Takara Bio USA

Terra™ Direct PCR Polymerase Mix User Manual

Cat. Nos. 639269, 639270, 639271
(102224)

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I. Introduction

Terra Direct PCR Polymerase Mix contains a novel enzyme developed for optimal, direct amplification from tissue samples, crude extracts, and dirty templates. It's perfect for amplifying short DNA targets (up to 2 kb), regardless of GC content or template purity. Terra Direct PCR is a highly sensitive enzyme that allows amplification of targets from small amounts of template. Moreover, the enzyme comes pre-blended with a monoclonal antibody that suppresses polymerase activity up to 98°C, allowing automatic hot start PCR.

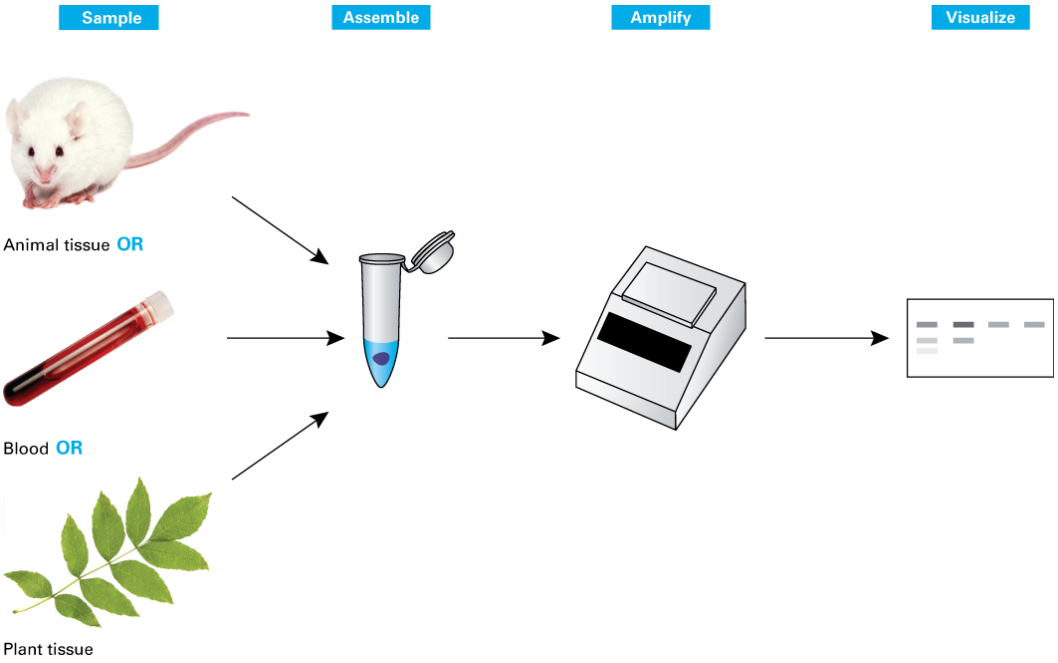


Figure 1. Direct PCR with Terra Direct PCR Polymerase Mix requires no sample prep. Just add a small portion of your tissue of interest directly to the PCR reaction and amplify your target.

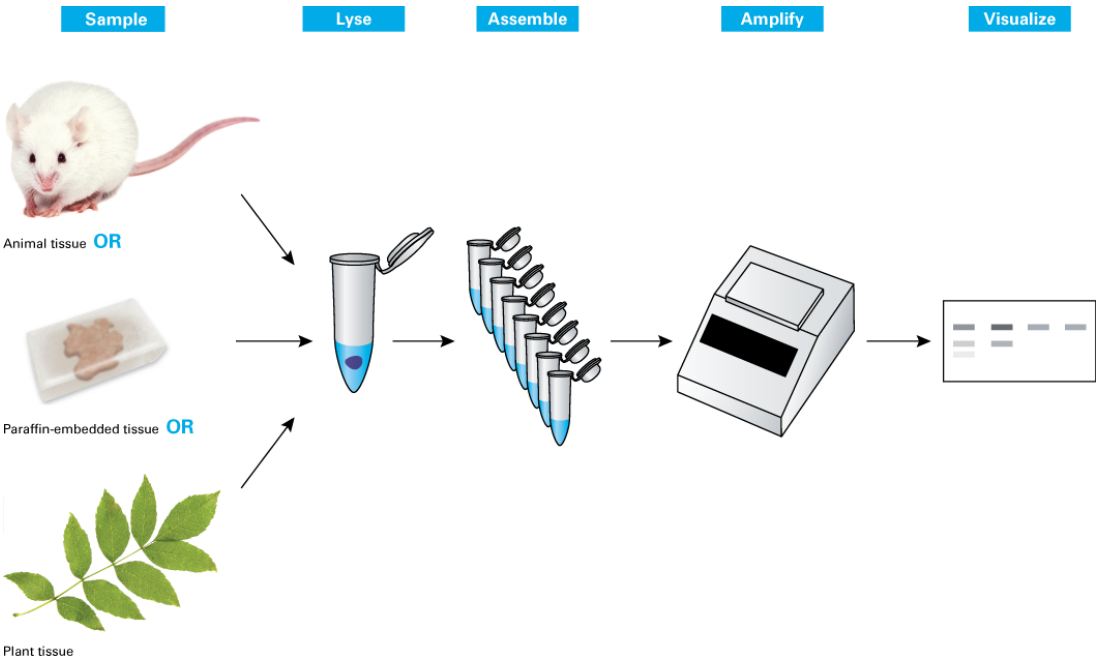


Figure 2. Amplification from crude tissue extracts requires no purification steps. Just add a small portion of your crude tissue extract to one or more PCR reactions and amplify your target with Terra Direct PCR Polymerase Mix.

II. List of Components

Store all components at –20°C.

Terra Direct PCR Polymerase Mix (40 rxns; Cat. No. 639269)

- 40 µl Terra Direct PCR Polymerase Mix (1.25U/µl)
- 1 ml 2X Terra Direct PCR Buffer (with Mg^{2+}, dNTP)
- 100 µl Proteinase K

Terra Direct PCR Polymerase Mix (200 rxns; Cat. No. 639270)

- 200 µl Terra Direct PCR Polymerase Mix (1.25U/µl)
- 5 tubes 2X Terra Direct PCR Buffer (with Mg^{2+}, dNTP; 1 ml/tube)
- 3 tubes Proteinase K (100 µl/tube)

Terra Direct PCR Polymerase Mix (800 rxns; Cat. No. 639271)

- 4 each Terra Direct PCR Polymerase Mix (200 rxns; Cat. No. 639270)

III. Additional Materials Required

The following materials are required but not supplied:

- **Gene-specific PCR primers** with $T_m \geq 60°C$. Avoid using primers that contain inosine.
- **PCR-grade water**
  Avoid using autoclaved H_2O; the recycled steam in some autoclaves can introduce contaminants that may interfere with PCR.
- **PCR reaction tubes or plate**
- **Aerosol-resistant pipette tips**, preferably equipped with hydrophobic filters.

NOTE: Terra Direct PCR Polymerase lacks 5’ to 3’ exonuclease activity; therefore, it cannot be used in probe-based (5’ nuclease) assays.

IV. Terra Direct PCR Protocol

PLEASE READ ENTIRE PROTOCOL BEFORE STARTING.

A. Setting up Terra Direct PCR Assays

Terra Direct PCR Polymerase Mix is designed to amplify targets from whole tissues and tissue extracts (see Section VI for examples). 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 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 ≤ 5µl 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 that 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 1 M Tris-HCl (pH 8.0).
iii. Add ≤ ***5 µl of the crude extract to the PCR reaction*** (see Table I, below).

1. On ice, combine the reagents shown in Table I in a PCR tube.
   - Thaw each reagent, and make sure that each reagent is homogeneous before adding to reactions. For example, vortex at low speed or pipet up and down until mixed thoroughly.
   - Prepare a master mix for all reactions plus one by combining the following reagents:

Table 1. Recommended Reagent Volumes

Reagent	Amount	Final concentration
2X Terra Direct PCR Buffer (with Mg^{2+}, dNTP) [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
Sterile Water	to 50 µl	
Total volume per reaction	50 µl [d]	

[a] A precipitate may be visible in the buffer. Mix the buffer until no precipitate is visible and the buffer is homogeneous. This may require slight warming of the tube and mixing until homogeneous.

[b] The final concentration of Mg^{2+} is 2 mM and the final concentration of each dNTP is 400 µM.

[c] See Option A and Option B in the shaded boxes 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. The maximum recommended amount of tissue sample/extract remains the same.

2. Mix the contents of each tube by tapping the bottom of the tube, pipetting up and down, or gently vortexing. Then centrifuge briefly.
3. Program your thermal cycler with the following cycling conditions:
   - **3-Step PCR** (for amplification of standard targets < 2 kb):

     98°C 2 min*
     98°C 10 sec
     60°C 15 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.

   - **2-Step PCR** (for amplification of targets that are GC-rich or ≥ 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.

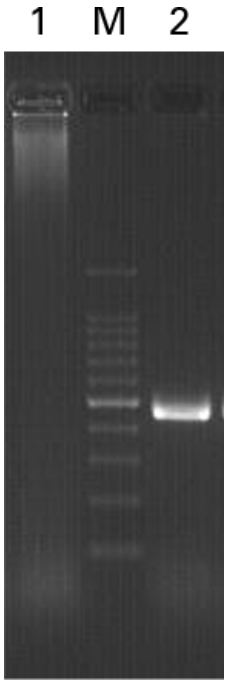
B. Post-PCR Considerations

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 amplified directly from animal tissue (e.g. mouse tail) do not always resolve well when electrophoresed on agarose gels; the products can become trapped in cell debris, which prevents them from migrating correctly through the gel. To prevent this, add proteinase K to the gel loading buffer. You may choose not to add proteinase K for sample types that have minimal cellular debris or protein after lysis, such as blood.
   a. Add 5 µl of Proteinase K to 50 µl 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.
3. PCR products produced by Terra Direct PCR contain 3′ A-overhangs, making them compatible with T/A cloning.

V. Troubleshooting

A. Terra Visual Troubleshooting

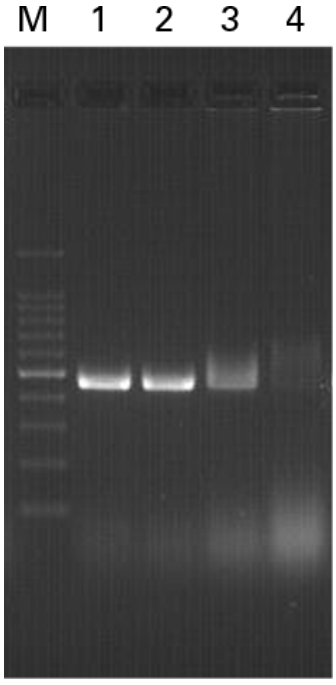
Problem: The Terra-amplified PCR product is stuck in the well, or does not migrate properly into the gel



- **Explanation:** PCR products amplified directly from animal tissues can become trapped in cell debris, which prevents them from migrating correctly through the gel.
- **Solution:** Before you load your sample(s) onto a gel, add proteinase K as described in the protocol.

Figure 3. Use Proteinase K if your PCR product is stuck in the gel. The Mapk6 gene was amplified using the Terra Direct PCR Red Dye Premix. Lane 1: 1 mm tail section (no Proteinase K). Lane M: 100 bp ladder. Lane 2: 1 mm tail section (with Proteinase K).

Problem: PCR band is diffused, or there is no PCR band



- **Explanation:** The PCR reaction could be overloaded. Samples contain impurities that include PCR inhibitors.
- **Solution:** Try reducing the amount of starting material, thereby reducing the concentration of PCR inhibitors.

Figure 4. Reduce amount of starting material to improve direct PCR results. The Mapk6 gene was amplified using the Terra Direct PCR Red Dye Premix. Lane M: 100 bp ladder. Lane 1: 1 mm tail section (recommended sample input).Lane 2: 2 mm tail section. Lane 3: 4 m mm tail section. Lane 4: 8 mm tail section.

Problem: It is difficult to cut samples small enough to get the recommended-size pieces

- **Explanation:** Higher sample input does not yield higher product output; in fact, it may *decrease* yield due to the higher level of PCR inhibitors present in a larger sample. It is best to use the recommended input amounts when possible.
- **Solution:** If you have to use larger samples because the samples are difficult to cut, use the Terra Genotyping Kit (Cat. No. 639285). Its extraction buffer and process provide a higher tolerance against the effects of PCR inhibitors.

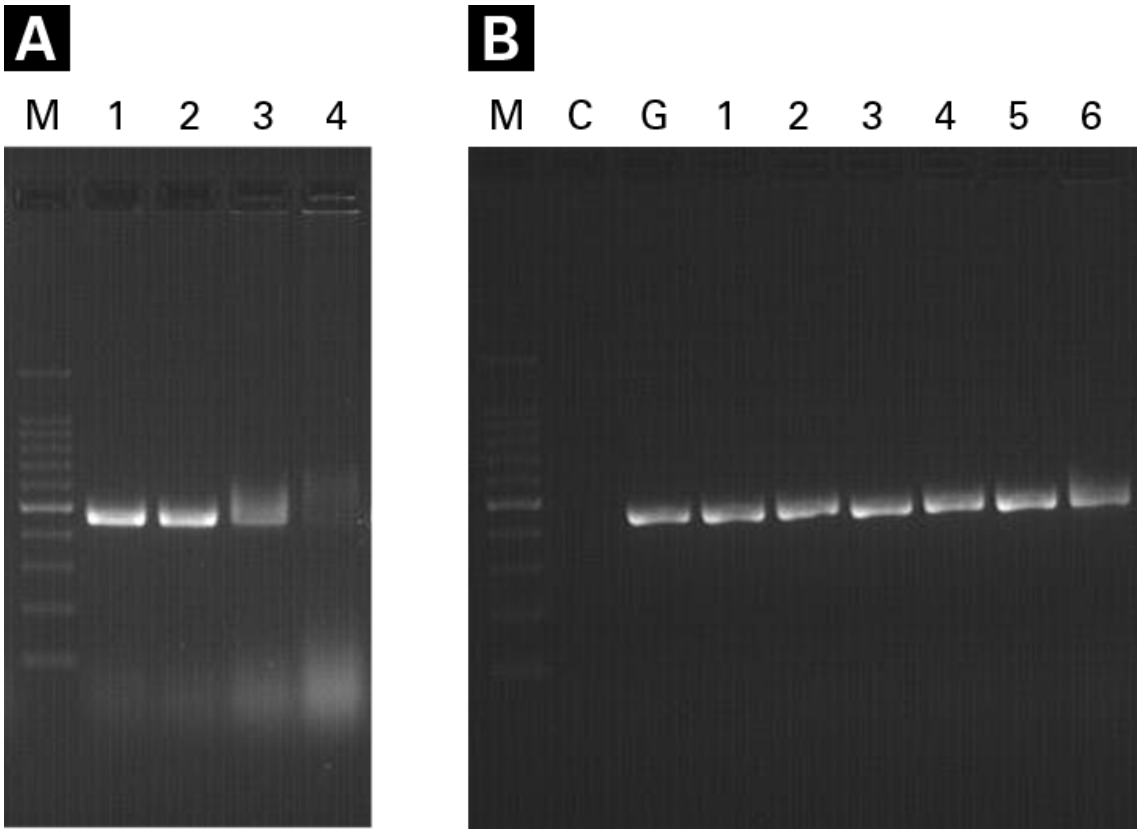


Figure 5. If your samples are large, use the Terra Direct PCR Genotyping Kit. The *Mapk6* gene was amplified from 1, 2, 4, and 8 mm tail sections using the Terra Direct PCR Red Dye Premix (**Panel A**) and from 2, 4, and 8 mm tail sections using the Terra Direct PCR Genotyping Kit (**Panel B**). The Genotyping Kit showed better results with larger samples.

Panel A. Terra Direct PCR Red Dye Premix	Panel B. Terra Direct PCR Genotyping Kit
Lane M: 100 bp ladder	Lane M: 100 bp ladder
Lane 1: 1 mm tail section*	Lane C: Negative control
Lane 2: 2 mm tail section	Lane G: 50 ng gDNA
Lane 3: 4 mm tail section	Lane 1: 2 mm tail section; 5 µl extraction mixture
Lane 4: 8 mm tail section	Lane 2: 2 mm tail section; 10 µl extraction mixture
	Lane 3: 4 mm tail section; 5 µl extraction mixture
	Lane 4: 4 mm tail section; 10 µl extraction mixture
	Lane 5: 8 mm tail section; 5 µl extraction mixture
* Recommended sample input	Lane 6: 8 mm tail section; 10 µl extraction mixture

Problem: PCR is nonspecific; multiple bands or a smear are observed

- **Explanation:** Primers are most likely annealing non-specifically. This often happens with templates that have higher than 70% GC content.
- **Solution:** Try increasing annealing stringency by using 2-step PCR, so that the primers anneal and extend at the same temperature.

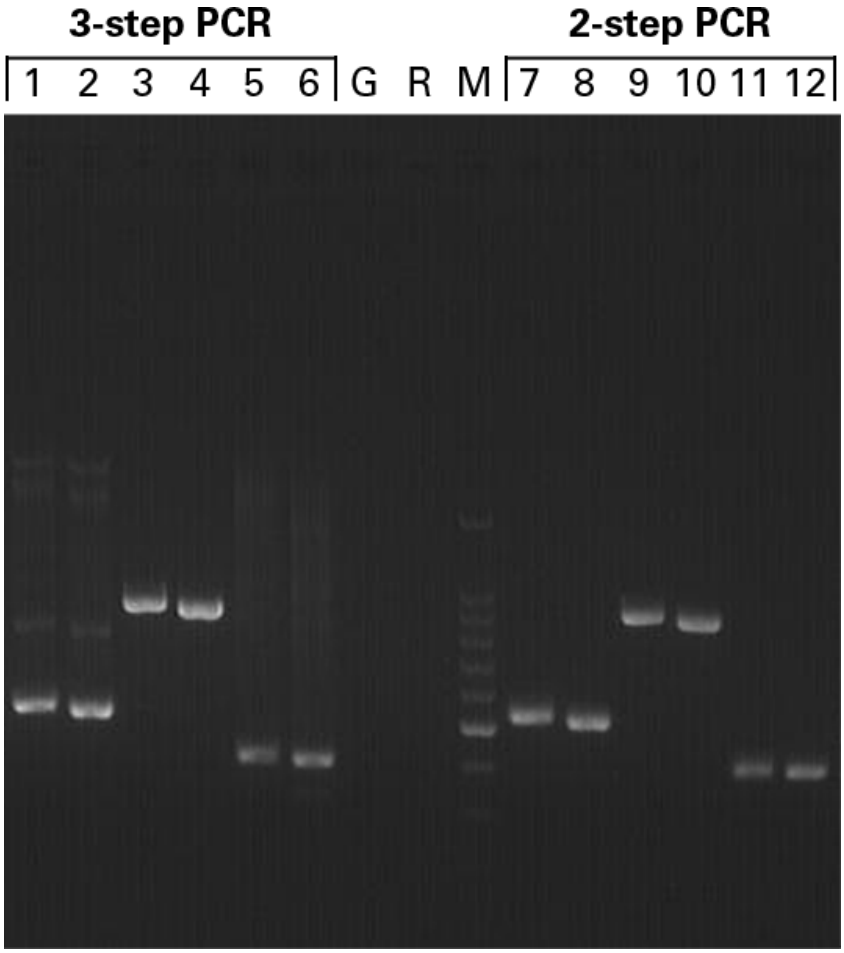


Figure 6. Increase specificity by doing 2-step PCR instead of 3-step PCR. The *Jund, Foxd1 and Ywhaz* genes were amplified from mouse genomic DNA using the Terra Direct PCR Red Dye Premix or Terra Direct PCR Genotyping Kit with either 3-step or 2-step PCR, as noted below. Smears and multiple bands observed for *Jund* and *Ywhaz* in the 3-step PCR products were eliminated by doing 2-step PCR.

Lane	Cycling Program	Target	% GC	Terra Direct PCR Product
1	3-step PCR	*Jund*	72%	Genotyping Kit
2	3-step PCR	*Jund*	72%	Red Dye Premix
3	3-step PCR	*Foxd1*	72%	Genotyping Kit
4	3-step PCR	*Foxd1*	72%	Red Dye Premix
5	3-step PCR	*Ywhaz*	74%	Genotyping Kit
6	3-step PCR	*Ywhaz*	74%	Red Dye Premix
G	Negative control			Genotyping Kit
R	Negative control			Red Dye Premix
M	100 bp ladder			
7	2-step PCR	*Jund*	72%	Genotyping Kit
8	2-step PCR	*Jund*	72%	Red Dye Premix
9	2-step PCR	*Foxd1*	72%	Genotyping Kit
10	2-step PCR	*Foxd1*	72%	Red Dye Premix
11	2-step PCR	*Ywhaz*	74%	Genotyping Kit
12	2-step PCR	*Ywhaz*	74%	Red Dye Premix

B. General PCR Troubleshooting

Table 2. General PCR Troubleshooting

Problem	Explanation	Solution
No PCR product is obtained Or Amplification efficiency is low	Primer T_m is not optimal	Redesign your primers.
	Cycling conditions are not optimal	If you used 3-Step PCR, try 2-Step PCR. If you used 2-Step PCR, try 3-Step PCR.
	Annealing temperature is too high	Decrease the annealing temperature in 2°C increments.
	Insufficient number of PCR cycles	Increase the number of PCR cycles, up to a maximum of 40 cycles.
	Increase extension time in 15 sec increments.	Insufficient extension time
	Need more/less template or sample	Reduce or increase the amount of template or tissue sample used.
	Sample/template preparation method is not effective	Re-evaluate/optimize your sample/template preparation method.
High background/ amplification products are nonspecific	Primer T_m is too low	Redesign your primers.
	Nonspecific primers	Redesign your primers.
	Cycling conditions are not optimal	If you used 3-Step PCR, try 2-Step PCR.
		Use 25−30 cycles.
	Sample/template preparation method is not optimal	Re-evaluate/optimize your sample/ template preparation method.
	Annealing temperature is too low	Increase the annealing temperature in 2° increments.
	Too much template	Try again with less template. Refer to Options A and B in section IV. A, Setting up Terra Direct PCR Assays

VI. Examples—Direct Amplification from Animal and Plant Tissues

A. Direct Amplification of Targets from Mouse Blood Treated with EDTA or Heparin

Terra Direct PCR was used to amplify the cyclin D2 gene (*Ccnd2*; 0.5 kb) and the transferrin receptor gene (*TfrC;* 2 kb) from blood treated with either EDTA or heparin. PCR (containing 5% blood) was performed using a 3-step protocol (30 cycles); 3 µl of each reaction was electrophoresed and the products compared (Figure 7). Terra Direct PCR was able to amplify the targets from both blood samples.

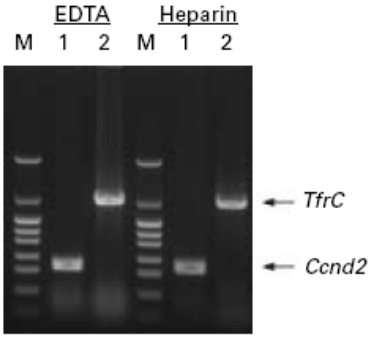


Figure 7. Direct amplification of *Ccnd2* and *TfrC* from mouse blood treated with EDTA. The cyclin D2 gene (*Ccnd2*, 0.5 kb; Lane 1), and the transferrin receptor gene (*TfrC*, 2 kb; Lane 2) were amplified from mouse blood treated with either EDTA or heparin. Terra Direct PCR was able to amplify the targets from both blood samples.

B. Direct Amplification of Targets from Mouse Tail and Ear Tissue

Terra Direct PCR was used to amplify the tyrosine 3/tryptophan 5-monooxygenase activation protein, zeta polypeptide gene (*Ywhaz1*; 1 kb) directly from a 1 mm mouse tail biopsy and a 1.5 mm^2 mouse ear biopsy. The tissues were placed directly into 50 µl PCR reactions and subjected to a 3-step (30 cycle) amplification protocol. Before the samples were run on an agarose gel (Figure 8), 4 µl of each was mixed with gel loading buffer that either lacked or contained proteinase K (Lanes 1 and 2, respectively). As can be seen, the PCR products treated with proteinase K ran as expected (Lane 2), whereas those that were not treated with proteinase K got stuck in the wells of the gel (Lane 1).

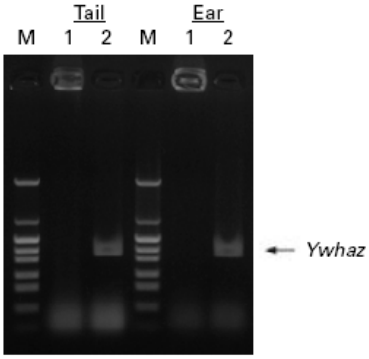


Figure 8. Direct amplification of *Ywhaz* from mouse tail and ear samples. Terra Direct PCR was used to amplify the tyrosine 3/tryptophan 5-monooxygenase activation protein, zeta polypeptide gene (*Ywhaz1*) directly from a 1 mm mouse tail biopsy and 1.5 mm^2 mouse ear biopsy; the tissues were added directly to the PCR reactions. Before the reactions were visualized on a gel, a portion of each was mixed with gel loading buffer lacking (Lane 1) or containing (Lane 2) proteinase K. The PCR products treated with proteinase K ran normally (Lane 2), while those that were untreated never left the wells (Lane 1).

C. Direct Amplification of Targets from Tomato and Spinach Leaves

Terra Direct PCR was used to amplify the cytochrome c oxidase gene (*cox1*; 0.5 kb) directly from tomato and spinach leaf samples (0.50 mm and 1.2 mm discs cut with a hole punch). The leaf discs were placed directly into PCR using a 3-step (30 cycle) amplification protocol; 5 µl of each reaction was then run on an agarose gel (see Figure 9). Terra PCR was able to amplify *cox1* from both the small and large leaf samples.

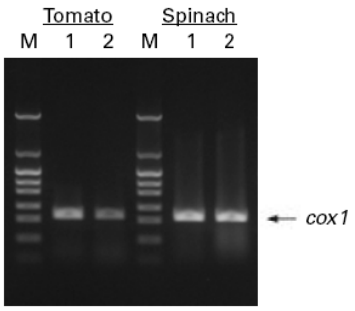


Figure 9. Direct amplification of *cox1* from tomato and spinach leaf samples. Terra Direct PCR was used to amplify the cytochrome c oxidase gene (*cox1*) from 0.5 mm (Lane 1) or 1.2 mm (Lane 2) tomato or spinach leaf cuttings added directly to the PCR reactions. Terra Direct PCR amplified *cox1* from all of the samples.

D. Amplification of Targets from Crude Extracts of Various Mouse Tissues

Crude extracts of a variety of mouse tissues were obtained by adding 180 µl of 50 mM NaOH to 5–10 mg of mouse tail, liver, spleen, thymus, or brain, and incubating for ten minutes at 95°C. Each extract was then neutralized with 20 µl of 1 M Tris-HCl (pH 8.0). Terra Direct PCR was then used to amplify the transferrin receptor gene (*Tfrc*; 2 kb) from 2.5 µl of each extract (in a 25 µl reaction, with a 2 step [30 cycle] protocol); 3 µl of each reaction was electrophoresed and the products compared (Figure 10). Terra Direct PCR produced high yields of *Tfrc* from all of the lysates.

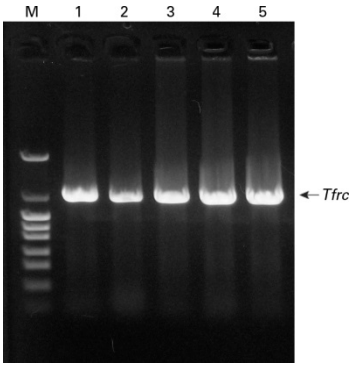


Figure 10. PCR amplification of *Tfrc* from a variety of crude mouse tissue extracts. Terra Direct PCR was used to amplify the transferrin receptor gene (*Tfrc*; 2 kb) from 2.5 µl of mouse tail (Lane 1), liver (Lane 2), spleen (Lane 3), thymus (Lane 4), and brain (Lane 5) extracts. Terra Direct PCR produced high yields of Tfrc from all of the lysates.

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