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Hydractinia_Nanos2      -PLVHSNTPQVPPRSSTS-----LQVCVFCRNNG-----ESESVYTSHVLK 40
Clytia_Nanos2          -PLPPS-QPQNRGASTNSNNNGVQVCVFCRNNG-----ESESVYTSHVLK 44
Hydra_cnno2           -TLYNS-HDSLTLRASN-----VCVFCRNNG-----ESENVYASHVLK 37
Nematostella_nanos2  --LGKP-TARSSAPGANR-----QVCVFCRNNG-----ESEEVYASHVLK 37
Hydra_cnno1          --QQIQSKALKNLKTS-----VCVFCRNNG-----ESREFYSSHTLK 37
Hydractinia_Nanos1   --PPLSKDSRLSKANKTT-----VCVFCRNNG-----ESKEFYSSHTLK 37
Clytia_Nanos1        --PFHSSHHRKNAAKAT-----VCVFCRNNG-----ESREFYSSHTLK 37
Nematostella_nanos1  ---NRENKKNRAN-----VCVFCRNNG-----ESKVVYSSHTLK 32
Ephydatia_nanos      ---TFSKLPQQPIKQQ-----VCVFCRNNG-----ESESFYTSHYLK 36
Mus_nanos1           ---ARLLKPELQV-----CVFCRNNK-----EVALYTTHTLK 30
Homo_nanos1          ---ARLLKPELQV-----CVFCRNNK-----EAMALYTTHTLK 30
Xenola_xcat2        ---ESVGHKG-----CGFCRSNR-----EALSLYTSRHLR 27
Homo_nanos3          -----ESSAPER-----LCSFCKHNG-----ESRAIYQSHVLK 29
Mus_nanos3           -----ESSAPER-----LCSFCKHNG-----ESRAIYQSHVLK 29
Danio_nanos          -----PKSSPAERK-----FCSFCKHNG-----ETEAVYTSHYLK 30
Homo_nanos2          -----PGANGGLT-----LCNFCCKHNG-----ESRHVYSSHTLK 30
Mus_nanos2           -----EGYPGCLPT-----ICNFCCKHNG-----ESRHVYSSHTLK 30
Platynereis_nanos    LLLTIITRSKHKAPTGKKN-----ICVFCCKTNG-----EGEVIYTSHTLK 39
Botryllus_nanos     ALLPSSPNARTQILPVAR-----FIGCSFCKNNK-----EVKEVYMSHTLK 41
Dugesia_nanos        --LLHKVRTS-NQIRKESH-----IELCVFCRNNN-----EPFEMYVSHVKV 39
Haliotis_nanos      --RGDVTQLQVQKSKK-----LICVFCCKNNK-----EPHVVYTGHTLK 38
Helobdella_nanos    ---GKSGEPAL-----VCVFCRNNK-----EPECVANSHLVK 29
Drosophila_sim_nanos ---YKRYN-SKAKEIS-----RHCVFCENNN-----EPEAVINSHSVR 34
Drosophila_nanos    ---YKRYN-SKAKEIS-----RHCVFCENNN-----EPEAVINSHSVR 34
Musca_nanos          ---QKRYNGPKNEKYSS-----AKHCVFCENNN-----EPDAVVKSHAVR 37
Chironomus_nanos    ---KKMDKNSIKKKMD-----DHCVFCCKNNG-----ADEILYKSHTVK 37
Anopheles_nanos     ---KCRN-KSTCELD-----HCVFCFNK-----ADREVVYSHRCK 32
Celegans_nanos1     ---PRGNPPHFLC-----CCFCFGTASEFARLHTLPAPRKDRGWPMSDHCSK 44
Celegans_nanos2     ---VPSLFKRREYG-----CGYCRSVG-----YMRWETHTRK 29

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Hydractinia_Nanos2      DTEGRTACPILRAYT-CPICKANGDGSHTIKYCPNLQNTN-----AGVMGQP- 87
Clytia_Nanos2          DTDGRTSCPILRAYT-CPICKANGDNSHTIKYCPMNQARMGQNGNNGNAGLFFRPPF 103
Hydra_cnno2           DTDGRTSCPILRAYT-CPICKANGDNSHTIKYCPMNQAR-----SASTFNGLS- 85
Nematostella_nanos2  SADGKTTCPILRAYT-CPICKASGDDSHTIKYCPNQQTQ-----GNGQLPPFP- 85
Hydra_cnno1          DNEGNTMCPILRAYT-CPLCKSHGNQSHTIKYCPKYTKPK-----KTDKLLGIS- 85
Hydractinia_Nanos1   DNEGNTTCPILRAYT-CPLCKANGDNSHTIKYCPKYTKPK-----KADKLLGIS- 85
Clytia_Nanos1        DSEGNTSCPILRAYT-CPLCKANGDSSHTIKYCPKYTKPKV-----KAEKLLHLN- 85
Nematostella_nanos1  DAEGNTTSCPILRAYT-CPLCKASGQSHTIKYCPKNKNGS-----KLQAKV- 77
Ephydatia_nanos      DAEGKVTCPVLRAYT-CPLCGANGDAHTIKYCPENSQSV-----RNGIGKRQ- 84
Mus_nanos1           GPDGRVLCVLRRYT-CPLCGASGDAHTIKYCPKSKVPPPTVRP-----PPRSNRDLSL 84
Homo_nanos1          GPDGRVLCVLRRYT-CPLCGASGDAHTIKYCPKSKVPPPPARP-----PPRSARDGPP 84
Xenola_xcat2        ALDGRVLCVLRGYT-CPLCGANGDAHTIMRYCPLRRLRD-----PQSNNSNP- 75
Homo_nanos3          DEAGRVLCPILRDYV-CPQCGATREHAHTRRFCPLTQGYTS-----VYSHTTRNSA 80
Mus_nanos3           DEAGRVLCPILRDYV-CPQCGATQEAHTRRFCPLTQGYTS-----VYCYTTRNSA 80
Danio_nanos          NRDGDVMCPYLRQYK-CPLCGATGAKAHTKRFCPMVDKNYCS-----VYAKSTW- 78
Homo_nanos2          TPDGVVVCPILRHYV-CPVCGATGDAHTLKYCPLNGG-QQS-----LYRRSRGNSA 80
Mus_nanos2           TPEGVVVCPILRHYV-CPLCGATGDAHTLKYCPLNNS-QQS-----LYRRSRGNSA 80
Platynereis_nanos    EKNGRVCCPILRAYK-CPNCGAGHDAHTLKYCPLSVENQKR-----LRRPPGFIF- 89
Botryllus_nanos     NNAGKVTCVLRQYE-CPLCEATGDAHTIGHCPLNPNRHS-----LPLAIRSKAN 92
Dugesia_nanos        DLNGKVTCVLRNYT-CPLCNSTGDAHTIKYCPISNSKSS-----LVE----- 83
Haliotis_nanos      DSRGYTACVLRKYP-CPICQATGDAHTIKYCPNNDSEFR-----TSPLRTRM 90
Helobdella_nanos    DEKGQVTCPILYIYT-CPICGATGKAHTIKYCPYNTGERFYVPP-----LTRKTGNRSQ 83
Drosophila_sim_nanos DNFNRVLCPKLRTYV-CPICGASGDAHTIKYCPKPKIIT-----MEDAIKAESF 83
Drosophila_nanos    DNFNRVLCPKLRTYV-CPICGASGDAHTIKYCPKPKIIT-----MEDAIKAESF 83
Musca_nanos          DSMGRVLCPKLRTYI-CPICKASGDKAHTVKYCPKPKIIT-----MEDAVNAESF 86
Chironomus_nanos    DLKGRVLCPKLRAYQ-CPICGADGQSHTVKYCPKPKIIVT-----MEDLKKLDAS 86
Anopheles_nanos     DEAGNVTCVPLQTFV-CMRCKATGTAHTAKYCPKPKIIT-----PEDCLAME- 79
Celegans_nanos1     K-RGRVVCVPLRSMV-CGICGATGDAHTTKHLEAFGDD-----EDFSRDFENRRF 82
Celegans_nanos2     K-----CDKLSLAPCKICGARGEMNHTETCYCPMKPSSQLFFN-----EDFSRDFENRRF 79

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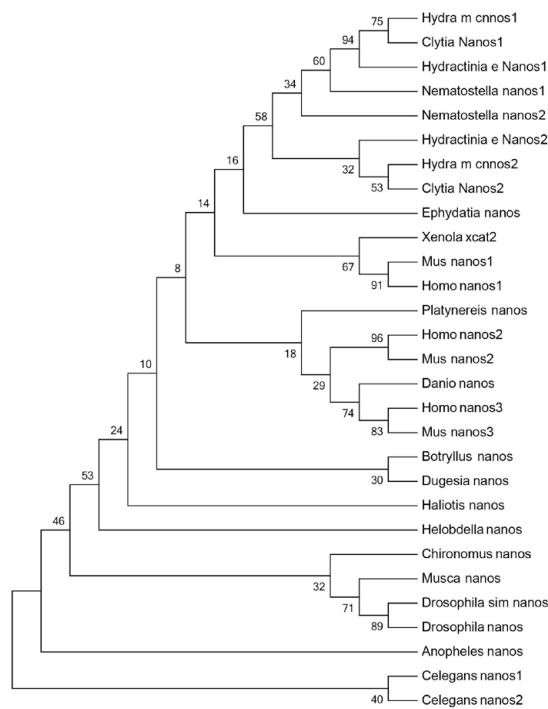


Fig. S1. Alignment of Nanos proteins from various animals and inferred phylogenetic tree. Bootstrap values are given.

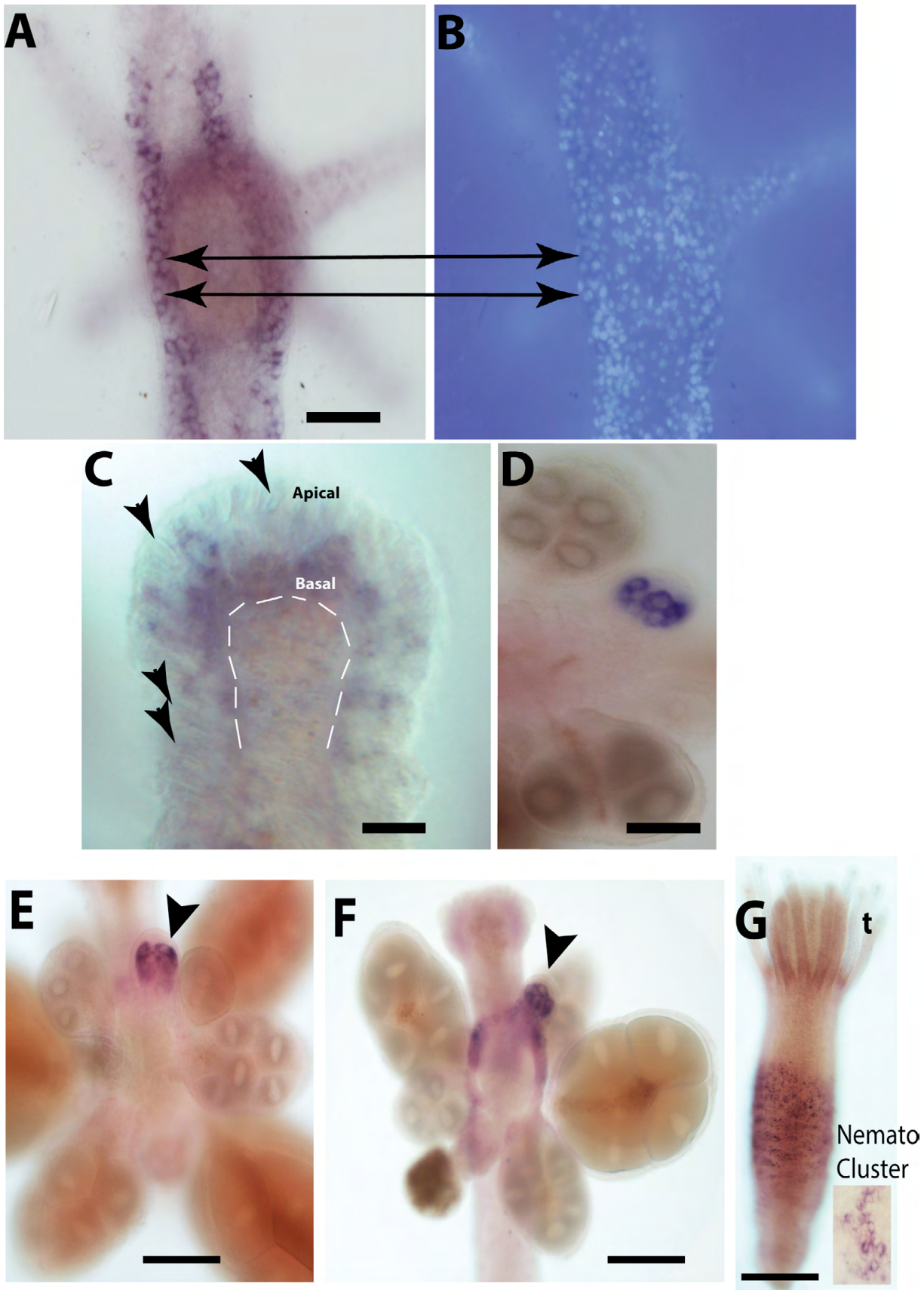


Fig. S2. *Nanos2*, *Nanos1* and *Pumilio* expression. (A-D) *Nanos2*. (A) Stolon of primary polyp, viewed from below; bright light. (B) The same animal as (A) but under UV light showing DAPI positive nuclei. Double headed arrows point to the same cells. (C) A tentacle. Mature nematocytes, apically mounted in battery cells and ready to discharge, are marked by arrows. *Nanos2*⁺ cells are only basally located. Dashed line represents the position of the mesoglea. (D) Sexual polyp. *Nanos2*⁺ developing oocytes are visible. (E) *Nanos1* expressing oocytes. (F) *Pumilio* expression in oocytes. (G) *Pumilio* expression in nematoblasts in a mature feeding polyp. Inset shows nematoblasts cluster. Scale bars 50 μ m in (A); 10 μ m in (C); 200 μ m in (D-G).

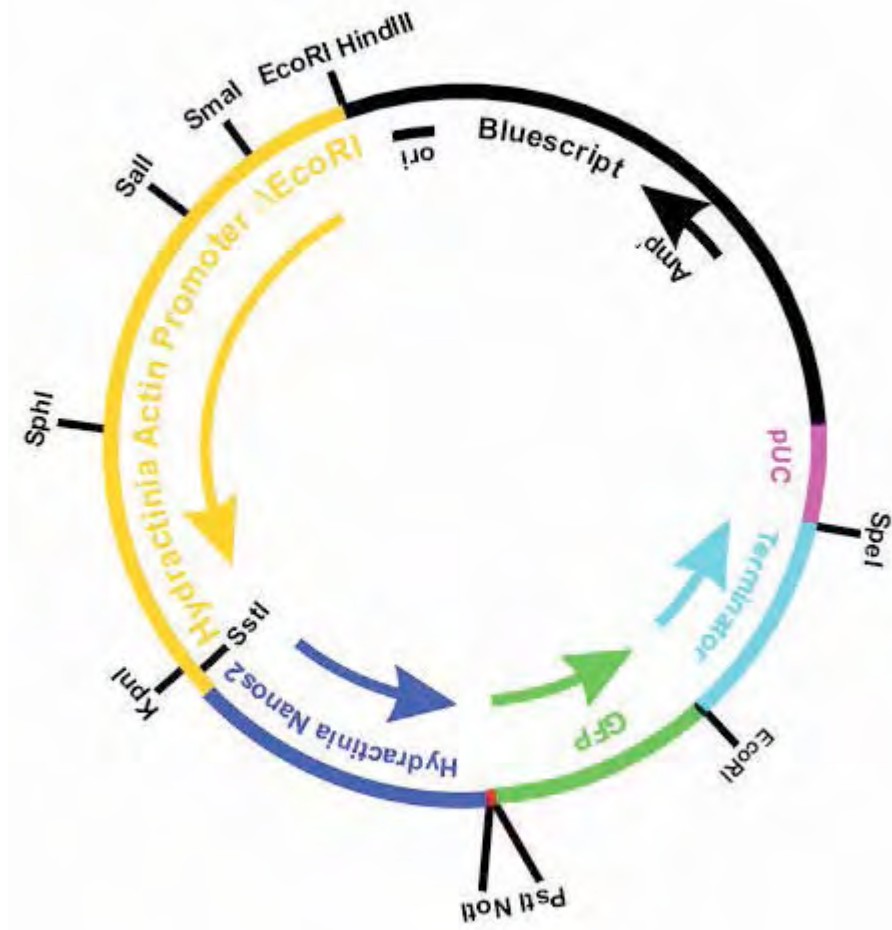


Fig. S3. Structure of the *Nanos2* ectopic expression construct.

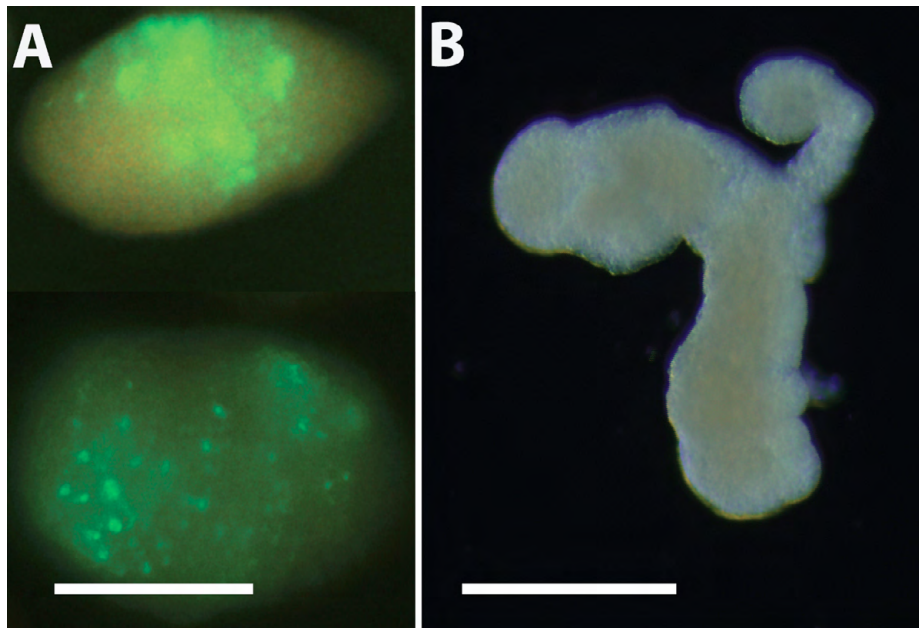


Fig. S4. (A) Transgenic, mosaic larvae expressing *Nanos2*-GFP. Scale bar represents 100 μ m. (B) Aberrantly developed transgenic *Nanos2* embryo. Scale bars 100 μ m.

Table S1. Oligonucleotides used during the study

Oligo name	Oligo sequence
Nanos1ATG-T7-fwd	gatcataatcgactcactatagggatgttcaaggcaatgtatagcaatgtt
Nanos1Sp6-rev	tagcaatttagtgacactatagaaggtgttttggccttagacaat
Nanos2fwd-ATG	atgtcgttgagtgatgttatgcta
Nanos2revRNAi	aatcctgaataaggaaaaatcacttgc
Nanos2fwdRNAiT7	ggatcctaatacgactcactatagggatgtcgttgagtgatgttatgc
Nanos2revRNAisp6	gatcctaatttagtgacactatagaaaatcctgaaataaggaaaaatc
Nanos2revpro1	tatgcccgccactcttcttagaaattcttttcaacaacaa
Nanos2rev-TAA	catcctaaccttccatgctggtt
Nanos2-RT-fwd2	acagcaaatcaacagccgaat
Nanos2-RT-rev2	gaaggcgttgcagga
Nanos2promoterrev-NotI	tatgcccgcccttcttagaaattcttttcagcaac
Promoter_insertionUFPrev-FseI	aatggccggcactggcctgttttcaaca
Nonos2promoterfwd-FseI	taaggccggccttctgtgttgctggtg
Splinkerout	cgaatcgtaaccgttcgtacgagaa
Splinkerin	tctgacgaaatcgtctcctctcc
Ash-T7-fwd	gatcataatcgactcactatagggcaccgattaacaatgatgca
Ash-sp6-rev	tagcaatttagtgacactatagaataacagcgaaataattacaatcta
RFamide-T7fwd	gatcataatcgactcactatagggatgttaatcatggcttcaaggc
RFamide-sp6rev	tagcaatttagtgacactatagaacttaacagtcctctctctgtgttg
Ncol1-T7 fwd	gatcataatcgactcactatagggcgtccaggaccaccaggagta
Ncol1-Sp6 rev	tagcaatttagtgacactatagaactggcaacagatgtggacaaga
Pumilionestrev1	ggtcctgttccagttagaactgaagat
Pumilionestfwd1	aagcattggagactataccaccagaa
Pumilio ATG start fwd	atgctcgacatcttggcaaca
Pumilio RACE rev1	ggattcttccaatgatttcaaaagtaaacat
Pumilio stop-TGA-rev	aactctcaatcaacatagaacgttcagc
Pumilio rev3	catttaacataggaataaccacatttgacc
PumilioRACE rev2	ttcgaaactgagtagtttatccccag
Pumilio rev4	atgaaaagagttggtctctgattcat
Pumilio T7-fwd	gatcataatcgactcactatagggatcggaggctccttcagctact
Pumilio sp6-rev	tagcaatttagtgacactatagaatacatgtattgtcatttctgggtgat
T7 Promoter	taatacgactcactataggg
Sp6 Promoter	atttaggtagactatagaa
pGEM-T RNAi Fwd	ggcctttctcatgctcac
pGEM-T RNAi Rev	taccgggttgactcaagac
pGEM-T RNAi fwd 2	cgacaggactataaagataccaggc
T7pGEM-T RNAi fwd2	atcctaatacgactcactatagggcagaggactataaagataccaggc
GFP-TAGstop-rev	ctattgtatgttcatccatgccat
Spliced leader	actcacactatttcaagcctgag
Splice leader long	aacgccccgagataaaaaaacacactatttcaagcctgagtttaag
Smart UPM long	ctaatacgactcactatagggcaagcagtggtatcaacgcagagt
Smart UPM short	ctaatacgactcactatagggc
Nanos2 morpholino	gcataacatcctcaacgacattt
Control morpholino	cctcttaccctcagttacaattata