Appendix 1

PCR cleanup protocol:

1) Remove ExoSAP-IT reagent from -20°C freezer and keep on ice throughout this procedure.
2) Mix 5µl of a post-PCR reaction product with 2µl of ExoSAP-IT reagent for a combined 7µl reaction volume. Note: When treating PCR product volumes greater than 5µl, simply increase the amount of ExoSAP-IT reagent proportionally.
3) Seal plate securely with self-adhesive film; centrifuge sample briefly to make sure everything is at the bottom of the wells.
4) Incubate at 37°C for 15 minutes to degrade remaining primers and nucleotides.
5) Incubate at 80°C for 15 minutes to inactivate ExoSAP-IT reagent.
6) The PCR product is now ready for use in DNA sequencing, SNP analyses, or other primer-extension applications. Treated PCR products may be stored at -20°C until required.

Big Dye Terminator Cycle Sequencing:

1) Generate a master mix based on the following per sample requirements (taking into account the total number of reactions needed; numbers are µl volumes)

UltraPure Water = 3.75
5X Sequencing Buffer = 1.75
F primer (0.8pmol/µl) = 2
Big Dye = 0.5
DNA = 2
Total = 10

2) Seal plate securely with self-adhesive film; centrifuge sample briefly to make sure everything is at the bottom of the wells.
3) Perform cycle sequencing in thermocyclers using the following protocol:

---

i – 96°C for 5 minutes
ii – 96°C for 10 seconds
iii – 50°C for 5 seconds
iv – 60°C for 4 minutes
****repeat steps ii-iv an ADDITIONAL 24 times**
v – hold at 4°C indefinitely

Purification by Ethanol Precipitation:

1) Centrifuge briefly to make sure contents of wells are at the bottom; remove self-adhesive film.

2) Add 5µl of 125mM EDTA (pH 8.0) to each sample. Make sure the EDTA reaches the bottom of the wells.

3) Add 30µl of 100% ethanol to each sample and pipet up and down 5 times to thoroughly mix contents of each well.

4) Incubate at room temperature for 15 minutes. Keep in a dark place (i.e. a drawer) to protect the samples.

*****use this time to chill the centrifuge down to 4°C**

5) Centrifuge at max speed (2250g [rcf]) at 4°C for 30 minutes.

*****proceed to next step immediately; if this is not possible, continue centrifuging the plate until you are ready to perform the next step**

6) Gently, remove plate from centrifuge, lay down paper towels in centrifuge, and invert plate onto the paper towels. Centrifuge with plate inverted at 180g [rcf] for 1 minute.

7) Add 30µl of 70% ethanol to each sample.

8) Centrifuge plate at 1650g [rcf] for 15 minutes.

9) Gently, remove plate from centrifuge, lay down paper towels in centrifuge, and invert plate onto the paper towels. Centrifuge with plate inverted at 180g [rcf] for 1 minute.

10) Continue to air dry until all visible ethanol has evaporated.

11) Add 20µl formamide to each sample and load on the sequencer