

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	30046
MMRRC Strain Name	C.129S4(B6)- <i>Prkdc</i> ^{tm1Get} /Mmnc
Gene Name(s)	Protein kinase, DNA activated, catalytic polypeptide (<i>Prkdc</i>)
Breeding Protocol(s)	Sib-mating
Protocol Date	9/3/13

MMRRC #30046 PCR Reaction

Amplification of DNA-PKcs wild type allele

	<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: 61 °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

Taq: Apex and Chromataq 5X Buffer

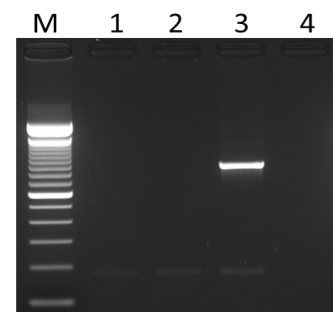
Primer sequences 5' to 3':

Primers are 10 μM with respect to each primer.
 MB 705: GAGCCTATGTGCTAATGTACAG
 TM-MB 708: GTGGCTGATGCACAGCAGTGC

Bands expected: WT: 906 bp
 DNA-PKcs^{-/-} mice: no band

Run on 1% agarose gel in TAE.

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Lane 1, 2: BALB/c DNA-PKcs^{-/-}; Lane 3: WT;
 Lane 4: H₂O; M: 100 bp DNA ladder Invitrogen