- Announcements
- Lab Quiz
- Pre-lab Lecture
  - DNA extraction (miniprep)
  - Diagnostic gel review
  - Intro to tissue culture
  - Today in Lab: M1D5

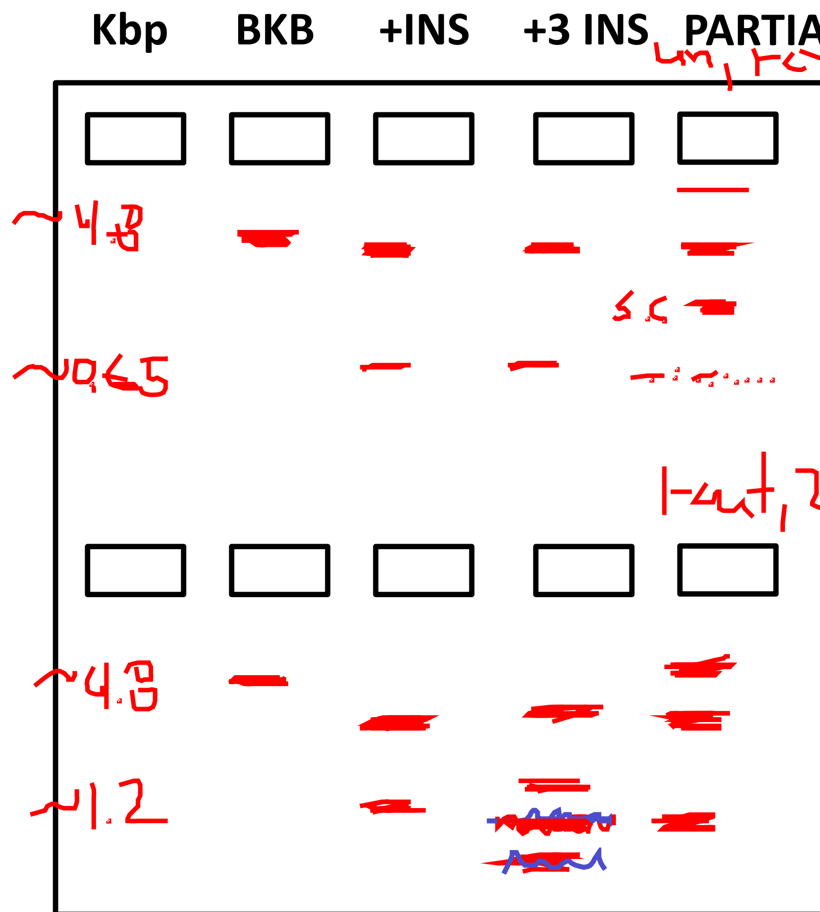
Announcements

- Next time: lab practical (and oral defense for some)
  - can bring laboratory notebook – including Day 1 primer design printout
  - *change*: CAN bring pre-lab notes also
- OH next week: Mon 12:30-1:30, Tue 4-5 pm
- Vacuum aspirators contain bleach for biohazardous waste (i.e., cells)
  - after bleach treatment, these go down the sink
- Chemical waste and sink-safe chemicals (w/out cells) should NOT be aspirated
  - the former is a safety risk, the latter just a hassle

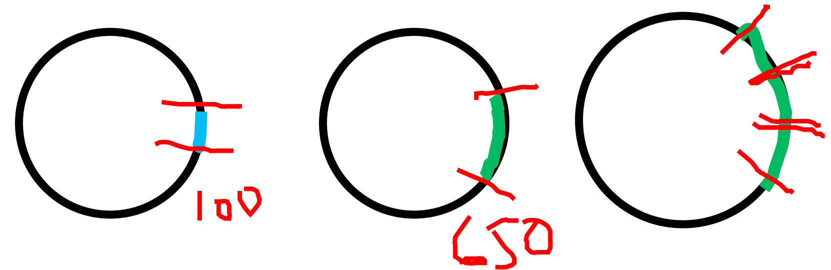
Extracting DNA from XL1-Blue

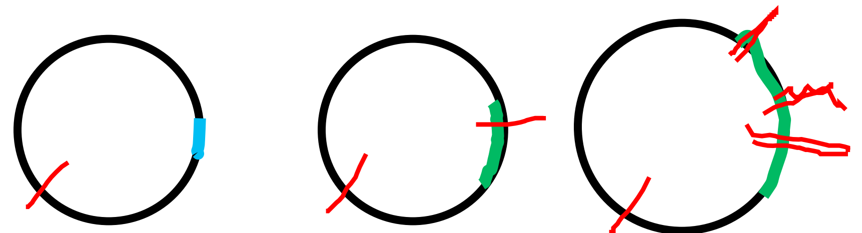
Step	Contains	Purpose
Prepare	EDTA Buffer, glucose	– weakens cell envelope – keep otherwise stable
Lyse	SDS Na^+ NaOH	–disrupt/solubilize lipid membranes, proteins – denatures ds → ss DNA
Neutralize	Acetic acid/KAc	neutralize pH; precipitate SDS renature genomic DNA crashes out
Transfer	N/A	use supernatant
Wash, collect	A) EtOH B) dry, water	–precipitates DNA – EtOH interferes w/ digest

Diagnostic DNA gels



Choosing restriction sites for digest





Practice tissue culture (TC)

- MES = murine embryonic stem cells
- Adherent cells
- Add trypsin to remove from dish
- Re-plate at lower density

  → "passage" cells
- Practice counting
- More about mammalian cells next time (review)

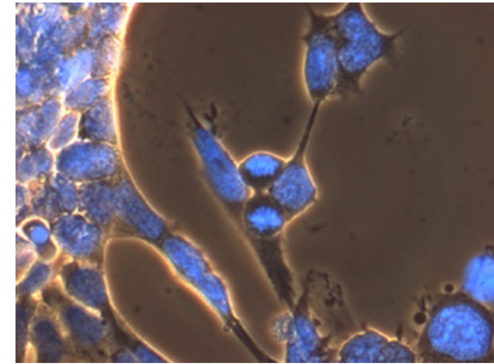


Image from http://www.stemcellresources.org/library_images.html

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 (all together)
  - don't need notebook, just a piece of scrap paper

Interpreting your ligation results

Consider…

- Why might some groups not have gotten many exptl colonies?
- What does the *no ligase* vs. the *no inse*rt sample control for? Which one do you expect to have more colonies?
- How big is the variation for identical samples, and what are the possible sources of error causing the variation?