- Announcements
- Lab Quiz
- Pre-lab Lecture
  - Writing a figure/caption
  - In vitro transcription
  - Choosing column conditions
  - Today in Lab: M1D3

Announcements

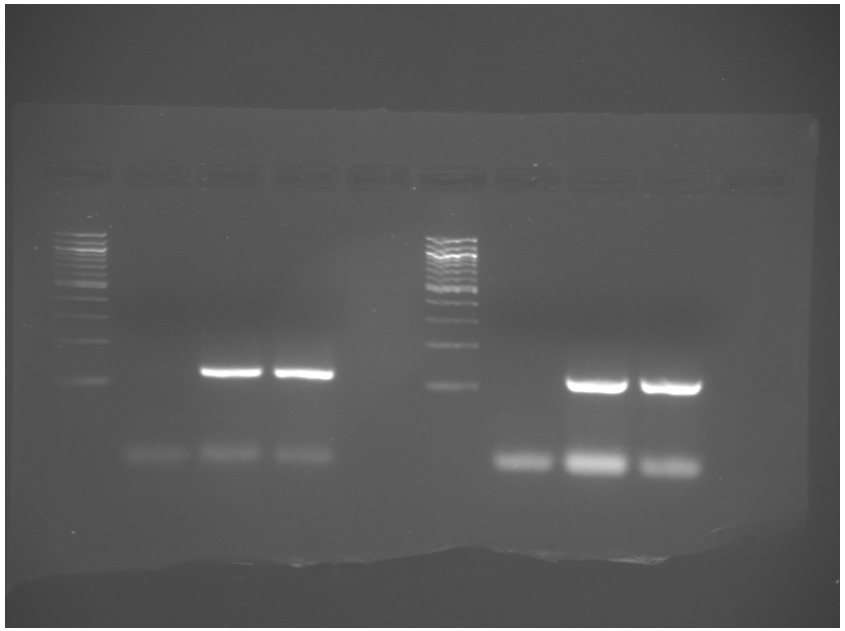
- Next time in lab is *packed*
  - Very short quiz + pre-lab lecture

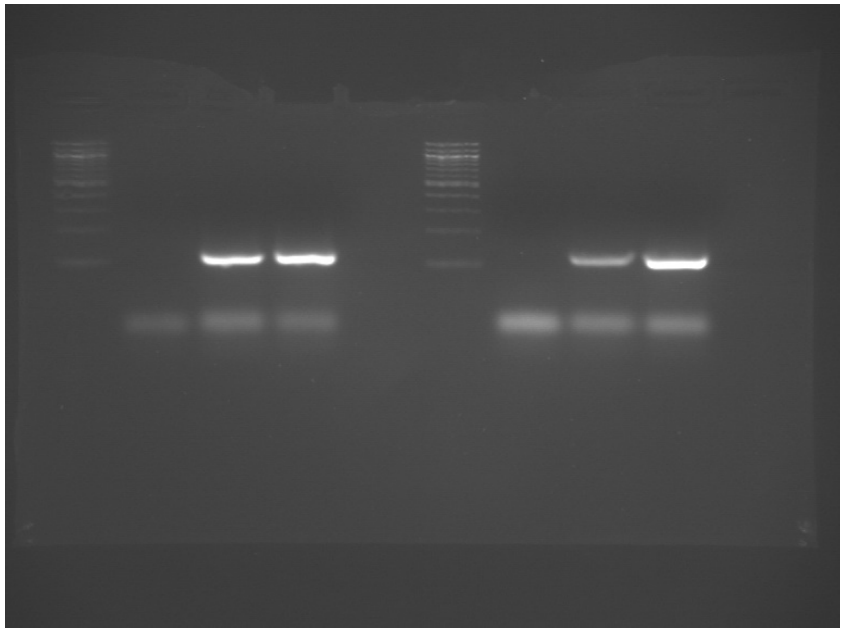
n+ class T/W

- FNT: Lots! Reading and calculations for Day 4, draft intro, practice figure/caption/results, another computational piece.
- Paper list will be finalized by Monday.
- About that last quiz…
  - Low average means our bad
  - Keep in mind: we won't ask you things you can't know
  - Always ask yourself what were the 1-2 main topics from pre-lab *and* lecture to be familiar with

+ sample names in notebook

Interpreting gel data





Second band?

What do relative intensities suggest?

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

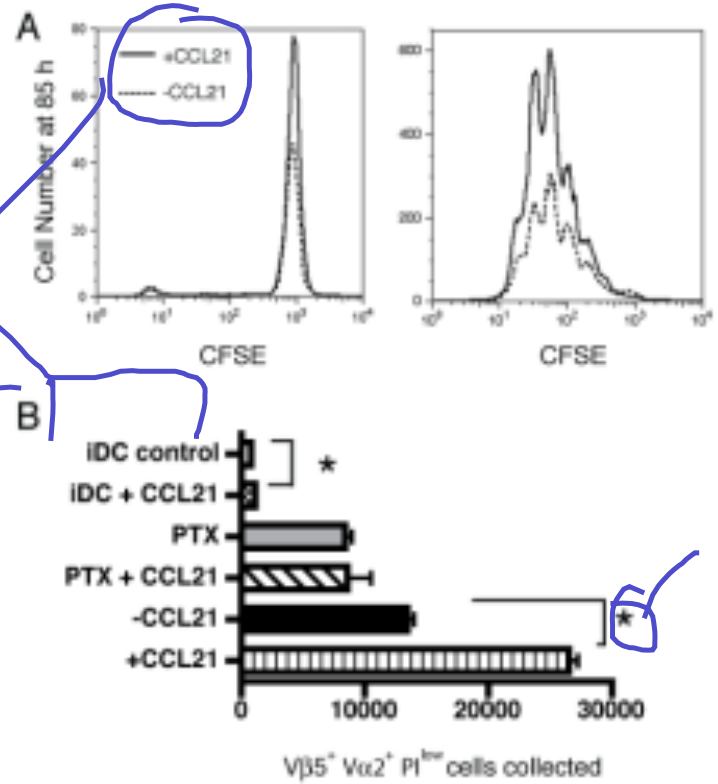
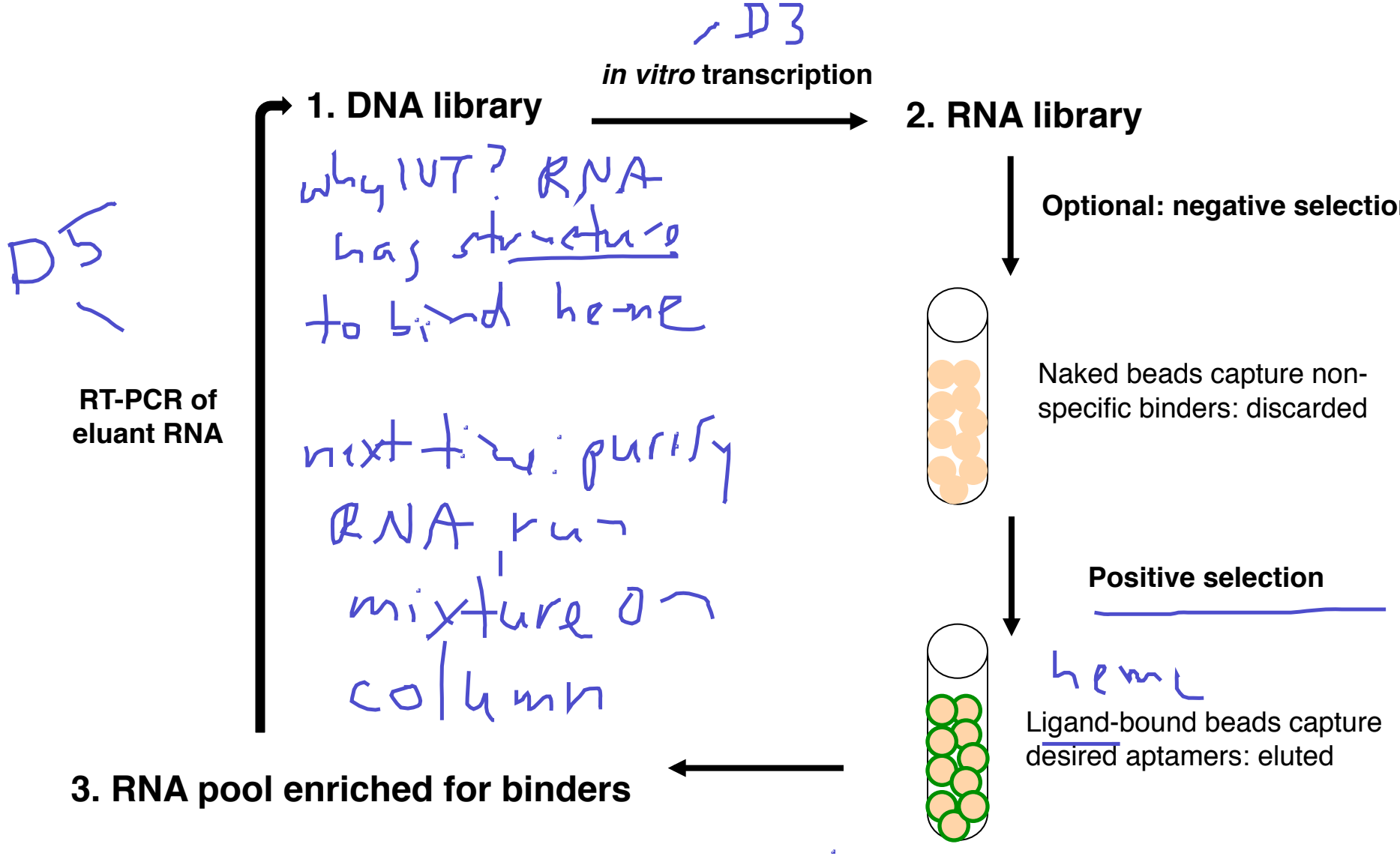
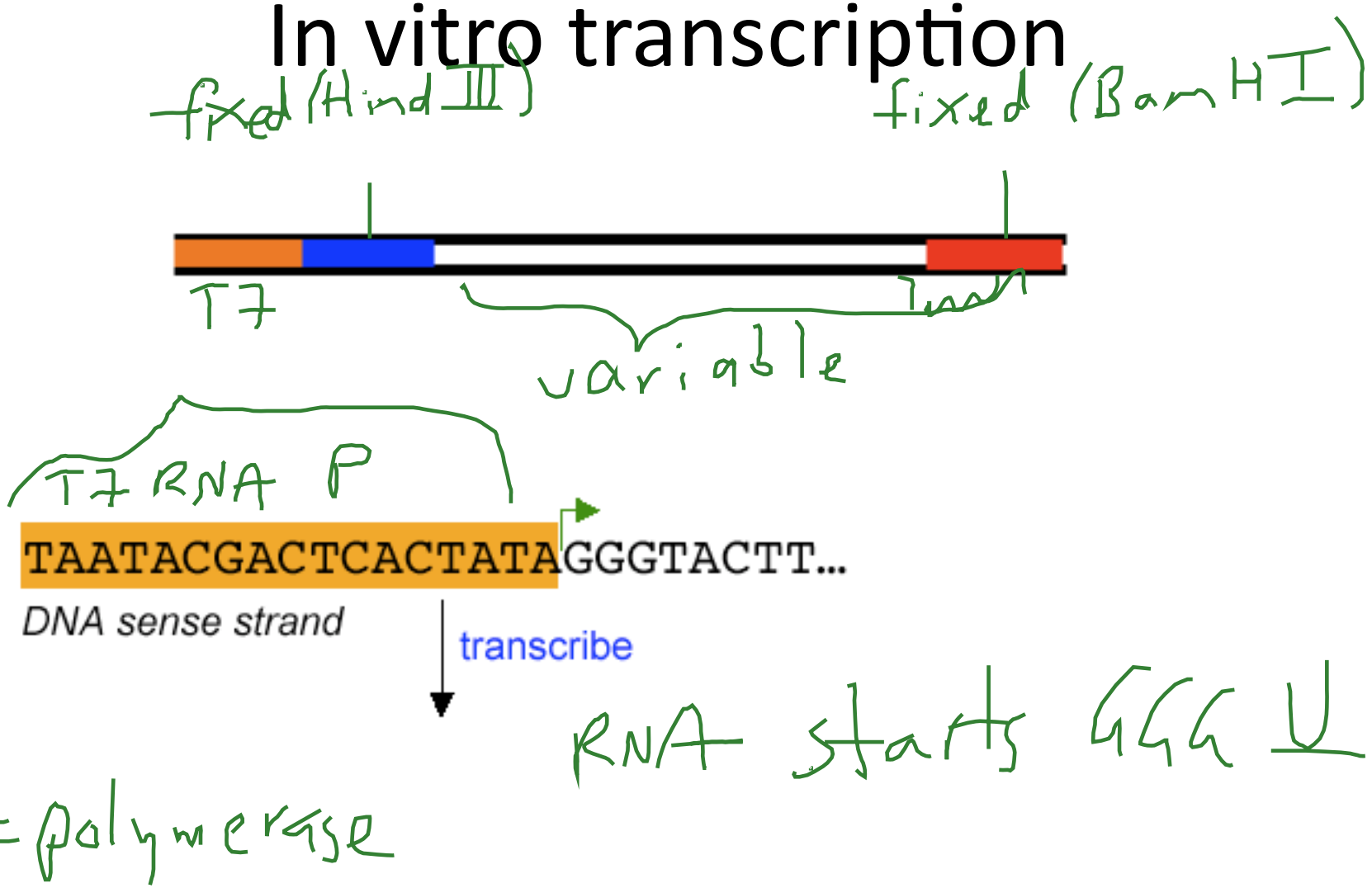


Figure 3 **CCL21 impacts naïve T cell proliferation under conditions of rare Ag-specific T-DC encounters.** Co-cultures comprising 9% OVA-specific OT-II CD4+ T cells, 81% C57Bl/6 CD4+ T cells, 5% OVA-mDC and 5% iDC with/without CCL21 were analyzed by flow cytometry at 85 h. (A) Sample CFSE histograms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

SELEX Overview



In vitro transcription



Cartoons from Niles Lecture 2.

PCR vs. IVT

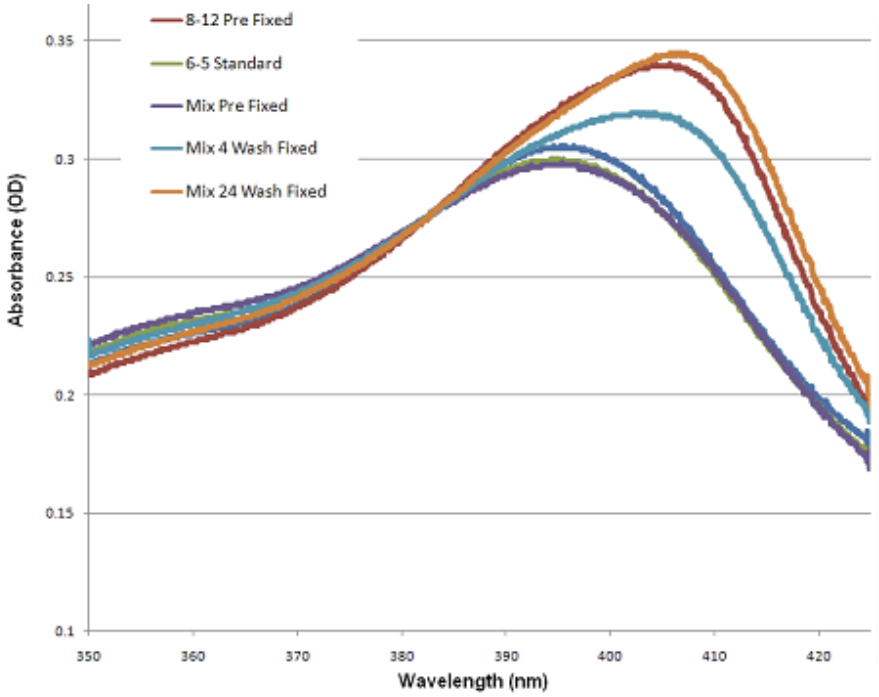
PCR	IVT
DNA plasmid template	linear fragment
Primers	N/A
dNTPs	NTPs
Taq DNA polymerase	T7 RNAP
Buffer, Mg ions	Similar

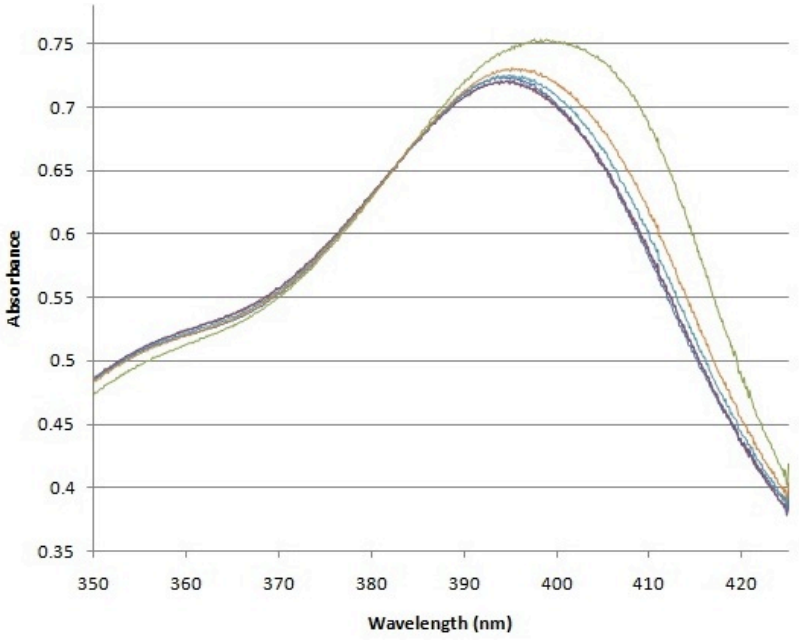
Summary data from spring 2010

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

See any trends?

Sample raw data, S10 and S11





shift from ~395 to 410 nm

shift from ~395 to 400 nm

Summary data from spring 2011

TABLE 1
Percentage 8-12 RNA aptamer yielded under varying wash and library conditions

Group Number	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
Group 1	0.25	1.21	4	24	16.3	32.3
Group 2	0.5	N/A	4	24	N/A	N/A
Group 3	1	N/A	4	24	N/A	N/A
Group 4	2	N/A	4	24	N/A	N/A
Group 5	2	2.9	4	32	40	47
Group 6	2	N/A	2	48	0	92.1
Group 7	2	2.88	4	24	57.31	69.42
Group 8	10	N/A	8	32	49.6	57.8
Group 9	10	15.72	2	34	96.05	78.53
Group 10	50	N/A	4	24	N/A	N/A
Group 11	50	51.9	8	16	69.62	79.75
Group 12	50	56.27	8	16	70.68	81.62
Group 13	50	42.47	4	24	69.86	54.79

Anything interesting to follow up on?

Options for this year

- Half of you will *repeat* an exp from S10 or S11
  - give rationale for why it's worth repeating
- Half of you will try a *new* condition
  - If you change composition, do 4/24 washes.
  - If you change washes, choose 2, 10, or 50 % 8-12.
  - No more than 2 groups total can use 50% 8-12!
- Optional: 1 group per day may pitch an idea to change yet a different parameter
  - well, more can pitch, but only one can be accepted

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 ~2:50
- Journal article discussion ~ 3:45