

Engineering Antibody Affinities via Yeast Surface Display

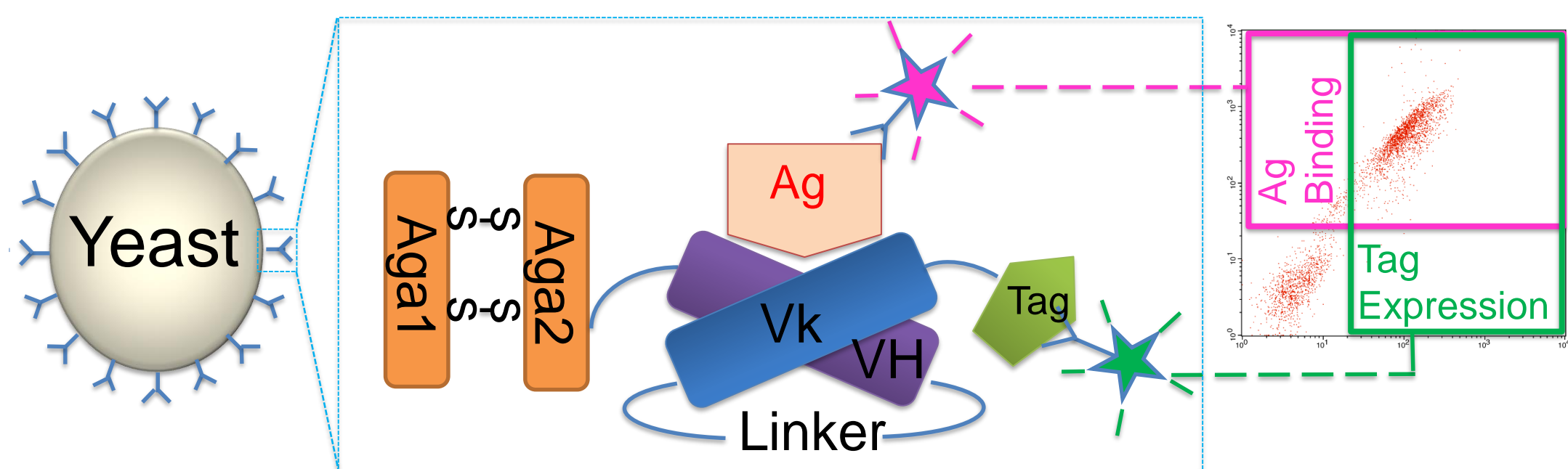
Yong Wang*; Chunyao Xia; Yi Zheng; Dan Qin; Hoa Giang; Raphael Levy; Jordon Wang
LakePharma, 520 Harbor Blvd, Belmont, CA 94002, USA, United States *Corresponding author. Tel: +1 650 288 4891 (ext 229) E-mail address: yong.wang@lakepharma.com

ABSTRACT

Yeast surface display platforms have become valuable tools for antibody engineering applications, such as antibody discovery from immune libraries, affinity maturation, and target epitope mapping. The ability to generate larger libraries via yeast electroporation and high-throughput clonal screening using flow cytometry and surface plasmon resonance has enabled efficient quantitative selection and screening of single-chain variable fragments (scFvs) that bind to target antigens with distinct affinities. Here we describe a case study where the affinity of a parental antibody was “detuned” via yeast surface display, resulting in clones of lower or higher binding affinities. Six CDR libraries were generated via soft mutagenesis, followed by magnetic (MACS) and flow cytometry (FACS) sorting. Enriched pools were subsequently combined and subjected to additional rounds of FACS sorting in order to further improve their affinities. Individual clones were identified with picomolar affinities, a 10-fold improvement over the parental counterpart. Additional clones were also identified with binding affinities that were 4- to 50-fold lower than the parental counterpart. This case study demonstrates that through yeast display, a panel of antibody candidates with a wide range of affinities can be rapidly generated in an efficient and directed manner.

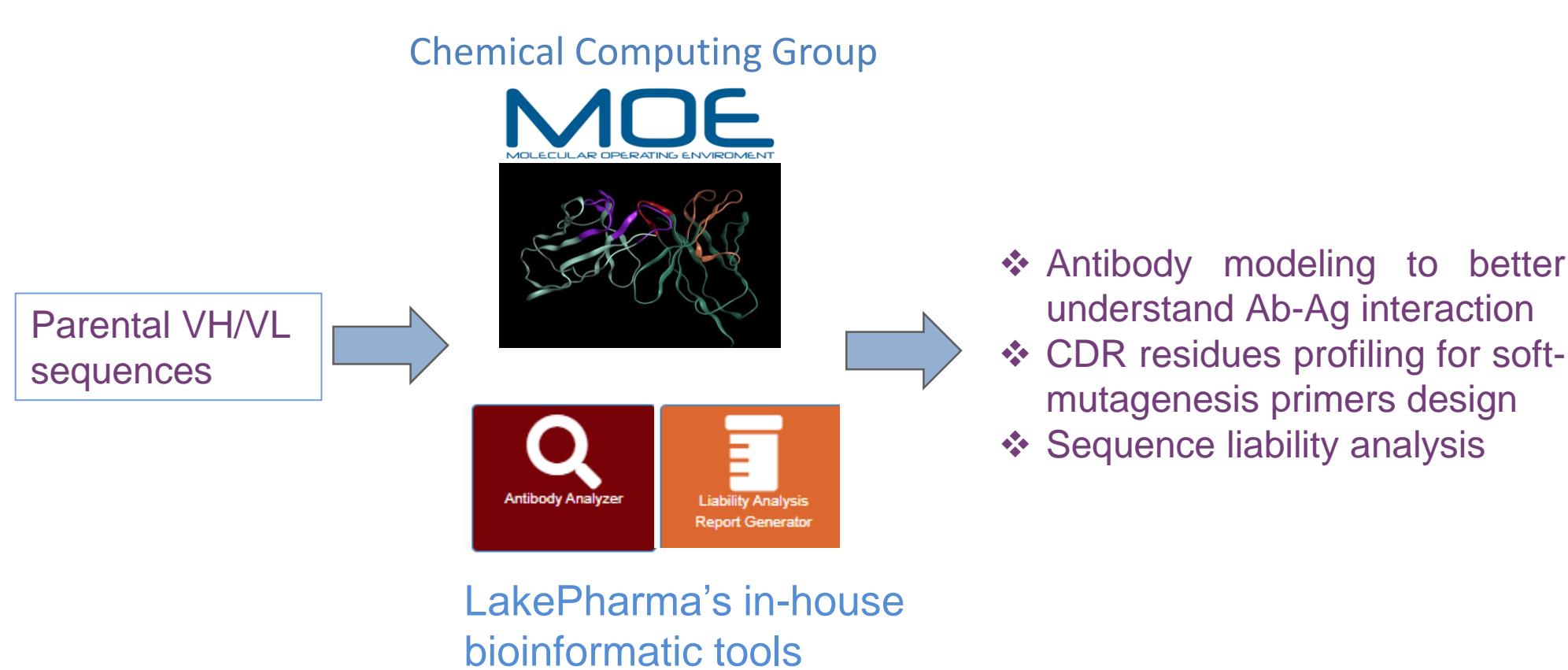
INTRODUCTION

- LakePharma offers eukaryotic-based yeast display platforms for *in vitro* antibody discovery, engineering, and target epitope mapping.
- LakePharma's yeast platform displays scFv/VHH, which is fused to Aga2 with a variety of tag selection (Myc, Flag, V5 or His tag)
- LakePharma's unparalleled *in vitro* antibody discovery capabilities can be bundled with unique lead optimization, characterization, and downstream large-scale GMP production, providing the entire spectrum of early- to late-stage antibody drug generation services.
- A case study is reported in which the affinity of a parental antibody was modified and screened via yeast surface display, resulting in clones of lower or higher binding affinities

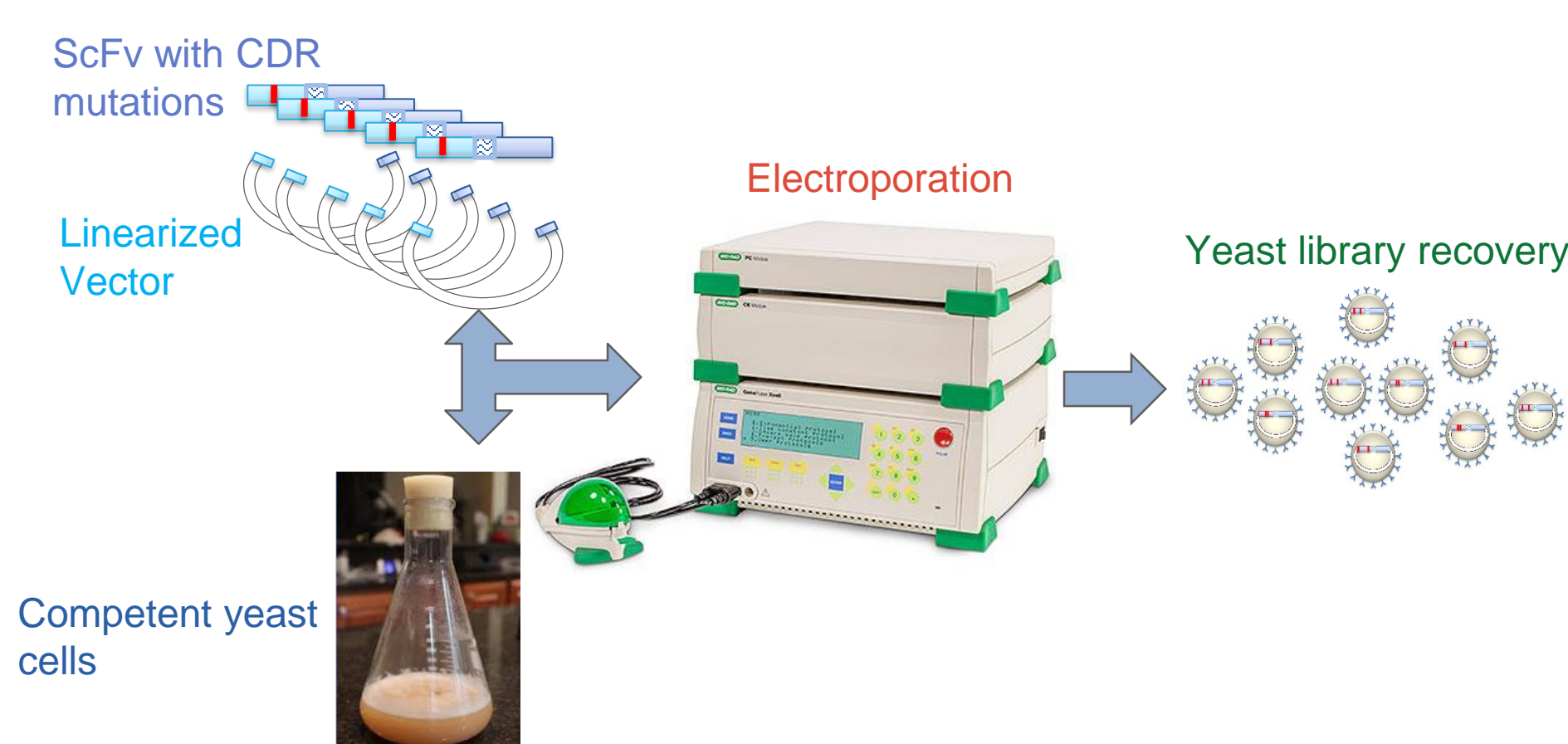


WORKFLOW

Bioinformatic Tools Including MOE Aid Affinity Maturation Method Design



Yeast Electro-transformation for Library Construction [1]



- The CDR-focused mutagenesis scFv was generated via overlap PCR and recombined with the vector via gap repair by transforming yeast competent cells
- Six libraries were generated: soft randomization of CDRH1, H2, H3, L1, L2, L3 region

Yeast Library Selection, Screening and Clone Ranking



RESULTS

Figure 1. Yeast Library Flow Cytometry Analysis

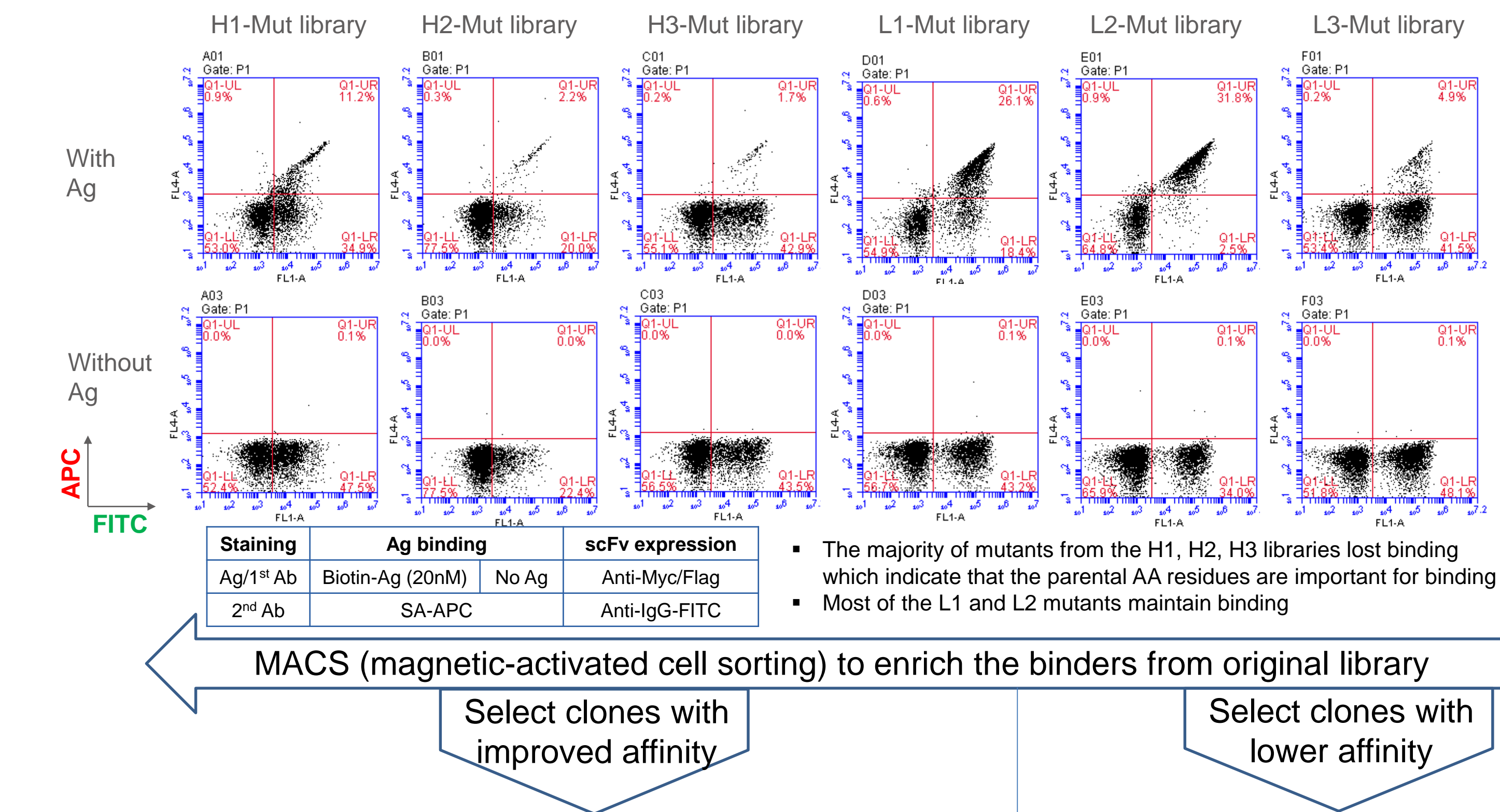


Table 1. Yeast Library QC

Library	Size	QC # of clones	Unique	%
H1-Mut	1.4 x 10 ⁷	26	26	100%
H2-Mut	2.4 x 10 ⁸	31	31	100%
H3-Mut	1.8 x 10 ⁸	28	28	100%
L1-Mut	4.6 x 10 ⁸	30	28	93.3%
L2-Mut	5.3 x 10 ⁸	28	27	96.4%
L3-Mut	4.0 x 10 ⁸	28	28	100%

Figure 2A. Sorting Strategy for Higher Affinity Clones

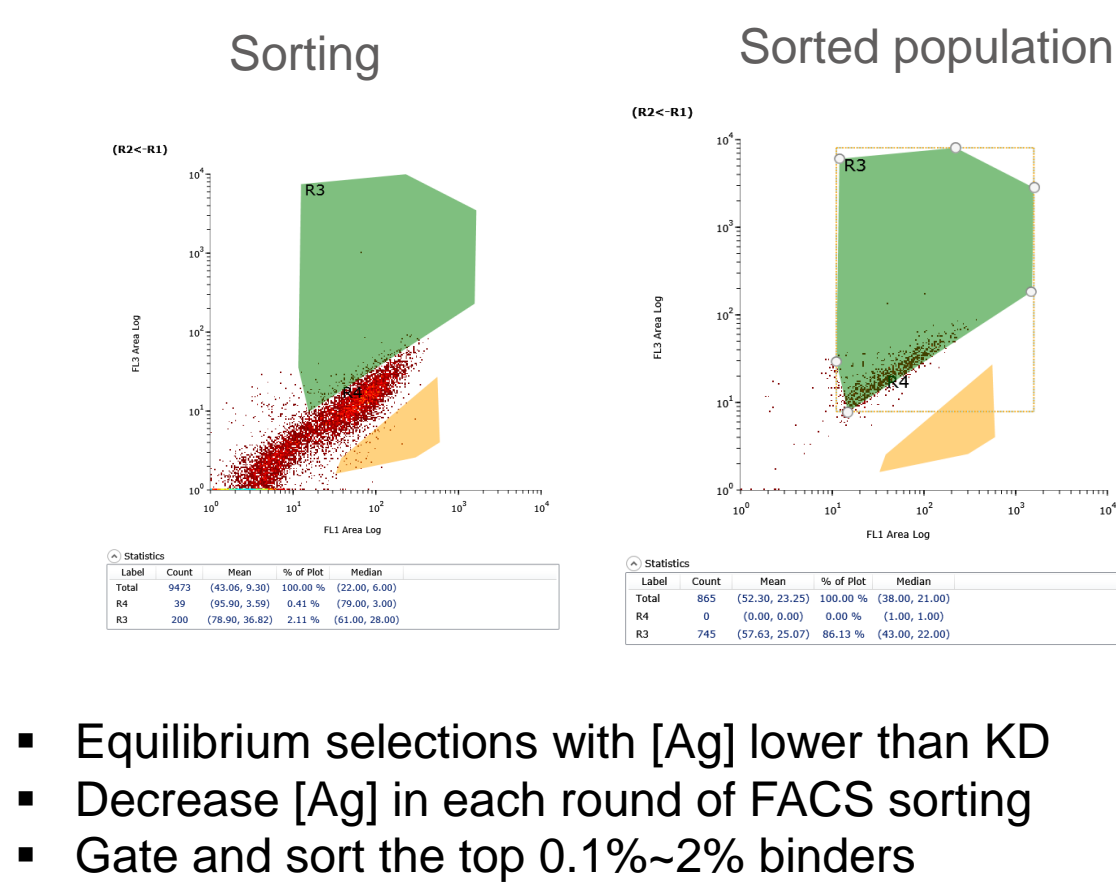


Figure 2B. Sorting Strategy for Lower Affinity Clones

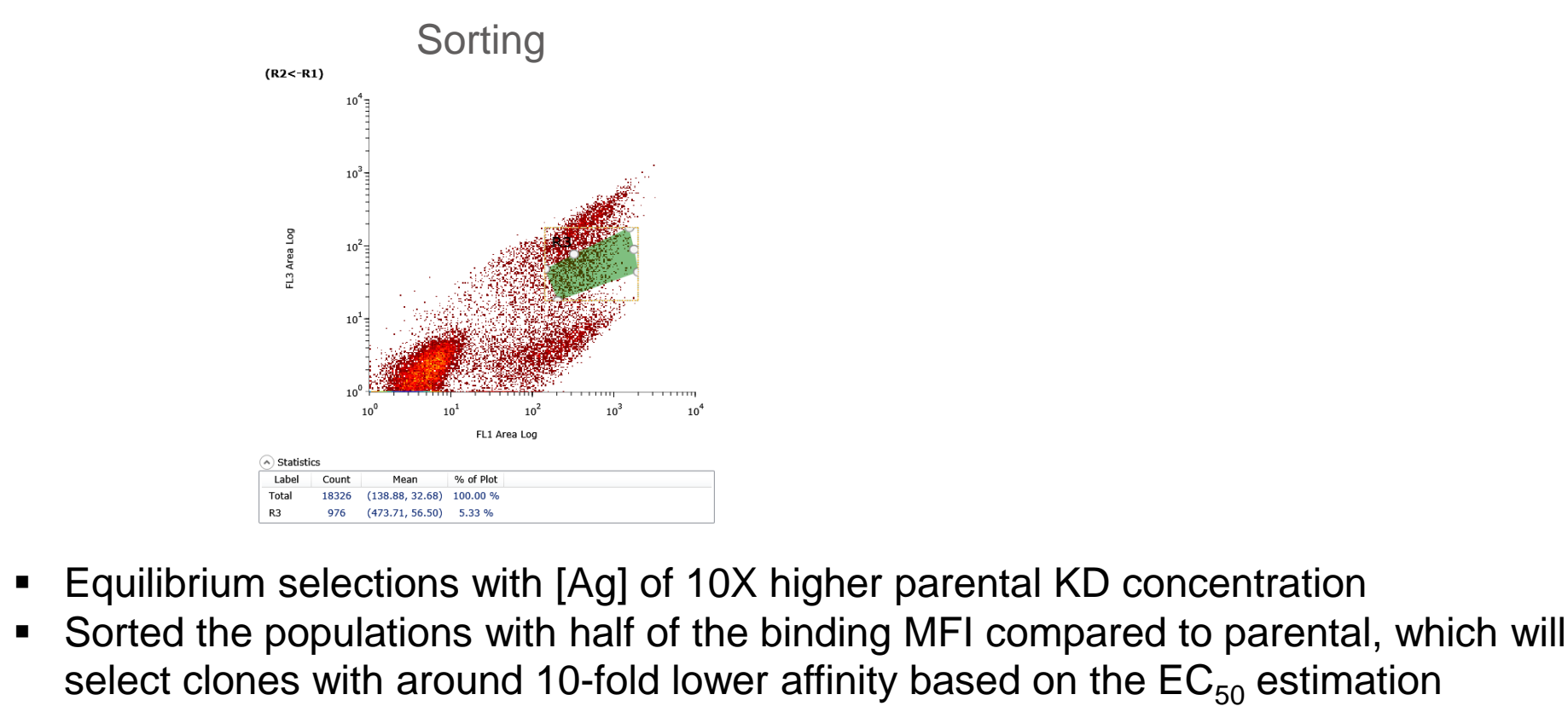


Figure 3A. Validation of Higher Binders from Sorted Library

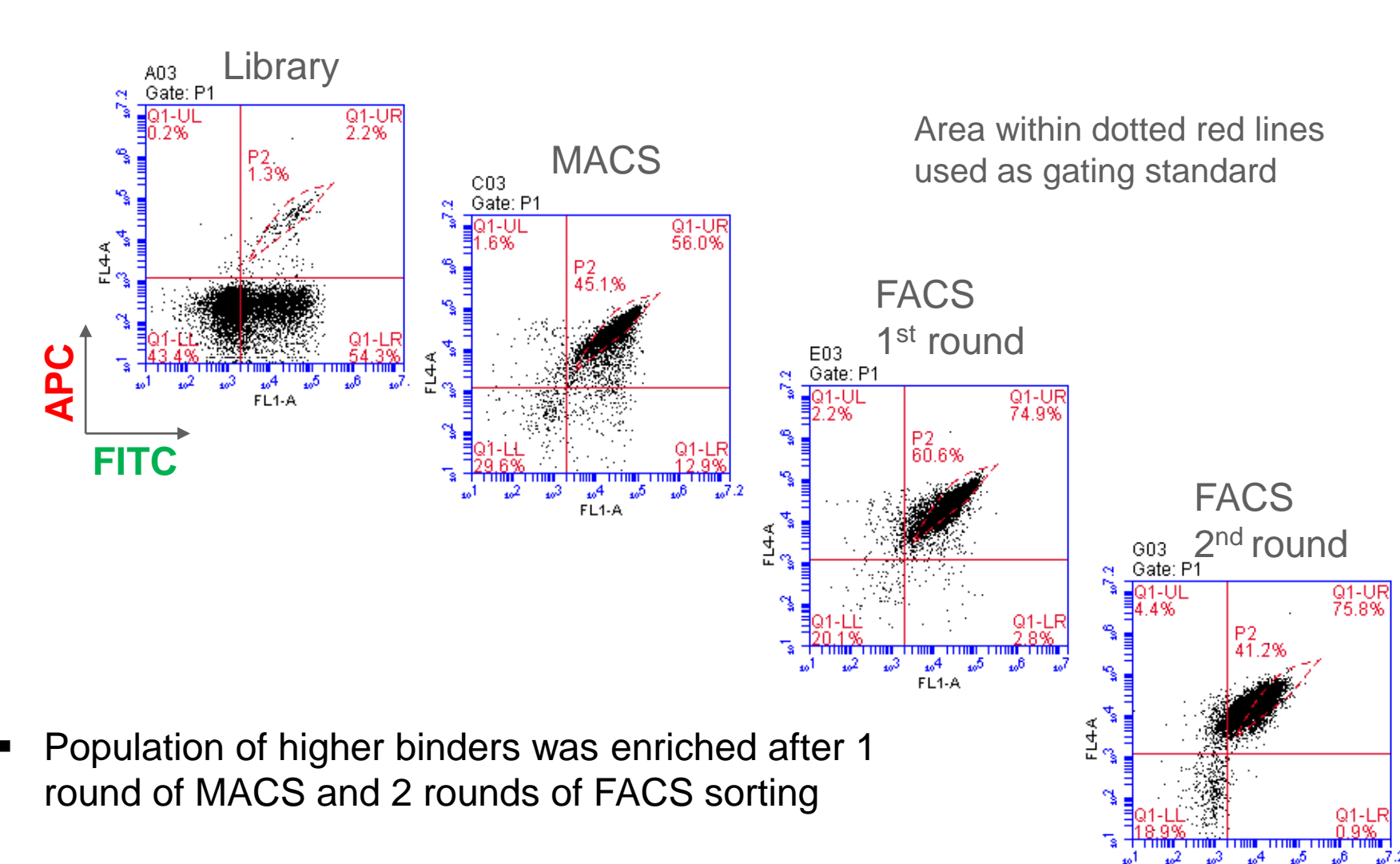


Figure 3B. Validation of Lower Binders from Sorted Library

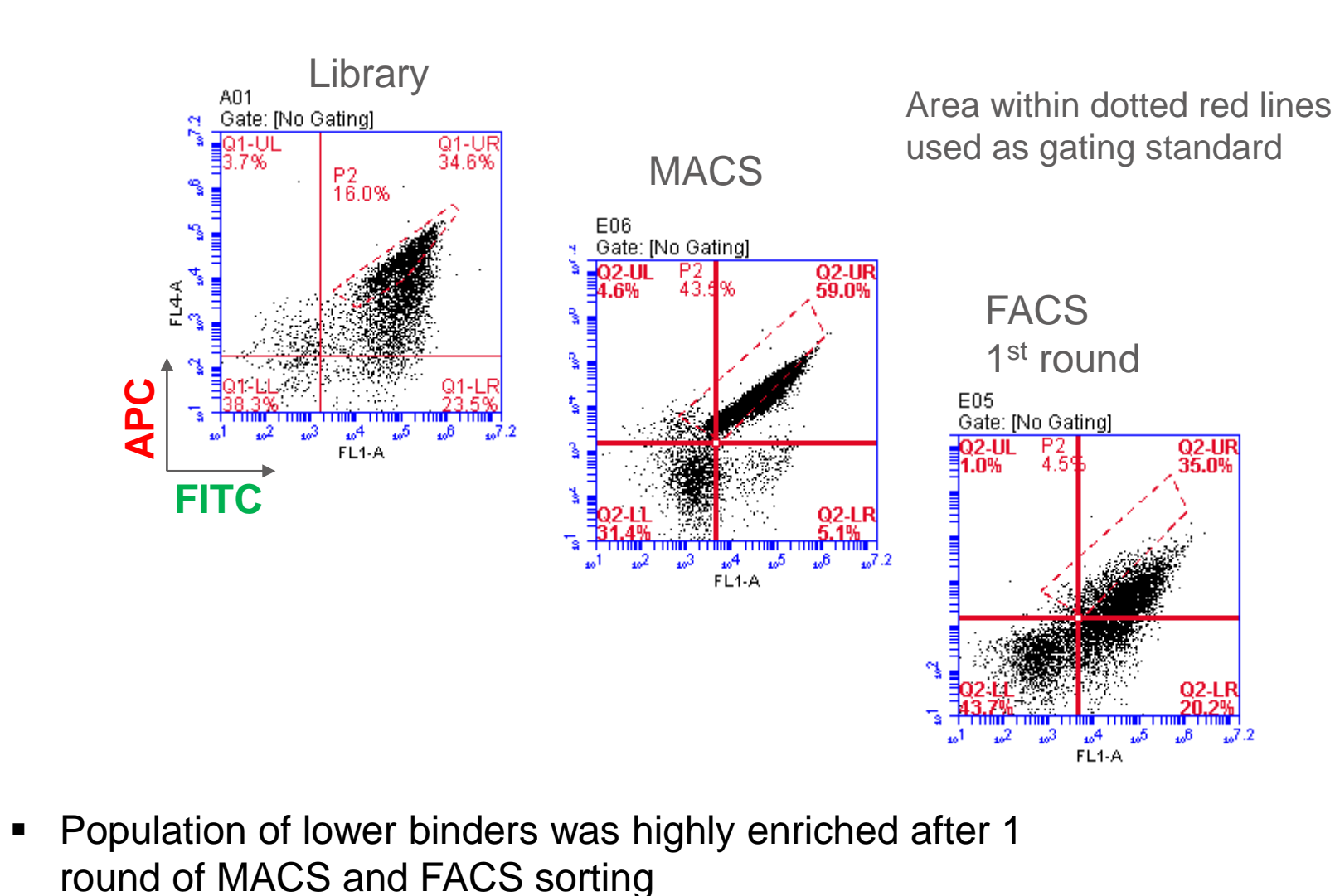
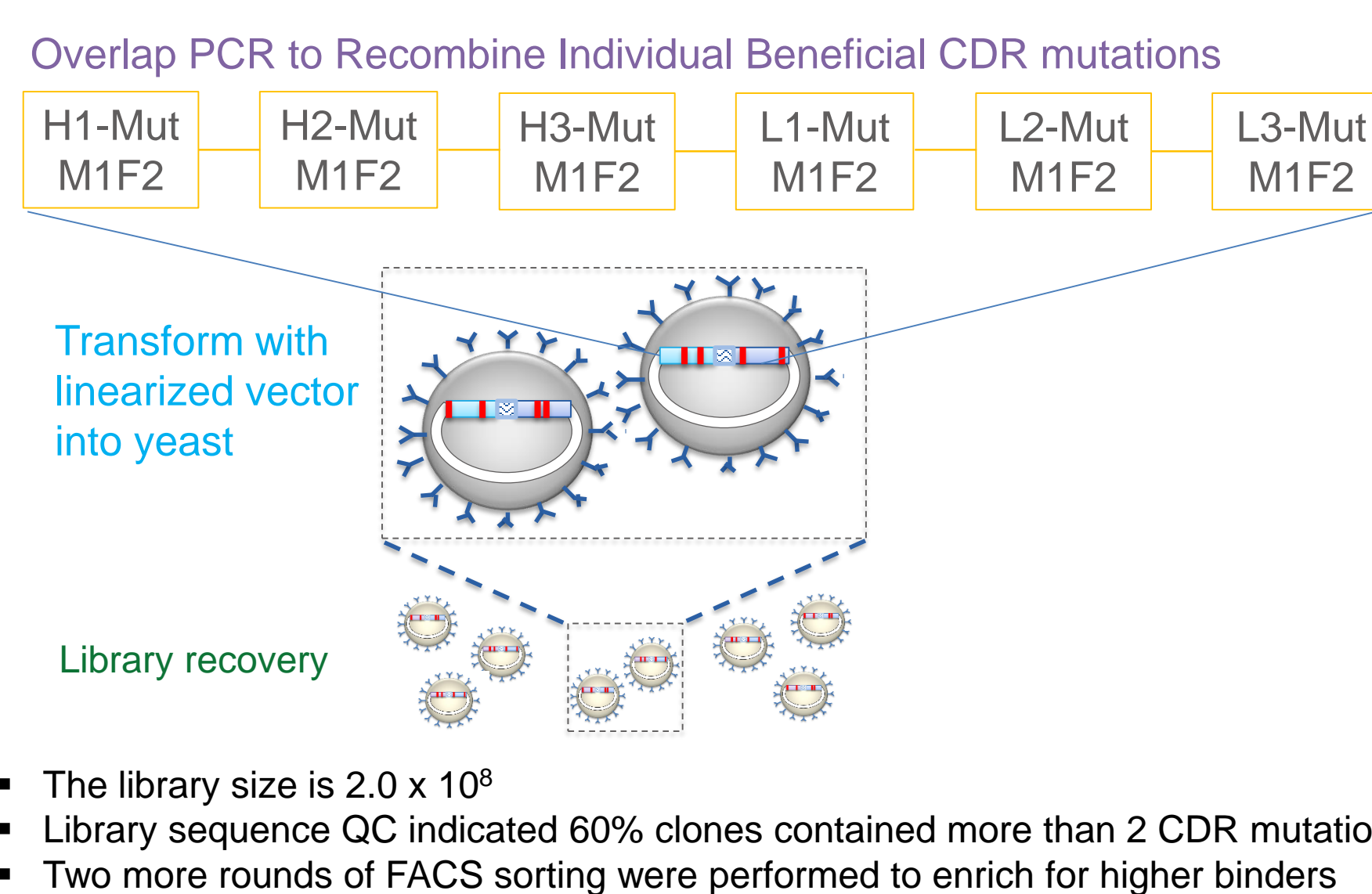


Figure 4. A New Library of Recombined CDR Mutants Was Generated to Further Improve Affinity



- The library size is 2.0 x 10⁸
- Library sequence QC indicated 60% clones contained more than 2 CDR mutations
- Two more rounds of FACS sorting were performed to enrich for higher binders

Figure 5A/B. Screening and Ranking of High Affinity Clones Via FACS EC₅₀ Assay

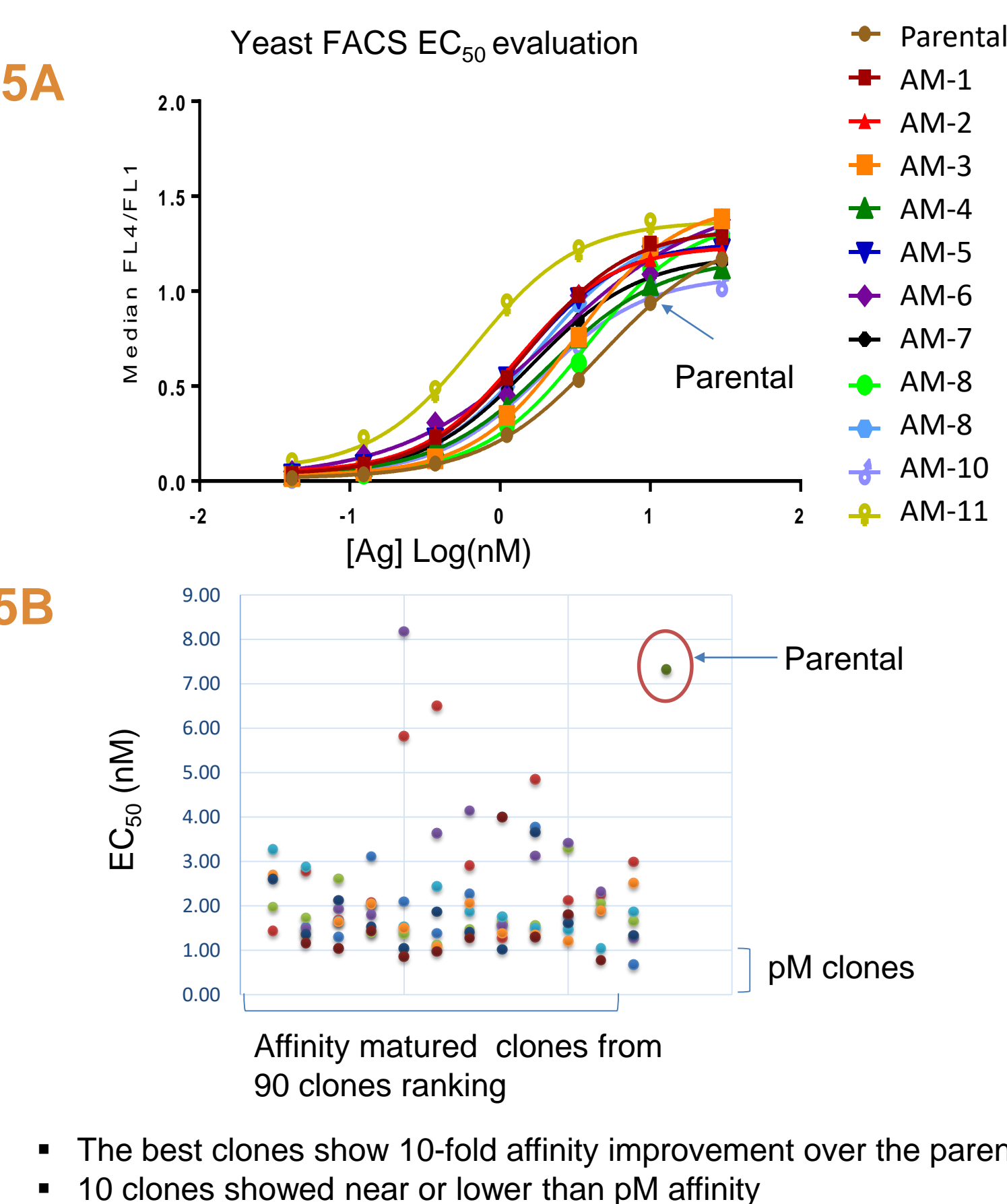
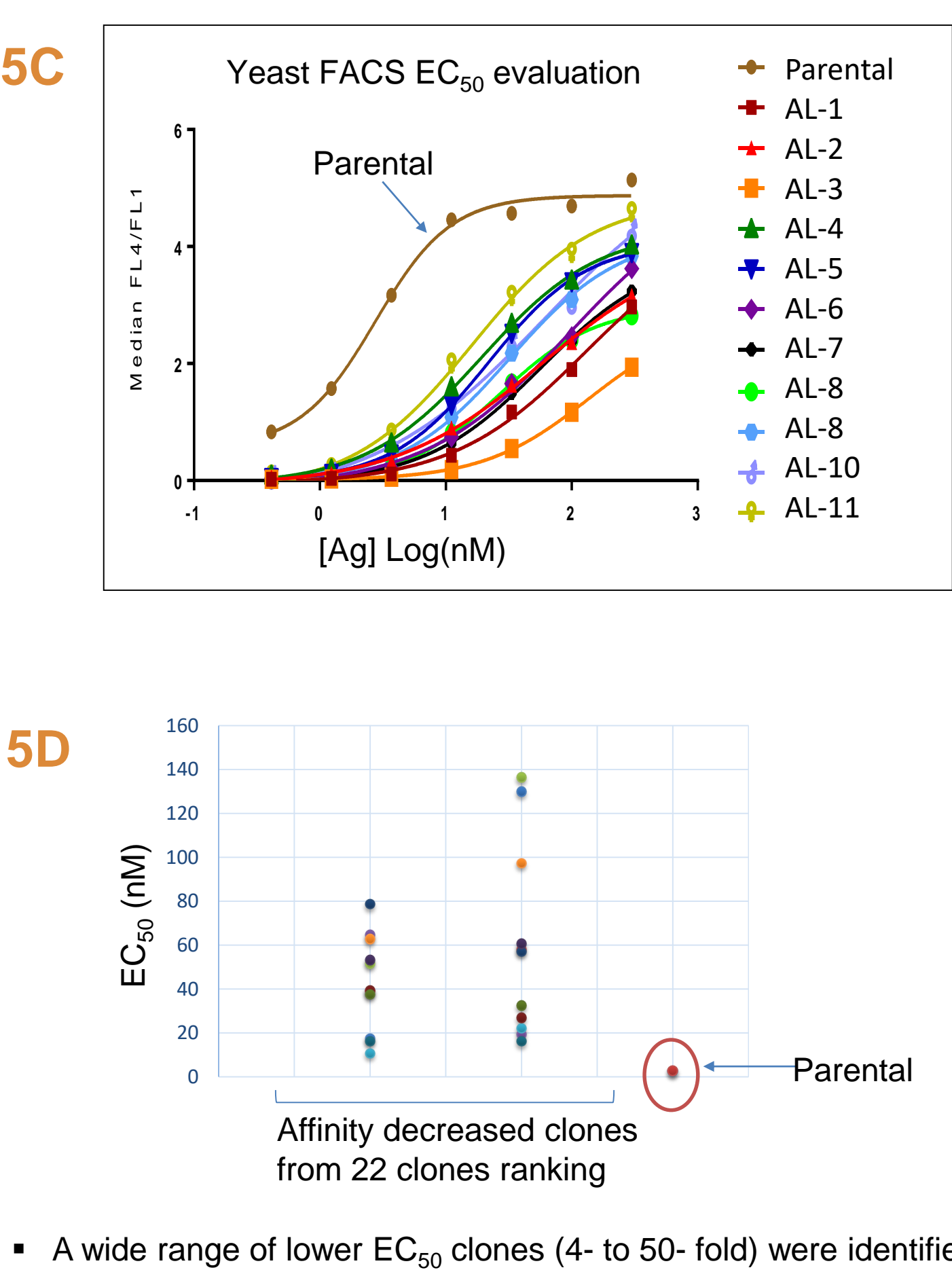


Figure 5C/D. Screening and Ranking of Lower Affinity Clones Via FACS EC₅₀ Assay



CONCLUSIONS

- Using different selection strategies via yeast display, we were able to engineer a wide range of affinities to a parental antibody
- Affinity maturation increased the affinity to picomolar levels, around 10-fold higher than the parental counterpart
- Affinity “detuning” can lower the parental clone’s affinities 4- to 50-fold, which will provide a broad selection for applications including affinity-function research, CAR or bi-specific design

REFERENCES

[1] Lorenzo *et al.* An improved yeast transformation method for the generation of very large human antibody libraries. PEDS, 2010, 23(4), 155-159.