Intoduction to sizing DNA on Agarose Gel

HC70AL
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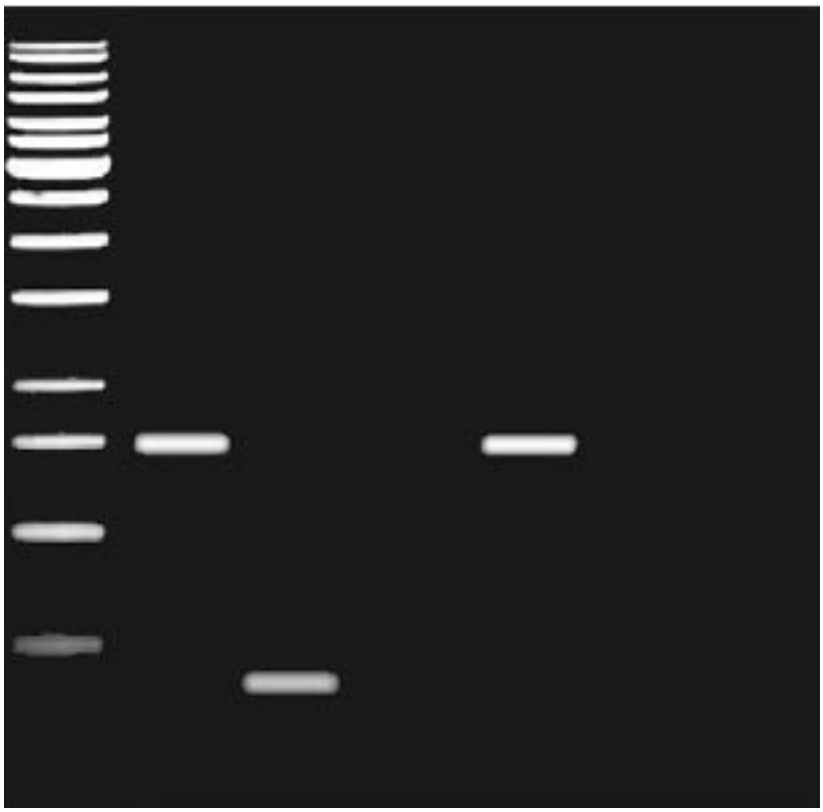
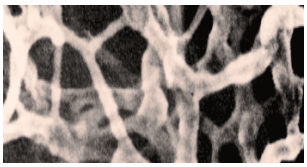
How do we determine the size of a DNA fragment?

- Agarose gel electrophoresis
  - Load DNA sample along with a DNA ladder and supply electric current
  - Compare the migration distance of the DNA sample to that of the DNA ladder

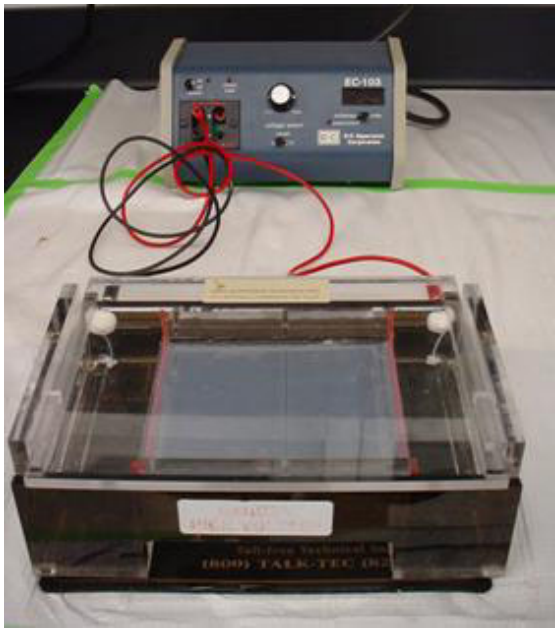


What is agarose gel?

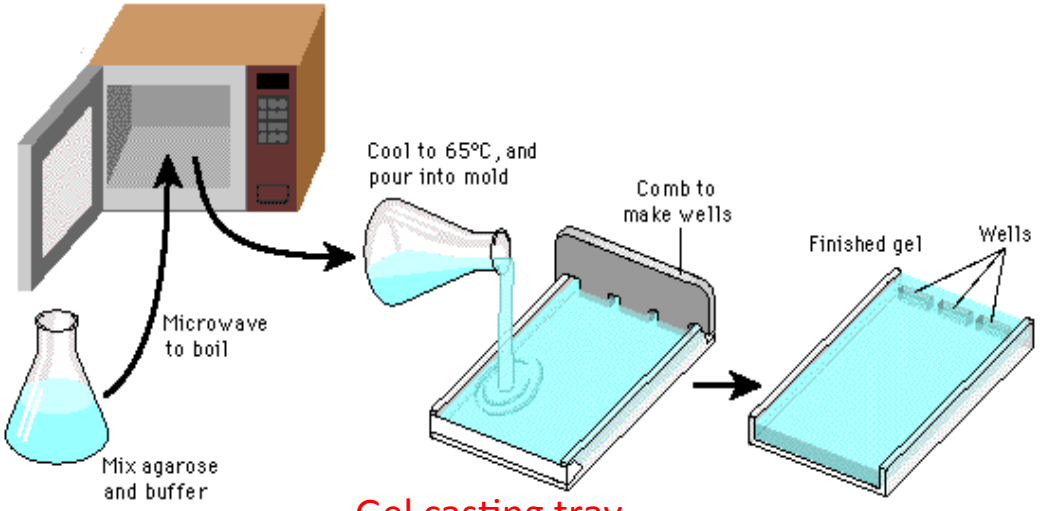
- An agarose gel is a semi-solid matrix with many pores



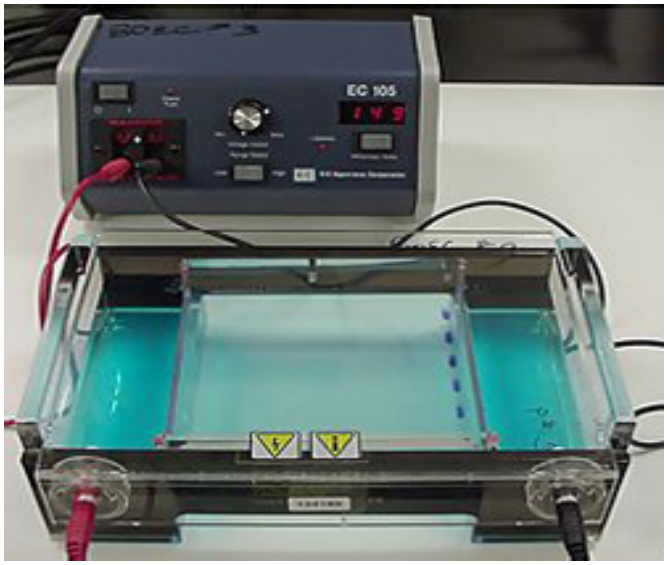
- Mix of agarose and electrophoresis buffer.
- The gel is immersed into an electrophosesis buffer.
  - Buffers help maintain a constant pH
- What type of buffer is used in this lab?
  - TAE (1x): composed of Tris-Acetate and EDTA



How do we prepare an agarose gel?



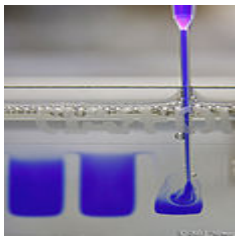
Gel casting tray



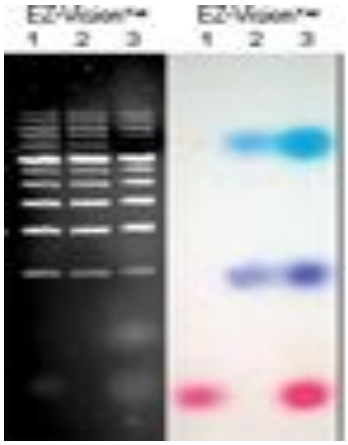
Gel box

How do we prepare the DNA sample for gel electrophoresis?

- Loading buffer contains:
  - A dense material (e.g. glycerol) to allow the DNA sample to "fall" into the sample wells



  - One or two tracking dyes which migrate in the gel and allow visual monitoring of how far the electrophoresis has proceeded
- What types of loading buffers do we use in this lab?

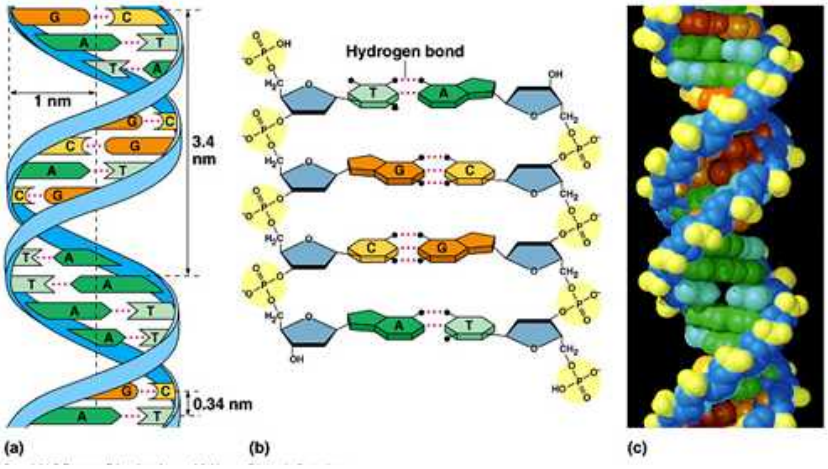


Xylene cyanol FF
- About 4kb in TAE

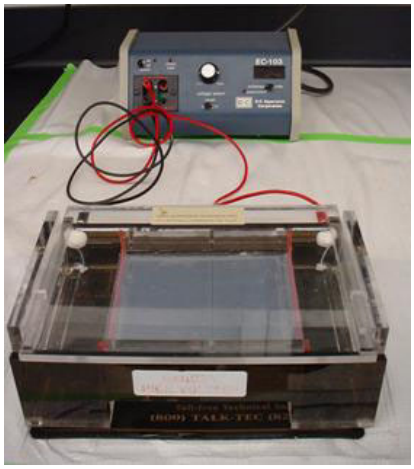
Bromophenol blue
- About 400bp In TAE

What causes the DNA fragments to move through the gel?

- Electrophoresis – refers to electromotive force (which creates an electric current) that is used to move molecules through the gel matrix
- Cathode – negatively charged end
- Anode – positively charged end
- Why does supplying an electric current cause DNA to move in a certain direction?

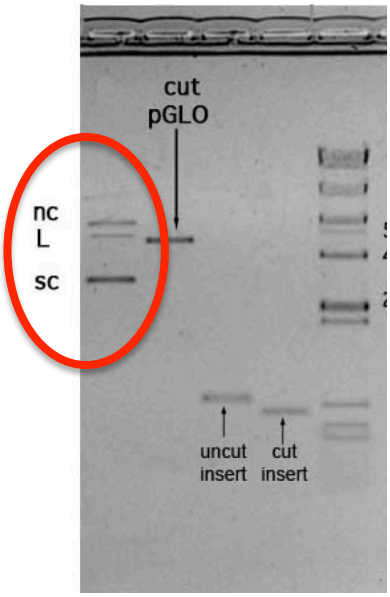


- Gel box and power supply



What factors affect the rate of DNA migration in agarose gels?

1. Size of DNA fragment
2. Conformation of DNA
   - Superhelical circular
   - Linear
   - Nicked circular

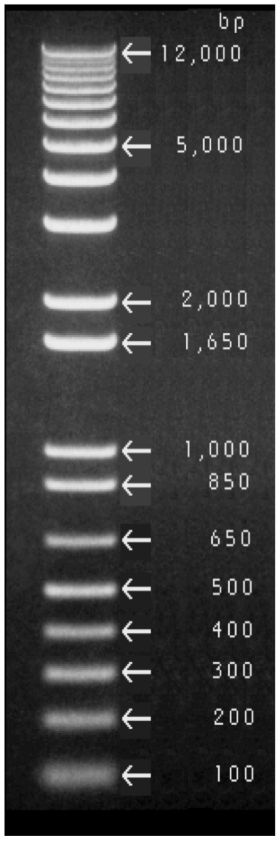


3. Agarose concentration
   - The higher the concentration, the denser the gel, and the better the gel can separate small molecules

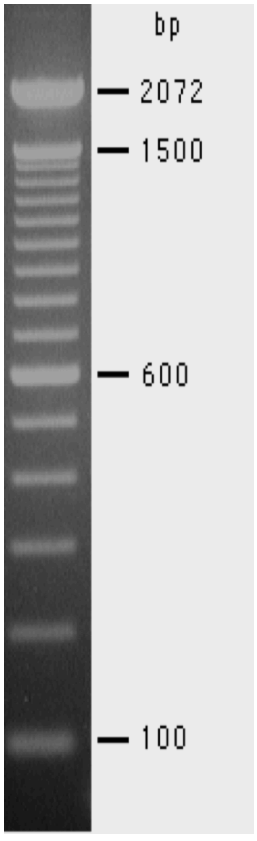
4. Composition of the electrophoresis buffer
   - Composition and ionic strength of the buffer
   - TAE (1x)
5. Applied voltage
   - At low voltage – rate of migration $\propto$ applied voltage
   - At high voltage – rate is not proportional to voltage

How do we determine the size of the DNA fragment?

- A DNA ladder is used as a reference to estimate the size of an unknown DNA fragment
- DNA ladder - A DNA solution composed of many DNA fragments of different sizes with known lengths
- What DNA ladders do we use in this lab?



• for sizing dsDNA fragments from 100bp – 12Kb
• the range from 2,000bp – 12,000 increases by 1,000bp increments



• for analyzing bases from 100bp – 1500
• the range from 100bp – 1,500 increases by 100bp increments

How do we visualize DNA?

- Stain the gel
  - EtBr
  - SYBR Safe gel stain
    - How does it work?
    - What are the advantages of using SYBR over EtBr?
      - Less mutagenic
- Expose to UV light

What are the steps in the gel electrophoresis process?

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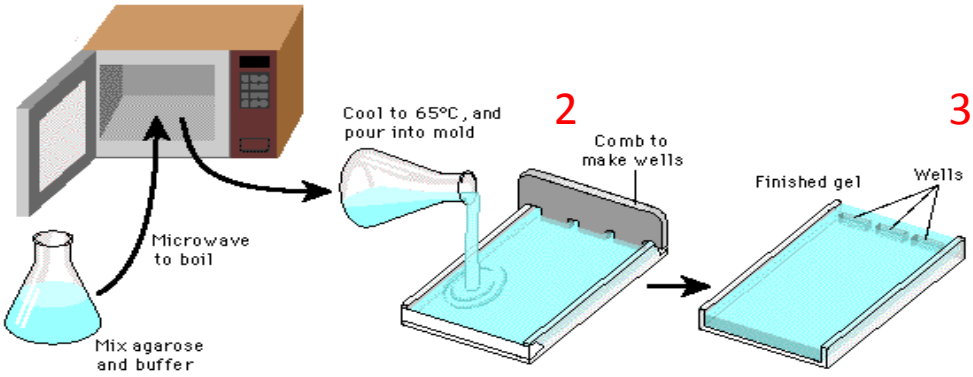

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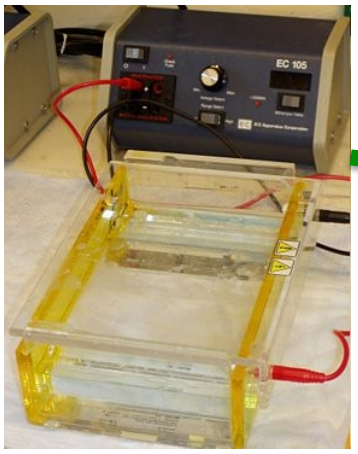

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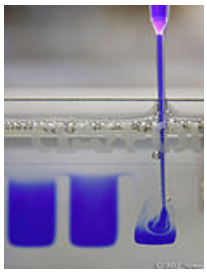

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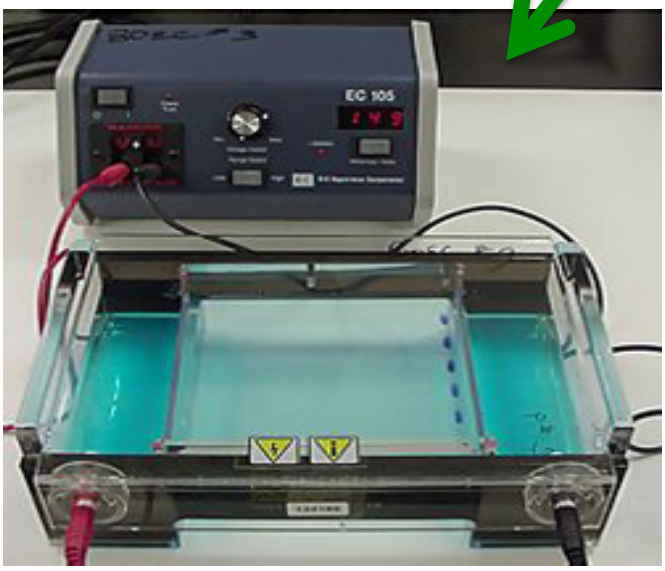

What are the steps in the gel electrophoresis process? (continued)

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