

RBRC 01474

Strain name B6.129-Fas^{tm1Osa}/OsaRbrc

| Primer | primer_name | primer_seq | length | Reference |
|--------|-------------|-------------------------|--------|------------------------------|
| 1 | intron 8 | CTCACCTAGCGCAGATGTGAAC | 22 | RIKEN BRC |
| 2 | exon 9 | CTGTCCATCAATGGAAGGTCTTC | 23 | RIKEN BRC |
| 3 | Neo | GATTCGCAGCGCATCGCCTT | 20 | Nature 443, 998-1002 (2006). |
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Reaction Components

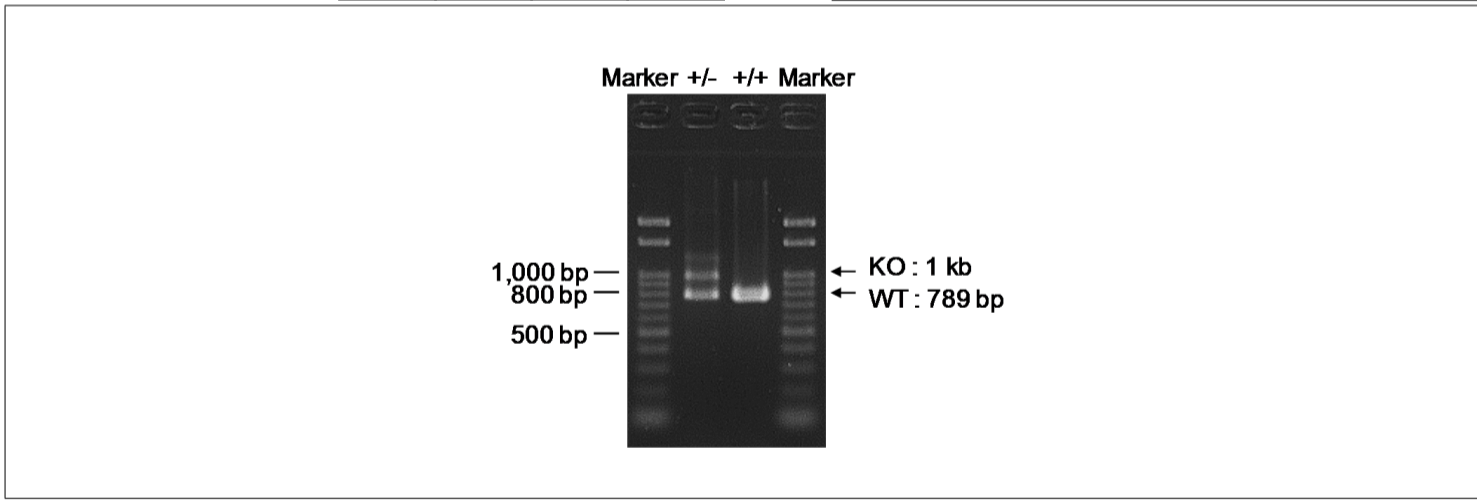
Enzyme TOYOBO KOD FX (Code.No : KFX-101)

Reaction Conditions

| step | temp (°C) | time (sec) | note |
|------|-----------|------------|-----------------------|
| 1 | 94 | 120 | |
| 2 | 98 | 10* | |
| 3 | 60 | 30* | |
| 4 | 68 | 60* | *Repeat for 30 cycles |
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| Reagent | Conc | Reaction 1 | 2 | 3 | 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 | | | |
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| total | | 20 | | | |

Marker Gene Ladder 100 (Wako, Cat. 316-06951)
Gel 2% agarose in 1 X TAE (100 V, 30 min)



| primer_set | product_size | product (allele type) | |
|------------|--------------|-----------------------|----|
| primer 1 | primer 2 | 789 bp | WT |
| primer 2 | primer 3 | 1 kb | KO |
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Comment Standard PCR

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

RBRC 01474

Strain name B6 Fas-KO

| Primer | primer_name | primer_seq | length | Reference |
|--------|---------------|----------------------------|--------|--------------------|
| 1 | Fas sense | GCTTGAGTAAATACATCCCG | 20 | Depositor's primer |
| 2 | Fas antisense | TGTCCATCAATGGAAGGTCT | 20 | Depositor's primer |
| 3 | NeoR primer | GATTCGCAGCGCATCGCCTTCTATCG | 26 | Depositor's primer |
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Reaction Components

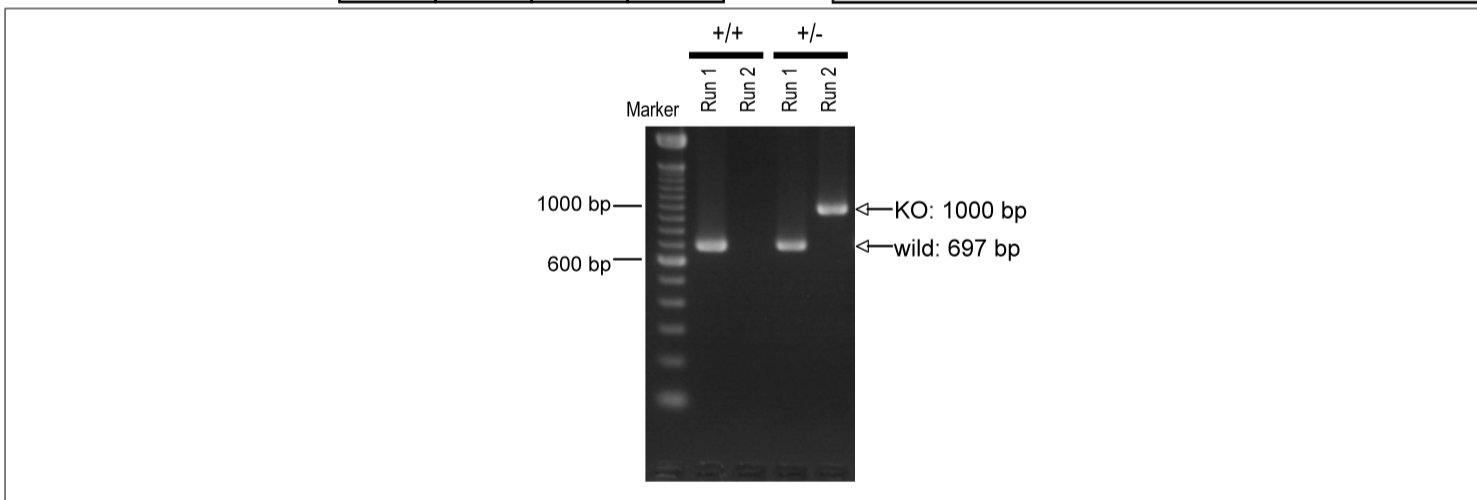
Enzyme TaKaRa LA Taq with GC buffer (Cat. No. RR02AG)

Reaction Conditions

| step | temp (°C) | time (sec) | note |
|------|-----------|------------|-----------------------|
| 1 | 94 | 180 | |
| 2 | 94 | 30* | |
| 3 | 57 | 30* | |
| 4 | 72 | 60* | *Repeat for 30 cycles |
| 5 | 72 | 300 | |
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| Reagent | Conc | Reaction 1 | 2 | 3 | 4 | (μ L) |
|--------------|------------------|------------|------|---|---|------------|
| H2O | | 4.8 | 4.8 | | | |
| PCR buffer | 2 X GC I | 10.0 | 10.0 | | | |
| dNTP | 2.5 mM | 2.0 | 2.0 | | | |
| primer 1 | 10 pmol/ μ l | 1.0 | 0 | | | |
| primer 2 | 10 pmol/ μ l | 1.0 | 1.0 | | | |
| primer 3 | 10 pmol/ μ l | 0 | 1.0 | | | |
| DNA | | 1.0 | 1.0 | | | |
| Taq | | 0.2 | 0.2 | | | |
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| total | | 20 | 20 | | | |

Marker 100 bp ladder (Invitrogen, Cat. 15628-050)
Gel 2% agarose in 1 X TAE (100 V, 35 min)



| primer_set | product_size | product (allele type) | |
|------------|--------------|-----------------------|----|
| primer 1 | primer 2 | 697 bp | WT |
| primer 2 | primer 3 | 1000 bp | KO |
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Comment Separated PCR