

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	54
MMRRC Strain Name	STOCK <i>Pkd1</i> ^{m1Bei} /Mmnc
Gene Name(s)	Polycystic Kidney disease 1 homolog (<i>Pkd1</i>)
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	1/24/14

MMRRC #54 PCR Reaction

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10 μM Primer F	1
10 μM Primer R	1
Taq	0.5
DNA	2

Note: This genotyping requires *Fat1* restriction digestion.

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 60°C for 30 sec
 Step 4: 72°C for 45 sec
 Step 5: 35 X from step 2 to step 4
 Step 6: 72°C for 7 min

Taq: Apex and Chromataq 5X Buffer

Primer sequences 5' to 3': Primers are 10 μM with respect to each primer.

Com-R (54): AACAGTCTCCATAATCACCAGACAGGGT

WT-Anti-Com (54): TCTGTGGCCTCTATACATCCCTGTG

Bands expected: 589 bp

***Fat1* restriction digestion mix for each PCR sample:**

Digestion total volume 20ul

14 μl H₂O

2.0 μl NEB Buffer 2, 10X

0.2 μl BSA 100X

2.0 μl *Fat1* (2,000 units/ml)

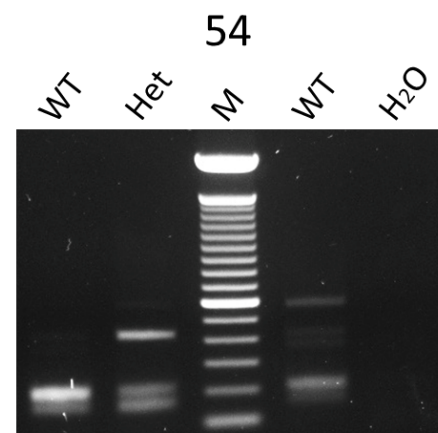
Add 18μl Mix to each 2 ul PCR product: incubate at 55 °C for overnight. Then 80°C for 20 minutes

Expected Results:

Het: 162 bp, 208 bp, 219 bp and 427 bp

WT: 162 bp, 208 bp, 219 bp

Run on a 2% agarose gel.



M: 100 bp DNA ladder (Invitrogen)