

Agarose Gel Electrophoresis and Extraction

Preparing 0.8% agarose gels

1. Set up the gel mold apparatus with the appropriate wells.
2. Weigh out 24 mg of **agarose** in a 250 mL Erlenmeyer flask.
3. Add 30 mL of 1X TAE buffer.
4. Microwave the mixture for 1 minute to dissolve the agarose.
5. Let the solution cool until warm to the touch.
6. Pour solution into mold. Remove any air bubbles. Leave gel to completely solidify before removing combs.

Running gels

1. Ensure that the gel is fully submerged in TAE buffer in Bio-Rad Gel Run apparatus.
2. If there is no dye in the reactions, add 1 μL of 6X DNA loading dye (New England Biolabs) for every 5 μL of reaction. Mix thoroughly by pipetting.
3. Pipette the full volume of each reaction (or the maximum volume the wells can hold) into a well.
4. Mix ~ 2 μL of DNA ladder to 8 μL of deionized water on a parafilm bead into a well and load onto the gel.
5. Set the power supply to 100 mV, 400 mA and constant voltage. Run for 30 minutes.
6. Prepare staining solution of 0.2 $\mu\text{g}/\text{mL}$ EtBr in 1xTAE buffer to completely submerge the gel.
7. After the run submerge the gel in the staining solution for 15 minutes.
8. Place the gel on a UV transilluminator and observe under 300 nm illumination. Bands will appear bright orange on a pale orange background.

Gel Slice and PCR Product Preparation

1. Following electrophoresis, excise DNA band from gel and place gel slice in a 1.5 mL microcentrifuge tube.
2. Add 10 μL Membrane Binding Solution (Promega) per 10 mg of gel slice. Vortex and incubate at 65°C until gel slice is completely dissolved. For PCR amplification product, add an equal volume of Membrane Binding Solution to the PCR amplification
3. Insert SV Minicolumn into Collection Tube.
4. Transfer dissolved gel mixture or prepared PCR product to the Minicolumn assembly. Incubate at room temperature for 1 minute.
5. Centrifuge at $16,000 \times g$ for 1 minute. Discard flowthrough and reinsert Minicolumn into Collection Tube.
6. Add 700 μL Membrane Wash Solution (ethanol added). Centrifuge at $16,000 \times g$ for 1 minute. Discard flowthrough and reinsert Minicolumn into Collection Tube.
7. Repeat Step 4 with 500 μL Membrane Wash Solution. Centrifuge at $16,000 \times g$ for 5 minutes.
8. Empty the Collection Tube and re-centrifuge the column assembly for 1 minute with the microcentrifuge lid open (or off) to allow evaporation of any residual ethanol.
9. Carefully transfer Minicolumn to a clean 1.5 mL microcentrifuge tube.
10. Add 50 μL of Nuclease-Free Water to the Minicolumn. Incubate at room temperature for 1 minute. Centrifuge at $16,000 \times g$ for 1 minute.
11. Discard Minicolumn and store DNA at 4°C or -20°C .

Restriction Enzyme Double Digestion For BioBrick

Set up reaction as follows:	
COMPONENT	50 μl REACTION
DNA	1 μg

10X NEBuffer 2.1	5 µl (1X)
EcoRI-HF	1.0 µl (or 10 units)
PstI	1.0 µl (or 10 units)
Nuclease-free Water	to 50 µl

Incubate at 37°C for 15 minutes.

Ligation Protocol with T7 DNA Ligase

1. Set up the following reaction in a microcentrifuge tube on ice.

COMPONENT	20 µl REACTION
T7 DNA Ligase Reaction Buffer (2X)*	10 µl
Vector DNA (4 kb)	50 ng (0.020 pmol)
Insert DNA (1 kb)	37.5 ng (0.060 pmol)
Nuclease-free water	to 20 µl
T7 DNA Ligase	1 µl

2. Gently mix the reaction by pipetting up and down and microfuge briefly.
3. Incubate at room temperature (25°C) for 15-30 minutes.
4. Chill on ice and transform 1-5 µl of the reaction into 50 µl competent cells.
Alternatively, store at -20°C.
5. Do not heat inactivate – Heat inactivation dramatically reduces transformation efficiency.

DNA Purification by Centrifugation (Promega PureYield)

Prepare Lysate

1. Add 600µl of bacterial culture to a 1.5 mL microcentrifuge tube.

Note: For higher yields and purity use the alternative protocol below to harvest and process up to 3 mL of bacterial culture.

2. Add 100 µL of Cell Lysis Buffer (Blue), and mix by inverting the tube 6 times.

3. Add 350 μL of cold (4–8°C) Neutralization Solution, and mix thoroughly by inverting.
4. Centrifuge at maximum speed in a microcentrifuge for 3 minutes.
5. Transfer the supernatant (~900 μL) to a PureYield™ Minicolumn without disturbing the cell debris pellet.
6. Place the minicolumn into a Collection Tube, and centrifuge at maximum speed in a microcentrifuge for 15 seconds.
7. Discard the flowthrough, and place the minicolumn into the same Collection Tube.

Wash

8. Add 200 μL of Endotoxin Removal Wash (ERB) to the minicolumn. Centrifuge at maximum speed in a microcentrifuge for 15 seconds.
9. Add 400 μL of Column Wash Solution (CWC) to the minicolumn. Centrifuge at maximum speed in a microcentrifuge for 30 seconds.

Elute

10. Transfer the minicolumn to a clean 1.5 mL microcentrifuge tube, then add 30 μL of Elution Buffer or nuclease-free water directly to the minicolumn matrix. Let stand for 1 minute at room temperature.
11. Centrifuge for 15 seconds to elute the plasmid DNA. Cap the microcentrifuge tube, and store eluted plasmid DNA at –20°C.

Alternative Protocol for Larger Culture Volumes

1. Centrifuge 1.5 mL of bacterial culture for 30 seconds at maximum speed in a microcentrifuge. Discard the supernatant.
2. Add an additional 1.5ml of bacterial culture to the same tube and repeat Step 1.
3. Add 600 μL of TE buffer or water to the cell pellet, and resuspend completely.
4. Proceed to Step 2 of the standard protocol above.

DNA Purification by Centrifugation (Wizard® SV Gel and PCR Clean-Up System)

Gel Slice and PCR Product Preparation

A. Dissolving the Gel Slice

1. Following electrophoresis, excise DNA band from gel and place gel slice in a 1.5ml microcentrifuge tube.
2. Add 10 μ l Membrane Binding Solution per 10 mg of gel slice. Vortex and incubate at 50–65°C until gel slice is completely dissolved.

B. Processing PCR Amplifications

1. Add an equal volume of Membrane Binding Solution to the PCR amplification.

Binding of DNA

1. Insert SV Minicolumn into Collection Tube.
2. Transfer dissolved gel mixture or prepared PCR product to the Minicolumn assembly. Incubate at room temperature for 1 minute.
3. Centrifuge at 16,000 \times g for 1 minute. Discard flowthrough and reinsert Minicolumn into Collection Tube.

Washing

4. Add 700 μ L Membrane Wash Solution (ethanol added). Centrifuge at 16,000 \times g for 1 minute. Discard flowthrough and reinsert Minicolumn into Collection Tube.
5. Repeat Step 4 with 500 μ L Membrane Wash Solution. Centrifuge at 16,000 \times g for 5 minutes.
6. Empty the Collection Tube and recentrifuge the column assembly for 1 minute with the microcentrifuge lid open (or off) to allow evaporation of any residual ethanol.

Elution

7. Carefully transfer Minicolumn to a clean 1.5 mL microcentrifuge tube.
8. Add 50 μ L of Nuclease-Free Water to the Minicolumn. Incubate at room temperature for 1 minute. Centrifuge at 16,000 \times g for 1 minute.
9. Discard Minicolumn and store DNA at 4°C or –20°C.

Quantifying DNA using the BioTek Cytation 5

1. Wipe the plate with a clean Kimwipe.
2. On the computer, open the Cytation 5 software and choose Nucleic Acid Analysis.
3. Initialize the Nanodrop by adding 2 μ L of ddH₂O on a well and mark as Blank in software.

6. Add 2 μL of your sample to the other wells and press "Measure". Record the concentration, the A260/A280, and the A260/A230 values. Clean the nanodrop by dabbing ddH₂O on a Kimwipe and gently wiping off the sample from the plate.
7. Repeat step 6 for all samples.
8. Close the software. Clean the plate for the next user by dabbing ddH₂O on a Kimwipe and gently wiping.

M13 Titer

1. Inoculate 20 mL of LB with a swatch of *E. coli* ER2738 picked from LB/Tetracycline plates and incubate with shaking at 37°C for 4–8 hours to mid-log phase (OD₆₀₀ of 0.5).
2. While cells are growing, melt Top Agar in microwave and dispense 3 mL into sterile culture tubes, one per expected phage dilution. Maintain tubes at 45-50°C.
3. Pre-warm, for at least one hour, one LB/IPTG/Xgal plate per expected dilution at 37°C until ready for use.
4. Prepare dilutions using 10 to 10⁻³ fold serial dilutions of phage in LB for 1 mL final volume. Suggested dilution ranges: for amplified phage stocks and infected culture supernatants, 10⁸-10¹¹; for unamplified panning eluates, 10¹-10⁴.
5. When the culture in Step 1 reaches mid-log phase, dispense 200 μL into microcentrifuge tubes, one for each phage dilution.
6. To carry out infection, add 10 μL of each phage dilution to each tube and vortex to mix.
7. Transfer the infected cells one infection at a time to culture tubes containing warm Top Agar. Vortex briefly and IMMEDIATELY pour culture onto a pre-warmed LB/IPTG/Xgal plate. Gently tilt and rotate plate to spread top agar evenly.
8. Allow the plates to cool for 5 minutes, invert, and incubate overnight at 37°C.

9. Count plaques on plates that have approximately 100 plaques. Multiply each number by the dilution factor for that plate to get phage titer in plaque forming units (pfu) per volume.

LB Medium

- Per liter: 10 g Bacto-Tryptone, 5 g yeast extract, 5 g NaCl
 - IPTG/Xgal Stock
1. Mix 1.25 g of isopropyl- β -D-thiogalactoside (IPTG) and 1 g of 5-bromo-4-chloro-3-indoyl- β -D-galactoside (Xgal) in 25 mL dimethyl formamide (DMF). Solution can be stored at -20 °C.

LB/ IPTG/Xgal Plates

1. 1 litre of LB medium + 15 g/L agar.
2. Autoclave, cool to <70 °C, add 1 mL IPTG/Xgal stock per litre and pour.
3. Store at 4°C in the dark.

Top Agar

- Per litre:
 - Components of LB Medium
 - 7 g of Bacto-Agar (or electrophoresis grade agarose).
1. Autoclave, dispense into 50 ml aliquots.
 2. Store at room temperature, melt in microwave as needed.

M13 Amplification

1. Grow overnight *E. coli* ER2738 culture.

2. Inoculate a 20 mL culture in a 250 mL Erlenmeyer flask with 200 μ L overnight *E. coli* culture. Add 1 μ L of phage suspension. Shake flask at 37°C, 250 RPM for 4-5 hours.
3. Remove cells by centrifugation at 4500 xg for 10 minutes. Transfer supernatant to a fresh tube. Repeat centrifugation.
4. Transfer top 16 mL of supernatant to a new tube and add 4 mL of 2.5 M NaCl/20% PEG-8000 (w/v). Briefly mix. Precipitate phage for 1 hour at room temperature.
5. Pellet phage by centrifugation at 12,000 xg for 15 minutes at 4°C. Decant supernatant. Resuspend pellet in 1 mL TBS. Transfer to an eppendorf tube. Spin briefly to remove any cell debris.
6. Transfer supernatant to a fresh tube. Add 200 μ L of 2.5 M NaCl/20% PEG-8000. Incubate at room temperature for 1 hour.
7. Centrifuge at 14,000 xg for 10 minutes at 4°C. Discard supernatant. Spin again briefly and remove remaining supernatant with pipette. Resuspend pellet in 200 μ L TBS. For long-term storage at -20°C, add 200 μ L sterile glycerol.

Biopanning of Phage displayed peptide libraries (Ph.D. 12 and C7C libraries) on *Nosema ceranae* spores

Materials:

- Sterile 1X Tris buffered saline+0.5% Tween-20 (TBST)
- Sterile glycine-HCl (pH 2.2)
- Tris HCl (pH 9.1)
- *E.coli* strain ER2738 culture
- *Nosema ceranae* spores
- 1.7 mL eppendorf tubes
- Bleach

- Bunsen burner (A flame source)

Phage titering supplies

- Agar plates with IPTG + X-gal for titering
- Top agar
- Titering tubes
- 1.7 mL eppendorf tubes

Procedure:

1. Mix *Nosema ceranae* spores and phage (10^{11} phages for 10^9 spores) in 1 mL of sterile TBST for 10 minutes at room temperature.
2. Collect spore-phage complexes by centrifugation (12000 xg) for 10 minutes, remove the supernatant afterwards. Save the supernatant in a sterile 1.7 mL eppendorf tube for titering.
3. Wash the phage-spore complex for 5-6 times with 1 mL (each) of ice-cold TBST, with centrifugation of 12000 xg for 5 minutes after every wash.
4. Save the supernatant after each wash in a labelled sterile eppendorf tube for titering.
5. After a final wash, quickly resuspend the pellet in 1 mL of sterile 0.2 mM glycine-HCl (pH 2). Gently mix at room temperature for 5 minutes.
6. Centrifuge at 12000 xg for 5 minutes.
7. The supernatant will have the eluted phage. Quickly after centrifugation add 150 μ L of 1 M Tris-HCl.
 - a. N.B.: Acid treatment to phage **should not exceed 9 minutes**.
8. Titer the supernatants saved from the previous washes and use a small amount from the eluted phage.
9. Amplify the remainder of eluted phage in ER 2738 to obtain a phage stock for the second round of biopanning. Perform 3-4 rounds of biopanning. Extract phage DNA from plaques from the eluted phage of the final round and submit for

Sanger sequencing to determine the region of each genomic DNA encoding the peptide sequence.

Chemical Competent Cells Preparation

1. Inoculate 5 mL of LB with a colony of the transformation candidate strain and let it grow overnight at 37°C at 220 RPM. If the strain carries a plasmid, do not forget to add the respective antibiotics.
2. The next day, inoculate 250 mL of LB medium with 1 ml of the overnight culture and let it grow until it reaches an OD₆₀₀ of 0.6-0.7.
3. Cool the cells on ice for 10 minutes.
4. Centrifuge the cells for 10 minutes at 6000 xg and discard the supernatant.
5. Resuspend the pellet in 10 ml of prechilled 0.1 M CaCl₂.
6. Incubate the suspension for 20 minutes on ice.
7. Centrifuge again for 10 minutes at 6000 g and discard the supernatant.
8. Resuspend the pellet in 5 mL of cold 0.1 M CaCl₂ with 15% Glycerol
9. Freeze 50 µl aliquots of bacteria suspension in liquid nitrogen and store at -80 °C.

Preparation of Electrocompetent E. coli

Day 1

At the end of the day, streak out cells from the original stock in the -80°C onto an SOB (or LB) plate with no antibiotics. Use a sterile needle. Leave to grow overnight at 37°C

Day 2

Prepare SOB media (1.6 L – 3 x 500 mL + 100 mL)

SOB recipe – 32 g of tryptone, 8 g of yeast extract, 0.928 g of NaCl and 0.304 g of KCl. Adjust to pH 7.4-7.5

Autoclave the media, as well as glass bottles for the glycerol solution, 6 centrifuge bottles (half-filled with water) and 3 cans of 1.5 mL Eppendorf tubes.

Note: NEVER use white tubes.

At the end of the day, pick a colony and add it to the 100 mL culture. Grow overnight at 37°C

Day 3

1. Inoculate each of the three 500 mL cultures with 7 mL of bacteria from the 100 mL subculture. Leave to grow in the 37°C shaker for 3.5 hours.
2. Dilute 350 mL of glycerol into 3 L of ddH₂O. Filter sterilize and store at 4°C during the incubation.
3. Store three cans of autoclaved tubes at -20°C.
4. After 3.5 hours, place the three 500 mL cultures on ice for 30 minutes. Turn the centrifuge on and allow it to cool down to 4°C.
5. Spin the cells down at 4000 RCF for 8 minutes using the A-10 rotor.
6. Remove the supernatant and add ~50 mL of chilled glycerol solution to each centrifuge bottle. Shake on ice until the cells are fully resuspended.
7. Top up the glycerol (+ ~200 mL) and spin the cells down again at 4000 RCF for 8 minutes.
8. Remove the supernatant and repeat re-suspension and washing with glycerol again.
9. Get liquid nitrogen.
10. Perform the second wash, remove the supernatant and place the bottles on ice. Gently resuspend the cells in the leGover liquid using transfer pipettes.
11. Aliquot 50 µL of cells into the 1.5 mL tubes. Snap-freeze the tubes in liquid nitrogen and store at -80°C.

Transformation of *E. coli*

1. Get ice. Wipe the bench down with 70% ethanol. Turn on the Bunsen burner.
2. Retrieve 50 μL aliquots of competent cells from -80°C freezer and put on ice to slowly thaw.
3. Place cuvettes on ice to cool.
4. Add 1 μL of plasmid to the competent cells. For reaction transformations, please see the specific reaction for the reaction volume to be transformed.
5. Turn on the electroporator, and use the “bacteria” setting. Press Measurement button to “ms”.
6. Transfer the competent cells (all of it) to the cuvette.
7. Thoroughly dry the outside of the cuvette using a Kimwipe or paper towel.
8. Place cuvette into the holder with metal plates positioned to make contact with the electrodes.
9. Press pulse. Measurement readings should be 5 ms or higher without arcing.
10. Add 900 μL LB with no antibiotic to the cuvette to resuspend the bacteria. Move the resuspended bacteria into a labelled 1.5 mL tube (usually the original comp cells tube)
11. If the plasmid contains an ampicillin resistance gene, proceed to step 12. If not, shake the cells at 37°C for at least an hour.
12. For plasmids, use a sterile inoculation loop, streak out the resuspended bacteria on a plate with the appropriate additives. For reaction transformations, plate 60 μL of the transformed bacteria, add ~5 sterile glass beads to the plate, and gently shake the plate to spread the cells evenly over the surface of agar before dumping out the glass beads. Incubate the plate upside down at 37°C .
13. Turn the Bunsen burner. Store transformed competent cells at 4°C for up to a week.

Protein Expression and Purification in LB Media

1. Pick a single colony from a plate (the fresher the plate, the better) and inoculate an Erlenmeyer flask containing LB media supplemented with the appropriate antibiotics and inducer. Common culture volumes are 50 mL, 150 mL, 200 mL, 250 mL and 500 mL. For volumes larger than 500 mL, we recommend using multiple flasks.
2. Shake overnight at 250 RPM in 37°C. If incubating for one day, ensure that the culture is given at least 24 hours to incubate before proceeding to Step 4. If incubating for two days, move the culture to a 30°C incubator for at least 24 hours following the overnight 37°C incubation.
3. Transfer the cultures into 250 mL centrifuge bottles.
4. Spin at 8000 RPM for 10 minutes at 4°C using rotor A-10. Repeat as necessary to pellet all the cells down.
5. Discard supernatant and add 2 mL of B-PER reagent per gram of cell pellet. Pipette the suspension up and down until it is homogeneous.
6. Incubate 10-15 minutes at room temperature.
7. Centrifuge lysate at 15,000 ×g for 5 minutes to separate soluble proteins from the insoluble proteins.

Buffer Exchange/Protein Concentrator

1. Add 500 µL of Tris-saline Buffer to filter columns of appropriate size for excluding proteins smaller than desired product.
2. Centrifuge at 14,000 xg for 1 minute at 4°C.
3. Discard filtrate and load 500 µL of protein solution to the top of 20K Size Exclusion Column (protein must be bigger than 20 kDa).
4. Centrifuge at 4°C for 10 minutes at 14,000 xg. Discard flow through.
5. Transfer columns to collection tubes; place columns upside down.
6. Centrifuge for 2 minutes at 1,000 xg at 4°C.
7. Add 500 µL of Tris-saline Buffer to the collection tube.

8. Store at 4°C for a maximum of 2 weeks.

Histidine tag purification

1. Add GE Healthcare His GraviTrap on ice for 1 hour before use.
2. Equilibrate column using 10 mL Binding Buffer (20 mM sodium phosphate, 500 mM NaCl, 10 mM imidazole, pH 7.4) by running the buffer through the column.
3. Load protein sample onto the column and run the solution.
4. Wash the column by running 10 mL Wash buffer (20 mM sodium phosphate, 500 mM NaCl, 20 mM imidazole, pH 7.4) to remove unbound proteins from the column.
5. Perform an additional wash with 5 mL Wash Buffer after first wash run is complete.
6. Arrange microcentrifuge tubes below the column for aliquoting elution from the column.
7. Add 3 mL of Elution Buffer (20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4) to column and aliquot 1 mL into each microcentrifuge tube for a total of 3 tubes.

Collecting the Fluorescence and Absorbance Spectra of Isolated Product.

1. Prepare a stock solution of protein extracted in the same solvent system used during the extraction to use for comparison by serial dilution in the solvent.
2. Approximately normalize the concentration of the protein stock solution to the fluorescent standard by diluting the stock solution with more solvent such that the intensity of both colours look the same.
3. Transfer ~100 µL of both extracted and standard solution to a 96-well plate.
4. Perform an absorbance scan from 380 to 630 nm.

5. On the BioTek Cytation 5 in bottomless black NUNC plates, perform an emission scan from 450 to 700 nm (ex: determined from plot of absorbance for overlap) and an excitation scan from 350 to 580 nm (em: 605 nm).

Quantum Yield

Materials

- 10mm quartz cuvette with 4 polished windows
- Positive control and its buffer for baseline and dilutions Reference fluorophore and its buffer for baseline and dilutions Unknown fluorophore and its buffer for baseline and dilutions Syringe and syringe filter
- Appropriately sized eppendorf tubes, conical tubes, micropipettes, pipette tips, etc. for sample prep.

Protocol

1. Filter and/or centrifuge samples immediately before use.
2. Measure a baseline for absorption with appropriate blank buffer (Tris, NaOH etc.). Prepare a relatively concentrated stock sample (FP in 10mM TRIS pH 7.4; Fluorescein in 0.1N NaOH; Rhodamine6G in EtOH; Cresyl Violet in Methanol; 9-aminoacridine in H₂O) and very precisely measure its full absorbance spectrum with 1nm or 0.5nm step size (SBW = 2.0nm).
 - This ideally needs to be done in the same cuvette to be used for fluorescence spectrum measurement - that way, you can measure the absorbance, make the appropriate dilution then move the cuvette to the fluorimeter and measure the emission spectrum. It's best to do this in triplicate, at least (along with the reference samples and any controls).
 - Standard Recommendations:

- 1-aminoanthracene in cyclohexane (QY = 0.61 [Berlman 1971]): BFP, CFP
 - Fluorescein in 0.1M NaOH (QY = 0.85 [Berlman 1971]): GFP, YFP
 - Rhodamine B in EtOH (QY = 0.65 [Kubin 1982]): YFP, OFP
 - Cresyl Violet in EtOH (QY = 0.54 [Magde 1979]): RFP, Far-RFP
 - Berlman, I. B. Handbook of Fluorescence Spectra of Aromatic Molecules. (Academic Press, New York., 1971).
 - Kubin, R. F. & Fletcher, A. N. Fluorescence Quantum Yields of Some Rhodamine Dyes. J Lumin 27, 455-462 (1982).
 - Magde, D., Brannon, J. H., Cremers, T. L. & Olmsted, J. Absolute Luminescence Yield of Cresyl Violet - Standard for the Red. J Phys Chem-Us 83, 696-699 (1979).
3. Prepare a dilute sample solution and carefully measure its excitation spectrum.
- To account for any differences in measurement between the UV-Vis spectrophotometer and fluorimeter (absorbance vs. excitation), **scale the absorption spectrum to the excitation spectrum** to determine the appropriate dilution factor for the peak absorbance reading (Note: If the excitation curve has an unusual shape, try diluting your sample even more as high concentrations will alter the shape of the excitation curve). Since the fluorescence emission measurements rely on the excitation more than the absorbance - if these spectra do not completely overlap - this type of scaling will reduce errors in dilution.
 - When selecting an excitation wavelength, try to select areas that have the smallest change (slope) between wavelengths (the broad part of the absorption shoulder is a good place to start). It's also a good idea to make sure the excitation is in an area where the unknown and standard have matching trends in their absorption curves (i.e. both are increasing with increasing wavelength), as curves with opposite trends will maximize error in dilution prep and emission recordings. Also, to avoid inner-filter effects,

prepare sample dilutions so that the OD ≤ 0.05 at the peak excitation wavelength. The OD reading for the unknown and standard samples should be the same.

4. After you have determined the appropriate dilution factor for your desired OD reading, prepare the sample.
 - For FPs, it is critical to ensure the sample is mixed very well before attempting to record fluorescence emission. The protein does not solubilize in the buffer as quickly as the synthetic standards, and you will get unusually high emission readings if you do not thoroughly mix your sample.
 - The protein also tends to stick to the sides of any containers or pipets (especially plastic) it comes into contact with, so minimizing the number of containers it comes into contact with after measuring your stock solution will also improve the accuracy of emission readings. A good way to do this is to measure your stock solution in the cuvette, directly transfer it to a clean glass bottle or jar (2-3mL size) and directly pipet (from this jar) the protein and buffer into the cuvette for measurements. Mix the sample by pipetting when it is in the cuvette.
5. After your sample is prepped in the cuvette and thoroughly mixed, record the emission spectrum and use the software to calculate its integrated intensity.
 - The excitation wavelength and detection settings for the standard and unknown fluorophores **MUST BE KEPT THE SAME!** This is to ensure that there are no changes in the energy used to excite your fluorophore (ie. Shorter wavelength = higher energy vs Longer wavelength = lower energy etc.).
 - If your excitation cuts a little bit into your emission spectrum, you can 'guess' those values (they should be very close to 0 – avoid selecting wavelengths that significantly overlap with the emission). Do not collect a 'blank' emission spectrum and attempt to subtract this from your

fluorophore's emission spectrum as any scattering in your sample (especially FPs) will significantly increase the peak and introduce inaccuracies.

6. Plug in the **scaled** absorbance values and integrated intensities into the following formula to determine the quantum yield of your unknown:

$$QY_{\text{unknown}} = \frac{(A_{\text{standard}} \cdot AUC_{\text{unknown}})}{(A_{\text{unknown}} \cdot AUC_{\text{standard}})} QY_{\text{standard}}$$

where: A = absorbance

AUC = area under curve (integrated intensity)

QY = quantum yield

If: $A_{\text{standard}} = A_{\text{unknown}}$, then – $QY_{\text{unknown}} = (AUC_{\text{unknown}}/AUC_{\text{standard}}) \cdot QY_{\text{standard}}$

Harvesting and Purification of *Nosema ceranae* spores from Honey Bee Midguts

1. Worker honey bees are collected from the hive and placed in 50 mL Falcon tubes to be chilled on crushed ice for approximately 15 minutes.
2. The worker bees are decapitated with micro-scissors and the alimentary canals removed by pulling the terminal abdominal segment with forceps.
3. The canals are placed onto a sterile petri dish with Type I water.
4. The midguts are separated from hindguts and foreguts using micro-scissors.
5. The midguts were grinded in Type I water using mortar and pestle and filtered through a 40 μm cell strainer (cell strainer can be skipped if sample does not require further filtration).
6. The filtrate is centrifuged at 20,000 $\times g$ and resuspended in a 1 mL volume of Type I water.
7. Spore count was performed under a hemocytometer to determine spore load.

Hemocytometer spore count

1. Prepare hemocytometer and coverslip using clean isopropanol before use.
2. Moisten coverslip with water and affix to hemocytometer and check for Newton's refraction rings under coverslip.
3. Remove the coverslip and wipe off the hemocytometer.
4. Using a pipette take 100 μ L of crushed bee suspension and fill each of the two chambers.
5. Using a microscope, focus on the grid lines of hemocytometer using 40X objective lens magnification (400X final magnification with 10X ocular lens)
6. Using a hand tally counter count the spores in one set of the 16 squares. While counting create a system to count cells they are set within a square or on the right-hand of bottom boundary line.
7. Move the hemocytometer to the next set of 16 corner squares and carry on counting till all 4 sets of 16 corners are counted.
8. Take average cell count from each of the sets of 16 corner squares.
9. Multiply by 10,000 and the dilution factor for the suspension.
10. The final spore count is the spore count obtained from the crushed bee sample.