

genotyping assay:

Genotyping of offspring from Sufu het. breeding colony is based on three primers in one reaction. Best results are obtained when using AmpliTaq Gold or other activatable polymerases. Alternatively, Taq polymerase with Hot Start may be used. The F21/R6 and F21/neo F2 primer combinations may also be run in separate reactions.

genotyping assay primers: Sufu F21 CCCTTTTTGTCA ATAGTTCC
 Sufu R6 TGACAATAGACTCCGCCTCC
 Neo F2 GCCTTCTATCGCCTTCTTGAC

genotyping conditions (final concentrations):

1x AmpliTaq Gold buffer (w/o MgCl ₂)	2.5 mM
MgCl ₂	0.2 mM
dNTPs	0.4 μM
F21	0.2 μM
R6	0.2 μM
F2	1.25 units
AmpliTaq Gold	1 μl tissue biopsy lysate

genotyping cycle program:

STEP1 95°C 10 min (to activate AmpliTaq Gold)
STEP2 95°C 30 sec
STEP3 55°C 15 sec
STEP4 72°C 1 min+ 5 sec autoext./cycle
STEP5 repeat from STEP2 for additional 34 cycles

Results: wt allele (F21/R6) = 275 bp; mut allele (F21/F2) = 195 bp