- Announcements
- Lab Quiz
- Pre-lab Lecture
  - ❖ Plasmids: Basics + Gel EP
  - ❖ DNA Ligation
  - ❖ Bacterial Transformation
  - ❖ Today in Lab: M2D4

Announcements

- Module 1 drafts returned tonight
  - 2 wks to revise, due 11 am Thu/Fri
- Module 2 versus Module 1
  - growing sophistication and self-reliance
  - in lab work protocols will be less explicit
  - in report focus on core results (not each step)
  - this doesn't mean don't ask questions!

lac operon

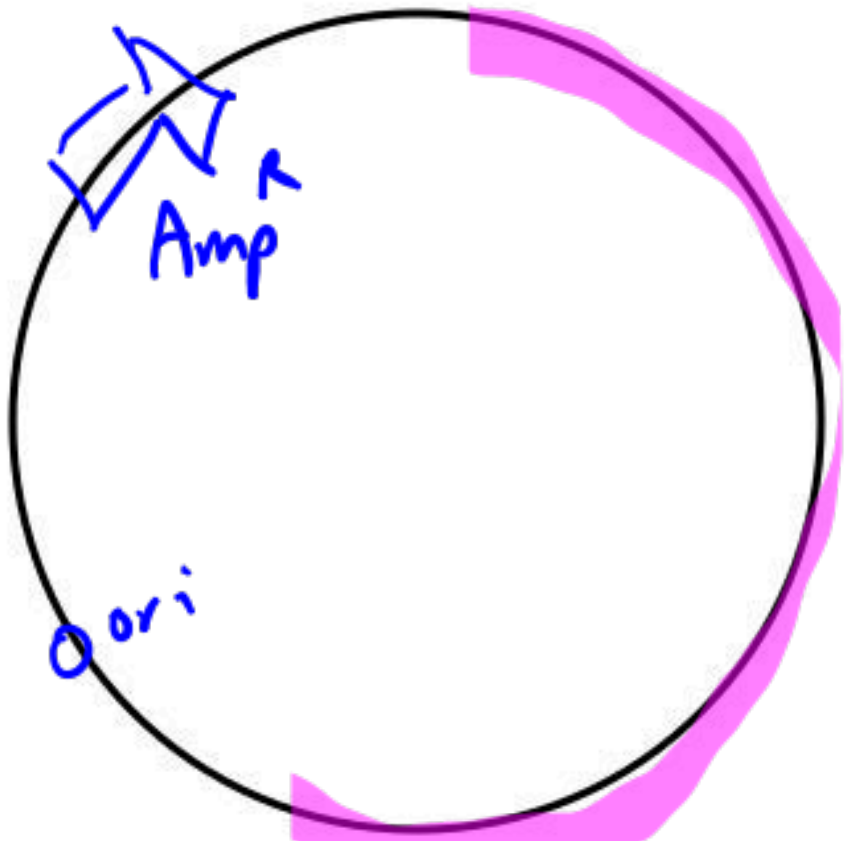
These three genes encode metabolic enzymes

Encodes a lac repressor protein that binds to P LAC (operator) turning it OFF.

IPTG

In turn, if lactose binds to the repressor, it is made inactive, turning ON expression of P LAC.

Plasmid Overview

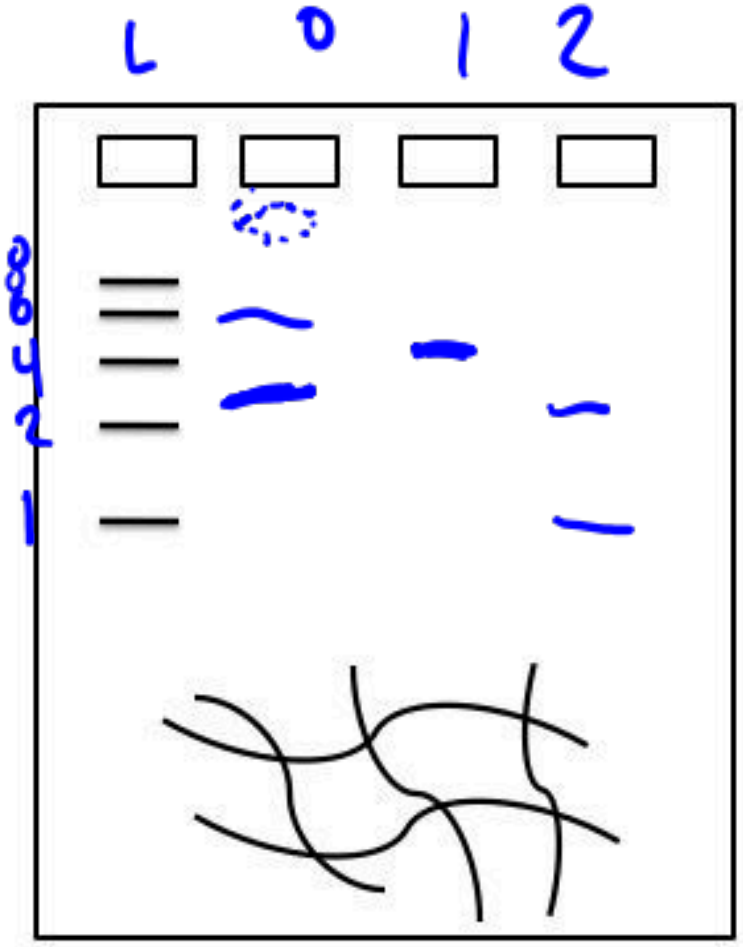


circular, ds, extrachromosomal

why? vector for inserting a gene into an organism

ampicillin resistance
→ select bacteria that have plasmid w/ Amp

DNA EP: Shape-dependence



Plasmid versus linear samples

e.g., 4kbp plasmid

1-cut: linear, run w/ ladder

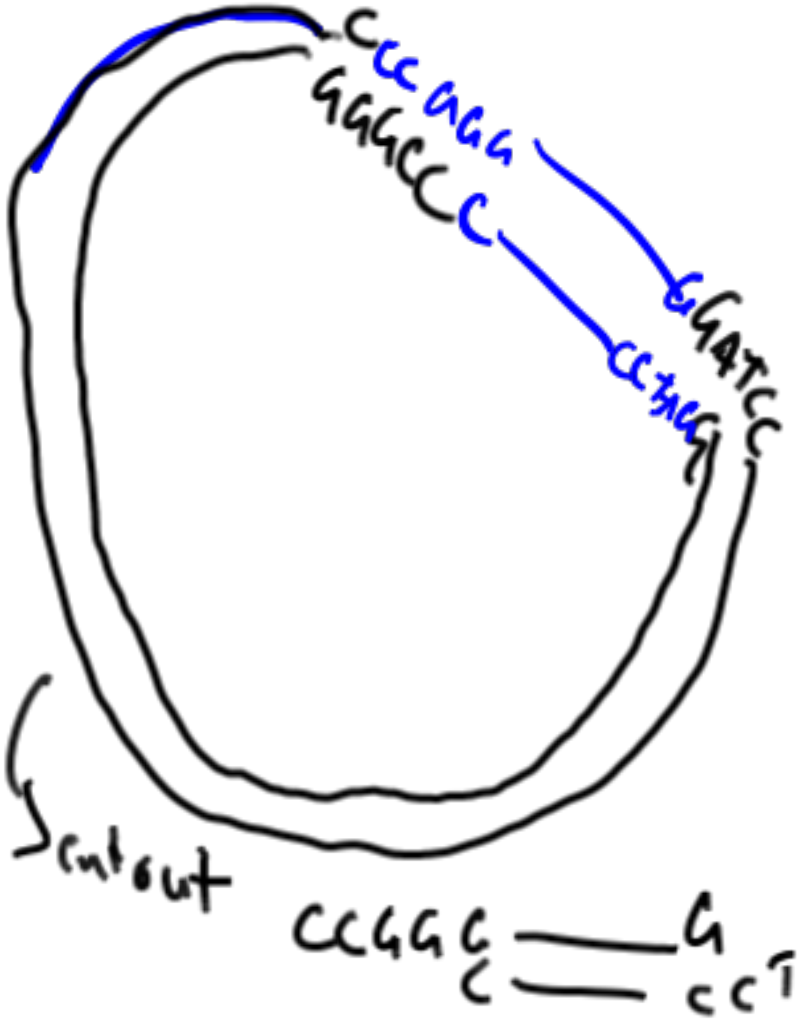
2-cut; sums to 4kp

uncut; supercoiled - faster

circular, (relaxed, nicked): slower

+ high mw concatemers

Restriction Enzymes for Cloning



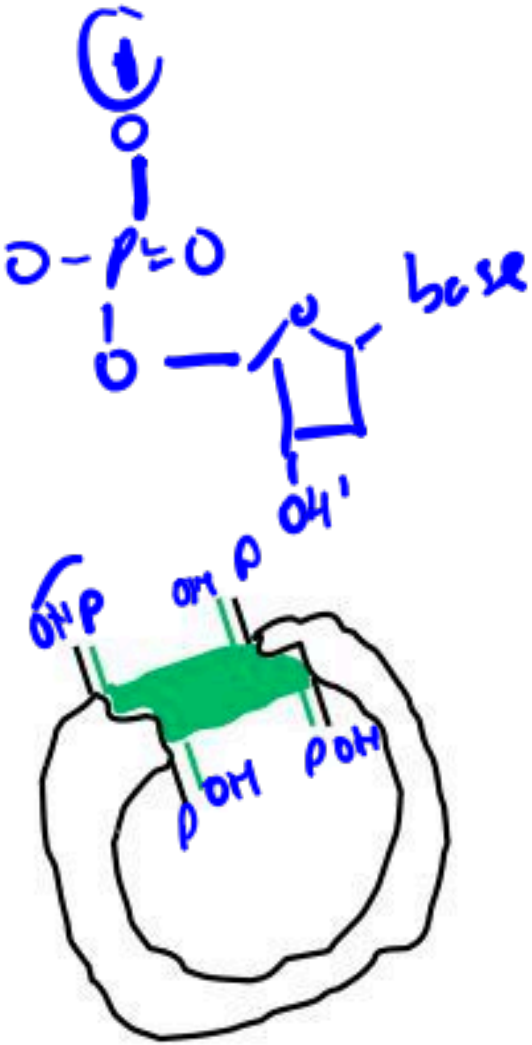
What if BamHI is 5' and XmaI is 3' on insert? insert reversed, non-coding product

What if BamHI on 5' *and* 3' ends? non-directional cloning, bkb re-close

Can you get multiple inserts?

-X X B BX X B B-

DNA Ligation



Reaction creates phosphodiester bond

Reaction requires ATP

What factors affect yield?

ratio of blunt:ins

[DNA], rxn. time

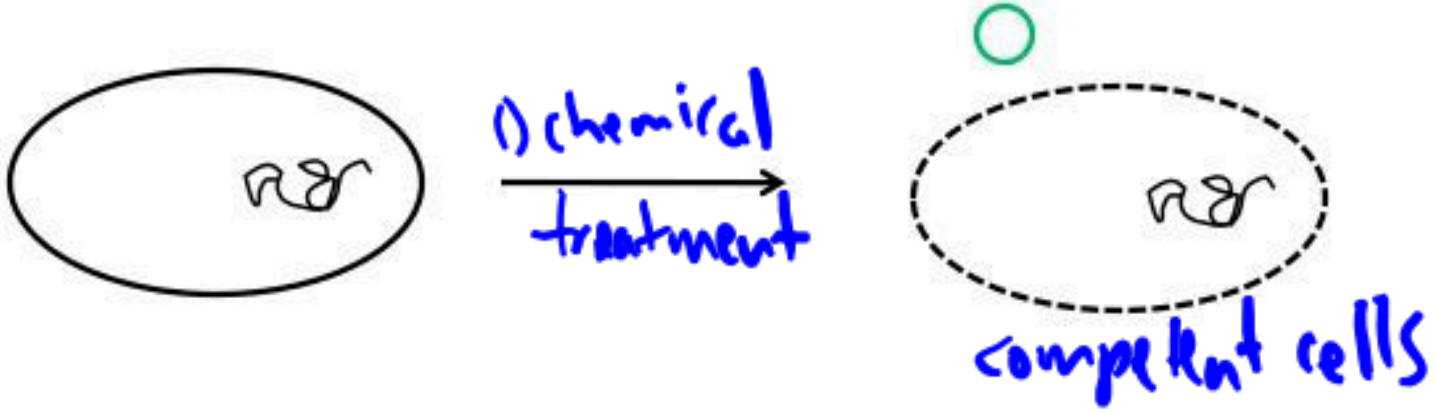
enzyme (conc., T, age, care)

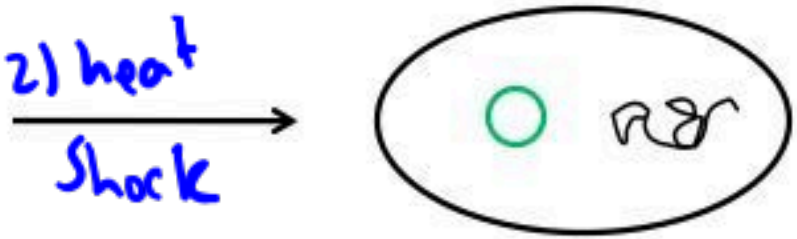
How do we assess if it worked?

sequencing

diagnostic digest if possible

Bacterial transformation



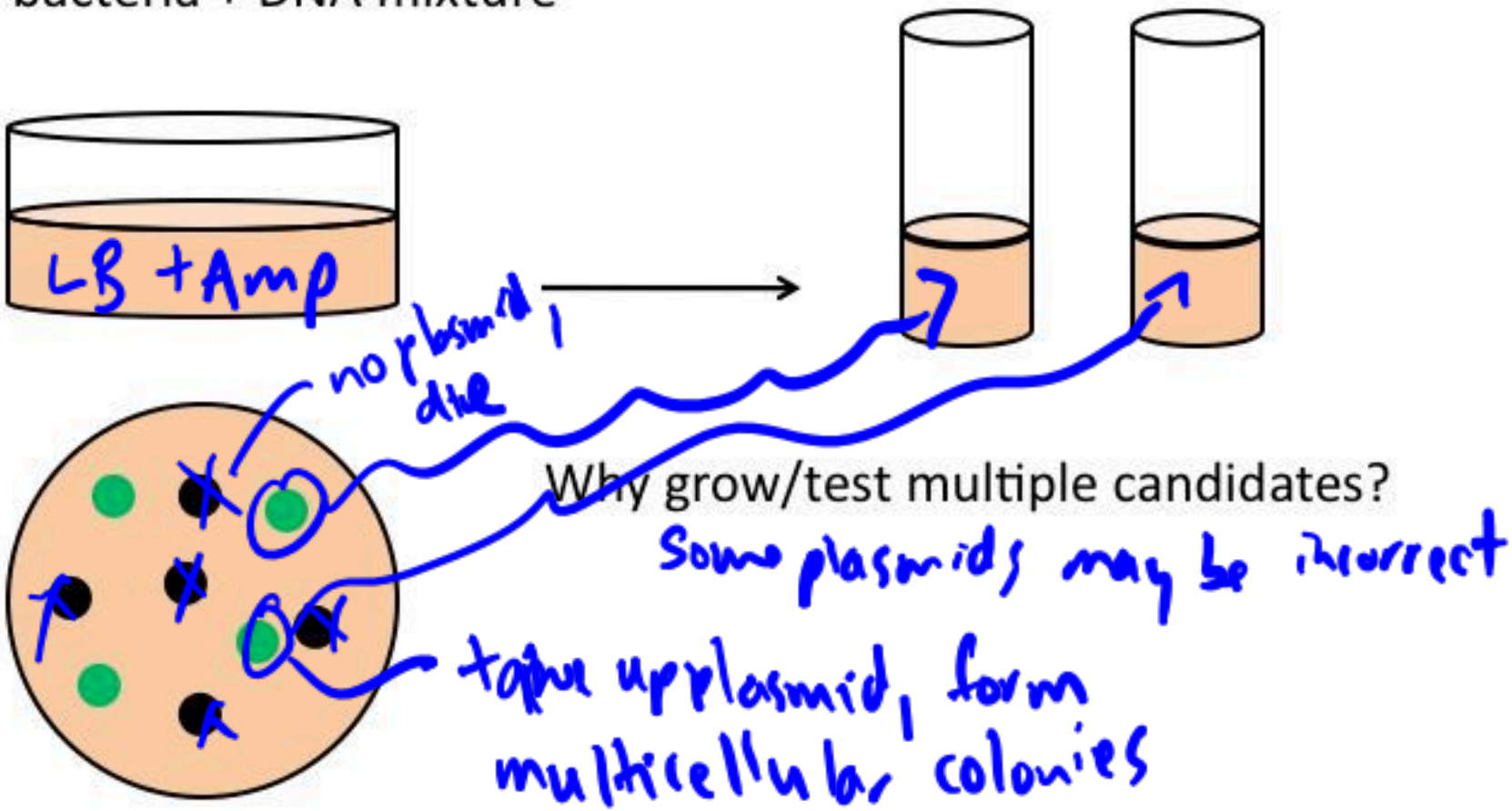


* keep cold
* don't vortex

other methods: electroporation, ballistics

DNA Amplification in Bacteria

Plate bacteria + DNA mixture



Transformation Controls + Outcomes

NEXT TIME

Sample	Expectation… What if?	Role

Miller assay calculation and result

$$\text{1 Miller Unit} = 1000 * \frac{(Abs420 - (1.75 * Abs550))}{\min(t * v * Abs600)}$$

x2 if plate assay

if stock OD600, use stock vol.
if diluted

What was the key finding from your first assay?
What do you expect from your new construct?

Today in Lab (M2D4)

- Decide on a workflow you like
  - Goal A: Ligation/transformation of pED-IPTG-YFD
  - Goal B: IPTG → lacZ transfer function (plate assay)
- FNT
  - I will send an email when it's finalized
  - Have a great spring break!