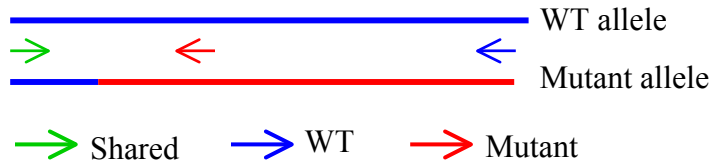


Primer sequence:

| | |
|---------------|--|
| Sod2MUT4U20 | GGGGCATCTAGTGGAGAAGT (shared primer) |
| Sod2MUT179L22 | ATCAATTGGAAGTAGCCGTTAT (mutant primer) |
| Sod2WT442L19 | GTCCCCCACCATTGAACTT (WT primer) |



Primer concentration: mix together shared (Sod2MUT4U20) and mutant (Sod2MUT179L22) primers at 10 μ M and WT primer (Sod2WT442L19) at 2.5 μ M to make a **10X** primer mix. Occasionally, we experience some inconsistency in the amplification of WT fragment. If that happens, increase the WT primer concentration to 5 μ M in the 10X mix.

PCR buffer: ThermoPol buffer (10X) from New England Biolab (ThermoPol reaction buffer pack B9004S \$10), BSA (100x) pack B9001S \$10. The buffer from NEB usually works well. However, we did run into some batches that were not quite satisfactory. Alternatively PC2 buffer (Barnes WM, PNAS 91: 2216-2220) prepared in-house almost always works well. Avoid freeze-thawing the buffer for more than 5 times – throw it away and get a fresh tube.

PCR reaction:

2.5 μ L 10X buffer
 2.5 μ L 10X primer mix
 0.25 μ L 100X BSA
 14.25 μ L H₂O
 0.5 μ L DNA
 20 μ l mineral oil overlay

Program the PCR machine to go up to 95°C for 5min. (“hot start”) then cool down to 80°C and hold at that temperature for 5 to 10 minutes for the addition of 5 μ l dNTP/Taq mix to each tube.

200 μ M dNTP (final concentration)
 0.5 U Tag polymerase
 H₂O to a total volume of 5 μ L per tube.

Once finish adding dNTP/Taq mix to every tube, start the PCR reaction for 30 cycles at:

94°C 30''
62 °C 30''
72°C 30''

We routinely use 1% agarose gel with TBE buffer to separate PCR fragments.

WT band is 457 bp and mutant band is 197 bp.

PC2 buffer (prepared as 10X solution)

200 mM Tris, pH 9.0

160 mM (NH₄)₂SO₄ (do not autoclave the stock solution, we usually keep the 1M stock
at -20°C).

35 mM MgCl₂

150 µg/ml BSA