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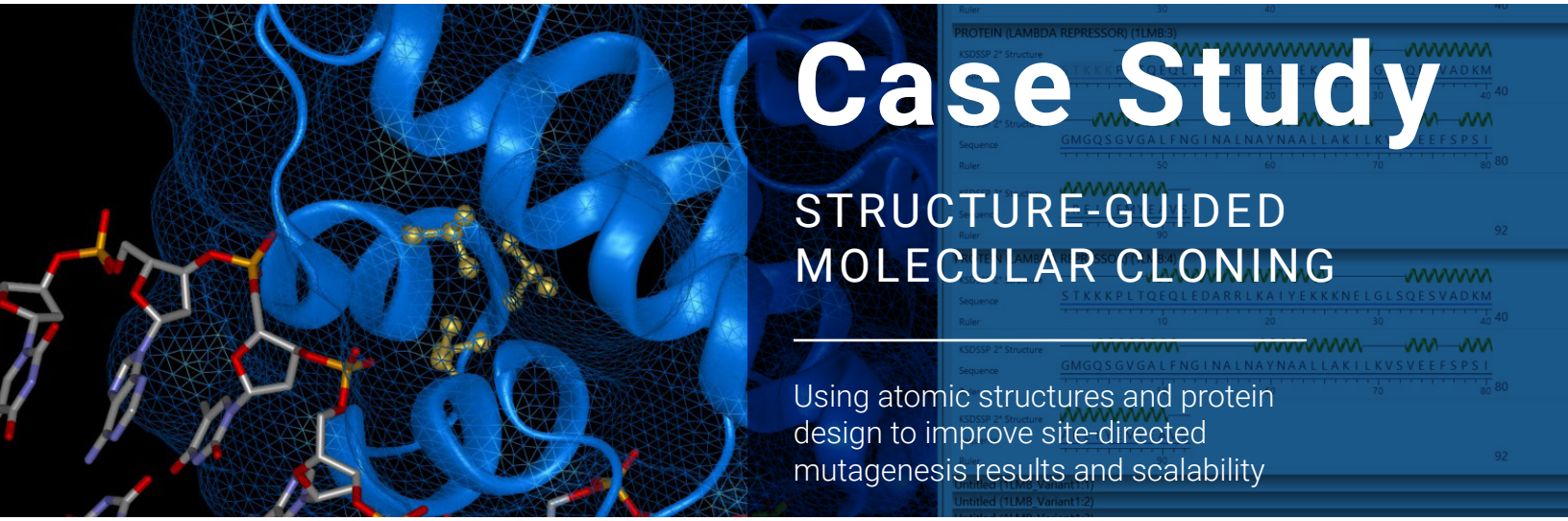


Case Study

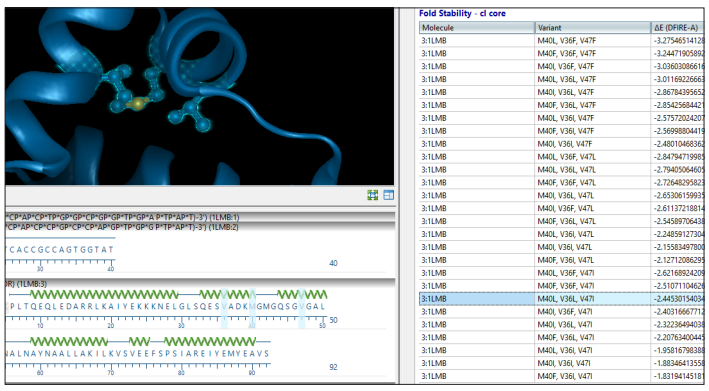
STRUCTURE-GUIDED MOLECULAR CLONING

Using atomic structures and protein design to improve site-directed mutagenesis results and scalability

DNASTAR LASERGENE: BRIDGING THE GAP BETWEEN PROTEIN SEQUENCE & STRUCTURE

Case Study Highlights

- The cI repressor protein was studied to understand how mutations in the protein core impact fold stability. The LLI variant was experimentally determined to be more stable than the wild type structure.
- The Lasergene Protein Design workflow reproduces this observation and suggests other mutations that could improve fold stability.
- The software can also be used to design cloning primers to synthesize the new molecule using an array of recombinant DNA technologies.



Overview

Protein stability is essential when optimizing the expression, purification, and formulation conditions for protein functional studies.

In this case study, we redesign the core of a protein to improve its fold stability and design cloning primers to synthesize the new molecule using an array of recombinant DNA technologies. The entire workflow can be completed within an hour using a Windows or macOS workstation.

About DNASTAR

DNASTAR is a global software company that has been meeting the sequence analysis needs of life scientists for 35 years. Our Lasergene software allows users to combine traditional and NGS sequence data with protein structure models and predictions, for greater insights into the effects of mutations.

Lasergene Packages

Molecular Biology

Perform sequence analysis, alignment, virtual cloning and primer design, and Sanger sequence assembly with our core molecular biology software.

Protein Modeling & Analysis

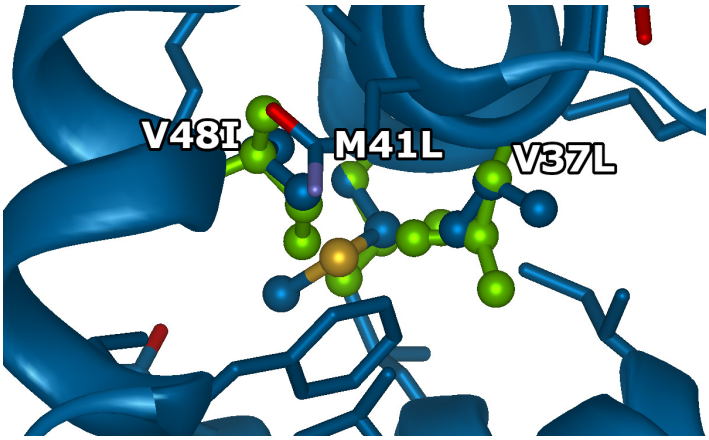
Analyze protein sequence and structure data, predict protein structures, design antibodies, model mutations, and perform hotspot scanning on protein structures.

Genomics

Assemble and analyze NGS data and integrate variant data with protein models to understand impact of mutation on structure and function.

Problem

Nature commonly evolves proteins to provide sufficient fold stability to optimize activity—not energetic stability. Buried core residues can be altered to improve a protein's developability and stability, but without protein structure data, molecular biologists are often left to trial and error approaches to site directed mutagenesis.



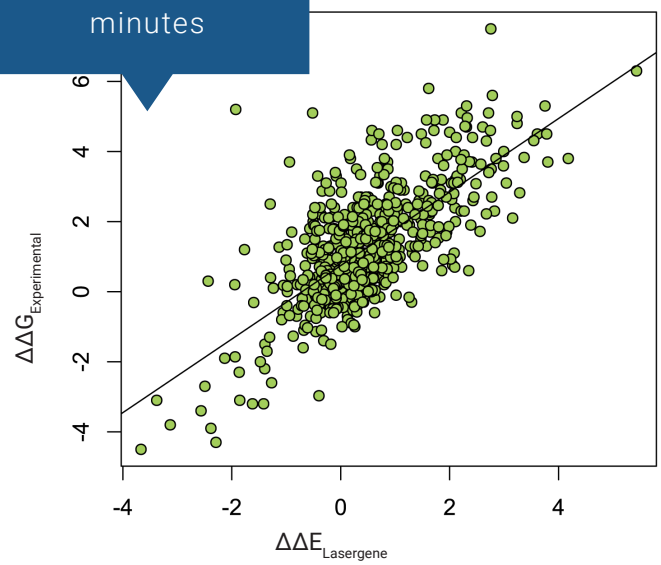
Benchmarks

The Fold-X Blind Test benchmark contains 664 substitution variants, each with an experimentally-determined change in free energy (ΔΔG) relative to wild type. The Lasergene Protein Design tool calculated the change in fold stability (ΔΔE) for the entire data set in less than 10 minutes on a single workstation with a Pearson's linear correlation coefficient (PCC) of 0.70. Backrub backbone flexibility was used and side chains of neighboring residues were repacked.

Time to model variants on 664 proteins:

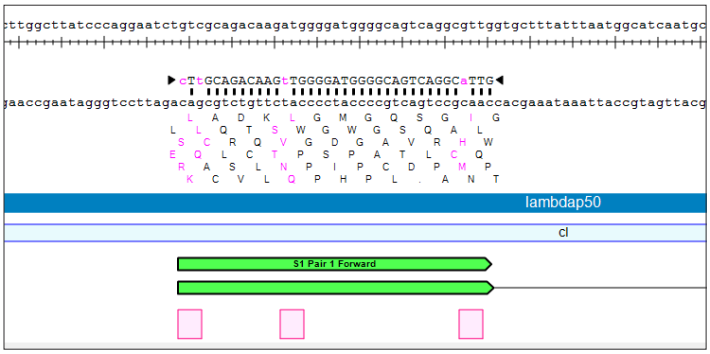
10

minutes



References

1. Lim, W. A., Hodel, A., Sauer, R. T. & Richards, F. M. (1994) Proc. Natl. Acad. Sci. USA 91, 423-427.
2. PDB ID: 1LMB. Beamer, L.J. Pabo, C.O. (1992) J Mol Biol 227, 177-196



Workflow

The following steps for the protein design and site directed mutagenesis workflow can be completed on virtually any Mac or Windows computer in under an hour.

01

Identify and test variants

Use the Protein Design workflow to scan for hot spots, improve fold stability, or create specific variants and assess resulting energy changes in the comprehensive reports.

02

Design and mutate primers

Create PCR primer pairs at the desired location and mutate primer sequences with our point-and-click tool. Primers can be further adjusted to improve Tm differences and minimize dimer formation.

03

Create virtual clones

Clone the mutated gene into an appropriate expression vector using multi-fragment PCR based cloning methods. View annotations and ORF predictions to confirm the gene is in-frame with the fusion protein.

Results

Buried core residues can be altered to improve a protein's developability and stability, as is the case with Repressor protein cI. The variant p.(Val37Leu; Met41Leu; Val48Ile) (LLI) confers 0.5 kcal/mol more stability than wild type[1]. Using the wild type structure[2], Lasergene reproduces this observation and suggests several other variant combinations that could provide even greater thermostability.

Free Trial

Obtain a fully functional, free trial version of Lasergene to try this workflow on your laptop or desktop computer.

www.dnastar.com/trial