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# MULTI & FAST: THE SECRET FOR A PROTEIN PRODUCTION FACILITY@ELETTRA

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Abstract — Expression, purification and structure determination of recombinant proteins is a fundamental requirement for a variety of biological applications, from exploratory research to pharmaceutical drug discovery. Exploiting the expertise and the strategic location of the laboratories of Elettra Synchrotron of Trieste, we have invested to create a dedicated center for protein production and characterization in the newly renovated structural biology lab of Elettra. The PROTEO project aims to offer a specialized support to research groups that need to obtain pure recombinant proteins for functional or structural studies. The core protein production unit is equipped to express/purify proteins with high efficiency in prokaryotic and eukaryotic systems and is setting up HT protocols. Currently, ten different projects are running in the lab. For each project, constructs design followed by fast multi-cloning approach has been performed leading to the combined evaluation of a number of different expression data: most adequate expression systems were chosen and the purification protocols were optimized to obtain correctly folded, functional and stable proteins for structural studies.
In this paper we present the organization of the PROTEO protein production platform and a number of studies ongoing.

**Index Terms — protocols were optimized to obtain correctly folded, functional and stable proteins for structural studies**

## 1 BACKGROUND

Expression and purification of recombinant proteins is a fundamental requirement for a variety of biological research applications, from exploratory research to pharmaceutical drug discovery. The past 10 years have witnessed a fast evolution of postgenomic technologies. One of the fields that was highly boosted by the "omics" initiatives is the production of recombinant proteins, which experienced

an extraordinary increase of throughput and results, fueling thus the comprehension of challenging scientific questions (1).

## 2 OBJECTIVES

Organize and validate a protein production lab by semi-automated approaches of cloning and expression of multiple protein targets in *E.coli*, insect cell and mammalian expression systems. Give specialized support to collaborators to get their protein of interest for functional and structural studies. Focus on proteins that are relevant for cancer diagnostic and therapy.

## 3 APPROACH & METHODS

### 3.1 General approach

The protein production workflow is schematized in Fig.1. Critical steps are the construct design and the parallel evaluation of multiple samples/variables. The "Multiple Cloning" and the "Multiple-Parameter Test-Expression" can be automated by set up of robotized protocols (Liquid Handling Freedom EVO 150 – TECAN).

### 3.2 Methods

Constructs design is based on bioinformatic analysis and experimental data. The defined sequences are PCR amplified and subcloned by Gateway® (Invitrogen) or LIC cloning methods that are suitable for parallel/robotic processes (2, 3).
Expression constructs are fused with different N-terminal tags accordingly with the project needs, and all contain an His6-tag to allow parallel affinity purification on methal chelated resin. Tags are cleaved by specific protease (TEV or Prescission protease). Purification optimization and scale up is done exploiting all chromatographic techniques available in the lab. Usually, the affinity chromatography step is followed by IEX and SEC. Quality control is done by SDS-PAGE and Western blot. When required, activity assay is performed. Thermal stability assay is done by RT-PCR using CFX96-BioRad (4).

## 4 RESULTS

Our production pipeline is currently composed of about 10 proteins, derived from active collaborations with PROTEO's partners and other external groups. These proteins are targets of pharmacological interest for cancer therapy.
Accordingly to the protein intrinsic features and the scope of the work, we can choose to express the protein in *E.coli*, insect or mammalian cells. Parallel evaluation of multivariate parameters (strains, induction mode, T°, t) leads to identification of optimal expression conditions. Below are shown some examples of results obtained for different proteins along the expression-purification workflow:

Evaluation of the expression levels of USP1 obtained by comparing different *E.coli* strains with various expression vectors (table 1)

- SDS-PAGE analysis from affinity-purification steps of three proteins (Fig. 2: a. Pin1; b. USP18; c. Her2)
- Thermofluor assay to evaluate the stability of purified proteins. In Fig. 3 is reported the thermal-shift plot of purified Pin1 WT, that results to have a Tm=53.8 °C ± 1.2°. Interestingly after preserving the

Fig. 1: Schematic description of the workflow of protein production platform.

protein for more than 3 weeks at 4°C, the Tm and the profile is almost unchanged, suggesting that the protein is stable in the storage buffer.

- Improve yields by growth in bioreactor: Pilot study to set up expression condition in a 2L bioreactor (Biostat B, Sartorius) has improved the yield of final purified Pin1, going form 20 mg/L to 80 mg/L (Fig. 4).

| | E.coli strains (T=16°C, o/n, auto-induction media) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | BL21 | BL21 RIPL | BL21 RP | BL21 pLysS | Rosetta2 | Rosetta2 pLysS | C 41 | C 43 | Arctic RIL | Arctic RP |
| pNIC28 | / | / | xxx | / | Xx | / | / | / | / | xxx |
| pNH-TrxT | xx | x | xxx | / | Xx | / | / | / | x | xxx |
| pGTvL2 | xx | x | xxx | / | Xx | / | / | / | x | xxx |
| pNIC-CTHF | / | / | / | / | / | / | / | / | / | / |
| pGTvL1 | / | x | xxx | x | xxx | xx | / | / | x | xx |

Tab1: Table of the total levels of expression otained for human USP1full-length expressed in different *E.coli* strain. RP strains express higher amount of proteins, although insoluble.

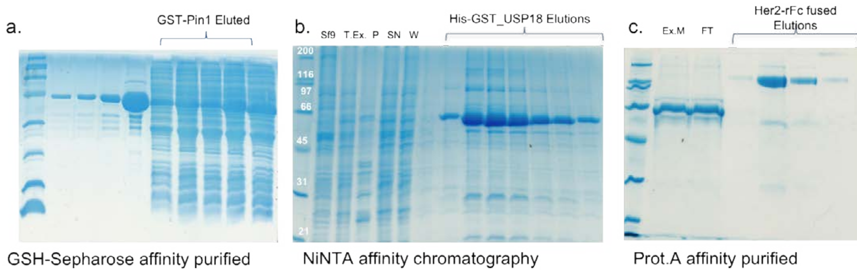


Fig. 2: SDS-PAGE analysis from affinity-purification steps of three proteins (a. Pin1; b. USP18; c. Her2). Pin1 expressed in *E.coli* (BL21); b. hUSP18 in insect cells (Sf9); c. Her2-Fc in mammalian cells (HEK293)

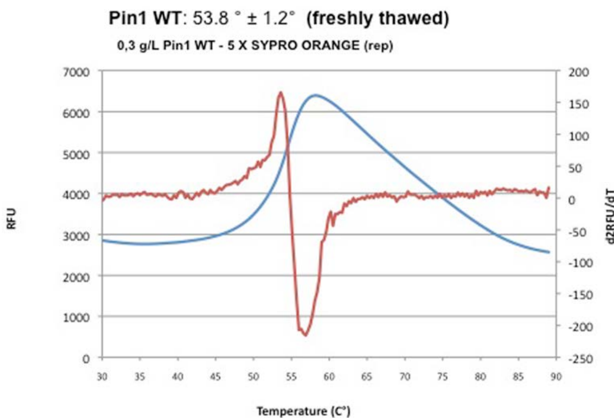


Fig. 3:Thermofluor assay to evaluate the stability of purified proteins. In the panel is reported the thermal-shift plot of purified Pin1 WT, that results to have a Tm=53.8 °C ± 1.2°.

| Parameters | |
|---|---|
| Volume | 2L |
| Stirring | 800 rpm |
| Foam control | Yes |
| pH control | Yes |
| $pO_2$ monitoring | OK |
| Gas availability | Air only |

Fig. 4: bioreactor Biostat B, (Sartorius) assembled with a 2L vessel, and parameters used to express Pin1.

## 5 POTENTIAL NEW PRODUCTS & SERVICES

The aim is to offer a specialized support to research laboratories (public or private) for a rapid identification of best conditions for recombinant protein production. Moreover, the close contact with synchrotron beam lines such as SAXS and X-ray diffraction, will facilitate access of biomedical researcher to structural biology approaches. On the long term our objective will be to create a tailored service that will enhance efficiency of basic and applied research in the local area.

## 6 CURRENT COLLABORATIONS

- Francesca Demarchi – LNCIB, Trieste (PP2, Proteo);
- Gianni Del Sal Lab – LNCIB, Trieste, (PP2, Proteo);
- Claudio Brancolini – University of Udine (PP5, Proteo);
- Ario de Marco – University of Nova Gorica (PP1, Proteo);
- Jan Mavri – COBIK, Ajdovščina (PP4, Proteo);
- Sabrina Pricl – University of Trieste;
- Gianmaria Severini – IRCCS Burlo Garofolo – Trieste;

## 7 CONTACT OR COLLABORATIONS NEEDED

The PROTEO protein production facility would like to collaborate with:
Protein chemistry experts for N-terminal sequencing and mass spectrometry ( ESI-MS, MALDI);
Pharmacologist and medicinal chemists, to develop small molecules that will interact/inhibit the protein targets we are interested in;
Anyone who needs to over-express and purify its protein and do not have the suitable resources.

## 8 FUNDS NEEDED

### 8.1 For basic research (investigation of biological mechanisms): 50.000 €

### 8.2 For applied research (solutions for real-world problems): 100.000 €

### 8.3 For pilot & demonstrator activities (to develop a prototype): 150.000 €

## 9 CONCLUSIONS

Thanks to the PROTEO project we could start to implement a new protein production facility at Elettra's Structural Biology Laboratory. Collaborating with external research laboratories we could set up the expression of different recombinant proteins in *E.coli*, insect and mammalian cells and validate several expression vectors and purification protocols. Some proteins have been obtained in quantity and quality suitable for further experiments (i.e. assay set up, crystallization trials, protein-protein interaction studies). Such a service will allow an increase in efficiency and scientific productivity, saving thus time and money.

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