# PCR protocol

Sheet ID PS_06421
Release date : 9/29/2016
Print date : 9/29/2016

**RBRC** 09295 **Strain name** B6.Cg-Unc5d<tm1.1>

| Primer | primer_name | primer_seq | length | | |
|---|---|---|---|---|---|
| 1 | F4-UncL | TCAAAGTTGAGCGAAACCTTGGTTTCCTGG | 30 | | Depositor's primer |
| 2 | R5-UncL | TCTAAGCTGGTATAAGCACCCGATAGGGAG | 30 | | Depositor's primer |

## Reaction Components

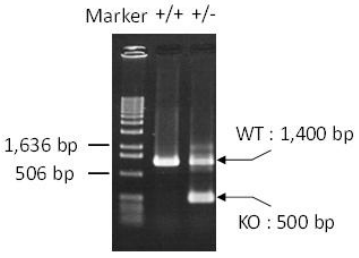
**Enzyme** TOYOBO KOD FX (Code.No : KFX-101)

| | Conc | Run 1 | Run 2 | Run 3 | Run 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 | | | |

## Reaction Conditions

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

Standard PCR




**Marker**

1 kb DNA ladder (Invitrogen, Cat. 15615-024)

**Gel**

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

**PCR products**

| primer_set | | product_size | product |
|---|---|---|---|
| primer 1 | primer 2 | 1400 bp | WT |
| primer 1 | primer 2 | 500 bp | KO |
| | | | |
| | | | |
| | | | |

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