

## Genotyping of Urokinase-type Plasminogen Actovator (uPA) Deficient Mice by PCR

**Purpose:** To identify C57BL/6 wild type mouse uPA gene (uPA +/+) from uPA deficient mouse gene (uPA -/-).

**Gene Information:** The mouse uPA gene is located on chromosome 14. It contains 11 exons and 10 introns. The neo cassette is inserted between exon 2 and exon 11.

### Primers:

- muPA-7p** = 5'-CTC CCG TGG CTG GGT AGT GG-3'  
(intron 10-11, 7719-7738 in mouse uPA gene, GenBank: M17922)
- muPA-4m** = 5'-AGA GGA CGG TCA GCA TGG GAA C-3'  
(exon 11, 8029-8008 in mouse uPA gene, GenBank:M17922)
- mPGK-2m** = 5'-GCC TTG GGA AAA GCG CCT C-3'  
(neo gene, 1092-1074 in mouse phosphoglycerate kinase-1 promoter, GenBank: X15339)

### PCR:

Reaction

1. Genomic DNA (1  $\mu$ l)
2. Promega PCR Master Mix (2x; 10  $\mu$ l)
3. Primers (10 pmol/ $\mu$ l; 1  $\mu$ l each)
4. Taq polymerase (0.1  $\mu$ l)
5. PCR water (5.9  $\mu$ l)

Total reaction volume is 20  $\mu$ l

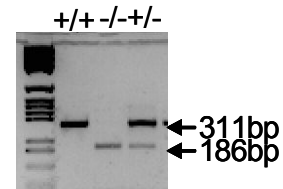
Program

1. 1 cycle - 95°C for 2 min
2. 35 cycles - 95°C for 1 min  
59°C for 1 min  
72°C for 1 min
3. 1 cycle - 72°C 5 min
4. Hold at 4°C.

### Expected Bands on TBE Agarose Gel Electrophoresis:

Resolve DNA bands on a 2% agarose gel.

uPA +/+	311 bp
uPA +/-	311 bp and 186 bp
uPA -/-	186 bp



### Screening Compound Deficient Mice:

This strategy may be used to detect the uPA alleles in compound deficient mice, as the primers are designed specifically for the uPA gene and its neomycin cassette.

### Reference:

Leif R Lund et al. EMBO J 2006; 25, 2686-2697

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