POGIL: Capillary Electrophoresis DNA Sequencing

Zhou, H., A. W. Miller, et al. (2000). "DNA Sequencing up to 1300 Bases in Two Hours by Capillary Electrophoresis with Mixed Replaceable Linear Polyacrylamide Solutions." *Anal. Chem.* **72**(5): 1045-1052.

MODEL 1 Electrophoretic Resolution

$$Rs = \frac{\sqrt{N}}{4}(\alpha - 1)\left(\frac{k'}{1+k'}\right) \qquad Rs = \frac{\sqrt{N}}{4}\left(\frac{\mu_1 - \mu_2}{\bar{\mu}}\right)$$

1. What does μ represent in the second equation?

2. The authors claim that it takes about two hours for a 1300 base nucleotide to travel through the column. Given an electric field of 125 V/cm and a 45 cm column, calculate the electrophoretic mobility.

3. The power of CE is that is can provide tremendous efficiencies (N) and very small plates heights (H). If the authors have system with H = 1 μm, how long a column do they need to get $N = 10^7$.

4. The authors claim that a resolution of 0.30 is adequate for separation of two nucleotide fragments. Given your answer to Q2 and an efficiency of $N = 10^7$, what minimum difference in mobilities is required to achieve this resolution.

5. The first equation describes the resolution in a liquid chromatography experiment while the second describes the resolution in a CE experiment. Hypothesize which terms are "equivalent" and which is one missing? Why might a term be missing?

MODEL 2 Sources of Diffusion

$$\sigma_{tot}^2 = \sigma_D^2 + \sigma_p^2 + \sigma_{\Delta T}^2 + \sigma_{eof}^2 + \sigma_{\det}^2 + \sigma_{inj}^2 + \ldots$$

1. What is electroosmotic flow? Why is it not an important source of diffusion in this experiment? What is the likely value of D_{eof}?

2. In order to determine if the ΔT term is significant, we need to calculate the power dissipated. The authors injected with electrical field strength of 9 V/cm and a current of 0.7 μA. What is the resistance of the capillary solution? How much current did they experience at 125 V/cm? How much power does this dissipate?

3. The authors claim that "thermal gradients across the column are minimized both by effective thermostating and by limiting electric current with a low conductivity buffer." What simple experiment do they perform that indicates that $D_{\Delta T} \sim 0$?

4. What role does the polymer solution play in the separation? According to Stellwagen, would the authors get any separation without such polymers?

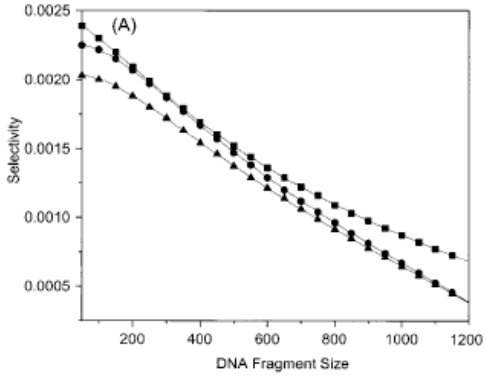
5. The authors claim that addition of a 50 kDa LPA polymer increased the thermostability of the network without significantly increasing the viscosity of the solution. Which term(s) does this affect in the equation above? How might it affect those terms?

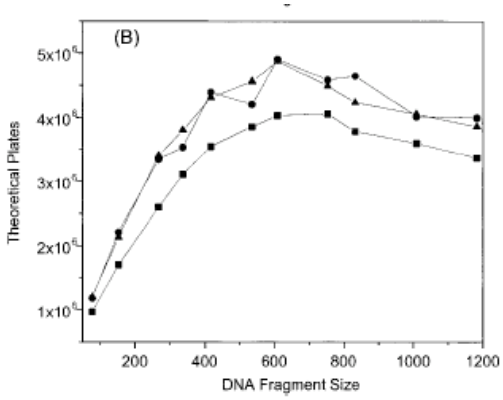
6. Suppose the authors could find a polymer solution that provides smaller H. What term(s) might this affect? How might you change the separation conditions to take advantage of this?

7. Diffusion due to detection is determined by the size of the peak relative to the detection width. Draw two "cartoons" that show a detector window much larger than the peak width and one with the detector window much smaller than the peak width. Explain using the two "cartoons" how detector broadening occurs and how you would minimize this experimentally.

8. Diffusion due to injection is often given by the equation: $\sigma_{inj}^2 = \frac{l^2}{12}$, where l = the injection length. What determines the injection "length"? How would you minimize the importance of this term experimentally? What tradeoffs might this entail?

MODEL 3 Experimental Results





1. Based on what you know about DNA duplex stability and the effects of temperature on diffusion, combined with Equations 4 -6 from the article, hypothesize the reasons for the differences between the circle (60°C) and square (70°C) results.

2. Using Equations 4 - 6 from the article, hypothesize reasons for the observed results between the triangles (lower MW polymer) and circles (higher MW polymers), both at 60°C. You may also include results from Table 3.

3. Using data from the Table 2, hypothesize why changes in the electric field have the observed effect on fragment size resolution. If the 100 V/cm result is best, why isn't it used in the optimal experiments?

Table 2. Effect of Electric Field Strength on DNA Sequencing Results with LPA 2% (w/w) 10 MDa/0.5% (w/w) 270 KDa at 70 °C[a]

electric field (V/cm)	migration time for base 1019 (min)	fragment size at resolution 0.3	read length[b] at 98.5% accuracy
250	44.0	871	927
200	55.6	995	1042
150	80.5	1060	1127
125	101.0	1168	1190
100	131.0	1236	1172

[a] Samples and other electrophoretic conditions were as in Figure 1. Values in the table are averages of three to five experiments. [b] Read length was defined as in footnote *b* in Table 1.

4. Evaluate this POGIL using the following criteria:

***Flow* of questions that lead to understanding** (10 points): The questions are structured in such a way that they lead the reader from an understanding of the simple but important topics to the more complex and important ideas in the paper.

Questions *focus* on the most relevant topic (10 points): In this case, the questions should focus the reader on understanding the basics of CE to a more detailed knowledge of DNA separations by CE in the context of things we learned in class. Reference to figures in the model is most useful.

Appropriate use of at least two *figures and/or tables* in questions (10 points): Questions lead the reader to better understand the *more difficult aspects* of the figures and their relation to the most important topics in the paper. Hypotheses that require thought beyond simple interpretation of the figures are given more credit.

***Challenging* questions** (10 points):
More probing questions that require thought beyond that required on the lower levels of Bloom's taxonomy are given higher credit than solely simple and obvious questions.

***Depth* of answers** (10 points):
Student answers their own questions with significant depth demonstrating that they understand more than simple paraphrasing of the paper discussion.