

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	34255
MMRRC Strain Name	129- <i>Spast</i> ^{tm1Jfnk} /Mmnc
Gene Name(s)	spastin (Spast)
Breeding Protocol(s)	Backcross to 129SvEv
Protocol Date	7/22/13

MMRRC #34255 PCR Reaction

Mutant Reaction:

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 66°C for 30 sec
 Step 4: 72°C for 45 sec
 Step 5: 30x from step 2 to step 4
 Step 6: 72°C for 7 min

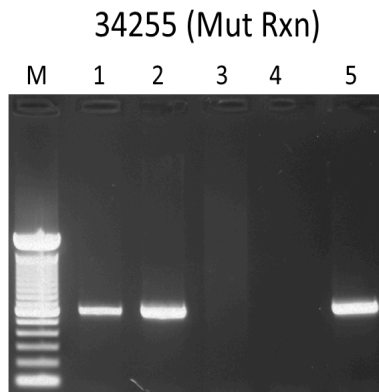
	<u>1X</u>
ddH ₂ O	13
5X Buffer	5
25mM MgCl ₂	2
10mM dNTPs	0.5
10 μM Primer Forward	1
10 μM Primer Reverse	1
Apex Taq	0.5
DNA	2

Taq: Apex and Chromataq 5X Buffer

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

Mut-F2 (34255): TGAACCAAATTTATGTCATGGGTCCC

Mut-R2 (34255): AAGGGGGAGGATTGGGAAGACAA



Lane 1: Het; Lane 2, 5: Hom; Lane 3: WT; Lane 4: H₂O; M: 100 bp DNA ladder (Invitrogen)

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WT Reaction:

Thermal Cycler:

Step 1: 94°C for 5 min

Step 2: 94°C for 30 sec

Step 3: 59°C for 30 sec

Step 4: 72°C for 60 sec

Step 5: 34x 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.

SpstWT R (34255): TGGCCAGGGAAAGTTAAACA

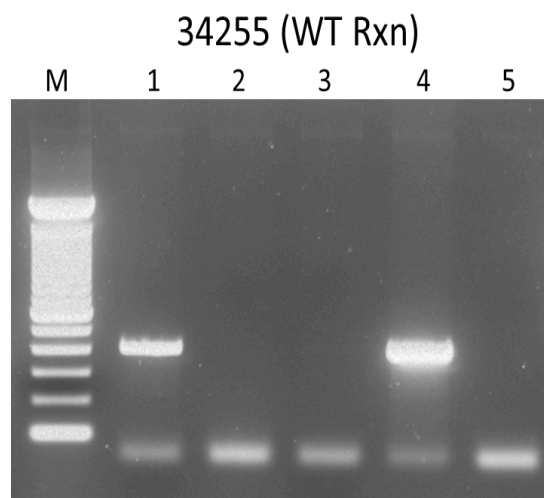
SpstWT F (34255): GTAACCCCTCACTCGCATGT

Bands expected:

SpstWT R (34255) + SpstWT F (34255): WT: 399 bp

Mut-F2 (34255) + Mut-R2 (34255): MUTANT: ~ 600 bp

Run on 1% agarose gel in TAE.



Lane 1: Het; Lane 2, 3: Hom; Lane 4: WT; Lane 5: H₂O; M: 100 bp DNA ladder (Invitrogen)