

RBRC 04714

Strain name B6;129S2-Spesp1<tm10sb>

| Primer | primer_name | primer_seq | length | Reference |
|--------|-------------|--------------------------------|--------|--------------------|
| 1 | #2882 | CTTTTCTCTCAGCTCTGATGGTACACGCTT | 30 | Depositor's primer |
| 2 | DT5' | GCCTTCTATCGCCTTCTTGACGAGTTCTTC | 30 | Depositor's primer |
| 3 | #3079 | GTGAGCCAGTGTGCGTCTAC | 20 | Depositor's primer |
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Reaction Components

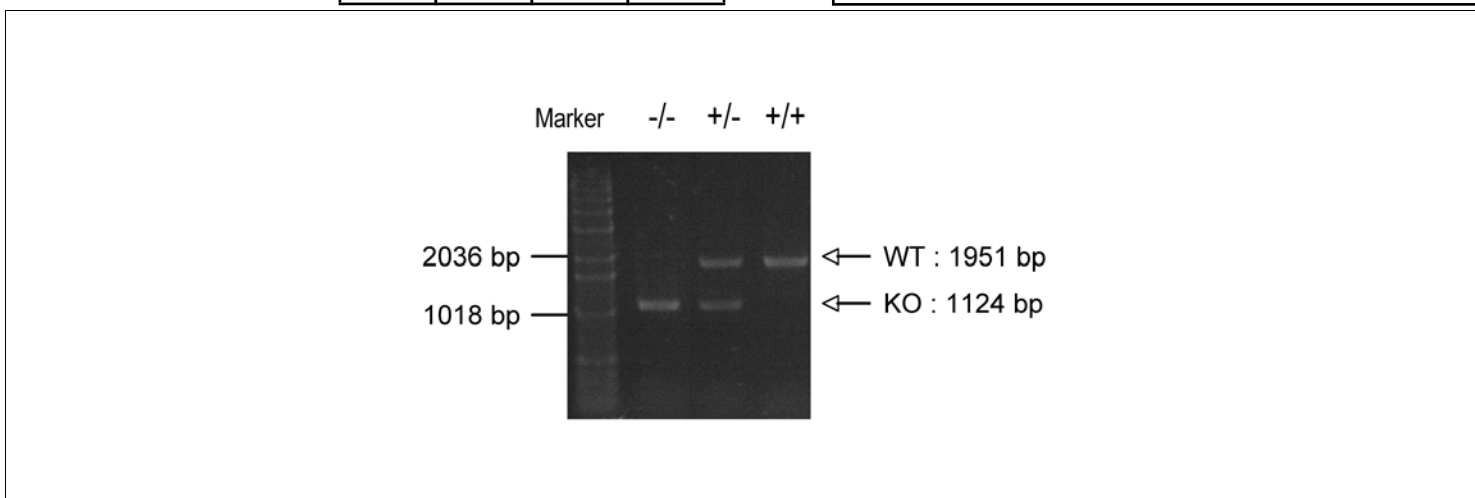
Enzyme TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)

Reaction Conditions

| step | temp (°C) | time (sec) | note |
|------|-----------|------------|-----------------------|
| 1 | 94 | 180 | |
| 2 | 94 | 30* | |
| 3 | 60 | 30* | |
| 4 | 72 | 120* | *Repeat for 30 cycles |
| 5 | 72 | 300 | |
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| Reagent | Conc | Reaction 1 | 2 | 3 | 4 | (μL) |
|--------------|------------|------------|---|---|---|------|
| H2O | | 3.9 | | | | |
| PCR buffer | 2 X GC I | 10.0 | | | | |
| 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 | 5 U/μl | 0.1 | | | | |
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| total | | 20 | | | | |

Marker 1 kb DNA ladder (Invitrogen, Cat. 15615-024)
Gel 1% agarose in 1 X TAE (100 V, 30 min)



| primer_set | product_size | product (allele type) | |
|------------|--------------|-----------------------|----|
| primer 1 | primer 3 | 1951 bp | WT |
| primer 2 | primer 3 | 1124 bp | KO |
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Comment Standard PCR