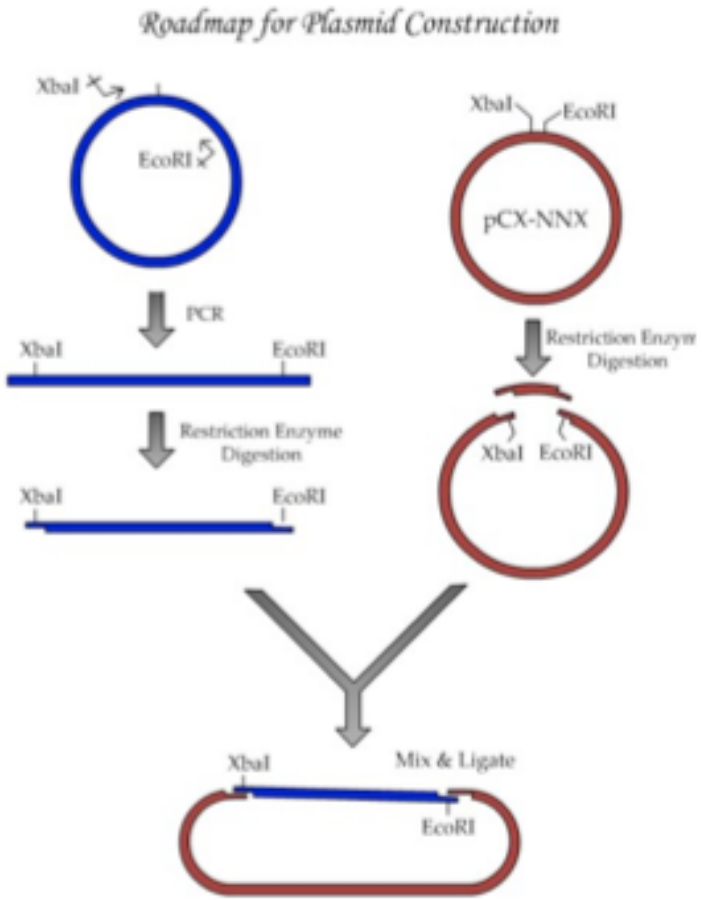
DNA Engineering
Mod 1 Day 5

Pre-lab Lecture

20.109 (F13)

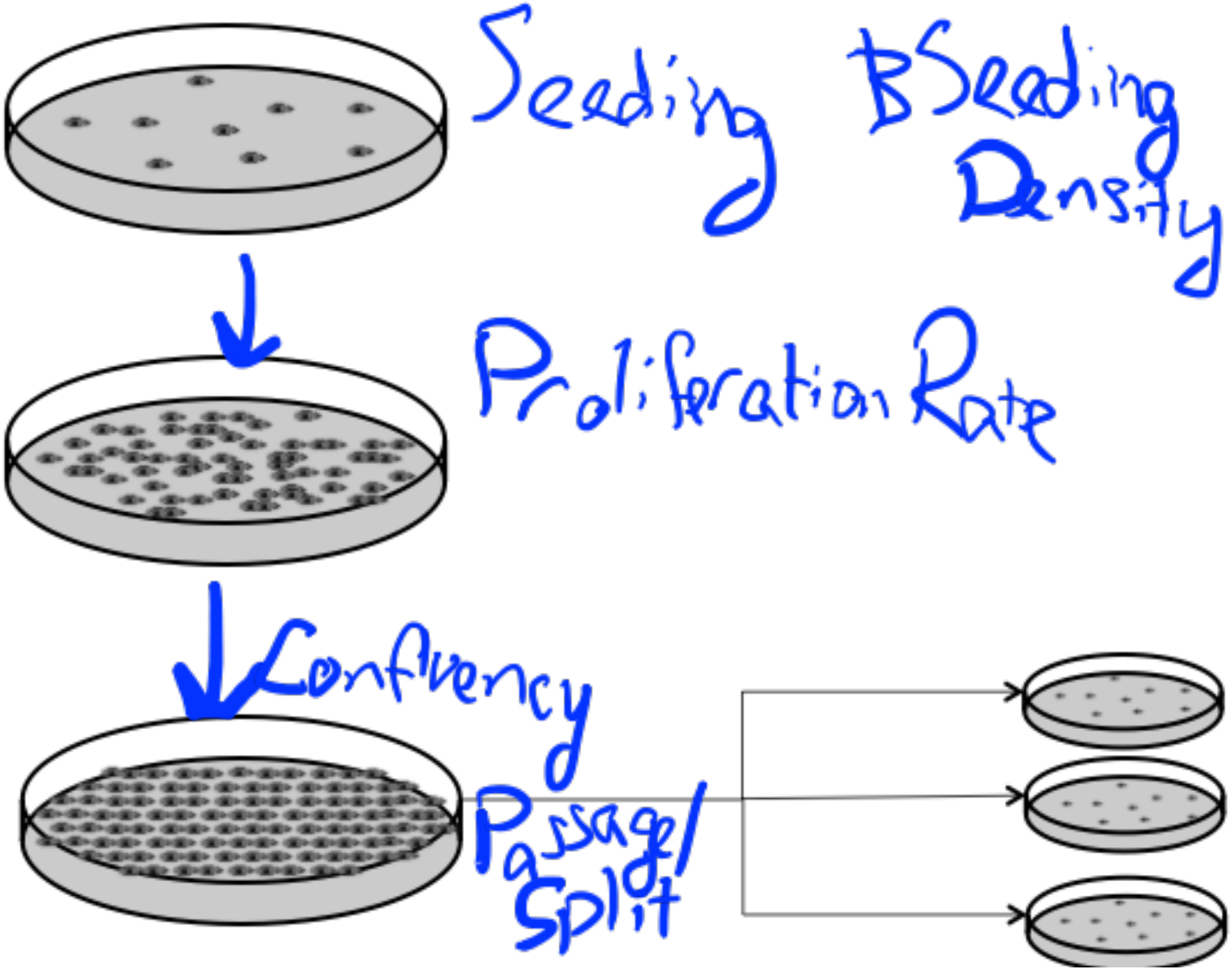
9/26/2013

M1D5



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

Tissue Culture!

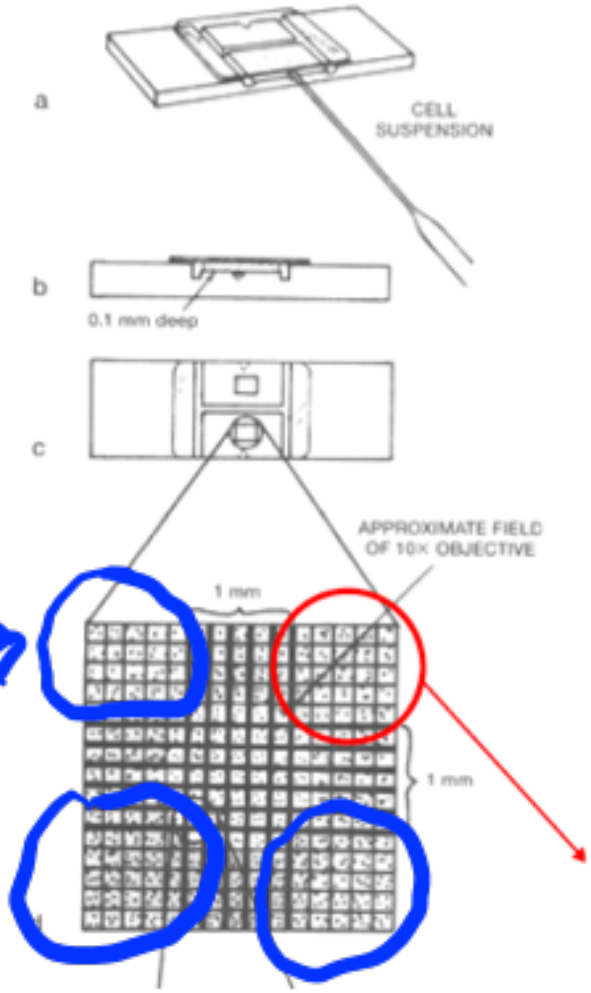


Hemocytometer!

RBC's

1:100

1:400



1 Square = 0.1μL

1. Count 4 corners
2. Avg
3. Calc Density (cells/mL)

Avg x 10^4

Review of Experiment + Controls

Experiment Condition	pCX-EGFP (5ng)	Backbone + Insert (no ligase)	Backbone + Ligase	Backbone + Insert + Ligase
Why?	Pos C.. Lots! ☺	Neg ∅	∅	Exp!
What if...	Fast -Low den. -[DB] 	-Uncut bKb -nbn Ecoli cont.	-Single digest	

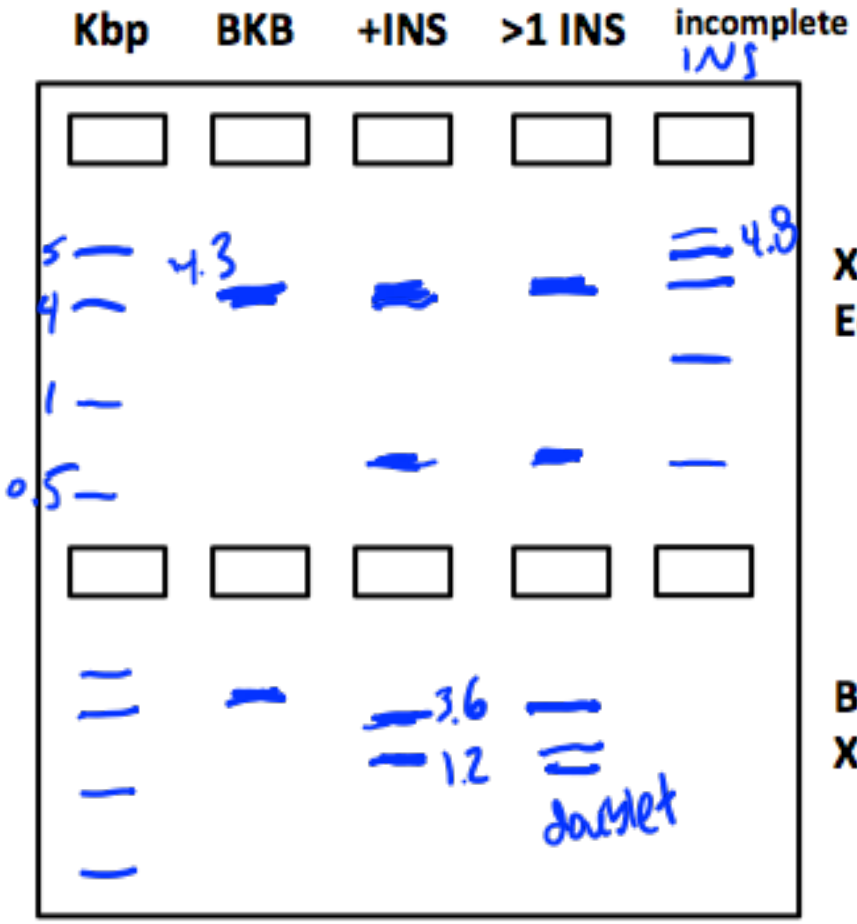
Variations in Experimental Samples

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

Extracting DNA from XL1-Blue

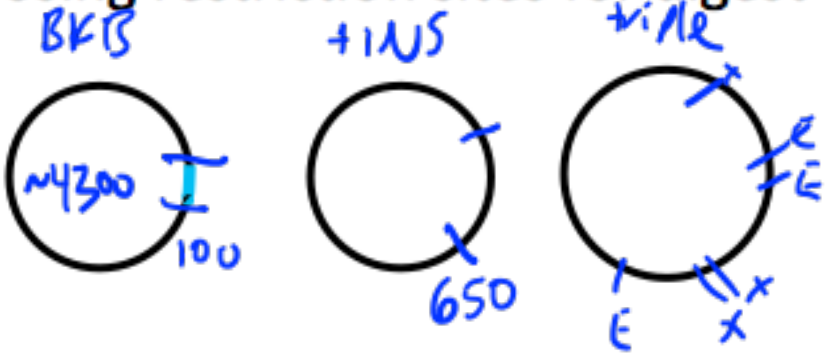
Step	Contains	Purpose
Prepare	EDTA Buffer, glucose	-weaken cell envelope - keep otherwise stable
Lyse	SDS Na^+ NaOH	- disrupt/solubilize lipid membranes, protein - denatures DS → SS DNA
Neutralize	Acetic acid/KAc	- neutralize pH, precipitate SDS  renature  genomic "crash out"
Transfer	N/A	keep Supernatant
Wash, collect	A) EtOH B) dry, water	-precipitate DNA -but EtOH interferes w/ digest

Diagnostic DNA gels

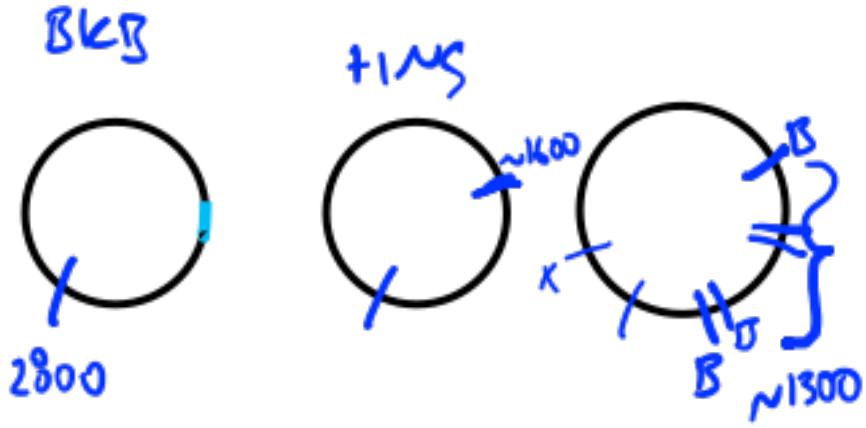


Choosing restriction sites for digest

XbaI
EcoRI



BamHI
XhoI



Today in Lab (M1D5)

- Miniprep three Δ5-EGFP candidates, and bacteria transformed with pCX-NNX
  - tip: orient tubes in centrifuge
  - pCX-NNX = control for technique
- 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