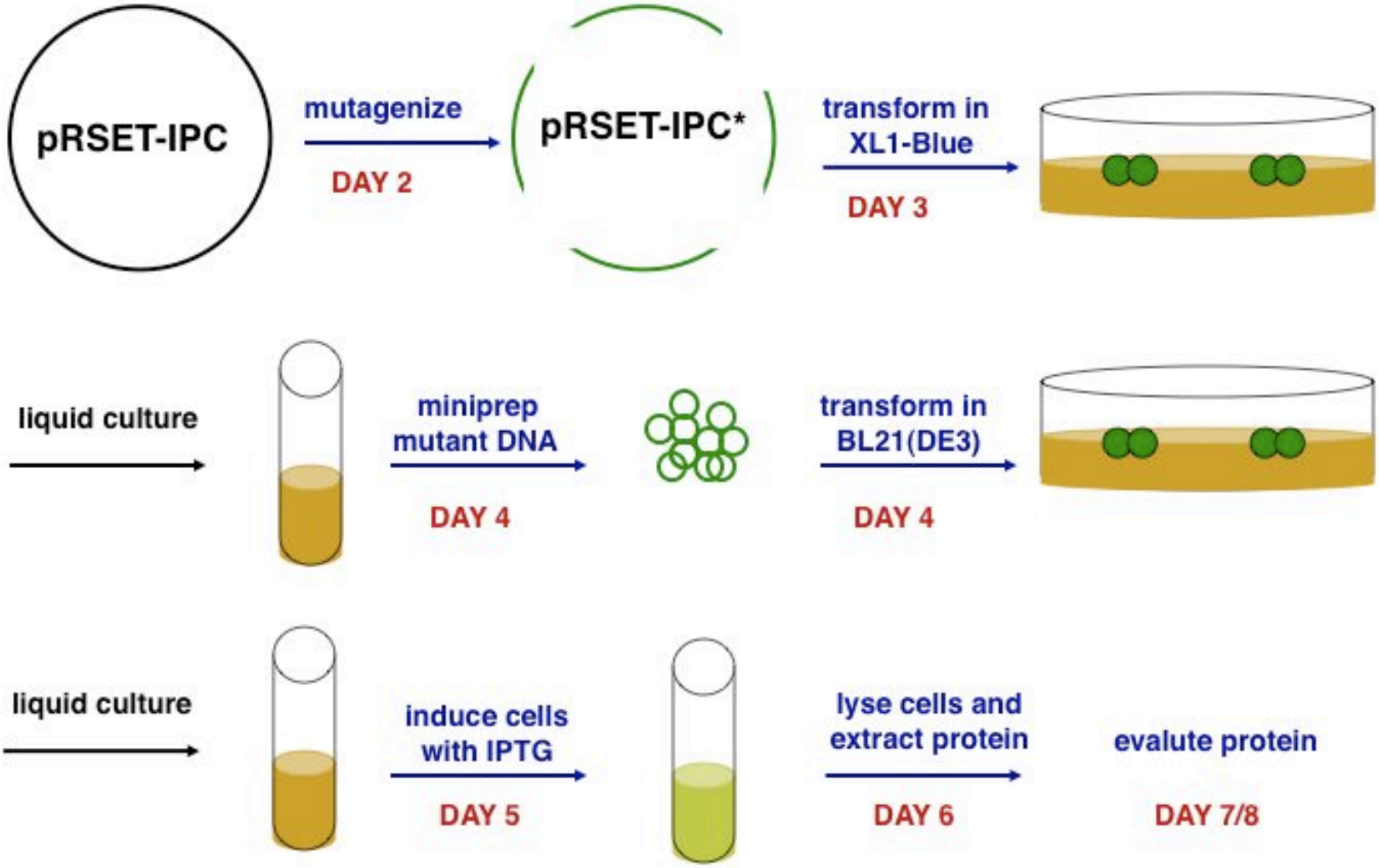
- Announcements
- Quiz
- Pre-lab Lecture
  - Genetic control elements
  - Sequencing recap
  - Writing a figure caption
  - Today in Lab (Mod 1 Day 5)

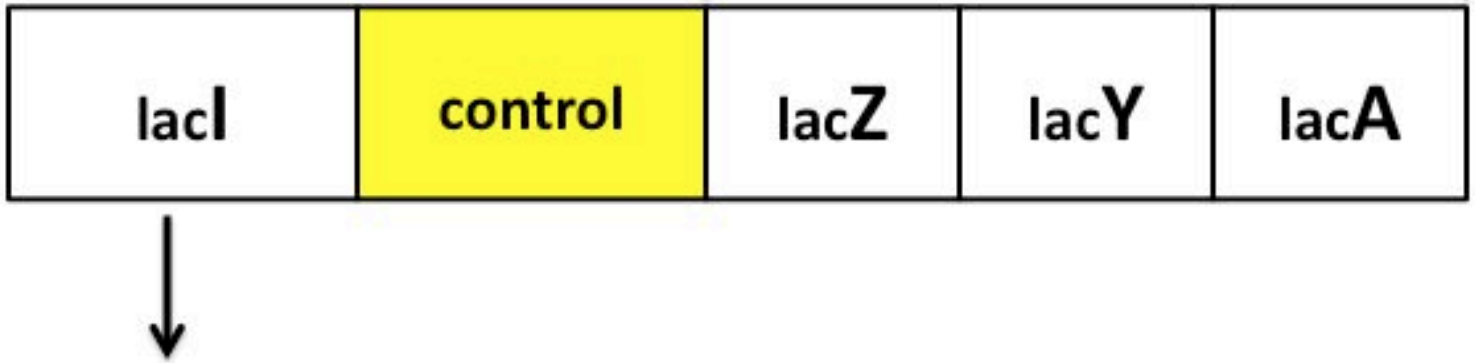
Announcements

- No quiz next time (full day)!
- Methods HW general comments:
  - Cite resources: Nagai and Watcut website
  - Give concentrations, not volumes (when possible)
  - Compositions available in "Reagent List" (Part 4) on each lab protocol


pRSET-IPC
mutagenize
DAY 2
pRSET-IPC*
transform in
XL1-Blue
DAY 3
liquid culture
miniprep
mutant DNA
DAY 4
transform in
BL21(DE3)
DAY 4
liquid culture
induce cells
with IPTG
DAY 5
lyse cells and
extract protein
DAY 6
evalute protein
DAY 7/8

lac operon

These three genes encode metabolic enzymes



Encodes a repressor protein that binds to control area turning it OFF.

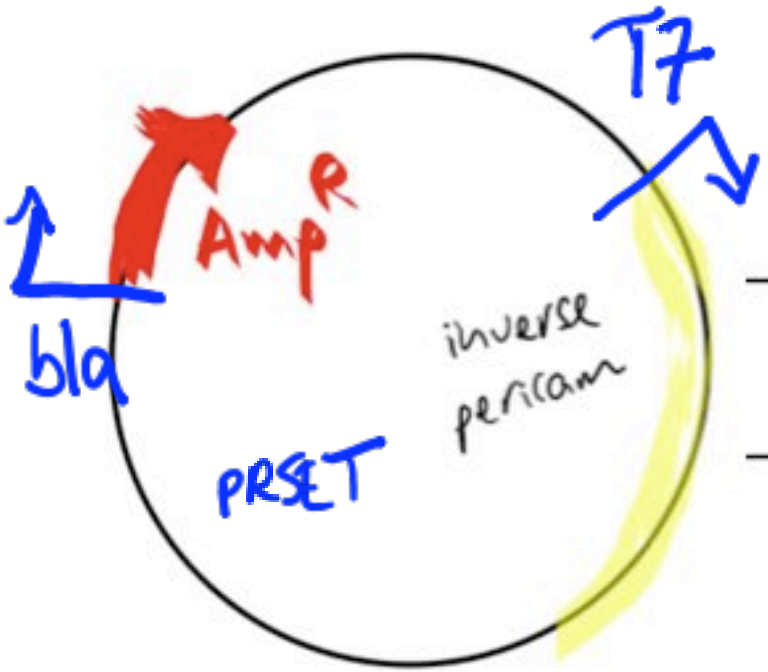
In turn, if lactose binds to the rep. protein, it is made inactive, turning ON expression of Z, Y, A.

Induction of a chosen protein

lacI	control	T7RNAP (polymerase)	lacZ

T7RAP gene is expressed in presence of lactose or analogue.

$[IPTG]_0 = [IPTG]_t$



bla promoter is constitutively on.

T7 is turned on in presence of T7RNAP.

BL21(DE3) bacterial strain

DE3: bacteriophage (virus) used to integrate lac construct into E. coli

pLysS: protein that produces lysozyme, which binds to T7RNAP, reducing "leaky" expression. Retained by chloramphenicol selection.

Sequencing reactions

Dideoxy method: no 3' OH → can't elongate
Run 4 rxns: (d)dT, dA, dG, dC and 3 others

A' G'' C''' T''''
different fluor.

Reactions ddT	ddA
TAAATT	TAAATT
AT*	A*
ATT*	ATTTA*
ATTT*	ATTTAA*
	ATTTAAA*

Gel

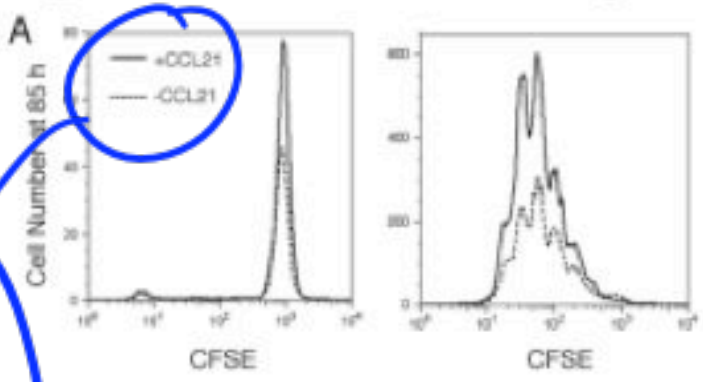
ddT	ddA

* = radioactive or fluorescent label

Figures: Style and Scope

- Title: concise, informative → gives overall result/goal.
  often similar to Results sub-headings.
- Caption: give context for result, from big to small.
  - Introduce what we are looking at.
  - Include just enough Methods to understand results.
  - Define all elements (eg., DNA ladder)
  - Cover primarily facts (results and/or expectations),
    limiting complex interpretations for Discussion.
- Aesthetics simplicity, clarity
  → at-a-glance labeling

Figures: Example



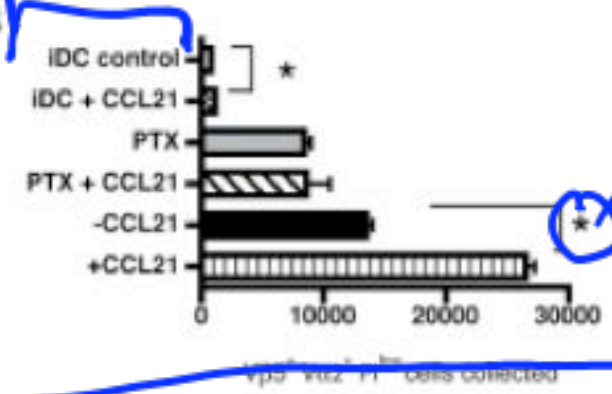


Figure 3 **CCL21 impacts naïve T cell proliferation under conditions of rare Ag-specific T DC encounters.** Co-cultures comprising 9% OVA-specific OT-II CD4+ T cells, 81% C57Bl/6 CD4+ T cells, 5% OVA-mDC and 5% iDC with/without CCL21 were analyzed by flow cytometry at 85 h. (A) Sample CFSE histograms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

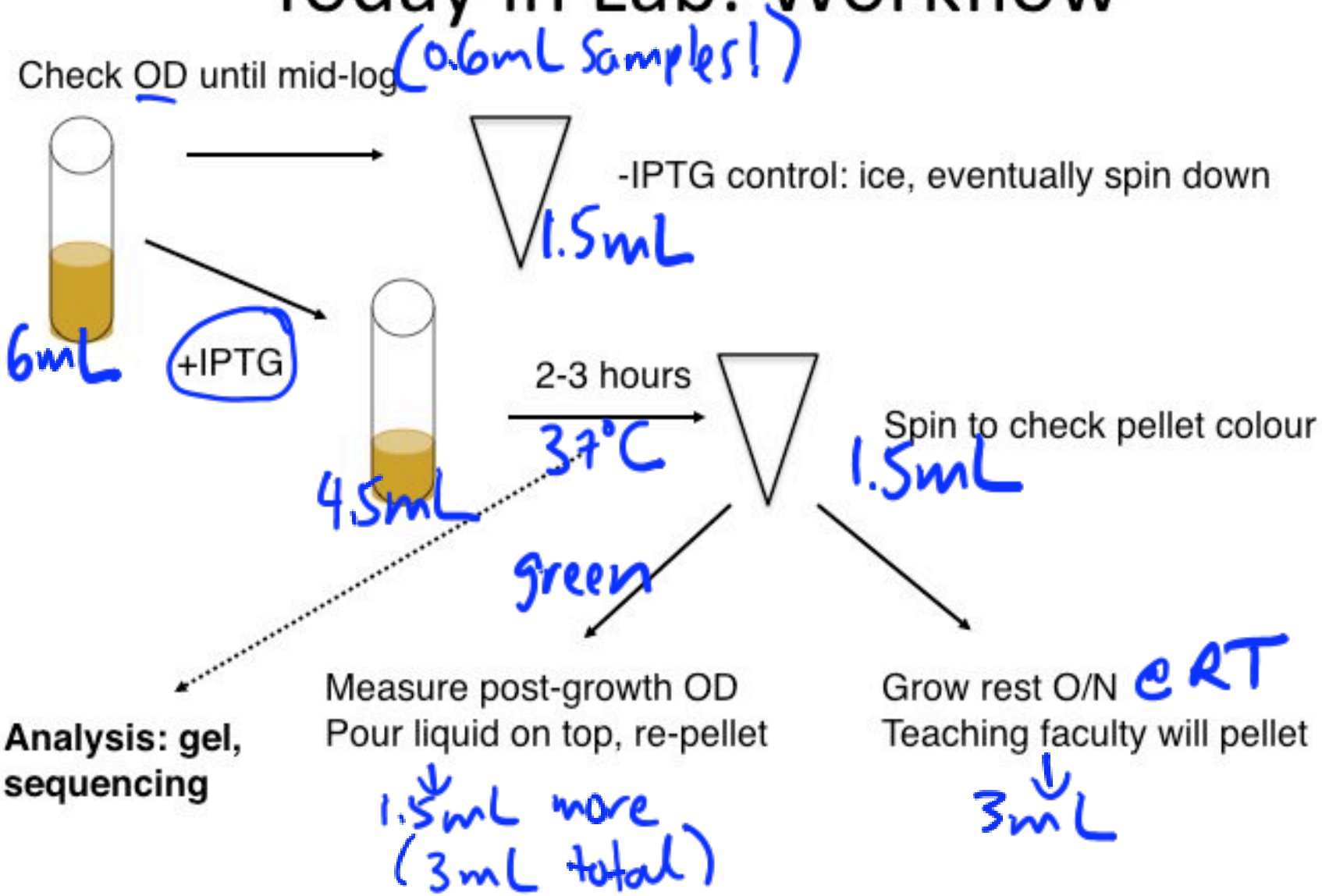
Figure Captions: Practice

Fig. N functional title descriptive caption

- Agarose gel title ideas:
  Gel analysis to verify (and measure) fragments after SD-mutagenesis
- Overview sentence topics:
  introduce idea of SDM to IPC
- Supporting detail topics:
  - expected and observed band sizes
  - what are samples
- Methods to include or not: almost none

* label a few bands in ladder

Today in Lab: Workflow



Today in Lab: Samples

- Start with four DE3 samples carrying plasmid
  - WT
  - S101L
  - X#Z candidates 1 and 2
- After gel and sequencing analysis, pick just one X#Z to continue working with
- End of day, "hand in" 6 pellets, or (3 pellets, 3 cultures, and 3 eppendorfs) to teaching faculty