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LiDirect™ Lightning Genotyping Kit

Cat. #: M0015 Size: 80/400 rxns

Introduction

LiDirect™ Lightning Genotyping Kit is designed for fast extraction of tissue DNA and subsequent PCR verification. The procedure is fast and simple, amenable to automation and does not use hazardous chemicals such as phenol, chloroform or guanidine isothiocyanate. There is no need for mechanical disruption, organic extraction, column purification, or precipitation of the DNA. LiDirect™ Genotyping DNA extraction buffer contains all the reagents needed to rapidly extract genomic DNA from mouse tails, yeast and plant cells, or any other tissues, cells, hair shafts, and saliva. The included LiTaq™ Plus PCR Master Mix ensures highly specific and robust DNA amplification from the DNA extract. The entire procedure can be completed within an hour.

Package Information

Components	M0015-01	M0015-04
LiDirect™ Extraction Buffer*	8 ml	40 ml
LiDirect™ Stop Solution	8 ml	40 ml
2× LiTaq™ Plus PCR Master Mix	1 ml	5 ml

* **Warning**: Alkaline, corrosive

Storage

PCR Master Mix: -20°C; Extraction Buffer & Stop Solution: 4-25°C

Protocol

All steps are carried out at room temperature unless otherwise noted. The optimum usage depends on the experiment and specimen, please optimize the sample and PCR template volume by each condition.

1. Place a 1~2 mm piece of mouse tail, or 5~10 mg of tissue, or ~1×10^6 cells into a PCR micro tube.

IMPORTANT: DO NOT add more than 10 mg of tissue per 100 µl of LiDirect™ Extraction Buffer. Too much tissue may inhibit downstream PCR reactions.

2. Add 100 µl of LiDirect™ Extraction Buffer.

3. Incubate at 98°C for 15 min on a thermocycler.

4. Add 100 µl of LiDirect™ Stop Solution.

Note: Tissues will not be completely digested at the end of the incubations. This is normal and will not affect performance.

5. Set up 25 µl of LiTaq™ Plus PCR reaction mix:

2× LiTaq™ Plus Master Mix	12.5 µl
Forward Primer (10 µM)	0.5 µl
Reverse Primer (10 µM)	0.5 µl
ddH_2O	10.5 µl
DNA extract (Template)	1 µl

6. Perform PCR reaction and analyze both reactions on an 1-1.5% agarose gel.

Thermocycling Conditions for a Routine PCR:

95°C	15 sec (Pre-denaturation)	
98°C	5 sec	35 cycles
55°C*	10 sec	
72°C**	5 sec/100bp	
72°C	3 min (final extension)	
4°C	Hold	

* Annealing temperature is based on the Tm of the primer pair and is typically 1-2°C below the calculated Tm.

** If performing multiplex PCR, please add 10 sec to the extension time calculated for the longest product.

Troubleshooting

No product at all or low yield

1. If positive control works, maybe too much tissue added, perform 1:5 serial dilution with ddH_2O to find out the best dilution factor.

2. Increase PCR cycles.

3. Increase primer concentration.

4. If positive control is not working, perform gradient PCR and increase extension time.

5. Try use LiTaq™ HS series of enzyme (M0031) if haven't already done so.

6. Centrifuge DNA extract, and purify the supernatant with LiPure™ Gel Extraction Kit (M0035).

Non-specific products - High molecular weight smears

1. Decrease enzyme concentration.

2. Decrease extension time.

3. Reduce the total number of cycles.

4. Increase annealing temperature or try 2-step protocol.

5. Vary denaturation temperature.

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