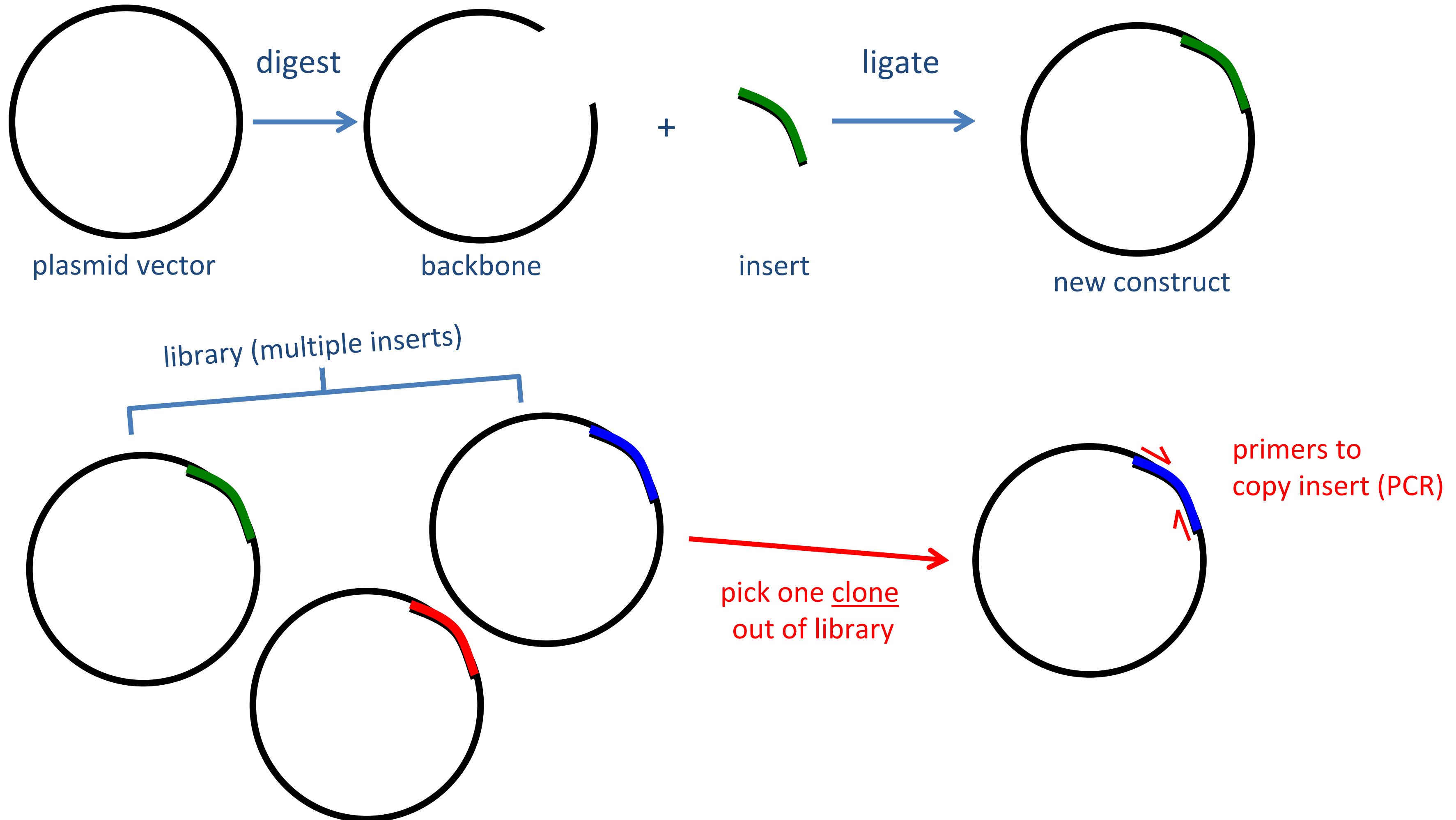
- Announcements
- Lab Quiz
- Pre-lab Lecture
  - Where are we/going?
  - Today in Lab: M1D5

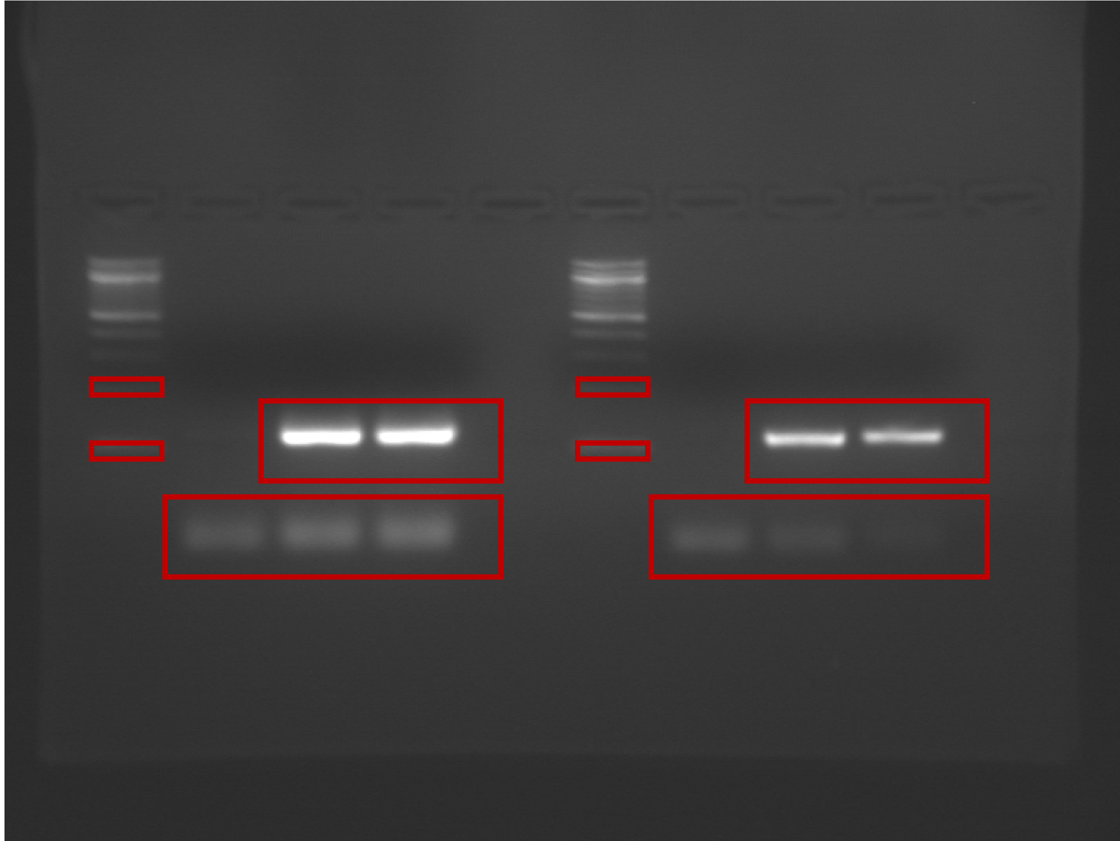
Announcements

- HW: MEME exercise
  - No one selected the correct clones
  - Where can you learn which are unique?
- Next time
  - Meet here to set up IVT
  - Move to 16-336 for talks (~1:30/45 pm)
- FNT: Mfold analysis, (due D7 if you present D6)

Preparing DNA library



PCR results and clarifications



What product size is expected?

How do you know?

What might the lower band be?

Note: along with main cycles, PCR has initial melting and final extension steps that are longer. Why?

IVT and purification

DNA:

T7 RNA Polymerase: binds to T7 promoter (orange)

Purpose of pyrophosphatase: increase efficiency (break down Ppi)

Purify on polyacrylamide column (cf. DNA on silica)

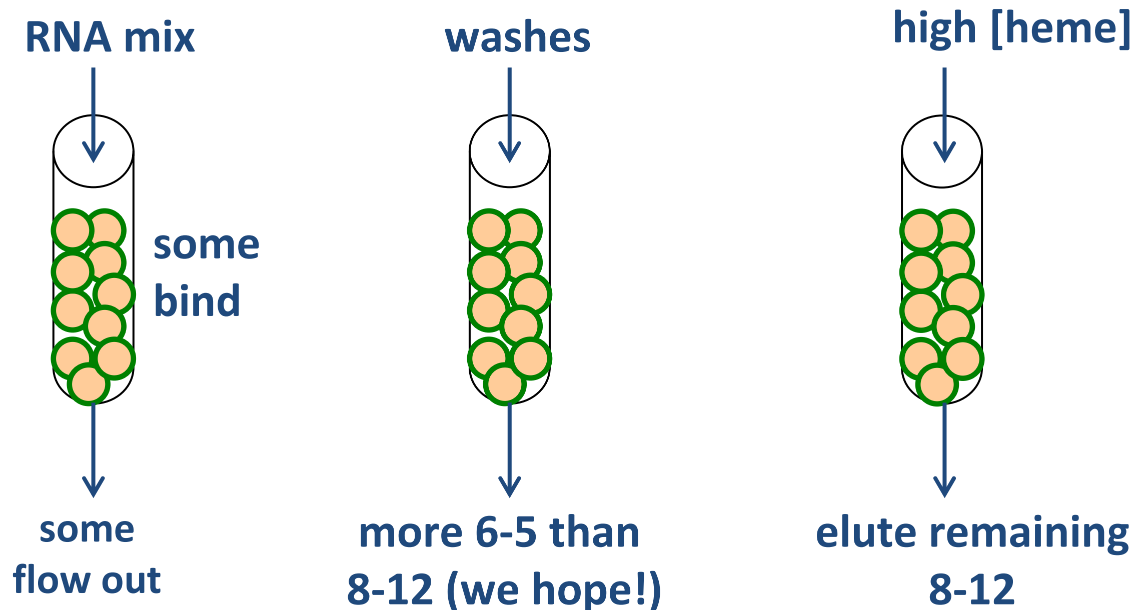
size

chemical, size

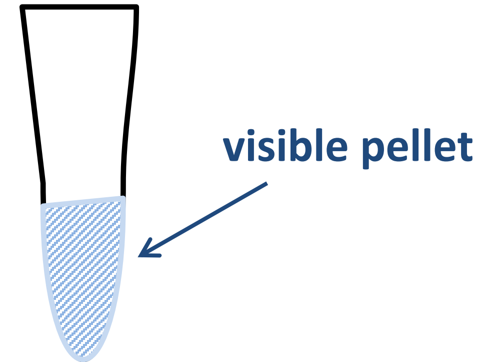
Cartoon from Niles Lecture 2.

Column purification

- Why did we heat the RNA first?
- What might varying wash # do?
- What about varying 8-12%?



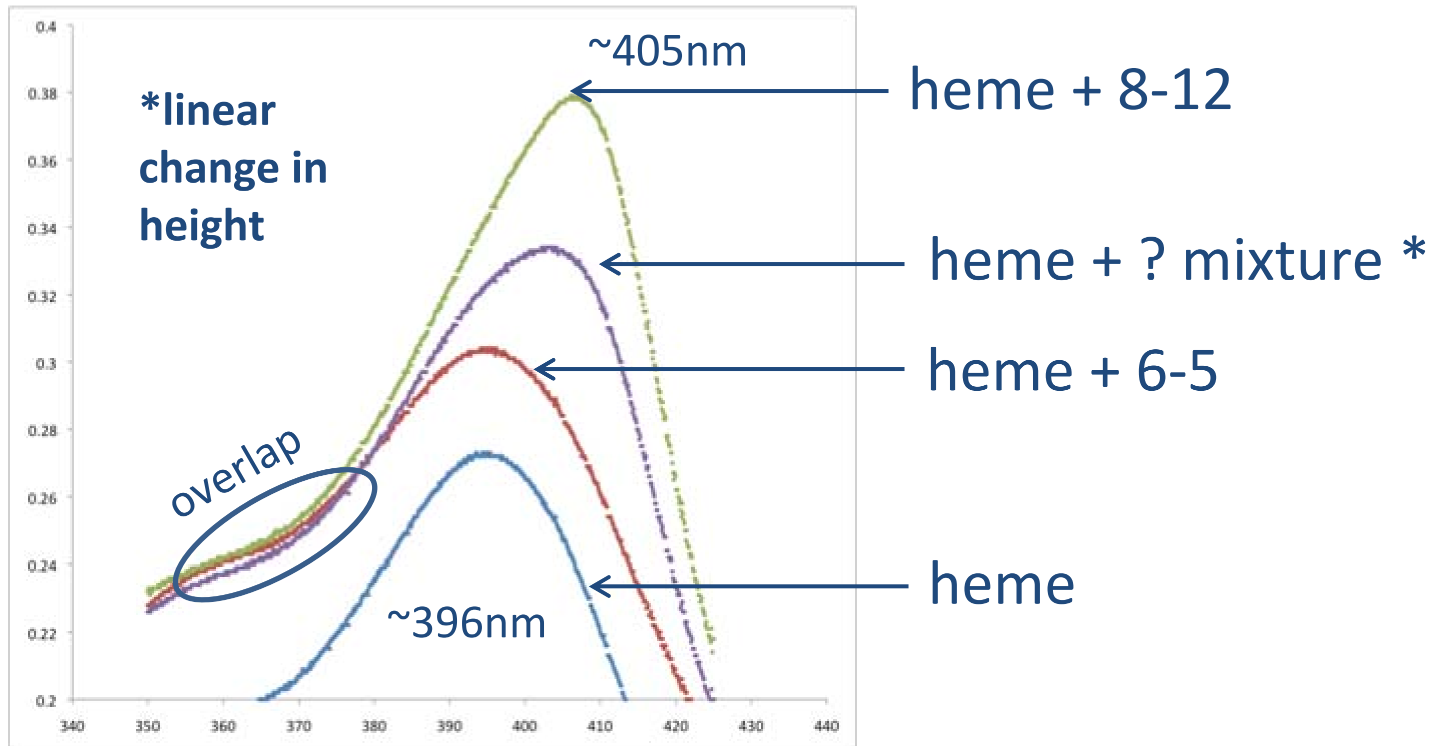
RNA precipitation and RT-PCR



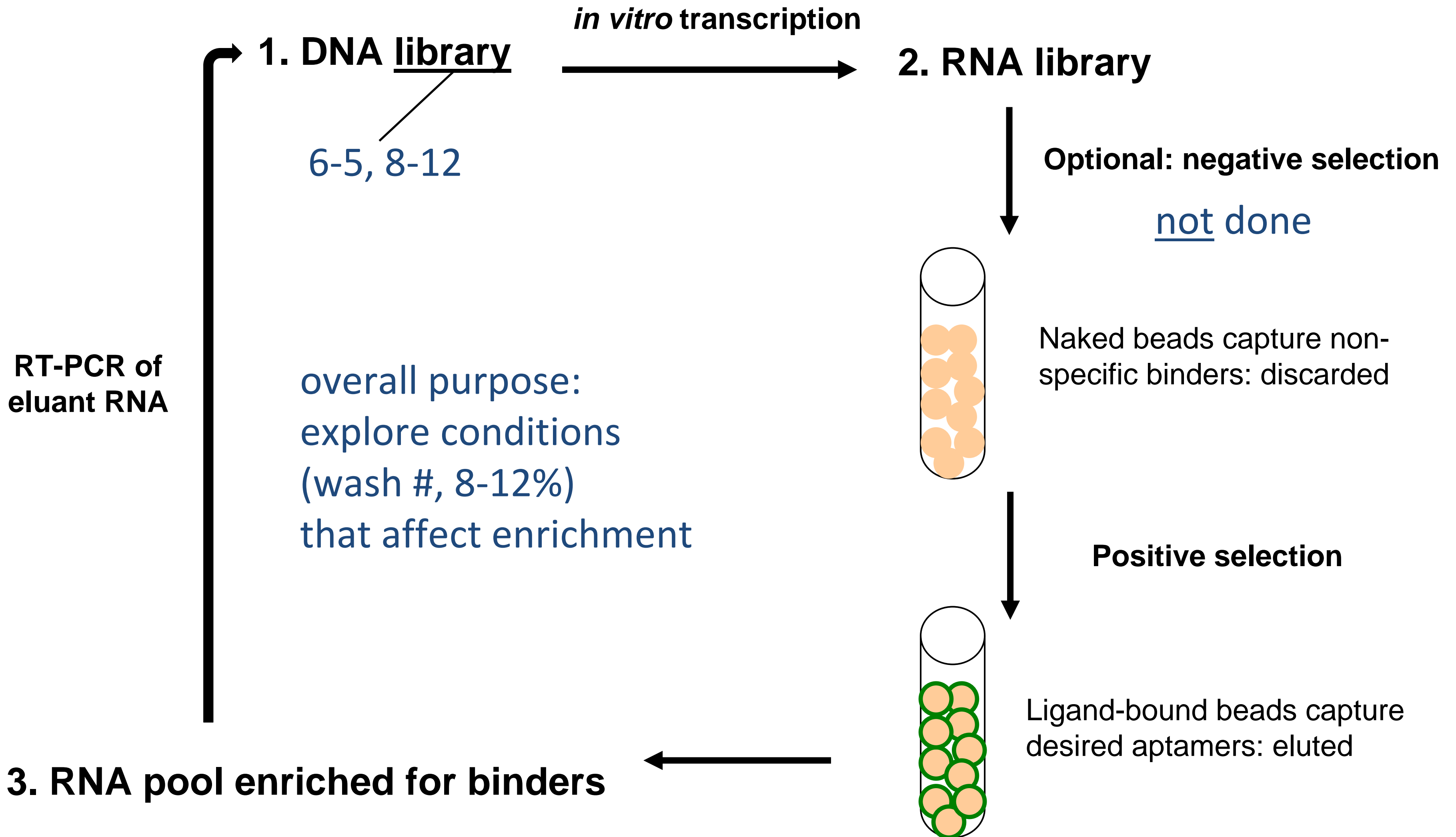
- RNA precipitation
  - Glycogen co-precipitate “carrier”
  - Ethanol lower screening
  - Salt bind/precipitate the RNA
- RT-PCR
  - Step 1: RT RNA → DNA s.s.
  - Step 2: PCR amplify DNA
  - Assume: same rate of amplification for 6-5 & 8-12
  - Purpose of BSA: prevent heme from interfering

Preview of binding assay

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



SELEX Overview



Today in Lab

- Still working with RNA (at first) – careful!
- Collect precipitated RNA, set up RT-PCR
- WAC session
  - Journal club presentations
  - Scientific writing and editing
- Prepare samples to run on gel

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