Reference gene validation for quantification of gene expression during ovarian development of turbot (*Scophthalmus maximus*)

Yunhong Gao[a,b] , Yuntao Gao[a,b], Bin Huang[a], Zhen Meng[a], Yudong Jia[a]*

[a] Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences; Qingdao Key Laboratory for Marine Fish Breeding and Biotechnology, Qingdao 266071, China

[b] College of Fisheries and Life Science, Shanghai Ocean University, Shanghai 201306, China

* Corresponding author

Supplementary table 1. The yield and purity of total RNA in hypothalamus, pituitary, liver, and ovary during turbot ovarian development. Prevtg: previtellogenesis; Evtg: early vitellogenesis; Latvtg: late vitellogenesis; Mig-nucle: migratory nucleus; Atre: atresia.

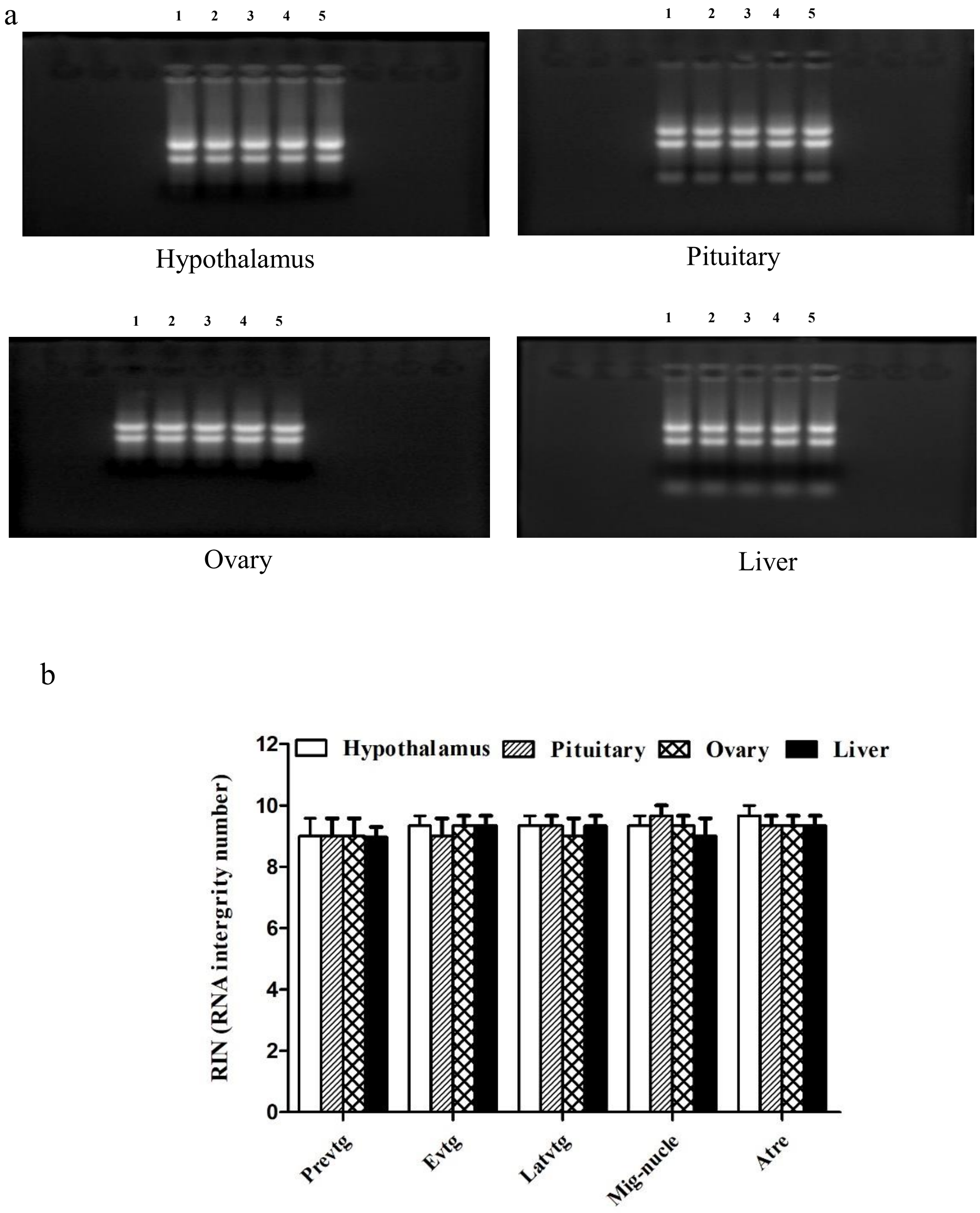
Tissues	Stages	Yield (ng/μl)	Purity (260/280)
Hypothalamus	Prevtg	678.3	1.96
	Evtg	635.1	1.89
	Latvtg	1079.5	1.98
	Mig-nucle	737.5	1.97
	Atre	535.2	1.98
Pituitary	Prevtg	308.9	1.93
	Evtg	322.2	1.85
	Latvtg	273.1	1.95
	Mig-nucle	342.6	1.96
	Atre	362.5	1.97
Ovary	Prevtg	479.4	1.86
	Evtg	515.5	1.92
	Latvtg	466.7	1.98
	Mig-nucle	354.0	1.94
	Atre	547.2	1.88
Liver	Prevtg	681.3	1.99
	Evtg	718.9	2.00
	Latvtg	446.2	1.91

Mig-nucle	492.6	1.96
Atre	822.4	1.97

Supplementary table 2. Standard deviations (SD) and correlation coefficient (*r*) of the *ub* and *rsp4* based on their quantification cycle values analyzed by Bestkeeper. The standard deviation (SD) > 0.95 and discarded from the calculation of correlation coefficient.

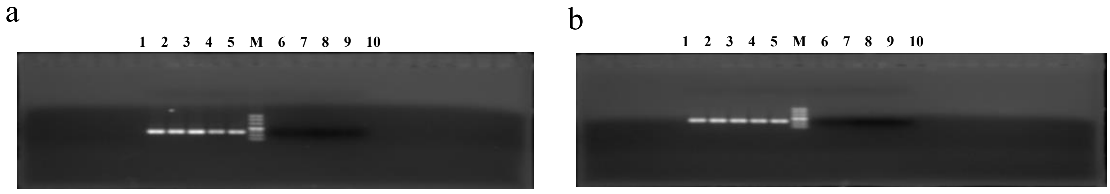
Tissue	Factor	UB	RP4
Hypothalamus	SD	2.14	1.61
	r	-	-
Pituitary	SD	1.45	1.53
	r	-	-
Ovary	SD	1.49	1.77
	r	-	--
Liver	SD	1.40	1.87
	r	-	-

Supplementary Figure 1.



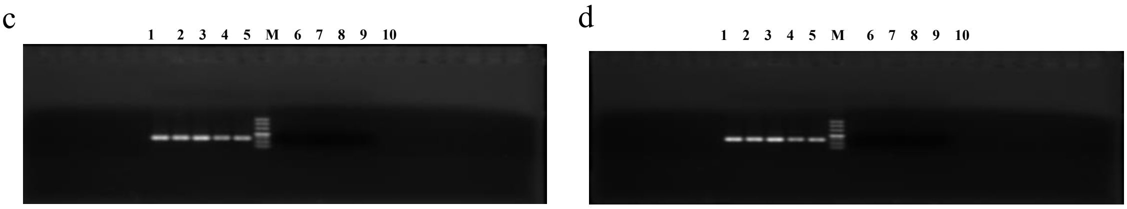
Supplementary Figure 1: The integrity of total RNA (a) agarose gel electrophoresis (b) RIN number in hypothalamus, pituitary, liver, and ovary during turbot ovarian development. RIN: RNA integrity number. Lanes from 1 to 5 are previtellogenesis, early vitellogenesis, late vitellogenesis, migratory nucleus and atresia stages, respectively. Other lanes were not used.

Supplementary Figure 2



Hypothalamus

Pituitary

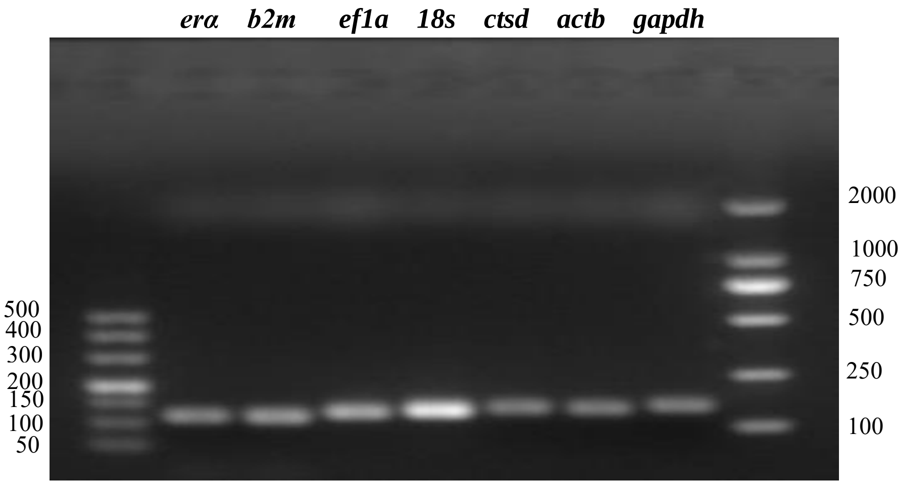


Liver

Ovary

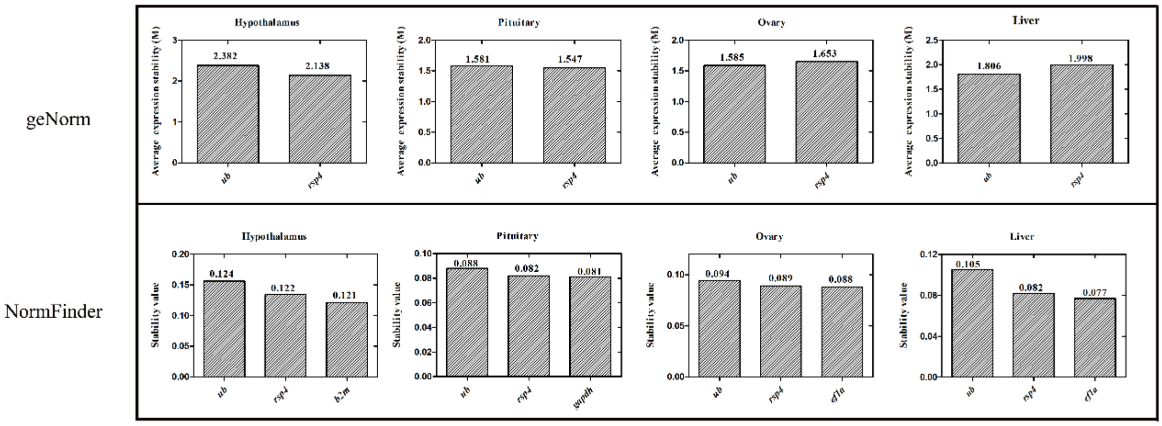
Supplementary Figure 2: The negative control without cDNA included in (a) hypothalamus, (b) pituitary, (c) liver, and (d) ovary during turbot ovarian development via qRT-PCR. 2% typical agarose gel stained with ethidium bromide showing the amplification of 18s. Left lanes from 1 to 5 are previtellogenesis, early vitellogenesis, late vitellogenesis, migratory nucleus and atresia stages, respectively. Right lanes from 6 to 10 are negative control. M: mark (50, 100, 150, 200, 300, 400, 500bp). Other lanes were not used.

Supplementary Figure 3



Supplementary Figure 3: 2% typical agarose gel stained with ethidium bromide showing the amplification of reference genes. The product size of *erα* (115bp), *b2m* (112bp), *ef1α* (123bp), *18s* (130bp), *ctsd* (137bp), *actb* (138bp) and *gapdh* (144bp) on gel via agarose gel electrophoresis.

Supplementary Figure 4



Supplementary Figure 4: Expression stability of *ub* and *rsp4* analyzed by geNorm and NormFinder. The M value of *ub* and *rp4* above 1.5, while exhibited the high stability value (the value negatively correlated to gene stability). Meanwhile, standard deviation (SD) > 0.95 in **Supplementary table 2** data. Thus, these two genes considered the unstable genes and not include in the current study.