Purification of Plasmids for Sequencing

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

When purifying recombinant plasmids in bacteria, plate out the transformants to obtain isolated colonies. Select a single colony and re-streak it on a plate. Select an isolated colony from that plate to obtain plasmids with the desired insert. The optimal procedure for preparing a particular plasmid depends on the particular bacterial strain and the yield of each construct. Good sequencing data has been obtained from the following methods:

* ABI PRISM ™ Plasmid Miniprep Kit (P/N 402790 or 402791)
* Cesium chloride (CsCl) banding
* Modified alkaline lysis/PEG precipitation method (see below)
* PureGene DNA Isolation Kit (Gentra Systems, Inc., P/N D-5500A)
* QIAGEN Plasmid Kits (http://www.qiagen.com):
– Mini (P/N 12123, 25 reactions; 12125, 100 reactions)
– Midi (P/N 12143, 25 reactions; 12144, 50 reactions; 12145, 100 reactions)
- Maxi (P/N 12162, 10 reactions; 12163, 25 reactions; 12165, 100 reactions)

Modified Alkaline Lysis/PEG Method

Reagents required:
* Chloroform (or chloroform:isoamyl alcohol (24:1)).
* Deionized water
* Ethanol, 70%
* GET buffer (50 mM glucose, 10 mM EDTA, 25 mM Tris, pH 8.0)
* Isopropanol, 100% (anhydrous)
* PEG 8000, 13% (sterilized by autoclaving, rather than by filtration)
* Potassium acetate, 3 M, pH 4.8
* RNase A (DNase-free), 10 mg/mL
* Sodium chloride (NaCl), 4 M
* Sodium hydroxide (NaOH), 0.2 N, with 1% SDS (freshly made)
Note To minimize shearing of contaminating chromosomal DNA, **do not use a vortex during this procedure.**

1. Pellet 1.5-mL aliquots of culture for 1 minute in a microcentrifuge at maximum speed.
Note: A total culture volume of 4.5 mL can be spun down per tube without changing volumes in the procedure. This allows you to achieve a threefold increase in yield while eliminating the need for extra tubes and additional handling.
2. Remove the supernatant by aspiration.
3. Resuspend the bacterial pellet in 200 µL of GET buffer by pipetting up and down.
4. Add 300 µL of freshly prepared 0.2 N NaOH/1% SDS. Mix the contents of the tube by inversion. Incubate on ice for 5 minutes.
5. Neutralize the solution by adding 300 µL of 3.0 M potassium acetate, pH 4.8. Mix by inverting the tube. Incubate on ice for 5 minutes.
6. Remove cellular debris by spinning in a microcentrifuge at maximum speed for 10 minutes at room temperature. Transfer the supernatant to a clean tube.
7. Add RNase A (DNase-free) to a final concentration of 20 µg/mL. Incubate the tube at 37 °C for 20 minutes.
**See Pat's Note, below.*
8. Extract the supernatant twice with chloroform:
    a. Add 400 µL of chloroform.
    b. Mix the layers by inversion for 30 seconds.
    c. Centrifuge the tube for 1 minute to separate the phases.
    d. Transfer the upper aqueous phase to a clean tube.
9. Add an equal volume of 100% isopropanol. Mix the contents of the tube by inversion.
10. Spin the tube in a microcentrifuge at maximum speed for 10 minutes at room temperature.
11. Remove the isopropanol completely by aspiration.
12. Wash the DNA pellet with 500 µL of 70% ethanol. Dry under vacuum for 3 minutes.
13. Dissolve the pellet in 32 µL of deionized water.
14. Add 8.0 µL of 4 M NaCl, then 40 µL of autoclaved 13% PEG 8000.
15. Mix thoroughly, then leave the sample on ice for 20 minutes.
16. Pellet the plasmid DNA by spinning in a microcentrifuge at maximum speed for 15 minutes at 2–6 °C.
17. Carefully remove the supernatant. Rinse the pellet with 500 µL of 70% ethanol.
18. Resuspend the pellet in 20 µL of deionized water. Store at –15 to –25 °C.

Pat's Note: After step 7 the procedure may be modified to include a phenol extraction:
7a. Add an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) to the tubes, and mix by inversion until an emulsion forms.
7b. Centrifuge the mixture for 1 min. in a microfuge at room temperature. Use a pipette to transfer the aqueous phase to a fresh tube. Discard the interface and organic phase,
7c. Repeat this extraction. (Continue on to step 8.)