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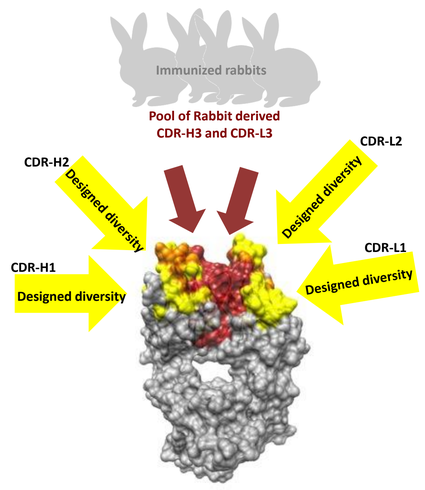
How affinity translates into a selective advantage: A Comparison of Phage and Yeast Display for Rabbit Mass Humanization

Michael Tomaszowski, Peter Milovník, Jacob Glanville (Distributed Bio), Remko Griep, Ahmad Trad and Vera Molkenthin

Introduction

AbCheck's Mass Humanization approach generates highly diverse human antibody libraries from immunized rabbits by batch cloning of the *in vivo* generated CDR3 diversity into prepared humanization landscapes with designed CDR1/2 diversity. Here, we directly compared Phage panning under different conditions and FACS sorting of a Yeast Display library regarding their efficiencies in the subsequent selection of high affinity binders. The analysis of sequences obtained from both methods clearly revealed overlapping findings, but also significant differences regarding the overall sequence diversity and binding affinity levels detected amongst the isolated hit candidates.

Figure 1. Rabbit Mass Humanization (RMH) libraries



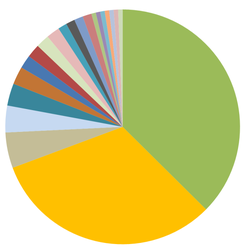
- 100% human VH/VL framework regions
- Free of biochemical liabilities
- Designed diversity in CDR1/2
- Combined with CDR-H3 and CDR-L3 diversity from immunized rabbits

Figure 2. High hit rate and large sequence diversity obtained from low stringency phage selections

Results of Sanger sequencing of 300 hits:

- >100 different CDR-H3 sequences
- 21 CDR-H3 sequence families
- Multiple CDR-L3 sequences per CDR-H3
- Varying CDR1/2 mutations

Two enriched CDR-H3 sequence families:



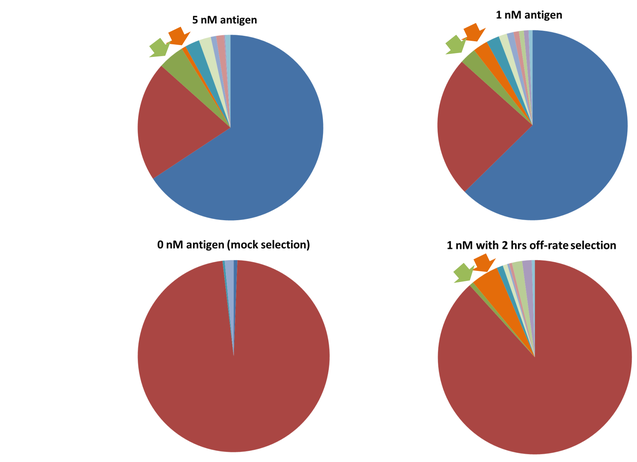
Consensus sequence of the two enriched CDR-H3 sequence families:

CARESGYY*A*DYAG*Y*GYAYGMDP

CARVYDDYGDDYF*N*I

Figure 3. Off-rate phage selection combined with NGS reveals the selective advantage of CDR-H3 sequences

Relative frequencies of CDR-H3 sequences after three phage panning rounds under varying conditions:



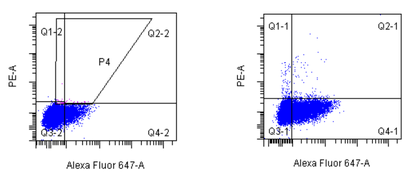
CARESGYYPDYAGYGYAYGMDP

CARVYDDYGDDYFDI

Figure 4. FACS sorting of a Yeast Display library yields a 100% binding population in 4 sorting rounds

Sorting round 1: 14 µM antigen

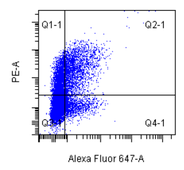
Sorting round 2: 14 µM antigen



- Yeast cells displaying library sequencing
- X-axis: Staining for displayed antibodies
- Y-axis: Staining for antigen binding

Sorting round 3:

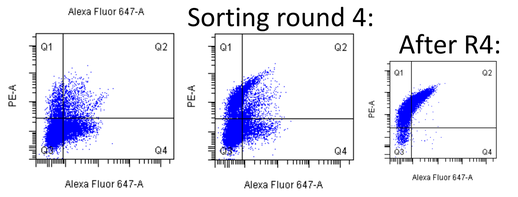
Sorting round 3a: 14 µM antigen



Sorting round 4:

After R4:

Sorting rounds 3b and 4b: 140 nM antigen



After R4:

Sorting rounds 3c and 4c: 14 nM antigen

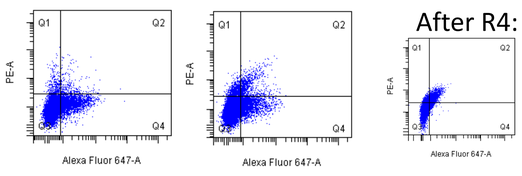
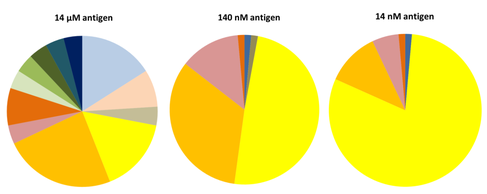


Figure 5. FACS sorting at low antigen concentration reveals selective advantage

Distribution of CDR-H3 sequences found in Sanger sequencing of hits after FACS sorting:



CARESDYYSDYAGYGYAYGMDP

CARESGYYADYAGLGYAYGMDP

Figure 6. Properties of selected sequences:

Sequence	IgG KD	% of V-domain identical to human germline
RMH-yeast 1	5,45E-11 M	93
RMH-yeast 2	1,60E-10 M	93
RMH-phage	1,49E-10 M	92

Conclusion:

- Phage panning and Yeast FACS sorting are both well suited to identify high affinity (subnanomolar) binders from a displayed Rabbit Mass Humanization library
- Based on sequencing, Phage Panning yielded in a broader sequence diversity which is likely to lead to an extended epitope spectrum and affinity panel of hit sequences compared to Yeast FACS sorting
- One particular CDR-H3 sequence group got selectively enriched in both Phage and Yeast, with a significantly better enrichment of high affinity binders after Yeast FACS sorting

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