Absolute Rat Telomere Length and Mitochondrial DNA Copy Number Dual Quantification qPCR Assay Kit (ARDQ)

Catalog #R8958
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. 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.

Mitochondrial DNA (mtDNA) is circular, multicopy genome DNA located in mitochondrion, a cellular organelle that plays a key role in the energy production. The capacity for energy production in a cell depends on both mtDNA integrity and copy number.

Evidence suggests that telomere length and mitochondrial dysfunction are positively correlated in cellular aging and other age-related disorders such as cancer, diabetes and neurodegenerative diseases. A possible mechanistic link between them has been proposed through the PPAR signaling pathway. However, the specifics of the mechanism still have to be elucidated.

ScienCell's Absolute Rat Telomere Length and Mitochondrial DNA Copy Number Dual Quantification qPCR Assay Kit (ARDQ) is designed to simultaneously quantify the average telomere length and mtDNA copy number of a rat cell population using qPCR. The telomere primer set recognizes and amplifies telomere sequences. The mtDNA primer set recognizes and amplifies one of the most conserved regions on rat mtDNA and will not amplify any off-target sequence on nuclear genomic DNA. The single copy reference (SCR) primer set recognizes and amplifies a 100 bp-long region on rat chromosome 17, and serves as reference for data normalization. The reference genomic DNA sample with known telomere length and mtDNA copy number serves as a reference for calculating the telomere length and the mtDNA copy number 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. The 2X GoldNStart TaqGreen qPCR Master Mix (Cat #MB6018a-1) is a SYBR®Green dye-based qPCR master mix with a "hot-start" property. It contains SYBR®Green, dNTPs, Taq DNA polymerase, and an inert gold-color loading indicator in a single tube. The "hot-start" property achieved through ScienCell's unique chemically modified Taq DNA polymerase provides maximal inhibition of primer dimer formation. The advanced buffer formulation provides superior specificity and efficiency with a

wide linear dynamic range. The inert gold-color loading indicator allows for better visualization and tracking of sample loading in qPCR plates or tubes.

Kit Components

Cat #	Component	Quantity	Storage
MB6018a-1	2X GoldNStart TaqGreen qPCR master mix, 1 mL	3 vials	-20°C
8958a	Telomere primer set, lyophilized	1 vial	-20°C
R8958b	Rat mtDNA primer set, lyophilized	1 vial	-20°C
R8958c	Rat single copy reference (SCR) primer set, lyophilized	1 vial	-20°C
8958d	Nuclease-free H_2O	6 mL	4°C
R8958e	Reference Rat genomic DNA sample (Lot #33599, telomere length: 6.32 ± 0.45 Mb per diploid cell mtDNA copy number: 937 ± 20 copies per diploid cell)	100 µL	-20°C

Additional Materials Required (Materials Not Included in Kit)

Component	Recommended
DNA isolation kit	SpeeDNA Isolation Kit (ScienCell, Cat #MB6918)
genomic DNA template	Customers' samples
qPCR plate or tube	

Quality Control

The specificity of the primer sets is validated by qPCR with melt curve analysis. The PCR products are analyzed by gel electrophoresis. The efficiency of the primer sets is validated by template serial dilution (See **Appendices 1 through 3**). The telomere length and mtDNA copy number of reference genomic DNA sample are determined by qPCR standard curve method (See **Appendix 4**).

Product Use

ARDQ 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 GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1) in the dark at -20°C in a manual defrost freezer, store the primers (Cat #8958a, R8958b and R8958c) and the reference genomic DNA sample (Cat #R8958e) at -20°C in a manual defrost freezer, and nuclease-free H_2O (Cat #8958d) at 4°C. Once thawed, do NOT refreeze GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1), and keep in the dark at 4°C or on ice at all times.

Procedures

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

1. Prior to use, allow vials (Cat #8958a, R8958b and R8958c) 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 #8958d) to telomere primer set (lyophilized, Cat #8958a) 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 #8958d) to mtDNA primer set (lyophilized, Cat #R8958b) to make mtDNA primer stock solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.

5. Add 200 µl nuclease-free H2O (Cat #8958d) to SCR primer set (lyophilized, Cat #R8958c) to make SCR primer stock solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.

6. For the reference genomic DNA sample (Cat #R8958e), prepare three qPCR reactions, one with telomere primer stock solution, one with mtDNA primer stock solution, and one with SCR primer stock solution. Prepare 20 µl qPCR reactions for one well as shown in Table 1. The reference genomic DNA sample (Cat #R8958e) included in the kit is enough for 30 qPCR reactions with each of the primer sets.

Table 1.

Reference genomic DNA sample (Cat #R8958e)	1 µl
Primer stock solution (Telomere, mtDNA or SCR)	2 µl
2X GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1)	10 µl
Nuclease-free H_2O (Cat #8958d)	7 µl
Total volume	***20 µl***

7. For each genomic DNA sample, prepare three qPCR reactions, one with telomere primer stock solution, one with mtDNA 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 (0.5 – 5 ng/µl)	1 µl
Primer stock solution (Telomere, mtDNA or SCR)	2 µl
2X GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1)	10 µl
Nuclease-free H_2O (Cat #8958d)	7 µl
Total volume	***20 µl***

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

9. Refer to Table 3 for qPCR program setup. The 2X GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1) contains SYBR®Green as the reporter dye and 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.

   Note: The primary factors that determine optimal annealing temperature are the primer length and primer composition. Based on the properties of Telomere, mtDNA and SCR primer sets (Cat #8958a, #R8958b and #R8958c), 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	32
Annealing	52°C	20 sec	
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.

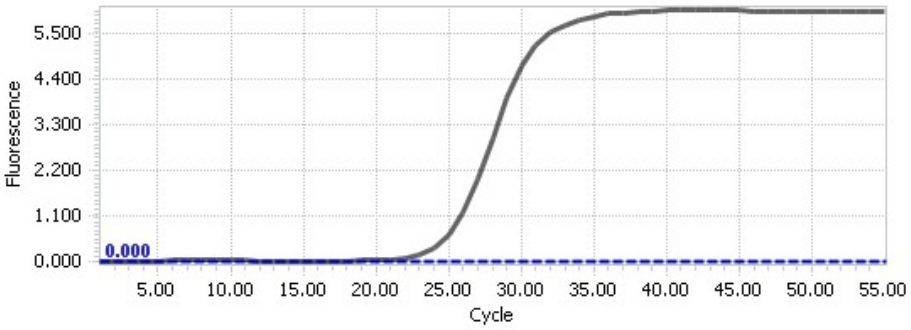
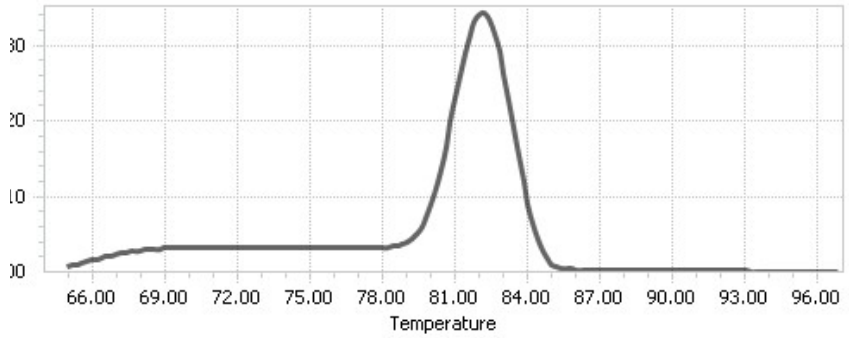


Figure 2. A typical melting peak of a qPCR product.



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.

   $$\Delta Cq\ (TEL) = Cq\ (TEL, target\ sample) - Cq\ (TEL, reference\ sample)$$

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

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

   $$\Delta Cq\ (mtDNA) = Cq\ (mtDNA, target\ sample) - Cq\ (mtDNA, reference\ sample)$$

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

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

   $$\Delta Cq\ (SCR) = Cq\ (SCR, target\ sample) - Cq\ (SCR, reference\ sample)$$

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

4. $\Delta\Delta Cq\ (TEL) = \Delta Cq\ (TEL) - \Delta Cq\ (SCR)$

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

6. The total telomere length of the target sample

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

7. $\Delta\Delta Cq\ (mtDNA) = \Delta Cq\ (mtDNA) - \Delta Cq\ (SCR)$

8. Relative mtDNA copy number of the target sample to the reference sample (fold)

   = $2^{-\Delta\Delta Cq\ (mtDNA)}$

9. The mtDNA copy number of the target sample

   = Reference sample mtDNA copy number x $2^{-\Delta\Delta Cq\ (mtDNA)}$

Example Calculations: Comparative ΔΔCq (Quantification Cycle Value) Method

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

Primer set	*Target sample*	*Reference sample*
TEL	14.62	16.68
mtDNA	16.41	17.23
SCR	24.64	26.10

ΔCq (TEL) = Cq (TEL, target sample) - Cq (TEL, reference sample)
= 14.62 - 16.68
= -2.06

ΔCq (mtDNA) = Cq (mtDNA, target sample) - Cq (mtDNA, reference sample)
= 16.41 - 17.23
= -0.82

ΔCq (SCR) = Cq (SCR, target sample) - Cq (SCR, reference sample)
= 24.64 - 26.10
= -1.46

- ΔΔCq (TEL) = ΔCq (TEL) - ΔCq (SCR)
= -2.06- (-1.46)
= -0.60

Relative telomere length of the target sample to the reference sample (fold)
$= 2^{-\Delta\Delta Cq\ (TEL)}$
$= 2^{0.60}$
= 1.52

The total telomere length of the target sample per diploid cell
= Reference sample telomere length x $2^{-\Delta\Delta Cq}$
= (6.32 ± 0.45 Mb) x 1.52
= 9.61 ± 0.68 Mb

There are 84 chromosome ends in rat one diploid cell, therefore, average telomere length on each chromosome end = (9.61 ± 0.68 Mb) / 84

= 114 ± 8 kb

- $\Delta\Delta Cq$ (mtDNA) = ΔCq (mtDNA) - ΔCq (SCR)

  = -0.82- (-1.46)

  = 0.64

  Relative mtDNA copy number of the target sample to the reference sample (fold)

  $= 2^{-\Delta\Delta Cq}$

  $= 2^{-0.64}$

  = 0.64

  The mtDNA copy number of the target sample per diploid cell

  = Reference sample mtDNA copy number x $2^{-\Delta\Delta Cq}$

  = (937 ± 20) x 0.64

  = 600 ± 13

Conclusions:

- The average telomere length of target genomic DNA sample is 9.61 ± 0.68 Mb per diploid cell, or 114 ± 8 kb per chromosome end.
- The average mtDNA copy number of target genomic DNA sample is 600 ± 13 per diploid cell.

Appendix 1: Quality assessment of Telomere primer set

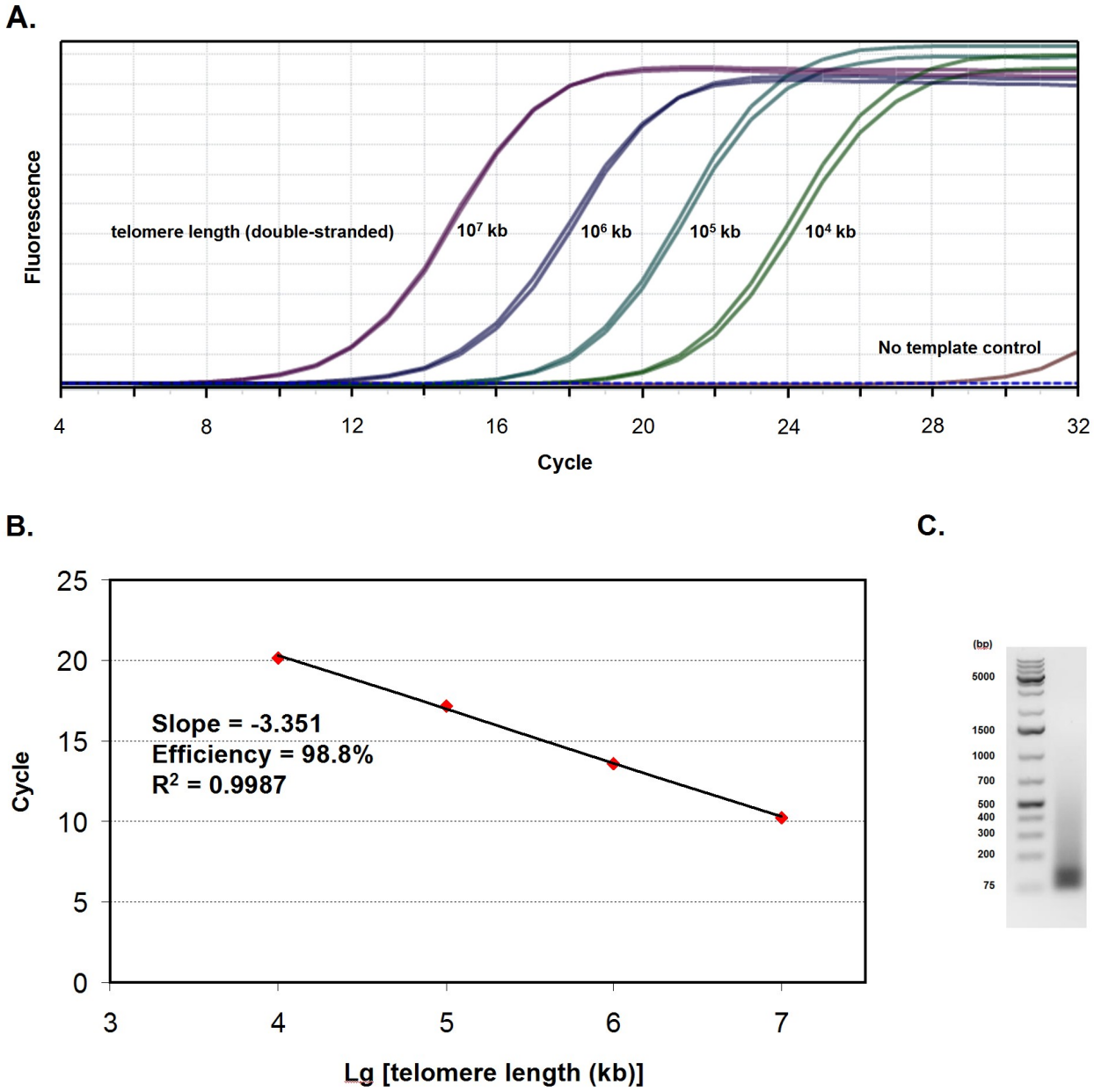


Figure 3. Quality assessment of Telomere primer set. (A) qPCR amplification curves using serially diluted telomere repeats as template. **(B)** Derivation of qPCR efficiency of Telomere primer set. **(C)** Separation of Telomere qPCR product by gel electrophoresis. A smeared band is observed as expected.

Note: Due to the tandemly repeated nature of telomere sequences, Telomere primer set may exhibit trace amounts of primer dimer formation in No Template Control (NTC) reactions. A Cq value of 28 or greater indicates the primer dimer formation in a NTC reaction and can be treated as a negative result. If the Cq value for a telomere reaction using a test sample is less than 20, then the formation of primer dimers will NOT affect the quantification of telomere length.

Appendix 2: Quality assessment of rat mtDNA primer set

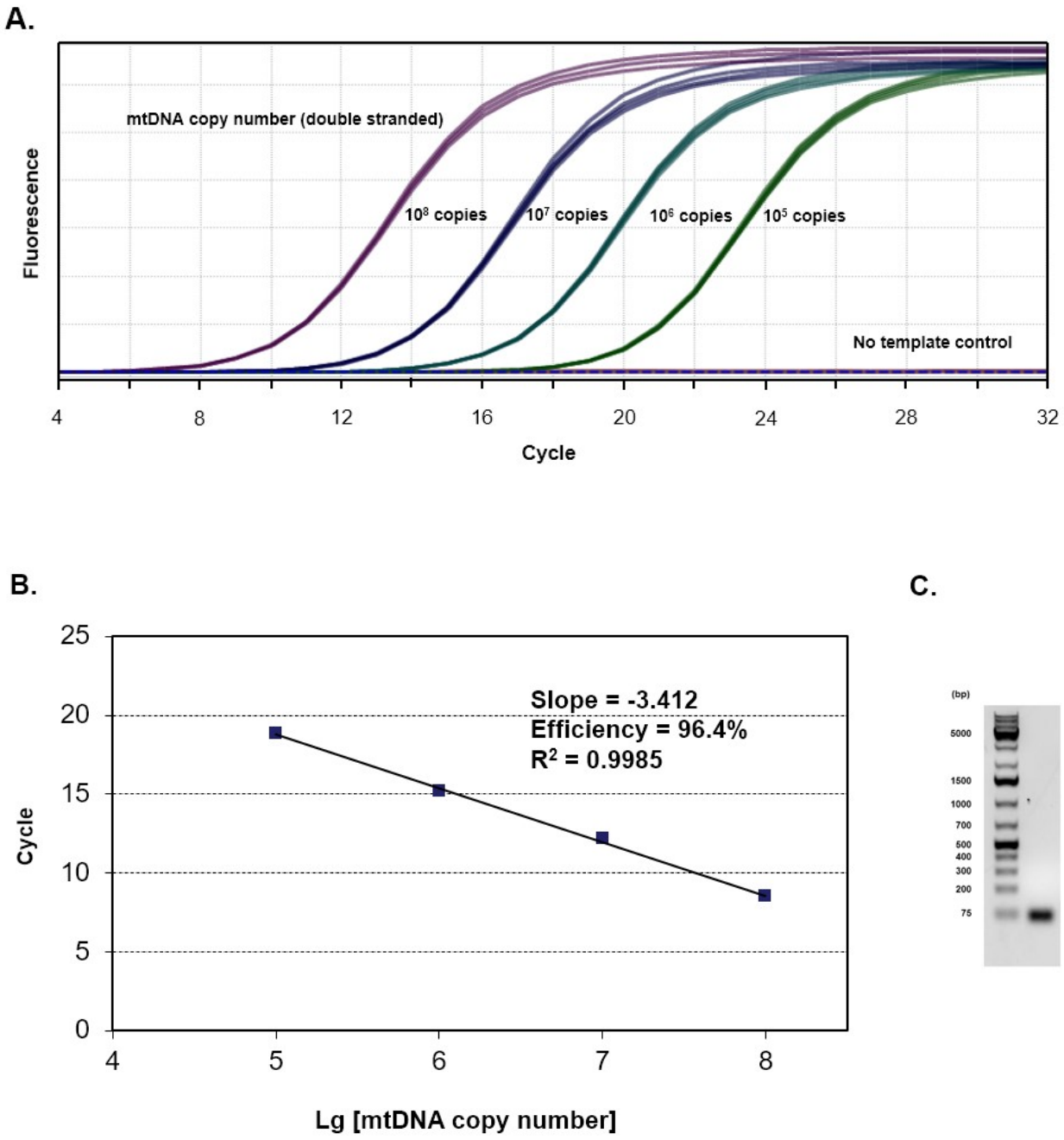


Figure 4. Quality assessment of rat mtDNA primer set. (A) qPCR amplification curves using serially diluted mtDNA template. **(B)** Derivation of qPCR efficiency of mtDNA primer set. **(C)** Separation of mtDNA qPCR product by gel electrophoresis.

Appendix 3: Quality assessment of rat single copy reference (SCR) primer set

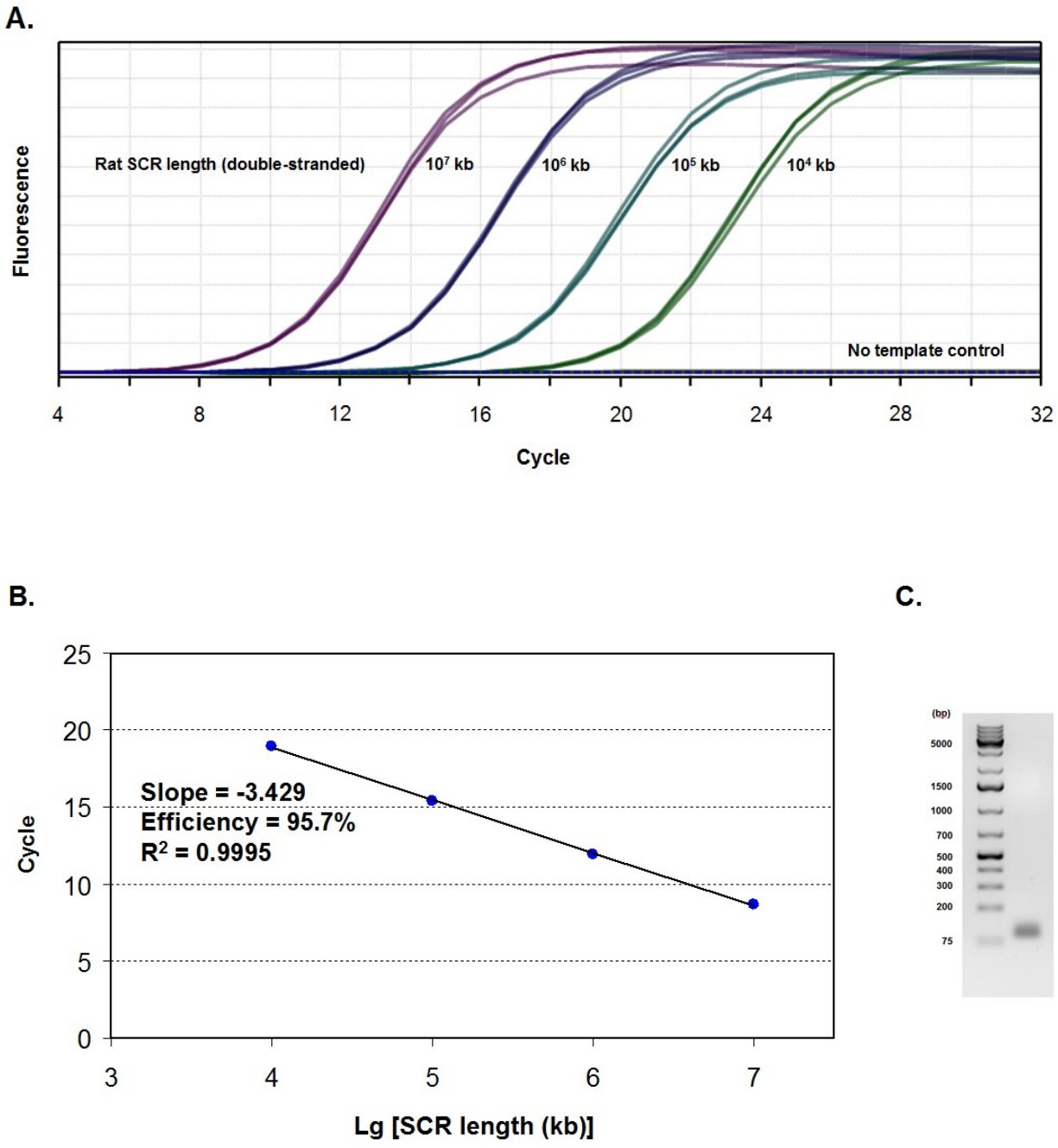


Figure 5. Quality assessment of rat single copy reference (SCR) primer set. (A) qPCR amplification curves using serially diluted SCR template. **(B)** Derivation of qPCR efficiency of SCR primer set. **(C)** Separation of SCR qPCR product by gel electrophoresis.

Appendix 4: Method for quantifying reference rat genomic DNA sample (Cat #R8958e)

To quantify the reference rat genomic DNA sample (Cat #R8958e), 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, 2 and 3, the telomere length, the mtDNA and SCR copy number of reference genomic DNA sample are determined to be:

Total telomere length (double-stranded): $(2.51 \pm 0.18) \times 10^4$ Mb

Total mtDNA copy number (double-stranded): $(372 \pm 8) \times 10^3$ copies

Total SCR length (double-stranded): 79.4 ± 1.4 kb

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

Total number of diploid cells = $(79.4 \pm 1.4 \text{ kb}) / 0.2 \text{ kb} = 397 \pm 7$ cells

Telomere length per diploid cell (double-stranded) = $(2.51 \pm 0.18) \times 10^4$ Mb / (397 ± 7)
= 6.32 ± 0.45 Mb

There are 84 chromosome ends in one rat diploid cell, therefore,

Average telomere length on each chromosome end = $(6.32 \pm 0.45 \text{ Mb}) / 84$
= 75.2 ± 5.4 kb

mtDNA copy number per diploid cell (double-stranded)
= $(372 \pm 8) \times 10^3$ copies / (397 ± 7)
= 937 ± 20 copies

Conclusions:

- The average telomere length of reference genomic DNA sample (Cat #R8958e) is 6.32 ± 0.45 Mb per diploid cell, or 75.2 ± 5.4 kb per chromosome end.
- The average mtDNA copy number of reference genomic DNA sample (Cat #R8958e) is 937 ± 20 copies per diploid cell.