

## 1. Multiple sequence alignment (CLUSTALW) used to design the pair of primer for the three selected target genes.

### CLUSTALW used to design the pair of primers for *P53* (underlined sequences)

Reference numbers of the NCBI sequences used:

- P53-Bos: *Bos taurus* tumor protein p53 (TP53), mRNA [NCBI Reference Sequence: NM\_174201.2]
- P53-Delphinapterus: *Delphinapterus leucas* P53 (p53) mRNA, complete cds [GenBank: AF475081.1]
- P53-Sus: *Sus scrofa* tumor suppressor p53 (p53) mRNA, complete cds [GenBank: AF098067.1]
- P53-Homo: *Homo sapiens* tumor protein p53 (TP53), transcript variant 7, mRNA [NCBI Reference Sequence: NM\_001126117.1]

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P53-Bos          TAACAGCTCCTGCATGGGGGGCATGAACCGGGCGGCCATCCTCACCATCATCACACTGGA 893
P53-Delphinapterus TAACAGCTCCTGCATGGGAGGCATGAACCGGGCGGCCATCCTCACCATCATCACACTGGA 755
P53-Sus          CAACAGCTCCTGCATGGGGGGCATGAACCGGGCGGCCATCCTCACCATCATCACACTGGA 754
P53-Homo          TAACAGTTCTGCATGGGGCGCATGAACCGGAGGCCATCCTCACCATCATCACACTGGA 655
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P53-Bos          AGACTCTTGTGGTAACTGCTGGGACGGAAACAGCTTTGAGGTGCGTGTGTTGTGCCGTGCC 953
P53-Delphinapterus AGACTCCAATGGTAATCTGCTGGGACGGAAACAGCTTTGAGGTGCGTGTGTTGTGCCGTGCC 815
P53-Sus          AGACGCCAGTGGCAACTTGTGCTGGGACGGAAACAGCTTTGAGGTGCGTGTGTTGTGCCGTGCC 814
P53-Homo          AGACTCCAGTGGTAATCTACTGGGACGGAAACAGCTTTGAGGTGCGTGTGTTGTGCCGTGCC 715
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P53-Bos          TGGGAGAGACCGCCGCACTGAGGAAGAAAATCTTCGCAAGAAGGGGCAGTCTTGCCCCGA 1013
P53-Delphinapterus TGGGAGAGACCGCCGCACAGAGGAAGAAAATTTCCACAAGAAGGGGCAGTCTTGCCCTGA 875
P53-Sus          TGGGAGAGACCGCCGCACTGAGGAAGAAAATTTCTCAAGAAGGGGCAGTCTTGCCCCGA 874
P53-Homo          TGGGAGAGACCGCCGCACAGAGGAAGAAAATCTTCGCAAGAAGGGGAGCCTCACCACGA 775
*****

P53-Bos          GCCACCCCCTAGGAGCACTAAGCGAGCACTGCCTACCAACACCAGCTCCTCTCCACAGCC 1073
P53-Delphinapterus GCTGCCCACTGGGAGCGCTAAGCGAGCACTGCCTACCGGCACCAGCTCCTCTCCACCGCA 935
P53-Sus          GCCGCCCCCTGGCAGCACTAAGCGAGCACTGCCCCACCAGCACCAGCTCCTCGCCAGTGCA 934
P53-Homo          GCTGCCCCAGGGAGCACTAAGCGAGCACTGCCCAACAACACCAGCTCCTCTCCACAGCC 835
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### CLUSTALW used to design the pair of primers for *KIN* (underlined sequences)

Reference numbers of the NCBI sequences used:

- KIN-Pan: PREDICTED: *Pan troglodytes* KIN, antigenic determinant of recA protein homolog (mouse), transcript variant 1 (KIN), mRNA [NCBI Reference Sequence: XM\_001148768.2]
- KIN-Equus: PREDICTED: *Equus caballus* KIN, antigenic determinant of recA protein homolog (mouse) (KIN), mRNA [NCBI Reference Sequence: XM\_001916725.2]
- KIN-Mus: *Mus musculus* antigenic determinant of rec-A protein (Kin), mRNA [NCBI Reference Sequence: NM\_025280.2]

- KIN-Homo: *Homo sapiens* cDNA FLJ58083 complete cds, highly similar to Homo sapiens KIN, antigenic determinant of recA protein homolog, mRNA [GenBank: AK301789.1]
- KIN-Bos: *Bos taurus* KIN, antigenic determinant of recA protein homolog (mouse), mRNA (cDNA clone MGC:159891 IMAGE:8492887), complete cds [GenBank: BC146082.1]

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KIN-Pan          TCATCAGAGACAACCTATTGCTGGCTTCAGAAAATCCTCAGCAGTTTATGGATTATTTTTC 257
KIN-Equus       TCATCAGAGACAACCTATTGCTGGCTTCAGAAAATCCTCAGCAGTTTATGGATTATTTTTC 206
KIN-Mus        TCATCAAAGACAACCTGTTGCTGGCTTCAGAAAACCTCAGCAGTTTATGGATTATTTTTC 225
KIN-Homo       TCATCAGAGACAACCTATTGCTGGCTTCAGAAAATCCTCAGCAGTTTATGGATTATTTTTC 300
KIN-Bos        TCATCAGAGACAGCTGTTGCTGGCTTCAGAAAATCCTCAGCAGTTTATGGATTATTTTTC 247
*****  *****  **  *****  *****  *****  *****  *****  *****
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KIN-Pan          AGAGGAATTCGAAATGACTTTCTAGAACTTCTCAGGAGACGCTTTGGCACTAAAAGGGT 317
KIN-Equus       AGAGGAATTCGAAATGACTTCTGGAACTTCTCAGGAGCGCTTTGGCACTAAGAGAGT 266
KIN-Mus        AGAGGAATTCGAAATGACTTTCTGGAACTTCTGAGGCGACGCTTTGGCACTAAAAGGGT 285
KIN-Homo       AGAGGAATTCGAAATGACTTTCTAGAACTTCTCAGGAGACGCTTTGGCACTAAAAGGGT 360
KIN-Bos        AGAGGAATTCGAAATGACTTCTAGAACTTCTCAGGAGACGCTTTGGCACCAAGAGGGT 307
*****  *****  **  *****  ***  *  *****  **  **  **
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### HSP70 primers design

For HSP70, we designed the pair of primers directly using a sequence of *HSP70* from a right whale (see reference below).

The CLUSTALW below shows where the primers (underlined sequences) were designed on the right whale sequence. It also shows the alignment of our sequence (HSP70-Balaenoptera) with the right whale sequence and the sequence of a dolphin (see reference numbers below).

Reference numbers of the NCBI sequences used:

- HSP70-Eubalaena: est\_e\_glacialis2590 RightWhaleSkin *Eubalaena glacialis* cDNA, mRNA sequence [GenBank: ES556841.1]
- Tursiops: *Tursiops truncatus* Contig167074, whole genome shotgun sequence [GenBank: ABRN02157953.1]
- HSP70-Balaenoptera: *Balaenoptera musculus* Heat shock protein 70 (*HSP70*) mRNA, partial cds (sequence obtained for this study)

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HSP70-Eubalaena      CCAAACCTTGCGGCCGATCAGCCGCTTCGCGTCAAGCACGGTGTCTGTGGGTTTCAGCGCC 491
HSP70-Balaenoptera  -----GTC AAGCACGGTGTCTGTGGGTTTCAGCGCC 31
Tursiops             CCAAACCTTGCGGCCGATCAGCCGCTTCGCGTCAAGCACGGTGTCTGTGGGTTTCAGCGCC 4980
                    *** * *****

HSP70-Eubalaena      ACCTGGTTCTTGCCCGCGTCGCCGATGAGCCGCTCGGTGTCCGTGAAGGCCACGTAGCTG 551
HSP70-Balaenoptera  ACCTGGTTCTTGCCCGCGTCGCCGATGAGCCGCTCGGTGTCCGTGAAGGCCACGTAGCTG 91
Tursiops             ACCTGGTTCTTGCCCGCGTCGCCGATGAGCCGCTCGGTGTCCGTGAAGGCCACGTAGCTG 5040
                    *****

HSP70-Eubalaena      GGGGTGGTGCGGTTGCCCTGATCGTTGGCGATGATCTCTACTTTGCCGTGCCGTAACAC 611
HSP70-Balaenoptera  GGGGTGGTGCGGTTGCCCTGATCGTTGGCGATGATCTCTACTTTGCCGTG----- 141
Tursiops             GGGGTGGTGCGGTTGCCCTGATCGTTGGCGATGATCTCTACTTTGCCGTGC-TGGAACAC 5099
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## 2. Gene sequences included in the analyses

The following sequences correspond to the section of the genes used in the qPCR analyses. The primers are underlined.

>Seq1 [organism=*Balaenoptera physalus*] Tumour protein 53 (*P53*) mRNA, partial cds for Seq1

CTCACCATCATCACACTGGAAGACTCCAGTGGTAATCTGCTGGGACGGAACAGCT  
TTGAGGTGCGTGTTTGTGCCTGTCCTGGGAGAGACCGCCGCACAGAGGAAGAAA  
ATTTCCGCAAGAAGGGGCAGTCTGGCCCTGAGCCGCCTCCTGGGAGCGCTAAGCG  
AGCACTGCCTA

>Seq2 [organism=*Balaenoptera musculus*] Heat shock protein 70 (*HSP70*) mRNA, partial cds for Seq2

GTCAAGCACGGTGTCTGTGGGTTTCAGCGCCACCTGGTTCTTGGCCGCGTCGCCG  
ATGAGCCGCTCGGTGTCCGTGAAGGCCACGTAGCTGGGGGTGGTGC GGTTGCCCT  
GATCGTTGGCGATGATCTCTACTTTGCCGTG

>Seq3 [organism=*Balaenoptera physalus*] (*KIN17*) mRNA, partial cds for Seq3

TGCTGGCTTCAGAAAATCCTCAGCAGTTTATGGATTATTTTCAGAGGAATCCGAA  
ATGACTTTCTAGA ACTTCTCAGGAGACGCTTTGGAACCAAGAG

>Seq4 [organism=*Balaenoptera physalus*] Ribosomal protein S18 (*RPS18*) mRNA, partial cds for Seq4

CAATTAAGGGTGTGGGGCGAAGATATGCTCATGTGGTGTGAGGAAAGCAGACA  
TCGACCTACCAAGAGGGCAGGAGAGCTCACTGAGGATGAGGTGGAACGTGTGA  
TCACCATTATGCAGAATCCACGCCAATAACAAGA

>Seq5 [organism=*Balaenoptera physalus*] Ribosomal protein L4 (*RPL4*) mRNA, partial cds for Seq5

CAGACCTTAGCAGAATCTTGAAAAGCCCAGAGATCCAAAGAGCCCTCCGAGCAC  
CACGCAAGAAGATTCATCGCAGAGTCTGAAGAAGAATCCACTGAAAAACCTGA  
GAATCATGTTGAAGCTAAACCCATATGCAAAGACCATGCGCCGGAACACCATTCT  
TCGCCAGG

>Seq6 [organism=*Balaenoptera musculus*] Succinate dehydrogenase (*SDHA*) mRNA, partial cds for Seq6

TGTTTCCCACCAGGTCACACACTGTCGCAGCCCAGGGAGGGATCAACGCCGCCCT  
GGGGAACATGGAGGAGGACA ACTGGAGGTGGCACTTCTACGACACCGTGAAGGG  
CTCCGACTGG

>Seq7 [organism=*Balaenoptera physalus*] Phosphoglycerate kinase 1 (*PGK1*) mRNA, partial cds for Seq7

ACAATGGAGCCAAGTCAGTTGTTCTTATGAGCCACCTGGGCCGGCCTGATGGTGT  
CCCCATGCCTGACAAGTACTCCTTGACAGCCAGTTGCTGTAGA ACTCAAATCTCTG  
CTGGGCAAGGATGTTTTGTTCTTGAAGGACTGCGTG

2. Supplementary Figures

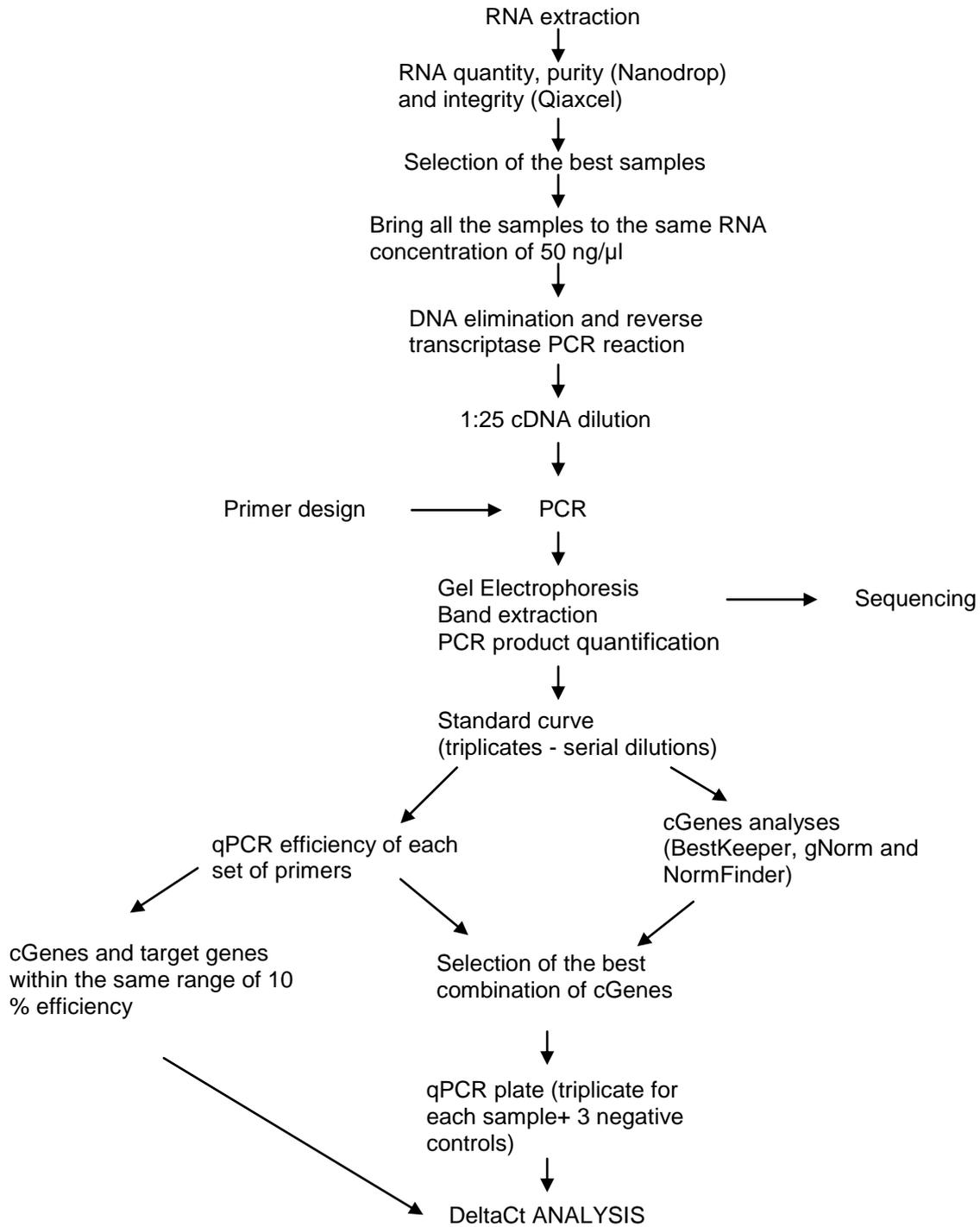
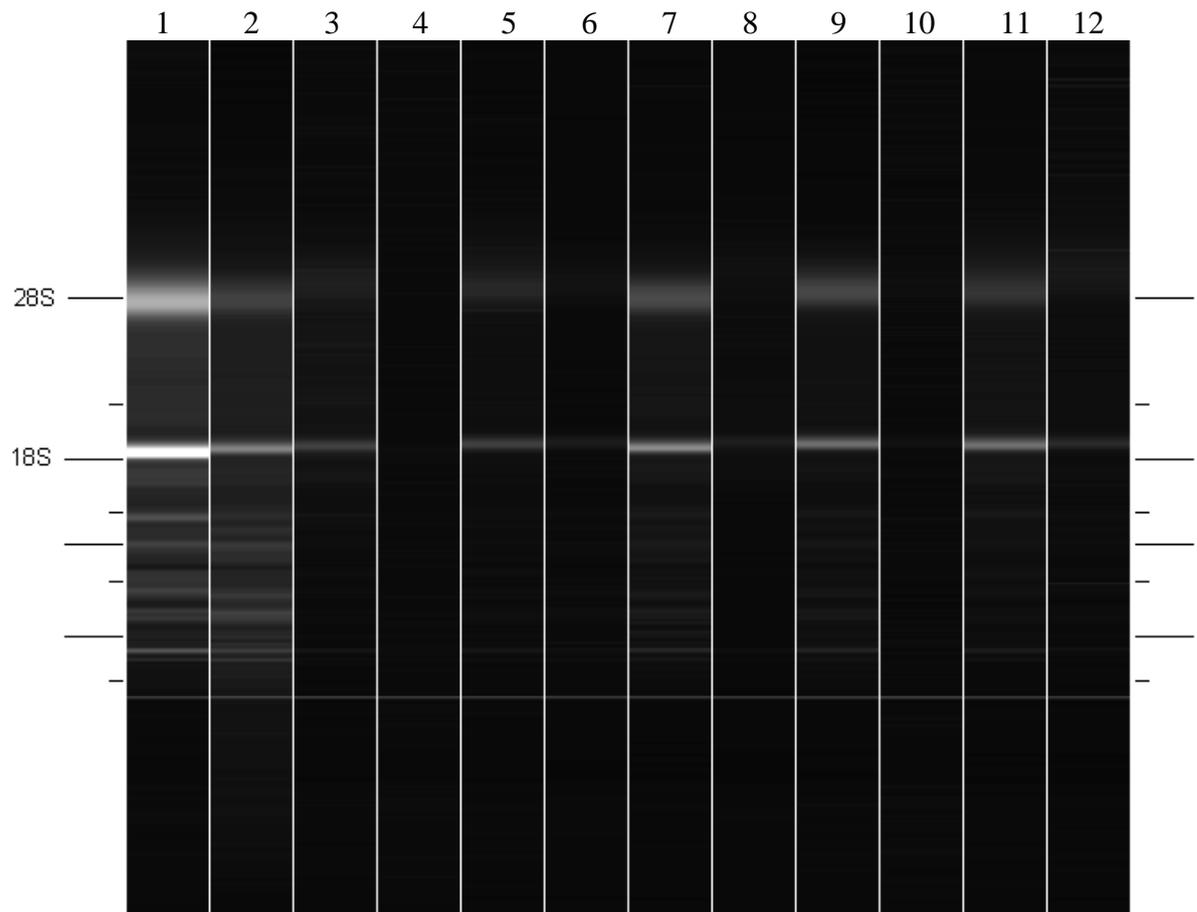
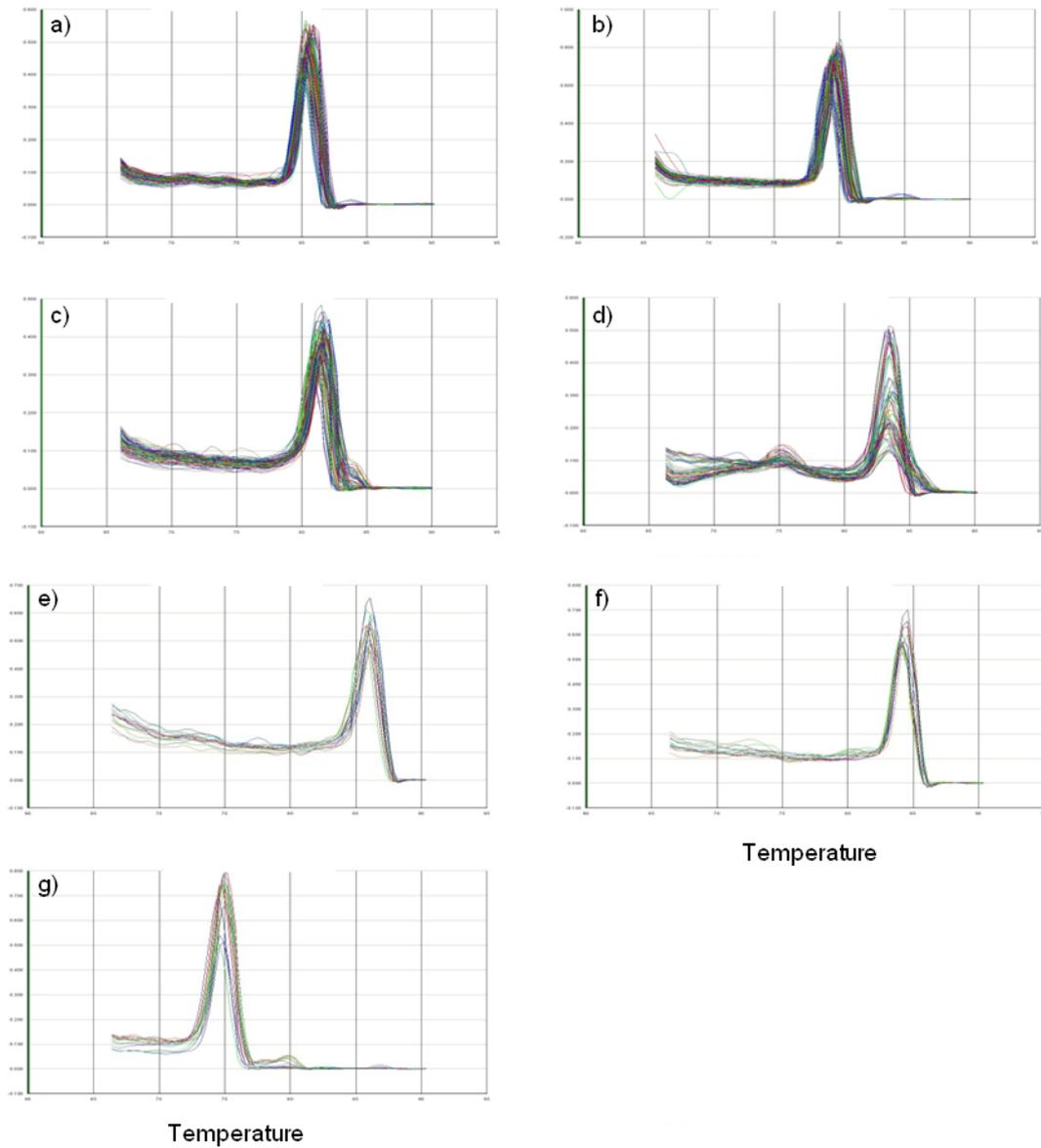


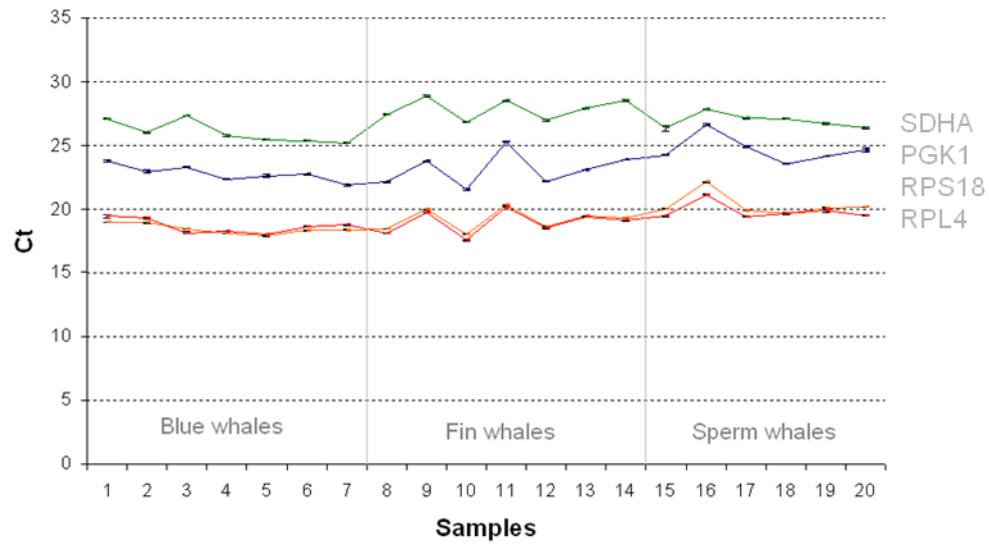
Figure S1. Schematic representation of the gene expression protocol



**Figure S2. RNA integrity using the QIAxcel system.** Intact RNA subunits 28S and 18S were observed (wells 1, 2, 3, 5, 6, 7, 9, 11 and 12) on an automated capillarity-electrophoresis system, the QIAxcel system (Qiagen,UK).



**Figure S3. qPCR dissociation curves of the genes used in the analyses.** Melting curve analyses images obtained with the 7300 Real-Time PCR System (Applied Biosystems) of the genes a) *RPS18*, b) *RPL4*, c) *PGK1*, d) *SDHA*, e) *HSP70*, f) *P53* and g) *KIN* showing unique product amplification. d) mild primer-dimer formation ( $T_m=75^\circ\text{C}$ ) due to the lower level of expression of *SDHA* compared to the other genes.



**Figure S4. Expression levels of the internal control gene candidates.** Mean Ct-values (left axis) for 20 skin samples of blue, fin and sperm whales ( $n = 7, 7$  and  $6$ , respectively).  $RPS18 = 19.25 \pm 0.23$  SE,  $RPL4 = 19.12 \pm 0.19$  SE,  $PGK1 = 23.47 \pm 0.28$  SE,  $SDHA = 26.93 \pm 0.24$  SE. Bars represent  $\pm$  SE.