OMV Purification

Introduction

Purpose: OMV purification from JC8031 (no antibiotic) and DeLisa ClyA-GFP (Cm+) fusion
OMVs were purified as described in the SBI protocol - 2 columns (one for GFP OMVs and ones for JC8031 no GFP)

Materials

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Procedure

To Purify OMVs

1. Prepare clarified supernatant from bacterial culture
2. Culture the bacteria in its growth medium overnight at 37 deg.
3. Spin down bacteria at 5000xg for 15 minutes at 4 deg C
4. 4000xg for 17 minutes - centrifuge would not go any higher
5. Transfer the supernatant to a new flask and filter through a 0.45um filter
6. Spin down the supernatant at 5000xg for 15 minutes at 4 deg C
7. Transfer the supernatant to a new flask and filter through a 0.22um filter. The filtered supernatant is now ready for OMV isolation
8. Pack the column/bind OMVs
9. Pipette 200 mL of the resin onto the column
10. Equillibrate by adding 1mL of the Binding Buffer and allow the solution to flow through. Discard the flow through
11. Place the yellow cap onto the bottom of the column
12. Add 10mL of the clarified bacterial supernatant (prepared in step 1) to the resin and incubate on a rotating rack at 4 deg C for 3-4 hours to allow for OMV binding
13. Used centrifuge at a very low speed.

OMV Elution

14. Place the column onto a rack and allow the resin/supernatant to flow through (collect the flow through for analysis if desired)

15. Wash the resin with 10mL Binding Buffer 2 times. Discard the flow through.
16. Add 500uL Elusion Buffer and collect in in 1.5ml Eppendorf tube
17. Repeat the elusion step for a total of 5 times in separate tubes

Analysis

18. Perform downstream analysis of the five separate elutions (or pool if desired)