

MMRRC:011951-UCD, SWR-Tg(Tcrb3A)1Skd/Mmucd

Sigma REExtract-N-Amp Tissue Kit Screening Protocol

EXTRACTION

1. Pipette 20 μ l of Extraction solution into a PCR tube. Add 5 μ l of Tissue Preparation Solution to the tube and pipette to mix.
2. Cut a small piece of tail (1-2 mm) and place into the Solution. Mix by pipetting or vortexing.
3. Incubate tube at room temperature for 10 minutes
4. Incubate samples at 95°C for 3 minutes.
5. Add 20 μ l of Neutralization Solution. Mix thoroughly by pipetting or vortexing.
6. Store the tissue extract at 4°C or use immediately for PCR.

PCR AMPLIFICATION

1. Add the following to a thin-walled PCR tube:

dH ₂ O	4	μ l
REExtract-N-Amp PCR Reaction Mix	10	μ l
Forward primer (stock 200 μ g/ml)	1	μ l
Reverse primer (stock 200 μ g/ml)	1	μ l
Tissue Extract (DNA template)	4	μ l
Total volume	20	μ l

2. Mix gently.
3. Perform thermal cycling using the following profile:

Step	Temp	Time	Cycles
Initial Denaturation	94°C	5:00 min	1
Denaturation	94°C	0:40 min	35
Annealing	64°C	1:00 min	
Extension	72°C	1:30 min	
Final Extension	72°C	10:00 min	1
Hold	4°C	∞	

(Electrophoresis in 1% agarose gel --Size of expected PCR product ~ 400bp)

MATERIALS NEEDED

Primers:

For screening MMRRC# 11951 = SWR.Nuc3A-Tg (TCRb) smd

V β 4-5' Cla I

CGC TTG TAT CGA TTT CTA CAA TTA TCT TGA GGA T

J β 2-3' Not I

AAA ATT GCG GCC GAG AGA TGT GAA TCT TAC CTA AA

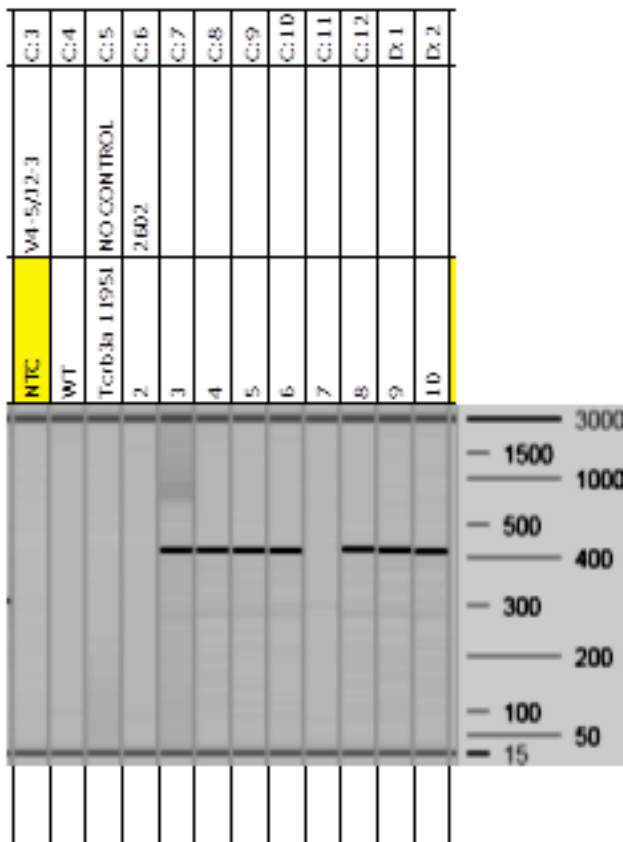
Sigma REExtract-N-Amp Tissue PCR Kit (Catalog# XNAT) Contains:

Sigma Extraction Solution (Catalog #E7526)

Sigma Tissue Preparation Solution (Catalog #T3073)

Sigma Neutralization Solution (Catalog #N3910)

Sigma REExtract-N-Amp PCR Reaction Mix (Catalog #R4775)



MMRRC:011951-UCD, SWR-Tg(Tcrb3A)1Skd/Mmucd

Sigma REDExtract-N-Amp Blood Kit Screening Protocol

EXTRACTION

1. Collect blood onto a small piece of Whatman filter paper and place into a microfuge tube. Store at 4°C or -20°C or use immediately.
2. Add 20 μ l of Lysis Soltuion to the tube. Mix thoroughly by pipetting or vortexing.
3. Incubate the sample at room temperature for 5 minutes.
4. Add 180 μ l of Neutralization Solution. Mix thoroughly by pipetting or vortexing.
5. Store the blood extract at 4°C or use immediately for PCR.

PCR AMPLIFICATION

1. Add the following to a thin-walled PCR tube:

dH ₂ O	6	μ l
REDExtract-N-Amp Blood PCR Reaction Mix	10	μ l
Forward primer (stock 200 μ g/ml)	1	μ l
Reverse primer (stock 200 μ g/ml)	1	μ l
Blood Extract (DNA template)	2	μ l

Total volume	20	μ l
--------------	----	---------

2. Mix gently.

3. Perform thermal cycling using the following profile:

Step	Temp	Time	Cycles
Initial Denaturation	94°C	5:00 min	1
Denaturation	94°C	0:40 min	35
Annealing	64°C	1:00 min	
Extension	72°C	1:30 min	
Final Extension	72°C	10:00 min	1
Hold	4°C	∞	

(Electrophorese in 1% agarose gel --Size of expected PCR product ~ 400bp)

MATERIALS NEEDED

Primers:

For screening MMRRC# 11951 = SWR.Nuc3A-Tg (TCRb) smd

V β 4-5' Cla I

CGC TTG TAT CGA TTT CTA CAA TTA TCT TGA GGA T

J β 2-3' Not I

AAA ATT GCG GCC GAG AGA TGT GAA TCT TAC CTA AA

Sigma Lysis Solution for Blood (Catalog #L3289)

Sigma Neutralization Solution for Blood (Catalog #N9784)

Sigma REDExtract-N-Amp Blood PCR Reaction Mix (Catalog #P8240)

Whatman Filter Paper (#3)