

DNA Engineering: M1D5 Lab Talk

20.109 (F12)

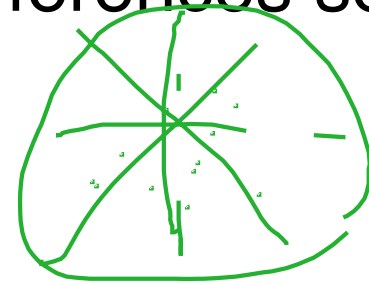
09.27.12

Team Color	5 ng pCX-EGFP	bkb+ insert, no ligase	bkb + ligase	bkb+ insert, + ligase*
RED				
ORANGE				
YELLOW				
GREEN				
BLUE				
PINK				
PURPLE				

**list each plate*

Interpreting ligation results

- Did the positive control work for many groups?
- No ligase results?
- Bkb + ligase tells you?
- What variations may be giving differences seen for experimental samples?



(bkb: insert)

(incubation time, salt in ligation
recovery NOT equal), (cells)
(Not on
re)

From cells on a plate to a tube of DNA....



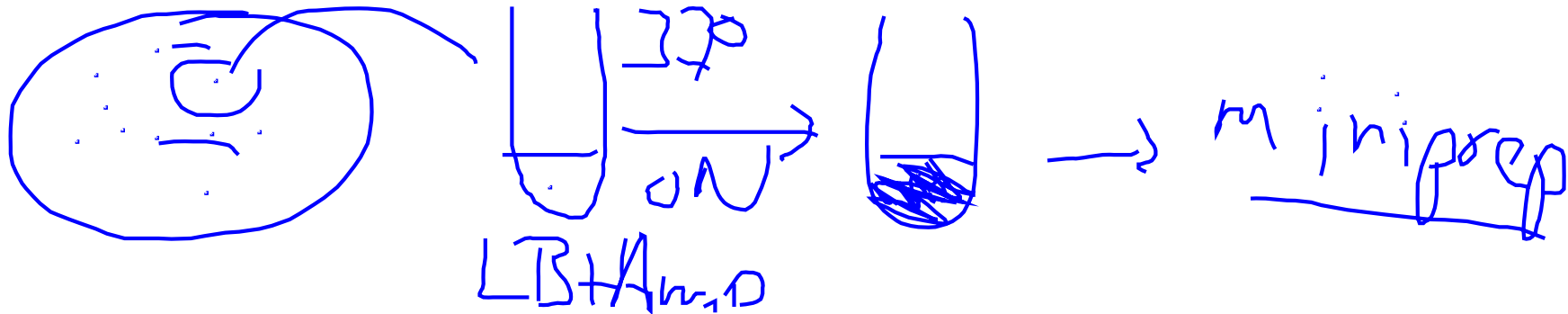
GENEWIZ, Inc.
115 Corporate Boulevard
South Plainfield, NJ 07080
p (877) GENEWIZ (436-3949)
f (908) 333-4511
www.genewiz.com

GENEWIZ DNA Preparation Report #10-206466626

Date: 9/26/2012 Customer: Natalie Kuldell
Reported by: Caraline Egan Institution: MIT

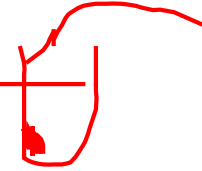
All prepped samples are in TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0)

Sample Name	Prep Size	Endo Free	Copy Number	Yield (µg)	Conc. (ng/µl)	Vol. (µl)	OD 260/280	OD 260/230	Anti-biotics	Sequencing	Glycerol stock
pBNAN	Maxi	No	Medium	518.5	1037	500	1.94	2.34	Amp	No	No



Isolating DNA from bacteria: DNA Miniprep

- Harvest cells + aspirate media



- Resuspend them

Soln I Tris buffer
EDTA

- Lyse them + raise pH

Soln II

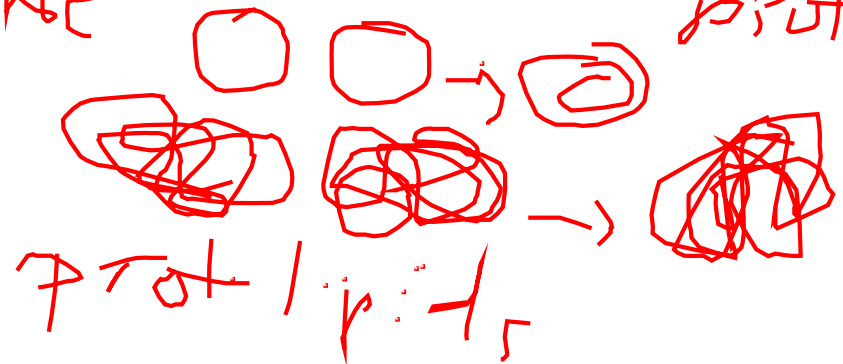
NaOH - denature DNA

- Restore pH

SDS - dissolve lipids
KAc - precipitate

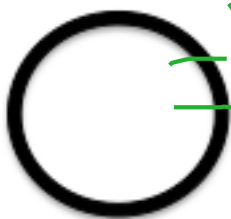
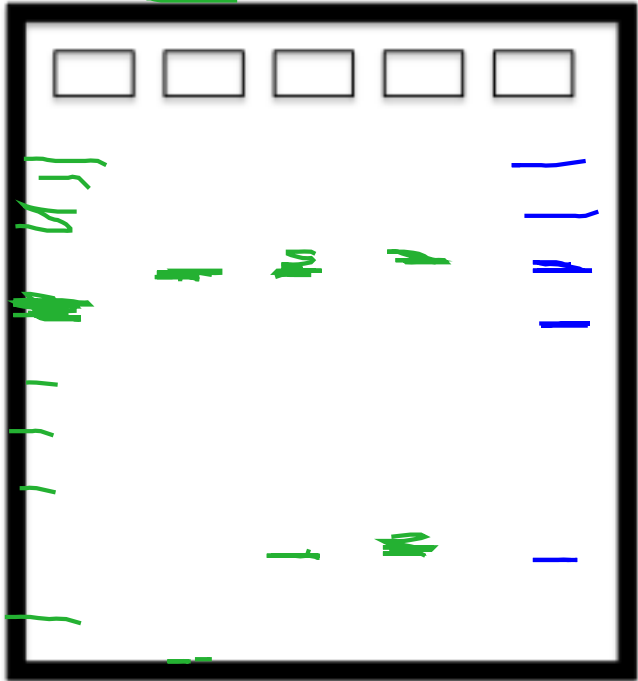
Soln III

- Concentrate + wash

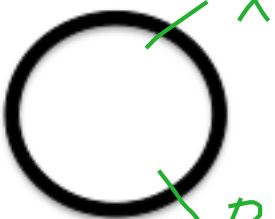


Diagnostic Digests: XbaI and RI?

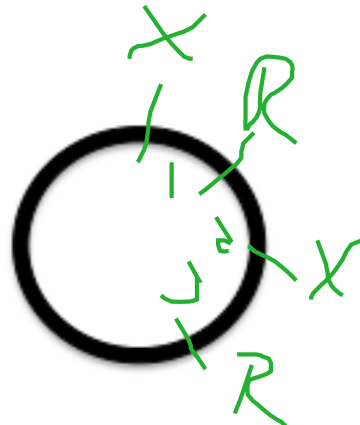
M - T + partial



- insert



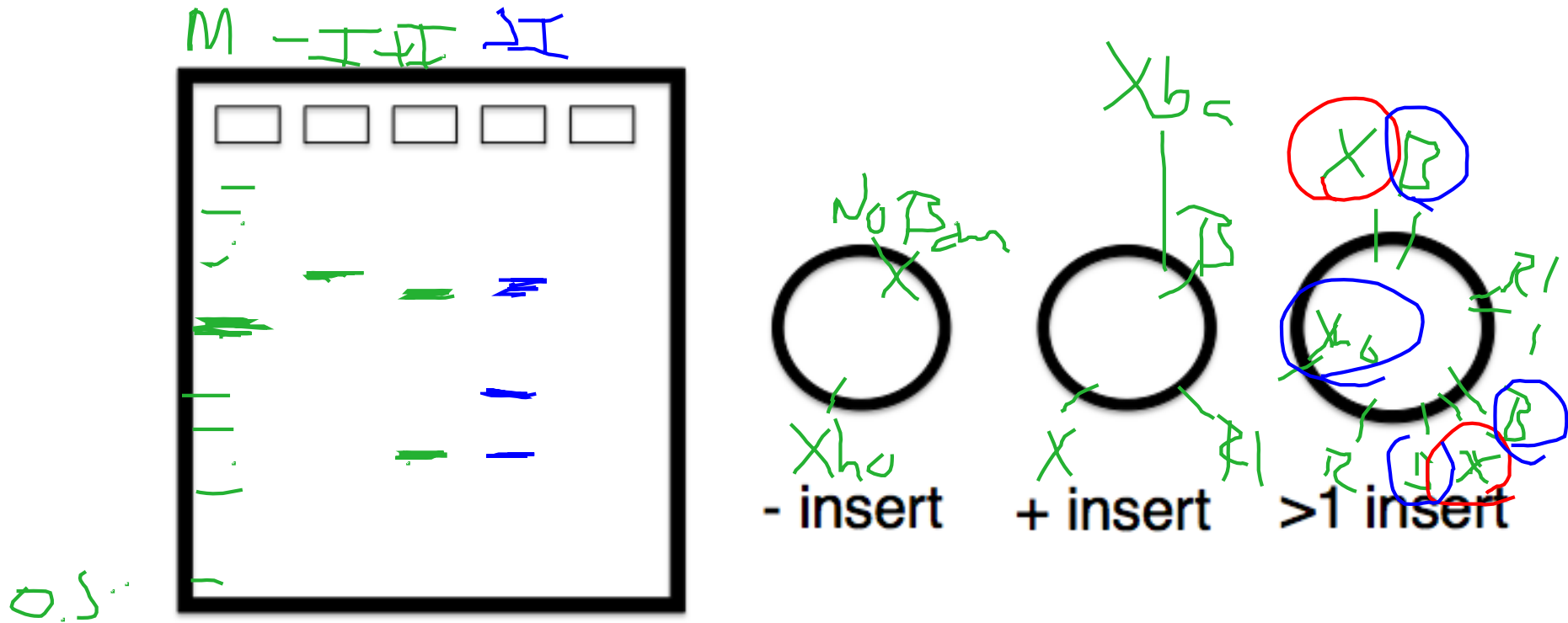
+ insert



>1 insert

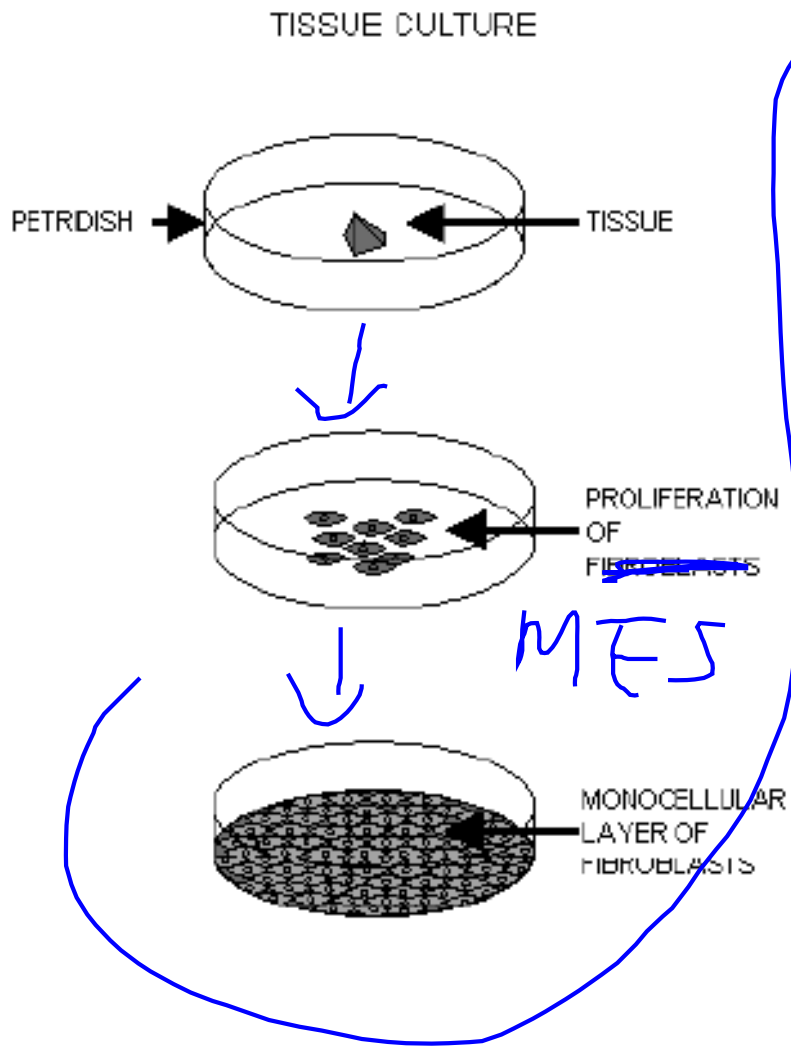
pCX-NAX

Diagnostic Digests: BamHI and XhoI?



Also consider: compatible buffers, reaction temperatures, results if one enzyme doesn't cut

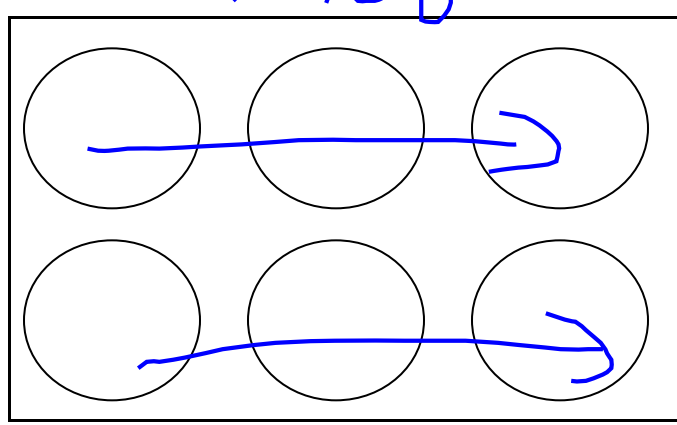
Also today: TC for fun....



split / passage

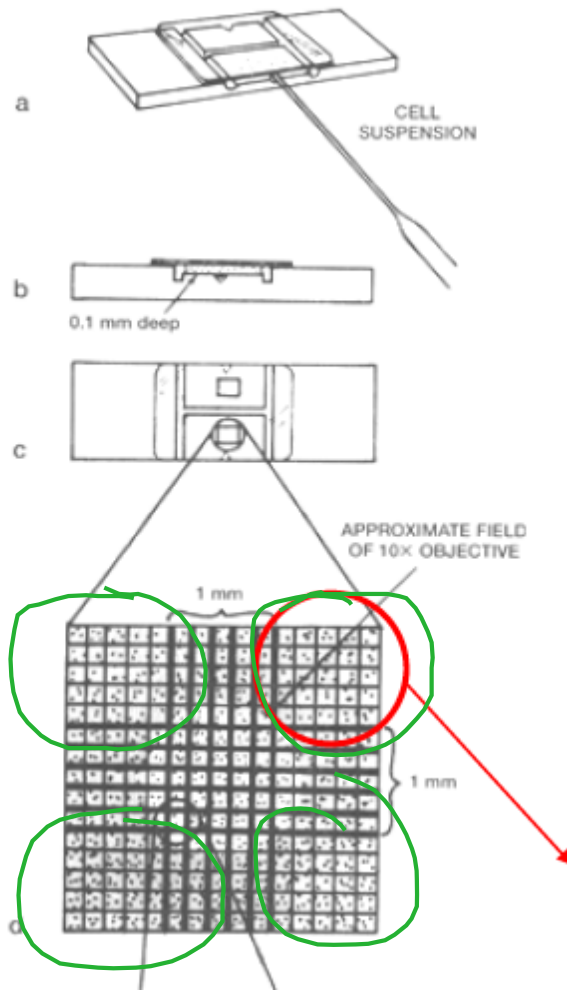
trypsin

1:100



1:400

Figuring out how many cells you have

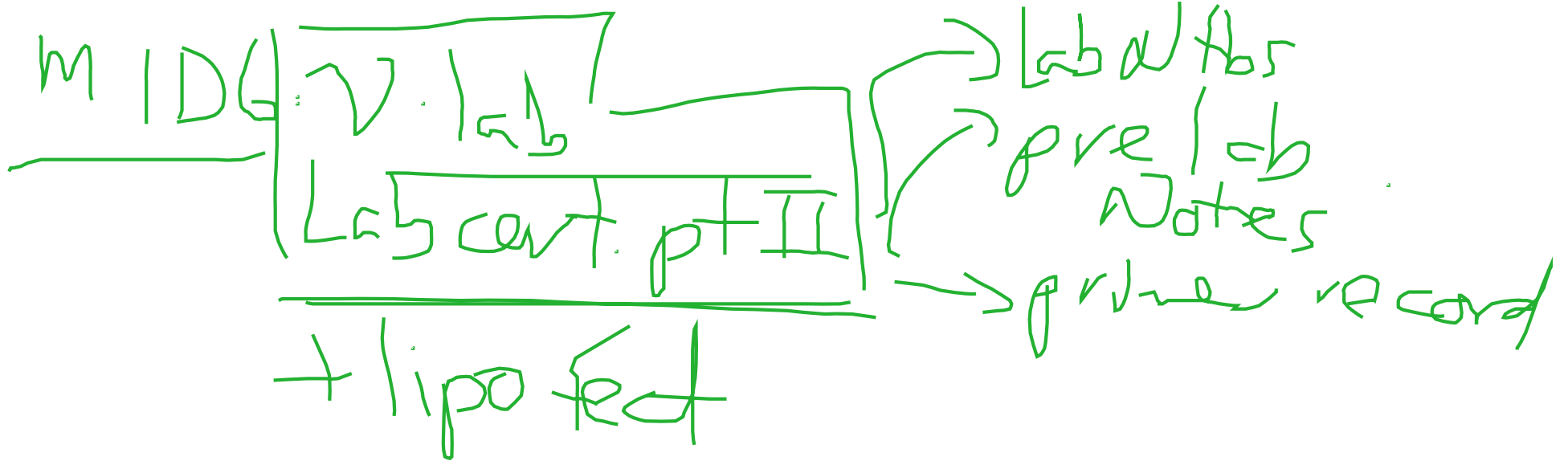


Count cells in
4 + 4 corners

Avg $\times 10,000$

cells / ml

Mod 1: what's left?



MID2: FACS

10.11.12

11A

Team Color	5 ng pCX-EGFP	bkb+ insert, no ligase	bkb + ligase	bkb+ insert, + ligase*
RED	2500	20	50	600, 1640
ORANGE	1920	11	22	69, 91
YELLOW	4464	7	113	360, 320
GREEN	5152	12	1096	1640, 2032
BLUE	3660	6	466	138, 256
PINK	2192	128	4144	3152, 308
PURPLE	6480	11	252	1008, 876

*list each plate

✓

✓

✓

✓

✓

✓