

Freeze-Squeeze Project

A) Materials

a) A list of P.C.R. samples that did not work during the Cycle-Seq. Rxn. was given.

b) This samples are shown in the agarose gel in the following order:

- 1) D4-25/ENVA
- 2) D4-19/ENVA
- 3) D4-14/3'NRT
- 4) D4-2/3'NRT
- 5) D4-3/3'NRT
- 6) D4-5/3'NRT
- 7) D4-25/ENVA
- 8) D4-35/ENVA
- 9) D4-12/3'NRT
- 10) D4-6/3'NRT

B) Procedures

a) Electrophoresis was done to the samples. Low melting point agarose (1.2%) was prepared and left over 4hrs. to polymerize.

b) Samples ran through the gel at 80 V for 45 mins.

c) Once the electrophoresis was over, a photograph was taken of the gel. The photo clearly shows contaminants in the samples. These contaminants have similar B.P. weight as the target band.

d) After the photo was taken, the process of "Freeze Squeeze" began. Samples were introduced in the freezer at -80°C for 5 mins; then remove at room temp. for 15 mins. These steps were repeated 4 times.

e) Then the samples were centrifuged for 15 mins at 10°C (14,000 rev/min)

f) A very small amount of supernatant was removed (barely 2.5 uL).

g) Following Owen's recommendation, the process of "Freeze Squeeze" was repeated.

h) The difference was not that much, still, the amount of supernatant was minimum.

i) Despite of this, 1uL of supernatant was used to perform an agarose gel electrophoresis.

j) Again, the gel was run at 80 V for 45 mins. The results showed almost no concentration of D.N.A.