**ROSA26-LSL-APPswe/ibe Homozygous/Heterozygous Genotyping Protocol**

(Updated Apr 2022)

The ROSA26-LSL-APPswe/ibe transgenic line expresses human APP695 rather than the mouse/human chimeric APP695 and therefore uses distinct primers for amplification compared with the lab's other APP transgenic lines (ie APP/PS1, tetO-APP, etc). This genotyping protocol distinguishes homozygous, heterozygous, and non-transgenic mice.

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| **Reagent** | **Volume/rxn (ul)** |
| ddH2O | 6.1 |
| 50 μM ROSA26 Forward | 0.4 |
| 50 μM ROSA26 Reverse | 0.1 |
| 50 μM ROSA26-APP Reverse | 0.4 |
| 2x PCR PreMix (Green Dye) (Syd Labs, MB067-EQ2G-L) | 10 |
| Tail DNA | 3 |

**PCR Program: R26 (ABI SimpliAmp)**

1. 94°C for 3 minutes
2. A. 94°C for 30 seconds

B. 58 °C for 1 minute

C. 72°C for 1 minute

D. Repeat 2A-C for 40 cycles

3. 72°C for 4 min

4. Hold at 4°C

**Primer Sequences**

ROSA26 Forward (5’ targeting arm): GTC GCT CTG AGT TGT TAT CAG T

ROSA26 Reverse (3’ targeting arm): CAC ACA CCA GGT TAG CCT TTA

ROSA26-APP Reverse (CAG promoter): GAC GTC AAT GGA AAG TCC CTA T

**Gel Percentage:** 2% Agarose Gel with 10uL of 10mg/mL ethidium bromide (30min at 175V in 1x SB)

**Ladder:** TrackItTM 100 bp Ladder (Invitrogen, Catalog number: 10488058)

**Expected Products:** Wild type (non-transgenic) animals should exhibit a single band at 251 bp indicating amplification of the native ROSA26 locus. Heterozygous animals should exhibit the 251 bp band and a band at 374 bp indicating amplification of both the native ROSA26 locus and the ROSA26-LSL-APP transgene. Homozygous animals should exhibit a single band at 374 bp indicating amplification of the ROSA26-LSL-APP transgene.