# I. List of Components

- Terra Direct PCR Polymerase Mix (1.25 U/µl)
- 2X Terra Direct PCR Buffer (with $Mg^{2+}$, dNTPs)—For Genotyping
- Terra Direct PCR—Tissue Extraction Buffer
  **NOTE:** Store at room temperature after thawing.
- Proteinase K.
- 5X Loading Dye—For Genotyping

# II. Additional Materials Required

- PCR-grade water (Avoid using autoclaved H2O; the recycled steam in some autoclaves can introduce contaminants that may interfere with PCR.)
- Gene-specific PCR primers (with Tm ≥ 60°C; avoid using primers that contain inosine)
- PCR reaction tubes or plate
- Aerosol-resistant pipette tips preferably equipped with hydrophobic filters.

# III. General Considerations

Follow the recommendations in Option A if you are performing direct PCR with the whole-tissue samples and Option B if you are performing PCR with crude tissue extracts:

**Option A**

**Direct PCR with Whole-Tissue Samples**

Use the following as a guide to help you determine the appropriate amount of whole-tissue sample to ***add directly to*** your PCR reaction.

When using:

- mouse tail biopsies, add ≤ 1 mm of tissue.
- mouse ear biopsies, add ≤ 2 mm diameter disc of tissue.
- mouse organs, add ≤ 1.5 $mm^3$.

**Option B**

**Direct PCR with Crude Tissue Extracts**

For tissue extracts, you may use any sample preparation method that is appropriate for your sample type.

We have found the following method works well for mouse tissue extracts:

i. Add 20 µl of Terra Direct PCR—Tissue Extraction Buffer* to the mouse tissue.
ii. Add 0.5 µl Proteinase K and mix by vortexing briefly.
iii. Incubate the reaction for 5 min at room temp, and then for 2 min at 98°C.
iv. Use ≤ 5 µl of the crude extract in the PCR reaction (see Table 1, next page).

* Perform the above procedure at room temperature. If a precipitate has formed in the Tissue Extraction Buffer, heat the tube at 60oC and invert gently until the precipitate has completely dissolved.

# IV. Protocol

## A. PCR Reaction Set-Up

1. Prepare the PCR reaction on ice by adding each component indicated in Table 1.
2. Briefly spin the tube in a microcentrifuge, and begin thermal cycling using the guidelines provided below.

**Table 1. PCR Reaction Components**

| Reagent | Amount | Final Conc. |
|---|---|---|
| Sterile $H_2O$ | Up to 50 µl | — |
| 2X Terra Direct PCR Buffer—For Genotyping[a] | 25 µl | 1X[b] |
| Primer 1 | 15 pmol | 0.3 µM |
| Primer 2 | 15 pmol | 0.3 µM |
| Tissue Sample/Extract | ≤ 5 µl[c] | |
| Terra Direct PCR Polymerase Mix | 1 µl | 1.25 U |
| **Total volume** | 50 µl[d] | |

[a] The buffer may turn white when left at room temperature; this is not a problem as it will become clear when placed on ice.

[b] In the PCR reaction, the final concentration of Mg2+ is 2 mM and the final concentration of each dNTP is 400 mM.

[c] See the 'General Considerations' section, above, for suggested amounts of different sample types.

[d] For 25 µl reactions, be sure to add only half the amount indicated for each reagent.

## B. Recommended Cycling Conditions

Use the following cycling conditions when setting up your initial experiments. These are general guidelines—the optimal conditions may vary.

**3-Step PCR (recommended)**

- 98°C for 2 min*
- 30–40 cycles:
  98°C for 10 sec
  60°C for 15 sec
  68°C for 1 min/kb

**2-Step PCR**

- 98°C for 2 min*
- 30–40 cycles:
  98°C for 10 sec
  68°C for 1 min/kb

* The initial denaturation step must be performed at 98°C for 2 min in order to denature the hot start antibody.

## C. Post-PCR Considerations

1. When running your samples on agarose gels, **we recommend using the 5X Loading Dye—For Genotyping supplied in the kit.** This loading dye is formulated to provide improved sample resolution on agarose gels.
2. Use TAE running buffer when visualizing your PCR products by agarose gel electrophoresis. The use of TBE is not recommended as it causes spreading of the DNA bands toward the bottom of the gel.
3. PCR products produced by Terra Direct PCR contain 3′ A-overhangs, making them compatible with T/A cloning.

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