M1D5: Examine Candidate Clones & Tissue culture

9/30/15

1. Pre-lab discussion
   - M1D4 HW follow up
   - Prep for M1D6 HW: Methods re-write & discussion of "Rad51-deficient vertebrate cells accumulate chromosomal breaks prior to cell death."
2. ½ to tissue culture, ½ purify DNA (plasmid mini-prep).
3. Measure mini-prep DNA concentration
4. Set up diagnostic digests

Follow-up on homework due M1D4

- Comments and scores for figures and captions returned M1D6

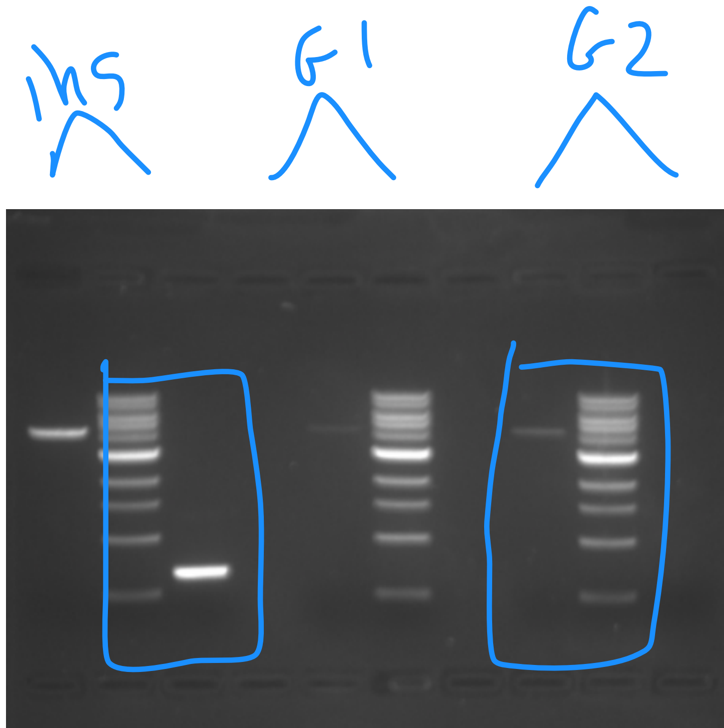


Figure 2) Title - Say something about what yo did!

caption: details needed understand image

A) digest B) blkb C) insert

rewrite Sat 5pm make up 1/2 points lost

Results section bullets

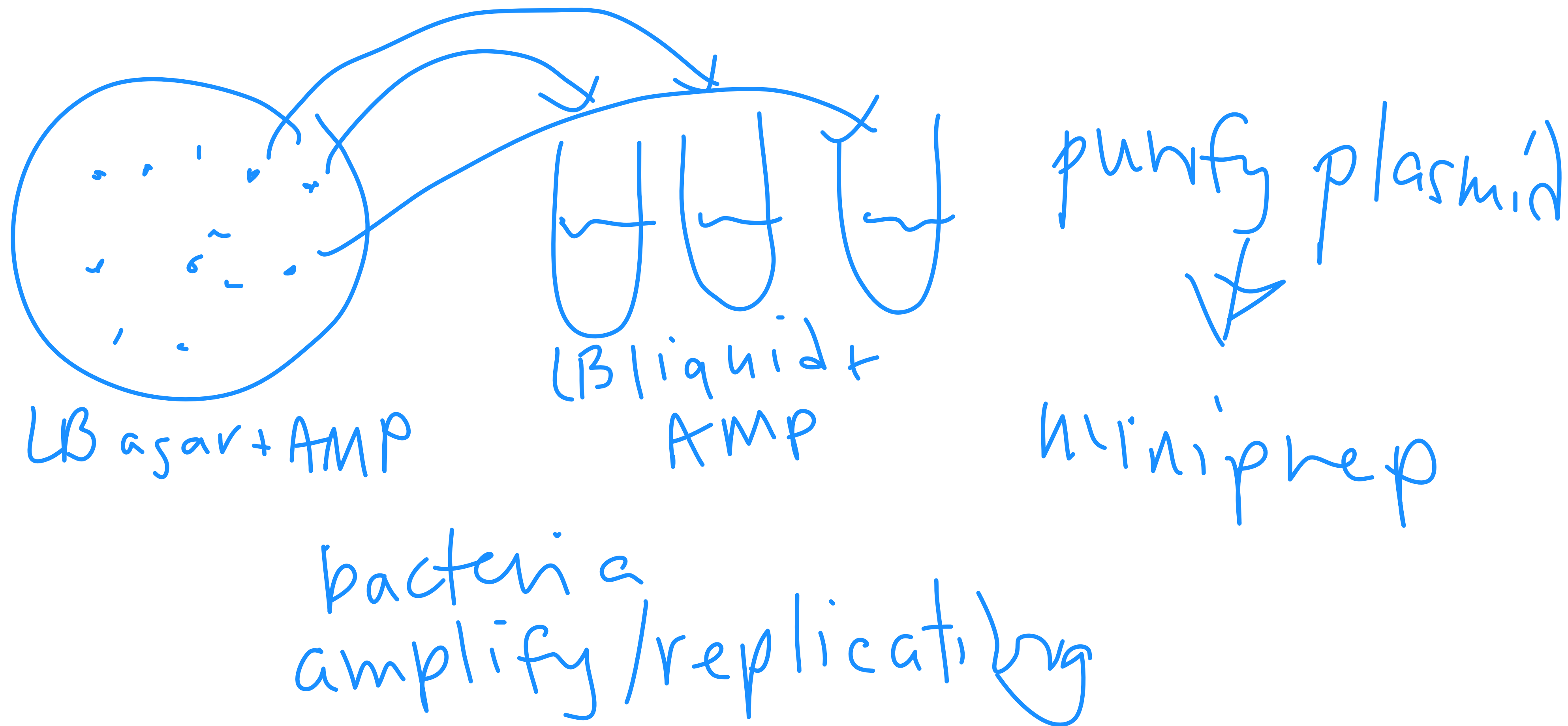
1. What was the overall goal of these data? State concisely in introductory sentence.
2. What was your expected result? State this concisely in a bullet point.
   – What are the expected band sizes on your gel?
3. What evidence do you have that your result is correct or incorrect?
   – What controls did you perform and did they work as expected?
4. What was the result? State this concisely in one or two bullets.
   – Were bands of the expected size present? Why or why not?
5. In sum, what do these data suggest or indicate? Think about how the data were used.
6. What does this motivate you to do next?

Thank you, Shannon

Homework due M1D6

- Incorporate edits from homework due M1D3
  - Eliminate 109 specific details
  - Report concentrations (NOT volumes)
  - Do not include details about tubes and water
  - Avoid repeating information
- Draft remaining methods in outline format
  - Use sub-section titles
  - Include topic sentences
  - Bulleted list of procedures

What happened while you were away…



Post transformant counts to M1D5 Discussion page

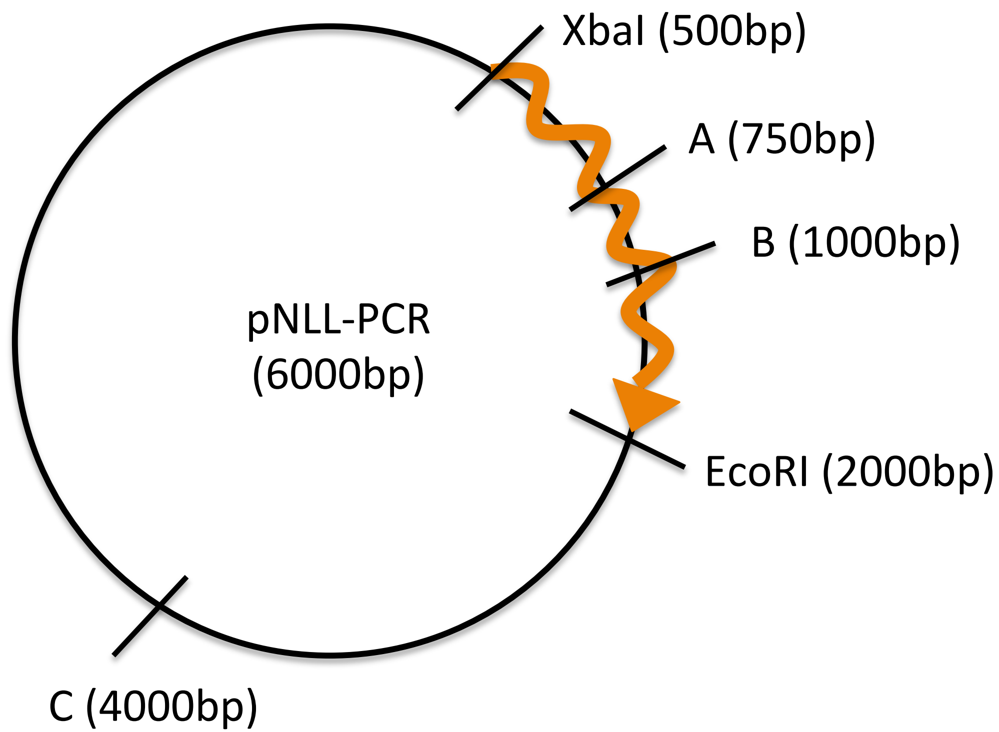
Overview of Mini-prep (Plasmid Purification)

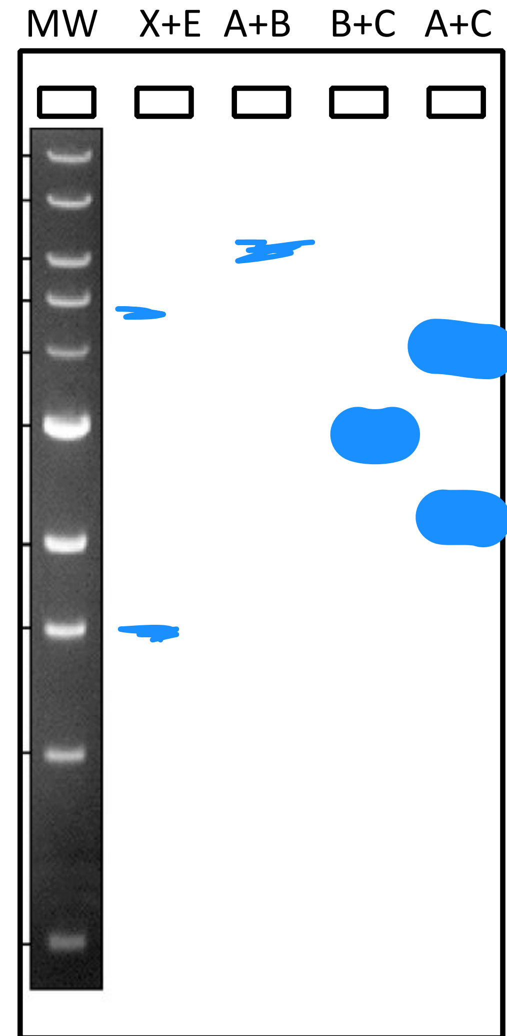
Pellet bacteria, remove media

Step	Contents	Purpose
Prepare	Tris & EDTA buffer	weaken membrane
Lyse	SDS NaOH	lyse + solubilize protein + denature DNA
Neutralize	Acetic Acid Potassium acetate	renature short DNA, precipitate genomic
Concentrate	Spin	* pellet protein + genomic
Wash	EtOH, dry	remove contaminants

resuspend in sterile H_2O

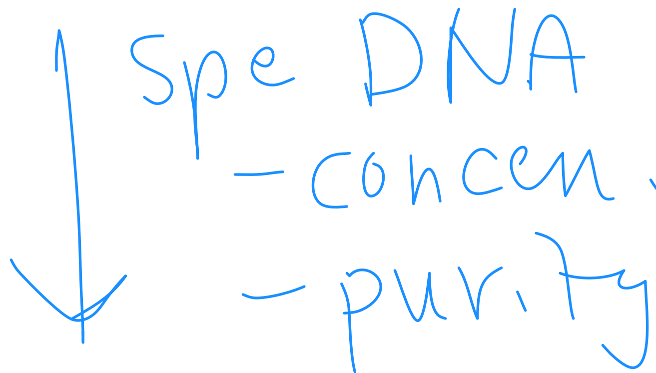
Confirmation of your clones





Diagnostic digest Set-up

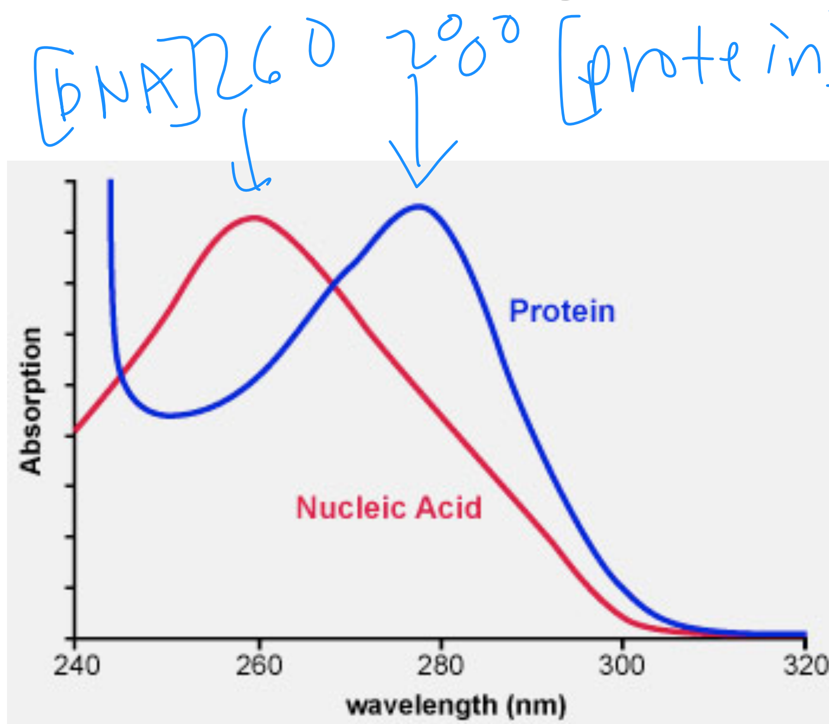
- You will miniprep **four** clones – 3 Δ5EGFP candidates and 1 pCX-NNX.



- You will set-up **three** diagnostic digests with **one** enzyme combination – 2 Δ5EGFP candidates (choose based on DNA purity) and 1 pCX-NNX.

Measuring DNA concentration

[DNA] 260   280 [protein]



1.0 OD @ 260 nm = 50 ng/ml ds DNA

$[DNA] = (OD_{260})(50\ \frac{ng}{ml})(dilution)$

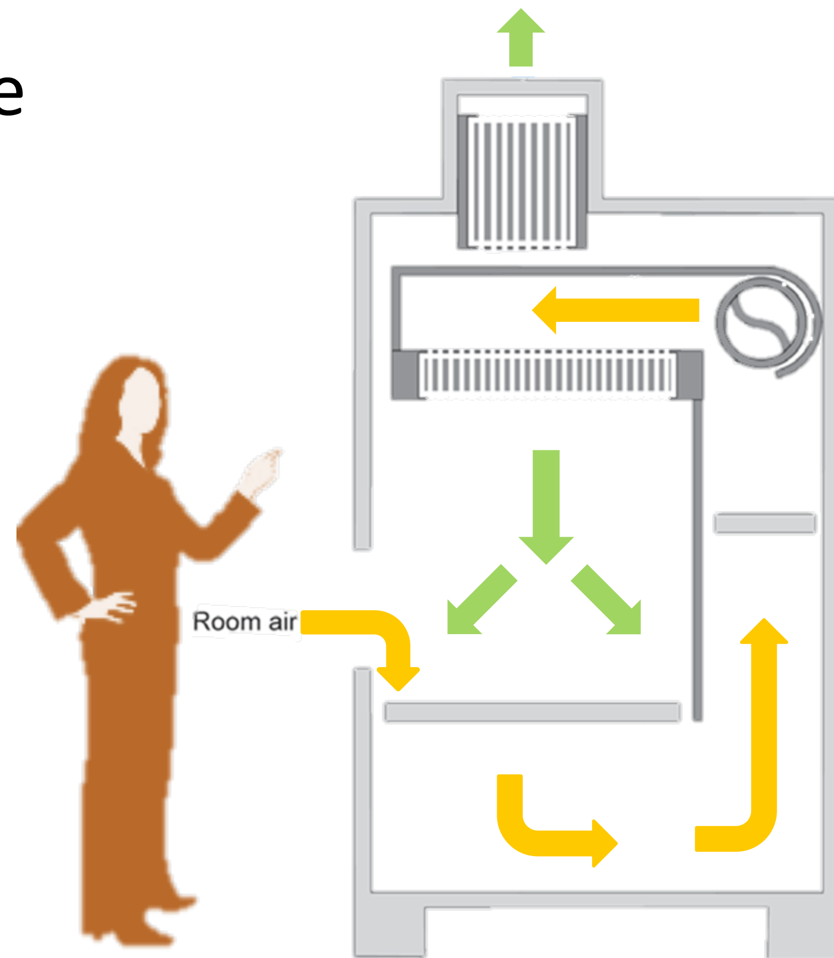
purity 260/280 = 1.8 - 2. high purity

Select two mini-preps to use in diagnostic digests

lower 1.8 = not pure

Mammalian cell culture: Biosafety Cabinet or “hood”

- Wipe cabinet with 70% EtOH before and after use
- Wipe everything that enters the cabinet with 70% EtOH
- Do not block grille or airflow slots
- Minimize side-to-side arm movements



Mammalian cell culture: Tissue culture medium

What do cells need to survive?

Food:

NEAA, growth factors

Non essential amino acids

DMEM

Amino Acids
Vitamins
Salts
pH ≈ 7

Non-food:

antibiotics

FBS

gen. growth factors
Cytokines
lipids
cholesterols

Mammalian cell culture: "Splitting" cells (lots of jargon!)

Why?

1. Look at cells, estimate confluence

   estimate growth, health

2. Rinse with PBS

   wash α-trypsin agent away

3. Detach cells with trypsin

   breaks cell/substrate adhesions

4. Count cells

   specific #

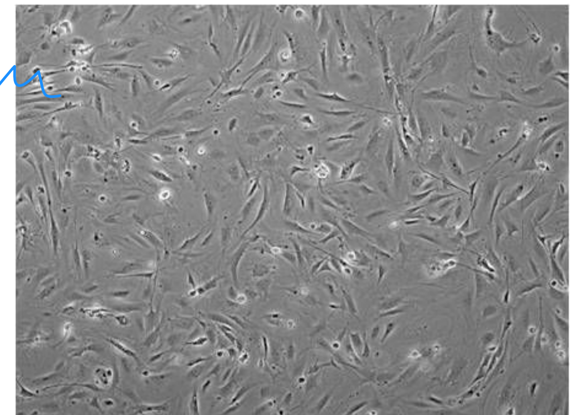
5. Seed new culture vessel

   have room to grow

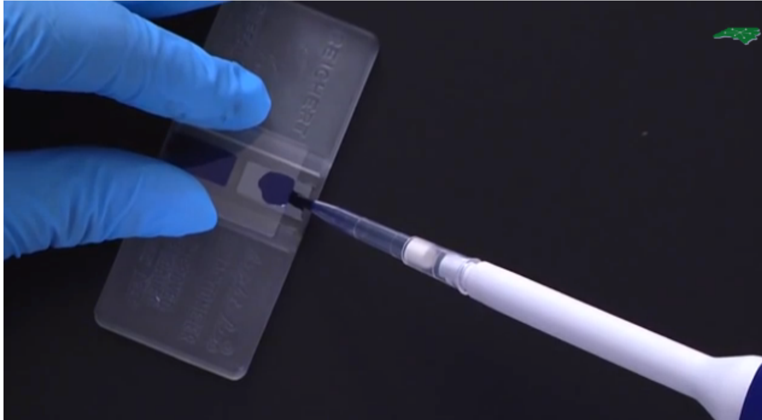
50K

100K

100%

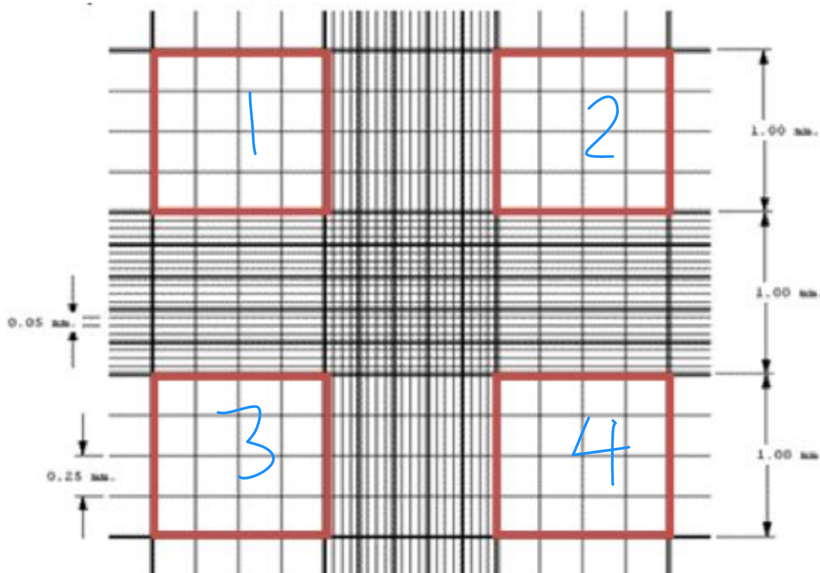


Mammalian cell culture: "Plating" cells



hymocytometer

10 ul



count 4 sq = average

average $\times 10^4$ = #cells/mL

Things to keep in mind today:

- Safety-related information:
  - Vacuum aspirators contain bleach for biohazardous waste (cells).
  - Chemical waste and sink-safe chemicals (w/o cells) should NOT be aspirated.
- Tissue Culture
  - Protocols printed for TC use, no need to move laptops etc.
  - Do not wear PPE in or out of TC room!