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Rapid cell-free expression and purification screen through digital microfluidics on eProtein Discovery™

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Introduction

Expressing VEGF in *E.coli* is challenging due to an inherent cysteine-knot motif that can result in protein misfolding and formation of inclusion bodies. Soluble VEGF expression in *E.coli* is only possible when the protein is expressed in specialized strains such as Origami 2 (DE3) in the presence of a solubility fusion tag[1]. Optimization to obtain sufficient yields requires:

- cloning into various expression plasmids
- testing numerous solubility tags, cell lines & expression conditions

Using the eProtein Discovery™ platform, we determined the ideal DNA construct and conditions to express soluble VEGF and obtain µg quantities in less than 48 hours, with < 2 hours hands-on time starting with DNA prepared for the system.

Objective: The aim of this study was to obtain soluble, active VEGF within 48 hours using the eProtein Discovery™ platform.

Methods

eProtein Discovery™ workflow

The eProtein Discovery system is an integrated platform that enables rapid protein access in a simple 3-step workflow (Figure 1) which involves:

- Cell-free protein expression in customizable conditions
- Solubility assessment of protein constructs with varying solubility fusion tags
- Soluble expression yield quantitation
- Bead-based protein purification
- Purified protein yield determination

The platform automates screening of up to 24 constructs in 8 cell-free expression systems for a total of 192 expression profiles within 24 hours allowing researchers to identify the best soluble expression and purification yields for various proteins. Soluble expression is detected via fluorescent complementation (Figure 2). Following this step, protein scale-up occurs the next day, right on the benchtop, providing reliable protein samples (up to the mg scale) in less than 48 hours.

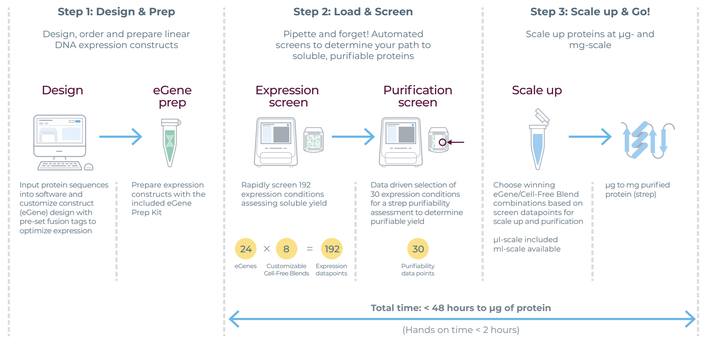


Figure 1. The eProtein Discovery™ workflow.

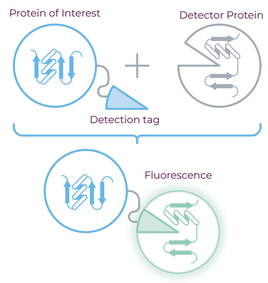


Figure 2. Fluorescence complementation technology used within the Smart Cartridge to detect soluble protein expression.

Step 1: Construct design and preparation

The VEGF protein sequence was uploaded onto the eProtein Discovery software where the protein sequence was converted to a codon optimized DNA sequence. Gene fragments were ordered using the optimized sequence. The final linear DNA was generated via PCR using megaprimers supplied in the eGene Prep Kit; which adds pre-determined solubility, purification and detection tags to each construct (Figure 3a, table).

Step 2: Expression and purification screening

Expression yield for all eight VEGF eGene constructs were determined using 3 types of cell-free blends. The three highest expressing candidates were purified on the cartridge and the purification yields were reported.

- Blend-2 consists of standard cell-free synthesis reagent
- Blend-3 consists of standard cell-free reagent + additives that promote disulfide bond formation
- Blend-4 consists of standard cell-free reagent + chaperone mix

Step 3: Scale-up expression, protein purification & characterization

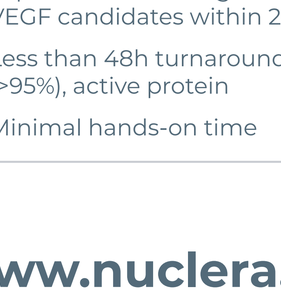
The winning construct shown to produce the highest purified yield was chosen for scale-up using an eProtein Discovery™ Scale-Up Kit. eGene was added to the cell-free blend in a reaction tube and incubated overnight. Expressed protein was captured on functionalized beads, eluted in 100 µL elution buffer and analyzed by SDS-PAGE. Activity of purified VEGF was quantified using the cell-based PathHunter® Bevacuzimab Bioasssay (Eurofins DiscoverX).

Results

Step 2A: Expression screening identifies the highest-expressing eGene-Blend combinations

The highest VEGF expression was achieved with construct A5 (SUMO_VEGF_STREP_DET) in Blend-3 (Figure 3). This combination promotes solubility through the presence of both the SUMO solubility fusion tag and additives that support disulfide bond formation. The result is consistent with published research showing that VEGF expresses well with a solubility fusion tag in an *E. coli* strain that helps disulfide bond formation[1].

a)



Label	Description
A1	STREP_VEGF_DET
A2	DET_VEGF_STREP
A3	DET_STREP_VEGF
A4	VEGF_STREP_DET
A5	SUMO_VEGF_STREP_DET
A6	TRX_VEGF_STREP_DET
A7	VEGF_STREP_SUMO_DET
A8	VEGF_STREP_TRX_DET

b)

Figure 3. Expression screen data. (a) Fluorescence from expressed and complemented proteins (bright spots) on the Smart Cartridge. (b) Yield of expressed proteins. Green dashed box highlights candidates selected for on-cartridge purification.

Step 2B: Subsequent purification screen reveals the "winning combination"

Soluble candidates yielding the highest expression were SUMO_VEGF_STREP_DET expressed in Blend-2, Blend-3 and Blend 4. These were automatically selected by the system for on-cartridge purification (Figure 4). The results demonstrate that the protein is soluble in all three blends and can be purified.

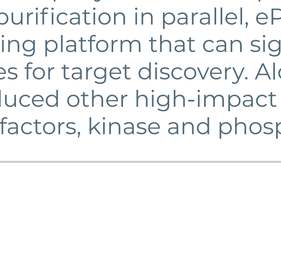


Figure 4. On-cartridge purification performed in duplicates

Side-by-side comparison of yields for soluble expression and purification (Figure 5) shows that SUMO_VEGF_STREP_DET in Blend-3 performed best in both assessments, making it the "winning combination" to take forward for off-cartridge scale-up.

Figure 5. On-cartridge expression and purification yield comparison.

Step 3: Scale-up expression produces VEGF of high purity and activity

Off-cartridge scale-up expression and purification of SUMO_VEGF_STREP_DET yielded 52 µg soluble protein at >95% purity (Figure 6).

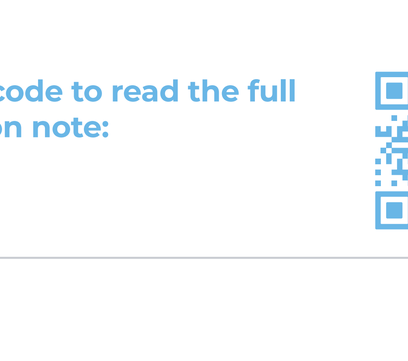


Figure 6. Off-cartridge scale-up expression and purification of SUMO_VEGF_STREP_DET.

The protein was functionally active in the cell-based PathHunter® Bevacuzimab Bioassay, with an EC50 of 12.49 ng/mL (Figure 7).

Figure 7. Dose-dependent activity of VEGF produced with eProtein Discovery. RLU: relative light units.

Conclusions

- Expression screening and selection of soluble, purifiable VEGF candidates within 24h
- Less than 48h turnaround time from DNA to 52 µg of pure (>95%), active protein
- Minimal hands-on time

With the power to rapidly screen multiple constructs for expression and purification in parallel, eProtein Discovery™ is a highly enabling platform that can significantly reduce turnaround times for target discovery. Along with VEGF, the system has produced other high-impact drug targets such as transcription factors, kinase and phosphatases.

Scan QR code to read the full application note:

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Reference 1: Nguyen, M. *et al.* Prokaryotic Soluble Overexpression and Purification of Human VEGF165 by Fusion to a Maltose Binding Protein Tag. PLOS ONE 11(5): e0156296. doi:10.1371/journal. | Version number: 08