# PCR protocol

Sheet ID PS_08555
Release date : 12/25/2023
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**RBRC** 12089 **Strain name** C57BL/6-F9<tm1.1(hF9_R338L)Tsuka>

| Primer | primer_name | primer_seq | length | | Reference |
|---|---|---|---|---|---|
| 1 | F9 KI long-F1 | GTACTTTAATAACAAGCCCTGACCA | 25 | | Depositor's primer |
| 2 | F9 KI short-R1 | AGGCCTCTGAGTATTATGGTTCTCT | 25 | | Depositor's primer |

**Reaction Components**

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

| Reagent | Conc | Reaction 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| H2O | | 2.8 | | | |
| 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 | | | |
| DNA | | 1.0 | | | |
| Taq | 1 U/μl | 0.2 | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | total | 20 | | | |

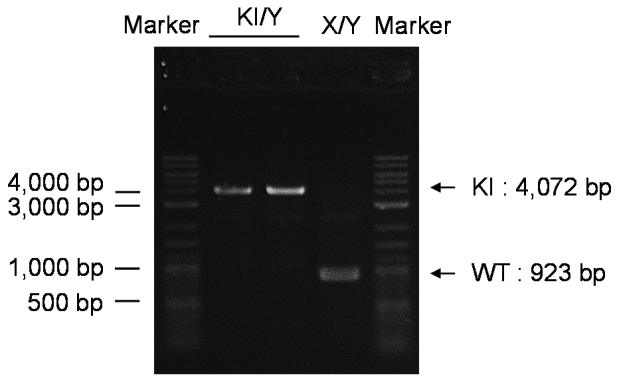
(μL)

**Reaction Conditions**

| step | temp (℃) | time (sec) | note |
|---|---|---|---|
| 1 | 94 | 120 | |
| 2 | 98 | 10* | |
| 3 | 65 | 30* | |
| 4 | 68 | 300* | *Repeat for 30 cycles |

**Marker** 2-Log DNA Ladder (NEB, Cat. N3200)

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




| primer_set | | product_size | product (allele type) |
|---|---|---|---|
| primer 1 | primer 2 | 923 bp | WT |
| primer 1 | primer 2 | 4,072 bp | KI |
| | | | |
| | | | |
| | | | |
| | | | |

**Comment**

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