Table S1. Detailed record of the lumpfish (*Cyclopterus lumpus*) samples in this study. Including the sample ID, phenotypic sex, sampled location, family (if known), and the amplification result of each primer set. The “+” indicates the presence of the anticipated band, and “-“ indicates the absence of the anticipated band

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sample ID | Sex | Original Region | Family | AMH1\_E3I6 (presence/absence) | AMH2\_I6E4 (presence/absence) | AMH1+3\_E6I6 (no. of band) |
| B1M | Male | British Isles | N/A | + | + | 2 |
| B2M | Male | British Isles | N/A | + | + | 2 |
| B3M | Male | British Isles | N/A | + | + | 2 |
| B4M | Male | British Isles | N/A | + | + | 2 |
| B5M | Male | British Isles | N/A | + | + | 2 |
| B6M | Male | British Isles | N/A | + | + | 2 |
| B7M | Male | British Isles | N/A | + | + | 2 |
| B8M | Male | British Isles | N/A | + | + | 2 |
| B9M | Male | British Isles | N/A | + | + | 2 |
| B10M | Male | British Isles | N/A | + | + | 2 |
| B11M | Male | British Isles | N/A | + | + | 2 |
| B12M | Male | British Isles | N/A | + | + | 2 |
| B13M | Male | British Isles | N/A | + | + | 2 |
| B1F | Female | British Isles | N/A | − | − | 1 |
| B2F | Female | British Isles | N/A | − | − | 1 |
| B3F | Female | British Isles | N/A | − | − | 1 |
| B4F | Female | British Isles | N/A | − | − | 1 |
| B5F | Female | British Isles | N/A | − | − | 1 |
| B6F | Female | British Isles | N/A | − | − | 1 |
| B7F | Female | British Isles | N/A | − | − | 1 |
| B8F | Female | British Isles | N/A | − | − | 1 |
| B9F | Female | British Isles | N/A | − | − | 1 |
| B10F | Female | British Isles | N/A | − | − | 1 |
| B11F | Female | British Isles | N/A | − | − | 1 |
| B12F | Female | British Isles | N/A | − | − | 1 |
| B13F | Female | British Isles | N/A | − | − | 1 |
| B14F | Female | British Isles | N/A | − | − | 1 |
| B15F | Female | British Isles | N/A | − | − | 1 |
| B16F | Female | British Isles | N/A | − | − | 1 |
| B17F | Female | British Isles | N/A | − | − | 1 |
| B18F | Female | British Isles | N/A | − | − | 1 |
| B19F | Female | British Isles | N/A | − | − | 1 |
| B20F | Female | British Isles | N/A | − | − | 1 |
| B21F | Female | British Isles | N/A | − | − | 1 |
| B22F | Female | British Isles | N/A | − | − | 1 |
| B23F | Female | British Isles | N/A | − | − | 1 |
| B24F | Female | British Isles | N/A | − | − | 1 |
| B25F | Female | British Isles | N/A | − | − | 1 |
| B26F | Female | British Isles | N/A | − | − | 1 |
| B27F | Female | British Isles | N/A | − | − | 1 |
| B28F | Female | British Isles | N/A | − | − | 1 |
| B29F | Female | British Isles | N/A | − | − | 1 |
| B30F | Female | British Isles | N/A | − | − | 1 |
| B31F | Female | British Isles | N/A | − | − | 1 |
| B32F | Female | British Isles | N/A | − | − | 1 |
| B33F | Female | British Isles | N/A | − | − | 1 |
| B34F | Female | British Isles | N/A | − | − | 1 |
| I1M | Male | Iceland | N/A | + | + | 2 |
| I2M | Male | Iceland | N/A | + | + | 2 |
| I3M | Male | Iceland | N/A | + | + | 2 |
| I4M | Male | Iceland | N/A | + | + | 2 |
| I5M | Male | Iceland | N/A | + | + | 2 |
| I6M | Male | Iceland | N/A | + | + | 2 |
| I7M | Male | Iceland | N/A | + | + | 2 |
| I8M | Male | Iceland | N/A | + | + | 2 |
| I9M | Male | Iceland | N/A | + | + | 2 |
| I10M | Male | Iceland | N/A | + | + | 2 |
| I11M | Male | Iceland | N/A | + | + | 2 |
| I12M | Male | Iceland | N/A | + | + | 2 |
| I13M | Male | Iceland | N/A | + | + | 2 |
| I14M | Male | Iceland | N/A | + | + | 2 |
| I15M | Male | Iceland | N/A | + | + | 2 |
| I16M | Male | Iceland | N/A | + | + | 2 |
| I17M | Male | Iceland | N/A | + | + | 2 |
| I18M | Male | Iceland | N/A | + | + | 2 |
| I19M | Male | Iceland | N/A | + | + | 2 |
| I20M | Male | Iceland | N/A | + | + | 2 |
| I21M | Male | Iceland | N/A | + | + | 2 |
| I22M | Male | Iceland | N/A | + | + | 2 |
| I1F | Female | Iceland | N/A | − | − | 1 |
| I2F | Female | Iceland | N/A | − | − | 1 |
| I3F | Female | Iceland | N/A | − | − | 1 |
| I4F | Female | Iceland | N/A | − | − | 1 |
| I5F | Female | Iceland | N/A | − | − | 1 |
| I6F | Female | Iceland | N/A | − | − | 1 |
| I7F | Female | Iceland | N/A | − | − | 1 |
| I8F | Female | Iceland | N/A | − | − | 1 |
| I9F | Female | Iceland | N/A | − | − | 1 |
| I10F | Female | Iceland | N/A | − | − | 1 |
| I11F | Female | Iceland | N/A | − | − | 1 |
| I12F | Female | Iceland | N/A | − | − | 1 |
| I13F | Female | Iceland | N/A | − | − | 1 |
| I14F | Female | Iceland | N/A | − | − | 1 |
| I15F | Female | Iceland | N/A | − | − | 1 |
| I16F | Female | Iceland | N/A | − | − | 1 |
| I17F | Female | Iceland | N/A | − | − | 1 |
| N1M | Male | Norway | Family 1 | + | + | 2 |
| N2M | Male | Norway | Family 1 | + | + | 2 |
| N3M | Male | Norway | Family 1 | + | + | 2 |
| N4M | Male | Norway | Family 1 | + | + | 2 |
| N5M | Male | Norway | Family 1 | + | + | 2 |
| N6M | Male | Norway | Family 1 | + | + | 2 |
| N7M | Male | Norway | Family 1 | + | + | 2 |
| N8M | Male | Norway | Family 1 | + | + | 2 |
| N9M | Male | Norway | Family 2 | − | − | 1 |
| N10M | Male | Norway | Family 2 | + | + | 1 |
| N11M | Male | Norway | Family 2 | + | + | 2 |
| N12M | Male | Norway | Family 2 | + | + | 2 |
| N13M | Male | Norway | Family 2 | + | + | 2 |
| N14M | Male | Norway | Family 2 | + | + | 2 |
| N15M | Male | Norway | Family 2 | + | + | 2 |
| N16M | Male | Norway | Family 2 | + | + | 2 |
| N17M | Male | Norway | Family 2 | + | + | 2 |
| N18M | Male | Norway | Family 2 | + | + | 2 |
| N19M | Male | Norway | Family 3 | − | − | 1 |
| N20M | Male | Norway | Family 3 | + | + | 2 |
| N21M | Male | Norway | Family 3 | + | + | 2 |
| N22M | Male | Norway | Family 3 | + | + | 2 |
| N23M | Male | Norway | Family 3 | + | + | 2 |
| N24M | Male | Norway | Family 3 | + | + | 2 |
| N25M | Male | Norway | Family 3 | + | + | 2 |
| N26M | Male | Norway | Family 3 | + | + | 2 |
| N27M | Male | Norway | Family 4 | + | + | 2 |
| N28M | Male | Norway | Family 4 | + | + | 2 |
| N29M | Male | Norway | Family 4 | + | + | 2 |
| N30M | Male | Norway | Family 4 | + | + | 2 |
| N31M | Male | Norway | Family 4 | + | + | 2 |
| N32M | Male | Norway | Family 4 | + | + | 2 |
| N1F | Female | Norway | Family 1 | − | − | 1 |
| N2F | Female | Norway | Family 1 | − | − | 1 |
| N3F | Female | Norway | Family 1 | − | − | 1 |
| N4F | Female | Norway | Family 1 | − | − | 1 |
| N5F | Female | Norway | Family 1 | − | − | 1 |
| N6F | Female | Norway | Family 1 | − | − | 1 |
| N7F | Female | Norway | Family 2 | − | − | 1 |
| N8F | Female | Norway | Family 2 | − | − | 1 |
| N9F | Female | Norway | Family 2 | − | − | 1 |
| N10F | Female | Norway | Family 2 | − | − | 1 |
| N11F | Female | Norway | Family 2 | − | − | 1 |
| N12F | Female | Norway | Family 2 | − | − | 1 |
| N13F | Female | Norway | Family 3 | − | − | 1 |
| N14F | Female | Norway | Family 3 | − | − | 1 |
| N15F | Female | Norway | Family 3 | − | − | 1 |
| N16F | Female | Norway | Family 3 | − | − | 1 |
| N17F | Female | Norway | Family 3 | − | − | 1 |
| N18F | Female | Norway | Family 3 | − | − | 1 |
| N19F | Female | Norway | Family 4 | − | − | 1 |
| N20F | Female | Norway | Family 4 | − | − | 1 |
| N21F | Female | Norway | Family 4 | − | − | 1 |
| N22F | Female | Norway | Family 4 | − | − | 1 |
| N23F | Female | Norway | Family 4 | − | − | 1 |
| N24F | Female | Norway | Family 4 | − | − | 1 |
| N25F | Female | Norway | Family 4 | − | − | 1 |

>*Cyclopterus lumpus* - AMH1\_E3I6(male)

GCGCTGCTCTGGGCATTTAAAAGGCCCCCCACTGGGAGGAATCCTGGACGTCACTTTCACCAGCCAGTCACTGCATCCTCACACACAGGTAAAGATGATGAATGAAGGGAAATCTGTCCTGAAGTTCCTGAATCTGTTGCGCACAATTTGGTCACAGATATAGCTGTTGTTTCTGTGTCTTTTCTAGACTGTGTGCATCTTGGGAGAAACGCGGTACATACTGCTGACGGGAAAAGCACAAGAGGGCAACGTTCACCTGAAATGGAGGATTTCTGTTGAGATGAAATCCCCCGATATGAGTAAGATATGAGTACGGTGTAATTTCTGATTCATGAGCACCCTCCTCACATTTTCCTCTCTTTGCAGGCCAAAGCCTCAAAGATATCCTTATTGGTGGAAAATCAGGAAGTAACATCAGCATGGTTCCACTTCTGCTTTTCATTTGGGAGAGAGGAACTGATACAAGGTTGTTAATGGCTTTTATGTTCCCTTTCGAAATATCACTCCTGTCAGAGCGGTGCTAAGCAACTTATTTGTTTTAATATTCTCTTTTTTAGATATCATCATAGTTCATCCTTGATCTCTTCCCATACCTCCTTTCTCTGTGAGCTGAAGCGGTTCATGGGTGACGTCCTGCGTCAGGACCCCCGTGAGTCCTCTTCGCTCCTGCAGGACTCCTTGCACTCCCTGCCTCCCCTGACGTTGGGCTTATCCTCCAGTGACGCTCTGCTGGCGGGACTAATCAACTCCTCCTCCATCACCATCTTCTCGTTCAGTAGCTGGAGCTCCGTGTTTCAGTTGCACCGCGGAGAATTGGCCCTTTCTCCTGCACTGCTGGAGGAGCTCAAGCAGAGGTTGGAGCAGACCGTGGGGAAGATAATGAAGGTCATACGGGAGGAGAAAGTGGGACAGAGGGCCAACAAGAGTCTGGGAAGGCTCAAGGACCTCAGTGCGTTCCCGTTGAAAGAACCGGCAGCAGGTGATGTGTCCAAGAAAACTTCTAAAAGCAGTTGTTCCCAAACTAATTAAAGTGGATTTTATTGAACCGTCAATGGTGTAAAAGTGGATATATTTTATCATGCAATTGGTCTGTTTAGATAGGTTTATGTTTGCATTCACACTACTACTACTTCAACCTTTCATTCATTACTTTAAACTAATTATTTAAATAGTTTATCCMCTACTTCCAGCTGTTATACATAACAGCTGGAAACATTGCAAACAGCAATCATGAATCAAGACAGCTAGTAGGTCCTCTCTGAAAGTAGAAAGGCTGGACAAGCTGATCTTGGTCTCACTTCAGGGACTCTAATCCCAAGACTTCAATGC

>*Cyclopterus lumpus* - AMH2\_I6E4(male)

GGATTCTGTTGAGATGAATCCCCCGATATGAGTAAGATATGAGTACGGTGTAATTTCTGATTCATGAGCACCCTCCTCACATTTTCCTCTCTTTGCAGGCCAAAGCCTCAAAGATATCCTTATTGGTGGAAAATCAGGAAGTAACATCAGCATGGTTCCACTTCTGCTTTTCATTTGGGAGAGAGGAACTGATACAAGGTTGTTAATGGCTTTTATGTTCCCTTTCGAAATATCACTCCTGTCAGAGCGGTGCTAAGCAACTTATTTGTTTTAATATTCTCTTTTTTAGATATCATCATAGTTCATCCTTGATCTCTTCCCATACCTCCTTTCTCTGTGAGCTGAAGCGGTTCATGGGTGACGTCCTGCGTCAGGACCCCCGTGAGTCCTCTTCGCTCCTGCAGGACTCCTTGCACTCCCTGCCTCCCCTGACGTTGGGCTTATCCTCCAGTGACGCTCTGCTGGCGGGACTAATCAACTCCTCCTCCATCACCATCTTCTCGTTCAGTAGCTGGAGCTCCGTGTTTCAGTTGCACCGCGGAGAATTGGCCCTTTCTCCTGCACTGCTGGAGGAGCTCAAGCAGAGGTTGGAGCAGACCNGTGGGGAAGATAATGAAGGTCATACGGGAGGAGAAAGTGGGACAGAGGGCCAACAAGAGTCTGGGAAGGCTCAANGGACCTCAGTGCGTTCCCGTTGAAAGAACCGGCAGCAGGTGATGTGTCCAAGAAAACTTCTAAAAGCAGTTGTTCCCAAACTAATTAAAGTGGATTTTATTGAACCGTCAATGGTGTAAAAGTGGATATATTTTATCATGCAATTGGTCTGTTTAGATAGGTTTATGTTTGCATTCACACTACTACTACTTCAACCTTTCATTCATTACTTTAAACTAATTATTTAAATAGTTTATCCACTACTTCCAGCTGTTATACATAACAGCTGGAAACATTGCAAACAGCAATCATGAATCAAGACAGCTAGTAGGTCCTCTCTGAAAGTAGAAAGGCTGGACAAGCTGATCTTGGTCTCACTTCAGGGACTCTAATCCAAGACTTCAATGCTGAATGGCCAATAGATCTGTGGTGGGAATCAACACGAGGGCATAGAATGCCAACGTGTGAATGGGAGAGAACAAACAAAAAAGAAACCAGCTGCGCTGCGAAGTTTCCAGGAATTGAACATGGACACAATAACTGCTAACACTTCAAATTAAGATTATGTAAAGCAGTGGTCACCAACCTTTTTAACCCGAAGATCCCTGACCTCTGCCTTGGTGACAGGCAAGATCTCCCTATTGAAGCGTTGAGAGAAAAACACTGTCCAGACTGGACTTACAACTTGAGGCTTTTTATTTGCCCTCATTATGATTGAATGAAATCAAACACTTTGGTCCAGCTGTCAAATTGATGTGACAAAGAACACACTAAATGACTTAATTTCAGTGCAACATAAAAAGTAAATTTGTTGTTAACCCCACACTATATGAAGAATAAAATCACAGTTGAAATGAGCAGACTGGATGAACTACCAATATTAATGTTGTATTTATCAACTGAAGCTACAACACAACACTGACAACATGTTCAGTCAGTTACTAATGTCAGTTCAGTTCAGCTGTGTGTCCCATCACAATGCCATCAGAAGTCATGTGACTCCAGTCAGTGAGCTGTCTGTGACTGAACACTGTCATGTGAGCTTGTAGTTGGGCTCATTAGCTACAGACAGCCAGTC

>*Cyclopterus lumpus* - AMH3\_E3I6 (male)

TCTGCTCCCCCGTCTCCTTTGCTCAAGCTGAGCCTGCACTGCTCCTNGGAGTTTGAAAGCCCCCCCACTGGAGGAATCCTGGACGTCACTTTCACCAGTCAGTCGCTGCATCCTCACGCACAGGTGAAGATGATGCATGAAGGGAAATCTGTCCTAAAGTTCCCGAGTCTGTTGCGCATGATTTGGTCACAGATATTGCTGTTGTTTCCTTTATTTTTTCCTAGACTGTGTGCATCTTGGGAGAAACGCGGTACCTACTGCTGACGGGAAAGGCAGCAGAGGGCAACCTTCACCAGAAATGGAGGATTTCTGTTGAGACGAAATCCCCCGATATGAGTAAGATATGAGCACGGTGTGATTTCTGATTCATGAGCACCCTCCTCACATGTTCCTCTTTTTTCAGGCCAAAGCCTCAAAGACATCCTTATTGGTGGAACATCAGGAAGTAACATCAGCATGGTTCCACTTCTGCTTTTCTCTGGGGAGACAGGAACTGATACAAGGTTTGAATGGCTATTGTGTTCCCTATTGAAGTGTCACTCCTGTCAGAGCGGTGCTCAGCAACCTTTTTTTCTTTTTTTTACATTATCTTTTTCAGATATCATCACAGTTCATCCTTGACCTCTTCCCAGACCTCCTTCCTCTGTGAGCTGAAGCGCTTCCTGGGTGACGTCCTGCCCCAGGACCCCCGTGAGTCCTCTCCGCTCCTGCGGGACTCCTTGCAGTCCCTGCCTCCCCTGACGCTGGGCTTATCCTCCAGCGACGCAATGCTGGCGGGACTGATCAACTCCTCCTCCATCACCATCTTCTCCTTCAGTAGCTGGAGCTCCGTGTTTCAGGTGCACCGCGGAGAATTGACCTTTTCTCCTGCGCTGCTGGACGAGCTCAAGCAGAGGTTGGAGCACACCGTGGGGAAGATTACGGAGGTCATACGGGAGGAGCAAGTGGGCCAGAGGGCCACGGAGAGGCTGGGGAGGCTCAAGGACCTCAGTGCTTTCCCGTTGAAGGAACCGGCATCAGGTGACATGTCCAAGAAAACTCCTAAAGCGGTGGTTCCCAGACATTGTTGTCAGACCCACGGCCCTGTCAGGATAACTCAGGACTCTATT

>*Cyclopterus lumpus* - AMH3\_E3I6 (female)

TTTGCTCCCACCGTCTCCTTTGCTCAAGCTGAGCCNTGCACTGCTCCTGGAGTTTGAAAGCCCCCCCACTGGAGGAATCCTGGACGTCACTTTCACCAGTCAGTCGCTGCATCCTCACGCACAGGTGAAGATGATGCATGAAGGGAAATCTGTCCTAAAGTTCCCGAGTCTGTTGCGCATGATTTGGTCACAGATATTGCTGTTGTTTCCTTTATTTTTTCCTAGACTGTGTGCATCTTGGGAGAAACGCGGTACCTACTGCTGACGGGAAAGGCAGCAGAGGGCAACCTTCACCAGAAATGGAGGATTTCTGTTGAGACGAAATCCCCCGATATGAGTAAGATATGAGCACGGTGTGATTTCTGATTCATGAGCACCCTCCTCACATGTTCCTCTTTTTTCAGGCCAAAGCCTCAAAGACATCCTTATTGGTGGAACATCAGGAAGTAACATCAGCATGGTTCCACTTCTGCTTTTCTCTGGGGAGACAGGAACTGATACAAGGTTTGAATGGCTATTGTGTTCCCTATTGAAGTGTCACTCCTGTCAGAGCGGTGCTCAGCAACCTTTTTTTCTTTTTTTTACATTATCTTTTTCAGATATCATCACAGTTCATCCTTGACCTCTTCCCAGACCTCCTTCCTCTGTGAGCTGAAGCGCTTCCTGGGTGACGTCCTGCCCCAGGACCCCCGTGAGTCCTCTCCGCTCCTGCGGGACTCCTTGCAGTCCCTGCCTCCCCTGACGCTGGGCTTATCCTCCAGCGACGCAATGCTGGCGGGACTGATCAACTCCTCCTCCATCACCATCTTCTCCTTCAGTAGCTGGAGCTCCGTGTTTCAGGTGCACCGCGGAGAATTGACCTTTTCTCCTGCGCTGCTGGACGAGCTCAAGCAGAGGTTGGAGCACACCGTGGGGAAGATTACGGAGGTCATACGGGAGGAGCAAGTGGGCCAGAGGGCCACGGAGAGGCTGGGGAGGCTCAAGGACCTCAGTGCTTTCCCGTTGAAGGAACCGGCATCAGGTGACATGTCCAAGAAAACTCCTAAAGCGGTGGTTCCCAGACATTGTNGTCAGACCCACGSCCCTGTCAGGATAACTCAGGACTCT

>*Cyclopterus lumpus* - AMH1+3\_E6I6 (male; autosomal, upper band)

GGTGTTATCCGGGAGGAACAAGGTGTTGTCATACGGGAGGAGCAAGTGGGCCAGAGGACCACGGAGARGCTGGGGANGCTCAAGGACCTCAGTGCTTTCCCGTTGAAGGARCCGGCATCAGGTGACATGTCCAAGAAAACTCCTAAAGCGGTGGTTCCCAGACATTGTTGTCAGACCCACGKCCCTGTCAGGATAACTCAGGAGCTCCTTTAGTCCACATCCCCTCGCCACGGTCCAAGTGTGTTCACTTGAACTAATTAAAGTGGATTTTATTGAACCGTTAATGGTATAAAAGTGGATATATTTTTATCATACAATTGGTCTGTTGCAGGAGGTAGAGGCTTCAACCTTTCATTCATTACTTTAAGCTTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATATTATATATAACAGCTGGAAACATTGCAAACAGTAATCAAGAATCAAGACAGTTCAAAGGTCATCTCTGAAAGTAGAGAGGATGGACAAGCTGATCTTGKTCTCRCTTTATCGACCCCCCGTCCAATACTTCAATGCTGAATGGCCGATAGATCTGTGGGGGGAACGAG

>*Cyclopterus lumpus* - AMH1+3\_E6I6 (male; male-specific, lower band)

TTGTCATACGGGAGGAGCAAGTGGGACAGAGGGCCAACAAGAGTCTGGGAAGGCTCAAGGACCTCAGTGCGTTCCCGTTGAAAGAACCGGCAGCAGGTGATGTGTCCAAGAAAACTTCTAAAAGCAGTTGTTCCCAAACTAATTAAAGTGGATTTTATTGAACCGTCAATGGTGTAAAAGTGGATATATTTTATCATGCAATTGGTCTGTTTAGATAGGTTTATGTTTGCATTCACACTACTACTACTTCAACCTTTCATTCATTACTTTAAACTAATTATTTAAATAGTTTATCCACTACTTCCAGCTGTTATACATAACAGCTGGAAACATTGCAAACAGCAATCATGAATCAAGACAGCTAGTAGGTCCTCTCTGAAAGTAGAAAGGCTGGACAAGCTGATCTTGGTCTCACTTCANGGACTCTAATCCAAGACTTCAATGCTGAATGGCCAATAGATCTGTGGTGGGAAC

>*Cyclopterus lumpus* - AMH1+3\_E6I6 (female)

GGCTGGGGGAAGGCTCAGGACTCAGTGCTTTCCCGTTGAAGGAACCGGCATCAGGTGACATGTCCAAGCAAAACTCCTAAAGCGGTGGTTCCCAGACATTGTTGTCAGACCCACGGCCCTGTCAGGATAACTCAGGAGCTCCTTTAGTCCACATCCCCTCGCCACGGTCCAAGTGTGTTCACTTGAACTAATTAAAGTGGATTTTATTGAACCGTTAATGGTATAAAAGTGGATATATTTTTATCATACAATTGGTCTGTTGCAGGAGGTAGAGGCTTCAACCTTTCATTCATTACTTTAAGCTAATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATATTATATATAACAGCTGGAAACATTGCAAACAGTAATCAAGAATCAAGACAGTTCAAAGGTCATCTCTGAAAGTAGAGAGGAAGGAGCTGATCTTGGTCTCMCTTTATGGACCCCCCCCCCACCACTACTTTGATGATTGGTGGATAAATCAGCGGTGGTGACAAGAAGATATCACTACCTGAATTAGCTAGAGCTATTACTATACTTTTAGCAGATCGAAATTTTAATACTTCTTTCTTTGATCCAGCATGACGAGCACACCCAATTCTATATCAACACTTATTTTTATTTTTG

>*Aptocyclus ventricosus* - AMH1+3\_E6I6 (male, autosomal, upper band)

TTTTTGTCATACGGGAGGAGCAAGTGGGCCAGAGGGCCACGGAGAGGCTGGGGAGGCTCAAGGACCTCAGTGCGTTCCCGTTGAAGGAACCGGCAGCAGGTGACGTGTCCAAGAAAACTCCCAAAGCGGTTGTTCCCAAACATTGTTGTCAGACCCTGTCAGGATAACTCAGGAGCTCCTTTAGTCAACATCCCCTCGCCACGGTCCAAGTGTGTTCACTTGAACTAATTAAAGTGGATTTTATTGAACCGTTAAGGGCATAAAAGTGGATATATTTTATTGTGCAATTGGTCTGTTGCAGGAGGTAGAGGCTTCAACCTTTCATTCATTACTTTAAGCTAATTATTTAAATTGTTTATCCACTACTTGGAGTTATATATAACAGCTGGAAACATTGCAAACAGTAATCAAGAATCAAGACAGTTCAAAGGTCATCTCTGAAAGTAGAGAGGATGGACAAGCTGATCTTGGTCTCACTTTAGGACCCCCACTCCAAGAATGGCC

>*Aptocyclus ventricosus* - AMH1+3\_E6I6 (male, autosomal, lower band)

TGTCATACGGGGAGGAGCAAGTGGGCCAGAGGGCCACGGAGAGGCTGGGGAGGCTCAAGGACCTCAGTGCGTTCCCGTTGAAGGAACCGGCAGCAGGTGACGTGTCCAAGAAAACTCCTAAAGCGGTTGTTCCCAAACATTGTTGTCAGACCCACGGCCCTGTCAGGATAACTCAGGAGCTCCTTTAGTCCACATCTCAACGCCACGGTCCAAGTGTGTTCACTTGAACTAATTAAAGTGGATTTTATTGAACCGTTAATGGTATAAAAGTGGATATATTTTATCGTGCAATTGGTCTGTTGCAGGAGGTAGAGGCTTCAACCTTTCATTCATTACTTTAAGCTGATCTTGGCCTCACTTTANGGACCCCCAGTCCAAGAATGGCCAATAGATCTGTGGTGGGGAACGAGA

>*Aptocyclus ventricosus* - AMH1+3\_E6I6 (female, autosomal,upper band)

GCTGGGGAGGCTCAGGACCTCAGTGCGTTCCCGTTGAAGGAACCGGCAGCAGGTGACGTGTCCAAGAAAACTCCCAAAGCGGTTGTTCCCAAACATTGTTGTCAGACCCACGGCCCTGTCAGGATAACTCAGGAGCTCCTTTAKTCCACATCTCAACGCCACGGTCCAAGTGTGTTCACTTGAACTAATTAAAGTGGATTTTATTGAACCGTTAAGGGCATAAAAGTGGATATATTTTATTGTGCAATTGGTCTGTTGCAGGAGGTAGAGGCTTCAACCTTTCATTCATTACTTTAAGCTAATTATTTAAATTGTTTATCCACTACTTGGAGTTATATATAACAGCTGGAAACATTGCAAACAGTAATCAAGAATCAAGACAGTTCAAAGGTCATCTCTGAAAGTAGAGAGGATGGACAAGCTGATCT

>*Aptocyclus ventricosus* - AMH1+3\_E6I6 (female, autosomal, lower band)

AAGTGGGCCAGAGGGCCACGGAGAGGCTGGGGAGGCTCAAGGACCTCAGTGCGTTCCCGTTGAAGGAACCGGCAGCAGGTGACGTGTCCAAGAAAACTCCTAAAGCGGTTGTTCCCAAACATTGTTGTCAGACCCACGGCCCTGTCAGGATAACTCAGGAGCTCCTTTAGTCCACATCTCAACGCCACGGTCCAAGTGTGTTCACTTGAACTAATTAAAGTGGATTTTATTGAACCGTTAATGGTATAAAAGTGGATATATTTTATCGTGCAATTGGTCTGTTGCAGGAGGTAGAGGCTTCAACCTTTCATTCATTACTTTAAGCTGATCTTGGCCTCACTTT

>*Eumicrotremus taranetzi* - AMH1+3\_E6I6 (male)

GGGAGAGCAAGTGGGCCAGAGGGCCACGGAGAGGCTGGGGAGGCTCAAGGACCTCAGTGCGTTCCCGTTGAAGGAACCGGCAGCAGGTGACGTGTCCACGAAAACTCCTAAAGCGGTTGTTCCCAAACATTGTTGTCAGACCCACGGCCCTGTCAGGATAACTCAGGAGCTCCTTTAGTCCCCATCCCCTCGCCACGGTCCAAGTGCGTTCACTTGAACTAATTAAAGTGGATTTTATTGAACCGTTAATGGTATAAAAGTGGATATATTTTATGATTCAATTGGTCTGTTGCAGGAGGTAGAGGCGTCAACCTTTCATTCATTACTTTAAGCTAATTATTTAAATTGTTTATCCACTACTTGGAGTTAWATATAACAGCTGGAAACATTGCAAACAGTAATCAAGAATCACGACAGTTCAAAGGTCATCTCTGAAAGTAGAGAGGATGGACAAGCTGATCTTGGTCTCACTTCAGGGACCCCCAGTCCAAGAATGGCCAATAGATCTGTGTGGGGGAACGAG

>*Eumicrotremus taranetzi* - AMH1+3\_E6I6 (female)

CGGGAGAGCAAGTGGGCCAGAGGGCCACGGAGAGGCTGGGGAGGCTCAAGGACCTCAGTGCGTTCCCGTTGAAGGAACCGGCAGCAGGTGACGTGTCCACGAAAACTCCTAAAGCGGTTGTTCCCAAACATTGTTGTCAGACCCACGGCCCTGTCAGGATAACTCAGGAGCTCCTTTAGTCCCCATCCCCTCGCCACGGTCCAAGTGCGTTCACTTGAACTAATTAAAGTGGATTTTATTGAACCGTTAATGGTATAAAAGTGGATATATTTTATGATTCAATTGGTCTGTTGCAGGAGGTAGAGGCGTCAACCTTTCATTCATTACTTTAAGCTAATTATTTAAATTGTTTATCCACTACTTGGAGTTATATATAACAGCTGGAAACATTGCAAACAGTAATCAAGAATCACGACAGTTCAAAGGTCATCTCTGAAAGTAGAGAGGATGGACAAGCTGATCTTGGTCTCACTTCAGGGACCCCCAGTCCAAGAATGGCCAATAGATCTGTGTGGGGAAAACCAAGA

>*Eumicrotremus asperrimus* - AMH1+3\_E6I6 (male)

GGGAGGAGCAAGTGGGCCAGAGGGCCACGGAGAGGCTGGGGAGGCTCAAGGACCTCAGTGCGTTCCCGTTGAAGGAACCGGCAGCAGGTGACGTGTCCCAGAAAACTCCTAAAGTGGTTGTTCCCAAACATTGTTGTCAGACCCACGGCCCTGTCAGGATAACTCAGGAGCTCCTTTAGTCCACATCCCCTCGCCACGGTCCAAGTGCGTTCACTTGAACTAATTAAAGTGGATTTTATTGAACCGTTAATGGTATAAAAGTGGATATATTGTATCATGCAATTGGTCTGTTCCAGGAGGTAGAGGCGTCAACCTTTCATTCATTACTTTAAGCTAATTATTTAAATTGTTTATCCACTACTTGGAGTTATATATAACAGCTGGAAACATTGCAAACAGTAATCAAGAATCTCGACAGTTCAAAGGTCATCTCTGAAAGTAGAGAGGATGGACAAGCTGATCTTGGTATCACTTTAGGGACCCCCGGTCCAAGAATGGCCAATAGATCTGTGGTGGGGAACGAGA

>*Eumicrotremus asperrimus* - AMH1+3\_E6I6 (female)

TGTCATACCGGGAGGAGCAAGTGGGCCAGAGGGCCACGGAGAGGCTGGGGAGGCTCAAGGACCTCAGTGCGTTCCCGTTGAAGGAACCGGCAGCAGGTGACGTGTCCCAGAAAACTCCTAAAGTGGTTGTTCCCAAACATTGTTGTCAGACCCACGGCCCTGTCAGGATAACTCAGGAGCTCCTTTAGTCCACATCCCCTCGCCACGGTCCAAGTGCGTTCACTTGAACTAATTAAAGTGGATTTTATTGAACCGTTAATGGTATAAAAGTGGATATATTGTATCATGCAATTGGTCTGTTGCAGGAGGTACAGGCGTCAACCTTTCATTCATTACTTTAAGCTAATTATTTAAATTGTTTATCCACTACTTGGAGTTATATATAACAGCTGGAAACATTGCAAACAGTAATCAAGAATCTCGACAGTTCAAAGGTCATCTCTGAAAGTAGAGAGGATGGACAAGCTGATCTTGGTATCACTTTAGGGACCCCCGGTCCAAGAATGGCCAATAGATCTGTGGTGGGGAACGAGA

Figure S1. DNA sequences of the amplicons amplified from primer sets in Table 1. The species, primer set, sex, the band’s sex specificity, and the band’s placement on the gel (Figure 2) are described on the top of each sequence. Note that the bases of *amh2*-related sequences have been reverse complemented for the consistent orientation.



Figure S2. PCR amplification results of the primer set utilised for estimating the upstream coverage of the male-specific region in lumpfish. Lanes with blue labels represent the amplification results of the phenotypic males (M1-6); Lanes with red labels represent the amplification results of the phenotypic females (F1-6); Lanes with the “B” labels represent blanks as the negative control. (A) Results of primer set 4K\_Up. (B) Results of primer set 6K\_Up. (C) Results of primer set 10K\_Up. The ladder used is GeneRuler 1 kb DNA Ladder (Thermo Fisher Scientific, USA). The samples were run on ethidium bromide-stained 2.0 % agarose gel at 80 V for 30 min.



Figure S3. PCR amplification results of the primer set utilised for estimating the downstream coverage of the male-specific region in lumpfish. Lanes with blue labels represent the amplification results of the phenotypic males (M1-6); Lanes with red labels represent the amplification results of the phenotypic females (F1-6); Lanes with the “B” labels represent blanks as the negative control. (A) Results of primer set 4K\_Down (B) ) Results of primer set 14K\_Down (C) Results of primer set 16K\_Down. The ladder used is GeneRuler 1 kb DNA Ladder (Thermo Fisher Scientific, USA). The samples were run on ethidium bromide-stained 2.0 % agarose gel at 80 V for 30 min.