CASE STUDY: University of Chicago's Staley Lab

THE UNIVERSITY OF CHICAGO

CL7/Im7 Purification System Drives Efficiency and Cost Savings for Researchers at University of Chicago's Staley Lab

OVERVIEW

Pre-mRNA splicing occurs in two steps and results in the joining of coding regions, known as exons, and the removal of non-coding regions, known as introns. Splicing is facilitated by a large ribonucleoprotein machine known as the spliceosome. The spliceosome contains 5 snRNAs and over 100 proteins that undergo dramatic changes and rearrangements throughout the splicing cycle.

The Staley Lab, located at the University of Chicago, uses a combination of molecular genetics, structural biology, transcriptomics and biochemistry to unlock the complex functions of the spliceosome. Using budding yeast and mammalian cells to study pre-mRNA splicing, Staley Lab researchers are developing a better understanding of the mechanism, fidelity and transcriptome-wide effects that splicing can have on the regulation of gene expression.

THE CHALLENGE

Widely used in academic, life science and pharmaceutical research, protein purification remains a cumbersome and inefficient process. Common tag-based purification methods, which have been essentially unchanged for decades and often require multiple purification steps, take several days to run and lead to loss of time and end-product. The result can be a research bottleneck, especially for challenging or complex proteins.

Cody Hernandez, Staley Lab researcher and Ph.D. candidate, is a graduate student studying RNA splicing using in vitro and in vivo approaches. He specifically studies the mechanism of helicases for ensuring splice site fidelity. Hernandez has always been on the lookout for different tags and strategies to facilitate simpler, more efficient protein purification to better support his lab's research.

"I've done a lot of purifications in my time as an undergraduate and graduate student, and one thing that really bothered me was having to do multiple rounds of purification just to get the protein to be 80 percent pure," explained Hernandez. "Sometimes it worked and sometimes it didn't, so it was quite frustrating."

Hernandez's purifications range from purified proteins to purified spliceosome complexes. Traditionally, the purification usually involves tandem affinity purification (TAP) through a protein-A tag and calmodulin binding protein tag. The Staley lab has used a number of conventional protocols, including His-tag, one of the most widely used tags for recombinant protein expression and purification. His-tag works reasonably well for many of Staley's research applications, with minimal loss of target protein. However, a major disadvantage is that high-affinity binding leads to nonspecific binding of proteins to the IMAC column. The lab has also used glycerol gradient ultracentrifugation – which takes 18 to 36 hours to run – as well as FLAG, HA, and GST tag systems.

"There are quite a few tools available, and I have tried probably every mainstream tag out there, but to no avail," said Hernandez, who continued searching. "Most researchers have figured out their protein purification routines and accept that it's a long process, but one that is reliable and eventually gets the job done."

"I just wanted something higher throughput, reproducible, and time efficient."

- Cody Hernandez, Staley Lab

THE SOLUTION

As Hernandez continued his search for a protein purification process that could better meet the unique needs of his nucleic acid lab, he discovered a Proceedings of the National Academy of Sciences (PNAS) article titled Efficient, ultra-high-affinity chromatography in a one-step purification of complex proteins.

"I ran across something online about a single-step affinity tag system, and that led me to the PNAS paper," relayed Hernandez. "I read through the article and was struck by three things, the binding affinity, reusability and cost efficiency. It seemed too good to be true, but I've since used it for different purifications, and it's the real deal."

The PNAS paper details the TriAltus ultra-high-affinity CL7/Im7 purification system. CL7/Im7 is a novel affinity tag system that allows for one-step, high-affinity purification of a range of challenging biological molecules, including eukaryotic, membrane, toxic and DNA/RNA-binding proteins and complexes. The benefits for Hernandez and his lab's research efforts include:

- Single-step purification
- Quality, purity and yield
- Reproducibility
- Recyclability
- Binding capacity
- Salt stability

> ***"It removes having to be good at biochemistry to do biochemistry."***
>
> **- Cody Hernandez, Staley Lab**

For Hernandez and others at Staley Lab, the biggest difference between working with the CL7 system, and working with any other system to date, is its simplicity and time savings.

"I can throw my lysate over a column, hit it with a few high-salt washes to remove a lot of nonspecific molecules (RNA's or other proteins) and still have my target protein on the column – it's a huge advantage," Hernandez emphasized. "Furthermore, using an FPLC column, I have the ability to purify a protein in a couple of hours. The usability is great. It removes having to be good at biochemistry to do biochemistry."

THE RESULT

Following several successful runs, Hernandez is working to convert everyone to the new CL7 tag system. For Hernandez and an increasing number of his colleagues, the CL7/Im7 method is proving to be a more efficient, rapid and less costly approach to purifications (Figure 1). Hernandez has purified three proteins, two helicases and one mutant helicase using the CL7 system. He is currently purifying more mutants, and another lab on the floor is purifying several other proteins.

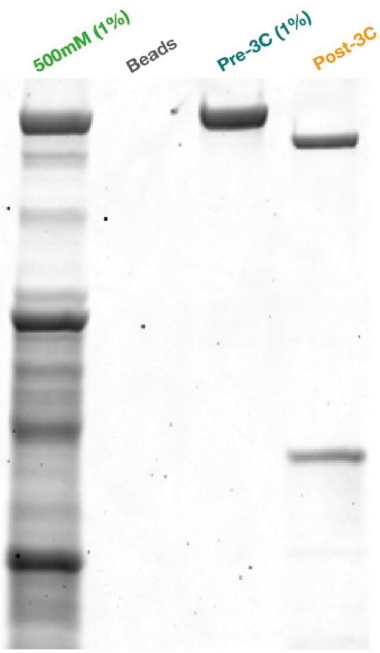

Figure 1. *Helicase X. The protein is larger than 150 kDa, meaning that it is highly susceptible to protease cleavage during expression and lysis. Since cleaved products can still bind the column, the lysate is put over Ni-NTA for an initial purification step and then eluted stepwise with increasing amounts of imidazole.*

Lanes:
500mM – Contains all of the protein product and is then flowed over an Im7 column. Note: high amounts of imidazole do not seem to interfere with protein binding to Im7 column.
Beads – Clean lane showing that everything is eluted from Ni-NTA beads after the 500 mM elution. Resin was boiled and supernatant removed and run as "Beads" lane.
Pre-3C – Total protein bound to 1% of the beads. 20 uL/2000 uL resin was removed for analysis to determine purity by R3C cleavage overnight (see Post-3C).
Post-3C – Eluted protein after overnight cleavage.

"There has been some resistance, but much of that simply comes from having to deal with the unknowns of a new system and a comfort level with current purification methods, even if they're only 80 percent effective," said Hernandez. "Converting an entire floor with four labs is a challenge, but all I have to do is demonstrate the powerful advantages of the CL7 system to my colleagues. The amount of time it takes to purify these proteins is minimal – just a few hours – so a lot of us see this system as the way forward in protein purification strategies."

Although each lab is generally in charge of its own ordering, switching to the CL7/Im7 system will bring cost savings to the lab, including a low cost-per-item and reusability of the columns. Hernandez estimates he is saving about 25% on each protein prep.

"I use about a quarter of the amount of culture for the preps, which means I'm using less reagents for prep and also not doing a glycerol gradient anymore, so that really speeds things up," Hernandez noted. "The cost-per-item is amazing. I've reused the resin multiple times and haven't seen any loss of binding."

Benefits to the CL7/Im7 system

Several hours vs. several days of work

Low resin cost-per-item

Save ~25% cost on each prep

Resin reusability without loss of binding

Reproducible results

Hernandez has been particularly impressed by the system's reproducibility using the same column. After running preps on the exact same proteins, the CL7 tag system results in gels that look identical nearly every single time. The Im7 resin is capable of up to 100 reactivations, and TriAltus has recently more than doubled the binding capacity from 15 – 20 mg/ml to 35 – 40 mg/ml of CL7-tagged protein.

The TriAltus CL7/Im7 system continues to be validated by research groups around the globe as it is applied to new areas of study.

About TriAltus Bioscience

TriAltus Bioscience, LLC (www.trialtusbioscience.com) provides life scientists with tools for production and purification of genetically engineered proteins. Our novel CL7/Im7 affinity tag system enables high-yield, high-purity and high-activity (HHH) protein purification with 97 to 100 percent purity in a single step.

TriAltus offers a limited free sample program for researchers to validate the CL7/Im7 system in their own labs. Learn more about how the CL-7 affinity tag can transform your institution's protein-based research by making it easier and more affordable to obtain high quality proteins. Visit our website at www.trialtusbioscience.com, call 205-453-8242 or email us at info@trialtusbioscience.com.

About Cody Hernandez

Cody Anthony Hernandez is a Ph.D. candidate in the University of Chicago Department of Molecular Genetics & Cellular Biology in the lab of Professor Jonathan Staley. He is particularly interested in the function of Prp22 in 3'ss fidelity during pre-mRNA splicing. Hernandez is a Howard Hughes Medical Institute (HHMI) Gilliam Fellow and Co-Founder of the Graduate Recruitment Initiative Team, an organization working to enhance diversity and inclusion across 18 graduate programs in the Biological Sciences Division (BSD) and Physical Sciences Division (PSD) at the University of Chicago and across the country.