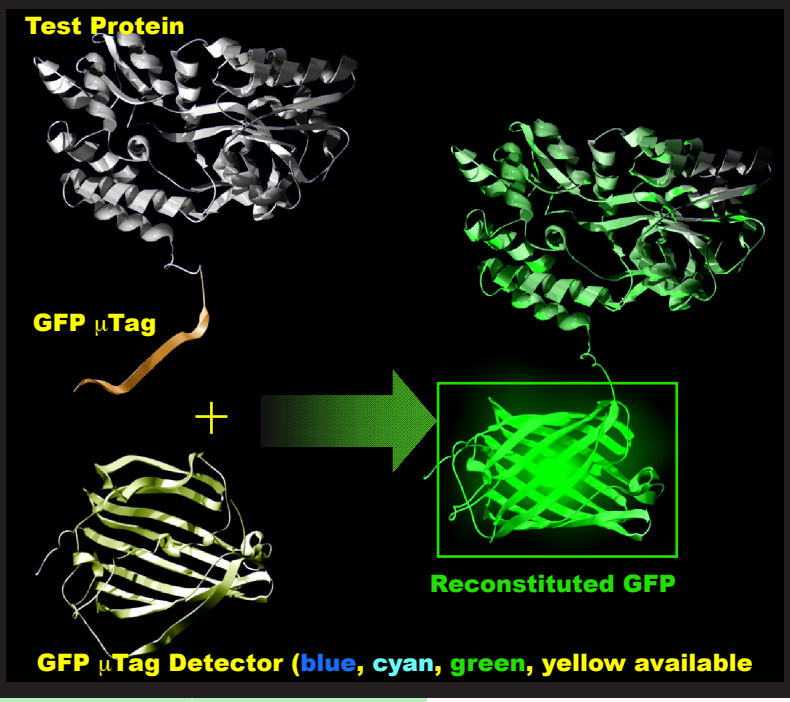
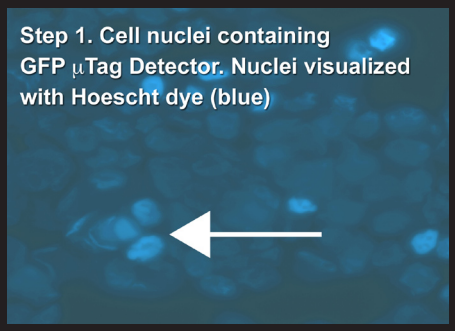
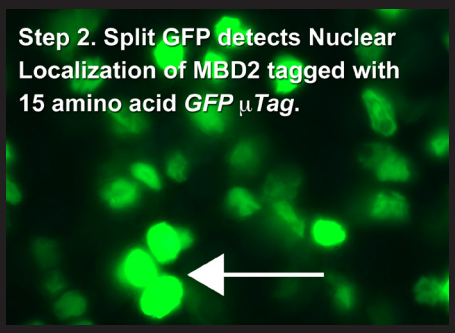
Licensable Technologies

Green Fluorescent Protein Toolbox







Applications:

- Protein tagging and detection in living cells and in the test tube
- Easy monitoring of protein folding and stability
- Protein-protein interactions by FRET and PCA
- Studying effects of DNA, protein, and cofactors on protein folding
- Quantification of protein binding assays
- Cell-cell protein trafficking, cell contact, and protein exchange
- Discovering soluble mutants and finding domains of proteins
- Evolving proteins for solubility in a high throughput format
- Increasing thermal stability and folding of industrial proteins

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Summary:

Proteins are essential parts of all living organisms serving as the workhorses of the cell, participating in every process within a cell. Consequently, protein dysfunction, which causes many human diseases, is a crucial problem to solve.
Los Alamos National Laboratory's (LANL's) Green Fluorescent Protein (GFP) Toolbox was designed to help scientists understand and solve the mysteries of protein dysfunction, including misfolding, aggregation, and abnormal movement.

GFPs were first isolated from luminescent jellyfish in the 1960s. The LANL GFP Folding Reporter has been widely used since its inception nearly 10 years ago, while researchers have been steadily developing new GFP tools. Our breakthrough innovations have numerous uses in the pharmaceutical and biotechnology industries.

The GFP Toolbox includes four GFP-based tools: Folding Reporter, SuperFolder, Insertion, and Split GFP. This suite enables researchers to perform experiments impossible to do with conventional GFPs. The toolbox is simple to use, versatile, and each component has only one moving part. The toolbox includes genetically encoded tags as short as 15 amino acids, with little or no effect on passenger protein localization, behavior, folding, or solubility.

Folding Reporter reports on the success of a target protein's folding and solubility. Used by over 100 labs and cited in over 300 publications in fields as diverse as the engineering of soluble proteins for structural genomics, finding drug inhibitors for Alzheimer's, and identifying protein complexes, folding partners, and chaperones. Folding Reporter GFP is attached to a target protein, reflecting its folding success. Folding Reporters fluorescence is lower when the target folds incorrectly, correlating with how well the target protein is folded.

SuperFolder GFP, which reports on a target protein's expression, is a more robustly folded version of Folding Reporter. SuperFolder fusion fluorescence is unaffected by the fusion partner's misfolding and is directly proportional to total expression regardless of the solubility of the fusion. SuperFolder is more tolerant

Benefits:

- Simple system
- Does not require external reagents
- Provides sensitive analytical signal with pmol detection limits
- Enables experimentation both *in vivo* and *in vitro*
- Does not affect target protein function or folding
- Amenable to high-throughput assays

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For information about GFP:

www.lanl.gov/projects/gfp

to circular permutation and random mutagenesis. The toolbox includes blue, cyan, green, yellow, and red fluorescent Superfolders, perfect for FRET to detect protein interactions. Protein fusions can be up to 50 fold brighter with Superfolder compared with conventional, enhanced GFPs. It has been successfully used to monitor protein localization in the hyperthermophile *T. thermophilus* at 80°C.

Insertion GFP, a suite of four unique GFP variants, is the most advanced protein-folding reporter tool available. It can be used to avoid tagging problems and eliminate false positives created by internal ribosome binding sites and early stop codons. Insertion GFP does this by attaching at both the N and C-termini of a target protein. In addition, the sensitivity of Insertion GFP to target protein misfolding is easily tuned to maximize sensitivity and productivity during screening.

Split GFP: Tagging target proteins with GFP has always been problematic because of the bulky GFP that can perturb protein behavior. *In vivo* tools for monitoring protein aggregation or screening protein libraries for soluble variants were hit-or-miss. *In vitro* tagging systems were slow, costly, and complicated. Split GFP solves these problems by tagging the target with GFP µTag, a small, 15 amino acid fragment of the GFP, strand no. 11. In living cells, expressing the tagged protein alone, then expressing GFP µTag Detector (the rest of the GFP molecule) gives the user a direct readout of soluble protein. Using the same cells and purified GFP µTag Detector, the tagged protein can be expressed alone and studied in the test tube without recloning or interfering with its folding and solubility. An added feature of the Split GFP is its capability to measure cell-surface protein interactions and cell-cell protein contacts without changing the behavior of the target proteins.

The toolbox includes a novel protein-interaction detector using 15 amino acid tags (one on each interactor), the least perturbing system available, perfect for protein-interaction studies under natural conditions. The Split GFP suite for high throughput screening of proteins, localization, and protein interactions is one of the most advanced tools in the GFP Toolbox.

Development Stage: Proven technology

Patent Status: Four patents pending; published documents available upon request. Foreign rights available for Split GFP and Insertion Vectors.

Licensing Status: Available for license, seeking partners for commercialization.