- Announcements, Review HW
- Lab Quiz
- Pre-lab Lecture
  - Writing a Figure caption
  - DNA Extraction (Miniprep)
  - Diagnostic Gel Review
  - Safety + Technical Tips

Announcements, old HW

- Can one group come to Tue Oct. 28 class? yellow
- Today at 3 pm, presentation on biosafety
- Your lab report is due in one week
  - OH (room TBA) on: Sun 7:30-9 pm
    Tue 4-5 pm
- Please post your colony counts in *talk* page table before leaving – we'll discuss them next time

* show me your process, from raw data

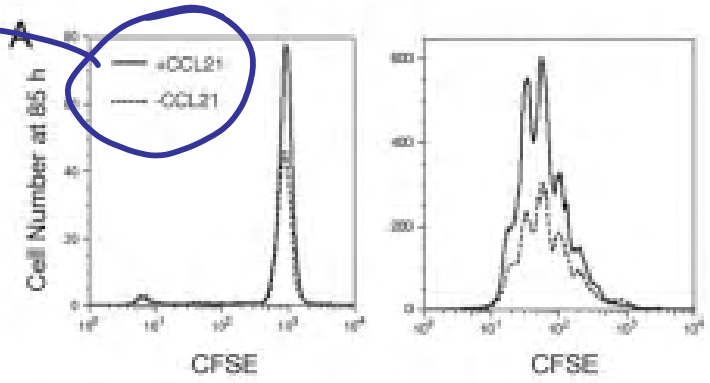
ladder = standards = known DNA lengths (bp)

Figures: Style and Scope

- Title: concise, informative give overall result/goal.
  - often similar to that Results sub-section heading
- Caption: give context for result, from big to small.
  - Introduce what we are looking at.
  - Include only as much Methods as needed to understand result.
  - Define all elements (e.g., DNA ladder)
  - Cover primarily facts (results or expectations), limiting more complex interpretations → go in Discussion
- Aesthetics simplicity, clarity
  - → at-a-glance labeling when possible

Figures: Example

at-a-glance labeling



* tell a story *

title gives result

(w/out over-interpreting)

defined in caption

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.

} overview of exp.

} details of results

Figure Captions: Practice

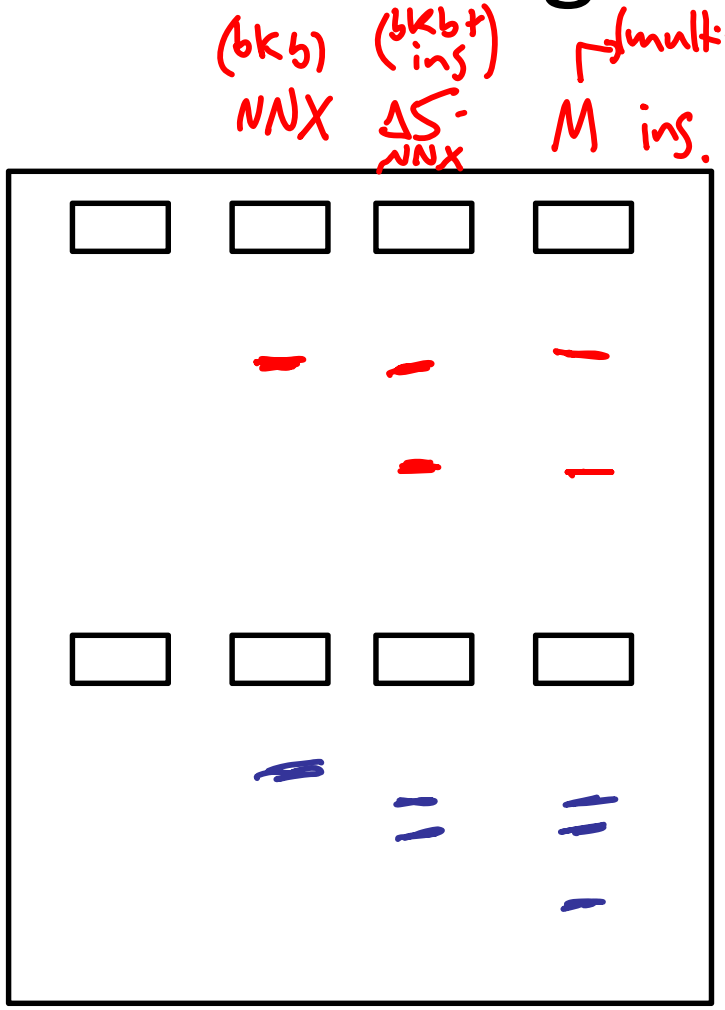
Fig. 3 **Fig. N functional title** descriptive caption

- Agarose gel title ideas: restriction digest, gel analysis of λ Restriction digest to {: confirm and extract} DNA fragments for cloning
- Overview sentence topics: define pCX-NNX, D32GFP (eV/ expected sizes)
- Supporting detail topics: all observed sizes = results and what they mean, label reference bands in ladder
- Methods to include or not: ~~45 min.~~ ~~12 V~~ (1% agarose) volume ~~of loading~~ dye

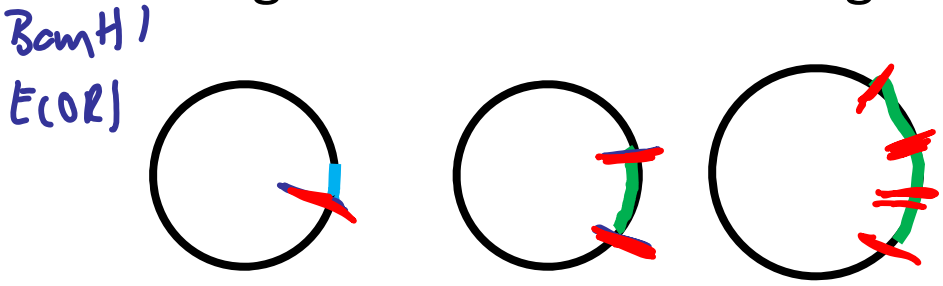
DNA Extraction from Bacteria

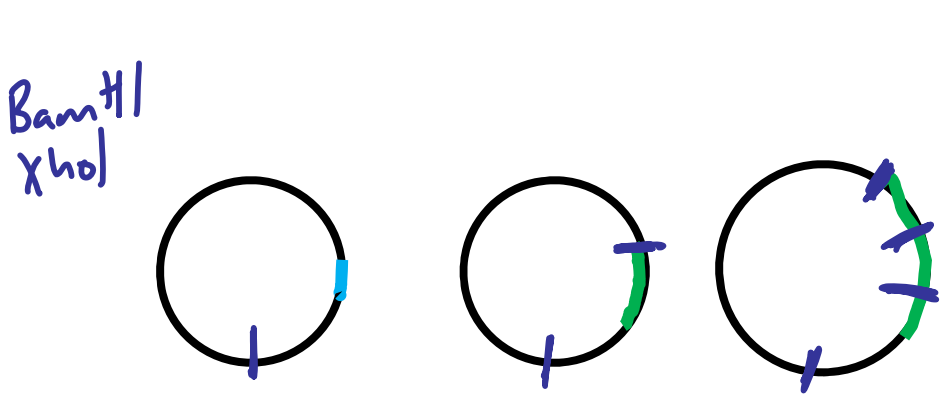
Step	Contains	Purpose
Soln. I	EDTA buffer, glucose	→ weaken cell envelope → otherwise stable
Soln. II	SDS mNa^+ NaOH	→ disrupt membrane, solubilize lipids/proteins → ds DNA → ss DNA
Soln. III	acetic acid/ KAc.	→ neutralize pH, precipitates SDS ↙ genomic DNA "crash" ↘ plasmid renature
Transfer	N/A purpose: isolate plasmid	
Final steps	EtOH, H_2O, drying	→ precipitating DNA, washing away salts, etc. → ethanol would interfere w/ digest

Diagnostic DNA Gels



Choosing restriction sites for digest





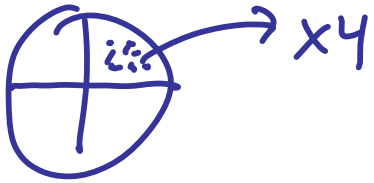
* choose one site uniquely on insert, one on backbone
* consider what data would like look like if

Today in Lab

- Miniprep three Δ5-EGFP candidates, and bacteria transformed with pCX-NNX

control for your technique

- Count and post colony #s



- Visit from Rhonda O'Keefe, EHS 3pm
- Set up digests
  - We will add loading dye if lab runs late