Single-Tube PCR Genotyping of Ptprca (JAXBoy, CD45.1) and Ptprcb (C57BL/6J, CD45.2)

Abdullah Lab, IMMEI, University Hospital Bonn

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Version: v1.0
Author: Dillon Corvino

Purpose

This protocol describes a **validated single-tube endpoint PCR assay** for distinguishing:

- **C57BL/6J (Ptprc^b/b, CD45.2)**
- **JAXBoy (Ptprc^a/a, CD45.1)**
- **Heterozygous (Ptprc^a/b)**

The method is derived directly from **Supplementary Tables S1, S4–S10 and Figures S1–S3** of Ryan et al. 2024.

Background

The Ptprc locus contains SNPs that differentiate the CD45.1 (Ptprc^a) and CD45.2 (Ptprc^b) alleles.

A single SNP assay using:

- Ptprc_a_F3cT3 (long-tailed allele-specific forward primer)
- Ptprc_b_F3d (allele-specific forward primer)
- Ptprc_R3 (common reverse primer)

provides robust genotype resolution in one reaction tube.

Materials

Reagents

- HotSHOT buffers
- DreamTaq Green 2× Master Mix
- Primers as detailed below
- Nuclease-free water

Equipment

- Thermocycler
- 0.2 mL PCR tubes
- Agarose gel system

Primer Sequences

Ptprc_b_F3d

```
TTG TTT GGA GTG GAA AAC GA
```

Ptprc_a_F3cT3
(81-nt tail + allele-specific region)

```
cat ctg ata ttt gaa aga ccc aag ccc tct cat acg cga cac gag atc tac act
ctt tcc cta cac gac gct ctt ccg atc TGT TTG GAG TGG AAA ACC A
```

Ptprc_R3

```
CGT TGT GAA TTT GTT TCA GTG C
```

Ready-to-Use Primer Mix (Total 100 µL)

- Ptprc_a_F3cT3 (100 µM): 13.3 µL
- Ptprc_b_F3d (100 µM): 10 µL
- Ptprc_R3 (100 µM): 10 µL
- Water: 66.7 µL

Use **0.5 µL per 10 µL PCR reaction.**

Genomic DNA Preparation (HotSHOT)

1. Add 50–75 µL alkaline buffer to ear/tail biopsy.
2. Heat 95°C × 30 min.
3. Chill and neutralize.
4. Spin briefly.
5. Use 1–2 µL supernatant per PCR.

PCR Reaction Setup (10 µL total)

- 2 µL HOTSHOT DNA
- 5 µL 2× DreamTaq Green
- 0.5 µL Primer Mix
- 2.5 µL Water

PCR Cycling Conditions

Standard cycling (recommended):

1. 94–95°C × 2 min
2. 36 cycles of:
   - 95°C × 30 s
   - **56°C × 30 s**
   - 72°C × 60 s
3. 72°C × 5 min

Expected Amplicon Sizes

Genotype	Size
Ptprc^b/b	**243 bp**
Ptprc^a/a	**323 bp**
Ptprc^a/b	243 + 323 bp

Gel Electrophoresis

- 2–3% agarose
- Run until 200–350 bp resolves
- Load 5–10 µL PCR product

Controls

Include known:
- Ptprc^b/b
- Ptprc^a/a
- Ptprc^a/b
- NTC

Troubleshooting

- Weak bands → increase template or cycles.
- Allele dropout → check 56°C annealing temperature.
- Non-specific bands → small increase in annealing temperature.

Version History

Version	Date	Description
v1.0	2025-12-01	Initial release.

Citation

Ryan CE et al. (2024). Single-tube Ptprc SNP genotyping assay.