Protocol

PCR Product Clean-Up Prior to Sequencing

This protocol is for the PCR Product Clean-Up Prior to Sequencing

The clean-up reaction removes unincorporated primers and degrades unincorporated nucleotides. The resulting PCR product is ready to use for sequencing without additional purification, e.g. using column purification kits.

1. Prepare the following reaction mixture:

PCR mixture (directly after completion of PCR)	5 µl
Exonuclease I (Exo I)	0.5 µl (10 u)
FastAP™ Thermosensitive Alkaline Phosphatase or Shrimp Alkaline Phosphatase (SAP)	1 µl (1 u)

2. Mix well and incubate at 37°C for 15 min.
3. Stop the reaction by heating the mixture at 85°C for 15 min.

Note

- Up to 5 µl of purified PCR products can be used directly for DNA sequencing without further purification.
- For reliable sequencing results there should not be non-specific PCR products.
- The protocol may be applied for clean-up of PCR products, generated by any thermophilic DNA polymerase or polymerase mix.
- The procedure is not recommended for downstream cloning applications.

Reference

1. Werle, E., et al., Convenient single-step, one tube purification of PCR products for direct sequencing, *Nucleic Acids Res.*, 22, 4354-4355, 1994.

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