

DNA Engineering: M1D5 Lab Talk

20.109 (F11)

09.29.11

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?

Cells not on ice

Loss of DAA

Ratio Bkb: insert

Isolating DNA from bacteria: DNA Miniprep

- Harvest cells+ aspirate media



- Resuspend them

Soln I

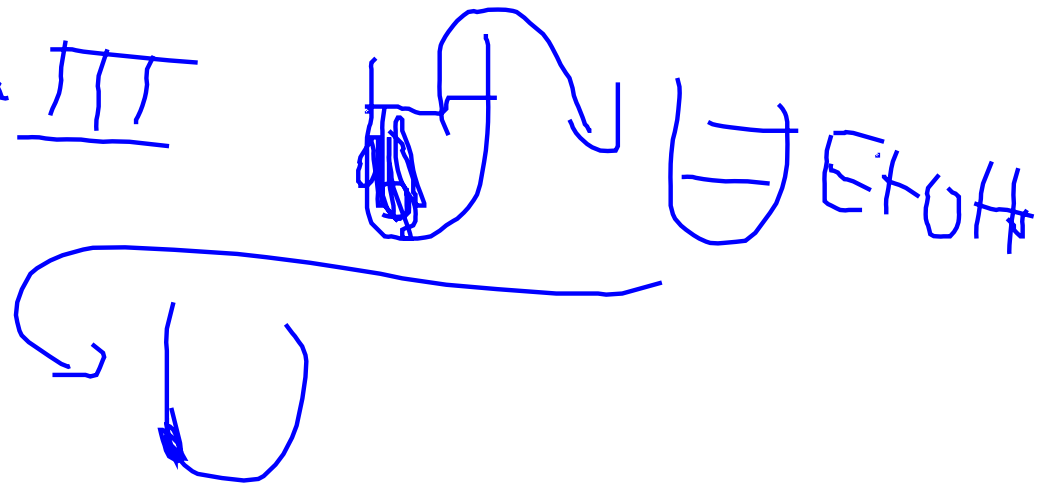
- Lyse them + raise pH

Soln II NaOH
SDS

- Restore pH

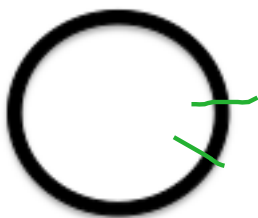
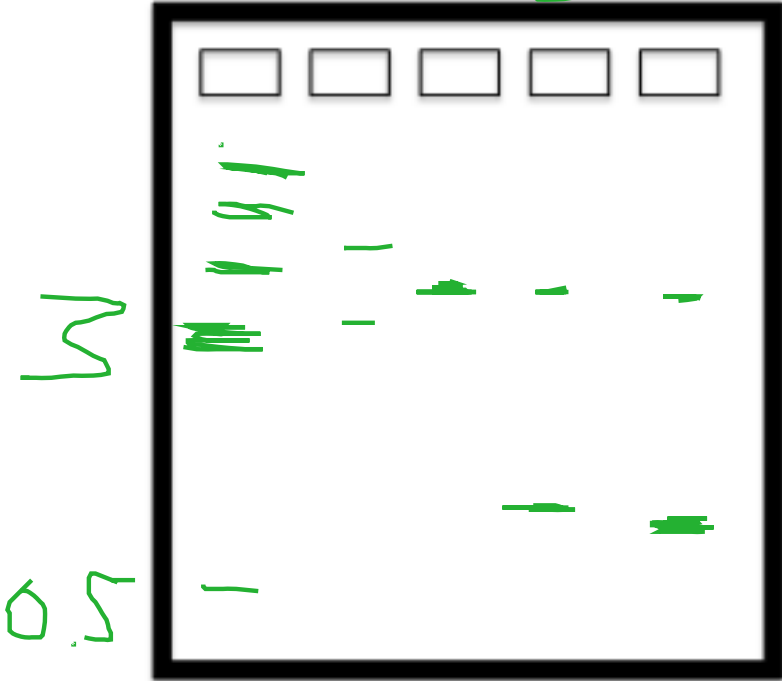
Soln III

- Concentrate + wash

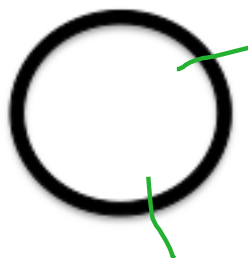


Diagnostic Digests: XbaI and RI?

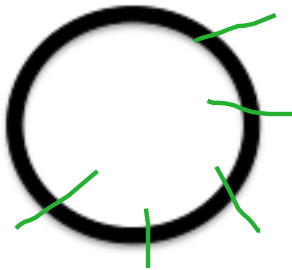
M 0 (-) (+) >1



- insert

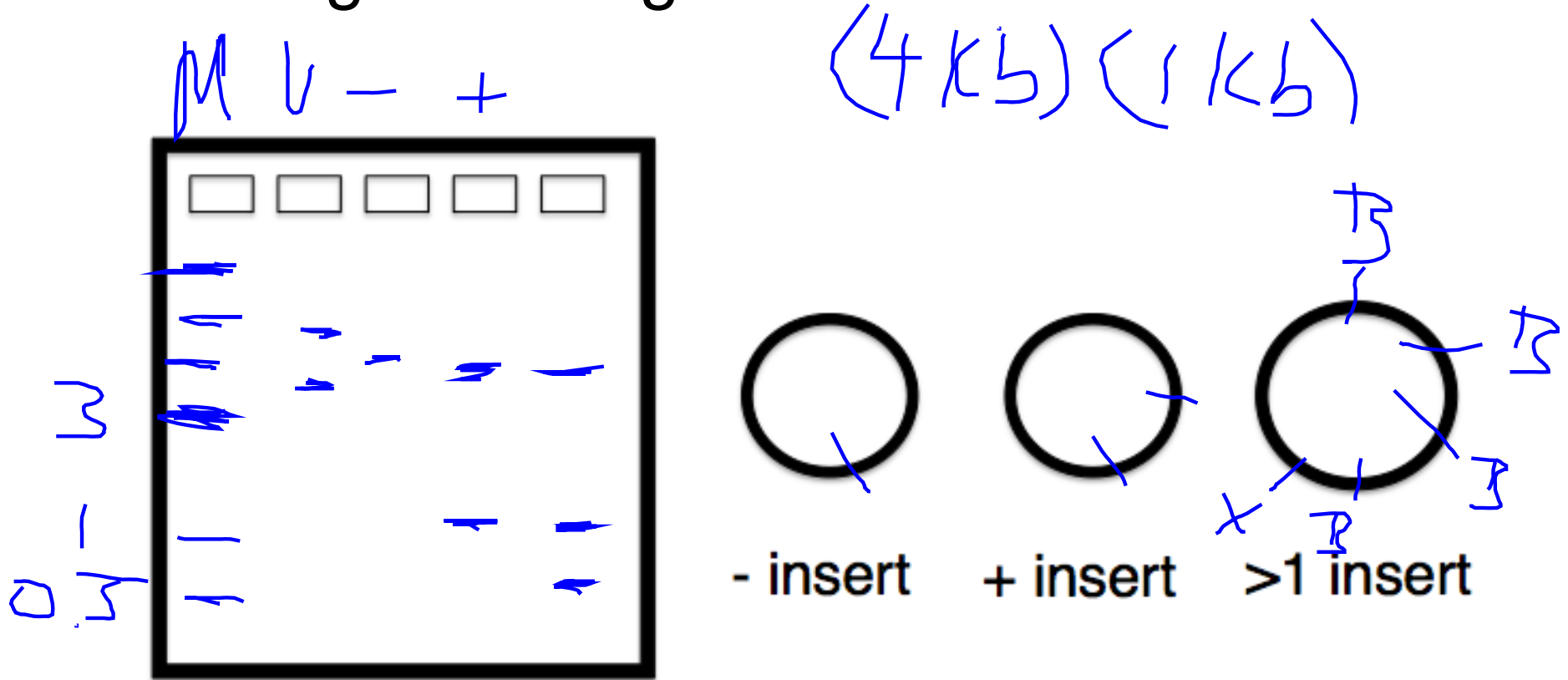


+ insert



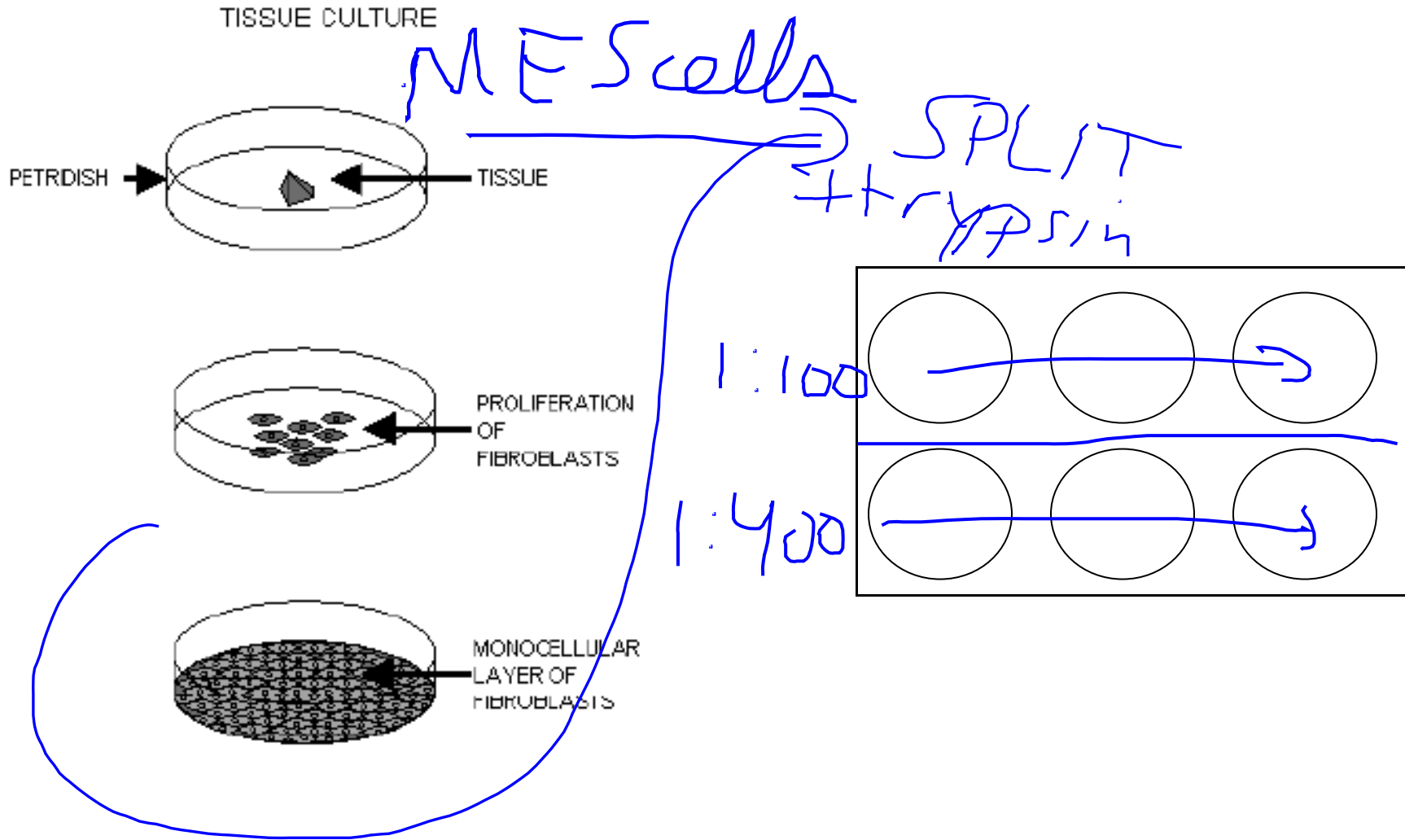
>1 insert

Diagnostic Digests: BamHI and XhoI?

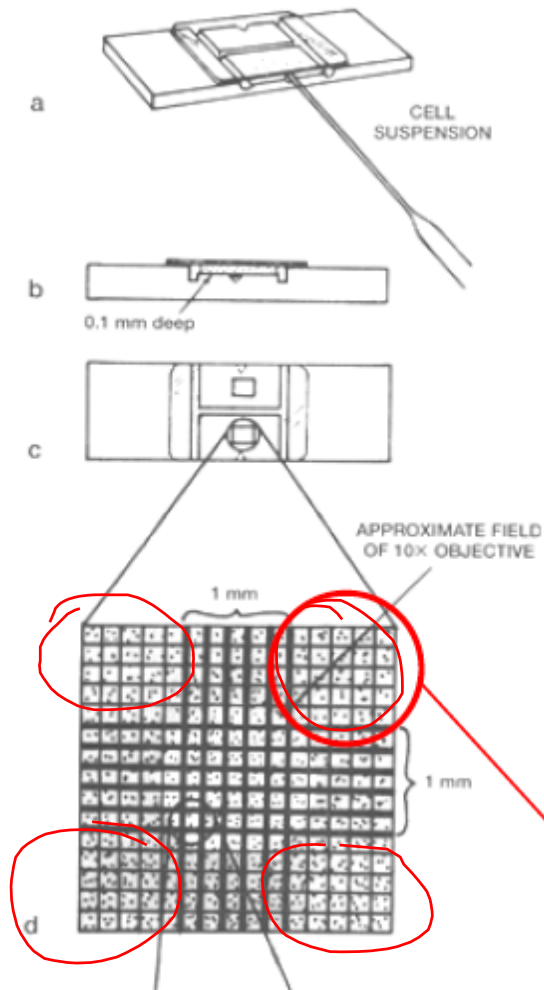


Also consider: compatible buffers, reaction temperatures, results if one enzyme doesn't cut

Also today: TC for fun....



Figuring out how many cells you have



Count #
cells in
4x4 corners

Avg x 10,000
cells/ml

Mod 1: what's left?

T Lipofect + L.P.

R FACS

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Team Color	5 ng pCX-EGFP	bkb+ insert, no ligase	bkb + ligase	bkb+ insert, + ligase*
RED	~2296	0	8	49,43
ORANGE	~884	0	0	2,0
YELLOW	~4200	0	12	4,0
GREEN	~1200	0	0	0,1
BLUE	~5800	1	4	172,166
PINK	~3072	1	7	183,221
PURPLE	0	40	20	1,1

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*list each plate