

Single-Tube PCR Genotyping of Ptp^{ca} (JAXBoy, CD45.1) and Ptp^{cb} (C57BL/6J, CD45.2)

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Protocol ID: MUS-PTPRC-GENO-001

Version: v1.0

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Purpose

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

- C57BL/6J (Ptp^{cb}/b, CD45.2)
- JAXBoy (Ptp^{ca}/a, CD45.1)
- Heterozygous (Ptp^{ca}/b)

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

Background

The Ptp^{ca} locus contains SNPs that differentiate the CD45.1 (Ptp^{ca}/a) and CD45.2 (Ptp^{cb}/b) alleles.

A single SNP assay using:

- Ptp^{ca}_a_F3cT3 (long-tailed allele-specific forward primer)
- Ptp^{cb}_b_F3d (allele-specific forward primer)
- Ptp^{ca}_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 $95^{\circ}\text{C} \times 30 \text{ min}$.
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 \times DreamTaq Green
- 0.5 μL Primer Mix
- 2.5 μL Water

PCR Cycling Conditions

Standard cycling (recommended):

1. $94\text{--}95^{\circ}\text{C} \times 2 \text{ min}$
2. 36 cycles of:
 - $95^{\circ}\text{C} \times 30 \text{ s}$
 - **$56^{\circ}\text{C} \times 30 \text{ s}$**
 - $72^{\circ}\text{C} \times 60 \text{ s}$
3. $72^{\circ}\text{C} \times 5 \text{ min}$

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:

- $\text{Ptp}^{\text{b/b}}$
- $\text{Ptp}^{\text{a/a}}$
- $\text{Ptp}^{\text{a/b}}$
- NTC

Troubleshooting

- Weak bands \rightarrow increase template or cycles.
- Allele dropout \rightarrow check 56°C annealing temperature.
- Non-specific bands \rightarrow 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 Ptpc SNP genotyping assay.