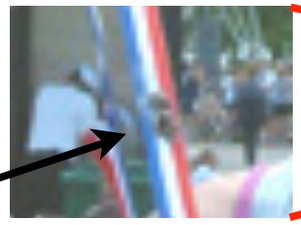
M1D3: PCR and Paper Discussion

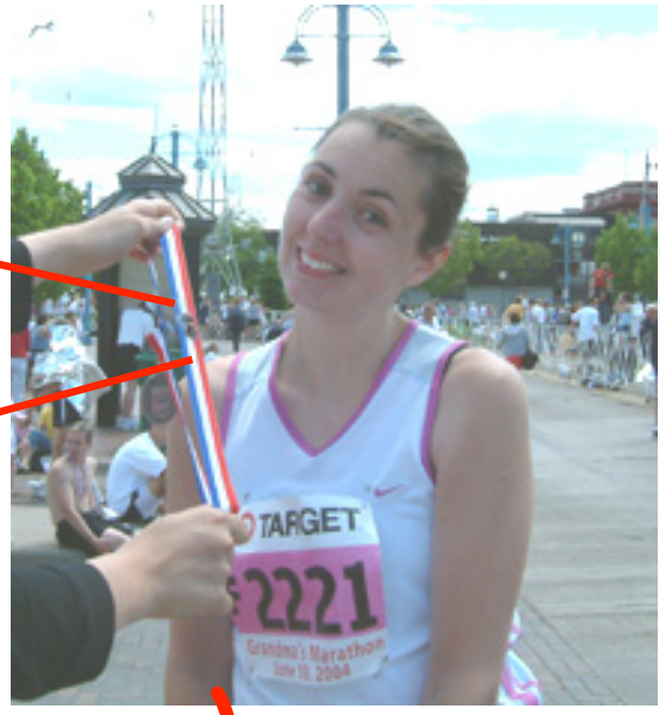
2/13/14

Announcements

- First lab treat:



Another source of DNA
for microbiota studies



- FNT -- long due to Holiday

No Lab on Tuesday! (or lecture)

- PCR Review, Gel Electrophoresis & Cloning basics
- Set up PCR
- Atissa will be here, then journal club!

FNT Assignment

1. Larger than usual — put that long weekend to good use!
   - Gull microbiota experiment — your own experimental schematic diagram (to be included in your *Abstract & Data Summary* assignment)
   - Microsporidia detection experiment — a publishable table including your primer sequence and details (to be included in your *Memo* assignment) + summary paragraph
   - MIDY ligation practice calculation
2. Office hours next week: Monday, noon (16-429b) ~~and 9pm (lab)~~

Bird Microbial Communities -- Experimental Overview

Bird Cloacal sample

extract DNA

DAY 1

Complex DNA pool (many microbial DNAs)

PCR

DAY 3

Simpler DNA pool (bacterial 16S DNA)

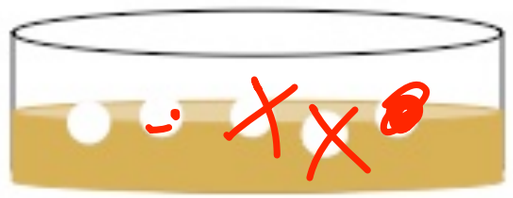
smaller DNA pool

gel purify, clone and transform

DAY 4

Many different 16S amplicons

Individual 16S cell colonies



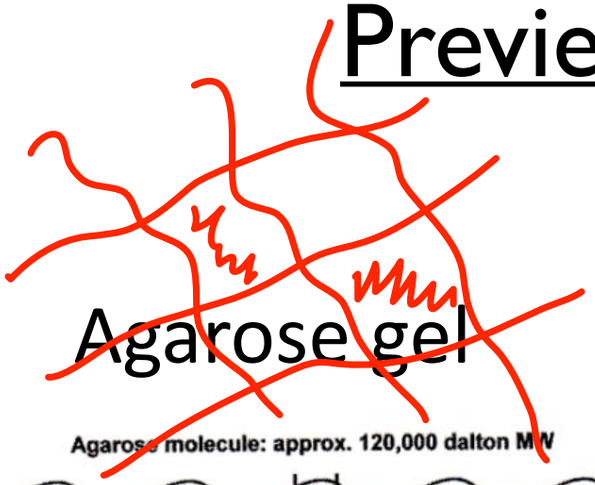
Amplificati

MID3: 16S rRNA gene amplification — PCR

Polymerase chain reaction

Component	Function
template → DNA purified	Original Copy
Polymerase pfu - high fidelity - hot start	Catalyzes DNA addition
dNTPs	Building Blocks
primers	Select and initiate new sequence
salts Mg^{2+} BSA	Optimal chemical environment

Preview of M1D4: DNA Electrophoresis



Agarose gel

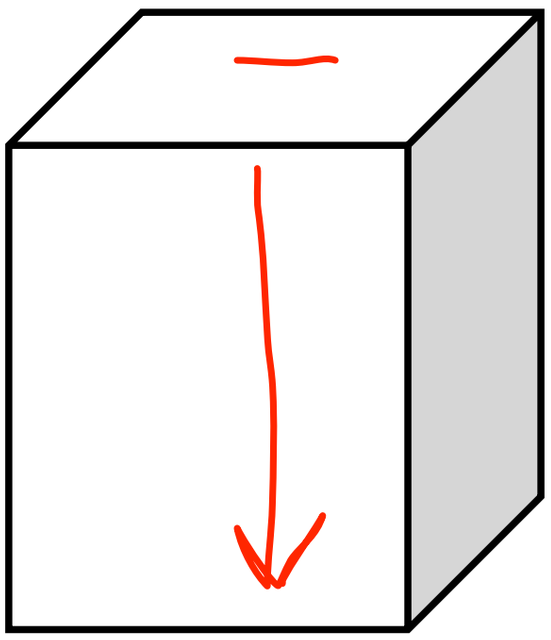
DNA ~

Agarose and DNA are both polymers

Driving force for separation: charge

DNA moves − to + because of phosphate groups

Separation is according to: size



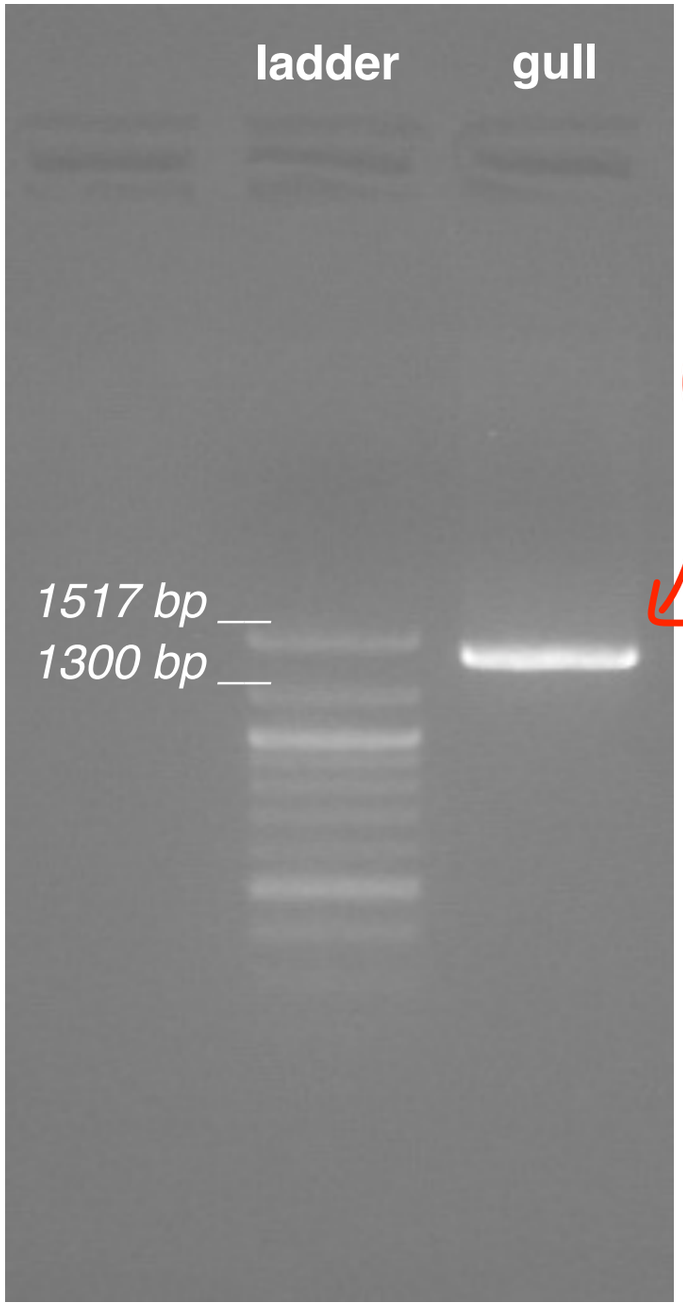
"Run to Red"

Small ____ DNA moves faster because

porosity

Thanks to Agi for this slide!

How do we visualize the DNA?



double-stranded DNA

UV activated

- ethidium bromide
- Sybr Green

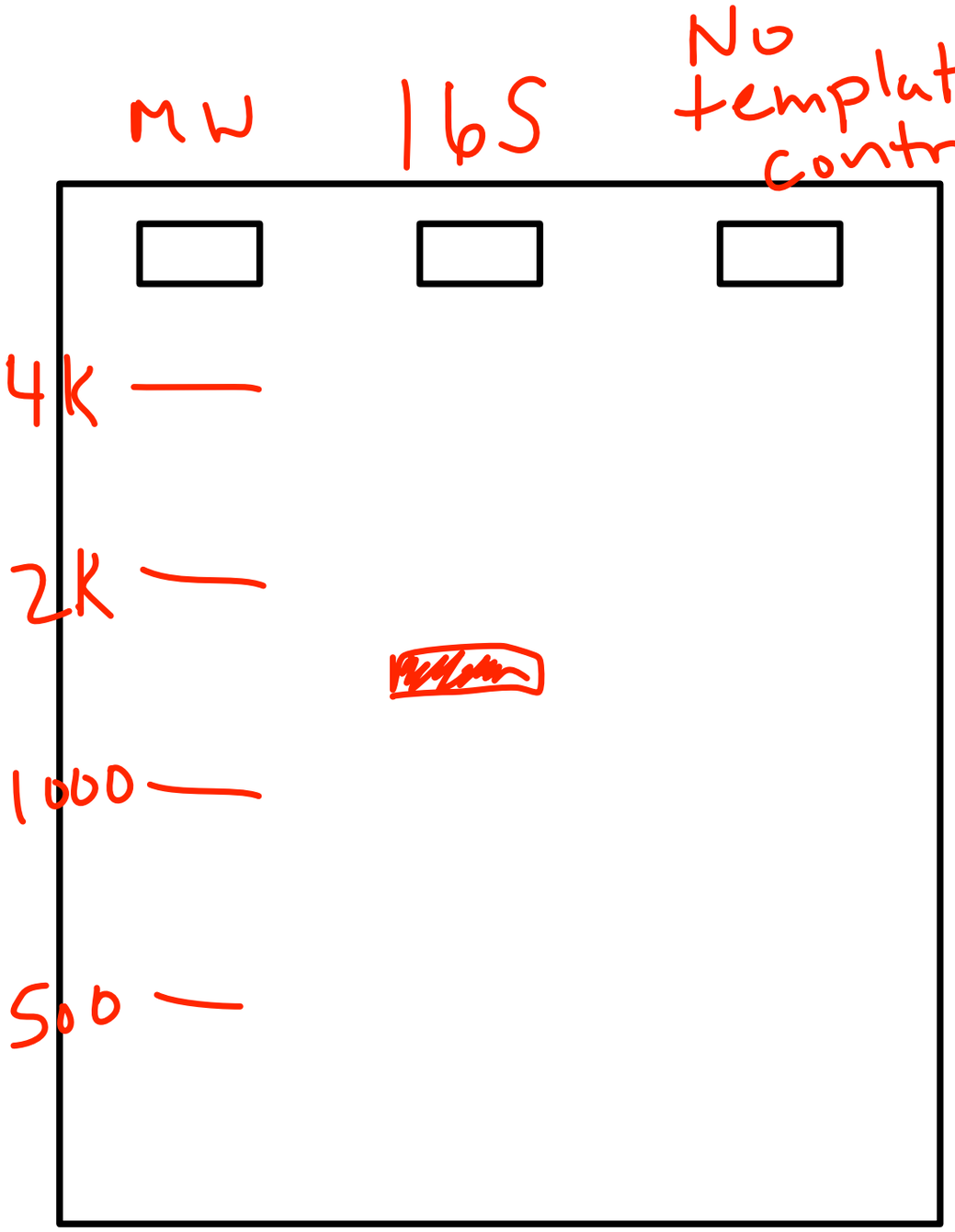
loading dye

- BPB
- glycerol
- salts

www.base-asia.com

http://en.wikibooks.org/wiki/Structural_Biochemistry/DNA_Gel_Electrophoresis

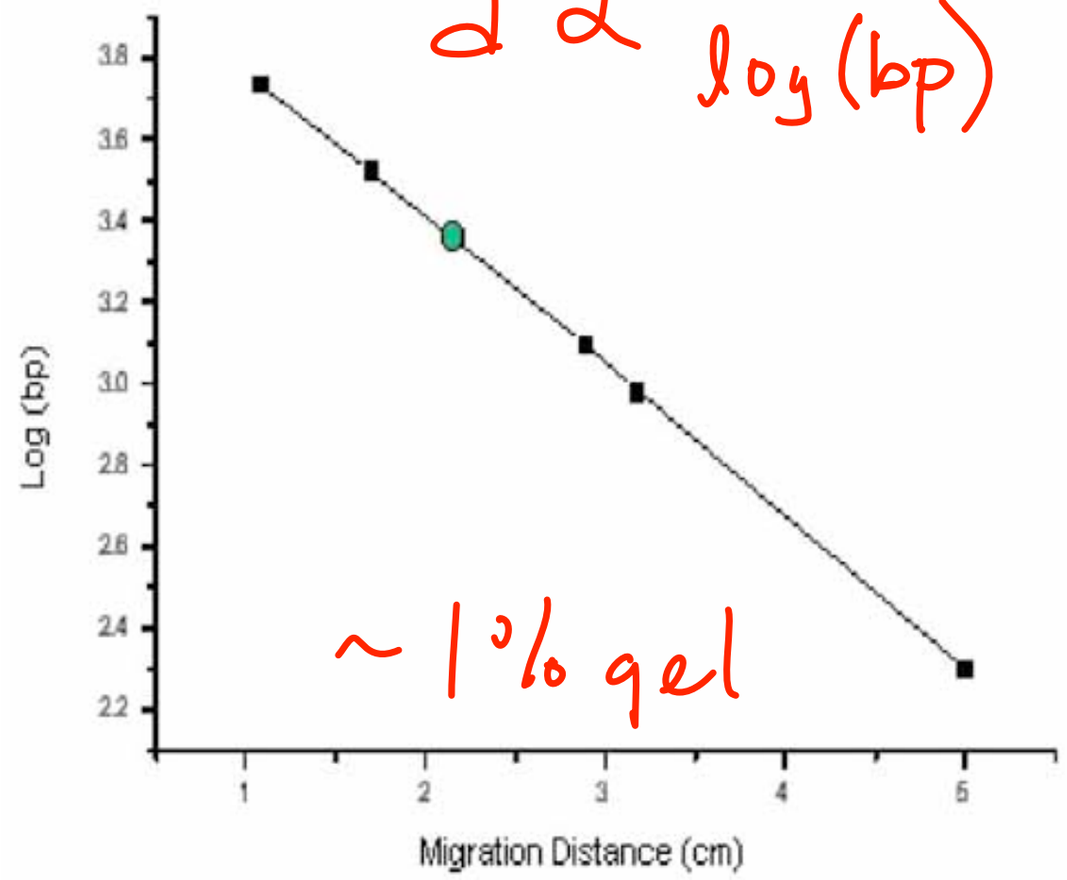
Preview of M1D4: Analysis



DNA ladder: Known sizes

Relationship:

$d \propto \frac{1}{\log(bp)}$

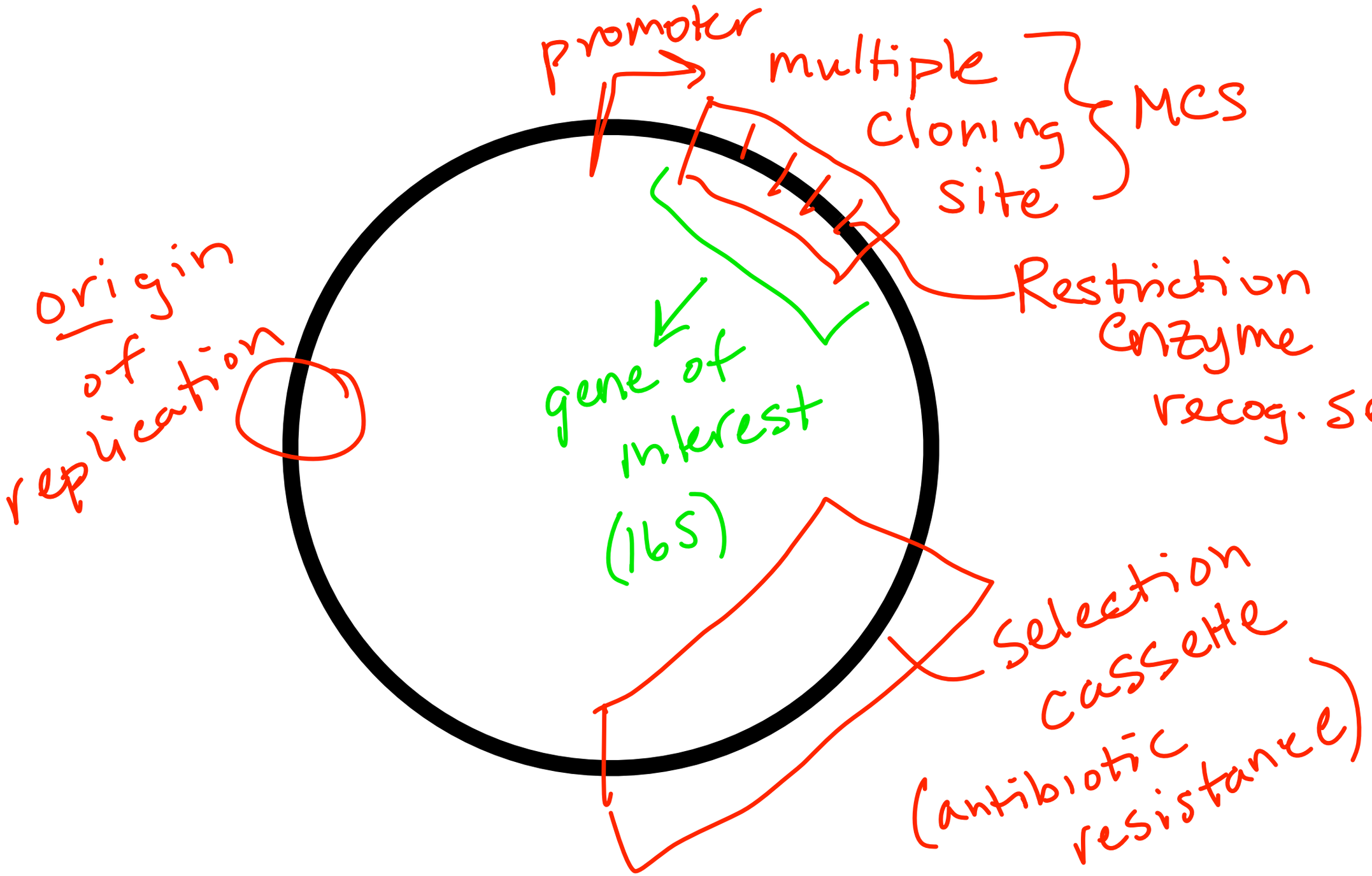


From MIT OCW

Preview of M1D4: Cloning

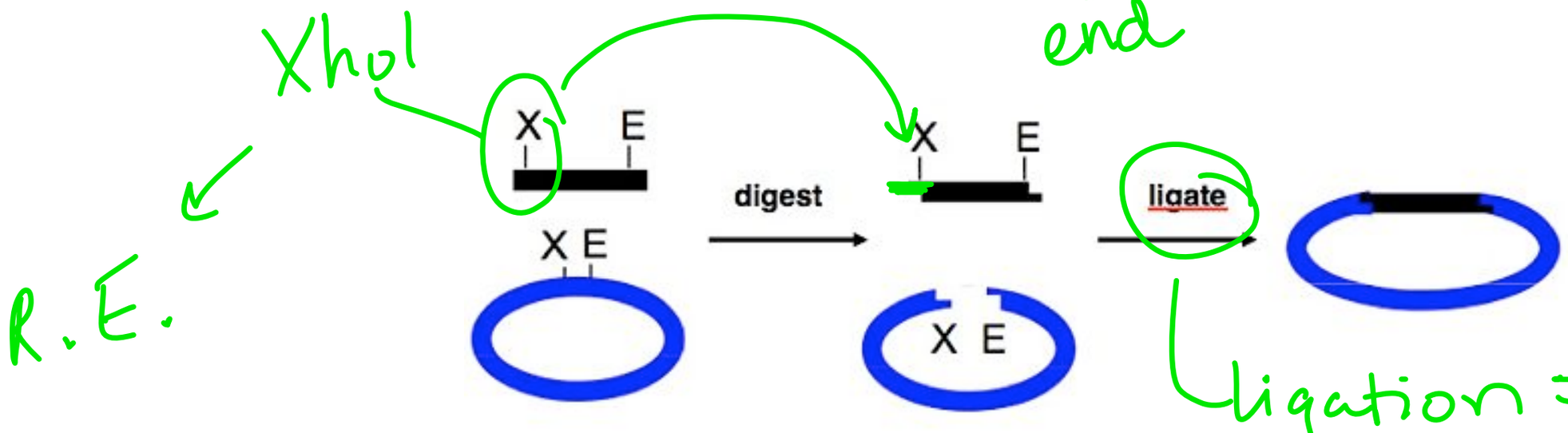
Recombinant DNA

Vector = Plasmid = Circular DNA

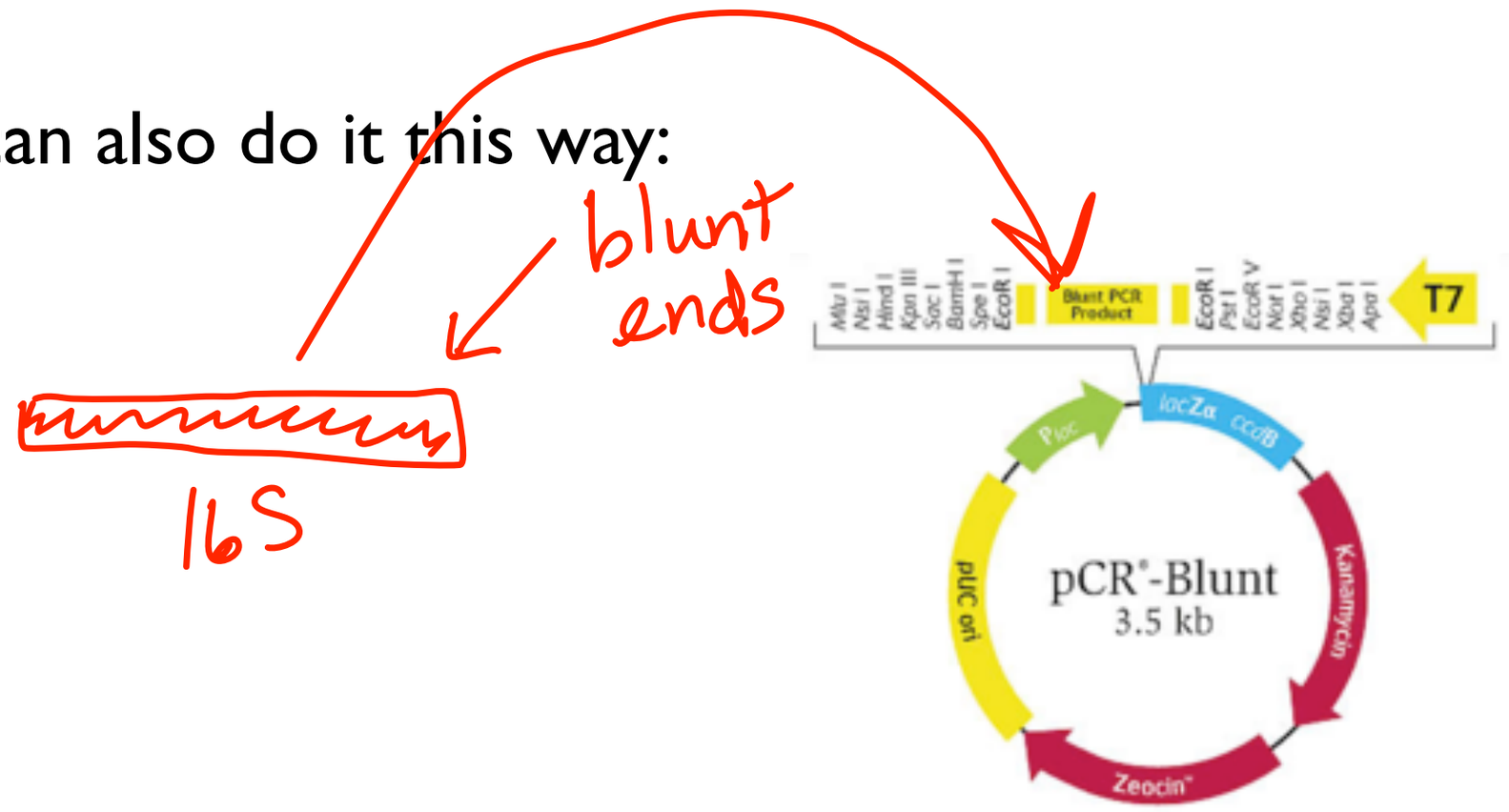


Preview of M1D4: Cloning

You may have done this before:



You can also do it this way:



Bird Microbial Communities -- Review of Overview

Bird Cloacal sample

extract DNA

DAY 1

Complex DNA pool (many microbial DNAs)

PCR

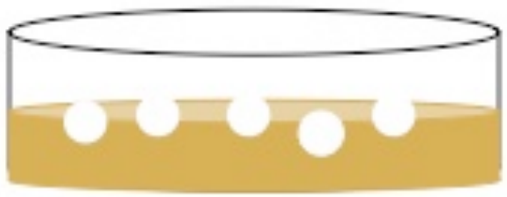
DAY 3

Simpler DNA pool (bacterial 16S DNA)

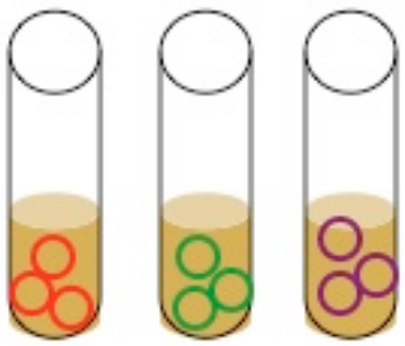
(gel purify); clone and transform

DAY 4

Individual 16S cell colonies



liquid culture



isolate DNA clones from bacteria

DAY 5

Individual 16S DNA plasmids

identify, relate, and compare sequences

DAY 7

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 ~2:20
- Presentation on giving talks from Atissa ~2:20
- Polish your slide ~ 3:15 ~2:35
- Discuss paper from technical POV *and* get feedback about your slide ~ 3:30-5 pm

Thanks to Agi for this slide!