

RBRC 11926

Strain name C57BL/6N-Nfyav1<em1Nbo>

| Primer | primer_name | primer_seq | length | Reference |
|--------|-------------|-------------------------|--------|--------------------|
| 1 | 5'Nfyav-F2 | GAGTCCCAAGCCACTGATGA | 20 | Depositor's primer |
| 2 | 5'Nfyav-R | ACCATGGATGAAGGAAGCTAGCC | 22 | Depositor's primer |
| 3 | 3'Nfyav-R | TCCTGCCTCCATATCCCAAC | 20 | Depositor's primer |
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Reaction Components

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

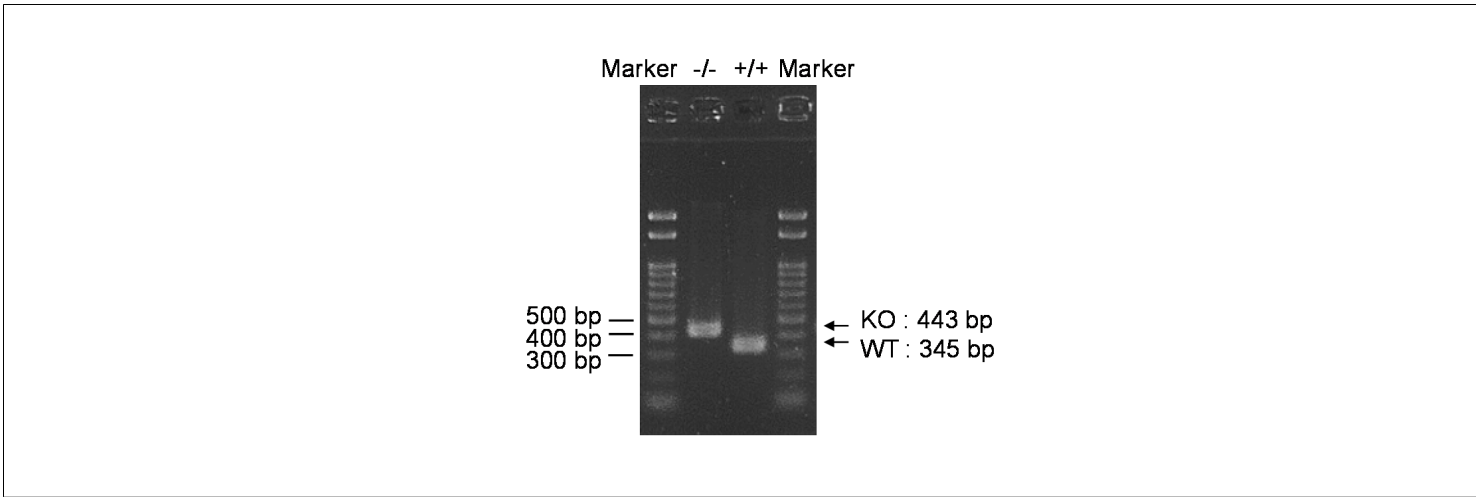
Reaction Conditions

| 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, 40 min)



| primer_set | product_size | product (allele type) |
|------------|--------------|-----------------------|
| primer 1 | primer 2 | 345 bp WT |
| primer 1 | primer 3 | 443 bp KO |
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Comment

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