

Effect of PCR amplification efficiency on reference gene selection in beluga blood
(*Delphinapterus leucas*)

I-Hua Chen¹, Jiann-Hsiung Wang¹, Shih Jen Chou¹, Yeong-Huey Wu², Tsung-Hsien Li³, Ming-Yih Leu³, Wen-Been Chang³, Wei-Cheng Yang^{1*}

¹Department of Veterinary Medicine, National Chiayi University, Chiayi, 600, Taiwan, ROC.

²Department of Veterinary Medicine, National Pingtung University of Science and Technology, Pingtung, 912, Taiwan, ROC.

³National Museum of Marine Biology and Aquarium, Pingtung, 944, Taiwan, ROC.

* Corresponding author: jackywc@gmail.com

PCR Efficiency evaluation is an essential marker in gene quantification procedure. The correction has been suggested in experimental designs employing standardization with housekeeping genes. Here we showed the effect of efficiency on reference gene selection in beluga blood. Sixty blood samples were taken monthly from 4 beluga whales in National Museum of Marine Biology and Aquarium and preserved in *RNAlater* immediately. Total RNA was extracted then following by cDNA synthesis and qPCR for 13 candidate HKGs. The stability value of the HKGs were determined by four different algorithms: geNorm, NormFinder, BestKeeper and comparative delta CT method. The results showed that RPL4, PGK1 and B2M are the most stable HKGs in beluga whale blood when the efficiency values of all candidate genes were 95-101%. However, the most stable HKGs changed into ACTB, PGK1, B2M if the efficiency ranges from 90-110%. It revealed that the correction for efficiency, as performed in efficiency corrected mathematically models, is strongly recommended and results in a more reliable estimation of reference gene selection compared to no-efficiency correction data.