

# PCR protocol

Sheet ID PS\_07341  
Release date : 8/23/2017  
Print date : 8/23/2017

RBRC 10047

Strain name B6(D2)-Sufu<Rgsc2070>

| Primer | primer_name | primer_seq                | length |                                  |
|--------|-------------|---------------------------|--------|----------------------------------|
| 1      |             | TCAGCTGGGAAGTCTGTCCT      | 20     | PLoS One. 10(3):e0119455 (2015). |
| 2      | Rgsc2070_WT | CTCCACAGAGAACACCTTCTGTATC | 25     | RIKEN BRC                        |
| 3      | Rgsc2070_MT | CTCCACAGAGAACACCTTCTGTATA | 25     | RIKEN BRC                        |
|        |             |                           |        |                                  |
|        |             |                           |        |                                  |
|        |             |                           |        |                                  |
|        |             |                           |        |                                  |
|        |             |                           |        |                                  |
|        |             |                           |        |                                  |
|        |             |                           |        |                                  |

## Reaction Components

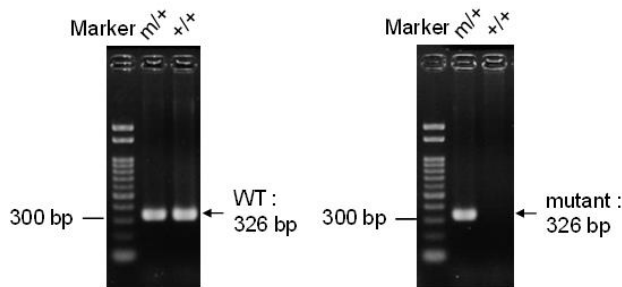
|        |                                            |
|--------|--------------------------------------------|
| Enzyme | QIAGEN Multiplex PCR Kit (Cat. No. 206143) |
|--------|--------------------------------------------|

## Reaction Conditions

| step | temp (°C) | time (sec) | note                  |
|------|-----------|------------|-----------------------|
| 1    | 95        | 900        |                       |
| 2    | 94        | 30*        |                       |
| 3    | 65        | 90*        |                       |
| 4    | 72        | 60*        | *Repeat for 30 cycles |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |
|      |           |            |                       |

|            | Conc       | Run 1 | Run 2 | Run 3 | Run 4 |
|------------|------------|-------|-------|-------|-------|
| H2O        |            | 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        |            | 1.0   | 1.0   |       |       |
| Master Mix | 2 X        | 5.0   | 5.0   |       |       |
|            |            |       |       |       |       |
|            |            |       |       |       |       |
|            |            |       |       |       |       |
|            |            |       |       |       |       |
|            |            |       |       |       |       |
|            |            |       |       |       |       |
|            |            |       |       |       |       |
|            |            |       |       |       |       |
|            |            |       |       |       |       |
| total      |            | 10    | 10    |       |       |

## Separated PCR



## 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   | 326 bp       | WT      |
| primer 1   | 326 bp       | mutant  |
|            |              |         |
|            |              |         |
|            |              |         |
|            |              |         |

## Comment