

For Research Use

TAKARA

**Transgene Detection Primer Set
for Real Time (Mouse)**

Product Manual

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I. Description

This kit is a primer set for Real-Time PCR to detect GFP (EGFP, AcGFP1) and lacZ, which are widely used for gene marker introduced in transgenic mouse. It is possible to screen of transgenic mouse by detecting these marker genes with Real-Time PCR.

The primer sets (Ywhaz, Raver2) for detecting genes on mouse genomic DNA are also included as reference. Therefore, it is possible to make a comparison of the transgene contents between samples by relative quantification method.

II. Components (for 100 reactions*1 in each primer)

Following 6 types of primer pair are included.

	Concentration	Amount	Product size
1. GFP_primer-1*2	2 μ M each	500 μ l	127 bp
2. GFP_primer-2*3	2 μ M each	500 μ l	162 bp
3. lacZ_primer-1*4	2 μ M each	500 μ l	96 bp
4. lacZ_primer-2*4	2 μ M each	500 μ l	141 bp
5. Reference_primer-1*5	2 μ M each	500 μ l	115 bp
6. Reference_primer-2*6	2 μ M each	500 μ l	89 bp

- * 1 Frequency when using TB Green® *Premix Ex Taq*™ II with 25 μ l reaction volume.
- * 2 GFP_primer-1 is designed for consensus sequence with EGFP and AcGFP1.
- * 3 GFP_primer-2 is designed for EGFP. It is not used for AcGFP1 detection.
- * 4 lacZ_primer-1 and -2 are designed for lacZ (β -galactosidase) gene and positioned at different sites.
- * 5 Reference_primer-1 is for a part of Ywhaz gene on mouse chromosome 15.
- * 6 Reference_primer-2 is for a part of Raver2 gene on mouse chromosome 4.

III. Materials Required but not Provided

- Real-Time PCR reagent
 - TB Green *Premix Ex Taq* II (Tli RNaseH Plus) (Cat. #RR820A/B)*1
 - TB Green *Premix Ex Taq* (Tli RNaseH Plus) (Cat. #RR420A/B)*1
 - 1.5 ml microtube
 - Micropipette and tips
 - Real-Time PCR instrument
 - Thermal Cycler Dice™ Real Time System II (Cat. #TP900)*2
- * 1 We have begun the process of changing the names for Takara Bio's intercalator-based real-time PCR (qPCR) products to the "TB Green series". These products can be used the same way as before, as only the names are changing. Catalog number and product performance are unaffected by this transition.
- * 2 Not available in all geographic locations. Check for availability in your area.

IV. Storage - 20 °C

V. Protocol

1. Genomic DNA extraction

Genomic DNA can be extracted from tips of mouse tail, for example. To quantify accurately by Real-Time PCR, it is recommended to use genomic DNA with high purity, which is purified by column-using method, like NucleoSpin Tissue, or by phenol/chloroform extraction method. For determining qualitatively the existence of transgene, genomic DNA extracted by simple method can also be used (see VII. Appendix).

2. Real-Time PCR

Prepare master mixture containing components other than primer and dispense aliquots of the mixture to each tube. At the final step, add each primer pair. If reaction mixture is prepared by this order, it would suppress dispersion of the template volume, resulting in obtaining stable result.

< Example of Real-Time PCR >

Reagent : TB Green *Premix Ex Taq* II (Tli RNaseH Plus)

Real-Time PCR instrument : Thermal Cycler Dice Real Time System //

- 1) Prepare the PCR reaction mixture shown in below. When using all the 6 types of primer pair contained in this kit, prepare master mixture for 7 reactions by using components other than primer. Add 20 μ l of this mixture to each tube and add 5 μ l of each primer pair.

Reagent	< for 1 reaction >	< for 7 reactions >
TB Green <i>Premix Ex Taq</i> II (2 \times)	12.5 μ l	87.5 μ l
Primer (2 μ M each)	5.0 μ l	-
Genomic DNA *	2.0 μ l	14.0 μ l
Sterile purified water	5.5 μ l	38.5 μ l
Total	25 μ l	

- * If genomic DNA is added too much, background fluorescence of intercalator might become higher. In such case, reduce contents of genomic DNA either to 1/10 or 10 - 20 ng.

2) Perform Real-Time PCR

95 $^{\circ}$ C 1 min Initial denaturation

↓
 95 $^{\circ}$ C 5 sec
 60 $^{\circ}$ C 30 sec] 40 cycles

Dissociation curve analysis

- * PCR reaction condition should be followed by the standard condition of the TB Green *Premix Ex Taq* II. Change the initial denaturation time to 1 min.

VI. Experimental Example

Determination of homozygous/heterozygous genotype by Real-Time PCR using mouse genomic DNA as template

Genomic DNA were prepared from tail of the transgenic mouse (homozygous or heterozygous)*, which carries 1 copy of EGFP gene and lacZ gene. Then both genes were detected by performing Real-Time PCR

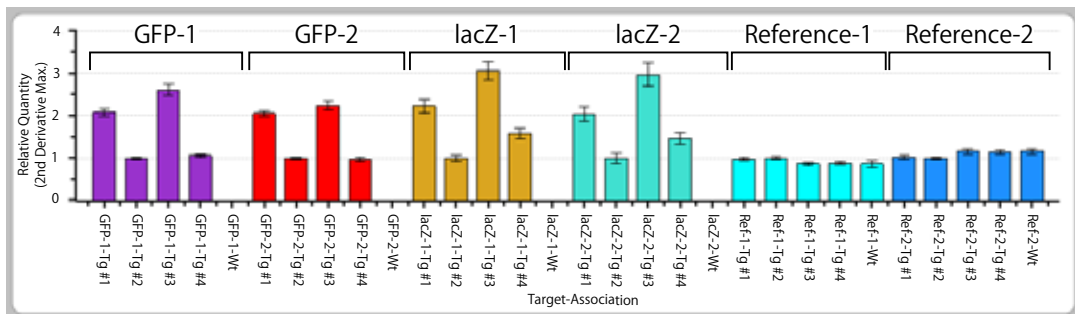
- * The tails of mouse were kindly provided by Dr. Ikenaka and Dr. Takebayashi, National Institutes of Natural Sciences in Japan.
The 5 samples used in this experiment were determined by Zygoty (homo/hetero) of the mouse mating as following; Tg#1 and Tg#3 are homo, Tg#2 and Tg#4 are hetero. Wt is a mouse which does not contain both EGFP and lacZ.

【 Procedure 】

Genomic DNA were prepared from mouse tail by using NucleoSpin Tissue. 1 μl of the obtained DNA were used for Real-Time PCR as template. The primer of this product and TB Green Premix Ex Taq (Tli RNaseH Plus) were used for Real-Time PCR reaction. Relative quantification analysis was performed by using 2 types of gene as reference.

【 Result 】

Relative quantification analysis using ΔΔCt method shows that the sample Tg#1 and Tg#3 contain twice more copy number of EGFP and lacZ than that of the Tg#2 and Tg#4. This result is consistent with actual genetic type of each sample. Furthermore, neither EGFP nor lacZ was detected in Wt, which is also consistent with the genetic type.



VII. Appendix

Simplified preparation method of genomic DNA by alkaline heat extraction

1. Cut 2 - 3 mm of mouse tail tips and place it to 1.5 ml screw cap tube.
2. Add 100 μ l of NaOH solution (0.025 N NaOH, 2 mM EDTA).
3. Incubate for 20 - 30 minutes at 100°C
4. Spin-down briefly, add 100 μ l of 40 mM Tris-HCl (pH 7.5 - 8.0) and close the cap immediately.
5. Vortex to mix.
6. Centrifuge for 30 seconds, and use 0.5 - 1.0 μ l of the supernatant for Real-Time PCR as template.

VIII. Related Products

NucleoSpin Tissue (Cat. #740952.10/.50/.250)

TB Green® *Premix Ex Taq*™ II (Tli RNaseH Plus) (Cat. #RR820A/B)

TB Green® *Premix Ex Taq*™ (Tli RNaseH Plus) (Cat. #RR420A/B)

Thermal Cycler Dice™ Real Time System II (Cat. #TP900)*

* Not available in all geographic locations. Check for availability in your area.

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