Technical Manual

One Hour Mouse Genotyping Kit

Catalog# GF1004

Description: The Gene-Foci One Hour Mouse Genotyping Kit includes DNA extraction buffer, Stop solution and Super Fast Taq DNA polymerase. This kit provides a fast and reliable method for digesting mouse tail snips and extracting DNA in 30 minutes. The Super Fast Taq DNA polymerase included is a deeply engineered version of Taq DNA polymerase, this enzyme can amplify 1Kb DNA in 10 seconds, compared with 1 minutes/kb required for regular Taq. The Super Fast Taq DNA polymerase is also resistant to polymerase inhibitors in the tail digest broth, and is ideal for genotyping PCR.

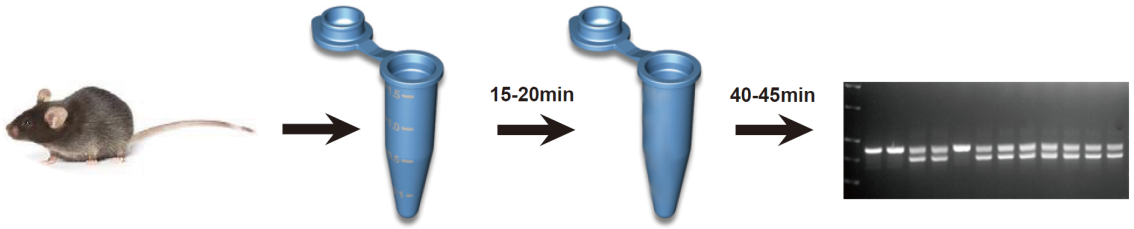
Features: Digest and extract DNA from mouse tails in 25 minutes, compared with overnight digestion of traditional methods.

Suitable for digesting mouse tail in 1.5 ml tubes or 96-well plates.

Super Fast Taq DNA polymerase minimizes the time required for PCR reactions and increases yield.

Storage : Store the DNA extraction buffer and Stop solution at 4°C. Store the 2xPCR Master Mix at -20°C.

From Tail to Genotype in One Hour



Tail Digestion Protocol:

I. Tail digestion in 1.5 ml tubes:

1. Add 200 µl of DNA Extraction Buffer to a 1.5 ml centrifuge tube.
2. Add mouse tail snips (~2-3mm) to the tube.
3. Incubate at 98°C for 20 minutes IN A SCREW CAP 1.5 ML TUBE (regular tube may pop upon heating. If the tail does not get digested completely, elongate the incubation time.
4. Cool down the tube on ice, add 25 µl of Stop Solution,mix well.
5. Spin at 13,000 rpm for 2-5 minutes to settle debris.
6. Use 1-2µl of supernatant as template for 25 ul PCR, setup PCR reaction as described below.

II. Tail digestion in 96-well plates:

1. Add 200 µl of DNA Extraction Buffer to wells in a 96-well PCR plate.
2. Add mouse tail snips (~2-3mm) to individual wells.
3. Incubate in a PCR machine at 98°C for 20 minutes.
4. Take out plate from PCR machine, Add 25 µl of Stop Solution.
5. Spin plates at 2000 rpm for 7 minutes to settle debris.
6. Use 1-2µl of supernatant as template for 25 µl PCR, setup PCR reaction as described below.

PCR Protocol:

1. To setup a 25 µl PCR reaction, add the following components into a DNase-free thin-wall PCR tube:

Components	Volume	Final Concentration
Super Fast Taq (5U/µl)	0.5 µl	0.1U/ µl
10xSuper Fast Taq Buffer	2.5 µl	1X
dNTP (10 mM)	0.5 µl	200 µM
Extracted DNA	1~2 µl	0.02~20 ng/ul
Forward Primer (10 µM)	0.5 µl	0.2 µM
Reverse Primer (10 µM)	0.5 µl	0.2 µM
ddH_2O	to 25 µl	

2. Gently mix the components by pipetting up and down several times, avoiding bubbles.
3. Briefly spin down the components in the PCR tubes or plate.
4. Set up the program for a regular PCR as follows:

Template denature before cycling:	94°C, 3 minutes
30-40 cycles:	94°C, 20~30 seconds
	42~60°C, 15-30 sec
	68~72°C, 10s-1 min/kb
Extension :	72°C, 5~10 minutes
Hold at:	4°C.

5. The PCR products can be directly loaded onto agarose gel for analysis.