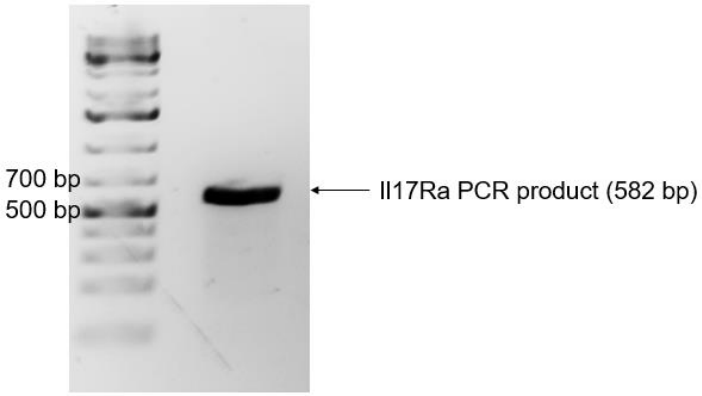
Il17ra^{em1} Mouse Genotyping Protocol



	Sequence (5'->3')	Length
Il17ra Fwd1	CCTTCTCCCCAAACATTCCT	21 bp
Il17ra Rev1	CCACTTGCCTTTTTCTTCCTGTG	23 bp
PCR product length	582 bp WT, 500 bp for 82 bp deletion mutant	582 bp or 500 bp

20 µL PCR reaction

2x Econotaq ready mix (Lucigen)	= 10.0 µL
10 µM Il17ra Fwd	= 1.0 µL
10 µM Il17ra Rev	= 1.0 µL
Nuclease-free water	= 7.0 µL
mouse tail DNA lysate	= 1.0 µL

PCR program

1. 94ºC, 2 minutes
2. 94ºC, 30 seconds
3. 62ºC, 30 seconds
4. 72ºC, 30 seconds
5. Go to 2, 35X
6. 72ºC, 7 minutes
7. 12ºC, ∞

Mouse Tail Digest Protocol: TMESCSR

Tail lysis buffer (500 ml):

500 mM KCl (83 ml, 3M stock)
100 mM TrisHCl pH 8.3 (50 ml of 1M stock)
0.1 mg/ml gelatin (50 mg)
1% NP40 (5 ml)
1% Tween 20 (5 ml)
Water to 500 ml volume (357 ml)

- For each tail clip (2-3 mm) add 100 ul tail lysis buffer and 0.1 ul 20 mg/ml Proteinase K
- Incubate overnight in 56°C water bath
- Heat inactivate at 100°C for 10 minutes and centrifuge about 2 minutes at 10,000 rpm or higher.
- This method of DNA isolation has been compatible with all polymerases tested. Further clean-up of DNA can be performed with a phenol/cloroform extraction followed by ethanol precipitation.