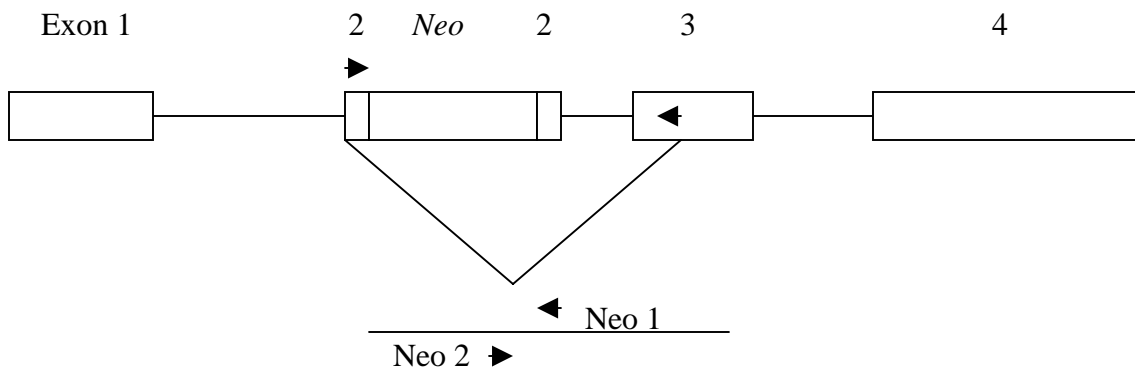
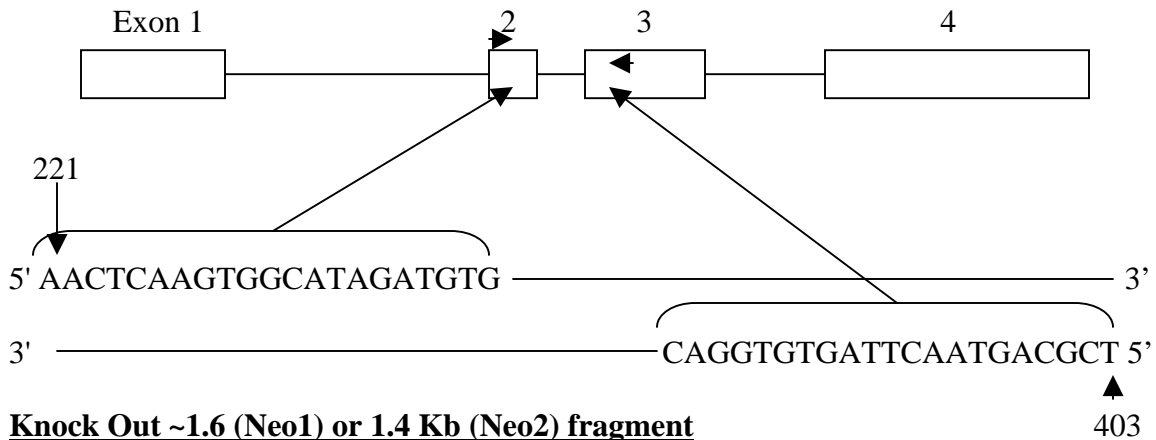


PCR Screening of Interferon- ζ $-/-$ Mice

Principle: The murine interferon gamma gene contains four exons and is located in mouse chromosome 10 (GenBank Acc. #NT_039501, NCBI gene ID 15978). The gene has been disrupted by insertion of a 2.0 Kb Neomycin (*Neo*) cassette (NCBI gene ID U43612) sequence into the end of exon 2. The screen, depending on which *Neo* primer is used, amplifies a section of the exon and the *Neo* cassette to yield a unique band specific to the KO. It is also useful to note that if the neo cassette is not present, a wild-type band will be found at ~227bp.

Wild type ~227 bp fragment



Oligos:

- 1.) DX2839=Exon-2 Sense 5'-AACTCAAGTGGCATAGATGTG-3'
- 2.) DX2840=Exon-3 Antisense 5'-TCGCAGTAACTTAGTGTGGAC-3'
- 3.) Neo 1= 781-800 Sense 5'-CTTGGGTGGAGAGGCTATTC-3'
- 4.) Neo 2= 1060-41 Antisense 5'-AGGTGAGATGACAGGAGATC-3'

PCR:

- Incubation
1. Genomic DNA (2 : 1)
 2. dNTPs (Invitrogen 2.5 mM mix; 4 : 1)
 3. Buffer D (Invitrogen; 5X; 4 : 1)
 4. Oligos, DX2839 & DX2840 0.1 : 1 each add both together
Add 1: 1 of either Neo1 or Neo2.
 5. Add Taq DNA Polymerase (0.3 : 1) Promega
 6. Total reaction volume - 20 : 1 brought up with PCR water

Program IFN γ

NO HOT START NEEDED.

1. 1 cycle 94°C for 30seconds
2. 25 cycles - 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 1 minute.
3. 1 cycle - 72°C for 5 min
4. Hold at 4°C 4.

Expected bands on agarose gel electrophoresis (2.0% TBE gel)

1. WT allele - 227bp
2. Disrupted allele - . 1.6 Kb with Neo1 and 1.4 Kb with Neo2.

Screening for double KO including IFN- γ .

The strategy is the same as above. There are no false bands in apoE $-/-$, LDL-R $-/-$ or IL-4 $-/-$ mice (SER. data 9.16.96.). Therefore, genomic DNA from a putative double KO may be analyzed for the presence of native or disrupted IFN- γ genes using the method as above. If using a double KO with two identical *neo* cassettes, the preceding procedure will only have a band on the IFN- γ KO and will not interact with the ApoE or any other *neo*, due to the DX2839 and 40 specificity for the IFN- γ sequence.

Reference

Dalton et al, Science (1993) 259:1740

File name: \adlab\protocol\mouse\ifngpcr.wpd

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