I. List of Components

- Terra PCR Direct Polymerase Mix (1.25 U/µl)
- 2X Terra PCR Direct Buffer—For FFPE Samples
  NOTE: The 2X Buffer contains 4 mM Mg^{2+} and 800 µM each dNTP.
- Terra PCR Direct—DNA Recovery Buffer
  NOTE: Store at room temperature after thawing.
- Proteinase K.

II. Protocol

A. Recovery of DNA from Paraffin-Embedded Tissue Sections

NOTE: Perform the procedure at room temperature. If a precipitate has formed in the DNA Recovery Buffer, heat the tube at 60°C and invert gently until the precipitate has completely dissolved.

1. Using a sterile spatula, scrape a 1–1.5 cm^2 paraffin-embedded tissue section from a slide, and place in a PCR tube containing 100 µl Terra PCR Direct—DNA Recovery Buffer and 1 µl Proteinase K.

NOTE: If a larger paraffin section is used, increase the volumes of DNA Recovery Buffer and Proteinase K.

2. In a thermal cycler, incubate the sample at 60°C for 15 minutes, then at 98°C for 5 min.
3. Briefly centrifuge the sample at room temperature to collect the precipitate at the bottom of the tube. The resulting supernatant will contain the DNA template for the PCR reaction in the next step. To save the DNA, transfer the supernatant to a separate tube and store at –20°C.

B. PCR Reaction Set-Up

1. Prepare your PCR reaction(s) on ice by adding each component indicated in Table 1.

NOTES:

i. The 2X Terra PCR Direct Buffer—For FFPE Samples may turn white when left at room temperature; this is not a problem as it will become clear when placed on ice.

ii. The supernatant containing the DNA template (see previous section, Step 3) must be returned to room temperature before use. The amount of supernatant added to the PCR reaction should be no more than the 1/10th the total volume of the reaction.

Table 1. PCR Reaction Components

Reagent	Amount	Final Conc.
PCR-Grade H_2O	Up to 50 µl	—
2X Terra PCR Direct Buffer—For FFPE	25 µl	1X
Primer 1	15 pmol	0.3 µM
Primer 2	15 pmol	0.3 µM
DNA template; room temperature (supernatant from Step 3, above)	≤ 5 µl	
Terra PCR Direct Polymerase Mix	1 µl	1.25 U
Total volume	50 µl	

2. Briefly spin the tube in a microcentrifuge, and begin thermal cycling using the guidelines provided below.

C. Recommended Cycling Conditions

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

NOTE: Formalin-fixed, paraffin-embedded tissues typically yield DNA that has been fragmented by the fixation and embedding process. The fragmented DNA is unsuitable as a template when PCR- amplifying long targets; therefore, limit your amplicon size to ≤ 500 bp.

The following cycling conditions are suggested for targets ≤ 500 bp:

3-Step PCR (recommended)

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

2-Step PCR

- 98°C for 2 min*
- 40 cycles:
  98°C for 10 sec
  68°C for 30 sec

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

D. Post-PCR Considerations

1. 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.
2. PCR products produced by Terra PCR Direct contain 3’ A-overhangs, making them compatible with T/A cloning.

Contact Us	
Customer Service/Ordering	**Technical Support**
tel: 800.662.2566 (toll-free)	tel: 800.662.2566 (toll-free)
fax: 800.424.1350 (toll-free)	fax: 800.424.1350 (toll-free)
web: www.clontech.com	web: www.clontech.com
e-mail: orders@clontech.com	e-mail: tech@clontech.com

Notice to Purchaser