- Announcements
- Pre-lab Lecture
  - Ligation and cloning: basic
  - Ligation and cloning: TOPO
  - Bacterial transformation
  - Today in Lab: M1D4
  - LATER: Writing a figure caption

Announcements

- Reminder: quiz next time
- Another long(ish) FNT:
  - gel figure
  - results section opening
  - results section outline
  - overview schematic
- Journal club coming up in a week! (And two)
- First notebook M1D7
  - D2, 4, or 5 collected * our choice

	TUE/WED	THU/FRI
	27 M1D5 1p Quiz 3: M1D3-4 (W/F) 2p M1D4 FNT due, W/F	28 M1D6 1p Journal Club I (T/R)
	6 M1D7 1p Quiz 4: M1D5-6 (W/F) 2p Reflection 1 (W/F JCI) +2 more FNT 1 notebook	7 M1D8 1p Journal Club II (T/R)
	13 11 Module 1 draft 11 am 1p Reflection 1 (W/F JCII	14
	20 11 Primer summary due 1p Quiz 5: M2D1-2 (W/F)	21
	27	28

where we are/going

Bird stool sample

extract DNA

DAY 2

Complex DNA pool (many pathogen DNAs)

PCR

DAY 3

Simpler DNA pool (bacterial 16S DNA)

gel purify, clone and transform

DAY 4

Individual 16S cell colonies

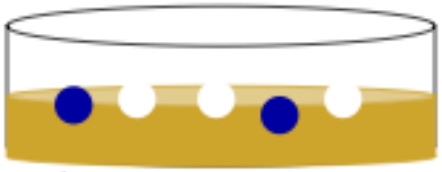
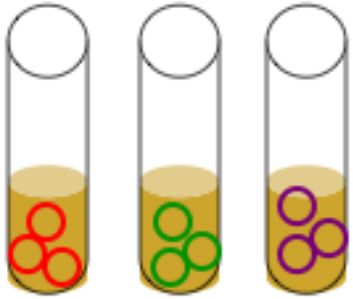


plate today → grow o/n

liquid culture

we pick ~colonies, grow o/n



isolate DNA clones from bacteria

DAY 5

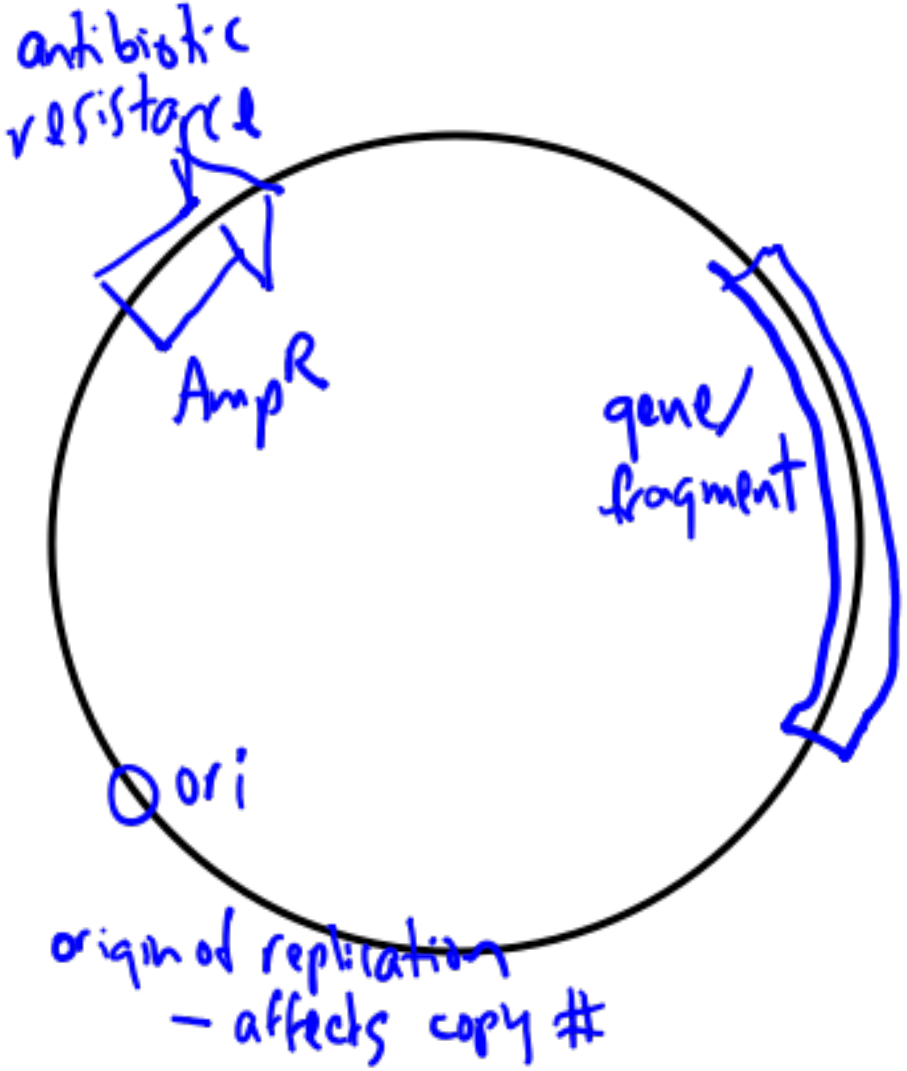
Individual 16S DNA plasmids

identify, relate, and compare sequences

DAY 7

Voilà!

Plasmid overview

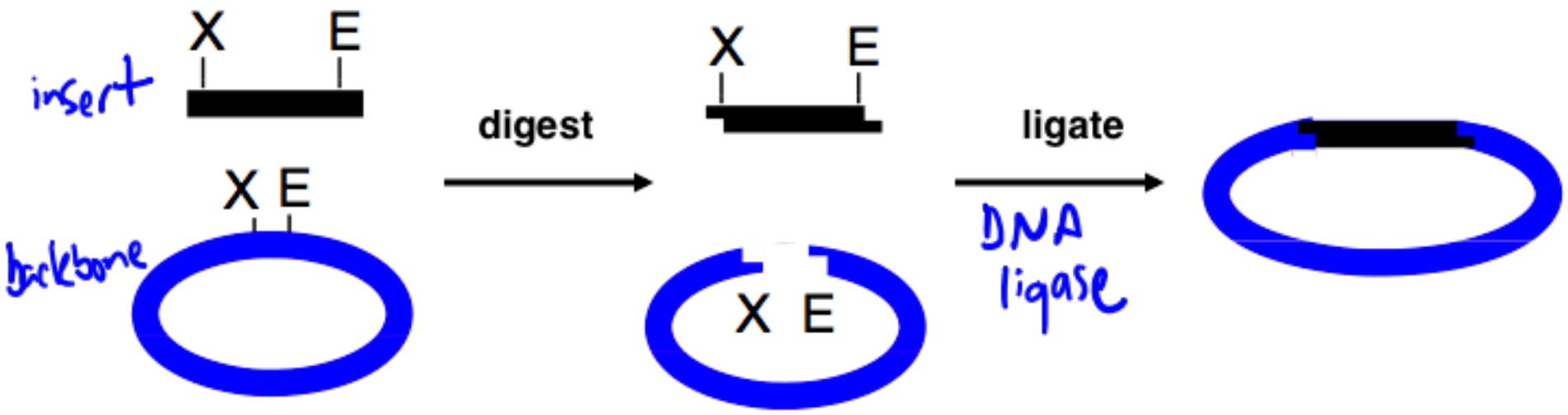


— ds, circular extrachromosomal

why? vector to introduce foreign gene/fragment into cells

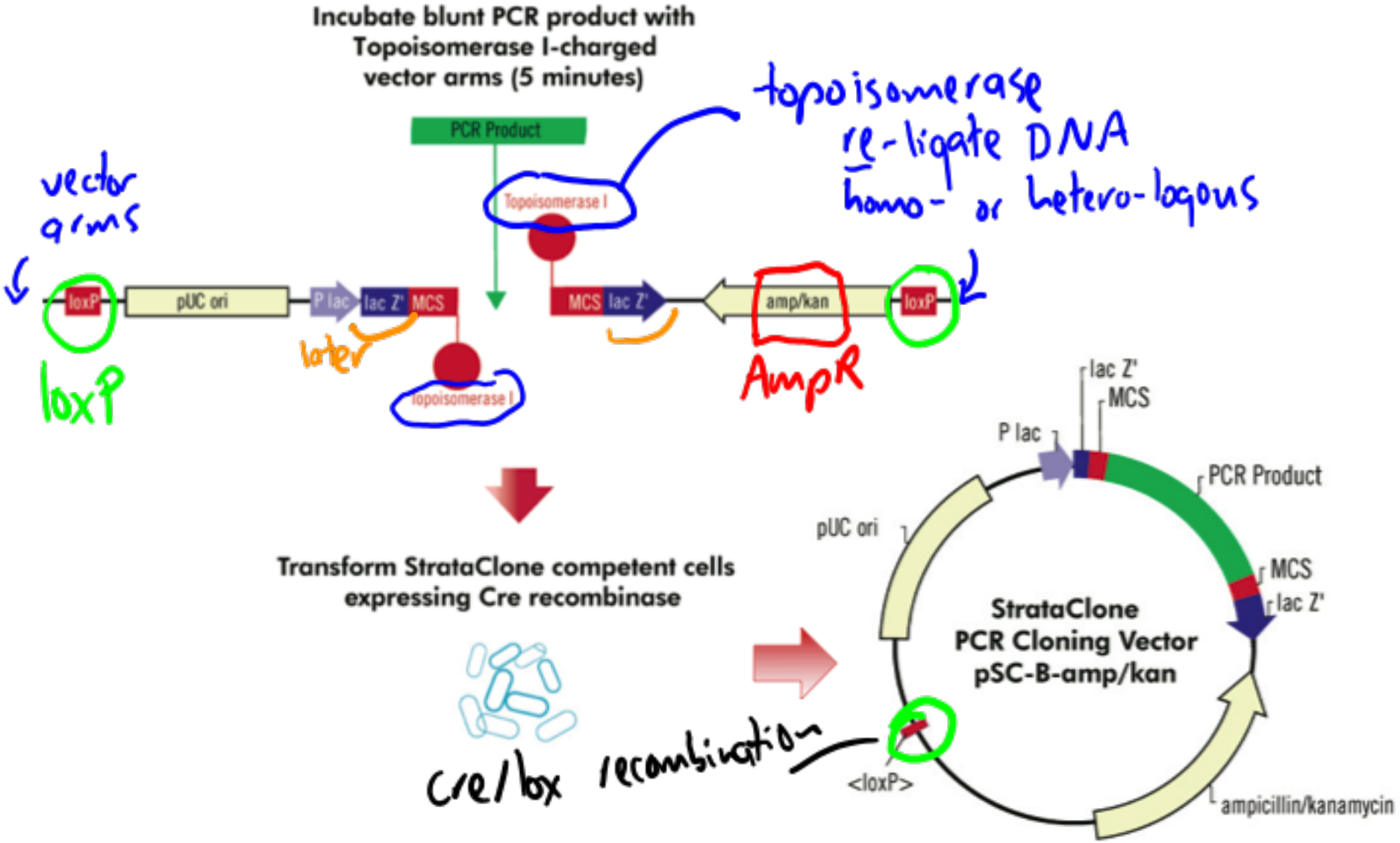
Ampicillin resistance → can select bacteria that have plasmid on ampicillin plates

Ligation and cloning: basic approach



X and E = restriction enzyme sites = DNA sequences recognized and cleaved by rest. enzymes (more in Mod 2!)

Ligation and cloning: TOPO approach



topoisomerase
re-ligate DNA
homo- or hetero-logous

vector arms

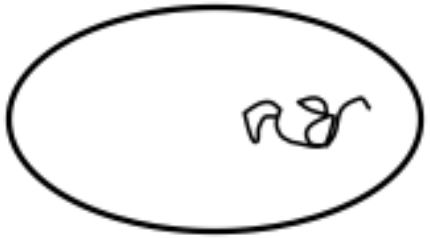
loxP

later

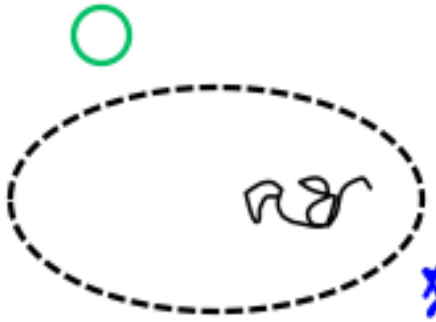
AmpR

cre/lox recombination

Bacterial transformation



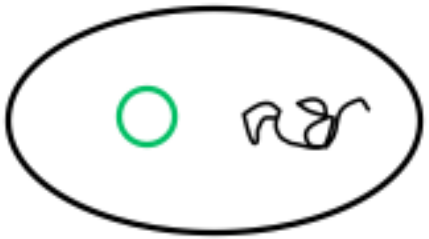
1. chemical treatment



"competent" cells

* Keep cold
* never vortex
fragile!

2. heat shock



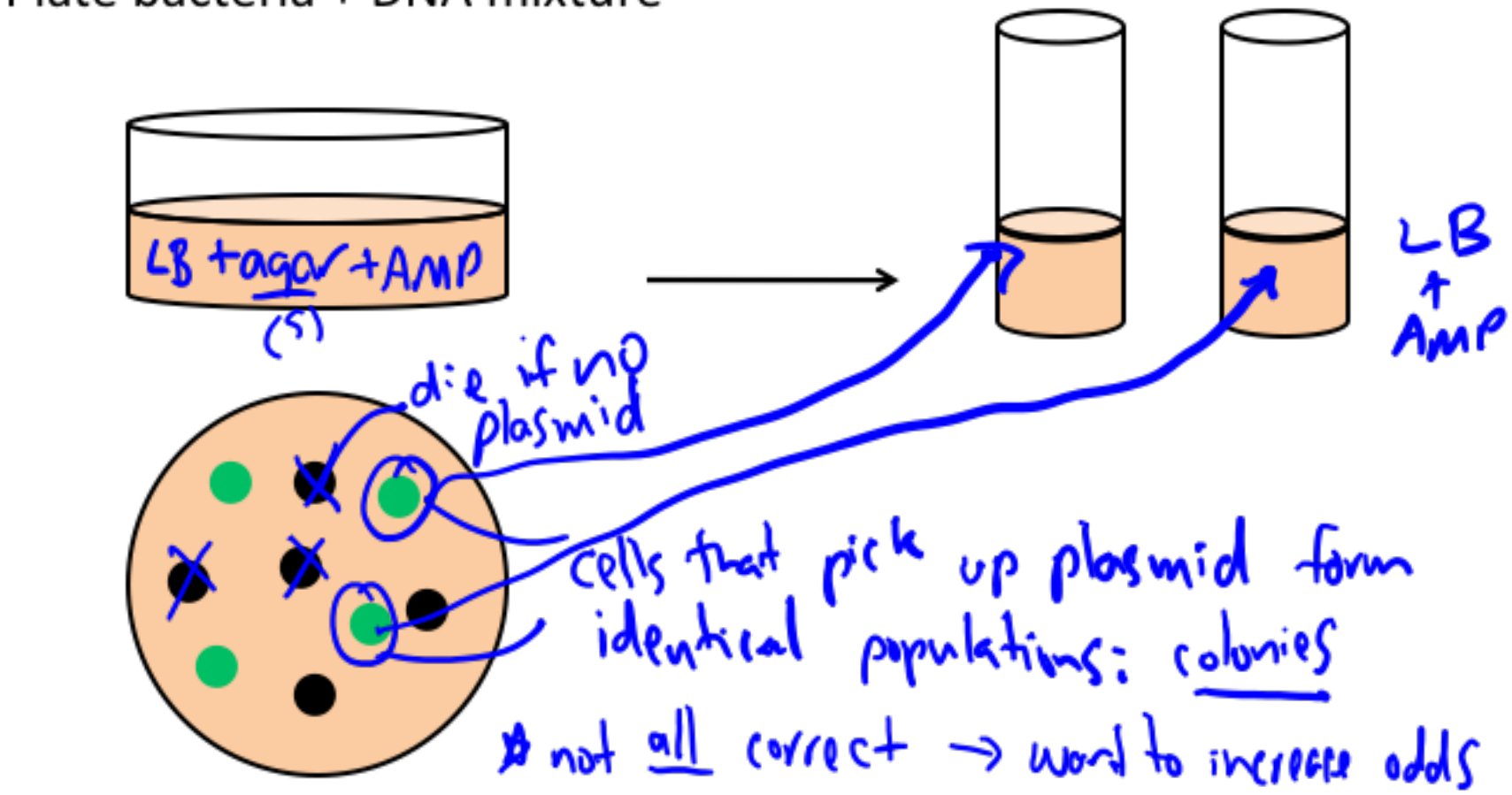
"transformed" cells

other methods

electroporation
ballistics

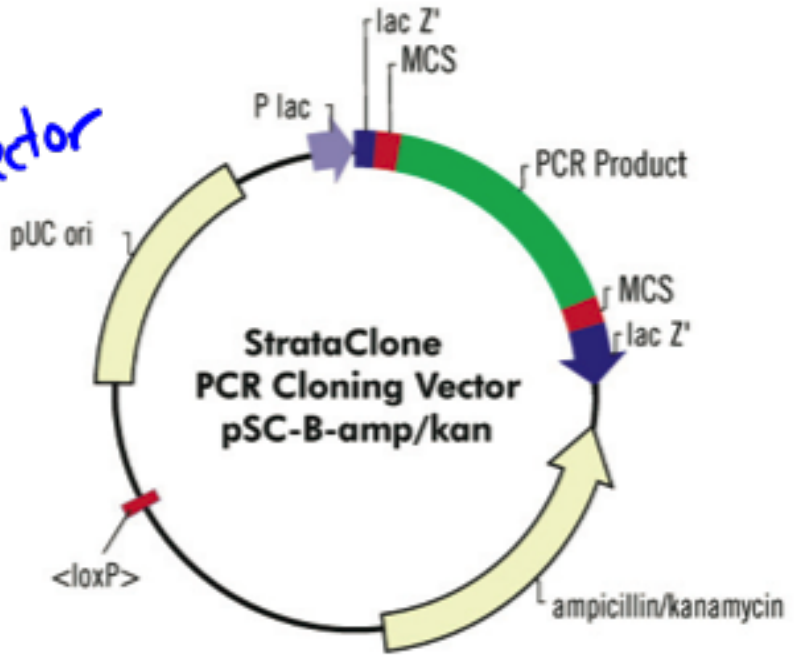
DNA amplification in bacteria

Plate bacteria + DNA mixture



"Blue-white screening" for insert selection

- lacZ encodes β-galactosidase
- inactive mutant can be rescued by peptide fragment
- production of complete peptide disrupted by insert
- X-gal = β-gal analogue
- blue color → active β-gal
- want:



DNA EP: clean-up and safety

- Use nitrile gloves when handling DNA gels and all equipment used for gels.
- Gels and gel-contaminated papers are disposed of in solid chemical waste.
- Wear amber glasses (blue light) or face shields (UV) when cutting DNA bands out of a gel.

Today in Lab (M1D4)

- Gels w/PCR products run 45 min. Meanwhile,
  - 15 min on figure caption best practices
  - prepare for next steps etc.
- Gel purify IFF multiple products; share if no product.
- Surprise! You each get to do a ligation. No re-pairing.
  - filter tips for prepping ligation reaction
- During 1 hr incubation
  - transformation demo (X-gal prep)
  - prepare tubes for liquid O/N culture
  - prepare primer stocks for μsporidia PCR

over for figure discussion

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 expected sizes
- Aesthetics: simplicity, clarity → at-a-glance

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

Figures: example

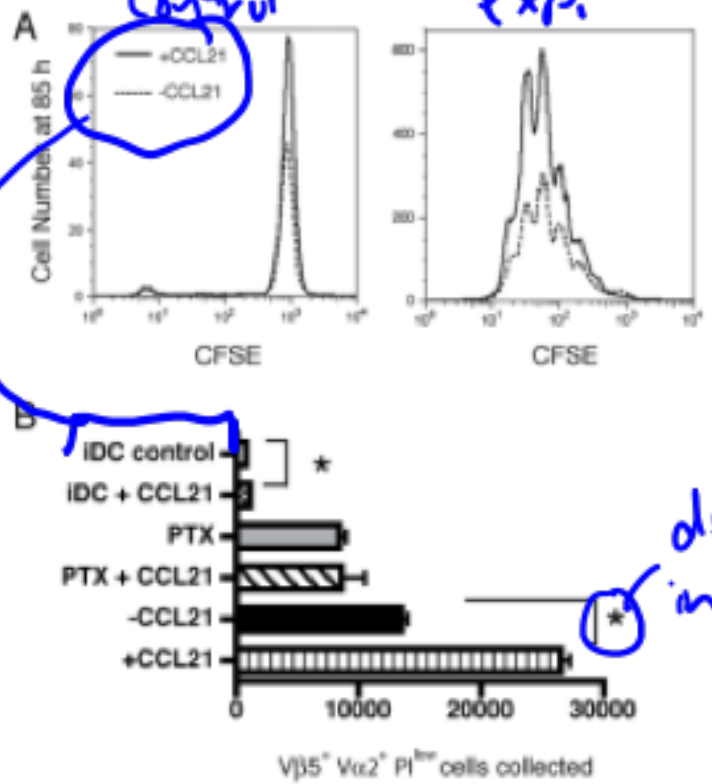


Figure 3 **CCL21 impacts naïve T cell proliferation under conditions of rare Ag-specific T-DC encounters.** Co-cultures comprising 9% OVA-specific OT-II CD4+ T cells, 81% C57Bl/6 CD4+ T cells, 5% OVA-mDC and 5% iDC with/without CCL21 were analyzed by flow cytometry at 85 h. (A) Sample CFSE histograms 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.