

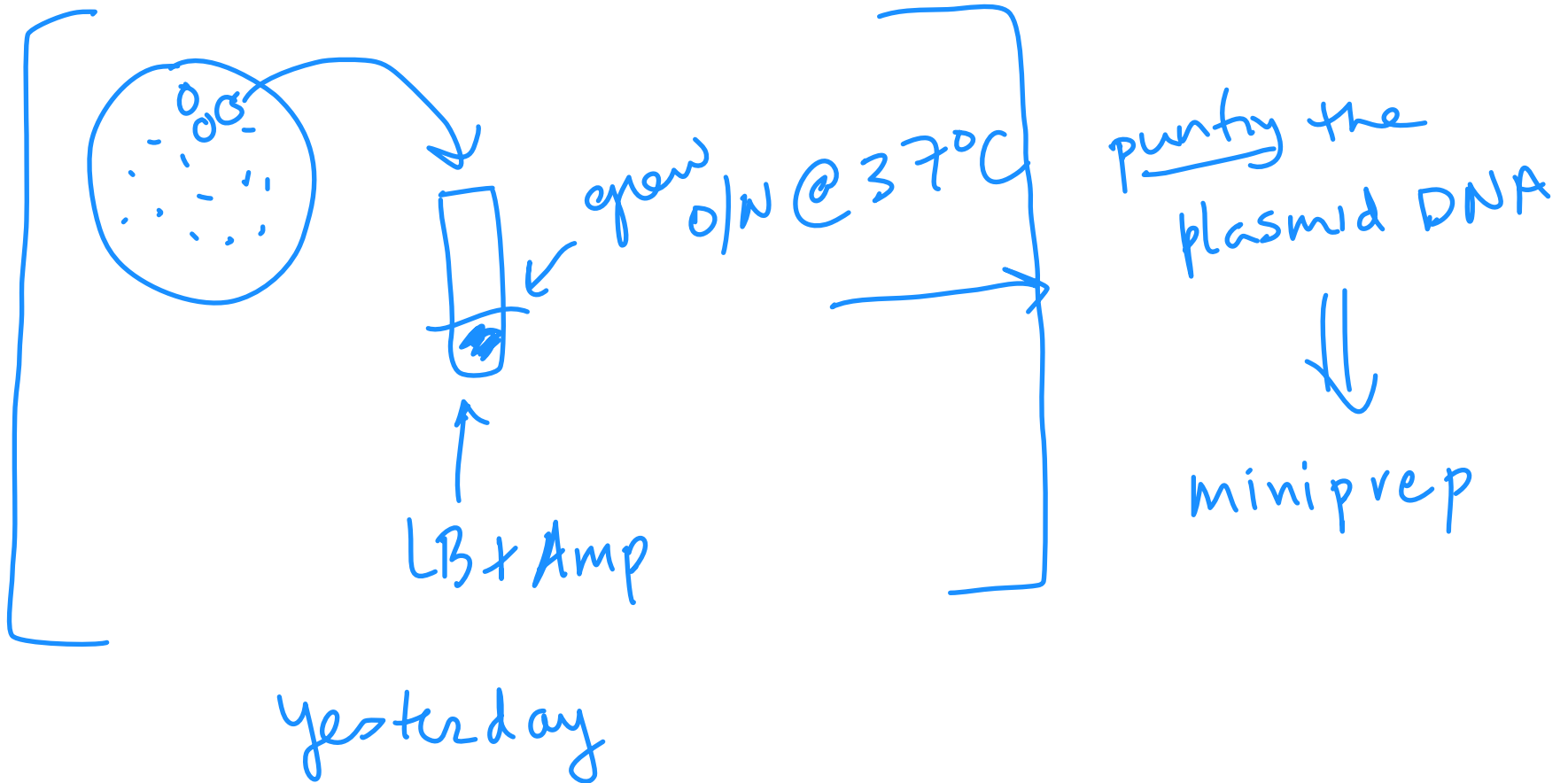
M1D5: Miniprep & Tissue culture

9/25/14

purple,
pink,
blue } to TC
first

- Pre-lab discussion
- $\frac{1}{2}$ to TC, $\frac{1}{2}$ purify DNA and set-up diagnostic digests
- Evaluate transformations
- Hint: start minipreps right away.

Review: what happened while you were away



Overview: Plasmid Purification -- Miniprep

Clean it up!

pellet the bacteria + remove the media

Step	Contents	Purpose
Prepare	Tris & EDTA Buffer	- resuspend → weaken the cell membrane
Lyse	SDS NaOH	→ solubilize protein → denature DNA
Neutralize	Acetic Acid/KAc	Renature ^{short} DNA → precipitate genomic DNA
Concentrate	10 min Spin all	→ pellet unwanted DNA
Wash	EtOH, dry	* Similar to MIDY

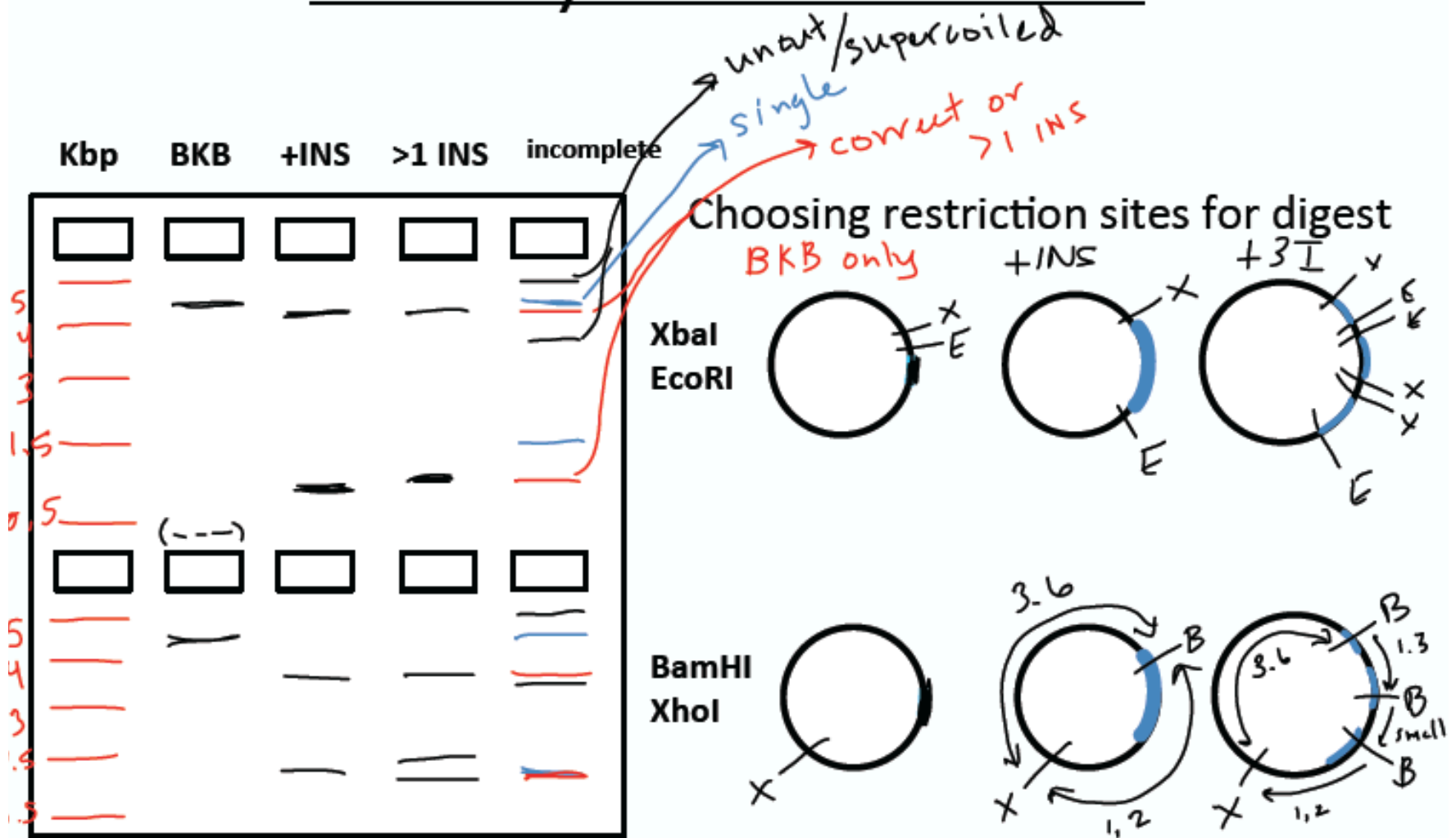
Note:

You will miniprep **three** clones – 3 experimental.

↓ spec your
DNA

You will set-up **three** diagnostic digests – 2 experimental and 1
pCX-NNX.

How do you know it worked?



TEAM COLOR	pCX-EGFP (5ng)	Backbone + Insert (no ligase)	Backbone + Ligase	Backbone + Insert + Ligase
RED				
TIGER				
YELLOW				
GREEN				
BLUE				
PINK				
PURPLE				

Mammalian cell culture — Tissue culture medium

What do cells need to survive?

Food(s):



— NEAA

Non-food(s):



— Antibiotics
— Antimycotics

DMEM
- amino acids
- vitamins
- [salt]



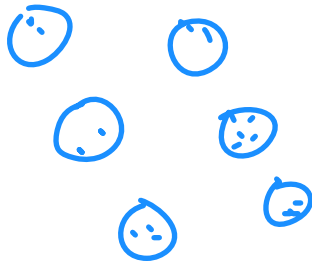
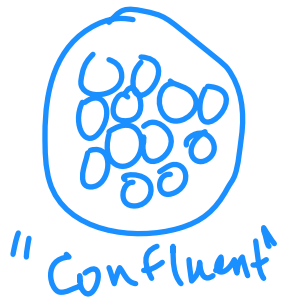
phenol red (pH)

• growth factors +
• cytokines
• lipids
• cholesterol
(5-10%)

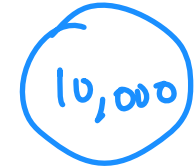


Tissue culture – lots of jargon

"Sub-culturing" → "splitting" a lot of cells → small #



1:100 or 1:400

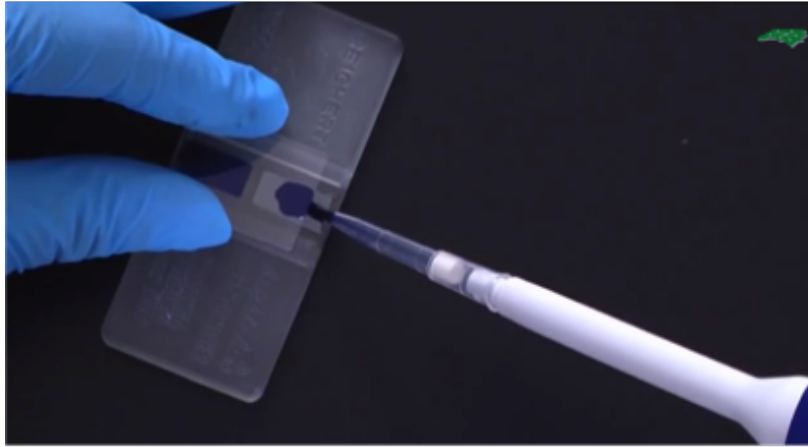


10mL

1. Rinse with PBS — why? Serum has anti-trypsin agents
2. Detach cells — why? Trypsin → break cell: substrate bonds
3. Count cells — why?
4. Add to new culture vessel — why? cells have room to grow

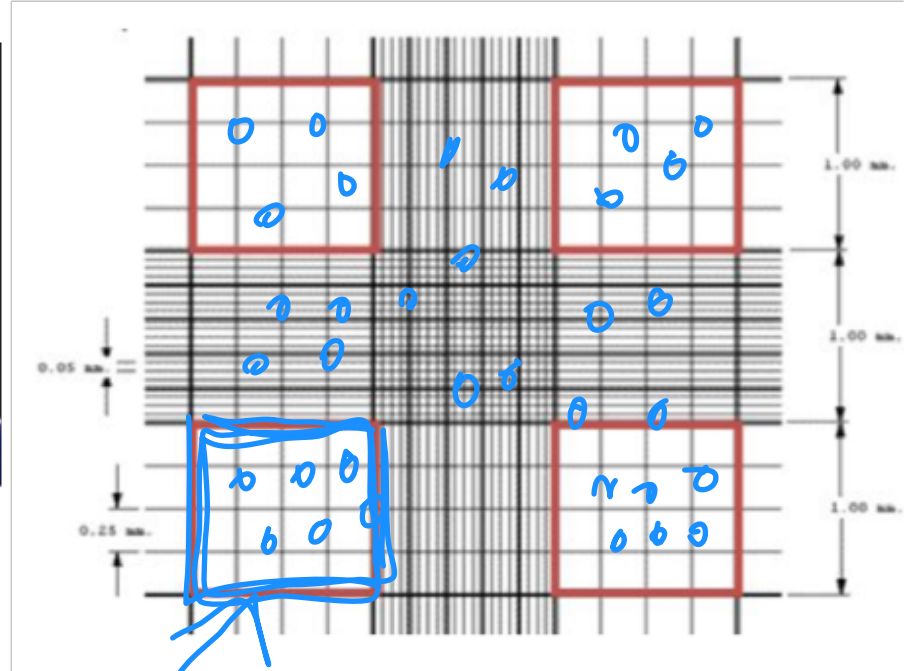
Mammalian cell culture — 'Plating' cells

hemocytometer



cell concentration:

$$\text{Avg} \times 10^4 = \frac{\text{cells}}{\text{mL}}$$



Count all four corners → find avg

Things to keep in mind today:

- Safety-related information:
 - Vacuum aspirators contain bleach for biohazardous waste (cells). Can go down sink afterwards.
 - Chemical waste and sink-safe chemicals (w/o cells) should NOT be aspirated.
- Upload Transformation results to M1D5 Talk page
- Tissue Culture
 - Protocols printed for TC use, no need to move laptops etc.