JAX Stock No. 009062 and 009089

DNA and PCR Conditions for Genotyping from Rachel Wevrick 12-8-2008

(modified from Andrew Lab protocol)

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- confirms this is a "generic lacZ protocol"

- expected product sizes are:
LacZ 480bp
Dlxin 313bp

- also provides a specific PCR that distinguishes between the two mutant alleles (see PCR COMBINED file)

Preparing DNA from Ear Notch Biopsies:

1. Add 100 µL Further Modified STE buffer per tube.
2. Add 6 µL proteinase K.
3. Incubate at 55°C for 1 hour to overnight.
4. Heat inactivate 95°C for 10 min.
5. Add 900 µL MilliQ water per tube.
6. Use 2 µL for PCR.

Further Modified STE Buffer:

	Per 100 mL
50mM Tris pH 7.5	5 mL 1M Tris pH 7.5
1 mM EDTA	0.2 mL 0.5M EDTA
100 mM NaCl	2 mL 5M NaCl
0.10% SDS	0.5 mL 20% SDS

0.20 µm filter, store at room temperature

Proteinase K solution >600 mAU/mL (from Qiagen DNeasy kit stored at room temperature) or
Andrew Lab uses 20 mg/mL Invitrogen 25530-015 ≥20U/mg (100 mg) stored at -20°C

PCR Conditions:

	Per reaction	
10XPCR Buffer	2 µL	
2 mM dNTPs	2 µL	
10 µM LacZF754 (RW3768)	1 µL	
10 µM LacZR1153 (RW3769)	1 µL	402 b.p. product
10 µM Dlxin 1F (RW3461)	0.5 µL	
10 µM Dlxin 2R (RW3462)	0.5 µL	313 b.p. product
50 mM $MgCl_2$	1 µL (2.5 mM final conc.)	
MilliQ H_2O	9.9 µL	
Taq	0.1 µL	
Total	18 µL	
	+2 µL DNA (or MilliQ H_2O)	

Program 57, 30X30 PCR Machine #3
94°C 3 min.
94°C 30s, 57°C 30s, 72°C 30s x 30 cycles
72°C 10 min., 4°C hold

LACZ 754F	CGT GAC TAC CTA CGG GTA AC
LACZ 1153R	AGT TGT TCT GCT TCA TCA GC
DLXIN-1F	CCT TGC TTG TGC AGA CCT TG
DLXIN-2R	GGC AGC ATG TGG ACC TTT AG