Experiment

Figla test expression #4

Infect Sf9 cells with virus to express figla protein

Notebook	SGC-UNC TH FIGLA
Created	Tammy Havener thavener@unc.edu 2023-07-20 14:12:39 GMT
Modified	Tammy Havener thavener@unc.edu 2023-08-11 14:19:03 GMT

Status: Active

Content

07/13/23 start up Sf9 cells

Thaw 1 vial of Sf9 cells from LN.

Use Sf9 6/8/22, 1 x 10^7 cell/vial

Add directly to 250ml sterile Erlenmeyer flask containing 35ml SFMIII media + 350ul Anti/Anti

Count = 2.92 x 10^5 cell/ml, 91.9% viable

Place on shaker at 27^{o}C, 105rpm, covered w foil.

Allow to grow for 7 days

07/18/23 check cells

Count = 1.5 x 10^6 cell/ml, 96.4% viable

Allow them to continue to grow

07/20/23 set up infection of Sf9

Count = 4.17 x 10^6 cell/ml, 95.1% viable.

Set up 2 250ml Erlenmeyer flasks at 2 x 10^6cell/ml. Each flask total of 40ml. Add 19ml of Sf9 cells + 21ml of SFMIII media containing 2%FBS .

Add 200ul P2 PBC38 C2.1 (red) virus to each flask.

Place on shaker at 120rpm, 27^{o}C, cover with foil and allow to grow for 4 days.

07/24/23 check infected cells

1. Count cells in each flask.
    - Flask 1 = 2.28 x 106, 79.6%, diam 17.47
    - Flask 2 = 1.51 x 106, 82.6%, diam 17.68
2. Places on ice. Remove 2ml of each to lyse and look for Figla. Then spin down remaining cells at 3000rpm, 5min.
3. Wash with ice cold PBS, spin again at 3000rpm, 5min.
4. Weigh pellets
    - 1 = 0.30g
    - 2 = 0.24g

Store pellets at -80

08/2/23 test sample for figla

I had 2 pellets from the infected cells (7/24/23) to use for a test expression to look for Figla.

Run a mini purification:

1. Wash x2 with ice cold PBS
2. combine pellets into one tube and wash again with ice cold PBC x2
3. Resuspend in 200ul Lysis buffer, sonicate (as before 5sec on/off, 35%) on ice
4. Centrifuge 3000xg, 30min, 4ºC
5. Remove the supernatant and add to 100ul of pre-washed (in lysis buffer) Ni-agarose
6. Inc on rocker for 30min, 4ºC
7. Centrifuge and wash x3 in lysis buffer
8. Elute with 200ul elution buffer.
9. Run on SDS-PAGE

08/2/23 gel plan

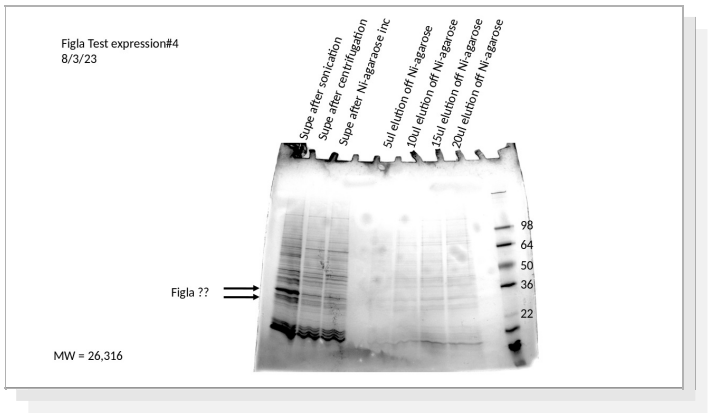
4-20% Novex, 10 well, Tris/Gly

Prepare samples with 2X Sample buffer and 10X reducing agent

1	1ul supernatant after sonicating
2	1ul supe after centrifuge of sonicated supe
3	2ul supernatant after spin out of Ni-agarose
4	
5	Elution off Ni-agarose, 5ul
6	Elution off Ni-agarose, 10ul
7	Elution off Ni-agarose, 15ul
8	Elution off Ni-agarose, 20ul
9	
10	SeeBlue2 MW marker, 7ul

figla test expression4.pptx

Modified: 2023-08-03 16:47:12 GMT Size: 1.52 MB (1,593,812) File type: Microsoft PowerPoint



08/3/23 start more cultures

It looks like the test expression has Figla. Sf9 cells have been expanded and growing so start more infections. There are 5 flasks ~50ml each

Flask counts:

1. 9.79x10^6	91.8% viab	14.83 diam
2. 1.82x10^6	93.8%	14.87
3. 1.87x10^6	94.2%	15.26
4. 1.77x10^6	94.5%	14.96
5. 1.46X10^6	95.8%	15.11

Pool all of the flasks and recount since there are enough cells:

3.16x10^6	95.2%	14.65

Set up 10 total 250ml Erlenmeyer flasks:

31.65ml Sf9 cells + 19ml SFMIII media containing 2%FBS

Place on shaker at 27^{o}C, 120rpm, covered in foil

Allow to shake for 2-3hrs before adding virus.

- Add 200ul P2 PBC38 C2.1 (red) virus to each flask
- Place back on shaker at 27^{o}C, 120rpm, covered in foil and allow to grow for 4 days

08/7/23 spin down cells

Pool all of the sf9 cells into one flask and count.

- ~500ml of cells, 1.82x10^6cell/ml, 80.2% viab, 18.4 diam.

1. Place cells on ice to allow to cool. Remove 1000ul for test gel. Spin remainder at 3000rpm, 5min
2. Wash with ice cold PBS x2
3. Weigh final pellet in conical tube. 4.57g
4. Add 8ml lysis buffer and place into -80. Also wash and store test pellet (resuspended in 100ul PBS) at -80.

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

08/09/23 Wblot of expr#4

1. Thaw test pellet in -80 from 8/7/23
2. Resuspend in another 300ul PBS (400ul total). Remove 100ul
3. Remove another 400ul and pellet. Add 200ul lysis buffer. Pellet and save supernatant.
4. Run samples on 4-20% Tris/Gly Novex, 10-well, SDS-PAGE (2XSB, 10X reducing,2 min 70^{o}C)

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

Figla "+" control = FiglaP2(red) protein (3/9/23) = 98.8mg/ml

Gel plan:

1	25ul initial pellet in PBS
2	20ul initial pellet in PBS
3	10ul initial pellet in PBS
4	5ul initial pellet in PBS
5	25ul supe after pellet lysis
6	20ul supe after pellet lysis
7	10ul supe after pellet lysis
8	5ul supe after pellet lysis
9	2ul positive control figla protein
10	7ul SeeBlue2 MW marker

Transfer to PVDF on iBlot, P3 (20v, 7min)

08/09/23 antibody

blot

- Block in 1%Milkfat/TBST for 1 hr at rt. Add Anti-Figla (CA151-6A10) Ab in 5%BSA/TBST, 1:500dil and incubate overnight at 4ºC.
- wash
- Goat anti-mouseHRP in 5%Milkfat/TBST, 1:2000dil, rt, 1 hr
- wash
- View with Pierce fempto reagent and view on iBright Imager

figla test exp4 wblot.pptx

Modified: 2023-08-11 14:19:02 GMT Size: 149.53 KB (153,120) File type: Microsoft PowerPoint