Cologne Center for Genomics

Source: http://portal.ccg.uni-koeln.de/ccg/service/sequencing

Exo-SAP PCR cleanup protocol (prior sequencing)

Before PCR products can be sequenced, you first must remove residual nucleotides. The following protocol is as simple as cheap!

It is very important to have a high quality PCR template. In any case you should check that PCR protocols produce clear and sharp single bands in gel electrophoresis. Otherwise it will result in sequence background signals!

… and quantify it before putting template to your BigDye reaction!

	Volume in µl
PCR-product	7,0
ExoI* (20U/µl)	0,15
SAP* (1U/µl)	0,9
H2O	1,95
TOTAL VOLUME	**10,0**

- Incubate samples at **37˚C for 20 minutes** and then at **85˚C for 15 minutes** in a PCR machine.
- Store samples at 4˚C or -20˚C until needed.

*Exonuclease I (15000U) from Neo Lab
*SAP (Shrimps Phosphatase Alkali) (500U) from Promega

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