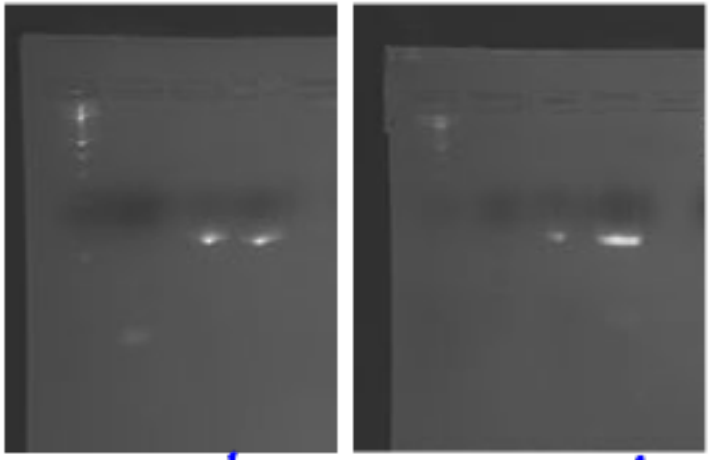
- Announcements
- Lab Quiz
- Pre-lab Lecture
  - Writing a Figure/Caption
  - In Vitro Transcription
  - Choosing Column Conditions
  - Today in Lab: M1D3

Announcements

- Next time in lab is *packed*
  - Very short quiz + pre-lab lecture
- FNT: Lots! Reading and calculations for Day 4, practice figure/caption/results, WAC letter.
- Paper list will be finalized by Monday morning.

On troubleshooting

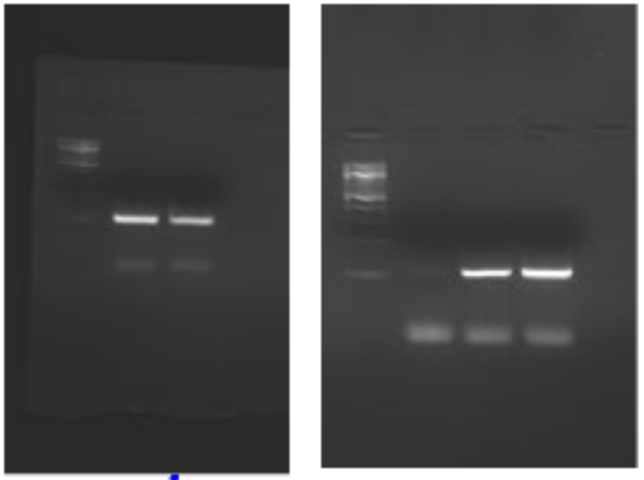
T/R



38 !°

6/17 °

TA Sample and W/F



40-50 ng/mL

Why might T/R gels have run strangely?

smaller/blocked wells, refrigeration (buffer, set time, etc.)

DNA recovery vs. DNA appearance

partly student technique; EtBr warped if in 4R@4°C; saturated columns

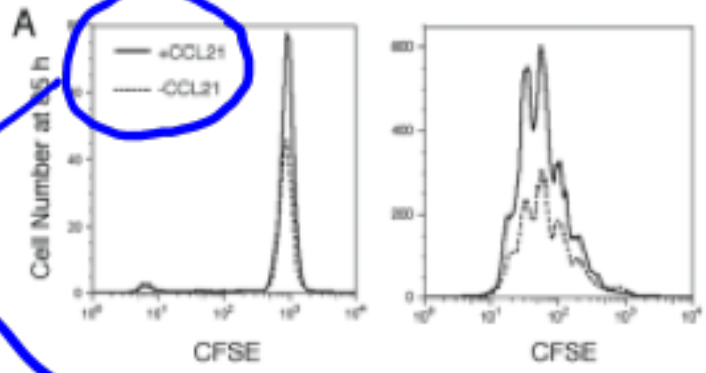
Figures: Style and Scope

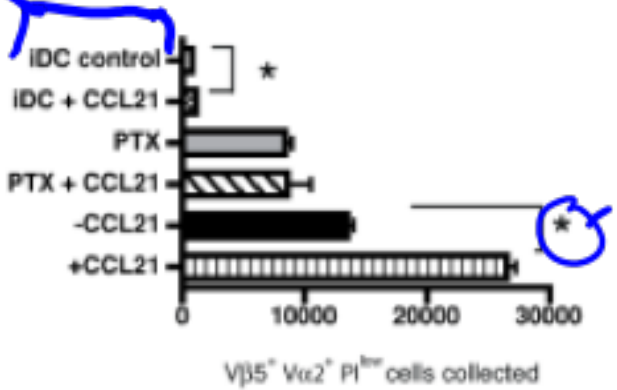
- Title: concise, informative, tells overall goal/result
- Caption: gives context for result from big → small
  - Introduce what we are looking at
  - Include just enough methods to understand result
  - Define all elements (e.g., DNA ladder)
  - Cover primarily facts, not interpretation

    e.g., observed and expected sizes
- Aesthetics: simplicity, clarity → at-a-glance

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

Figures: Example





at-a-glance labeling

states result, without over-interpreting

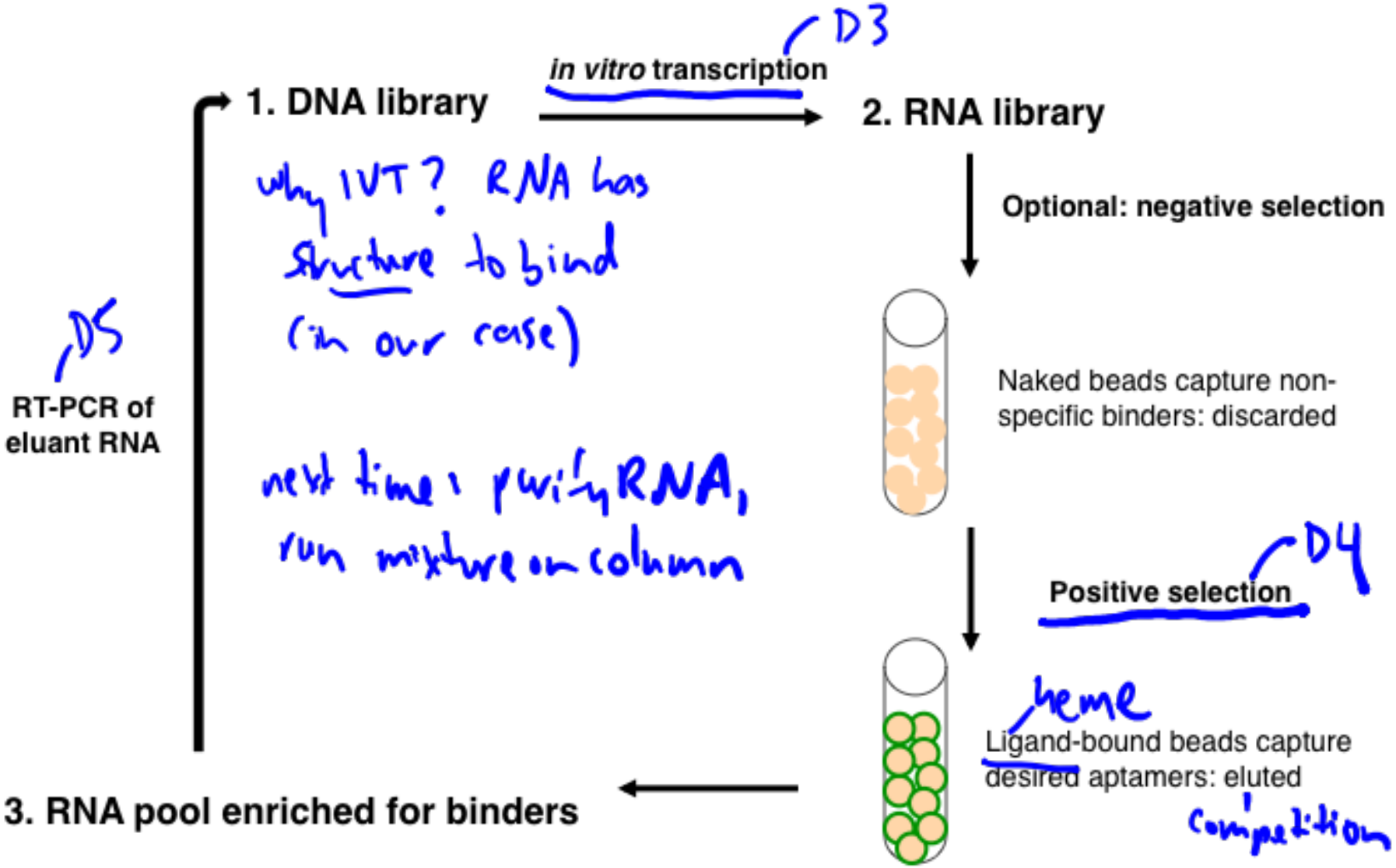
defined in caption

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.

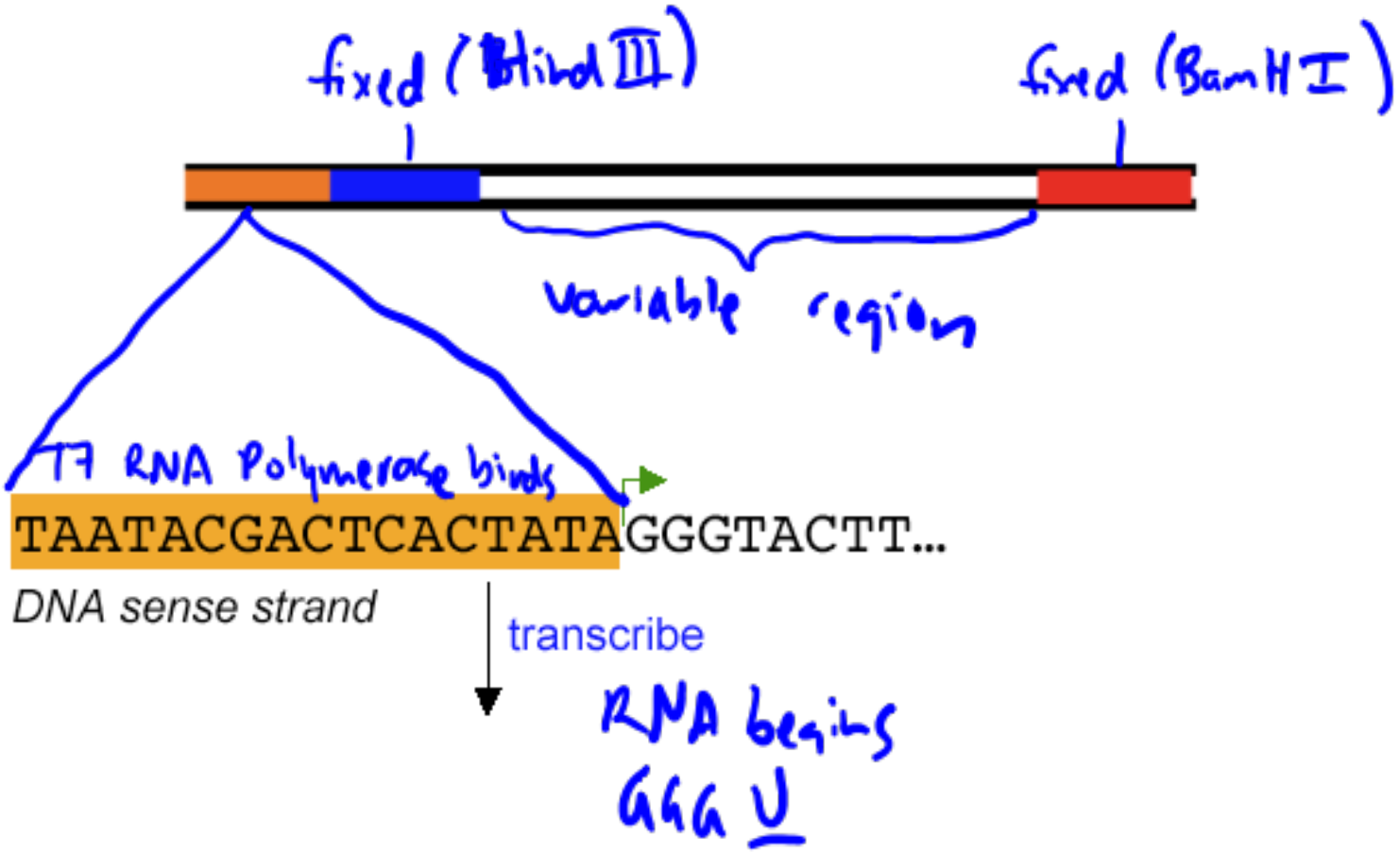
overview of exp.

walkthrough of data

SELEX Overview



In vitro transcription



Cartoons from Niles Lecture 2.

PCR vs. IVT

PCR	IVT
DNA plasmid template	DNA fragment (linear)
Primers	N/A
dNTPs	NTPs
Taq DNA polymerase	T7 RNAP
Buffer, Mg ions	Similar

Data from last year

Supposed Pre-Selection 8-12%	Actual Pre-Selection 8-12%	Lower # of washes	Higher # of washes	8-12% after fewer washes	8-12% after more washes
2	1.0	4	24	66.7	108
2	5.4	8	16	41.8	37.6
10	13	4	24	57.1	90.9
10	14	8	16	67	106
10	12	8	16	64	61
10	14	8	16	82.5	80.9
50	10	4	24	53.5	92.4
50	52	8	16	80.4	71.7

as washes ↑, enrichment ↑

See any trends?

even w/ 2% O_2, can still get close to 100

Options for this year

- Half of you will *repeat* an exp from last year
  - you choose which condition
  - give rationale for why it's worth repeating
- Half of you will try a *new* condition
  - If you change composition, do 4/24 washes.
  - What compositions might be most interesting? low
  - If you change washes, choose 2, 10, or 50 % 8-12.
  - What wash conditions might be most interesting? extremes (high)

Today in Lab

- Working with RNA
  - Gloves on, keep area and equipment clean
- Set up IVT rxns
  - Run for 4 hrs, note your start time up front
  - Stored frozen till next time
  - Return the rest of your DNA, too!
- Condition sign up and discussion prep ~2:15
- Presentation on giving talks from Atissa ~2:45
- Journal article discussion ~ 3:40