

Genotyping of Low Density Lipoprotein (LDL) Receptor^{-/-} Mice by PCR

Purpose: To identify LDL receptor deficient mouse gene from wild type gene.

Gene Information: The LDL receptor is located on Chromosome 9. LDL receptor^{-/-} mice were created by insertion of the *neo* cassette into exon-4 so a truncated non-functional protein is detectable.

Primers:

LDLr14 = 5'-AGGTGAGATGACAGGAGATC-3'
LDLr332 = 5'-AGGATGACTTCCGATGCCAG-3'
LDLr696 = 5'-GCAGTGCTCCTCATCTGACTTG-3'

Reaction:

1. Genomic DNA (3 µl)
2. Promega PCR Master Mix (2X; 10µl)
3. Primers (0.1µl)
4. Taq DNA polymerase (Promega, 0.1 µl)
5. PCR Water (6.6µl)

Program:

LDL receptor

NO HOT START NEEDED.

1. Cycle 1: (35X) Step 1 - 95°C for 1 min
 Step 2 - 50°C for 1 min
 Step 3 - 72°C for 2 min
2. Cycle 2: (1X) Step 1 - 72°C for 5 min
3. Cycle 3: (1X) Step 1 - Hold at 4.0°C for ∞

Expected bands by TBE agarose gel electrophoresis:

LDL receptor +/+ = 383 bp
LDL receptor -/- = 800 bp

Screening for Double Deficient Mice:

This strategy works well for LDL-receptor^{-/-} mice and should work well for double-KO mice also

References: Ishibashi et al. *J Clin Invest* 1993; **92**: 883-893.

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Date of last update: 11-21-06

Person updating: DR