# PCR protocol

**RBRC** 02761 **Strain name** B6.Cg-Atg3<tm1>

| Primer | primer_name | primer_seq | length |
|---|---|---|---|
| 1 | forward | CTATTCAGTGTGACAGTGCTAGGG | 24 |
| 2 | reverse | CTGCATGGGTGAACTGAACACATAG | 25 |
| 3 | | CCTTCTATCGCCTTCTTGACGAG | 23 |

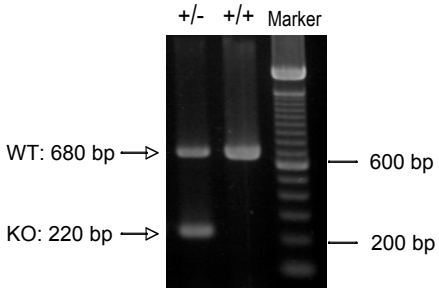
## Reaction Components

**Enzyme** TaKaRa Ex Taq (Cat. No. RR001A)

| | Conc | Run 1 | Run 2 | Run 3 | Run 4 |
|---|---|---|---|---|---|
| H2O | | 16.3 | | | |
| PCR buffer | 10 X | 2.5 | | | |
| dNTP | 2.5 mM | 2.0 | | | |
| 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 | | 0.2 | | | |
| | total | 25 | | | |

## Reaction Conditions

| step | temp (℃) | time (sec) | note |
|---|---|---|---|
| 1 | 94 | 180 | |
| 2 | 94 | 30* | |
| 3 | 60 | 30* | |
| 4 | 72 | 60* | *Repeat for 30 cycles |
| 5 | 72 | 300 | |




**Marker**

100 bp ladder (Invitrogen, Cat. 15628-050)

**Gel**

2% agarose in 1 X TAE (100 V, 30 min)

## PCR products

| primer_set | | product_size | product |
|---|---|---|---|
| primer 1 | primer 2 | 680 bp | WT |
| primer 1 | primer 3 | 220 bp | KO |
| | | | |
| | | | |
| | | | |

**Comment**