Automated High-throughput Protein Purification with PhyTip® (PhyNexus) at Micro-scale

Hoangdung D. Ho, Simon W. Lee, Sandra Wang, Alan Kutach, David Shaw, Manjiri Ghate and Jim Barnett
Discovery Sciences and Technologies, Roche Palo Alto, CA, USA

Abstract

High-throughput protein expression and purification have been successful tools to accelerate the finding of new targets in structural and functional studies in our group. We have routinely cloned and expressed varied constructs of a target protein simultaneously in different expression systems to rapidly identify the forms with highest expression levels, stability and/or potency. Thus, an automated high-throughput micro-purification method was developed to process such numerous culture samples. The current methodology is based on robotic pipet tips - PhyTip® (PhyNexus) - prepacked with different types of chromatography resins (affinity, ion-exchange, desalting etc.). The PhyTips are programmed to be transferred by a robot from protein samples to different purification buffers. Within a day, many plates of protein samples can be purified and analyzed by SDS-PAGE, thus decisions can be made quickly on constructs to scale up. We show here how the Biomek® FX robot and PhyTips are set up; how we routinely use this technology to purify tagged proteins from different cell cultures to check for expression levels and stability ; and how quickly we can determine the forms and buffer conditions for scaling up.

Materials and Methods

• Sample preparations:

- For intracellular proteins, cell pellets from 3-5ml *E.coli* or Baculoviral cultures were harvested and lysed with 200µl lysis buffers (Novagen). For secreted proteins, culture fluids from 3-5ml Baculoviral or Mammalian cultures were concentrated to 200µl. Lysates and culture fluids were clarified by centrifugation, then transferred to 96-well round bottom plates (Greiner) for PhyTips purification.

• PhyTips purification method:

- PhyTips (Ni-NTA, Protein A…) were washed with 200µl binding buffers, then transferred to samples (200-300µl/tip) for binding proteins. Phytips were washed twice with 200µl washing buffers, then proteins were eluted in 50µl eluting buffers. Protein samples (10µl) were analyzed by SDS-PAGE gels stained with different staining buffers.

- Standard buffers for different types of PhyTips were prepared manually. A matrix of buffers was prepared in 96-deep well blocks by using Hummingbird robot when many buffer conditions were screened for best protein bindings and/or stability.

Results

1. Expression levels of many different protein constructs expressed from different cell lines were screened rapidly and simultaneously using robotic PhyTips purification method (figures 1, 2 and 5).
2. Multiple PhyTip purifications of one protein yielded reasonable amount and purity for activity level comparison and protein sequence confirmation (figure 3).
3. Many different buffer conditions can be screened for best protein captures and/or stability to determine scale up purification conditions (figure 4).

Screening the Expression Levels of Various Proteins Expressed from Different Cell Lines

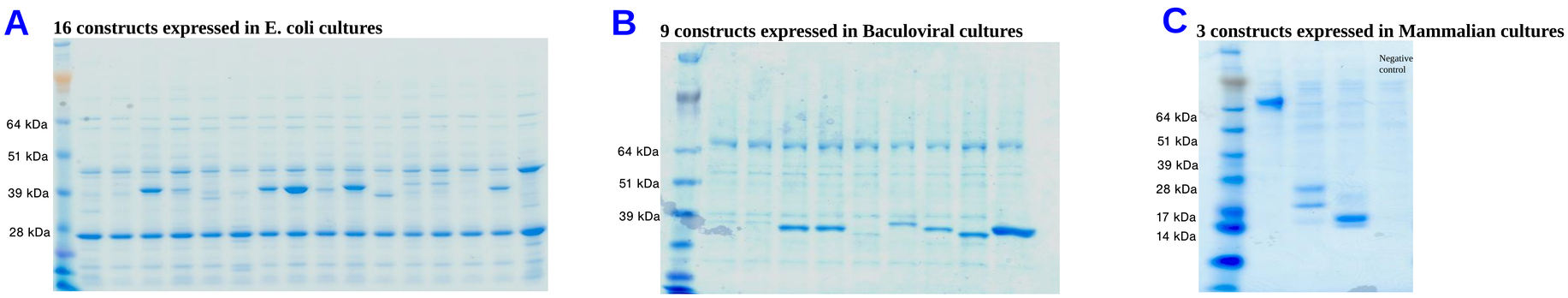

Figure 1. Cell pellets from 3ml E. coli cultures (Panel A) and Baculoviral cultures (Panel B) were lysed in 200µl lysis buffers (Novagen). Culture fluids from 3ml Mammalian cultures (Panel C) were concentrated to 200µl. Lysates and culture fluids were clarified by centrifugation and purified using IMAC PhyTips. Proteins were eluted in 50µl eluting buffers and 10µl samples were analyzed by 4-12% Bis-Tris SDS-PAGE. Expression levels for different constructs were shown.

Different PhyTips for Different Protein Tags

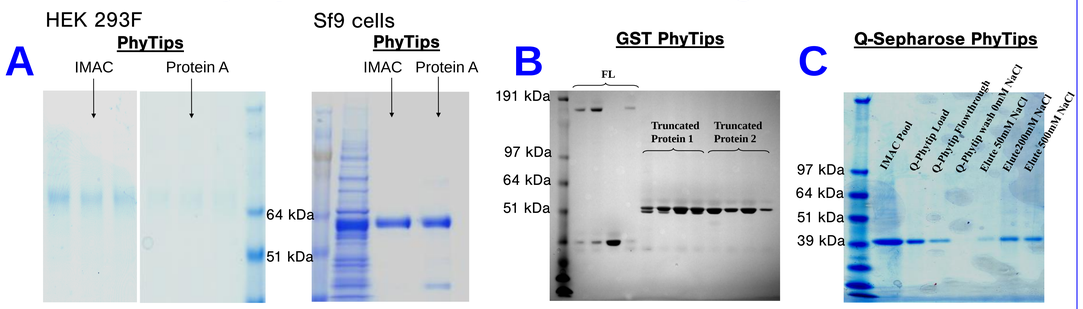

Figure 2. Panel A showed PhyTips purification of a double tagged secreted protein expressed in two different systems. Baculovirus system expressed protein with higher yield and less glycosylation and aggregation than Mammalian system. Panel B showed PhyTips purification of full length and truncations of an intracellular protein with GST tag. Different Baculovirus clones yielded different expression levels. Panel C showed custom made Q-Sepharose PhyTips bound and eluted protein at different NaCl concentrations.

Mid-Scale PhyTips Purification of Different Proteins for Activity Level Comparison

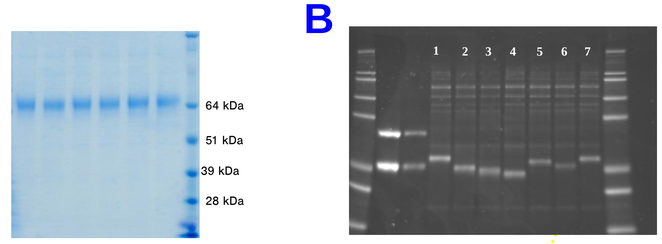

Figure 3. Panel A showed the performance of 6 IMAC PhyTips purification of the same protein culture fluid. Purified proteins from these Phytips were combined to send for activity screening. Many constructs can be purified simultaneously at mid-scale using multiple PhyTips for activity comparison studies as shown in Panel B. Seven intracellular proteins were purified from 72ml Sf9 cell pellets using 12 IMAC PhyTips for each.

Screening Buffer Conditions for Scaling up

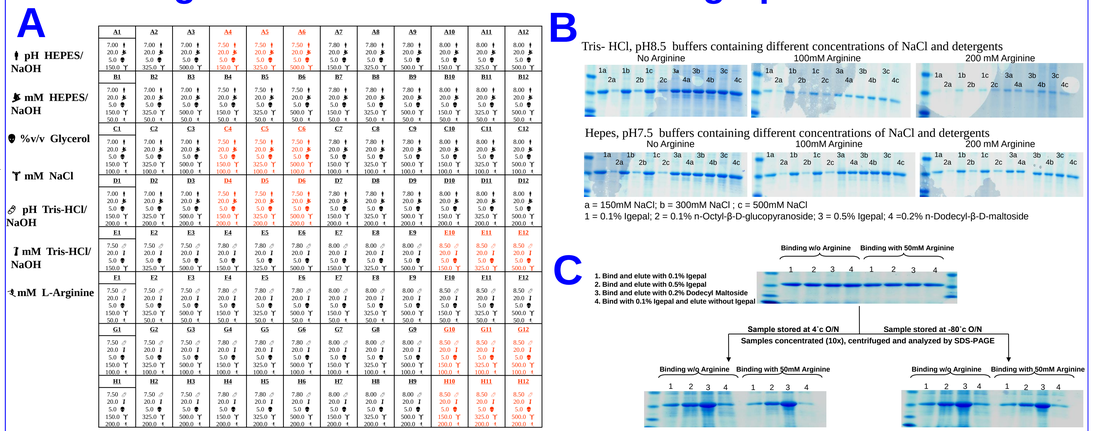

Figure 4. Panel A showed a matrix of buffers prepared in a 96-deep well block by a Hummingbird robot. A few buffer conditions were selected for screening binding affinity of a His-tagged protein on IMAC PhyTips at different pHs, NaCl concentrations and in the presence of different detergents which were added manually (Panel B). The best binding conditions then were used to screen for protein stability as shown in Panel C. This protein was shown to have best binding affinity and to be most stable in Tris-HCl, pH8.5 without Arginine + 0.2% n-Dodecyl-β-D-maltoside.

PhyTips and Deck Layout on Biomek FX

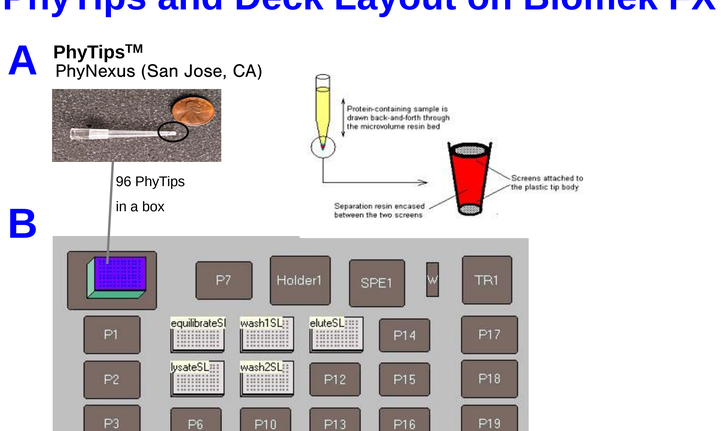

Figure 5. A Phytip picture is shown in Panel A. These PhyTips are packed with 5ul of resin. There are 2 porous screens at two ends to keep resin in place. The standard purification protocol includes 5 steps as shown in Panel B and the program time is 30-60 minutes depending on the flowrates.

Conclusions:

Automated high-throughput purification using PhyTips method is very easy and fast to set up. Within a day, many proteins can be purified at micro-scale under many different buffer conditions to screen for protein expression levels and stability. Within a week, the expressed proteins can be purified at mid-scale to screen for activity level and to confirm the protein sequence. Within a month, many active constructs can be purified at large scale for structural and functional studies.