

## Introduction

**LiDirect™ Genotyping DNA Extraction Buffer** is designed for fast extraction of tissue DNA for PCR amplification only. 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, mouse tails, yeast, plant cells and other animal tissues, cells, hair shafts, and saliva. The resulting extract can be used directly in PCR reactions using any of our DNA polymerase enzymes and master mixes (M0020-M0025).

## Package Information

Components	M0014
LiDirect™ Extraction Buffer*	150 ml
LiDirect™ Stop Solution	250 ml

\* **Warning:** Alkaline, corrosive

## Storage

Store at 4°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  $\sim 1 \times 10^6$  cells into a PCR micro tube.

**IMPORTANT:** DO NOT add more than 10 mg of tissue per 75  $\mu$ l of LiDirect™ Extraction Buffer. Too much tissue may inhibit downstream PCR reactions.

2. Add 75  $\mu$ l of LiDirect™ Extraction Buffer.

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

4. Add 125  $\mu$ l 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 50  $\mu$ l of PCR reaction mix and split in half.

6. Take 1  $\mu$ l of the extract supernatant and mix into the first vial of PCR solution, then take 1  $\mu$ l of the resulting mix and serial dilute into the second vial.

7. Perform PCR reaction according to the respective manual, and analyze both reactions on an 1-1.5% agarose gel.

## Trouble Shooting

### No product at all or low yield

1. If positive control works, maybe too much tissue added, perform 1:2 serial dilution with ddH<sub>2</sub>O 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 LiSpark™ Max series of enzyme (M0020-M0022) if haven't already done so.

### 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.
6. Decrease primer concentration.