

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	15204
MMRRC Strain Name	STOCK <i>Plk3^{tm1Pjs}/Mmnc</i>
Gene Name(s)	Polo-like kinase 3 (Plk3)
Breeding Protocol(s)	Backcross
Protocol Date	03/28/14

MMRRC #15204 PCR Reaction

Thermal Cycler:

Step 1: 94°C for 5 min
 Step 2: 94°C for 30 sec
 Step 3: 58°C for 30 sec
 Step 4: 72°C for 45 sec
 Step 5: 35x from step 2 to step 4
 Step 6: 72°C for 7 min

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

WT-F (15204): CAGGACCATCTGTGGCACTCCC

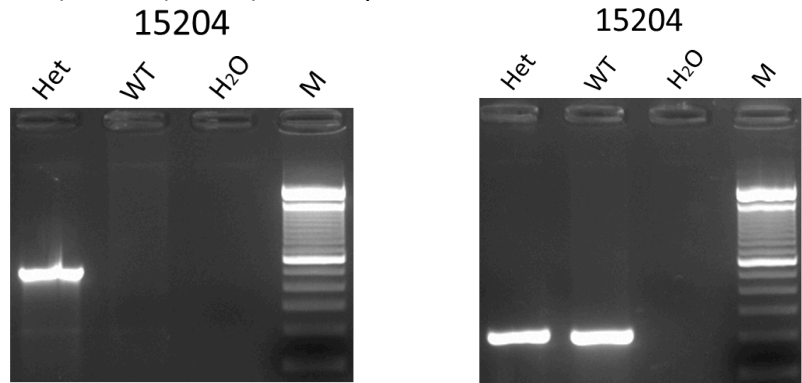
WT-R (15204): CACAGGAGCGTGTACCTATGCC

Neo (15204): CGCTCCCGATTGCGAGCGCATCGCC

Bands expected: WT: WT-F (15204) + WT-R (15204), 206 bp

Mut: WT-R (15204) + Neo (15204), ~500 bp

Run on 2% agarose gel in TAE.



Primers: WT-R (15204) + Neo (15204)
 M: 100 bp DNA ladder (Invitrogen)

Primers: WT-F (15204) + WT-R (15204)
 M: 100 bp DNA ladder (Invitrogen)