Histological and transcriptome analyses of testes from Duroc and Meishan boars

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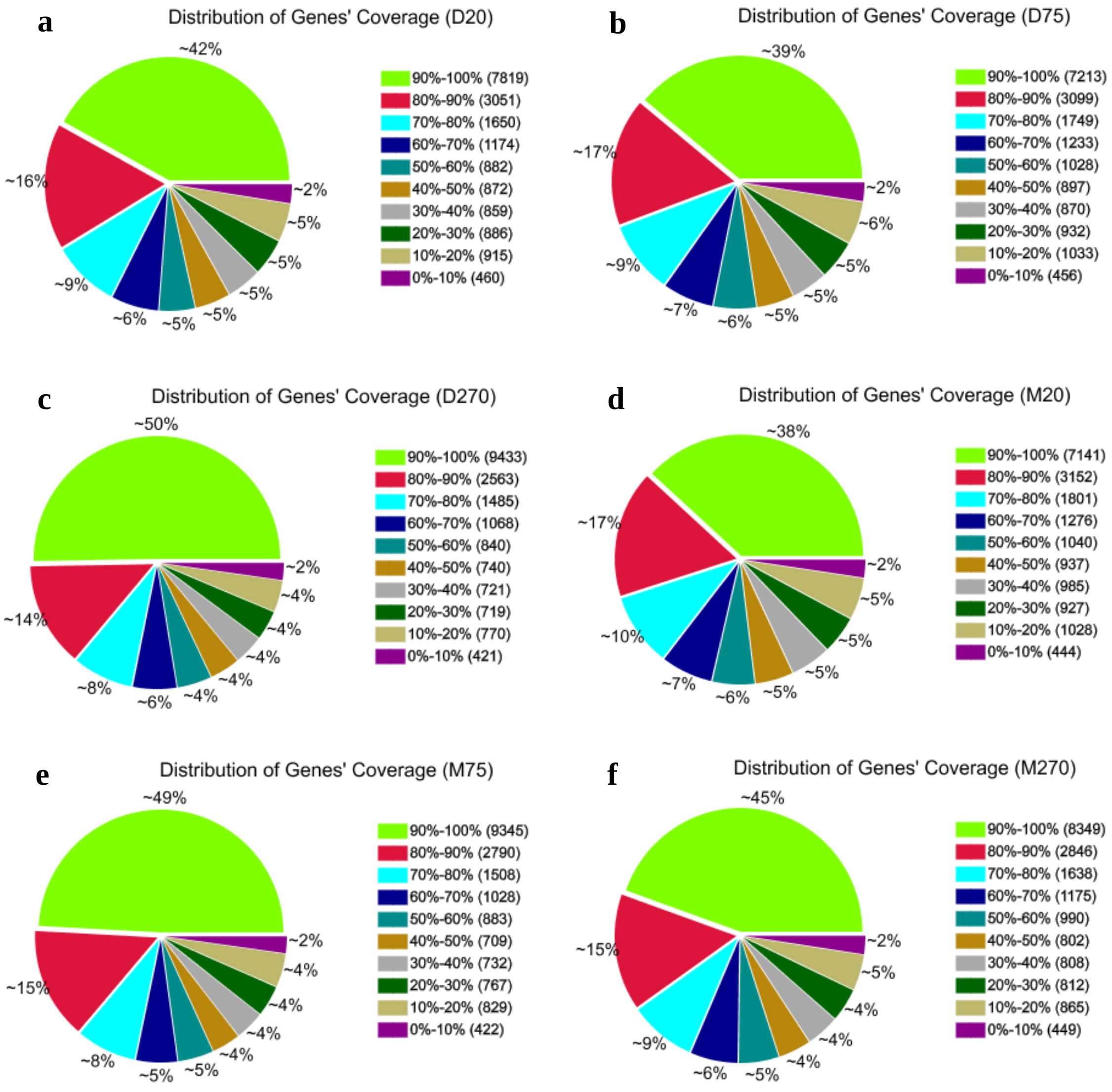
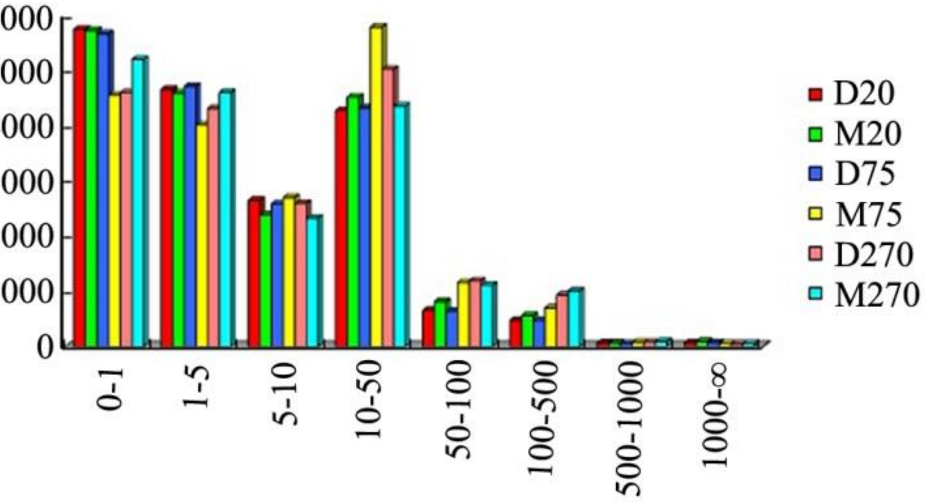
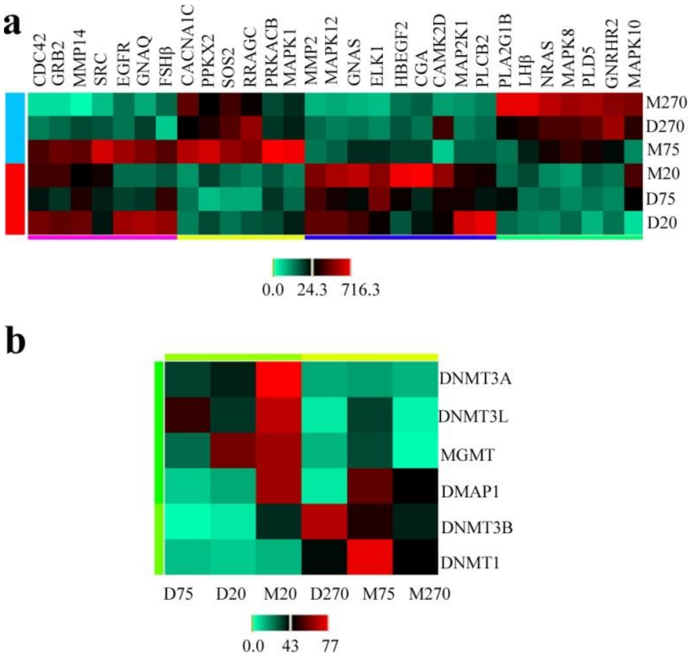


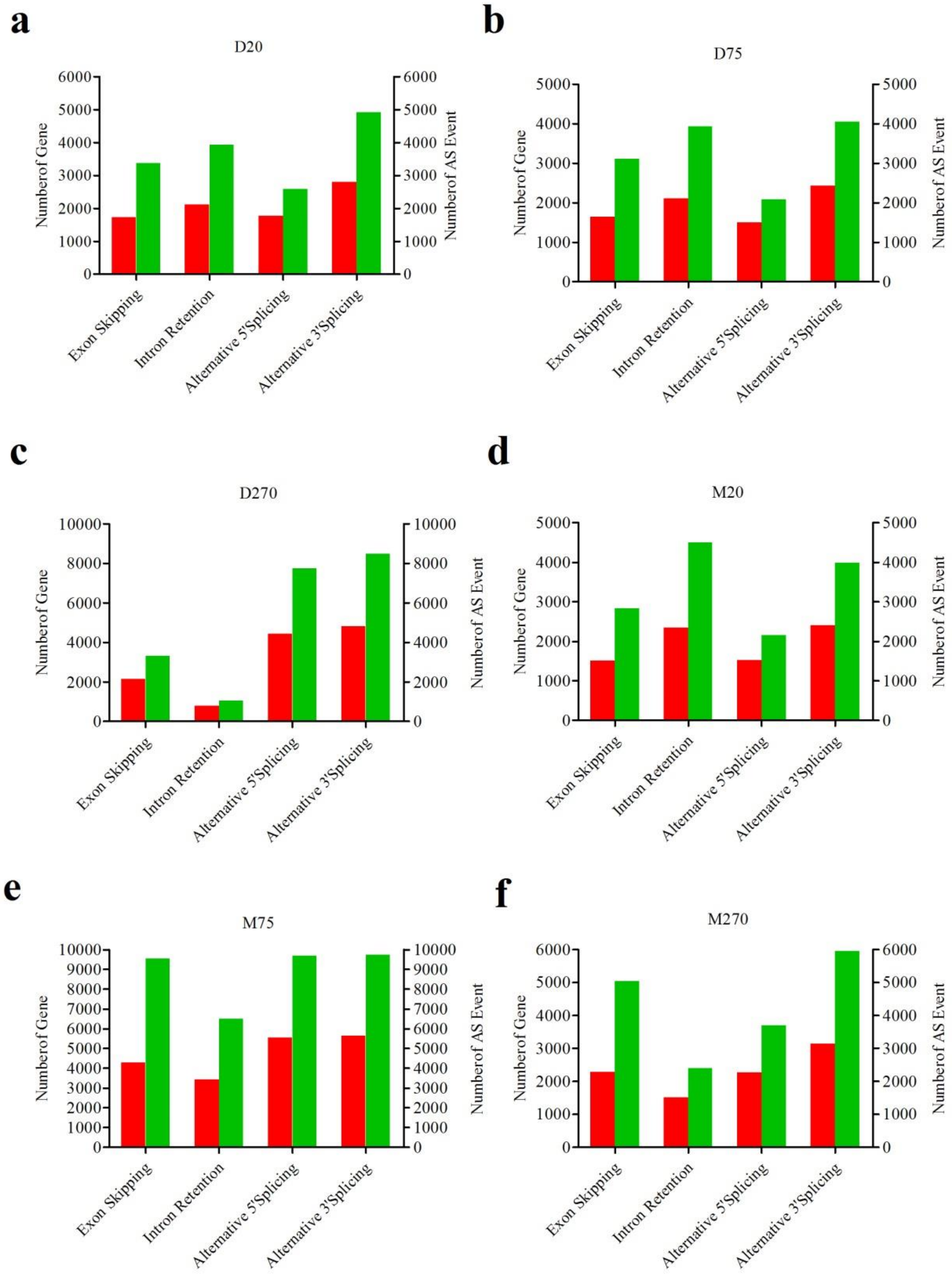
Supplementary Figure S1. Distribution of read coverage in transcripts detected. (a) D20. (b) D75. (c) D270. (d) M20. (e) M75. (f) M270.



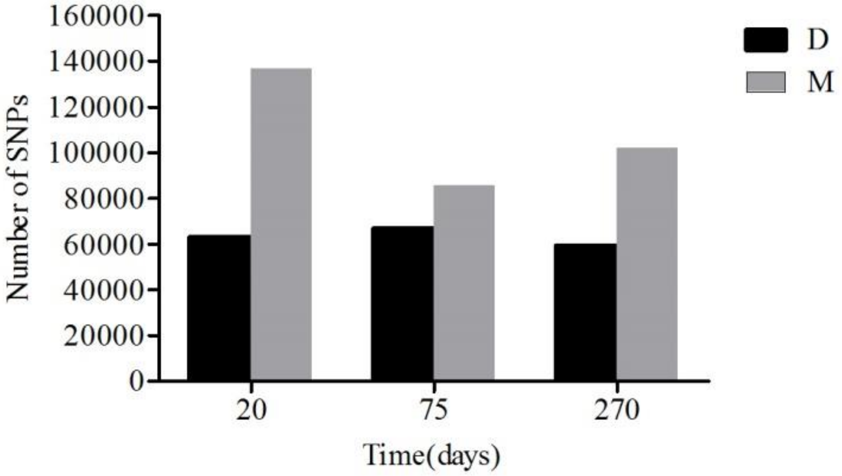
Supplementary Figure S2. Abundant distribution of genes. 0-10, low expression genes. 10-500, moderate expression genes. >500, high expression genes.



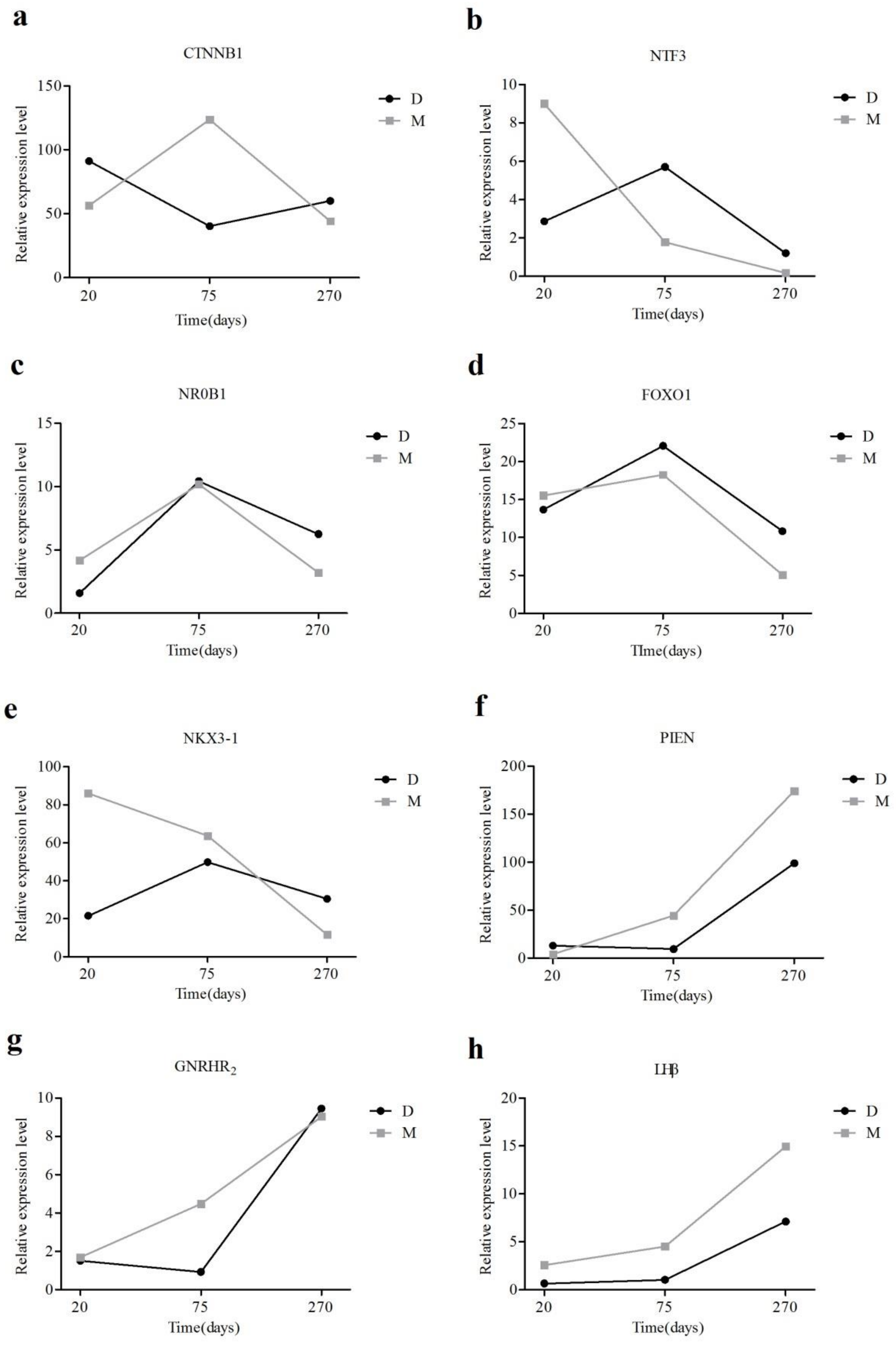
Supplementary Figure S3. Heatmap results. (a) Heatmap of the subset DEGs in the GnRH pathway from D20-vs-M20, which is critical to reproduction regulation. (b) Heatmap of DEGs encoding key enzymes in epigenetic regulation during spermatogenesis.



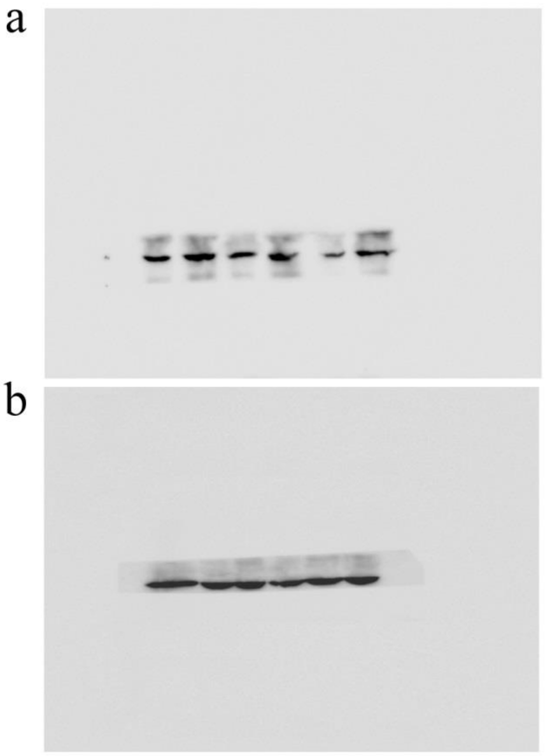
Supplementary Figure S4. The number of alternative splicing event and genes of six samples. (a) D20. (b) D75. (c) D270. (d) M20. (e) M75. (f) M270.The red bars indicated the number of genes and the green bars indicated the number of alternative splicing events. AS, alternative splicing.



Supplementary Figure S5. The number of SNPs in six samples. The black bars show the number of SNPs in Duroc boars, and the gray bars show the number of SNPs in Meishan boars. D indicates Duroc boars; M indicates Meishan boars.



Supplementary Figure S6. The results of RNA-seq between the two breeds. (a) CTNNB1. (b) NTF3. (c) NR0B1. (d) FOXO1. (e) NKX3-1. (f) PTEN. (g) GNRHR$_2$. (h) LHβ. D indicates Duroc boars; M indicates Meishan boars.



Supplementary Figure S7. Full image of Western Blotting. (a) NTF3 was probed with anti-NTF3 antibody. (b) β-actin was probed with anti-β-actin antibody. Lanes from left to right represent the testes tissues of 20-day-old, 75-day-old, 270-day-old Duroc boars and 20-day-old, 75-day-old and 270-day-old Meishan boars.

Supplementary Table S1. Primers for real-time qPCR. [1]F, forward primer. [2]R, reverse primer.

Primer	Sequence of primers (5'-3')	Tm(°C)	Size (bp)
CTNNB1-F[1]	TAAACTCCTACATCCACCATC	57	141
CTNNB1-R[2]	CGAACCAGCAACTGAACT		
NKX3-1-F	AGGGAGGAAGAGGCAGCG	65	128
NKX3-1-R	GTCCAGCAGATAAGTCTCAAGGTG		
PTEN-F	TATTCCCAGTCAGAGGCG	61	215
PTEN-R	CGAACTTGTCTTCCCGTC		
FOXO1-F	CAGAGGGAGGCAAGAGTG	59	211
FOXO1-R	GTCCAGTTATCAAAGTCATCG		
NR0B1-F	CTTCCTTGCCAAGTGCTGGA	63	179
NR0B1-R	GGTACCCCCTGTGTGTCATC		
NTF3-F	TATCACCCTGCCCAAAGC	62	196
NTF3-R	TCCCACATAATCCTCCACG		
GnRHR2-F	CAGTTTCCTTCTTGCCTTGCC	61	106
GnRHR2-R	TTGAGCCTTGAAGCTGCCTT		
LHβ-F	TAGGGACTGCTGTTGTGGCT	62	146
LHβ-R	ATGCTGGTGGTGAAGGTGAT		
β-actin-F	CCAGGTCATCACCATCGG	60	158
β-actin-R	CCGTGTTGGCGTAGAGGT		

Supplementary Table S2. Quality evaluation of transcriptome library. (a) RNA quality detection for samples. RNA samples (n=3) of each library were mixed together when measured. (b) Statistics of transcriptome library in each sample. (c) The number of reads mapping to reference genome and reference gene from each sample.

Sample	Concentration （(ng/ μL）	Quality (μg)	RIN	28S/ 18S	OD260/280	Test results
D20	1593.0	55.76	9.0	1.6	1.98	A type
D75	2210.0	55.25	8.7	1.6	1.96	A type
D270	1110.0	61.05	8.0	1.8	2.00	A type
M20	2610.0	78.30	8.0	1.6	2.00	A type
M75	3069.0	138.11	8.7	2.0	1.99	A type
M270	444.0	22.20	7.5	1.6	2.00	A type

(a)

Sample	Raw reads	Clean reads
D20	88239426	82204520 (93.16%)
D75	82447852	78732460 (95.49%)
D270	84540534	79277080 (93.77%)
M20	88198764	82280956 (93.29%)
M75	75184178	70130324 (93.28%)
M270	89026326	78512750 (88.19%)

(b)

Sample	Total reads	Map to Genome		Map to Gene	
		Total mapped	Unique mapped	Total mapped	Unique mapped
D20	82204520	49006496（59.62%）	45637349（55.52%）	48794808（59.36%）	46030981（56.00%）
D75	78732460	45998100（58.42%）	42530620（54.02%）	41090470（52.19%）	38812571（49.30%）
D270	79277080	42098395（53.10%）	38237952（48.23%）	42366329（53.44%）	39639577（50.00%）
M20	82280956	42409303（51.54%）	38399404（46.67%）	37433323（45.49%）	34597892（42.05%）
M75	70130324	35668778（50.86%）	32257507（46.00%）	39153887（55.83%）	36486454（52.03%）
M270	78512750	39422130（50.21%）	35531534（45.26%）	41166231（52.43%）	38340413（48.83%）

(c)

Supplementary Table S3. The reads mapping to reference genome and reference gene from six samples. (a) D20. (b) D75. (c) D270. (d) M20. (e) M75. (f) M270.

D20	Map to Genome		Map to Gene	
	reads number	percentage	reads number	percentage
Total Reads	82204520	100.00%	82204520	100.00%
Total BasePairs	7398406800	100.00%	7398406800	100.00%
Total Mapped Reads	49006496	59.62%	48794808	59.36%
perfect match	34381852	41.82%	37004468	45.02%
<=5bp mismatch	14624644	17.79%	11790340	14.34%
unique match	45637349	55.52%	46030981	56.00%
multi-position match	3369147	4.10%	2763827	3.36%
Total Unmapped Reads	33198024	40.38%	33409712	40.64%

(a)

D75	Map to Genome		Map to Gene	
	reads number	percentage	reads number	percentage
Total Reads	78732460	100.00%	78732460	100.00%
Total BasePairs	7085921400	100.00%	7085921400	100.00%
Total Mapped Reads	45998100	58.42%	41090470	52.19%
perfect match	34340094	43.62%	33746382	42.86%
<=5bp mismatch	11658006	14.81%	7344088	9.33%
unique match	42530620	54.02%	38812571	49.30%
multi-position match	3467480	4.40%	2277899	2.89%
Total Unmapped Reads	32734360	41.58%	37641990	47.81%

(b)

D270	Map to Genome		Map to Gene	
	reads number	percentage	reads number	percentage
Total Reads	79277080	100.00%	79277080	100.00%
Total BasePairs	7134937200	100.00%	7134937200	100.00%
Total Mapped Reads	42098395	53.10%	42366329	53.44%
perfect match	30844157	38.91%	34042799	42.94%
<=5bp mismatch	11254238	14.20%	8323530	10.50%
unique match	38237952	48.23%	39639577	50.00%
multi-position match	3860443	4.87%	2726752	3.44%
Total Unmapped Reads	37178685	46.90%	36910751	46.56%

(c)

M20	Map to Genome		Map to Gene	
	reads number	percentage	reads number	percentage
Total Reads	82280956	100.00%	82280956	100.00%
Total BasePairs	7405286040	100.00%	7405286040	100.00%
Total Mapped Reads	42409303	51.54%	37433323	45.49%
perfect match	23973246	29.14%	23137340	28.12%
<=5bp mismatch	18436057	22.41%	14295983	17.37%
unique match	38399404	46.67%	34597892	42.05%
multi-position match	4009899	4.87%	2835431	3.45%
Total Unmapped Reads	39871653	48.46%	44847633	54.51%

(d)

M75	Map to Genome		Map to Gene	
	reads number	percentage	reads number	percentage
Total Reads	70130324	100.00%	70130324	100.00%
Total BasePairs	6311729160	100.00%	6311729160	100.00%
Total Mapped Reads	35668778	50.86%	39153887	55.83%
perfect match	24172082	34.47%	28600977	40.78%
<=5bp mismatch	11496696	16.39%	10552910	15.05%
unique match	32257507	46.00%	36486454	52.03%
multi-position match	3411271	4.86%	2667433	3.80%
Total Unmapped Reads	34461546	49.14%	30976437	44.17%

(e)

M270	Map to Genome		Map to Gene	
	reads number	percentage	reads number	percentage
Total Reads	78512750	100.00%	78512750	100.00%
Total BasePairs	7066147500	100.00%	7066147500	100.00%
Total Mapped Reads	39422130	50.21%	41166231	52.43%
perfect match	25595635	32.60%	29722518	37.86%
<=5bp mismatch	13826495	17.61%	11443713	14.58%
unique match	35531534	45.26%	38340413	48.83%
multi-position match	3890596	4.96%	2825818	3.60%
Total Unmapped Reads	39090620	49.79%	37346519	47.57%

(f)

Supplementary Table S4 . The QTLs related to reproduction traits and their chromosome positions

Traits	QTL	chromosome
Age at puberty	AGEP	chr1, chr2, chr3, chr4, chr5, chr6, chr7, chr8, chr9, chr10, chr11, chr12, chr13, chr14, chr15, chr16, chr17, chr18
Epididymis weight	EPIDW	chr2, chr3, chr7, chrX
Seminiferous tubule diameter	SEMTD	chr5, chr13, chr16, chrX
Ejaculation duration	EJDUR	Chr6, chr17
Semen PH	SEPH	chr2, chr6, chr9, chr12
Sperm concentration	SPCON	chr1, chr5, chr17
Sperm motility	SPMOT	chr1, chr5
Sperm per ejaculate	SPEEJ	chr1, chr2
Testicular weight	TWSTIWT	chr1, chr3, chr5, chr7, chrX
Semen volume	SEMVOL	chr1, chr3, chr15, chr18
Ejaculation times	EJTIME	chr6, chr16, chr17
Sperm abnormality rate	SPABR	chr4, chr5, chr9