ScienCell™
Research Laboratories

Absolute Mouse Telomere Length Quantification qPCR Assay Kit (AMTLQ)
Catalog #M8918
100 reactions

Product Description
Telomeres are repetitive nucleotide elements at the ends of chromosomes that protect chromosomes from degradation and genetic information loss. Normal diploid cells lose telomeres with each cell cycle. Telomere length, therefore, decreases over time and may predict lifespan. Telomere shortening has negative effects on health conditions and has been linked to many health issues including aging and cancer. Accurate and consistent quantification of telomere length is important in many aspects of cell biology such as chromosomal instability, DNA repair, senescence, apoptosis, cell dysfunctions, and oncogenesis.

ScienCell's Absolute Mouse Telomere Length Quantification qPCR Assay Kit (AMTLQ) is designed to directly measure the average telomere length of a mouse cell population. The telomere primer set recognizes and amplifies telomere sequences. The single copy reference (SCR) primer set recognizes and amplifies a 100 bp-long region on mouse chromosome 10, and serves as reference for data normalization. The reference genomic DNA sample with known telomere length serves as a reference for calculating the telomere length of target samples. The carefully designed primers ensure: (i) high efficiency for trustworthy quantification; and (ii) no non-specific amplification. Each primer set has been validated by qPCR with melt curve analysis and gel electrophoresis for amplification specificity and by template serial dilution for amplification efficiency.

Kit Components

Cat #	Component	Quantity	Storage
8918a	Telomere primer set, lyophilized	1 vial	-20°C
M8918b	Single copy reference (SCR) primer set, lyophilized	1 vial	-20°C
8918c	Nuclease-free H$_2$O	4 mL	4°C
M8918d	Reference Mouse genomic DNA sample (Lot #24556, telomere length: 3.92 ± 0.16 Mb per diploid cell)	100 μL	-20°C

Additional Materials Required (Materials Not Included in Kit)

Component	Recommended
genomic DNA template	Customers' samples
qPCR plate or tube	
qPCR master mix	FastStart Essential DNA Green Master (Roche, Cat #06402712001)

Quality Control
The specificity of the primer sets are validated by qPCR with melt curve analysis. The PCR products are analyzed by gel electrophoresis. The efficiency of the primer sets are validated by template serial dilution (See **Appendices 1 and 2**). The telomere length of reference genomic

DNA sample is determined by qPCR standard curve method (See **Appendix 3**). The AMTLQ kit is validated using C57BL/6 and CD1 mouse samples.

Product Use
AMTLQ is for research use only. It is not approved for human or animal use, or for application in clinical or *in vitro* diagnostic procedures.

Shipping and Storage
The product is shipped on dry ice. Upon receipt, store the primers (Cat #8918a and M8918b) and the reference genomic DNA sample (Cat #M8918d) at -20°C in a manual defrost freezer, and nuclease-free H_2O (Cat #8918c) at 4°C.

Procedures

*Important: **Only** use polymerases with hot-start capability to prevent possible primer-dimer formation. **Only** use nuclease-free reagents in PCR amplification.*

Note: The quality of the qPCR master mix is a critical element for successful qPCR analyses. AMTLQ is optimized using FastStart Essential DNA Green Master (Roche, Cat #06402712001) and is highly recommended. Use of other qPCR master mixes may compromise results.

1. Prior to use, allow vials (Cat #8918a and #M8918b) to warm to room temperature.

2. Centrifuge the vials at 1,500x g for 1 minute.

3. Add 200 µl nuclease-free H_2O (Cat #8918c) to telomere primer set (lyophilized, Cat #8918a) to make telomere primer stock solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.

4. Add 200 µl nuclease-free H_2O (Cat #8918c) to SCR primer set (lyophilized, Cat #M8918b) to make SCR primer stock solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.

5. For the reference genomic DNA sample (Cat #M8918d), prepare two qPCR reactions, one with telomere primer stock solution, and one with SCR primer stock solution. Prepare 20 µl qPCR reactions for one well as shown in Table 1.

Table 1.

Reference genomic DNA sample	1 µl
Primer stock solution (Telomere or SCR)	2 µl
2x qPCR master mix	10 µl
Nuclease-free H_2O (Cat #8918c)	7 µl
Total volume	*20 µl*

6. For each genomic DNA sample, prepare two qPCR reactions, one with telomere primer stock solution, and one with SCR primer stock solution. Prepare 20 µl qPCR reactions for one well as shown in Table 2.

Table 2.

Genomic DNA template	0.2 – 2 ng
Primer stock solution (Telomere or SCR)	2 µl
2x qPCR master mix	10 µl
Nuclease-free H_2O (Cat #8918c)	variable
Total volume	*20 µl*

7. Seal the qPCR reaction wells. Centrifuge the plates or tubes at 1,500x g for 15 seconds. For maximum reliability, replicates are strongly recommended (minimum of 3).

8. For qPCR program setup, refer to Table 3 when using FastStart Essential DNA Green Master (Roche, Cat #06402712001). This master mix does not contain a ROX passive

reference dye. If the qPCR instrument being used has a "ROX passive reference dye" option, please deselect this option. When using other qPCR master mixes, the qPCR program may require optimization with Table 3 as a starting protocol.

Note: The primary factors that determine optimal annealing temperature are the primer length and primer composition. Based on the properties of telomere and SCR primer sets (Cat #8918a and #M8918b), we highly recommend an annealing temperature of 52°C as shown in Table 3:

Table 3.

Step	Temperature	Time	Number of cycles
Initial denaturation	95°C	10 min	1
Denaturation	95°C	20 sec	
Annealing	52°C	20 sec	32
Extension	72°C	45 sec	
Data acquisition		Plate read	
Optional		*Melting curve analysis*	1
Hold	20°C	Indefinite	1

Figure 1. A typical amplification curve showing the amplification of a qPCR product.
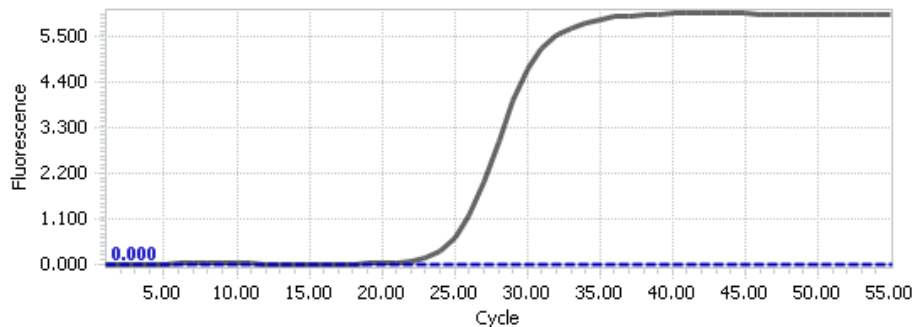
Figure 2. A typical melting peak of a qPCR product.
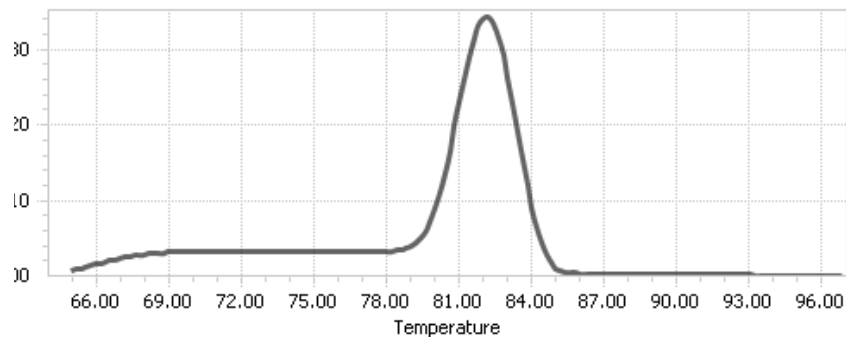
Quantification Method: Comparative ΔΔCq (Quantification Cycle Value) Method

Note: Please refer to your qPCR instrument's data analysis software for data analysis. The method provided here serves as guidance for quick manual calculations.

1. For telomere (TEL), ΔCq (TEL) is the quantification cycle number difference of TEL between the target and the reference genomic DNA samples.

    ΔCq (TEL) = Cq (TEL, target sample) - Cq (TEL, reference sample)

    Note: the value of ΔCq (TEL) can be positive, 0, or negative.

2. For single copy reference (SCR), ΔCq (SCR) is the quantification cycle number difference of SCR between the target and the reference genomic DNA samples.

    ΔCq (SCR) = Cq (SCR, target sample) - Cq (SCR, reference sample)

    Note: the value of ΔCq (SCR) can be positive, 0, or negative.

3. ΔΔCq = ΔCq (TEL) - ΔCq (SCR)

4. Relative telomere length of the target sample to the reference sample (fold) = $2^{-\Delta\Delta Cq}$

5. The total telomere length of the target sample

    = Reference sample telomere length x $2^{-\Delta\Delta Cq}$

Example Calculations: Comparative ΔΔCq (Quantification Cycle Value) Method

Table 3. Cq (Quantification Cycle) values of telomere qPCR (TEL) and single copy reference qPCR (SCR) obtained for three genomic DNA samples.

Primer set	Target sample	Reference sample
TEL	11.87	14.03
SCR	24.70	26.66

ΔCq (TEL) = Cq (TEL, target sample) - Cq (TEL, reference sample)

= 11.87 - 14.03

= -2.16

ΔCq (SCR) = Cq (SCR, target sample) - Cq (SCR, reference sample)

= 24.70 - 26.66

= -1.96

$$\Delta\Delta Cq = \Delta Cq\ (TEL) - \Delta Cq\ (SCR)$$
$$= -2.16 - (-1.96)$$
$$= -0.20$$
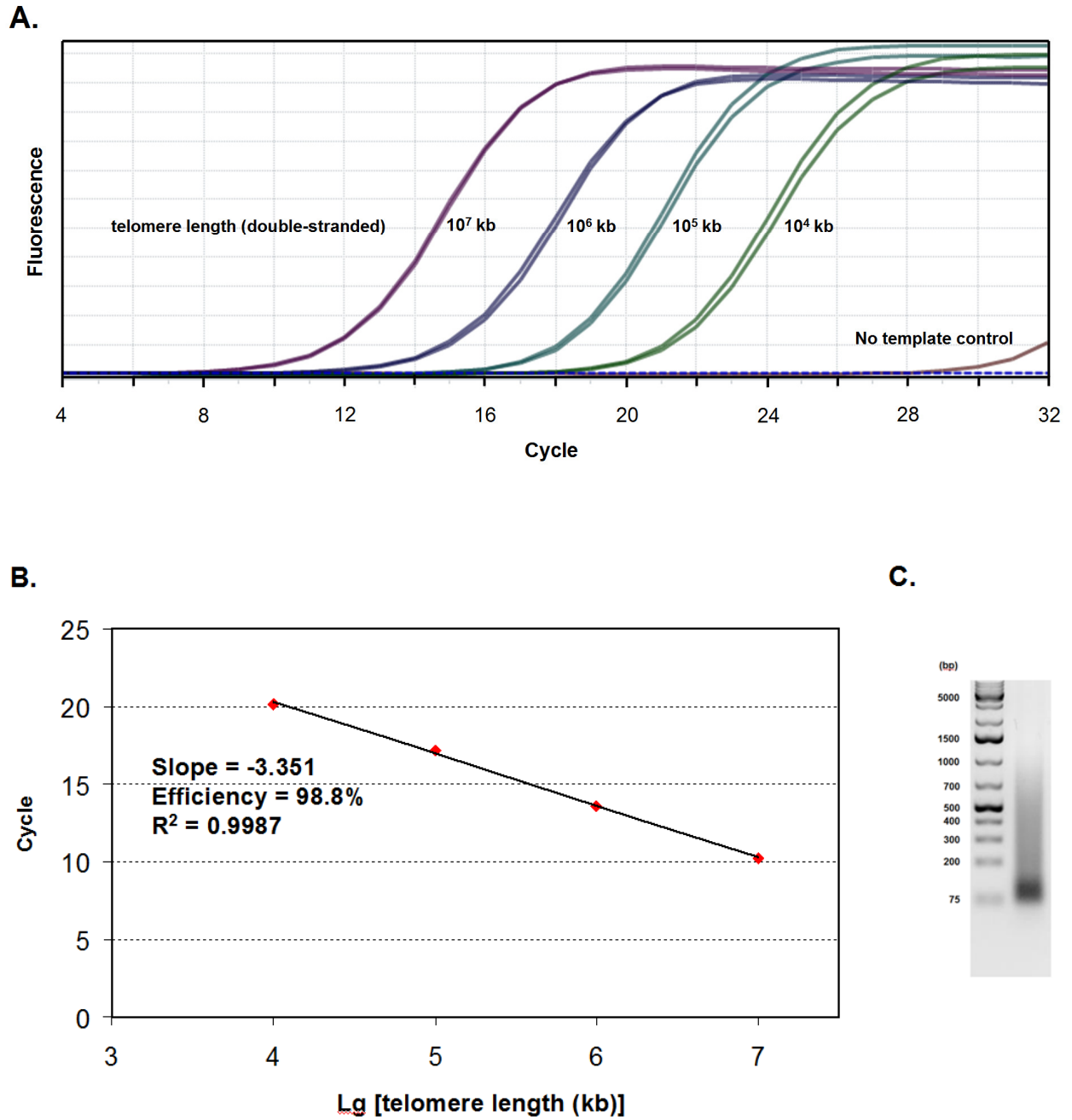
Relative telomere length of the target sample to the reference sample (fold)
$$= 2^{-\Delta\Delta Cq}$$
$$= 2^{0.20}$$
$$= 1.15$$

The total telomere length of the target sample per diploid cell
$$= \text{Reference sample telomere length} \times 2^{-\Delta\Delta Cq}$$
$$= (3.92 \pm 0.16\ \text{Mb}) \times 1.15$$
$$= 4.51 \pm 0.18\ \text{Mb}$$

There are 80 chromosome ends in one mouse diploid cell, therefore, average telomere length on each chromosome end $= (4.51 \pm 0.18\ \text{Mb}) / 80$
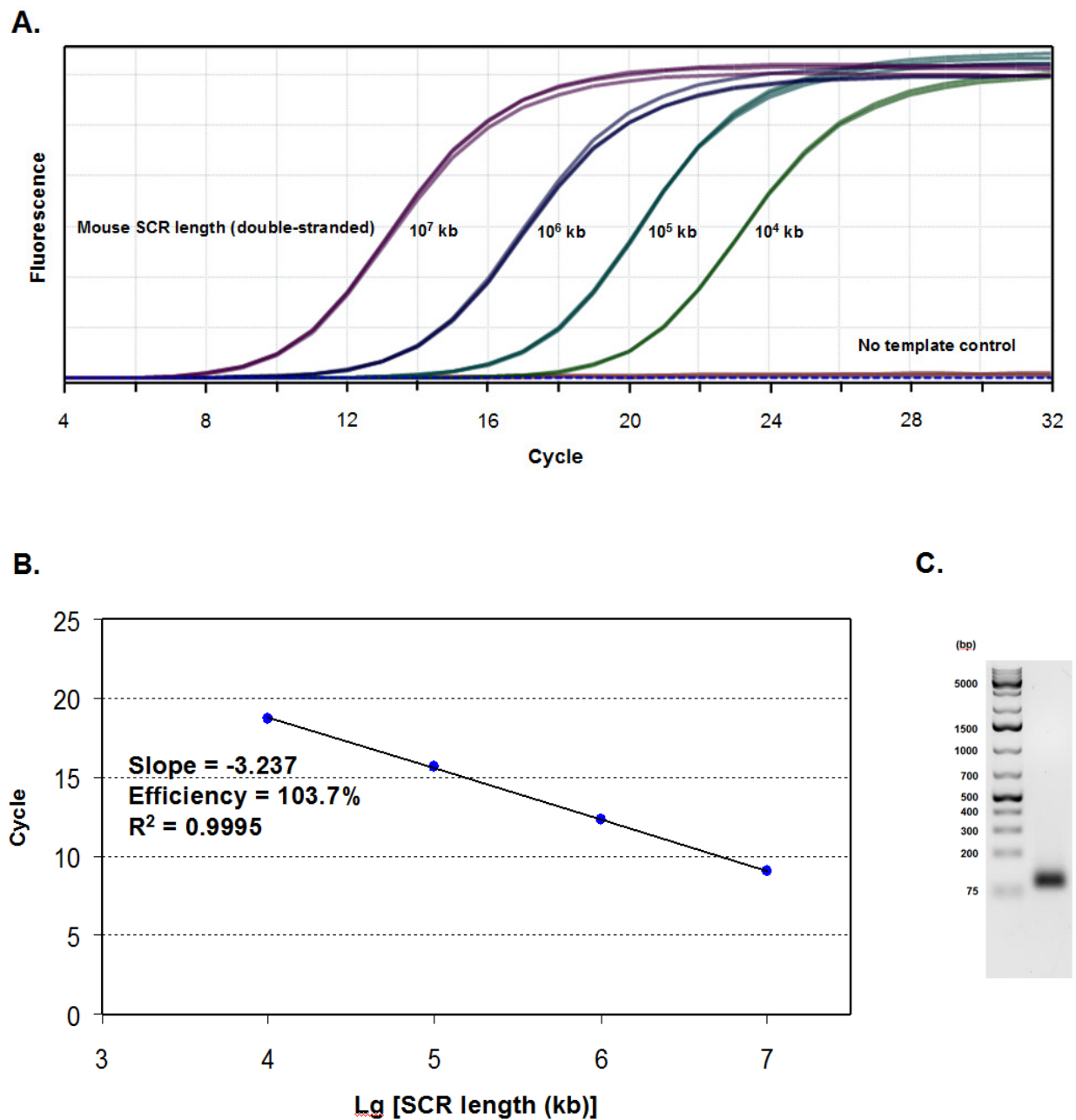$$= 56.4 \pm 2.3\ \text{kb}$$

Conclusions: The average telomere length of target genomic DNA sample is 4.51 ± 0.18 Mb per diploid cell, or 56.4 ± 2.3 kb per chromosome end.

A.

B.

C.

Figure 3. Quality assessment of Telomere primer set. (A) qPCR amplification curves using serially diluted telomere repeats as template. **(B)** Derivation of qPCR efficiency of Telomere primer set. **(C)** Separation of Telomere qPCR product by gel electrophoresis. A smeared band is observed as expected.

A.

B.

C.

Slope = -3.237
Efficiency = 103.7%
$R^2 = 0.9995$

Figure 4. Quality assessment of Single copy reference (SCR) primer set. (A) qPCR amplification curves using serially diluted SCR template. **(B)** Derivation of qPCR efficiency of SCR primer set. **(C)** Separation of SCR qPCR product by gel electrophoresis.

Appendix 3: Method for quantifying reference genomic DNA sample (Cat #M8918d)

To quantify the reference genomic DNA sample (Cat #M8918d), a qPCR analysis using it as the template was performed. All experiments were performed in triplicates under the same conditions and repeated at least twice.

Derived from the standard curves in appendices 1 and 2, the telomere and SCR length of reference genomic DNA sample in each qPCR reaction is determined to be:

Total telomere length (double-stranded): $(1.71 \pm 0.07) \times 10^3$ Mb

Total SCR length (double-stranded): 87.2 ± 2.8 kb

The SCR template is 100 bp long, therefore, there are 0.2 kb SCR per diploid cell.

Total number of diploid cells = $(87.2 \pm 2.8 \text{ kb}) / 0.2 \text{ kb} = 436 \pm 14$ cells

Telomere length per diploid cell (double-stranded) = $(1.71 \pm 0.07) \times 10^3$ Mb / (436 ± 14)
$= 3.92 \pm 0.16$ Mb

There are 80 chromosome ends in one mouse diploid cell, therefore,

Average telomere length on each chromosome end = $(3.92 \pm 0.16 \text{ Mb}) / 80$
$= 49.0 \pm 2.0$ kb

Conclusions: The average telomere length of reference genomic DNA sample (Cat #M8918d) is 3.92 ± 0.16 Mb per diploid cell, or 49.0 ± 2.0 kb per chromosome end.