Terra™ PCR Direct Red Dye Premix Protocol-At-A-Glance

I. List of Components

- 2X Terra PCR Direct Red Dye Premix
- dH_2O (PCR-grade)
- 2 tubes Proteinase K (100 µl/tube)

II. Additional Materials Required

- Gene-specific PCR primers (with $T_m \geq 60°C$; avoid using primers that contain inosine)
- PCR reaction tubes or plate
- Aerosol-resistant pipette tips preferably equipped with hydrophobic filters.

III. General Considerations

The red dye in the premix serves two functions as a visual aid. First, the colored dye aids positioning during the loading process. Second, the dye separates into two colored components to aid in monitoring the progress of electrophoresis. Follow the recommendations in Option A (below) if you are performing direct PCR with whole-tissue samples and Option B if you are performing PCR with crude tissue extracts.

NOTE: While there is a tolerance in the amount of material added, the maximum recommended amounts listed here are more than sufficient to obtain good signal. In fact, *less sample may provide a higher signal*.

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 use in your direct PCR reaction.

When using:

- blood treated with EDTA or heparin, add ≤ 5 µl of blood ***directly to*** your PCR reaction.
- mouse tail biopsies, add ≤ 1 mm of tissue ***directly to*** your PCR reaction.
- mouse ear biopsies, add ≤ 1.5 mm^2 of tissue ***directly to*** your PCR reaction.
- plant leaves (e.g., tomato or spinach), add a ≤ 1.2 mm diameter disc ***directly to*** your PCR reaction.

Option B

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 180 µl of 50 mM NaOH to 5–10 mg of mouse tissue (e.g., tail, liver, spleen, thymus, or brain) and incubate for ten minutes at 95°C.

ii. Neutralize the extract by adding 20 µl of 1M Tris-HCl (pH 8.0).

iii. Add ***≤ 5 µl of the crude extract to the PCR reaction*** (see Table 1, below).

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. Recommended Reagent Volumes

Reagent	Amount	Final concentration
2X Terra PCR Direct Red Dye Premix	25 µl	1X
Primer 1	15 pmol	0.3 µM
Primer 2	15 pmol	0.3 µM
Tissue Sample/Extract	≤ 5 µl [a]	
dH_2O	to 50 µl [b]	
Total volume per reaction	50 µl [c]	

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

[b] In the PCR reaction, the **final concentration** of Mg^{2+} is 2 mM and the final concentration of each dNTP is 400 mM.

[c] 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
(For amplification of standard targets)

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

2-Step PCR
(For amplification of GC-rich targets)

98°C	2 min*	
98°C	10 sec	30–40 cycles
68°C	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

- Use TAE running buffer for agarose gel electrophoresis. TBE is not recommended as it causes spreading of the DNA bands toward the bottom of the gel. Load 3−5 µl of each reaction on the gel after cycling.
- PCR products amplified directly from animal tissue (e.g. mouse tail) do not always resolve well when electrophoresed on agarose gels. To improve band resolution on agarose gels, add 1 µl Proteinase K to the entire 50 µl PCR sample **after** thermal cycling. You may choose not to add Proteinase K for sample types that have minimal cellular debris or protein after lysis, such as blood.
- PCR products produced by Terra PCR Direct contain 3′ A-overhangs, making them compatible with T/A cloning.
- Please refer to the Terra Polymerase Mix User Manual for the Terra and General PCR troubleshooting guides.

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