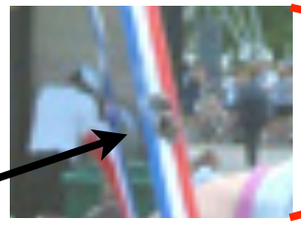
M1D3: PCR and Paper Discussion

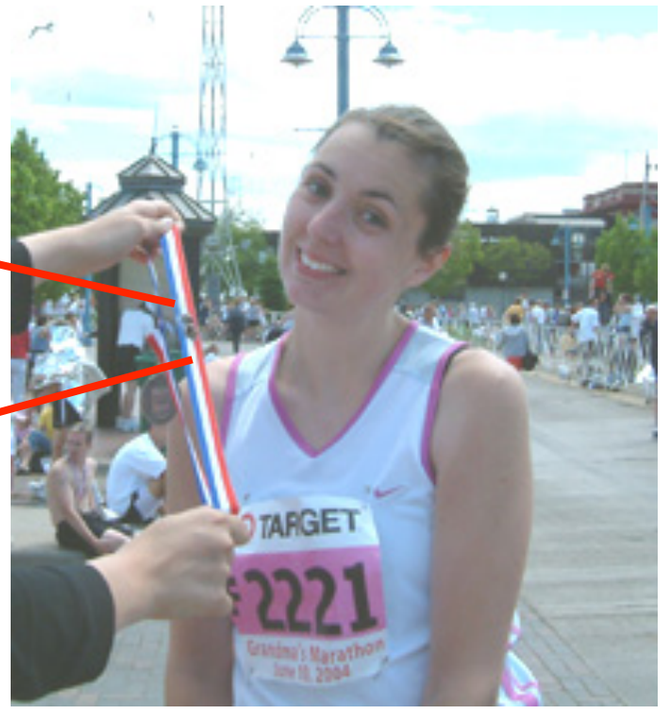
2/15/13

Announcements

- First lab treat:



Another source of DNA
for microbiome studies



- FNT -- long due to Holiday

- PCR Review and Gel Electrophoresis

- Set up PCR

- Atissa will be here, then journal club!

FNT Assignment

1. Larger assignment than usual. Changed a bit since Wednesday.
   - Draft methods section on M1D2-M1D3
   - Partial introduction for microbiome work
   - Primer design table + thoughts
   - Note: Methods & Intro due on Stellar

2. I will be out of town this weekend -- contact me through gmail account

3. Office hours next week: Monday, 8pm & Tuesday, 11am

HOMEWORK →

Methods Section Tips

- Divide into sub-sections!

  Put in a logical order -- list primer THEN list PCR reagents, etc

  Start with a overview sentence "16S rRNA genes were amplified from each sample...."

- Methods are clear and concise explanations

  The methods section is not a benchtop protocol

  Space-wise, avoid tables/lists when a sentence will do

  Sentence-wise, avoid extra words

  Content-wise, cover what's needed and only that needed to understand and replicate your work

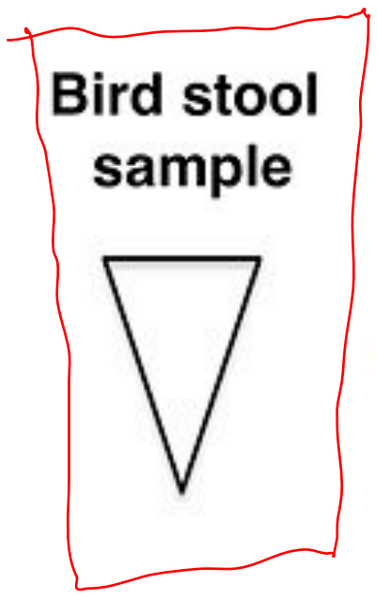
- Think about the most flexible units -- concentration vs volume. (For replication!)

Methods section exercise

- Consider the following passage: “Template DNA (5 ng) and primers were mixed with 20 uL of 2.5X Master Mix in a PCR tube. Water was added to 50 uL. A tube without template was prepared and labeled control. ”
  - What information is missing?
  - What information can be cut?

Thanks to Agi for this slide!

Bird Microbial Communities -- Experimental Overview



-20°C

extract DNA

DAY 2

Complex DNA pool
(many pathogen DNAs)

PCR

DAY 3

Simpler DNA pool
(bacterial 16S DNA)

gel purify, clone and transform

DAY 4

~1400bp

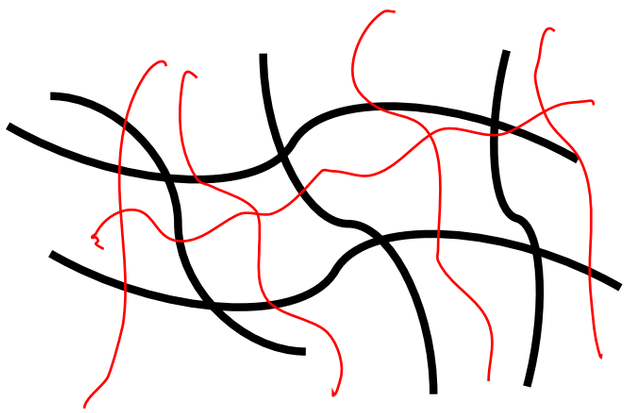
PCR

Component	Function
template DNA	Original Copy
polymerase Taq Pfu	Catalyzes DNA addition
dNTPs	Building Blocks
primers	☆ Select and initiate new sequence
Buffer Mg^{2+} BSA	Optimal chemical environment

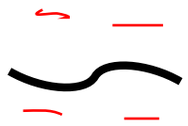
☆ Hot start

DNA Electrophoresis (EP): Principle

Agarose gel



DNA

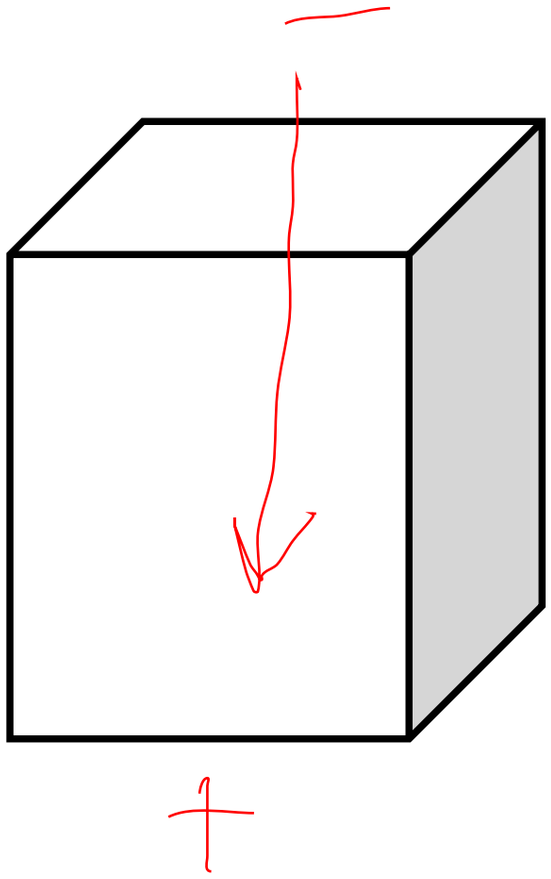


Agarose and DNA are both polymers

Driving force for separation:

DNA moves to + because of phosphate groups

Separation is according to: size

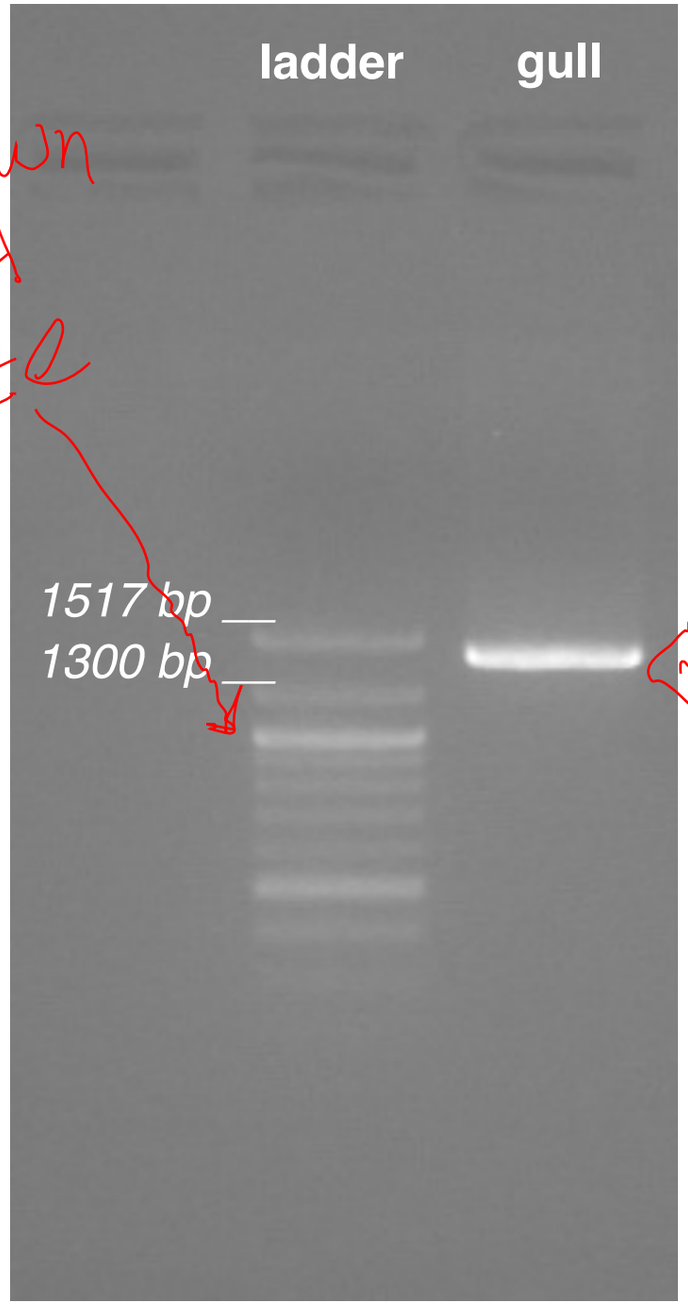


Smaller ____ DNA moves faster because

density

Thanks to Agi for this slide!

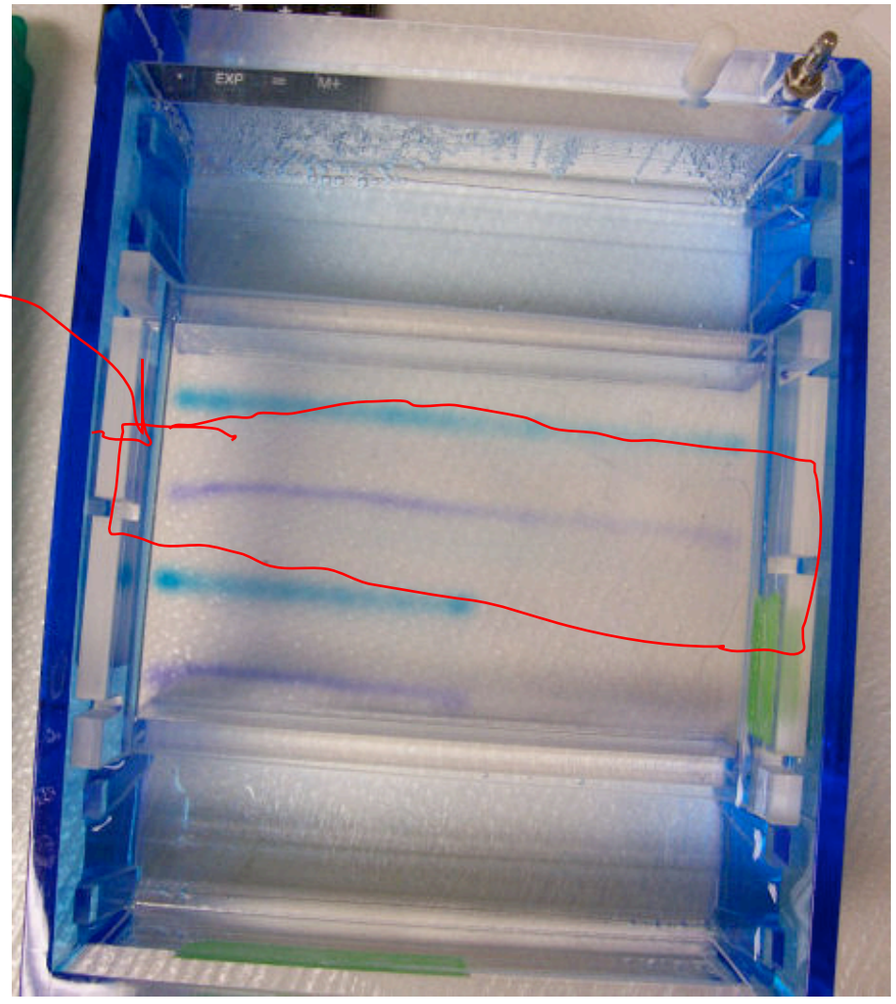
How do we visualize the DNA?



Known DNA size

Loading dye
- glycerol
- c
- xylene cyanol
- RNase

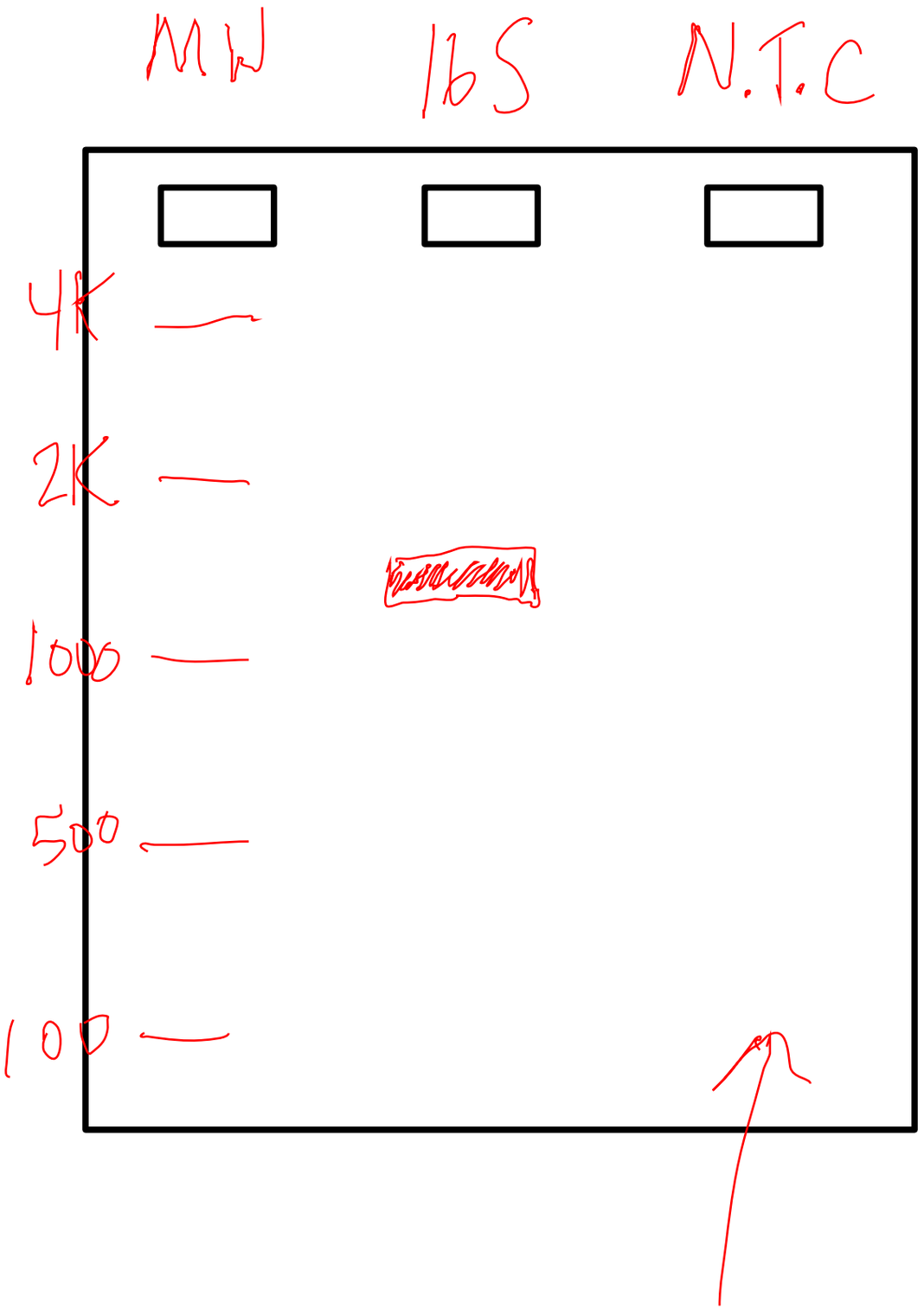
← Sybr green



sr.wikipedia.org

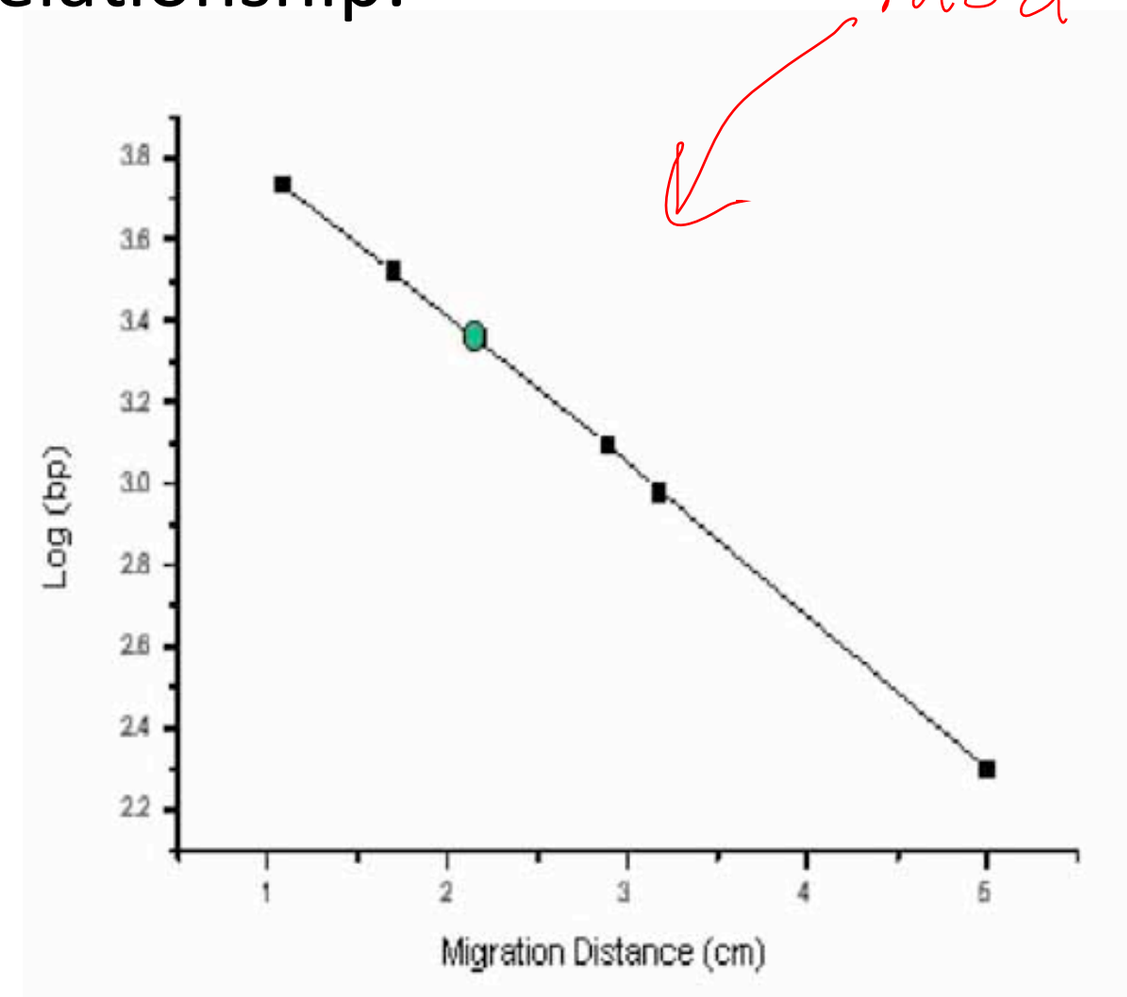
awkward photo with your advisor

DNA EP: Analysis



DNA ladder:

Relationship:



From MIT OCW

About *V corneae*… and your primers

- Gave you poor reference sequence
  - Broad = shotgun sequence, avoided it for simplicity
  - DaSilva complete: 3 mismatch, 1 gap
  - Baker complete: 2 mismatch, 25(!) gaps
- Gave you wrong direction
  - Target Ta, not Tm, of 58 °C is best (matches V1/PMP2)
- What we will do
  - revise your primers to Broad seq., checked new Tm & G/C
- Your options now
  - accept our revision (we will run a second PCR at 53 °C)
  - revise your primers to a Tm of 63 °C

Thanks to Agi for this slide!

Today in Lab (M1D3)

- Set up PCR rxns
  - Change pipet tips between samples, primers, etc.
  - Keep PCR tubes cold!
  - Write small *directly* on the PCR tubes – do not put sticky labels in the PCR machine.
- Discuss paper from writing POV ~2 pm
- Presentation on giving talks from Atissa ~2:20
- Polish your slide ~ 3:15
- Discuss paper from technical POV *and* get feedback about your slide ~ 3:30-5 pm

Thanks to Agi for this slide!