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Mouse Direct PCR Kit DE-070• DE-071

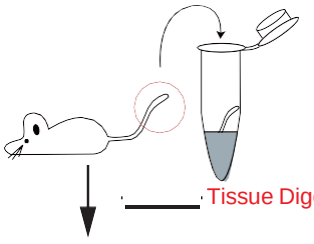
Description:

The Mouse Direct PCR Kit provides a fast preparation and PCR amplification that is specifically designed for mouse genotyping. The Buffer L and Protease Plus rapidly digest mouse **tail**, **ear** and **toe** to release intact genomic DNA that can be used directly as the template for PCR amplification. By using this kit, the digestion process only takes **15 min**. In addition, the 2x M-PCR OPTI Mix (which includes an optimized Taq Polymerase) ensures high amplification efficiency of target DNA.

Storage

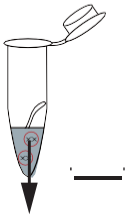
Buffer L should be stored at 4°C. Other reagents should be stored at -20°C. All reagents can be stored for 2 years.

Protocol



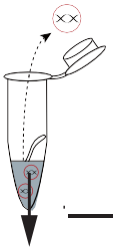
Add protease mixture to the tissue tub

Tissue Digestion



Incubate at 55°C for 15 min to release genomic DNA

Protease Inactivation



After digestion, incubate at 95°C for 5 min to inactivate the proteases

PCR

After centrifuge, 12,000 rpm for 10 min, the supernatant of digested solution can be used as the template for PCR

Components

Contents	DE-070 (200 rxns)	DE-071 (500 rxns)
Buffer L	20 mL	50 mL
Protease Plus	0.4 mL	1 mL
2 x M-PCR OPTI™ Mix[a]	2 mL	5 mL

a. Includes more powerful DNA polymerase, dNTPs, Mg^{2+}, and DNA Loading Dye.

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1. Tissue digestion

Prepare tissue digestive solution according to the number of mice. The proportion of reagents was as follows:

	Per sample
Protease Plus	2 µL
Buffer L	100 µL

Prepare the tissue digestive solution when using and mix thoroughly.

1.2 Add 100 µL[b] fresh tissue digestive solution to each EP tube containing mouse tissue samples[c] and digest at 55°C water bath or metal bath for 15 min[d]. It is necessary to immerse the tissues completely in the digestive solution. After digestion, the sufficient amount of genomic DNA has been released, even if the tissue appears still intact, which does not affect the subsequent PCR experiments.

b. If DNA was extracted from mouse over 3 months, increase the digestive buffer up to 200 µL or prolonged the digestive time.

c. How to get the proper tissue samples of mouse?

Tail or toe: 1-2 mm or 3-5 mg; ear: less than 5 mm^2 or 3-5 mg; different organs: less than 20 mm^2.

d. If the target gene is difficult to amplify, it is recommended to prolong digestion time to 30 min.

1.3. Incubate the samples in a water bath/metal bath at 95°C for 5 min to inactivate the proteases. Centrifuge the supernatant at 12000 rpm for 5 min as the template for PCR. The digested supernatant can be stored at -20°C for 3 months[e].

e. In order to guarantee the amplification efficiency of hard-to-amplify genes, the template of PCR should be used as soon as possible after extraction.

2. PCR amplification

2.1. PCR reaction system: prepare the reaction system in ice bath to ensure the efficiency and specificity of PCR amplification.

PCR Reaction Components	20 µL Reaction Volume (µL)	50 µL Reaction Volume (µL)
ddH_2O	8	21
Forward Primer (10 µM)	0.5	1
Reverse Primer (10 µM)	0.5	1
Template[f]	1	2
2 x M-PCR OPTI™ Mix	10	25

f. Change the amount of template properly in order to increase the brightness of target bands or reduce the non-specific bands.

2.2 PCR Program setting

Temperature (°C)	Time	Cycles
94	5 min	1
94	20 sec	35
50-65	30 sec	
72	X min (2 kb/min)	
72	5 min	1
12	--	1

1. Agarose gel electrophoresis

The 2 x M-PCR OPTITM Mix Contains bromophenol blue dyes, PCR products can be directly loading into the agarose gel electrophoresis. The PCR products can be cloned directly into T vector for DNA sequencing because their 3' terminal contains base A.

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Trouble Shooting

Problem	Potential Cause(s)	Suggestion(s)
No amplification product in test or control samples	Improper storage was led to loss of activity of PCR reagents	Replace all components with fresh reagents
	Primers were not optimal and did not anneal	Redesign primers
	Too high annealing temperature	It is suggested that annealing temperature is set every 2°C for a gradient to find the optimum conditions
	The extension time or cycles were not enough	Increase the extension time or set the cycle number at 36-40
Amplification worked in the control samples, but not in test samples	Digestion was incomplete	Extend digestion time up to 30 min at 55°C
	Too many templates inhibit the PCR reaction	Reduce the amount of template
	Incomplete inactivation of the protease activity	Heat the digestion products at 95°C for 5 min
Non-specific amplification product(s)	The incorrect preparation of PCR primers	Redesign primers
	The incorrect preparation of PCR system (The concentration of Primers or DNA template was too high)	Reduce the amount of primers or DNA template
	The incorrect setting condition of PCR reaction (The annealing temperature was too low or the cycle number was too high)	Increase the annealing temperature or lower the cycle number
	High ambient temperature during the preparation of the PCR reaction system or too long delay time at ambient temperature after the preparation	Prepare the PCR reaction system in ice bath and use as soon as possible
The target band appeared in the negative control	The contamination of PCR Reagents or experimental Tools	Use new reagents or re-sterilize ddH_2O and PCR products at high pressure; Operate gently to avoid contaminate other samples.
Poor repeatability of the results	Unstable Reagent Activity or DNA Template Degradation	Preserve all reagents at low temperature or extract fresh genome DNA
	The cross contamination of the DNA samples	Before the next sample is taken, the sampler should be washed in 75% alcohol or 2% sodium hypochlorite solution and then cleaned with a clean paper towel. Of course, one-time samplers are better.
	The cross contamination of the PCR products	Operate gently to avoid contaminate other samples. Avoid excessive sample loading and change pipette tips for different samples.

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