

08.05.10 MS
11.06.15 MLS

Genotyping Protocol: **MMRRC 131**

Strain Characteristics: Rearranged Valpha-11.1 and Vbeta-8.2 T-cell receptor chain genes isolated from a T-cell hybridoma specific for type II collagen (CII) using an internal promoter. They were ligated together, resulting in one construct. Details can be found in Osman et al (1998) Int Immunol 10(11):1613-22.

Assay Type: PCR - can not distinguish heterozygous animals from homozygous animals

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy Blood and Tissue kit (Cat#69506). Kit directions for animal tissues were performed with a few minor modifications as follows: repeat AW1 and AW2 wash steps one time, elute in 200µl of AE buffer once.

Primer Information:

- | | |
|---------------------|---|
| 1) Name: V alpha 5' | Sequence: 5`-GGT TCT GCT CTG AGA TGC ATT TTT-3` |
| 2) Name: V alpha 3' | Sequence: 5`-GAT GTC CCC TGC CAA ACA C-3` |
| 3) Name: V beta 5' | Sequence: 5`-AAC AGG AGG AAA GGT GAC ATT GAG-3` |
| 4) Name: V beta 3' | Sequence: 5`-AGC TTG GTT CCA TGA CCG A-3` |

Primer locations: V-alpha 5' = *Mus musculus* V-11.1 T-cell receptor alpha chain mRNA
V-alpha 3' = *Mus musculus* V-11.1 T-cell receptor alpha chain mRNA
V-beta 5' = *Mus musculus* V-8.2 T-cell receptor beta chain mRNA
V-beta 3' = *Mus musculus* V-8.2 T-cell receptor beta chain mRNA

Assay Names: M131 Tg PCR

PCR Master Mix Components:

Master Mix #1: (TCR VA)

component	manufacturer	concentration	µl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
V-alpha 5'	IDT	25µM	0.3
V-alpha 3'	IDT	25µM	0.3
FastStart <i>Taq</i>	Roche (Cat#12032953001)	5 U/µl	0.2
sterile water			13

PCR Setup:

Final Reaction: 19µl master mix & 1µl DNA template (10-20ng/µl)

All reactions were performed in 200µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:

- | | | |
|----|--|--------------------------------|
| 1) | 94°C | 5 minutes |
| 2) | 94°C | 30 seconds |
| 3) | 58°C | 30 seconds |
| 4) | 72°C | 1 minute |
| 5) | Repeat steps 2-4 34 times for a total of 35 cycles | |
| 6) | 72°C | 7minutes |
| 7) | 4°C | hold until refrigerate product |

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Product Analysis:

All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

Alignment Marker: QX Alignment Marker 15bp/3Kb (Cat# 929522)

Size Marker: QX DNA Size Marker 100-3Kb (Cat# 929553)

Method: AM320

Injection: 10s at 5KV

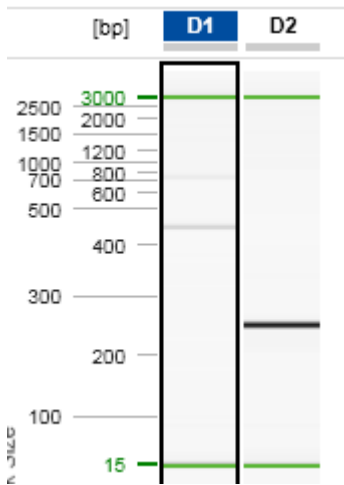
Separation: 320s at 6KV

Expected products:

Transgene positive: ~260 bp

Transgene negative: no product

Example gel:



Lane D1 displays a transgene negative sample (no product).
 two nonspecific bands are visible with this assay
 Lane D2 displays a transgene positive sample (~260bp product)

Please note: the 15bp and 3kb bands are reference markers specific to the QIAxcel method and do not represent expected products.

Master Mix #2: (TCR VB)

component	manufacturer	concentration	µl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
V-beta 5'	IDT	25µM	0.3
V-beta 3'	IDT	25µM	0.3
FastStart Taq	Roche (Cat#12032953001)	5 U/µl	0.2
sterile water			13

PCR Setup:

Final Reaction: 19µl master mix & 1µl DNA template (10-20ng/µl)

All reactions were performed in 200µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:

- 1) 94°C 3 minutes
- 2) 94°C 30 seconds
- 3) 64°C 30 seconds
- 4) 72°C 1 minute
- 5) Repeat steps 2-4 34 times for a total of 35 cycles
- 6) 72°C 7minutes
- 7) 4°C hold until refrigerate product

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Product Analysis:

All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

Alignment Marker: QX Alignment Marker 15bp/3Kb (Cat# 929522)

Size Marker: QX DNA Size Marker 100-3Kb (Cat# 929553)

Method: AM320

Injection: 10s at 5KV

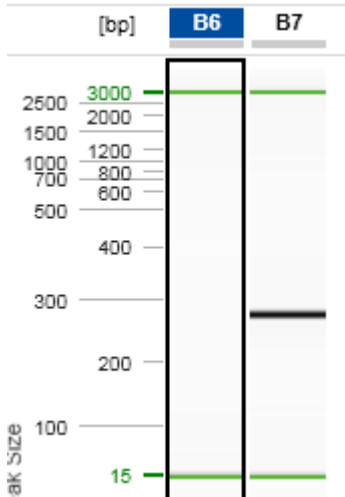
Separation: 320s at 6KV

Expected products:

Transgene positive: ~290 bp

Transgene negative: no product

Example gel:



Lane B6 displays a transgene negative sample (no product).

Lane B7 displays a transgene positive sample (~290bp product)

Please note: the 15bp and 3kb bands are reference markers specific to the QIAxcel method and do not represent expected products.