

RBRC 06174

Strain name C57BL/6N-Ppp4r2<tm2b(EUCOMM)Hmgu>/E07

| Primer | primer_name | primer_seq | length | Reference |
|--------|-------------|-------------------------|--------|-----------|
| 1 | | CTCTTGATTTGCTGTGATGGCGG | 24 | RIKEN BRC |
| 2 | | CGGACCCTTAAGCTGAAGTGCTC | 23 | RIKEN BRC |
| 3 | | GGCACATGGCTGAATATCGACGG | 23 | RIKEN BRC |
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Reaction Components

Enzyme TOYOBO KOD FX (Code.No : KFX-101)

| Reagent | Conc | Reaction 1 | 2 | 3 | 4 |
|--------------|------------|------------|---|---|---|
| H2O | | 1.8 | | | |
| PCR buffer | 2 X | 10.0 | | | |
| dNTP | 2.0 mM | 4.0 | | | |
| primer 1 | 10 pmol/μl | 1.0 | | | |
| primer 2 | 10 pmol/μl | 1.0 | | | |
| primer 3 | 10 pmol/μl | 1.0 | | | |
| DNA | | 1.0 | | | |
| Taq | 1 U/μl | 0.2 | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| total | | 20 | | | |

(μL)

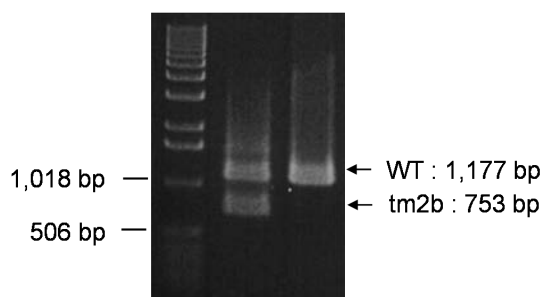
Reaction Conditions

| step | temp (°C) | time (sec) | note |
|------|-----------|------------|-----------------------|
| 1 | 94 | 120 | |
| 2 | 98 | 10* | |
| 3 | 60 | 30* | |
| 4 | 68 | 120* | *Repeat for 30 cycles |
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Marker 1 kb DNA ladder (Invitrogen, Cat. 15615-024)

Gel 1% agarose in 1 X TAE (100 V, 30 min)

Marker tm2b/+ +/+



| primer_set | product_size | product (allele type) |
|------------|--------------|-----------------------|
| primer 1 | primer 2 | 1,177 bp WT |
| primer 2 | primer 3 | 753 bp tm2b |
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| | | |

Comment Standard PCR

Novagen KOD Xtreme™ Hot Start DNA Polymerase can be purchased from EMD.

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Strain name C57BL/6N-Ppp4r2<tm2b(EUCOMM)Hmgu>/E07

| Primer | primer_name | primer_seq | length | Reference |
|--------|-------------|----------------------------|--------|-----------|
| 1 | | GCACACAACCTTTGGAACTTGAGTGG | 26 | RIKEN BRC |
| 2 | | CGGACCCTTAAGCTGAAGTGCTC | 23 | RIKEN BRC |
| 3 | | GGCACATGGCTGAATATCGACGG | 23 | RIKEN BRC |
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Reaction Components

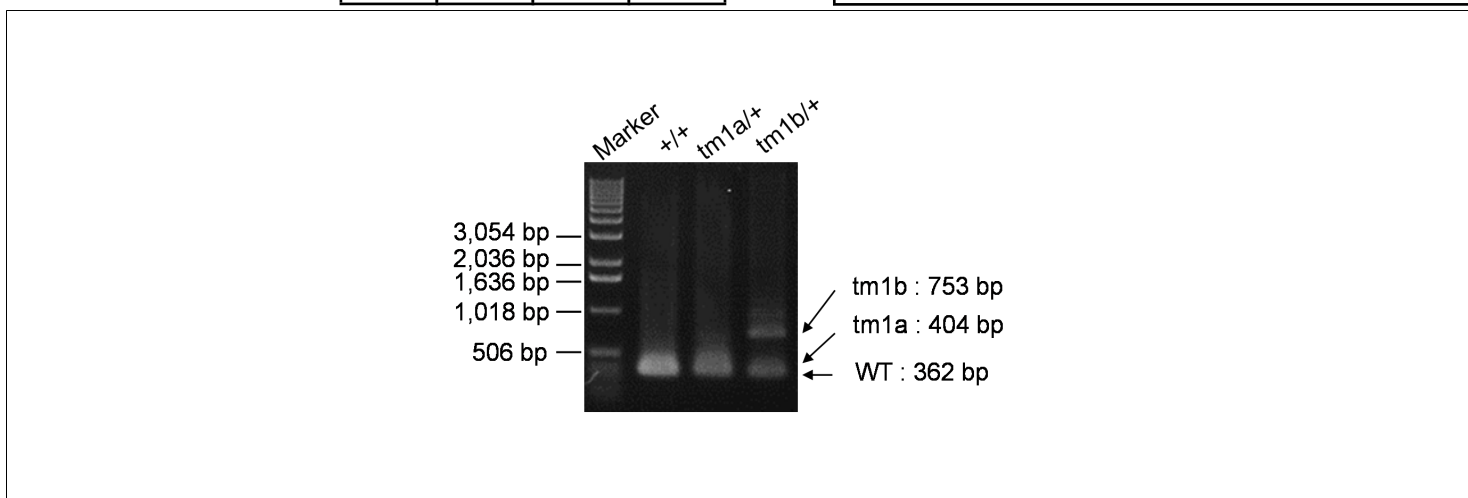
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

| step | temp (°C) | time (sec) | note |
|------|-----------|------------|-----------------------|
| 1 | 94 | 120 | |
| 2 | 98 | 10* | |
| 3 | 60 | 30* | |
| 4 | 68 | 300* | *Repeat for 30 cycles |
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| Reagent | Conc | Reaction 1 | 2 | 3 | 4 | (µL) |
|--------------|------------|------------|---|---|---|------|
| H2O | | 1.8 | | | | |
| PCR buffer | 2 X | 10.0 | | | | |
| dNTP | 2.0 mM | 4.0 | | | | |
| primer 1 | 10 pmol/µl | 1.0 | | | | |
| primer 2 | 10 pmol/µl | 1.0 | | | | |
| primer 3 | 10 pmol/µl | 1.0 | | | | |
| DNA | | 1.0 | | | | |
| Taq | 1 U/µl | 0.2 | | | | |
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| | | | | | | |
| total | | 20 | | | | |

Marker 1 kb DNA ladder (Invitrogen, Cat. 15615-024)
Gel 1% agarose in 1 X TAE (100 V, 30 min)



| primer_set | product_size | product (allele type) |
|------------|--------------|-----------------------|
| primer 1 | primer 2 | 362 bp WT |
| primer 1 | primer 2 | 404 bp flox (tm1a) |
| primer 2 | primer 3 | 753 bp lacZ(tm1b) |
| | | |
| | | |
| | | |

Comment [Standard PCR](#)

Novagen KOD Xtreme™ Hot Start DNA Polymerase can be purchased from EMD.