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, #M0293) degrades the residual PCR primers and Shrimp Alkaline Phosphatase (rSAP, #M0371) dephosphorylates the remaining dNTP. It enables direct downstream applications, such as Sanger sequencing, NGS, genotyping, SNP analysis and nested PCR etc. These two enzymes are added directly to the PCR reaction after thermal cycling, without changing buffer condition or additional additives. These enzymes are 100% compatible with all commonly used PCR reaction buffers.

Materials

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

Protocol

1. Add 0.5 µl of Exo I and 1 µl of rSAP to 5 µl of PCR product.
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

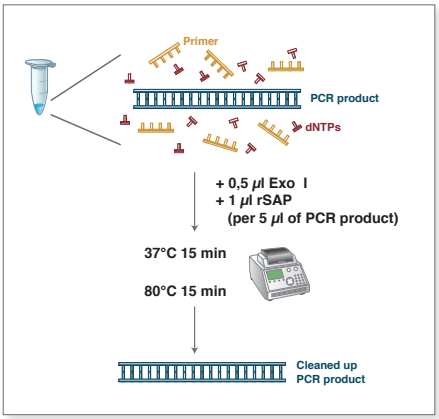


Figure 1: Enzymatic cleanup workflow diagram

Results

A PCR amplicon of 1,5 kb length was produced using One*Taq*® 2X Master Mix with Standard Buffer #M0482 according to protocol recommendations. The PCR product was treated and untreated with Exo I and rSAP and analyzed by Sanger sequencing (Figure 2) and agarose gel electrophoresis (Figure 3). Treatment resulted in significant improvement in overall sequence quality.

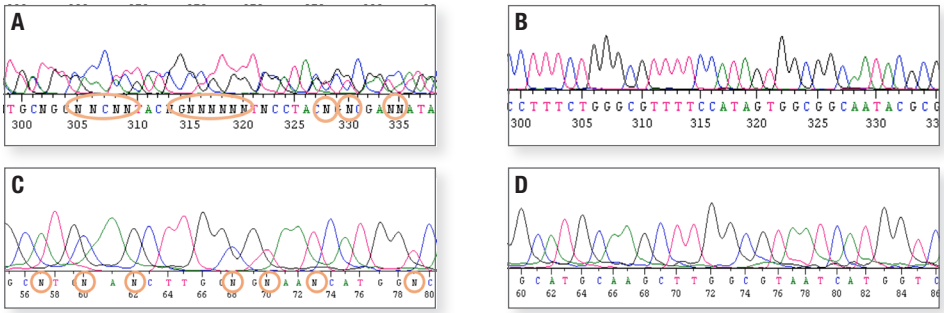
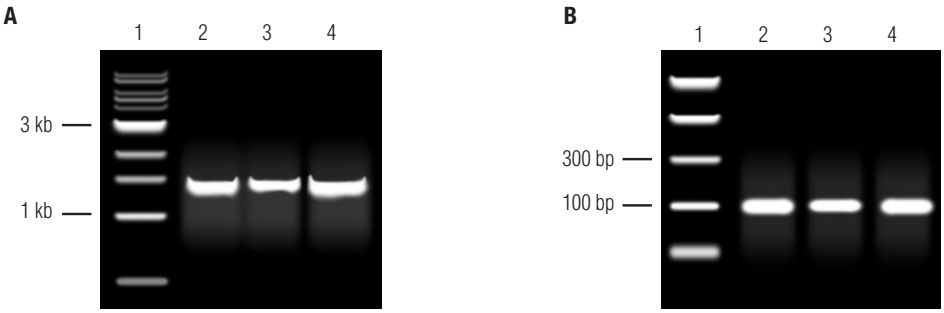


Figure 2: Sequencing results with and without enzymatic cleanup. Panel A and C show untreated samples of 1,5 kb and 150 bp PCR products, resp. Panel B and D show the same PCR products treated with ExoI and rSAP before sequencing.



1: Quick-Load® Purple 1 kb DNA Ladder (#N0552)
2: No cleanup
3: Column cleanup
4: Enzymatic cleanup

1: PCR Marker (#N3234)
2: No cleanup
3: Column cleanup
4: Enzymatic cleanup

Figure 3: Agarose gel analysis of PCR products (A: 1,5 kb; B: 150 bp) that 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	PRICE
Exonuclease I	M0293S/L	3.000/15.000 units	68 € / 272 €
Shrimp Alkaline Phosphatase (rSAP)	M0371S/L	500/2.500 units	59 € / 236 €

COMPLEMENTARY PRODUCTS	NEB #	SIZE	PRICE
One*Taq* 2X Master Mix with Standard Buffer	M0482S/L	100/500 rxns	38 € / 152 €
Quick-Load® Purple 1 kb DNA Ladder	N0552S	125 gel lanes	56 €
PCR Marker	N3234S/L	100/500 gel lanes	59 € / 236 €

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