

7.003 Spring 2022
Day 21 In-Lab Questions

1) What protocol modifications did you incorporate into the three magnetic bead selections you performed today? How do you predict your protocol modifications will affect the sensitivity and specificity of the bead selection protocol?

2) What information will you need to know to be able to calculate the sensitivity and specificity of your bead selection protocols? How will you be able to determine this information from your plate results next week?

3) Why did you have to prepare serial dilutions of each final enriched bead selection sample before plating them (Steps 9 – 15)? Why didn't you just directly plate the final enriched bead selection without making any dilutions?

4) The table below summarizes the preparation of the serial dilutions of your final enriched bead selections (Steps 9 – 15). Fill in the missing information in the table.

Guide to preparing serial dilutions for bead selections				
Tube	Volume of PBSA	Volume & source of yeast cells	Dilution of original bead selection	Fraction plated of original undiluted bead selection if you plated 20 μL
#1	None	200 μ L of undiluted bead selection		
#2	180 μ L PBSA	20 μ L of cells from the "#1" tube		
#3	180 μ L PBSA	20 μ L of cells from the "#2" tube		
#4	180 μ L PBSA	20 μ L of cells from the "#3" tube		

5) What is the distinction between a dilution and a fraction? When calculating specificity and sensitivity of your bead selections, which will be more important to know: the dilution of the original bead selection that was plated in each plate quadrant or the fraction of the original bead selection that was plated in each plate quadrant?

6) In flow cytometry, when an object (cell/particle) is passing through the interrogation point (laser beam), the object refracts and scatters light at all angles. Optics can detect forward scatter (FSC) and side scatter (SSC). FSC is the amount of light scattered in the forward direction. SSC is the light scattered and detected at 90 degree (perpendicular to the laser).

A) Which one (FSC or SSC) do you believe is roughly proportional to the object size? Explain.

B) Which one (FSC or SSC) do you think is proportional to the internal complexity of a cell? Explain.

7) We are sorting with fluorescent labels (AF488 & AF647) similar to the diagram below. The yeast surface protein binds to your purified COVID-19 biomarker target, which has both a biotin and His-tag. The yeast surface protein has both a c-Myc and HA-tag.

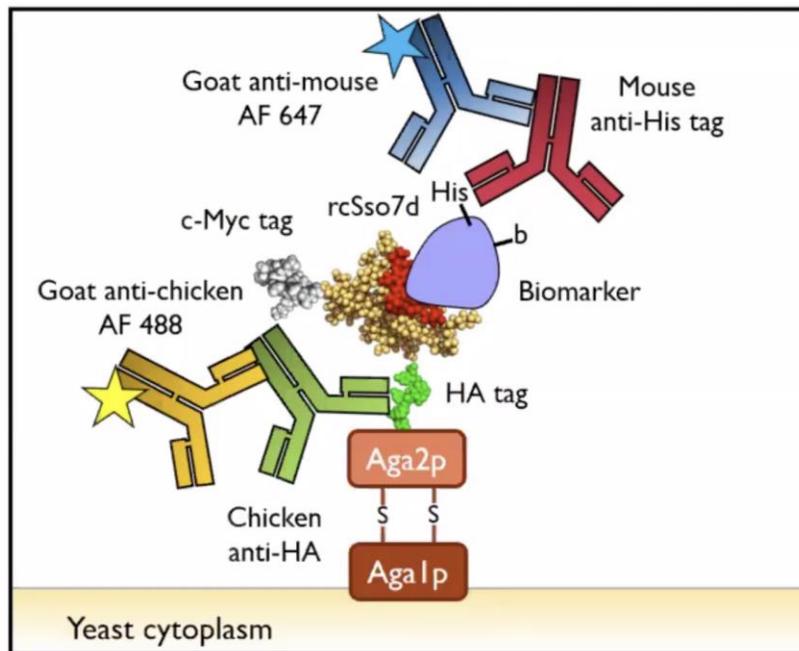


Figure 2C in: Miller, EA, et al. "Beyond Epitope Binning: Directed in Vitro Selection of Complementary Pairs of Binding Proteins" *ACS Combinatorial Science* 2020 22 (1), 49-60. DOI: [10.1021/acscombsci.9b00176](https://doi.org/10.1021/acscombsci.9b00176). Copyright © 2019 American Chemical Society. This is an unofficial adaptation of a figure in an article that appeared in an ACS publication. ACS has not endorsed the content of this adaptation or the context of its use.

A) How is the AF488 or AF647 detection in flow cytometry different than the FSC or SSC detection?

B) What do the AF488 signal and the AF647 signal each represent in the surface display system?

C) What kinds of controls do we need to remove the fluorescent signals from the background and non-specific binding?

D) Three different FACS controls are depicted below. Define the scenario represented in each case.

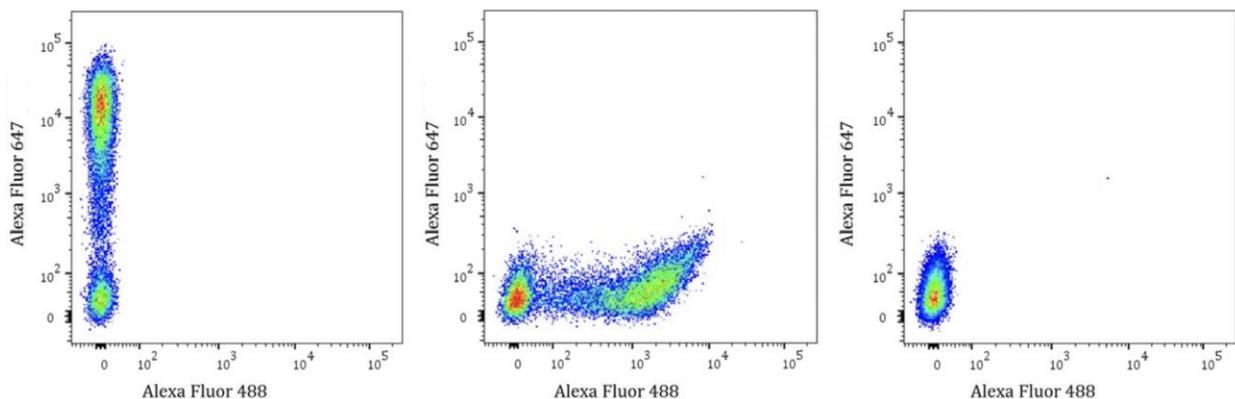


Figure S2: Representative cytometry plots from the single-color controls (AF-647 and AF-488, respectively), and the secondary binding control. From [Supplementary Info](#). In: Activity-based assessment of an engineered hyperthermophilic protein as a capture agent in paper-based diagnostic tests. E. A. Miller, M. W. Traxlmayr, J. Shen and H. D. Sikes, *Mol. Syst. Des. Eng.*, 2016, 1, 377 [DOI: 10.1039/C6ME00032K](https://doi.org/10.1039/C6ME00032K). © The Royal Society of Chemistry 2016. All rights reserved.

8) Negative fluorescence controls help us to define quadrants, where (i) a dot plot can be dissected and (ii) double positive fluorescence signals can be defined and counted. The image below depicted how quadrants and gating are set and their respective percentage of events in each area.

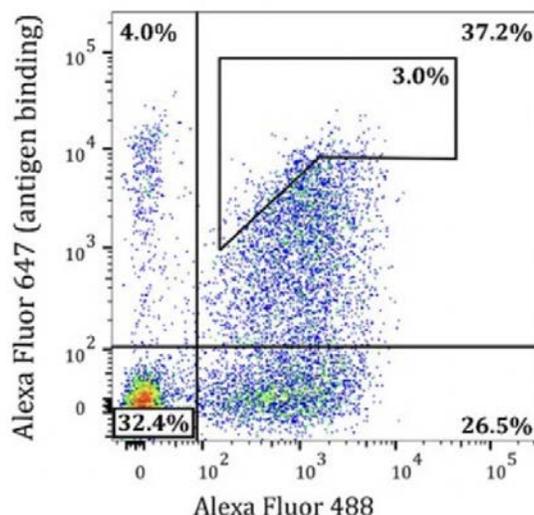


Figure S1: Cytometry plots from Rounds 1-5 of FACS library screening. From [Supplementary Info](#). In: Activity-based assessment of an engineered hyperthermophilic protein as a capture agent in paper-based diagnostic tests. E. A. Miller, M. W. Traxlmayr, J. Shen and H. D. Sikes, *Mol. Syst. Des. Eng.*, 2016, 1, 377 [DOI: 10.1039/C6ME00032K](https://doi.org/10.1039/C6ME00032K). © The Royal Society of Chemistry 2016. All rights reserved.

A) Intuitively, we draw a sorting gate and collect the library population with high affinity binding of our biomarker. Describe the advantage of the gating strategy to collect 3.0% of the population captured in the diagram.

B) If you wanted to make your FACS selection more specific, how might you adjust your overall gate? How would you predict your new gate would affect the sensitivity of your FACS selection?

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