Please read the User Manual before using this Protocol-at-a-Glance. This abbreviated protocol is provided for your convenience, but is not intended for first-time users.

- 1. Terra Direct PCR Polymerase Mix is designed to amplify targets from whole tissues and tissue extracts. Use the sample amount—and/or preparation method—that is appropriate for your sample type.
- 2. On ice, combine the reagents shown in Table I in a PCR tube. Make sure you use enough of each reagent for all of your reactions plus an additional reaction to accommodate pipetting errors.

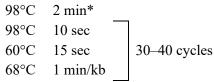
8		
Reagent	Volume	<b>Final concentration</b>
2X Terra Direct PCR Buffer (with Mg <sup>2+</sup> , dNTP)	25 ml	1X
Primer 1	15 pmol	0.3 μΜ
Primer 2	15 pmol	0.3 μM
Tissue Sample/Extract	$\leq 5 \mu l *$	
Terra Direct PCR Polymerase Mix	1 µl	1.25 U
Sterile Water	to 50 µl	
Total volume per reaction	50 µl †	

Table 1. Recommended Reagent Volumes

\* When using: 1) crude extracts from various animal tissues, add  $\leq 5 \mu$ l; 2) blood treated with EDTA or heparin, add  $\leq 5 \mu$ l; 3) mouse tail, add  $\leq 1 \text{ mm}$ ; 4) mouse ear, add  $\leq 1.5 \text{ mm}$ 2; or 5) plant leaves (e.g., tomato or spinach), add  $\leq 1.2 \text{ mm}$  diameter disk.

 $\dagger$  For 25  $\mu l$  reactions, be sure to add only half the amount indicated for each reagent.

- 3. Mix the contents of each tube by tapping the bottom of the tube, then centrifuge briefly.
- 4. Program your thermal cycler with the following cycling conditions:
  - **3-Step PCR** (for amplification of standard targets < 2 kb):



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

- **2-Step PCR** (for amplification of targets that are GC-rich or  $\geq 2$  kb):
  - 98°C 2 min\* 98°C 10 sec 68°C 1 min/kb

30-40 cycles

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

## 5. Post-PCR Considerations

- Use TAE running buffer when visualizing your PCR products by agarose gel electrophoresis.
- Add proteinase K to the gel loading buffer.
  - a. Add 5  $\mu$ l of Proteinase K to 50  $\mu$ l of 5–6X loading buffer.
  - b. Before loading your samples onto a gel, add 15 μl of the loading buffer-proteinase K mixture to the entire 50 μl PCR reaction, or add 1 μl of the loading buffer-proteinase K mixture to 4 μl of the PCR reaction.

## Terra<sup>™</sup> Direct PCR Polymerase Mix Protocol-At-A-Glance

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This document has been reviewed and approved by the Quality Department.