M1D5:
Examining clones and tissue culture

9/29/15

Lab business

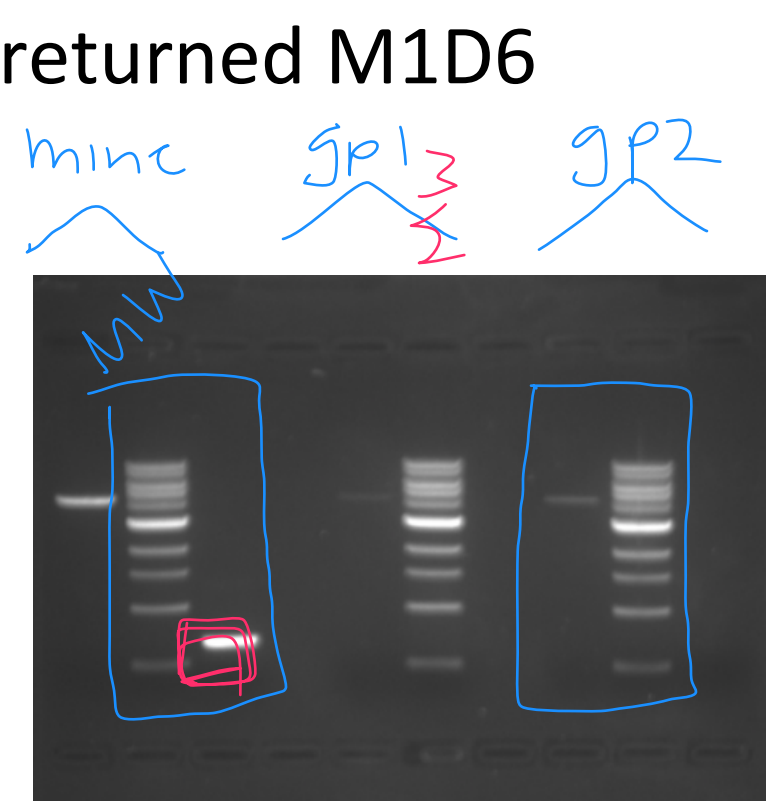
- Follow-up on homework due M1D4
- Homework due M1D6
  - Review methods write-up
- Prep for next time…
  - Paper discussion with Bevin

The EMBO Journal Vol.17 No.2 pp.598–608, 1998

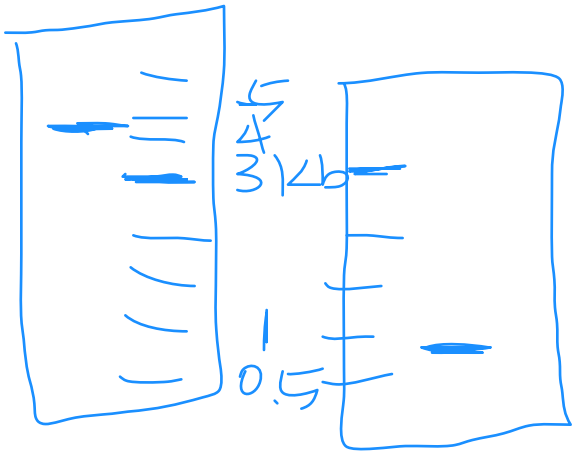
Rad51-deficient vertebrate cells accumulate chromosomal breaks prior to cell death

Follow-up on homework due M1D3

- Comments and scores for figures and captions returned M1D6



A: digest
B insert gel
C. Vector gel



Results section bullets

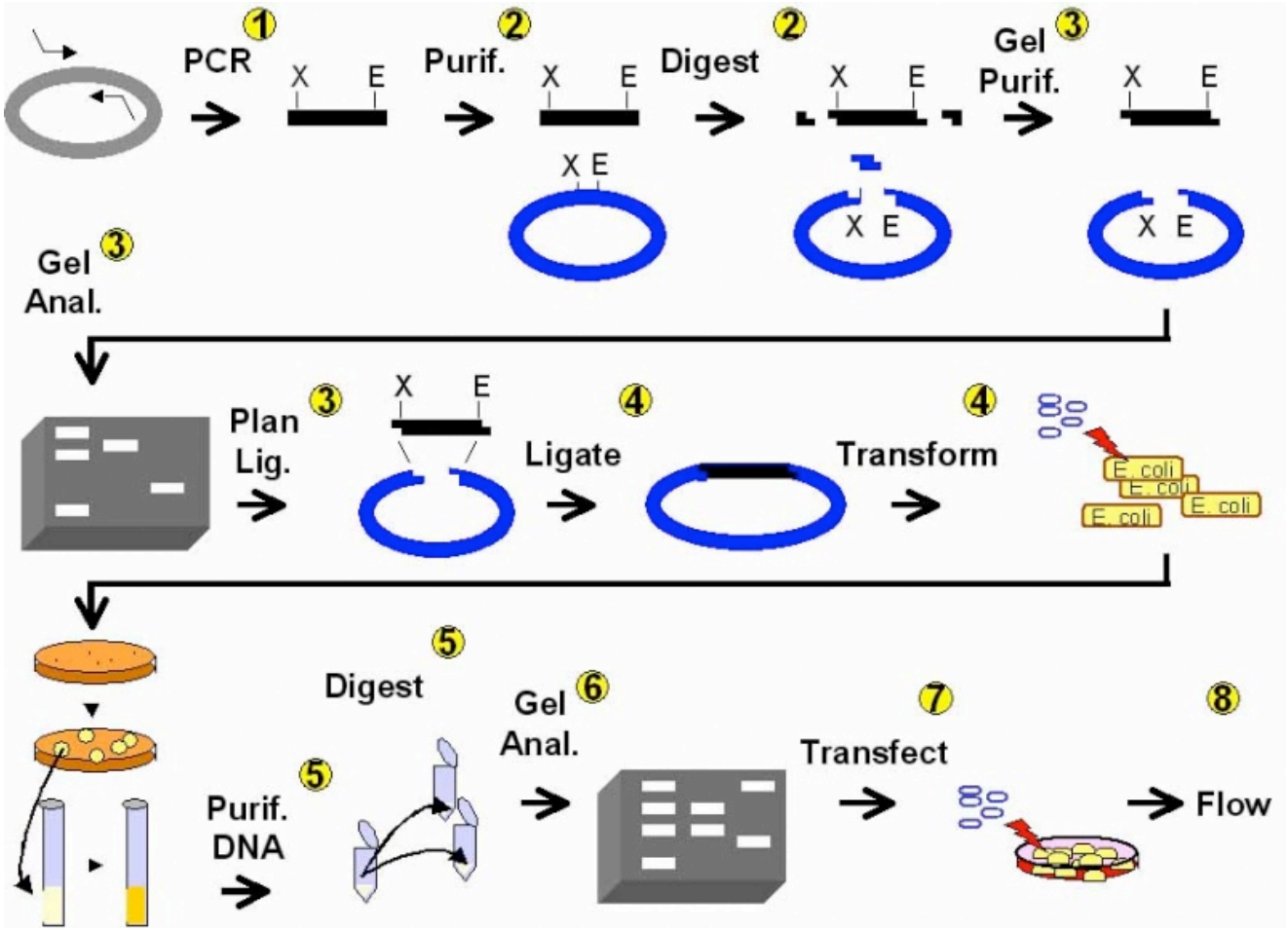
1. What was the overall goal of these data? State concisely in introductory sentence.
2. What was your expected result? State this concisely in a bullet point.
   - What are the expected band sizes on your gel?
3. What evidence do you have that your result is correct or incorrect?
   - What controls 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

Mod 1 overview



From last time…

- ligation ΔS EcoRI + pCR-NNX

- transformation

  ⇒ think mentally back to discussion about controls

Post transformant counts to M1D5 Discussion page

While you were away…

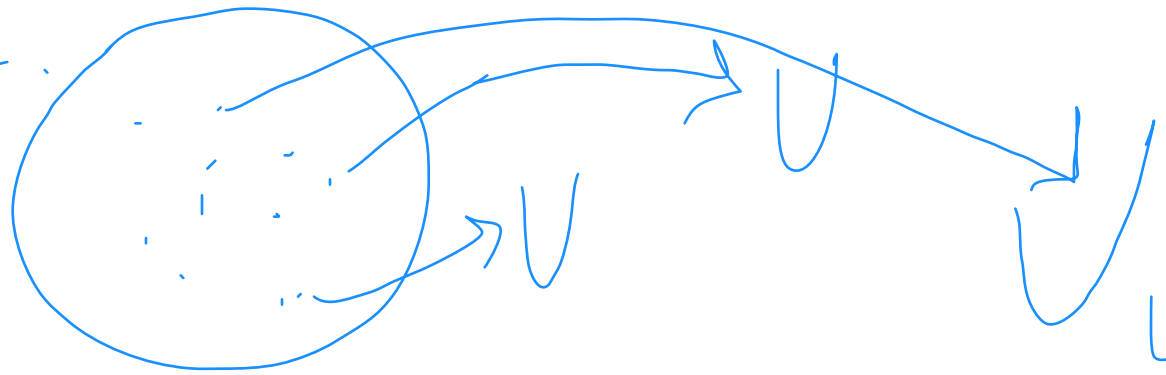
1. troubleshooting

repeated transformation

w/ running low

of your ligation

2.



LB amp

LB amp

Inubated 37C

ON

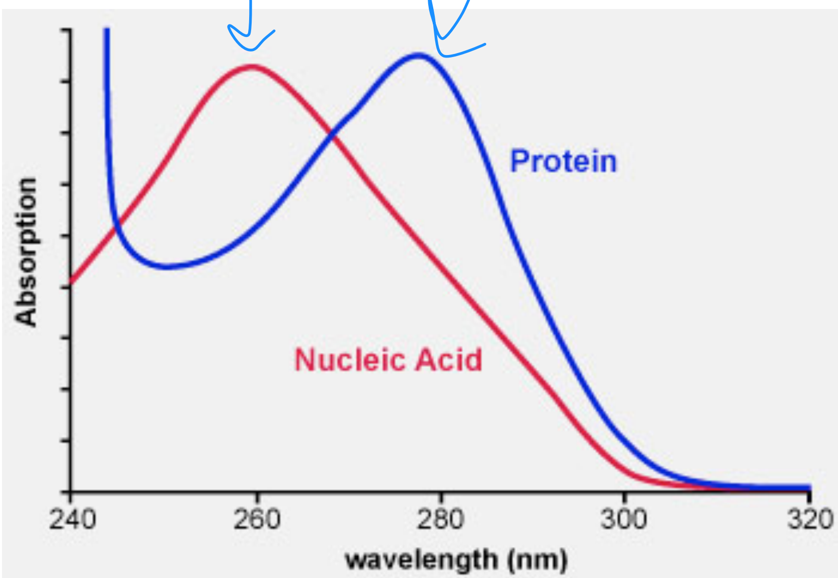
Mini-prep to examine candidate clones

pellet cells then discard media

Step	Contents	Purpose
Prepare	Tris / EDTA buffer	resuspends cells weakens membrane
Lyse	SDS NaOH	solubilize proteins denature DNA
Neutralize	Acetic Acid Potassium acetate	renature short DNA precipitates long DNA
Concentrate	Spin	pellet garbage
Wash	EtOH	Similar to Column

Measuring DNA concentration

260nm 280nm



260nm for calc. [DNA]

1.0 OD @ 260nm = 50ng/ul dsDNA

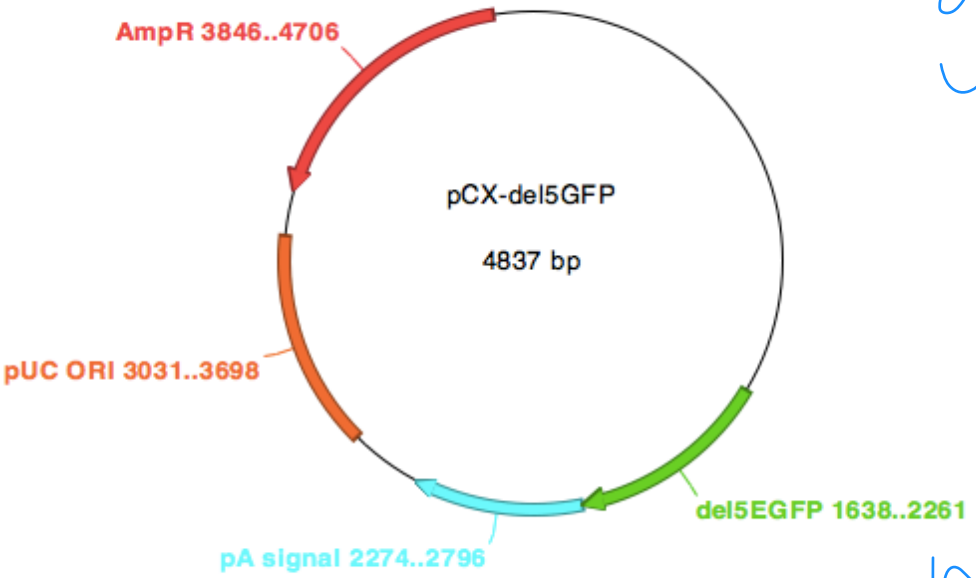
[DNA] = OD_{260} · 50 ng/ul · dilution factor

purity 260nm/280nm

1.8-2.0 ↑ purity

Select two mini-preps to use in diagnostic digests

Diagnostic digest reactions



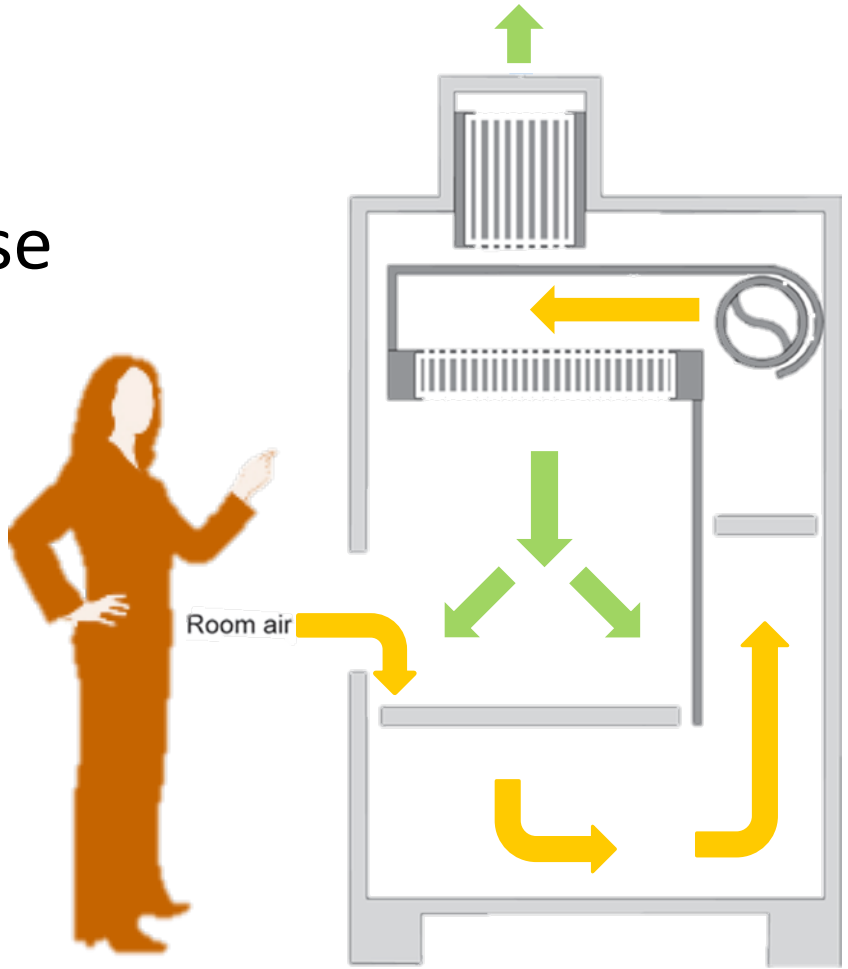
guidelines
→ cut sites
→ fragment sizes

which enzymes we have

Why digest candidate clones ***and*** pCX-NNX?

Tissue culture overview

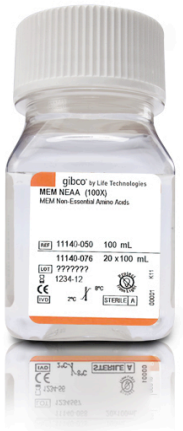
- Wipe cabinet with 70% EtOH before and after use
- Wipe everything that enters the cabinet with 70% EtOH
- Do not block grille or airflow slots
- Minimize side-to-side arm movements



Mammalian cell culture medium

DMEM

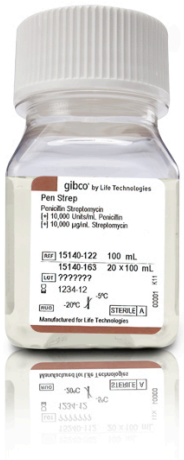
- amino acids
- vitamins
- salt



NEAA

FBS

= growth factor
- lipids
cholesterol



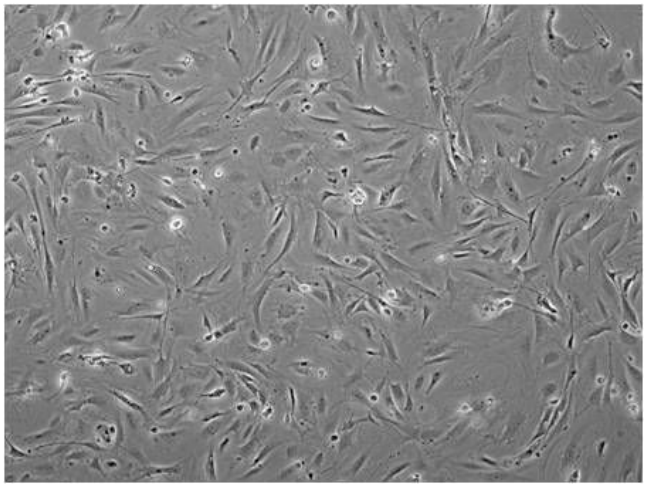
antibiotics

antimycotics

Mammalian cell culture jargon

- Confluence

  density



- Splitting

  "sub-culturing"

- Seeding

Seeding your J1 cells

1. Rinse with PBS

   removes α-trypsin agents

2. Detach with trypsin

   breaks cell-substrate bond

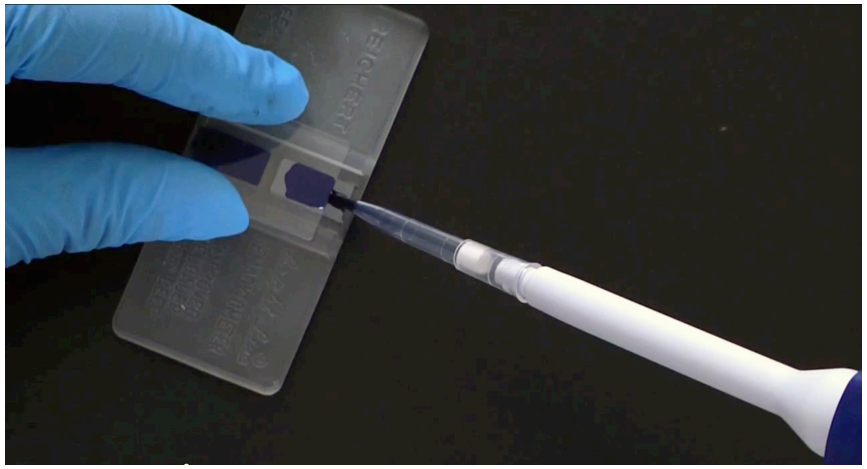
3. Calculate number of cells

   hemocytometer

4. Seed new culture dish

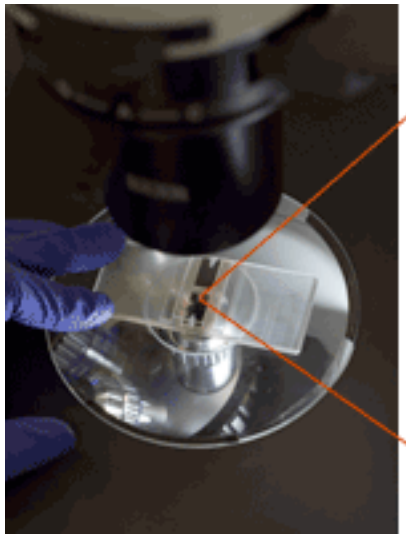
   50K
   100K (75K)

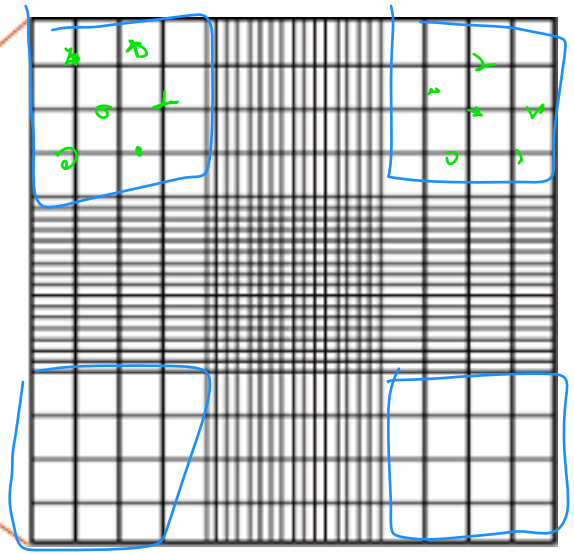
Calculating number of cells



count # of cells in each corner

determine avg





Avg · 104

= cells/mL

Today

- Class split between mini-preps and tissue culture
  - Red and Orange Teams begin in TC
- For tissue culture work
  - Printed protocols are at each hood
- For mini-prep work
  - Aspirate cell culture waste (aspirator contains bleach)
  - Do not aspirate chemicals