Annex B13
Clean-up of PCR Products

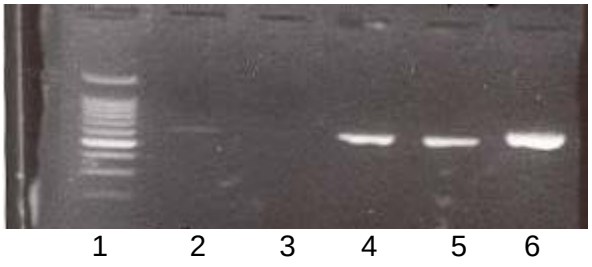
Purpose
The following protocol provides recommendations for the purification of DNA fragments from positive PCR reactions. Excess nucleotides, primers and enzyme must be removed from the reaction. The purified DNA can be used as a template for sequencing.
Many different kits for clean-up of PCR products are commercially available. It is recommended to use a commercially available kit rather than methods such as ethanol precipitation, because the concentration of recovered DNA is higher and more reproducible when using kits. Good results have been obtained with the following kits:

QiaQuick PCR Purification kit (Qiagen, catalog # 28104)
Charge-Switch PCR Clean-up kit (Life Technologies, catalog # CS12000)
PureLink PCR Purification kit (Life Technologies, catalog # K3100-01)

Other kits are acceptable if they produce purified PCR products suitable for sequencing. Please refer to the kit manual for instructions.

Template gel
The purified DNA must be analyzed by agarose gel electrophoresis to assess the recovery of DNA. It is recommended to run an agarose gel as described in the Agarose Gel Electrophoresis protocol. 2 µl of purified PCR product should be loaded on the gel.
After electrophoresis, bands should be easily visible. If bands are faint, the amount of template for sequencing can be increased. Very faint bands indicate insufficient quantities for sequencing.

Example for template gel with purified PCR products
The bands in lanes 4, 5, 6 indicate sufficient quantities of DNA for sequencing.



Lane 1: Marker
Lanes 2-6: purified PCR products.