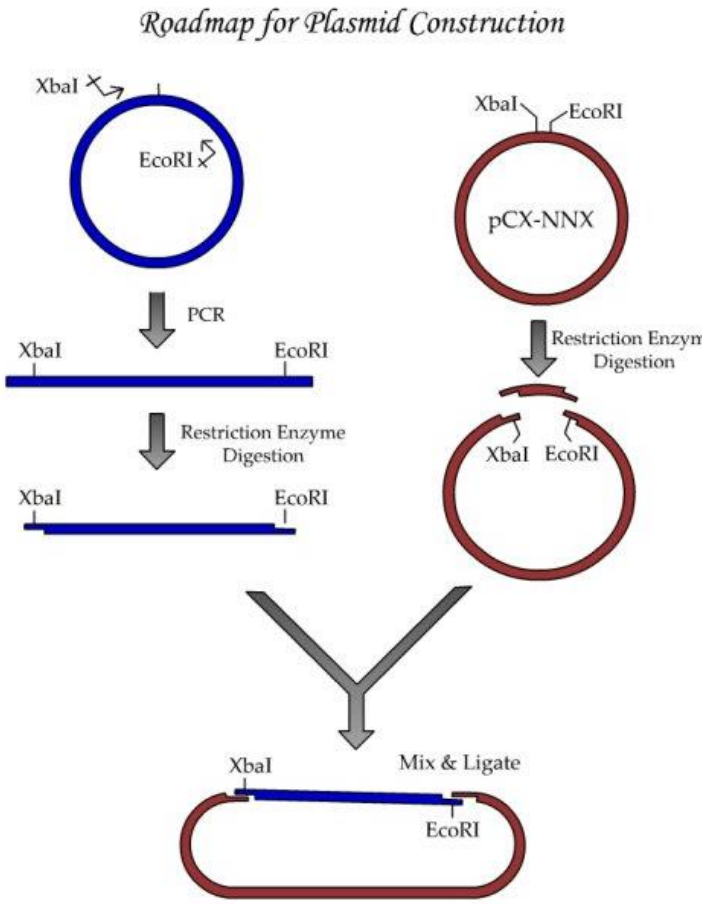
DNA Engineering
Mod 1 Day 5

Pre-lab Lecture

20.109 (F13)

9/26/2013

M1D5

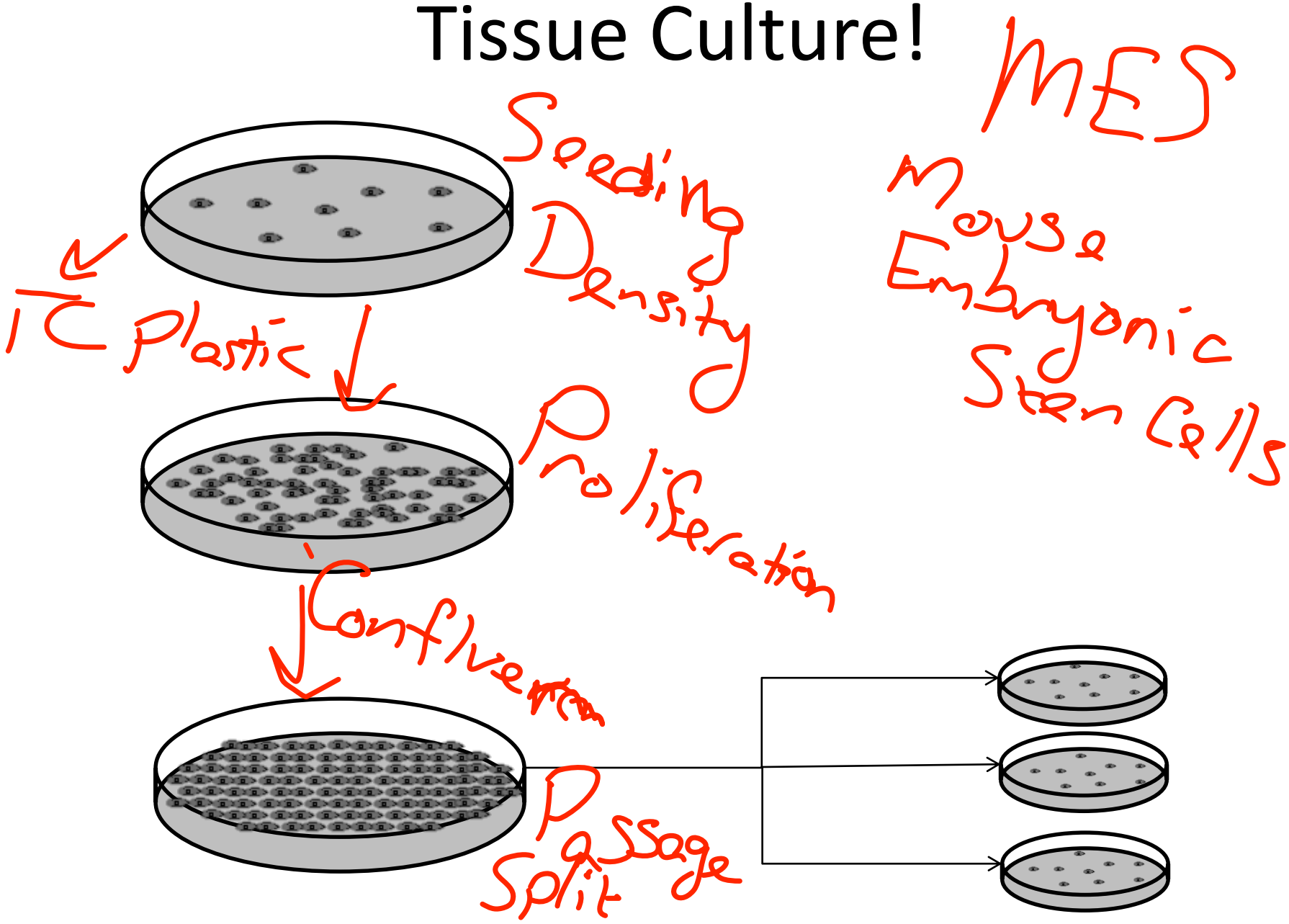


- Quiz
- Pre-lab discussion
- ½ class to TC
- Purify clones
- Set-up diagnostic digests
- Evaluate transformation

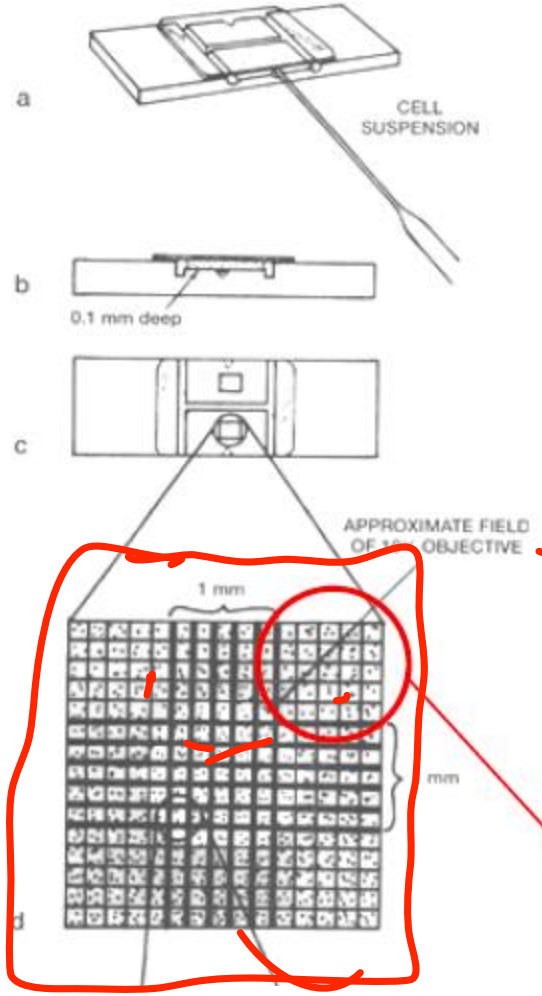
Announcements

- Important Stuff:
  - 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.

Tissue Culture!



Hemocytometer!



RBC's

1. Count 4 corners

2. Avg

3. Calculate

Avg × 10^4 = Cells/mL

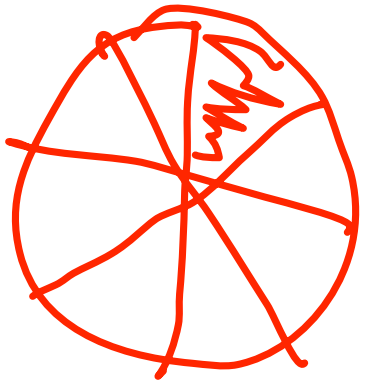
1 Square = 0.1 µL

Review of Experiment + Controls

Experiment Condition	pCX-EGFP (5ng)	Backbone + Insert (no ligase)	Backbone + Ligase	Backbone + Insert + Ligase
Why?	Pos ☺	Neg ϕ	Neg ϕ	Exp!
What if…	[Amp] Temp Cell Competency	Uncut Plasmid →		

Variations in Experimental Samples

- Backbone-to-Insert ratio
- Incubation time
- Salt in ligation
- Unequal recovery



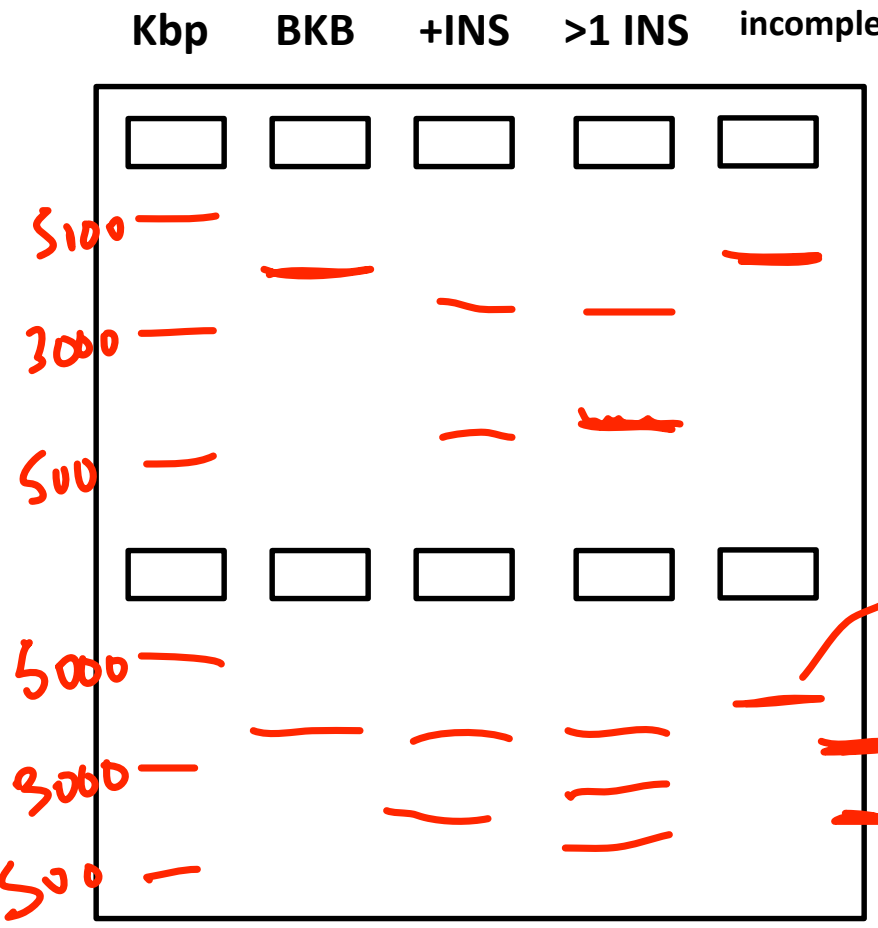
TEAM COLOR	pCX-EGFP (5ng) —	Backbone + Insert (no ligase)	Backbone + Ligase	Backbone + Insert + Ligase
RED	480	0	2	50, 44
ORANGE	~640	0	20	0, 0
YELLOW	688	8	5	560, 680
GREEN	210	0	24	15, 6
BLUE	880	4	14	232, 376
PINK	1040	1	9	142, 96
PURPLE	600	0	110	960, 1040
PLATINUM	1048	2	6	296, 480
WHITE	~784	2	6	166, 173

Overview: Plasmid Purification -- Miniprep

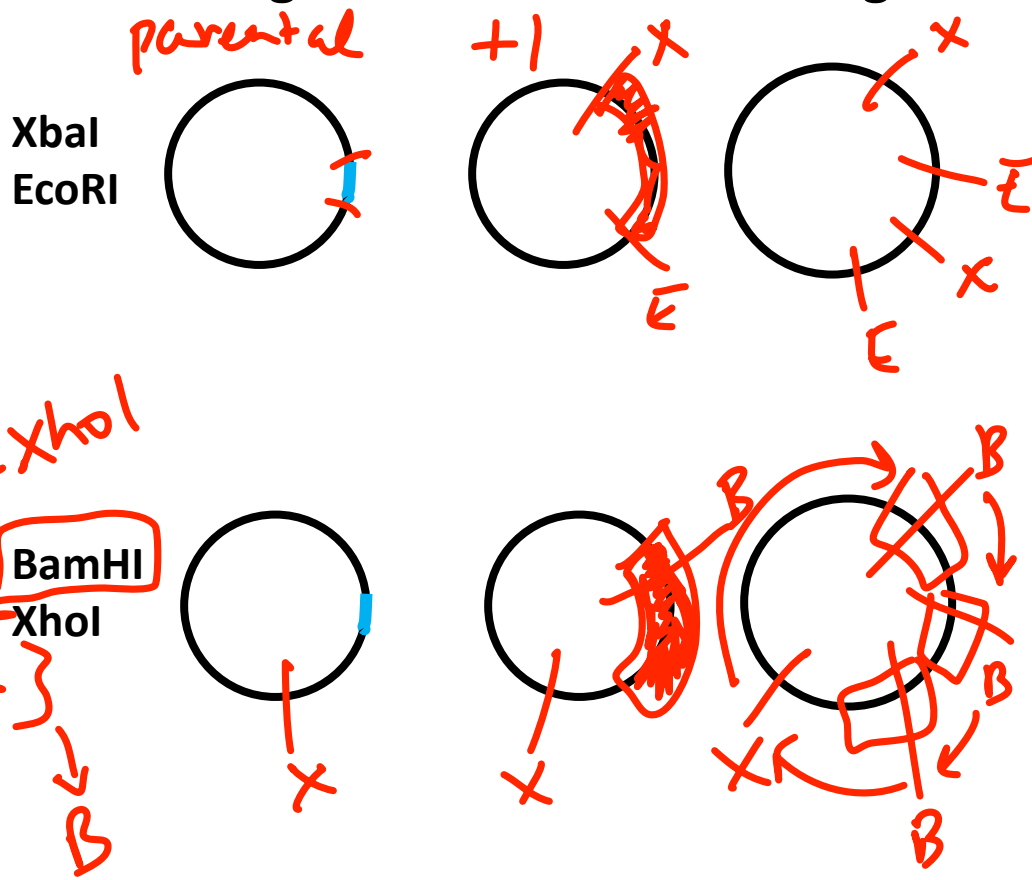
Clean it up!

Step	Contents	Purpose
Prepare	Tris & EDTA Buffer	- resuspension - weaken cell membrane
Lyse	SDS NaOH	solubalize membrane + protein denature DNA
Neutralize	Acetic Acid/KAc	Renature DNA (plasmid)
Concentrate	Spin all	pellet unwanted material
Wash	EtOH, dry	**Keep the Sup ? pree + clean it up

Diagnostic DNA gels



Choosing restriction sites for digest



Today in Lab (M1D5)

- Miniprep three Δ5-EGFP candidates, and bacteria transformed with pCX-NNX
  - tip: orient tubes in centrifuge
  - pCX-NNX = control for
- Set up digests
  - tip: make reaction cocktail → efficiency
  - add loading dye before leaving lab
- Count and post colony #s on M1D5 *Talk* page
  - we will discuss briefly before heading to TC
- TC practice session: don't need notebook, just a piece of scrap paper