Bien 155: Biotechnology Lab
Thursday Lab Section 021

Group 1
pET28(b)-CyPet-SUMO1

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Introduction

CyPet
- Cyan fluorescent protein
- derived from Aequorea victoria (also called: crystal jelly)

SUMO1 Gene
- Ubiquitin-like modifier, small
- SUMOylation

Top10 Electrocomp™ E.coli (Invitrogen) bacterial cells
- E.coli is not naturally competent-> electroporation
- Fast: No lacI^{q} gene

BL_{21} cells
- Transforms plasmid DNA into protein expressed bacterial cells
- Isopropyl-β-D-thio-galactoside (IPTG)
- T7 RNA polymerase

[1]**Figure 1:** SUMO1 gene ribbon structure

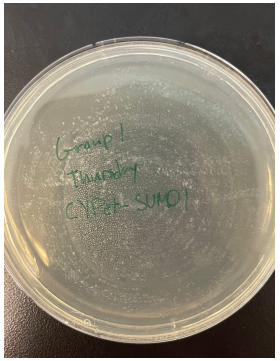


Figure 2: Lab 1 cell culture results

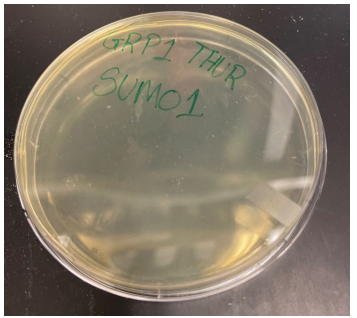


Figure 3: Lab 4 transformation plate results

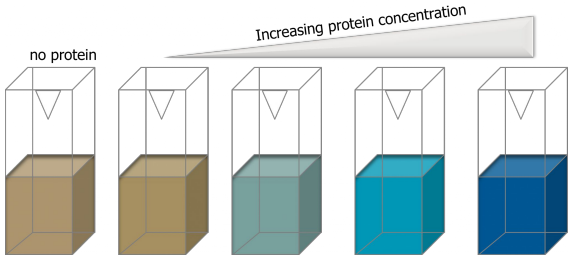
Introduction

Bradford Assay (Coomassie Blue Dye)

- Colorimetric method of protein quantitation (concentration)
- Spectral shift
  - Free dye:
    - Abs max at 470 nm (brown color)
  - Protein bound:
    - Abs max at 595 nm (blue color)
- Coomassie dye ligands
  - Proportional amount to positive charges on protein
  - Bind to basic amino acids (arginine, lysine)

Förster Resonance Energy Transfer (FRET) Assay

- [Donor to acceptor] Non-radiative energy transfer phenomenon
- Emission and excitation spectrum-overlap significantly
  - Fluorescent emission always has a longer wavelength (lower energy state) than excitation



[2]Figure 4: Bradford Assay

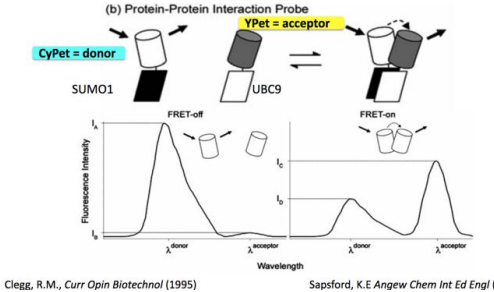


Figure 5: Overview of FRET Assay

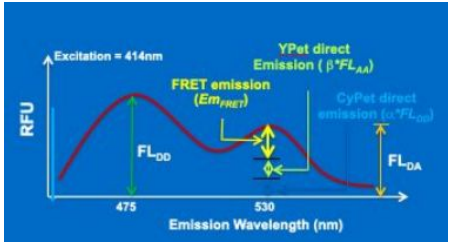
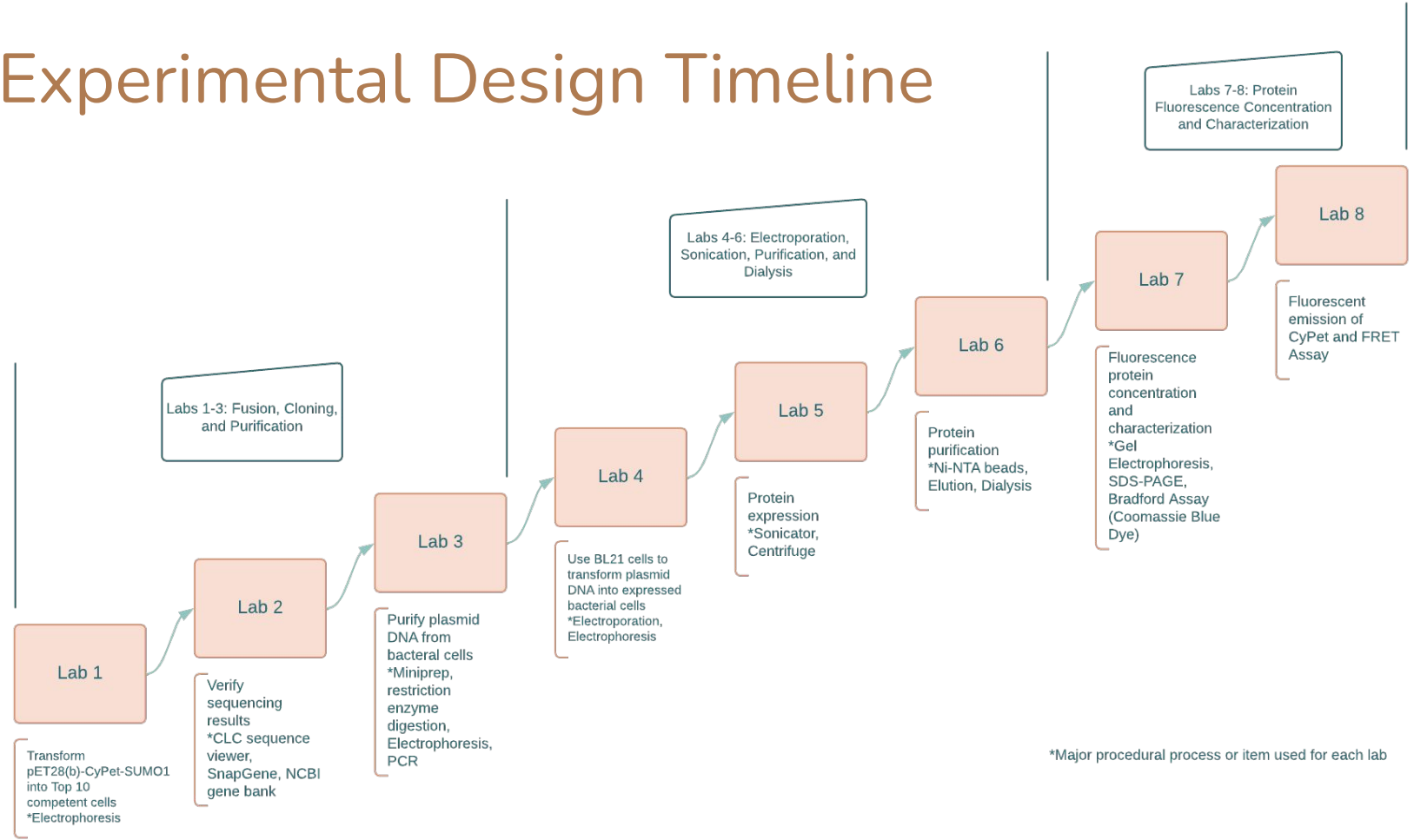


Figure 6: FRET Assay Analysis

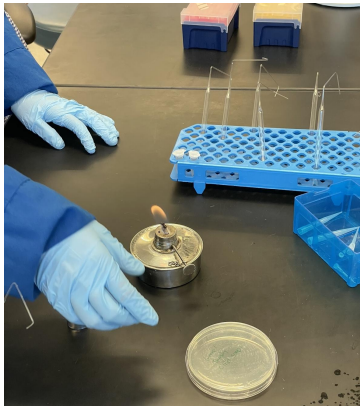
Experimental Design Timeline

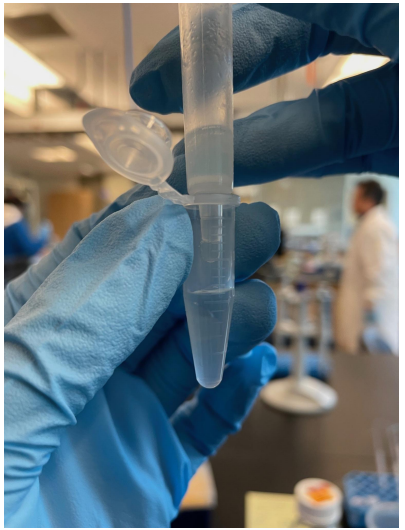


Experimental Procedure

Week 1 -Week 3 CyPet-SUMO1 plasmid DNA preparation

- Mix competent cells with plasmid DNA and perform eletroporation.
- Incubate the cells on LB agar plate overnight then pick up colonies into medium with Kanamycin.
- Lyse bacteria and clear it by centrifuge then the plasmid is ready to purification.
- QIAprep membrane only absorb DNA after washing and elution the high-quality plasmid is made.
- Check the gene clones by digestion and PCR.
- Retrieve sequences of SUMO1 from NCBI and assemble it with the sequence of CyPet.
- Use CLC Sequence Viewer and SnapGene/4peak to verify the sequence.

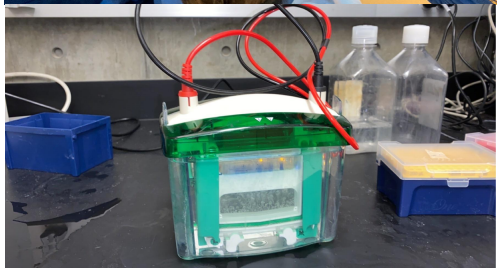




Experimental Procedure

Week 4 -Week 8 **Protein Extraction and determination**

- Perform electroporation with BL21 cells and CyPet-SUMO1 plasmid DNA mixture.
- Incubate the mixture on LB agar plate with kanamycin.
- Lysing cells by sonication 5 sec on and 5 sec off intervals.
- Centrifuge to separate DNA and proteins.
- Pour the target DNA solution into column with washed Ni-NTA beads inside.
- Wash the protein with 3 different buffers.
- Inject the protein into dialysis bag after elute it.
- Put the dialysis bag in PBS overnight.



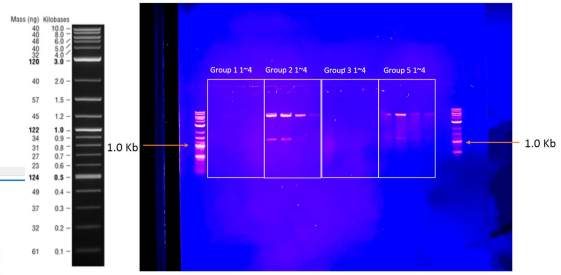
- Prepare BSA standards and make standard curve on laptop.
- Calculate the concentration of our sample and perform electrophoresis with 5 microgram of sample.
- Check the fluorescent emission intensities of CyPet at 475 nm and use the given standard curve to identify the concentration of the fluorescent proteins.
- Compare CyPet-SUMO1 with YPet-Ubc9 by FRET assay.

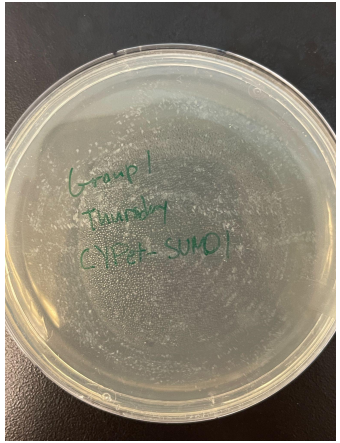
Results

Digestion Checking:

Cuts DNA with restriction enzymes

Digest checking 1

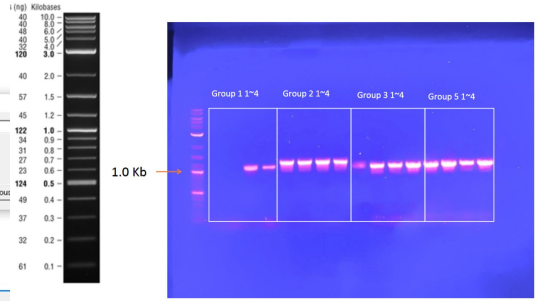




Cell Culture

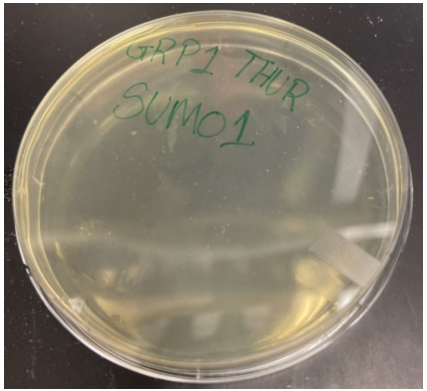
Mutation in DNA sequence

PCR checking 1

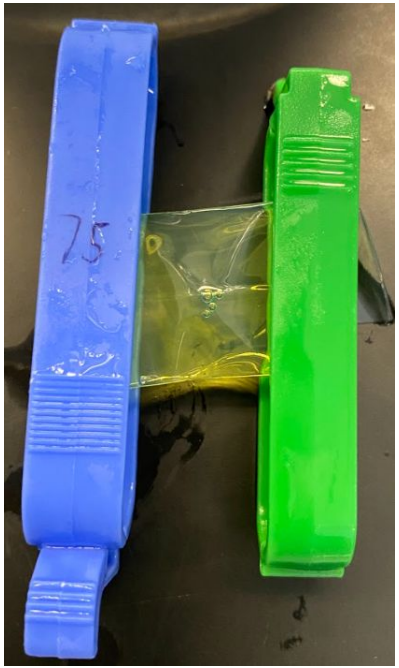


PCR Checking:
BP band in the PCR run

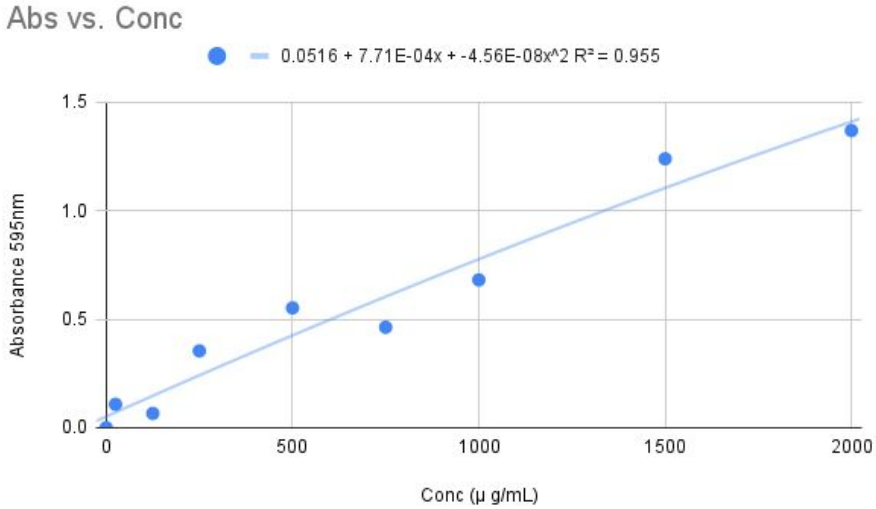
Results



Week 4: LB Agar Plate after incubation



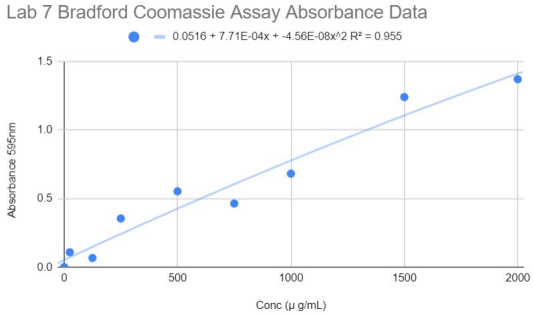
Week 6: Protein transferred into dialysis bag for being dialyzed overnight

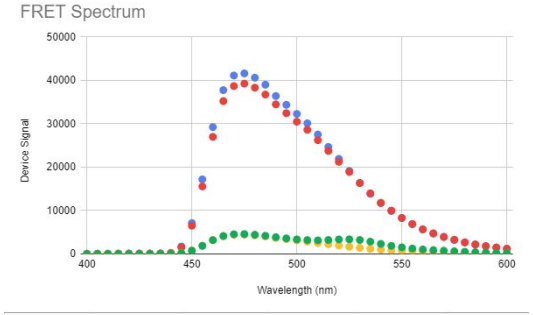


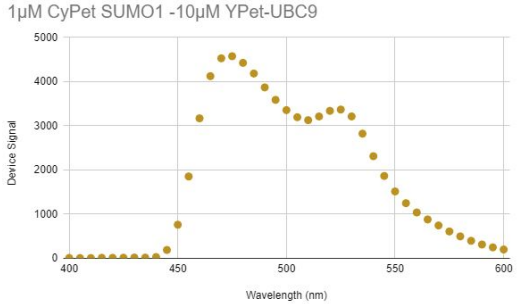
BSA standard to measure concentration of unknown protein and total protein concentration was 1932 µg/mL

Results

Week 8: Analysis of produced proteins via FRET Assay







$$Fluorescence\ Protein\ Concentration = \frac{63979.469}{4370.9} = 14.63\mu M$$

$$Bradford\ Essay\ Concentration\ Conversion: \frac{1.932 g/L}{38000\ g/mol} = 50.84\mu M$$

$$Protein\ Purity\ Ratio = \frac{14.63\mu M}{50.84\mu M}\ x\ 100 = 29.1\%$$

$$FRET\ Signal = \frac{Emission\ 530/414}{Emission\ 475/414} = \frac{3211.78}{4574.59} = .70209$$

Conclusion

Labs 1-3

- Cloned CyPet-SUMO1 via E.Coli transformation and selection
- Used sequenced data to identify errors in sequence as device error or genuine mutations
- Performed Agarose Gel Electrophoresis to visualize DNA bands and verify cloning progress

Labs 4-6

- Transformed CyPet-SUMO1 plasmid DNA into Bl21(DE3) E.Coli cells for protein expression
- Extracted recombinant proteins and utilized Ni-His tag purification

Labs 7-8

- Quantified yield using Bradford Assay and FRET
- Obtained purity ratio (29.1%) and FRET signal (.70207)

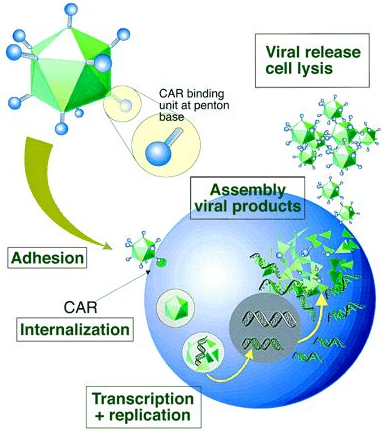
Future Directions

FRET:

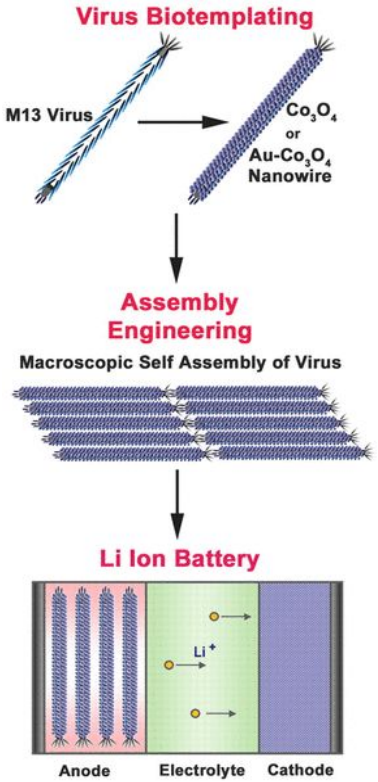
- Potential use in measuring and building protein charge transfer systems
- Analytical method in construction of large synthetic protein structures

Post translational modification systems:

- Potential use in Adenoviral Vector and Lentiviral vector manufacturing
    - Emergence of viral therapeutics



Source: Signa Laboratories



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