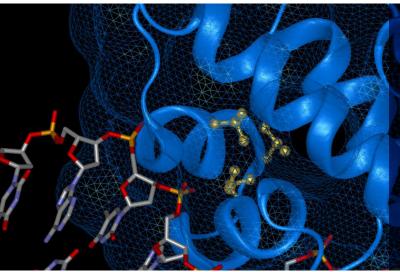


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STRUCTURE-GUIDED

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 cl 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 cloningprimers to synthesize the new molecule using an array of recombinant DNA technologies.

	Fold Stability - cl core		
	Molecule	Variant	ΔE (DFIRE-A)
	3:1LMB	M40L, V36F, V47F	-3.27546514128
	3:1LMB	M40F, V36F, V47F	-3.24471905892
	3:1LMB	M40I, V36F, V47F	-3.03603086616
	3:1LMB	M40L, V36L, V47F	-3.01169226665
	3:1LMB	M40I, V36L, V47F	-2.86784395652
	3:1LMB	M40F, V36L, V47F	-2.8542568442
	3:1LMB	M40L, V36I, V47F	-2.57572024207
	3:1LMB	M40F, V36I, V47F	-2.56998804419
	3:1LMB	M40L V36L V47F	-2.48010468363
	3:1LMB	M40L, V36F, V47L	-2.8479471998
	3:1LMB	M40L, V36L, V47L	-2.79405064605
	3:1LMB	M40F, V36F, V47L	-2.7264829582
	3:1LMB	M40I, V36L, V47L	-2.6530615993
	3:1LMB	M40I, V36F, V47L	-2.61137218814
<pre>'CP'AP'CP'TP'GP'GP'CP'GP'GP'TP'GP'A P'TP'AP'T)-3') (1LMB:1) CP'AP'CP'CP'CP'CP'AP'GP'TP'GP'G P'TP'AP'T)-3') (1LMB:2)</pre>	3:1LMB	M40F, V36L, V47L	-2.5458970643
CPTAPTCPTCPTCPTCPTCPTCPTCPTCPTCPTCPTCPTCPTCP	3:1LMB	M40L, V36I, V47L	-2.24859127304
CACCGCCAGTGGTAT	3:1LMB	M40(, V36(, V47L	-2.15583497800
40	3:1LMB	M40F, V36L V47L	-2.12712086295
20 20	3:1LMB	M40L, V36F, V47I	-2.62168924209
R) (1LMB:3)	3:1LMB	M40F, V36F, V47I	-2.51071104626
	3:1LMB	M40L, V36L, V47I	-2.44530154034
PLTQEQLEDARRIKATYEKKKNELGISQESVADKNGMGQSGVGAL	3:1LMB	M40I, V36F, V47I	-2.40316667712
10 20 30 50	3:1LMB	M40L V36L, V47I	-2.3223649403
	3:1LMB	M40F, V36L, V47I	-2.2076340044
IA L NA Y NA A L L A K I L K V S V E E F S P S I A R E I Y EM Y EA V S	3:1LMB	M40L, V36I, V47I	-1.95816798388
92	3:1LMB	M40L V36L V47I	-1.88346413558
60 70 80 90 92	3:1LMB	M40F, V36I, V47I	-1.8319414518

<u>Overview</u>

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.

<u>Lasergene Packages</u>

<u>Molecular Biology</u>

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

Protein Modeling & Analysis

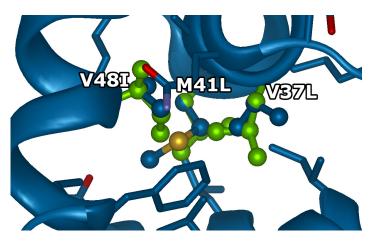
Analyze protein sequence and structure data, predict protein structures, design antibodies, model mutations, and perform hotspot scanning on protein structures.

<u>Genomics</u>

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

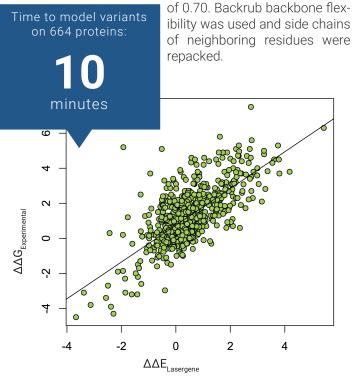
<u>Problem</u>

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.



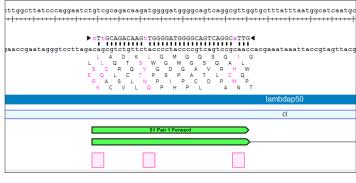
<u>Benchmarks</u>

The Fold-X Blind Test benchmark contains 664 substitution variants, each with an experimentally-determined change in free energy ($\Delta\Delta G$) relative to wild type. The Lasergene Protein Design tool calculated the change in fold stability ($\Delta\Delta E$) for the entire data set in less than 10 minutes on a single work-station with a Pearson's linear correlation coefficient (PCC)



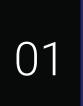
<u>References</u>

- 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



<u>Workflow</u>

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.



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.

Design and mutate primers Create PCR primer pairs at the de

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.



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Create virtual clones

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

<u>Results</u>

Buried core residues can be altered to improve a protein's developability and stability, as is the case with Repressor protein cl. The variant p.(Val37Leu; Met41Leu; Val48lle) (LLI) confers 0.5 kcal/mol more stability than wild type¹. Using the wild type structure², 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