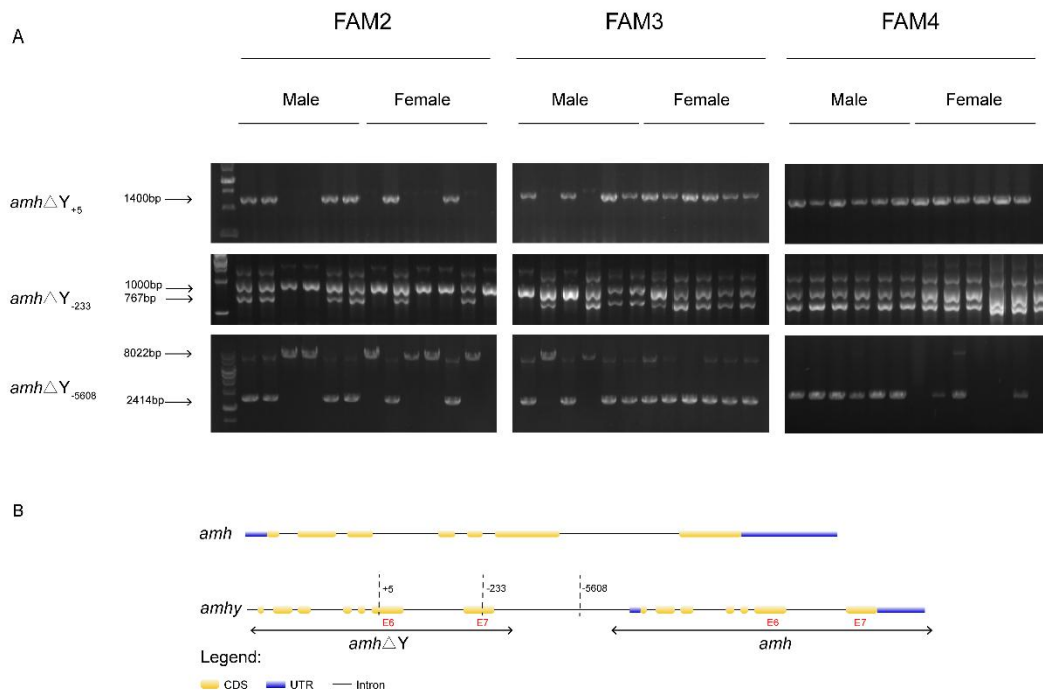
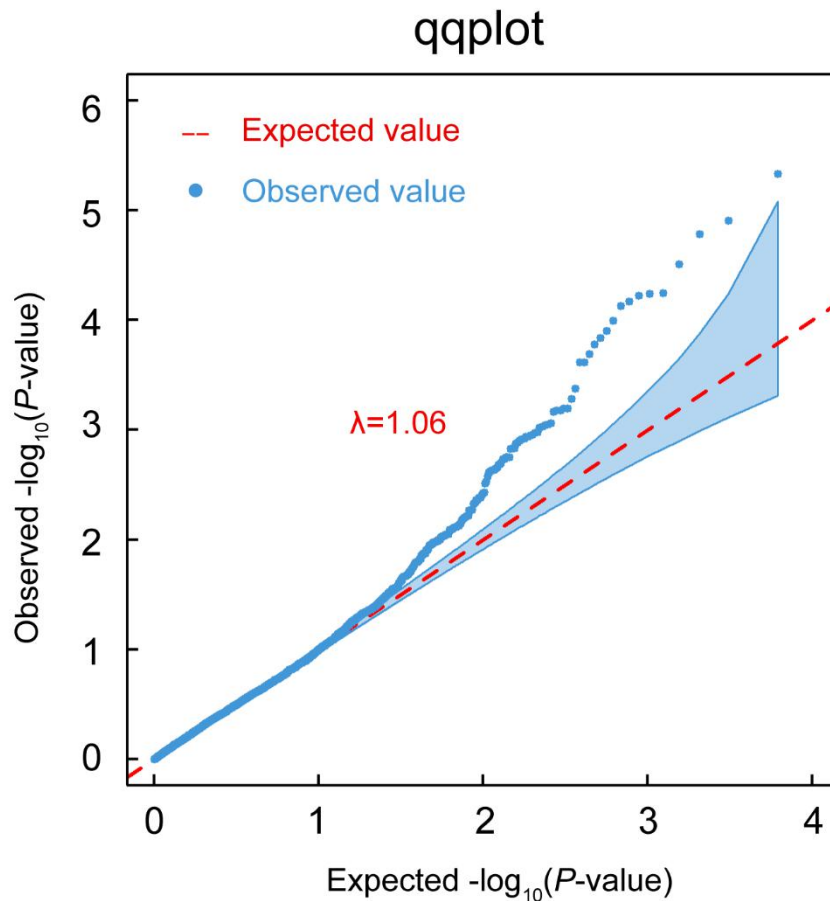


## Supplementary Materials



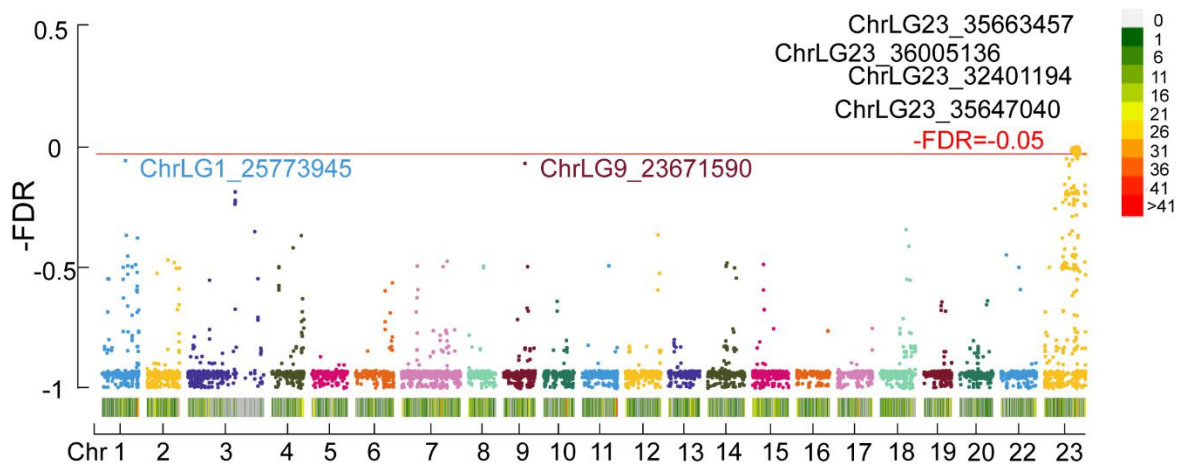
### Supplementary Figure S1. PCR amplification of *amhy* in male red tilapia.

A: Y-specific duplicate of *amh* gene was amplified in male red tilapia by *amh* Y chromosome markers. All individual genotypes are presented. B: Structures of *amh* and *amhy* genes in previous studies.



**Supplementary Figure S2. QQ plot of  $P$ -values for FAM3 from GWAS analysis using MLM with 11 PCA components.**

Observed negative base 10 logarithm of  $P$ -values was plotted against expected observed negative base 10 logarithm of  $P$ -values under assumption that  $P$ -values follow a uniform  $[0,1]$  distribution. Red dotted line represents expected value and blue dots represent observed value. Inflation factor lambda ( $\lambda$ ) is 1.06.



**Supplementary Figure S3. Genome-wide significant QTL intervals identified by GWAS for sex in FAM3.**

X-axis indicates chromosome numbers labeled with different colors. Y-axis is -FDR value for each SNP marker by GWAS, corrected by FDR. Corrected genome-wide significant threshold ( $-q(\text{FDR})=0.05$ ) is indicated. There are four SNPs on chrLG23 above the corrected threshold level. Legend on upper right indicates density of SNPs across genome, with green indicating low density and red indicating high density.

**Supplementary Table S1. Primer list used in qRT-PCR and marker development**

Primer name	Species	Chromosome	Forward primer (5'-3')	Reverse primer (3'-5')	PCR product size	T <sub>m</sub>
<i>sox14</i>	Red tilapia	ChrLG23	CCTGCTCAAGAAGGACCGTT	AGCTGAACTGACTTGGGTCG	176bp	55°C
<i>dot11</i>	Red tilapia	ChrLG23	CAGCTACCTATGCAGAGACCA	CTCTGGACCAAAGGCAAAGT	173bp	52.3°C
<i>scly</i>	Red tilapia	ChrLG23	CTTCACATCAGCTTACAAGGGTA	GGGCTTCAGAAATGGCCTGA	185bp	55.8°C
<i>atp10a</i>	Red tilapia	ChrLG23	GACTGCGAGTTTGACCCCTT	GTAGCAGTCAGCGTCTTTGC	179bp	55.8°C
<i>slco2a1</i>	Red tilapia	ChrLG23	CCCCTACAGGATTGGGGAGT	ATGTACATGGGGTTGTGCGT	162bp	57.9°C
<i>oca2</i>	Red tilapia	ChrLG23	AACCTGCCAGTTAAACCGCT	GGGGAATCCCAGCTTGAAGA	162bp	55.8°C
<i>amh</i>	Red tilapia	ChrLG23	GACACACAGTGCTGCTATTTT	TGGGAGTGAGTACTCGGTTA	220bp	53.8°C
<i>lpp</i>	Red tilapia	ChrLG23	GAAGCTAGCAGTTTGTGTGCC	AGACGGAGATGCTCGCACTA	179bp	56.1°C
<i>gpr17</i>	Red tilapia	ChrLG23	TGTTCCGCCTACTGCTTGTT	ATGCGCACGCACATTTGATT	194bp	53.8°C
<i>ube3a</i>	Red tilapia	ChrLG23	CAGCGTCCATCGGCCTTATT	GCAGTCGCTCTGTTCATTCG	204bp	55°C
<i>tp63</i>	Red tilapia	ChrLG23	GCTGAGGATGCCCCATTAG	AGGGTCCCTGAGTATGTCTGA	150bp	55.3°C
<i>cog4</i>	Red tilapia	ChrLG1	ATCTTCCCTCTTCTGGGCCT	GCGTTAGCGTGTCTGCAAAT	160bp	60°C
SSR141	Red tilapia	ChrLG1	TTTTCACACCACTGAGTCGCAA	AAGTGTGTTTTCTGTCAAGTCTG	270bp	60.14°C
SSR147	Red tilapia	ChrLG1	TACTCTCCCAACACCCACA	TGCTGCTTACACACCTTGTGT	203bp	58.60°C
SSR3204	Red tilapia	ChrLG23	GACAGTGCAGACGCAACAGT	CAGGGTCTTGCTGCCTTTAG	189bp	60.10°C
SSR3293	Red tilapia	ChrLG23	CGGTTGCCGTGTAAGATT	TGAGCATGGACTGACAGGAG	217bp	59.98°C
<i>amh</i> Δ <sub>Y+5</sub>	Red tilapia	ChrLG23	AAACCTCCTTCTTTGTGAATGTC	CTAGCGGCATCCACACTCCCTCAC	1400bp	58.2°C
<i>amh</i> Δ <sub>Y-233</sub>	Red tilapia	ChrLG23	CGGTCCCAGTGACCTATGAG	AAGTACACGTGGTGTATTGTAATTGA	767bp	57.9°C
<i>amh</i> Y <sub>-5608</sub>	Red tilapia	ChrLG23	GAAAGGGGTGTTTTGGTGTGCGC	ACCCAGGAAGCGTTTCATCTCA	2414bp	60.2°C

**Supplementary Table S2. Summary of QTL-seq analysis data for FAM1**

<b>Chromosome</b>	<b>Window number</b>	<b>Average SNP number/window</b>	<b>SNP range/window</b>	<b>Average <i>Fst</i>/window</b>	<b><i>Fst</i> range/window</b>
ChrLG1	3168	37.6	21-378	0.087	0.010-0.269
ChrLG2	3769	42.3	21-303	0.041	0.012-0.141
ChrLG3	1887	51.5	21-289	0.049	0.007-0.178
ChrLG4	2994	41.5	21-381	0.053	0.006-0.185
ChrLG5	1976	40.3	21-262	0.054	0.008-0.174
ChrLG6	4600	40.6	21-306	0.050	0.010-0.163
ChrLG7	6052	41.4	21-321	0.052	0.005-0.158
ChrLG8	4838	46.0	21-260	0.048	0.009-0.123
ChrLG9	1644	42.5	21-230	0.052	0.009-0.133
ChrLG10	1224	40.8	21-243	0.048	0.010-0.152
ChrLG11	1370	38.0	21-183	0.049	0.008-0.134
ChrLG12	3164	38.1	21-207	0.048	0.009-0.128
ChrLG13	3160	40.2	21-277	0.056	0.007-0.171
ChrLG14	4380	41.7	21-301	0.057	0.011-0.154
ChrLG15	3467	47.6	21-439	0.051	0.011-0.143
ChrLG16	4435	41.6	21-488	0.051	0.010-0.156
ChrLG17	4552	42.3	21-479	0.056	0.006-0.164
ChrLG18	3925	42.4	21-431	0.053	0.010-0.150
ChrLG19	3303	41.3	21-271	0.049	0.012-0.147
ChrLG20	2846	41.1	21-228	0.048	0.010-0.157
ChrLG22	2687	42.0	21-278	0.052	0.008-0.161
ChrLG23	1505	39.3	21-265	0.062	0.009-0.175
Average	3225	41.9	–	0.053	–

**Supplementary Table S3. Distribution of SNP markers among chromosomes in FAM2**

<b>Chromosome</b>	<b>Physical distance (bp)</b>	<b>Number of SNP markers</b>	<b>LOD value range</b>	<b>% Expl. range</b>
ChrLG1	40673430	42	0.79-5.01	3.5-20.1
ChrLG2	36523203	55	0-0.47	0-2.1
ChrLG3	87567345	87	0-1.18	0-5.2
ChrLG4	35549522	138	0-0.83	0-3.6
ChrLG5	39714817	122	0-1.57	0-6.8
ChrLG6	42433576	41	0-0.36	0-1.6
ChrLG7	64772279	85	0-1.09	0-4.7
ChrLG8	30527416	42	0-0.64	0-2.8
ChrLG9	35850837	39	0-0.94	0-4.1
ChrLG10	34704454	97	0-0.52	0-2.3
ChrLG11	39275952	132	0-1.89	0-8.1
ChrLG12	38600464	146	0.01-0.51	0-2.2
ChrLG13	34734273	69	0-1.62	0-7
ChrLG14	40509636	50	0-0.46	0-2.1
ChrLG15	39688505	86	0-0.72	0-3.2
ChrLG16	36041493	64	0-1.04	0-4.6
ChrLG17	38839487	39	0.01-0.76	0-3.3
ChrLG18	38636442	60	0.01-0.65	0.1-2.9
ChrLG19	30963196	135	0-0.6	0-2.6
ChrLG20	37140374	41	0-1.17	0-5.1
ChrLG22	39199643	82	0-0.79	0-3.5
ChrLG23	45655644	72	0-0.68	0-3
Total	—	1724	0-5.01	0-20.1

**Supplementary Table S4. Information on all SNP markers at genome-wide significance on ChrLG1 or ChrLG23 of FAM2 and FAM4**

Chromosome	Population name	Genetic distance (cM)	Position (bp)	LOD value	% Expl.	SNP annotation
ChrLG1		31.654	21096680	5.01	20.1	<i>LOC112847427</i> Upstream:3011
ChrLG1		31.654	21096664	5.01	20.1	<i>rpl13</i> Exon:2:6
ChrLG1		31.654	21096727	5.01	20.1	<i>LOC100712533</i> Downstream:3692
ChrLG1		31.678	20761648	5.01	20.1	
ChrLG1		31.678	20761731	5.01	20.1	
ChrLG1		31.678	20761592	5.01	20.1	
ChrLG1		8.106	18807025	4.56	18.4	<i>EIF3J</i> Downstream:1239
ChrLG1		29.246	22632441	4.56	18.4	<i>COG4</i> Intron:7:18
ChrLG1	FAM2	29.246	22632627	4.56	18.4	<i>COG4</i> Intron:6:18
ChrLG1		29.246	22632634	4.56	18.4	<i>COG4</i> Intron:6:18
ChrLG1		32.457	19010360	4.56	18.4	<i>LOC100698737</i> Intron:1:29
ChrLG1		32.457	19010394	4.56	18.4	<i>LOC100698737</i> Intron:1:29
ChrLG1		10.936	23971887	4.13	16.9	<i>LOC100702345</i> Upstream:315
ChrLG1		10.936	23971966	4.13	16.9	<i>LOC100702345</i> Upstream:394
ChrLG1		33.236	15071526	4.13	16.9	<i>LOC100693785</i> Intron:2:3
ChrLG1		33.242	16696311	4.13	16.9	<i>LOC100710764</i> Intron:5:8
ChrLG1		33.242	15630254	4.13	16.9	<i>LOC100693248</i> Intron:5:29
ChrLG1		26.79	25773889	3.73	15.4	<i>LOC100699231</i> Upstream:3251
ChrLG23		47.362	32948918	27.71	37.5	<i>LPP</i> Intron:6:10
ChrLG23		47.189	32970125	26.82	36.6	<i>LPP</i> Intron:6:10
ChrLG23	FAM4	49.508	33386273	26.25	36	<i>ATP10A</i> Upstream:2733
ChrLG23		45.974	32512288	23.95	35	<i>SNED1</i> Exon:2:31
ChrLG23		59.246	35555909	22.11	30.9	<i>NFIA</i> Upstream:1924

ChrLG23		58.935	35555920	21.53	30.3	<i>nfia</i>	Upstream:1913
ChrLG23		60.44	35934888	18.86	27	<i>tubgcp5</i>	Intron:12:22
ChrLG23		28.801	32904594	16.46	31.9	<i>LOC100694679</i>	Intron:4:11
ChrLG23		68.444	43270127	16.38	28.7	<i>LOC100706092</i>	Downstream:25
ChrLG23		69.018	43738701	16	27.1	<i>ttc39a</i>	Intron:1:20
ChrLG23		69.018	43738643	15.7	26.6	<i>ttc39a</i>	Intron:1:20
ChrLG23		71.821	41301803	14.88	21.9	<i>LOC100710029</i>	Intron:1:7
ChrLG23		73.795	44949883	14.51	21.5	<i>LOC112843737</i>	Downstream:3671
ChrLG23		71.513	45206551	14.41	21.8	<i>p3h2</i>	Intron:1:14
ChrLG23		70.028	44487138	14.27	23.1		
ChrLG23		70.13	45161161	14.15	22.7	<i>cpox</i>	Upstream:84
ChrLG23		73.518	45235481	13.91	20.6	<i>ccdc50</i>	Downstream:2015
ChrLG23		71.019	44798015	12.83	19.2	<i>LOC100702158</i>	Downstream:3498
ChrLG23		70.686	44859705	12.78	19.5	<i>LOC100701890</i>	Intron:2:63
ChrLG23		22.974	24533428	11.52	17.7		
ChrLG23		21.098	26908972	10.39	16.1	<i>fbxo3</i>	Downstream:3154
ChrLG23		20.646	22039918	9.67	15	<i>unc80</i>	Upstream:3901
ChrLG23		18.786	20736202	7.53	12	<i>LOC100702007</i>	Intron:7:7
ChrLG23		16.812	19361070	7.33	11.5		
ChrLG23		17.908	20123698	7.32	11.5	<i>LOC100705760</i>	Exon:5:9
ChrLG23		16.664	19761461	6.98	11	<i>LOC100707622</i>	Intron:2:10
ChrLG23		15.502	19959935	6.41	10.4	<i>nabl</i>	Intron:3:8
ChrLG23		14.962	16846754	5.89	9.4	<i>jagn1</i>	Upstream:3990
ChrLG23		13.034	15608750	5.59	9		
ChrLG23		8.405	15608749	4.54	7.6		
ChrLG23		8.456	15608704	4.54	7.6		
ChrLG1	FAM4	33.065	25773945	8.03	12.7	<i>LOC100699231</i>	Exon:1:9
ChrLG1		39.292	23290008	7.69	12.8	<i>emc8</i>	Downstream:2149



ChrLG1	42.752	22511506	7.63	12.2	<i>rab27a</i>	Upstream:1180
ChrLG1	20.229	28756890	7.47	14.4		
ChrLG1	20.229	28756856	7.47	14.4		
ChrLG1	42.335	22632460	7.47	11.7	<i>cog4</i>	Exon:7:19
ChrLG1	42.484	22632424	7.41	11.7	<i>cog4</i>	Intron:7:18
ChrLG1	42.264	22632442	7.37	11.6	<i>cog4</i>	Intron:7:18
ChrLG1	42.77	22511490	7.31	11.7	<i>rab27a</i>	Intron:1:5
ChrLG1	42.651	22632627	7.27	11.6	<i>cog4</i>	Intron:6:18
ChrLG1	42.651	22632441	7.25	11.6	<i>cog4</i>	Intron:7:18
ChrLG1	34.123	26818125	7.04	11.2	<i>greb1</i>	Upstream:3727
ChrLG1	83.032	7435430	6.62	14.6	<i>csrp3</i>	Intron:4:5
ChrLG1	82.844	4817995	6.61	14.5	<i>adamts17</i>	Intron:2:19
ChrLG1	83.713	8894478	6.6	14.9	<i>celfl</i>	Upstream:2492
ChrLG1	83.318	785106	6.33	14.2		
ChrLG1	15.307	30566255	6.25	11.2		
ChrLG1	15.005	30566230	6.21	11.1		
ChrLG1	8.001	32999490	5.94	10.2	<i>LOC100695615</i>	Exon:1:9
ChrLG1	8.001	32999334	5.94	10.2	<i>LOC100695615</i>	Intron:1:8
ChrLG1	9.094	32734592	5.92	10.1	<i>csk</i>	Intron:1:12
ChrLG1	10.749	32999326	5.88	9.4	<i>LOC100695615</i>	Intron:1:8
ChrLG1	6.236	34046099	5.82	10.1	<i>eprs</i>	Upstream:1218
ChrLG1	5.673	35481932	5.79	10.1		
ChrLG1	5.673	35481952	5.74	10		
ChrLG1	12.415	30920174	5.57	9.1	<i>chd9</i>	Intron:1:37
ChrLG1	11.923	32999472	5.55	8.9	<i>LOC100695615</i>	Exon:1:9
ChrLG1	42.77	22511492	4.82	13.7	<i>rab27a</i>	Intron:1:5
ChrLG1	42.77	22511461	4.82	13.7	<i>rab27a</i>	Intron:1:5

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ChrLG1	43.92	22442553	4.77	12.8	<i>nedd4</i>	Intron:6:24
ChrLG1	0.933	38584440	4.73	7.9		
ChrLG1	43.37	22552880	4.7	12.9	<i>LOC102081663</i>	Intron:12:16
ChrLG1	1.878	37435805	4.69	7.8	<i>LOC100709838</i>	Intron:22:23
ChrLG1	1.162	38525302	4.67	7.8		
ChrLG1	1.253	39553955	4.67	7.8		
ChrLG1	2.235	38525449	4.65	7.7		
ChrLG1	79.607	10859022	4.52	8.9	<i>neol</i>	Exon:10:28

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**Supplementary Table S5. SNP distributions across genome of FAM3 from GWAS analysis**

<b>Chromosome</b>	<b>Number of SNPs</b>	<b>Physical distance (bp)</b>
ChrLG1	275	40673430
ChrLG2	290	36523203
ChrLG3	339	87567345
ChrLG4	273	35549522
ChrLG5	245	39714817
ChrLG6	238	42433576
ChrLG7	552	64772279
ChrLG8	251	30527416
ChrLG9	258	35850837
ChrLG10	229	34704454
ChrLG11	306	39275952
ChrLG12	230	38600464
ChrLG13	259	34734273
ChrLG14	244	40509636
ChrLG15	360	39688505
ChrLG16	271	36041493
ChrLG17	239	38839487
ChrLG18	281	38636442
ChrLG19	213	30963196
ChrLG20	166	37140374
ChrLG22	232	39199643
ChrLG23	406	45655644
Total	6157	

**Supplementary Table S6. SNP distribution among chromosomes in FAM4**

<b>Chrom</b>	<b>Physical distance (bp)</b>	<b>SNP marker number</b>	<b>LOD value range</b>	<b>% Expl. range</b>
ChrLG1	40673430	62	2.62-8.03	4.3-12.7
ChrLG2	36523203	57	0.02-2.53	0-4.3
ChrLG3	87567345	65	0-2.65	0-4.3
ChrLG4	35549522	72	0.08-1.3	0.1-2.1
ChrLG5	39714817	58	0-1.54	0-2.6
ChrLG6	42433576	34	0-1.54	0-3.4
ChrLG7	64772279	136	0-3.11	0-5.1
ChrLG8	30527416	77	0-1.58	0-2.6
ChrLG9	35850837	38	0.07-1.88	0-4
ChrLG10	34704454	67	0-3.12	0-5.1
ChrLG11	39275952	120	0-1.01	0-1.7
ChrLG12	38600464	37	0.21-2.06	0.4-3.5
ChrLG13	34734273	27	0-1.39	0-2.3
ChrLG14	40509636	100	0-1.62	0-2.8
ChrLG15	39688505	72	0.04-0.66	0.1-1.2
ChrLG16	36041493	35	0.08-1.01	0.2-2
ChrLG17	38839487	51	0.21-1.68	0.4-2.8
ChrLG18	38636442	59	0.71-2.58	1.2-4.3
ChrLG19	30963196	57	0.12-1.07	0.2-1.8
ChrLG20	37140374	61	0.4-1.82	0.7-3
ChrLG22	39199643	60	0.18-1.86	0-3.3
ChrLG23	45655644	77	0.9-27.71	1.5-37.5
Total	–	1422	0-27.71	0-37.5