Module Overview

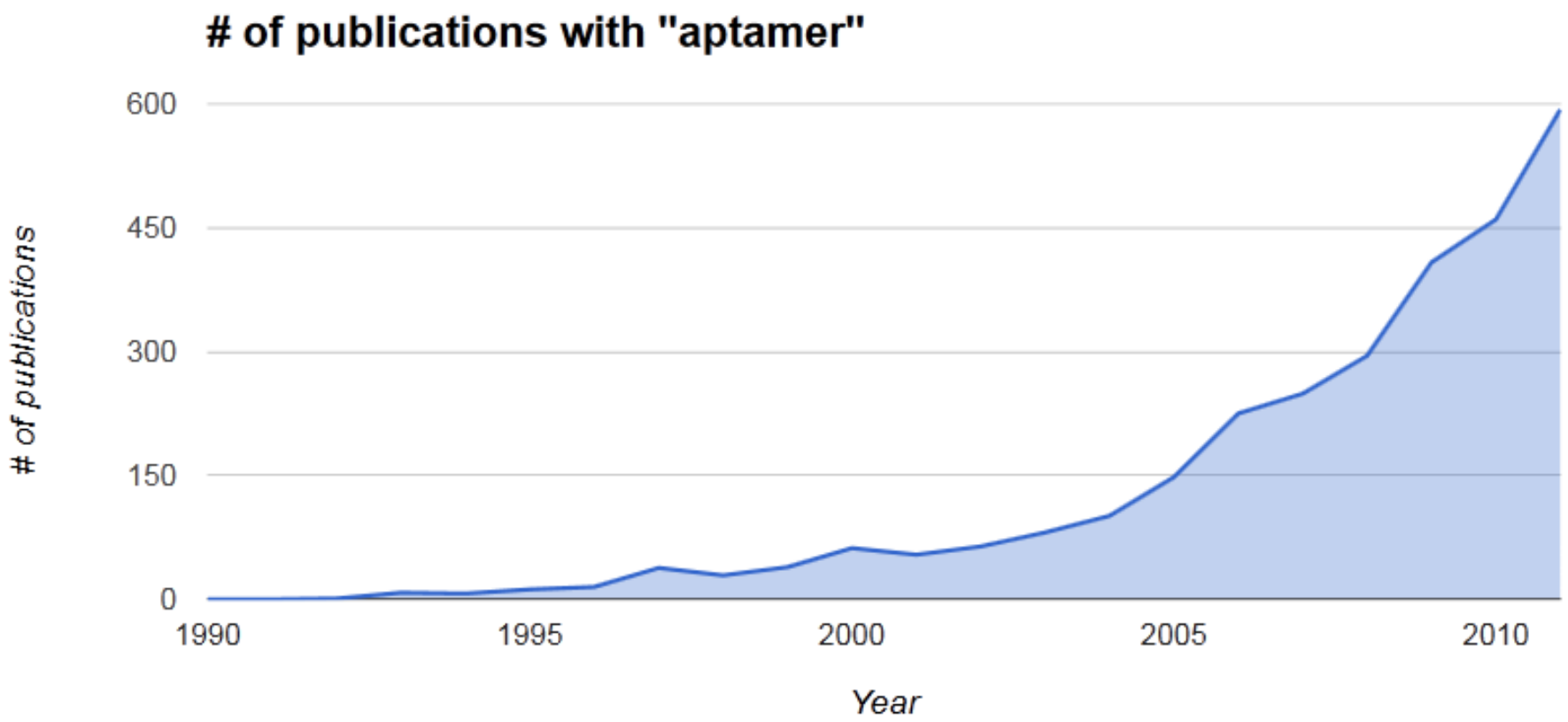
Day	Lecture	Lab
1	Introduction	DNA library synthesis (PCR)
2	SELEX I: Building a Library	DNA library purification (agarose gel electrophoresis)
3	SELEX II: Selecting RNA with target functionality	RNA library synthesis (*In vitro* transcription = IVT)
4	SELEX III: Library deconvolution, problem-solving & technical advances	RNA purification and heme affinity selection
5	Characterizing aptamers	RNA to DNA by RT-PCR
6	Introduction to porphyrins: chemistry & biology	Post-selection IVT Journal Club 1

SELEX III

20.109 Lecture 4

23 February, 2012

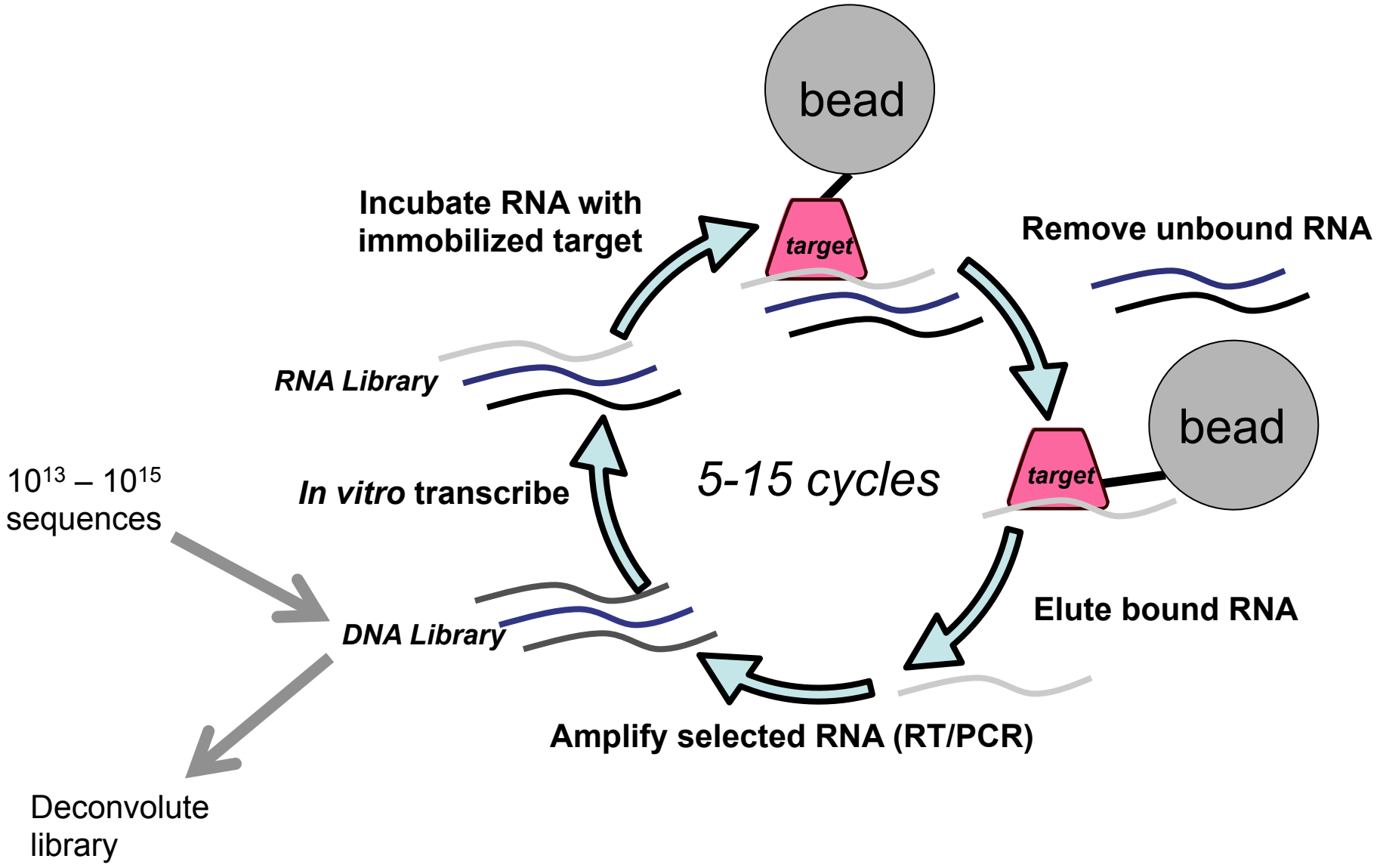
Aptamers are gaining popularity



Today's Objectives

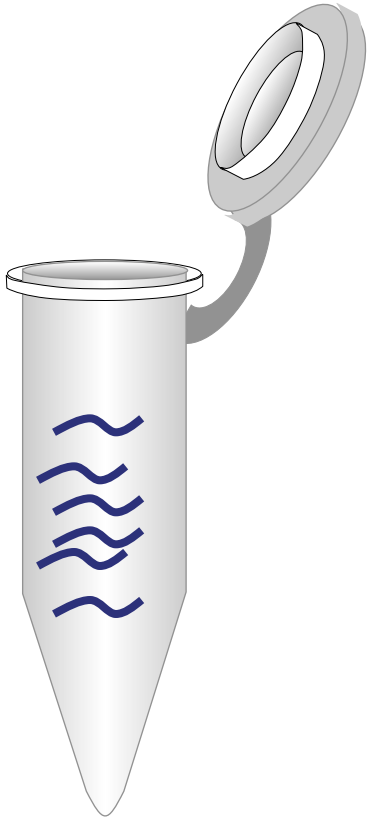
- Deconvoluting a SELEX library
- How do you know you've succeeded (or failed)?
- Conceptualizing selection stringency
- Things to consider if/when SELEX fails

Typical SELEX



Deconvoluting your selected library

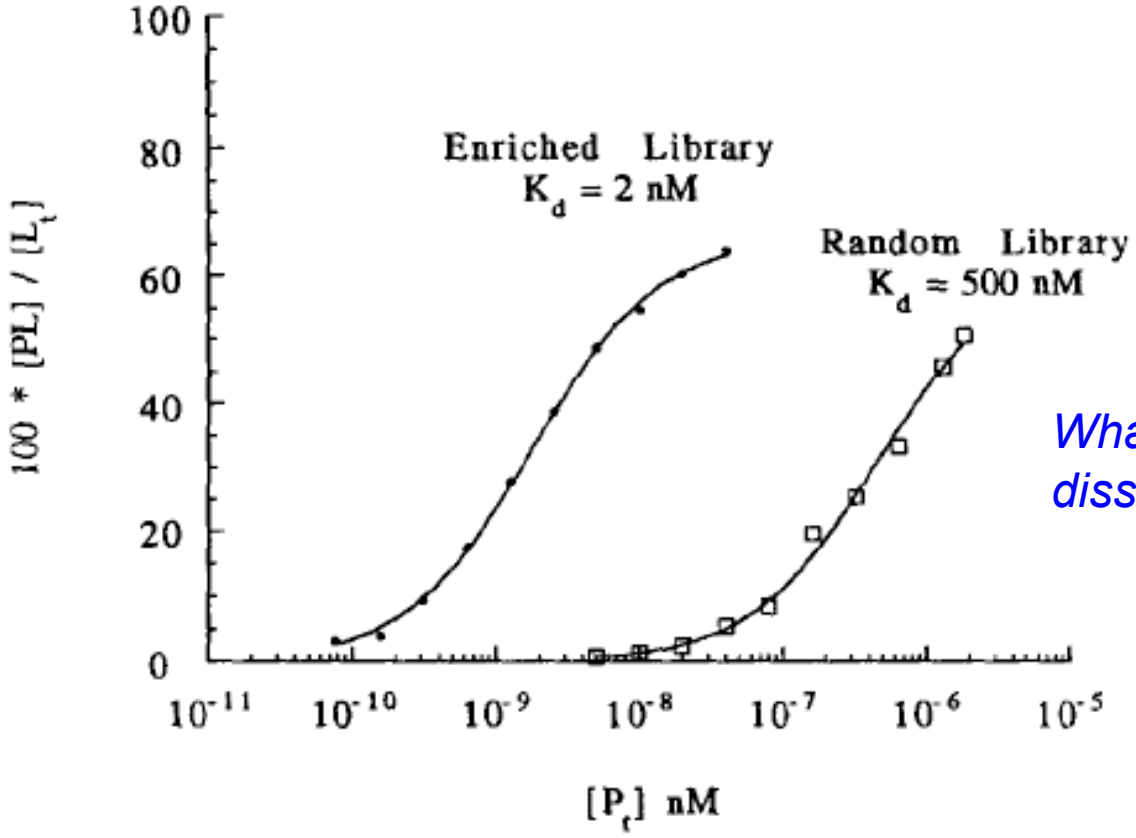
- Was SELEX successful???
  - *How test?*



- If your SELEX was successful:
  - How identify the individual library members?
  - How identify conserved sequences/motifs?

Determining the success of your SELEX experiment

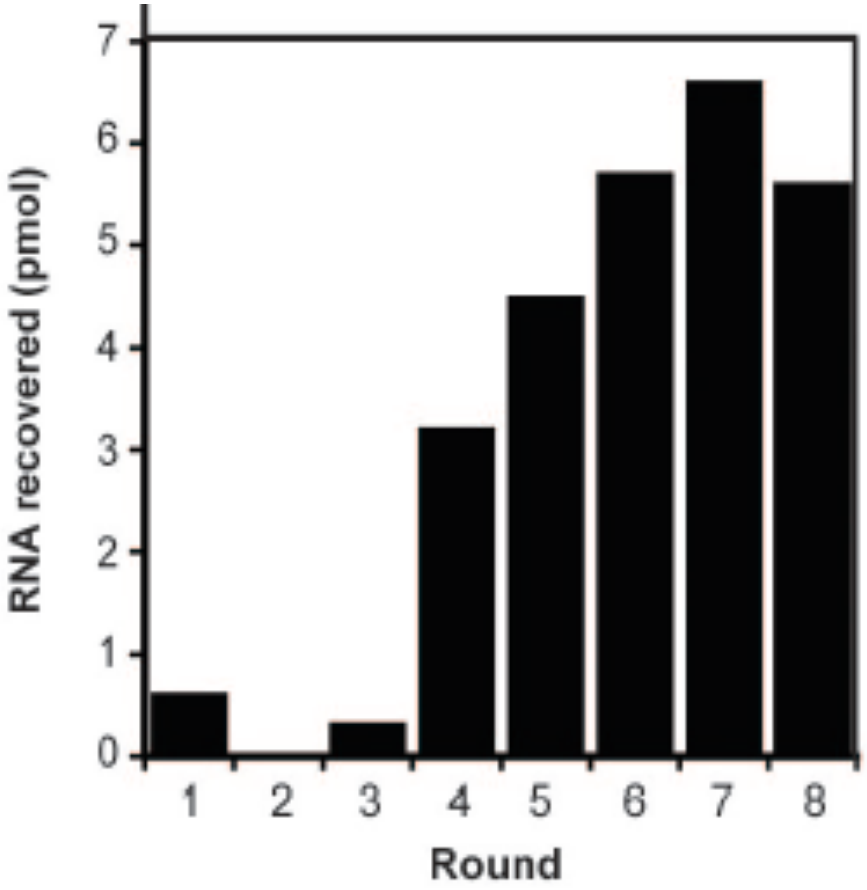
- Compare library dissociation constants pre- and post- SELEX



What does it mean to have a larger dissociation constant or K_d?

Schneider *et al*, ***FASEB J***,, 7(1), 201-207, 1993

Determining the success of your SELEX experiment

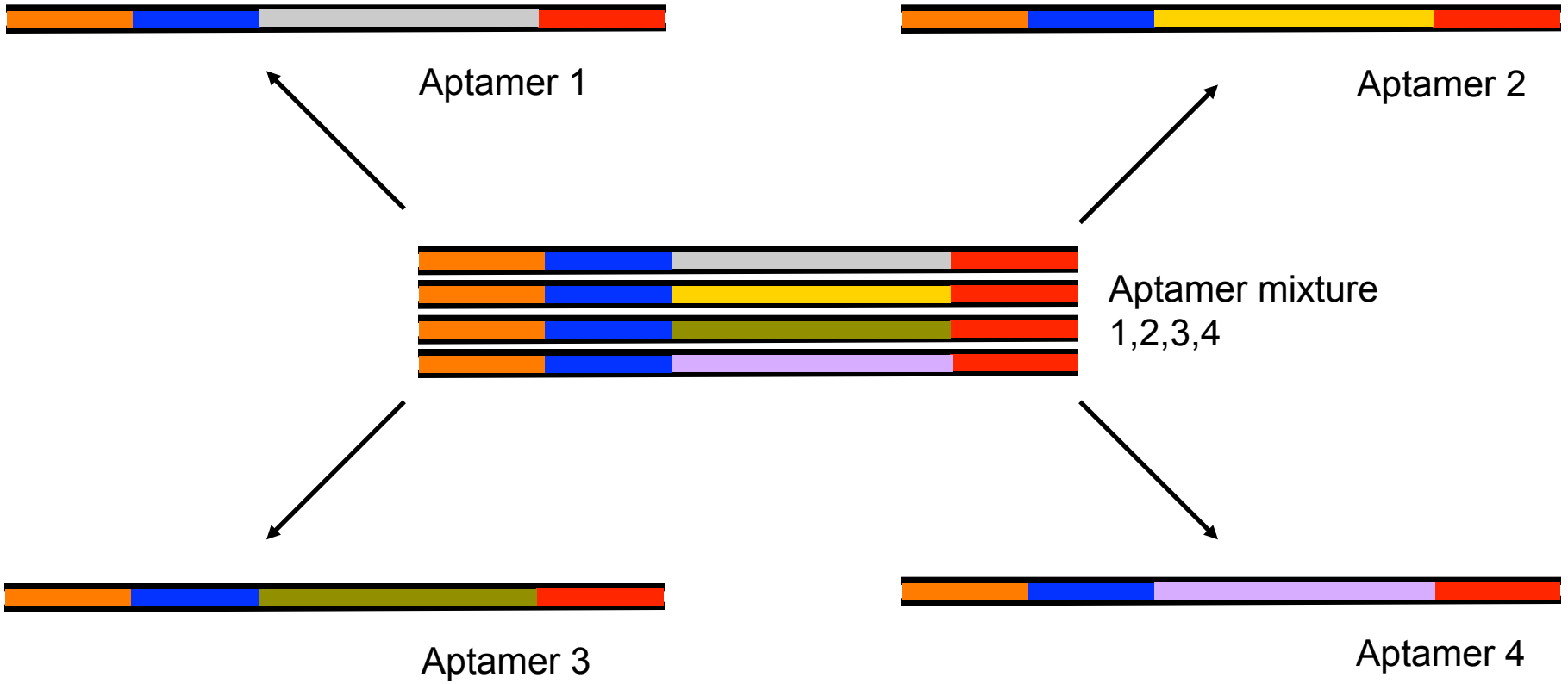


- Measure RNA recovered after each round of selection (quantitative PCR)

- **Advantages**:
  - Determine progress in real time
  - Facilitates rapidly knowing the impact of changing SELEX conditions

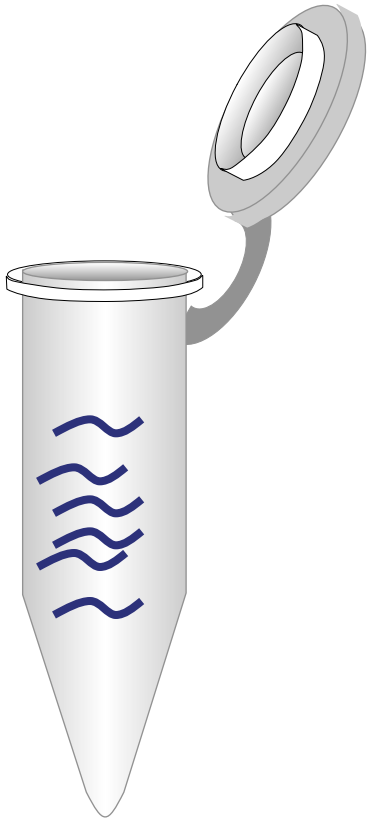
Library deconvolution

– Isolate individual aptamers for:
-> Sequencing (identification)
-> Characterization (binding, etc)

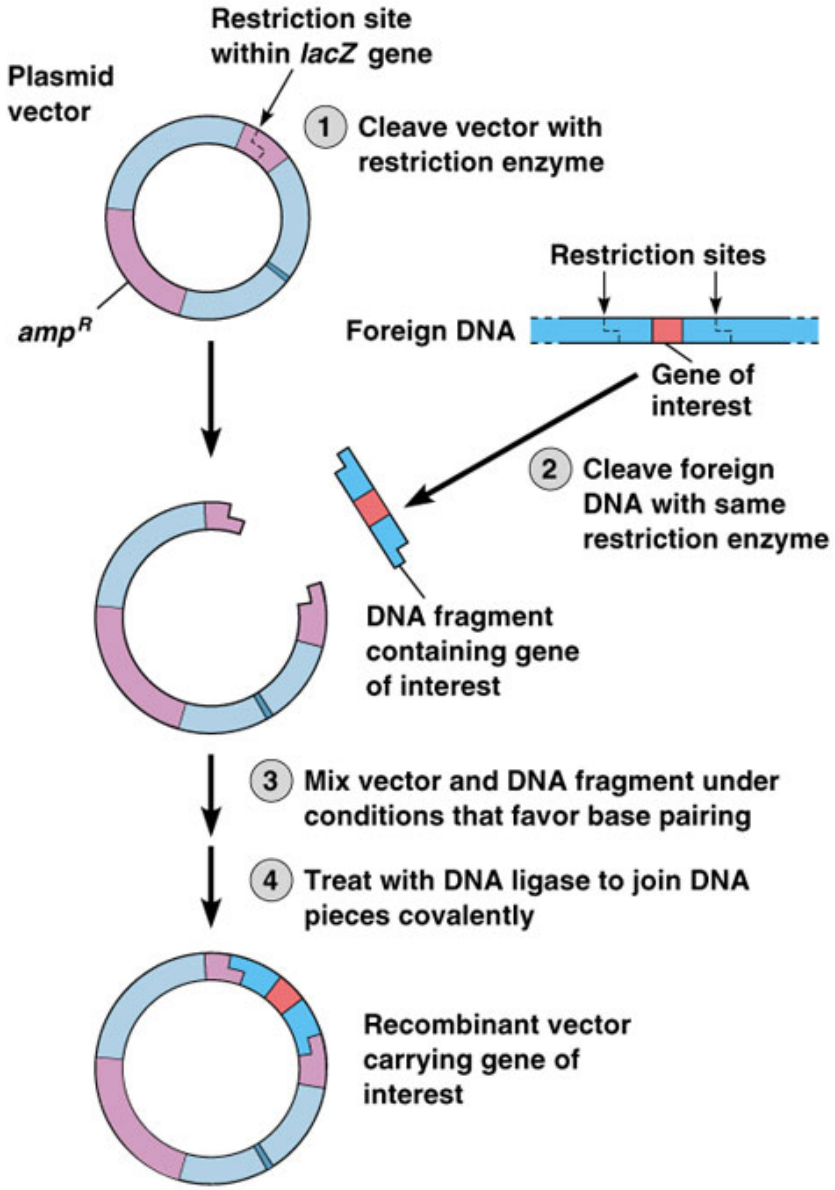


Library deconvolution

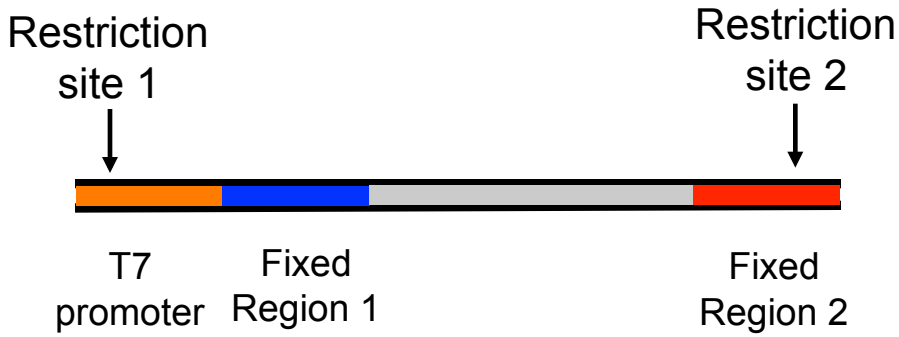
- You observe binding of your bulk selected library to the target
  - ~ 10^{14} unique members in starting library
  - *How many are present at the end?*
- Identifying individual aptamers in your library
  - *How would you do this?*
- One option: **Exactly how you'd clone a new gene!**



Cloning the aptamer library

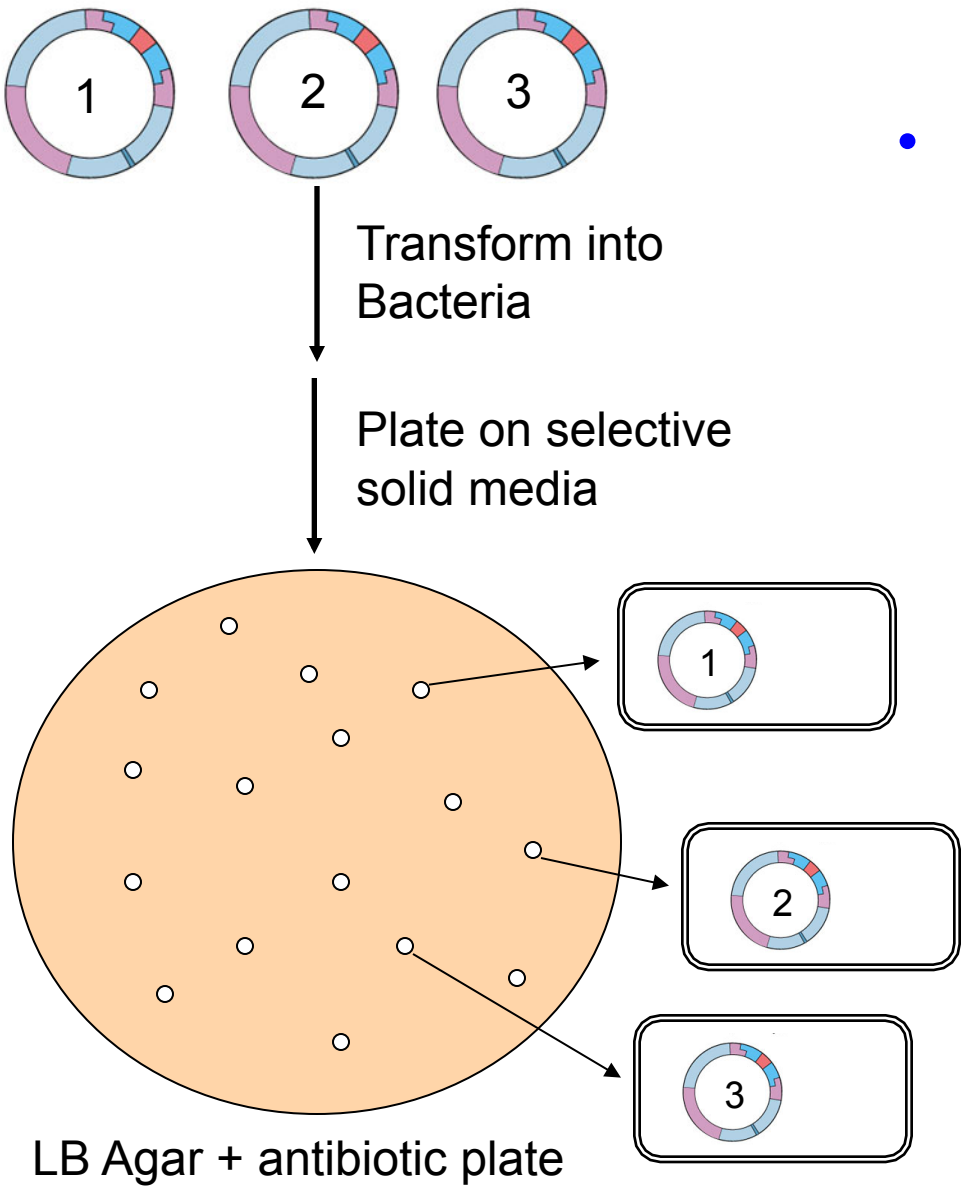


Addison Wesley Longman, Inc.



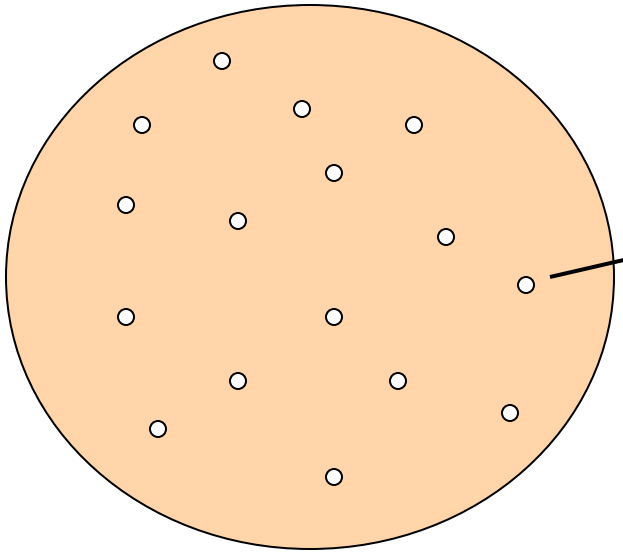
- **Single hit conditions**:
  - One insert on average incorporated into one plasmid
  - Each plasmid now encodes a single aptamer
- **Problem**
  - You have a mixture of plasmids
  - *How do you isolate clonal plasmids?*

Cloning the aptamer library



- **Bacterial transformation**
  - Single hit conditions:
    - On average: ≤1 plasmid per bacterial cell
  - Plating on selective media:
    - Single colony derived from a single bacterial cell
    - Each colony contains many bacterial cells, each carrying the identical plasmid

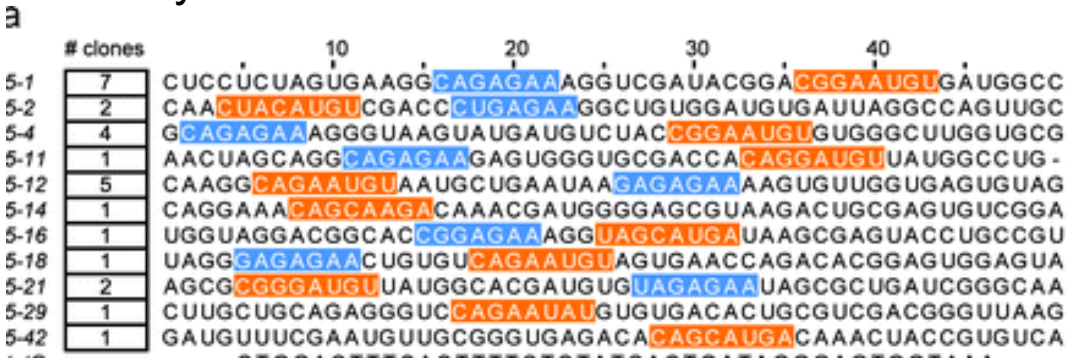
Aptamer library now encoded in plasmid library



Glycerol stocks
(storage @ -80ºC)

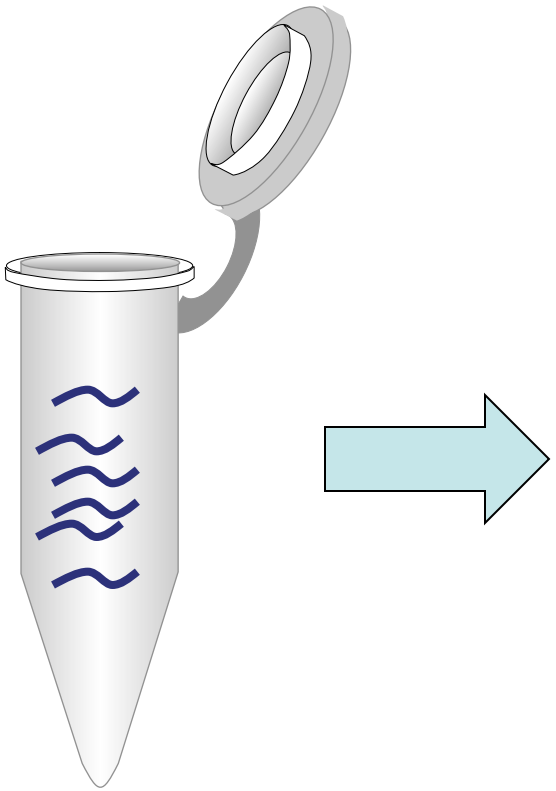
Mini-prep to isolate plasmid
- Aptamer sequencing
- *In vitro* transcription to obtain aptamer

- Achieved:
  - Mixture of aptamers in selected library resolved into a plasmid library of individual aptamers
  - Preserved ability to manipulate library
  - Library archive



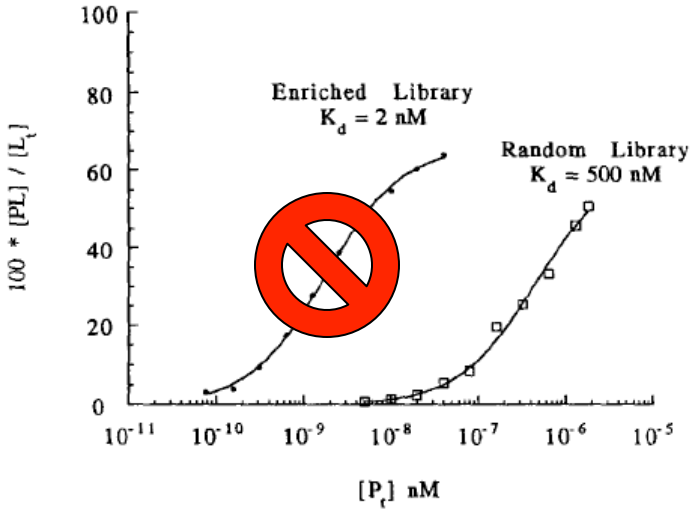
The future of deconvolution

- High-throughput sequencing
  - Same instruments sequence genomes
  - Useful for early rounds



…but what went wrong with my SELEX? some common scenarios

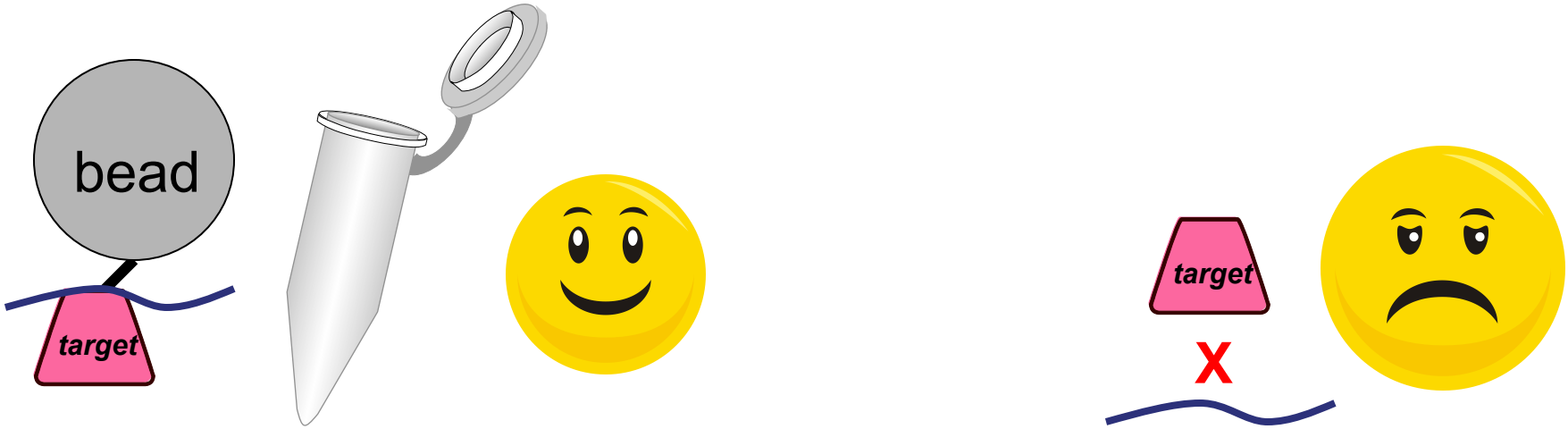
1. No detectable binding to target



- Why might this occur?
  - Problem with your binding assay
    - *How might you assess this?*
  - Too few rounds of selection completed
    - *How would you determine this?*
  - Your selection process went awry
    - Poor choice of selection stringency conditions
    - Sequences selected based on amplification efficiency, **NOT** target binding
      - PCR, RT, *in vitro* transcription

…but what went wrong with my SELEX?
Some common scenarios

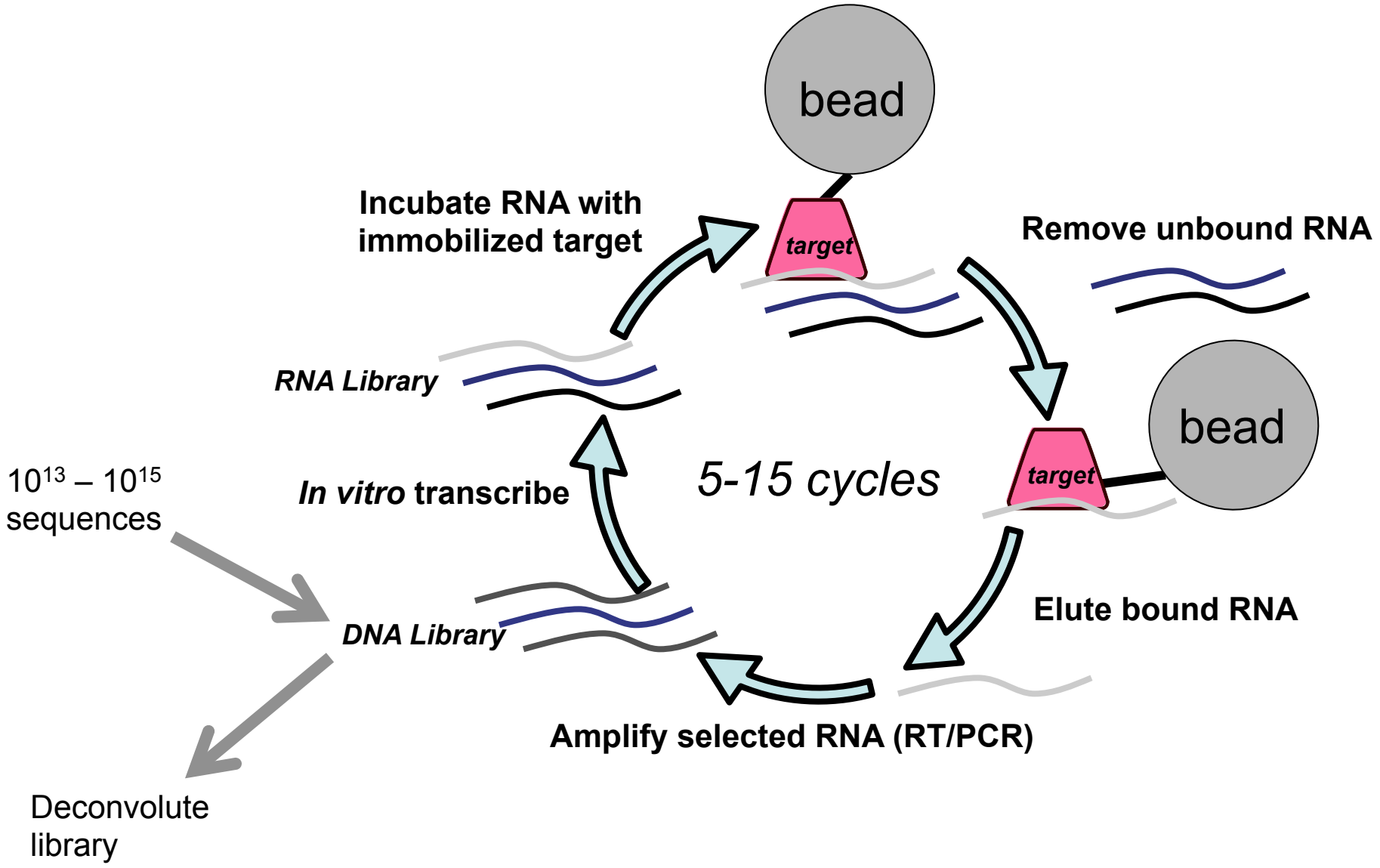
2. Aptamers bind target, but ONLY when immobilized in the format used during SELEX



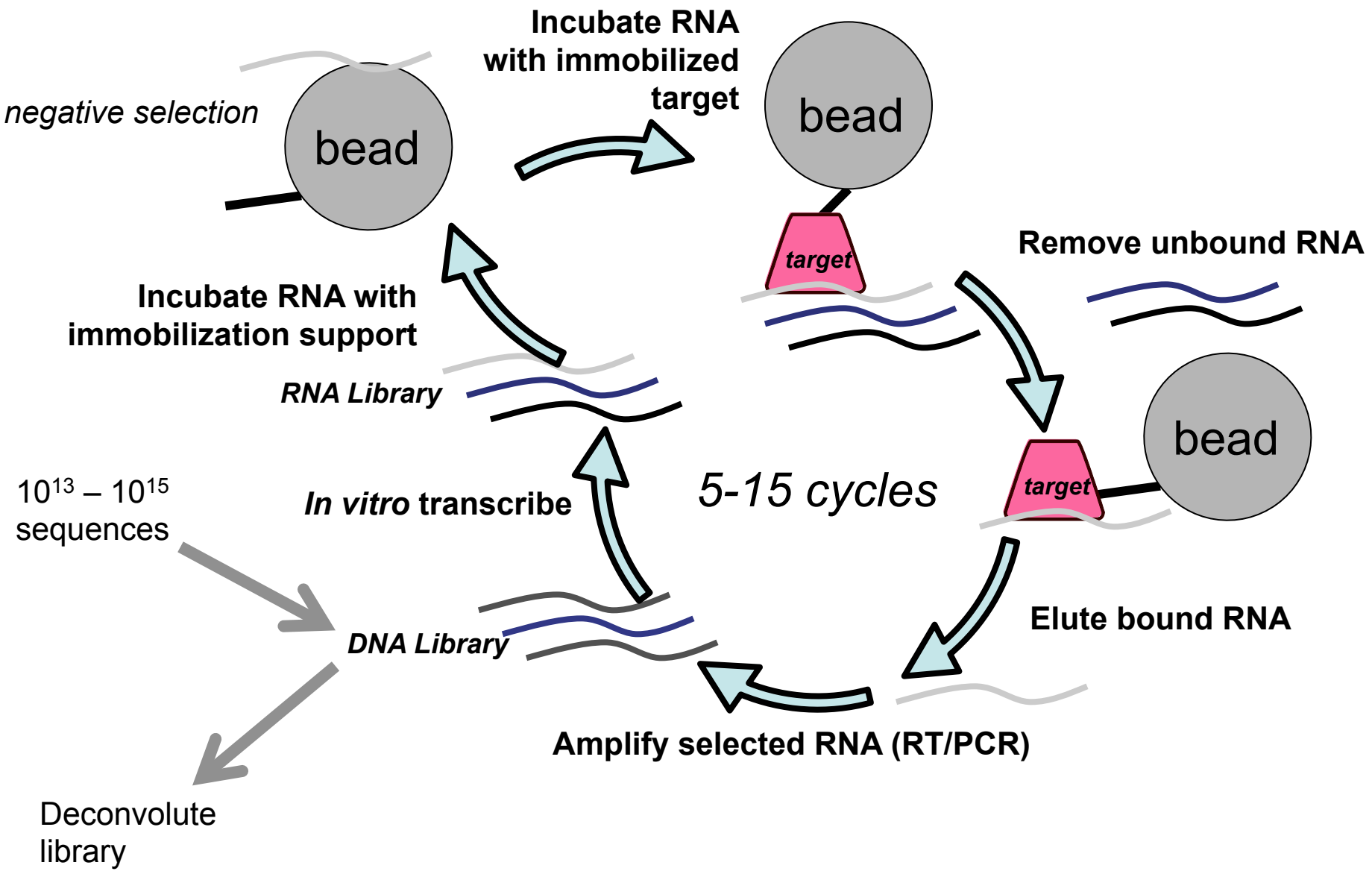
- *Why might this arise?*
  - Aptamers partially or completely recognize and bind to the solid support!

- *How would you change your selection format to avoid this?*

Typical SELEX



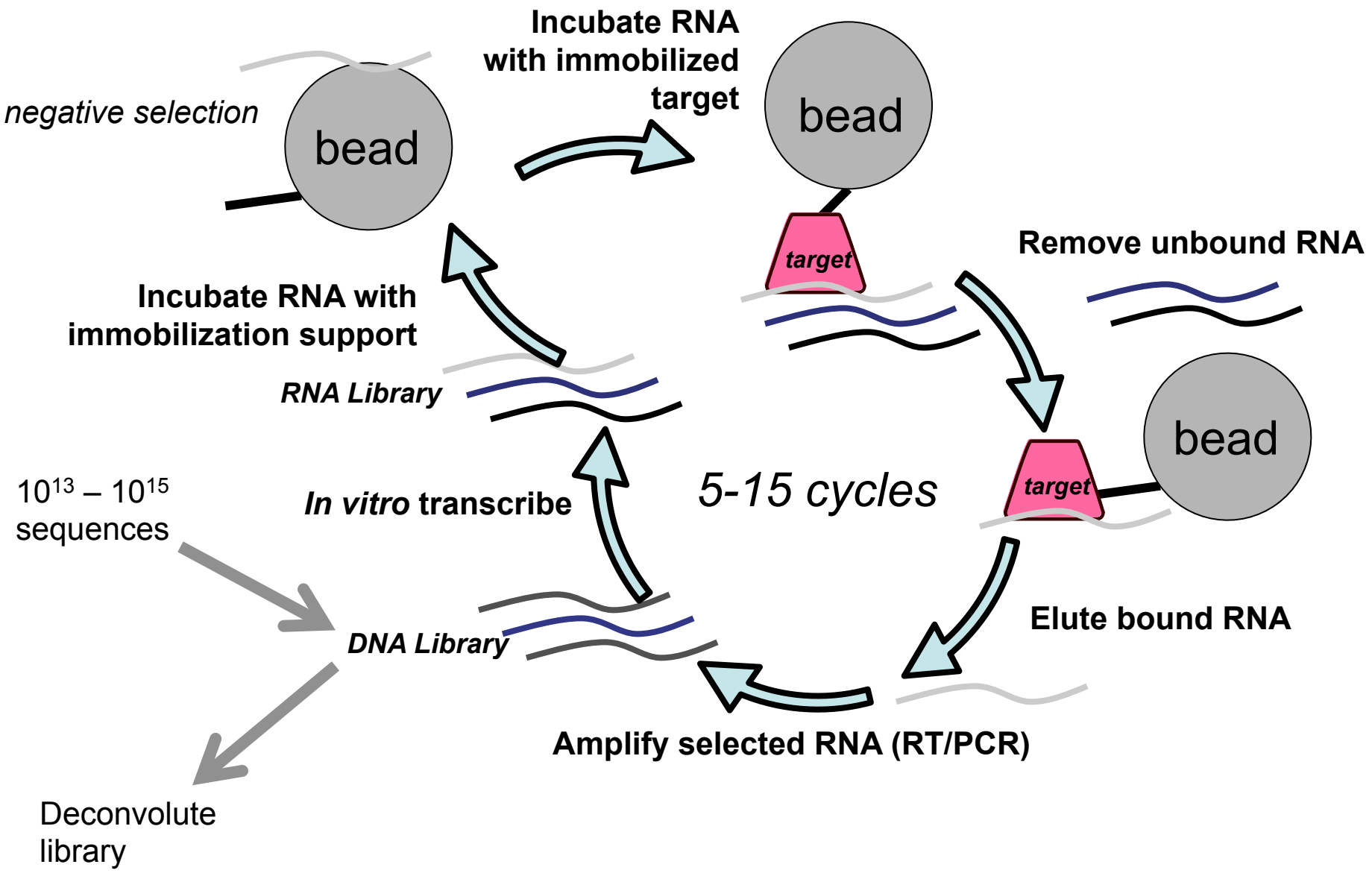
Negative selection step



Maximizing SELEX efficiency

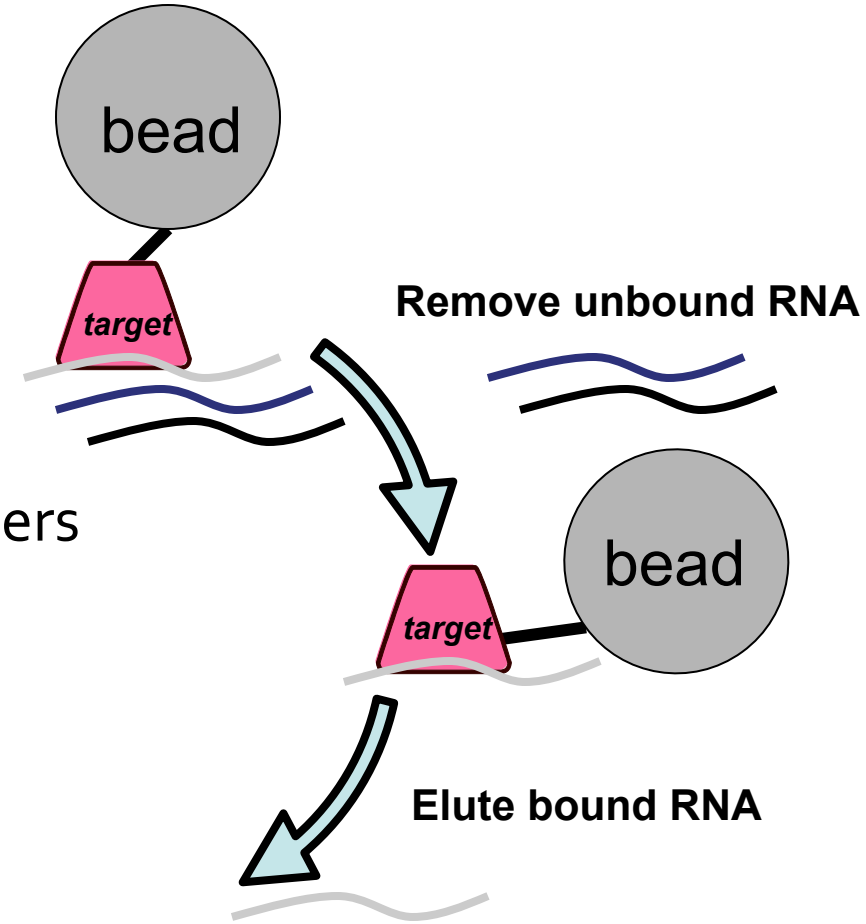
- **Design specifications**
  - Obtain target aptamers on first try
  - Fewest possible number of rounds
  - High affinity

SELEX


negative selection
bead
Incubate RNA with immobilized target
bead
target
Remove unbound RNA
Incubate RNA with immobilization support
RNA Library
bead
target
10^13 – 10^15 sequences
In vitro transcribe
5-15 cycles
DNA Library
Elute bound RNA
Amplify selected RNA (RT/PCR)
Deconvolute library

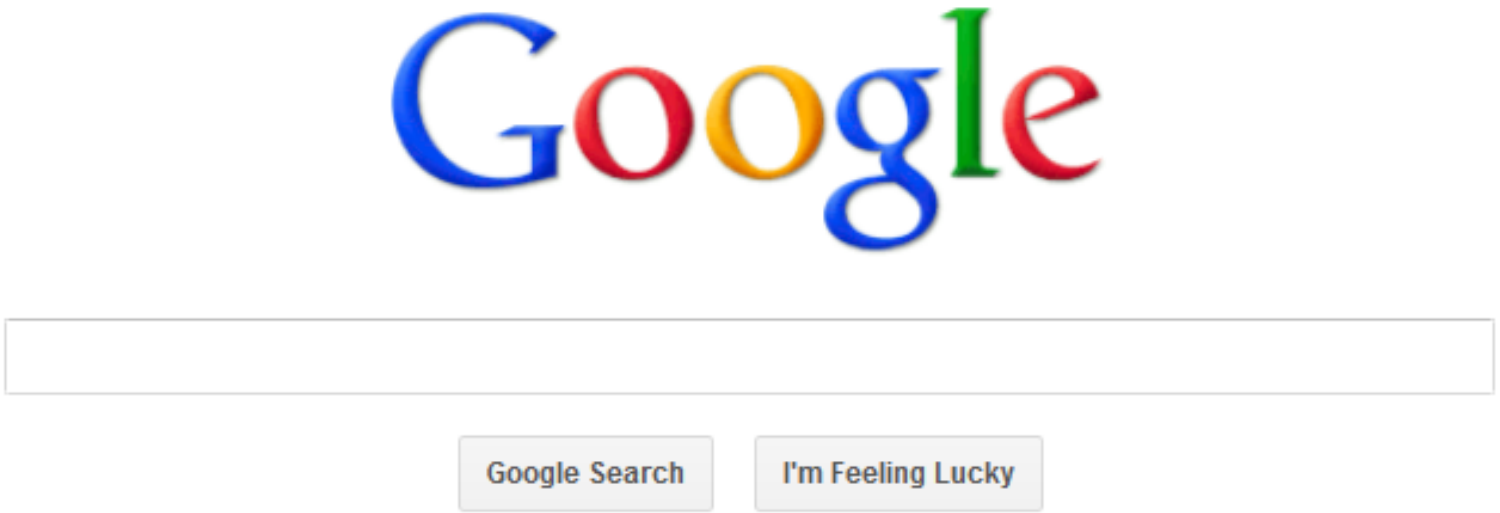
Maximizing SELEX efficiency: stringency

- **Design specifications**
  - Obtain target aptamers on first try
  - Fewest possible number of rounds
  - High affinity



- Keep binders, remove non-binders

Stringency: everyday example



- Not too broad, not too focused…just right

puppy

Brian Belmont 0 + Share

About 286,000,000 results (0.27 seconds)

SafeSearch

Related searches: **cute** puppy **beagle** puppy **puppies for sale** **dogs and puppies** **puppies wallpaper**

puppy white small

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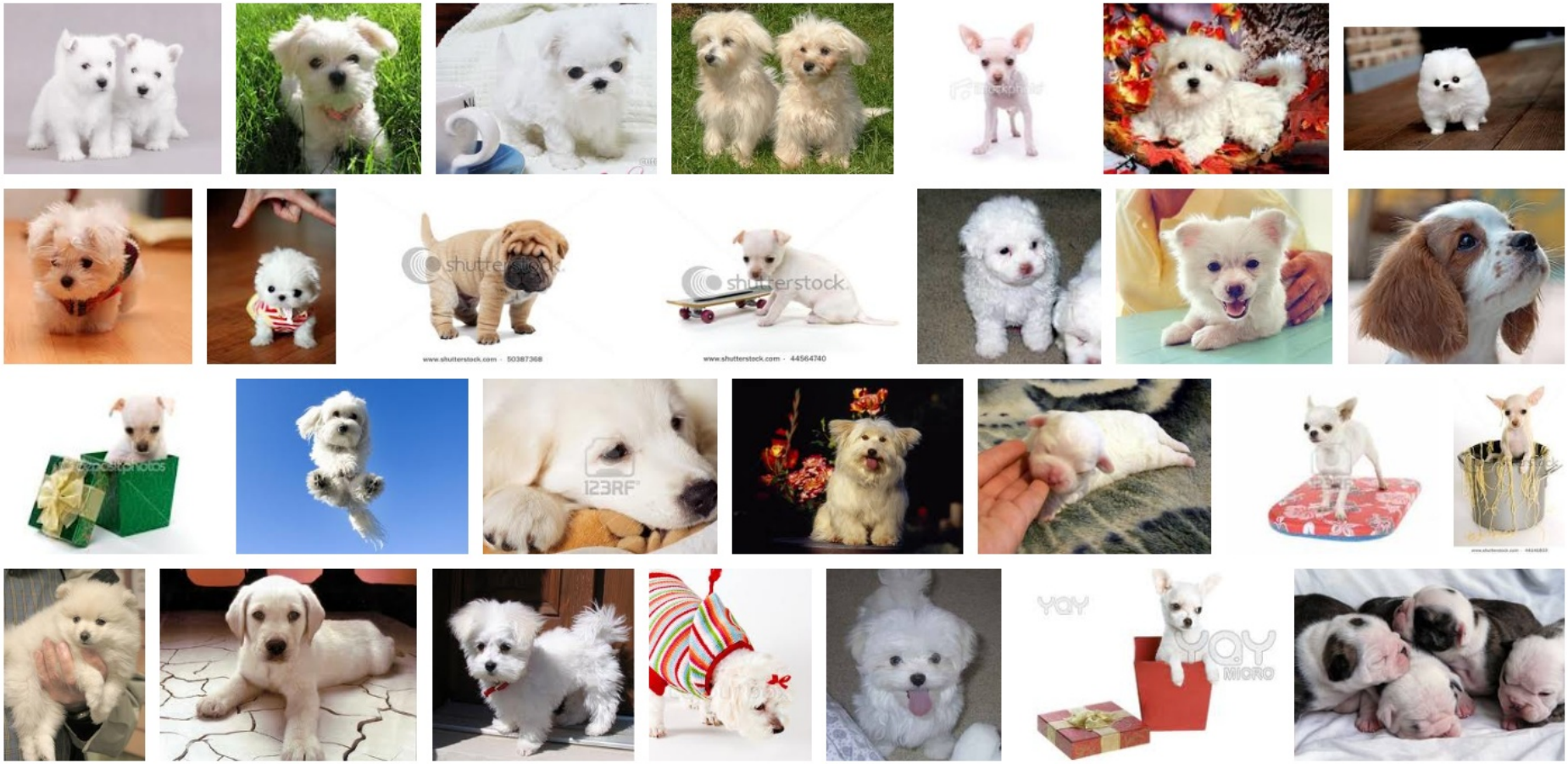
About 32,500,000 results (0.44 seconds)

SafeSearch



puppy white small "Cambridge, MA"

Brian Belmont 0 + Share

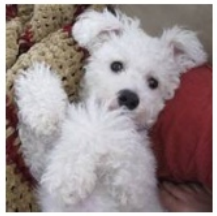
About 109,000 results (0.55 seconds)

SafeSearch















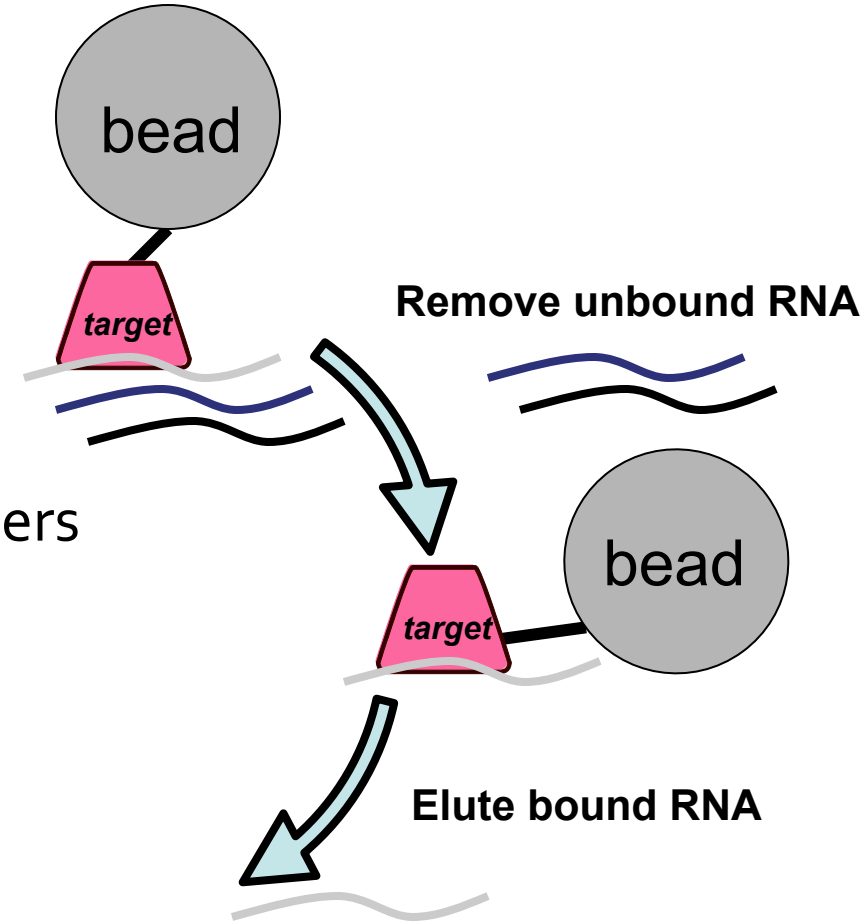






Maximizing SELEX efficiency: stringency

- **Design specifications**
  - Obtain target aptamers on first try
  - Fewest possible number of rounds
  - High affinity



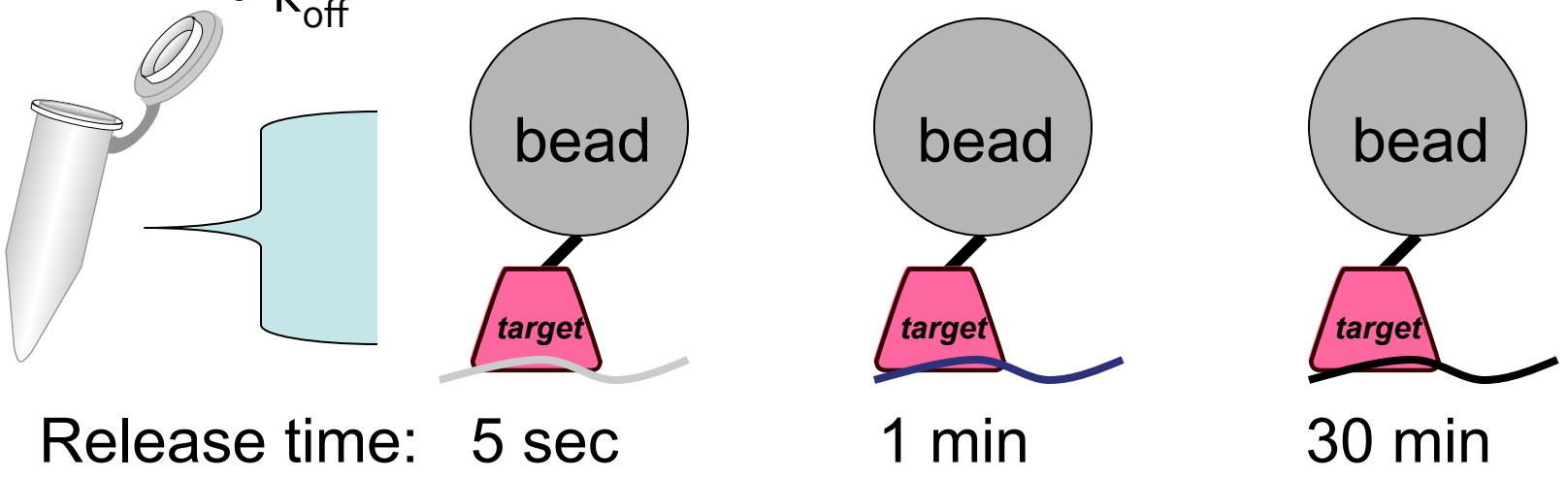
- Keep binders, remove non-binders

- *How adjust stringency???*

SELEX stringency: washing

- Washing
  - Higher stringency --> more/longer washes
  - Lower stringency --> fewer/shorter washes

  - Specifies thermodynamics
    - Dissociation constant
    - k_{off}

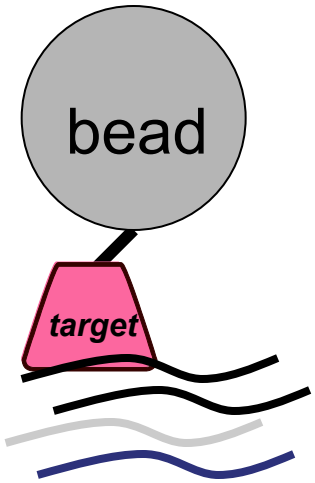


SELEX stringency: [RNA]:[target] ratio

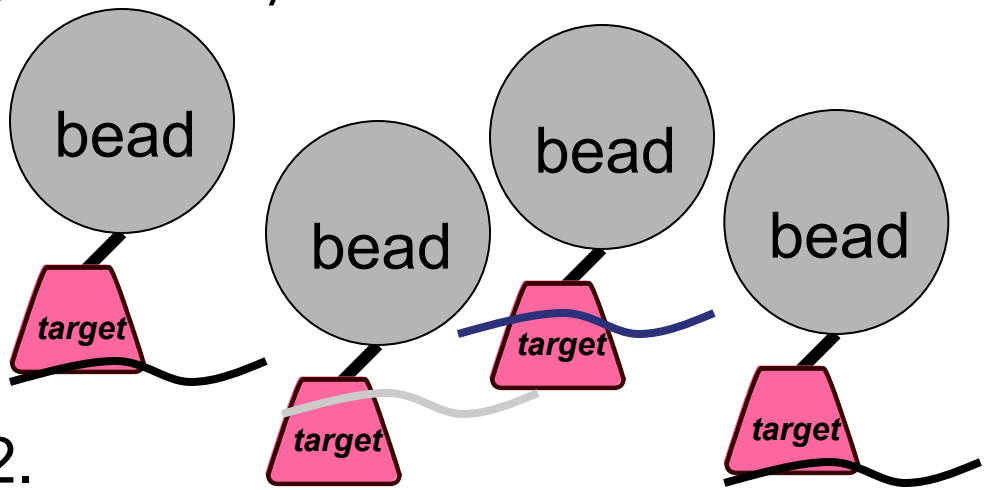
- [RNA]:[target] ratio
  - Higher stringency --> higher ratio
  - Lower stringency --> lower ratio

  - Specifies thermodynamics
    - Dissociation constant
    - Favor recovering higher affinity RNA

1.

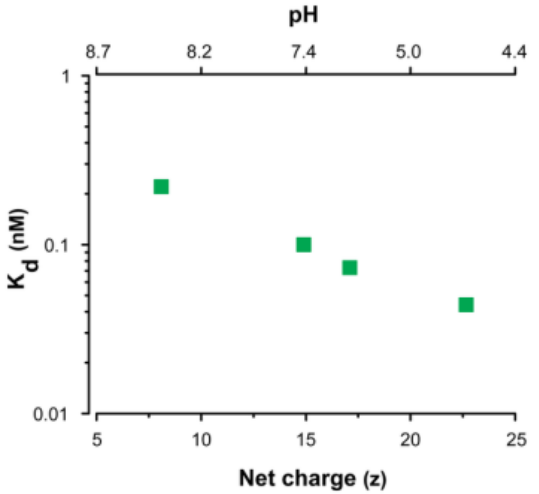


2.

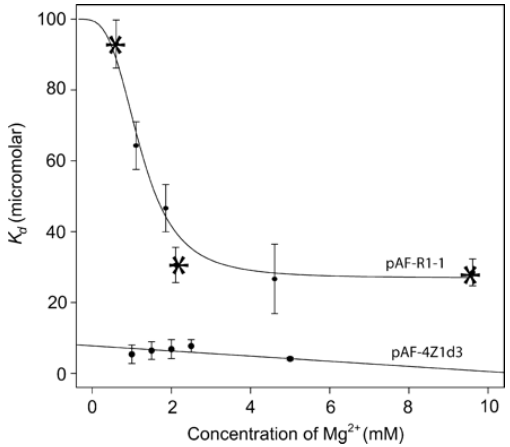


SELEX stringency: buffer

- Buffer components/additives
  - pH
  - tRNA (nucleic acid)
  - BSA (protein)
  - Salt concentrations



Ahmad, K. M. *et al.* Probing the Limits of Aptamer Affinity with a Microfluidic SELEX Platform. *PLoS ONE* **6**, e27051 (2011).



Carothers, J. M., Goler, J. A., Kapoor, Y., Lara, L. & Keasling, J. D. Selecting RNA aptamers for synthetic biology: investigating magnesium dependence and predicting binding affinity. *Nucl. Acids Res.* **38**, 2736–2747 (2010).

My parameter optimization space is HUGE…help!?

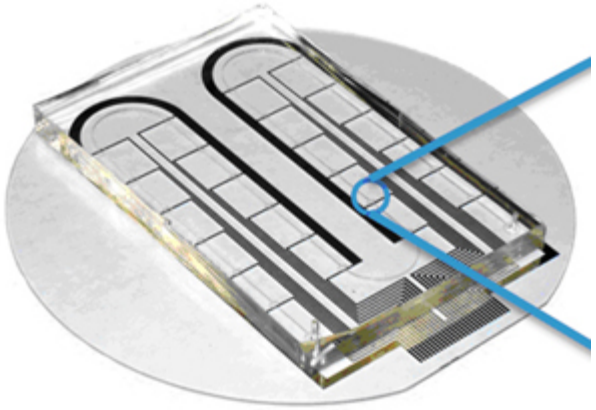
- **Vary**:
  - Wash number
  - [Library]:[target] ratio
  - Buffer conditions
    - pH
    - [salt]
    - tRNA
    - BSA (protein)

- Where do I start my SELEX?

- Which variable(s) do I change if it fails?

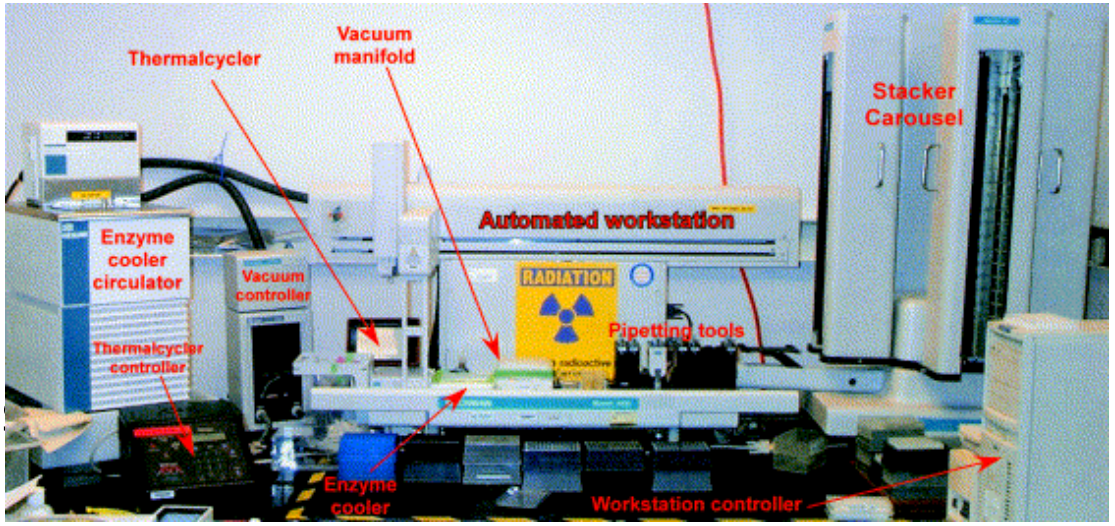
Automating SELEX

- Library synthesis (DNA synthesizer)



microfluidics

- Enzymatic reactions
  - PCR (thermal cycler)
  - RT (thermal cycler)
  - *In vitro* transcription (thermal cycler)
- Binding reactions
  - 96-well plates (shakers)
- Inter-process sample tran
  - Liquid handling robots



Cox & Ellington, ***Bioorganic & Medicinal Chemistry***, 9(10), 2525-2531, 2001

Leaders and future of SELEX

- Diagnostics

SomaLogic

  - >1000 aptamers to blood proteins

- Contract discovery

AptaMatrix

Base Pair BIOTECHNOLOGIES The Aptamer Discovery Company

Aptagen

Summary

- Determine library characteristics
- Define individual sequences
- Adjust SELEX stringency