- Announcements → AP - 20109
  UG research session 7:30
  HW: don't omit or add to DNA product
- Lab Quiz
- Set up gels to run 45 min
- Pre-lab Lecture
  - ❖ Progress report comments
  - ❖ Making a figure/caption
  - ❖ DNA Electrophoresis
  - ❖ DNA Ligation, part 1

Progress report comments

- Progress report should be concise and complete
  - Space-wise, avoid lists/tables when a sentence will do
  - Sentence-wise, avoid extra (or confusing) words
  - Content-wise, cover what's needed (and only that) to understand and replicate your exp.
- Use introductory sentences wisely (goal of exp)
- About expt'l methods:
  - concentrations are more useful than volumes
  - or you can state masses/moles, plus total volume

Comments from Natalie

- Consider motivation for PhD interim report
  - What is your purpose?
  - Who is your audience?
- Use passive voice
  - Emphasize substantive content, not procedure
- Avoid (or concisely explain) lab jargon
- Don't write a recipe or a diary
- Use past tense
- See also her slides on the wiki for examples!

Writing exercises

- How can I more elegantly express "We started our primer by writing the landing sequence, which annealed to EGFP after 32 amino acids, then added XbaI on the left side of it."

  The 5' primer was designed to anneal to the 97th-107th base pairs of EGFP, and with an XbaI restriction site upstream of the coding sequence.

- How can I more concisely express "1 mL of protein (1 g/mL concentration) in 45 mL of water and 5 mL of 10X buffer B"?

  1g protein in 50mL (aqueous) buffer π

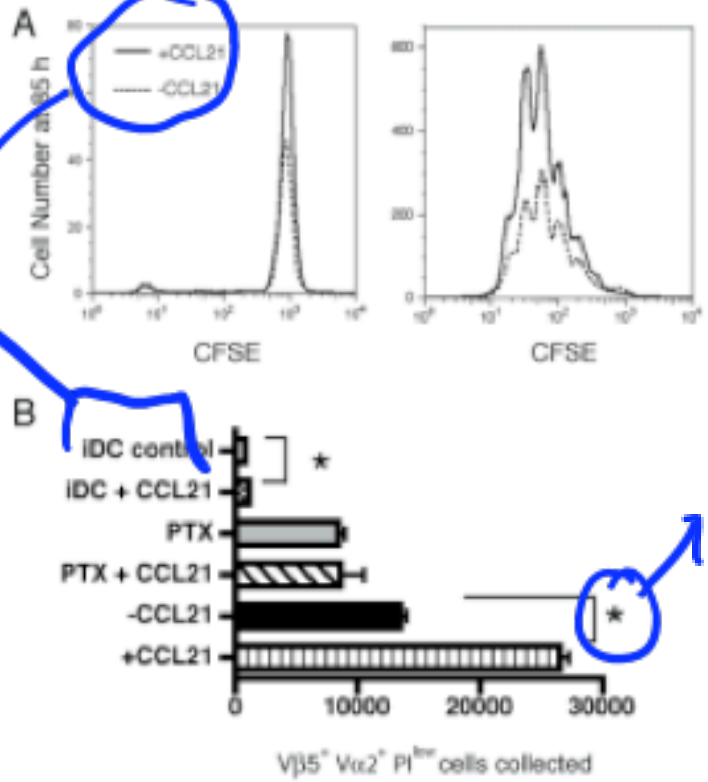
  2% protein in buffer B

  .02 g/mL = 20mg/mL

Figures: Style and Scope

- Title: concise, informative → gives overall exp. goal (or result). (mark in bold)
- Caption: gives context for result, from big to small
  - Introduce what we are looking at
  - Include just enough methods to understand result
  - Define all elements (eg., DNA ladder)
  - Cover primarily facts, not interpretation. observed sizes (maybe expected)
- Aesthetics simplicity, clarity
  → at-a-glance labeling

Figures: Example



a-a-glance labelling

title states result without 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.

walk through figure

stand alone text

DNA Electrophoresis (EP): Principle

Agarose gel

DNA

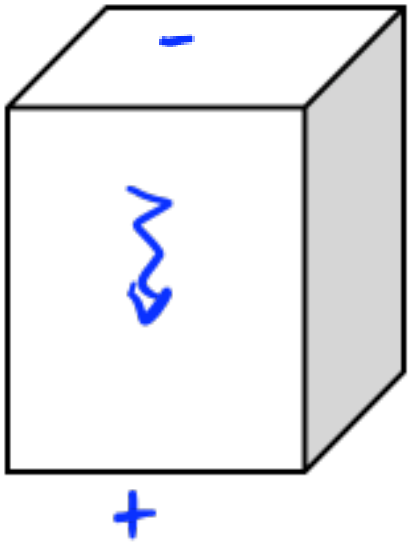
Agarose and DNA are both bio polymers

molecular entanglements (pasta)

repeat unit

Driving force for separation: charge

DNA moves − to + because of phosphate groups



Separation is according to: size

Smaller DNA moves faster because

entanglements compete w/ charge

DNA EP: Visualization

Loading dye: glycerol → make DNA sink

xylene cyanol (XC) → real-time tracking dye

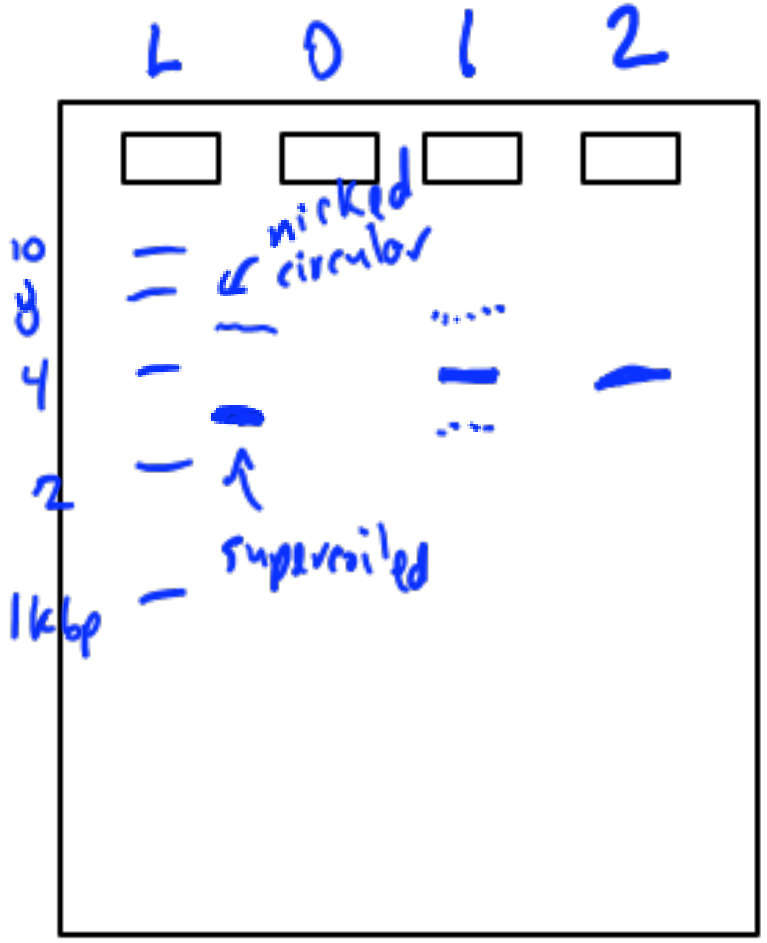
(so don't run off edge)

Ethidium bromide:

shows up under UV, but only fluoresces

if bound to DNA (π-stacking

DNA EP: Analysis



DNA ladder: standards of known size

Controls:

uncut plasmid → 2 forms

single-cut plasmid → linear

Sample:

for collection (purification) + analysis → DS

Relationship: distance $\propto \frac{1}{\log(MW)}$

DNA EP: Clean-up and Safety

- Use nitrile gloves when handling DNA gels and all equipment used for gels.
- Wear eye protection/face shields when cutting DNA bands out of the gel.
- Gels and gel-contaminated papers are disposed of in solid chemical waste.

DNA extraction from agarose gel

- Another Qiagen kit: similar principles but different buffers
  - In addition to buffer composition, size of the silica beads can affect what is retained

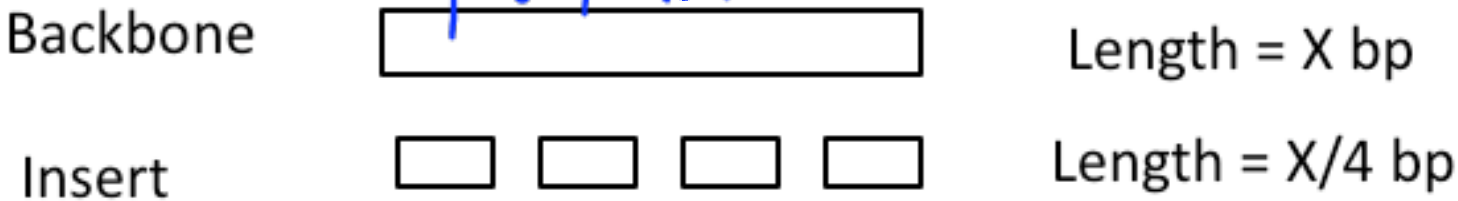
- Mixture should ideally look yellow, not blue

Preparing for DNA ligation

Ethidium intensity reflects ***absolute DNA amount***.

1 molec./mole bk b # pairs

↑ weight, ran insert



Equal intensity of insert and backbone means that the DNA amounts in the two lanes are equal. This means an equal mass ratio and unequal molar ratio of DNA.