Purification of PCR products for Sequencing

The following protocols for preparing good quality DNA templates for sequencing are recommended by Applied Biosystems and can be found online in their Automatic DNA Sequencing Guide at:
http://www.pebiodocs.com/pebiodocs/04305080.pdf

PCR products can be sequenced directly using cycle sequencing methods, as long as there is only one product present and the primers and excess dNTP's have been removed. Other components such as competing enzymes or buffer components could also cause problems. Therefore the product must be purified before it is sequenced.

There are several methods for purifying PCR products:

*Column purification
*PEG precipitation
*Gel purification
*Ethanol precipitation

IMPORTANT: If more than one PCR product is present, neither column purification nor ethanol precipitation will isolate the desired product. PEG precipitation might work, but for the best results use gel purification to isolate the desired product or reoptimize the PCR to obtain a single product.

I. Column Purification

Commercially available products for PCR product purification usually give good results. A protocol for one such product is listed below, but in general, use the manufacturer's protocol:

* Centricon ® -100 columns (P/N N930-2119)
These columns contain an ultrafiltration membrane that separates primers and dNTPs from larger PCR products. However, they may not work as well for short PCR products (<125 bases).
To purify PCR fragments by ultrafiltration:

1. Assemble the Centricon-100 column according to the manufacturer's recommendations.
2. Load 2 mL deionized water onto the column.
3. Add the entire sample to the column.
4. Spin the column at 3000 x g in a fixed-angle centrifuge for 10 minutes.
Note The manufacturer recommends a maximum speed of 1000 x g, but 3000 x g has worked well in Applied Biosystems laboratories. If you are following the manufacturer's guidelines, increase the time to compensate.
5. Remove the waste receptacle and attach the collection vial.

6. Invert the column and spin it at 270 x g for 2 minutes to collect the sample. This should yield approximately 40–60 µL of sample.
7. Add deionized water to bring the purified PCR fragments to the original volume.

Other products include:

* QIAquick PCR Purification Kits (QIAGEN: P/N 28104, 50 reactions; 28106, 250 reactions)
These kits work well for PCR products ranging from 100 bp–10 Kbp.

* QIAquick Gel Extraction Kits (QIAGEN: P/N 28704, 50 reactions; 28706, 250 reactions)
These kits are used to purify PCR fragments from agarose gels. The kits work well for DNA ranging from 70 bp–10 Kbp. Fragments larger than this should be extracted with the QIAEX II Gel Extraction Kits (QIAGEN: P/N 20021, 150 reactions; 20051, 500 reactions). Refer to the manufacturer's instructions for the procedures

II. PEG Purification of PCR Fragments

It is acceptable to purify your PCR fragments for sequencing by PEG precipitation. After running your PCR, ethanol precipitate your fragment (the method below works well), then:

1. Dissolve the precipitated fragment in 32 µL of reagent grade (milli-Q) water
2. Add 8 µL of 5.0 M NaCl (final concentration 0.5 M)
3. Add 40 µL of 22% PEG 8000 and MIX
(11% PEG will precipitate all DNA fragments larger than 180 bp)
4. Incubate on ice at least 20 min.
5. Centrifuge at 4°C for 5 to 10 min.
6. Aspirate and discard the supernatant.
7. Dissolve the pellet in 20 µL 0.3 M NaOAc, and add 2.5 volumes of 95% ethanol.
   MIX. Leave on ice for about 15 min, then spin in the microfuge for 15 min.
8. Carefully aspirate and discard the supernatant. Rinse the pellet with 250 µL of 70% ethanol. Spin for 5 min. in the microfuge, then carefully aspirate and discard the supernatant. Dry the pellet for 3 min. under vacuum, or air dry.
9. Resuspend the pellet in 20 µL of deionized water, and store at – 20°C.

For a good explanation of PEG precipitation see "*Fractionation of DNA Fragments by Polyethylene Glycol Induced Precipitation*" by John T. Lis in Methods in Enzymology, Vol. 65, 1980, pp 347 to 353.