Agilent Tools for Synthetic Biology

David Weiss
Field Application Scientist
Agilent Technologies
Agilent’s Story
"Innovating the HP Way"

iLab Solutions
A part of Agilent Technologies

STRATAGENE
An Agilent Technologies Division

Cartagenia
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VARIAN

Seahorse Bioscience
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Dako
An Agilent Technologies Company

lab901
More time for discovery™

Multiplicom

LaserGen

Agilent Technologies
Agilent Genomics

- **Expertise in Oligo Library Synthesis (OLS) & Precision Instruments**
  - Market leaders in microarrays and scanners
  - Market leaders in NGS target enrichment (SureSelect & HaloPlex)
  - Market leading Bioanalyzers and Workflow Automation Solutions
  - Market leading Mass Spec and LC-MS

- **Custom Manufacturing of Oligonucleotides**
  - Cost-effective customization of customer designs
  - Microarray fabrication facility offers ability to manufacture oligonucleotides in a massively parallel way independent of sequence
  - High fidelity, longest oligos commercially available

- **Enzyme and Protein Engineering Capabilities**
  - Specialty polymerase enzymes developed with licensed directed evolution technology can be leveraged in specialty applications
  - Mutagenesis methods for developing proteins with programmed genetic changes
  - Cloning and protein expression products

- **Creating a New Legacy in Synthetic Biology**
  - Ongoing collaborations with leading synthetic biology institutions
  - Several new and up-coming product offerings
    - SureVector Next-Generation Cloning
    - QuikChange HT protein engineering kit
    - SureGuide CRISPR/Cas9 Nuclease kit
Agilent leverages the commercial microarray manufacturing capabilities to generate High quality Oligo libraries (OLS) by cleaving and collecting the oligos from the glass slide. OLS uses the 244,000 format for all designs.
Chemical Synthesis: Achieving High synthesis efficiency

1) Coupling
2) Oxidation
3) Deblock

Long length synthesis is achieved by improved cycle yield:
- ↑ coupling efficiency
- ↓ depurination
- ↑ consistency

Depurination side reaction

Repeat n times

150mer complex library

PCR

For Research Use Only. Not for use in diagnostic procedures.
A Goal of Synthetic Biology: Accelerate the SynBio engineering cycle

Agilent participates in all steps:

- **Design**: Databases, algorithms & software
- **Build/Edit**: Gene assembly & genome editing
- **Measure**: Genomics, proteomics, metabolomics
- **Analyze**: Databases, algorithms & software
- **Integration**: Enabling scientific advancement through partnerships and collaborations
Development of Three Key Genomics Platforms for Synthetic Biology

- **Next-Gen Molecular Biology Tools**
  - SureVector
  - QuikChange HT

- **Genome Editing and Engineering**
  - Cas9 Nuclease Kit
  - gRNA Libraries

- **DNA/RNA synthesis**
  - Synthetic Genes and Constructs (partners)
  - OLS Collaborations
SureVector: The world’s first modular vector kit

Assemble up to 7 fragments into a circularized plasmid in a single, 20 minute reaction
SureVector Modular Vector Assembly

- Each component is a substitutable fragment
- Extensive set of standard parts and custom fragments
- Use 1 reaction and 1 system for all of your cloning projects
- Amenable to automation
What comes in the SureVector kit?

• **Core Kit**
  - All DNA fragments shown on the previous page
  - SureVector Enzyme Blend & Buffers
  - XL1-blue Competent Cells
  - 15 reaction kit

• **Entry Kit**
  - Select DNA fragments
  - SureVector Enzyme Blend & Buffers
  - 5 reaction kit

• **Expansion Kits**
  - SureVector E. coli N- and C-terminal Expansion Kits
    - 4 promoters and 6 affinity tags for protein expression in E. coli
  - SureVector E. coli N- and C-terminal Tag Kits
    - 6 solubility and affinity tags for use in bacterial cells for each (N- & C-)
  - SureVector E. coli Promoter Kit
    - 4 promoters for E. coli
  - SureVector Mammalian N- and C-terminal Expansion Kits
    - 3 promoters, 5 tags, and 3 selection markers for use in mammalian cells
  - SureVector Yeast N- and C-terminal Expansion Kits
    - 3 promoters, 5 tags and 3 selection markers for use in yeast cells
A complete cloning solution

<table>
<thead>
<tr>
<th>SureVector System Fragments &amp; Part Numbers</th>
<th>E. coli</th>
<th>Mammalian</th>
<th>Yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Promoters</strong></td>
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<tr>
<td>T7 (G7515A-B)</td>
<td>CMV (G7516A-B)</td>
<td>GAL1 (G7517A-B)</td>
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<tr>
<td>Trp (G7515A-B, G7518B-C)</td>
<td>SV40 (G7516A-B)</td>
<td>CUP1 (G7517A-B)</td>
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<td>Tac (G7515A-B, G7518B-C)</td>
<td>EF-1a (G7516A-B)</td>
<td>ADH1 (G7517A-B)</td>
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<td>Rhamnose (G7515A-B, G7518B-C)</td>
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<td><strong>Tags</strong></td>
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<td>GST (n-term only) (G7515A, G7518D)</td>
<td>6xHis (G7516A-B)</td>
<td>6xHis (G7517A-B)</td>
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<tr>
<td>6xHis (G7515A-B, G7518D-E)</td>
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<td>3xHA (G7517A-B)</td>
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<tr>
<td>CBP (G7515A-B, G7518D-E)</td>
<td>SBP (G7516A-B)</td>
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<td>Thioredoxin (c-term only) (G7515B, G7518E)</td>
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<td>c-Myc (c-term only) (G7515B, G7518E)</td>
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<tr>
<td>HA (c-term only) (G7515B, G7518E)</td>
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<td><strong>Bacterial Selection</strong></td>
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<td>CamR (G7514A, G7518A)</td>
<td>CamR (G7514A, G7518A)</td>
<td>CamR (G7514A, G7518A)</td>
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<td>KanR (G7514A, G7518A)</td>
<td>KanR (G7514A, G7518A)</td>
<td>KanR (G7514A, G7518A)</td>
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<td><strong>Bacterial Origins of Replication</strong></td>
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<td>pUC (G7514A, G7518A-G)</td>
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<td>p15A (G7514A)</td>
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<td>pBR322 (G7514A)</td>
<td>pBR322 (G7514A)</td>
<td>pBR322 (G7514A)</td>
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<td><strong>XP1 Fragments</strong></td>
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<td>XP1 (G7514A, G7518A-G)</td>
<td>yARS (G7514A)</td>
<td>XP1 (G7514A, G7518A-G)</td>
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<td><strong>XP2 Fragments</strong></td>
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<td>LacI (G7514A, G7518A-G)</td>
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<td>URA3 (G7517A)</td>
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<td>Gentamicin (G7516A)</td>
<td>HIS3 (G7517A)</td>
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<tr>
<td></td>
<td>Puromycin (G7516A)</td>
<td>Hygromycin (G7517A)</td>
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<td></td>
<td>NeoR (G7514A)</td>
<td>LEU2 (G7517A)</td>
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<td><strong>Promoter-Tag Fusions</strong></td>
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<tr>
<td>His-T7 (G7514A)</td>
<td>His-CMV (G7514A)</td>
<td>His-GAL1 (G7514A)</td>
<td></td>
</tr>
</tbody>
</table>

Over 100,000 possible combinations!
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SureVector Mechanism

Restriction/ligation methods

Type II restriction enzyme based (Golden Gate)

- Cut with restriction enzyme in the presence of ligase and target vector
- Ligated product accumulates

- Robust
- Requires RE recognition sequence
- Lower efficiency

Overlap annealing-based methods

Gibson assembly

- Recess ends with 5′-3′ exonuclease
- Anneal, extend with polymerase and ligate

SureVector chemistry

- Melt and anneal

- Fast - Isothermal
- Exonuclease optimization has large influence on efficiency

- Fast and most efficient
- Standardized conditions
- Repeat sequences can create multi-insertions

Agilent Technologies
SureVector Assembly Protocol

I. Standard parts: DNA fragments designed for orthogonal assembly

II. Seamless Assembly

III. Transform *E. coli*

IV. Analysis: pick colonies, isolate the assembled DNA construct and analyze by restriction digest, sequencing and PCR
SureVector Design Software

- Explore the possible configurations that can be made with SureVector
- Export reports and sequence files for SureVector constructs for use in external analysis tools
- Determine what SureVector kits you’ll need to build a desired vector
- Order SureVector components using our e-Commerce system

www.agilent.com/genomics/surevectordesign

Vector component selection from drop-down menus, upload or paste your GOI

Vector visualization, sequence, & download (FastA, GenBank, PDF)
<table>
<thead>
<tr>
<th>Available solutions</th>
<th>Service providers</th>
<th>Catalog Vectors</th>
<th>Next-Gen assembly reagents</th>
<th>SureVector</th>
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<tbody>
<tr>
<td>Price Per Vector</td>
<td>$$$$$</td>
<td>$$</td>
<td>$$</td>
<td>$</td>
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<tr>
<td>Already Optimized</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Number vectors available</td>
<td>Unlimited</td>
<td>~1700</td>
<td>Unlimited</td>
<td>&gt;100,000 (unlimited with custom fragments)</td>
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<tr>
<td>Time to new vector</td>
<td>2-6 weeks</td>
<td>2-7 days</td>
<td>3-4 days</td>
<td>&lt;1 day</td>
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<tr>
<td>Number of fragments</td>
<td>-</td>
<td>-</td>
<td>Up to 5</td>
<td>6-11</td>
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<tr>
<td>Web design tool</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Integrated workflow</td>
<td>No</td>
<td>Varies</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>Validated system</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Open IP on end use</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Simultaneous assembly of multiple constructs</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
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</tbody>
</table>
YOUR VISION.
YOUR VECTORS.
Introducing SureVector
Next Gen Cloning Kits

Fast and Flexible
• Less than a day from design to vector
• Assemble new vectors as experimental requirements change

Easy to Use
• Fits cleanly into existing workflows
• Single tube assembly method

Reliable and Precise Assembly
• Extensively tested
• Guaranteed to assemble into a functional vector

www.agilent.com/genomics/surevector
## Mutagenesis

From rational design to random mutagenesis, solutions for any application

Rapidly create point mutations, amino acid substitutions, insertions and deletions

<table>
<thead>
<tr>
<th>Product</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>QuikChange Lightning</strong></td>
<td>- Site-directed mutagenesis&lt;br&gt;- Specially engineered enzymes reduce thermocycling time 75%&lt;br&gt;- More efficient with improved colony yields&lt;br&gt;- One kit for both short and long templates (4-14 Kb)</td>
</tr>
<tr>
<td><strong>QuikChange Lightning Multi</strong></td>
<td>- Fast, reliable and easy protocol&lt;br&gt;- Mutate up to 3 sites simultaneously in a single reaction&lt;br&gt;- Linear amplification strategy reduces errors</td>
</tr>
<tr>
<td><strong>GeneMorphII</strong></td>
<td>- Random Mutations&lt;br&gt;- Mutazyme II DNA polymerase, a novel error prone PCR enzyme blend&lt;br&gt;- More uniform mutational spectrum</td>
</tr>
</tbody>
</table>
QuikChange Lightning Site-directed mutagenesis

1. **Mutant Strand Synthesis**
   Thermal cycling to denature DNA template, anneal Mutagenic Primers, extend primers and ligate nicks with QuikChange enzyme

2. **DpnI Digestion of template**
   Digests methylated and hemimethylated DNA

3. **Transformation**
   Transform mutated ssDNA into XL10-Gold ultracompetent cells, which repairs nicks and synthesizes the complementary strand
Whole protein Single Amino Acid scanning
- Identify functional regions of uncharacterized proteins

Whole protein Codon Saturation Scanning
- Allows precise mapping of functional features at the atomic level

Targeted combinatorial mutagenesis
- Rationally design combinations
- Quick optimization of specified combinations (protein expression & activity)

Codon optimization
Target multiple entire domains in one or more proteins
QuikChange HT
Whole protein single amino acid scanning

QuikScan 1 determines relevant sites for structure, function and stability:

• Separately replaces each amino acid in the wild type mutational region with a particular amino acid
• Often used for Alanine Scanning to quickly identify key functional or structural amino acids
QuikChange HT
Whole protein codon saturation scanning

QuikScan 19 identifies single codon replacements that improve binding, function, or stability:

Codon saturation scanning:
• Systematically replaces each amino acid in the wild type mutational region with all 19 other amino acids
• Results in 19 mutagenic oligos for each amino acid position in the mutational region

50AA x 19mut = 950oligos  1 QuikChange reaction
QuikChange HT
Targeted combinatorial mutagenesis

Use QuikCombine to discover a multisite mutant with improved structure, function, and stability:

- Combine multiple mutants in groups of 1-4 position with defined variation at each site.
- Make up to $1.2 \times 10^5$ libraries for a single 50AA set or combine a few identified variants and validate functional relevance.

**Example 1:** 10 positions 5aa variants per site, all combinations in Groups of 1...4 paired mutations

<table>
<thead>
<tr>
<th>Groups</th>
<th>Variants</th>
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</thead>
<tbody>
<tr>
<td>4</td>
<td>131250</td>
</tr>
<tr>
<td>3</td>
<td>15000</td>
</tr>
<tr>
<td>2</td>
<td>1125</td>
</tr>
<tr>
<td>1</td>
<td>50</td>
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</tbody>
</table>

**Example 2:** 20 positions 19aa variants per site, all combinations in Groups of 1...4 paired mutations

<table>
<thead>
<tr>
<th>Groups</th>
<th>Variants</th>
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</thead>
<tbody>
<tr>
<td>4</td>
<td>6.31E+08</td>
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<tr>
<td>3</td>
<td>7819260</td>
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<td>2</td>
<td>68590</td>
</tr>
<tr>
<td>1</td>
<td>380</td>
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</tbody>
</table>
QuikChange Applications & Publications

Synthetic Biology

Quikgene: a gene synthesis method integrated with ligation-free cloning.
Mao Y1, Lin J, Zhou A, Ji K, Downey JS, Chen R, Han A.

Epigenetics

Accelerated chromatin biochemistry using DNA-barcoded nucleosome libraries.
Nouwen UT1, Billova L2, Müller MM2, Fierz B1, David Y2, Houck-Lecomis B3, Feng Y2, Dann GE2, Muir TW2.

Infectious Disease

RNA viruses can hijack vertebrate microRNAs to suppress innate immunity.
Troyansky DW1, Gardner CL1, Sun G1, Hardow AD2, Wang E2, Chippindale E3, Milner A4, Weaver SQ2, Ryman KD4, Klimstra WJ1.

Cancer

Recurrent activating mutation in PRKACA in cortisol-producing adrenal tumors.
Goh G1, Scholl UP1, Haake JM1, Choi M1, Prasad ML1, Nelson-Williams G1, Kunsman JW1, Korah R1, Sultorp AC1, Dietrich D1, Haase R1, Willenberg HS1, Sidransky E1, Heilman PM1, Akerblom H1, Bölkund P1, Carlino TM1, Liffon RP4.

Genetic Disease

Mutations in TUBG1, DYNC1H1, KIF5C and KIF2A cause malformations of cortical development and microcephaly.

Genome Engineering

CRISPR RNA-guided activation of endogenous human genes.
Maeder ML1, Linder SJ, Casper VM, Fu Y, Ho QH, Joung JK.

Structural Biology

Structural basis of kynurenine 3-monooxygenase inhibition.
Amaral M1, Lew C, Heves DJ, Lama P, Outiero TF, Giordini F, Levs D, Scrutton NS.

Cell Signaling

DNA-repair scaffolds dampen checkpoint signalling by counteracting the adaptor Rad9.
Ohou PY1, Bastos de Oliveira Fil, Liu Y, Ma CJ, Smolka MS.

QuikChange Publications

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PR7000-0240
SureGuide CRISPR Genome Editing Solutions

- **Cas9 Nuclease Kits**
  - Customizable endonuclease specificity

- **High quality, pre-cloned gRNA libraries**

- **Custom gRNA libraries**

- **gRNA Synthesis Kits**
  - Validated synthetic system with enzymes and reagents optimized for use with Cas9 nuclease

- **Online Design Tool**
  - Find gRNAs that target CRISPR sites within any sequence
  - Identify potential off-target candidate sites in the input or selected background
  - Download ready-to-order DNA sequences
1. New invader enters

(A) Adaptation

2. Spacer acquired

Cas genes → CRISPR

3. CRISPR array expanded

- Repeats
- Spacers
How Does CRISPR/Cas9 Work

CRISPR/Cas Schematic Function
How Does CRISPR/Cas9 Work (continued)

NHEJ
(non homologous end joining)

Homologous recombination

CRISPR-A/I
Enabling Life Sciences Research in Complex Diseases

- Cancer
- Cardiovascular disease
- Neurological disorders
- Metabolic disorders
- Autoimmune disorders

Genome sequencing has yielded 1000s of disease associated candidate genes

Understanding the functions and roles of these genes has been challenging

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SureGuide *in vitro* offering

Programmable nuclease use in lieu of Restriction Enzymes and PCR cloning
Discovery Using Guide RNA Libraries

Systematically find phenotypes associated with genes or combination of genes

Arrayed libraries

- Each position contains a different guide (RNA or genetically encoded)
- Targeted gene identified by position
- Only as many wells as there are different guides
- May allow identification of “synthetic lethals”

- Large (genome wide) libraries are difficult
- Likely to require robotics
- When unselected transfection is used as delivery form (e.g. direct delivery of synthetic guide RNA or charged CAS9 complexes) variations between wells need to be monitored

Pooled Libraries

Introduce guide cassettes into host cell

Select for phenotype

- Genes identified by selection for phenotype and sequencing of gRNA
- Requires genetic delivery of guide RNA
- guide RNA / Cas9 expression maintained through selection process
- Integrated guide cassettes lead to secondary mutation event
- Requires large libraries and screens to reduce sampling bias

Current Agilent SureGuide CRISPR Approach
CRISPR Pooled Functional Screening Library Workflow

LIBRARY GENERATION

Library Design
- List of Guide Sequences
  - Pool of Cell Library (DNA)
  - Pooled Guide Library

SureVector Cloning
- Plasmid Library Containing Guides
  - Lentiviral Particles Introduced with Cas9

Transduced Cells
- Lentiviral sgRNA Library
  - Engineered Pool of Cells
    - Cells Passing Selection Prepped for Sequencing

Readout by NGS
- Read Abundance Mapped to Guide Targets
  - Hit ID

CRISPR-Cas9 SCREEN
- Screen Assay
  - VEHICLE
  - TREATMENT
Lentiviral Delivery Workflow

- Viral delivery can efficiently introduce CRISPR sequences into cells
- Requires integration for expression
- Can be titered to Multiplicity of Infection (MOI) of 1 or less

Lentiviral Preparation

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SureGuide CRISPR gRNA Libraries

**Ready-to-Package**

- Catalog gRNA Libraries
  - Plasmid Library
  - GeCKOv2
  - Human and Mouse
  - Cloned into lentivirus vector with hU6 promoter

**Ready-to-Clone**

- Custom Human & Mouse gRNA Libraries
  - Pre-amplified oligo library
  - User defined subset or designed
  - Human and mouse
  - Compatible with SureVector cloning

**Ready-to-Amplify**

- Custom User Defined Libraries
  - Unamplified oligo pool
  - Any species, any cloning method
  - Entirely custom by user design

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The Agilent Advantage

Fidelity

Per cycle yields during synthesis

- Fewer errors with longer constructs
- Improves library quality
- Reduces screening time and false negatives

CRISPR Libraries

- More correct constructs
- 92% vs. 77%
- Fewer avg. errors/kb
- 4 vs. 12

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The Agilent Advantage

Improved Representation

- Fewer missed guides
- Less under-represented population

Plasmid libraries with 90/10 ratios < 3
- Find all the hits in your screen, not just the over-represented ones

<table>
<thead>
<tr>
<th>Libraries</th>
<th>Missed Guides</th>
<th>90th/10th percentile</th>
<th>95th/5th percentile</th>
<th>99.5th/0.5th percentile</th>
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<td>2.32</td>
<td>3.64</td>
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<tr>
<td>pSGL-008J (NF)</td>
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<td>2.47</td>
<td>3.38</td>
<td>11.06</td>
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<tr>
<td>pSGL-009J (V2)</td>
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<td>2.38</td>
<td>3.19</td>
<td>3.83</td>
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<tr>
<td>pSGL-128J-dc (SJJ)</td>
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<td>1.99</td>
<td>2.64</td>
<td>8.39</td>
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<tr>
<td>GeCKO (Broad)</td>
<td>7</td>
<td>8.73</td>
<td>16.00</td>
<td>NA</td>
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<tr>
<td>GeCKO (Competitor)</td>
<td>39</td>
<td>5.29</td>
<td>9.03</td>
<td>68.40</td>
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<tr>
<td>GeCKO (Competitor) expanded</td>
<td>204</td>
<td>6.09</td>
<td>11.98</td>
<td>332.90</td>
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</table>
YOUR VISION. YOUR VECTORS.

Assembly

Transformation

Selectable marker
Expansion slot 1
Gene of interest
Origin of replication
Expansion slot 2
Promoter/Tag

annealing
flap cleavage
ligation

Novel overlap-based assembly method using flap cleavage-mediated strand joining
Comparison of Gibson and SureVector cloned libraries

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<thead>
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<th></th>
<th>OLS</th>
<th>Gibson</th>
<th>SureClone</th>
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<td>meas.</td>
<td>hypoth.</td>
<td>meas.</td>
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<tr>
<td>missed guides</td>
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<td>0</td>
<td>9</td>
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<tr>
<td>10th percentile</td>
<td>56</td>
<td>63</td>
<td>29</td>
</tr>
<tr>
<td>20th percentile</td>
<td>62</td>
<td>67</td>
<td>38</td>
</tr>
<tr>
<td>80th percentile</td>
<td>88</td>
<td>83</td>
<td>84</td>
</tr>
<tr>
<td>90th percentile</td>
<td>94</td>
<td>87</td>
<td>99</td>
</tr>
<tr>
<td>10/90 ratio</td>
<td>1.679</td>
<td>1.381</td>
<td>3.414</td>
</tr>
<tr>
<td>20/80 ratio</td>
<td>1.419</td>
<td>1.239</td>
<td>2.211</td>
</tr>
</tbody>
</table>

Missed Guides: 0.01%

10/90 ratio: 2.5
Subsequent Workflow: Compilation of Sequencing

<table>
<thead>
<tr>
<th>cloning</th>
<th>OLS</th>
<th>Gibson plasmid</th>
<th>SureClone plasmid</th>
</tr>
</thead>
<tbody>
<tr>
<td>total clones</td>
<td>N/A</td>
<td>1.17E+06</td>
<td>4.13E+06</td>
</tr>
<tr>
<td>fraction empty</td>
<td>N/A</td>
<td>0.100</td>
<td>0.043</td>
</tr>
<tr>
<td>projected total complete clones</td>
<td>N/A</td>
<td>1.05E+06</td>
<td>3.95E+06</td>
</tr>
<tr>
<td>average clones/guide</td>
<td>N/A</td>
<td>16.3</td>
<td>61.2</td>
</tr>
</tbody>
</table>

83% no mismatches
140 bases
61%
77%

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Subsequent Workflow: Plasmid Libraries - Transformation and Amplification

- Assembly reaction
- Cleanup (SPRI beads)
- Transformation (electroporation)
- Selection in very low melt agar (2-3 days at 30 °C)
- Harvest by centrifugation

Expansion in liquid culture
The Agilent Advantage

Easy Customization

- Catalog quality, pricing and lead-times
- Any site, any species, any application
- Ready-to-clone libraries for mammalian system (with design service)
- Ready-to-amplify libraries for total flexibility
Get to your oligo pools in one easy step.
# Catalog Libraries

<table>
<thead>
<tr>
<th></th>
<th>Human GeCKOv2</th>
<th>Mouse GeCKOv2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part Number</td>
<td>G7553A</td>
<td>G7554A</td>
</tr>
<tr>
<td>Format</td>
<td>Ready-to-package (plasmid library)</td>
<td>Ready-to-package (plasmid library)</td>
</tr>
<tr>
<td>Delivery</td>
<td>Lentiviral (pSGLenti)</td>
<td>Lentiviral (pSGLenti)</td>
</tr>
<tr>
<td>Targets</td>
<td>Human exons</td>
<td>Mouse exons</td>
</tr>
<tr>
<td>Application</td>
<td>Genome-wide knock-out screening</td>
<td>Genome-wide knock-out screening</td>
</tr>
<tr>
<td>Content</td>
<td>Pre-defined (no user design input)</td>
<td>Pre-defined (no user design input)</td>
</tr>
<tr>
<td>Promoter</td>
<td>U6</td>
<td>U6</td>
</tr>
<tr>
<td>Reporter</td>
<td>GFP</td>
<td>GFP</td>
</tr>
<tr>
<td>Selection</td>
<td>Puromycin</td>
<td>Puromycin</td>
</tr>
</tbody>
</table>

Sequence-verified plasmid library in pSGLenti vector
Ready-to-package into lenti-particles for mammalian cell delivery

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# GeCKOv2 Libraries

<table>
<thead>
<tr>
<th></th>
<th>Wang et al. 2 library</th>
<th>Shalem et al. 1 GeCKOv1 library</th>
<th>Koike-Yusa et al. 3 library</th>
<th>GeCKOv2 human library</th>
<th>GeCKOv2 mouse library</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Species</strong></td>
<td>Human</td>
<td>Human</td>
<td>Mouse</td>
<td>Human</td>
<td>Mouse</td>
</tr>
<tr>
<td><strong>Genes targeted</strong></td>
<td>7,114</td>
<td>18,080</td>
<td>19,150</td>
<td>19,050</td>
<td>20,611</td>
</tr>
<tr>
<td><strong>Targeting constructs per gene</strong></td>
<td>10</td>
<td>variable (typically 3 or 4)</td>
<td>variable (typically 4 or 5)</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td><strong>miRNA targeted</strong></td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>1,864</td>
<td>1,175</td>
</tr>
<tr>
<td><strong>Targeting constructs per miRNA</strong></td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td><strong>Control (nontargeting) sgRNA</strong></td>
<td>100</td>
<td>None</td>
<td>None</td>
<td>1,000</td>
<td>1,000</td>
</tr>
<tr>
<td><strong>Total sgRNA constructs</strong></td>
<td>73,151</td>
<td>64,751</td>
<td>87,897</td>
<td>123,411</td>
<td>130,209</td>
</tr>
<tr>
<td><strong>Viral plasmid vector</strong></td>
<td>Dual Vector: sgRNA only</td>
<td>Single Vector: Cas9 &amp; sgRNA (lentiCRISPRv1)</td>
<td>Dual Vector: sgRNA only</td>
<td>Dual Vector: sgRNA only</td>
<td>Dual Vector: sgRNA only</td>
</tr>
</tbody>
</table>
## Custom Libraries (Ready-to-clone)

<table>
<thead>
<tr>
<th></th>
<th>Human/Mouse 10k</th>
<th>Human/Mouse 30k</th>
<th>Human/Mouse 60k</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Part Number</strong></td>
<td>G7555A#010</td>
<td>G7555A#030</td>
<td>G7555A#060</td>
</tr>
<tr>
<td><strong>Format</strong></td>
<td>Ready-to-clone (linear DNA, pre-amplified)</td>
<td>Ready-to-clone (linear DNA, pre-amplified)</td>
<td>Ready-to-clone (linear DNA, pre-amplified)</td>
</tr>
<tr>
<td><strong>Delivery</strong></td>
<td>Designed for lentivirus (pSGLenti)</td>
<td>Designed for lentivirus (pSGLenti)</td>
<td>Designed for lentivirus (pSGLenti)</td>
</tr>
<tr>
<td><strong>Targets</strong></td>
<td>User defined</td>
<td>User defined</td>
<td>User defined</td>
</tr>
<tr>
<td><strong>Application</strong></td>
<td>Custom knock-out screening, CRISPRa/i, other CRISPR applications</td>
<td>Custom knock-out screening, CRISPRa/i, other CRISPR applications</td>
<td>Custom knock-out screening, CRISPRa/i, other CRISPR applications</td>
</tr>
<tr>
<td><strong>Number of guides</strong></td>
<td>Up to 10,000</td>
<td>10,001-30,000</td>
<td>30,001-60,000</td>
</tr>
<tr>
<td><strong>Content</strong></td>
<td>User defined (design service available)</td>
<td>User defined (design service available)</td>
<td>User defined (design service available)</td>
</tr>
</tbody>
</table>

**Compatible with SureVector library cloning kit (G7556A)**

*Cloning kit includes all reagents to generate an ultra-high quality plasmid library*
Custom Amplified Library Configuration

TAACTTGAAGTATTTCGAATTCTGGCTTTATATATCTTGTGGAAAGGACGAAACACCG
nnnnnnnnnnnnnnnnnnnn
GTTTTAGAGCCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAGT

U6 overlap
Designable region (up to 50 nucleotides)
Scaffold

Up to 50
60 bps Cloning overlap
PCR amplify
60 bps Cloning overlap
Purify (SPRI beads)

[FU]

0 100 200

4 20 40 80 150 [nt]
## Custom Libraries (Ready-to-amplify)

<table>
<thead>
<tr>
<th></th>
<th>Custom 5k</th>
<th>Custom 25k</th>
<th>Custom 50k</th>
<th>Custom 100k</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part number</td>
<td>G7555V#005</td>
<td>G7555B#025</td>
<td>G7555B#050</td>
<td>G7555B#100</td>
</tr>
<tr>
<td>Format</td>
<td>Ready-to-amplify (oligo pool)</td>
<td>Ready-to-amplify (oligo pool)</td>
<td>Ready-to-amplify (oligo pool)</td>
<td>Ready-to-amplify (oligo pool)</td>
</tr>
<tr>
<td>Delivery</td>
<td>Any (user defined)</td>
<td>Any (user defined)</td>
<td>Any (user defined)</td>
<td>Any (user defined)</td>
</tr>
<tr>
<td>Targets</td>
<td>Any (user defined)</td>
<td>Any (user defined)</td>
<td>Any (user defined)</td>
<td>Any (user defined)</td>
</tr>
<tr>
<td>Application</td>
<td>Any (user defined)</td>
<td>Any (user defined)</td>
<td>Any (user defined)</td>
<td>Any (user defined)</td>
</tr>
<tr>
<td>Number of guides</td>
<td>Up to 5,000</td>
<td>5,001-25,000</td>
<td>25,001-50,000</td>
<td>50,001-100,000</td>
</tr>
<tr>
<td>Content</td>
<td>Any (user defined)</td>
<td>Any (user defined)</td>
<td>Any (user defined)</td>
<td>Any (user defined)</td>
</tr>
<tr>
<td>Available length</td>
<td>Up to 200 bp</td>
<td>Up to 200 bp</td>
<td>Up to 200 bp</td>
<td>Up to 200 bp</td>
</tr>
</tbody>
</table>

Includes amplification kit with all required reagents to generate a ready-to-clone library.
What matters in the end – what fraction of my library is correct?

- ≈ 90% of all clones contain no detectable error
- ≈ 5% of clones contain a deletion
- ≈ 2% of clones may contain a point mutation
- For retroviral/lentiviral vectors the recombination rate across the LTRs is ≈ 5%

<table>
<thead>
<tr>
<th></th>
<th>Average</th>
<th>Plasmid Libraries</th>
<th>OLS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td>Stdev</td>
</tr>
<tr>
<td>n</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>deletion across LTR</td>
<td>5.1%</td>
<td>2.3%</td>
<td>NA</td>
</tr>
<tr>
<td>no error</td>
<td>87.8%</td>
<td>1.1%</td>
<td>92.6%</td>
</tr>
<tr>
<td>point mutation</td>
<td>2.1%</td>
<td>0.4%</td>
<td>2.2%</td>
</tr>
<tr>
<td>deletions</td>
<td>4.9%</td>
<td>0.8%</td>
<td>5.2%</td>
</tr>
</tbody>
</table>

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When pooled together it’s a fantastic pool

- Fidelity
- Representation
- Customization
When pooled, it's a fantastic pool

Representation is key to Agilent pooled libraries
Sample CRISPR Applications

Gene Therapy 2.0

Disease Model Building

Discovery

What’s Important

Specificity
- Guides will generate cleavage events at off-target sites
- The targeted cell may differ from the reference genome

Efficacy
- Not all guides are created equal. Some guides fail to generate measurable cleavage events

• Choose better guides (algorithms to predict off-site cleavage events)
• Keep exposure times short
• Complementation-dependent cleavage
• Modify guides
• Alternate CAS9 systems

• Choose better guides (algorithms to predict off-site activity and cleavage efficiency)