

Identification and Characterization of MicroRNAs involving in Initial Sex Differentiation of *Chlamys farreri* Gonads

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Table S1: Specific primers used for RT-qPCR of the miRNAs

miRNA	Forward primer sequence (5'-3')
novel_miR5	TTACCCTGTAGAACCGAGCGAGT
novel_miR7	CCCGTGATAGCTCAGTTGGTAG
novel_miR54	GATCTCTCCAAGGCCTTCGG
novel_miR65	GCAGGAGTTGTCTTGTCTGTT
novel_miR79	TGAGTAGCAGCCGGGACC
miR-87a-3p_1	GTGAGCAAAGTTTCAGGTGTG
miR-124a	TAAGGCACGCGGTGAATGCC
miR-124-3p_4	TAAGGCACGCGGTGAATGCCA
miR-375_1	TTTGTTTCGTTTCGGCTCGCGTTA
miR-375-3p_1	TTTGTTTCGTTTCGGCTCGCGTTATGTGC
miR-1985	CATTTTTATCAGTCACTGTG
5S rRNA	TATCACGTTGAAAACACCGGTTCTCG

The reverse primer was a universal primer (Takara, Dalian, China)

Table S2: Specific primers for amplifying the target gene fragments

Target gene	Primer Sequence (5'-3')
<i>Foxl2</i> -WT-S	<u>CGAGCT</u> CAACAGTATCCGCCACAACCT
<i>Foxl2</i> -WT-AS	CCT <u>CGAGAG</u> TGGTTGTCAGCAAATAAAGG
<i>Foxl2</i> -MT-S	GGTGAACGGAAAGGAAG <u>TCCTC</u> GGACTTGGA
<i>Foxl2</i> -MT-AS	<u>GAGGACT</u> TCCTTTCCGTTACCTCCACCTTCCCTCGGCA
<i>Klf4</i> -WT-S	<u>CGAGCT</u> CCTCCCATCTGAAAGCACACT
<i>Klf4</i> -WT-AS	CCT <u>CGAGG</u> TGGCACTGGAAAGGACGAT
<i>Klf4</i> -MT-S	GATGCGGCTGGAAAT <u>CCATCT</u> GCTCTGATGAAC
<i>Klf4</i> -MT-AS	<u>AGATGG</u> ATTTCCAGCCGCATCCCTTCCATGCGC

WT: wild-type; MT: mutant type; S: sense; AS: antisense; Single underline: cleavage sites of restriction enzymes; Double underline: mutant bases.

Table S3: The DEMs between testis and ovar

miRNAs	Testis (TPM)	Ovary (TPM)	Log ₂ (fold change)	p-value	mature miRNAs sequence
miR-124a	71.1125516840033	5.93560650481348	3.5826368662642	0.018463927	UAAGGCACGCGGUGAAUGCC
miR-124-3p_4	766.810265851032	268.214396432376	1.51548297544166	0.01440008	UAAGGCACGCGGUGAAUGCCA
miR-1985	17491.3396364478	8001.10215998119	1.12837013590651	1.14E-05	UGCCAUUUUUATCAGUCACUGUG
miR-375_1	1648.50526430289	3968.69846821903	-1.26750746005321	5.45E-05	UUUGUUCGUUCGGCUCGCGUUA
miR-375-3p_1	2433.17741785888	5279.77768604446	-1.11763566391377	0.001216834	UUUGUUCGUUCGGCUCGCGUUAU
miR-87a-3p_1	119.888137867487	444.997115730411	-1.8921070644632	0.042434472	GUGAGCAAAGUUUCAGGUGUG
novel_miR5	1905.42516714067	199.343983935073	3.25678097791346	1.53E-08	UUACCCUGUAGAACCGAGCGAGU
novel_miR54	74.4307965107132	8.63342185017998	3.10789528588565	0.047379671	GAUCUCUCCAAGGCCUUCGG
novel_miR65	418.93997433732	20.2413341945236	4.37136725698746	0.004382968	CAGGAGUUGUCUUGUCUGUU
novel_miR7	238.380178479512	1480.41700967009	-2.63466743255438	0.000801096	CCCGUGAUAGCUCAGUUGGUAG
novel_miR79	8.420054401	103.75193373645	-3.62316486105696	0.019962792	UGAGUAGCAGCCGGGACCGCCCU