# PCR protocol

Sheet ID PS_00075
Release date : 6/6/2006
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RBRC 00758 Strain name MKK6 Knockout mice

| Primer | primer_name | primer_seq | length | | |
|---|---|---|---|---|---|
| 1 | 00758 | TCTAGACAGTCTCTCTTTCGAC | 22 | | RIKEN BRC |
| 2 | MKK6 WT | ACCTTCCTGAGCCTTGGTAG | 20 | | Depositor's primer |
| 3 | PGKF | GCGCCTACCGGTGGATGTGGAATGTGTGCG | 30 | | |

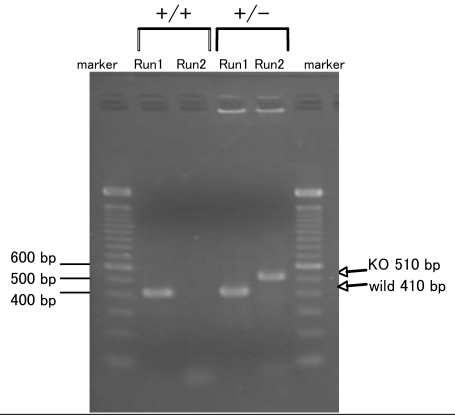
## Reaction Components

Enzyme TaKaRa Ex Taq (Cat. No. RR001A)

| | Conc | Run 1 | Run 2 | Run 3 | Run 4 |
|---|---|---|---|---|---|
| H2O | | 16.3 | 16.3 | | |
| PCR buffer | 10 X | 2.5 | 2.5 | | |
| dNTP | 2.5 mM | 2.0 | 2.0 | | |
| primer 1 | 10 pmol/μl | 1.0 | 1.0 | | |
| primer 2 | 10 pmol/μl | 1.0 | 0 | | |
| primer 3 | 10 pmol/μl | 0 | 1.0 | | |
| DNA | | 2.0 | 2.0 | | |
| Taq | | 0.2 | 0.2 | | |
| | total | 25 | 25 | | |

## Reaction Conditions

| step | temp (℃) | time (sec) | note |
|---|---|---|---|
| 1 | 94 | 300 | |
| 2 | 94 | 45 | * |
| 3 | 60 | 30 | * |
| 4 | 72 | 120 | * *Repeat for 38 cycles |
| 5 | 72 | 300 | |




## Marker

100 bp ladder (Invitrogen, Cat. 15628-050)

## Gel

2% agarose in 1 X TAE (100 V, 30 min)

## PCR products

| primer_set | | product_size | product |
|---|---|---|---|
| primer 1 | primer 2 | 410 bp | WT |
| primer 1 | primer 3 | 510 bp | KO |

## Comment