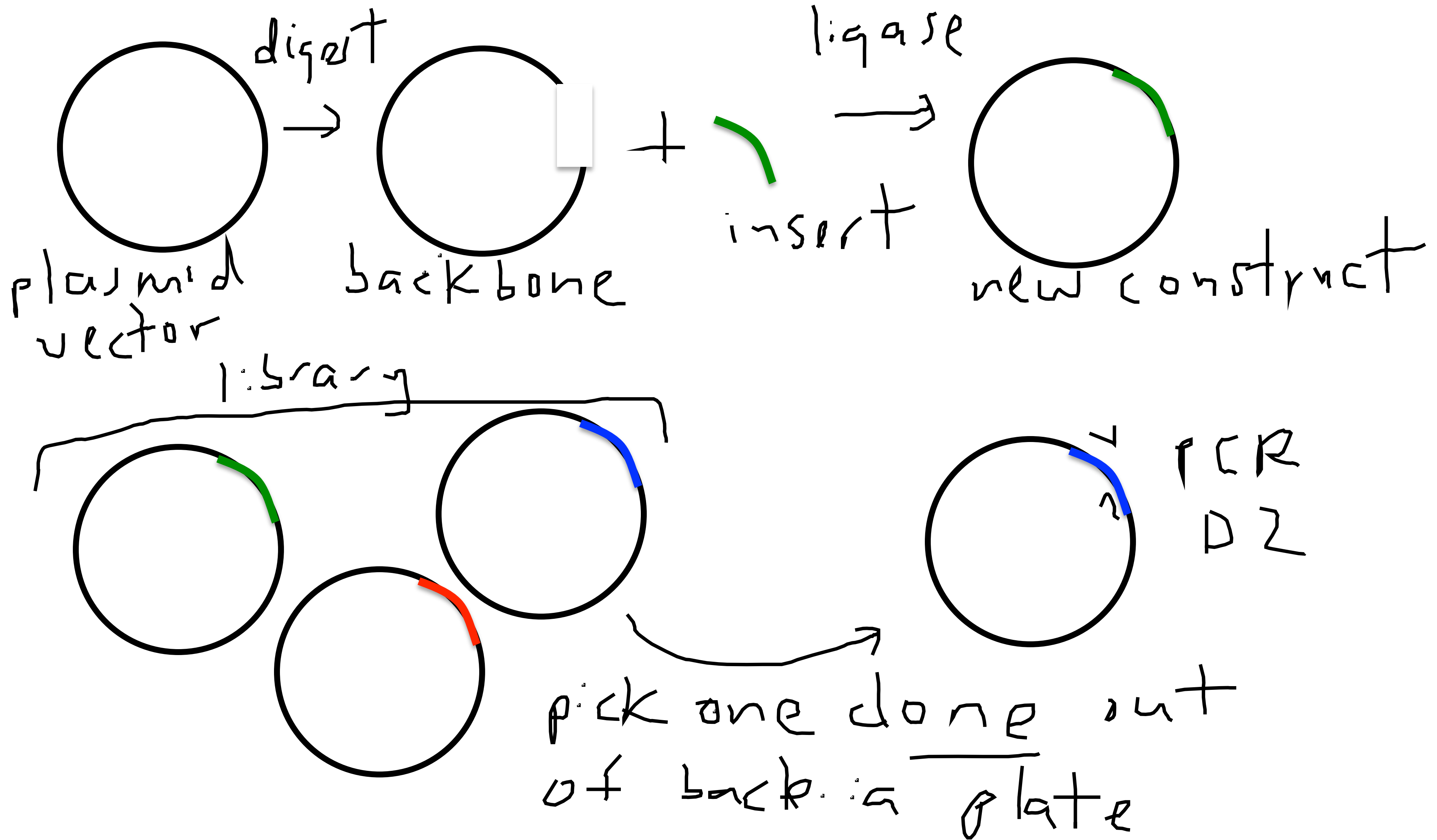
- Announcements
- Lab Quiz
- Pre-lab Lecture
  - Where are we/going?
  - Today in Lab: M1D5

Announcements

- FNT feedback
  - MEME exercise: very few folks selected the correct clones; how can you learn which ones are unique?
  - Intro: please read "notes to all", which apply to most folks
  - Figure/Results: set context, then describe or interpret fully
- Next time: M1D6
  - 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 due D6 or D7 depending on your j. club day
  - First *required* reflection is j. club self-eval

+ writing help
+ OH plan

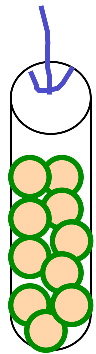
Preparing DNA library



Column separation

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

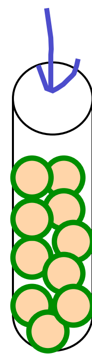
1. RNA mix



some bind

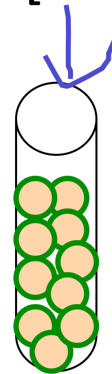
some elute (of each)

2. Wash (SB)



(elute) more 6–5 than 8–12 (we hope!)

3. High [heme]



elute 8–12

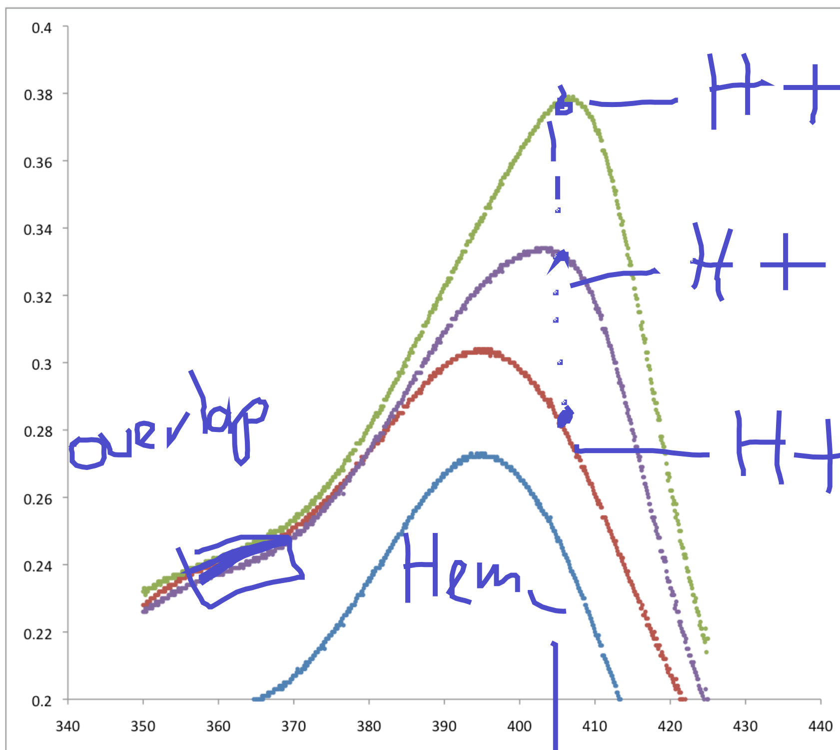
RNA precipitation and RT-PCR

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

- RT-PCR
  - Step 1: RT RNA → DNA s.s.
  - Step 2: PCR amplify DNA d.s.
  - Assume: same copy rate for 6-5 + 8-12
  - Purpose of BSA: prevent heme interference

Preview of binding assay

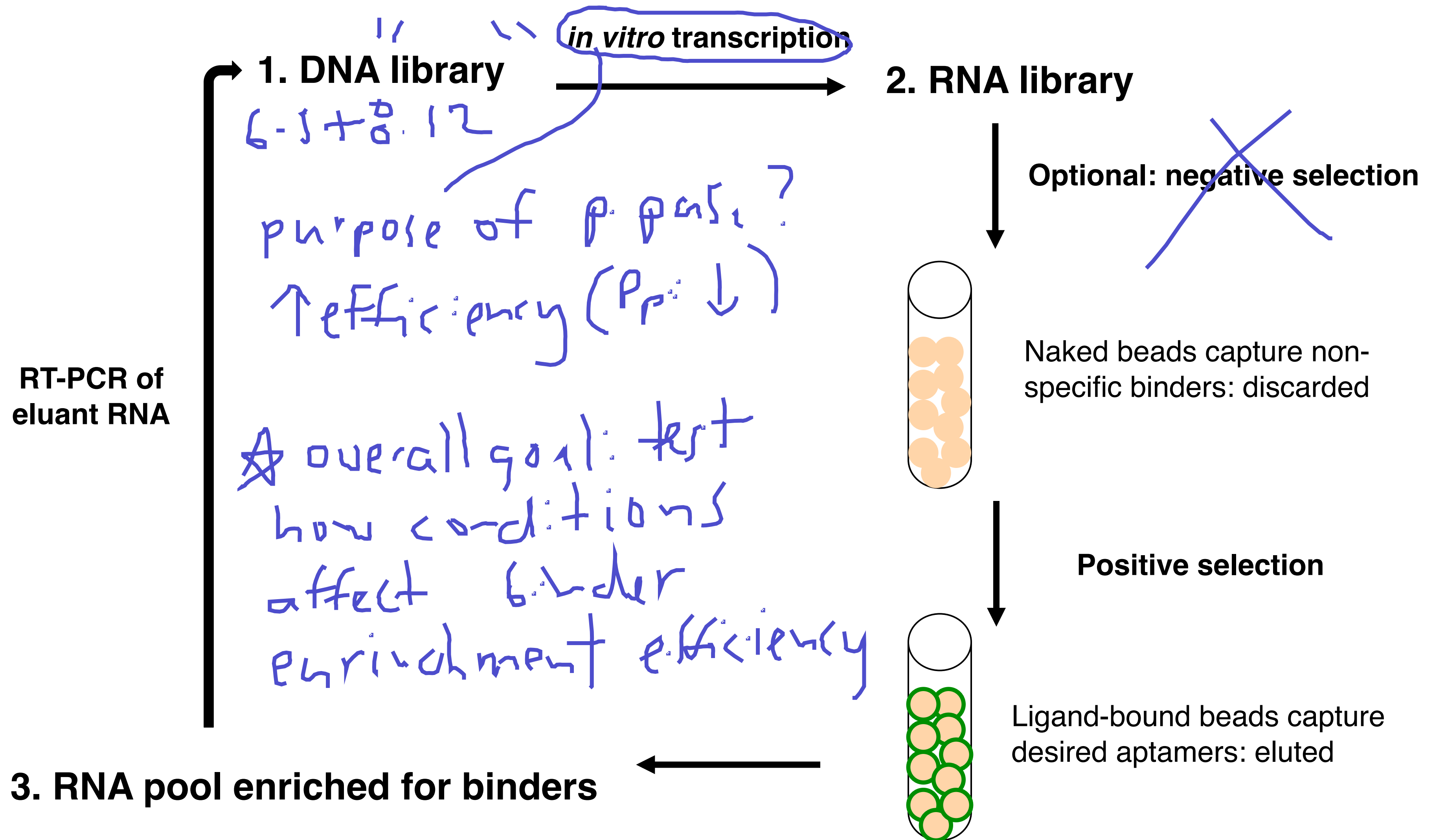
- Mix RNA and heme ~ 1:1
- Measure peak shift



linear Δ in height

50%

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 ~3p
  - Workshop using student examples
- Biosafety talk by Martha Adams from EHS ~4p
- Prepare samples to run on gel