

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	30624
MMRRC Strain Name	B6;129S6- <i>aspb</i> /Mmnc
Gene Name(s)	axonal spheroid body (<i>aspb</i>)
Breeding Protocol(s)	Sib-mating
Protocol Date	8/12/13

MMRRC #30624 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

Thermal Cycler:

- Step 1: 94°C for 5 min
- Step 2: 94°C for 30 sec
- Step 3: 54 °C for 30 sec
- Step 4: 72°C for 30 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.

M-VC10F: GCCTTTGAGCCAGTCAAGACA

VC10R: CCAGTCTGGACTTGTGAATGA

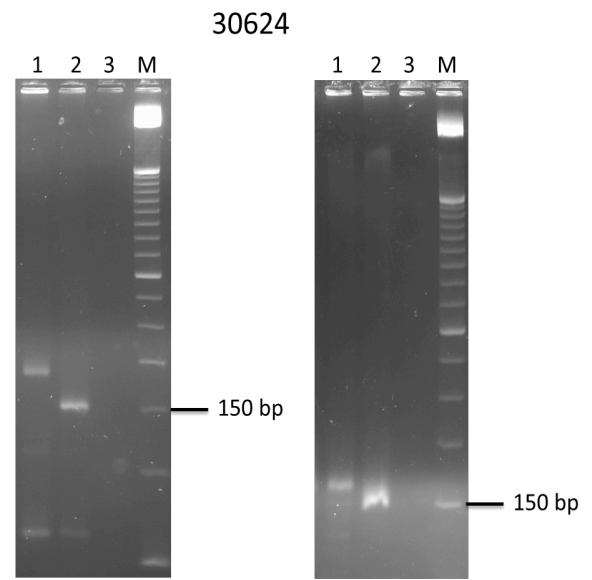
VC11.1F: TCAAAGTCAAGGTGGGCAG

VC11.1R: ACTTCCAGATGTTGGGCAC

Bands expected:

- Primers M-VC10F + VC10R: 129 (affected): 184bp
- B6 (unaffected): 150 bp
- Primers VC11.1F + VC11.1R: 129 (affected): 160 bp
- B6 (unaffected): 147 bp

Run on 4% agarose gel in TAE.



Primers: M-VC10F + VC10R

Primers: VC11.1F + VC11.1R

Lane 1: 129 affected; Lane 2: B6 unaffected; Lane 3: H₂O; M: 50 bp DNA ladder (Invitrogen)