VLP Expression/ Purification/Conjugation Protocol

Last Updated: 4/22/19 SR

Recipes:

2xYT Media

- 31g/L of premade 2xYT powder **(WITH TRYPTONE NOT PEPTONE)**
- Wrap top of (baffled) flask with Aluminum foil and Autoclave using "Liq 20"

Lysis Buffer

- 20mM Tris HCl
- 150mM NaCl
- 75mM Imidazole
- 0.1% Tween 20
- pH adjusted to 7.8
- Stored at 4C

Wash Buffer

- 50mM Tris HCl
- 150mM NaCl
- 100mM Imidazole
- 0.1% Tween 20
- pH adjusted to 7.8
- Stored at 4C

Elution Buffer

- 2M Imidazole
- 50mM Glycine
- 25mM Na Citrate
- 0.1% Tween 20
- pH adjusted to 8.5
- Stored at 4C

10x Dialysis buffer **(MAKE 500mL FRESH 1X EACH TIME)**

- 500mM Glycine
- 250mM Na Citrate
- 1% Tween 20 (I add when I make 1x)
- pH adjusted to 8.0
- Stored at 4C

Other Notes:

- **Keep unconjugated VLPs on ice or +4C AT ALL TIMES*****
- Make sure to plan ahead and sign up for all equipment including shakers, cell disruptor, and purification systems/columns/pumps in advance
- Also, be aware VLP expression is known for being very inconsistent

Transformation (Day 1):

- C41 (DE3) overexpressed competent cells → (red box in upright -80C)
- Plasmid (PGN SPY Catcher CP3)
- SOC media
- LB Carb agar plate (100 µg/µL)

1. Remove cells from -80C, thaw on ice
2. Add 0.5 µL of plasmid
3. Gently flick tube 4-5 times
4. Ice 3 min
5. Heat shock 10 seconds at 42C
6. Ice 2 min
7. Add 950 µL SOC Media
8. Plate 200 µL and incubate at 37C overnight

*Plate should be good up to ~4 weeks but it's best practice to use a fresh plate each expression

Culture Growth (Days 2 and 3):

- 2xYT media – 50 mL for O/N culture, 1 L for expression
- IPTG
- Lysis buffer

(Day 2)

1. Pick a single colony, eject tip into a pre-autoclaved flask with 50mL 2xYT+carb
2. Grow O/N at 37C, 220RPM

(Day 3)

3. Spin culture down in 50 mL Falcon tube at 4,000G for 5 min
4. Resuspend in 4mL of 2xYT
5. Aliquot cells into 1L 2xYT+carb in baffled flask(s)
6. Grow at 200 RPM, 37C until cells reach log phase (OD~0.5) (~5-6 hrs)
    i. Use Jensen Lab's Spectrophotometer with 1.5 mL cuvettes. Blank with 1 mL of media
7. Take a small aliquot before inducing and store until later for the gel
8. Induce with 0.42mM IPTG (420 µL of 1M IPTG found in Freezer #5)
9. Grow at 200 RPM, 30C for 5 hours
10. Take a small aliquot before spinning
11. Spin down the cells at 6,000G for 15-20 min at 4C
12. Resuspend each pellet with 20 mL Lysis buffer
13. Pour each resuspended pellet into 50 mL Falcon tubes
14. Freeze in liq NO_2 and store -80C

i. These pellets can be stored long term. ***Once purified and before conjugated, the SPY catcher-VLPs are unstable***, so it is recommended to not purify until needed. Quality and yield of VLPs begins decreasing immediately after purification and will require extra filtering
ii. If using next day, can just store pellet in -20C

15. Run a 4-20% SDS-PAGE gel
    i. Prepare samples to be roughly the same amount of cells per vol (doesn't need to be exact)
    ii. Heat at 90C for 5 min with red/non red dye. This will lyse the cells and free the protein
    iii. Run for 40 min at 200V
    iv. Develop using Instant Blue for ~15 minutes
    v. Wash the gel, hold in water, and image

The gel will tell us if we have VLPs in our expression and will save us the time of spending an entire day purifying an unsuccessful expression.

This is a good place to stop if necessary. Pellets can be frozen and stored long term in the -80C and thawed and used as needed.

Purification (Day 1):

- PMSF
- DNase
- RNase
- 0.45 micron Surfactant-free cellulose acetate filter device (Nalgene)
- 5 mL Ni-NTA His trap column from PEC (BH-# label, email Leesa, return once finished)
- Pall Acrodisc Syringe Filter 0.2 um Supor Membrane Low Protein Binding (PN4602)
- Buffers

1. Thaw the pellet, preferably on ice but +37C water bath for a short while is fine too
2. Add a scoop each of DNase and RNase (Freezer #7)
3. Run cells through cell disruptor (PEC)
    a. Sign up on PEC website and sign in on the sign in sheet at the door
    b. Turn on machine ~10 min before use. Water should be cooled to +5C.
    c. Fill mouth with MilliQ water, press Run Continuous on screen, press orange button, and press start on screen. Make sure beaker is placed under the valve
    d. Adjust pressure to 30 kPSI. Keep at 30 kPSI the entire run
    e. Run TBS through (in the fridge across from the cell disruptor)
    f. Place a 125 micron Nylon DeVilbiss filter in the mouth
    g. Filter cells through and run. Collect using a 100 mL bottle
    h. Run cells through again
    i. Rinse with ~15 mL of TBS and collect the first 2 spurts, everything else goes into waste. Rinse again with TBS
    j. Clean walls with squirt bottle and wipe sides with a Kimwipe, and rinse with water. Use 70% EtOH spray bottle to get rid of bubbles
    k. Spray with EtOH and wipe with Kimwipe
    l. Rinse with 70% EtOH and store in EtOH
    m. Waste goes in sink, and rinse beaker with water
4. Add PMSF (stock 100x) (prewarmed/thawed in water bath)
5. Add a scoop each of DNase and RNase
6. Spin lysates in 250 mL bottles at 21,000G for 30 min at 4C (“Lysis” program)
7. Filter using a 0.45 micron surfactant-free cellulose acetate filter device (Nalgene)
8. Benchtop/cold room His-tag purification
    a. Wash loop with ~5 mL water
    b. Hook up one 5 mL Ni-NTA His trap column
    c. Equilibrate with ~20-25 mL Lysis buffer. Set up falcon tube for FT collection
    d. Run filtered lysate over column at 1mL/min
    e. Wash with ~25-30 mL of wash buffer
    f. Elute in ~25 mL of Elution buffer
    g. Check the concentration on the nanodrop. Should be ~2-4 mg/mL w/ high A260
9. Concentrate elution to ~7-8 mL using a 30kD/100kD concentrator at 3800g
    a. This will take an incredibly long time. Roughly 3 hours.
    b. Check the concentration on the nanodrop. Should be ~5-10 mg/mL w high A260

10. SEC purification on Rosalind using HiLoad 16/600 Superdex 200 (Pos 7) and a 10 mL loop
    a. Filter the VLPs using a 0.2 micron syringe filter unit
    b. Wash 130 mL Dialysis buffer at 1 mL/min
    c. Rinse port (water) and loop (20 mL)
    d. Load sample (leave in load mode before beginning program)
    e. Run SDX200 1660 program in Alex's folder
    f. VLPs should elute somewhere between A10-B9 ish (~45-50 mL)
    g. Run a nonreducing and reducing gel of the fractions to determine which to pool
        i. 7.5 µL of sample and 7.5 µL of dye
        ii. 200V for 40 min

Quantification – Biorad Protein Assay (Day 2)

1. "Microtiter plate protocol – standard"
2. Spin VLPs at 4000g for 5 min and filter using a 0.2 micron syringe filter if necessary (purified more than 1 day prior)
3. Make standards for the assay:
    a. Make 200uL of a 1 mg/mL stock which is then used to make
    b. 0.5, 0.4, 0.3, 0.2, 0.1, 0.05 mg/mL stocks, 100 µL of each (save the rest!!)
4. Add 10 µL the stds and VLPs into an ELISA plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BUFFER - blank	BUFFER - blank	VLP undiluted	VLP undiluted								
B	0.5 mg/mL	0.5 mg/mL	VLP 1:1	VLP 1:1								
C	0.4 mg/mL	0.4 mg/mL	VLP 1:4	VLP 1:4								
D	0.3 mg/mL	0.3 mg/mL										
E	0.2 mg/mL	0.2 mg/mL										
F	0.1 mg/mL	0.1 mg/mL										
G	0.05 mg/mL	0.05 mg/mL										
H												

5. Dilute 1 mL of the dye in 4 mL of water
6. Add 100 µL of dye into each well
7. Wait 5 minutes and no more than 1 hour for it to develop
8. Analyze the plate using the Tecan plate reader at 595 nm
9. Plot the data and determine the concentration (Use the Excel quantification sheet)

The concentration *usually* is close to the A_{280} measurement. ~1.1 ratio of A_{280}/[VLP] from assay

Conjugation (Day 2)

$MW_{VLP\text{-}SC\ (AP205)}$ = 28300
$MW_{1a053E2core\text{-}SPY}$ = 26185
$MW_{1a053E2ecto\text{-}SPY}$ = 31422
$MW_{1a157E2core\text{-}SPY}$ =26166
$MW_{1a157E2ecto\text{-}SPY}$ = 31442

1. If necessary, spin VLPs down and filter as before
    a. If refiltering, you must recheck the concentration using the Biorad assay
2. Begin equilibrating the Superose 6 10/300 Increase column (column Pos 1) on Rosalind using PBS* 30-40mL, flow rate 0.5 mL/min, and high alarm set at **3.7**
3. Use a 1:1 molar ratio of VLP:Ag. Use the Excel spreadsheet in box to perform all calculations
4. Add SPY-tagged antigen into a labeled Eppendorf tube, and then add VLP. Pipette up and down a few times to mix
5. Incubate at room temperature for 2 hours
6. Remove the port on Rosalind and use a syringe to wash with 10 mL of water. Reattach the port
7. Prepare syringe with sample and insert in port
8. Wash the loop with minimum 2-5x the loop volume
9. Load mode, inject sample, leave in "Load"
10. Run program in Andrew's folder: Superose 6 10300
    a. When beginning the program, it will give an error message about the high-pressure limit. Just clear the error – it is because the AKTA program does not have this column as an option and thinks the pressure limit should be 1.5 instead. The program will proceed as normal though
11. Run a non-reducing and reducing gel – 7.5 µL of VLP from fractions and 7.5 µL dye. 200V for 40 min
    a. Controls are VLP – 1 µg, and Antigen – 500 ng

*If using these VLPs for animal experiments, you *must* use the special endotoxin-free PBS in the cold room. Filter only what is needed using a complete sterile 0.2 micron filter/bottle device