# PCR protocol

Sheet ID PS_08254
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**RBRC** 11509 **Strain name** B6.B6D2-Amy1<em1(luc/mVenus)Matam>

| Primer | primer_name | primer_seq | length | | Reference |
|---|---|---|---|---|---|
| 1 | Amy1-F | TGAAATTCTTCCTGCTGCTTTCC | 23 | | Depositor's primer |
| 2 | Amy1-R | TGTCTTACCTGCACACCTGC | 20 | | Depositor's primer |
| 3 | GFP-Full-R | CTTGTACAGCTCGTCCATGCCGAG | 24 | | RIKEN BRC |

**Reaction Components**

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

| 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 | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | **total** | 20 | | | |

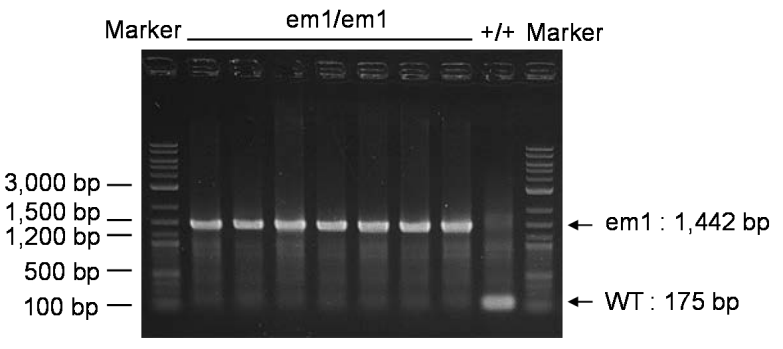
(μL)

**Reaction Conditions**

| step | temp (℃) | time (sec) | note |
|---|---|---|---|
| 1 | 94 | 120 | |
| 2 | 98 | 10 | * |
| 3 | 60 | 30 | * |
| 4 | 68 | 180 | * *Repeat for 30 cycles |

**Marker** 2-Log DNA Ladder (NEB, Cat. N3200)

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




| primer_set | | product_size | product (allele type) | |
|---|---|---|---|---|
| primer 1 | primer 2 | 175 bp | WT | +/+ (wild-type): 175 bp |
| primer 1 | primer 3 | 1,442 bp | em1 | em1/em1 (homozygous): 1,442 bp |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

**Comment** Standard PCR

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