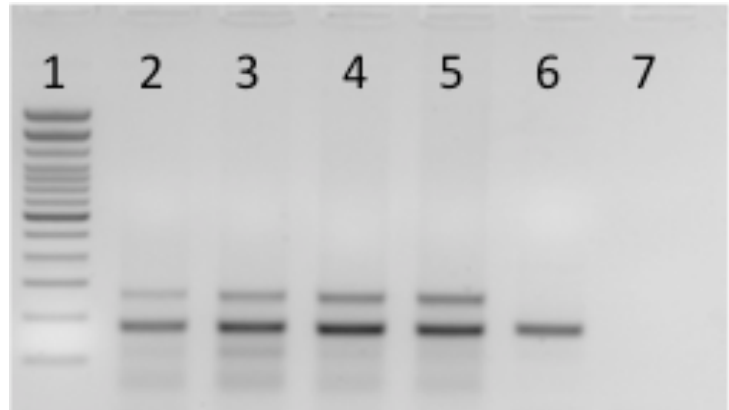


MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	30022
MMRRC Strain Name	CBACa.129S4(Cg)- <i>Coch</i> ^{tm1Mrtn} /Mmnc
Gene Name(s)	Cochlin
Breeding Protocol(s)	Backcross
Protocol Date	12/13/19

PCR Reaction

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



Thermal Cycler:

Step 1: 95C, 5min
 Step 2: 95C, 45sec
 Step 3: 53C, 45sec
 Step 4: 72C, 60sec
 Step 2 to 4 Cycles: 30
 Step 5: 72C, 7min

1. 100bp Marker (Invitrogen)
 2. Heterozygous Sample
 3. Heterozygous Sample
 4. Heterozygous Sample
 5. Heterozygous Sample
 6. Wild-type Control
 7. Negative Control

Taq: Denville Taq and Chromataq 5X Buffer

Bands: WT: 175bp MUTANT: 275bp

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

Coch(30022)F: TCAACCATGGGACAGAGTCAC

Coch(30022)R: TTGATGTATTTGAAAAGCAC