Experiment
# Figla test expression #3

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|---|---|
| **Notebook** | SGC-UNC TH FIGLA |
| **Concept** | |
| **Labhead** | |
| **Program** | |
| **Created** | Tammy Havener<br>thavener@unc.edu<br>2023-03-08 16:31:44 GMT |
| **Modified** | Tammy Havener<br>thavener@unc.edu<br>2023-03-14 16:41:26 GMT |

## Status: Active

# Content

## Background

Trying another larger culture expression even though we don't quite have the right equipment to purify

## 03/03/23 sf9 cells

1.  Set up 4 250ml flasks of ~50ml Sf9 cells at 2x10$^6$ cell/ml in SFMIII media + 2%FBS

count = 1.17x10$^7$, 87.5%viab, 14.25 diam

Therefore, add 8.5ml cells into 41.5ml media

2.  Add 250ul of virus  P2 PBC38 C2.1 (red) to each flask

Place in 27$^o$C shaker, 120rpm, covered in foil

Grow for 4 days

## 03/06/23 pellet lysis

Check cell counts

flask 1 = 4.26x10$^6$, 85.2%, 17.49 diam

flask 2 = 3.96x10$^6$, 79%, 17.88

flask 3 = 3.56x10$^6$, 84.6%, 17.58

flask 4 = 3.73x10$^6$, 83%, 17.66

1. Place flasks on ice and allow to cool.  Spin cells at 3000rpm, 5 min

2. Wash each pellet with ice cold PBS and spin again to pellet

3. Remove the supernatant and save on ice to aliquot some as P3 virus to use later

4. Weigh pellet  = 2.07g

5.  Resuspend pellet in 10ml Lysis buffer.  Then sonicate each on ice

> ALSO **thaw sample from 2/27/23 that is in -80 and purify at the same time**

- 5min total with 5seconds on, 10 seconds off, 35% (save 10ul to use on gel later)

6.  Centrifuge after sonication at 15,000rpm, 20min, 4$^o$C

7.  Carefully remove the supernatant and add to 500ul of Ni-agarose (was 1000ul Ni-agarose slurry pre washed with H$_2$0 and lysis buffer x3) which is in a Biorad disposable poly prep column.

> NOTE ** samples were both too viscous and would not flow through the columns.  Tried to load in cold room for an hour and wouldn't flow.   Ended up adding more Lysis buffer to each sample for a final volume of 35ml.   Decided to spin it down 3000rpm, 5 min and remove the supernatant.   Then take the remaining Ni-agarose and add it to the Biorad columns using lysis buffer.  Then proceed.

8. Wash x5 column volumes in lysis buffer

- Lysis buffer = 50mMHepes/500mMNaCl/10%glycerol/10mMImidazole + fresh 0.5mMTCEP and complete EDTAfree protease table

9. Elute x5 column volumes in Elution buffer.

- Elution buffer = 50mMHepes/500mMNaCl/10%glycerol/300mMImidazole + fresh 0.5mMTCEP

10. Do one final elution with 1.0ml 1M Imidazole and collect

11. Run samples on SDS-PAGE, 4-20% Novel Tris/Gly using 10X reducing agent and 2xSB

| 1 | 1ul total cell |
|---|---|
| 2 | 1ul total lysate |
| 3 | 5ul clarified lysate (after sonication) |
| 4 | 15ul flow thru off Ni-agarose |
| 5 | 15ul W1 |
| 6 | 15ulW2 |
| 7 | 15ul E1 |
| 8 | 15ul E2 |
| 9 | 15ul E3 |
| 10 | 15ul E4 |
| 11 | 15ul E5 |
| 12 | 15ul E6 |
| 13 | empty |
| 14 | empty |
| 15 | 10ul Thermo MW marker |

# 03/08/23 figla protein samples

1. Pool samples to store for later purification

02/27/23 prep - pool elution

03/06/23 prep - pool elution

2. OD on nanodrop using ext coef = 11.59 and MW = 26316.62
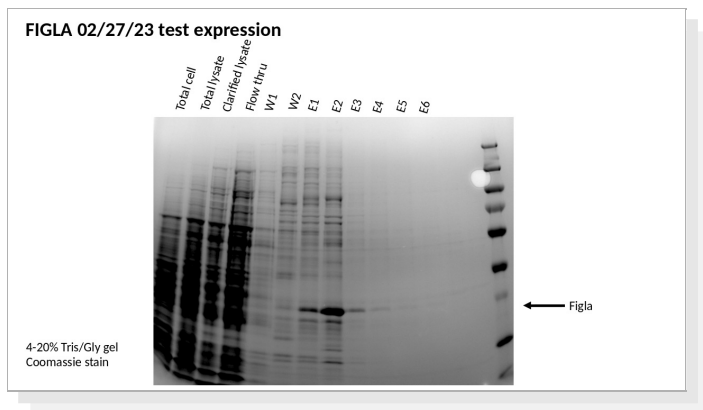
02/27/23 prep

03/06/23 prep

3. Dialyze in 50mMHepes, pH7.4

## 02/27/23 prep coomassie gel

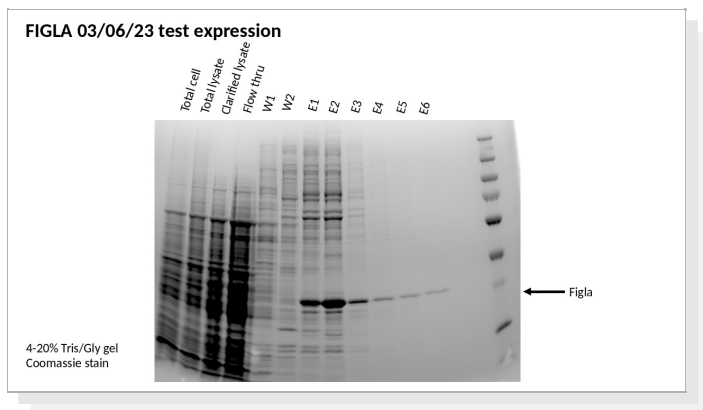Modified: 2023-03-08 16:31:47 GMT      Size: 2.61 MB  (2,736,694)      File type: Microsoft PowerPoint



## 03/06/23 prep coomassie gel

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## 03/08/23 pool dialyze samples

Make up 1.5L of 50mMHepespH7.5/150mMNaCl/10%glycerol/1mMTCEP and place in cold room to chill.

Make 3 samples to dialyze in 10K MWCO 16nm tubing

1 = E2 from 2/27 prep

2 = E1, E2 from 3/06 prep

3 = E3, E4, E5 from 3/06 prep

Add each to tubing and allow to dialyze overnight in cold room.

# image off ibright 022723 prep

# image off ibright 030623 prep

## 03/09/23 OD of samples

1. Remove samples from dialysis and OD protein on NanodropOne

2. Use ext coef = 11.59 and MW = 26316.62

      Sample 1 = 98.8mg/ml, A280 = 0.43, A260/280 = 1.31.  Have ~1.2ml

      Sample 2 = 25.8mg/ml, A280 = 0.11, A260/280 = 1.77.  Have ~1.0ml

      Sample 3 = 18.68mg/ml, A280 = 0.08, A260/280 = 1.42.  Have ~1.6ml

3. Aliquot each set of samples, snap freeze and store in -80°C.  Top shelf, box 1, row3

## 03/09/23 western of samples

Run 1ug and 10ug of each of the Figla P2 samples to probe with antibodies.

1. use 4-20% Tris/Gly wedge well, 10well.  With 2xSB and 10xreducing agent.
2. Transfer to PVDF on iBlot

**blot 1**

- Block in 1%Milkfat/TBST for 1 hr at rt.   Add Anti-Figla (PA5-72033) Ab in 5%BSA/TBST, 1:1000dil,  o/n at 4°C.
- Wash, add goat anti-rabbitHRP in 5%Milkfat/TBST, 1:5000dil, rt, 1 hr
- Wash, then view with Pierce fempto reagent and view on iBright Image

**blot 2**

- Block in 1%Milkfat/TBST for 1 hr at rt.   Add Anti-Figla (CA151-6A10) Ab in 5%BSA/TBST, 1:500dil,  o/n at 4°C.
- Wash, add goat anti-mouseHRP in 5%Milkfat/TBST, 1:2000dil, rt, 1 hr
- Wash, then view with Pierce fempto reagent and view on iBright Imager

| 1 | 1ug P2 Red tube 1 |
|---|---|
| 2 | 10ug P2 Red tube 1 |
| 3 | |
| 4 | 1ug P2 Red tube 2 |
| 5 | 10ug P2 Red tube 2 |
| 6 | |
| 7 | 1ug P2 Red tube 3 |
| 8 | 10ug P2 Red tube 3 |
| 9 | |
| 10 | Seeblue 2 MW marker |

# 03/10/23 Wblot monoclonal and polyclonal

Modified: **2023-03-10 18:53:09 GMT**          Size: **1.61 MB**  (1,689,177)          File type: **Microsoft PowerPoint**
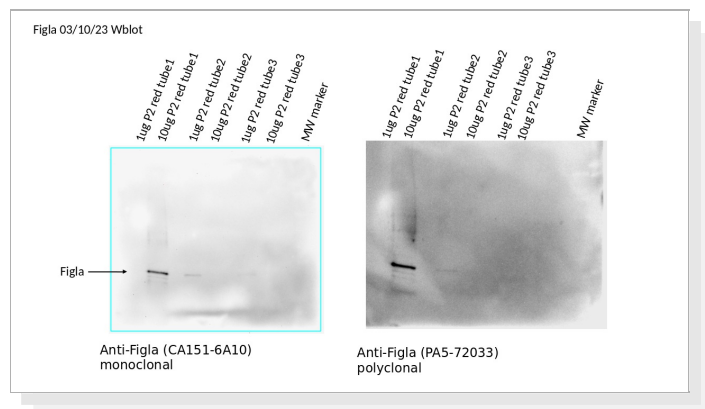
# 03/13/23 Wblot with HEK293

Run Wblot of Figla P2 protein with HEK293 lysate

1.  Use HEK293 lysate from 8/8/22 = 1200ug/ml in RIPA buffer and Figla protein P2 (red) 3/9/23 = 98.8mg/ml

    - Add 20ug of HEK293 +/- 2ul of Figla protein

2. Run on 4-20% Tris/Gly Wedgewell, 10 well, 2XSB, 10X Reducing agent

| 1 | HEK293 only |
|---|---|
| 2 | HEK293 + Figla |
| 3 | Figla only |
| 4 | blank |
| 5 | SeeBlue2 MW |
| 6 | HEK293 only |
| 7 | HEK293 + Figla |
| 8 | Figla only |
| 9 | blank |
| 10 | SeeBlue2 MW |

3.  Transfer to PVDF using iBlot, P3 (20v, 7min).  Cut membrane in half after lane 5

**blot 1 (lanes 1-5)**  (polyclonal)

- Block in 1%Milkfat/TBST for 1 hr at rt.   Add Anti-Figla (PA5-72033) Ab in 5%BSA/TBST, 1:2000dil + anti-mouseGAPDH, 1:1000dil, o/n at 4ºC.
- Wash, add goat anti-rabbitHRP in 5%Milkfat/TBST, 1:2000dil + IRDye anti-mouse, 1:15,000dil rt, 1 hr
- Wash, then view with IRDye filter 800 and then Pierce fempto reagent on iBright Imager

**blot 2 (lanes 6-10)**  (monoclonal)

- Block in 1%Milkfat/TBST for 1 hr at rt.   Add Anti-Figla (CA151-6A10) Ab in 5%BSA/TBST, 1:500dil, + anti-rabbitGAPDH, 1:2000dil,  o/n at 4ºC.
- Wash, add goat anti-mouseHRP in 5%Milkfat/TBST, 1:2000dil, + IRDye anti-rabbit 1:15,000 rt, 1 hr
- Wash, then view with IRDye filter 680 and then Pierce fempto reagent on iBright Imager

# 03/14/23 Wblot slide

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