# PCR protocol

Sheet ID PS_08059
Release date : 10/1/2020
Print date : 10/1/2020

RBRC 10893

Strain name C57BL/6N-Slc5a10<em1Rbrc>

| Primer | primer_name | primer_seq | length | | Reference |
|---|---|---|---|---|---|
| 1 | ck_MGE216-F | CAGTTGCTGGACTTTGAGTTGTTAC | 25 | | RIKEN DNA Bank |
| 2 | ck_MGE216-R | CACACACGCCCCTCCTGCTTTGCTG | 25 | | RIKEN DNA Bank |

**Reaction Components**

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

| Reagent | Conc | Reaction 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| H2O | | 2.8 | | | |
| 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 | | | |
| DNA | | 1.0 | | | |
| Taq | 1 U/μl | 0.2 | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | total | 20 | | | |

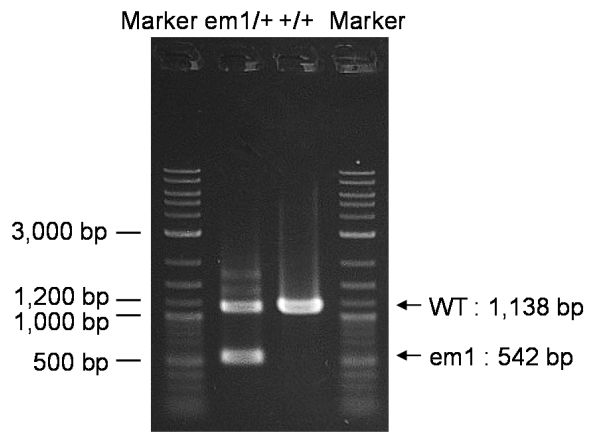
(μL)

**Reaction Conditions**

| step | temp (℃) | time (sec) | note |
|---|---|---|---|
| 1 | 94 | 120 | |
| 2 | 98 | 10* | |
| 3 | 60 | 30* | |
| 4 | 68 | 90* | *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 | 1,138 bp | WT |
| primer 1 | primer 2 | 542 bp | em1 |
| | | | |
| | | | |
| | | | |
| | | | |

+/+ (wild-type): 1,138 bp
em1/+ (heterozygous): 1,138 bp and 542 bp

**Comment** Standard PCR

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