Selection of Reference Genes for Quantitative RT-PCR Studies in Blood of Beluga Whales (Delphinapterus leucas) and Bottlenose Dolphins (Tursiops truncatus) Chen, I-Hua¹; Li, Tsung-Hsien²; Zhan, Molly³; Chang, Wen-Been²; Jen, I-Fan⁴; Wang, Jiann-Hsiung¹; Chou, Shih-Jen¹; Wu, Yeong-Huey⁵; Yang, Wei-Cheng¹



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Introduction

• Quantitative RT-PCR is often used for research in gene expression, and it • The purpose of this study is to determine stably-expressed HKGs in blood, is vital to choose appropriate housekeeping genes (HKGs) as reference which can be the appropriate reference genes in relative quantification in gene expression research. It may have a contribution to preventive medicine genes to obtain correct result. • Blood is the most common sample and easy to get from captive cetaceans. and early diagnosis in captive cetaceans through mRNA relative • To date, however, no clear direction has emerged to choose appropriate quantification.

housekeeping gene to serve as reference genes using cetaceans blood.

EDTAanticoagulated blood preserved in **RNAlater**[®] (Ambion).



Farglory of Marine Biology **Captive place** Ocean Park and Aquarium (FOP) (NMMBA)

Samples were taken from November 2011 to May 2013.

Comprehensive gene stability

Materials and Methods



Total RNA was extracted using Ribo-Pure[™]-Blood kit (Ambion) following the manufacturer's instructions. **RNA concentration was adjusted to 100** µg/mL following by cDNA synthesis using **QuantiTect® Reverse Transcription kit** (Qiagen).

Sequences of 15 candidate HKGs (ACTB, B2M, GAPDH, HPRT1, LDHB, LDHA, SDHA, PGK1, RPL4, RPL8, RPL18, RPS9, RPS18, TFRC, YWHAZ) of cetaceans were obtained from GenBank. Primers and corresponding probes from Roche Universal ProbeLibrary (UPL) of the genes mentioned above were designed using Roche UPL design software (ProbeFinder, v.2.49).

Unive	ersal ProbeLi	ibrary for	Other orga	anism	1	Roche Applied Sci							
Probe	Finder has desi	gned the o	ptimal real-t	ime P	CR assay	for:							
gene 1	1												
Assay	details:												
	Use Universal ProbeLibrary probe: #65, cat.no. 04688643001												
	Primer Length Position Tm %GC Sequence												
	Left Primer	25	97 - 121	59	40	ttgaaacatcttcagtgtctagcag							
	Right Primer	21	146 - 166	59	38	tgctttgagcaacatttagca							
	Amplicon (7	70 nt)											

 ${\tt ttgaaacatcttcagtgtctagcagaagaactcaaacctctggaggatgtgctaaatgtt}$

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The Cq values of the candidate genes of each sample were obtained by real-time PCR, and the stability values of the HKGs were determined by 4 software: delta CT method, Bestkeeper, geNorm and NormFinder via **RefFinder website.**



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	Hi	gh ex	kpres	sion	, low	Cqv	value				

Comprehensive gene stability

Least stable genes ==>

ACTB B2M GAPDH HPRT1 LDHB PGK1 RPL4 RPL8 RPL18 RPS18 RPS9

Cq value of 13 candidate HKGs in 60 beluga whales. **Points represent** mean Cq value and whiskers represent

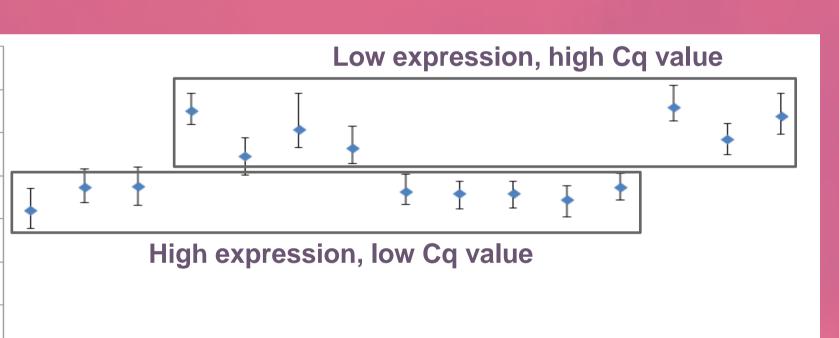
Results

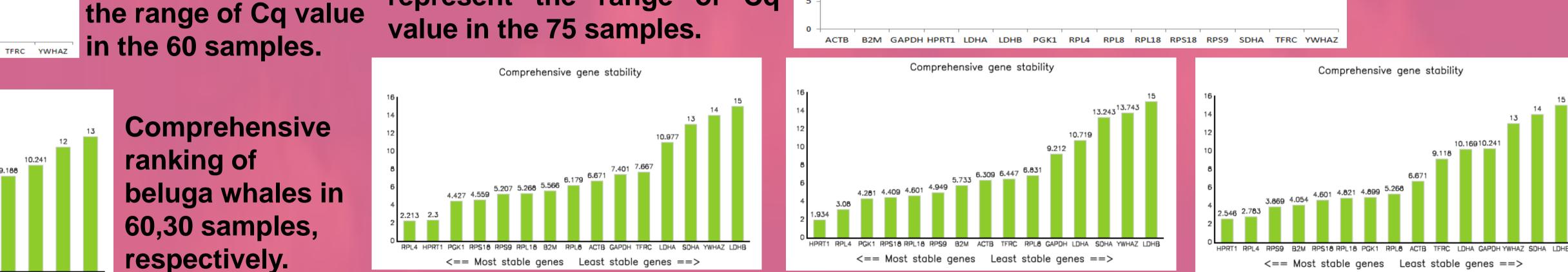
geteaaagea

Cq value of candidate 15 HKGs 75 bottlenose in dolphins. Points represent mean Cq value and whiskers represent the range of Cq

Least stable genes ==>

<== Most stable genes





Comprehensive ranking of bottlenose dolphins in 75, 55, 35 samples, respectively.

Results of 4 different softwares obtained from RefFinder in 60 beluga whales.

	60 samples in beluga whales ranking order (BetterGoodAverage)														
Method	1	2	3	4	5	6	7	8	9	10	11	12	13		
Delta CT	RPL4	PGK1	ACTB	B2M	RPL18	RPS18	HPRT1	RPS9	RPL8	GAPDH	LDHB	TFRC	YWHAZ		
BestKeeper	RPL8	RPS18	B2M	ACTB	RPL18	RPL4	LDHB	PGK1	HPRT1	RPS9	GAPDH	TFRC	YWHAZ		
Normfinder	RPL4	PGK1	ACTB	B2M	HPRT1	RPL18	RPS9	RPS18	GAPDH	RPL8	LDHB	TFRC	YWHAZ		
Genorm	ACTB B2M		RPL4	PGK1	RPS18	RPL8	RPL18	RPS9	HPRT1	GAPDH	LDHB	TFRC	YWHAZ		
Recommended comprehensive ranking	RPL4	АСТВ	B2M	PGK1	RPS18	RPL8	RPL18	HPRT1	RPS9	LDHB	GAPDH	TFRC	YWHAZ		

75 samples in bottlenose dolphin ranking order (BetterGoodAverage)															
Method	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Delta CT	HPRT1	PGK1	RPL4	ACTB	GAPDH	RPS18	RPS9	B2M	RPL8	LDHA	RPL18	TFRC	SDHA	YWHAZ	LDHB
BestKeeper	RPL4	TFRC	RPS9	HPRT1	B2M	RPL8	RPL18	PGK1	RPS18	GAPDH	ACTB	LDHA	SDHA	YWHAZ	LDHB
Normfinder	HPRT1	RPL4	PGK1	B2M	ACTB	GAPDH	RPS9	RPS18	RPL8	RPL18	LDHA	TFRC	SDHA	YWHAZ	LDHB
Genorm	RPS18 RPL18		RPL8	RPL4	RPS9	B2M	HPRT1	PGK1	ACTB	GAPDH	LDHA	TFRC	SDHA	YWHAZ	LDHB
Recommended															
comprehensive	RPL4	HPRT1	PGK1	RPS18	RPS9	RPL18	B2M	RPL8	ACTB	GAPDH	TFRC	LDHA	SDHA	YWHAZ	LDHB
ranking															



Most stable genes

Least stable genes

Beluga whales

RPL4, ACTB, B2M, PGK1 GAPDH, TFRC, YWHAZ

Acknowledgement

A special thanks to the staff at Farglory Ocean Park and National Museum of Marine Biology and Aquarium for all their support.

Results of 4 different softwares obtained from RefFinder in 75 bottlenose dolphins.



RPL4, HPRT1, RPS18, PGK1 SDHA, YWHAZ, LDHB

•This is the first reference gene research of in cetaceans blood. •According to the results, it may indicate that about 30 samples is enough for reference gene selection research.



• Spinsanti G, Panti C, Lazzeri E, Marsili L, Casini S, Frati F, Fossi CM. 2006. Selection of reference genes for quantitative RT-PCR studies in striped dolphin (Stenella coeruleoalba) skin biopsies. BMC Mol Biol 7: 32. • Lee CS, Sitt T, Bowen L, Blanchard MT, Smith BR, Stott JL. 2009. Transcriptional gene signatures in *Tursiops truncatus*: an aid in differential diagnostics? IAAAM 40th Annual **Conference Proceedings, San Antonio, Texas; Pp. 51-52.** RefFinder: http://www.leonxie.com/referencegene.php