Protocol for Dideoxy Sequencing Using Sequenase

1. Rxn Mixture : DNA - 4µl (~6µg)
   H_2O - 16µl
   2M NaOH } 2µl
   2mM EDTA }

   22µl

   - incubate at 85°C for 5 mins.

2. Put on ice. Add 10µl of 0.9M NaOAc
3. Add 1µl Primer (~20ng * can dilute 100µM 12mer 1/10 use 1µl) on the side and mix well
4. Add 95µl 100% EtOH. Incubate @ -80°C 15 mins
5. Spin in cold room 20 mins top speed.
6. Rinse with 70% EtOH (100µl). Spin 5 mins. Dry in Speed Vac.
7. Resuspend in 10µl 1X buffer for Sequenase. Mix well.
8. Add : DTT - 1µl
   dLab. Mix - 2µl (1:5) 1/1 to read close to primer
   ATP^{35}S - 0.6µl
   Sequenase - 2µl (1:8 in enz. dil. buffer) mix well.

   15µl Incubate @ 37°C (R. temp) for 5 mins.
9. Label G,A,T,C tubes (use 0.5ml Tubes)
10. Add 2.5µl of ddNTP to each tube
11. Add 3.5µl extension mix to each tube (G, A, T, C) and mix well.
12. Incubate @ 37°C 5 mins. Add 4µl Stop Sol'n. Freeze

For SS DNA Sequencing : template DNA - 7µl
5x Seq. Buff - 2µl
-40 primer - 1µl

10µl

* Heat @ 65°C 2 mins - in 500ml beaker then cool down in cold room. Proceed from Step 8.