- Announcements
- Lab Quiz
- Pre-lab Lecture
  - PCR and gel electrophoresis
  - Writing a methods section
  - Today in Lab: M1D3

Announcements

- FNT: Lots!
  - substantially revised yesterday (old FNT outline, sorry!)
  - report: draft D2-D3 methods and partial intro
  - primer design summary: comparison table plus a few sentences about design choices
- A few FNTs are due on Stellar
- Questionnaire got modified ☹
  - please leave your # with me

HOMEWORK →

General
edit topic – delete topic – add a

Due M1D4, T/R edit – delete
Due 21 February 2013 1:05 p.m.

Due M1D4, W/F edit – delete
Due 22 February 2013 1:05 p.m.

Due M1D5, T/R edit – delete
Due 26 February 2013 1:05 p.m.

Due M1D5, W/F edit – delete
Due 27 February 2013 1:05 p.m.

Due M1D7, T/R edit – delete
Due 05 March 2013 1:05 p.m. Pc

M1D7, W/F edit – delete
Due 06 March 2013 1:05 p.m. Pc

Next few steps in lab

Complex DNA pool
(many pathogen DNAs)

PCR

DAY 3

Simpler DNA pool
(bacterial 16S DNA)

Gel electrophoresis and cloning

discuss today

DAY 4

PCR

Component	Function
dNTPs	building blocks for new DNA
polymerase ~~Taq~~ *Pfu - error rate	catalyzes DNA extension
DNA template	sequence to copy
primers	select and initiate new DNA sequence
buffer; Mg^{++} → co-factor for enzyme	right chem. environment

"hot" * start specifity

+BSA! efficiency

DNA Electrophoresis (EP): Principle

Agarose gel

DNA

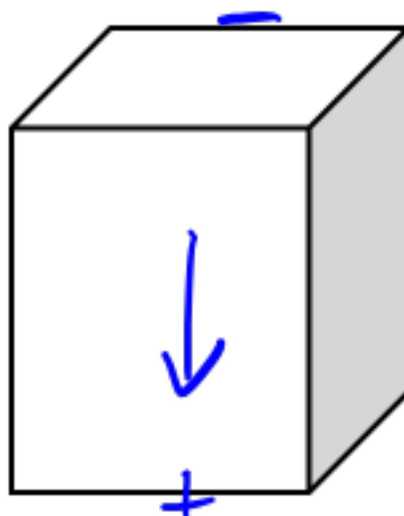
Agarose and DNA are both polymers.

∴ have molec. entanglements

Driving force for separation: mass : charge

DNA moves − to + because of phosphate groups

Separation is according to: size



Smaller DNA moves faster because entanglements ↑ size

note: as wt% gel ↑, pore size ↓

DNA EP: Visualization

Loading dye: glycerol – sink into wells

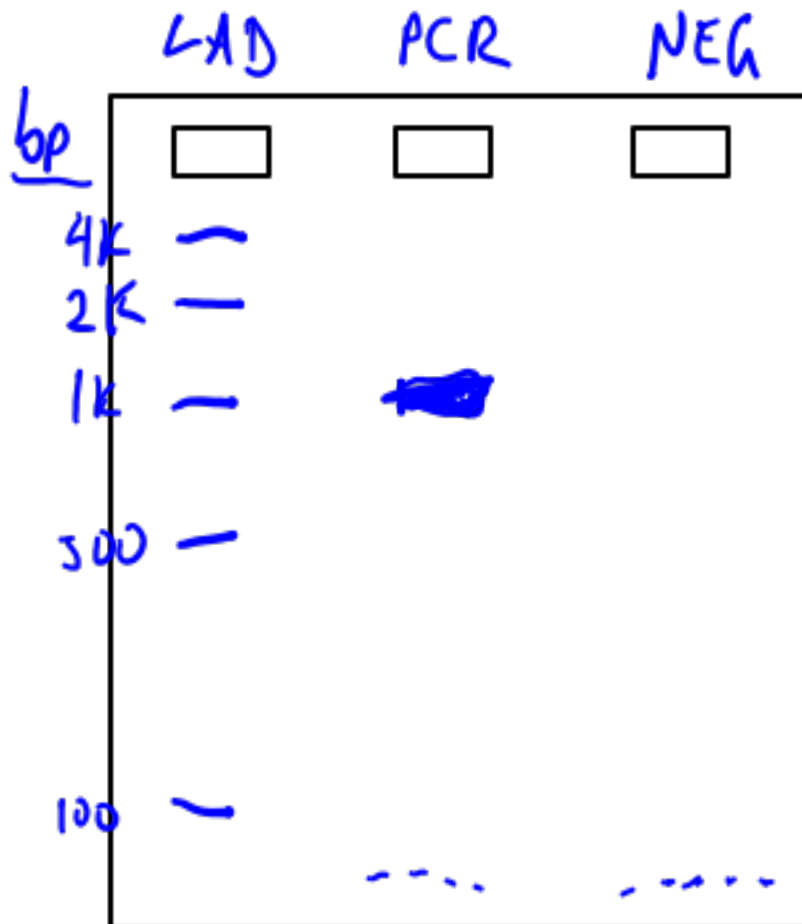
xylene cyanol – real-time visual tracking dye

– single band

RNase

Ethidium bromide (or SYBR Safe, etc.): fluoresces under UV/blue light IFF DNA-bound

DNA EP: Analysis



DNA ladder: Standards of known length and concent.

Relationship:

distance $\propto \frac{1}{\log(\text{size})}$

more in Mod 2!

Methods section tips

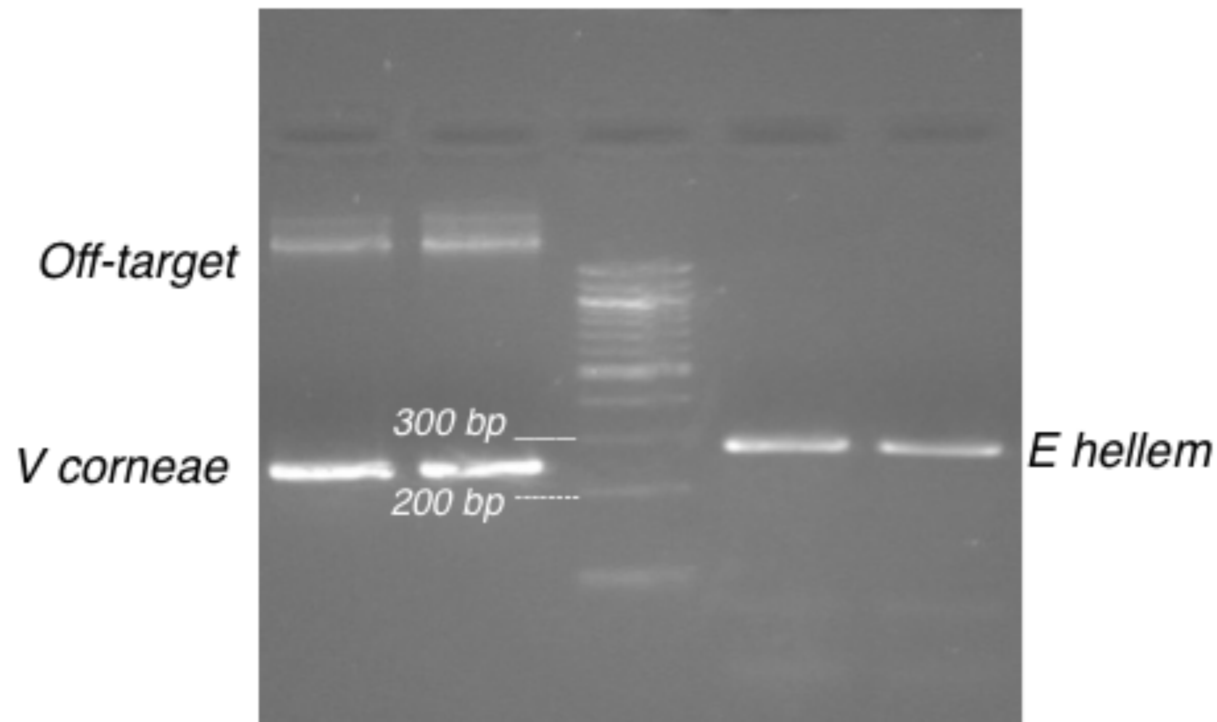
- Organizing sub-sections
  - Often start with an overview/introductory sentence (*what*, not why) → then give step-by-step details
- Methods should be concise and complete
  - Space-wise, avoid tables/lists when a sentence will do
  - Sentence-wise, avoid extra words
  - Content-wise, cover what's needed and only that needed to understand and replicate your work
- Concentrations are more useful than volumes; or you can state amounts, plus total volume.

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?

Microsporidia preview (D4+5)

D4: you set up primers → we'll run PCR → D5: you run gel



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 I've done
  - revised your primers to Broad seq., checked new Tm & G/C
- Your options now
  - accept my revision (we will run a second PCR at 53 °C)
  - revise your primers to a Tm of 63 °C

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