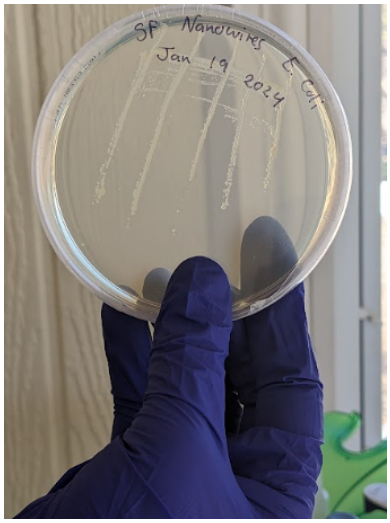
Master Plate

Plate E. coli from glycerol stock stored in -80

Streek E. coli on LB Agar plate with Kanamycin ~ every 3 weeks for continuous use.

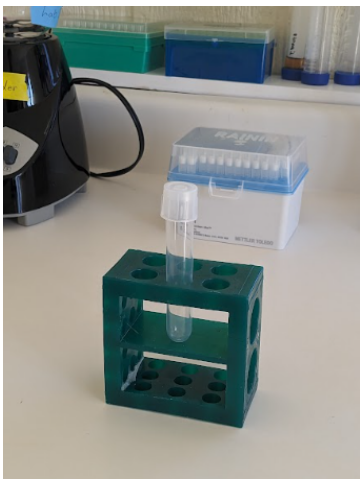
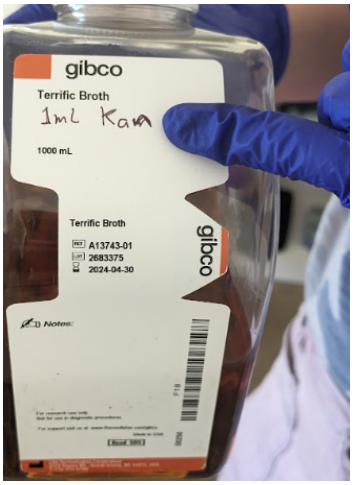
- Plates originated from Teknova
- LB Agar Plates with Kanamycin - 50



Starter Culture - 3mL

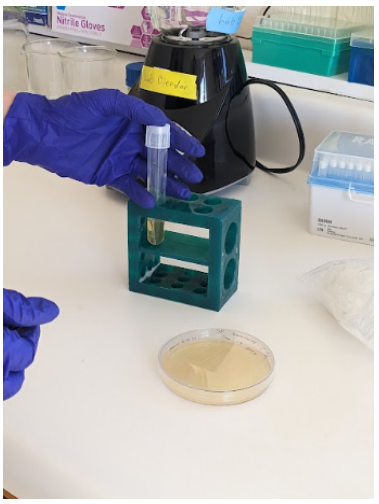
Use Terrific Broth from Teknova as media
Use 50ug/mL kanamycin as antibiotic
Shake for ~ 2-3 hours
Incubate at 37°C

1. Pipet 3mL Terrific Broth media into a 14mL round bottom culture tube with 50ug/mL kanamycin.

2. Pick a colony from the master plate with your micropipette tip and release the tip with the colony into the 3mL of Terrific Broth media. Leave the tip inside the 14mL tube.





3. Place liquid starter culture colony into the shaker at ~ 220rpm for ~ 2-3 hours at 37°C.

Batch Production - 100mL

Use Terrific Broth from Teknova as media
Use 50ug/mL kanamycin as antibiotic
Autoinduction with 30uM IPTG
Shake overnight for ~ 16 hours
Incubate at 30°C

1. Pipette 500uL of starter culture into 100mL Terrific Broth with 50ug/mL kanamycin in a 500mL Erlenmeyer baffled flask.

2. Add 30uM (30uL/L) IPTG to colony
3. Place Erlenmeyer baffled flask into shaker overnight at ~ 220rpm for ~16 hours at 30°C.


PilA Protein Extraction - 100mL:

Use 150mM ethanolamine, pH 10.5 as buffer
Use (353uL of Triton X100) to 6mM as detergent

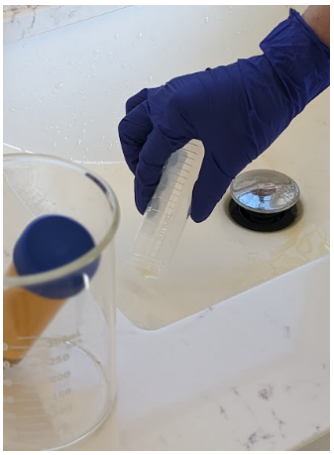
1. Collect the E. coli cells from Terrific Broth by dividing the 100mL Terrific Broth culture EQUALLY into two 50mL Falcon tubes and ensure they are all equal weight

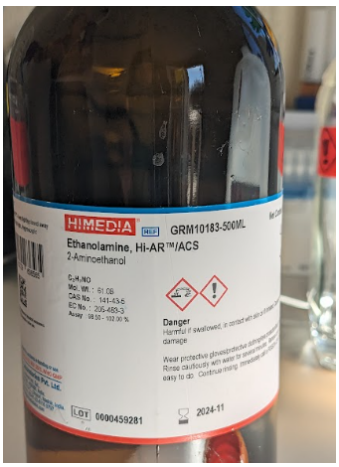


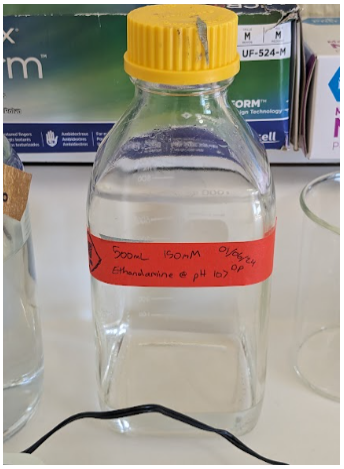
2. Centrifuging them at 4000rpm for 15 minutes at 4°C. The cells will form a pellet at the bottom of the tubes.



3. Pour off supernatant into the sink careful not to disturb the pellet and add 30mL of 150mM ethanolamine, pH 10.5 to resuspend and pour into the blender.





4. Wash the tube three times with 20mL of 150mM ethanolamine, pH10.5 and pour into the blender and blend (90mL) for 2 minutes on low speed.

5. Transfer everything to a centrifuge bottle and rinse the blender with 10mL ethanolamine and add to the bottle.

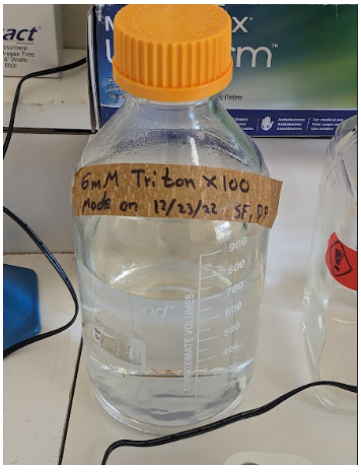
6. Centrifuge at 5000 x g for 30 minutes at 4°C.



7. Pour supernatant into a medium sized glass jar with a lid that can screw on.



8. Pipet detergent (353uL of Triton X100) to 6mM concentration to the supernatant in the glass jar.
   Give the jar a quick swirl.
9. Shake at 100rpm at room temperature for 45 minutes.



PilA Protein Purification - Vacuum Filtration:

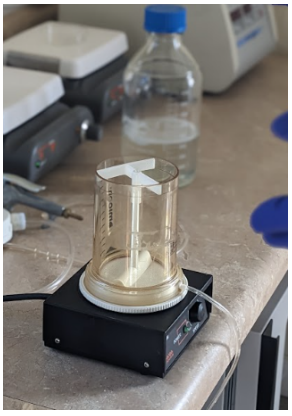
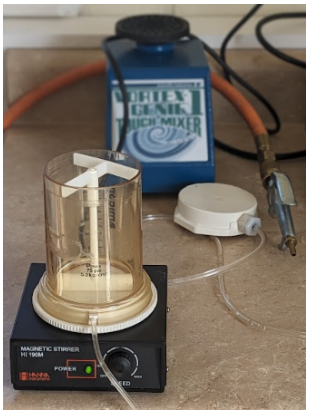
Vacuum filter system used - EMD Millipore 5124 Amicon Stirred Cell Model 8400, 400 mL for 76 mm Filters; 1/Pk
Filter membrane used - 100 kDa MWCO Pall Omega Membrane Discs
Used Nitrogen Gas at 10 psi
Wash with H2O

1. Set up the stirred filter system with 100 kDa MWCO Omega membrane filter (modified PES).
2. Prepare 100 KDa MWCO Omega membrane filter
    a. Wear gloves.
    b. Use a pair of tweezers that are not pointy to pick up the filter
    c. Set up a small beaker (~500mL) with water (~ 300mL) to hydrate the filter
    d. There is a shiny and a non shiny side. You want the shiny side up!
    e. Dip the filter into water with tweezers letting it get all wet.
    f. You will be scraping the pili off of the shiny side with a cell scraper after the filtering process is over.
    g. Disassemble the filter system so that the bottom portion is free from the O-ring and place filter facing up (shiny side of top). Then place the O-ring back into position and assemble the filter system.
    h. Be very careful that the O-ring is in the correct place.
    i. Place the middle plastic portion over the bottom and screw the bottom part tightly into place.
    j. Pour water ~ 300mL from small beaker into filter system
    k. Insert stir unit that has the magnet within. It should snap into place.
    l. Squeeze on the top of the filter system into place. There should also be a top O-ring that is a different size from the bottom.
    m. Push the entire system into black stand. This should be on top of the magnetic stir hot plate without any heat on.
    n. Turn the stir plate on first before turning on gas so that it has a nice even consistent speed and spin.
    o. Turn gas on the tank (nitrogen at 10 psi) then flip the switch on black stand.
    p. The water should pour through the unit coming out of the bottom tube.
    q. Make sure you have a bucket to catch the water coming out!

r. The water is washing the filter. This will take some time! But will be faster than the protein once added.

3. Dilute sample down to 2mM detergent by addition of 200mL of 150mM ethanolamine, pH 10.5.


4. Run through the filter system (nitrogen at 10 psi).
    a. Turn off gas!
    b. Gently push down the filter system out of the black stand to pop it out.
    c. Open top and pour in pili that have been shaking and now diluted with more ethanolamine.
    d. You want the foam, but you can get in the rinse of the bottle
    e. Pop top on, place into black stand, make sure the waste tube is going into the waste fluid bucket.
    f. Turn on stir plate
    g. Turn on nitrogen gas on the tank (nitrogen at 10 psi) then open the back filter system switch on the back of the device.
    h. Should see a nice steady stream of waste fluid.


5. Use 100mL ethanolamine to wash pili sample bottle used on shaker to get foam, vortex, and apply to the filter system.
6. Run through the filter system until it has all come out as waste. There will be some foam left in the filter system that will get rinsed with water.
7. Use 100mL water to wash the filter 4 times.
    a. For every water wash:

    b. Turn the speed of the hot plate spinner up higher than before, still keeping a nice even smooth pace.
    c. This will mix up the pili on the filter a bit to clean them.
    d. Set the timer for 2 mins.
    e. Add the next water wash.
    f. On the last wash let the water go out completely. It will not look like there is anything on the filter.
8. Collect the pilA protein from the filter with a cell scraper and 300uL water and repeat three times (for 0.9mL total volume).
    a. Disassemble filter
    b. Use tweezers again to remove bottom O-ring and filter membrane being careful to only grab the edge of the filter membrane.
    c. Place the filter membrane with pili into a petri dish with pili facing up.
    d. Pipet the first 300uL of water onto filter paper to rehydrate a bit.
    e. Hold onto the filter membrane with tweezers and lift up one side as you gently scrape off the pili from the filter the petri dish collecting little water droplets in the petri dish with pili.
    f. You can place the filter on the petri lid as you collect the droplets.
    g. Pipet first 300uL droplets of water and pili into 1.5mL falcon tube. They might be slightly brown from any concentrated ethanolamine.
    h. Pipet the next 300uL water onto the filter membrane.
    i. Scrape the filter membrane and collect water droplets with pili pipetting them into the same 1.5mL falcon tube.
    j. Repeat this process for the last 300uL of water.
    k. There should be a nice squeaky sound of the plastic of the cell scraper rubbing against the shiny side of the filter membrane.
    l. Label 1.5mL Falcon tube if you have not already leaving space for the concentration of pili.
9. Store pili in the fridge on ice if you are not using right away.

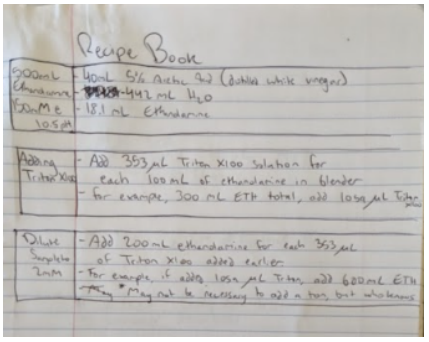
Calculating Concentration of Purified Protein - Beer's Lambert Law:

Collect absorbance at 280nm using spectrophotometer - DeNovix

1. Use the Absorbance using spectrophotometry
2. This is the NCBI link to the pilA protein https://www.ncbi.nlm.nih.gov/protein/534285959
3. Calculate extinction coefficient here - https://web.expasy.org/protparam/

4. PilA extinction coefficient = 4470 $M^{-1}cm^{-1}$
5. Concentration = Absorbance/4470. This will give you concentration in molarity. Multiply by 1000 to get the concentration in millimolar or 1E6 to get micromolar
6. This protein does not contain any Trp residues. This could result in more than 10% error in the computed extinction coefficient.
7. If needed the molecular weight of pilA is 6572.56 g/mol

How to Prepare Dilutions



Ethanolamine Recipe:

500 ml ethanolamine 150mMe, 10.5pH

- 40ml 5% acetetic acid (diluted white vinegar)
- 442ml H2O
- 18.1ml ethanolamine

Adding Triton Recipe Guideline:

- Add 353ul triton x 100 solution for each 100ml of ethanolamine in blender
    - For example - 300ml TH total - add 1050ul Triton

Diluting Supernatant:

- Add 200ml ethanolamine for each 353ul of Triton x100 added earlier
    - For example: if added 1050ul Triton add 600ml ETH