

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	+	+	2
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

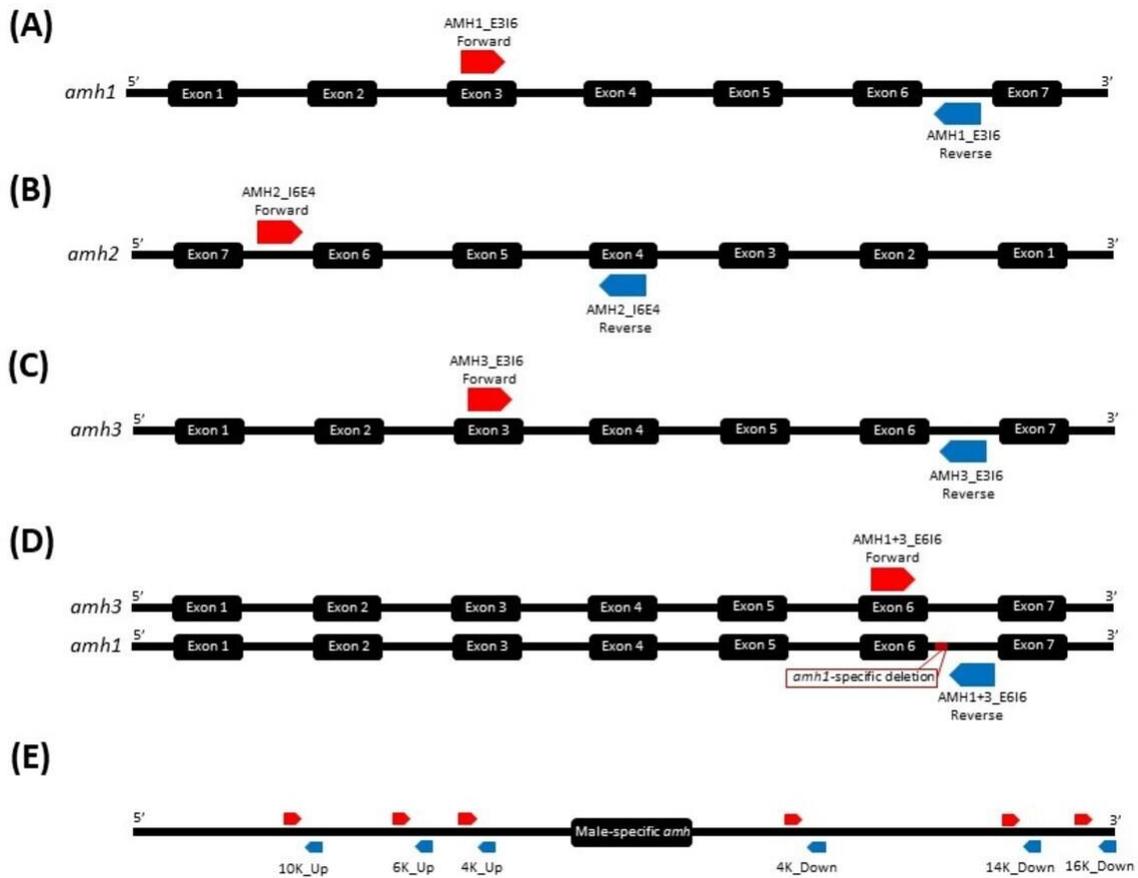


Figure S1. Binding sites of the primers utilised in this study to amplify *anti-Müllerian hormone (amh)* genes of lumpfish. Red pentagons represent forward primer. Blue pentagons represent reverse primers. (A) The expected binding sites of the primer set AMH1_E316 on *amh1*. (B) The expected binding sites of the primer set AMH2_I6E4 on *amh2*. (C) The expected binding sites of the primer set AMH3_E316 on *amh3*. (D) The expected binding sites of the primer set AMH1+3_E616 on the shared region of *amh1* and *amh3* which contain *amh1*-specific 91 bp deletion. (E) The expected binding sites of the primer set 4K_Up, 6K_Up, 10K_Up, 4K_Down, 14K_Down, and 16K_Down, which located adjacent to the male-specific *amh*.

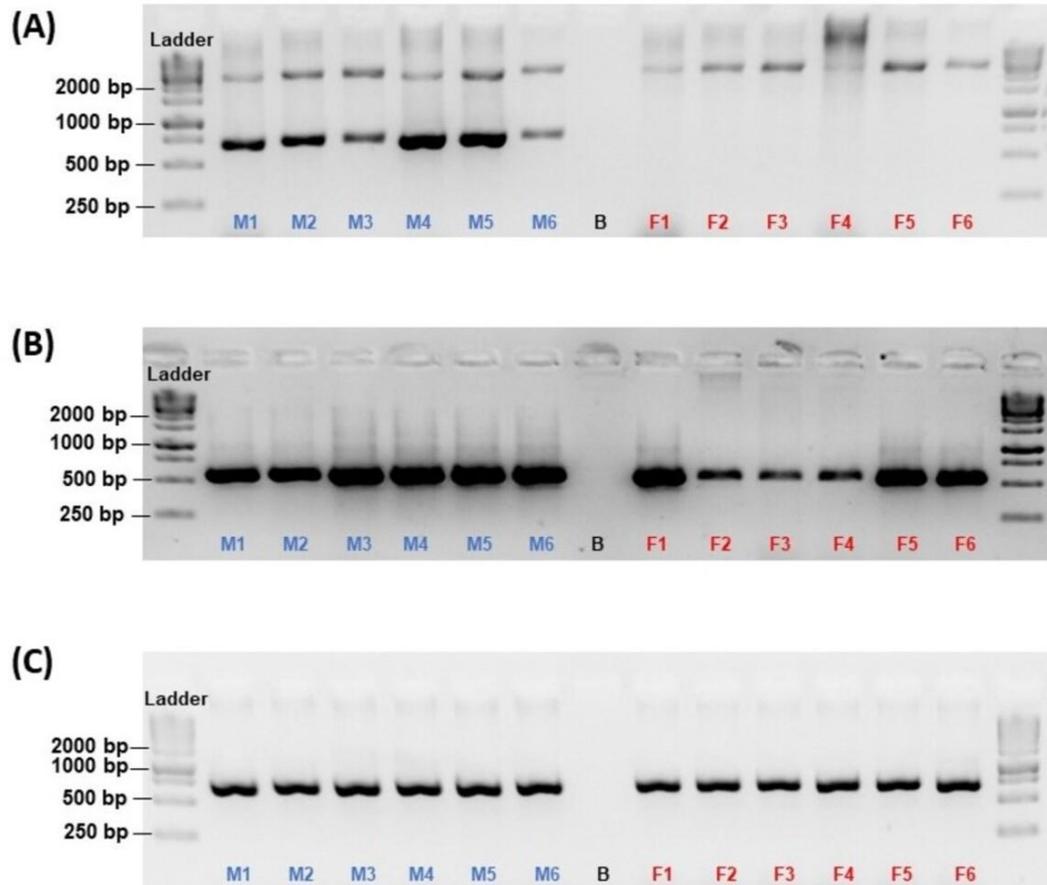


Figure S2. PCR amplification results of the primer set utilised for estimating the upstream coverage of the male-specific region in lumpfish. Lanes with blue labels represent the amplification results of the phenotypic males (M1-6); Lanes with red labels represent the amplification results of the phenotypic 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, Waltham, Massachusetts, USA). The samples were run on ethidium bromide-stained 2.0 % agarose gel at 80 V for 30 min.

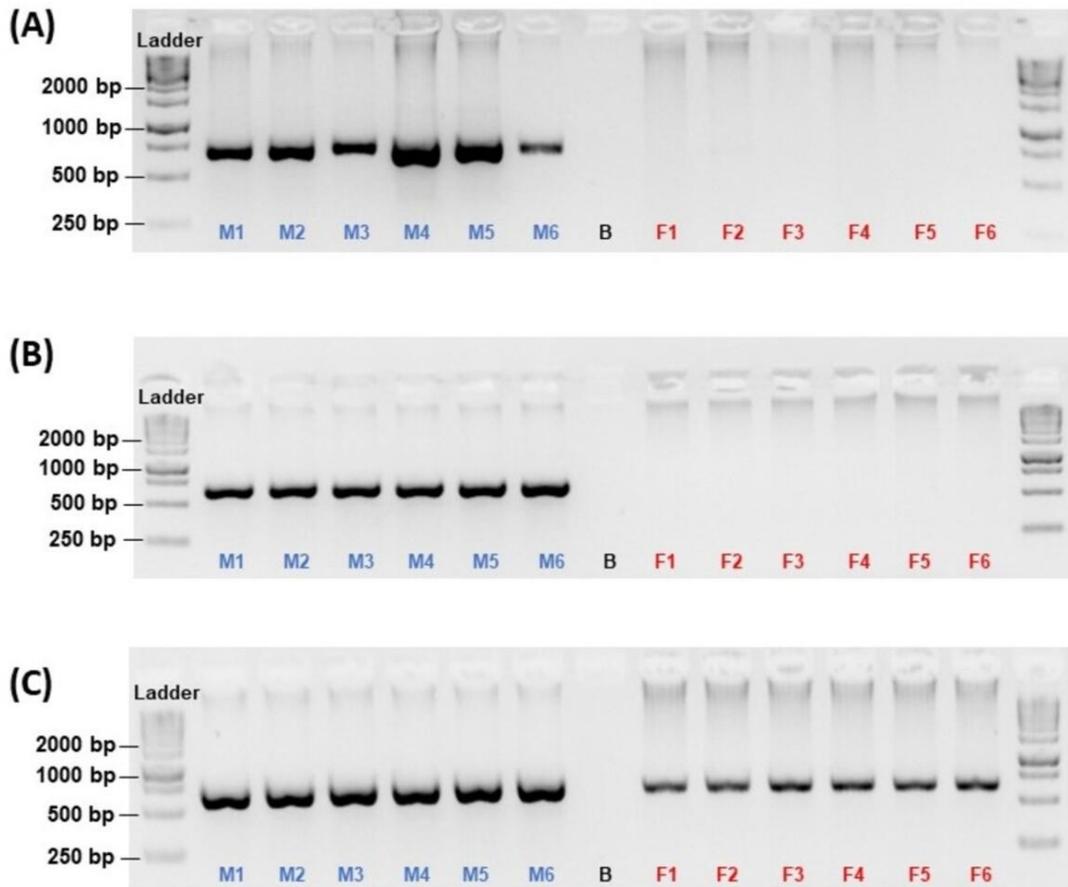


Figure S3. PCR amplification results of the primer set utilised for estimating the downstream coverage of the male-specific region in lumpfish. Lanes with blue labels represent the amplification results of the phenotypic males (M1-6); Lanes with red labels represent the amplification results of the phenotypic 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, Waltham, Massachusetts, USA). The samples were run on ethidium bromide-stained 2.0 % agarose gel at 80 V for 30 min.