# BIEN155 Presentation

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# Introduction

**Purpose:** Explore different ways to test and analyze protein expression and purification

**Proteins: Ypet-Ubc9** and CyPet-SUMO1

- These  two proteins play an essential role in the SUMOylation process.
- Yellow and Cyan Fluorescent Proteins

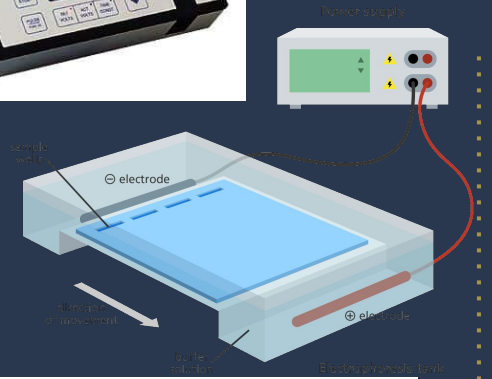
# Experimental Design

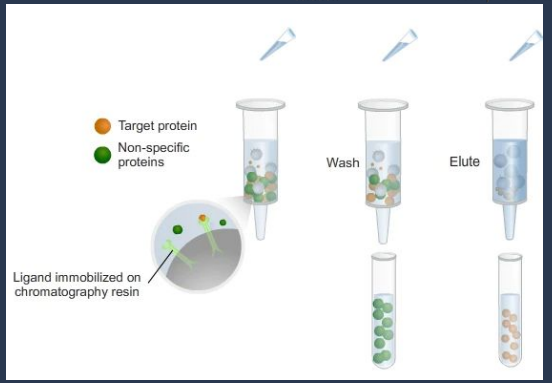
**Electroporation:** Used to increase the permeability of the E. coli cells so we could transform our plasmid into the cells

**Affinity Chromatography:** Used to purify the protein by having it bind to the beads and washing everything else off

**Gel Electrophoresis**: Used to separate molecules by size and charge in order to verify if a protein is being expressed

**FRET (Förster Resonance Energy Transfer)**: Characterize the spectral properties of the donor and acceptor in protein-protein interactions

# Experimental Techniques

***Lab 1-3:* Molecular Cloning Techniques for Protein Synthesis**

- *E. coli* transformation via electroporation
- Plasmid Purification
- DNA digestion with restriction enzymes
- PCR
- Gel Electrophoresis
- DNA sequencing and analysis

***Lab 4-6:* Protein Expression and Purification**

- Inducible protein expression
- Protein purification via affinity-based chromatography

***Lab 7-8:* Understand protein quantification and characterization, determine protein-protein interactions**

- Bradford Assay
- SDS-PAGE
- FRET Assay

# Experimental Procedure

Lab 1-3: cDNA construct qualification and validation

(1) Bacterial transformation and antibiotic selection
(2) DNA qualification via PCR and Endonuclease
(3) Validation of DNA Sequence

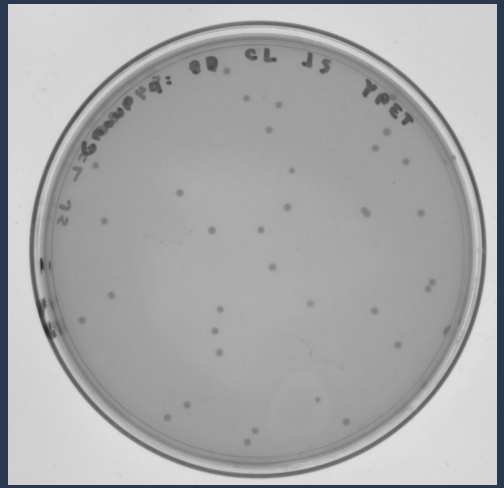
Lab 4-6: Expression, isolation, and purification of genes

(1) Controlled expression of tagged genes CyPet-SUMO1 or YPet-Ubc9
(2) Perform IMAC via Nickel-NTA and 6xHis tag

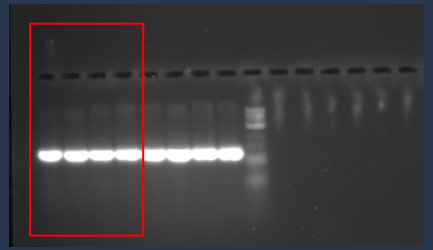
Lab 7-8: Validation of SUMO1-Ubc9 Interaction utilizing FRET principles

(3) Qualify purified proteins through SDS-PAGE gel and Coomassie Staining
(4) Characterization of fluorescent proteins
(5) Measure FRET signal and resolve FRET signal through fluorescent data

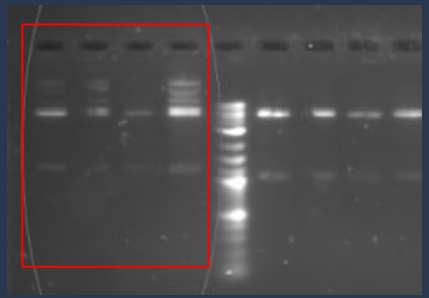
# Experimental Results Lab 1-3



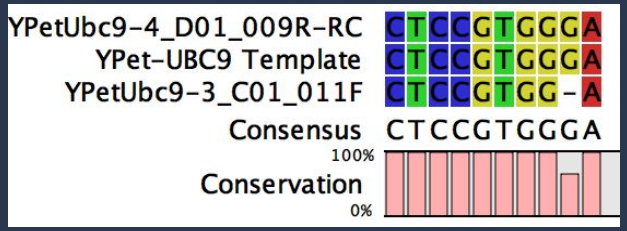
TOP10 *E.Coli* Transformation with Kanamycin Resistance

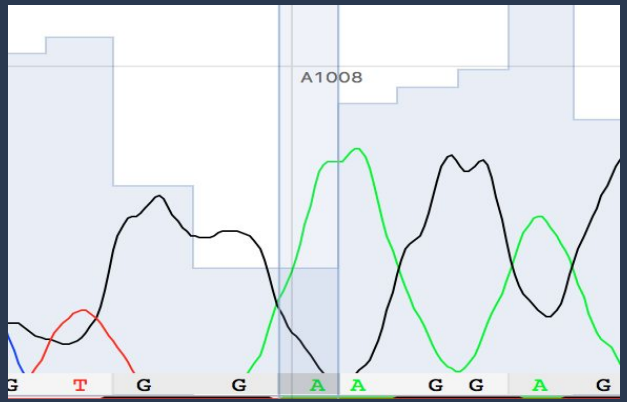


Electrophoresis results for gene check by PCR showing a band at the 1.2 kb mark



Electrophoresis results for gene check by restriction enzyme digestion showing a band at the 1.2 kb mark

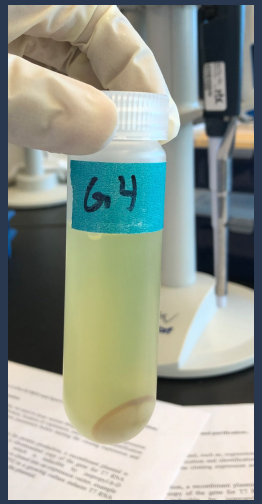



CLC sequence viewer (top) and 4Peaks (bottom) used to compare our sequence with literature template, which showed a machine error

# Experimental Results Lab 4-6


Bacterial culture of $BL_{21}(DE_3)$ *E. Coli*

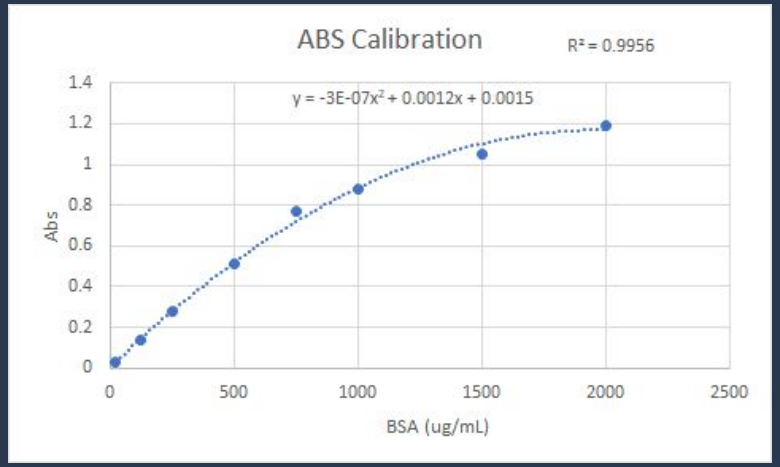



Supernatant and the pellet containing unwanted cellular debris, etc. (After sonication and centrifugation)

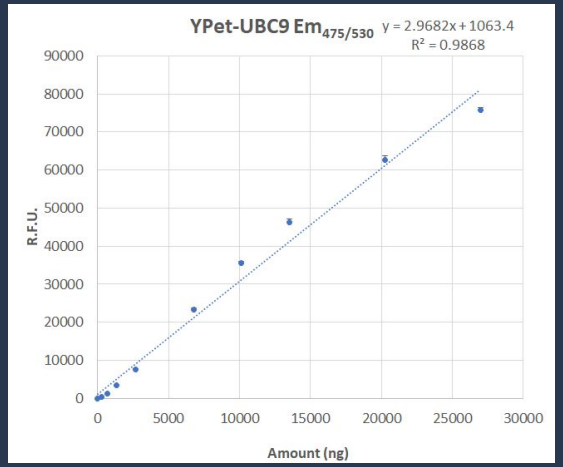

Supernatant containing protein of interest

Isolated protein prepared for dialysis

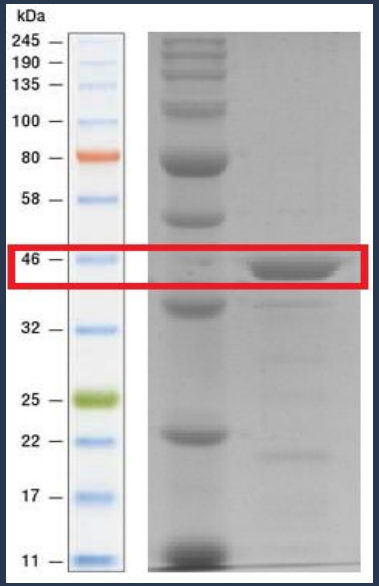
# Experimental Results Lab 7-8




Calibration curve of BSA standards used to find concentration of our unknown protein - 11029 ng/µL




Fluorescence Standard Curve used to calculate the fluorescence concentration of our protein - 13772 ng/µL

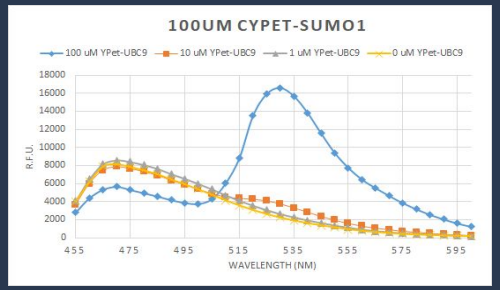


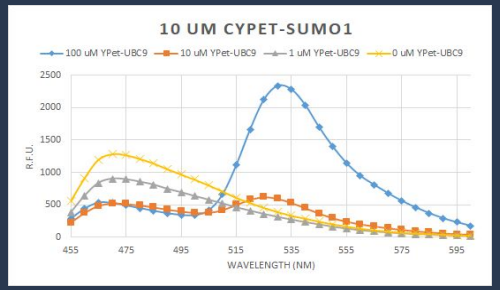

SDS-PAGE results showing a band at 45 kDa indicating the presence of YPet

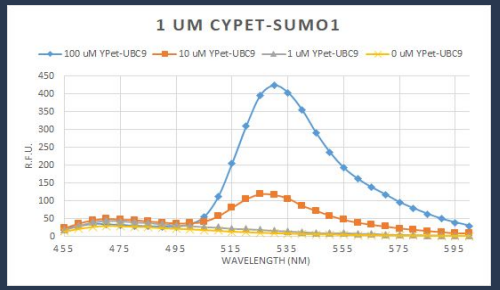
$$\frac{Fluorescence\ Concentration}{Total\ Protein\ Concentration\ from\ Bradford\ Assay}$$

$$\frac{13772.6978\ \frac{ng}{\mu L}}{11029\ \frac{ng}{\mu L}} \times 100\% = 124.88\%$$

# Experimental Results Lab 7-8




R.F.U. of mixtures containing 100 μM, 10 μM, and 1 μM of CyPet-SUMO1 with differing concentrations of YPet-Ubc9 at wavelengths from 455 nm to 600 nm

| CyPet-SUMO1 | YPet-UBC9 | FRET Ratio |
|---|---|---|
| 100 uM | 100 uM | 3.127402 |
| 100 uM | 10 uM | 0.490876 |
| 100 uM | 1 uM | 0.312801 |
| 100 uM | 0 uM | 0.283376 |

| CyPet-SUMO1 | YPet-UBC9 | FRET Ratio |
|---|---|---|
| 10 uM | 100 uM | 4.7392 |
| 10 uM | 10 uM | 1.15659 |
| 10 uM | 1 uM | 0.351589 |
| 10 uM | 0 uM | 0.309615 |

| CyPet-SUMO1 | YPet-UBC9 | FRET Ratio |
|---|---|---|
| 1 uM | 100 uM | 13.04637 |
| 1 uM | 10 uM | 2.450828 |
| 1 uM | 1 uM | 0.384866 |
| 1 uM | 0 uM | 0.32944 |

FRET Ratios (calculated by dividing the peak emission value at 530 nm by peak emission value at 475 nm)

# Conclusion & Future Directions

## Conclusion

- The SUMOylation process is a post-translational modification involved in many cellular functions. Many diseases have abnormal expression of proteins involved in SUMOylation and use SUMOylation for disease progression.
- Various methods, including FRET technology are used to elucidate the noncovalent interactions between the two proteins from the SUMOylation process, SUMO1 and UBC9

## Future Directions

- The expression and purification methods from this lab can be used for:
  - Future cancer research and technologies
  - Drug delivery techniques
  - Gene therapies