

Supplementary Information

Title: Identification of stable reference genes for quantitative PCR in koalas

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Supplementary Table S1: Demographic information of koalas used in this study

ID	Zone	Sex	Body weight	Tooth wear (Martin)	Body Score (1-5)	Clinical condition (based on pathological examination)	RNA value (A260/A280)	RNA value (A260/A230)
Q1	Queensland	F	5	4	4	Liver mass	2.1	2.21
Q2		F	4.8	4	3	Urogenital Chlamydiosis	2.04	2.12
Q3		M	5.1	4	1.5	Severe pyogranulomatous nephritis; mild choroiditis	2.06	2.14
Q4		M	6.4	4	2.5	Ocular Chlamydiosis	2.09	2.23
Q5		F	5	4	0	Urogenital Chlamydiosis	2.14	2.29
Q6		M	7.7	5	1	Ocular Chlamydiosis	2.11	2.31
Q7		M	6.8	4	3.5	Ocular Chlamydiosis	2.01	2.14
Q8		M	4.9	4	0.5	Multifocal fibrosis	2	2.11
Q9		M	6	4	0	Myocardial fibrosis	2.17	2.23
Q10		M	7.6	4	2	Chronic keratitis	2.13	2.24
Q11		M	7	4	0.5	Pleural fibrosis	2.04	2.17
S1	South Australia	M	7.4	5	3	Osteomyelitis; Oxalate nephrosis	2.11	2.23
S2		M	7	2	3	Osteomyelitis	2.09	2.21
S3		F	4.7	4	2	Oxalate nephrosis	2.05	2.17
S4		M	7.8	2	3	Gastrointestinal perforation	2.14	2.26
S5		M	8	4	3	Ocular Chlamydiosis	2.01	2.14
S6		F	3.6	1	5	Scoliosis and kyphosis, pneumonia	2.11	2.25
S7		M	7.27	2	3	Oxalate nephrosis	2.05	2.11
S8		F	4.46	3	2	Oxalate nephrosis	2.04	2.16

Supplementary Table S2: Descriptive statistics of RT-qPCR CT values of the analysed genes (a) Queensland population (b) South Australia population and (c) Combination of all samples from both populations

(a)

	Candidate Reference Gene												
	ACTB	Smap2	Sec22b	Bet1	Grk2	Hmg20a	Nckap1l	Ndufaf3	Pdap1	Stx12	Chchd2	Tmem97	GAPDH
Minimum	13.67	27.8	27.85	19.36	20.94	21.27	20.48	21.62	22.38	20.91	23.63	23.19	24.2
25% Percentile	13.77	28.29	28.45	20.26	21.52	22.2	20.54	22.62	23.24	21.21	24.15	23.48	24.7
Median	14.12	30.18	28.98	20.69	22.18	22.7	20.73	22.94	23.57	21.6	24.56	23.73	25.34
75% Percentile	14.87	30.95	30.35	21.19	22.58	22.85	21.47	23.32	25.2	22.25	25.24	24.69	25.55
Maximum	15.85	33.25	30.8	21.63	24.08	24.32	23.75	24.76	27.74	23.33	27.27	25.99	26.44
Mean	14.29	29.95	29.26	20.65	22.23	22.69	21.18	23.09	24.17	21.75	24.92	24.12	25.25
Std. Deviation	0.6865	1.668	1.015	0.6311	0.9222	0.8309	0.953	0.882	1.591	0.7312	1.167	0.8813	0.6845
Std. Error of Mean	0.207	0.5029	0.3059	0.1903	0.2781	0.2505	0.2873	0.2659	0.4797	0.2205	0.352	0.2657	0.2064
Lower 95% CI of mean	13.83	28.83	28.57	20.23	21.61	22.14	20.54	22.5	23.1	21.26	24.13	23.52	24.79
Upper 95% CI of mean	14.75	31.07	29.94	21.08	22.85	23.25	21.82	23.69	25.24	22.24	25.7	24.71	25.71
Geometric mean	14.28	29.9	29.24	20.64	22.21	22.68	21.16	23.08	24.12	21.74	24.89	24.1	25.24
Geometric SD factor	1.048	1.057	1.035	1.031	1.042	1.037	1.044	1.039	1.066	1.034	1.047	1.037	1.027

(b)

	Candidate Reference Gene												
	ACTB	Smap2	Sec22b	Bet1	Grk2	Hmg20a	Nckap1l	Ndufaf3	Pdap1	Stx12	Chchd2	Tmem97	GAPDH
Minimum	13.17	28.62	27.54	20.5	20.65	21.43	20.5	22.24	22.55	21.16	24.09	23.02	24.37
25% Percentile	13.86	29.14	28.04	20.86	21.74	22.31	20.59	22.42	23.25	21.46	24.15	23.53	24.83
Median	14.53	31.04	29	21.41	22.74	23.2	22.04	23.44	24.3	21.96	24.87	24.26	25.66
75% Percentile	15.21	31.59	30.6	22.11	23.03	23.55	22.22	24.06	26	23.21	26.23	24.67	26.37
Maximum	15.37	32.82	31.28	22.35	23.89	24.42	23.02	24.62	27.11	24.13	28.22	26.94	26.99
Mean	14.48	30.72	29.24	21.43	22.46	23.01	21.65	23.36	24.53	22.3	25.36	24.38	25.63
Std. Deviation	0.7836	1.449	1.414	0.6827	1.008	0.9183	0.937	0.8611	1.597	1.03	1.45	1.183	0.8844
Std. Error of Mean	0.277	0.5122	0.4999	0.2414	0.3565	0.3247	0.3313	0.3044	0.5646	0.3643	0.5125	0.4181	0.3127
Lower 95% CI of mean	13.83	29.51	28.06	20.85	21.62	22.24	20.87	22.64	23.19	21.44	24.14	23.39	24.89
Upper 95% CI of mean	15.14	31.93	30.42	22	23.31	23.78	22.43	24.08	25.86	23.16	26.57	25.37	26.37
Geometric mean	14.46	30.69	29.21	21.42	22.44	22.99	21.63	23.35	24.48	22.28	25.32	24.36	25.62
Geometric SD factor	1.056	1.049	1.049	1.032	1.046	1.041	1.044	1.038	1.067	1.047	1.057	1.048	1.035

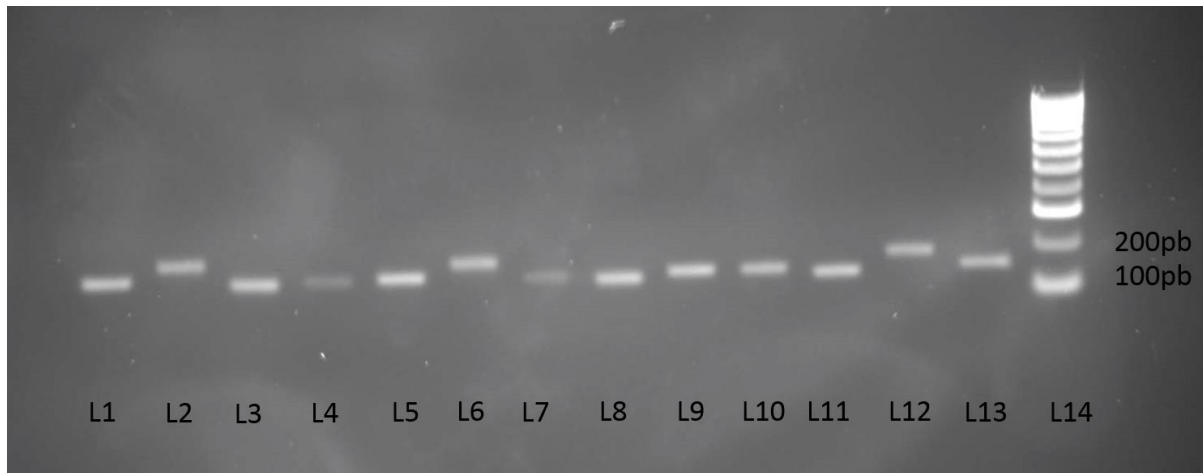
(c)

	Candidate Reference Gene												
	ACTB	Smap2	Sec22b	Bet1	Grk2	Hmg20a	Nckap1l	Ndufaf3	Pdap1	Stx12	Chchd2	Tmem97	GAPDH
Minimum	13.17	27.8	27.54	19.36	20.65	21.27	20.48	21.62	22.38	20.91	23.63	23.02	24.2
25% Percentile	13.77	28.62	28.16	20.5	21.65	22.23	20.54	22.62	23.24	21.37	24.15	23.48	24.75
Median	14.22	30.63	28.98	20.93	22.49	22.77	21.3	23.08	23.81	21.73	24.56	23.87	25.46
75% Percentile	14.99	31.24	30.35	21.63	23.02	23.48	22.07	23.9	25.2	22.61	26.14	24.69	26.11
Maximum	15.85	33.25	31.28	22.35	24.08	24.42	23.75	24.76	27.74	24.13	28.22	26.94	26.99
Mean	14.37	30.27	29.25	20.98	22.33	22.83	21.38	23.21	24.32	21.98	25.1	24.23	25.41
Std. Deviation	0.714	1.587	1.162	0.7457	0.9392	0.8586	0.9502	0.8597	1.559	0.8869	1.274	0.9966	0.7757
Std. Error of Mean	0.1638	0.364	0.2665	0.1711	0.2155	0.197	0.218	0.1972	0.3577	0.2035	0.2924	0.2286	0.178
Lower 95% CI of mean	14.03	29.51	28.69	20.62	21.88	22.41	20.92	22.79	23.57	21.55	24.49	23.75	25.04
Upper 95% CI of mean	14.72	31.04	29.81	21.34	22.78	23.24	21.84	23.62	25.07	22.41	25.72	24.71	25.79
Geometric mean	14.35	30.23	29.23	20.97	22.31	22.81	21.36	23.19	24.27	21.97	25.07	24.21	25.4
Geometric SD factor	1.051	1.054	1.04	1.036	1.043	1.038	1.045	1.038	1.065	1.04	1.051	1.041	1.031

Supplementary Table S3: Overall ranking of candidate reference genes analysed separately in Queensland, South Australia and both populations combined

		Ranking order												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Queensland	Method													
	Delta CT	Tmem97	Stx12	Hmg20a	ACTB	GAPDH	Nckap1l	Ndufaf3	Grk2	Sec22b	Chchd2	Bet1	Smap	Pdap1
	BestKeeper	Bet1	ACTB	Stx12	GAPDH	Hmg20a	Ndufaf3	Nckap1l	Tmem97	Grk2	Sec22b	Chchd2	Pdap1	Smap
	Normfinder	Tmem97	Stx12	ACTB	Nckap1l	Hmg20a	GAPDH	Grk2	Ndufaf3	Sec22b	Chchd2	Bet1	Smap	Pdap1
	Genorm	Hmg20a Ndufaf3		GAPDH	Grk2	Nckap1l	ACTB	Tmem97	Stx12	Sec22b	Chchd2	Bet1	Smap	Pdap1
	Recommended comprehensive ranking	Tmem97	Hmg20a	Stx12	ACTB	Ndufaf3	GAPDH	Nckap1l	Bet1	Grk2	Sec22b	Chchd2	Smap	Pdap1
		Ranking order												
		1	2	3	4	5	6	7	8	9	10	11	12	13
South Australia	Method													
	Delta CT	Nckap1l	Tmem97	Ndufaf3	Hmg20a	Stx12	GAPDH	Grk2	ACTB	Chchd2	Bet1	Smap	Sec22b	Pdap1
	BestKeeper	Bet1	ACTB	Ndufaf3	Hmg20a	GAPDH	Tmem97	Grk2	Nckap1l	Stx12	Smap	Chchd2	Sec22b	Pdap1
	Normfinder	Tmem97	Stx12	Nckap1l	Ndufaf3	ACTB	Hmg20a	GAPDH	Chchd2	Grk2	Bet1	Smap	Sec22b	Pdap1
	Genorm	Grk2 Hmg20a		GAPDH	Ndufaf3	Nckap1l	Stx12	Tmem97	Chchd2	ACTB	Bet1	Smap	Sec22b	Pdap1
	Recommended comprehensive ranking	Tmem97	Hmg20a	Nckap1l	Ndufaf3	Grk2	Stx12	GAPDH	ACTB	Bet1	Chchd2	Smap	Sec22b	Pdap1
		Ranking order												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Combination of Both populations	Method													
	Delta CT	Tmem97	Stx12	Nckap1l	Hmg20a	Ndufaf3	GAPDH	ACTB	Grk2	Chchd2	Bet1	Sec22b	Smap	Pdap1
	BestKeeper	Bet1	ACTB	GAPDH	Hmg20a	Ndufaf3	Stx12	Tmem97	Nckap1l	Grk2	Sec22b	Chchd2	Smap	Pdap1
	Normfinder	Tmem97	Stx12	Nckap1l	ACTB	Hmg20a	Ndufaf3	GAPDH	Grk2	Chchd2	Bet1	Sec22b	Smap	Pdap1
	Genorm	Hmg20a Ndufaf3		GAPDH	Grk2	Nckap1l	Tmem97	Stx12	ACTB	Chchd2	Sec22b	Bet1	Smap	Pdap1
	Recommended comprehensive ranking	Tmem97	Hmg20a	Ndufaf3	Stx12	Nckap1l	GAPDH	ACTB	Bet1	Grk2	Chchd2	Sec22b	Smap	Pdap1

Supplementary Figure S1: Agarose gel electrophoresis view of corresponding reference genes (negative contrast); 100 bp ladder (bioline) used as a DNA marker. L1 = *ACTB*, L2 = *Stx12*, L3 = *Chchd2*, L4 = *Tmem97*, L5 = *Bet1*, L6 = *Ndufaf3*, L7 = *Nckap1l*, L8 = *Grk2*, L9 = *Hmg20a*, L10 = *Pdap1*, L11 = *Smap2*, L12 = *Sec22b*, L13 = *GAPDH* and L14= 100bp ladder



Supplementary Figure S2: Melt-curve profiles of the respective reference genes in Sybr green qPCR.

