The Industry Expert in Gene Synthesis Solutions

Using Synthesis to Build Multi-Site Libraries and Gene Variants to Improve Protein and Antibody Function

A Better Way to Discovery

Existing tools

New methods

New technologies

BlueHeron® The Gene Synthesis Company

ORIGENE Your Gene Company

BlueHeron®

Founded in 1999 to automate labor-intensive reagent production by combining expertise in:

- Molecular biology
- Chemistry
- Informatics

2001 launched GeneMaker®

- Patented multi-technology platforms established
- Production scale custom gene synthesis services

2007-2010 Gold Standard Industry leader

- The first company to synthesize and deliver a 52KB gene
- The primary supplier for the 1st bacterial genome
- The sole DNA source for the 1st synthetic life by J. Craig Venter Inst.- 1 mega base of DNA synthesized in 1 month

2010 Joined OriGene as a wholly owned subsidiary

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Goal: Efficient pathway to target discovery

Improve Protein Function

Improve Antibody Affinity

Discover New Targets

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Site Saturation vs. Directed Evolution

doi:10.1016/j.jmb.2005.07.020 J. Mol. Biol. (2005) 352, 621–628

JMB

Available online at www.sciencedirect.com

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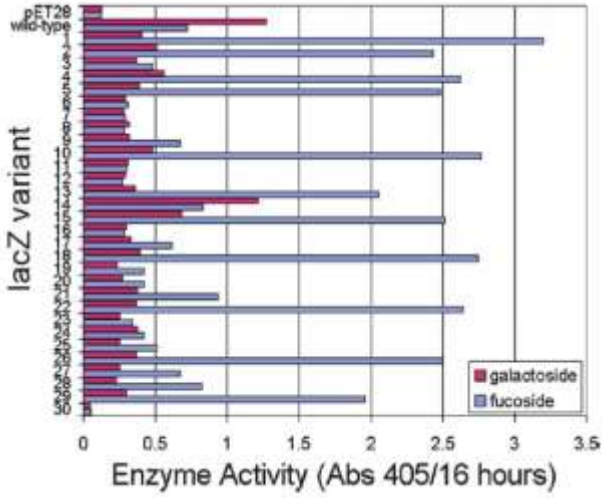
ELSEVIER

Site-saturation Mutagenesis is more Efficient than DNA Shuffling for the Directed Evolution of β-Fucosidase from β-Galactosidase

Monal R. Parikh and Ichiro Matsumura*

Evaluation of Beta-gal activity on a non-native substrate

- **Directed Evolution**
  - 7 iterated cycles of DNA shuffling and screening
  - 39-fold increase in non-native activity
  - 1,000-fold improved discrimination
- **Saturation Mutagenesis**
  - Saturated 3 residues in active site
  - H540V 225-fold higher activity on non-native
  - ~100,000-fold improved discrimination



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Problem: What path to take?

Method	Roadblocks
• **Random mutagenesis** →	Inexpensive but low specificity Methods: error Prone PCR, MutS E. coli strains
• **Recombination** →	Need clones, limited by PCR, limit to mutation loci Method: PCR Shuffling (Stemmer, *et al*. Maxygen)
• **Degenerate oligonucleotides** →	Creates biased pools

Synthesis: A better option

New Option

- **Synthetic gene variants**
  - **Codon optimization**
  - **Simple base substitutions**
  - **Amino acid substitutions**
  - **Variable region substitutions**
  - **Defined Multi-site libraries**

Advantages

Can introduce changes ANYWHERE

Enables ability to encode specific changes (e.g. codon-based)

Individual clones received in an expression vector

Defined timeline and costs

Goal: Efficient pathway to target discovery

Improve Protein Function

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Codon Optimization of Gene Sequence for Protein Expression

Submit:

Amino Acid Sequence

Include or exclude specific DNA motifs

GENEMAKER®

Codon Optimization

Codon usage match

Expression Optimization

Secondary RNA structure minimization

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Goal: Efficient pathway to target discovery

Improve Antibody Affinity

Discover New Targets

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Creating Variants

- **Simple Variants**
  - SNPs, adding new 5’ or 3’ tag or promoter
- **Complex Variants**
  - Multi-site base changes
  - Amino Acid scans and substitutions (R&D development)
- **Variants for Antibody Research**
  - Single Region Variable H/L chain single cassette
  - Dual Region Variable H/L chain swap
- **Variant Libraries- (R&D development)**
  - Complex Defined Variant Pooled Libraries
    - Multi-site, close proximity amino acid libraries
- **Dual Region swaps for antibody discovery**

Complex Variants

Multi-site base changes

ATGTCGAGATCGATTAGAGCGCTCGAATCGATAGCTTAG

ATGTGGAGCTCGATTAGAGCGCTCCAATCGTTAGCTTAG

Varying methods for improving protein function/antibody affinity

- **Structure-based engineering**
  - Requires structure and highly detailed models of the proteins function
- **Mutagenesis and screening or selection**
  - Error-prone PCR
- **Directed Evolution**
  - Mix diversity from a family of native proteins
- **Synthetic Defined Multi-site Libraries**

Antibody Improvement

PEDS Advance Access published May 13, 2008

Protein Engineering, Design & Selection pp. 1–11, 2008
doi:10.1093/protein/gzn027

Rapid discovery and optimization of therapeutic antibodies against emerging infectious diseases

J. Rogers[1†], R.J. Schoepp[2], O. Schröder[1†], T.L. Clements[2], T.F. Holland[1†], J.Q. Li[1†], J. Li[1†], L.M. Lewis[1†], R.P. Dirmeier[1], G.J. Frey[1†], X. Tan[1], K. Wong[1†], G. Woodnutt[1†], M. Keller[1†], D.S. Reed[3], B.E. Kimmel[1†] and E.C. Tozer[1,4]

Traditionally, protection from pathogens can be achieved by either active or passive immunization. Active immunization in which a vaccine is administered to elicit a protective immune response is generally the desired therapeutic goal. Unfortunately, vaccine development can be slow and expens-

- Site saturation mutagenesis of 67 light and heavy chain CDR amino acids = ~1,350 clones
- Multi-site library with five best variants
- Best multi-site clone had two changes,
  - 40-fold higher affinity
  - Neutralized at an 8-fold lower concentration of antibody

Amino Acid Substitutions

- **Non-complex**

MTGPAGCTPTLLACPCGSCULCSLTPATRLCSTLPACGGPLGC

Amino Acid Substitution: Alanine ⟶ Cystein

- **Moderately-Complex**

MTGPAGCTPTLLACPCGSCULCSLTPATRLCSTLPACGGPLGC

Replace each with given number of amino acids (19, 10, 5, etc.)

- **Complex-** R&D Technology Development

MTGPAVCTPTLLACPCGSCULCSLTPATRLCSTLPACGGPLGC

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SINGLE REGION VARIANTS

Fast and economical antibody optimization and design

Figure 1: Define/Design the Variable Region

-Modify amino acid sequence

-Modify nucleotide sequence

-Scalable Projects make a few to hundreds of variants

Figure 2: Newly Synthesized Plasmid

-New Variable Regions will be inserted as requested to form the final plasmid ready for expression or screening

-Backbone vector can be a Customer-provided expression vector

-Delivered as individual, sequence verified clones

Applications:

Antibody Screening

Variant Screening

Benefits:

Design Freedom

Economical Solution

Fast Turnaround

Figure 1:

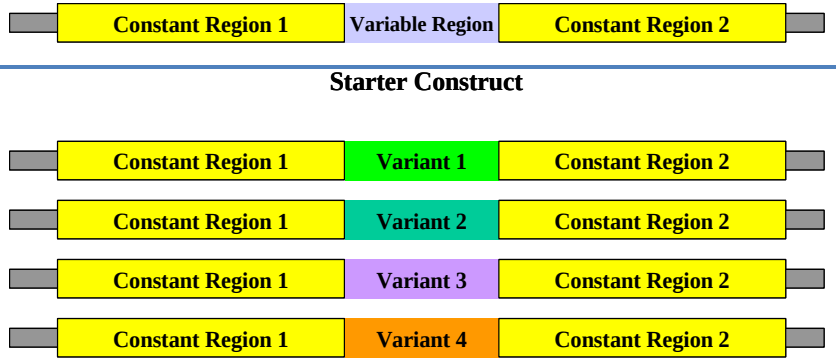
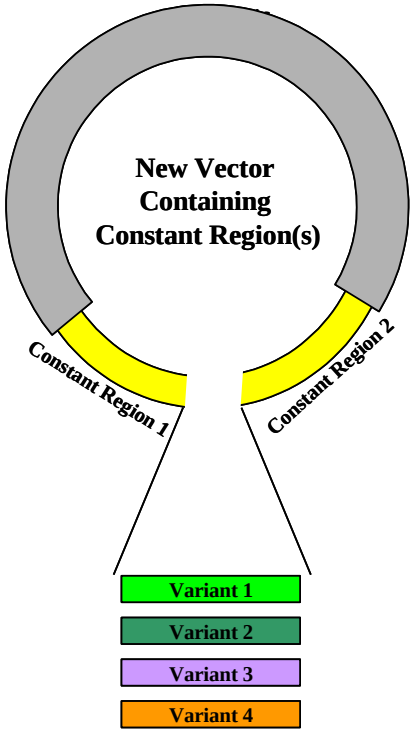


Figure 2:



Your variants in the New Vector containing constant region(s)

BlueHeron® Biotech-Synthesis Application: Multi-site Defined Mutagenesis

Figure 1:

Choose the Amino Acid positions to replace or choose full saturation

Figure 2/3:

Each amino acid will be replaced synthetically

Delivered as individual, sequence verified clones or pooled groups

Advantages:

-Define 1 to 19 amino acids at single or multiple positions

-Sequence verified as individual clones

-Equal representation as pooled groups

-Ability to further define position changes downstream using an existing template for synthesis

-Fast (4-6 weeks)

-Economical

Figure 1: Wild Type

Figure 2: Single amino acid changes

Figure 3: Multiple amino acid changes per location:

ASAKVSCKASGYTFTCSVTAAPQVSAAVSTTLVLQP

AS**L**KVSCKASGYTFTC**L**VTAAPQVSAAVST**L**LVLQP

AS**C**KVSCKASGYTFTC**C**VTAAPQVSAAVST**C**LVLQP

AS**G**KVSCKASGYTFTC**G**VTAAPQVSAAVST**G**LVLQP

AS**K**KVSCKASGYTFTC**K**VTAAPQVSAAVSTT**K**VLQP

AS**T**KVSCKASGYTFTC**T**VTAAPQVSAAVSTT**T**VLQP

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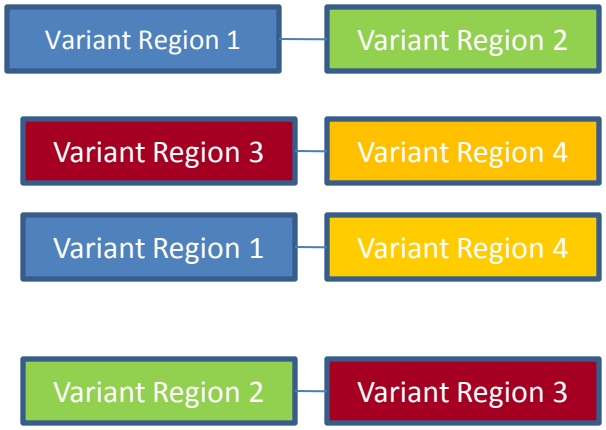
Dual Region Swaps

- Codon Optimize synthesized fragments.
- Synthesize with linkers, tags, promoter regions.
- Assemble in various configurations
- Blue Heron Bio adds synthetic ends to allow for cassette assembly
- Clone into customer-provided vector or BHB standard vector
- Delivered as individual clones- sequence verified

Parts- Synthesized individually

Fragments assembled in various configurations



Defined Multi-site Libraries

- Choose 50-300 amino acid positions in your protein
- Blue Heron delivers a library: 16-19 clones for each position
  - Customer defines number of amino acid or codon substitutions at each position
    - Example- 950 clones for a 50 position library
- Each is cloned and sequence-verified

Advantages of Using synthesis to create a multi-site library

- **Minimize the number of assays**
  - No need for 10X coverage to ensure that you assay each variant once
- **Maximize the value of the information**
  - Assay in triplicate
- **Optimize the time to results**
  - High quality clones + reproducible assays = high quality data

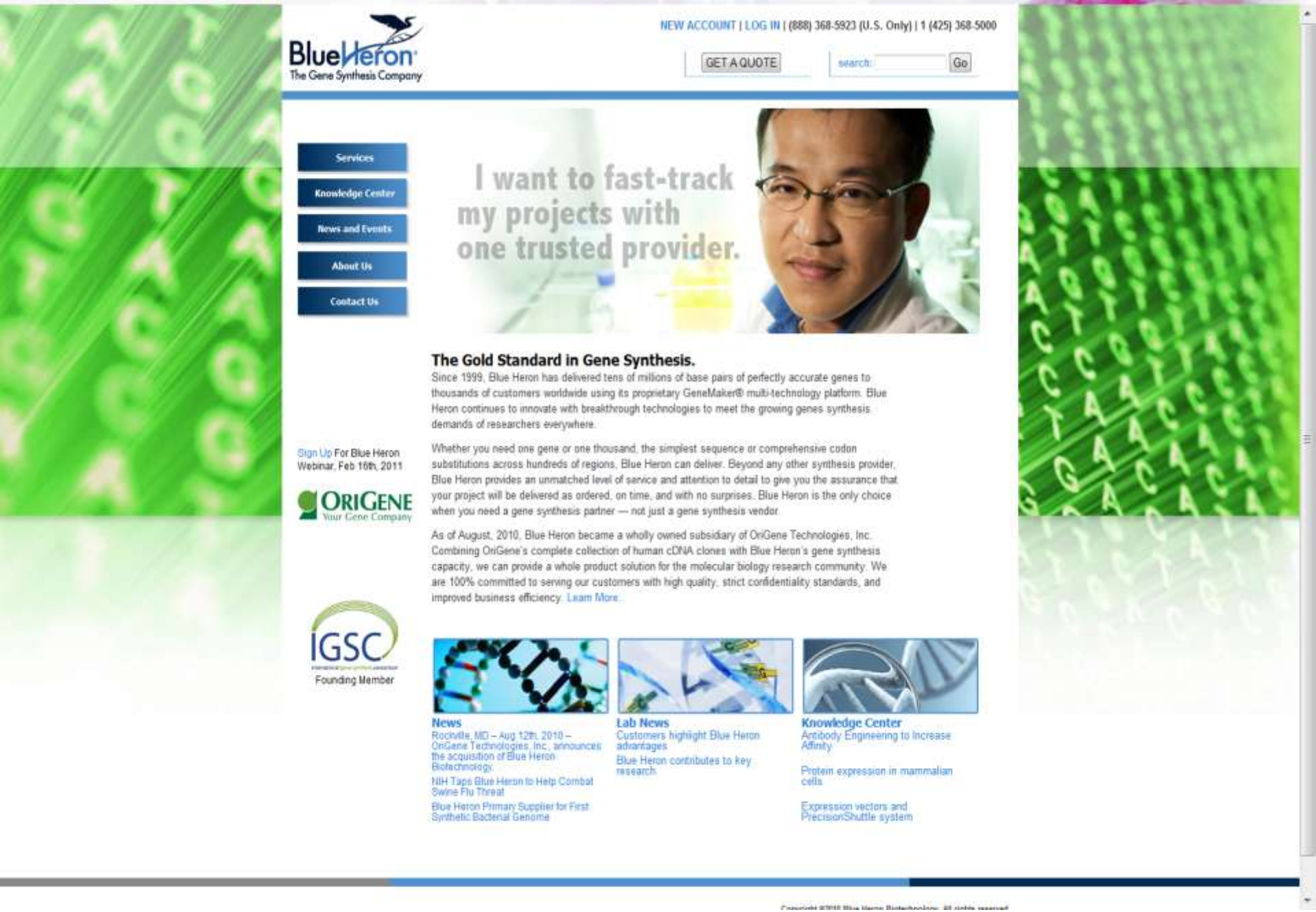
Timeline to Protein Improvement

	Blue Heron	Customer
Defined Saturation Mutagenesis	6-8 weeks	
Perform Assays, analyze data		2-4 weeks
Multi-Site Library	4-6 weeks	
Assay, choose best protein		2-4 weeks

- Improved protein in 3-5 months
- Predictable cost and well-defined results

Well-Defined Path to Improvement

- Small and well-defined number of highly-informative assays
- Crisp decision points
  - Single change improves the activity?
  - Multi-site results positive?
- Predictable costs and timeline
- If a protein is worth your scientists' time and effort, isn't it worth doing the definitive experiment?



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