Sanger Sequencing Protocol by using magnetic beads (Agencourt)
A.R. Del Valle-Echevarria's Lab Notebook (July 23rd, 2012)
Modified from the UW Biotech website

Before getting started

After completing the miniprep of your cloned amplicon, take a microliter of pGEM purified plasmid that is at 200 ng/μl and run a gel with one microliter your minipreped samples. This pGEM sample will be your reference for intensity.

Suggestion	
Sample	1μl
6X Loading Dye	2μl
Gel Green 1:100	1μl
Water	7μl
Total Volume	10μl

Sequencing Reaction

Big Dye Buffer	1.5 μl
Big Dye Enzyme Mix	1.0 μl
Sequencing Primer	2.0 μl (~3 pmol/μl) *
Miniprep DNA	- μl (100-200 ng for < 20 kb plasmid)
Milli Q Water	- μl
Total Reaction Volume	10μl

*This varies based on the concentration of the primer and remember that pmol/μl is **equal** to μM. In other words, **3 pmol/μl *is* 3 μM**.

Sequencing PCR Protocol: AVBIOTECH

This is the cycling protocol the UW Biotech Center uses when they do all the procedure.

96°C for 2 minutes
96°C for 10 sec, 52°C for 15 sec and 60°C for 3 minutes; 35 cycles
72°C for 1 minute (Final Extension)

Cleanup

1. Add 5 μl of CleanSeq beads to the 10 μl reaction.
2. Add 40 μl of 85% ethanol and mix seven times.
3. Put on magnetic plate for 3 minutes
4. Remove the liquid and discard. (IN MAGNETIC PLATE)
5. Add 65 μl of 85% ethanol. (IN MAGNETIC PLATE)
6. Remove the ethanol as much as possible. (IN MAGNETIC PLATE)
7. Let it air dry for 10 minutes. (IN MAGNETIC PLATE)

8. Remove it from the magnetic plate and add 50 μl of Milli Q water and let it incubate for a minimum of 5 minutes.
9. Put the reaction tubes in the magnetic plate and wait 3 minutes so the beads get separated. Meanwhile, prepare the set of tubes where the reaction is going to be transferred.
10. CAREFULLY, remove the solution and pass it to the new tubes. Make sure that the beads don't get into the solution because it could jam the machine.
11. Go to biotech and submit the order. The option is "Striptubes $2 a sample", when in doubt, ASK!