TNF GENOTYPING

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BACKGROUND

This protocol is used to genotype TNF knockout ($TNF^{-/-}$), TNF heterozygote ($TNF^{+/-}$), and wild type ($TNF^{+/+}$) mice from the Bowdish Lab Litter Mate Heterozygote (LMH) breeding colony.

NOTES

- Expected results
  - Wild type: 183 bp
  - TNF mutant: 318 bp
  - Heterozygote: 183 bp and 318 bp
- Protocol adapted from Stock Number: 003008 from the Jackson Laboratory https://www2.jax.org/protocolsdb/f?p=116:5:0::NO:5:P5_MASTER_PROTOCOL_ID,P5_JRS_CODE:22433,003008
- Additional PCR tips can be consulted at: https://www.jax.org/jax-mice-and-services/customer-support/technical-support/genotyping-resources/general-pcr-reminders-dos-and-donts

MATERIALS

- PCR grade water
- 10X PCR buffer (Invitrogen, 10342020)
- 50µM $MgCl_2$ (Invitrogen, 10342020)
- 10µM dNTP mix (ThermoFisher Scientific, 18-427-088)
- 100µM Common TNF genotyping forward primer (IDT, oIMR4182, 5' TAG CCAGGA GGG AGA ACA GA 3')
- 100µM Wild type reverse TNF genotyping primer (IDT, oIMR4183, 5' AGT GCC TCT TCT GCC AGT TC 3')
- 100µM Mutant reverse TNF genotyping primer (IDT, oIMR7297, 5' CGT TGG CTA CCC GTG ATA TT 3')
- Genomic DNA
- Taq DNA polymerase, recombinant (ThermoFisher Scientific, 10342020)
- 8-Strip PCR Tube (DiaMed, DIATEC420-1377)
- 100bp + 3K DNA ladder (SmoBio, DM2300)
- 6x Purple gel loading dye (New England Biolabs, B7024S)

PROTOCOL

1. Prepare a PCR master mix comprised of PCR grade water, 10X PCR buffer, 50µM $MgCl_2$, 10µM dNTPs, 10µM common TNF genotyping forward primer, 10µM wild type reverse TNF genotyping primer, and 10µM mutant reverse TNF genotyping primer. Add Taq polymerase last. Mix by vortexing. Keep on ice.
   a. A single PCR reaction contains the following components at the following volumes:
      i. PCR grade water 16.3µl

ii.	10X PCR buffer	2.5µl
iii.	50µM $MgCl_2$	0.75µl
iv.	10µM dNTP mix	0.50µl
v.	Common TNF forward genotyping primer	1.25µl
vi.	Wild type reverse TNF genotyping primer	1.25µl
vii.	Mutant reverse TNF genotyping primer	1.25µl
viii.	Genomic DNA	1µl
ix.	Taq DNA polymerase	0.2µl

2. Add 24µl of the PCR master mix to sterile 8-strip 0.2mL PCR tubes
    a. Mix by vortexing with each new 8-strip
    b. Keep 8-strip 0.2mL PCR tubes on ice

3. Add 1µl genomic DNA to sterile 8-strip 0.2mL PCR tubes containing PCR master mix
    a. Gently mix by flicking the 8-strip 0.2mL PCR tubes
    b. Keep 8-strip 0.2mL PCR tubes on ice

4. Briefly pulse centrifuge 8-strip 0.2mL PCR tubes containing genomic DNA and PCR master mix

5. Place samples in thermocycler (Eppendorf, Mastercycler, Pro S) and run the following touchdown amplification program:
    a. 94°C 5 minutes
    b. 94°C 45 seconds
    c. 65°C 30 seconds
    d. 68°C 40 seconds x10 cycles: Tm decreases by 0.5° every cycle
    e. 94°C 45 seconds
    f. 55°C 30 seconds
    g. 72°C 40 seconds x35 cycles
    h. 72°C 7 minutes
    i. 4°C Infinite hold

6. Remove samples from thermocycler
    a. Add 4µl 6x purple gel loading dye to each sample
    b. Gently mix by flicking the 8-strip 0.2mL PCR tubes
    c. Keep 8-strip 0.2mL PCR tubes on ice

7. Briefly pulse centrifuge 8-strip 0.2mL PCR tubes containing PCR products and loading dye

8. Separate PCR products by gel electrophoresis on a 1.5% agarose gel
    a. Small gel:
        i. 50mL 1X TAE buffer
        ii. 0.75g agarose
        iii. 2.5µl RedSafe nucleic acid staining solution
    b. Medium gel:
        i. 100mL 1X TAE buffer
        ii. 1.5g agarose

iii. 5µl RedSafe nucleic acid staining solution

c. Run at: Voltage: 120V, Duration: 25 minutes

d. Large gel:

i. 350mL 1X TAE buffer

ii. 5.25g agarose

iii. 15µl RedSafe nucleic acid staining solution

e. Run at: Voltage: 120V, Duration: 60-75 minutes