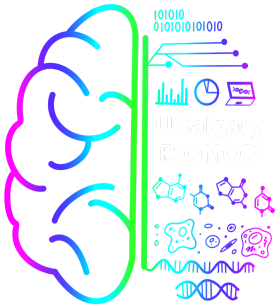


BIOMOD

Protocol Compilation

Part 1: PCR

Preparing DEPC-treated Water (UltraPure)

Objective: To create 500 mL of DEPC-treated water through covalent modifications of histidine residues.

Materials

- 0.5 mL DEPC
- 499.5 mL Milli-Q Water
- Orbital shaker
- Magnetic stir bar
- 1L glass container with a lid
- Autoclave

Methods

DEPC-treatment [1]:

1. Add 0.1% (v/v) (500 uL) of DEPC to 499.5 mL of Milli-Q Water to a 1L glass container
    a. Shake to gently mix
    b. Be careful to not mix with reagents with an amine group
2. Shake for 1 hour to overnight on orbital shaker
    a. Alternative Method: Mix with a magnetic stirrer for 30 minutes
3. Autoclave for 45 minutes at 15 PSI on liquid cycle

PCR Amplification

Objective: To amplify desired DNA sequences (primers: 5seqT7ApFwd, 5seqT7ApRev, 5seqT7ApTMSDTARRev)

Materials

- Preparation of PCR Oligo Stock
    - 7 Eppendorf (1.5mL) tubes
    - Sharpie
    - P10 pipette and pipette tips
    - P200 pipette and pipette tips
    - Ice box
    - RNase free water (200µL)
    - Stock tubes of
        - 5seqT7ApFwd
        - 5seqT7ApRev
        - 5SEQT7APLRev
        - 5SEQT7APTMSDTARRev
        - 5SEQT7AP
        - 5SEQT7APL
        - 5SEQT7APTMSDTAR
- **PCR**
    - PCR SuperMix
    - Template DNA
    - Fwd primers
    - Rev primers
    - DEPC/UltraPure Water
    - RNase-free microcentrifuge tubes
    - RNase-free PCR tubes
    - 96-well PCR tube holder
    - Ice bucket
    - Thermal cycler
    - -4C and/or -20C storage

Methods

Preparation of PCR Oligo Stock [2,3]

1. Thaw components of PCR MasterMix, briefly centrifuge to collect at the bottom.
2. Set up a PCR Master mix in a 1.5mL microcentrifuge tube, mix by pipetting up and down, spin down to combine everything
3. Suspend primers in 1mL of UltraPure/DEPC-treated water
4. Label PCR tubes, determine the components that will go in each. This was done with a label for the respective primers as seen in the table below:

Table #1: Ordered DNA Primers and Their Melting Point, Size, and Mass

Primers	Tm (°C)	Size (nmol)	Mass (mg)
5seqT7ApFwd	62	104.1	0.76
5seqT7ApRev	59.2	68.4	0.53
5seqT7ApLRev	58.1	69.5	0.56
5seqT7ApTMSDTARRev	57.8	77	0.58

5. Determine the volume of stock solution and the volume of water required to obtain the desired final concentration (approximately 10 µM), which was determined according to the table below:

Table #2: Preliminary Volumes of Various Primers for PCR

Primer	Concentration ($\frac{\mu mol}{L}$)	Total Volume of Stock and Water Needed Total (μL)	Volume needed from Stock of Primer (μL)	Volume of Water Needed (μL)
5seqT7APFwd	104.1	3	0.288	2.711
5seqT7APRev	68.4	1	0.200	1.169
5seqT7APLRev	69.5	1	0.200	1.188
5seqT7APTMSDTARRev	77.0	1	0.200	1.338

PCR Amplification

1. Place a 96-well plate into an ice bucket, and place the labeled PCR tubes inside
2. Pipette 45uL Master Mix into PCR tubes except for negative control (see step 3)

3. Set up negative control in a separate PCR tube:
    a. Add all the same reagents as Master Mix except for the template DNA
    b. Add 3 uL of DEPC-treated water in place of the template DNA
4. Add Template DNA
5. Centrifuge down briefly to combine
6. Cap PCR tubes and place onto thermal cycler
7. Set thermal cycler to the setting labeled "BIOMOD" and run as follows:

    Thermal cycler settings:
    a. Initial denaturation: 94C, 2 min
    b. 25-35 PCR cycles:
        i. Denature: 94C, 15s
        ii. Anneal: 62C (based primer Tm and template Tm), 30s
        iii. Extend: 72C, 1 min/kb
    c. Hold: 4C, indefinite

8. Four 50uL reactions were performed, with 30 PCR cycles
    a. One for each of the three template sequences
    b. One for negative control
    c. Lid temp was set for 105°C
    d. 3uL of template DNA for the forward primer was made, 1uL of the primer was used in each reaction
    e. 1uL of the template DNA for the reverse primers was used in the reaction
9. After the program runs, store PCR tubes at 4°C for few days or -20°C for several months
10. Run samples on through a Nanodrop to determine the final concentrations
11. Use 10µL of PCR product for gel electrophoresis to test if amplification of the desired product occurred

DNA agarose gel electrophoresis

Objective: To analyze various DNA samples such as PCR results.

Materials

- **Preparation of the gel**
  - Agarose
  - Erlenmeyer flask (150mL)
  - 1X TBE
  - Microwave
  - SYBR Safe dye
  - Gel casting/running apparatus
- **Sample preparation and running gel**
  - PCR tubes
  - Gel loading dye
  - DNA to be run
  - 1X TBE
  - DNA size marker (DNA ladder)
- Observing results
  - Paper towels
  - UV transilluminator
  - Gel documentation system

Methods

Preparation of the gel [4]

1. Weigh out a 2% agarose gel in an erlenmeyer flask.
   a. 3 grams of agarose for a 150mL gel.
2. Add the correct volume of running buffer (150 mL 1X TBE) to the agarose-containing flask. Swirl to mix.
   a. (1X) TBE buffer
      i. Use milli-q water to dilute
   b. 150mL
3. Melt the agarose/buffer mixture in a microwave, at 30 s intervals, remove the flask and swirl the contents to mix well. Repeat until the agarose has completely dissolved.

4. Wait until the flask is cooled down enough to touch the skin, Add 15µL of SYBR Safe dye.
5. Clean and place the gel tray into the casting apparatus. Place an appropriate comb into the gel mold to create the wells.
6. Pour the molten agarose into the gel mold. Allow the agarose to set at room temperature.
7. Remove the comb and place the gel in the gel tank.
    a. Place in a tank with the direction of DNA running from negative to positive.

Sample preparation and running gel [4]

1. Add loading dye to the DNA samples to be separated. Use 10µL of DNA products.
    a. Gel loading dye is typically made at 6X concentration
    b. 2uL of loading dye to 10uL of sample
2. Program the power supply to a constant 120V
    a. (Could change to somewhere between 1-5V/cm between electrodes).
3. Add enough running buffer to cover the surface of the gel.
    a. Same running buffer as the one used to make the gel, approx 700ml
4. Attach the leads of the gel box to the power supply. Ensure everything is running properly.
5. Remove the lid. Carefully load the DNA sample(s) into the gel.
    a. Including an appropriate DNA size marker (DNA ladder) should always be loaded along with experimental samples. Use 12uL of DNA ladder.
6. Replace the lid to the gel box. Double check the leads are connected properly.
7. Turn on the power. Run the gel until the dye has migrated to an appropriate distance (at least halfway). This will take approximately 30 minutes.

Observing results [4]

1. When the gel has run, turn off power and remove the gel from the tank, drain off excess buffer.
2. Place the gel tray on paper towels to absorb the extra buffer.
3. Use a UV transilluminator to look at the gel.
4. Image the gel using a gel documentation system (Gel Doc).
5. Dispose of gel and running buffer properly.

PCR Purification

Objective: To analyze purify PCR product through column purification

Materials:

- Monarch® PCR & DNA Clean up Kit
  - Monarch® DNA Cleanup Columns (5 µg)
  - Monarch® Collection Tubes (2 ml)
  - Monarch® DNA Wash Buffer
  - Monarch® DNA Cleanup Binding Buffer
  - Monarch® DNA Elution Buffer
- PCR Product to be purified

Methods [5]:

1. Dilute sample with DNA Cleanup Binding Buffer (ensure that isopropanol has been added, as indicated on the bottle label)* according to the table below

Sample Type	Ratio of Binding Buffer:Sample	Example
dsDNA > 2 kb (plasmids, gDNA)	2:1	200µL:100µL
dsDNA < 2 kb (amplicons, fragments)	5:1	500µL:100µL
ssDNA > 200 nt	7:1	700µL:100µL

2. Mix well by pipetting up and down or flicking the tube. **Do not vortex**.
3. Insert column into collection tube and load sample onto column and close the cap. Spin for 1 minute, then discard flow-through.
4. Re-insert column into collection tube. Add 200 µl DNA Wash Buffer and spin for 1 minute. Discarding flow-through is optional
5. Repeat Step 4
6. Add ≥ 6 µl of DNA Elution Buffer to the center of the matrix. Wait for 1 minute, then spin for 1 minute to elute DNA.
7. Spin the tubes one more time for 1 minute

In-Vitro Transcription (IVT) and Purification

Objective: To allow for transcription of the RNA transcript of the aptazyme sequence from DNA

Materials:

- IVT/Control
  - DEPC-treated water
  - 5X TranscriptAid Reaction Buffer
  - ATP/CTP/GTP/UTP mix (NTP)
  - Template DNA (1,2,3,5)
  - TranscriptAid Enzyme Mix
  - Control DNA
- Removal of Template DNA
  - DNase I, RNase-free, 1U/µL
  - Chloroform
  - Thermocycler
- Purification of RNA Transcripts
  - 96+% Ethanol
  - 70% ethanol
  - DEPC water
  - Chloroform
  - RNA to be Purified

Methods:

In vitro transcription [6]:

1. Thaw all frozen reaction components, mix and briefly centrifuge to collect everything at the bottom.
2. Keep TranscriptAid Enzyme Mix and nucleotides on ice (Keep 5X TranscriptAid Reaction buffer at room temp)
3. Combine equal volumes of the four NTP solutions in one tube
4. Combine the following reaction components at room temp in the given order:
   - DEPC-treated water: To 20µL
   - 5X TranscriptAid Reaction Buffer: 4µL
   - ATP/CTP/GTP/UTP mix: 8µL
     - Notes: Equal amounts of the four NTP solutions should be used.
   - Template DNA: 5µL
     - 2-5µL of PCR mixture can be directly used in 20 µL of in vitro transcription reaction

- TranscriptAid Enzyme Mix: 2µL

5. Mix thoroughly by pipetting, spin to collect
6. Incubate at 37°C for 2hrs.
7. Proceed to purification of RNA transcripts

Control reaction [6]:

1. Combine the following components at room temp in the given order for a total volume of 20µL:
   - DEPC-treated water: 4µL
   - 5X TranscriptAid Reaction Buffer: 4µL
   - ATP/CTP/GTP/UTP mix: 8µL
   - Control DNA (0.5µg/µL): 2µL
   - TranscriptAid Enzyme Mix: 2µL
2. Mix thoroughly by pipetting up and down, briefly spin to collect
3. Incubate at 37 C for 2hrs.
4. Proceed to purification of RNA transcripts.

Removal of Template DNA after transcription [7]:

1. Add 2U of DNase I, RNase-free per 1µg of template DNA directly to the transcription reaction mixture.
   a. The DNase I from the kit comes in a concentration of 1U/µL.
   b. This amount can also be adjusted as seen with empirical results.
2. Incubate at 37°C for 15 min.
3. Inactivate DNase I by chloroform extraction or inactivate by adding 2µL of 0.5M EDTA, pH 8.0 and incubated at 65°C for 10 min.

Purification of RNA Transcripts [8]:

1. Extract three times with an equal volume of chloroform.
   a. Use chemical grade, if issues arise try DEPC treating the chloroform.
2. Collect the aqueous phase and transfer to a new tube.
3. To XµL of reaction mixture, add 5.75XµL of DEPC-treated water and 0.75XµL of 3M sodium acetate solution (pH 5.2; provided). Mix by pipetting up and down.
   a. To 20µL of reaction mixture, add 115µL of DEPC-treated water and 15µL of 3M sodium acetate solution (pH 5.2; provided). Mix by pipetting up and down.
4. Precipitate RNA by adding 2 volumes of 96+% ethanol, incubate at -20C for at least 30 minutes

5. Centrifuge and carefully remove the supernatant, rinse the pellet with 500µL of cold 70% ethanol
6. Centrifuge again for 3:00 min at 21130 rpm
7. Resuspend the RNA in 20µL of DEPC-water
8. Run Nanodrop on the RNA mixtures to determine concentration
9. Store the RNA at -20C short-term or -70C long-term
10. Run a formaldehyde denaturing agarose gel to analyze transcripts

RNase-Free Formaldehyde Denaturing Gel Electrophoresis

Objective: To prepare and run denaturing gel to test the success of transcription products

Materials:

- Agarose
- Deionized water
- 10X TBE
- 37% Formaldehyde
- SYBR Safe
- 500mL graduated cylinder
- 250mL erlenmeyer flask

Methods:

Prepare 1X TBE running buffer:

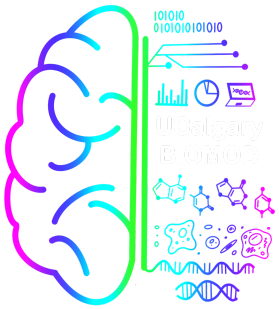
1. Add 450mL of RNase free deionized water to 50mL of 10X TBE in a 500mL tube
2. Mix and set aside until needed

Gel preparation [9]:

1. Wipe down all the gel casting and running equipment (gel casting tray, comb, gel holder, and gel apparatus) using ethanol and RNase Zap. Set aside this equipment in the fume hood
2. Weigh out 1g of agarose
3. Add 35mL of deionized water to a 250mL erlenmeyer flask
4. Heat the flask at 15 second intervals until completely dissolved
5. In a 25mL cylinder, add 5mL of 10X TBE and 9mL of 37% formaldehyde under the fume hood. Mix well and set aside.
6. Once the gel is warm to the touch, add the TBE and formaldehyde mixture.
7. Add 5µL of SYBR Safe and mix well
8. Pour the gel, position the comb and let the gel set

Gel storage [9]:

1. Use 70% ethanol and RNAZap to clean an empty micropipette tip box
2. Transfer some 1X TBE buffer to the tip box
3. Add the casted gel
4. Cover the remainder of the gel with 1X TBE buffer



BIOMOD

Protocol Compilation

Part 2: TMSD

Toehold Mediated Strand Displacement (TMSD) Sample Preparation

Objective: To prepare 200uM stock of TMSD oligos and 20uM working solutions for TMSD

Materials:

- Ice
- Pipettes
- TMSD oligos
    - TMSDTAR
    - TMSDKEY
    - TMSDNAN
    - TMSDNANL
- Deionized DNAase free- water
- Vortex
- Centrifuge

Methods:

1. Prepare and resuspend samples to 200uM (keep all samples inside an ice bath) as follows:

Sample	Label	nmoles	Mass (mg)	Volume (uL) to resuspend in:
TMSDTAR	TMSDTAR	28.7	0.35	143.3
TMSDKEY	KEY	85.1	1.30	426.5
TMSDNAN	NAN	29.5	0.30	146.1
TMSDNANL	NANL	102.3	1.31	513.3

    i. Centrifuge at 10000 for 30 seconds or 3000xg
    ii. Add deionized water
    iii. Vortex and centrifuge
2. Prepare 20uM of 100 uL
    i. Add 90uL to each Eppendorf tube and add 10uL of stock
3. Store all Oligo stocks in the -20C fridge

TMSD: Non-SDS Native PAGE Part 1

Objective: To examine whether the TMSD reaction from literature is working as intended in its original form and in the form where the nanopore strand has been extended by a linker.

Materials:

- Centrifuge
- Samples (20uM of 100 uL)
    - TMSDNAN
    - TMSDNANL
    - TMSD TAR
    - TMSD KEY
- 10X TBE
- DNA ladder
- DNA loading dye (6X)
- Incubator
- Aluminum foil
- Deionized water
- Ethanol
- PAGE minigel assembly
    - 4 glass plates (2 should be 1mm and the other 2 should be standard)
    - Electrical equipment
- TEMED
- Disposable centrifuge tube (50mL)
- 10% Ammonium Persulfate (APS)
- 30% (29:1) Acrylamide/bisacrylamide solution

Methods [10,11,12]:

1. Prepare 1xTBE running buffer
    a. Add 100mL of 10X TBE to 900mL DEPC-treated water or Milli-Q water into a 1L autoclaved bottle
2. Prepare samples for experiment 1 and experiment 2
    a. Transfer 10uL of each sample to labeled eppendorf tubes
    b. Incubate all samples for 30 minutes at 25 C
    c. Add 2uL of DNA loading dye to each sample
3. Prepare ladder
    a. Thaw, centrifuge briefly, and add 2uL of loading dye to 10uL of ladder
4. Prepare PAGE apparatus and check for leakage
    a. Clean plates and spacers with deionized water, ethanol, dry, and assemble the apparatus
    b. Assemble 2 Hoeffer minigels of 1mm thickness

c. Add DEPC-water and check for leakage (leave the set up for 20 minutes)
    i. If no leakage is observed then drain the water in the sink and use a folded paper towel to dry the casting apparatus as much as possible making sure not to move the glass plates in the process.
d. Prepare a 15% gel solution of 12mL according to Table 1 for 1 running gel and Table 2 for 2 running gels.

Table 1. PAGE% preparation for 12mL. [CITE]

%	**30% Acrylamide/Bisacrylamide (29:1) (mL)**	**H20 (Milli-Q or DEPC-treated) (mL)**	**5X TBE (mL)**	**10%APS (uL)**
10	4.0	5.4	2.4	200
14	5.6	3.8	2.4	200
15	6.0	3.4	2.4	200
16	6.4	3	2.4	200

Table 2. PAGE% preparation for 16mL

%	**30% Acrylamide/ Bisacrylamide (29:1) (mL)**	**H20 (Milli-Q or DEPC-treated) (mL)**	**5X TBE (mL)**	**10%APS (uL)**
15	8	5.4	2.4	200

e. Add SYBRSafe according to gel preparation volume
    i. 1.2 uL for 12mL of gel preparation or;
    ii. 1.6 uL for 16mL of gel preparation
f. Vortex the solution
g. Add 10uL of TEMED
    i. Do this in a fume hood as TEMED is toxic
h. Immediately vortex for 2 seconds and pour the gel into the casting apparatus
    i. Make sure to cast two gels to complete the circuit for PAGE
    ii. You cannot perform PAGE with only one casted gel
i. Insert a comb and let the gel polymerize for 20-30 minutes
    i. A good way to estimate polymerization is to see whether the left over gel in the gel preparation centrifuge tube has solidified or not.

ii. The polymerized gel can be placed in 1X TBE and stored in 4C for overnight storage

j. Remove the comb and set the running PAGE apparatus by adding the gel (with both glass slides) to the electrophoresis tank. Cover the gels with 1X TBE buffer

k. Prepare samples according to the experiment being performed.Incubate each sample for 30 minutes at 25C. Do not incubate the samples with the DNA ladder.
   i. For experiment 1 (seeing whether the reaction from literature works or not) prepare samples according to Table 3.
   ii. For experiment 2 (seeing whether the reaction works when the nanopore strand of TMSD is modified) prepare samples according to Table 4.
   iii. For experiment 3 (seeing whether the reaction works when the target strand of TMSD is modified) prepare samples according to Table 5.
   iv. For experiment 4 (seeing whether the reaction works when the nanopore strand and aptazyme strands of TMSD are modified) prepare samples according to Table 6.

Table 3. TMSD Experiment 1 (original reaction)

Tubes	Content	Amount (uL)
1	TMSDNAN	10
2	TMSDTAR	10
3	TMSDKEY	10
4	TMSDNAN + TMSDTAR	5 , 5
5	TMSDNAN + TMSDTAR + TMSDKEY	3.33, 3.33, 3.33
6	TMSDNAN + TMSDKEY	5, 5
7	TMSDTAR + TMSDKEY	5, 5
8	LADDER	10
9	LADDER	20

Table 4. TMSD Experiment 2 (modified nanopore strand)

Tubes	Content	Amount (uL)
1	TMSDNAN	10
2	TMSDNANL	10
3	TMSDTAR	10
4	TMSDKEY	10
5	TMSDTAR + TMSDNAN	5, 5
6	TMSDTAR + TMSDNANL	5,5
7	LADDER	10
8	TMSDTAR+ TMSDNANL + TMSDKEY	3.33, 3.33, 3.33
9	TMSDNANL + TMSDKEY	5, 5
10	LADDER	20

Table 5. TMSD Experiment 3 (modified target strand)

Tubes	Content	Amount (uL)
1	TMSDNAN	10
2	TMSDTAR	10
3	TMSDKEY	10
4	TMSDNAN + TMSDTAR	5 , 5
5	TMSDNAN + TMSDTAR + TMSDKEY	3.33, 3.33, 3.33
6	TMSDNAN + TMSDKEY	5, 5
7	TMSDTAR + TMSDKEY	5, 5

8	LADDER	10
9	LADDER	20

Table 6. TMSD Experiment 4 (modified nanopore and target strands)

Tubes	Content	Amount (uL)
1	TMSDNAN	10
2	TMSDTAR	10
3	TMSDKEY	10
4	TMSDNAN + TMSDTAR	5 , 5
5	TMSDNAN + TMSDTAR + TMSDKEY	3.33, 3.33, 3.33
6	TMSDNAN + TMSDKEY	5, 5
7	TMSDTAR + TMSDKEY	5, 5
8	LADDER	10
9	LADDER	20

l. Add 2uL of loading dye to each sample and briefly centrifuge the samples down before loading 12uL of each sample into the wells.
m. Run gel at 1V/cm - 8V/cm until the dye front has reached the end of the gel or washed past the gel length
    i. If no current is detected, run the gel at 120V
    ii. Run at constant volts; do not change the other parameters
n. Turn the apparatus off, discard the buffer in a bottle and image the gels
o. If no bands are visible then perform post-run staining either with SYBRSafe or with Ethidium Bromide
    i. Refer to (add link for post-run staining here) for protocol and notes

5. Resuspend gels in 50mL of 1X TBE and store overnight at 4C

TMSD: Non-SDS Native PAGE Part 2

Objective: To visualize TMSD experimental bands

Materials:

- Pipette boxes (2)
- Aluminum foil
- Separate solid waste, liquid waste, and gloves waste for Ethidium Bromide
- Rocking platform
- Ethidium bromide (10 mg/mL)
- UV transilluminator

Methods:

1. Dilute 10 mg/mL Ethidium bromide 1:10 000 or 1:20 000 in buffer for 0.5-0.1ug/mL of final concentration. Add 2.5uL of EtBr in 50mL of the 1X TBE buffer that the gels are soaked in.
    a. Dispose of gloves that have come into EtBr in an EtBr-specific waste container in the fume hood
    b. Keep all pipettes and pipette tips outside of the fume hood
2. Cover the gel box with aluminum foil and avoid exposing the box to direct light
3. Transfer gels to a rocking platform and agitate them for 30 minutes at room temperature
    a. Staining time could be increased to 60 minutes or 90 minutes.
4. Image the gel using a transilluminator
    a. Place plastic wrap on the UV transilluminator to avoid as much contact of EtBr with communal equipment as possible
5. If there is high background observed, destain the gel in deionized water for 5-15 minutes to remove excess dye
    a. Dispose of all liquid waste that has come into contact with anything with EtBr in an EtBr labeled liquid waste container located inside the fume hood.
6. Post-stain image processing
    a. Open the gel image in *ImageJ.* Click on image on the top panel> color > split channels. Three windows should appear. Select the window with the green for the best results.

Ethidium Bromide Gel Staining

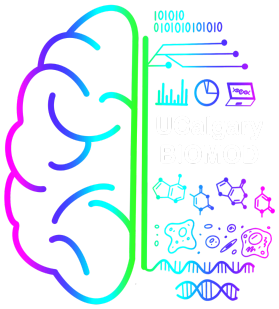
Objective: to stain various gels without SYBR Safe

Materials:

- Pipette boxes (2)
- Aluminum foil
- Separate solid waste, liquid waste, and gloves waste for Ethidium Bromide
- Rocking platform
- Ethidium bromide (10 mg/mL)
- UV transilluminator

Methods:

1. Stain the gel for 30 minutes by adding 2.5uL of EtBr to 50mL of 1xTBE and rocking the gels for 30 minutes
2. Image gel



BIOMOD

Protocol Compilation

Part 3: Aptazyme

Aptazyme Stability Assay

Objective: to test the stability of aptazyme samples in different buffer solutions

Materials:

- PCR Tubes
- Thermal Cycler
- Pipette/Pipette tips
- Aptazyme Samples
    - AP and APT (Supermix 3) were from RNA Purification and Quantification
    - APL was from In Vitro Transcription, Purification, and Quantification
- RNA Loading Dye (includes formamide)
- 10X TBE
- 10X TAE
- DEPC-Water
- RNase-free water

Methods [13]:

1. Prepare the appropriate amount (to a final volume of 110 uL) of mediums in labeled PCR tubes as follows:
    a. 1X TAE diluted with DEPC-water
    b. 1X TBE diluted with DEPC-water
    c. DEPC-water
    d. RNase-free water

Determined Aptazyme Sample Concentrations

Aptazyme Sample	Concentration ng/uL
AP (S3.1)	205.5
APL	164.0
APT (S3.3)	304.8

2. Transfer the appropriate volume of 110 ng Aptazyme samples into labeled PCR tubes according to the following table:

Volumes of Aptazyme Sample and Medium to Add to Labeled PCR Tubes

Aptazyme Sample	Volume of Aptazyme Sample for 110 ng	Volume of Medium to Add (μL)

	(μL)	
AP (S3.1)	0.54	109.46
APL	0.67	109.33
APT (S3.3)	0.36	109.64

3. Transfer 10 uL of RNA Loading dye into PCR tubes that will be used to store the aliquots before collecting
4. Withdraw 10 uL aliquots at the specified times from 30s to 48h and transfer into prepared PCR tubes containing RNA Loading Dye (See Note 2):
    a. 30s, 15 min, 30 min, 45 min, 1 hr, 2 hr, 4 hr, 8 hr, 24 hr, 48 hr
    b. As the aliquots are being collected, freeze prior to gel loading until the last aliquot has been collected
5. Load aliquots into 3% (non-denaturing) Agarose gel using previous protocols for gel preparation

Aptazyme Activity Assay General Procedure:

Objective: to test aptazyme activity

Materials:

- Preparing 10X MOPS Running buffer
    - MOPS-free acid
    - Sodium Acetate–$3H_2O$
    - DEPC-treated dH_2O
    - Na_2EDTA
    - 10M NaOH or Acetic Acid
- In Vitro Cleavage Assay
    - Theophylline (target molecule)
    - Aptazyme (ribozyme)
    - 0.5mL PCR tube
    - 50 mM Tris-HCl, pH 8.0
    - Heat block
    - Ice box
    - MgCl2
    - RNase inhibitor
- 2% Agarose/Formaldehyde/TBE Gel Electrophoresis
    - Fumehood
    - Gel Doc System

- Thermocycler
- Incubator
- Gel Electrophoresis Components:
  - Gel Caster, Gel Comb, Gel Box
- HHR samples
- 1M $MgCl_2$
- RNase inhibitor (RNaseout from Life Technologies – 40 U needed per reaction)
- RNA Gel-Loading Buffer
  - 95 mL Formamide, 18 mL EDTA (pH 7.5), 0.025% SDS, 0.05g bromophenol blue, and 0.05g xylene cyanol (add DEPC-treated water to have a final volume of 100 mL and autoclave after mixing)
- Agarose
- RNase-Eliminating Solvent
- 37% Formaldehyde
- DEPC-Water

Methods [14,15]:

10X MOPS running buffer preparation

1. Prepare 10x MOPS running buffer (if 10mL isn't readily available):
   a. Add 41.85 g 4-morpholino propanesulfonic acid (MOPS-free acid) and 6.80 g sodium acetate–$3H_2O$ to 800 mL DEPC-treated dH_2O and stir until completely dissolved.
   b. Add 20 mL of a DEPC-treated 0.5 M Na_2EDTA solution and adjust pH to 7.0 with 10 M sodium hydroxide or glacial acetic acid
   c. Adjust volume to 1 L with DEPC-treated dH_2O
2. If MOPS 10x is not available, TBE buffer can also substitute.

Aptazyme Activity Assay: Modified Target Concentrations

1.
2. Moles of aptazyme in each tube is approximately 21.08 pmoles.
3. Aptzyme and target were diluted in 50mM Tris-HCl (pH 8.0)

Tube	Ratio (AP: Tar)	Aptazyme (ng/uL)	Aptazyme (pmoles)	Aptazyme (uM)	Volume of aptazyme solution (uL)	Target (uM)	Volume of target solution	50mM Tris-HCl pH 8.0	Total Vol. (uL)
Negative control	1:0	50	~21.08	~2.108	10	0	0	10	20
Acti	1:1	50	~21.08	~2.108	10	2.108	10	0	20

vity test									

1. First, prepare PCR tubes of of target concentrations without adding aptazyme samples using the following table above:
    a. There should be negative controls for each activity sample
2. Add the correctly diluted Aptazyme solutions.
    a. Do this on ice.
3. Incubate PCR tubes of aptazyme for 30 minutes at 37°C
    a. Will only use AP samples for now
4. Take 5 uL aliquot from the PCR tubes for gel, quench immediately with the same volume of 2X RNA Loading dye.
    a. Also prepare Low Range Riboruler RNA ladder similarly
5. IMMEDIATELY place the rest of the solution on ice or freeze to save
    a. Avoid cooling this solution to prevent annealing
6. Incubate all samples at 70°C for 10 min.
    a. Place on ice for a few minutes after
7. Run samples on a gel to analyze

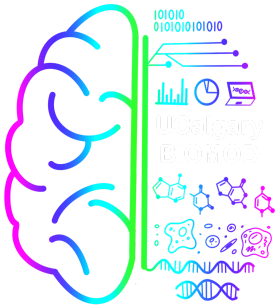
Concentration:

- Aptazyme
    - 25 pM
    - TBD
- Target
    - 10 pM
    - Caffeine
        - Powder
    - Theophylline
        - Powder
    - Adenosine
        - Powder
- MgCl
    - 0mM
    - 14mM
    - powder
- Tris-HCl pH 8
    - 50mM

2.5 % Agarose/Formaldehyde/TBE Gel Electrophoresis [1]

1. Rinse gel box, combs, and caster with millipure water and ensure all salt deposits are removed prior to undergoing electrophoresis
3. Air dry the components
    a. a 50°C incubator can be used for this step if needed
4. Wipe components with RNase-eliminating solvent (RNase Zap).

    a. Rinse with miliQ water
5. Melt agarose for a gel:
    a. Melt 0.8 grams of RNase-free agarose in 28 mL of RNase-free ddH_2O n a 250 mL erlenmeyer flask
6. cool down to 60°C, or just until you can hold the flask to bare skin.
    a. In a fumehood, add 8 mL of 37% formaldehyde and 4 mL of 10X (MOPS or TBE) buffer to the agarose solution
    b. Add 4uL SYBR Safe dye.
7. Cast the gel in the fumehood by placing the gel comb in the gel caster and pouring the melted agarose/formaldehyde/buffer solution in the gel caster.
    a. Cover and allow the gel to cool and polymerize.
8. Heat the samples to be loaded to 70°C for 10 minutes
9. Load each of each sample into each well of the gel
10. Run the gel in 1x TBE buffer for approximately 12 minutes at 0.10 A (constant)
    a. Voltage should be set to 50V
    b. Recirculate the running buffer while the gel runs if possible, otherwise stop the gel halfway and mix manually by pipetting the buffer from one side to the other.
11. Photograph the gel using a gel doc system
    a. destain more if necessary.
    b. Get densitometry analysis image, in TIFF file format



BIOMOD

Protocol Compilation

Part 4: Nanopore + LUV

Nanopore: 10X Folding Buffer Prep

Objective: to prepare buffer for nanopore folding

Materials:

- Magnesium chloride hexahydrate
- Ultrapure 10X TAE Buffer

Methods:

1. Weigh out ~0.028g of $MgCl_2$
2. Add it to 10mL of Ultrapure 10X TAE buffer solution
3. Add prepared solution to the centrifuge tubes
4. Label centrifuge tubes and store at room temperature

Nanopore Synthesis:

Objective: to synthesize the nanopore

Materials:

- Tilibit folding kit basic, type p7249
- 500 µl 100 nM single-stranded scaffold DNA, type p7249
- 500 µl tilibit 10x folding buffer XM
- 1M $MgCl_2$
- Eppendorf tubes
- PCR tubes
- Thermal Cycler
- P10 Pipette & tips
- P200 Pipette & tips
- Mineral oil
- Centrifuge
- Vortex
- RNAse free deionized water

Methods [16]:

1. Take out 10 PCR tubes and label each tube
2. Add following components as listed in Table 1.
3. Add 20 uL mineral oil to all tubes

4. Place PCR tubes into the thermal cycler and choose the setting titled “NANO-GP” which should follow the following protocol: (NOTE: the thermal cycler protocol will run for 8 days as follows)
    a. From 80°C to 60°C at a rate of 1°C per 5 min;
    b. Followed by 60°C to 20°C at a rate of 1°C per 300 min (5 hours).
    c. Infinite hold at 20°C
5. The nanopore samples were set up as followed:

NANOPORE RUN 5	Staple (150nM)	Scaffold (30nM)	Folding buffer (1X)	Water	Total Volume
0.0 MgCl2	9.55	6	2	2.45	20
1.4 MgCl2	9.55	6	2	2.45	20
1.4 MgCl2	9.55	6	2	2.45	20
2.1 MgCl2	9.55	6	2	2.45	20
2.1 MgCl2	9.55	6	2	2.45	20
	47.75	30		12.25	
0.0 MgCl2	28.7	18	6	7.3	60
1.4 MgCl2	28.7	18	6	7.3	60
1.4 MgCl2	28.7	18	6	7.3	60
2.1 MgCl2	28.7	18	6	7.3	60
2.1 MgCl2	28.7	18	6	7.3	60
	143.5	90		36.5	

1. The PCR tubes were labeled as follows:
    a.

Tube content	Tube Label for 20uL reactions	Tube label for 60uL reactions
0.0 MgCl2	1	2
1.4MgCl2	2	3
1.4 MgCl2	3	4
2.1 MgCl2	4	5
2.1 MgCl2	5	6

2. Run DNA agarose gel electrophoresis on the products everyday for all 8 days (temporarily remove the samples from the thermocycler, but do not stop the protocol. Place the samples back into the thermocycler and continue the protocol).

LUV Synthesis:

Objective: to dehydrate Lipid samples at the proper ration

Materials:

- 4 thin glass vials
- 2 glass vials
- Hamilton syringes
- Nitrogen Gas
- Vacuum
- DOPE
- DOPC

Methods [17]:

Cleaning Procedure:

1. Wash from most polar to least polar, rinse glass vial or tin foil in descending order of organic solvent listed above. This also applies to Hamilton Syringes (See note 1):
    a. Acetone
    b. Methanol
    c. Hexanes
    d. Chloroform

Lipid Film Preparation:

1. Using clean tweezers, bend 4 solvent washed tin foil into a "boat-like" shape which will fit into the opening of a clean (also 4 solvent washed) glass vial
    a. Lipids are found on the second shelf of the -20°C freezer
2. Place tin foil on Sartorius Microbalance MC 5 analytical scale, and weigh by difference approximately 1mg of lipid (or less)
3. Transfer the lipids to the clean vial, attempting to deposit as much lipid as possible towards the bottom of the vial
4. Reweigh the tin foil to determine the amount of lipid deposited into the vial (See note 2):
    Example
    a. 0.000 mg tin foil (scale tared)
    b. 1.062 mg POPC + tin foil
    c. 0.056 mg Tin Foil following the lipid transfer
    d. 1.006 mg of lipid deposited
5. Record the amount of lipid used
6. Dissolve the lipids in chloroform to approximately 1 mg/mL (See Note 3)
7. Briefly Sonicate to ensure dissolution of lipids (30-60 seconds) (See Note 4)

8. Evaporate solvent in the fume hood, by using a steady and smooth stream of argon gas – Be sure to avoid too much force as this will cause solution to spill and continue this process until there is little to no solvent remaining
    a. There should be a noticeable formation of lipid film after evaporation
9. Place the vial in the vacuum overnight at ~20 inHg to ensure complete evaporation of the organic solvent

Nanopore Gel-Shift Assay:

Objective: to run gel and gather data on the nanopore samples

Materials:

- Thermal cycler
- Agarose
- 1X TAE
- 1000 µl tilibit 6x gel loading dye
- DNA Ladder
- Nanopore samples
- P10 Pipette & Tips
- Gel electrophoresis apparatus
- Ethidium bromide
- Shaker
- 100uM Cholesterol
- PAGE appartus

Methods:

Preparation of PAGE Gel:

1. Prepare PAGE apparatus and check for leakage
    a. Clean plates and spacers with deionized water, ethanol, dry, and assemble the apparatus
    a. Assemble 2 hoeffer minigels of 1mm thickness
    b. Add water and check for leakage (leave the set up for 20 minutes)
        i. If no leakage is observed then drain the water in the sink and use a folded paper towel to dry the casting apparatus as much as possible making sure not to move the glass plates in the process.
2. Prepare a 15% gel solution of 12mL according to Table 1 for 1 running gel and Table 2 for 2 running gels.

Table 1. PAGE% preparation for 14mL (2 GELS)

%	30%	H20 (Milli-Q or	10X TAE	10%APS

	Acrylamide/Bisacrylamide (29:1) (mL)	DEPC-treated) (mL)	(mL)	(uL)
15	7.0	4.4	2.4	200

3. Load the samples into the gel
4. Load DNA ladder into the gel
5. Load the running buffer into the gel
    a. 950mL of 1X TAE was added
        i. 700mL originally added
        ii. 250mL added afterwards
6. Set the gel to run at a constant 120V.

Image Gel According to the Ethidium Bromide Procedure

DLS Liposome Analysis:

Objective: to determine the polydispersity and size of the liposomes

Materials:

- Lipid samples from the 27/08/2024
- Glass vials
- Acetone, methanol, hexane, and chloroform
- Cuvettes
- Kimwipes

Methods:

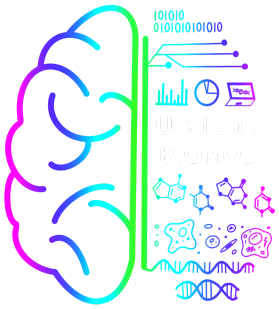
1. LUV only (20/08/2024)
    a. Diluted the sample by adding 100uL of sample and 900uL of buffer 7
    b. Used a new cuvette. Washed this cuvette with water and buffer 7 twice
2. LUV+ FITC (20/08/2024) diluted
    a. 100uL sample, 900uL buffer 7
    b. Used a new cuvette. Washed this cuvette with water and buffer 7 twice
3. LUV +FITC (20/092024) not diluted
    a. 1000uL sample
    b. Washed this cuvette with water, ethanol, twice with water and then twice with buffer 7
4. LUV +SRB (20/092024) not diluted
    a. 1000uL sample

b. Washed this cuvette with water, ethanol, twice with water and then twice with buffer 7
c. Results: Attenuation: 10 (shows that sample is dilute)
    i. Run 1:
        1. Polydispersity: 0.205
        2. Size: 308.9

    ii. Run 2:
        1. Polydispersity: 0.240
        2. Size: 371.2

    iii. Run 3:
        1. Polydispersity: 0.223
        2. Size: 334.6

5. LUV +SRB+FITC (20/092024) not diluted
    a. 1000uL sample
    b. Washed this cuvette with water, ethanol, twice with water and then twice with buffer 7
6. Keep all samples on ice and wrap each 4 solvent washed glass vial with parafilm and aluminum foil



BIOMOD

Protocol Compilation

Part 5: Dye-Flux

Dye Flux (1.1):

Objective: Observe the fluorescence of SRB with differing concentrations in buffer solution

Materials:

- SRB dye
- Buffer
- Eppendorf Tubes
- Pipettes/Pipette Tips
- 96 well plate – white w/ clear bottom
- Plate Reader

Methods:

Preparation of SRB Solutions

1. Prepare a buffer solution with 1X TAE, 0.5M NaCl, and 16 mM $MgCl_2$
2. Prepare 2 Stock Solutions with the following components:
    a. In an eppendorf tube dissolve 19.6 mg of SRB in 350 uL of buffer solution to make a 100 mM stock solution
    b. Pipette 10 uL of the 100 mM stock solution into a separate eppendorf tube and mix with 90 uL of buffer solution to make a 10 mM stock solution
3. To prepare the solutions for Stage 1.1 of dye flux, mix the following components onto a 96 well plate:

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1	H2	H3	H3	H5	H6	H7	H8	H9	H10	H11	H12

A1 → 100 uL Buffer Solution
A2 → 1 uL 10 mM SRB and 99 uL Buffer Solution
A3 → 10 uL 10 mM SRB and 90 uL Buffer Solution
A4 → 20 uL 10 mM SRB and 80 uL Buffer Solution
A5 → 50 uL 10 mM SRB and 50 uL Buffer Solution

A6 → 10 uL 100 mM SRB and 90 uL Buffer Solution (See Note 1)
A7 → 20 uL 100 mM SRB and 80 uL Buffer Solution
A8 → 30 uL 100 mM SRB and 70 uL Buffer Solution
A9 → 40 uL 100 mM SRB and 60 uL Buffer Solution
A10 → 50 uL 100 mM SRB and 50 uL Buffer Solution
A11 → 75 uL 100 mM SRB and 25 uL Buffer Solution
A12 → 100 uL 100 mM SRB

Navigating the Plate Reader Software

1. Open the 96 well plate tray by pressing the "PLATE" button on the plate reader
2. Place the 96 well plate on the tray without the lid and close by pressing the "PLATE" button on the plate reader
3. Press the "Settings" button on the Plate Reader Window and configure to the following specifications below:
    a. Read Mode: FL (fluorescence)
    b. Read Type: Spectrum
    c. Wavelengths: Excitation = 544 nm / Emission = 564 - 700 nm / Step = 9 nm
    d. Plate Type: 96 Well Standard clrbtm*
    e. Read Area: *Highlight the follow wells that were populated in the 96 well plate*
4. Press "OK" and "READ"
5. The plate reader should initialize before reading the signals from the samples – let the plate reader finish completely before moving on to the next step

Exporting Data

1. Press the button with the "*96 Well Plate in a circle"* on the very top left corner
2. On the drop down menu, press "EXPORT"
3. On the pop up window, select the experiment to be exported and configure to the following specifications:
    a. Plate Data Options: Both
    b. Output Format: Plate (.txt or .xls)
4. Press "Okay" and rename the exported file

Dye Flux (1.2):

Objective: To observe the fluorescence and absorbance of SRB with differing concentrations in buffer solution and old dye load vesicles with and without Triton X100

Materials:

- SRB dye
- SRB 0 ← Encapsulated dye vesicle sample from first run through (1st samples)
- SRB 1 ← Encapsulated dye vesicle sample from second run through (1st samples)
- No Dye ← Vesicles with no dye (1st samples)
- Buffer
- Eppendorf Tubes
- Pipettes/Pipette Tips
- 96 well plate – white w/ clear bottom
- Plate Reader

Methods:

Preparation of SRB Solutions

1. Prepare a buffer solution with 1X TAE, 0.5M NaCl, and 16 mM $MgCl_2$
2. Prepare 2 Stock Solutions with the following components:
    a. Pipette 150 uL of the 50 mM SRB stock solution into a eppendorf tube and mix with 100 uL of buffer solution to make a 30 mM stock solution
    b. Pipette 6.67 µL of the 30 mM stock solution into a separate eppendorf tube and mix with 193.33 µL of buffer solution to make a 1 mM stock solution
3. To prepare the solutions for Stage 1.2 of dye flux, mix the following components onto a 96 well plate:

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1	H2	H3	H3	H5	H6	H7	H8	H9	H10	H11	H12

A1 → 100 uL Buffer Solution
A2 → 0.01 mM: 1 µL 1 mM SRB and 99 µL Buffer Solution
A3 → 0.02 mM: 2 µL 1 mM SRB and 98 µL Buffer Solution
A4 → 0.05 mM: 5 µL 1 mM SRB and 95 µL Buffer Solution
A5 → 0.1 mM: 10 µL 1 mM SRB and 90 µL Buffer Solution
A6 → 0.5 mM: 50 µL 1 mM SRB and 50 uL Buffer Solution
A7 → 1 mM: 100 µL 1 mM SRB
A8 → 2 mM: 6.67 µL 30 mM SRB and 93.33 µL Buffer Solution
A9 → 5 mM: 16.67 µL 30 mM SRB and 83.33 µL Buffer Solution
A10 → 10 mM: 33.33 µL 30 mM SRB and 66.67 µL Buffer Solution
A11 → 15 mM: 50 µL 30 mM SRB and 50 µL Buffer Solution
A12 → 30 mM: 100 µL 30 mM SRB

B1 → Unruptured Non-Loaded Dye Vesicles
B2 → Ruptured Non-Loaded Dye Vesicles

C1 → Unruptured SRB#0 Vesicles
C2 → Ruptured SRB#0 Vesicles (w/ Triton X100)

D1 → Unruptured SRB#1 Vesicles
D2 → Ruptured SRB#1 Vesicles (w/ Triton X100)

Navigating the Plate Reader Software (Fluorescence)

1. Open the 96 well plate tray by pressing the “PLATE” button on the plate reader
2. Place the 96 well plate on the tray without the lid and close by pressing the “PLATE” button on the plate reader
3. Press the “Settings” button on the Plate Reader Window and configure to the following specifications below:
    a. Read Mode: FL (fluorescence)
    b. Read Type: Spectrum
    c. Wavelengths: Excitation = 544 nm / Emission = 564 - 700 nm / Step = 9 nm
    d. Plate Type: 96 Well Standard clrbtm*
    e. Read Area: *Highlight the follow wells that were populated in the 96 well plate*
4. Press “OK” and “READ”
5. The plate reader should initialize before reading the signals from the samples – let the plate reader finish completely before moving on to the next step (See Note 1)

Navigating the Plate Reader Software (Absorbance)

1. Open the 96 well plate tray by pressing the “PLATE” button on the plate reader

2. Place the 96 well plate on the tray without the lid and close by pressing the “PLATE” button on the plate reader
3. Press the “Settings” button on the Plate Reader Window and configure to the following specifications below:
    a. Read Mode: ABS (Absorbance)
    b. Read Type: Spectrum
    c. Wavelengths: Start - 230 nm/End - 800 nm/Step - 10 nm
    d. Plate Type: 96 Well Standard clrbtm*
    e. Read Area: *Highlight the follow wells that were populated in the 96 well plate*
4. Press “OK” and “READ”
5. The plate reader should initialize before reading the signals from the samples – let the plate reader finish completely before moving on to the next step

Exporting Data

1. Press the button with the “*96 Well Plate in a circle”* on the very top left corner
2. On the drop down menu, press “EXPORT”
3. On the pop up window, select the experiment to be exported and configure to the following specifications:
    a. Plate Data Options: Both
    b. Output Format: Plate (.txt or .xls)
4. Press “Okay” and rename the exported file

Notes

(1) Absorbance was performed first and due to time limitations, was left to run by itself for 2 hours. When the reading was finished, the tray opened, leaving the dye molecules vulnerable to light for an unspecified amount of times. The samples were still tested for fluorescence.

Dye Flux (1.3):

Objective: To observe the fluorescence of FITC with differing concentrations in buffer solution

Materials:

- FITC dye
- Buffer
- Eppendorf Tubes
- Pipettes/Pipette Tips
- 96 well plate – white w/ clear bottom
- Plate Reader

Methods:

Preparation of SRB Solutions

1. Prepare a buffer solution with 1X TAE, 0.5M NaCl, and 16 mM $MgCl_2$
2. Prepare one stock solution for a serial dilution
    a. In an eppendorf tube dissolve 2.4 mg of FITC in 120 uL of buffer solution to make a 1 mM stock solution
    b. Pipette 111.111 uL of the stock solution into well A8
3. To prepare the solutions for Stage 1.3 of dye flux, mix the following components onto a 96 well plate:
    a. Pipette 11.11 uL of solution from well A8 into A7 and mix with 100 uL of Buffer
    b. Pipette 11.11 uL of the mixed solution in A7 into well A6 and mix with 100 uL of Buffer
    c. Continue serial dilution until well A2 – there should be a total volume of 111.11 uL into well A2
    d. Pipette 11.11 uL of solution from well A2 and discard

A1	A2	A3	A4	A5	A6	A7	A8
B1	B2	B3	B4	B5	B6	B7	B8
C1	C2	C3	C4	C5	C6	C7	C8
D1	D2	D3	D4	D5	D6	D7	D8
E1	E2	E3	E4	E5	E6	E7	E8
F1	F2	F3	F4	F5	F6	F7	F8
G1	G2	G3	G4	G5	G6	G7	G8
H1	H2	H3	H3	H5	H6	H7	H8

A1 → 100 uL Buffer Solution (Blank)
A2 → 100 uL 1 nM FITC
A3 → 100 uL 10 nM FITC
A4 → 100 uL 100 nM FITC
A5 → 100 uL 1 uM FITC
A6 → 100 uL 10 uM FITC
A7 → 100 uL 100 uM FITC
A8 → 100 uL 1 mM FITC

Navigating the Plate Reader Software (Fluorescence)

1. Open the 96 well plate tray by pressing the "PLATE" button on the plate reader
2. Place the 96 well plate on the tray without the lid and close by pressing the "PLATE" button on the plate reader
3. Press the "Settings" button on the Plate Reader Window and configure to the following specifications below:
    a. Read Mode: FL (fluorescence)
    b. Read Type: Spectrum
    c. Wavelengths: Excitation = 490 nm and 480 nm / Emission = 500 - 700 nm / Step = 5 nm
    d. Plate Type: 96 Well Standard clrbtm*
    e. Read Area: *Highlight the follow wells that were populated in the 96 well plate*
4. Press "OK" and "READ"
5. The plate reader should initialize before reading the signals from the samples – let the plate reader finish completely before moving on to the next step

Navigating the Plate Reader Software (Absorbance)

1. Open the 96 well plate tray by pressing the "PLATE" button on the plate reader
2. Place the 96 well plate on the tray without the lid and close by pressing the "PLATE" button on the plate reader
3. Press the "Settings" button on the Plate Reader Window and configure to the following specifications below:
    a. Read Mode: ABS (Absorbance)
    b. Read Type: Spectrum
    c. Wavelengths: Start = 300 nm/End = 800 nm/Step = 10 nm
    d. Read Area: *Highlight the follow wells that were populated in the 96 well plate*
4. Press "OK" and "READ"
5. The plate reader should initialize before reading the signals from the samples – let the plate reader finish completely before moving on to the next step

Exporting Data

1. Press the button with the "*96 Well Plate in a circle"* on the very top left corner
2. On the drop down menu, press "EXPORT"
3. On the pop up window, select the experiment to be exported and configure to the following specifications:
    a. Plate Data Options: Both
    b. Output Format: Plate (.txt or .xls)
4. Press "Okay" and rename the exported file

Dye Flux (2.1):

Objective: To observe the fluorescence of SRB and FITC with differing and same concentrations in buffer solution

Materials:

- SRB Dye
- FITC dye
- Buffer
- Eppendorf Tubes
- Pipettes/Pipette Tips
- 96 well plate – white w/ clear bottom
- Plate Reader

Methods:

Preparation of Buffer and Dye Stocks

1. Prepare a buffer solution with 1X TAE, 0.5M NaCl, and 16 mM $MgCl_2$
2. Prepare 4 Stocks of different SRB concentrations:
    a. 40 µL of 50 mM SRB
        i. Obtain from vesicle preparation stock
    b. 145 µL of 1 mM SRB
        i. 2.9 µL of 50 mM SRB
        ii. 142.1 µL of Buffer
    c. 75 µL of 5 µM SRB
        i. 0.375 µL of 1 mM SRB
        ii. 74.625 µL of Buffer
    d. 25 µL of 50 nM SRB
        i. 0.25 µL of 5 µM SRB
        ii. 24.75 µL of Buffer
3. Prepare 3 stocks of different FITC concentrations:
    a. 140 µL of 1 mM
        i. 2.8 mg of FITC

ii. 140 µL of Buffer

b. 175 µL of 2 µM FITC
   i. 0.35 µL of 1 mM FITC
   ii. 174.65 µL of Buffer

c. 50 µL of 50 nM FITC

4. 1.25 of 2 µM FITC
5. 48.75 µL of Buffer

Preparation of Different SRB and FITC Concentrations

1. Prepare SRB and FITC solutions at the following concentrations and pipette into wells A1 - A10:

A1:

- Total Volume 100 µL
  - 100µL of Buffer

A2:

- Total Volume 100 µL
- SRB Concentration: 500 nM
  - Add 10 µL of 5 µM SRB
  - Add 40 uL of Buffer
- FITC Concentration: 1 nM
  - Add 2 µL of 50 nM FITC
  - Add 48 µL of Buffer

A3:

- Total Volume 100 µL
- SRB Concentration: 1 µM
  - Add 20 uL of 5 µM SRB
  - Add 30 uL of Buffer
- FITC Concentration: 10 nM
  - Add 20 µL of 50 nM FITC
  - Add 30 µL of Buffer

A4:

- Total Volume 100 µL
- SRB Concentration: 10 µM
  - Add 1 µL of 1 mM SRB
  - Add 49 µL of Buffer
- FITC Concentration: 100 nM
  - Add 5 µL of 2 µM FITC
  - Add 45 µL of Buffer

A5:

- Total Volume 100 µL
- SRB Concentration: 50 µM
    - Add 5 µL of 1 mM SRB
    - Add 45 µL of Buffer
- FITC Concentration: 500 nM
    - Add 25 µL of 2 µM FITC
    - Add 25 µL of Buffer

A6:

- Total Volume 100 µL
- SRB Concentration: 100 µM
    - Add 10 uL of 1 mM SRB
    - Add 40 uL of Buffer
- FITC Concentration: 1 µM
    - Add 50 µL of 2 µM FITC

A7:

- Total Volume 100 µL
- SRB Concentration: 500 µM
    - Add 50 µL of 1 mM SRB
- FITC Concentration: 10 µM
    - Add 1 µL of 1 mM FITC
    - Add 49 µL of Buffer

A8:

- Total Volume 100 µL
- SRB Concentration: 1 mM
    - Add 2 µL of 50 mM SRB
    - Add 48 µL of Buffer
- FITC Concentration: 50 µM
    - Add 5 µL of 1 mM FITC
    - Add 45 µL of Buffer

A9:

- Total Volume 100 µL
- SRB Concentration: 5 mM
    - Add 10 µL of 50 mM SRB
    - Add 40 µL of Buffer
- FITC Concentration: 100 µM
    - Add 10 µL of 1 mM FITC
    - Add 40 µL of Buffer

A10:

- Total Volume 100 µL
- SRB Concentration: 10 mM

    - Add 20 µL of 50 mM SRB
    - Add 30 µL of Buffer
- FITC Concentration: 500 µM
    - Add 50 µL of 1 mM FITC

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
H1	H2	H3	H3	H5	H6	H7	H8	H9	H10

Preparation of the Same SRB and FITC Concentrations

1. Prepare SRB and FITC solutions at the following concentrations and pipette into wells B1 - B10:

   B1:
   - Total Volume 100 µL
       - 100µL of Buffer

   B2:
   - Total Volume 100 µL
   - SRB Concentration: 1 nM
       - Add 2 µL of 50 nM SRB
       - Add 48 uL of Buffer
   - FITC Concentration: 1 nM
       - Add 2 µL of 50 nM FITC
       - Add 48 µL of Buffer

   B3:
   - Total Volume 100 µL
   - SRB Concentration: 10 nM
       - Add 20 µL of 50 nM SRB
       - Add 30 µL of Buffer
   - FITC Concentration: 10 nM
       - Add 20 µL of 50 nM FITC
       - Add 30 µL of Buffer

   B4:

- Total Volume 100 µL
- SRB Concentration: 100 nM
    - Add 2 µL of 5 µM SRB
    - Add 48 µL of Buffer
- FITC Concentration: 100 nM
    - Add 5 µL of 2 µM FITC
    - Add 45 µL of Buffer

B5:

- Total Volume 100 µL
- SRB Concentration: 500 nM
    - Add 10 µL of 5 µM SRB
    - Add 40 µL of Buffer
- FITC Concentration: 500 nM
    - Add 25 µL of 2 µM FITC
    - Add 25 µL of Buffer

B6:

- Total Volume 100 µL
- SRB Concentration: 1 µM
    - Add 20 µL of 5 µM SRB
    - Add 30 µL of Buffer
- FITC Concentration: 1 µM
    - Add 50 µL of 2 µM FITC

B7:

- Total Volume 100 µL
- SRB Concentration: 10 µM
    - Add 1 µL of 1 mM SRB
    - Add 49 µL of Buffer
- FITC Concentration: 10 µM
    - Add 1 µL of 1 mM FITC
    - Add 49 µL of Buffer

B8:

- Total Volume 100 µL
- SRB Concentration: 50 µM
    - Add 5 µL of 1 mM SRB
    - Add 45 µL of Buffer
- FITC Concentration: 50 µM
    - Add 5 µL of 1 mM FITC
    - Add 45 µL of Buffer

B9:

- Total Volume 100 µL

- SRB Concentration: 100 µM
    - Add 10 µL of 1 mM SRB
    - Add 40 µL of Buffer
- FITC Concentration: 100 µM
    - Add 10 µL of 1 mM FITC
    - Add 40 µL of Buffer

  B10:
- Total Volume 100 µL
- SRB Concentration: 500 µM
    - Add 50 µL of 1 mM SRB
- FITC Concentration: 500 µM
    - Add 50 µL of 1 mM FITC

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
H1	H2	H3	H3	H5	H6	H7	H8	H9	H10

Dye Flux (2.2):

Objective: ?

Materials:

- SRB Dye
- FITC dye
- Buffer
- Eppendorf Tubes
- Pipettes/Pipette Tips
- 96 well plate – white w/ clear bottom
- Plate Reader

Methods:

Preparation of SRB Solutions:

A: Control – SRB concentrations test gradient in used buffer

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12

A1:

- Total Volume 100 µL
    - 100µL of Buffer

A2:

- Total Volume 100 µL
- SRB Concentration: 10 µM
    - Add 1 µL of 1 mM SRB
    - Add 99 µL of Buffer

A3:

- Total Volume 100 µL
- SRB Concentration: 50 µM
    - Add 5 µL of 1 mM SRB
    - Add 95 µL of Buffer

A4:

- Total Volume 100 µL
- SRB Concentration: 100 µM
    - Add 10 µL of 1 mM SRB
    - Add 90 µL of Buffer

A5:

- Total Volume 100 µL
- SRB Concentration: 250 µM
    - Add 25 µL of 1 mM SRB
    - Add 75 µL of Buffer

A6:

- Total Volume 100 µL
- SRB Concentration: 500 µM
    - Add 50 µL of 1 mM SRB
    - Add 50 µL of Buffer

A7:

- Total Volume 100 µL
- SRB Concentration: 750 µM
    - Add 1.5 µL of 50 mM SRB
    - Add 98.5 µL of Buffer

A8:

- Total Volume 100 µL
- SRB Concentration: 1 mM

    - Add 2 µL of 50 mM SRB
    - Add 98 µL of Buffer

A9:

- Total Volume 100 µL
- SRB Concentration: 2.5 mM
    - Add 5 µL of 50 mM SRB
    - Add 95 µL of Buffer

A10:

- Total Volume 100 µL
- SRB Concentration: 5 mM
    - Add 10 µL of 50 mM SRB
    - Add 90 µL of Buffer

A11:

- Total Volume 100 µL
- SRB Concentration: 7.5 mM
    - Add 15 µL of 50 mM SRB
    - Add 85 µL of Buffer

A12:

- Total Volume 100 µL
- SRB Concentration: 10 mM
    - Add 20 µL of 50 mM SRB
    - Add 80 µL of Buffer

Preparation of FITC Solutions:

B: Control – FITC concentrations test gradient in used buffer

B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12

B1:

- Total Volume 100 µL
    - 100µL of Buffer

B2:

- Total Volume 100 µL
- FITC Concentration: 2.5 µM
    - Add 5 µL of 50 µM FITC
    - Add 95 µL of Buffer

B3:

- Total Volume 100 µL
- FITC Concentration: 12.5 µM
    - Add 25 µL of 50 µM FITC
    - Add 75 µL of Buffer

B4:

- Total Volume 100 µL
- FITC Concentration: 25 µM
    - Add 50 µL of 50 µM FITC
    - Add 50 µL of Buffer

B5:

- Total Volume 100 µL
- FITC Concentration: 62.5 µM
    - Add 6.25 µL of 1 mM FITC
    - Add 93.75 µL of Buffer
        - 93 + 0.75

B6:

- Total Volume 100 µL
- FITC Concentration: 125 µM
    - Add 12.5 µL of 1 mM FITC
        - 10 + 2.5
    - Add 87.5 µL of Buffer

B7:

- Total Volume 100 µL
- FITC Concentration: 187.5 µM
    - Add 18.75 µL of 1 mM FITC
        - 9 + 9 + 0.75
    - Add 81.25 µL of Buffer
        - 81 + 0.25

B8:

- Total Volume 100 µL
- FITC Concentration: 250 µM
    - Add 25 µL of 1 mM FITC
    - Add 75 µL of Buffer

B9:

- Total Volume 100 µL
- FITC Concentration: 500 µM
    - Add 50 µL of 1 mM FITC
    - Add 50 µL of Buffer

Preparation of SRB + Mixed Dye Solutions (Varied Concentration):

C: SRB+FITC Mixed Dye Test (Varied Concentration) – SRB concentrations test gradient in used buffer mixed with FITC concentration test gradient

C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12

C1:

- Total Volume 100 µL
    - 100µL of Buffer

C2:

- Total Volume 100 µL
- SRB Concentration: 10 µM
    - Add 1 µL of 1 mM SRB
- FITC Concentration: 2.5 µM
    - Add 5 µL of 50 µM FITC
- Buffer: 94 µL

C3:

- Total Volume 100 µL
- SRB Concentration: 50 µM
    - Add 5 µL of 1 mM SRB
- FITC Concentration: 12.5 µM
    - Add 25 µL of 50 µM FITC
- Buffer 70 µL

C4:

- Total Volume 100 µL
- SRB Concentration: 100 µM
    - Add 10 µL of 1 mM SRB
- FITC Concentration: 25 µM
    - Add 50 µL of 50 µM FITC
- Buffer 40 µL

C5:

- Total Volume 100 µL
- SRB Concentration: 250 µM
    - Add 25 µL of 1 mM SRB
        - 20 µL + 5 µL
- FITC Concentration: 62.5 µM
    - Add 6.25 µL of 1 mM FITC
        - 6 + 0.25
- Buffer: 68.75 µL

C6:

- Total Volume 100 µL
- SRB Concentration: 500 µM
    - Add 50 µL of 1 mM SRB
- FITC Concentration: 125 µM
    - Add 12.5 µL of 1 mM FITC
        - 10 + 2.5
- Buffer: 37.5 µL

C7:

- Total Volume 100 µL
- SRB Concentration: 750 µM
    - Add 1.5 µL of 50 mM SRB
- FITC Concentration: 187.5 µM
    - Add 18.75 µL of 1 mM FITC
        - 9 + 9 + 0.75
- Buffer: 79.75 µL
    - 79 + 0.75

C8:

- Total Volume 100 µL
- SRB Concentration: 1 mM
    - Add 2 µL of 50 mM SRB
- FITC Concentration: 250 µM
    - Add 25 µL of 1 mM FITC
- Buffer: 73 µL

C9:

- Total Volume 100 µL
- SRB Concentration: 2.5 mM
    - Add 5 µL of 50 mM SRB
- FITC Concentration: 500 µM
    - Add 50 µL of 1 mM FITC
        - 13 µL + 37 µL
- Buffer: 45 µL

C10:

- Total Volume 100 µL
- SRB Concentration: 5 mM
    - Add 10 µL of 50 mM SRB
- FITC Concentration: 500 µM
    - Add 50 µL of 1 mM FITC
- Buffer: 40 µL

C11:

- Total Volume 100 µL
- SRB Concentration: 7.5 mM
    - Add 15 µL of 50 mM SRB
        - 10 + 5
- FITC Concentration: 500 µM
    - Add 50 µL of 1 mM FITC
- Buffer: 35 µL

C12:

- Total Volume 100 µL
- SRB Concentration: 10 mM
    - Add 20 µL of 50 mM SRB
- FITC Concentration: 500 µM
    - Add 50 µL of 1 mM FITC
- Buffer: 30 µL

Dye Flux (2.3):

Objective: to test the dilutions of vesicles and their dye concentrations alongside various dye dilutions

Materials:

- SRB Dye
- FITC dye
- Buffer
- Eppendorf Tubes
- Pipettes/Pipette Tips
- 96 well plate – white w/ clear bottom
- Plate Reader
- X-Triton
- No-Dye Vesicles

Methods:

Preparation of SRB Solutions:

A: Control – SRB concentrations test gradient in used buffer

	A1	A2	A3	A4	A5	A6	A7	A8
Stock SRB (mM)	0	1	1	1	1	50	50	50
Final SRB (uM)	0	10	50	100	500	1 000	5 000	10 000
Buffer (uL)	100	99	95	90	50	98	90	80
SRB (uL)	0	1	5	10	50	2	10	20

Preparation of FITC Solutions:

B: Control – FITC concentrations test gradient in used buffer

	B1	B2	B3	B4	B5	B6	B7	B8
Stock FITC (uM)	0	100	100	100	1 000	1 000	1 000	1 000

Final FITC (uM)	0	1	5	10	50	100	500	1 000
Buffer (uL)	100	99	95	90	95	90	50	0
FITC (uL)	0	1	5	10	5	10	50	100

Preparation of SRB + FITC Mixed Solutions (Varied Concentration):

C: SRB+FITC Mixed Dye Test (Varied Concentration) – SRB concentrations test gradient in used buffer mixed with FITC concentration test gradient

	C1	C2	C3	C4	C5	C6	C7	C8
Stock FITC (uM)	0	100	100	100	100	1 000	1 000	2 000
Final FITC (uM)	0	1	5	10	50	100	500	1 000
Stock SRB (mM)	0	1	1	1	1	50	50	50
Final SRB (uM)	0	10	50	100	500	1 000	5 000	10 000
Buffer (uL)	100	98	90	80	0	88	40	30
FITC (uL)	0	1	5	10	50	10	50	50
SRB (uL)	0	1	5	10	50	2	10	20

Preparation of Vesicle + Triton Solutions:

	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
Buffer (uL)	1	0	1	1	1	0	1	0	1	0
FITC-2mM (uL)	0	0	0	0	0	0	99	99	49.5	49.5
FITC-dilute-100uM (uL)	0	0	0	0	99	99	0	0	0	0
SRB-not diluted -50mM (uL)	0	0	99	99	0	0	0	0	49.5	49.5
Vesicles Containing	99	99	0	0	0	0	0	0	0	0

no Dye (uL)										
X-Triton (uL)	0	1	0	0	0	1	0	1	0	1
Total Volume (uL)	100	100	100	100	100	100	100	100	100	100

Dye Flux (2.4):

Objective: ?

Materials:

- SRB Dye
- FITC dye
- Buffer
- Eppendorf Tubes
- Pipettes/Pipette Tips
- 96 well plate – white w/ clear bottom
- Plate Reader
- No-Dye Vesicles
- X-Triton

Methods:

Preparation of SRB Solutions:

A: Control – SRB concentrations test gradient in used buffer

	A1	A2	A3	A4	A5	A6	A7	A8
Stock SRB (mM)	0	1	1	1	1	50	50	50
Final SRB (uM)	0	10	50	100	500	1 000	5 000	10 000
Buffer (uL)	100	99	95	90	50	98	90	80
SRB (uL)	0	1	5	10	50	2	10	20

Preparation of FITC Solutions:

B: Control – FITC concentrations test gradient in used buffer

	B1	B2	B3	B4	B5	B6	B7	B8

Stock FITC (uM)	0	100	100	100	1 000	1 000	1 000	1 000
Final FITC (uM)	0	1	5	10	50	100	500	1 000
Buffer (uL)	100	99	95	90	95	90	50	0
FITC (uL)	0	1	5	10	5	10	50	100

Preparation of SRB + FITC Mixed Dye Solutions (Varied Concentrations):

C: SRB+FITC Mixed Dye Test (Varied Concentration) – SRB concentrations test gradient in used buffer mixed with FITC concentration test gradient

	C1	C2	C3	C4	C5	C6	C7	C8
Stock FITC (uM)	0	100	100	100	100	1 000	1 000	2 000
Final FITC (uM)	0	1	5	10	50	100	500	1 000
Stock SRB (mM)	0	1	1	1	1	50	50	50
Final SRB (uM)	0	10	50	100	500	1 000	5 000	10 000
Buffer (uL)	100	98	90	80	0	88	40	30
FITC (uL)	0	1	5	10	50	10	50	50
SRB (uL)	0	1	5	10	50	2	10	20

Preparation of Vesicle + Triton Solutions (Not Dilute):

	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
Buffer (uL)	1	0	1	1	1	0	1	0	1	0
FITC-Not dilute-2mM(uL)	0	0	0	0	0	0	99	99	49.5	49.5
FITC-dilute-100uM (uL)	0	0	0	0	99	99	0	0	0	0
SRB-not diluted -50mM (uL)	0	0	99	99	0	0	0	0	49.5	49.5

Vesicles Containing no Dye (uL)	99	99	0	0	0	0	0	0	0	0
X-Triton (uL)	0	1	0	0	0	1	0	1	0	1
Total Volume (uL)	100	100	100	100	100	100	100	100	100	100

Preparation of Vesicle + Triton Solutions (Dilute):

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Buffer (uL)	1	0	1	0	1	0	1	0	1	0
FITC-Not dilute-2mM(uL)	0	0	0	0	0	0	0	0	99	99
SRB-diluted -10mM (uL)	0	0	0	0	99	99	0	0	0	0
SRB-not diluted -50mM (uL)	0	0	0	0	0	0	99	99	0	0
Vesicles Containing no Dye (uL)	0	0	99	99	0	0	0	0	0	0
Vesicles Containing no Dye-DILUTED (uL)	99	99	0	0	0	0	0	0	0	0
X-Triton (uL)	0	1	0	1	0	1	0	1	0	1
Total Volume (uL)	100	100	100	100	100	100	100	100	100	100

Dye Flux (3.1):

Objective: to test the prepared nanopores with dye vesicles containing SRB

Materials:

- SRB Dye
- FITC dye
- Buffer
- Eppendorf Tubes
- Pipettes/Pipette Tips
- 96 well plate – white w/ clear bottom
- Plate Reader
- X-Triton
- No-Dye Vesicles
- Nanopore Samples 3 and 4, with/without Cholesterol

Methods:

Preparation of SRB Solutions:

A: Control – SRB concentrations test gradient in used buffer

	A1	A2	A3	A4	A5	A6	A7	A8
Stock SRB (mM)	0	1	1	1	1	50	50	50
Final [SRB] (uM)	0	10	50	100	500	1 000	5 000	10 000
Buffer (uL)	100	99	95	90	50	98	90	80
SRB Added (uL)	0	1	5	10	50	2	10	20

SRB Dilution Series: 10 mM, 5 mM, 1 mM, 500 µM, 100 µM, 50 µM, 10 µM, 0 µM

Preparation of FITC Solutions:

B: Control – FITC concentrations test gradient in used buffer

	B1	B2	B3	B4	B5	B6	B7	B8
Stock FITC (uM)	0	100	100	100	1 000	1 000	1 000	1 000
Final FITC (uM)	0	1	5	10	50	100	500	1 000

Buffer (uL)	100	99	95	90	95	90	50	0
FITC (uL)	0	1	5	10	5	10	50	100

FITC Dilution Series: 1 mM, 500 µM, 100 µM, 50 µM, 10 µM, 5 µM, 1 µM, 0 µM

Preparation of SRB + FITC Mixed Dye Solutions (Various Concentrations):

C: SRB+FITC Mixed Dye Test (Varied Concentration) – SRB concentrations test gradient in used buffer mixed with FITC concentration test gradient

	C1	C2	C3	C4	C5	C6	C7	C8
Stock FITC (uM)	0	100	100	100	100	1 000	1 000	2 000
Final FITC (uM)	0	1	5	10	50	100	500	1 000
Stock SRB (mM)	0	1	1	1	1	50	50	50
Final SRB (uM)	0	10	50	100	500	1 000	5 000	10 000
Buffer (uL)	100	98	90	80	0	88	40	30
FITC (uL)	0	1	5	10	50	10	50	50
SRB (uL)	0	1	5	10	50	2	10	20

SRB Dilution Series: 10 mM, 5 mM, 1 mM, 500 µM, 100 µM, 50 µM, 10 µM, 0 µM
FITC Dilution Series: 1 mM, 500 µM, 100 µM, 50 µM, 10 µM, 5 µM, 1 µM, 0 µM

Preparation of Vesicle + Triton Solutions (Not Dilute):

	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
Buffer (uL)	1	0	1	1	1	0	1	0	1	0
FITC-Not dilute-2mM(uL)	0	0	0	0	0	0	99	99	49.5	49.5
FITC-dilute-100uM (uL)	0	0	0	0	99	99	0	0	0	0
SRB-not diluted -50mM (uL)	0	0	99	99	0	0	0	0	49.5	49.5

Vesicles Containing no Dye (uL)	99	99	0	0	0	0	0	0	0	0
X-Triton (uL)	0	1	0	0	0	1	0	1	0	1
Total Volume (uL)	100	100	100	100	100	100	100	100	100	100

Preparation of Vesicle + Triton Solutions (Dilute):

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Buffer (uL)	1	0	1	0	1	0	1	0	1	0
FITC-Not dilute-2mM(uL)	0	0	0	0	0	0	0	0	99	99
SRB-diluted -10mM (uL)	0	0	0	0	99	99	0	0	0	0
SRB-not diluted -50mM (uL)	0	0	0	0	0	0	99	99	0	0
Vesicles Containing no Dye (uL)	0	0	99	99	0	0	0	0	0	0
Vesicles Containing no Dye-DILUTED (uL)	99	99	0	0	0	0	0	0	0	0
X-Triton (uL)	0	1	0	1	0	1	0	1	0	1
Total Volume (uL)	100	100	100	100	100	100	100	100	100	100

Preparation of Vesicle + Nanopore Solutions:

	G1	G2	G3	G4	G5	G6	G7

Buffer (uL)	50	45.8	45.8	49	45.8	45.8	49
Nanopore Sample 3 (uL)		4.2					
Nanopore Sample 3-WITH CHOLESTEROL(uL)			4.2				
Nanopore Sample 4 (uL)				4.2			
Nanopore Sample 4-WITH CHOLESTEROL(uL)					4.2		
SRB Vesicles (uL)	50	50	50	50	50	50	50
X-Triton (uL)				1			1
Total Volume (uL)	100	100	100	100	100	100	100

Dye Flux (3.2):

Objective: to test nanopore insertion and dye leakage

Materials:

- SRB Dye
- FITC dye
- Buffer
- Eppendorf Tubes
- Pipettes/Pipette Tips
- 96 well plate – white w/ clear bottom
- Plate Reader
- Cholesterol
- SRB Libosomes
- X-Triton

Methods:

Gradients A-H Were Created According to the Table Below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	Blank	non-encapsulated	non-encapsulated	non-encapsulated	R6S3 + Chol +10mM SRB liposome	R6S3 + Chol +10mM SRB liposome	R6S3 + Chol +10mM SRB liposome	R6S9 + Chol +10mM SRB liposome	R6S9 + Chol +10mM SRB liposome	R6S9 + Chol +10mM SRB liposome
B	10uM Cholesterol	10uM Cholesterol	10uM Cholesterol	10mM SRB liposome	10mM SRB liposome	10mM SRB liposome	R6S3 + Chol +10mM SRB liposome +Triton	R6S3 + Chol +10mM SRB liposome +Triton	R6S3 + Chol +10mM SRB liposome +Triton	R6S9 + Chol +10mM SRB liposome +Triton	R6S9 + Chol +10mM SRB liposome +Triton	R6S9 + Chol +10mM SRB liposome +Triton
C	1% Triton	1% Triton	1% Triton	Triton + non-encapsulated	Triton + non-encapsulated	Triton + non-encapsulated	R6S7 + Chol +10mM SRB liposome	R6S7 + Chol +10mM SRB liposome	R6S7 + Chol +10mM SRB liposome	R6S6 + Chol +10mM SRB liposome	R6S6 + Chol +10mM SRB liposome	R6S6 + Chol +10mM SRB liposome
D	1uM SRB	1uM SRB	1uM SRB	Triton + 10mM SRB liposome	Triton + 10mM SRB liposome	Triton + 10mM SRB liposome	R6S7 + Chol +10mM SRB liposome + Triton	R6S7 + Chol +10mM SRB liposome + Triton	R6S7 + Chol +10mM SRB liposome + Triton	R6S6 + Chol +10mM SRB liposome +Triton	R6S6 + Chol +10mM SRB liposome +Triton	R6S6 + Chol +10mM SRB liposome +Triton
E	100uM SRB	100uM SRB	100uM SRB									
F	500uM SRB	500uM SRB	500uM SRB									
G	1mM SRB	1mM SRB	1mM SRB									
H	10mM SRB	10mM SRB	10mM SRB									

SRB Dilutions:

D1-3: 1 µM SRB

- Total Volume 100 µL
- SRB Concentration: 1 µM
- 1 µL of 100 µM SRB
- 99 µL of Buffer

E1-3: 100 µM SRB

- Total Volume 100 µL
- SRB Concentration: 100 µM
- 1 µL of 10 mM SRB
- 99 µL of Buffer

F1-3: 500 µM SRB
- Total Volume 100 µL
- SRB Concentration: 500 µM
- 5 µL of 10 mM SRB
- 95 µL of Buffer

G1-3: 1 mM SRB
- Total Volume 100 µL
- SRB Concentration: 1 mM
- 10 µL of 10 mM SRB
- 90 µL of Buffer

H1-3: 10 mM SRB
- Total Volume 100 µL
- SRB Concentration: 10 mM
- 100 µL of 10 mM SRB

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