7.003 Spring 2022
Day 20 In-Lab Questions

1) Draw a diagram of a yeast cell from a surface display library bound to a target-coated magnetic bead. Indicate any relevant reagents/proteins or relevant components on plasmids.

2) Surface display technologies utilize the concept of "linking phenotype to genotype." What is the advantage of using a surface display technology to find a binder to a target (as compared to simply mixing a collection of antibodies with a target)?

3) What is meant by performing a "negative selection" when screening a surface display library? How would one do a negative selection with magnetic bead sorting and under what circumstances might one want to perform a negative selection?

4) When performing a magnetic bead sorting protocol, it is important to consider sensitivity and specificity.

A) In the context of magnetic bead sorting, define what sensitivity and specificity represent.

B) What are some ways to alter the conditions of your magnetic bead sorting protocol that could potentially affect the sensitivity and/or specificity of the overall selection?

C) You have two different magnetic bead selection protocols. Protocol #1 has a high percent sensitivity but a low percent specificity. Protocol #2 has a high percent specificity but a low percent sensitivity. Which protocol (#1 or #2) might be better to use for your very first round of screening a surface display library for potential binders to a target? Explain.

5) On the next lab day (Day 21), you and your lab partner together will perform a set of three magnetic bead selections, with the goal of trying to optimize the protocol conditions to achieve both high sensitivity and specificity in your selections. Read the Background Information and Steps (A) – (E) for Lab Day 21 (pages 119 – 120 of the lab manual). Steps (A) – (E) summarize the overall steps for performing a magnetic bead selection using "standard" protocol conditions.

You and your lab partner together will perform a total of three different magnetic bead selections on Day 21. Determine what specific modifications (if any) you will make to the standard bead selection protocol for each of your three bead selections. You might choose to modify the same parameter(s) to varying degrees for all three selections or you might choose to design completely different conditions for each of the three selections – the choice is up to you and your lab partner.

Before you leave lab today, you must check with Vanessa or Eric to confirm your bead selection protocol conditions – the teaching staff will need this information in advance of Day 21 so that they can set up the appropriate reagents properly for your lab group based on the protocol modifications you chose.

*****Please be sure to do this before you leave (it will count towards your lab participation score for today!).*

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