Fine-tuning yeast surface display selection for efficient antibody discovery

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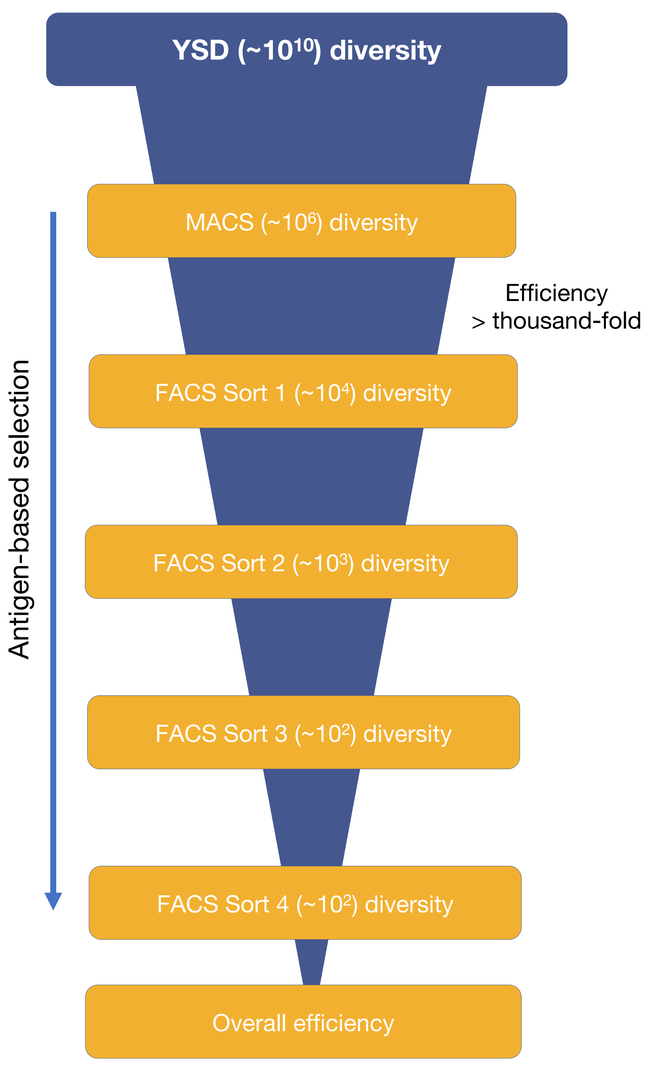
Introduction

Yeast surface display (YSD) remains an attractive approach for antibody discovery, affinity maturation and directed protein evolution studies. The fusion and display of antibodies or proteins using the Aga1P-Aga2P system allows separation of cells of interest. In an antibody discovery campaign, yeast cells recognizing a target of interest are screened and enriched by utilizing magnetic- and fluorescence-activated cell sorting (MACS/FACS). However, in a newly designed YSD library, the efficiency of these steps remains obscure. Here, we used anti-PDL1 antibodies (discovered in-house and licensed out) to understand the efficiency of MACS and FACS steps in our overall antibody discovery process. Yeast cells expressing anti-PDL1 were labeled with Carboxyfluoroscein Succinimidyl Ester (CFSE) and spiked in at 1,000 or 100,000 copies into our YSD library (1E+10 yeast cells). Our results showed greater than a thousand-fold enrichment of anti-PDL1 clones when we spiked in 1,000 or 100,000 copies at MACS. Adalimumab was used as a specificity control. We recovered all five spiked clones when we added 100 copies and four when we added 25 copies into our YSD library (4.5E+10 yeast cells). These clones were recovered with varying degrees of NGS read counts. These studies will allow further optimization of the antibody discovery process.

Materials & Methods

Schematic of antibody discovery at IPI



We start our process by selecting among 10 billion different binding sequences on our MACS machines, which quickly reduces the binding population to a few million cells. We use FACS to further the filtering process on our flow cytometry machines. We then use NGS to determine which antibodies to produce.

Efficiency of MACS

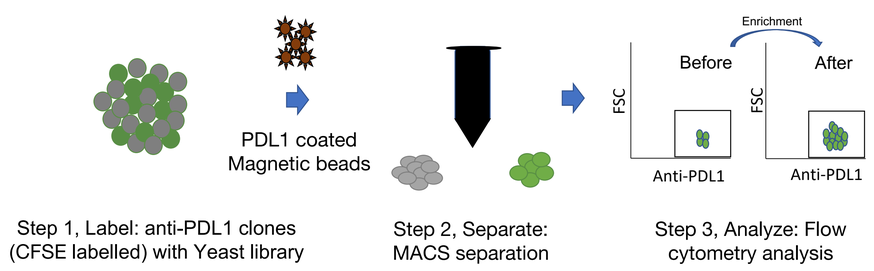
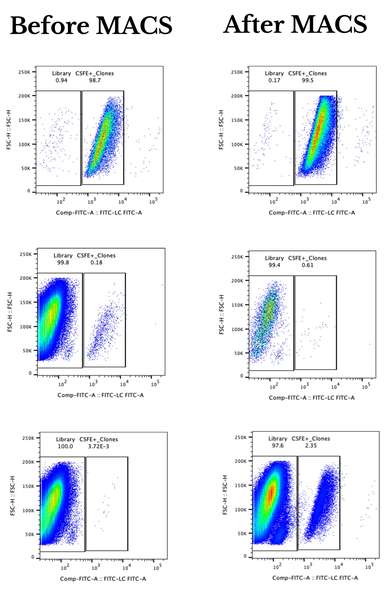


Figure 1: anti-PDL1 clones were spiked in to determine efficiency.

A known number of CFSE-labeled anti-PDL1 yeast clones were spiked into the library, and MACS was performed. We compared output with a sample not purified using MACS (before MACS) or after MACS purification to calculate the enrichment scores per FACS analysis.



Positive Control

1E8 of anti-PDL1 clone alone was used as a positive control. As expected, mostly PDL1 binders were measured before and after MACS selection.

Specificity Control

1E5 of Adalimumab specificity control spiked into 1E8 of our IPI yeast library, demonstrating de-enrichment.

1E5 anti-PDL1 clone spiked into 1E10 Library

An example result. We spiked 1E5 anti-PDL1 into 1E10 of our IPI library. Note enrichment of PDL1 clones in the right panel of the "After MACS" sample.

Figure 2: A sample dataset showing scatterplot between CFSE vs. FSC (forward scatter) before and after MACS enrichment.

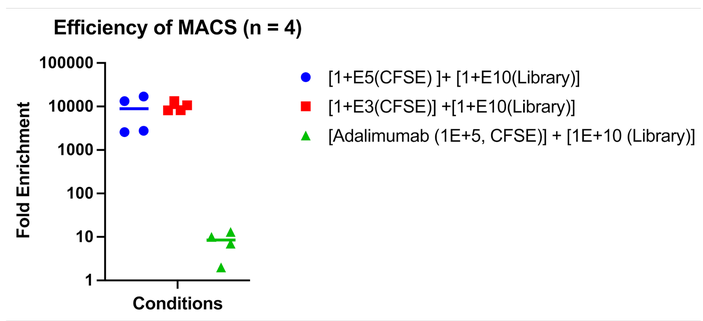


Figure 3: A dot plot showing Fold Enrichment* for different conditions.

$$\textbf{Fold Enrichment*} = \frac{A}{B}$$

A = After MACS [CFSE+ Clone Count / FSC Singlets Count]

B = Before MACS [Input Clone Amount / Input Library Amount]

Overall Efficiency

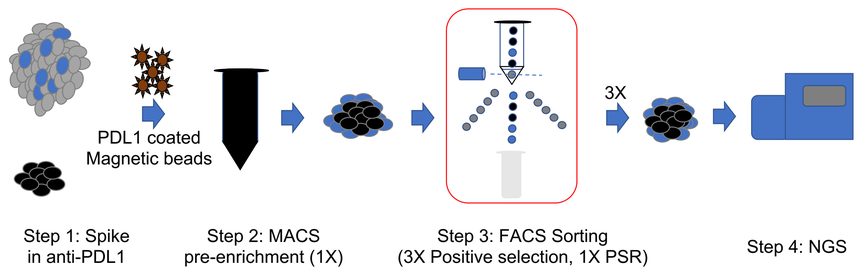


Figure 4: The efficiency of our pipeline was assessed by spiking in multiple anti-PDL1 clones, which undergo the same selection process as an IPI antibody discovery campaign. The process begins with MACS, followed by multiple rounds of positive FACS sorts and one negative FACS sort (polyspecficity reagent sort).

In these experiments, we used 25 or 100 copies of the known anti-PDL1 binders (five clones) which were later recovered after the NGS step to further validate our process and calculate the overall efficiency.

Block104	Concentration	4nM	1nM	
TAB ID	Sort Round	Aff2	Aff3	KD
TAB0000640	RVDWEDY	7220	26182	9.80E-09
TAB0000645	DERGVDRWG	6075	22623	8.80E-09
TAB0000652	TGPVVFGYLFG	3496	6082	5.50E-09
TAB0000660	VIFPYIFGRQGD	2183	9623	3.10E-08
TAB0000661	GPLYAAR	1897	3309	6.10E-08

Table 1: NGS of 100 copy spike in
NGS results when 100 copies of each clone (x5) were spiked into our IPI yeast library of 45 billion clones. All clones were recovered. The kinetic values (KD) shown were derived using surface plasmon resonance (SPR).

Block110	Concentration	100nM	20nM	PSR1	4nM	
TAB ID	Sort Round	Aff1	Aff2	PSR1	Aff3	KD
TAB0000640	RVDWEDY	97	603	561	1513	9.80E-09
TAB0000645	DERGVDRWG					8.80E-09
TAB0000652	TGPVVFGYLFG	900	8340	5250	24068	5.50E-09
TAB0000660	VIFPYIFGRQGD	69	849	693	2341	3.10E-08
TAB0000661	GPLYAAR	82	1153	6048	7894	6.10E-08

Table 2: NGS of 25 copy spike in
NGS results when 25 copies of each clone (x5) were spiked into our IPI yeast library of 45 billion clones. One clone was not recovered. The kinetic values (KD) shown were derived using surface plasmon resonance (SPR).

Summary

After staining our licensed PDL1 binder with CFSE dye, we used it to determine a larger than thousand-fold enrichment of our binders and approximate the efficiency of our MACS machine. We also show that we can spike our PDL1 clone into our library at a copy number of 25 clones and still recover it using our IPI pipeline process. We aim to use this analysis process to determine the kinetic properties of each clone in the NGS data; improve our antibody selection process; and determine better MACS or FACS methodologies, thereby improving the efficiency of the Antibody Discovery Platform.

Want To Collaborate With Us?

We have generated antibodies to more than 200 cell surface receptor targets. We are open to collaborations from industry and academia. Contact us at **https://proteininnovation.org/receptor-engineering/** or via **rob.meijers@proteininnovation.org**

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