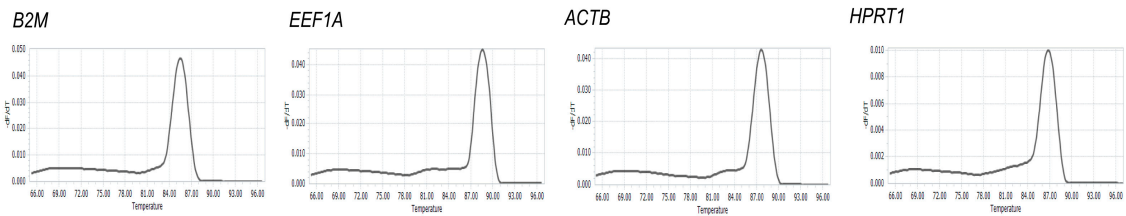


Supplementary:

Supplementary Figure S1. The melting curves of the cDNA samples amplified with candidate genes in ovary.



Supplementary Figure S2. The melting curves of the cDNA samples amplified with candidate genes in pituitary.

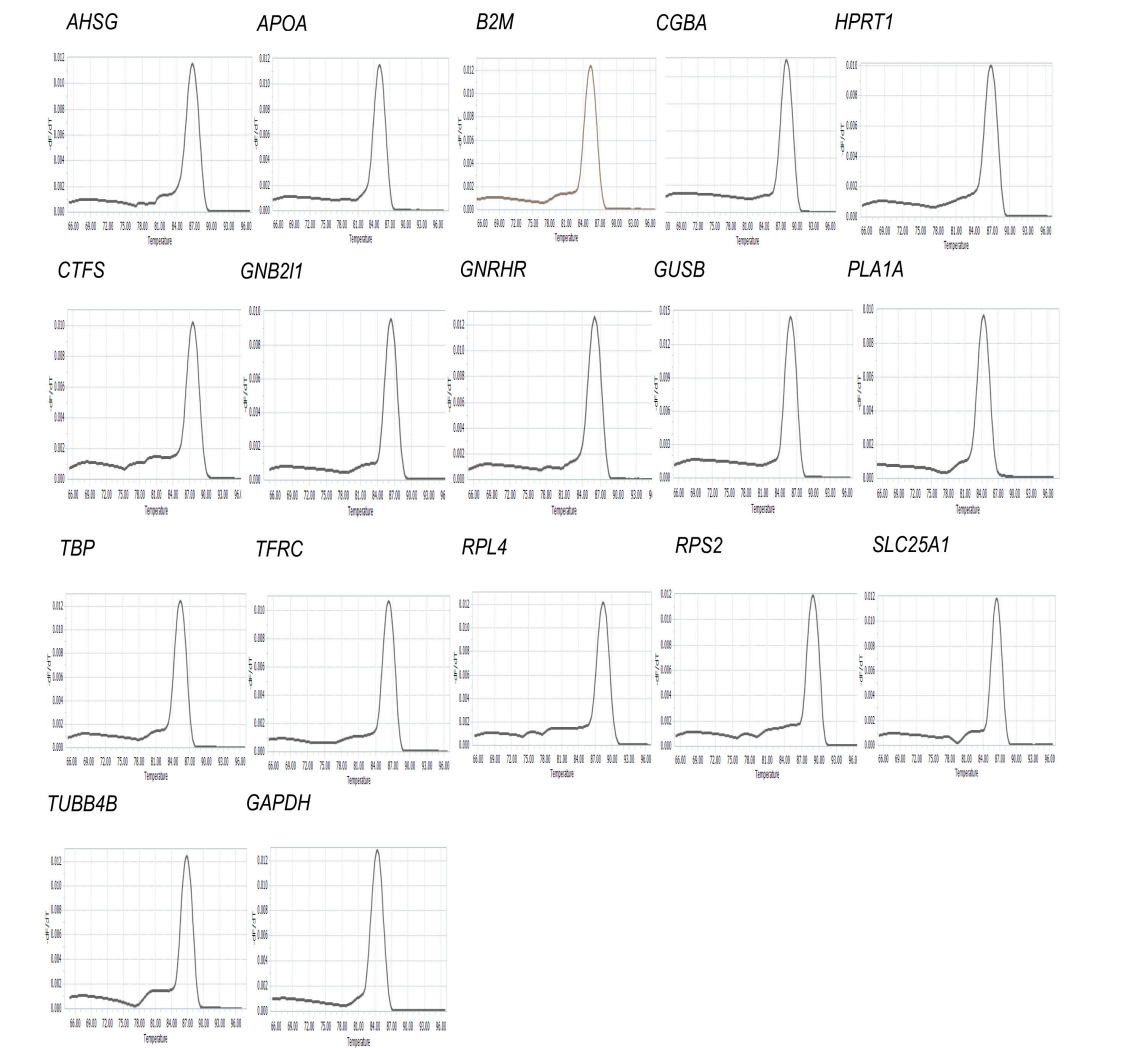


Table S1. Ovarian stages of the 20 female spotted scat

Ovarian stages	Fish numbers	Fish IDs
Stage II	6	A3,A7,A9,A12,A17,A20
Stage III	3	A13A15,A18
StageIV	11	A1,A2,A4,A5,A6,A8,A10,A11,A14,A16,A19

Table S2. Expression stability analysis of each candidate reference gene in the ovary based on five softwares.

tissues	Stages	Rank	GeNorm		NormFinder		$\Delta$ Ct		BestKeeper		Comprehensive Ranking	
			gene	stability	gene	stability	gene	stability	gene	stability	gene	stability
Ovary	Stage II	1	EEF1A	0.694	EEF1A	0.347	EEF1A	0.77	ACTB	0.63	EEF1A	1.19
		2	HPRT1	0.694	HPRT1	0.702	HPRT1	1.01	EEF1A	0.73	HPRT1	1.73
		3	ACTB	0.839	ACTB	0.915	ACTB	1.1	HPRT1	0.81	B2M	2.45
		4	B2M	1.019	B2M	1.07	B2M	1.2	B2M	1.17	ACTB	4
	Stage III	1	ACTB	0.245	ACTB	0.123	ACTB	0.35	HPRT1	0.71	EEF1A	1.41
		2	HPRT1	0.245	HPRT1	0.177	HPRT1	0.4	EEF1A	0.75	B2M	1.73
		3	EEF1A	0.25	EEF1A	0.344	EEF1A	0.44	ACTB	0.87	HPRT1	2.06
		4	B2M	0.473	B2M	0.68	B2M	0.7	B2M	1.22	ACTB	4
	Stage IV	1	EEF1A	0.42	EEF1A	0.073	EEF1A	0.62	B2M	0.64	EEF1A	1.57
		2	HPRT1	0.42	HPRT1	0.334	HPRT1	0.68	HPRT1	0.75	HPRT1	1.73
		3	ACTB	0.561	ACTB	0.615	ACTB	0.79	EEF1A	0.89	B2M	1.86
		4	B2M	0.764	B2M	0.884	B2M	0.97	ACTB	1.11	ACTB	4
	All stages	1	EEF1A	0.604	HPRT1	0.19	HPRT1	0.91	B2M	1.01	EEF1A	1.57
		2	HPRT1	0.604	EEF1A	0.446	EEF1A	0.92	HPRT1	1.62	HPRT1	1.73
		3	ACTB	0.679	ACTB	0.685	ACTB	1.01	ACTB	1.85	B2M	1.86
		4	B2M	1.083	B2M	1.411	B2M	1.49	EEF1A	1.96	ACTB	4

Table S3. Expression stability analysis of each candidate reference gene in the pituitary based on five softwares.

tissues	Stages	Rank	GeNorm		NormFinder		$\Delta$ Ct		BestKeeper		Comprehensive Ranking	
			gene	stability	gene	stability	gene	stability	gene	stability	gene	stability
Pituitary	Stage II	1	TUBB4B	0.155	TBP	0.174	RPL4	0.81	GNB211	0.14	GNB211	2.34
		2	GAPDH	0.155	GNB211	0.265	PLA1A	0.82	PLA1A	0.15	RPL4	2.78
		3	PLA1A	0.177	CTFS	0.272	GNB211	0.82	RPL4	0.19	PLA	3.13
		4	RPL4	0.199	RPS2	0.297	GAPDH	0.83	RPLP0	0.24	GAPDH	3.31
		5	GNB211	0.254	RPL4	0.309	TUBB4B	0.83	GAPDH	0.24	TUBB4B	3.96
		6	CTFS	0.305	GAPDH	0.32	TBP	0.83	TUBB4B	0.27	TBP	4.41
		7	TBP	0.334	TUBB4B	0.322	CTFS	0.85	CTFS	0.27	CTFS	5.24
		8	RPS2	0.35	PLA1A	0.36	RPS2	0.85	RPS2	0.28	RPS2	6.73

	9	RPLP0	0.393	TFRC	0.487	RPLP0	0.97	TBP	0.33	RPLP0	7.73
	10	B2M	0.437	APOA1	0.527	B2M	1.01	B2M	0.42	B2M	10.47
	11	APOA1	0.49	RPLP0	0.585	TFRC	1.03	APOA1	0.54	APOA1	10.98
	12	TFRC	0.543	B2M	0.595	APOA1	1.03	GUSB	0.57	TFRC	11.15
	13	GUSB	0.587	GUSB	0.625	GUSB	1.07	TFRC	0.62	GUSB	12.74
	14	HPRT1	0.666	HPRT1	1.246	HPRT1	1.44	HPRT1	0.85	HPRT1	14
	15	SLC25A1	0.804	SLC25A1	1.561	SLC25A1	1.77	SLC25A1	1.24	SLC25A1	15
	16	GNRHR	0.996	GNRHR	2.321	GNRHR	2.42	GNRHR	1.51	GNRHR	16
	17	CGBA	1.166	CGBA	2.324	CGBA	2.44	CGBA	1.63	CGBA	17
Stage III	1	GNB211	0.16	GAPDH	0.06	TUBB4B	0.72	RPL4	0.14	RPL4	2.45
	2	PLA1A	0.16	TUBB4B	0.097	GAPDH	0.73	GNB211	0.2	TUBB4B	2.91
	3	RPL4	0.216	RPS2	0.225	RPL4	0.75	B2M	0.2	GNB211	3.08
	4	TBP	0.308	RPL4	0.308	RPS2	0.77	TBP	0.2	GAPDH	3.15
	5	B2M	0.336	SLC25A1	0.51	GNB211	0.83	PLA1A	0.31	PLA	3.81
	6	TUBB4B	0.427	GNB211	0.554	PLA1A	0.85	TUBB4B	0.42	RPS2	5.26
	7	GAPDH	0.501	PLA1A	0.577	SLC25A1	0.85	RPS2	0.44	TBP	5.3
	8	RPS2	0.534	GUSB	0.595	GUSB	0.9	GAPDH	0.44	B2M	6.51
	9	SLC25A1	0.58	B2M	0.732	TBP	0.94	CGBA	0.46	SLC25A1	7.67
	10	CGBA	0.628	CGBA	0.737	B2M	0.95	RPLP0	0.59	GUSB	9.78
	11	GUSB	0.672	TBP	0.741	CTFS	1.01	SLC25A1	0.64	CGBA	10.19
	12	CTFS	0.722	CTFS	0.771	CGBA	1.02	GNRHR	0.75	CTFS	12.2
	13	GNRHR	0.768	APOA1	0.85	APOA1	1.07	GUSB	0.76	GNRHR	13.69
	14	APOA1	0.813	HPRT1	0.972	HPRT1	1.14	CTFS	0.84	APOA1	13.73
	15	HPRT1	0.851	GNRHR	0.995	GNRHR	1.17	APOA1	0.86	RPLP0	14.23
	16	RPLP0	0.896	RPLP0	1.197	RPLP0	1.29	HPRT1	1	HPRT1	14.73
	17	TFRC	0.973	TFRC	1.465	TFRC	1.55	TFRC	1.26	TFRC	17
Stage IV	1	RPL4	0.273	GNB211	0.346	RPL4	0.83	RPL4	0.19	RPL4	1.32
	2	TUBB4B	0.273	RPS2	0.35	GNB211	0.85	GNB211	0.2	GNB211	2
	3	PLA1A	0.308	RPL4	0.354	RPS2	0.86	GAPDH	0.28	RPS2	3.46
	4	GNB211	0.336	PLA1A	0.422	PLA1A	0.86	RPS2	0.3	TUBB4B	3.94
	5	GAPDH	0.357	RPLP0	0.432	TUBB4B	0.89	B2M	0.31	PLA	4.28
	6	RPS2	0.377	CGBA	0.478	TBP	0.89	TUBB4B	0.35	GAPDH	5.54
	7	TBP	0.392	TBP	0.518	GAPDH	0.91	PLA1A	0.37	TBP	7.17
	8	B2M	0.428	TUBB4B	0.526	B2M	0.96	APOA1	0.49	B2M	7.52
	9	APOA1	0.474	GAPDH	0.529	CGBA	0.99	TBP	0.53	RPLP0	8.8
	10	CTFS	0.51	B2M	0.566	RPLP0	0.99	RPLP0	0.54	CGBA	9.19
	11	CGBA	0.547	APOA1	0.642	APOA1	1.01	CTFS	0.62	APOA1	9.66
	12	RPLP0	0.579	CTFS	0.742	CTFS	1.04	CGBA	0.71	CTFS	11.22
	13	GUSB	0.635	GUSB	0.789	GUSB	1.17	GUSB	0.84	GUSB	13
	14	TFRC	0.737	TFRC	1.111	TFRC	1.46	TFRC	0.99	TFRC	14
	15	HPRT1	0.823	HPRT1	1.337	HPRT1	1.57	HPRT1	1.18	HPRT1	15
	16	SLC25A1	0.971	SLC25A1	1.757	SLC25A1	1.96	SLC25A1	1.31	SLC25A1	16
	17	GNRHR	1.172	GNRHR	2.599	GNRHR	2.68	GNRHR	2	GNRHR	17
All stages	1	RPL4	0.247	RPL4	0.248	GNB211	0.2	GNB211	0.2	RPL4	1.19
	2	GNB211	0.247	RPS2	0.294	RPL4	0.21	RPL4	0.21	GNB211	1.57
	3	PLA1A	0.294	GNB211	0.353	GAPDH	0.3	B2M	0.33	RPS2	3.66
	4	TUBB4B	0.325	PLA1A	0.398	PLA1A	0.32	GAPDH	0.3	PLA	4.36
	5	GAPDH	0.35	TUBB4B	0.399	RPS2	0.32	RPS2	0.32	TUBB4B	4.6
	6	RPS2	0.381	GAPDH	0.4	B2M	0.33	PLA1A	0.32	GAPDH	4.61
	7	TBP	0.429	B2M	0.587	TUBB4B	0.37	TUBB4B	0.37	B2M	6.96
	8	B2M	0.463	CTFS	0.588	RPLP0	0.51	RPLP0	0.51	TBP	8.18
	9	CTFS	0.506	TBP	0.593	CTFS	0.54	CTFS	0.54	CTFS	9
	10	RPLP0	0.552	APOA1	0.622	TBP	0.55	TBP	0.55	RPLP0	9.46
	11	APOA1	0.595	RPLP0	0.662	APOA1	0.67	APOA1	0.67	APOA1	11
	12	GUSB	0.658	GUSB	0.799	GUSB	0.78	GUSB	0.78	GUSB	12
	13	TFRC	0.747	TFRC	1.053	TFRC	0.93	TFRC	0.93	TFRC	13
	14	HPRT1	0.871	HPRT1	1.52	HPRT1	1.31	SLC25A1	1.41	HPRT1	14

		15	SLC25A1	1.009	SLC25A1	1.748	SLC25A1	1.41	HPRT1	1.31	SLC25A1	15
		16	GNRHR	1.165	GNRHR	2.145	CGBA	1.67	GNRHR	1.82	CGBA	16
		17	CGBA	1.302	CGBA	2.164	GNRHR	1.82	CGBA	1.67	GNRHR	17
tissues	Stages	Rank	GeNorm		NormFinder		$\Delta Ct$		BestKeeper		Comprehensive Ranking	
			gene	stability	gene	stability	gene	stability	gene	stability	gene	stability
Ovary	Stage II	1	EEF1A	0.694	EEF1A	0.347	EEF1A	0.77	ACTB	0.63	EEF1A	1.19
		2	HPRT1	0.694	HPRT1	0.702	HPRT1	1.01	EEF1A	0.73	HPRT1	1.73
		3	ACTB	0.839	ACTB	0.915	ACTB	1.1	HPRT1	0.81	B2M	2.45
		4	B2M	1.019	B2M	1.07	B2M	1.2	B2M	1.17	ACTB	4
	Stage III	1	ACTB	0.245	ACTB	0.123	ACTB	0.35	HPRT1	0.71	EEF1A	1.41
		2	HPRT1	0.245	HPRT1	0.177	HPRT1	0.4	EEF1A	0.75	B2M	1.73
		3	EEF1A	0.25	EEF1A	0.344	EEF1A	0.44	ACTB	0.87	HPRT1	2.06
		4	B2M	0.473	B2M	0.68	B2M	0.7	B2M	1.22	ACTB	4
	Stage IV	1	EEF1A	0.42	EEF1A	0.073	EEF1A	0.62	B2M	0.64	EEF1A	1.57
		2	HPRT1	0.42	HPRT1	0.334	HPRT1	0.68	HPRT1	0.75	HPRT1	1.73
		3	ACTB	0.561	ACTB	0.615	ACTB	0.79	EEF1A	0.89	B2M	1.86
		4	B2M	0.764	B2M	0.884	B2M	0.97	ACTB	1.11	ACTB	4
	All stages	1	EEF1A	0.604	HPRT1	0.19	HPRT1	0.91	B2M	1.01	EEF1A	1.57
		2	HPRT1	0.604	EEF1A	0.446	EEF1A	0.92	HPRT1	1.62	HPRT1	1.73
		3	ACTB	0.679	ACTB	0.685	ACTB	1.01	ACTB	1.85	B2M	1.86
		4	B2M	1.083	B2M	1.411	B2M	1.49	EEF1A	1.96	ACTB	4