

Supplementary Material For:

[Letter to the editor]

PCR prescreen for genotyping the Ts65Dn mouse model of Down syndrome

Materials and methods

Mice

Female B6EiC3Sn a/A-Ts(17¹⁶)65Dn (Ts65Dn) and male B6 × C3H/HeJ F₁ (B6C3F1) mice were originally obtained from The Jackson Laboratory (Bar Harbor, ME, USA). Offspring from Ts65Dn × B6C3F1 matings were produced in our colonies at Indiana University-Purdue University Indianapolis (IUPUI) and the Johns Hopkins University School of Medicine (JHUSOM). All male/female pairs were kept in a 12 h day/12 h night light cycle with food and water administered ad libitum in a temperature- and humidity-controlled room. Animal use and protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at IUPUI and JHUSOM.

DNA Isolation

Tissue was obtained from the offspring of Ts65Dn mothers at postnatal day zero (P0), 5–10 (P5–P10) or 21–30 (P21–P30), and pups were marked for subsequent identification. DNA was isolated using standard protocols (overnight Proteinase K digestion and isolation the following day by salting out). In our hands, the commonly used “HotSHOT” (25) method did not reliably produce DNA suitable for the subsequent PCR.

PCR

PCR was utilized to amplify a 246-bp segment with a single nucleotide polymorphism (SNP) in the *Zdhbc14* gene (rs48029645 GenBank) using the primers 5'-AAATAGTAGCATCTCATGAGTG-3' (forward) and 5'-CATAGTGCATCTTAGACAAGC-3' (reverse) ($T_m = 60^\circ\text{C}$) (Invitrogen, Carlsbad, CA) (Figure 1). For a single reaction, 1× PCR Rxn Buffer (Invitrogen), 2.5 mM MgCl₂ (Invitrogen), and 0.02 μg/μL *Taq* DNA polymerase (Bioline, Taunton, MA, USA) were used with 2 μL DNA (~100 ng/μL) in a 25 μL reaction. DNA was amplified using the following program.

- 1) 94°C for 1 min
- 2) 94°C for 30 s

- 3) 65°C for 20 s
- 4) 72°C for 20 s
- 5) Repeat steps 2–4 for 5 cycles
- 6) 94°C for 30 s
- 7) 65°C for 30 s (-1 degree per cycle)
- 8) 72°C for 1 min
- 9) Repeat steps 6–8 for 10 cycles
- 10) 94°C for 30 s
- 11) 55°C for 30 s
- 12) 72°C for 20 s
- 13) Repeat steps 10–12 for 10 cycles
- 14) 72°C for 1 min
- 15) 72°C hold.

Afterward, 8 μL of the PCR can be removed and a 246-bp band visualized on a 1% agarose gel (Figure 1).

Restriction digest

The *SacI* enzyme was used to discern the SNP in PCR products. For a single reaction, 2 μL Buffer I (New England BioLabs, Ipswich, MA, USA), 2 μL 10× BSA, 7 μL sterile water, and 1 μL *SacI* restriction enzyme were combined in a 1.5 mL Eppendorf tube to which 8 μL of the PCR product was added. Samples were incubated in a 37°C water bath for 2.5 h and resolved on a 3% agarose gel. Products were visualized using SYBR Safe DNA gel stain (Invitrogen) or ethidium bromide and a UV source.

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