

Figure A2. Amplification plots of the zebra finch *Taeniopygia guttata* and red-billed chough *Pyrhcorax pyrrhcorax* *GAPDH* reference gene using eight commercial qPCR master mixes.

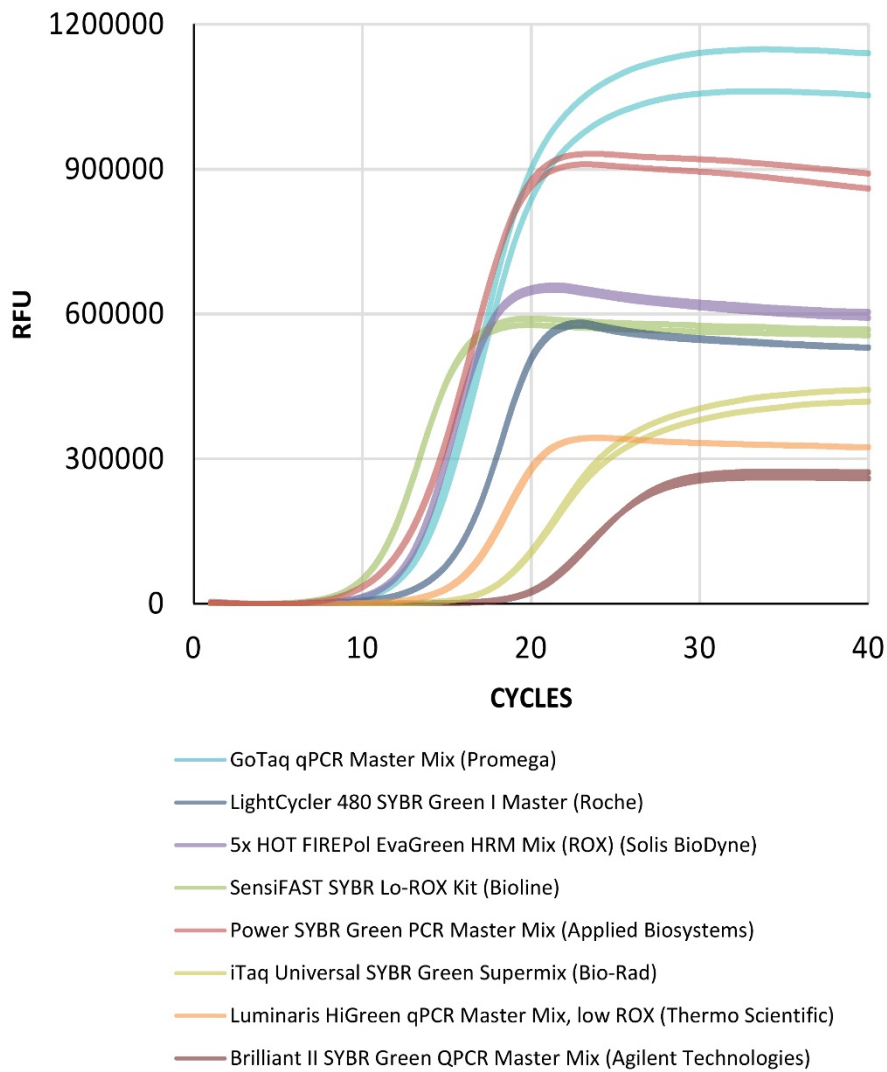


Figure A3. Variability in the amplification of the synthetic telomeric oligo using different qPCR master mixes.

Table A1. Standard curve values and mean amplification Ct of *GAPDH* obtained using eight qPCR master mixes.

Master mix	Zebra finch				Red-billed cought			
	Slope	R <sup>2</sup>	Efficiency (%)	Mean Ct (samples)	Slope	R <sup>2</sup>	Efficiency (%)	Mean Ct (samples)
LightCycler 480 SYBR Green I Master (Roche)	-3.300	0.982	100.906	23.97 ± 0.21	-3.319	0.993	100.127	24.76 ± 0.21
Brilliant II SYBR Green QPCR Master Mix (Agilent Technologies)	-3.256	0.993	102.840	23.70 ± 0.17	-3.318	0.985	100.181	24.68 ± 0.21
iTaq Universal SYBR Green Supermix (Bio-Rad)	-3.289	0.994	101.410	23.20 ± 0.20	-3.269	0.973	102.257	24.23 ± 0.19
GoTaq qPCR Master Mix (Promega)	-3.260	0.996	102.660	25.88 ± 0.33	-3.321	0.991	100.043	26.92 ± 0.24
Luminaris HiGreen qPCR Master Mix, low ROX (Thermo Scientific)	-3.319	0.996	100.122	24.30 ± 0.19	-3.317	0.998	100.223	25.25 ± 0.16
SensiFAST SYBR Lo-ROX Kit (Bioline)	-3.318	0.988	100.152	23.80 ± 0.22	-3.254	0.968	102.906	24.90 ± 0.24
Power SYBR Green PCR Master Mix (Applied Biosystems)	-3.237	0.980	103.693	24.95 ± 0.16	-3.316	0.981	100.256	26.13 ± 0.17
5x HOT FIREPol EvaGreen HRM Mix (ROX) (Solis BioDyne)	-3.284	0.995	101.620	25.18 ± 0.25	-3.245	0.988	103.300	26.35 ± 0.21

Table A2. Mean amplification Ct values of telomeric sequences in zebra finch *Taeniopygia guttata* samples (S1-S8) using different qPCR master mixes.

	S1	S2	S3	S4	S5	S6	S7	S8
GoTaq qPCR Master Mix (Promega)	17.17	16.71	16.53	16.30	15.32	15.71	13.87	15.64
LightCycler 480 SYBR Green I Master (Roche)	16.65	16.47	16.24	16.44	16.27	15.91	13.95	15.60
5x HOT FIREPol EvaGreen HRM Mix (ROX) (Solis BioDyne)	14.63	14.31	14.30	14.14	13.35	13.72	12.04	13.55
SensiFAST SYBR Lo-ROX Kit (Bioline)	13.75	13.15	13.12	12.97	12.06	12.30	10.56	12.17
Power SYBR Green PCR Master Mix (Applied Biosystems)	25.79	26.56	26.45	26.44	26.22	26.19	26.99	26.31
iTaq Universal SYBR Green Supermix (Bio-Rad)	19.91	19.45	19.84	19.51	18.19	18.46	16.39	18.41
Luminaris HiGreen qPCR Master Mix, low ROX (Thermo Scientific)	21.29	20.65	20.91	20.75	19.50	19.73	17.40	19.58
Brilliant II SYBR Green QPCR Master Mix (Agilent Technologies)	24.65	23.92	23.86	23.78	22.76	23.50	21.65	22.80

Table A3. Mean amplification Ct values of telomeric sequences in red-billed chough *Pyrhcorax pyrrhcorax* samples (S1-S8) using different qPCR master mixes.

	S1	S2	S3	S4	S5	S6	S7	S8
GoTaq qPCR Master Mix (Promega)	16.69	16.45	16.53	15.44	16.90	16.56	16.17	16.41
LightCycler 480 SYBR Green I Master (Roche)	17.32	16.48	16.45	15.52	16.36	16.18	15.91	15.90
5x HOT FIREPol EvaGreen HRM Mix (ROX) (Solis BioDyne)	14.93	14.54	14.67	14.05	15.00	14.54	14.07	14.27
SensiFAST SYBR Lo-ROX Kit (Bioline)	13.61	12.99	13.03	12.35	13.28	13.21	12.83	12.98
Power SYBR Green PCR Master Mix (Applied Biosystems)	21.43	20.63	22.08	21.00	21.82	20.22	19.94	20.16
iTaq Universal SYBR Green Supermix (Bio-Rad)	19.28	18.95	19.05	18.59	19.58	19.26	18.58	18.72
Luminaris HiGreen qPCR Master Mix, low ROX (Thermo Scientific)	20.30	20.04	20.31	19.62	20.58	20.82	20.37	20.93
Brilliant II SYBR Green QPCR Master Mix (Agilent Technologies)	22.49	21.77	22.28	21.12	22.50	21.73	22.77	22.38

Table A4. Pair-wise correlation values between amplification Ct of the telomeric sequences in the same samples with different qPCR master mixes for zebra finch (below diagonal) and red billed chough (above diagonal). p-values are shown in parentheses. Significant correlations are shown in bold.

	GoTaq qPCR Master Mix (Promega)	LightCycler 480 SYBR Green I Master (Roche)	5x HOT FIREPol EvaGreen HRM Mix (ROX) (Solis BioDyne)	SensiFAST SYBR Lo-ROX Kit (Bioline)	Power SYBR Green PCR Master Mix (Applied Biosystems)	iTaq Universal SYBR Green Supermix (Bio-Rad)	Luminaris HiGreen qPCR Master Mix, low ROX (Thermo Scientific)	Brilliant II SYBR Green QPCR Master Mix (Agilent Technologies)
GoTaq qPCR Master Mix (Promega)	1	0.705 (0.051)	<b>0.843 (0.009)</b>	<b>0.904 (0.002)</b>	0.310 (0.455)	<b>0.815 (0.014)</b>	0.667 (0.071)	0.630 (0.094)
LightCycler 480 SYBR Green I Master (Roche)	<b>0.904 (0.002)</b>	1	<b>0.822 (0.012)</b>	<b>0.873 (0.005)</b>	0.463 (0.248)	0.656 (0.077)	0.118 (0.781)	0.408 (0.316)
5x HOT FIREPol EvaGreen HRM Mix (ROX) (Solis BioDyne)	<b>0.994 (&lt;0.001)</b>	<b>0.928 (0.001)</b>	1	<b>0.849 (0.008)</b>	0.686 (0.060)	<b>0.944 (&lt;0.001)</b>	0.265 (0.527)	0.335 (0.418)
SensiFAST SYBR Lo-ROX Kit (Bioline)	<b>0.997 (&lt;0.001)</b>	<b>0.920 (0.001)</b>	<b>0.993 (&lt;0.001)</b>	1	0.283 (0.497)	<b>0.796 (0.018)</b>	0.555 (0.153)	0.568 (0.142)
Power SYBR Green PCR Master Mix (Applied Biosystems)	-0.658 (0.076)	<b>-0.720 (0.044)</b>	-0.670 (0.069)	-0.680 (0.064)	1	0.564 (0.145)	-0.259 (0.535)	0.060 (0.888)
iTaq Universal SYBR Green Supermix (Bio-Rad)	<b>0.980 (&lt;0.001)</b>	<b>0.912 (0.002)</b>	<b>0.988 (&lt;0.001)</b>	<b>0.985 (&lt;0.001)</b>	-0.603 (0.114)	1	0.360 (0.381)	0.227 (0.589)
Luminaris HiGreen qPCR Master Mix, low ROX (Thermo Scientific)	<b>0.985 (&lt;0.001)</b>	<b>0.936 (0.001)</b>	<b>0.993 (&lt;0.001)</b>	<b>0.992 (&lt;0.001)</b>	-0.659 (0.076)	<b>0.996 (&lt;0.001)</b>	1	0.552 (0.156)
Brilliant II SYBR Green QPCR Master Mix (Agilent Technologies)	<b>0.977 (&lt;0.001)</b>	<b>0.878 (0.004)</b>	<b>0.973 (&lt;0.001)</b>	<b>0.980 (&lt;0.001)</b>	-0.688 (0.059)	<b>0.953 (&lt;0.001)</b>	<b>0.963 (&lt;0.001)</b>	1