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How Affinity Translates into a Selective Advantage: A Comparison of Phage and Yeast Display for Rabbit Mass Humanization

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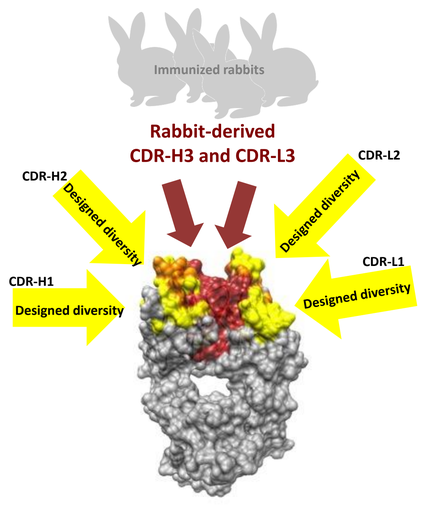
Introduction

AbCheck's Mass Humanization provides highly diverse human antibodies from immunized non-transgenic rabbits in shorter time compared to hybridoma screens, followed by CDR grafting and antibody engineering. Mass Humanization libraries are generated by batch cloning of the *in vivo* generated CDR3 diversity into human frameworks with designed CDR1/2 diversity, and human antibodies are selected from these libraries. Here, we directly compared Phage panning under different conditions and FACS sorting of a Yeast Display library with respect to their efficiencies in the selection of human high affinity binders from such libraries. The analysis of sequences obtained from both methods revealed overlapping findings, but also significant differences regarding the overall sequence diversity and binding affinity.

Figure 1. Rabbit Mass Humanization (RMH) libraries



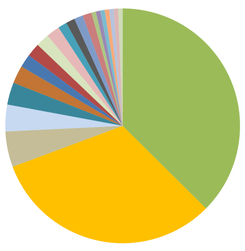
- 100% human VH/VL framework regions
- No biophysical liabilities
- Designed diversity in CDR1/2
- Combined with CDR-H3 and CDR-L3 diversity from immunized rabbits

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:



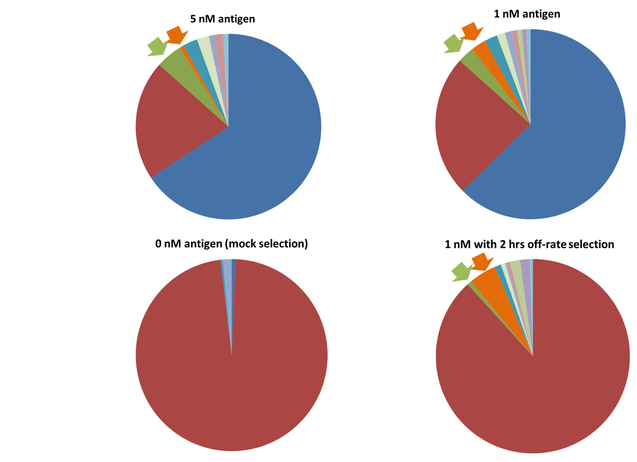
Consensus sequence of the two enriched CDR-H3 sequence families:

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:



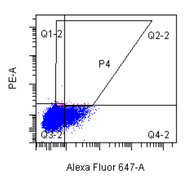
CARESGYYPDYAGYGYAYGMDP

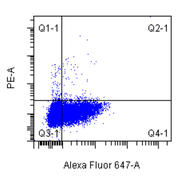
CARVYDDYGDDYFDI

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

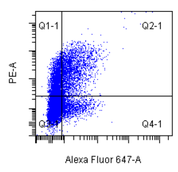




- **Yeast cells displaying library sequencing**
- **X-axis: Staining for displayed antibodies**
- **Y-axis: Staining for antigen binding**

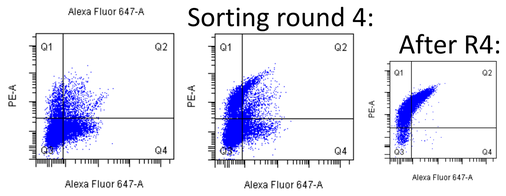
Sorting round 3:

Sorting round 3a: 14 µM antigen



Sorting round 4:

Sorting rounds 3b and 4b: 140 nM antigen



Sorting rounds 3c and 4c: 14 nM antigen

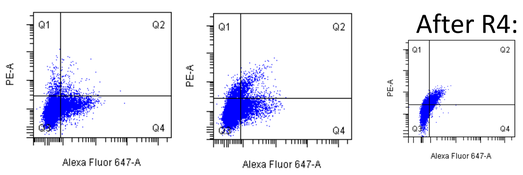
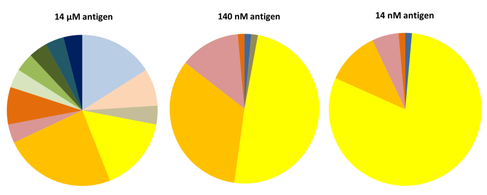


Figure 5. FACS sorting at low antigen concentration reveals selective advantage

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 (sub-nanomolar) binders from a displayed Rabbit Mass Humanization library
- Based on sequencing, Phage panning yielded 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 was selectively enriched in both Phage and Yeast with a significantly better enrichment of high affinity binders out of a highly diverse humanized immune repertoire after Yeast FACS sorting

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