# PCR protocol

Sheet ID PS_07397
Release date : 8/23/2017
Print date : 8/23/2017

**RBRC** 10082 **Strain name** B6-Kcnj11<tm2.1Sse>

| Primer | primer_name | primer_seq | length | | |
|---|---|---|---|---|---|
| 1 | Kir6.2-loxP 9418F | ATGTAGAGTGGTGGGGTGCG | 20 | | Depositor's primer |
| 2 | Kir6.2-loxP 11606Rwt | CCAAGACGGGCTTTGAGACC | 20 | | Depositor's primer |
| 3 | promoter | ATGGCCTGGCTGAGGACAGTG | 21 | | RIKEN BRC |

## 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 | 2.0 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 | | | |

## Reaction Conditions

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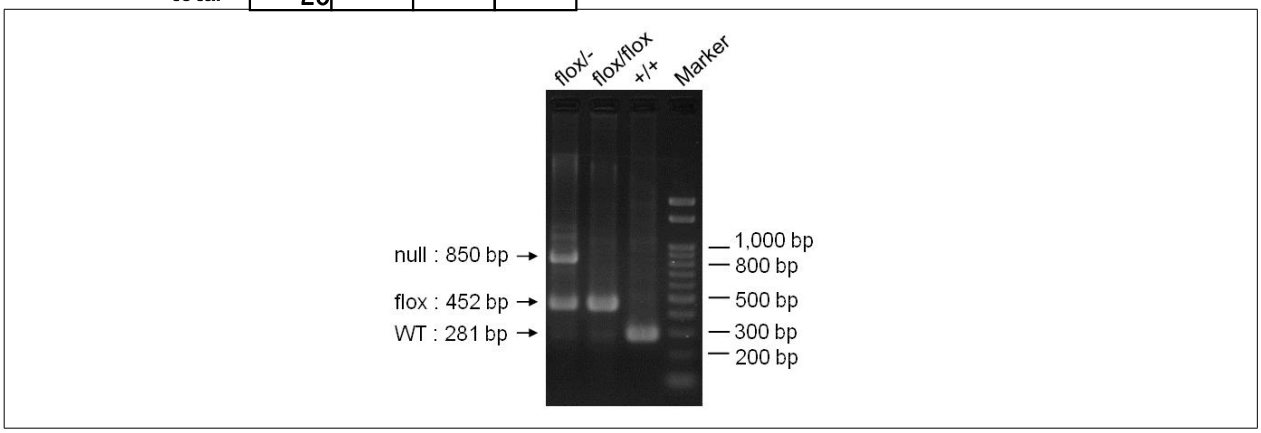
Standard PCR




**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 | 281 bp | WT |
| primer 1 | primer 2 | 452 bp | flox |
| primer 2 | primer 3 | 850 bp | null |
| | | | |
| | | | |
| | | | |

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