#### Selection of Reference Genes for Quantitative RT-PCR Studies in Blood of Beluga Whales (Delphinapterus leucas) I-Hua Chen,<sup>1</sup>\* Tsung-Hsien Li,<sup>2</sup> Molly Zhan,<sup>3</sup> Wen-Been Chang,<sup>2</sup> Jiann-Hsiung Wang,<sup>1</sup> Shih-Jen Chou,<sup>1</sup> Yeong-Huey Wu<sup>4</sup> and Wei-Cheng Yang<sup>1</sup>

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• Quantitative RT-PCR is often used for research in gene expression, and it is vital to choose appropriate housekeeping genes (HKGs) as reference genes to obtain correct result. • Blood is the most common sample and easy to get

30 samples Ranking Order (BetterGoodAverage)													
	1	2	3	4	5	6	7	8	9	10	11	12	13
Delta CT	ACTB	RPL4	PGK1	RPL18	B2M	RPS18	S9	HPRT1	RPL8	GAPDH	LDHB	TFRC	YWHAZ
BestKeeper	RPL8	RPS18	RPL18	RPL4	ACTB	B2M	PGK1	S9	LDHB	TFRC	HPRT1	GAPDH	YWHAZ
Normfinder	ACTB	RPL4	PGK1	B2M	RPL18	HPRT1	S9	RPS18	RPL8	GAPDH	LDHB	TFRC	YWHAZ
Genorm	ACTB /B2M		RPL4	PGK1	RPS18	RPL8	RPL18	S9	HPRT1	GAPDH	LDHB	TFRC	YWHAZ
Recommended comprehensive ranking	ACTB	RPL4	B2M	PGK1	RPL18	RPS18	RPL8	S9	HPRT1	LDHB	GAPDH	TFRC	YWHAZ

60 samples Ranking Order (Better--Good--Average

from captive cetaceans.

• To date, however, no clear direction has emerged to choose appropriate housekeeping gene to serve as reference genes using cetaceans blood.

• The purpose of this study is to determine stablyexpressed HKGs in blood, which can be the appropriate reference genes in relative quantification in gene

expression research. It may have a contribution to preventive medicine and early diagnosis in captive cetaceans through mRNA relative quantification.

## Introduction

# Materials

	1	2	3	4	5	6	1	8	9	10	11	12	13
Delta CT	RPL4	ACTB	PGK1	B2M	RPL18	RPS18	RPL8	S9	HPRT1	GAPDH	LDHB	TFRC	YWHAZ
BestKeeper	RPL8	RPS18	B2M	RPL18	RPL4	ACTB	LDHB	PGK1	TFRC	S9	HPRT1	GAPDH	YWHAZ
Normfinder	RPL4	ACTB	PGK1	B2M	RPL18	RPS18	HPRT1	S9	RPL8	GAPDH	LDHB	TFRC	YWHAZ
Genorm	B2M / RPL4		ACTB	PGK1	RPS18	RPL8	RPL18	S9	HPRT1	GAPDH	LDHB	TFRC	YWHAZ
Recommended comprehensive ranking	RPL4	B2M	ACTB	PGK1	RPS18	RPL8	RPL18	S9	HPRT1	LDHB	GAPDH	TFRC	YWHAZ

It showed that RPL4, ACTB, B2M and PGK1 are the most stable HKGs in beluga whale blood while GAPDH, TFRC, and YWHAZ are the most unstable genes.

> Furthermore, the result using 30 randomly selected and all 60 samples are essentially identical, indicating that only 30 samples is enough for reference gene selection research.

> > Results

### Conclusion

 Blood can serve as an 200 indication of health status in cetaceans because changes Methods of gene expression in blood is prior to hematology and chemistry findings. • Sixty EDTA-anticoagulated blood samples were taken

This research provides recommendation Of from 4 beluga whales (Delphinapterus leucas) in reference gene selection, which may contribute to National Museum of Marine Biology and Aquarium from further mRNA relative quantification research in the November 2011 to May 2013, and were preserved in peripheral blood leukocytes in beluga whales. **RNAlater<sup>®</sup> (Ambion).** • We are going to design appropriate primers and Total RNA was extracted using Ribo-Pure<sup>™</sup>-Blood kit probes for target genes, using relative quantification to (Ambion) following the manufacturer's instructions. study gene expression in beluga whales. RNA concentration was adjusted to 100 µg/mL

**Reverse Transcription kit (Qiagen).** 

following by cDNA synthesis using QuantiTect<sup>®</sup> A special thanks to the staff at National Museum of Marine Biology and Aquarium for all their support.

 Sequences of 13 candidate HKGs (ACTB, B2M, GAPDH, HPRT1, LDHB, 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). • 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.

#### References

**Acknowledgement** 

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