Differential Centrifugation, Antibiotic and Filtration Purification Protocol

Adapted from "A combination approach for rapid and high yielding purification of bacterial minicells" by Jivrajani, et al., 2013

1. Centrifuge 200 mL culture, grown in LB broth with appropriate antibiotic, for 10 minutes at 2000xg to pellet parent bacteria. Approximately 90% of the parent strain will be separated from the minicells by initial differential centrifugation.
2. Centrifuge the supernatant for 10 minutes at 10,000xg to pellet minicells.
3. Resuspend the minicell pellet in 50 mL of LB broth and incubate at 37ºC, 180 rpm for 45 minutes to facilitate reinitiation of cell growth.
4. Add Ceftriaxone at a dose of 100µg/mL and incubate again at 37ºC, 180 rpm for 45 minutes. This dose of Ceftriaxone is sufficient to cause cell lysis without having any detrimental effect on minicells.
5. Centrifuge the broth at 400xg for 5 minutes to remove cell debris and dead cells.
6. Centrifuge the supernatation at 10,000xg to pellet minicells.
7. Wash minicells with 50 mL fresh broth.
8. Filter minicells through 0.45 µm dead end filter (Millipore) to remove any residual parent strain.
9. Filter minicells in 0.22 µm cross-flow filter (Millipore) to remove any cell debris and free endotoxins.

Sucrose Gradient Purification Protocol

Adapted from "Grazing Rate of Bacterioplankton via Turnover of Genetically Marked Minicells" by Johan Wikner in Handbook of Methods in Aquatic Microbial Ecology, 1993

1. Grow strains overnight in 10 mL of LB containing appropriate antibiotic.
2. Inoculate the 10 mL overnight culture into 400 mL of fresh LB prewarmed to 37ºC with proper antibiotics. Grow at 37ºC for approximately 5 h until an optical density measured at 600 nm of 0.8 is reached.
3. Centrifuge 10 min in 250-mL centrifuge tubes at 27,000 x g and 4ºC.
4. Resuspend cells in 10 mL of 1 x BSG.
5. Vortex the 250-mL bucket while disrupting the pellet with a 10 mL pipette. Suck the suspension in an out of the pipette five times. Transfer to a 60 mL centrifuge tube.
6. Pellet the cells 10 min at 20,000 x g and 4ºC.
7. Resuspend the cells in 2 mL 1 x BSG using a vortexer and a 5 mL pipette for at least 60 sec.
8. Layer the cell suspension on an ice cold sucrose gradient in 60 mL centrifuge tubes, by slowly pouring the suspension along the side of the tube.
9. Centrifuge in a cold swing-out rotor at 4100 x g for 20 min at 4ºC.
10. A could of minicells should be seen in the middle of the tube. Pull out minicell cloud with a syringe (approximately 12 mL). Suspend in 10 mL 1 x BSG
11. Pellet cells at 20,000 x g and 4ºC for 13 mins.
12. Discard supernatant and resuspend the pellet in 1 mL 1 x BSG. Use vortexer and a 1 mL pipette for 60 sec.
13. Layer the minicells on a sucrose gradient in a 15 mL centrifuge tube.

14. Centrifuge 20 min at 4100 x g and 4ºC with rubber adaptor.
15. Take out eh minicell cloud and squeeze into a 60 mL centrifuge tube with 10 mL of minicell medium.
16. Centrifuge 13 min at 20,000 x g and 4ºC.
17. Discard the supernatant and resuspend the pellet in 2 mL of MM by vortexting at least 60 sec (no pipetting). Add 10 mL MM and mix carefully.
18. Centrifuge 13 min at 20,000 x g and 4ºC.
19. Resuspend pellet in 2 mL of MM by vortexing for 30 sec. Add an appropriate volume of MM to give a final OD of 0.25 (optional).