_lucayanum_NOSC	-----	-----	-----	-----	-----
Ciona_intestinalis_NOS	DEEVACFVRT	APT FHLPLKD	NIP CILVGGP	TGIAPFRSFW	QQR LAEIRKG
Ciona_savignyi_NOS	EEEVPCFVRM	APT FHLPTKE	DAP CILVGGP	TGIAPFRSFW	QHRISQIQKG
Strongylocentrotus_purpuratus_NOSa	DEVIPAFVSS	APYFHLPADT	SVPVIMVGGP	SGVAPFRSFW	QDRQTDLRQG

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Homo_sapiens_nNOS	GMCPMVLVFG	CROSKIDHIY	REETLQAKNK	GVFRELYTAY	SREPDKPKKY
Homo_sapiens_iNOS	GVGRMTLVFG	CRRPDEDHIY	QEEMLEMAQK	GVLHAVHTAY	SRLPGKPKVY
Homo_sapiens_eNOS	GLTPMTLVFG	CRCSQLDHLIY	RDEVQNAQQR	GVFGRVLTAF	SREPDPNPKTY
Branchiostoma_floridae_NOSA	TTGDMTLVFG	CROSRLDHIY	KEETAQARRD	GALKDHTAL	SREPDTTKTY
Branchiostoma_floridae_NOSB	NHGDMTLVFG	CROSQIDHIY	KEETAQAKSD	GALTDIHTAY	SREPGTPKTY
Branchiostoma_floridae_NOSC	DPGQMTLVFG	CROSKMDDIY	KHETAQAKED	GALTEVYTAL	SREPGVPKSY
Branchiostoma_belcheri_NOSA	SNGDMTLVFG	CROSRVDHIY	KEETAQARRD	GALTDIYTAL	SREPGTNKTY
Branchiostoma_belcheri_NOSB	DHGDMMLVFG	CROSETDHIY	REETAAQQT	GALTDIYTAY	SREPGRPKTY
Branchiostoma_belcheri_NOSC	DPGQMTLVFG	CROSKMDDIY	KHETAQAKED	GALTEVYTAL	SREPGVPK--
Branchiostoma_lanceolatum_NOSA	TTGDMTLVFG	CROSRVDHIY	KEETAQARRD	GALTDLYIAL	SREPGTTKTY
Branchiostoma_lanceolatum_NOSB	NHGDMTLVFG	CROSQIDHIY	KETAQAKTD	GALTDIYTAY	SREPDTPKTY
Branchiostoma_lanceolatum_NOSC	DPGQMTLVFG	CROSKMDDIY	KHETAQAKED	GALTEVYTAL	SREPGVPKSY
Asymmetron_lucayanum_NOSA	TNGDMTLVFG	CROSKLDHIY	KEEVAQAERD	GALSDIHTAL	SREPGTPKMY
Asymmetron_lucayanum_NOSB	KIGDMTLVFG	CROSKMDHIY	QEETARAKKD	GALTDIYTAY	SREPPTPKTY
Asymmetron_lucayanum_NOSC	-----	-----	-----	-----	-----
Ciona_intestinalis_NOS	AHCTMALLFG	CRNP NHDHLY	KQEVAAAMKE	GAI SHAYTAF	SREK GKPKRY
Ciona_savignyi_NOS	DR-TMALLFG	CRNP QDHLIY	KDEVE TAMKQ	GAI SHAYTAF	SREEGKPKQY
Strongylocentrotus_purpuratus_NOSa	EGGDMTLVYV	CRNAKED-LY	KGETSVAKDE	GALTAVLTGY	SREPGKPKRY

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Homo_sapiens_nNOS	VQDILQEQLA	ESVYRALEQG	GHIYVCGDVT	MAADV LKAIQ	RIMTQQGKLS
Homo_sapiens_iNOS	VQDILRQQLA	SEVLRVLKEP	GHLYVCGDVR	MARDVAHTLK	QLVAAKLLKN
Homo_sapiens_eNOS	VQDILRTELA	AEVHRVLLER	GHMFVCGDVT	MATNVLQTVQ	RILATEGDME
Branchiostoma_floridae_NOSA	VQDIIRQQMP	RKVLDLVRDG	GHVYVCGDVT	MATDVCETIQ	RILVEHGGMS
Branchiostoma_floridae_NOSB	VQHVLYNQLS	EKVKELLENS	GHIYVCGDVA	MADDVCTTVQ	KILEEKSGMS
Branchiostoma_floridae_NOSC	VQNVILDLIP	EKVC DLLNHN	GHFYVCGDVS	MAADV CNTLE	KVMEKQQGMA
Branchiostoma_belcheri_NOSA	VQDILRERIP	RKVLDLVREG	GHVYVCGDVT	MATDVCETVQ	RILVKHGGMS
Branchiostoma_belcheri_NOSB	VQHILHNQLS	KRVQELLDSD	GHIYVCGDVA	MADDVCTTVQ	KILEENSAMT
Branchiostoma_belcheri_NOSC	--NVILDQIP	EKVC DLLKQN	GHFYVCGDVS	MAADV CNTLE	KVLEKQQGLT
Branchiostoma_lanceolatum_NOSA	VQDIIRQQIP	KKVLDLVKDG	GHIYVCGDVT	MATDVGETVQ	RILVEHGGMS
Branchiostoma_lanceolatum_NOSB	VQHVLYNQLS	ERVKELLKNN	GHIYVCGDVA	MADDVCTTVQ	KILEEKSAMT
Branchiostoma_lanceolatum_NOSC	VQNVILDLIP	EKVC DLLKHN	GHFYVCGDVS	MAADV CNTLE	KAMEKQQGMT
Asymmetron_lucayanum_NOSA	VQDILRQQIS	RKVRDLVRDG	GHIYVCGDVT	MATDVCTAVQ	KILEEHGGMS
Asymmetron_lucayanum_NOSB	VQHVLHNKLS	KRVQELLENN	GHIYVCGDVA	MADDVCTTVQ	KILEETS GMK
Asymmetron_lucayanum_NOSC	VQNVILDQIP	QKVC DLLKHN	GHFYVCGDVS	MAADV CNTLE	TVMEKEGGMT
Ciona_intestinalis_NOS	VQNVIMSEIP	DFVYDIVEKK	GHFYVCGDVT	MATEVNKTL	KILCIKGGMT
Ciona_savignyi_NOS	VQDVIMNDIS	EFVYDLITRN	GHFYVCGDVT	MAAEVHRTLK	DVLCINGGMT
Strongylocentrotus_purpuratus_NOSa	VQDILKDH-P	WLVDYVLRRO	GHIYVSGDVN	MVREVSNTIE	GIIAQFGRVG

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Homo_sapiens_nNOS	AEDAGVFISR	MRDDNRYHED	IFGVTLRTYE	VTNRLRSIEE	SKKDTIDE
Homo_sapiens_iNOS	EEQVEDYFFQ	LKSQKRYHED	IFGAVFPYEA	KKDRVA-VQP	SSLEMSA
Homo_sapiens_eNOS	LDEAGDVIQV	LRDQQRHED	IFGLTLRTQE	VTSRIRTFDP	PGSDTNS
Branchiostoma_floridae_NOSA	RARAEDFINT	MKDNNRYHED	IFGVTLKTHE	VENEARLVT	SDDEDTS
Branchiostoma_floridae_NOSB	STESEAFIRN	LKDSNRYHED	IFGVTLNTKA	VREAARSVEP	ADSIANG
Branchiostoma_floridae_NOSC	PNRAKDFVDK	LKDCNRYHED	IFGVTLRTQE	VTDRVRSVRE	VGENTLI
Branchiostoma_belcheri_NOSA	AARAEDFINN	MKDNNRYHED	IFGVTLKTHE	VEDAARKFTS	VNYHFSN
Branchiostoma_belcheri_NOSB	STESEAFIRS	LKDSNRYHED	IFGVTLNTKA	VREAARNTPE	SESTANA
Branchiostoma_belcheri_NOSC	PTKAKDFVDK	MKDCNRYHED	IFGVTLRTQE	VTDRVRSLSL	SSSLSST
Branchiostoma_lanceolatum_NOSA	VARAEDFINN	MKDNNRYHED	IFGVTLKTHE	VEDAARKISL	SEENTSL
Branchiostoma_lanceolatum_NOSB	STESEALIRM	LKDSNRYHED	IFGVTLNTKE	VREAARS---	---VESA
Branchiostoma_lanceolatum_NOSC	PNKAKDFVDK	LKDCNRYHED	IFGVTLRTQE	VTDRVRSVRE	SSSLSST
Asymmetron_lucayanum_NOSA	AARAEDFVNN	MKDNNRYHED	IFGVTLKTHE	VQDAARKLMV	SDGDSTR
Asymmetron_lucayanum_NOSB	ADESEAFIRN	LKDSNRYHED	IFGVTLNTTE	VREAARNVEN	GESLDNG
Asymmetron_lucayanum_NOSC	AKNAKDFVDK	LKDCNRYHED	IFGVTLRTQE	VTDRVRSVRE	SSSLSST
Ciona_intestinalis_NOS	KEKAQEFINS	MKTEERYHED	IFGVTLRTYE	VNRSVRLKEK	SESLSHK
Ciona_savignyi_NOS	PEQVEEYITA	MKSEDRYHED	IFGVTLRTYE	VNRSL-----	-----
Strongylocentrotus_purpuratus_NOSa	AAEAKDFVRR	IKDEDHYHEE	MYGLTLHVSE	VMEKIRAVRI	EEKAVII

Supplementary Table S6

List of primers used for *Branchiostoma lanceolatum* Nos cloning and real time PCR experiments.

Primer name	Primer sequence		PCR product length
Cloning			
<i>Nos-A</i>	FORWARD	CTGAAGTACGCCTACGATG	1076 bp
	REVERSE	GATTTGACCAAACCAGCTCC	
<i>Nos-B</i>	FORWARD	CGGTACAATCCAGAGAAACG	830 bp
	REVERSE	GTTCCAGTTCCAGGGGTACG	
<i>Nos-C</i>	FORWARD	TCGGCCGAACGTAATTGCCG	787 bp
	REVERSE	CAGCCAGTTCTTCATGCGGGC	
qPCR			
<i>Nos-A</i>	FORWARD	AGTACAGTCATCTCCAGAAC	F-R1 = 221 bp F-R2 = 258 bp
	REVERSE 1	TCTTGCAGCGCTTCTATCTG	
	REVERSE 2	CGTCCATGATTTGACCAAAC	
<i>Nos-B</i>	REVERSE	AGTTTACTCCCGGCGATCA	191 bp
	FORWARD	AGAACATGGCGGCAAACGC	
<i>Nos-C</i>	REVERSE	CAGGATTCTGCGGTTTGC	197 bp
	FORWARD	GGAGCTAGCCTCGTCATG	
L-32	REVERSE	GGCTTCAAGAAATTCCTCGTC	117 bp
	FORWARD	AATGAGTTTCCTCTTGCGTGA	