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: Technical advances & problem-solving	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
7	Aptamer applications in biology & technology	Aptamer binding assay
8	Aptamers as therapeutics	Journal Club 2

SELEX III

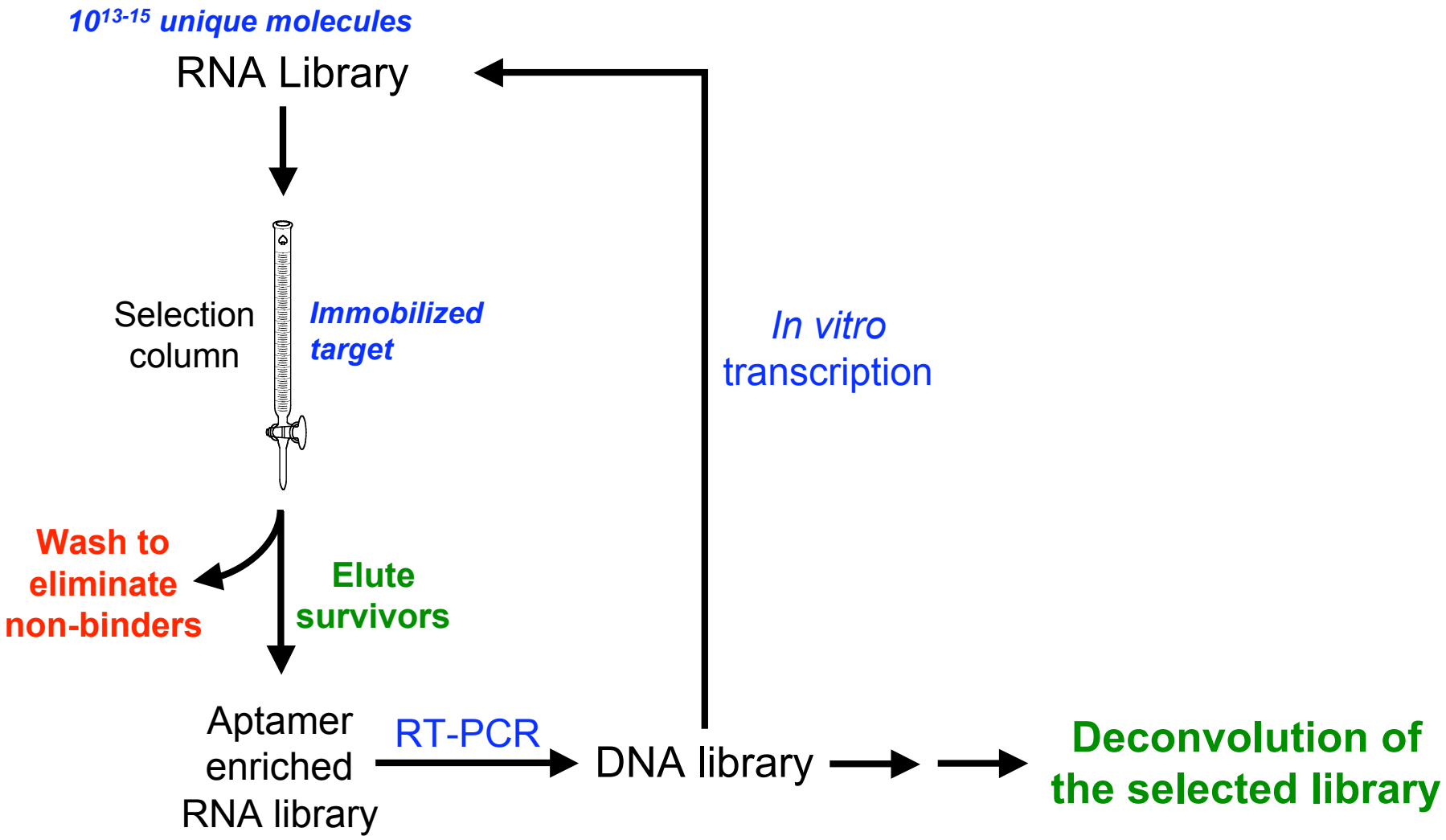
20.109 Lecture 4

18 February, 2010

Today's Objectives

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

A typical SELEX workflow



Deconvoluting your selected library

- Was your SELEX experiment successful?
  - Have you obtained your desired aptamers
    - *How do you determine this?*

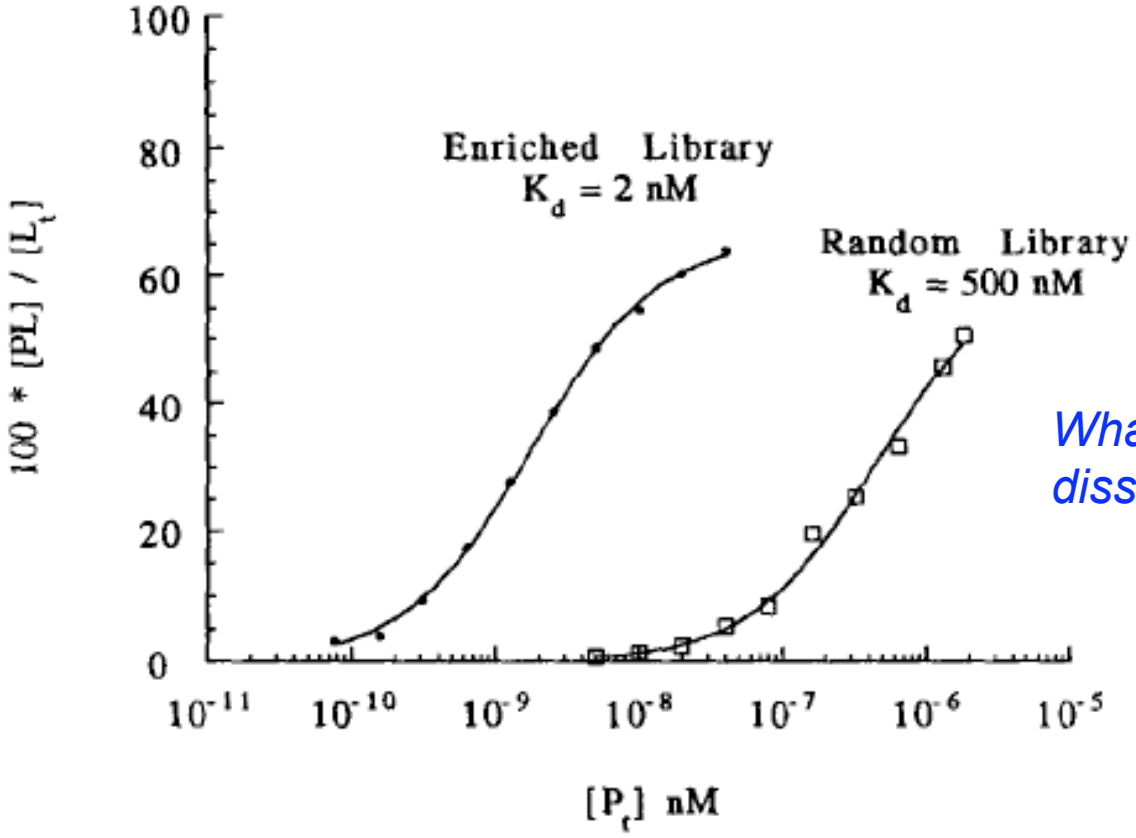
- If your SELEX was successful:

  - How do you identify the individual members of the selected library?
    - Are all members of your library competent for target binding?

    - Are there discernible, conserved features present in your aptamers?

Determining the success of your SELEX experiment

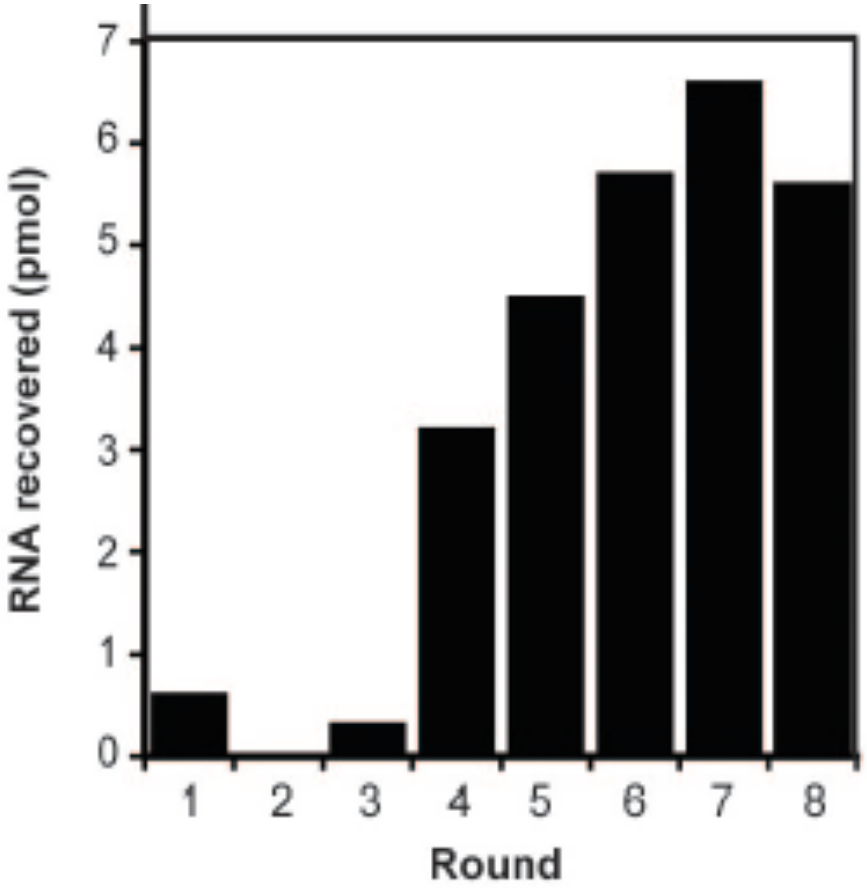
- Compare library dissociation constants pre- and post- SELEX



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



- Track the amount of RNA recovered at the end of each round of selection

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

- **Disadvantage**
  - Introduce radioactivity in your workflow

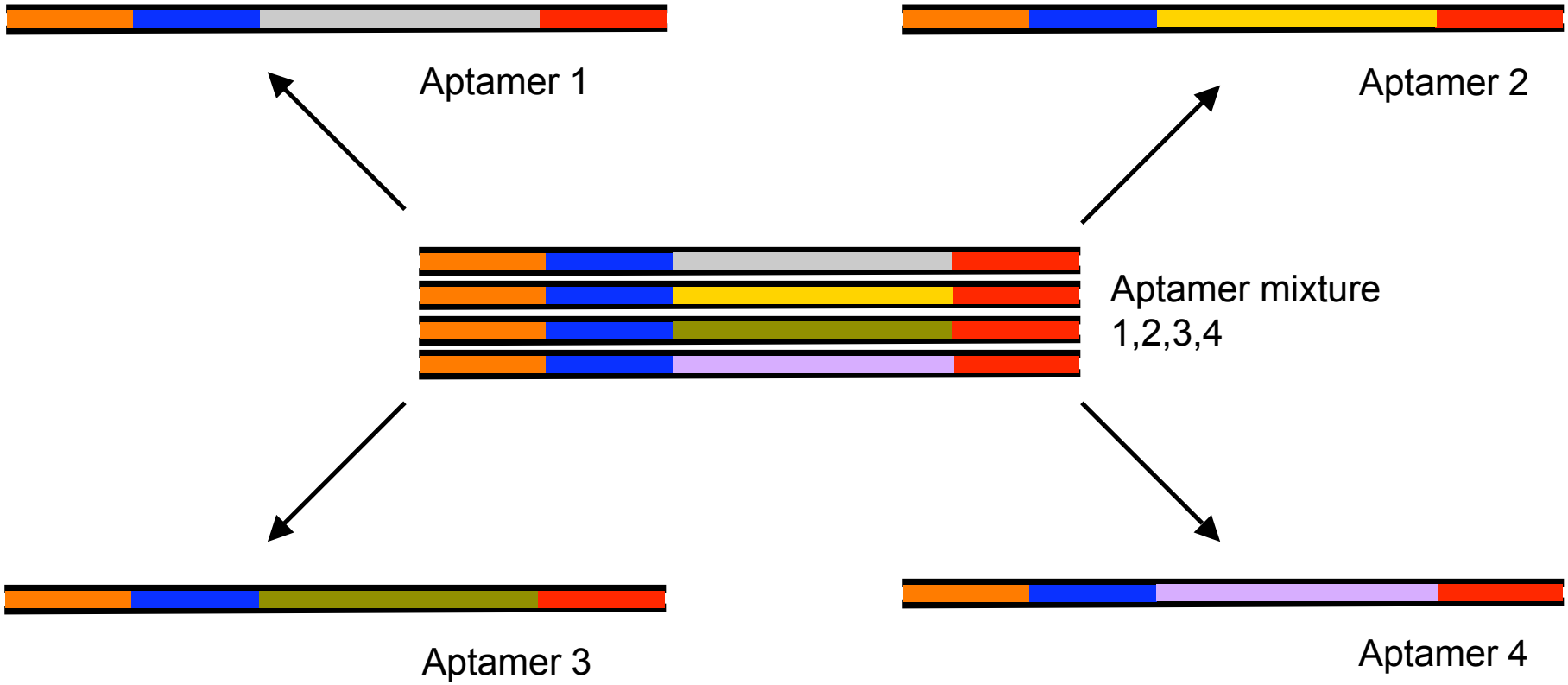
Library deconvolution

- **Achieve**:

– Isolation of individual aptamers to simultaneously facilitate:

-> Sequencing

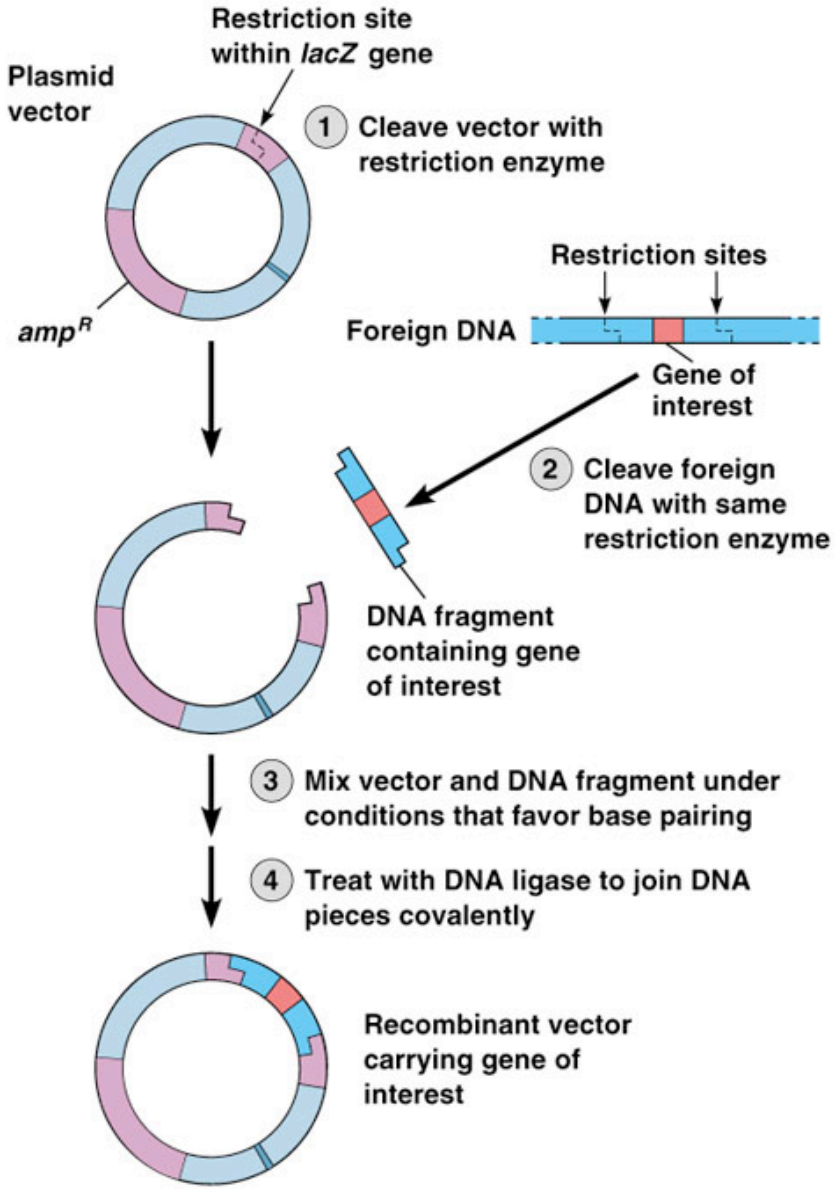
-> Characterization (binding, etc)



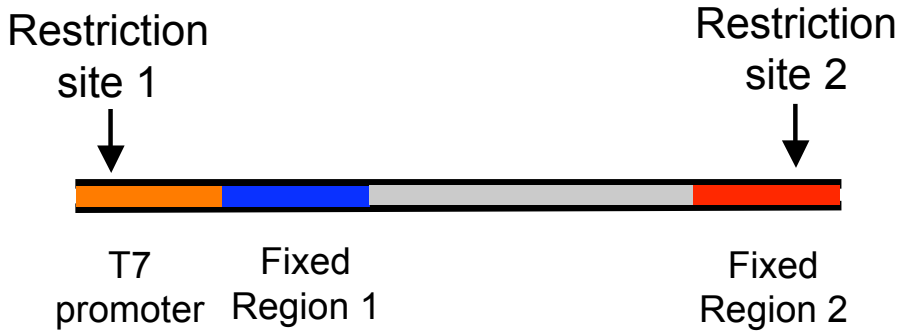
Library deconvolution

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

Cloning the aptamer library

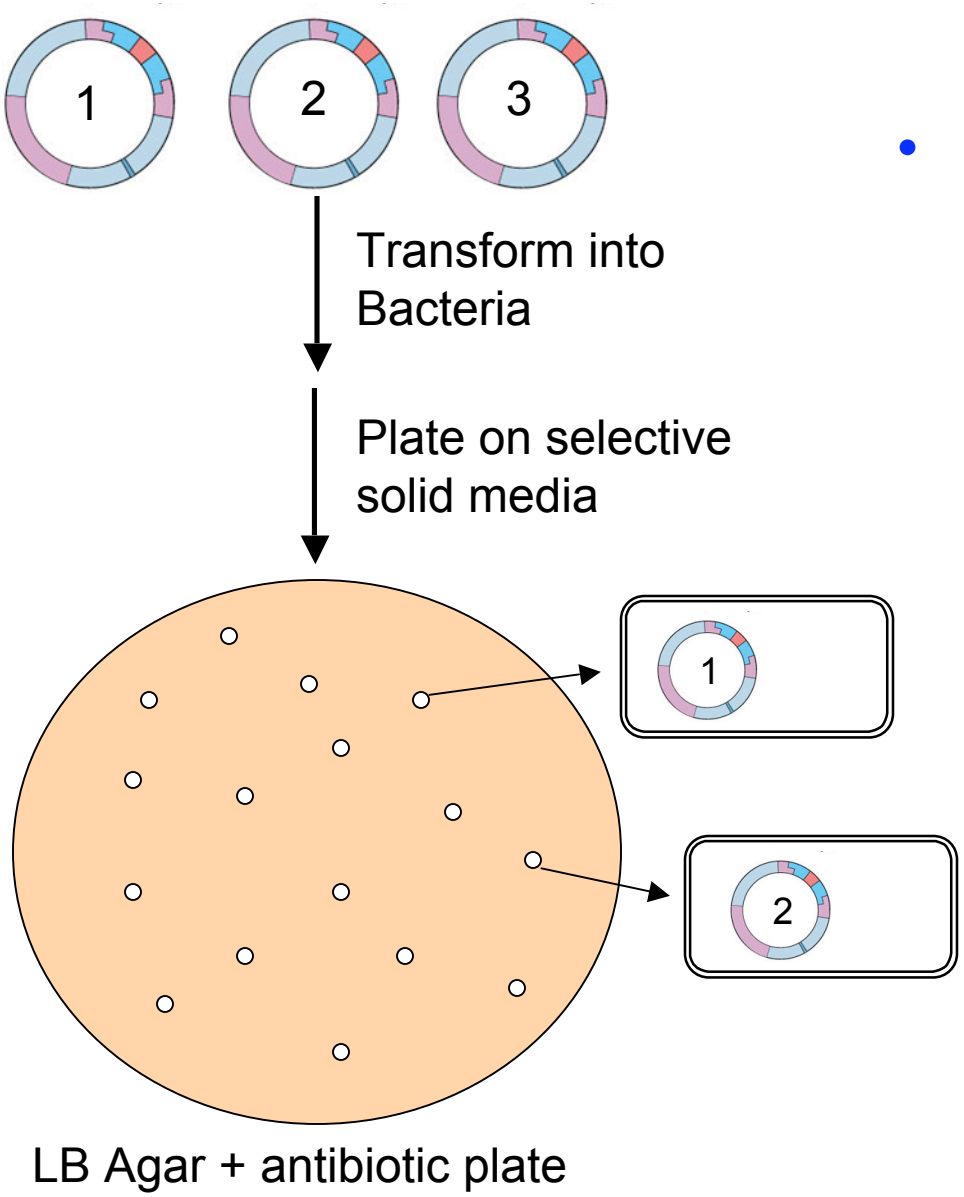


Addison Wesley Longman, Inc.



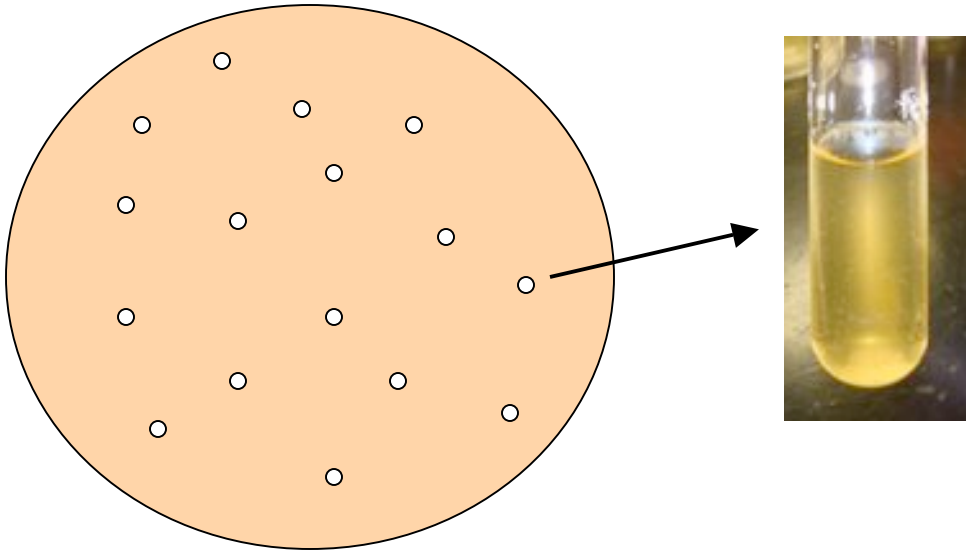
- **Single hit conditions**:
  - One insert on average incorporated into one plasmid
  - Each plasmid now encodes a single aptamer
- **Problem**
  - You have a mixture of plasmids
  - *How do you isolate clonal plasmids?*

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

…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

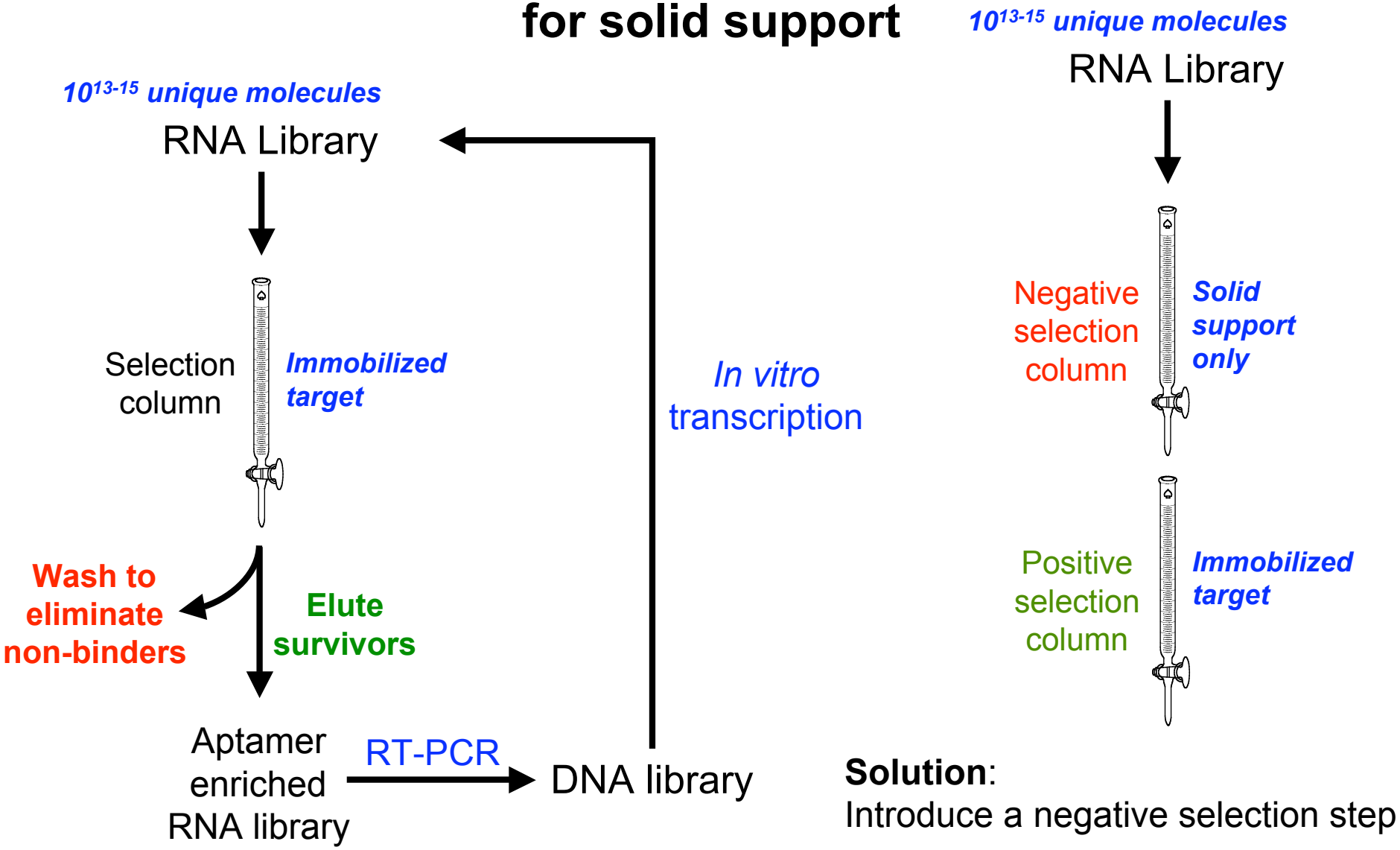
…but what went wrong with my SELEX?
Some common scenarios

2. Selected library and individual aptamers bind tightly to 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 counter this?*

Eliminating library members with high inherent affinity for solid support



Solution:
Introduce a negative selection step

Maximizing SELEX efficiency

- **Desirable**:
  - Obtain target aptamers on first try!
  - In the fewest possible number of rounds

- What is the best way to ensure achieving this?
  - Efficiently eliminate non-binders
  - Efficiently recover binders

- Driven by selection stringency!

Conceptualizing stringency during SELEX

Molecular targets
e.g. heme

Majors

20
1
2
3
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6
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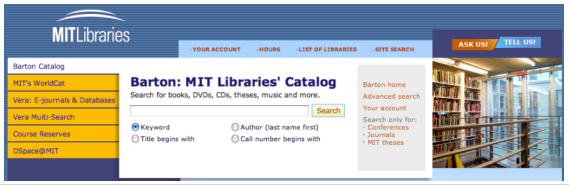
24

SELEX
Strategy for efficiently querying your RNA library



Barton
Strategy for efficiently querying the MIT Collections

MITLibraries

Book collection = RNA sequence collection

History
Science
Engineering
Philosophy

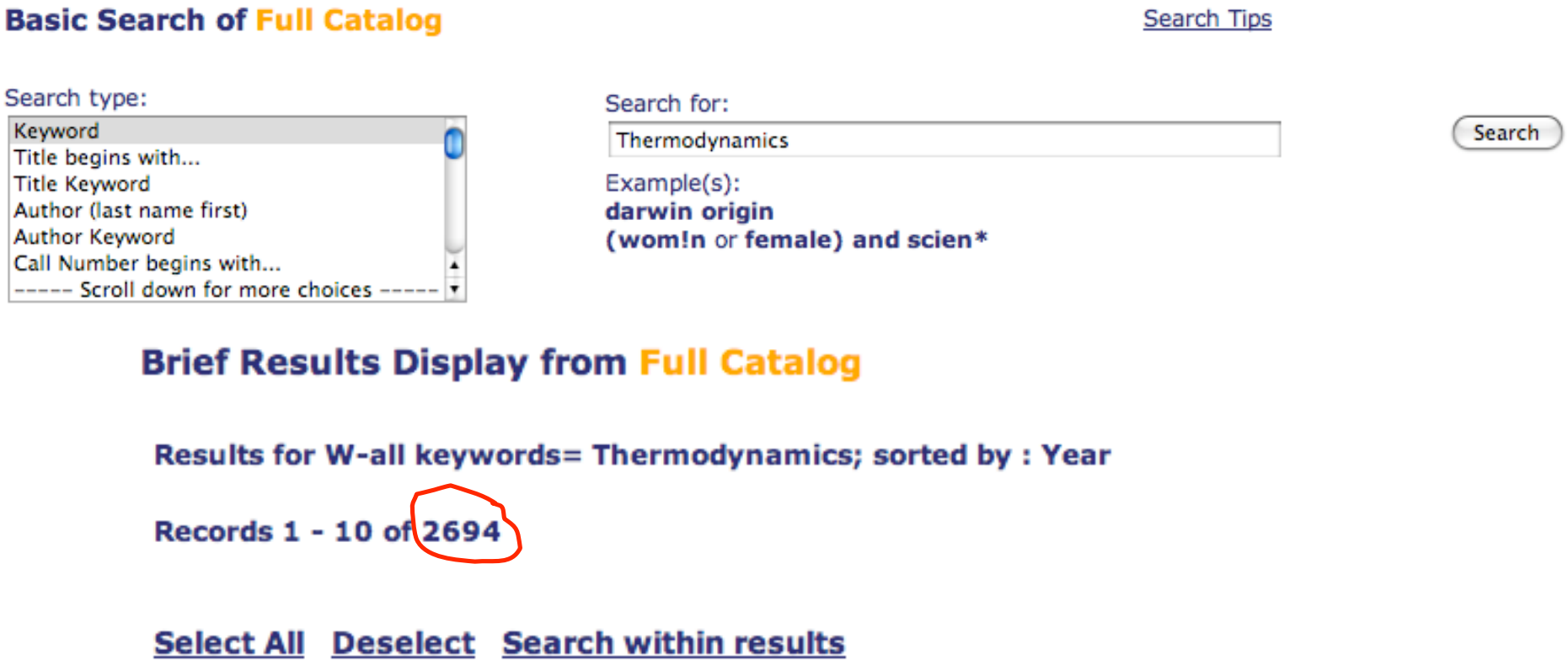
Book {x,y,z} = Aptamer

Sub-topic

Book X
Book Y
Book Z

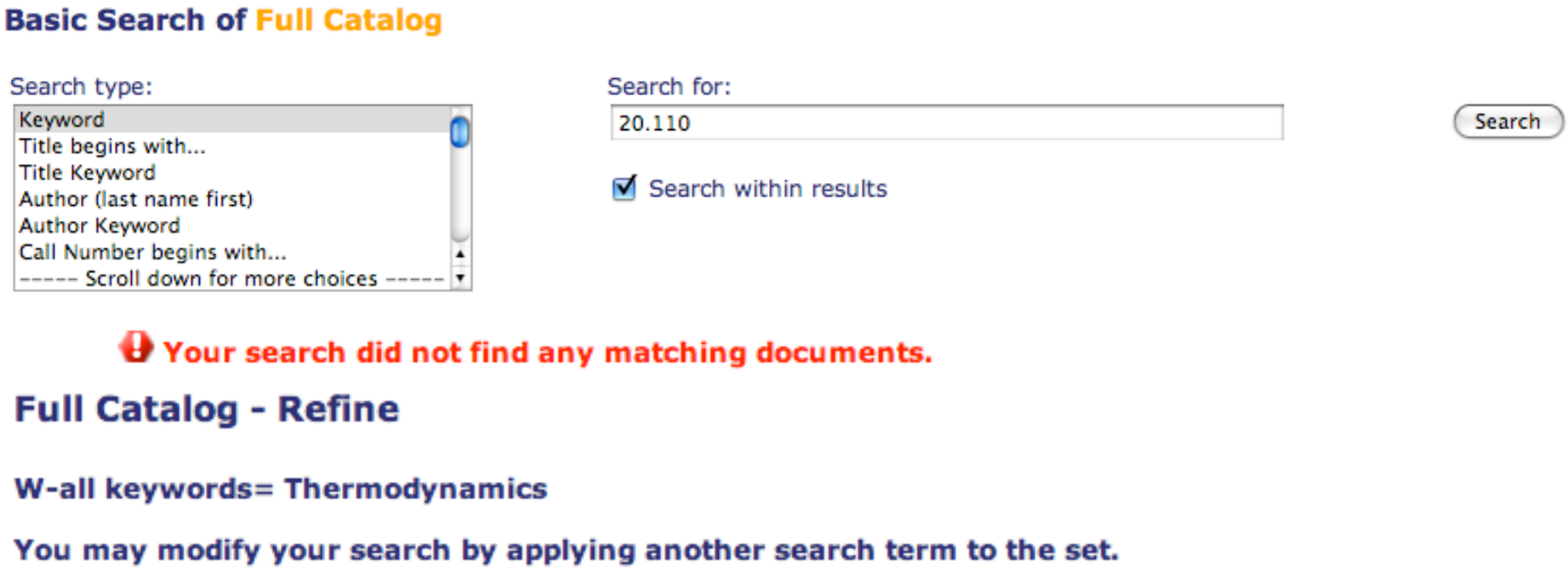
Conceptualizing stringency during SELEX

- Trying to locate *that* {Thermodynamics textbook} used in {20.110}
  - Limited specific information available
    - Perform a low stringency search



Conceptualizing stringency during SELEX

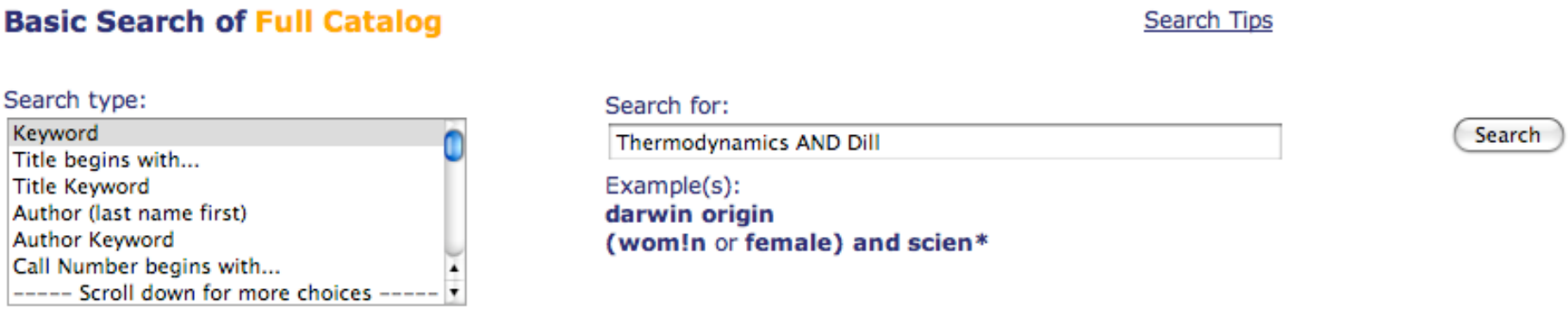
- Trying to locate *that* {Thermodynamics textbook} used in {20.110}
  - Limited specific information available
    - Narrow using available information



Use too narrowly defined a search term
Result: Lose your desired target!

Conceptualizing stringency during SELEX

- Trying to locate *that* {Thermodynamics textbook} authored by {Dill} used in {20.110}
  - Narrow using available information



Conceptualizing stringency during SELEX

- Trying to locate *that* {Thermodynamics textbook} authored by {Dill} used in {20.110}
  - Narrow using available information

Brief Results Display from Full Catalog

Results for W-all keywords= Thermodynamics AND W-all keywords= Dill; sorted by : Year

Records 1 - 2 of 2

[**Display full record**]

Author	Dill, Ken A.
Title	Molecular driving forces : statistical thermodynamics in chemistry and biology / Ken A. Dill, Sarina Bromberg ; with the assistance of Dirk Stigter on the electrostatics chapters.
Published	New York : Garland Science, c2003.
Format	Book
Subject	Statistical thermodynamics.
Availability	Click All items to check current status
Location	Barker Library - Stacks | QC311.5.D55 2003
Location	Hayden Library - Reserve Stacks | QC311.5.D55 2003
Location	Hayden Library - Stacks | QC311.5.D55 2003

More specific information about target available
Result: More efficient search and recovery!

Conceptualizing stringency during SELEX

MIT Libraries

- Trying to locate *that* {Thermodynamics textbook} used in {20.110}

RNA Library

- Trying to find the {RNA aptamers} that bind {target X}

- Very little information specified in initial query

  – Difficult to rationally restrict the search space

  – Searching is inherently inefficient

  – *How can we modulate information input to influence the outcome of our SELEX experiment?*

Modulating SELEX stringency--practically

1. Vary how extensively the selection column is washed to remove non-interacting RNAs
   - Higher stringency --> more washes
   - Lower stringency --> fewer washes

- Information content specified:
  - **Thermodynamics** (Dissociation constant)
    - The lifetime of the {aptamer-target} complex must exceed the time it takes to complete your washing
    - Sufficient complex must survive the dilution and extraction process associated with washing

Query: Find the {RNA aptamers} that bind {target X} with a {dissociation constant ≤ xx}.

Modulating SELEX stringency--practically

2. Alter the library-to-target ratio

- Higher stringency --> higher ratio
- Lower stringency --> lower ratio

- Information content specified:
  - **Thermodynamics** (Dissociation constant)
    - Limit the number of possible target binding sites
    - Favor recovering higher affinity library members (increased signal)
    - Fewer sites for non-specific and low affinity interactions (decreased noise)
      - E.g. Less solid support used when the amount of target used is decreased

Query: Find the {RNA aptamers} that bind {target X} with a {dissociation constant ≤ xx}.

Modulating SELEX stringency--practically

3. Using buffer additives to suppress undesired interactions

- pH
  - Consider target pI
  - pH too low --> target carried net positive charge --> encourage non-specific electrostatic interactions with negatively charged RNA
  - Raising pH increases stringency by reducing net positive charge on target since this reduces bulk library interactions with the target

- tRNA
  - Bind non-specific sites on solid support

- Salt concentration
  - Modulate electrostatic contributions during binding

- Major benefit is in reducing the “noise” during your selection

My parameter optimization space is HUGE…help!?

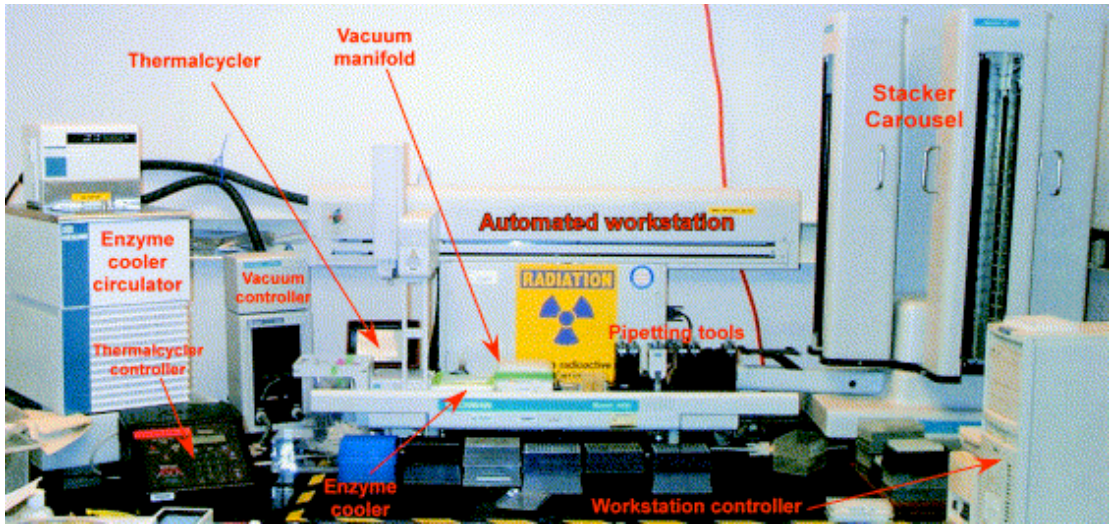
- **Vary**:
  - Wash number
  - Library-to-target ratio
  - Buffer conditions
    - pH
    - [salt]
    - tRNA
    - BSA (protein)

- Where do you start your SELEX?

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

Automating SELEX

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



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

Summary

- Selected aptamer libraries can be made into plasmid libraries
  - Using standard molecular biology methods
  - Each plasmid represents a specific aptamer in selected pool
  - Facilitate aptamer archival and further characterization
- Many factors can impact the success or failure of SELEX
  - Must carefully consider target properties in selecting your SELEX conditions
  - Establish your strategy for using stringency to control the efficiency of your selection
  - Selecting a stringency protocol is empirical
    - Insufficient initial knowledge to rationally decide best strategy beforehand
    - Altering stringency involves considering thermodyamic principles