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We use this protocol and it's working

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20 minute PCR Enzymatic Cleanup

Julian Liber[1]

[1]Department of Biology - Duke University

Julian Liber

ABSTRACT

A rapid microplate-compatible protocol for cleanup of PCR products prior to Sanger sequencing. Verify that the PCR produces a single band, and retain some PCR product for cleanup and sequencing. Set up at room temperature, and react on the thermocycler.

Mechanism: PCR products contain 1) primers and 2) dNTPs which will interfere with downstream Sanger sequencing reactions. Exonuclease I degrades ssDNA (primers) and calf intestinal phosphatase degrades dNTPs. Functions like NEB #E1050.

MATERIALS

- Quick CIP (5U/uL) NEB #M0525
- Thermolabile Exonuclease I (20U/uL) NEB #M0568
- rCutSmart buffer NEB #B6004 (or CutSmart buffer)
- NEBuffer r3.1 NEB #B6003 (or NEBuffer 3.1)
- PCR-grade water

Making the enzyme master mix

1 Add to a 1.5 mL sterile conical tube

- 760 uL water
- 94 uL 10x rCutSmart buffer
- 96 uL 10x Exonuclease I buffer (NEBuffer r3.1)
- 4 uL Quick CIP (5U/uL) NEB #M0525
- 10 uL Thermolabile Exonuclease I (20U/uL) NEB #M0568

2 Mix, then aliquot 200 uL into separate tubes. Store at -20C. Good for at least 2 years.

PCR Cleanup

3 Mix 1.2 uL of master mix with 1.5 uL PCR product in PCR strip tubes or plates.

> **Note**
>
> A 1:1 ratio of enzyme mix:PCR product can also be used. Pipetting 2 uL may be easier, and you can do equal parts in such a case. If doing a large number of reactions, I generally use 2 uL because it is faster to pipette that volume and ensure it stays in the tube.

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4 Seal tubes or plates and place on a thermocycler. React at 37ºC for 15 minutes, then 80ºC for 1 minute.

5 Add the product to Sanger sequencing reactions. 1 uL of product, 1 uL of 10 uM primer, and 10 uL of water per tube. If the band is faint, you can use 2 uL or more of product.

> **Note**
>
> Follow the sequencing facility's requirements for template DNA and primer concentration. The above amounts are recommendations based on what has worked for us.