

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 Mg²⁺ 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 cm² 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 ₂ O	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

Clontech® products are to be used for research purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, *in vitro* diagnostic purposes, therapeutics, or in humans. Clontech products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without prior written approval of Clontech Laboratories, Inc.

Your use of this product is also subject to compliance with any applicable licensing requirements described on the product's web page at <http://www.clontech.com>. It is your responsibility to review, understand and adhere to any restrictions imposed by such statements.

Clontech, the Clontech logo, and Terra are trademarks of Clontech Laboratories, Inc. All other marks are the property of their respective owners. Certain trademarks may not be registered in all jurisdictions. Clontech is a Takara Bio Company. ©2014 Clontech Laboratories, Inc.

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