

Sex-relevant genes in the embryo stage of Chinese soft-shelled turtles as revealed by RNA-Seq analysis

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Supplementary material

Table S1.

Summary of clean data using the Illumina Hiseq X Ten platform.

Stages	Sample	Clean reads pairs	Clean base (bp)	Length	Q20 (%)	Q30 (%)	GC (%)
Male in gonadal sex	A1	24,709,984	7,412,995,200	150;150	96.7;96.8	92.7;92.6	51.4;51.3
differentiation	A2	34,552,664	10,365,799,200	150;150	95.2;92.0	93.6;88.8	48.8;48.6
stages	A3	38,637,511	11,591,253,300	150;150	95.0;91.8	93.4;88.7	49.9;49.8
Female in gonadal	B1	24,313,629	7,294,088,700	150;150	96.5;96.3	92.2;91.6	55.8;55.8
sex differentiation	B2	24,843,892	7,453,167,600	150;150	96.4;96.3	92.0;91.8	56.7;56.7
stages	B3	23,315,748	6,994,724,400	150;150	97.1;95.7	95.6;93.7	55.5;55.5
Male in sex	C1	18,886,099	5,665,829,700	150;150	96.5;94.8	91.7;88.4	52.0;51.8
determination	C2	19,154,060	5,746,218,000	150;150	96.5;95.1	91.7;88.9	51.7;51.6
stages	C3	21,580,489	6,474,146,700	150;150	96.4;95.0	91.5;88.8	52.5;52.3
Female in sex	D1	26,366,637	7,909,991,100	150;150	96.5;94.4	91.7;87.5	51.9;51.7
determination	D2	21,256,419	6,376,925,700	150;150	96.5;95.3	91.7;89.4	52.9;52.7
stages	D3	19,449,892	5,834,967,600	150;150	96.6;95.5	91.7;89.6	52.4;52.2
Male in shelling	E1	27,690,009	8,307,002,700	150;150	97.1;95.7	93.2;89.5	52.7;52.6
stage	E2	29,140,514	8,742,154,200	150;150	97.2;95.8	93.4;89.8	54.1;54.0
	E3	26,368,246	7,910,473,800	150;150	97.2;95.9	93.3;90.1	55.1;55.1
Female in shelling	F1	29,425,992	8,827,797,600	150;150	97.0;96.2	93.1;90.9	54.5;54.4
stages	F2	28,082,744	8,424,823,200	150;150	97.2;95.9	93.3;89.9	52.6;52.6
	F3	26,227,180	7,868,154,000	150;150	97.2;96.2	93.4;90.7	54.3;54.3

Table S2.

Each sample aligned to the reference genome total reads of Chinese soft-shelled turtles.

Sample	Total reads	Total mapping rate	Left reads mapped	Left reads multiple mapped	Right reads mapped	Right reads multiple mapped
A1	49,419,968	31,158,210 (63.0%)	15,579,958 (63.1%)	494,087 (3.2%)	15,578,252 (63.0%)	504,151 (3.2%)
A2	69,105,328	42,469,949 (61.5%)	22,019,432 (63.7%)	573,605 (2.6%)	20,450,517 (59.2%)	524,469 (2.6%)
A3	77,275,022	44,728,979 (57.9%)	23,201,271 (60.0%)	684,662 (3.0%)	21,527,708 (55.7%)	625,690 (2.9%)
B1	48,627,258	26,114,355 (53.7%)	13,078,383 (53.8%)	348,154 (2.7%)	13,035,972 (53.6%)	352,172 (2.7%)
B2	49,687,784	25,278,526 (50.9%)	12,633,715 (50.9%)	430,030 (3.4%)	12,644,811 (50.9%)	438,033 (3.5%)
B3	46,631,496	25,435,971 (54.5%)	12,846,608 (55.1%)	384,222 (3.0%)	12,589,363 (54.0%)	382,014 (3.0%)
C1	37,772,198	22,273,692 (59.0%)	11,535,254 (61.1%)	240,786 (2.1%)	10,738,438 (56.9%)	225,310 (2.1%)
C2	38,308,120	22,580,943 (58.9%)	11,628,997 (60.7%)	251,778 (2.2%)	10,951,946 (57.2%)	236,924 (2.2%)
C3	43,160,978	24,569,679 (56.9%)	12,663,041 (58.7%)	276,160 (2.2%)	11,906,638 (55.2%)	261,152 (2.2%)
D1	52,733,274	31,298,635 (59.4%)	16,327,741 (61.9%)	339,239 (2.1%)	14,970,894 (56.8%)	315,695 (2.1%)
D2	42,512,838	24,584,209 (57.8%)	12,603,998 (59.3%)	266,521 (2.1%)	11,980,211 (56.4%)	255,334 (2.1%)
D3	38,899,784	22,838,520 (58.7%)	11,700,064 (60.2%)	249,632 (2.1%)	11,138,456 (57.3%)	237,722 (2.1%)

E1	55,380,018	31,543,075	16,104,160	311,499	15,438,915	297,984
		(57.0%)	(58.2%)	(1.9%)	(55.8%)	(1.9%)
E2	58,281,028	32,117,296	16,388,728	320,140	15,728,568	311,244
		(55.1%)	(56.2%)	(2.0%)	(54.0%)	(2.0%)
E3	52,736,492	27,951,571	14,231,253	359,773	13,720,318	352,240
		(53.0%)	(54.0%)	(2.5%)	(52.0%)	(2.6%)
F1	58,851,984	30,175,676	15,258,560	393,529	14,917,116	383,344
		(51.3%)	(51.9%)	(2.6%)	(50.7%)	(2.6%)
F2	56,165,488	32,423,269	16,529,416	363,604	15,893,853	350,080
		(57.7%)	(58.9%)	(2.2%)	(56.6%)	(2.2%)
F3	52,454,360	27,757,051	14,070,685	346,991	13,686,366	340,640
		(52.9%)	(53.6%)	(2.5%)	(52.2%)	(2.5%)

Table S3.

Summary of sample reads and their mapping status.

Sample	Total read pairs	Total mapped reads	Uniq mapped reads	Multiple mapped reads
A1	24,709,984	14,517,388 (58.75%)	3,456,903 (13.99%)	11,060,485 (44.76%)
A2	34,552,664	20,809,298 (60.22%)	4,596,356 (13.30%)	16,212,942 (46.92%)
A3	38,637,511	22,344,985 (57.83%)	5,175,927 (13.40%)	17,169,058 (44.44%)
B1	24,313,629	11,567,834 (47.58%)	2,592,375 (10.66%)	8,975,459 (36.92%)
B2	24,843,892	11,401,288 (45.89%)	2,677,789 (10.78%)	8,723,499 (35.11%)
B3	23,315,748	10,989,341 (47.13%)	2,553,959 (10.95%)	8,435,382 (36.18%)
C1	18,886,099	10,194,243 (53.98%)	2,067,839 (10.95%)	8,126,404 (43.03%)
C2	19,154,060	10,643,698 (55.57%)	2,246,555 (11.73%)	8,397,143 (43.84%)
C3	21,580,489	11,442,259 (53.02%)	2,342,090 (10.85%)	9,100,169 (42.17%)
D1	26,366,637	14,326,931 (54.34%)	2,818,323 (10.69%)	11,508,608 (43.65%)
D2	21,256,419	11,158,127 (52.49%)	2,391,190 (11.25%)	8,766,937 (41.24%)
D3	19,449,892	10,470,688 (53.83%)	2,113,241 (10.87%)	8,357,447 (42.97%)
E1	27,690,009	14,521,674 (52.44%)	3,655,293 (13.20%)	10,866,381 (39.24%)
E2	29,140,514	14,623,017 (50.18%)	3,225,883 (11.07%)	11,397,134 (39.11%)
E3	26,368,246	12,551,704 (47.60%)	3,065,246 (11.62%)	9,486,458 (35.98%)
F1	29,425,992	14,413,913 (48.98%)	3,511,737 (11.93%)	10,902,176 (37.05%)
F2	28,082,744	14,756,366 (52.55%)	3,476,046 (12.38%)	11,280,320 (40.17%)
F3	26,227,180	12,794,070 (48.78%)	2,919,686 (11.13%)	9,874,384 (37.65%)

Table S4.

Statistical analysis of differentially expressed genes.

Sample	Expressed genes	Total genes	0	0–1	1–3	3–15	15–60	>60
A1	31,202	47,265	16,063 (33.98%)	12,510 (26.47%)	5,141 (10.88%)	7,826 (16.56%)	4,097 (8.67%)	1,628 (3.44%)
A2	32,047	47,265	15,218 (32.20%)	13,320 (28.18%)	5,363 (11.35%)	7,728 (16.35%)	4,068 (8.61%)	1,568 (3.32%)
A3	32,237	47,265	15,028 (31.80%)	13,248 (28.03%)	5,372 (11.37%)	8,002 (16.93%)	4,012 (8.49%)	1,603 (3.39%)
B1	31,095	47,265	16,170 (34.21%)	11,302 (23.91%)	5,329 (11.27%)	8,189 (17.33%)	4,315 (9.13%)	1,960 (4.15%)
B2	31,888	47,265	15,377 (32.53%)	11,461 (24.25%)	5,692 (12.04%)	8,611 (18.22%)	4,298 (9.09%)	1,826 (3.86%)
B3	30,607	47,265	16,658 (35.24%)	10,935 (23.14%)	5,294 (11.20%)	8,128 (17.20%)	4,329 (9.16%)	1,921 (4.06%)
C1	29,129	47,265	18,136 (38.37%)	10,536 (22.29%)	4,661 (9.86%)	7,884 (16.68%)	4,304 (9.11%)	1,744 (3.69%)
C2	28,595	47,265	18,670 (39.50%)	10,528 (22.27%)	4,427 (9.37%)	7,679 (16.25%)	4,222 (8.93%)	1,739 (3.68%)
C3	29,821	47,265	17,444 (36.91%)	11,044 (23.37%)	4,845 (10.25%)	7,821 (16.55%)	4,294 (9.08%)	1,817 (3.84%)
D1	30,599	47,265	16,666 (35.26%)	11,636 (24.62%)	4,699 (9.94%)	7,982 (16.89%)	4,497 (9.51%)	1,785 (3.78%)
D2	30,075	47,265	17,190 (36.37%)	10,880 (23.02%)	4,900 (10.37%)	8,214 (17.38%)	4,304 (9.11%)	1,777 (3.76%)
D3	29,536	47,265	17,729 (37.51%)	10,519 (22.26%)	4,647 (9.83%)	8,109 (17.16%)	4,437 (9.39%)	1,824 (3.86%)
E1	27,812	47,265	19,453 (41.16%)	11,583 (24.51%)	4,575 (9.68%)	6,902 (14.60%)	3,263 (6.90%)	1,489 (3.15%)
E2	28,374	47,265	18,891 (39.97%)	11,623 (24.59%)	4,645 (9.83%)	6,932 (14.67%)	3,442 (7.28%)	1,732 (3.66%)
E3	28,013	47,265	19,252 (40.73%)	11,199 (23.69%)	4,915 (10.40%)	6,982 (14.77%)	3,275 (6.93%)	1,642 (3.47%)
F1	29,717	47,265	17,548 (37.13%)	11,922 (25.22%)	5,062 (10.71%)	7,647 (16.18%)	3,526 (7.46%)	1,560 (3.30%)
F2	30,234	47,265	17,031 (36.03%)	12,416 (26.27%)	5,111 (10.81%)	7,557 (15.99%)	3,565 (7.54%)	1,585 (3.35%)
F3	30,108	47,265	17,157 (36.30%)	11,669 (24.69%)	5,364 (11.35%)	7,711 (16.31%)	3,685 (7.80%)	1,679 (3.55%)

Table S5.

Statistics of significantly differentially expressed genes in Chinese soft-shelled turtle between male and female gonads on 15 to 23 days, 24 to 29 days and 33 to 45 days.

Differentially expressed genes	15 to 23 days	24 to 29 days	33 to 45 days
Upregulated in the female gonad	765	12	196
Downregulated in the female gonad	210	31	104
Total(up-plus downregulated)	975	43	300

Values indicate genes with $\log_2FC > 1$ or $\log_2FC < -1$ and false discovery rate (FDR)-corrected P value < 0.05 in males compared with females.

Table S6.

2206 genes in Subcluster-1 and annotated information.

[See AB-1542_Table S6.xlsx]

Table S7.

1006 genes in Subcluster-2 and annotated information.

[See AB-1542_Table S7.xlsx]

Table S8.

3689 genes in Subcluster-3 and annotated information.

[See AB-1542_Table S8.xlsx]