

Genotyping of Chemokine (C-C motif) Receptor 2 (CCR2) Deficient Mice by PCR

Purpose: To identify the CCR2 receptor deficient mouse gene from wild type gene.

Gene Information: The CCR2 receptor is located on chromosome 9. CCR2 receptor^{-/-} mice were created neo cassette insertion disrupting the entire coding region except first 39 nucleotides and 5' untranslated region.

Primers:

1. PIFC =Common Upstream Primer 5'-GTGTGTGCAGGTTCCAATGGA G-3'
2. GhpA =Knockout Downstream Primer 5'-GGAAGACAATAGCAGGCATGC-3'
3. WU =WT Downstream Primer 5'-CCTTCATCAAGCTCTTGG-3'

Reaction: Needs 2 PCR Reactions- one for neocassette and one for WT.

Reaction 1: For neocassette

1. Genomic DNA (1 µl)
2. Oligos, PIFC 0.25 µl +GhpA 0.25µl
3. PCR multimix (10 µl)
4. Taq (.1 µl)

Total reaction volume - 20 µl made up with PCR water

Reaction 2: For WT

1. Genomic DNA (1 µl)
2. Oligos, PIFC 0.25µl +WU 0.25 µl
3. PCR multimix (10 µl)
4. Taq (.1 µl)

Total reaction volume - 20 µl made up with PCR water

Program: Reaction 1

CCR2

NO HOT START NEEDED.

1. 1 cycle - 94°C for 2 min
2. 36 cycles - 94°C for 1 min
52°C for 1 min
72°C for 2 min
3. 1 cycle - 72°C for 5 min
4. Hold at - 4°C

Reaction 2

CCR2

NO HOT START NEEDED.

1. 1 cycle - 94°C for 2 min
2. 36 cycles - 94°C for 1 min
57°C for 1 min
72°C for 2 min
3. 1 cycle - 72°C for 5 min

4. Hold at - 4°C

Expected bands by TBE agarose gel electrophoresis:

1. PIFC+GHpA - About 1Kb for -/- allele
2. PIFC+WU - About 1Kb for +/+ allele

Screening for double KO mice:

This strategy works well for CCR2-receptor^{-/-} mice and should work well for double-KO mice also. (It has worked well in APOE^{-/-}-XCCR2^{-/-})

References:

Boring et al. J Clin Invest 1997 100: 2552-2561.

File name: Z:\AAA PPG\Rateri Core\PCR protocols\CCR2 PCR protocol.wpd

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Person updating: DR