Enzymatic PCR cleanup using Exonuclease I and Shrimp Alkaline Phosphatase

Introduction

Enzymatic PCR cleanup method offers an easy way to remove the remaining primers and dNTP left from a PCR reaction. Two enzymes are needed to complete the process: Exonuclease I (Exo I, NEB #M0293) which degrades the residual PCR primers, and Shrimp Alkaline Phosphatase (rSAP, NEB #M0371) which dephosphorylates the remaining dNTP. This method enables direct downstream applications, such as Sanger sequencing, NGS, genotyping, SNP analysis and nested PCR etc. The two enzymes are added directly to the PCR reaction after thermal cycling, without changing buffer condition or additional additives. Further, these enzymes are 100% compatible with all commonly used PCR reaction buffers.

Protocol

1. Add 0.5 µl of Exo I and 1 µl of rSAP to 5 µl of PCR product. (Note that the Quick Dephosphorylation Kit (NEB #M0508), which contains Quick CIP, can be used in the same volume).
2. Incubate the mix at 37°C for 15 minutes.
3. Inactivate both enzymes at 80°C for 15 minutes.
4. PCR products are ready for downstream application.

PCR product	5 µl
Exo I	+ 0,5 µl
rSAP	+ 1 µl

Results

Two PCR amplicons, 150 bp and 1.5 kb in length, were generated using One*Taq*® Hot Start Quick-Load 2X Master Mix with Standard Buffer (NEB #M0488) according to recommended protocols. The PCR product was treated with Exo I and rSAP, and analyzed by Sanger sequencing (Figure 2) and agarose gel electrophoresis (Figure 3). Control reactions were also set up with no enzymatic treatment. In both cases, enzymatic treatment resulted in significant improvement in overall sequence quality.

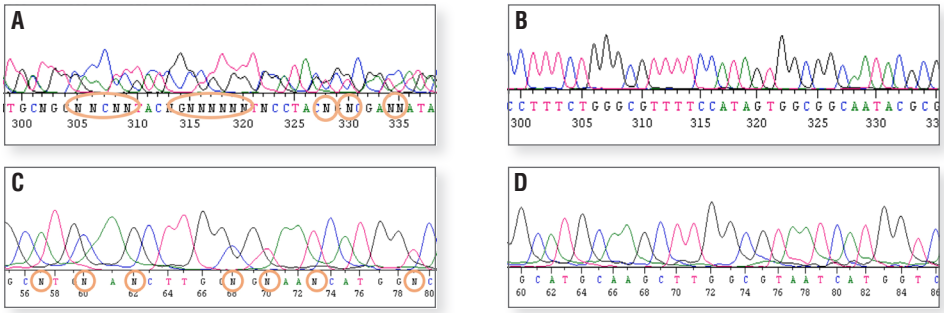

Figure 2: *Sequencing analysis of enzymatic cleanup. Panel A and C show untreated samples of 1.5 kb and 150 bp PCR products, respectively. Panel B and D show the same PCR products treated with ExoI and rSAP prior to sequencing.*

Materials

- Exonuclease I (Exo I)
- Shrimp Alkaline Phosphatase (rSAP)

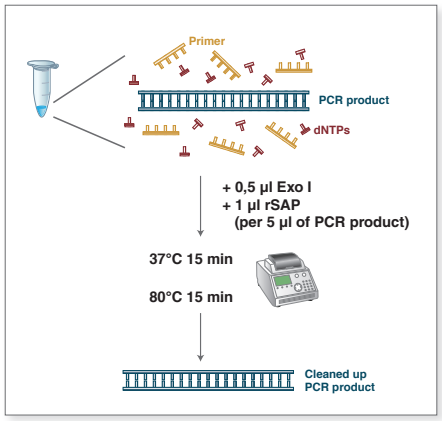

Figure 1: *Enzymatic cleanup workflow diagram*

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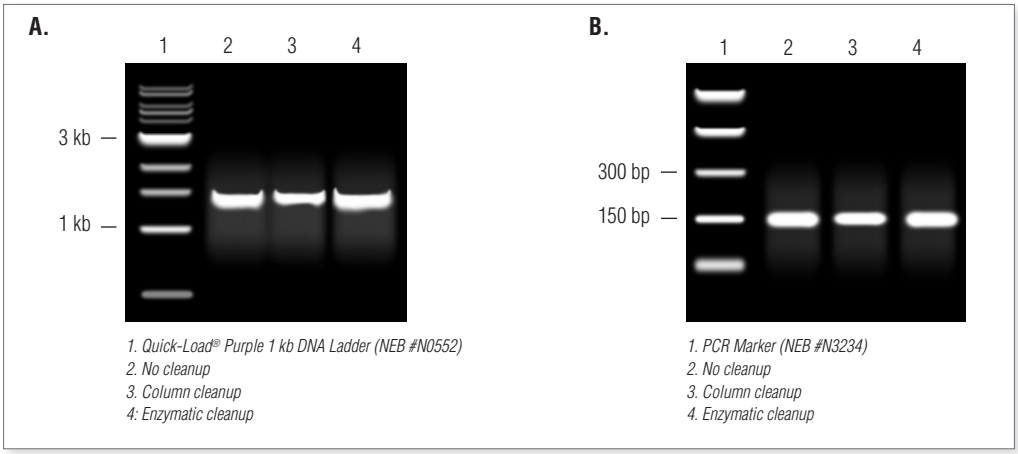

Figure 3: *Agarose gel analysis of enzymatic cleanup. 1.5 kb (A) and 150 bp (B) PCR products were cleaned by either a spin-column or enzymatic method. Results indicate no product loss during enzymatic cleanup process.*

Summary

Enzymatic cleanup with Exo I and rSAP is a convenient way of conditioning a PCR product for downstream applications or analysis. It combines minimal hands on time with virtually no sample loss, and enables high quality sequencing results.

Ordering Information

PRODUCTS	NEB #	SIZE
Exonuclease I	M0293S/L	3,00/15,000 units
Shrimp Alkaline Phosphatase (rSAP)	M0371S/L	500/2,500 units

COMPANION PRODUCTS	NEB #	SIZE
One*Taq* Hot Start Quick-Load 2X Master Mix with Standard Buffer	M0488S/L	100/500 reactions
Quick-Load® Purple 1 kb DNA Ladder	N0552S	125 gel lanes
PCR Marker	N3234S/L	100/500 gel lanes
Quick Dephosphorylation Kit	M0508S/L	100/500 reactions

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