

## STRAIN: EM04455

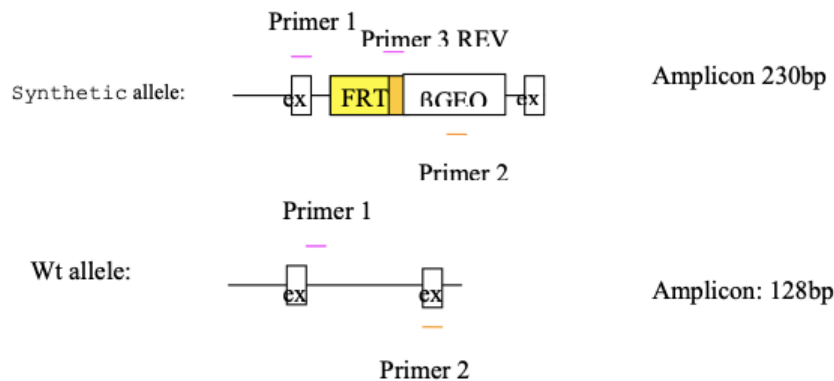
MUTATION: Mtch2

BLASTOCYST DONOR: C57BL/6J-Tyr<c-2J>

E.S. CELL: C57BL6/N JM8.F6

CLONE NAME: Mtch2 HEPD0522\_1\_C06

NOTE: Oligonucleotide construction in 5' homology near frt site.



**PRIMER MTCH2 FOR** 5'-TCT-CAT-CTA-GAC-CTG-ATG-CCT-CAG-3'  
**PRIMER MTCH2 REV** 5'-TCT-GTC-TCA-TTG-TCT-AGG-CTG-GCC-3'  
**PRIMER FTR REV** 5'-CCT TCC TCC TAC ATA GTT GGC AGT-3'

|                  |                          |          |
|------------------|--------------------------|----------|
| PRIMER MTCH2 FOR | 15pm / total volume 30ul | Tm: 72°C |
| PRIMER MTCH2 REV | 10pm / total volume 30ul | Tm: 72°C |
| PRIMER FTR REV   | 15pm / total volume 30ul | Tm: 72°C |

### PCR MIX

|                        |             |
|------------------------|-------------|
| DNTPS (2mM)            | 3ul         |
| PCR BUFFER 10x         | 3ul         |
| PRIMER MTCH2 FOR       | (0.5pm/ul)  |
| PRIMER MTCH2 REV       | (0.3pm/ul)  |
| PRIMER FTR REV         | (0.5pm/ul)  |
| H2O                    |             |
| TAQ POLYMERASE (5U/ul) | 0.3ul       |
| DNA                    | 2ul         |
| <b>TOTAL</b>           | <b>30ul</b> |

### PCR PROGRAM:

- 1) 95°C x 2'00''
- 2) 95°C x 0'30''
- 3) 46°C x 0'30''
- 4) 72°C x 1'00''
- 5) step 2 to step 5 x 50 repeats
- 6) 72°C x 10'00''
- 7) 4°C forever
- 8) End

PCR EXPECTED BAND (2-2,5% agarose gel):

WILDE TYPE : 1 (128bp)

Heterozygous: 2 (128bp, 230bp)

Homozygous : 1 (230bp)