Human ELOVL2 Promoter and Global DNA Dual Methylation Quantification qPCR Assay Kit (HEGDD)

Catalog #7018
100 reactions

Product Description

DNA methylation at CpG islands in promoters regulates gene expression. It is a dynamic epigenetic process affected by various genetic and environmental factors. Numerous diseases including cancer have been linked to aberrant global changes in genomic DNA methylation. It is generally thought that global genomic DNA methylation level can be represented by the methylation level at repetitive elements. One example of this includes the repetitive Long Interspersed Nuclear Element-1 (LINE-1), which compromises 17-18% of the human genome and is usually heavily methylated in most normal primary cells. Furthermore, the CpG island methylation level at human ELOVL2 gene promoter has been well-associated with chronological age in various populations, cell types, and tissues. ELOVL2 promoter methylation level is therefore a potential marker for human chronological age.

ScienCell's Human ELOVL2 Promoter and Global DNA Dual Methylation Quantification qPCR Assay Kit (HEGDD) is designed to simultaneously quantify the level of human ELOVL2 promoter and LINE1 promoter methylation. The reference DNA sample consists of a 1:1 ratio of methylated ELOVL2 promoter copies to non-methylated ELOVL2 promotor copies (both copies represent bisulfite converted promoter sequences) and a 1:1 ratio of methylated LINE1 promoter copies to non-methylated LINE1 promotor copies (both copies represent bisulfite converted promoter sequences), and serves as a reference for calculating the ratio of methylated to non-methylated ELOVL2 promotor and LINE1 promoter of target samples. The carefully designed primers ensure: (i) high efficiency for trustworthy quantification; and (ii) negligible 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
8998a	Methylated ELOVL2 promoter (MEP) primer set, lyophilized	1 vial	-20°C
8998b	Non-methylated ELOVL2 promoter (OEP) primer set, lyophilized	1 vial	-20°C
7008a	Methylated LINE1 promoter (MLP) primer set, lyophilized	1 vial	-20°C
7008b	Non-methylated LINE1 promoter (OLP) primer set, lyophilized	1 vial	-20°C
7018c	Nuclease-free H_2O	8 mL	4°C
7018d	Reference DNA sample	200 µL	-20°C

Additional Materials Required (Materials Not Included in Kit)

Component	Recommended
DNA isolation kit	DNeasy Blood & Tissue Kit (Qiagen, Cat #69504, 69506)
bisulfite conversion kit	EpiTect Bisulfite Kits (Qiagen, Cat #59104)
qPCR plate or tube	
qPCR master mix	FastStart Essential DNA Green Master (Roche, Cat #06402712001)

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 4**). The reference DNA sample is sequenced by Sanger sequencing.

Product Use

HEGDD 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 #8998a, #8998b, #7008a and #7008b) and the reference DNA sample (Cat #7018d) at -20°C in a manual defrost freezer, and nuclease-free H_2O (Cat #7018c) at 4°C.

Procedures

***Important:* Only** *use Taq DNA polymerase-based qPCR master mix, as Pfu DNA polymerase can NOT amplify uracil-containing templates.* **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. HEGDD 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. This kit works ONLY with bisulfite converted genomic DNA samples. For genomic DNA isolation and bisulfite conversion, we recommend using DNeasy Blood & Tissue Kit (Qiagen, Cat #69504, 69506) and EpiTect Bisulfite Kits (Qiagen, Cat #59104), respectively. Please follow manufacturer's instructions to obtain bisulfite converted genomic DNA samples.

2. Prior to use, allow vials (Cat #8998a, #8998b, #7008a and #7008b) to warm to room temperature. Centrifuge the vials at 1,500x g for 1 minute.

3. Add 200 µl nuclease-free H_2O (Cat #7018c) to MEP primer set (lyophilized, Cat #8998a) to make MEP 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 H2O (Cat #7018c) to OEP primer set (lyophilized, Cat #8998b) to make OEP 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 H_2O (Cat #7018c) to MLP primer set (lyophilized, Cat #7008a) to make MLP primer stock solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.

6. Add 200 µl nuclease-free H2O (Cat #7018c) to OLP primer set (lyophilized, Cat #7008b) to make OLP primer stock solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.

7. For the reference DNA sample (Cat #7018d), prepare four qPCR reactions, one with MEP primer stock solution, one with OEP primer stock solution, one with MLP primer stock solution, and one with OLP primer stock solution. Prepare 20 µl qPCR reactions for one well as shown in Table 1.

   Table 1.

Reference DNA sample (Cat #7018d)	1 µl
Primer stock solution (MEP, OEP, MLP or OLP)	2 µl
2x qPCR master mix	10 µl
Nuclease-free H_2O (Cat #7018c)	7 µl
Total volume	***20 µl***

8. For each bisulfite converted genomic DNA sample, prepare four qPCR reactions, one with MEP primer stock solution, one with OEP primer stock solution, one with MLP

primer stock solution, and one with OLP primer stock solution. Prepare 20 µl qPCR reactions for one well as shown in Table 2.

Table 2.

Bisulfite converted genomic DNA sample	5-20 ng
Primer stock solution (MEP, OEP, MLP or OLP)	2 µl
2x qPCR master mix	10 µl
Nuclease-free H_2O (Cat #7018c)	variable
Total volume	***20 µl***

9. 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).

10. 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 the primer sets (Cat #8998a, #8998b, #7008a and #7008b), we highly recommend an annealing temperature of 60°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	60°C	20 sec	
Extension	72°C	20 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.

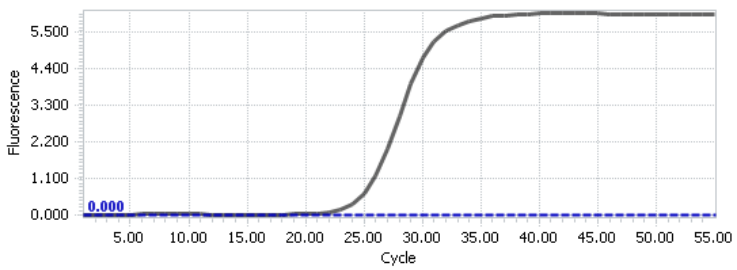
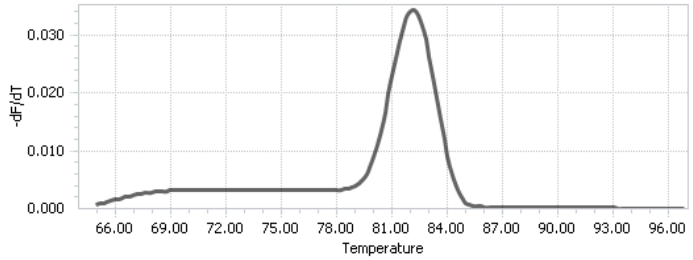


Figure 2. A typical melting peak of a qPCR product.



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 methylated ELOVL2 promoter (MEP), ΔCq (MEP) is the quantification cycle number difference of MEP between the target and the reference DNA samples.

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

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

2. For non-methylated ELOVL2 promoter (OEP), ΔCq (OEP) is the quantification cycle number difference of OEP between the target and the reference DNA samples.

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

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

3. $\Delta\Delta Cq,e = \Delta Cq\ (MEP) - \Delta Cq\ (OEP)$

4. The ratio of MEP to OEP of the target sample = $2^{-\Delta\Delta Cq,e}$

5. The percentage of methylated ELOVL2 promoter $= 2^{-\Delta\Delta Cq,e} / (2^{-\Delta\Delta Cq,e} + 1) \times 100\%$

6. For methylated LINE1 promoter (MLP), ΔCq (MLP) is the quantification cycle number difference of MLP between the target and the reference DNA samples.

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

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

7. For non-methylated LINE1 promoter (OLP), ΔCq (OLP) is the quantification cycle number difference of OLP between the target and the reference DNA samples.

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

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

8. $\Delta\Delta Cq,l = \Delta Cq\ (MLP) - \Delta Cq\ (OLP)$

9. The ratio of MLP to OLP of the target sample = $2^{-\Delta\Delta Cq,l}$

10. The percentage of methylated LINE1 promoter $= 2^{-\Delta\Delta Cq,l} / (2^{-\Delta\Delta Cq,l} + 1) \times 100\%$

Example Calculations: Comparative ΔΔCq (Quantification Cycle Value) Method

Table 4. Cq (quantification cycle) values obtained for the samples by qPCR using MEP, OEP, MLP and OLP primer sets.

Primer set	*Target sample*	*Reference sample*
MEP	25.08	20.99
OEP	25.64	20.80
MLP	24.74	21.33
OLP	23.98	21.51

- ΔCq (MEP) = Cq (MEP, target sample) - Cq (MEP, reference sample)
  = 25.08 - 20.99
  = 4.09

ΔCq (OEP) = Cq (OEP, target sample) - Cq (OEP, reference sample)
= 25.64 - 20.80
= 4.84

ΔΔCq,e = ΔCq (MEP) - ΔCq (OEP)
= 4.09- (4.84)
= -0.75

The ratio of MEP to OEP of the target sample
$= 2^{-\Delta\Delta Cq,e}$
$= 2^{0.75}$
= 1.7

The percentage of methylated ELOVL2 promoter
= 1.7/(1.7+1) x 100%
= 63%

- ΔCq (MLP) = Cq (MLP, target sample) - Cq (MLP, reference sample)
  = 24.74 – 21.33
  = 3.41

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

= 23.98 – 21.51

= 2.47

$\Delta\Delta$Cq,l = ΔCq (MLP) - ΔCq (OLP)

= 3.41- (2.47)

= 0.94

The ratio of MLP to OLP of the target sample

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

= $2^{-0.94}$

= 0.52

The percentage of methylated LINE1 promoter

= 0.52/(0.52+1) x 100%

= 34%

Conclusions: The percentage of methylated ELOVL2 promoter in the target sample is 63%, and the percentage of methylated genomic DNA in the target sample is 34%.

Appendix 1: Quality assessment of methylated ELOVL2 promoter (MEP) primer set

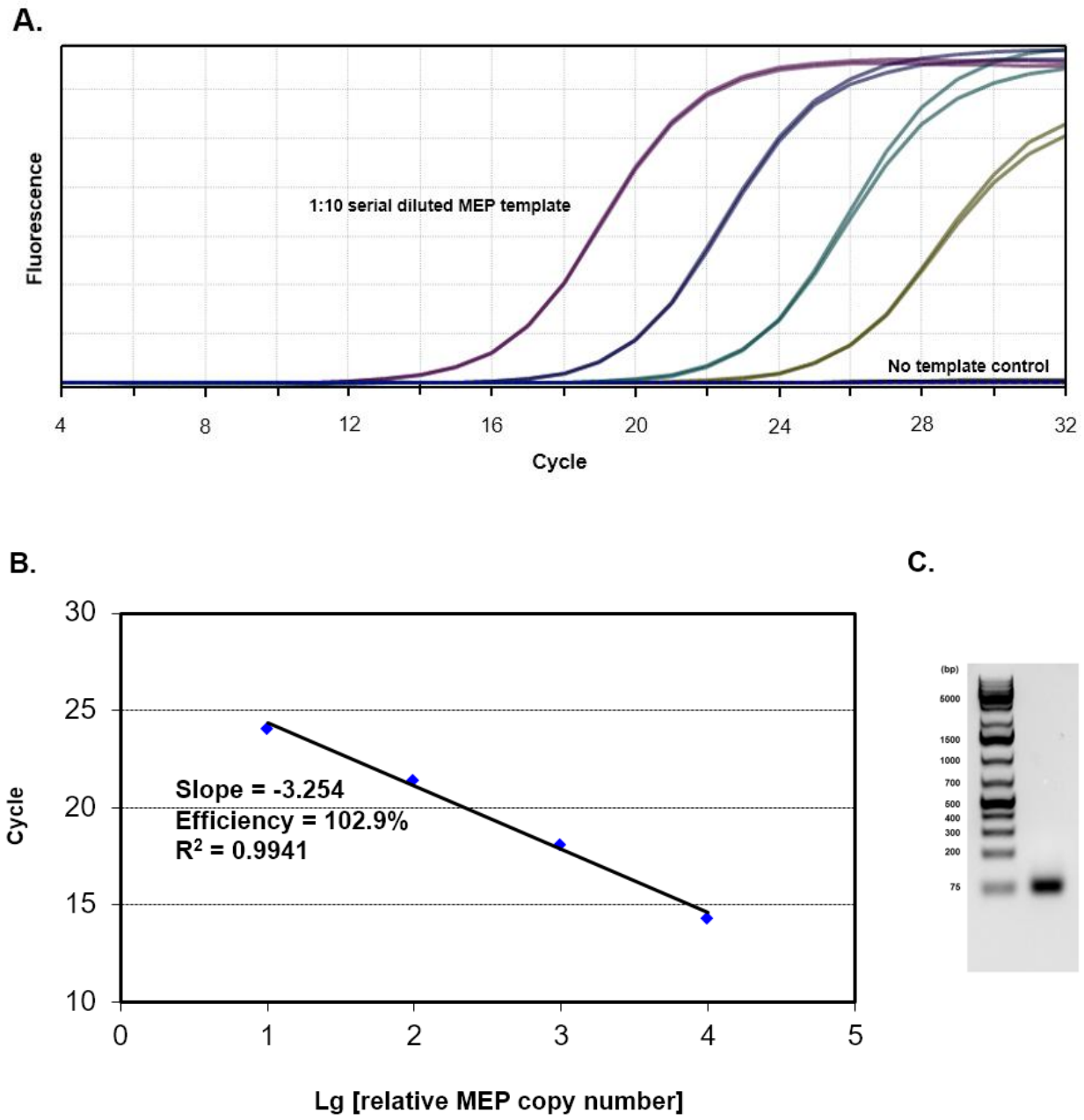


Figure 3. Quality assessment of MEP primer set. (A) qPCR amplification curves using serially diluted MEP repeats as template. **(B)** Derivation of qPCR efficiency of MEP primer set. **(C)** Separation of MEP qPCR product by gel electrophoresis.

Appendix 2: Quality assessment of non-methylated ELOVL2 promoter (OEP) primer set

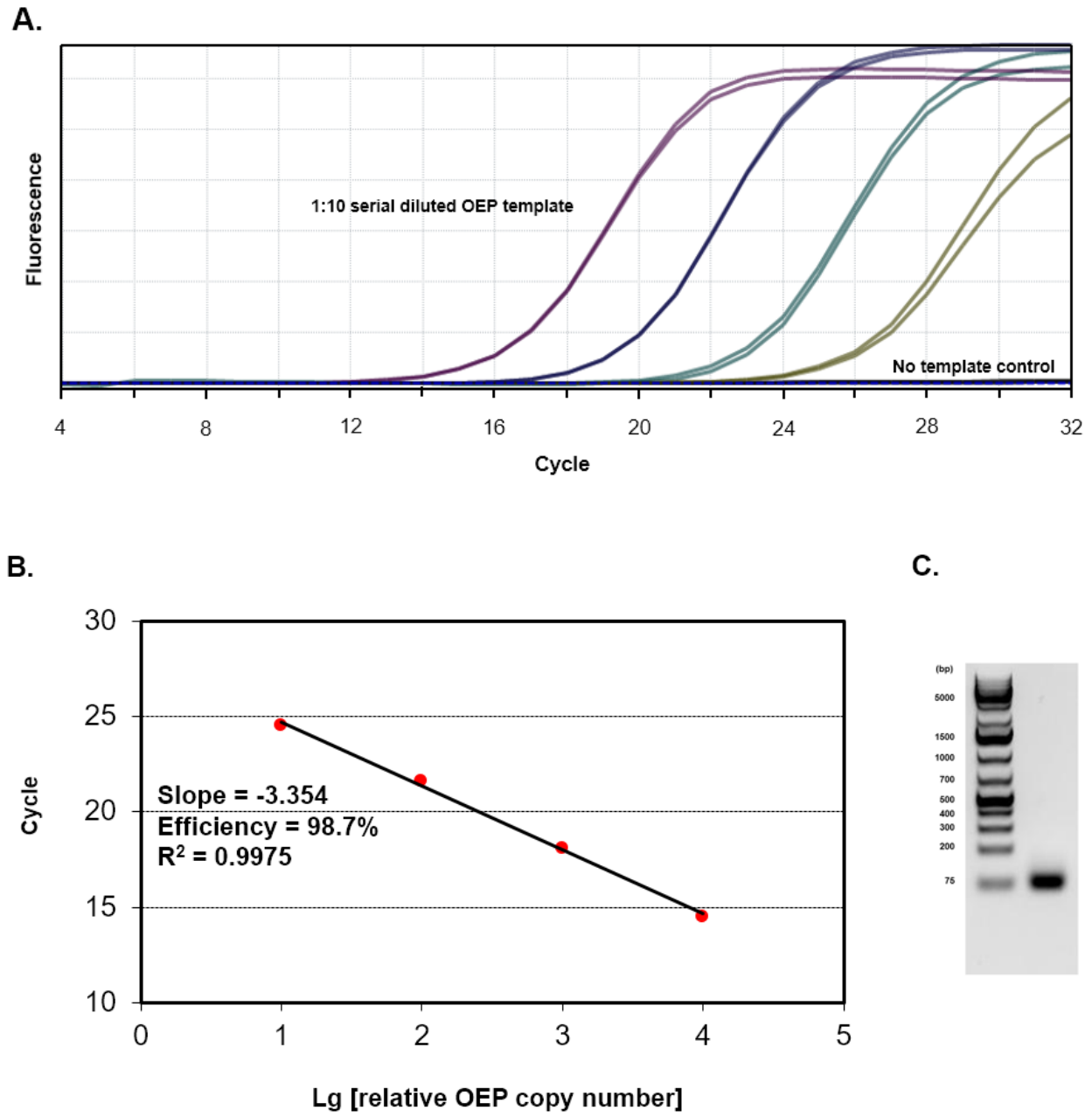


Figure 4. Quality assessment of Single copy reference (OEP) primer set. (A) qPCR amplification curves using serially diluted OEP template. **(B)** Derivation of qPCR efficiency of OEP primer set. **(C)** Separation of OEP qPCR product by gel electrophoresis.

Appendix 3: Quality assessment of methylated LINE1 promoter (MLP) primer set

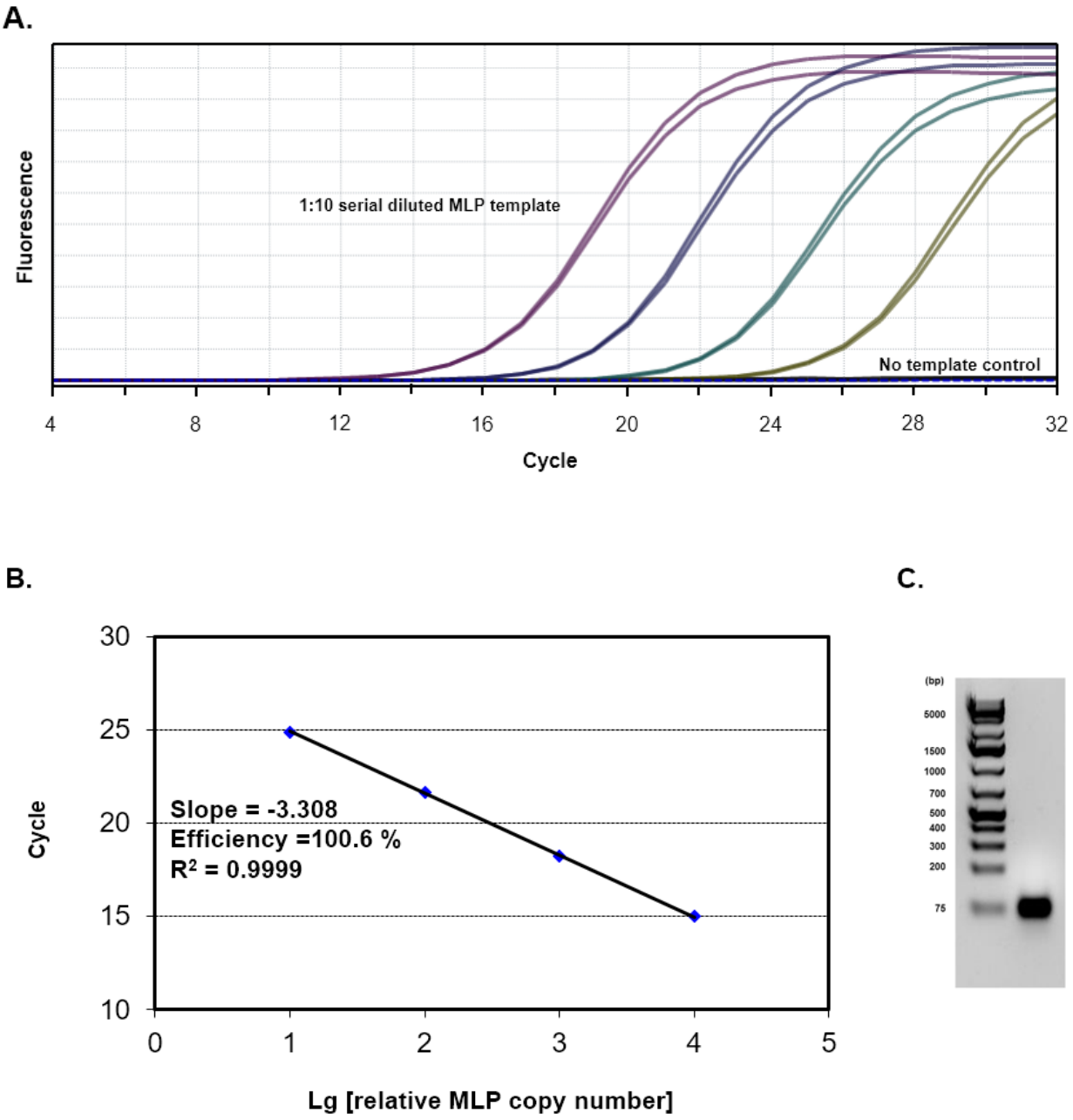


Figure 5. Quality assessment of MLP primer set. (A) qPCR amplification curves using serially diluted MLP repeats as template. **(B)** Derivation of qPCR efficiency of MLP primer set. **(C)** Separation of MLP qPCR product by gel electrophoresis.

Appendix 4: Quality assessment of non-methylated LINE1 promoter (OLP) primer set

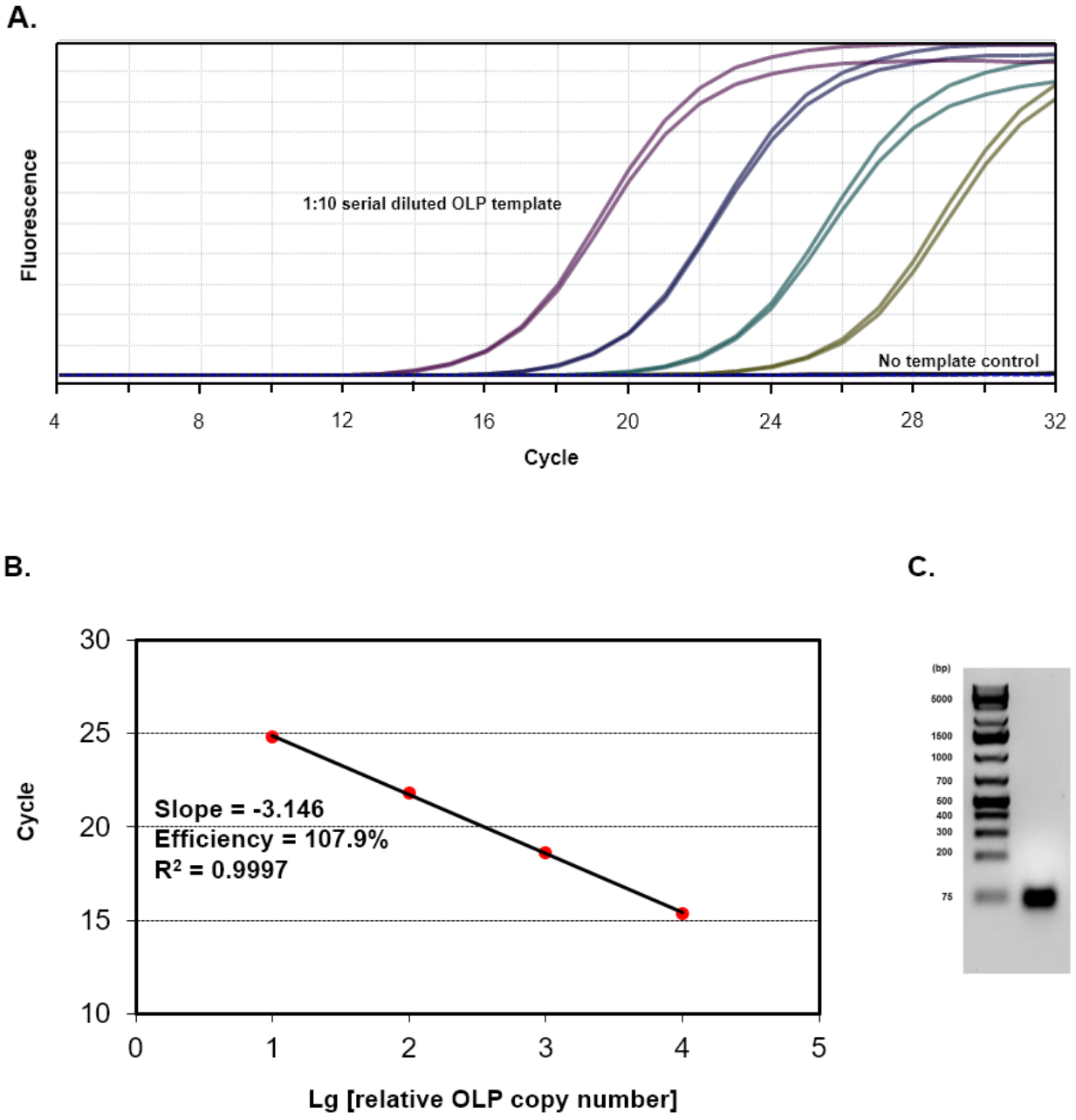


Figure 6. Quality assessment of Single copy reference (OLP) primer set. (A) qPCR amplification curves using serially diluted OLP template. **(B)** Derivation of qPCR efficiency of OLP primer set. **(C)** Separation of OLP qPCR product by gel electrophoresis.