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Fast rodent genotyping

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We use this protocol and it's working

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Abstract

This protocol describes a very fast method (approximately 2 h) to do genotyping from rodent tail clips and other samples.

Materials

Equipment

Operating Scissors (like Roboz Surgical Store RS-6828)
70% Ethanol mix (KOPTEC UN1170)
Heat block (like Thermo Fisher 88870002)
PCR thermocycler (like Bio-Rad MyCycler)
DNA electrophoresis system (like VWR Horizontal MINI M Gel Electrophorosis System)
DNA gel visualization device (like Licor Odyssey Fc Imager)
Mini centrifuge (like USA Scientific 2631-0006)
Microwave oven

Consumables

Platinum Direct PCR Universal Master Mix (Sigma Aldrich A44647100)
Eppendorf tubes (labForce 1149K01)
PCR tubes (Bio-Rad TWI0201)
Tris (Sigma Aldrich T6066)
EDTA (Sigma Aldrich E6511)
Glacial acetic acid (Sigma Aldrich AX0073)
Agarose (Thermo Fisher 16500-500)
DNA dye (Biotium 41011)
DNA ladder (like NEB N0550 or N0556)

Recipes

50x TAE buffer, 1L in ddH_2O
2 M Tris (242 g)
1 M Glacial acetic acid (60.05 mL)
50 mM EDTA (20.81 g)

Before start

The current protocol describes genotyping using rodent tail clips. This protocol has been verified using both rats (Sprague Dawley strain) and mice (C57BL/6J strain). It can also be applied to other types of biological samples, like blood, buccal swabs etc.
Example genotyping applied includes PARK20 mice and sexing of wt rats.
Operating scissors should be kept very sharp at all times, by cutting through aluminum foil or stainless steel sponges.

DNA extraction

10m

1 Tail clips from mice or rats of appropriate age should be snipped using sharp operating scissors and in compliance to local protocols by national and institutional regulatory organizations and placed in sterile Eppendorf tubes.

2 Note: This protocol has been tested using the smallest tail clips possible of ~ 1 mm. Always clean the scissors with [M] 70 % (v/v) Ethanol in between animals.

3 Add 20 µL of Lysis Buffer and 0.6 µL of Proteinase K in each sample tube.
Note: Preparing a master mix is suggested for several samples.

4 Incubate samples at Room temperature for 00:01:00 . 1m

5 Put samples in the heat block at 98 °C for 00:01:00 . 1m

6 Spin down the samples using a mini-centrifuge for 00:00:30 and transfer lysate to new tubes. 30s

PCR

1h 20m

7 Prepare PCR reactions at Room temperature as follows
Note: Preparing a master mix is suggested for several samples.

	A	B
	Component	Volume (ul)
	Platinum master mix	10
	DNA sample	1
	Primers	0.4+0.4
	Nuclease free water	8.2
	Total	20

8 Note: PCR reaction may need optimization. Primers should be used at a final concentration of [M] 0.2 micromolar (µM) . In this example, 0.4 µL is added for each of two primers from a [M] 10 micromolar (µM) stock.

9 Place PCR samples in a PCR thermocycler. Set up the thermocycler with the following program using a Hot Start function.

	A	B	C	D
	Step	Temperature (°C)	Time	Cycles
	Initial Denaturation	94	2 min	1
	Denaturation	94	15 s	35
	Annealing	55	15 s	35
	Extension	68	20 s/Kb	35
	Final Extension	68	2 min	1
	Hold	4	oo	1

10 Note: PCR program may need optimization.

DNA gel electrophoresis

30m

11 During the PCR, prepare an appropriate agarose percentage DNA gel.

12 Load PCR sampes along with an appropriate DNA ladder on the DNA gel.

13 Submit the DNA gel to electrophoresis.

14 Visualize gel using an appropriate device.

Protocol references

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