SEQUENCING REACTION : (SEQUENASE)

Preparation of samples to be sequenced:

- Denature DNA

NaOH (4M)	1uL
EDTA (4mM)	1uL
dH2O	15.5uL
DNA	2.5uL
Total	20.0uL

- Incubate at room temp. For 5 min.
- Ethanol precipitate to pellet the DNA

5M NH4OAc	4uL
2X vol. 100% EtOH	40uL

spin for 15 min., wash with 70% EtOH, spin and speed vac to dry.

Add 7uL dH2O to the pellet to resuspend.

- Heat the DNA at 95 C for 5 min.
- Quick cool by placing in ice water.

Annealing DNA to primer

DNA	7uL
Primer (5 pmol/uL)	1uL
Sequenase buffer	2uL
Total	10uL

- Incubate at 42 C for 20 minutes.

Labelling Reaction: (For 4 reactions)

Annealing reaction	10uL
DTT (0.1M)	1uL
dITP labelling mix (1:10 dilution)	2uL
35S-dATP	0.5uL

Sequenase (1:7 dilution in TE)	2uL
Total	15.5uL

Diluted Labelling Mix (1:10) (dITP)

- 1uL labelling mix
- 9uL dH2O

TOTAL= 10uL

Diluted sequenase (1:7)

- 1uL sequenase
- 0.5uL pyrophosphate
- 6.5uL cold TE

TOTAL= 8.0 uL

- Incubate at room temperature for 5 min.

Add 2.5uL each of G,A,T and C termination into separate tubes. (G,A,T and C that go with dITP labelling mix).

Termination Reaction:

Add 3.5uL of the labelling reaction to each of the tubes with G, A, T, and C.

- Incubate at 37 degrees C for 10 min.

Add 4uL of stop mix to each tube.

- Heat samples at 85 degrees C for 5 min. before loading onto the gel. (Gel was pre-run by Andrew and made by Andrew)

Loaded Samples

T7--------->	T7<--------(FO-Zc)
G A T C	G A T C

-gel was dried at 80 degrees C for 1.25 hours and placed on film for overnight exposure.