

1 **ADDITIONAL FILES**

- 2 **Additional File 1.A** Overview of the assays (assay name, gene symbol, gene ID, primer sequences,
- 3 amplicon lengths and annealing temperature).

Assay name	Forward primer sequence (5'-3')	Amplicon length(s)
Gene symbol (NCBI Gene ID)	Reverse primer sequence (5'-3')	Ta
UBC integrity assay	GCACCCTGTCHGACTACAACATCCAGAA	137/365/593/821 bp
UBC (multiple alignment)	ATGGTGTCRCTGGGCTCSACYTC	68°C
UBC bisulfite integrity assay	GAARGAGTTTATTTTGTATTT	105/333/561/789 bp
UBC (multiple alignment)	TCACTAAACTCMACYTCC	54°C
<i>Btau</i> SOD2	RAACACCCCAITTTCCCC	462 bp
SOD2 (ID 104973000)	GGTYGTTTAGTTTTAGTTTGAAAT	60°C
<i>Btau</i> MPO	TGAGTTGTTTAGAGAAGGA	442 bp
MPO (ID 511206)	TAACCCCACTACATAAACA	56°C

- 4
- 5 **Additional File 1.B** Overview of the PCR conditions (PCR mixes and cycling programs).

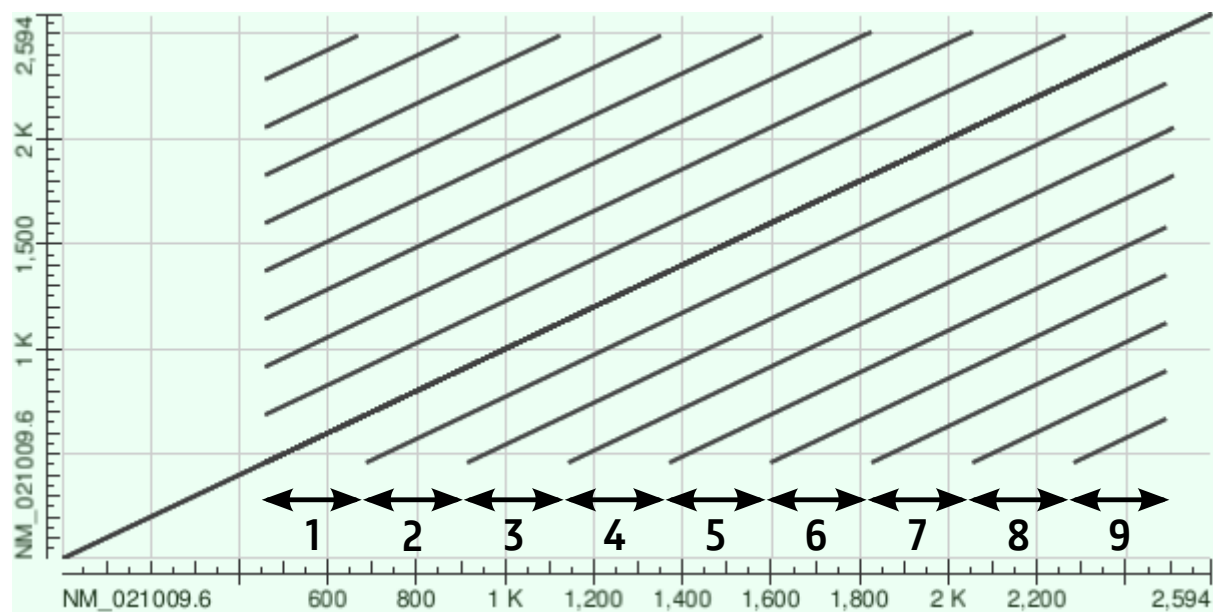
PCR mix (VWR)		Cycling program (VWR)		
5.7 µl	H2O	14'30''	95°C	] x40
1.0 µl	10x Key Buffer	00'30''	95°C	
1.0 µl	10 µM primers (5 µM each primer)	00'30''	Ta	
0.2 µl	40 mM dNTPs (10 mM each nucleotide)	02'00''	72°C	
0.1 µl	5 U/µl TEMPase Hot Start DNA Polymerase	02'00''	72°C	
2.0 µl	Template	Hold	15°C	
10.0 µl	Total volume			

- 6
- 7

8 **Additional File 2.A** Overview of the mammal UBC genes used in the *in silico* analysis (species,
9 Gene ID, GenBank ID) and number of UBC monomer tandem repeats (TR) found by intraspecies
10 pairwise sequence alignment.

Species	UBC Gene ID	UBC sequence	UBC monomer TR
Homo sapiens (<i>Hsap</i> , man)	7316	NM_021009.6	9
Mus musculus (<i>Mmus</i> , mouse)	22190	NM_019639.4	10
Sus scrofa (<i>Sscr</i> , pig)	396966	XM_003483411.3	7
Bos taurus (<i>Btau</i> , cow)	444874	NM_001206307.1	9
Equus caballus (<i>Ecab</i> , horse)	100059289	XM_005612652.2	5
Ovis aries (<i>Oari</i> , sheep)	101115230	XM_012097675.2	6
Canis familiaris (<i>Cfam</i> , dog)	610457	XM_847967.4	5
Felis catus (<i>Fcat</i> , cat)	101099660	XM_011287697.2	7

11
12 **Additional File 2.B** Dot matrix view of intraspecies pairwise sequence alignment of human UBC
13 sequence NM_021009.6, showing that it encodes 9 tandem repeats of the UBC monomer.



15 **Additional File 2.C** Nucleic acid (228 bp) and amino acid (76 aa) sequence of the first monomer of
16 the human UBC gene (NM_021009.6).

1	M	Q	I	F	V	K	T	L	T	G	K	T	I	T	L	E	V	E	P	S
1	ATGCAGATCTTCGTGAAGACTCTGACTGGTAAGACCATCACCCCTCGAGGTTGAGCCCAGT																			
21	D	T	I	E	N	V	K	A	K	I	Q	D	K	E	G	I	P	P	D	Q
61	GACACCATCGAGAATGTCAAGGCAAAGATCCAAGATAAGGAAGGCATCCCTCCTGACCAG																			
41	Q	R	L	I	F	A	G	K	Q	L	E	D	G	R	T	L	S	D	Y	N
121	CAGAGGCTGATCTTTGCTGGAAAACAGCTGGAAGATGGGCGCACCCCTGTCTGACTACAAC																			
61	I	Q	K	E	S	T	L	H	L	V	L	R	L	R	G	G				
181	ATCCAGAAAGAGTCCACCCTGCACCTGGTGCTCCGTCTCAGAGGTGGG																			

Forward primer: ----->

24 **Additional File 4** Human UBC EST profile (NCBI Unigene, consulted on 14-07-2017) [11],
25 showing that UBC is ubiquitously expressed.
26

EST Profile

Hs.520348 - UBC: Ubiquitin C

Breakdown by Body Sites

		Hs.520348
adipose tissue	2020	26 / 12866
adrenal gland	5403	178 / 32940
ascites	2209	88 / 39834
bladder	2511	75 / 29860
blood	3427	419 / 122252
bone	642	46 / 71618
bone marrow	1025	50 / 48737
brain	3990	4360 / 1092688
cervix	2083	101 / 48486
connective tissue	4346	648 / 149072
ear	559	9 / 16100
embryonic tissue	681	145 / 212896
esophagus	5507	111 / 20154
eye	521	109 / 208840
heart	2703	242 / 89524
intestine	2202	511 / 231981
kidney	2784	587 / 210778
larynx	894	21 / 23466
liver	983	202 / 205291
lung	1920	643 / 334815
lymph	338	15 / 44302
lymph node	211	19 / 89748
mammary gland	1501	227 / 151230
mouth	3038	201 / 66150
muscle	846	90 / 106371
nerve	2446	38 / 15535
ovary	1044	106 / 101488
pancreas	679	145 / 213440
parathyroid	194	4 / 20594
pharynx	270	11 / 40725
pituitary gland	484	8 / 16526
placenta	1282	363 / 283019
prostate	1677	318 / 189536
salivary gland	148	3 / 20265
skin	1361	287 / 210759
spleen	9607	513 / 53397
stomach	1881	180 / 95679
testis	3655	1591 / 435204
thymus	6173	492 / 79697
thyroid	1502	70 / 46583
tonsil	176	3 / 17021
trachea	5890	305 / 51780
umbilical cord	1888	26 / 13764
uterus	3795	881 / 232093
vascular	3620	187 / 51649

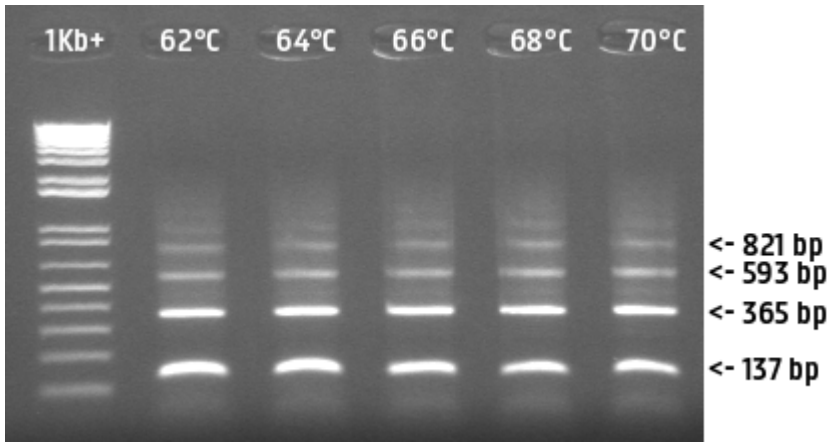
Breakdown by Health State

		Hs.520348
adrenal tumor	948	12 / 12655
bladder carcinoma	796	14 / 17584
breast (mammary gland) tumor	1557	145 / 93090
cervical tumor	2000	69 / 34484
chondrosarcoma	1255	104 / 82838
colorectal tumor	799	90 / 112517
esophageal tumor	5566	96 / 17245
gastrointestinal tumor	1417	168 / 118498
germ cell tumor	4437	1168 / 263230
glioma	2733	293 / 107194
head and neck tumor	724	97 / 133826
kidney tumor	2729	188 / 68872
leukemia	2138	202 / 94479
liver tumor	1635	157 / 96023
lung tumor	749	77 / 102765
lymphoma	664	48 / 72196
non-neoplasia	4346	420 / 96623
normal	2827	9411 / 3328811
ovarian tumor	1115	85 / 76185
pancreatic tumor	895	94 / 105004
primitive neuroectodermal tumor...	3748	476 / 127001
prostate cancer	211	22 / 103844
retinoblastoma	710	33 / 46439
skin tumor	566	71 / 125373
soft tissue/muscle tissue tumor	702	88 / 125265
uterine tumor	1720	155 / 90107

Breakdown by Developmental Stage

		Hs.520348
embryoid body	528	37 / 69969
blastocyst	341	21 / 61448
fetus	1640	914 / 556978
neonate	6469	201 / 31070
infant	2722	64 / 23511
juvenile	6531	363 / 55574
adult	1458	2803 / 1921829

29 **Additional File 5.A** Agarose gel showing amplicons generated with the UBC integrity assay by
30 gradient PCR on 100 ng of high quality porcine DNA.



32 **Additional File 5.B** Agarose gel showing amplicons generated with the UBC bisulfite integrity
33 assay by gradient PCR on 5 ng of bisulfite treated bovine DNA.

