- Announcements
- Lab Quiz
- Pre-lab Lecture
  - PCR
  - Gel electrophoresis
  - Cloning preview
  - Today in Lab: M1D3

Announcements (pre-quiz)

- No lecture/lab next Tue/Wed
- FNT: Lots!
  - Gull microbiota experiment: experimental overview schematic for *Abstract & Data Summary*
  - Microsporidia detection experiment: primer design table and paragraph summarizing design choices for *Memo*
  - Prepare Excel-based ligation calculator for M1D4 lab
  - Primer designs due (ideally sooner) mark as "FINAL"

+ a few Qairre not signed → today

Announcements (post-quiz)

- Standing office hours
  - Tuesday 4:30-5:30 pm
  - Monday better?? (1-2p?)

> let's talk over Doodle

- Music in the lab: results
  - country is really polarizing!
  - most people want music (then neutral, then opposed)
- Music in the lab: plan
  - concentration-heavy days: classical or none
  - repetitive mechanical days: variety → safe(ish) but not 100% bland choices, occasionally hitting solo despise category
  - TELL ME if anything starts to affect your work

Bird microbiota experiment: next steps

D1

Complex DNA pool (many microbial DNAs)

PCR

DAY 3

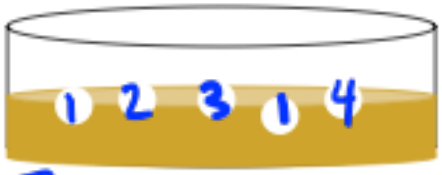
Simpler DNA pool (bacterial 16S DNA)

Gel electrophoresis and cloning

DAY 4

many different 16S amplicons

Individual 16S cell colonies



SEQ ↓ species

deconvolve individual sequences by cloning into bacteria

PCR eaction

Component	Function
dNTPs	building blocks for new DNA
polymerase < ~~Taq~~ Pfu { lower error rate, quasi hot start	catalyzes DNA extension
primers	select and initiate sequence to be copied
template	sequence to copy
buffer; BSA; Mg^{++} → cofactor for polymerase	right chem. environment

DNA Electrophoresis (EP): Principle

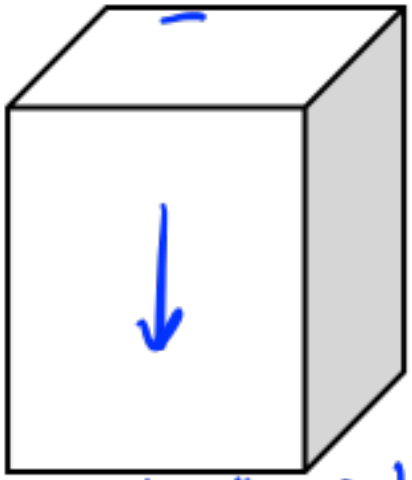
Agarose gel

DNA

Agarose and DNA are both polymers.
∴ have molecular 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/interactions ↑ with size

note: as wt. % gel ↑, pore size ↓

DNA EP: Visualization

Loading dye: • glycerol - sink into wells

• tracking dye(s) – real-time progress, independent of DNA

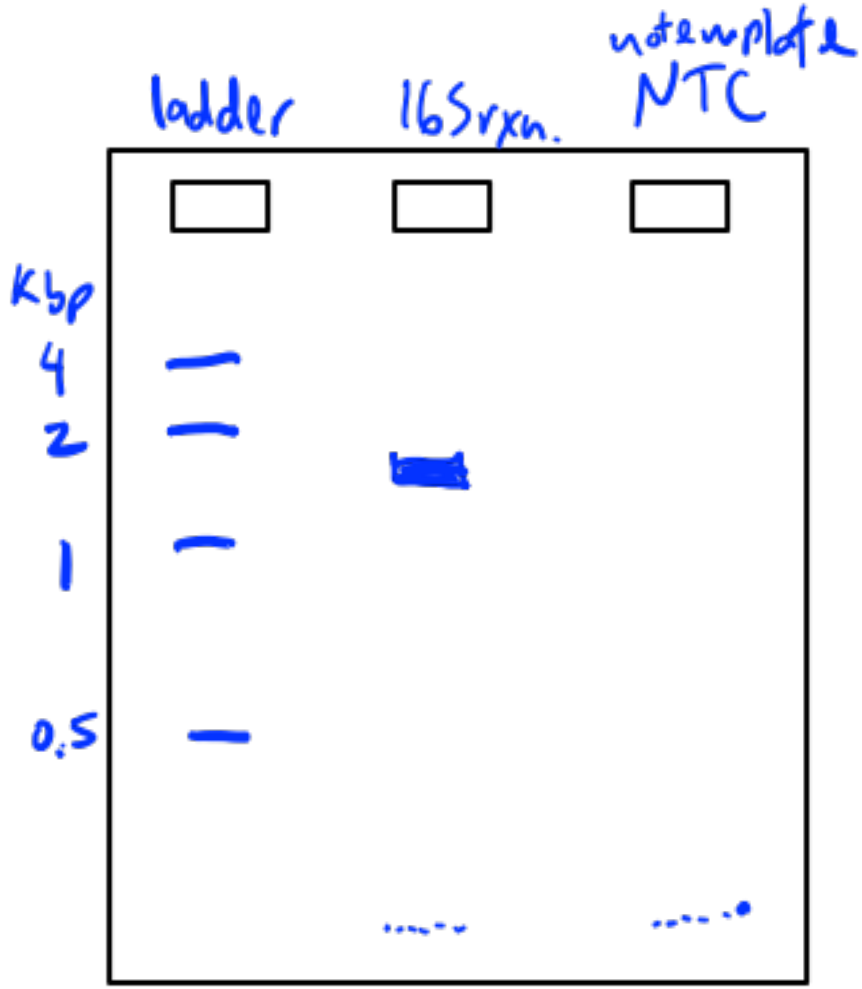
• sometimes: RNase, EDTA, buffer

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

IFF bound to DNA

intensity of # bp of mass? ~~moles~~?

DNA EP: Analysis



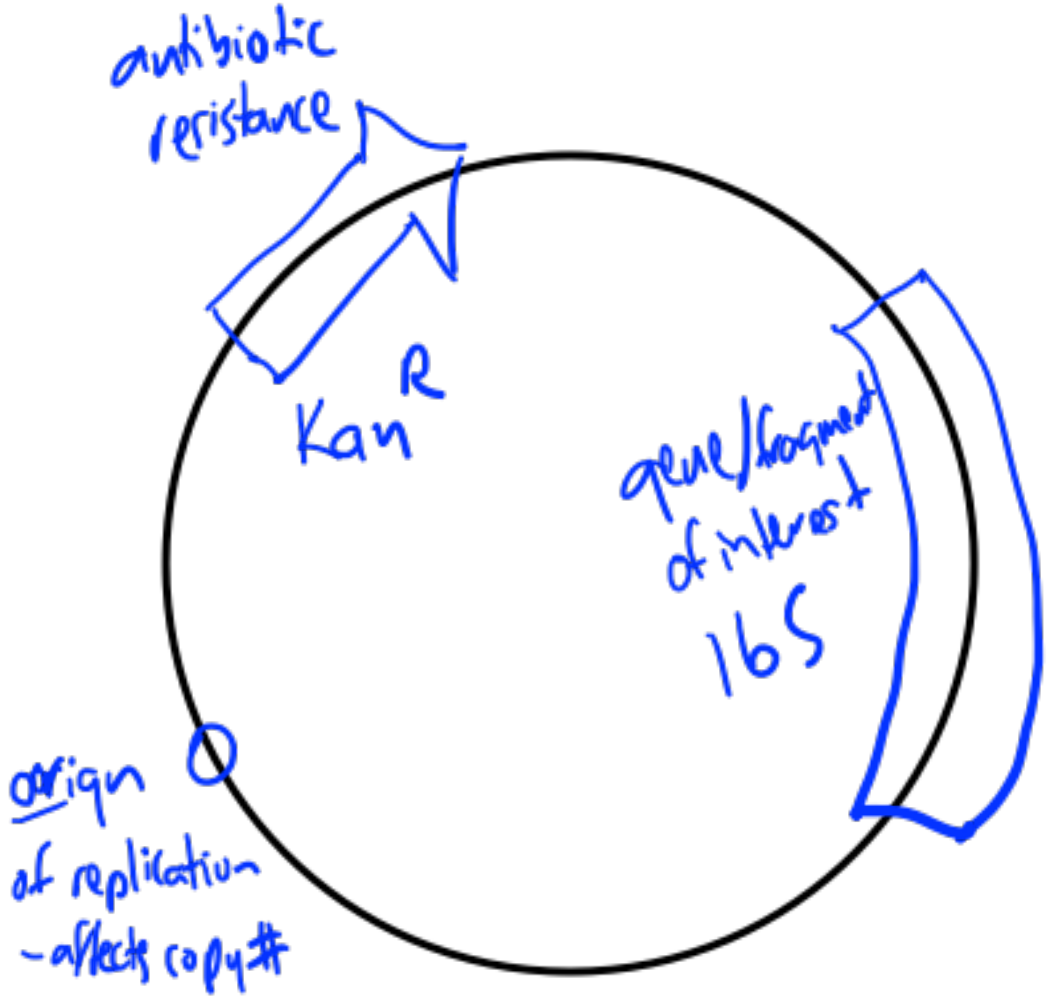
DNA ladder: standards of known size (and concentration)

Relationship:

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

more later...

Plasmid overview

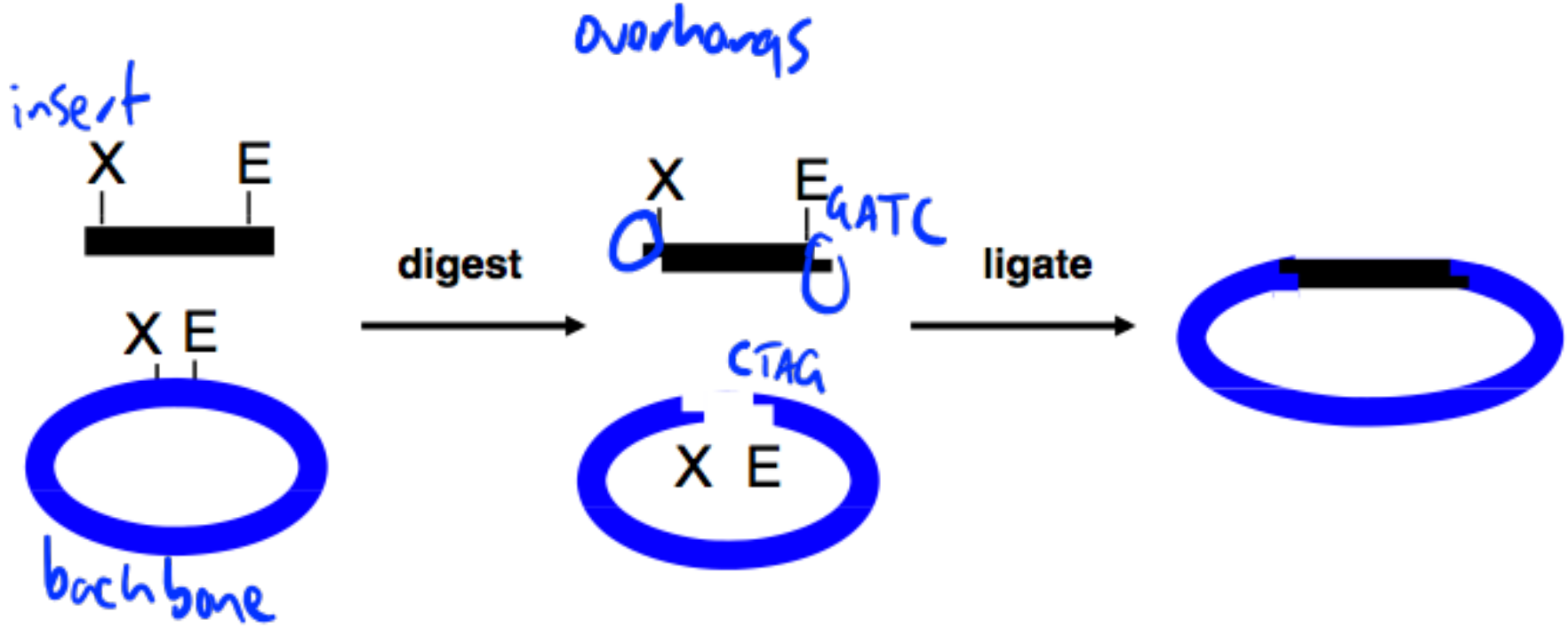


— ds, circular, extrachromosomal DNA

why? vector to introduce gene/fragment into cells

Kan^R → select plasmid-containing bacteria on antibiotic plate

Cohesive-end restriction cloning



X and E = restriction enzyme sites

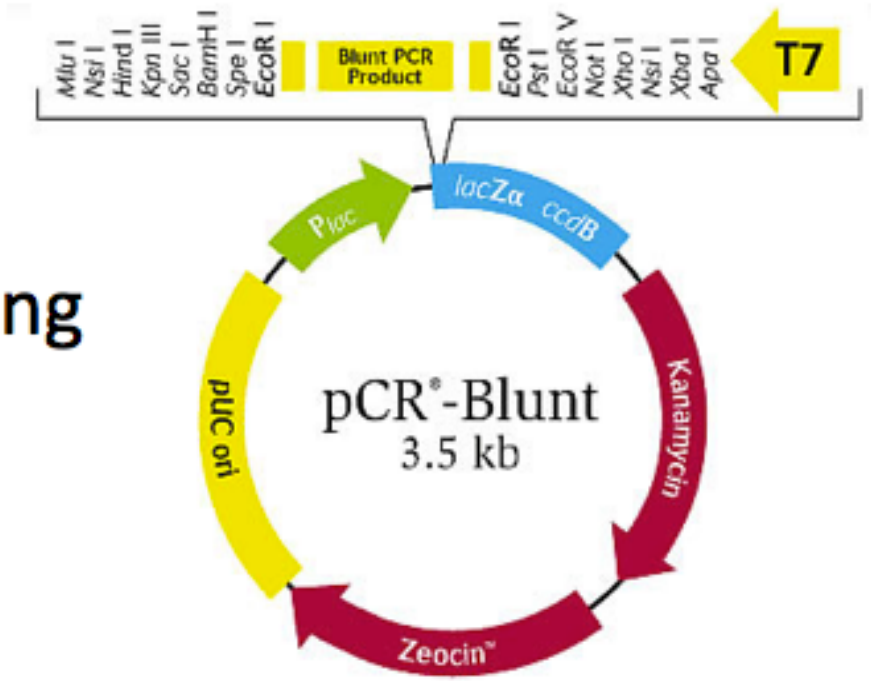
Blunt-end ligation and cloning

- No restriction digestion on our end
- Polymerase mustn't add overhangs

  Pfu doesn't ; Taq does
- Need efficient ligase

  no overhang H-bonding
- "Non-directional" cloning

  product can face in either direction



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 ~2:20 pm
- Presentation on giving talks from Atissa ~2:35
- Polish your slide ~ 3:40
- Discuss paper from technical POV *and* get feedback about your slide ~ 4-5 pm