Mouse genotyping with Thermo F170S Phire Tissue Direct PCR Kit (hair follicles)

DNA extraction mix, per sample:
10µL Dilution buffer + 0,5 µL DNA Release Additive.
Prepare the required volume of DNA extraction mix. Distribute 10µL in 8-tubes PCR stripes. Place 10-50 hair follicles (wipe the forceps with 70% EtOH paper tissue between sampling) into each tube. Incubate at room temperature 5 – 30 min. Heat inactivate 96ºC 2 min (on PCR cycler).
Prepare PCR mix (per 10 samples):

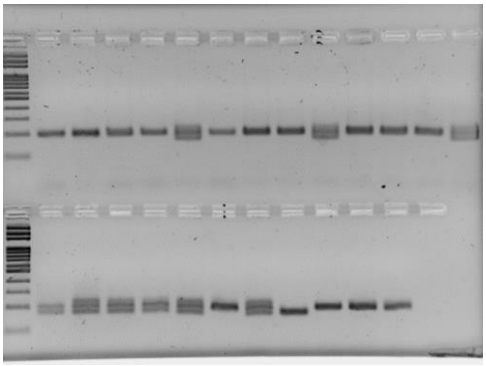
50µL Phire Tissue Direct PCR Master Mix (2X)
5µL Forward primer 10µM
5µL Reverse primer 10µM
30µL water (to 90µL)

Distribute 9µL per PCR tube and add 1µL from DNA extraction reaction. Run 33-35 cycles. For annealing temperature calculation use the online tool:
https://www.thermofisher.com/de/de/home/brands/thermo-scientific/molecular-biology/molecular-biology-learning-center/molecular-biology-resource-library/thermo-scientific-web-tools/tm-calculator.html

Run/detect/analyse PCR products on 2 – 3% agarose gel.

Alternative protocol (for robust PCR tests, to save 50% of PCR Master Mix (2X)).
PCR mix (per 10 samples):
25µL Phire Tissue Direct PCR Master Mix (2X)
5µL Forward primer 10µM
5µL Reverse primer 10µM
4µL dNTPs (2,5mM each) / 1µL dNTPs (10mM each)
5µL Taq Buffer x 10 (or 10µL Phusion/Q5 buffer x 5)
XµL water (to 90µL)

Hair sampling procedure



Example: 200bp (WT) and 178bp (mutant allele, 22bp deletion) fragments of tet3 gene.