

- Announcements
- Lab Quiz
- Pre-lab Lecture
 - ❖ Where are we/going?
 - ❖ Today in Lab: M1D5

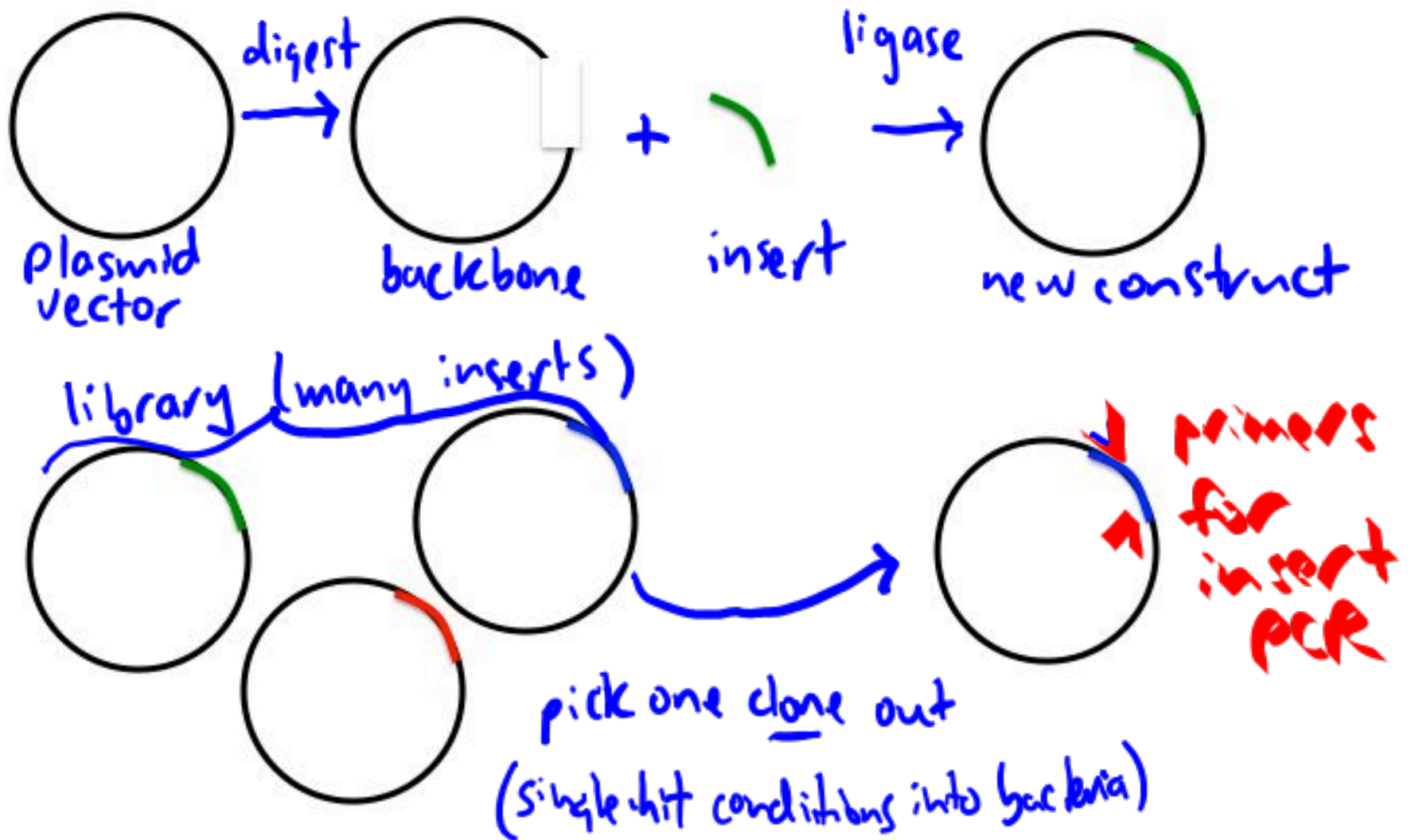
Announcements

- HW: MEME exercise
 - Very few folks selected the correct clones
 - Think about how you can learn which are unique
- Next time
 - One week from today
 - Meet here to set up IVT
 - Move to 16-336 for talks (~1:30/45 pm)
 - Check out j club rubric in advance!
- FNT: Mfold analysis, draft introduction.

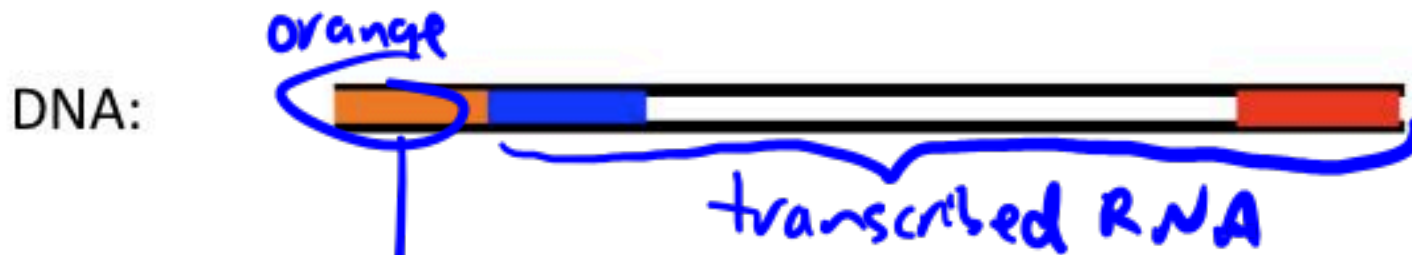
D2 FNT: Figure/Results

- D2 FNT can be picked up early next week, TBA
- For now, a few comments...
- Results
 - Set context in 1-2 sentences [read example]
 - Focus on results, not methods
 - Consider observations vs. expectations – be explicit
 - What do you know about band size?
- Figure
 - Still set a little context (e.g., the word aptamer)
 - Avoid writing “6-5” and “8-12” this way

Preparing DNA library



IVT and purification



T7 RNA Polymerase: binds to T7 promoter

Purpose of pyrophosphatase: ↑ efficiency (P_i breaks down)

Purify on polyacrylamide column (cf. DNA on silica)

↓
size

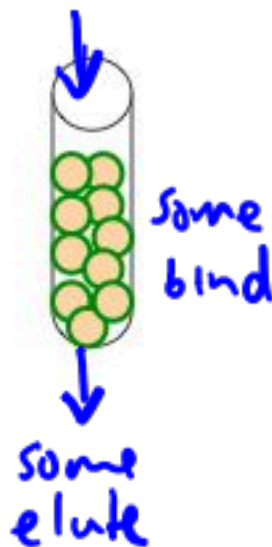
↓
size, chemical

Cartoon from Niles Lecture 2.

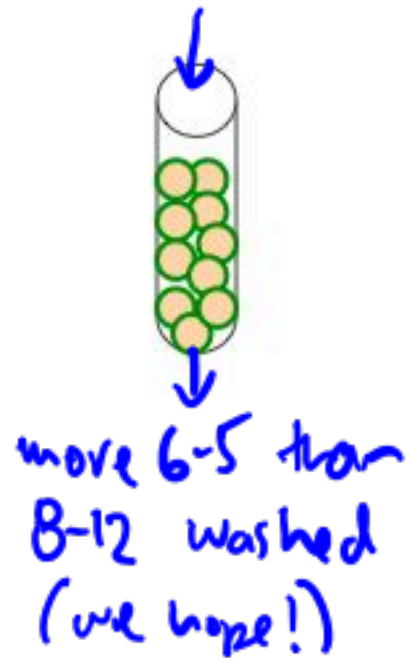
Column purification

- Why did we heat the RNA first? *structure*
- What happens at each step?

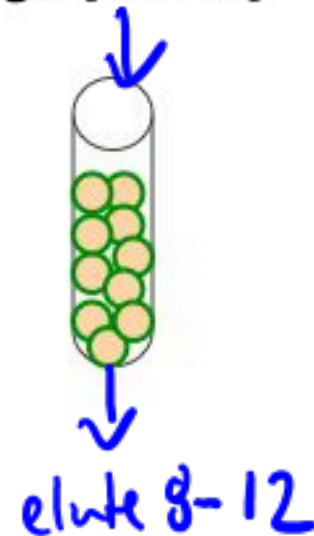
1. RNA mix



2. Wash (SB)



3. High [heme]



RNA precipitation and RT-PCR

- RNA precipitation

- Glycogen co-precipitate "carrier"
- Ethanol lowers charge screening
- Salt bind/precipitate RNA

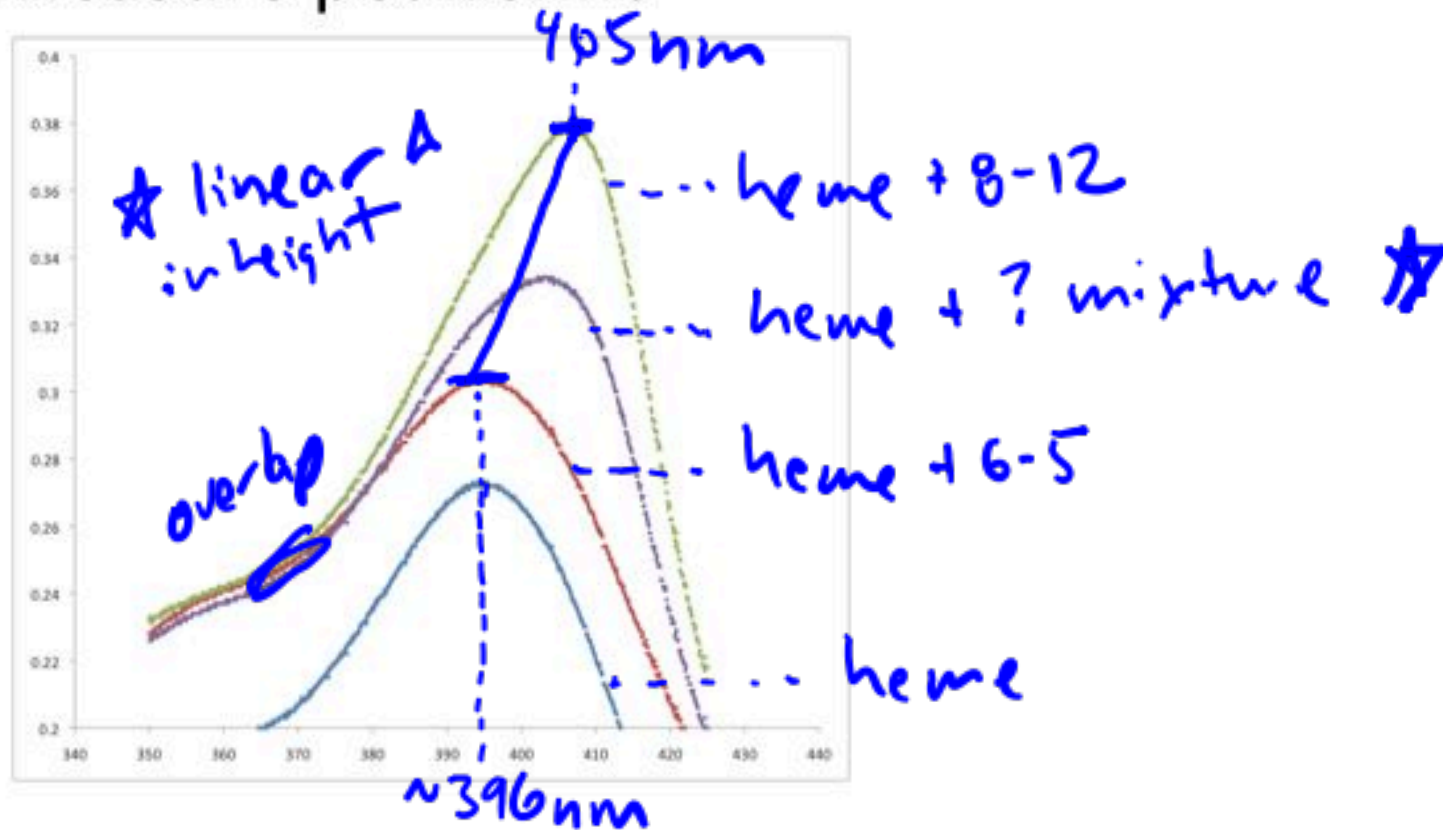


- RT-PCR

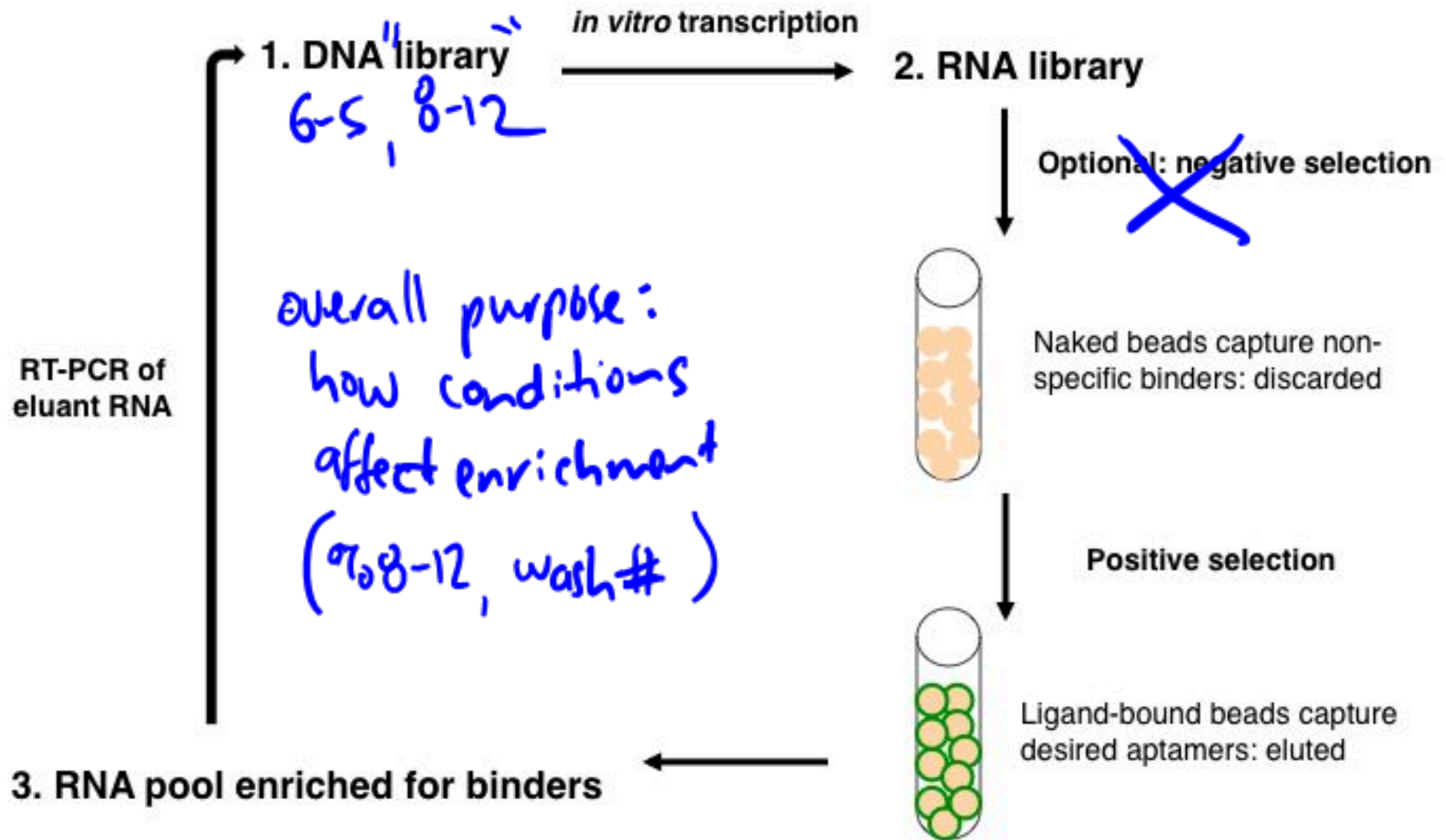
- Step 1: RT RNA \rightarrow DNA S.S.
- Step 2: PCR amplify DNA d.S.
- Assume: same copy rate for 6-5, 8-12
- Purpose of BSA: prevent hump interference

Preview of binding assay

- Mix RNA and heme $\sim 1:1$
- Measure peak shift



SELEX Overview



Today in Lab (M1D5)

- Still working with RNA (at first) – careful!
- Collect precipitated RNA, set up RT-PCR
 - check EtOH removal with us
- WAC session *~3pm*
 - Academic integrity and plagiarism
- Prepare samples to run on gel