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®

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
Goal: Efficient pathway to target discovery

Improve Protein Function

Improve Antibody Affinity

Discover New Targets
Site Saturation vs. Directed Evolution

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
Problem: What path to take?

Method

- **Random mutagenesis**

  Roadblocks: Inexpensive but low specificity

  Methods: error Prone PCR, MutS

  E. coli strains

- **Recombination**

  Roadblocks: Need clones, limited by PCR, limit to mutation loci

  Method: PCR Shuffling (Stemmer, *et al.* Maxygen)

- **Degenerate oligonucleotides**

  Roadblocks: 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
Codon Optimization of Gene Sequence for Protein Expression

Submit:
Amino Acid Sequence
Include or exclude specific DNA motifs

Codon Optimization
Codon usage match

Expression Optimization
Secondary RNA structure minimization
Goal: Efficient pathway to target discovery

Improve Antibody Affinity

Discover New Targets
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

ATGTCGAGATCGATTAGAGCGCTCGAATCGATAGCTTTAG

ATGTTGAGGCTCGATTAGAGCGCTCACAATCGTTTAG
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

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
  MTGPAGCTPTLLLACPCGSCULCSLTPATRLCSTLPACGGPLGC
  Amino Acid Substitution: Alanine → Cystein

• Moderately-Complex
  MTGPAGCTPTLLLACPCGSCULCSLTPATRLCSTLPACGGPLGC
  Replace each with given number of amino acids (19, 10, 5, etc.)

• Complex- R&D Technology Development
  MTGPAVCTPTLLLACPCGSCULCSLTPATRLCSTLPACGGPLGC
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
BlueHeron® Biotech-Synthesis Application: Multi-site Defined Mutagenesis

Figure 1: Wild Type
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

<table>
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<tr>
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<th>ALA</th>
<th>ALA</th>
<th>ALA</th>
<th>ALA</th>
<th>ALA</th>
<th>ALA</th>
</tr>
</thead>
</table>

Figure 2: Single amino acid changes

<table>
<thead>
<tr>
<th></th>
<th>Cys</th>
<th>Cys</th>
<th>Cys</th>
<th>Cys</th>
<th>Cys</th>
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</thead>
</table>

Figure 3: Multiple amino acid changes per location:

```
ASAKVSCASKASGYTFTCȘVTAAPQVSAAVSTțLVLQP
ASLKVSCASKASGYTFTCLVTAAPQVSAAVSTțLVLQP
ASCKVSCASKASGYTFTCCVTAAPQVSAAVSTțC'LVLQP
ASGKVSCASKASGYTFTCCVTAAPQVSAAVSTțGLVLQP
ASKKVSCASKASGYTFTCKVTAAPQVSAAVSTțKVLQP
ASTKVSCASKASGYTFTCTVTAAPQVSAAVSTțTVLQP
```
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

<table>
<thead>
<tr>
<th>Step</th>
<th>Blue Heron</th>
<th>Customer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defined Saturation Mutagenesis</td>
<td>6-8 weeks</td>
<td></td>
</tr>
<tr>
<td>Perform Assays, analyze data</td>
<td>2-4 weeks</td>
<td></td>
</tr>
<tr>
<td>Multi-Site Library</td>
<td>4-6 weeks</td>
<td></td>
</tr>
<tr>
<td>Assay, choose best protein</td>
<td></td>
<td>2-4 weeks</td>
</tr>
</tbody>
</table>

- 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?