Lyse n Amp™ Mouse Genotyping Kit

Catalog # PD101

GeneBio Systems, Inc.™

1. Introduction

Lyse n Amp™ Mouse Genotyping Kit (One Step Mouse Genotyping Kit) is specially designed for the rapid genotyping of mouse and rat. The kit contains a complete set of reagents for DNA extraction and PCR amplification.

This kit can be used for rapid extraction of genomic DNA from mouse tails, ears, toes, and other tissues with no need of homogenization, crushing, overnight digestion, phenol chloroform extraction, DNA precipitation or column purification operations, which greatly shortens the experimental time. The extracted genomic DNA can be used directly as template for facile PCR amplification. In this kit procedure, the tissue is soaked in lysis buffer with Proteinase K, and then incubated at 55 ℃ for 20 min for efficient cellular lysis and proteolysis, followed by 5 min heating at 95℃ to inactivate the Proteinase K. After a brief centrifugation step, the resulting lysate can be directly used as PCR template. Extensive testing has shown that fragments < 2 kb can be routinely amplified. In addition, multiplex PCR as many as four pairs of primers has been found to be successful. This Kit supplies a robust 2 × Taq Plus Master Mix (Dye Plus) which includes a high-performance Taq Plus DNA Polymerase, dNTP, and optimized buffer system, delivering high amplification efficiency of target DNA. There is dA overhang on each 3' end of the PCR product, thus the product can be cloned into any T vector.

2. Applications

- Mouse Genotyping, including transgenic mouse identification and mouse knockout analysis

3. Package Information

Components	Cat. # PD101-01 500 rxn (20 µl/rxn)	PD101-02 1000 rxn (20 µl/rxn)
1 × Mouse tissue Lysis Buffer	40 ml	2 x 40 ml
Proteinase K	800 µL	2 x 800 µL
2 × Taq Plus Master Mix (Dye Plus)	5 x 1 ml	10 x 1 ml
25 mM $MgCl_2$	500 µL	2 x 500 µL
5 × PCR Enhancer	500 µL	2 x 500 µL

4. Storage

Store 1 × Mouse tissue Lysis Buffer at 4 ℃; Store other components at -20 ℃.

5. Procedure

Notes:

- Rinse all tools to be used in the tissue separation process with 70% ethanol.
- Do not skip the step of the inactivation of Proteinase K (95 ℃, 5 min). Otherwise, residual activity of Proteinase K may inhibit the subsequent PCR reactions
. - Prepare the PCR reaction mixture in ice-water bath, in order to improve the specificity of the PCR amplification.

1. DNA Extraction

The recommended tissue amount:

- 3 ~ 5 mm^2 of mouse tail tip
- 5 ~ 10 mm^2 of mouse ears
- 1 ~ 2 of mouse toes

1.1 Prepare the Lysis Reagent based on the number of samples (N). The following table shows the volumes of reagents to prepare the Lysis Reagent for N samples.

	N samples	5 samples	10 samples
Proteinase K	1.6 x N µL	8 µL	16 µL
1 × Mouse tissue Lysis Buffer	80 x N µL	400 µL	800 µL

Note: The Lysis Reagent should be prepared freshly. Please mix the reaction throughly by vortexing.
Please note for N reactions, we recommend to prepare the Lysis Reagent with 5-10 % more than required to account for pipetting errors. For sample , for 5 samples, prepare 5.25 or 5.5 x master.

1.2. Place each mouse tissue sample, such as tail, ear, or toe, in a 1.5 ml microcentrifuge tube. To each sample, add 81µl of the Lysis Reagent to the tissue, mix by vortexing, and then incubate at 55 ℃ for 20 min. To improve the efficiency of DNA release, please try to make sure the Lysis Reagentr covers all the tissue in the tube. Incubation time may vary, depending on the size of the target DNA fragment. The recommended incubation time at 55 ℃ is as follows:

Size of the amplified fragment	Recommended incubation time at 55℃
~500 bp	10 min
~1000 bp	20 min
~1500 bp	30 min

Note: It is normal that tissue blocks are not digested completely. Incomplete digestion does not affect the follow-up experiment.

1.3. Incubate the samples in boiling water bath or at 95 ℃ for 5 min to inactivate Proteinase K.

1.4. Mix the lysate thoroughly by vortexing for 10 s. *Optional: centrifuge at 12000 rpm for 5 min.* The lysate or supernatant can now be directly used as a PCR template. The supernatant can be transferred into a new tube and stored at - 20 ℃ for at least three months.

2. PCR Amplification

2.1. General Reaction Setup for PCR:

Take the 2 × Taq Plus Master Mix (Dye Plus) from freezer, thaw it completely and invert it several time to mix. Primers are as required for your PCR. Please note, the two primers must be provided by the user and resuspended to 10μM each. Prepare the reaction system on ice as follows:

	N samples	5 samples	10 samples
2 × Taq Plus Master Mix (Dye Plus)	10 x N μL	50 μL	100 μL
Primer 1 (10 μM)	0.8 x N μL	4 μL	8 μL
Primer 2 (10 μM)	0.8 x N μL	4 μL	8 μL

Please note for N reactions, we recommend to prepare a master mix with 5-10 % more than required to account for pipetting errors. For sample, for 5 samples, prepare 5.25 or 5.5 x master.

Ailiquote11.6 ul into each PCR tube, or a well of PCR strip tube or plate.
To each tube, add 1 to 2 μl of lysate. Add nuclease-free water to top volume to 20 μl per tube/well.

2.2. Set up Thermocycling Program following the guidelines below. Start PCR:

94°C	5 min	(Pre-denaturation)
94°C	30 sec	35 Cycles
55°C*	30 sec	
72°C	30 sec/kb	
72°C	7 min (Complete extension)	

* The annealing temperature should be adjusted according to the Tm values of primers, preferably at a temperature that is 1-2 ℃ lower than the Tm values of primers. The PCR products contains pre-mixed gel loading dye, thus they can be loaded directly to agarose gels for electrophoresis, without addition of DNA Loading Buffer.

6. Troubleshooting

A. No amplification product in test or control samples
1. Amplification reaction was incorrectly set up: Ensure the proper reaction set up procedure in step 2 is followed;
2. Improper storage has led to loss of activity of PCR reagents: Replace all components with fresh reagents;
3. Primer design was not optimal, thus primers did not anneal: Redesign primers;

B. Amplification worked in the control samples, but not in test samples
1. Digestion was incomplete: Extend digestion time up to 60 min at 55 ℃;
2. Lysis solution was mixed with PCR mixture for too long: Collect fresh mouse tail samples for genomic DNA extraction and repeat procedure with proper digestion time. ;
3. The quantity of amplified product was not sufficient: Increase the number of PCR cycles to 35-40 to yield more amplification product.

C. Non-specific amplification product(s)
1. Annealing temperature was too low: Increase the annealing temperature;
2. The number of PCR cycles was too high: Decrease the number of cycles to 30-35;
3. Primer concentration was too high: Decrease primer concentration;
4. Template concentration was too high: Dilute template in purified H_2O or TE buffer.