# PCR protocol

Sheet ID PS_07298
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**RBRC** 09904 **Strain name** B6.129-Has1<tm1Nita>Has2<tm1.1Nita>

| Primer | primer_name | primer_seq | length | | |
|---|---|---|---|---|---|
| 1 | KOA 2 | AACATGGTGCTAGTGAGATTGTTGG | 25 | | |
| 2 | | CAGTACTTGACAGAGTCTCTCTTC | 24 | | RIKEN BRC |
| 3 | PGK forward 2 | AGTATTGTTTTGCCAAGTTCTAATTCCATC | 30 | | |
| 4 | HAS2CKO F-6 | TGTAAGAAAACAGTGTCTTGCCTGAGTGG | 29 | | Depositor's primer |
| 5 | HAS2 CKO-8 | CAGGGAGTTAAGAAGAGACAGAGCCTG | 27 | | Depositor's primer |
| 6 | intron 1 | CGTTCAGGTTGGACTTCAGCTGTTGG | 26 | | RIKEN BRC |
| | | | | | |
| | | | | | |
| | | | | | |

## Reaction Components

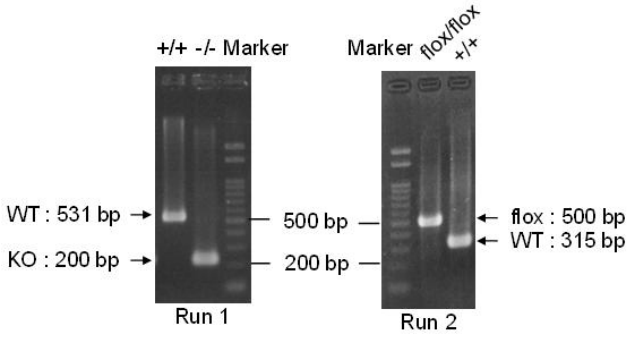
**Enzyme** TOYOBO KOD FX (Code.No : KFX-101)

| | Conc | Run 1 | Run 2 | Run 3 | Run 4 |
|---|---|---|---|---|---|
| H2O | | 1.8 | 1.8 | | |
| PCR buffer | 2 X | 10.0 | 10.0 | | |
| dNTP | 2.0 mM | 4.0 | 4.0 | | |
| primer 1 | 10 pmol/μl | 1.0 | 0 | | |
| primer 2 | 10 pmol/μl | 1.0 | 0 | | |
| primer 3 | 10 pmol/μl | 1.0 | 0 | | |
| primer 4 | 10 pmol/μl | 0 | 1.0 | | |
| primer 5 | 10 pmol/μl | 0 | 1.0 | | |
| primer 6 | 10 pmol/μl | 0 | 1.0 | | |
| DNA | | 1.0 | 1.0 | | |
| Taq | 1 U/μl | 0.2 | 0.2 | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | total | 20 | 20 | | |

## Reaction Conditions

| step | temp (℃) | time (sec) | | note |
|---|---|---|---|---|
| 1 | 94 | 120 | * | 【Run 1】 |
| 2 | 98 | 10 | * | |
| 3 | 60 | 30 | * | |
| 4 | 68 | 60 | * | *Repeat for 30 cycles |
| | | | | |
| 1 | 94 | 120 | * | 【Run 2】 |
| 2 | 98 | 10 | * | |
| 3 | 60 | 30 | * | |
| 4 | 68 | 180 | * | *Repeat for 30 cycles |
| | | | | |
| | | | | |
| | | | | |

Standard 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 | primer 2 | 531bp | WT (Has1) |
| primer 1 | primer 3 | 200 bp | KO (Has1) |
| primer 4 | primer 5 | 315 bp | WT |
| primer 4 | primer 5 | 500 bp | flox |
| primer 5 | primer 6 | 1.6 kb | null (After cre mediated excision) |
| | | | |

**Comment**

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