RaDR PCR Protocol – Whole ear lysate and PCR Supermix

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Ear Punch Digestion

1. Add 100 uL 50mM NaOH to ear punch in PCR tube
2. Heat at 100C for 15 minutes
3. Vortex and heat again at 100C for 15 minutes
4. Add 30 uL 1M Tris (pH 7.4) to neutralize and vortex
5. Use immediately or store at -20C

RaDR PCR

Primer Mix (in water)

Sequence	**Concentration**	**Identifies**
aaa gtc gct ctg agt tgt tat	6 uM	
gcg aag agt ttg tcc tca acc	4 uM	Mutant (RaDR positive)
gga gcg gga gaa atg gat atg	6 uM	Wt

PCR mix

Component	**Amount per sample**
Platinum Supermix	20 uL
Primer Mix	2 uL
Rediload (inert loading dye)	1 uL
Ear punch digested DNA	1 uL

Cycling conditions

Temp (C)	Time	# Cycles
94	3 min	1
94	45 sec	35
58	45 sec	
72	45 sec	
72	5 min	1
4	Hold	

Wt product is 577 bp
RaDR product is 313 bp

Genotyping RaDR Mice from Ear/Tail – Purified DNA and separate PCR ingredients

Joshua Corrigan

Reagents:

Lysis Buffer
Isopropanol
Proteinase K
Tris-Edta (TE)

Lysis Buffer:

100 mL of 1 M Tris Cl pH 8.0
10 mL of 0.5 M EDTA
10 mL of 20% SDS
40 mL of 5 M
NaCl 840 mL of
diH_2O

DNA Extraction Procedure:

Per sample:	Lysis Buffer	500 µL
	Proteinase K	3.2 µL

1. Mix lysis buffer and proteinase K to make digestion master mix.
2. Add 500 µL of digestion master mix to each ear/tail sample.
3. Incubate at 55ºC overnight.
4. The next day, remove tubes from the incubator and vortex for 15 seconds.
5. Spin samples for 10 minutes at max RPM.
6. Label and fill new Eppendorf tubes with 500 µL of isopropanol.
7. Remove samples from centrifuge and pour supernatant into the isopropanol, being careful not to dislodge the pellet.
8. Invert tubes 15-20 times to precipitate out the DNA.
9. Spin samples for 15 minutes at max RPM.
10. Remove samples from centrifuge. Pour off the supernatant and let the open tubes dry inverted on a paper towel for 20 minutes.
11. Add 100 µL of TE to each tube and let sit for 20 minutes.
12. Vortex briefly before use. Keep at 4ºC, store at -20ºC long term.

Primers:

RaDR 1	aaa gtc gct ctg agt tgt tat
RaDR 2	gcg aag agt ttg tcc tca acc
RaDR 3	gga gcg gga gaa atg gat atg

PCR Procedure (per sample):

	Per sample (ul)
H2O	13.7
buffer	2.5
RaDR 1	1.5
RaDR 2	1
RaDR 3	1.5
2mM dNTPs	2.5
Taq	0.3
DNA	2
Total	25

PCR Settings:

1. 1. 95C 5 min
2. 2. 95C 30 sec
3. 3. 62C 30 sec
4. 4. 72C 30 sec
5. 5. Repeat steps 2-4 (39x)
6. 6. 72C 5 min
7. 7. 10C Forever

Band Size:

WT ~577 bp
RaDR ~313 bp