

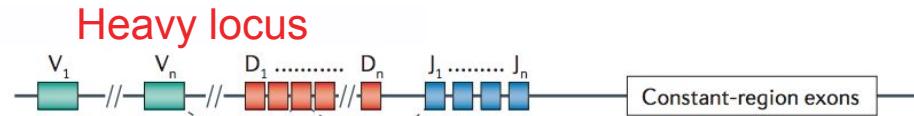
# **abseqR: Reporting functions and downstream analysis of antibody libraries Rep-Seq**

Harry Fong

# Outline

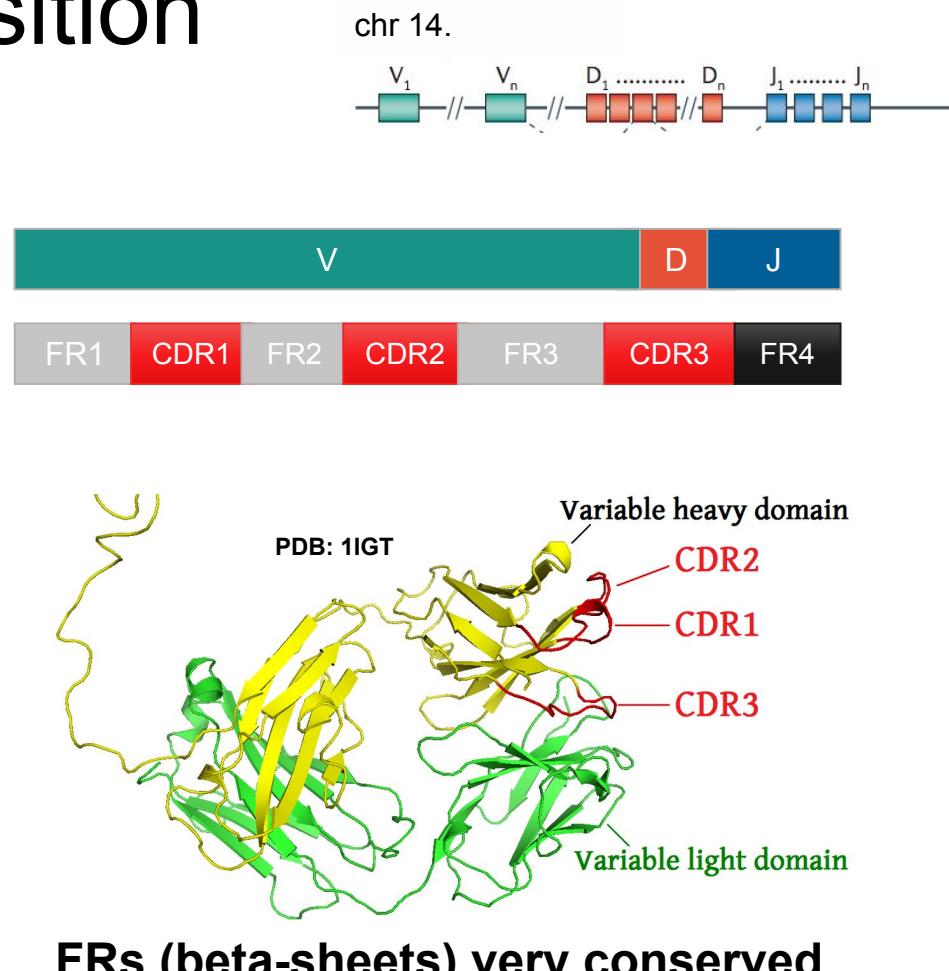
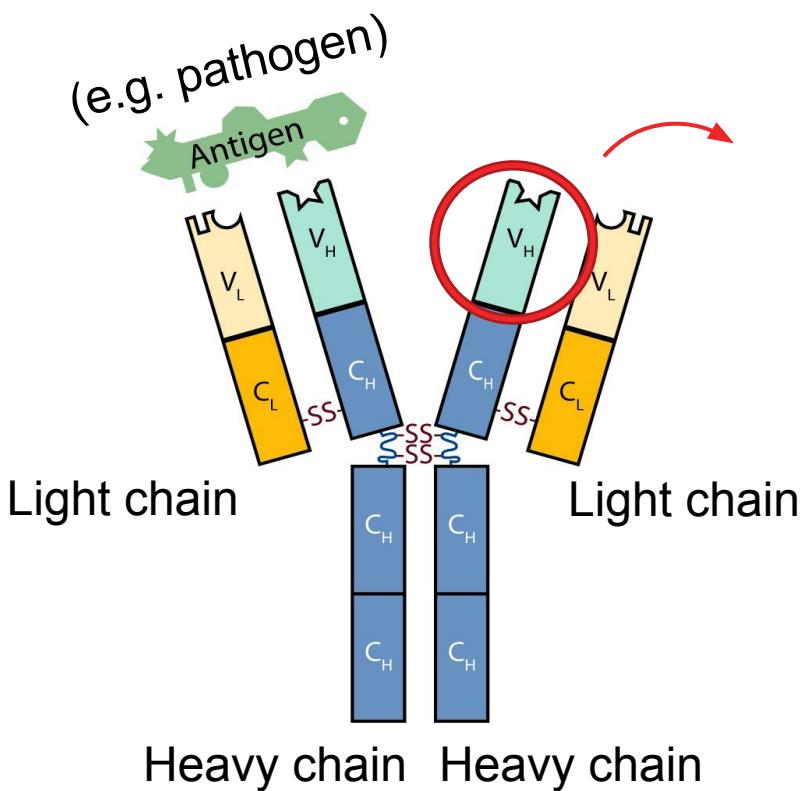
- Antibody composition and antibody libraries
- Application of antibody libraries in drug discovery
- AbSeq pipeline
- Case study: Using AbSeq to analyze biopanning campaigns

# Antibody composition

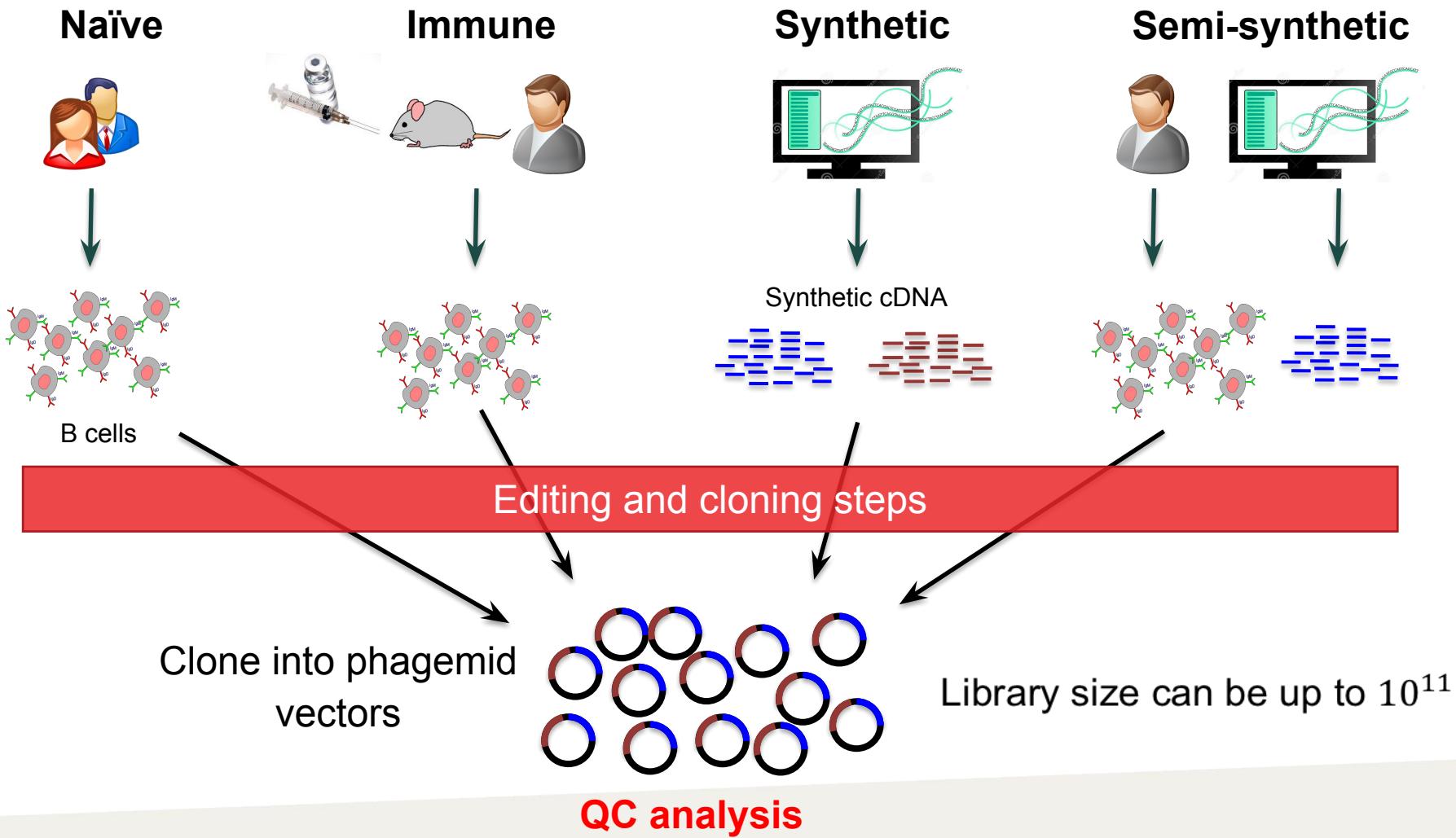


Nemazee, *Nature Reviews Immunology*, 2006

# Antibody composition

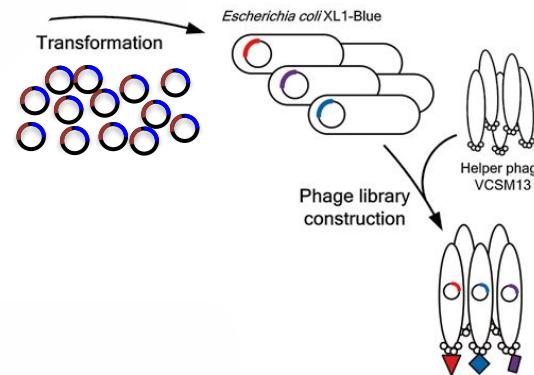


# Antibody libraries



# Application of antibody libraries in drug discovery

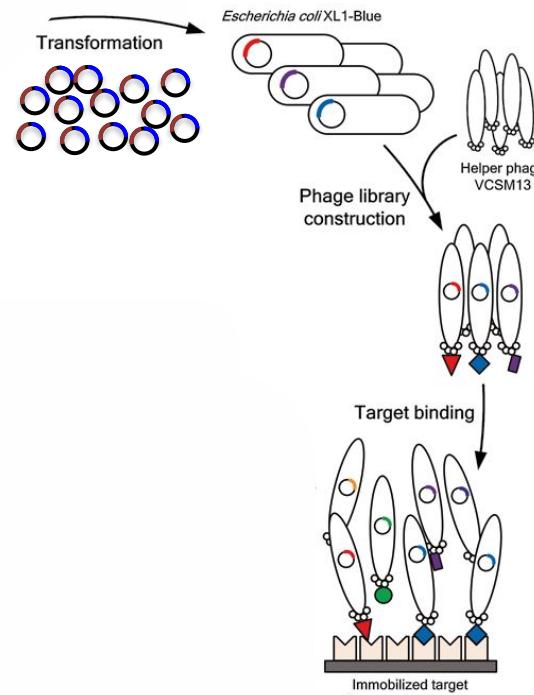
## Biopanning campaigns



Levisson et al., Protein Downstream Processing. *Methods in Molecular Biology*, 2014

# Application of antibody libraries in drug discovery

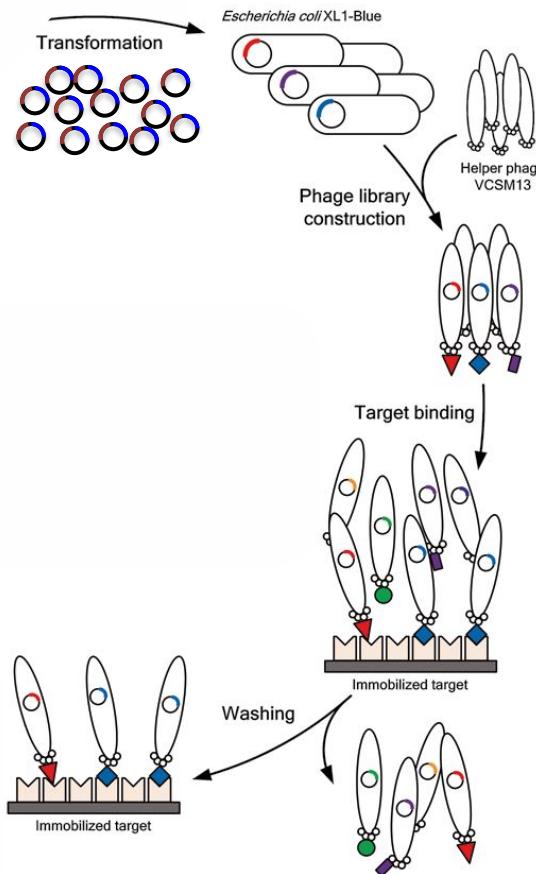
## Biopanning campaigns



Levisson et al., Protein Downstream Processing. *Methods in Molecular Biology*, 2014

# Application of antibody libraries in drug discovery

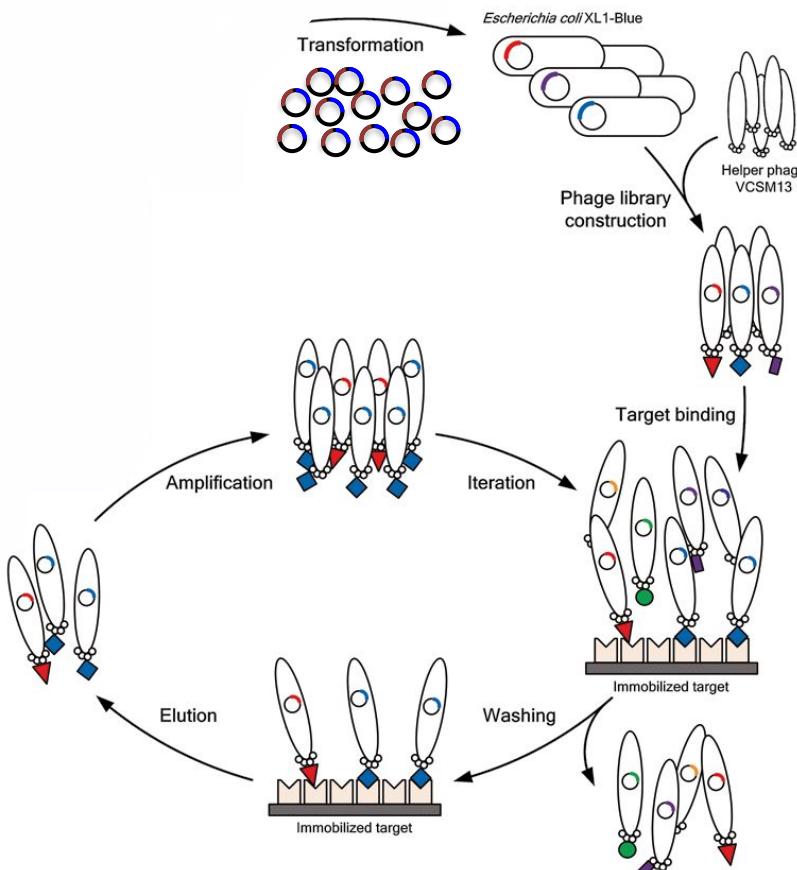
## Biopanning campaigns



Levisson et al., Protein Downstream Processing. *Methods in Molecular Biology*, 2014

# Application of antibody libraries in drug discovery

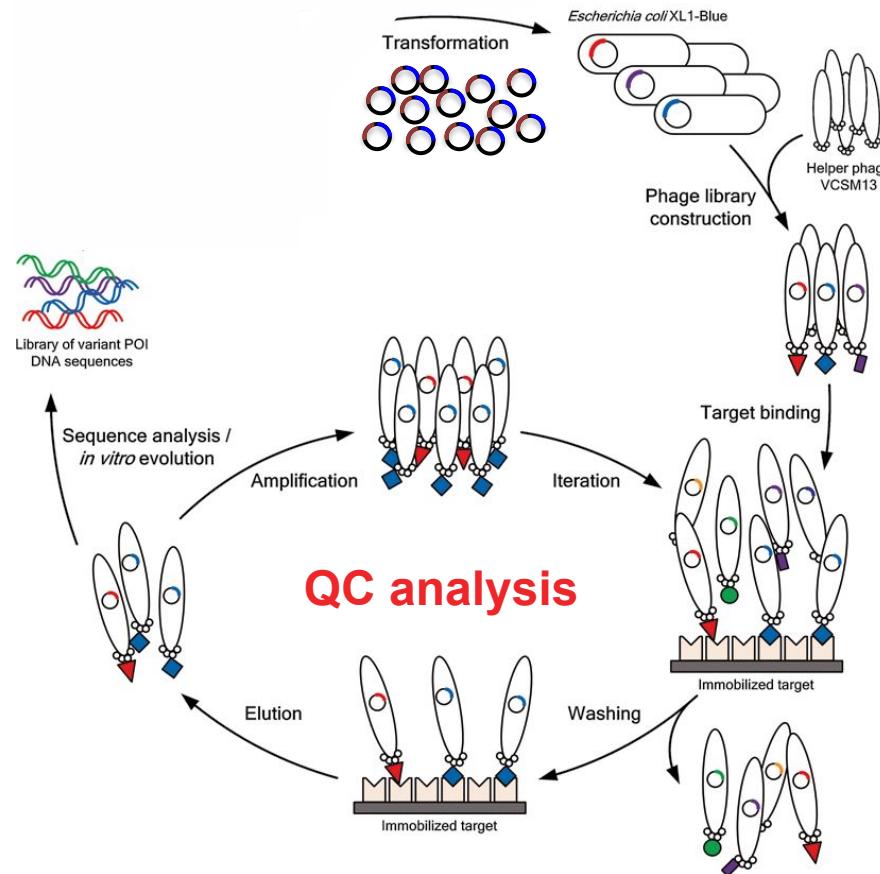
## Biopanning campaigns



Levisson et al., Protein Downstream Processing. *Methods in Molecular Biology*, 2014

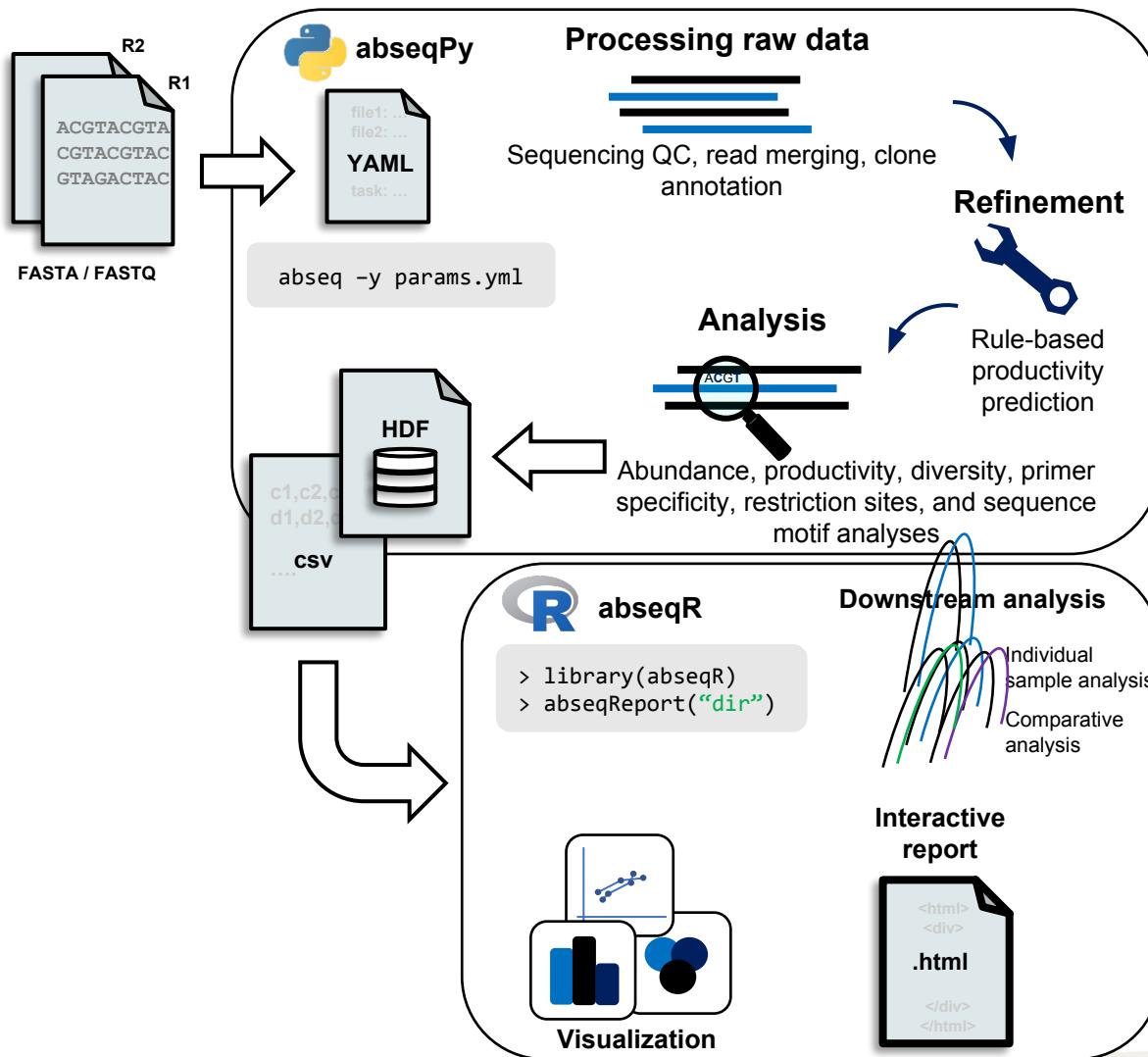
# Application of antibody libraries in drug discovery

## Biopanning campaigns



Levisson et al., Protein Downstream Processing. *Methods in Molecular Biology*, 2014

# AbSeq pipeline



# abseqR

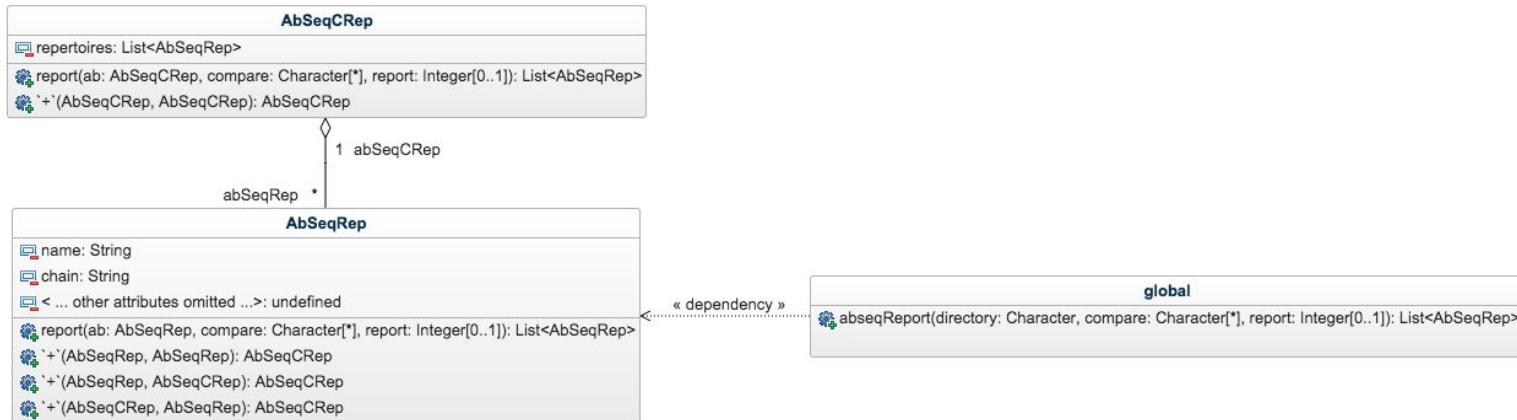
- Technical features:
  - **Visualizations** for:
    - Abundance analysis
    - Productivity analysis
    - Diversity analysis
    - Primer specificity analysis, restriction sites analysis, and sequence motif analysis
  - **Downstream analyses**
    - Sample-vs-sample comparison
  - **HTML reporting capabilities**
    - Collate plots and results

# abseqR

- Straightforward syntax:

```
abseqReport("path/to/dir", compare =  
"...")  
report(AbSeqRep, compare =  
"...")  
= AbSeqRep + AbSeqRep + ... AbSeqRep  
+  
+ AbSeqRep
```

returns **AbSeqRep**



# Case Study

# Case Study

## Analysis on a biopanning campaign

These clones are not guaranteed to be binders

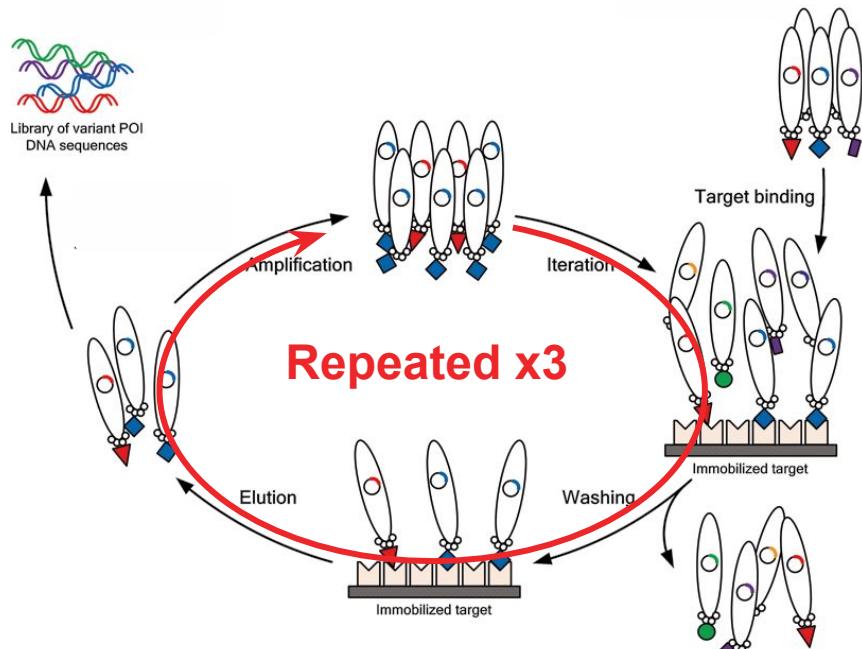
- Only a subset of these clones are tested to identify binders

### Question 1:

- Observable **differences** between the three rounds?

### Question 2:

- Does NGS identify **novel binding** clones?



Levisson et al., Protein Downstream Processing. *Methods in Molecular Biology*, 2014

# Using AbSeq

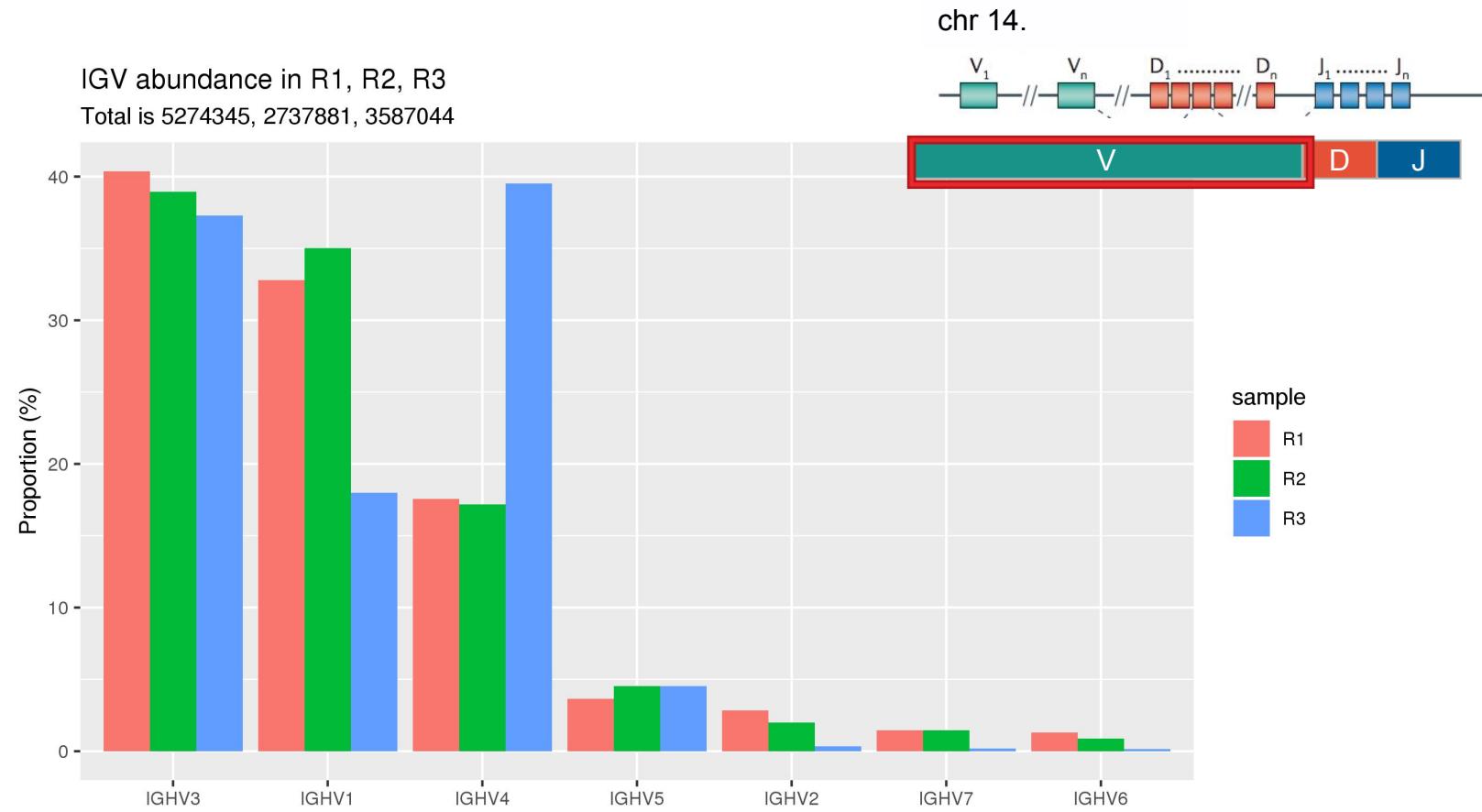
```
abseq -y biopanning_params.yml
```

```
library(abseqR)

biop <- abseqReport("biopanning_exp", compare = c("R1, R2, R3"))
names(biop)
#> [1] R1 R2 R3
```

# Comparison between R1, R2, and R3

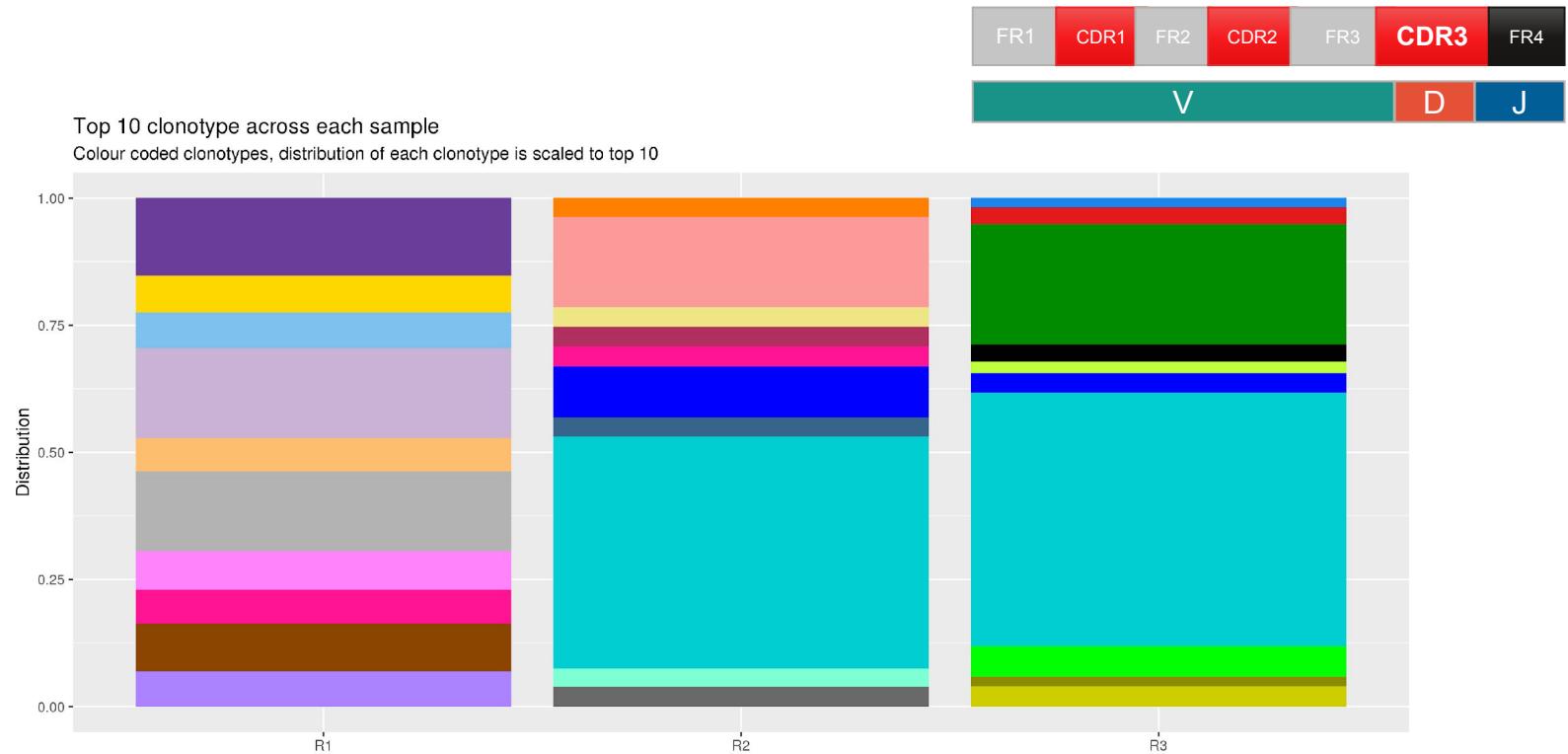
Subsequent panning cycles exhibit differences



Bias towards IGHV4 family in Round 3

# Comparison between R1, R2, and R3

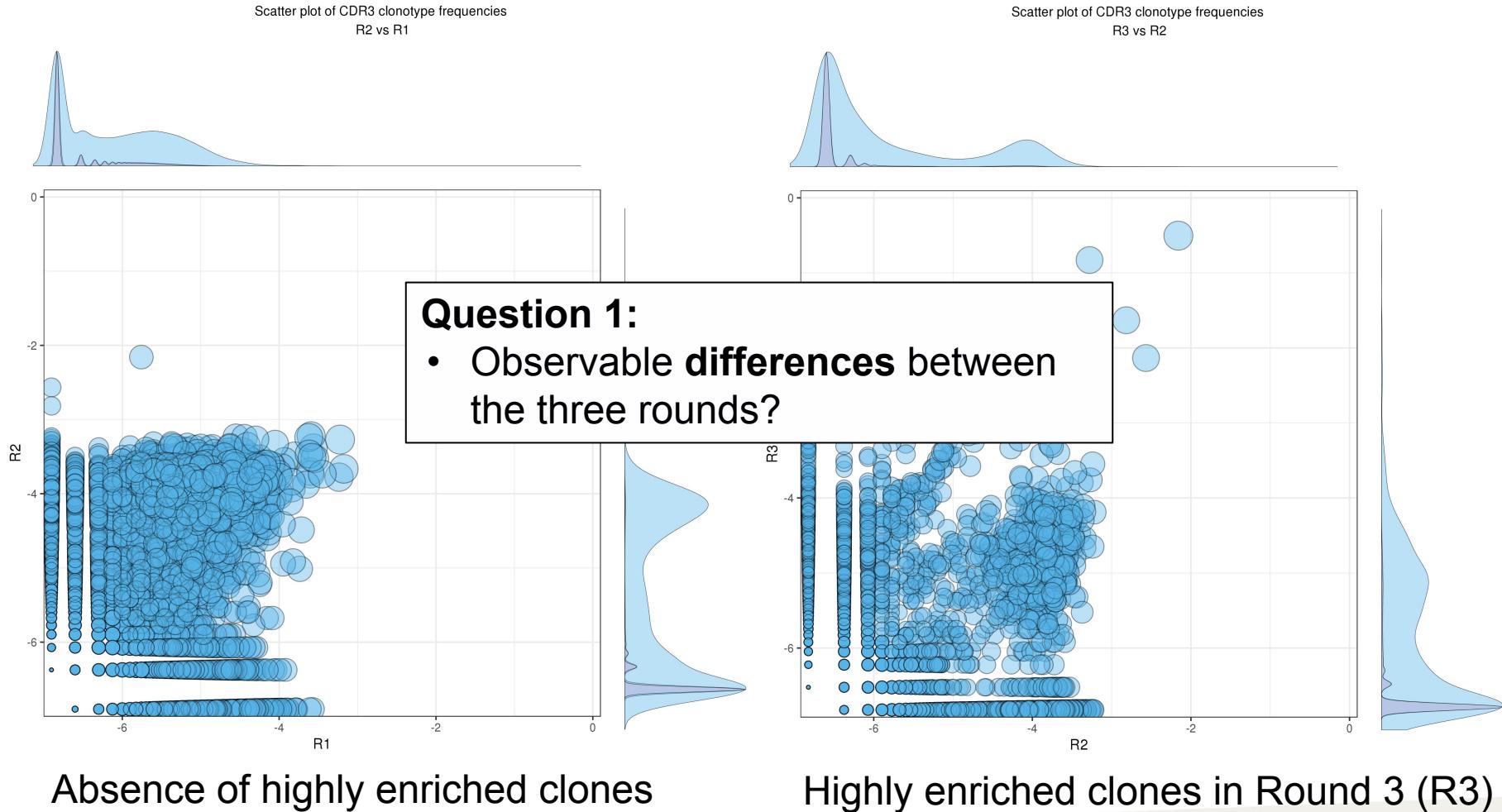
Subsequent panning cycles contain more over-represented clones



Evenly distributed clonotypes in Round 1, but enriched clonotypes in Rounds 2 and 3

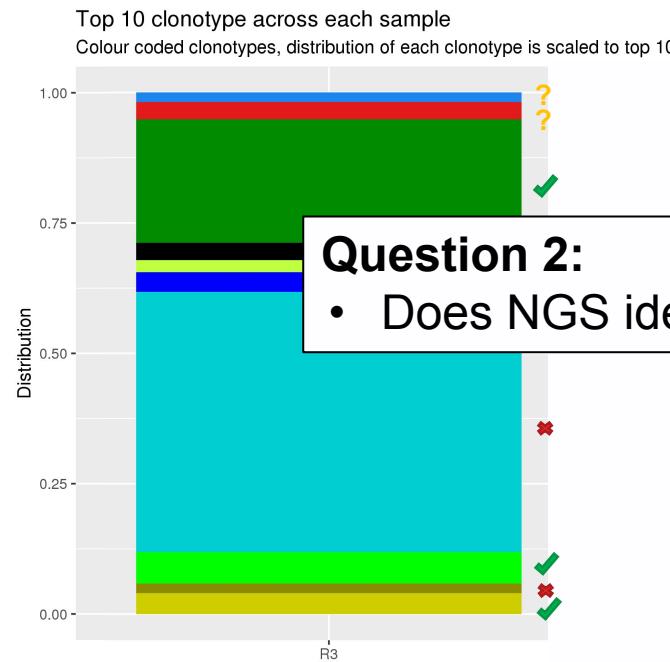
# Comparison between R1, R2, and R3

Subsequent panning cycles contain more over-represented clones



# Over-represented clones in Round 3 vs random sampling

Random sampling picked highly abundant clones in NGS dataset



Out of the 10 most abundant clones in Round 3 (R3):

## Question 2:

- Does NGS identify **novel binding** clones?

- 5/10 are non-binding clones
- 2/10 were not sampled for testing

# Summary

- **AbSeq** empowers scientists with the ability to validate the quality of their antibody libraries during construction and biopanning.
- **AbSeq** can be extended to incorporate additional analyses.

# Future research

- Clonotype differential expression analysis
- Clonotype tracking (biopanning campaigns)
- Implementing error correction
- Implementing clonotype clustering

# Acknowledgements

## **Antibody Technology Group**

Andrew Hammet

Georgina Sansome

Daria Kurtov

Kirsten Edwards

Chao-Guang Chen

## **Data Science Group**

Ingrid Lönnstedt

Monther Alhamdoosh

Milica Ng

# biopanning\_params.yml

```
1  -
2  defaults:
3    task: all
4    outdir: biopanning_exp
5    threads: 20
6    clonelimit: inf
7    bitscore: 350
8    alignlen: 260
9    sstart: 1-3
10   ---
11   name: R1
12   file1: fastq/PCR1_BR9V5_CAAAGACG-CGTGAT_L001_R1.fastq.gz
13   file2: fastq/PCR1_BR9V5_CAAAGACG-CGTGAT_L001_R2.fastq.gz
14   ---
15   name: R2
16   file1: fastq/PCR2_BR9V5_CTAGTACG-CTAGCT_L001_R1.fastq.gz
17   file2: fastq/PCR2_BR9V5_CTAGTACG-CTAGCT_L001_R2.fastq.gz
18   ---
19   name: R3
20   file1: fastq/PCR3_BR9V5_AGGCAGAA-TACAGC_L001_R1.fastq.gz
21   file2: fastq/PCR3_BR9V5_AGGCAGAA-TACAGC_L001_R2.fastq.gz
22   -
```