The iGEM Experience: Creating DIY, Low-cost Biology for the Masses

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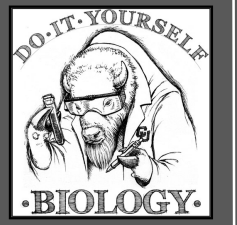
Introduction

The goal of the CU Boulder iGEM team was to help make synthetic biology accessible and affordable by creating novel Do It Yourself components and methods such as:



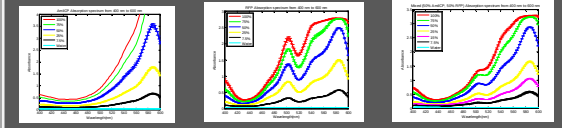
DIY

- Methods for protein purification:
  - Repeats in Toxin (RTX) tags
  - Elastin Like Protein (ELP) tags
  - Gel purification systems
- Biobrick and Freiburg compatible enzymes:
  - EcoRI
  - M.EcoRI
- Methods for recycling of Columns

Protein Purification with gel

We used a 0.5% agarose gel to perform protein purification. The separation was run at 180V, and then purified at 100V using folded paper. To find the best wavelength to use to calculate yield, and assess purity, along with what range the instrument works over, we generated spectrums from 400 nm to 600 nm for pure RFP, pure AmilCP and a 50:50 mixture between them

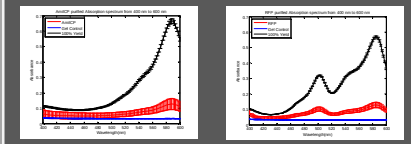
Figure 1



Based on these graphs the best way to measure RFP is at an absorbance of 502 nm since that is well clear of the AmilCP absorption at 588 nm. RFP has a higher absorbance at 584 nm and fluoresces at 607 nm. This is far too close to the AmilCP absorbance to tell them apart.

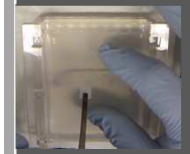
The figures below are the resulting spectrums for recovered RFP and AmilCP from an originally mixed sample. Based on the graphs it is obvious that the AmilCP only contains AmilCP and the same is true of RFP only containing RFP.

Figure 2



Yield	
RFP	34% +/- 5%
AmilCP	19% +/- 6%

Gel Purification of DNA



This method of DNA purification is much simpler, cheaper and faster than using a gel purification kit. Run a gel like you normally would to get separation.

Simply cut the gel right below the band, place a piece of filter paper backed by dialysis tubing in the cut, run for another 5 minutes and your DNA is now in the filter paper and tubing. To get it out simply place a small eppendorf tube with a hole in the bottom inside a larger eppendorf tube and centrifuge for 30 seconds. You now have DNA to use in the large tube and are finished.

Protein Purification Tags - Repeats in Toxin (RTX)

RTX is a structural motif consisting of a repeating set of amino acids that allows for precipitation in the presence of calcium. The RTX protein is intrinsically disordered under physiological conditions but undergoes a conformation change upon binding to calcium, otherwise known as ligand-induced disorder-to-order transition, which results in precipitation from solution. Our goal was to take advantage of this characteristic for the purpose of purifying proteins.

Figure 3A: **Precipitation of RTX with increasing concentrations of calcium**

Experimental results show that calcium concentrations of 10 mM or above result in effective precipitation and is capable of a high level of purification of a fused protein (in this case, GFP) without disrupting protein function (green fluorescence is present). Additionally, calcium concentrations below ~1 mM do not produce a useful amount of precipitate for the purpose of protein purification.

Figure 3B:
Lane 1: Clarified cell lysate
Lane 2: Sup. + 0mM CaCl2
Lane 3: Pellet + 0mM CaCl2
Lane 4: Sup. + 0.1mM CaCl2
Lane 5: Pellet + 0.1mM CaCl2
Lane 6: Sup. + 1mM CaCl2
Lane 7: Pellet + 1mM CaCl2
Lane 8: Sup. + 10mM CaCl2
Lane 9: Pellet + 10mM CaCl2
Lane 10: Sup. + 100mM CaCl2
Lane 11: Pellet + 100mM CaCl2

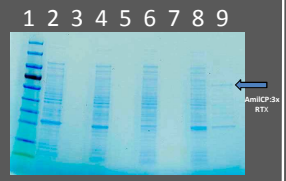


Figure 3C:
Lane 1: Ladder
Lane 2: 0xRTX Lysate
Lane 3: 0xRTX Pellet + 100mM CaCl2
Lane 4: 1xRTX Lysate
Lane 5: 1xRTX Pellet + 100mM CaCl2
Lane 6: 2xRTX Lysate
Lane 7: 2xRTX Pellet + 100mM CaCl2
Lane 8: 3xRTX Lysate
Lane 9: 3xRTX Pellet + 100mM CaCl2

Elastin Like Proteins (ELP)

Elastin-like proteins are oligomeric repeats of Val-Pro-Gly-Xaa-Gly (Xaa being any amino acid with the exception of proline). ELPs undergo reversible, inverse phase transitions at a transition temperature or after the addition of NaCl allowing for an inexpensive precipitation based purification system. Ideally, iGEM teams could create fusion proteins consisting of a protein of interest and an ELP tag that could be easily precipitated and re-solubilized allowing for inexpensive purification of proteins. We designed and submitted a Biobrick coding for an ELP that should precipitate at around 40 degrees Celsius and an NaCl concentration of about .2M. If successful, this method should only require a heat source and NaCl for efficient purification of tagged proteins.

Production of Biobrick compatible Restriction Enzymes: EcoRI, M.EcoRI

The biobrick standard allows researchers to cut and paste DNA elements using only four restriction enzymes. Our goal was to produce one of these enzymes, EcoRI, in addition to its methylase M.EcoRI. We created a Freiburg compatible EcoRI enzyme but our EcoRI methylase sequence contained a premature stop codon, potentially rendering the enzyme non-functional. However, we were able to reverse this through site directed mutagenesis and have confirmed its functionality.

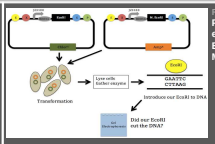


Figure 5: **Plan for expression of EcoRI and M.EcoRI:**

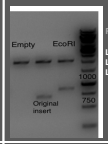


Figure 4: **EcoRI plasmid verification:**

Lane1: Empty vector
Lane2: Vector containing original insert
Lane3: Vector containing EcoRI sequence

Figure 6: **Gel using E. coli lysate expressing functional methylase.**

Lane 2: Background DNA from the lysate
Lane 3: Expected band sizes of the plasmid when cut twice. Lane 4: Tested purification methods, gel purifying the lysate and spiking in the plasmid.

Band at just under 3000bp indicates that the methylase in the whole cell lysate sufficient protects the plasmid from cleavage by EcoRI.

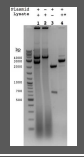
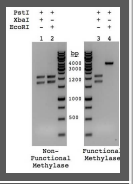


Figure 7: **The non-functional methylase compared to the functional methylase.**



DIY Cost Comparison

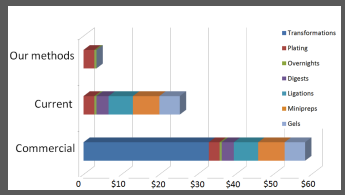


Figure 6: **Cost comparison between our developed methods and traditional standards**

- **Protein Purification Methods**
  - Gel purification using color tags
  - RTX – calcium precipitation
  - ELP – heat induced precipitation
- **DNA Purification Methods**
  - Homemade miniprep systems w/ mini-columns
  - Re-cycling commercial mini-columns
  - Gel purification using filter paper and dialysis tubing
- **Restriction Enzymes**
  - EcoRI – REase and MTase to produce your own EcoRI
- **Other Investigations**
  - SpeI, PstI, and ApoI restriction enzymes
  - Gel recycling

Conclusion & Outreach

One of the main focuses of our team was to make synthetic biology easier and more accessible to future iGEM teams. We had a lot of difficulty getting our experiments to work at the beginning of the summer, and the results from a survey of this year's iGEM teams suggested that others had experienced similar struggles. To accomplish this, we sent iGEM a proposal to initiate some changes that would allow teams to submit protocols to the registry as if they were parts, and to include this as an option for completing basic medal requirements.

Figure 7: **Compiled responses from iGEM teams concerning the protocol survey**

- DIY biology is critical for the future. Researchers who take advantage of DIY biology can create low cost solutions for day to day lab work..
- We formulated a plan that will help ensure future iGEM teams have reliable protocols, so that they will be able focus their time and effort toward developing the innovative projects that make the iGEM competition so special.

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