- Announcements, Review HW
- Lab Quiz
- Pre-lab Lecture
  - DNA Extraction (Miniprep)
  - Diagnostic Gel Review
  - Intro to Tissue Culture
  - Figure Captions (or at OH)
  - Today in Lab: M1D5

Results of pre-lab interaction survey

- Mostly #3 (hate talking in class), couple of straight up #1 (too easy) and #2 (too hard)
- Options/thoughts:
  - If the questions are fine, I could call on people instead of wait for volunteers… can be awkward though!
  - Alternatively you could talk in groups of four and then have one person speak for the group.
  - I will ask for others to speak up if only 1-2 people are talking; you don't need to self-censor.
  - Hearing things put another way (from a peer) can be very helpful to peoples' learning.
  - Explaining things yourself, out loud, is one of the best ways for *you* to learn!

Couple of HW notes

- PCR update
  - universal ORF finding
  - *minimum* primer length
  - reaction and cycling rules of thumb
- Figures/captions
  - need better labeling and full captions
  - will discuss in pre-lab (if time) or Monday OH

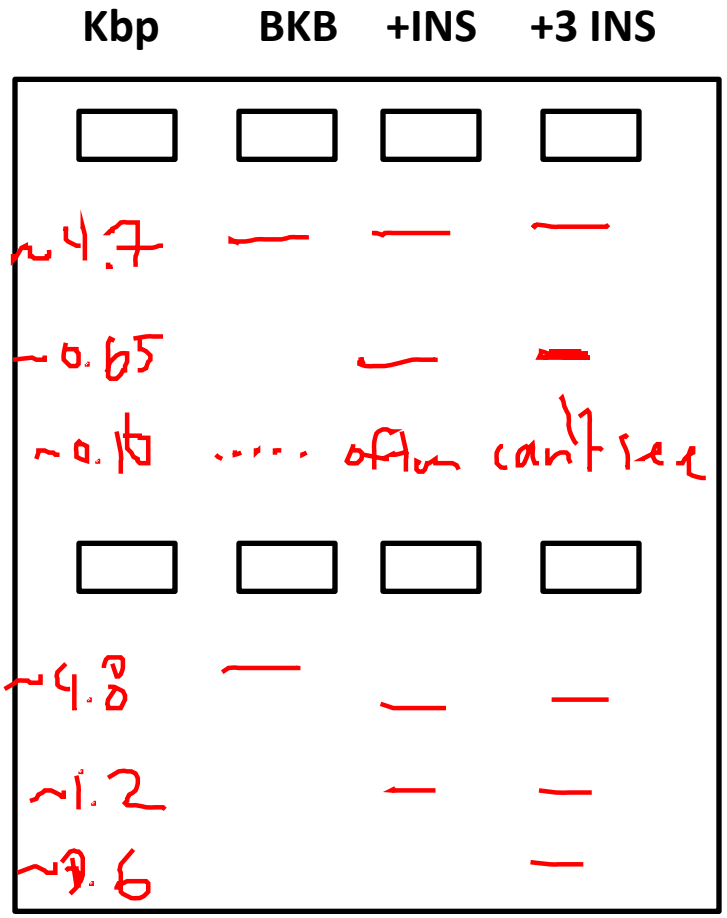
Announcements

- Lab practical next time! HW returned Monday.
- Please post your colony counts in *talk* page table before leaving – we'll discuss them next time
- 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

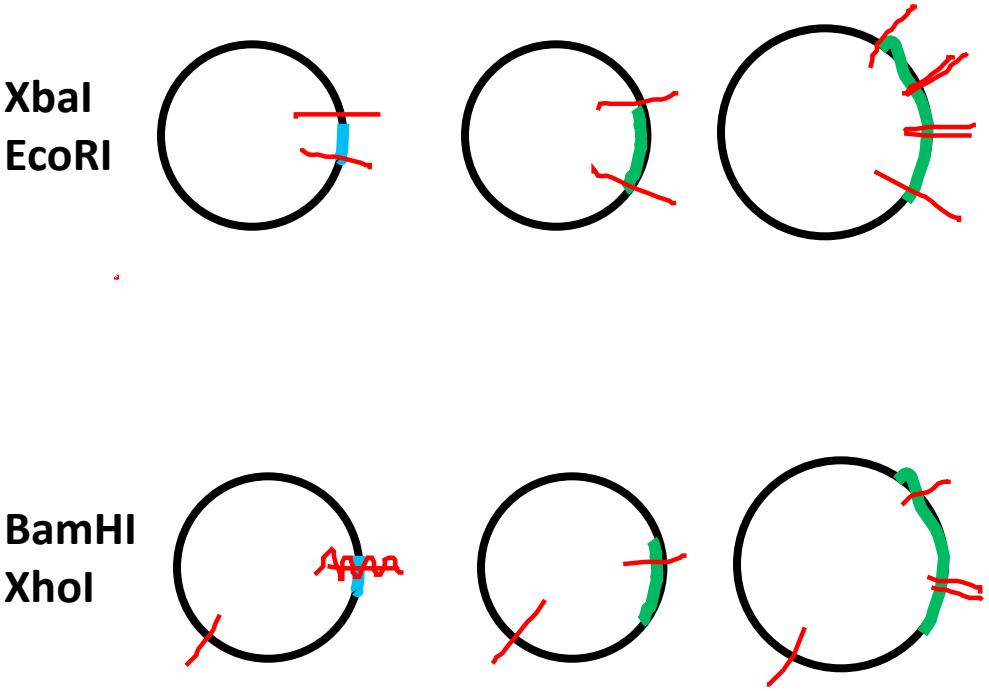
Extracting DNA from XL1-Blue

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

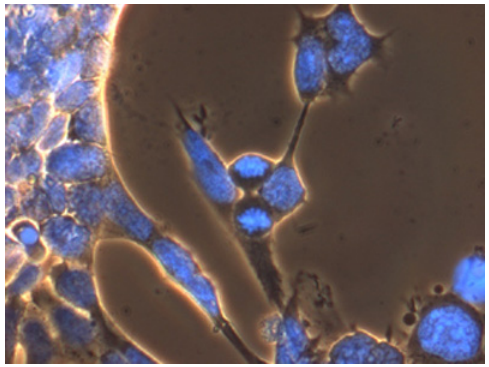


Image from http://www.stemcellresources.org/library_images.html

Figures: Style and Scope

- Title: concise, informative, tells overall goal/result
- Caption: gives context for result from big → small
  - Introduce what we are looking at
  - Include just enough methods to understand result
  - Define all elements (e.g., DNA ladder)
  - Cover primarily facts, not interpretation

    e.g., observed and/or expected sizes
- Aesthetics: simplicity, clarity → at-a-glance

  labeling (e.g., some ladder band sizes)

Figures: Example

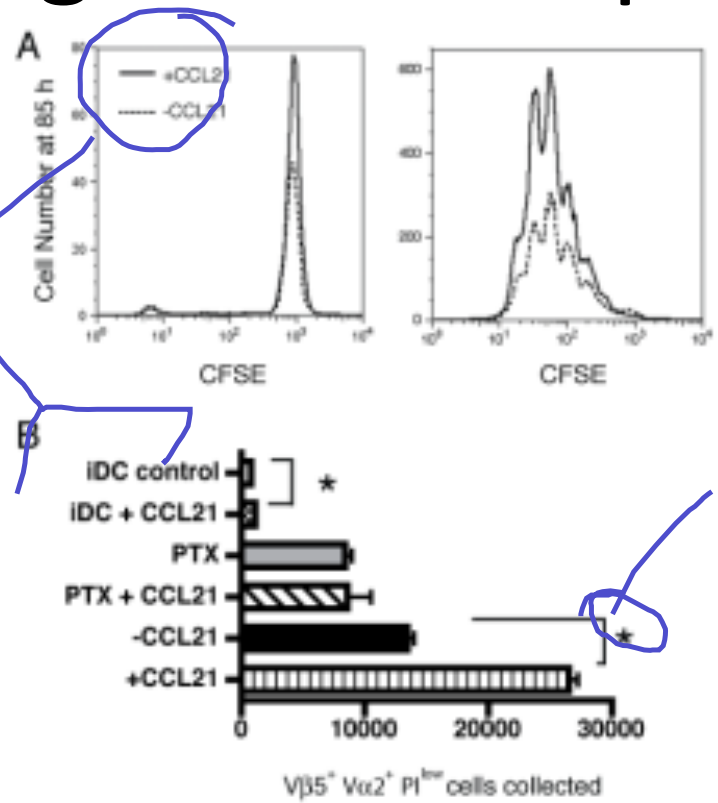
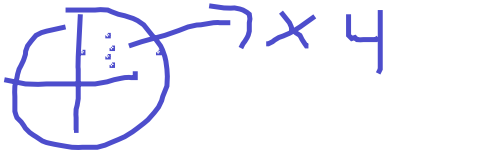


Figure 3 **CCL21 impacts naïve T cell proliferation under conditions of rare Ag-specific T-DC encounters.** Co-cultures comprising 9% OVA-specific OT-II CD4+ T cells, 81% C57Bl/6 CD4+ T cells, 5% OVA-mDC and 5% iDC with/without CCL21 were analyzed by flow cytometry at 85 h. (A) Sample CFSE histograms are shown for control (left, iDC only) and experimental (right, with OVA-mDC) conditions. (B) OTII cell recovery for all conditions is shown. Ave ± std. dev. for 3 wells per condition. [* indicates bracketed conditions statistically different ($p \leq 0.05$)] (A-B) are from 1 representative of 5 experiments.

Today in Lab

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



- Count and post colony #s
- TC practice session (half of class at a time)
  - don't need notebook, just a piece of scrap paper
- Set up digests
  - we will add loading dye if lab runs late
  - tip: make reaction cocktail → efficiency