Fast Protein Liquid Chromatography (FPLC): FPLC is a method of chromatography used to purify a specific protein of interest from a sample.

Materials/Reagents:

- AKTAxpress FPLC Machine
- 1L Binding buffer (300 mM NaCl, 10 mM imidazole, 20 mM Tris HCl, pH 8.0,)
- 1L Recharge buffer (0.1 M NiSO4)
- 1L Chelating buffer (50 mM EDTA pH8.0)
- 1L Water
- 1L 0.5 M NaOH
- 1L 20% Ethanol
- 1L Elution buffer 300 mM NaCl, 300 mM imidazole, 20 mM Tris HCl, pH 8.0
- 1L Dialysis buffer 100 mM NaCl, 20 mM Tris HCl, pH 7.5

Sample Preparation:

1. Cell pellets harvested by centrifugation and stored at -80 °C were resuspended in a lysis buffer (300 mM NaCl, 10 mM imidazole, 20 mM Tris HCl, pH 8.0)
2. The resuspended cells were sonicated for 10 minutes (with 10 second pulses and 50 seconds rest).
3. The lysate was then clarified by centrifugation at 40,000 x g for 45 minutes.

Load Buffers into Machine:

- Make sure all lines are fully submerged
- Swap out 92 Well Plate
- Check what line corresponds to which buffer:
    - File → Instant Run → select method → click next until summary page which lists what lines correspond to what buffer

Wash FPLC Machine:

1.) On System Control Application: Manual→ Pump:
    a.) Change these settings
        i.) Pump:
            (1) Flow: (Press Insert After)
                (a) Flow Rate: 5
                (b) Mode: Buffer
            (2) PumpWash: A1 (Press Insert After)
            (3) Loopwash: Bypass (Press Insert After)
        ii.) Flowpath:
            (1) InjectionValve: Waste (Press Insert After)
            (2) Column Position: Bypass (Press Insert After)
            (3) Outlet Valve: WasteF1 (Press Insert After)
            (4) InletValve: A1 (Press Insert After)
            (5) Loop Selection: Bypass (Press Insert After)

2.) Press Execute

Replace the column:

1. On System Control Application: Manual→ Pump:
    - Change these settings
        i. Pump:
            1. Flow:
                a. Flow Rate: 1 (Press Insert After)
                b. Mode: Buffer
            2. Flowpath:
                a. ColumnPosition: Position1 (Press Insert After)
                b. OutletValve: WasteF1 (Press Insert After)
                c. InletValve: A5 (DI H_2O) (Press Insert After)
2. Execute
3. Swapping the Column Out:
    - Minimize Exposure to Air
        i. Unscrew tube attached to top of old column and put cap on the column top (make sure there is dripage coming out of tube)
        ii. Remove cap from top of new column and attach line
        iii. Unscrew the old column from the system and cap bottom
        iv. Unscrew cap on the bottom of the new colon and screw into the machine

Wash Sample Line with DI H_2O:

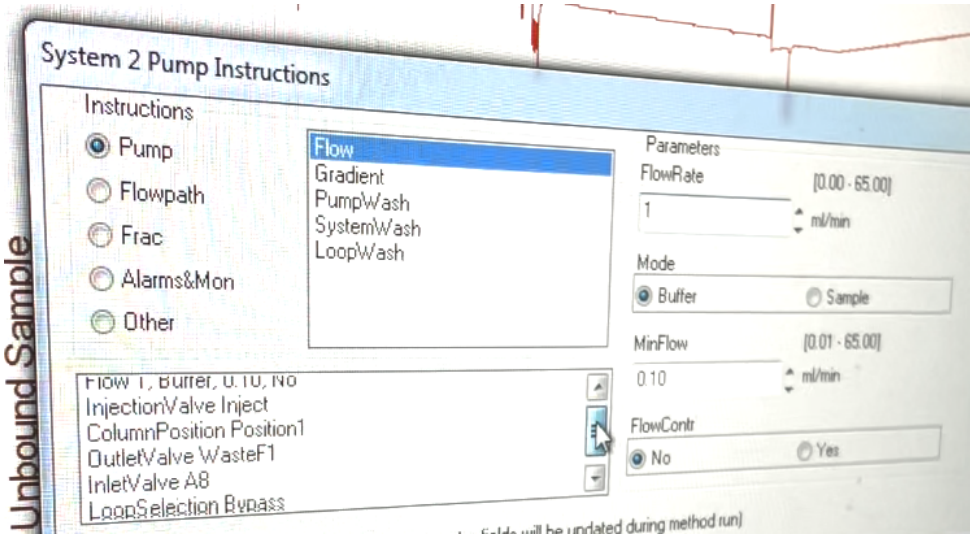
1.) On System Control Application: Manual→ Pump:
    a.) Change these settings
        i.) Pump:
            (1) Flow:
                (a) Flow Rate: 1 (Press Insert After)
                (b) Mode: Buffer
        ii.) Flowpath:
            (1) ColumnPosition: Position1 (Press Insert After)
            (2) OutletValve: WasteF1 (Press Insert After)
            (3) InletValve: A5 (DI H_2O) (Press Insert After)

Run Method:

1.) Instant run
    a.) Change plan to iGEM1 or the guy's we talked to.
    b.) Click Next.
    c.) Click on system 1 or 2 depending on what we use.
    d.) Summary should show which line goes into each buffer.

Pump Wash:

For cleaning up - line goes into ethanol (A8)



Regenerating Column:

2.) On System Control Application: Manual→ Pump:

a.) Change these settings

i.) Pump:

(1) Flow:

(a) Flow Rate: 1

(b) Mode: Buffer (Press Insert After)

(c) Pumpwash: B2 ($NiSO_4$) (Press Insert After)

ii.) Flowpath:

(1) ColumnPosition: Position1 (Press Insert After)

(2) OutletValve: WasteF1 (Press Insert After)

Replace Old Column:
(Same process as swapping out with new)

Dialysis: Membrane dialysis removes small molecular weight substances, or contaminants, via selective, passive diffusion through a semi-permeable membrane,

Materials

- Fisher Scientific SnakeSkin Dialysis Tubing
- 1 L Dialysis buffer (100 mM NaCl, 20 mM Tris HCl, pH 7.5)
- Dialysis Clips
- Beaker

1. Pull out the required length of SnakeSkin dialysis tubing as recommended by the manufacturer and cut it off.

2. Fold over the bottom end of the dialysis tubing twice, with two ¼ inch folds and use a clip to secure it.
3. Fill the open end of the tubing with your protein sample.
4. Seal the tubing with two ¼ inch folds and use a clip to secure it.
5. Suspend the tube filled with sample in a beaker filled with dialysis buffer.
6. Place a stir bar in the beaker and stir at 4 °C.
7. Change the dialysis buffer every 2-3 hours.
8. The last stage of dialysis can be stored overnight. Stir at 4 °C.
9. Collect samples from the dialysis bag.