Direct Genotyping Kit Plus

Product description

The Direct Genotyping Kit Plus is specifically designed for species genotype identification. This product contains lysis buffer and balance buffer, which can quickly digest tissues, release complete genomic DNA, and the DNA can be directly used as a PCR template without being extracted from the mixed solution. The kit contains modified Taq DNA Polymerase, dNTP and an optimized buffer system. The modified Taq DNA Polymerase has strong amplification performance and is compatible with a variety of complex templates. It is suitable for gene identification of various tissues or cells including plant, colony PCR and mice. This Master Mix operation only requires the addition of primers, templates and ddH_2O, which greatly simplifies the experimental steps, reduces personal errors and improves the repeatability of the results. This product contains blue dye, which means it can be directly subjected to electrophoresis after amplification without the need to add sample loading buffer. If agarose Gel electrophoresis of PCR products is required, our product SYBR Safe DNA Gel Stain (Item No. A8743) can be added to the gel.

Composition and storage conditions

Components \ Size	200 rxns	500 rxns	Storage
2× Taq PCR Master Mix Plus (with dye)	2×1 mL	5×1 mL	-20°C
Balance buffer	20 mL	50 mL	4°C
Lysis buffer	20 mL	50 mL	4°C
Proteinase K	200 µL	500 µL	-20°C
Shipping: Dry Ice	**Shelf life:** 2 years		

Experimental operation

1. Tissue digestion (taking mouse genotype identification as an example)

a) Place the mouse tails, toes or ears (~2mm) in 75 µL Lysis buffer and 0.75 µL Proteinase K for digestion, and incubate at 56 ° C for 15 minutes. Then incubate the mixed solution at 95 ° C for 10-60 minutes (undissolved tissues do not

interfere with PCR).

b) After heating, cool the samples to 4℃ and add 75 μL of Balance buffer to each sample.

c) For every 20 μL of PCR system, use 1 μL of the above-mentioned preparation solution as the template for the next PCR (the sample system can also be adjusted as needed).

***Note:** During the digestion process, shaking the tube several times will contribute to the release of genomic DNA. For most mouse tissue samples, incubation with protease K at 56°C for 15 minutes is sufficient to extract genomic DNA. The tissue may still seem intact, but cracking has occurred. The obtained genomic DNA can be applied to PCR amplification. If not used immediately, the sample can be centrifuged to collect the supernatant and stored at -20℃.

2. Reaction Setup

a) Thaw PCR mix, mix thoroughly and then centrifuge. Place on ice for later use.

b) Configure the reaction system according to the following table:

Reagent	50 μL Reaction	Final Concentration
2X Taq PCR Master Mix Plus (with dye)	25 μL	1×
Forward primer (10 μM)	1-2 μL	0.2-0.4 μM
Reverse primer (10 μM)	1-2 μL	0.2-0.4 μM
Template DNA	X μL	
ddH_2O	to 50 μL	

***Note:**

a. Generally, the final primer concentration is recommended to be 0.2-0.4 μmol/L, but it can also be adjusted within the range of 0.1-1.0 μmol/L as needed.

b. The optimal reaction concentrations for different templates vary. Taking the 50 μL system as an example, when the template is genomic DNA, the generally recommended template usage is 10-200 ng. When the template is plasmid or viral DNA, the generally recommended usage amount is 10 pg-5 ng. Excessive template quantity is prone to cause non-specific amplification.

c) Invert and mix well, or gently pipette or gently vortex to thoroughly mix the reaction solution, and briefly centrifuge to collect the liquid to the bottom of the tube.

3. PCR Cycling Conditions

Place the reaction tube on the PCR instrument and start the thermal cycle. Set the reaction program according to the following table:

Procedure	Temperature	Time	Cycles
Initial Denaturation	95°C	3-5 min	1
Denaturation	95°C	30 s	30-35
Annealing	55-65°C	30 s	
Extension	72°C	15-30 s/kb	
Final extension	72°C	5 min	1

***Note:**

a. When performing colony PCR, pre-denaturation for 10 min can fully break the cell walls, and both *Escherichia coli* and yeast can be efficiently amplified.

b. The annealing temperature should be set according to the Tm value of the primer. If necessary, it is recommended to establish a temperature gradient to find the optimal temperature for the combination of primers and templates. In addition, the annealing temperature directly determines the amplification specificity. If poor amplification specificity is found, the annealing temperature can be appropriately increased.

c. If the length of the target fragment is ＜3 kb, the extension time can be shortened to 15 s/kb. The length of the target fragment is > 3 kb, and the recommended extension time is 30 s/kb. To achieve the best amplification effect or higher yield, it is recommended to uniformly extend at a speed of 30 s/kb.

Notes

Primer Design Guidelines: The primers for qPCR experimental design need to have good amplification effects and few non-specific products. The following design guidelines can be followed:

1) The last base at the 3 'end of the primer is preferably G or C. The overall distribution of primers A, G, C and T should be as uniform as possible, and areas with high GC or AT content should be avoided.

2) The last 8 bases at the 3 'end of the primer should avoid consecutive mismatches and the formation of hairpin structures.

3) It is preferable that the difference between the Tm values of the forward Primer and the reverse primer does not exceed 1°C, and the Tm value should be adjusted to 55-65° C (It is recommended to calculate the Tm value of the primer using Primer Premier 5).

4) Additional sequences of primers, that is, sequences that are not paired with the template, should not be involved in the calculation of primer Tm values. The GC content of the primers is controlled between 40% and 60%.

5) Avoid having complementary sequences of more than five bases within the primers or between the two primers, and avoid having complementary sequences of more than three bases at the 3 'ends of the two primers.

After the primer design is completed, please use the NCBI BLAST function to search for primer specificity to avoid non-specific amplification.

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