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JOHNS HOPKINS MEDICINE

Hackam Lab
Genotyping By PCR, Ear Punches

- Use ear punch to mark ears and transfer punch into an Eppendorf tube containing 300µl 0.1M NaOH/1mM EDTA
- Boil ear punch for 10 mins and store at 4˚C. The ear punch will not completely dissolve but will release enough DNA for PCR.

PCR REACTION
Important: Set up PCR on ice. Use barrier tips.

PCR mix	/tube
H2O	19.0 µl
10X Taq buffer (Invitrogen)	2.5µl
5mM dNTP	0.5µl
10mM Primer mix (Forward+Reverese)	1.0µl
Taq (Invitrogen)	1.0µl

- Add 1µL DNA in the bottom of PCR tube
- Add ice cold PCR mix 24µl ice
  (just below ring (to avoid contamination, no need to change tips)
- Seal tops well, keeping reaction chilled
- Bio-rad PCR machine and set parameters to

94˚C/5min	x 1
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94 ˚C/30secs	
55˚C/30secs	x 35-40
72˚C/90secs	

25˚C	

- Start PCR run. WAIT until block reaches 94˚C, Pause run
- Transfer tubes to PCR machine
- Resume run
- Pour 2.5% agarose/TAE gel
- Once PCR run is complete add 5µl of 10X loading buffer to each sample.
- Run 20µl of each, run @300V (big gel takes 40-50 min) and photograph gel.