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

Announcements

- Common issues on quizzes
- Vacuum aspirators have bleach for biohazardous waste (i.e., cells) → eventually ends up in **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
  - *do* aspirate LB taken right off of the cells
- Heads up: travel 10/3-10/6. OH options:
  - Skype on Sat 10/4 (agi.ns)
  - office phone (617.324.1940) forwards to my cell
  - email any time

* Noreen has Mon AM office hours

10/5=wedding, 10/6=travel
* in SF, so Δt=3hr.

Extracting DNA from XL1-Blue

- pellet cells + aspirate LB

Step	Contains	Purpose
Prepare	EDTA Buffer, glucose	→ weaken cell envelope → keep otherwise stable
Lyse	SDS Na^+ ⌒ NaOH	→ disrupt/solubilize lipid membranes and protein → denatures DS → SS DNA
Neutralize	Acetic acid/KAc	→ neutralize, precipitate SDS 1) ○ ⇝ ◎ renature 2) genomic "crashes out" w/ SDS
Transfer	N/A	Keep supernatant
Wash, collect	A) EtOH B) dry, water	→ precipitates DNA → but EtOH interferes w/ digest, etc.

Tissue culture (TC) medium

↳ environment (37°C, etc.)

- What do cells need to survive?

Food: energy source
- glucose; L-glutamine; (sodium pyruvate)

building blocks or co-factors for cell rxns.
- essential amino acids
- non-essential amino acids → separate usually
- lipids, vitamins, minerals

pro-life and other signals (e.g., growth factors)
- cytokines

(←) serum ⇒ separately (~ blood)

Non-food: Antibiotics (Pen/Strep)
phenol red (track pH)

Passaging adherent cells

↳ "splitting" (or "sub-culturing")

- MES = murine embryonic stem cells
- Rinse w/PBS
  - media has trypsin inhibitors
- Add trypsin to remove from dish
  - proteolytic enzyme → breaks cell-matrix contacts
- Re-plate at lower density
  - why? contact inhibition and/or differentiation and/or nutrient & O_2 competition

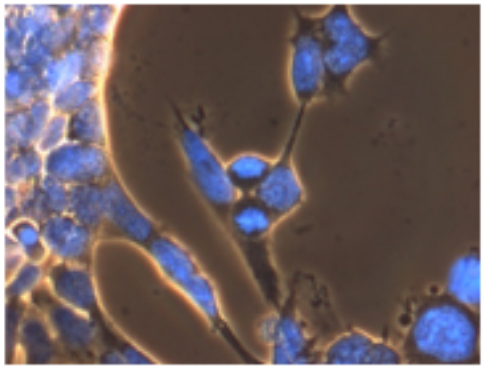
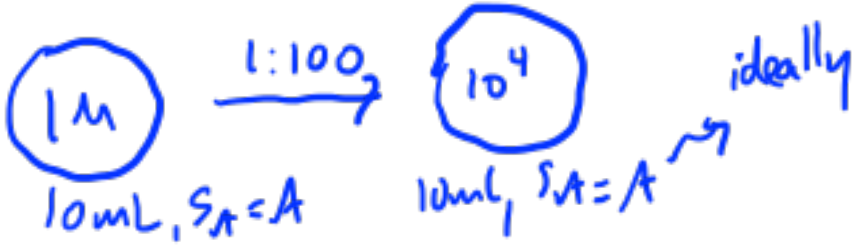


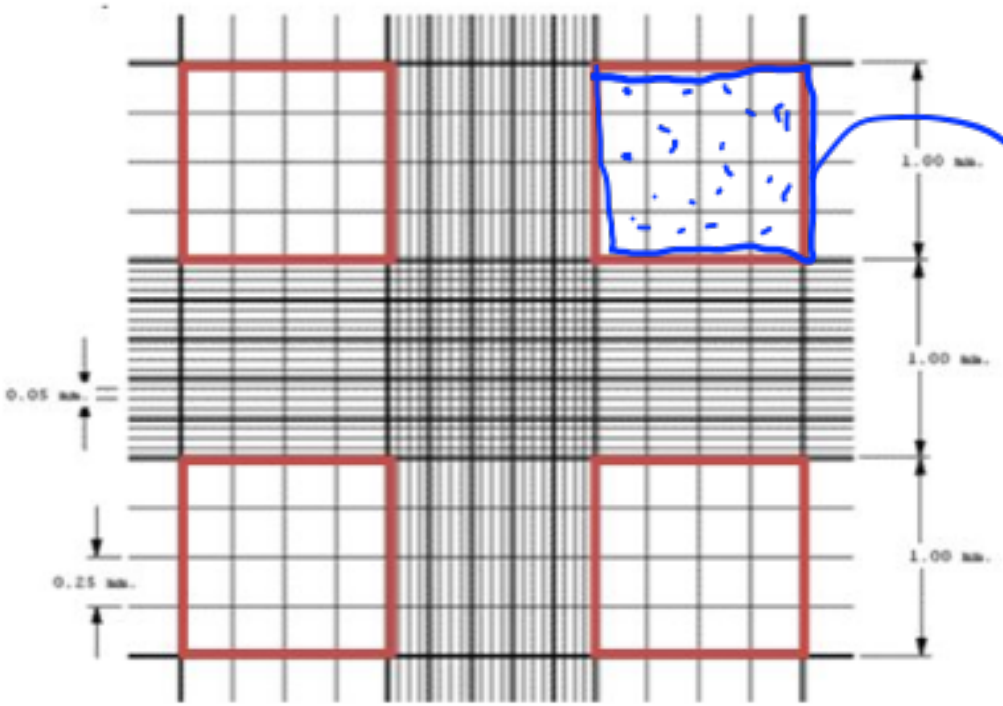
Image from http://www.stemcellresources.org/library_images.html

"confluent" (x 70)



Final steps: counting and plating cells

Hemocytometer



- in general: be able to plate a known amount

cells x 10,000 = cells/mL (+ any dilution factor)

average corners
- clusters
- avoid dbl count

Hemocytometer image: www.allcells.com/blog/how-to-count-fresh-primary-cells/
Handy well plate image from (thanks, Shannon!): www.cellsignet.com/media/templ.html

Today in Lab (M1D5)

- Let's all start with TC practice session
  - don't need notebook, just a piece of scrap paper
- Miniprep **3** Δ5-EGFP candidates, and **1** pCX-NNX
  - tip: orient tubes in centrifuge
  - pCX-NNX = control for miniprep technique
- Set up digests to evaluate candidate clones
  - pick **2** candidates, based on [DNA] and purity; + **1** pCX-NNX
  - tip: make reaction cocktail → efficiency
  - add loading dye next time
- Count and post colony #s on M1D5 *Talk* page
  - we will discuss next time

260 → [ ]
280 → purity