Protocol for DNAese Sequencing Using Sequenase

1. **Mix:**
   
   - DNA: 4 ml (0.6 μg)
   - H₂O: 16 ml
   - 2 M NaOH: > 2 ml
   - 2 M EDTA: 2 ml
   
   - Incubate at 85°C for 5 mins.

2. Put on ice. Add 10 μl of 0.9 M NaOAc

3. Add 1 μl Primer (in 100 μl of 10 μM (10X) use 1 μl) on the side and mix well

4. Add 95 μl 10X ETPH. Incubate E at 80°C 15 mins

5. Spin in Cold room 20 mins top speed.

6. Rinse with 70% EtOH (10 ml). Spin 5 mins. Dry in Speed Vac.

7. Resuspend in 10 μl 1X buffer for Sequenase. Mix well.

8. Add:
   - DTT: 1 μl
   - dNTP Mix: 2 μl (1:5) half to read close to primer
   - ATPs: 0.6 μl
   - Sequenase: 2 μl (1:8 in enz. diln. buffer) Mix well.

   - 15 μl Incubate E at 37°C (R. temp) for 5 mins.

9. Label G, A, T, C tubes (use 0.5 ml tubes)

10. Add 2.5 μl of ddNTP to each tube

11. Add 3.5 μl extension mix to each tube (G, A, T, C) and mix well.

12. Incubate E at 37°C 5 mins. Add 4 μl Stop Sol'n. Freeze

   For SS DNA Sequencing: template DNA: 7 μl
   - 5X Sequ. Buff: 2 μl
   - 40 μl Primer: 1 μl

   Heat E at 65°C 2 mins in 500 μl beaker then cool down in cold room. Proceed from Step 8.