NEB SEQUENCING (VENT POLYMERASE)--ALTERNATE METHOD PREPARING SAMPLES TO BE SEQUENCED

1. Vectors are double stranded and firstly the DNA must be denatured.

   For each tube:

4M NaOH	1.0uL
4mM EDTA	1.0uL
dH2O	15.5uL
DNA	2.5uL
Total	20.0uL

2. Incubate at room temp. For 5 min.
3. Ethanol precipitate solution to pellet DNA--gets rid of NaOH in solution.

   Using:

   - 4uL of 5 M NH4OAc
   - 2X the volume of EtOH(100%)=48uL EtOH (b/c 20uL solution +4 uL salt (NH4OAc) +24uL)
   - then spin for 15'
   - Keep the pellet (hard to see), discard supernatant.
4. Each tube containing pellet was filled with 6.5uL of dH2O to resuspend pellet.
5. Each tube is heated at 95 C for 5' (to denature DNA since NaOH has been removed.
6. After 5' the tubes are placed in an ice bath to "quick-cool" them. (This ensures denatured DNA will not reanneal)
7. Anneal DNA to primer.

DNA	6.5uL
10X vent buffer	1.5uL
primer (T7 5pmol/uL)	1.0uL
Total	9.0uL

8. Incubate at 42 C for 20'.
9. Extension reaction--add the following to the above;

Ext. Mix	2.0uL
35S dATP	2.0uL

Vent polymerase	1.0uL
DNA + primer solution	9.0uL
Total	14uL

10. Incubate at 42 degrees C for 5'.
11. GATC tubes were prepared for each clone. -add 3uL of each dNTP solution into its respective GATC tube.
12. Add 2.8uL of extension reaction (14uL) for each clone to its respective GATCset of tubes. -the tubes were then mixed by flicking with finger or vortex, and were then incubated at 72 degrees C for 10'. (The reaction proceeds very quicly ~ 30bp/sec)
13. The solution were spun and 4uL of STOP/LOADING solution was added to each solution. The tubes can then be frozen.
14. The samples were heated before loading at 72 degrees C for 2 min (or 7 min if previously frozen) , so that they are the same temp. as the gel.
15. The combs were inserted into the warming gels.
16. 2.0uL of each clones GATC tubes (whle still in the heat blacK) were quickly added to the wells.