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Supplementary material

SUPPLEMENTARY MATERIAL FOR:

Absolute standards as a useful addition to the avian quantitative PCR telomere assay

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Appendix 1: Primers, standards, and calculations

Supplementary material Appendix 1, Table 1

Table 1 - Details of primers and standards. Please note that all primers and standards should be ordered and diluted frequently, aliquoted and stored at -20°C. Failure to do so may result in drop of efficiency and accuracy of absolute standard curve. Primers were extracted using Reverse phase chromatography (Invitrogen), oligomer standards were extracted to PAGE purity (Invitrogen). *The *GAPDH* 53mer oligonucleotide contained the amplified region of the chicken *GAPDH* gene, however the amplified region for the zebra finch could also be used; AAA CCA GCC AAG TAC GAT GAC ATC AAG AGG GTA GTG AAG GCT GCT GCT GAT GG.

Type of sequence	*Purpose*	*Name*	*Sequence*	*Reference*
Primer	Amplification of telomere sequence	Tel1b	5'- CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT-3'	(Criscuolo et al. 2009, Roos et al. 2008)
Primer	Amplification of telomere sequence	Tel2b	5'- GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT- 3'	(Criscuolo et al. 2009, Roos et al. 2008)
Primer	Amplification of GAPDH sequence	GAPDH F	5'- AAA CCA GCC AAG TAC GAT GAC AT-3'	(Criscuolo et al. 2009)
Primer	Amplification of GAPDH sequence	GAPDH R	5'- CCA TCA GCA GCA GCC TTC A-3'	(Criscuolo et al. 2009)
Standard	Telomere oligomer standard	Telomere	84mer: TTAGGG repeated 14 times	(O'Callaghan et al. 2008)
Standard	GAPDH oligomer standard	GAPDH	53mer: AAA CCA GCC AAG TAT GAC GAC ATC AAG AGG GTA GTG AAG GCT GCT GCT GAT GG *	This study

Supplementary material Appendix 1, Calculations 1

Calculations 1 – calculating telomere lengths and number of diploid genomes from synthetic standards:

Telomere length (also see O'Callaghan et al. 2008):

The telomere 84mer has a molecular mass (m) of 26667.8. The weight of one molecule is m/Avagadro's number. Therefore, the weight of a telomere standard is: $26667.8/6.02 \times 10^{23} = 4.43 \times 10^{-20}$ g. The highest concentration used (Standard A) has 600 pg of telomere oligomer (600×10^{-12} g) per reaction. Therefore there are $600 \times 10^{-12}/4.43 \times 10^{-20} = 1.36 \times 10^{10}$ molecules of telomere sequence in standard A. To obtain the TL from the number of molecules we multiplied by 84 to get the number of bases (1.14×10^{12} bp) and then divided by 1000 to obtain the number of kb (1.14×10^{9} kb).

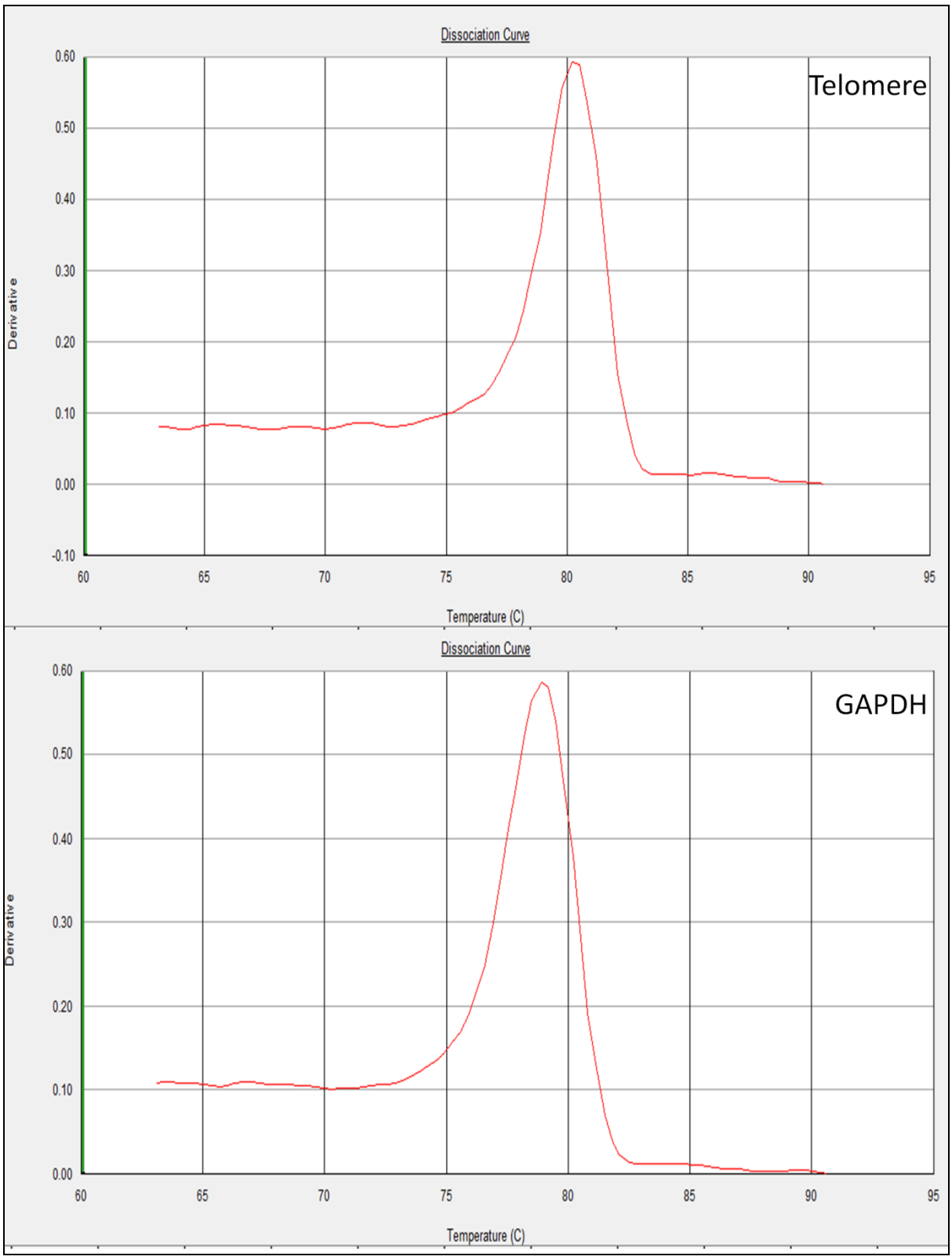
Number of diploid genomes

The *GAPDH* oligomer is 53 bp long with an m of 16489.6. The weight of one molecule is m/Avagadro's no. = 2.74×10^{-20} g. The highest concentration was 60 pg of *GAPDH* (60×10^{-12} g) per reaction. Therefore, there are 60×10^{-12} g/ 2.74×10^{-20} g = 2.19×10^{9} copies of *GAPDH* in standard A. To obtain the figure for a diploid genome, where there are 2 copies of *GAPDH* per genome, divide by 2. Standard A of *GAPDH* oligomer is equivalent to 1.10×10^{9} diploid genome copies of *GAPDH.* We plotted the C_q values of the serial dilutions of telomere and *GAPDH* oligomers against log values of kb length and number of diploid genomes, respectively.

Appendix 2: Dissociation (melt) curves

Supplementary material Appendix 2, Figure 1

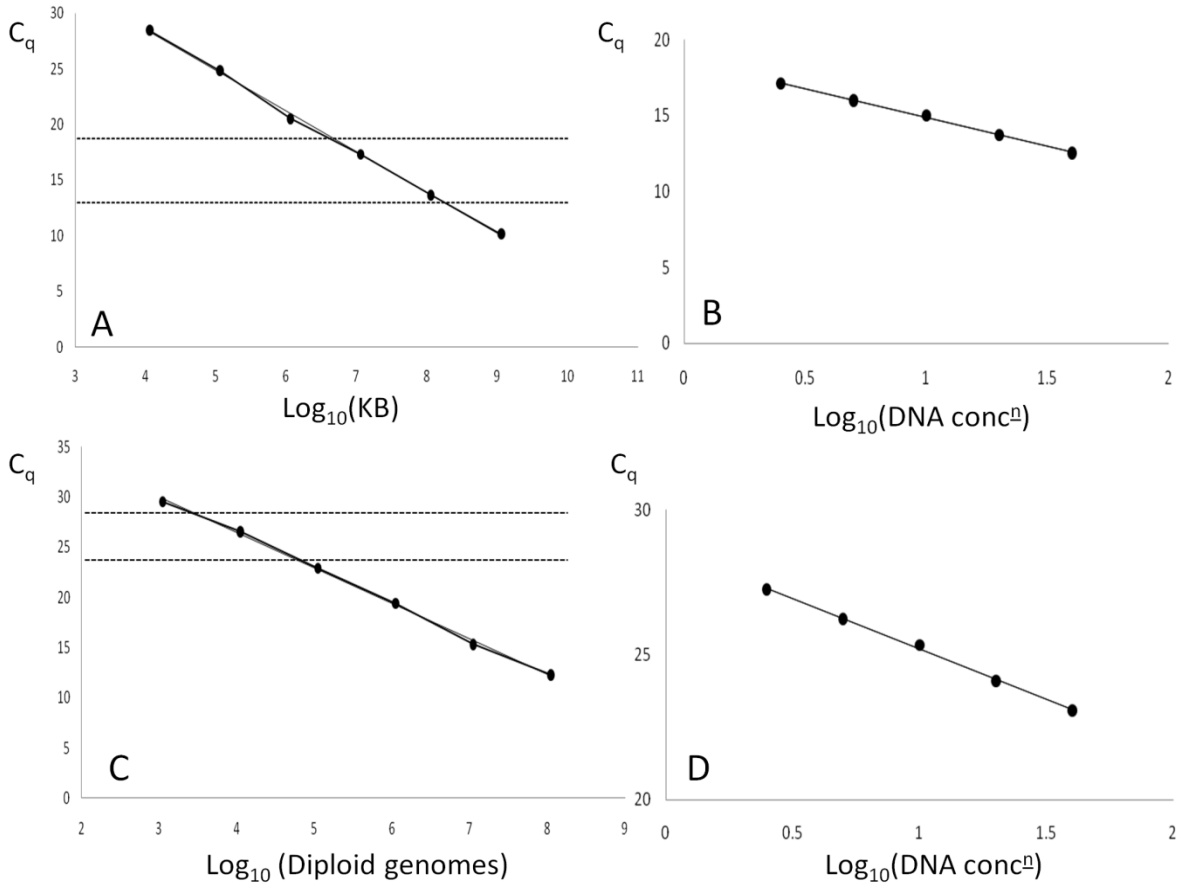
Figure 1. Dissociation curves of telomere and GAPDH amplification in the Seychelles warbler. Tm telomere = 80.0°C Tm GAPDH = 78.4°C, respectively.



Appendix 3: Standard curves

Supplementary material Appendix 3, Figure 2

Figure 2. Standard curves of oligomer standards and samples for telomere and GAPDH amplification. Standard curves of serial dilutions of oligomer standards (A & C) and Seychelles warbler DNA (B & D), when amplified with Telomere (A & B) or GAPDH (C & D) primers. (A) *Telomere oligomer standard*; R^2 = 0.9987, slope = -3.67, intercept = 43.18: (B) *Telomere sample standard;* R^2 = 0.9981, slope = -3.80, intercept = 18.68: (C) *GAPDH oligomer standard;* R^2 = 0.9984, slope = -3.53, intercept = 40.55: (D) *GAPDH sample standard;* R^2 = 0.9977, slope = -3.48, intercept = 28.68). Dashed lines in A and C represent the window in which sample Cq measurements fell after amplification with telomere and GAPDH primers, respectively.



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