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

1. Digest plasmid vector
2. Add backbone
3. Add insert
4. Ligase
5. New construct

Library (many inserts) → pick one clone out
(single shot conditions into bacteria)
IVT and purification

DNA:

Transcribed RNA

T7 RNA Polymerase: binds to T7 promoter

Purpose of pyrophosphatase: ↑ efficiency (Pi breaks down)

Purify on polyacrylamide column (cf. DNA on silica)

Cartoon from Niles Lecture 2.
Column purification

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

1. RNA mix  
   - Some bind
   - Some elute

2. Wash (SB)  
   - Move 6-5 than 8-12 washed (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
    \[ RNA \rightarrow DNA \text{ s.s.} \]
  - Step 2: PCR
    \[ \text{amplify DNA d.s.} \]
  - Assume: same copy rate for 6-51, 8-12
  - Purpose of BSA: prevent hem interference
Preview of binding assay

• Mix RNA and heme ~ 1:1
• Measure peak shift
SELEX Overview

1. DNA "library"
   6-5, 8-12
   \textit{in vitro} transcription
   \textbf{overall purpose:}
   \textbf{how conditions affect enrichment} (9, 8-12, wash #)

2. RNA library
   \textbf{optional: negative selection}
   Naked beads capture non-specific binders: discarded
   \textbf{positive selection}
   Ligand-bound beads capture desired aptamers: eluted

3. RNA pool enriched for binders

RT-PCR of eluant RNA
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