**Supplementary figures and table**

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**Fig. S1. Sequence alignment of 24 colonies from one F1 female of strain 1.** Among them, colony 6, 7, 10, 13, 15, 17, 18, 19, 22 and 24 (10 in total) contain both the 6-bp marker and mutation (red), colony 1, 2, 3, 4, 5, 8, 9, 12, 14, 16, 20, 21 and 23 (13 in total) contain neither the 6-bp sequence nor mutation, and the remaining one (colony 11) contains mutation but not 6-bp marker reflecting a low level of somatic recombination.

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**Fig. S2. *Bsm*I-based method to identify the 6-bp marker.** Amplicons of primer pair T-F2/T-R2 were digested with *Sac*I to detect the mutation. amplicons of primer pair P-F3/P-R0 were digested with *Bsm*I to detect the 6-bp insertion polymorphism. The amplicons from one strain were digested synchronously. Thick arrows indicate bands result from mutant or reference allele, thin arrows indicate bands result from WT or insertion allele.

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**Fig. S3. Primers used for survey of sex-linked marker and their locations.** The arrows indicate primer directions. Dashed box indicates the region containing the 6-bp polymorphism (red box). The blue and black boxes represent untranslated regions and coding sequences of *Nodal* gene, respectively. The thin black line represents either flanking or intronic sequences of *Nodal* gene.

P-F1/P-R0.4 (reverse strand)

female2 CATAGAACCACATTTAGTAATGCAGGGTAATCAATTACATTCATCACATYWTATAAACTWAAGCTTCTTTCAAGTGGGTTRGATTTTGAAAAAGAGACTTTTTTTTACATATTTTGTACATTCTCATCACAGTGACATACAATTGTGAACATCTGTACA[159]

female1 ...-.............................................Y.K.......WM..................W..........R....T.........W..MMWWW..............................................[159]

male2 ...C.............................................YW........W....................R..............................................................................[159]

female2 TCTATATCCTGCAACCACTGGAGCCAAAAGTACACTAAGTAGCTTTTATCATAATAGATCATTGTCATTGTCATTTATCCCAGAGTGMTGATAATCAAATCACTGTWAGGTACTATGYAAKGCTTGTCTACACTGTACTATGTCCCTGCATATATTCAA[318]

female1 ...................T...........................................................Y..K....A.................KW.............S............Y............YKY..........[318]

male2 .......................................................................................M..................W..........Y..K......................................[318]

female2 GTCCGTATCAGTTATGTATTGACCTTTTTTGGCCTAGATAAAATTTTGGGGGAAGCAGTTGTTTTCTMCTTTGACGCATCGTTTATCACTGT[410]

female1 ...M.....WT..W...W..................K..............R...Y.RY.K........C...........C.A.....G.A[410]

male2 ...................................................................M.............C.A........[410]

P-F1.1/P-R0.3 (reverse strand)

female1 GGGACGGGATATTGTCCGGTTTTCTGCTGCCGAGCTCCGGCGTTTGGGACGGGTGCTTATAAAGATCTGCAACTTCTTGTAAATCATCATCAAATAACAACATGTCACTGGTATTGGGACCTTTTTTGGGGGGKRSRRRWRWATAAAAACTATTTTAGA[159]

female2 ................................................G.........R......................G...G.......................R.......................KRYAWAAAA..TM.....W.......[159]

male1 ................................................GY........G......................G...........................A.................---.....TATAAAA..TC.....W.......[159]

male2 ................................................G.........R......................G...G.......................R................. .....KRYAWAAAA..TM.....W.......[159]

male3 ................................................GY........G.........R............G...R.......................A................K---.....TATAAAA..TC.....W.......[159]

male6 ................................................G.........G.........R............G...R.......................A................K---.....TATAAAA..TC.....W.......[159]

female1 CAAGCAACTTTGATCAAAATCACAATTTATTGAAAAYAATAMAGAATGATGAYCCAACCTACCTAYAGATTCATGGTATCAAATTTSTCAAGACAGCTCACAAAGTTTCAAGAAAGGARTAAYTCTCTCCTGAATCATATTATCRATCTATGGAGAACA[318]

female2 ....................................C....C................................R....S............................M...............................C...G..............[318]

male1 ....................................C....C................................A....S............................................................C...G..............[318]

male2 ....................................C....C................................W....S............................M...............................C...G..............[318]

male3 ....................................C....M....W...........................R....S......................................R...Y.................Y...R.........S....[318]

male6 ....................................C....M....Y...........................R....S......................................R...Y.................Y...R.........S....[318]

female1 TGCATYTTTCTRGGGATGAAATTTCMTWWGTGCATTYRTCATTGAGAGGTTTTAAGATAAGTACTGAARTATGAAAAATGCATTCAACAGATGCAATGT[417]

female2 .........................C..........TG.............................................................[417]

male1 .........................C..........TG.............................................................[417]

male2 .........................C..........TG.............................................................[417]

male3 .....Y.................K.C..........TR.............................................................[417]

male6 .....Y.................K.C..........TR.............................................................[417]

P-F1.2/P-R0.2 (reverse strand)

female5 TCTACAAGTTGATTTTACGGACGGCAAAGCGTTAACTTATGGCCAAGATCAAGGAGCTTTCATCGACAGGTTAACGCTCATAGTTTCCGTGCTGGCATTAGAGATCATTAAATCTTGAGACCATTAGCCCATGTTGAAAATTGCGCATCATCGCTTCCC[159]

female4 .....................................K..............K......................................................................................................Y...[159]

male3 ..................................R..K....Y..................................................................................................Y.................[159]

male4 .............................................................................................................................................Y.................[159]

female5 ACAAAACCAAACGCCAGTGTTTCAAGTCTGCRAAGGAGGCTTTATCTTTCACACAGCCCCGCTTAACGCTACCTCTACGGTGCTAGGAGTGTATCCGCCATCCAGTTCATTGGCGCAAGCCGGAGGAAATGTTACAGGCTTGCTTTTACCCGGAGGGCC[318]

female4 ....M.Y...MSSM..K......W.......................................................................................................................................[318]

male3 .............................................................................S.....M.......................................G......K.......S....................[318]

male4 ...............................R...................................................Y.......................................R...................................[318]

female5 AGTTAAACGATATCCTGCCACTGCAGACTTATGTTTAGTAGGTTGGGCCCGGTGAAATACCCATAAGCCATCCAGTAGAGAGTGGTGACCAGGCTATTTAAAGGTTGCGACTGTTGGTCAGTAATTACGAYTTTGCCC[456]

female4 .........................................................Y................................................................................[456]

male3 .......................M.............................R...C...M.........................................R..........................T.YYS...[456]

male4 .......................M.................................Y.............................................R..........................T.YYS...[456]

P-F2/P-R0.1 (reverse strand)

female1 ATGATAATAATGGTTTCAATTTTGGTAGACATTCCTGAAATGCCTTATTTATACTTGACATAGTTGTGTCCCAAAATTTGAGTTGWACACCTATTTCTTGGTGARGCTAAGTACTCACTGATGCCGACAATGGTTAGTATGATCAAGGACAGGAAGGAG[159]

female2 .............................................W.......................................T...................................................K.....................[159]

female3 .................................M...................................................T..................R..........................................R...........[159]

male2 .............................................A.......................................T...................................................G.....................[159]

female1 GGTGTATGATGGGAAGATATCGATCAGGGGATTAGGACTTCCATAAATGCCCTAAAATACCCTGCTTCCGCCAAACTTATCATAAAGAGCCCACCACACTTGCAACACAAAGAAACTTCATGACACACARGTATAGTATGCGAATTGCAAAATAACGCG[318]

female2 .................................................................................................................................R.............................[318]

female3 ...........................................................................................Y.....................................R.............................[318]

male2 .................................................................................................................................R.............................[318]

female1 TACTTGCGCTTAGTGTCCATTTTGAAGTTAAGAGTTAGGAATAAGACCCTTCTCTGAAACAAAAAATTAAGAAGTAACAGTGATAGAAGTTAAGTATTTTGGTCCCATGAGGGTTGAATTTCTCATTRATTGGTAAGATCTCCTTGGGAGGTACACAGC[477]

female2 ...............................................................................................R...............................................................[477]

female3 ...............................................................................................................................................................[477]

male2 ................................................................................................R..............................................................[477]

female1 TTAGTKGTGTTTTCACACCTAGGTTTATGTATCGTCAGTGGATCAACAAGATTAACTCGGTATCACCT[545]

female2 ...................................................W................[545]

female3 .....KS.............................................................[545]

male2 ...................................................W................[545]

P-F2.1/P-R0.0 (reverse strand)

female1 TCATTTTGGTCTTTTGATGGTATGGAAAGCAAACCGTGTCTTTSACGGGAAAATGACACACGATTTCAAAGTTCAAAATGATTTTTTGCTCCTCCAGAAACCTCATCACGTTGTAATTATCATCTTATCTTAATTACCCCATGACTGGTAATTAGCTCA[159]

female2 ...................S.......................S..R...........................................Y....................................................................[159]

male1 ...........................................G..R...........................................Y....................................................................[159]

male2 ...................S.......................S..R...........................................Y....................................................................[159]

female1 CCGTCCGGTTTTTAGACAAGCTAAGTGACTTGTCGATCGACCATTGACGGCCTATAAAAGAGCAATCAACAAACGCCATACAAACACGGTGAGTACACAGACAACACCGCCATGCTTTAGTTWCGGCTCTTCACAGGCACMTTATCACTCGGGTAATCT[318]

female2 ...............................................................................M..........................................W.................M..................[318]

male1 ...............................................................................M...........................S....K..............................................[318]

male2 ...............................................................................M..........................................W.................M..................[318]

female1 GCCATCTTTTACGCCTTTTGTTCACGGCGATCTCCCAACACCGTTAAGTACTTACTTTAACACCAT--TAAATATCCGGGCAAAAAGTCACTTCGAAAAAAA[420]

female2 ..........W.......................................................TA.................M................[420]

male1 ..........W.......................................................TA..................................[420]

male2 ..........W.......................................................TA..................................[420]

T-F1/T-R2

female1 TTTCTGACGGCTTCGGTCGCGTTCTTCGCCGTTCAACTCCTGCAGTGTGAATGCTTGTCGAACGAGCATACACTAGCGGCGTCGGGCCTACTTCGAGCGAGTGGCGGAGAGGGTCTGACGCTGGGAAGATACGAGCATGGCGACGGCGTGGTTAACGTG[159]

female2 ..........................................G....................................................................................................................[159]

female3 ...............................................................................................................................................................[159]

female4 ...............................................................................................................................................................[159]

female5 ...............................................................................................................................................................[159]

female6 ...............................................................................................................................................................[159]

male1 ..........................................G....................................................................................................................[159]

male2 ..........................................G....................................................................................................................[159]

male3 ...............................................................................................................................................................[159]

male4 ..........................................G....................................................................................................................[159]

male5 ...............................................................................................................................................................[159]

male6 ..........................................G....................................................................................................................[159]

female1 ACCATGCGGGGCATGGCCGTGGCGGGGAGGATGACTCCAGAGTTCATGCTCGACTTCTACCAGAGTCTGAGCTCGGGGACAGACCTCAACGTCACCCGGGAGGAAAACCAGGCGCTGCCGCTGCCGTCCGACACCGTCAGGAGTTTCGCACTAAAAGGT[318]

female2 ...............................................................................................................................................................[318]

female3 ............................................Y............................................................W..............................................R......[318]

female4 .........................................................................................................W.....................................................[318]

female5 .........................................................................................................W.....................................................[318]

female6 .........................................................................................................W.....................................................[318]

male1 ...............................................................................................................................................................[318]

male2 ...............................................................................................................................................................[318]

male3 ............................................Y...........................................................................................................R......[318]

male4 ...............................................................................................................................................................[318]

male5 ...............................................................................................................................................................[318]

male6 ............................................Y...........................................................................................................R......[318]

female1 CAGTCAGTTTTAACGGA[335]

female2 .................[335]

female3 ....Y.S..........[335]

female4 .................[335]

female5 .................[335]

female6 .................[335]

male1 .................[335]

male2 .................[335]

male3 ....Y.S..........[335]

male4 .................[335]

male5 .................[335]

male6 ....Y.S..........[335]

G-F1/G-R1

female3-6 CCGTCAGGAGTTTCGCACTAAAAGGTCAGTCAGTTTTAACGGA--ATTATCTGGAGCTTTGCAATTGAGATGAGGCAAACGATTTTTTTTTCGATTACCTTTGTATCGACAAGAAGAGAAAGGGGTAGACTCTTGTTGTAAGAAACATCCAAGTGTTTT[159]

female3-8 ...........................................--..................................................................................................................[159]

female3-15 ...............................G...........--..................................................................................................................[159]

female4-1 ...........................................--..................................T..--.........................................C.................................[159]

female4-4 ...........................................--.....................................-................................A.........C.................................[159]

female4-6 ...........................................--..................................T..-..........................................C..............C..................[159]

male4-3 ...........................................TT..................................T..-..........................................C.................................[159]

male4-8 ...........................................--..................................T..-..........................................C.................................[159]

male5-2 ...........................................TT..................................T..-..........................................C.................................[159]

male5-5 ...........................................--..................................T..-..........................................C.................................[159]

male6-4 ...........................................--..................................T.............................................C......................G..........[159]

male6-8 ...........................................--..................................T.............................................C......................G..........[159]

male6-10 ...........................................--..................................T.............................................C......................G..........[159]

female3-6 AGAAATTGCATCCCAAGTAGATCCATTGAGAGGTCGAAATGGTATTCAGAATTAAAAGTAGATATTTGGACGACGTTTCTACCAGTGCCACAGATGTTGTTCATCAACTAGTCTGTATGTGGCCTGTGGACACTTTCTGCTGTGCAATGGTACAAATTA[318]

female3-8 .......................................A..........G............................................................................................................[318]

female3-15 ...............................................................................................................................................................[318]

female4-1 ..................................................................................................................................T............................[318]

female4-4 ..................................................................................................................................T............................[318]

female4-6 ....G.............................................................................................................................T............................[318]

male4-3 ................A................................................................................C................................T............................[318]

male4-8 ...............................................................................................................................................................[318]

male5-2 ................A.................................................................................................................T............................[318]

male5-5 ..................................................................................................................................T............................[318]

male6-4 ...............................................................................................................................................................[318]

male6-8 .................................C.............................................................................................................................[318]

male6-10 ...............................................................................................................................................................[318]

female3-6 TCATTTGATTGCCAAATCCAATTTCATTACCTAATTTCGTCTGTTGTGTGGAATAAGATAAGACCACAGCTG--AAAAAAGGAGGGAAAAGGTGAAACAAAAATCGGTATCGATACAAGCGGTACCATACAAGAGTGTTGTTTTTCCTTTTACAGCAAA[477]

female3-8 ........................................................................--...G.................................................................................[477]

female3-15 ........................................................................--.....................................................................................[477]

female4-1 ...........................................C............................--..................................................................................-..[477]

female4-4 ...........................................C............................--..................................................................................-..[477]

female4-6 ...........................................C............................--..................................................................................-..[477]

male4-3 ........................................................................--..................................................................................-..[477]

male4-8 ........................................................................AA................................................................A......T...C....A.-..[477]

male5-2 ........................................................................--..................................................................................-..[477]

male5-5 ....................................C......C............................--.....................................................................C............-..[477]

male6-4 ........................................................................--..................................................................................-..[477]

male6-8 ........................................................................--..................................................................................-..[477]

male6-10 ........................................................................--..G...............................................................................-..[477]

female3-6 AAATGTGTATTGGGTCCGAGACGGTAATACACATCGGGTCCCACATCAAGCGTATCGGTCTACGGATCAGATAAGACTCTTTTCTTCACACCGATTCTACTCTGTTAGTGTTACAGCGAGGAACAAACGACCGTTCACACTGTTTGCAAAAACTCGGCA[636]

female3-8 ...............................................................................................................................................................[636]

female3-15 ...............................................................................................................................................................[636]

female4-1 .........................................................................................G............------......................................T............[636]

female4-4 .........................................................................................G............------......................................T............[636]

female4-6 .........................................................................................G............------................................C.....T............[636]

male4-3 ......................................................................................................------...........A..........................T............[636]

male4-8 ......................................................................................................------......................................T............[636]

male5-2 ......................................................................................................------...........A..........................T............[636]

male5-5 .........................................................................................G............------......................................T............[636]

male6-4 .....................................A................................................................------............................G......................[636]

male6-8 .....................................A................................................................------............................G......................[636]

male6-10 .....................................A................................................................------............................G......................[636]

female3-6 ATGTTTTCATTATGCATGTTGATCAGGCAACTCCTTCATCCGACTTGCGATA-TTTTTCTCGTATTTCTTTCAGTTCACGTAGTACACGTTT---AAAACTGTGCCCGGAAAGTTTATCAAGGGTGCCATTTTCTTTGTCCAGAAAATACTGGAAAAAA[795]

female3-8 ....................................................-.......................................---................................................................[795]

female3-15 ....................................................-.......................................---................................................................[795]

female4-1 .........................T..G...............A.......T.......................................AAA...............................................C................[795]

female4-4 .........................T..G...............A.......-.......................................AAA...............................................C................[795]

female4-6 .........................T..G...............A.......-.......................................AAA..................C............................C................[795]

male4-3 .........................T..G.......................-.......................................---................................................................[795]

male4-8 .........................T..G.......................-..............T........................---................................................................[795]

male5-2 .........................T..G.......................-................C......................---G...............................................................[795]

male5-5 .........................T..G...............A.......-.......................................AAA...............................................C.........A......[795]

male6-4 ....................................................-......................................AAAA................................................................[795]

male6-8 ....................................................-......................................AAAA................................................................[795]

male6-10 ....................................................-......................................AAAA................................................................[795]

female3-6 ATTTCCTTGGTAGATTCTGCTAGATTTGAAAGGCCGATCCGTTATCAACAGGAATGATCAGATGATAAATATGCACAAGAACCTCCCTTTGTCCGACGGAATCAATTTTCAAACACAAAACTCCCCAAAGCAGTTTCAAGTTGTTTTACAAGGCTTGGA[954]

female3-8 ...............................................................................................................................................................[954]

female3-15 ...............................................................................................................................................................[954]

female4-1 .......G............A..........................................................................................................................................[954]

female4-4 .......G............A..........................................................................................................................................[954]

female4-6 .......G............A................................................................................................................C.........................[954]

male4-3 .......G............A..........................................................................................................................................[954]

male4-8 .......G............A..........................................................................................................................................[954]

male5-2 .......G............A..........................................................................................................................................[954]

male5-5 .......G............A..........................................................................................................................................[954]

male6-4 .......G.......................................................................................................................................................[954]

male6-8 .......G.......................................................................................................................................................[954]

male6-10 .......G.......................................................................................................................................................[954]

female3-6 GAGGTGTAGAACACGCGCAAAGCATTCCAGAGTTATCATCATATTGGACAACATTTGACGAAACAAGTACGGAGTTTCTGAATAGGTGTAAAGGATTAATTAGCAAAACAGTGATAATTACTGATCGTAATCTCCATCTCTCCACTGGGGGCGTTTCTA[1113]

female3-8 ................................................................................................C..............................................................[1113]

female3-15 ...............................................................................................................................................................[1113]

female4-1 ..................................................................................................................................................-............[1113]

female4-4 ...............................................................................................................................................................[1113]

female4-6 ....................................................................G..........................................................................................[1113]

male4-3 ............................G..........A.......................................................................................................................[1113]

male4-8 ...............................................................................................................................................................[1113]

male5-2 ............................G..................................................................................................................................[1113]

male5-5 ...............................................................................................................................................................[1113]

male6-4 ...............................................................................................................................................................[1113]

male6-8 ...............................................................................................................................................................[1113]

male6-10 ..........................................................................................................................................C....................[1113]

female3-6 ATCCGAGGTATCCGCCCCATAATGACTCATTCACACCATGCATTCGCAAATAGCCACTCATGCATGCTGGTTGATACACAACAAGGAGGTTATTTTATCCTCCTTGATACACAATGAGACAAGACTCTCTACAGTCATGTAAAACGTCTCACTGGA-CT[1272]

female3-8 ............................................................................................................................................................-..[1272]

female3-15 ............................................................................................................................................................-..[1272]

female4-1 ............................................................................................................................................................C..[1272]

female4-4 ............................................................................................................................................................C..[1272]

female4-6 ............................................................................................................................................................C..[1272]

male4-3 ........................................................................................................C...................................................-.C[1272]

male4-8 ........................................................................................................C.........................................C.........-..[1272]

male5-2 ............................................................................................................................................................C..[1272]

male5-5 ............................................................................................................................................................-..[1272]

male6-4 .....................................................................................................G......................................................-.C[1272]

male6-8 .....................................................................................................G......................................................-.C[1272]

male6-10 .....................................................................................................G......................................................-.C[1272]

female3-6 GCAGCACGTTTGCGGAGCCGCTGTGTCCTATGTTAGACTTGTCAGAGAGAGG---------------------------------GATTTCAG------TCAGGCTACTACGACTTAATTACCCCTCTTGTGAGTCTCTTGTGCTATATAAAGGTCACA[1431]

female3-8 ....................................................---------------------------------........------............................................................[1431]

female3-15 ...........................................G........---------------------------------........------............................................................[1431]

female4-1 .................T..A...............................---------------------------------..C.....------............................................................[1431]

female4-4 .................T..A...............................---------------------------------..C.....------............................................................[1431]

female4-6 .................T..A...............................---------------------------------..C.....------............................................................[1431]

male4-3 ....................................................---------------------------------........------............................................................[1431]

male4-8 ..............................G.....................GAGGTAAACTGTCGGGTTGGCCGGTAGGACCGC.....T..TCGACA.........................-----------........................[1431]

male5-2 .................T..A...............................---------------------------------..C.....------............................................................[1431]

male5-5 ....................................................---------------------------------........------............................................................[1431]

male6-4 ..............................G.....................---------------------------------........------............................................................[1431]

male6-8 ..............................G.....................---------------------------------........------............................................................[1431]

male6-10 ..............................G.....................---------------------------------........------............................................................[1431]

female3-6 TTTCCAAACCCGGGCCGGTCGACCGAAATGGTCGTGGAAACGAAAATGTAAGTGTAAATCATAGAATATACACAGGTTATGGTCATGACTAATTTTATTACGTTGTTTGTGTTTTGTTTTCGATCTCGGAAGCTCCCCGACCTGGTCCCGGTTAATTCT[1590]

female3-8 ......................................................................................................................................A........................[1590]

female3-15 ..............................................................................................C.......................................A........................[1590]

female4-1 .............A...............................................................................................................C........A.....T..................[1590]

female4-4 .............A...............................................................................................................C........A.....T..................[1590]

female4-6 .............A...............................................................................................................C........A.....T..................[1590]

male4-3 .............A........................................................................................................................A........................[1590]

male4-8 .............A........................................................................................................................A........................[1590]

male5-2 .............A...............................................................................................................C........A.....T..................[1590]

male5-5 .............A........................................................................................................................A........................[1590]

male6-4 .............AA.........................................G.............................................................................A.....T..................[1590]

male6-8 .............AA.........................................G.............................................................................A.....T..................[1590]

male6-10 .............AA.........................................G...............................................................-.............A.....T..................[1590]

female3-6 ATTG-AAAACAATTCAAAACTGTATTTTCTGAGCAAGTCAAAAGAATGTTATGAACAAGAAAGATACCTTTATGATATAGATAATTTTGACATTCGAAAAGCGATAACTCAGTTAAGAATCAGTAGCCATAAATTGAATATTGAAACAGGCAGATGTTA[1749]

female3-8 ....-..........................................................................................................................................................[1749]

female3-15 ....-..........................................................................................................................................................[1749]

female4-1 ....-..................C.................................................................GA................................................................A...[1749]

female4-4 ....-..................C..................................................................A................................................................A...[1749]

female4-6 ....-..................C..................................................................A................................................................A...[1749]

male4-3 ....A....A.............C..................................................................A................................................................A...[1749]

male4-8 ....-..................C............................T.....................................A................................................................A...[1749]

male5-2 ....-..................C..................................................................A................................................................A...[1749]

male5-5 ....A....A.............C..................................................................A................................................................A...[1749]

male6-4 ....-..................C.............................................................C....A................................................................A...[1749]

male6-8 ....-..................C.............................................................C....A................................................................A...[1749]

male6-10 ....-..................C.............................................................C....A................................................................A...[1749]

female3-6 CAATGTAGCCCCTGACCAAAGGTTCTGCCCTTTCTGTCCAAAGCATATTGAAGATGAATTTCACTTTTTAATGGAATGTTCT--------AAATACACTATTTTACGTAATGAGCTATTCACATTTCTAGAATCTATTACAGCAGATTTCAAGAACCTC[1908]

female3-8 ..................................................................................--------.....................................................................[1908]

female3-15 ..................................................................................--------.....................................................................[1908]

female4-1 ..................................................................................--------.....................................................................[1908]

female4-4 ..................................................................................--------.....................................................................[1908]

female4-6 ..................................................................................--------.....................................................................[1908]

male4-3 .............................................C....................................--------.......................A.............................................[1908]

male4-8 ..................................................................................AAATAGTA.....................................................................[1908]

male5-2 ..................................................................................--------.....................................................................[1908]

male5-5 .............................................C....................................--------.......................A.............................................[1908]

male6-4 ..............G...................................................................--------.....................................................................[1908]

male6-8 ..............G...................................................................--------.....................................................................[1908]

male6-10 ..............G................A..................................................--------.....................................................................[1908]

female3-6 GACGCTAAAAACAGATTTGTATATATCTTTGGATGCCACAACTCCCATAATGCAAAGTTAGGCAAATATATCAAAGACTGCTTTGTTATTAGAAAGAATAATGAAAGCCATGATTAGCCCAACTCGATTGTAACATTATATCTTGTTTTAGCCCTTTTT[2067]

female3-8 ...............................................................................................................................................................[2067]

female3-15 ...............................................................................................................................................................[2067]

female4-1 ..T...........................A..................C.............................................................................................................[2067]

female4-4 ..T...........................A..................C.............................................................................................................[2067]

female4-6 ..T...........................A..................C.............................................................................................................[2067]

male4-3 ..T...........................A................................................................................................................................[2067]

male4-8 ..T...........................A...................................................................................A.......................C....................[2067]

male5-2 ..T....................G......A..................C.............................A...............................................................................[2067]

male5-5 ..T...........................A................................................................................................................................[2067]

male6-4 ..T...........................A..................C.......C.............................................................T.......................................[2067]

male6-8 ..T........................-..A..................C.......C.............................................................T.......................................[2067]

male6-10 ..T...........................A..................C.......C.............................................................T.......................................[2067]

female3-6 GCTATGTTAATAAGGATGTTAAGTCTTATTCTATACACCTATTTTGTACTTTGTGTAATAGTTGTTGCCATAC----------------ATGTCGTGCAATAAAGTTCTTCTATTATCTAAGCCGGCT---AAGCATCACGTTTCACCTGGTCACTTCT[2226]

female3-8 .....................C...................................................----------------.......................................---............................[2226]

female3-15 .........................................................................----------------.......................................---............................[2226]

female4-1 ...T............A...............G........C...............................ATTGTACCATGTACAA.......................................---............................[2226]

female4-4 ...T............A........................C...............................ATTGTACCATGTACAA.......................................---............................[2226]

female4-6 ...T............A........................C...............................ATTGTACCATGTACAA.......................................---............................[2226]

male4-3 .........................................................................ATTGTACCATGTACAA................-..................TTA.CTA............................[2226]

male4-8 ............................................................A............ATTGTACCATGTACAA..............................------------............................[2226]

male5-2 ...T............A........................C...............................ATTGTACCATGTACAA.......................................---............................[2226]

male5-5 ................................................T........................ATTGTACCATGTACAA.......................................---............................[2226]

male6-4 .........................................................................ATTGTACCATGTACAA..............................T........---............................[2226]

male6-8 .........................................................................ATTGTACCATGTACAA..............................T........---............................[2226]

male6-10 .........................................................................ATTGTACCATGTACAA..............................T........---............................[2226]

female3-6 AGATCCTCTATCGGAATTCCATCCTCTCCATGATCCAGCGTAAGCCCTAATCCGGAAGTGAACGTCACATCCGTTCCCACCCAGTATGATGGCTCATATTTTTGTGCAAGGCAGATCAACCAAGAGACTGTCTGACATGAATCTTGATGTAGCTTAACC[2385]

female3-8 ...............................................................................................................................................................[2385]

female3-15 ...............................................................................................................................................................[2385]

female4-1 ..........................C............................................................................................................T.......................[2385]

female4-4 .......................................................................................................................................T.......................[2385]

female4-6 .......................................................................................................................................T.......................[2385]

male4-3 .......................T...........................................................................C..............................C....T.......................[2385]

male4-8 ..................................................................................................................................C....T.......................[2385]

male5-2 .......................................................................................................................................T.......................[2385]

male5-5 ..................................................................................................................................C....T.......................[2385]

male6-4 ..................................................................................................................................C....T.C.....................[2385]

male6-8 .....................................................................................................................T............C....T.......................[2385]

male6-10 ..................................................................................................................................C....T.......................[2385]

female3-6 AAGGCTTTTCAAGGCAATAACCACGTTTCTGCCCTTCCGTCAATGCTTTGTGTGTGTGTATTGGGTGTGTATGTTGTGTGTAACAATACTCCTATCAGCGGAATTATATTTCTCTGG-TACTTATGTCGGTAAAAGATCCCATGCAAGAATTTTCAGAA[2544]

female3-8 .....................................................................................................................-.........................................[2544]

female3-15 .....................................................................................................................-.........................................[2544]

female4-1 ........................................................................................................C............T....................T....................[2544]

female4-4 ........................................................................................................C............T....................T..............C.....[2544]

female4-6 ........................................................................................................C............T....................T....................[2544]

male4-3 .......................................C.............................................................................-....................T....................[2544]

male4-8 ...................................................T.........C..............--..........................C............-....................T....................[2544]

male5-2 .G......................................................................................................C............T....................T....................[2544]

male5-5 .....................................................................................................................-....................T....................[2544]

male6-4 ................................................................A............A.......................................-....................T.......---------....[2544]

male6-8 ................................................................A....................................................-....................T.......---------....[2544]

male6-10 ................................................................A....................................................-....................T.......---------....[2544]

female3-6 TTTTTACATGTAGTCTTTCTTTAATTACTTTTGCCTAACTAAACTGAAAATTTCGTTGATTGGCGATGTAAAGTCAAAATCTTCTATAATCTTTCATTAGTTTCATTAGTTTGTATAGTCTTTAACTTTTTTTATCTACAGGAAAGCAGCTCGCGTGTG[2703]

female3-8 ...............................................................................................................................................................[2703]

female3-15 ..................................................................................C............................................................................[2703]

female4-1 ......................C........................................................................................................................................[2703]

female4-4 ......................C........................................................................................................................................[2703]

female4-6 ......................C........................................................................................................................................[2703]

male4-3 ......................C........................................................................................................................................[2703]

male4-8 ......................C.............................................C..........................................................................................[2703]

male5-2 ......................C........................................................................................................................................[2703]

male5-5 ......................C........................................................................................................................................[2703]

male6-4 ..........CG..........C........................................................................................................................................[2703]

male6-8 ..........CG..........C........................................................................................................................................[2703]

male6-10 ..........CG..........C................................................G.......................................................................................[2703]

female3-6 TTCTTGCCATATTTATATTTATGATATACCAGTAGTGTTCAATGGTTTCGCTAAGTATCAATATTATCTTACTCTCCATCCAGATGCCAAGCAGAAAGGTAGCA[2807]

female3-8 .......................................................................T................................[2807]

female3-15 .......................................................................T................................[2807]

female4-1 .......T.....C...............T.........................................T................................[2807]

female4-4 .......T.....C.....C.........T.........................................T....T...........................[2807]

female4-6 .......T.....C...............T.........................................T................................[2807]

male4-3 .............................T..........................................................................[2807]

male4-8 ...................................G...................................T................................[2807]

male5-2 .............................T.........................................T................................[2807]

male5-5 .............................T..........................................................................[2807]

male6-4 .............................T.........................................T........T.......................[2807]

male6-8 .............................T.........................................T........T.......................[2807]

male6-10 .............................T......................................C..T........T.......................[2807]

G-F2/G-R2

female3 GAGCTGCGCATGCGCGGTCCGCACTTCTCGCCTCCCCGCAGACTTTCCGTGCGTGTCTTCAGCCACGAGGAGAAAAAGTGCAAGGTGTGTCGGGTGTTCATCTTACAGCGATGAG----CMG-WACTWAAKAAGKWCATTCACTSTMTCTMGTAYKCAT[159]

female1 ..........................................................................G...............Y........................ATACT..T....................G...............[159]

female2 ..........................................................................G........................................ATACT..T....................G...............[159]

male1 ..........................................................................G......R.....K...........................MYWYW.KW..SWY.W.M...R.M.YY..KRY.Y..WS..YRS.Y[159]

male3 ..........................................................................G........................................MYWYW.KW..SWY.W.M.S.R.M.YY..GRY.YM.WS.MYRS.Y[159]

female3 TCGCAGCGACAGGASTYSKGRGTAATATGTCAAAGGTGAAGGCCCATGTCTGAACAGCACTCTAGTCTCGACCATTGTGTCGATTTGCATTTTAAAAAAAATTAGACGKGTTTTTTTTATGTCTCAGSGSCYYCCATCTYCAACATAGKSCCTATAATT[318]

female1 ...T..........-.......................M..................T.....C..............C.............-.......T.........-.......M.............Y...C........Y.T.A....G....[318]

female2 ...T..........-.......................M..................T.....C..............C.............W.......T.........-.....W.M.................C..........T.A....G....[318]

male1 ..KWMS....WSSR-KW..........K......R...C..................T.....C..............C.....................T.........-.....A...................C..........T.A....G....[318]

male3 ..KWMSM...WSSR-KWK.........KK.......K.C..................T.....C..............C.....................T.........-.....A...................C..........T.A....G....[318]

female3 TKGCACAKGKGTGCTCAGTACTGAAKGTTTTCATATGAGCATGTTAARAAGTTAAYTTATAAACGAACAGTTTCACCATTGTAGTCAGAACCAGCCTCTTGCTAGATCTGAACGAATAAAGACGTCAT--CAGACACGCTGTATTTTT-CACATCACTA[477]

female1 ..........T...G..T.G.........A...C.................C..T.........C............RR..G......................................C.......--.RRR..............YY........G[477]

female2 ..........T...G..T.G......R..A...C.................C..T.......RYC............RA..G......................................C.......AC...M............Y.Y.........G[477]

male1 ..........T...G..T.G......A..A...C.................C..T.......G.C.............A..G......................................C.......--..A...............T.........G[477]

male3 ..........T...G..T.G......A..A...C.................C..T.......G.C.............A..G......................................C.......--..A...............T.........G[477]

female3 ATAGAACCTTTGTATTTTATATATGCTGTTTTAGAAGAAGAAAGAAGGCGCCACGTGCACGGTTAAACACCGAATCGCCAGCTTTAAACACATCCAAGAAGACCTGACCGCGGACGGCTGGAGAGTGGTGGACTTGACGAAGAAACTCTCAAGGTATGT[636]

female1 ....MM...C.KK-........G...SS...........................................RR......................................................................................[636]

female2 ..M......C............G...C............................................................................Y.......................................................[636]

male1 .........C.T.TAA......G....C............................................G........T..........................................................................C..[636]

male3 .........C.T.TAA......G....C............................................G........T..........................................................................C..[636]

female3 AACATTTGGTAGTTTTATTTTCTAGTCAAAATCGAAAATGGTAATAATATCTGTTGTATGTTGTATAAAGAAAGGTCCAGGAACAGCCTGTTCCGATATGAGAGCACATGTGATATCATCAAAATTAAAAAAAAGTCAGCTGGCAATTCAAGATATATT[795]

female1 ..Y............................NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN.................................[795]

female2 .....................S........NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN.................................[795]

male1 .........................................---...C........C.........................G..................A.........................................................[795]

male3 .........................................---...C........C.........................G..................A.........................................................[795]

female3 TCATGCTAAGGAATTTTTTCTTCGTTTTGATAACATGTTTTTCTCTCTCAATCCTCACAGATGGGTGCGACAGAATGTCTCCGCGGACACCATTGAGATCCAGATCACGTCAAAACCGGAGAGGCGACGTCCCCGGGACCTGAGCACCGACGCCTCTGA[954]

female1 .....................................................T..............................A..........................................................................[954]

female2 .....................................................T.................................................................................T.......................[954]

male1 ...................A...........................C...............................................................................................................[954]

male3 ...................A...........................C...............................................................................................................[954]

female3 TTGGCTGATGCTGATGAAGAACATGTCCCCGGTTCGTGAGGAAAGGGACACGTCGTTTGACGAGAGTCATTCGGAAGACGAGGAAAGTGATTTCTATGGCACGGACGAACCTGATGCAGAATCTAGACAGAAACGAAGGGTGGAAATCAAACTGTCCGA[1113]

female1 ...............................................................................................................................................................[1113]

female2 ...............................................................................................................................................................[1113]

male1 ..............................................................RK...................................................................................M...........[1113]

male3 ..............................................................G....................................................................................M...........[1113]

female3 AGATGTGAGTCTGGTAGTGTTCTCCCAGGACCAAAAGGCCGACTTACTCCGAGACTCCCAGAAGGCCAARCGCGCACAACACCCCGTAAGACACGACCACCAAGCGGAAACGCGAATYGCGCGCTCCAAYAGRAGACGYCAGAAGAAGTCTAAAAAGGG[1272]

female1 .......................................................................................G.......................................................................[1272]

female2 .......................................................................................G.......................................................................[1272]

male1 .........Y.................R...........................................................G...................................Y.....Y........Y....................[1272]

male3 .........Y.................A.........................................R.................R..............R..............Y...........C..R.....Y....................[1272]

female3 AAAGACGGACAGTTCTAAGAMAGAAGACACTCCTTGCAAGAAAGTAGAGTTCTGGGTGGATTTCGACCACATCGGCTGGGGCACGTGGATCATTTATCCGAAGAGGTTCAACGCGTTTCGTTGTGAAGGCGTGTGTCCGACTCCGGTAGACCAGCTCTA[1431]

female1 ...........S........A..................................................................................................................Y.......................[1431]

female2 ....................A........................................................................................................................K.................[1431]

male1 ..............M.....A.........G...........R....................................................................................................................[1431]

male3 ....................A.........G...........R....................................................................................................................[1431]

female3 CCACCCCACYAACCACGCCGTCATGACCAGCATTTTGAACTTACACAAGCCGGGAAAGGCGCCCATGCCCTGCTGCATCCCCACTAAGTTAAAAGC[1527]

female1 .........Y................................R.....................................................[1527]

female2 .........T................................G.....................................................[1527]

male1 .........T......................................................................................[1527]

male3 .........T......................................................................................[1527]

**Fig. S4. Sequence alignment of *Nodal* genomic regions amplified from male and female amphioxus with primers indicated.** The numbers of males or females analyzed was indicated. The second numbers in the names of sequences from primer pair G-F1/G-R1 are colony numbers. Letter M (A/C), R (A/G), W (A/T), S (C/G), Y (C/T) and K (G/T) marked in yellow indicate single nucleotide polymorphism in two alleles. Fragments marked in green indicate deletion in one of the two alleles. Sequence denoted with N are not determined.

254KD-F1/254KD-R1 (reverse strand)

female1 TGCCCTACCATCTGCAGGAGGCAGGACTGGAAGACAGACTTGCAGACTTCTTAAAGAACAACAAGGACATCACAGAAAAGCACCGATGATCACTCAAAGTCAACCATGAAGAAAATATCATAGTGCCAGCATAAAAGTTGCAACTCTATTTTTGATACC[159]

female2 ...........................W.............................................................................---..............A..............................T.....[159]

male1 .........................................................................................................---..............A..............................T.....[159]

male2 .........................................................................................................---..............A..............................T.....[159]

male3 ...............................W.........................................................................---..............A..............................T.....[159]

female1 TTTTGATTTGTGACTTCAAGTTGATGTGGAACTCCATAATTTTCATGTAATCTGACCCCACAGAAAATCCCAAAGCTTGTATTGTTGTTGTCAATTGCATTTTTTGTTTCATAAAGAATTTGTAACAAATACAAAAGAGCATGGCTTGTTTTCA---TT[318]

female2 ...............................................................................................C..........................................................TAT..[318]

male1 ...............................................................................................C..........................................................TAT..[318]

male2 ...............................................................................................C..........................................................TAT..[318]

male3 ...............................................................................................C..........................................................TAT..[318]

female1 TGTATTTTCTAATGGGAAAGTAATGTTTATATAATGTAAGATGTTATTGGCATGTAAATGAATATTTTGTATTTTTCTATGACAGAATATAAAGTCTTTGAACACAACTTACAGCATAGTATTGAGTCTTTATTTTCCCATATTTACTACGTACTCTCT[477]

female2 .....................T............................................A.........A...........................................C...............T......................[477]

male1 .....................T............................................A.........A...........................................C...............T..............W.......[477]

male2 .....................T............................................A.........A...........................................C...............T......................[477]

male3 .....................T............................................A.........A....................................R......C...............T......................[477]

female1 TAATTCTAGACTTCATATTATTATTGATATTATTATTATCATTAATCTAGTTTGCAACATGCTACATTTATCTGATTTTCTTGACAATCCTTAGCAAGTAATCATTTCACACACAAACAGTAGAAATAACATGGTCATCTTAAGCCACAGAAATTTACC[636]

female2 ...................................................................................C..............C.......................................................G....[636]

male1 ...................................................................................C..............C...........................................T...........G....[636]

male2 ...................................................................................C..............C.......................................................G....[636]

male3 ...................................................................................C..............C...........................................W...........G....[636]

female1 TGTCCTCATTTCA[649]

female2 .............[649]

male1 .............[649]

male2 .............[649]

male3 .............[649]

185KD-F1/185KD-R1(reverse strand)

female1 TTCTGGTAACAGTGACTTAAAACCATCACGAAAGTTGGGGTTGAAGTAGCCGTATATGATAGGATTCACAGAACTGTTGAAGTAGGACAGCCAATGGGCAATGGGGAAAATATAGATGTACATGACCTGTCTTTGGAGGTAGGACAACTGGACAAAGTC[159]

female2 ..................................................................................................................R............................................[159]

female5 ..................................................................................................................R............................................[159]

male4 ..................................................................................................................................................R............[159]

female1 ATTCAGTAACAGGGTAGTGTGGAGAGGCAGCCAGAATAAGGCGAACACTACCACTACCACTATCAGCATCTTGACCACCTTTCTCTTCTTCTGCAAGGCTATGTTCTGGGCTTCCATGGACGCCTTCTGMACKCCTGGTGTGGCCTTGAACCAGATTCG[318]

female2 ...................................................................................................K..R........................................................[318]

female5 ...................................................................................................G..R.....................................................K..[318]

male4 .................................................................................................................................T..K..........................[318]

female1 ATGGCCGATGAGCACGTAGAGGACTAGGATGATGCCGAGAGGCACCAGGTAGCTGAGGATGAATAGCGAGAGGCTGTAATACTTGCTGACATCTCCGGCAGGCCATTGCTCGTCACAGTAATGGACGAGGAAGATGACGTCCCCTTCCGGGTCCGGGTA[477]

female2 ...R...........................................................K...........................................S.........R.........................................[477]

female5 ...R...........................................................G...........................................S.........R.........................................[477]

male4 ...............................................................K...............................................................................................[477]

female1 TTCCGTGTCGTGCTTGAAGATGATCAATGGCAGGCTCACGGACAAGGCGAACACCCAGATGGTGGTGACGGCCTTCACCATTTGGCTCACGCTCAGCTTTGGCTTCGTAGGAAGCACTACYGCATAGAATCTGTAAGGCAAARGCCAGAAAATGATGAA[636]

female2 ........................................................................................................................C.....................G................[636]

female5 ........................................................................................................................C.....................R..........R.....[636]

male4 ........................................................................................................................Y......................................[636]

female1 AACATTATTTTTCATGATAGCATTTATCTTATTTATCTATTGGTTTGGCTGTTTAGCACACACAGCAAGGGCTGCCCAGCAAGCCTGTGGCTGTGAAAGGATTTAG[742]

female2 ..NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN[742]

female5 ..-..Y.Y......KR...S.................................CR....................Y.....R.....................Y..[742]

male4 ..A.......................................................................................................[742]

147KD-F1/147KD-R1(reverse strand)

female1 TCATCTAGTCACGCGATTGAGA-TGGTCTACAGGTCCTTCTGCACCTGGTCTAGGAGCGGATTTTATTTCCATTTCAGGACGACGGGTAAGACATCAATTGATAAATGTAY---CACCGAAGTAAAAAGGCTGTTCCATAGCTTTTTTTAAAGAAGTAA[159]

female5 ......................G...............................................Y..........R.............................AAT..................................Y..........[159]

male1 ..............A.......-........................................................................................---.............................................[159]

male3 ..............A.......-.......................................................................................Y---.............................................[159]

female1 GGACAGTGCGTTGTTTGTTATTCACTGTCTAAGGCGGGGGCAGAGGGGGAACCCATCCCTCTCCTTCCACC--------MCCCCCCCCCTCCATGAACTCAGTACCTTTTCCAATGACACAAAGTCTAGCCAGAAATTCTAAAAAAGCAATTTGTCTAT[318]

female5 ....................................R................................TTTCCACCCA..........Y...................................................M...W............Y[318]

male1 ......................................R................................CCCCMC--................................................................................[318]

male3 ......................................RR...............................CCC-----................................................................................[318]

female1 ATTGAAAACAATGCAGCGTTGTTTCCCCATGTTTTGTTTAGAATGCACACAGATACCAACAACAAGCTGTAAGCAGCAACAGGACCTACAGGCACCATGCGGGTTCTTTCAACCACCTCCCCGCAGTGACGTGGACAGGCAGCCGTTAGTATGCGACTG[477]

female5 .........................Y............................R.............................................................................R..........................[477]

male1 ...................................................................................M...........................................................................[477]

male3 ...............................................................................................................................................................[477]

female1 GCACATATCACCTCCACCAGGAAACAAGGCGGTGCTAACGTTCCAAAACCTCAGTCTAACGGCGGACCAGAAAGGAGAGGACTTCATCTTAATCCAAGAGCTGGAAGACGATGGCACATTTCGAACAGTTCTGGATATACGGCAAAGTTATCCACTGTT[636]

female5 ...............R..............................................................................................................................M...........Y....[636]

male1 ..........................................................................................................................................................Y....[636]

male3 .........................................................R................................................................................................Y....[636]

female1 CTCAGTGGAGCTCAAAAGTTCGCAGGTGATGGTAAAGTTTGTCAAGGACAGAATAGG[693]

female5 .........................................................[693]

male1 ...................................R.....................[693]

male3 .........................................................[693]

110KD-F1/110KD-R1

female3 GTAGACGACGTGTTTCTGACTTGCTGACAGCTGAGTAAAGTCATTCAGCAACATGATCGTGTGCAATGGTAACCAGAATAATGCAAACAAGACCACCACAACAATGAGCATCTTGATGACYTTGATTTTCTTCTTCAAGAGTGCGTTTTGTGCCTCCAC[159]

female1 ........................................................................................................................C......................................[159]

male1 ................................................................................R.......................................C......................................[159]

male3 .....................................................................Y..................................................C......................................[159]

female3 AGATGCCCTCTGGCCTGGCGCTGATTTGTACCAGACTTGATATCCGATGATCATGTACAGGACGAGAATGATGCTGAGTGGYGCCAGGTAGCAGATGATGAAAAGGGCGAAGGAGTAATACTTGCTGATGTCGTCTTTTGTCCATGAACCTGTTGCGGG[318]

female1 .................................................................................Y..........................A..................................................[318]

male1 ...............................................................................................................................................................[318]

male3 .................................................................................Y.............................................................................[318]

female3 CCACTGTTCATCACAGTAATGTACCAGGTATGTAGCGTCCTGCTGAACGTCCTCGTATATCTCGTCACGGTTGAAGATCATCAGCGGCAGGCTGACCGTGATGGCGAACATCCAGATGGTGATGACTGCCTTGGTCATAACAGTGACCTTGAGTTTGGG[477]

female1 ...............................................................................................................................................................[477]

male1 ...............................................................................................................................................................[477]

male3 ...............................................................................................................................................................[477]

female3 TTTGGTAGGGAGGACAACWGCATAAAACCTGCAAC-AAAGAGCACCAACCTTAAGTTTTGTTTAGCTGCATCACTTTGATGCTATTCCAAGCAGAGCCAGAGGTTATGTTTGGGGGACTACTAGTGGTTGCTTAGAAGGAAAAATG--AAGAATCTTGA[636]

female1 ..................W................A...R........................................T.........A....A................................................C.TA...R.......[636]

male1 ..................W................-......M.....................................T.........R....R................................................C.TA...G.......[636]

male3 ..................A................-............................................K...............................................................Y.TA...R.......[636]

female3 GGCCTGCAAGCCCCCGACCAAAACYACTGTTTGGATAATATTTCAATATTGTTACAACTTTGCGACTCACTTAGATAGATAACGATAACATAGATAA[733]

female1 ........................C......................GC....G............................T.....A........[733]

male1 K................S......C..................S...GC....G............................Y..............[733]

male3 ........................C..................S...RY....A.R..........................Y.....M........[733]

71KD-F1/ 71KD-R1

female1 CTCAGAGGTCCGCCATCCGACAGACKTGGGGGAACGAGAACAACGTGCCAGGAACCGTCATTAAAACTSTCTTTGCGGTGGGGAAACYGGGMAAAYCCTCCATACAACACAGTCTGGRGGACGAAAACATGGTCCACRRGGAYATCATCCAAGAGGACT[159]

female2 .......A.................K...........R.................................................Y...M...Y...............................Y.........RR....................[159]

male1 .........................K..............................................................R..M...Y...............................Y.........R.....................[159]

male2 .......A.................K.............................................................Y...M...Y...............................Y.........RR....................[159]

female1 TCGTCGATTCCTATAAAAACTTGACTCTYAAGACCGTMATGTGTCTAAAATGGGCCTCCMAATTCTGTCCCARCGCCAAGTTCGTTATGAAAGCTGATGACGACACMTKTGTGAACATCTTYAACTTAGTAAAACGTTTGAARTTCACGGTGCCAGRGG[318]

female2 .....................................M.....................M............R...................R.............M.K............Y..................M.R................[318]

male1 ...........................................................M............R.................................M.K.........S..Y..................M.R.............R..[318]

male2 .....................................M.....................M............R...................R.............M..............Y..................M.R................[318]

female1 AATTTGTGACAGGGTATCGTTKTYACCAACRCRAGACCRRTTAGAGCYSKMKATGACCGTTGGTACGTAAGCGAGRAAGAGTATCCCCGGGAGACCTTCCCGAGATACCCMTGTGGATTCGCATACGTCATGTCCAATGACRTCACCGGCSTAATCTAC[477]

female2 .....................K.Y......R.R.....RR.......YSKMK.........................................................................................R........S........[477]

male1 .....................K.Y......R.R.....RR.......YSKMK.............Y.........R.................................................................R........S........[477]

male2 .....................K.Y......R.R.....RR.......YSKMK.........................................................................................R........S........[477]

female1 CRGACCTCTTTGACCYTGAARTACMTTTTTCTYGAAGAYGTKTTCTTGGGACTTTGCTTAGAAAAACTRGCAATCGACCCCGTGCACGACACTCGCTTCCACCATTCAGAGACAACGCCATCTTGCGAAACTGGTAAAGAATGGATYGCYTTTTCATTK[636]

female2 ...............Y....R...M.......Y.....Y..K..............Y...........R.....Y........R..............................................................-TG.Y.......-[636]

male1 .R.............Y....R...M.......Y.....Y..K..............Y...........R.....Y........R..............................................................-YG.Y.......-[636]

male2 ...............Y....R...M.......Y.....Y..K..............Y...........R.....Y........R...............................................................YG.Y.......-[636]

female1 TGATWAAAACTCR--KKACGRTATGR-TAAGGCKTGGCARGACGTCACCAGTTGCGASGMATCCCTGTCTWAGCTTGAATWAGATCGCCTTTAAAAGCTTTTATRACACYTTGCATGGGAAGATG[761]

female2 K...W........-RKK...R....RT......K.....R.................S.MR...................W.......................R....Y...............[761]

male1 K...W........-RKK...R....R-......K.....R.................S.MR.....K.........R...W.......................R....Y...............[761]

male2 K...W........ARKK...R....RT......K.....R.................S.MR...................W.......................R....Y...............[761]

32KD-F1/ 32KD-R1

female5 GTAAGTGCCATTAACCATCAGGAAAATGTTACAGCCAGCCATCACAGGTCATTCCACGCTATAATGGTTGTGAAATGCACATGTGGTGACACTACCGAAGTAAACATTAGCAAGACTCCATGTCCTTTTCTACAAACCTATTATTATTGCACTATATTA[159]

female2 ..........Y.......................T...G..................................C.....................................................................................[159]

male1 ..........C.......................T...G.............................A..........G...............................................................................[159]

male2 ..........Y.......................T...G..................................C.....................................................................................[159]

female5 TTAAGTAGATTCTATAATCTGTAACTTTAAACAGTCCCAAAAC----TATTGGATAGAGTATCGTATTTAGTTACATGCAGCGACCGAAATAAGTGACGATACCATATGCCTACTGCGTATTAGATACATTTCTTTTCTTTCTTATGATTGCTGGTAAC[318]

female2 .....-------.......C............G..........----.......A.............C.............................C..................................C.........................[318]

male1 .....-------.......C............G..........TATA.......A.............C.............................C..................................C.........................[318]

male2 .....-------.......C............G..........----.......A.............C.............................C..................................C.........................[318]

female5 ATCTGATTATTTCTGATTATTGTTATTAGTTTCACATATTGGAGAGGGCGAGCTCCCCTGCCCGCGGGACAGAACATAGAGAGCTGTGCTCTGATGCTGACGGGAAAATCAGAAGATTCACCCGAGACGAGATGGTTCACCGTGCCCTGTGACGATGGT[477]

female2 ........................................................T............................................T....C........C.........................................C.[477]

male1 ........................................................T......A.....................................TA............C.........................................C.[477]

male2 ........................................................T............................................T....C........C.........................................C.[477]

female5 GAGGCTACCCGGACCTACGTCTGCAAGGCCAAGGCCAGAGGTAGCTTGTCAGTATATCCTTAGTAGGTGATGAAAAGGCGTGCACGCGTGGTAAAAGTACCACATAGACATGGTCGTTGGTAAAATAGCCCATCCAGGTACACTAGCCTATTGGACTGT[636]

female2 ........M...............................................A......................................................................................................[636]

male1 ........A...............................................A......................................................................................................[636]

male2 ........M...............................................A......................................................................................................[636]

female5 GAACTACAATAGGGATCTATTCCGAATATCATGAGAATACGTCGTTTCTTTAATATCGATATCCTCTTTGAAAGGTTTTGACAAACACTTGTTGCGGCGTGTATGTCCGTTGACTACTGGACATACTATGAAATTCATAGTTTGTGTTGAAGCATATAT[795]

female2 ......................................C.............------.................................................................................................R...[795]

male1 ......................................C.............------.....................................................................................................[795]

male2 ......................................C.............------.................................................................................................R...[795]

female5 TCATCGTGGGACGTTTCTAAAATGATTTCTTTCTATACCAAGGCTGCAACAGGGGTTGGAGCATG[860]

female2 .A...............................................................[860]

male1 .A...............................................................[860]

male2 .A...............................................................[860]

48KU-F1/ 48KU-R1

female3 TAGGCAAGCCCGTTTTTAGCTGTCTCTATCAATTTCATTACGGGGAATTGTGCAATTGAAYAAGGGGTGCAATTGAATAATGTTAGAATATAATAATGGTGAACAAATATTCAAGCTAAGGTAAATACAAATGGGTGCTCCAATGATGGTACCAAGAAC[159]

female5 ..........................C.................................T.................R.................................................G..............................[159]

male1 ..........................K.................................T.................R.................................................R.......................Y......[159]

female3 GATATATGTAATCTATATAATGTAATCATGTATGAATCCCTTGCTTTAAGACGCACACATACTAGTATGTAAAAAGTCCATTTGTGCACATAGAGGTTTAGGCAGATTTATTGGAATCACTGTAGGAAAGCGCGTGGCATTTGCTGTAAGTCACACATG[318]

female5 ................R....................................A.........................................................................................T...............[318]

male1 .....................................................A.........................................................................................Y......Y........[318]

female3 GTAAGCCGTTTTAATGGTTATGTTACACTGGTGGTAGATTGGTAAGAAATTCAACCTGGTATTATTAGGCAGGCCTTCATCTGTCCGTTAGGTTAGTCTATGGTTCTACATGTAATATAC-AAAAAAAATGGAATTAAAAACTGCTTTACTCTGTACCT[477]

female5 .....................K..................................................................................................A......................................[477]

male1 .....................K..................................................................................................A......................................[477]

female3 TGGTAAGGAAACACTAGTACGGCGTTAGATCCTGGCAAACGACTA-TTTTTAGCTGTTGCCTTGACAACGGCACTTTGGCTACATTTTGGTCTGCGGGCGCTCCTTGAGCCACCTAGCGACCCCCTGTTGGGACTGCACGTTGTCCATGACCTTGGCGA[636]

female5 ................................C............T.................................................................................................................[636]

male1 ..............W.................Y............-.................................................................................................................[636]

female3 GG[638]

female5 ..[638]

male1 ..[638]

123KU-F1/123KU-R1

female3 AAGTTGGCCGCACTTGCGGAACGCATCAAAACGTACCCGGGCATAAAGGAGTGGCTGGCCAAACGTCCGAAGACACCATGGTGAACAGATAGACACCAGTCTCTTACGGCGGGCTCACACATATTAAAGCCCGTATGAGGTCCGTATGGAGTTCTTATT[159]

female5 ...............................................................................................................................................................[159]

female2 ..........................................................................................................Y..................W.................................[159]

male1 ...............................................................................................................................................................[159]

male3 ...............................................................................................................................................................[159]

male2 ..........................................................................................................Y..................W.................................[159]

female3 CTCTACAAGGATTCACATATCGCTGGAGAGAATAAGATATAAAATAGAATCAATTTTACCTTAAGACAACATTTAGACAATTCTCTGATTGGCTGACAGCTGGTTAGATCAGGCCACGGTCCCAAATTACGATGGAAACCTCTGTCCGTTTTAGCCGAG[318]

female5 ...............................................................................................................................................................[318]

female2 ............Y............................................................................................MR......Y....R................R.......................[318]

male1 ...............................................................................................................................................................[318]

male3 ...............................................................................................................................................................[318]

male2 ............Y............................................................................................MR......Y....R................R.......................[318]

female3 CTTCTCCCAGGGTATATTGTATAAGACGCAGGCGAGCTGTGTGTGC----------------AATTCAAAATTCTAAAGTAAAATGATAGAATGTTGAAGTAGTTGTACCTTTCCCATGACAGATAGATTGGTTCTGATAGAGATTAAGTGAATAAAAT[477]

female5 ..............................................----------------.................................................................................................[477]

female2 ...................Y..........................AAATGTTAAATTTTAG.................................................................................................[477]

male1 ..............................................----------------.................................................................................................[477]

male3 ..............................................----------------.................................................................................................[477]

male2 ...................Y..........................AAATGTTAAATTTTAG.................................................................................................[477]

female3 ACCATTGAAAATACATATCGTTTATACACATCTTTGATTCACCTTTTATTCAGTCAATAAAATATTTACAGACTTGTGG[556]

female5 ...............................................................................[556]

female2 ...............................................................................[556]

male1 ...............................................................................[556]

male3 ...............................................................................[556]

male2 ...............................................................................[556]

238KU-F1/238KU-R1

female4 GGGCCAGCACTGGCAGGAGGACAAATGCAACGCATCTACACTCACTGTGTGTGAATCAATATAACATATAAAACACATGAAAATGCAAACAAGCAGGAAGTGAATTTTTGATTATGTTTTGATAATTTTGCACCCTTCTTGCCCTGGCAGCCATCATGG[159]

female3 ...............................................................................................................................................................[159]

female1 .............................................--................................................................................................................[159]

male1 ...............................................................................................................................................................[159]

male3 ...............................................................................................................................................................[159]

female4 AGCGTAAGTGCAGCAAGGAGGGTCAGTACTGGCAGGAGGATGTCTGCACCAACTGCACCTGCCAGTCTGGGAAGGCCATCTGTGTCGCCCAGGCCTGCCCCCAGGTCCCCAGCGGCTGCAAGCCCATCTTCAGCGACTCCGAGTGCTGCCCGCGCTTCG[318]

female3 ...............................................................................................................................................................[318]

female1 ..................................................................................C............................................................................[318]

male1 ..................................................................................Y............................................................................[318]

male3 ..................................................................................Y............................................................................[318]

female4 AGTGCCACGACAGTACGTGTCTCCATTTCATTTTGATAGTAGAATATGATGTTTAAGGGTTGAGGACCTGCCCCAAGGTGTCATAACGCCTGGGCCTTAGCTATAACCACCGGATAAAACCACCGGGTGAGCGAAGCGGATTGCTGCCGTCGCTTTGAT[477]

female3 ............................................................................................................................................K....R.....A.......[477]

female1 ............................................................................C...............................................................G....A.............[477]

male1 ..........................................................................W.K...............................................................G..........R.......[477]

male3 ............................................................................................................................................G..........R.......[477]

female4 TGCCAGGACAGTGGGTGCCTCCTTTACATAATGATAGTAGAATATTTAAGGGTTCATGACCTACCCCAAGGTGTATATTGTGTCACAATGCCTGGTCCTTTGCTATAACCACCAAATAAAACCACTGAGTGAGCGAAGCAGAGTGCTGACCTCGCTTTG[636]

female3 ................................................................................................Y........................................................R...Y.[636]

female1 .........................................................................................................................................................A...C.[636]

male1 .............K..................................................................................T............................................................C.[636]

male3 ................................................................................................T........................................................R...C.[636]

female4 AGTGCCAGAACAGTGTGTGTCTCCTTTCCATTATAATAAA[676]

female3 ........................................[676]

female1 ........................................[676]

male1 ........................................[676]

male3 ........................................[676]

**Fig. S5. Sequence alignment of genomic regions near the *Nodal* gene amplified from male and female amphioxus with primers indicated.** The numbers of males or females analyzed was indicated. Letter M (A/C), R (A/G), W (A/T), S (C/G), Y (C/T) and K (G/T) marked in yellow indicate single nucleotide polymorphism in two alleles. Fragments marked in green indicate deletion in one of the two alleles. Sequence denoted with N are not determined.

T-F2/T-R2

female\_parent TTATAAGGAGTGGTAATCAGTTAATGCAGCAGGCAGGCCGAGACCAACACCGCGYCGGACATGCCAGCTCCGCGCCCARCCATGGGGACATTTCTGACGGCTTCGGTCGCGTTCTTCGCCGTTCAACTCCTGCAGTGTGAATGCTTGTCGAACGAGCAT[159]

male\_parent ......................................................T.......................G................................................................................[159]

female1 ......................................................T.......................G................................................................................[159]

female2 ......................................................T.......................G................................................................................[159]

female3 ......................................................T.......................G................................................................................[159]

male1 ......................................................Y.......................R................................................................................[159]

male2 ......................................................Y.......................R................................................................................[159]

female\_parent ACACTAGCGGCGTCGGGCCTACTTCGAGCGAGTGGCGGAGAGGGTCTGACGCTGGGAAGATACGAGCATGGCGACGGCGTGGTTAACGTGACCATGCGGGGCATGGCCGTGGCGGGGAGGATGACTCCAGAGTTCATGCTCGACTTCTACCAGAGTCTG[318]

male\_parent ...............................................................................................................................................................[318]

female1 ...............................................................................................................................................................[318]

female2 ...............................................................................................................................................................[318]

female3 ...............................................................................................................................................................[318]

male1 ...............................................................................................................................................................[318]

male2 ...............................................................................................................................................................[318]

female\_parent AGCTCGGGGACAGACCTCAACGTCACCCGGGAGGAAAACCAGGCGCTGCCGCTGCCGTCCGACACCGTCAGGAGTTTCGCACTAAAAGGTCAGTCAGTTTTAACGGATTATTATCTGGAGCTTTGCAATTGAGATGAGGCAAATGATTTTTTTTTCGAT[477]

male\_parent ..................................................................................................................................................-............[477]

female1 ..................................................................................................................................................-............[477]

female2 ..................................................................................................................................................-............[477]

female3 ..................................................................................................................................................-............[477]

male1 ...............................................................................................................................................................[477]

male2 ...............................................................................................................................................................[477]

female\_parent TACCTTTGTATCGACAAGAAGAGAAAGGGGCAGACTCTTGTTGTAAGAAACATSCAAGTGTTTTAGAAATTGCATCCCAARTAGATCCATTGAGAGGTCGAAATGGTATTCAGAATTAAAAGTAGATATTTGGACGACG[616]

male\_parent ...........................................................................................................................................[616]

female1 ...........................................................................................................................................[616]

female2 ...........................................................................................................................................[616]

female3 ...........................................................................................................................................[616]

male1 .....................................................S..........................R..........................................................[616]

male2 .....................................................S..........................R..........................................................[616]

P-F3/P-R0

female\_parent CCGTAACTAAAGCATGGCGGTGTTGTCTGTGTACTCACCGTGTTTGKATGGCGTTTGTTGATTGCTCTTTTATAGGCCGTCAATGGTCGATCGACAAGTCACTTAGCTTGTCTAAAAACCGGACGGTGAGCTAATTACCAGTCATGGGGTAATTAAGAT[159]

male\_parent ...............................................................................................................................................................[159]

female1 ...............................................................................................................................................................[159]

female2 ...............................................................................................................................................................[159]

female3 ...............................................................................................................................................................[159]

male1 ..............................................K................................................................................................................[159]

male2 ..............................................K................................................................................................................[159]

female\_parent AAGATGATAATTACAACGTGATGAGGTTTCTGGAGRAGCAAAAAATCATTTTGAACTTTGAAATCGTGTGTCATTTTCCYGTCAAAGACACGGTTTGCTTTCCATACCATCAAAAGACCAAAATGAAGACCGCTGAATGTCTGACACTTCATTGTCCCC[318]

male\_parent ...................................G...........................................................................................................................[318]

female1 ...................................G...........................................................................................................................[318]

female2 ...................................G...........................................................................................................................[318]

female3 ...................................G...........................................................................................................................[318]

male1 ...................................R...........................................Y...............................................................................[318]

male2 ...................................R...........................................Y...............................................................................[318]

female\_parent TATCACCTCCCGCCTTGCYTTTGTCTTTCGCCTCGCGTCATGAATATTAAAGAGGTGGAATSTGGGTTATAAGGAGTGGTAATCAGTTAATGCAGCAGGCAGGCCGAGACCAACACCGCGYCGGACATGCCAGCTCCGCGCCCAGCCAT[467]

male\_parent ..................C..........................................C.......................................................................................[467]

female1 ..................C..........................................C.......................................................................................[467]

female2 ..................C..........................................C.......................................................................................[467]

female3 ..................C..........................................C.......................................................................................[467]

male1 ..................Y..........................................S..........................................................Y............................[467]

male2 ..................Y..........................................S..........................................................Y............................[467]

P-F2/P-R0.1 (reverse strand)

female parent TGCGAGCATGATAATAATGGTTTCAATTTTGGTAGACATTCCTGAAATGCCTWATTTATACTTGACATAGTTGTGTCCCAAAATTTGAGTTGWACACCTATTTCTTGGTGARGCTAAGTACTCACTGATGCCGACAATGGTTAGTATGATCAAGGACAG[159]

male parent ....................................................T.......................................T..................R.....R.........................................[159]

female1 ....................................................T.......................................W..................R.....R.........................................[159]

female2 ....................................................T.......................................W..................R.....R.........................................[159]

female3 ....................................................T.......................................W..................R.....R.........................................[159]

male1 ....................................................W.......................................T........................R.........................................[159]

male2 ....................................................W.......................................T..................R...............................................[159]

female parent GAAGGAGGGTGTATGATGGGAAGATATCGATCAGGGGATTAGGACTTCCATAAATGCCCTAAAATACCCTGCTTCCGCCACGATAAACTTATCATAAAGAGCCCACCACACTTGCAACACAAAGAAACTTCATGACACACAAGTATAGTATGCGAATTG[318]

male parent ...............................................................................-----.........................................................R.................[318]

female1 ...............................................................................-----.........................................................R.................[318]

female2 ...............................................................................-----.........................................................R.................[318]

female3 ...............................................................................-----.........................................................R.................[318]

male1 .............................................................................................................................................R.................[318]

male2 ...............................................................................................................................................................[318]

female parent CAAAATAACGCGTACTTGCGCTTAGTGTCCATTTTGAAGTTAAGAGTTAGGAATAAGACCCTTCTCTGAAACAAAAAATTAAGAAGTAACAGTGATAKAAGTTAAGTATTTTGGTCCCATGAGGGTTGAATTTCTCATTRATTGGTAAGATCTCCTTGG[477]

male parent ........................................K..................................................................................................G..Y................[477]

female1 ...........................................................................................................................................R...................[477]

female2 ...........................................................................................................................................R...................[477]

female3 ...........................................................................................................................................R...................[477]

male1 .................................................................................................K.........................................G...................[477]

male2 ........................................K........................................................K.........................................G..Y................[477]

female parent GAGGTACACAGCTTAGTKGTGTTTTCACACCTAGGTTTATGTATCGT[524]

male parent ...............................................[524]

female1 .................K.............................[524]

female2 .................K.............................[524]

female3 .................K.............................[524]

male1 ...............................................[524]

male2 ...............................................[524]

**Fig. S6. Polymorphism analysis of *Nodal* genomic regions amplified from a pair of adult parents and their offspring.** One female parent and one male parent and their offspring (three females and two males) were analyzed. Letter M (A/C), R (A/G), W (A/T), S (C/G), Y (C/T) and K (G/T) marked in yellow indicate single nucleotide polymorphism in two alleles. Fragments marked in green indicate deletion in one of the two alleles. The female parent and offspring do not share the 6-bp insertion polymorphism (arrow indicates the location of 6-bp in other females which share it).

**Table S1 sequences of primers used in this study**

|  |  |
| --- | --- |
| Primer | Sequences (5’-3’) |
| T-F1 | GCAGGCCGAGACCAACACCGC |
| T-R1 | TTTAGTGCGAAACTCCTGACGG |
| T-F2 | CCGCCTTGCCTTTGTCTTTC |
| T-R2 | CACTGGTAGAAACGTCGTCCA |
| P-F3 | GCCTGTGAAGAGCCGTAACT |
| P-R0 | AACGGCGAAGAACGCGACCGA |
| P-F1 | ACAGTGATAGCGATGCGTCA |
| P-R0.4 | TCATCATTGAGAGGTTTTAAG |
| P-F1.1 | TGAATGTAATTGATTACC |
| P-R0.3 | ATTGACTGGTGGCTCTT |
| P-F1.2 | TATGTTGGTTTCTGCAC |
| P-R0.2 | ATCACCTGGTCTCACAGTGG |
| P-F2 | CCACTGTGAGACCAGGTGAT |
| P-R0.1 | CCATGTTCCCCTTCTCCCTTAC |
| P-F2.1 | GTAAGGGAGAAGGGGAACATGG |
| P-R0.0 | GAAAGACAAAGGCAAGGCGG |
| G-F1 | CCGTCAGGAGTTTCGCACTA |
| G-R1 | TGCTACCTTTCTGCTTGGCAT |
| G-F2 | ATGCCAAGCAGAAAGGTAGCA |
| G-R2 | CTACTGACAGCCGCATTCATCCA |
| 254KD-F1 | GACACCATCGCCAGTTGAAAT |
| 254KD-R1 | CACAAACGCCTGGCAGACTA |
| 110KD-F1 | TGGGATTGACGGAACTGTTGA |
| 110KD-R1 | CCCTTTCATGTCCACAGCGT |
| 123KU-F1 | TTCCTGTCCCTGTCTGACGA |
| 123KU-R1 | TGCTGCTATACTCCACAAGTCTG |
| 238KU-F1 | TGATGGTAACCCTTTTTGCCC |
| 238KU-R1 | AGACAGTGGCTTCTTCTCGC |
| 185KD-F1 | CATGCAGACCACCTCCGTTA |
| 185KD-R1 | ATGAACAGCGGCTTATGCGA |
| 147KD-F1 | TGGAGTTACAGCTTTGGTGGT |
| 147KD-R1 | TACTCGTATTGCCGGGAAGC |
| 71KD-F1 | CGGAAGTGACGTATTCCTGCT |
| 71KD-R1 | AAGCTTCAGCACATCTTCCCA |
| 32KD-F1 | CTACAGGCCAAGATGCACGA |
| 32KD-R1 | GGAGACAAGACTTCCCAGCC |
| 48KU-F1 | TGGGGAATTGTGCAGGGATA |
| 48KU-R1 | TCAACATGATTGGCTGGGTGA |