# PCR protocol

Sheet ID PS_05502
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RBRC 06037 Strain name C57BL/6-Pou5f1<tm1(Pou5f1/EGFP)Osb>

| Primer | primer_name | primer_seq | length | | |
|---|---|---|---|---|---|
| 1 | | GAGCTTATGATCTGATGTCCATCTC | 25 | | RIKEN BRC |
| 2 | Oct4-Ex5-Rev | GCTATCTACTGTGTGTCCCAGTCTTTATTT | 30 | | Depositor's primer |
| 3 | EGFP-Rev | TTACTTGTACAGCTCGTCCATGCCG | 25 | | Depositor's primer |

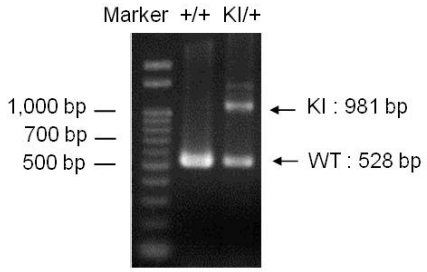
## Reaction Components

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 |




## 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 | 528 bp | WT |
| primer 1 | primer 3 | 981 bp | KI |

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