# 1- OMV Extraction Protocol

## Introduction

Creator: Arthur
Demonstrator: This is the general protocol for purifying OMVs. This has not been tested by this lab yet so parts may change. The protocol shown has been adapted from a previous iGEM team.
Edit log:

Arthur - Created File
Noah 15/09 - Tidied and Printed

At the end of this procedure you should end up with very few proteins in a cell free sample of OMVs larger than a 100kDa protein and smaller than ~220um.

## Materials

- Shaking Incubator
- Beckman Avanti Swinging bucket centrifuge
- 100kDa centrifugal filter units (Millipore)
- 0.2µm syringe filters (Millipore)
- Sterile 20ml syringes
- PBS

## Procedure

### Bacterial Growth

1. 100mL of LB with appropriate antibiotic (if required) was inoculated with each strain for analysis, and placed on a shaking incubator at 37$^{o}$C, shaken at 200 rpm
2. After 3 hours of incubation, any necessary inducing agents (IPTG at 0.5 mM) were added the cultures, which were then left on the shaking incubator at 37 degrees overnight

### Cell Extraction

3. The **next day**, the OD600 of each culture was measured before OMV harvest *(what is the point of this step?)*
4. Each culture was then centrifuged for 20 mins at 6000g in a fixed angle rotor to pellet the biomass
5. The supernatant was recovered and then passed through a 0.2µm syringe driven filter *(how much can be passed through a single filter?)*

### Protein Extraction And OMV Collection

6. 40mL of the syringe-filtered supernatant was then loaded into a 100kDa centrifugal filter unit *(this is letting most proteins filter through while retaining the OMVs in the retentate)*

7. The filter unit was then centrifuged at 3500g in a swinging bucket rotor for 15 minutes

8. The volume of the filtrate was recorded and then discarded

9. PBS of equal volume to the discarded filtrate was loaded into the filter unit on the retentate-side

10. The filter unit was then centrifuged at 3500g for 10 minutes in the same swinging bucket rotor

11. The filtrate was again discarded. The retentate was recovered by fitting a **retentate collection cap** to the filter unit and centrifuging with the filter in the opposite to normal orientation for 5 minutes at 1000g. This was recovered and the volume recorded. *(what is a rententate collection cap?)*

12. 400µL of PBS was loaded directly onto each of the 2 filter units of the filter unit *(Why? Presuming you then centrifuge again, given step 13?)*

13. The sample above was combined with the retentate collected in step 11

14. OMVs were stored at 4$^{o}$C until analysis *(N.B. store in cold room. How long do these last?)*