GeneCopoeia CRISPR sgRNA Libraries For Functional Genomics

GeneCopoeia, Inc.

Presenter:
Ed Davis, Ph.D.
Senior Application Scientist
GeneCopoeia, Inc.

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GeneCopoeia products & services
Outline

- Introduction: Functional genomics & CRISPR
- What are CRISPR sgRNA libraries?
- Development of previous libraries
- GeneCopoeia pathway- & gene group-specific CRISPR sgRNA libraries
Introduction: Functional genomics & CRISPR

- What are CRISPR sgRNA libraries?
- Development of previous libraries
- GeneCopoeia pathway- & gene group-specific CRISPR sgRNA libraries
1) Safe harbor knock-in ORF clones are used for transgene insertion at the human AAVS1 or mouse ROSA26 genomic sites using CRISPR/Cas-9 or TALEN. Safe harbor site integration ensures that transgenes will be transcriptionally active and expressed at consistent levels, and presents no known adverse effects on cells caused by disruption of the sites.

2) IndelChek™ kit for TALEN and CRISPR/Cas-9 functional validation.
CRISPR genome editing technology

CRISPR-Cas9: RNA-guided endonuclease

- 20 nt single guide RNA (sgRNA) guides Cas9 nuclease to target site.
- Requires NGG “PAM” site immediately downstream of sgRNA target sequence.
- Cas9-RNA complex makes DSB 3-4 nt upstream of PAM.
- Target almost any gene in any cell

Ran, et al. (2013). Nature Protocols 8, 2281
Targeted DNA editing by DSB induction
Applications for genome editing

Knockout via NHEJ

indels in human EMX1 locus

WT
5’-..GGAGGAAGGGCCTGAGTCCGAGCAGAAG-AAGAAGGGCTC-..-3’

D1
GGAGGAAGGGCCTGAGTCCGAGCAGAAG--AGAAGGGCTC

+1
GGAGGAAGGGCCTGAGTCCGAGCAGAAGAAAGAAGGGCTC

D2
GGAGGAAGGGCCTGAGTCCGAGCAGAAG---GAAGGGCTC

D3
GGAGGAAGGGCCTGAGTCCGAGCAGAAG----AAGGGCTC

D6
GGAGGAAGGGCCTGAGTCCGAGCAGAAG--------GGCTC

# GeneCopoeia CRISPR services

## Pathway & gene group sgRNA libraries

<table>
<thead>
<tr>
<th>Library name</th>
<th>Number of genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Innate kinases &amp; ubiquitin ligases</td>
<td>239</td>
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<td>Nuclear hormone receptors</td>
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</tr>
<tr>
<td>Custom</td>
<td>Made-to-order</td>
</tr>
</tbody>
</table>

Available as bacteria, DNA, or lentiviral particles either:
A. Pooled
B. Individually arrayed
What are CRISPR sgRNA libraries?

- Collections of hundreds-to-thousands of plasmids encoding sgRNAs
- In presence of Cas9, create DSBs intended to knock genes out
- Knock out many genes simultaneously
- Target whole genome or smaller groups of genes
Applications for CRISPR sgRNA libraries

Previously carried out using shRNA libraries

- Drug target discovery (e.g. Cooper & Brockdorff, 2013. *Development* **140**, 4110)

- Reporter assays (e.g. Sethi, *et al.*, 2012. *PLoS ONE* **7**.)


- Others (Drug target validation, *etc.*)
## CRISPR or RNAi?

### Knock out vs. Knock down

<table>
<thead>
<tr>
<th>Method</th>
<th>Change genetic code</th>
<th>Change expression level</th>
<th>Knock down</th>
<th>Knock out</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRISPR</td>
<td>✔</td>
<td>✔</td>
<td></td>
<td>✔</td>
</tr>
<tr>
<td>RNAi</td>
<td></td>
<td>✔</td>
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<td></td>
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</table>
How are CRISPR sgRNA libraries used?

**Important:** Need “readout” (phenotype or assay)

**A**
- Infect cell line with pooled sgRNA library lentiviral particles
- Screen plates for response of phenotype of interest
- PCR and/or sequence individual sgRNA(s)

**B**
- Infect cell line in single wells with individual sgRNA library lentiviral particles
- Screen plates for response of phenotype of interest
- Identity of each individual sgRNA in each well is known without PCR or sequencing

[GeneCopoeia](https://www.gene-copoeia.com)
How are CRISPR sgRNA libraries used?

**Example readout # 1: Cell death**

- Step 1: Infect cell line with pooled sgRNA lentiviral particles
- Step 2: After a few days of cell division, prepare DNA from mixed population of living cells and either:
  - Perform Sanger sequencing on selected sgRNAs
  - OR
  - Perform deep sequencing, analyze representation of all sgRNAs before and after infection
- sgRNAs that are under-represented in living cells represent candidates for genes necessary for biological process of interest.
- Step 3: Test individual candidate sgRNAs in assay to validate hits
How are CRISPR sgRNA libraries used?

Example readout # 2: Drug resistance

- Step 1: Infect cell line with pooled sgRNA lentiviral particles
- Step 2: Apply drug of interest to selected cells and allow cell division. Then either:
  - Perform Sanger sequencing on selected sgRNAs
  - Perform deep sequencing, analyze representation of all sgRNAs before and after infection

- sgRNAs that are over-represented in pool represent candidates for genes necessary for drug mechanism of action.
- Step 3: Test individual candidate sgRNAs in assay to validate hits
How are CRISPR sgRNA libraries used?

Example readout # 3: Reporter assay

- Step 1: Infect cell line with pooled sgRNA lentiviral particles
- Step 2: FACS sort cells to enrich for those expressing a fluorescent reporter (e.g. GFP). Then either:
  - Perform Sanger sequencing on selected sgRNAs
  - OR
  - Perform deep sequencing, analyze representation of all sgRNAs before and after infection
  - sgRNAs that are under- or over-represented in pool represent candidates for genes necessary biological process of interest.
- Step 3: Test individual candidate sgRNAs in assay to validate hits
Introduction: Functional genomics & CRISPR

What are CRISPR sgRNA libraries?

Development of previous libraries

GeneCopoeia pathway- & gene group-specific CRISPR sgRNA libraries
Previously-developed libraries

Genetic Screens in Human Cells Using the CRISPR-Cas9 System

Wang, et al. (2014). Science 343, 80

Genome-Scale CRISPR-Cas9 Knockout Screening in Human Cells

Shalem, et al. (2014). Science 343, 84

- Used pools of lentiviral-delivered sgRNAs for genome-scale forward mutagenesis screens in human cells
Previously-developed libraries

Pool of sgRNAs targeting 7,300 genes

Wang, et al. (2014). Science 343, 80
Previously-developed libraries

Plx drug resistance screen in A375 melanoma cells

All 6 validated individually for Plx resistance

Shalem, et al. (2014). Science 343, 84
Introduction: Functional genomics & CRISPR

- What are CRISPR sgRNA libraries?

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Pathway & gene group sgRNA libraries

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Available as bacteria, DNA, or lentiviral particles either:

A. Pooled
B. Individually arrayed
Advantages of the GeneCopoeia sgRNA libraries

- Individually constructed and cultured in *E. coli* before pooling. Makes possible use in pools or as individual sgRNAs.

- Pools limited to 150 sgRNAs, ensuring excellent representation of each sgRNA

- Sequence verification provides high quality of each sgRNA

- Small library sizes: Reduces time and cost of screening
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Library pooling strategy

1. Plasmids expressing individual sgRNAs constructed separately & sequenced
2. Plasmids expressing individual sgRNAs cultured in *E. coli* separately
3. *E. coli* cultures are pooled
4. Plasmid DNA is prepared from pooled *E. coli*
5. Lentiviral particles are prepared from pooled DNA
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Library pooling strategy

Number of sgRNAs in each pool is limited to 150
GeneCopoeia sgRNA libraries

“Dual-use” lentiviral vectors

pCRISPR-LvSG03
GeneCopoeia sgRNA libraries

sgRNA library considerations

Best to use Cas9-expressing stable cell line for transducing with sgRNA libraries
GeneCopoeia sgRNA libraries

sgRNA library considerations

Best to use Cas9-expressing stable cell line for transducing with sgRNA libraries

Cas9 integrated at AAVS1 with Hygro:

Cas9 integrated at AAVS1 with Neo:

Can purchase pre-made Cas9 cell lines, order custom Cas9 cell line, or purchase DIY Safe Harbor clones
How do I order sgRNA libraries from GeneCopoeia?
Related products & services

Premade Cas9-expressing stable cell lines

- Stable cell lines with constitutively-inducible-expressing Cas9
- Have pre-made lines, or can have us integrate Cas9 in your cell line
- Donor clones available for DIY stable cell line creation
- Cas9 integrated at Safe harbor locus for high expression and insertion without consequences
- Ideal for sgRNA library screening or validation
Related products & services

Safe Harbor cloning kits

Features

- Human AAVS1 & mouse Rosa26 sites ensure transcription-competency of the transgenes & present no known adverse effects on cells.

- Safe Harbor integration provides low copy number of transgene & close to physiological-level expression.

- Can use with TALEN or CRISPR.
Features

- Over 40,000 sequence-verified human & mouse ORFs available
- Inserted between AAVS1 or Rosa26 sites for ready safe harbor integration using TALEN or CRISPR
Some applications for Safe Harbor knockin ORF clones:

- Rescue of a knockout or mutagenesis phenotype
- Overexpression of a fusion tagged protein
- Expression of a gene from a different species (e.g. human gene in a mouse)
Related products & services

Lentiviral production service & packaging kits

- Packaging of lentiviral-based clones into ready-to-use particles
- Ideal for CRISPR lentiviral clones
- Can package individual CRISPR clones, or CCI/outside libraries
GeneCopoeia sgRNA libraries

GeneCopoeia Technical Note: sgRNA libraries

Genome Editing: Applications For GeneCopoeia CRISPR sgRNA Libraries

Ed Davis, Ph.D.

Biomedical researchers are enjoying a Renaissance in functional genomics, which aims to use a wealth of DNA sequence information—most notably, the complete sequence of the human genome—to determine the natural roles of the genes encoded by the genome. As a result, biochemical networks and pathways will be better understood, with the hope of leading to improved disease treatments.

A major approach of functional genomics is to ablate gene function, by either “knockdown” (reduction) or “knockout” (complete elimination). Since 2012, researchers have turned increasingly to CRISPR (clustered, regularly interspaced, short palindromic repeats) for functional genomics studies. CRISPR’s simple RNA-guided mechanism provides a quick, convenient, and relatively low-cost method for many applications, from gene knockout, in-frame fusion tagging, mutagenesis, and transgene knockin. Several groups recently adapted CRISPR for high-throughput knockout applications, by developing large-scale CRISPR sgRNA libraries. GeneCopoeia recently launched a number of smaller, pathway- and gene group-focused CRISPR sgRNA libraries, which offer several key advantages over the whole-genome libraries. In this Technical Note, we discuss the merits and applications for CRISPR sgRNA libraries, how to use...

Download from:
Summary

- CRISPR sgRNA libraries have tremendous potential for functional genomics screening.

- Applications for CRISPR sgRNA libraries include novel drug target discovery, drug target validation, phenotypic screening, reporter assays, & more.

- GeneCopoeia sgRNA libraries advantages over previously developed libraries include sequence validation, individual clone construction, and individual culturing in *E. coli*.

- GeneCopoeia CRISPR sgRNA libraries are available in a range of formats, and have many powerful companion products and services.
Upcoming webinar!

GeneCopoeia CRISPR & TALEN Technology For Genome Modification

Wednesday, May 13, 2015 9:00 am EDT (GMT-0:400)

Register here:

https://attendee.gotowebinar.com/register/4429962400977907202
Another upcoming webinar!

GeneCopoeia Genome Editing Stable Cell Line Services

Wednesday, May 27, 2015 12:00 pm EDT (GMT-0:400)

Register here:

https://attendee.gotowebinar.com/register/7724986543678467842
Thank you!

Any questions?

Call: 1-866-360-9531 x227
Email: edavis@genecopoeia.com
Or visit us on the web:
www.genecopoeia.com