EXPRESSO® SOLUBILITY AND EXPRESSION SCREENING SYSTEM

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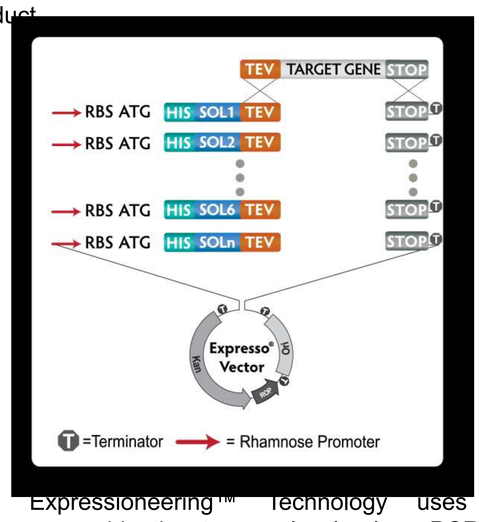
ABSTRACT

Many heterologous proteins are insoluble or poorly expressed in *Escherichia coli*. One solution to this problem is to fuse a "solubility tag" to the target protein. Several solubility tags are available from a variety of vendors, but the best tag for a given target protein can only be determined empirically. With current technology, tag selection is a time consuming trial-and-error process that lacks uniformity in promoter selection and cloning technologies. Lucigen has developed the **Expresso Solubility and Expression Screening System** to allow rapid screening of 7 different cleavable fusion partners in parallel. Expanded screening of up to 24 different N-terminal fusion partners in combination with a unique fluorescent C-terminal fusion partner is available as a service. This fluorescent tag, LucY (Lucigen Yellow) provides an instant visual readout of the amount of soluble protein, and also imparts solubility to target proteins for which an N-terminal fusion partner alone is insufficient.

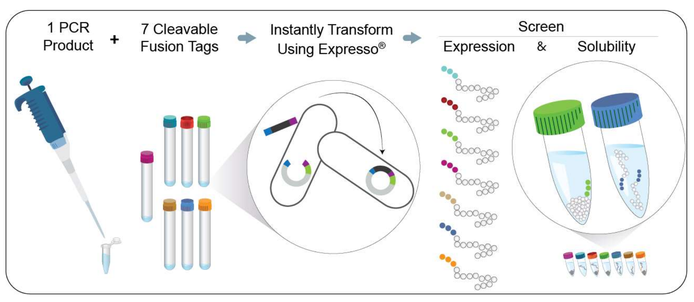
Introduction

Lucigen has developed the **Expresso Solubility and Expression Screening System** to enable the rapid, parallel screening of fusion tags for enhanced expression and solubility of difficult target proteins. A suite of vectors, each containing a unique fusion partner, enables instant parallel cloning of a single PCR product.



Lucigen's Expressioneering™ Technology uses *in vivo* homologous recombination to seamlessly clone PCR amplified Open Reading Frames into multiple expression vectors in parallel. The ability to quickly assess the solubility of multiple fusion proteins enables empirical tag optimization for a given protein.

Expresso Solubility & Expression Workflow



The **Expresso Solubility and Expression Screening System** includes 8 Expressioneering-ready vectors (7 different tags and 1 untagged control); chemically competent cells for cloning and expression, rhamnose and glucose solutions for controlled induction using the tunable $RhaP_{BAD}$ promoter, and SelecTEV Protease for tag removal.

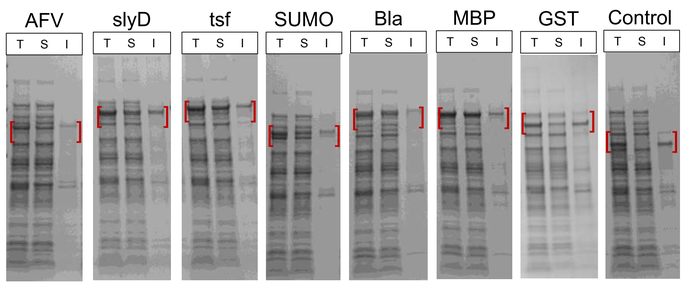


Figure 1: Empirical Evaluation of Fusion Partners for Soluble Expression of SMAD2.
SMAD proteins are recruited to the TGF-β receptors and mediate TGF-β signaling. Rhamnose-induced cultures were harvested, sonicated, and fractionated by centrifugation. Fractions of the total cell lysate (T), soluble supernatant (S) and insoluble pellet (I) were analyzed by SDS-PAGE. Each lane contains 0.05 OD equivalents. The Control clone, with only a His6 tag at the amino terminus, produces only insoluble SMAD2. The tsf and MBP tags produced the highest levels of soluble protein.

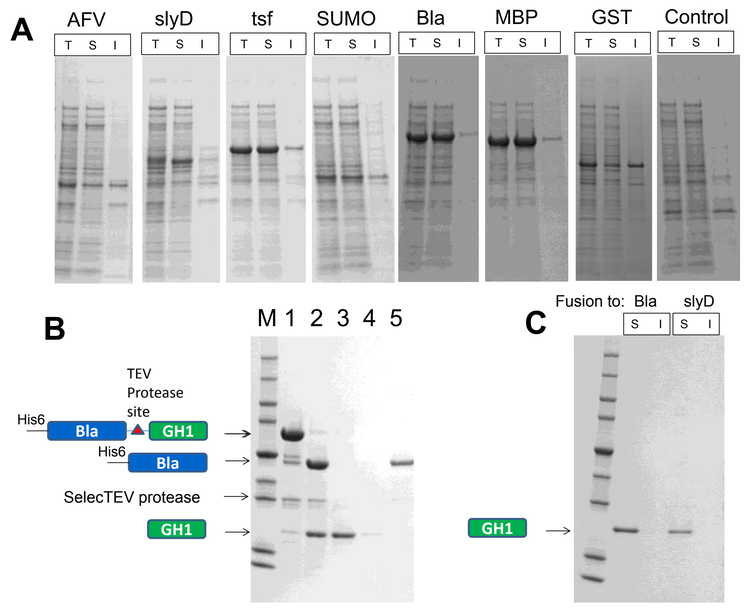


Figure 2. Expression and purification of growth hormone GH1.
GH1 is the form of human growth hormone expressed in the pituitary gland. **A.** Screening for soluble GH1 expression allowed high-level expression of soluble protein. **B.** Tag removal with SelecTEV protease and purification of de-tagged GH1. Lanes 1 and 2 show cleavage of IMAC-purified Bla-GH1 fusion protein with SelecTEV protease at time 0 and after 2 hr at room temperature. Lanes 3, 4 and 5 show flowthrough, wash and elution fractions from subtractive IMAC purification of the cleavage reaction. Free GH1 appears in the flowthrough, while the His6-tagged Bla fragment and His6-tagged SelecTEV protease bind to the column and are eluted with imidazole. **C.** Purified, de-tagged GH1 remains soluble upon centrifugation after 72 hrs at 4°C.

LucY: a fluorescent protein that enhances solubility

LucY (Lucigen Yellow) is a novel yellow fluorescent protein discovered by Lucigen scientists that is unrelated to GFP. Unlike EGFP, LucY can impart solubility when fused to the C-terminus of insoluble proteins such as tobacco etch virus (TEV) protease.

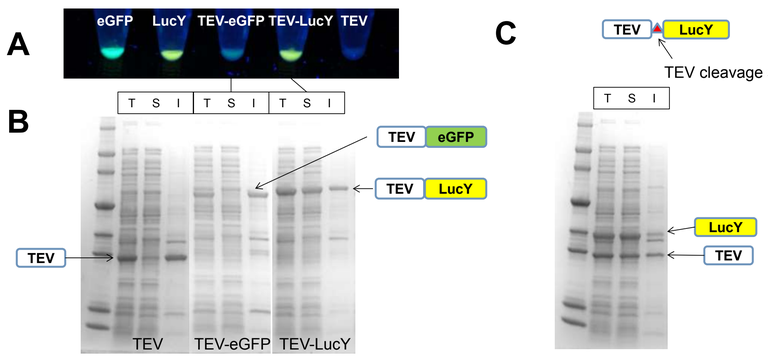


Figure 3. Enhanced solubility with C-terminal fusion to LucY.
A. Cell pellets expressing EGFP or LucY alone exhibit fluorescence under long-wavelength UV illumination. A TEV-LucY fusion protein shows similar fluorescence, but TEV-EGFP does not. **B.** TEV protease is >90% insoluble when expressed alone or as a fusion to EGFP, but >50% soluble when fused to LucY. **C.** Inclusion of a TEV protease autocleavage site between TEV and LucY produces cleaved, soluble TEV protease *in vivo*.

Dual-tag fluorescent solubility screening service

For deeper screening capability, we have assembled a panel of over 2 dozen fusion partners. Screening of these tags, with or without the fluorescent C-terminal fusion partner LucY, is performed as a service by Lucigen scientists.

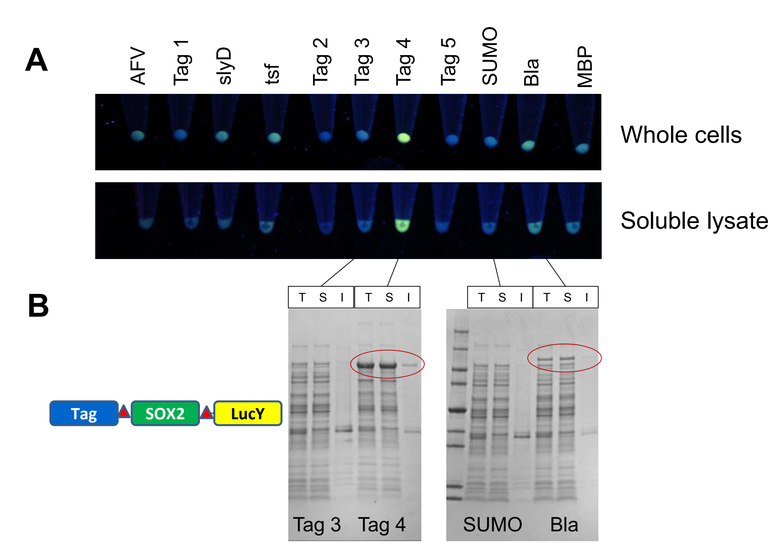
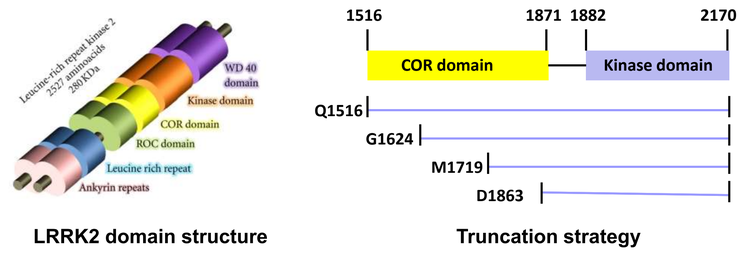


Figure 4. Soluble expression of SOX2 with dual fusion tags. SOX2 is an important transcription factor involved in maintenance of stem cell pluripotency. SOX2 could not be expressed in *E. coli* without a fusion partner or with an N-terminal fusion tag alone (not shown). Fusion to both N-terminal Tag 4 and C-terminal LucY produced high levels of soluble protein that could be easily detected by fluorescence in both whole cells and in the soluble fraction of cell lysate following centrifugation (panel **A**). Gel analysis (panel **B**) recapitulated the fluorescence results: Tag 4 yielded the highest levels of fluorescence and soluble fusion protein.

Parkinson's disease biomarker LRRK2 solubility trials

Missense mutations in the *leucine-rich repeat kinase 2* (*LRRK2*) gene are the most common known genetic cause of Parkinson's disease (PD). The *LRRK2* mutation G2019S occurs in the kinase domain and enhances both kinase activity and toxicity *in vitro.* LRRK2 is a very important target for PD therapeutics but obtaining milligram quantities of the protein for structural studies has been severely hampered due to lack of sufficient soluble protein expression in *E. coli.*



We focused on the kinase domain and screened a series of N-terminal truncations against the expanded Lucigen fusion tag screening panel, with and without fusion to C-terminal LucY. Modest expression but poor solubility was observed with several N-terminal tags alone. In conjunction with C-terminal fusion to LucY, a few tags showed dramatically increased soluble expression. A subset of the screening results is shown below.

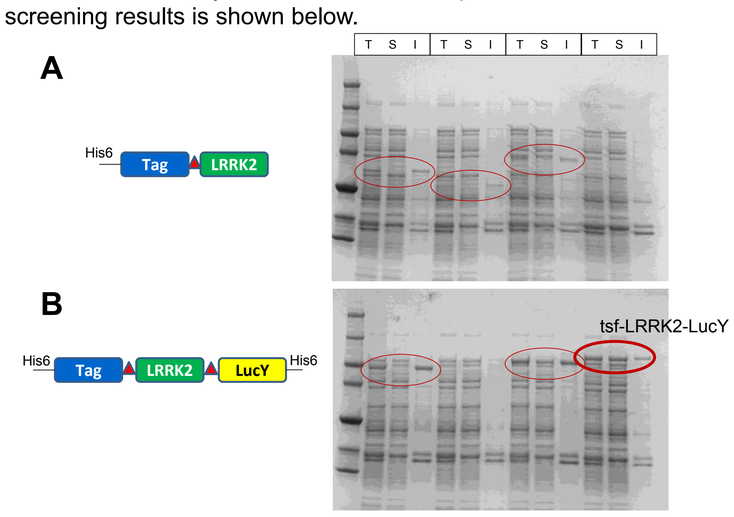


Figure 5. Expression of LRRK2 kinase domain truncation M1719
A. The LRRK2 kinase domain truncation M1719 was expressed as a fusion with several different N-terminal fusion partners.
B. The same constructs as in panel A were expressed with C-terminal fusion to LucY. The highest level of soluble fusion protein expression was observed with tsf as the N-terminal fusion partner.

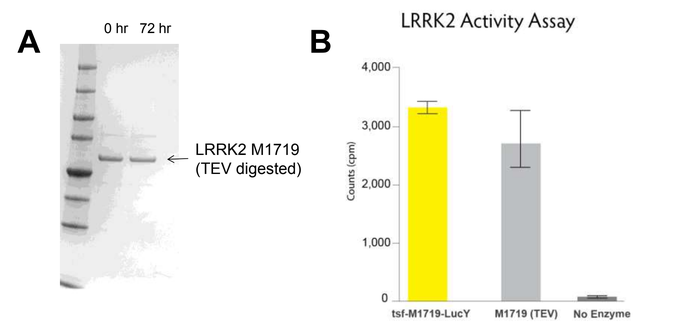


Figure 6. Isolated LRRK2 kinase domain is soluble and active.
Following purification of the tsf-LRRK2-LucY protein by IMAC and cleavage with SelecTEV protease, the LRRK2 fragment was isolated by subtractive IMAC to remove the His6-tagged tsf and LucY fragments as well as the His6-tagged SelecTEV protease. Upon centrifugation 72 hours later (21,000 x g, 45 min) , the LRRK2 kinase domain remained completely soluble (panel **A**). The isolated kinase domain exhibited kinase activity comparable to that of the doubly-tagged fusion protein (panel **B**).

CONCLUSIONS

Since target proteins respond uniquely and unpredictably to solubility tags, choosing a solubility tag for optimal soluble expression is a lengthy evidence-based process. The **Expresso Solubility and Expression Screening System** represents a game changing improvement in the process by which solubility tags are selected. Past methods of selecting fusion partners involved cumbersome cloning methods that were too frequently incompatible with insert sequences, and were thus ill-suited to testing multiple partners for the expression of important proteins. With the Expresso System, cloning and evaluation of 7 different tags can be completed in a matter of days. Alternatively, a unique dual tag approach can be employed in a custom service. This approach combines the C-terminal LucY fluorescent solubility enhancer with an expanded suite of up to 24 N-terminal fusion partners and dramatically increases the probability of obtaining functional, soluble protein.

Visit the Lucigen booth for more information or contact us at www.lucigen.com to learn how you can take advantage of these technologies.

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