

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	71305
MMRRC Strain Name	B6J.Cg- <i>Dlk1</i> ^{tm2.1Stadt} /Mmnc
Gene Name(s)	delta like non-canonical Notch ligand 1
Breeding Protocol(s)	Backcross
Protocol Date	9/24/2024

PCR Reaction

	<u>1X</u>
ddH ₂ O	12.5
5X Buffer	5
25mM MgCl ₂	2
10mM dNTPs	0.5
10uM Primer 1	1
10uM Primer 2	1
Taq	1
DNA	2

Note: 2,2 Primer Reactions (WF+CR for wild-type and MF+CR for mutant)

Thermal Cycler:

Step 1: 94C, 5min

Step 2: 94C, 45sec

Step 3: 57C, 45sec

Step 4: 72C, 60sec

Step 2 to 4 Cycles: 35

Step 5: 72C, 7min

Taq: **Apex Taq**

Bands: WT: 801bp MUTANT: 488bp

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

DLk(71305)WF: CATCCTGAAGGTGTCCATGA

DLtom(71305)MF: CTGTTCTGTACGGCATGG

DLk(71305)CR: TGATGTGAGTGTGCGTGAGA