

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	36474
MMRRC Strain Name	B6.129P2-Kdm3atm1.1Yzha/Mmnc
Gene Name(s)	lysine (K)-specific demethylase 3A (Kdm3a)
Breeding Protocol(s)	Backcross to C57BL/6J
Protocol Date	6/27/13

MMRRC #36474 PCR Reactions

NOTE: 2, 2 primer Reaction

Taq: **Denvill Taq and chromataq Buffer**

Primers are 10uM with respect to each primer

Primer sequences 5' to 3':

A-F(36474): ccaaatgcccaaggctttac

C-F (36474): gaatatgtgtgtgtggcagc

D-R(36474): ccacatacacaacttctcttag

	<u>1X</u>
ddH ₂ O	13
5X Buffer	5.0
25mM MgCl ₂	2
10mM dNTPs	0.5
10 uM Primer F	1
10 uM Primer R	1
Taq	0.5
DNA	2

WT Reaction

36474 (Primer C + D)

Thermal Cycler:

Step 1: 94°C for 5 min

Step 2: 94°C for 30 sec

Step 3: 60°C for 30 sec

Step 4: 72°C for 30 sec

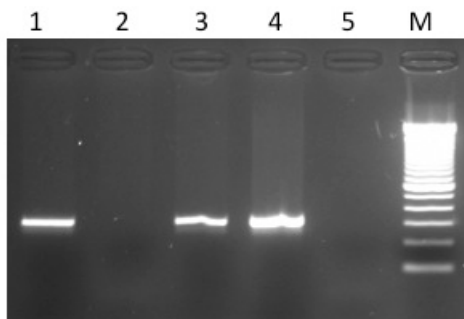
Step 5: Step 2 to 4 Cycles: 30

Step 6: 72°C for 7 min

“C-F + D-R” Bands:

WT: 316 bp

1lox(KO): no product



Lane 1: Het; Lane 2: Hom; Lane 3-4: WT; Lane 5: ddH₂O;
M: 100 bp DNA ladder Invitrogen

Mutant Reaction

Thermal Cycler:

Step 1: 94°C for 5 min

Step 2: 94°C for 30 sec

Step 3: 55°C for 30 sec

Step 4: 72°C for 30 sec

Step 5: Step 2 to 4 Cycles: 30

Step 6: 72°C for 7 min

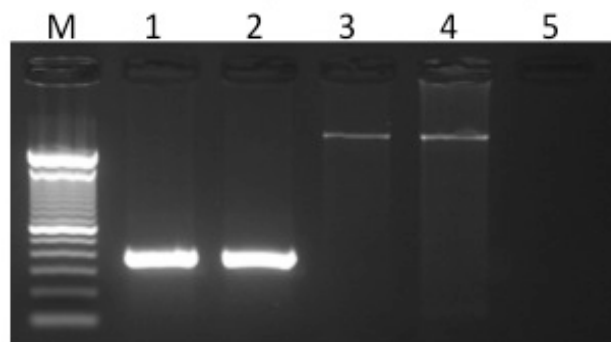
“A-F + D-R” Bands:

1lox(KO) - A-D: ~350bp

WT: ~2.8 kb, weak

Run on 1% agarose gel in TAE.

36474 (Primer A + D)



Lane 1: Het; Lane 2: Hom; Lane 3-4: WT; Lane 5: ddH₂O;
M: 100 bp DNA ladder Invitrogen