# PCR protocol

Sheet ID PS_05021
Release date : 12/20/2012
Print date : 7/31/2014

RBRC 04109 Strain name B6.129S6-Calca<tm1Hku>

| Primer | primer_name | primer_seq | length | | |
|---|---|---|---|---|---|
| 1 | EAD_4840C | GGTCCTGAGAGAGACATTTGCAC | 23 | | RIKEN BRC |
| 2 | 04109WT2 | TGCACCCTATGCCTCTTAGCAC | 22 | | RIKEN BRC |
| 3 | PGKF | GCGCCTACCGGTGGATGTGGAATGTGTGCG | 30 | | |

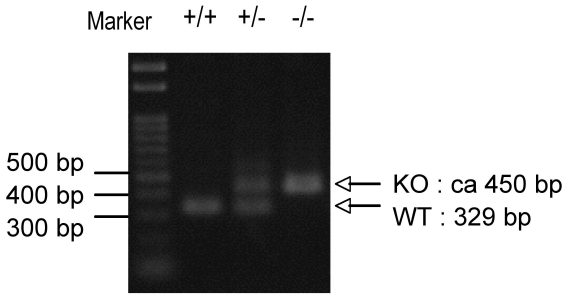
## Reaction Components

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

| | Conc | Run 1 | Run 2 | Run 3 | Run 4 |
|---|---|---|---|---|---|
| H2O | | 1.8 | | | |
| PCR buffer | 2 X | 10.0 | | | |
| dNTP | 2 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 | | | |

## Reaction Conditions

| step | temp (℃) | time (sec) | note |
|---|---|---|---|
| 1 | 94 | 120 | |
| 2 | 98 | 10 | * |
| 3 | 63 | 30 | * |
| 4 | 68 | 60 | * *Repeat for 30 cycles |




## Marker

Gene Ladder 100 (Wako, Cat. 316-06951)

## Gel

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

## PCR products

| primer_set | | product_size | product |
|---|---|---|---|
| primer 1 | primer 2 | 329 bp | WT |
| primer 2 | primer 3 | ca 450 bp | KO |

## Comment

# PCR protocol

Sheet ID PS_03642
Release date : 01/27/2011
Print date : 7/31/2014

RBRC 04109 Strain name B6.129S6-Calca<tm1Hku>

| Primer | primer_name | primer_seq | length | | |
|---|---|---|---|---|---|
| 1 | EAD_4840C | GGTCCTGAGAGAGACATTTGCAC | 23 | | RIKEN BRC |
| 2 | 04109WT2 | TGCACCCTATGCCTCTTAGCAC | 22 | | RIKEN BRC |
| 3 | PGKF | GCGCCTACCGGTGGATGTGGAATGTGTGCG | 30 | | |

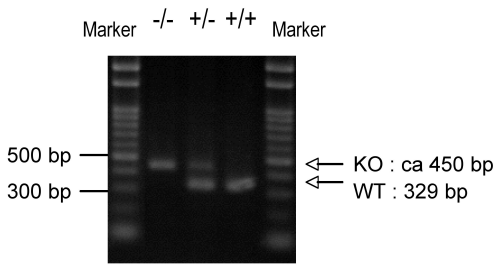
## Reaction Components

Enzyme: QIAGEN Multiplex PCR Kit (Cat. No. 206143)

| | Conc | Run 1 | Run 2 | Run 3 | Run 4 |
|---|---|---|---|---|---|
| H2O | | 1.0 | | | |
| Master Mix | 2 X | 5.0 | | | |
| primer 1 | 10 pmol/μl | 1.0 | | | |
| primer 2 | 10 pmol/μl | 1.0 | | | |
| primer 3 | 10 pmol/μl | 1.0 | | | |
| DNA | | 1.0 | | | |
| | total | 10 | | | |

## Reaction Conditions

| step | temp (℃) | time (sec) | note |
|---|---|---|---|
| 1 | 95 | 900 | |
| 2 | 94 | 30 | * |
| 3 | 60 | 90 | * |
| 4 | 72 | 90 | * *Repeat for 30 cycles |
| 5 | 72 | 600 | |




**Marker**

Gene Ladder 100 (Wako, Cat. 316-06951)

**Gel**

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

**PCR products**

| primer_set | | product_size | product |
|---|---|---|---|
| primer 1 | primer 2 | 329 bp | WT |
| primer 2 | primer 3 | ca 450 bp | KO |

**Comment**