GeneCopoeia CRISPR sgRNA Libraries For Functional Genomics

GeneCopoeia, Inc.

Presenter:

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

April 29, 2015

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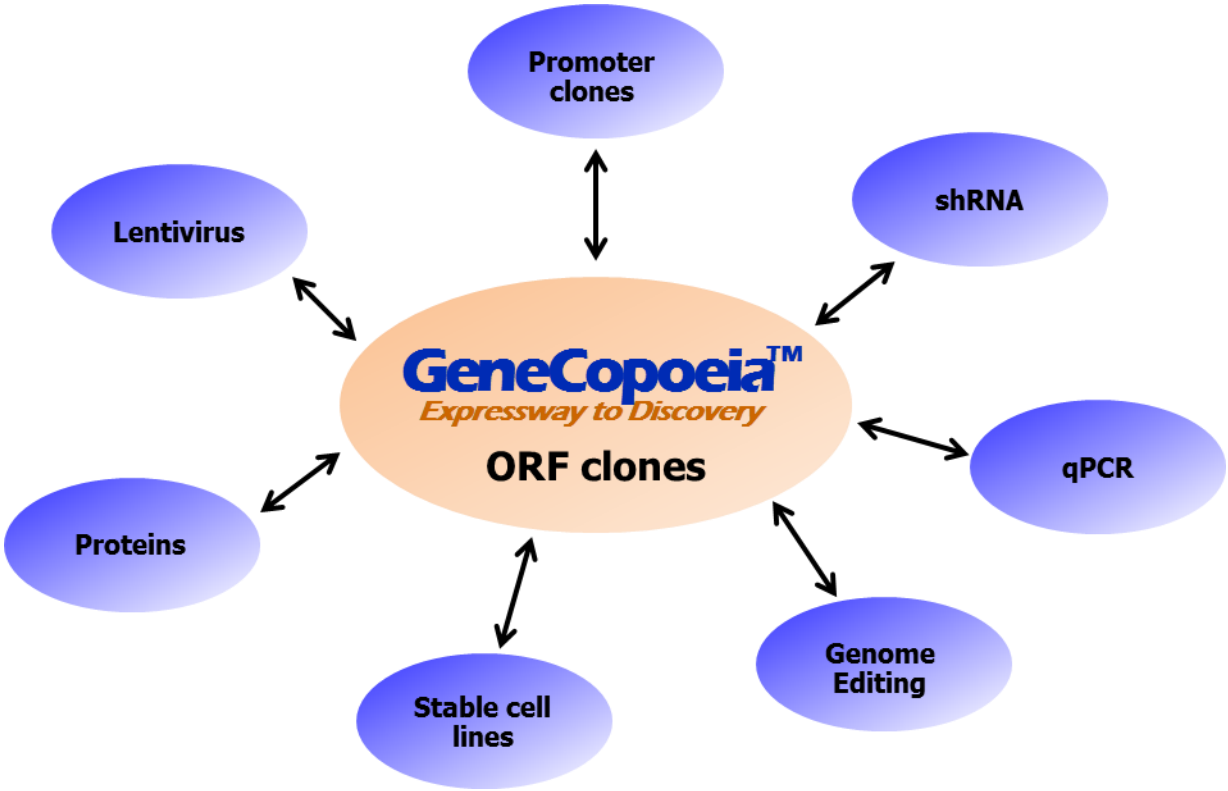
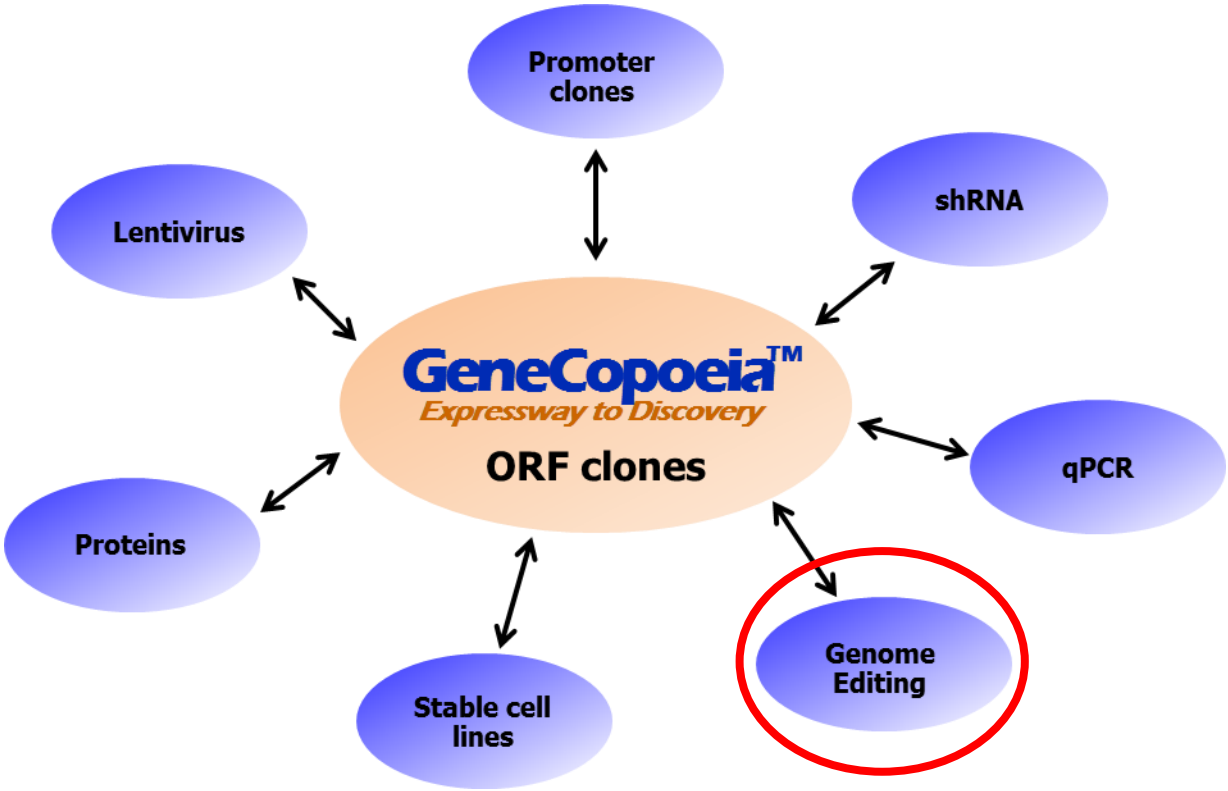
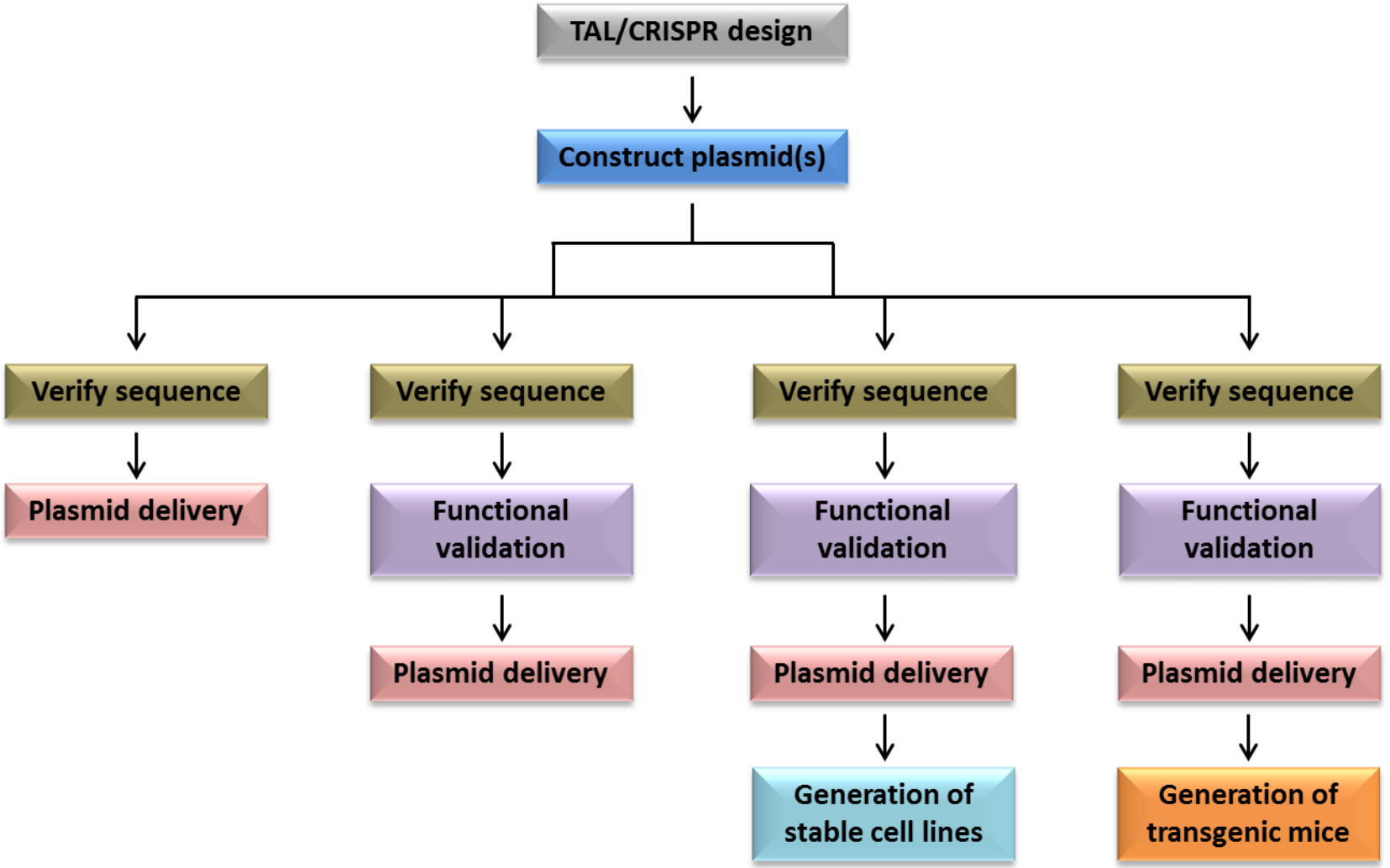
GeneCopoeia products & services



GeneCopoeia products & services



GeneCopoeia genome editing services



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Outline

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

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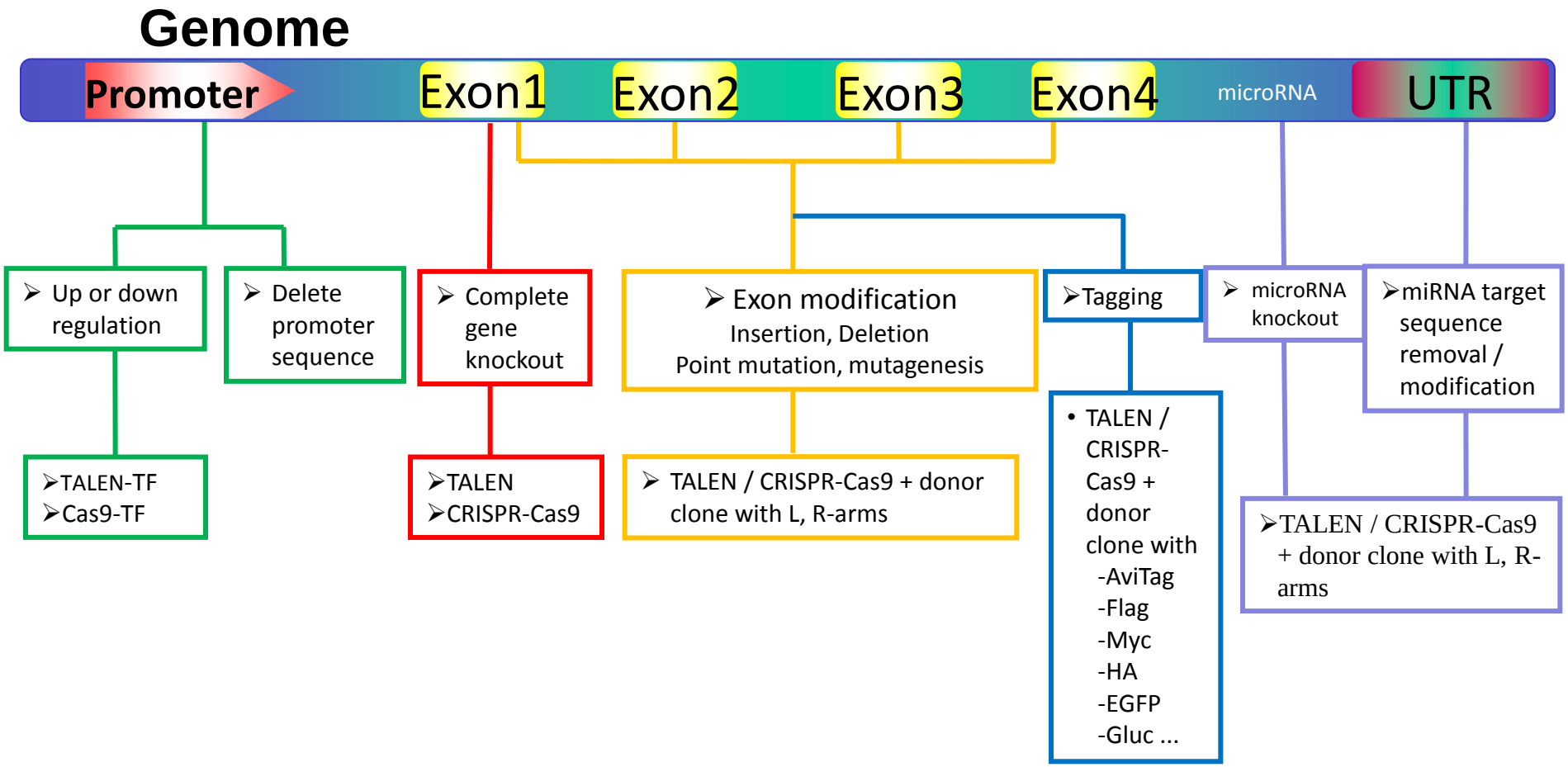
Functional genomics



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.

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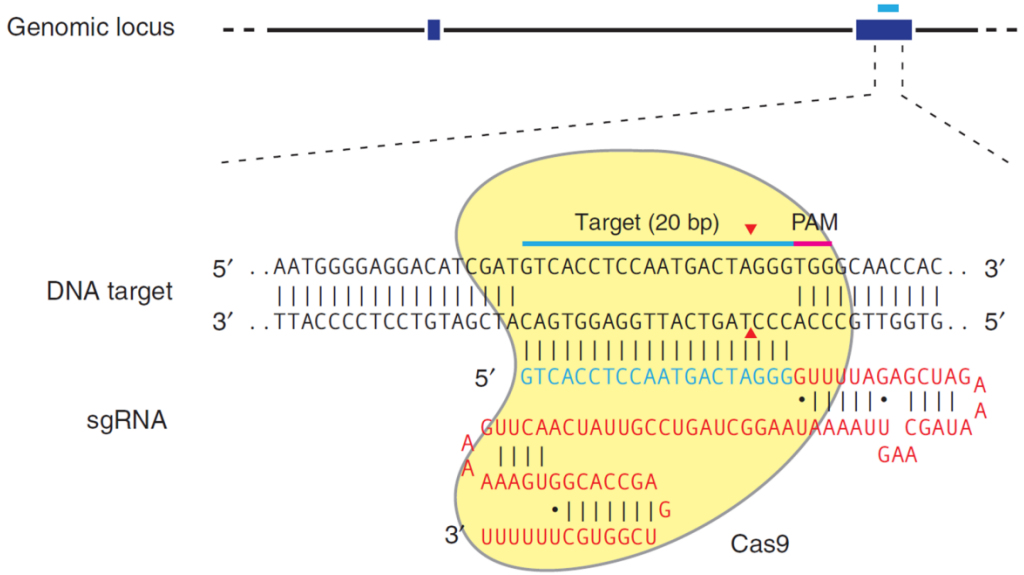
CRISPR genome editing technology

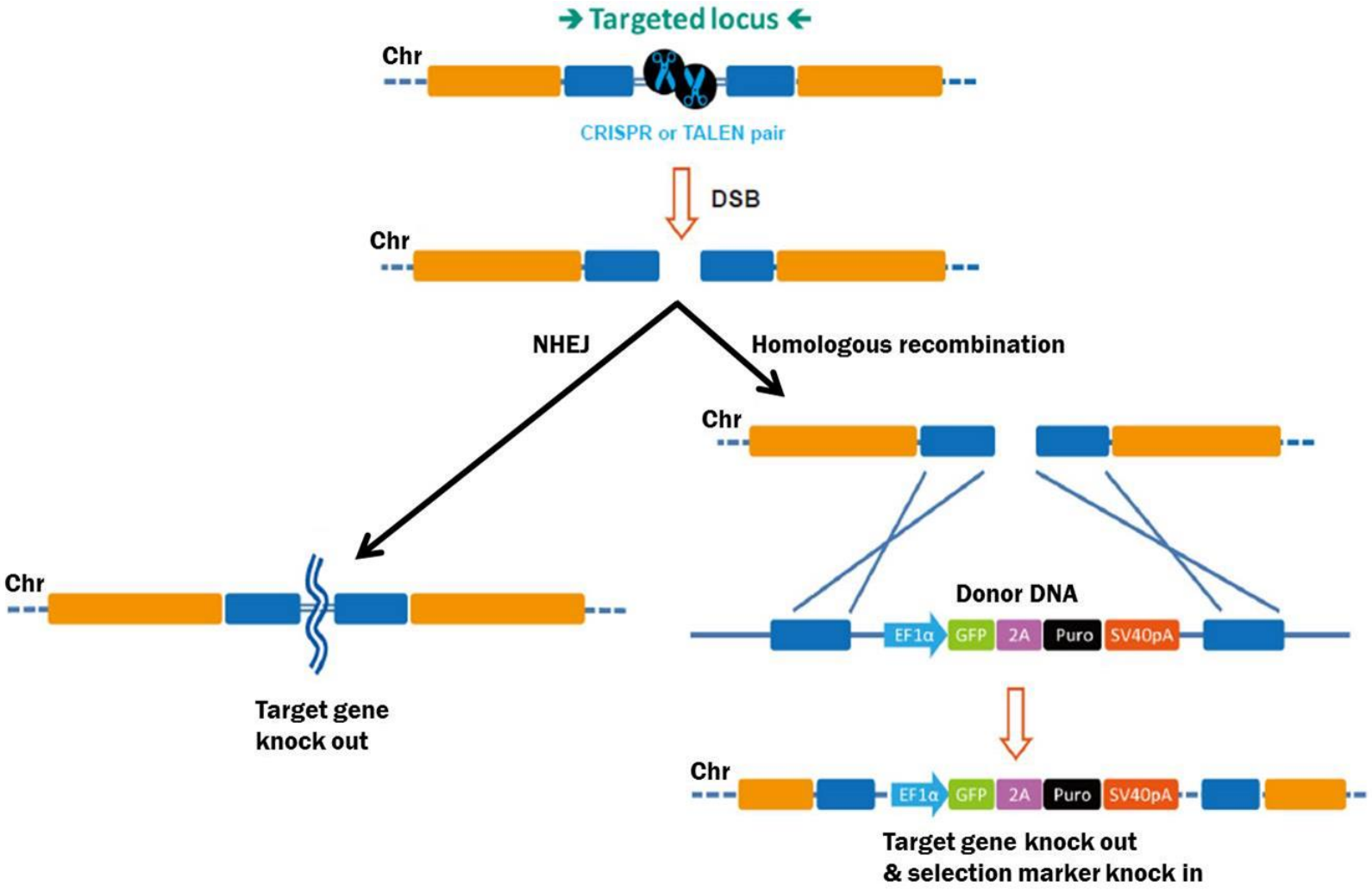
CRISPR-Cas9: RNA-guided endonuclease



Ran, et al. (2013). Nature Protocols 8, 2281

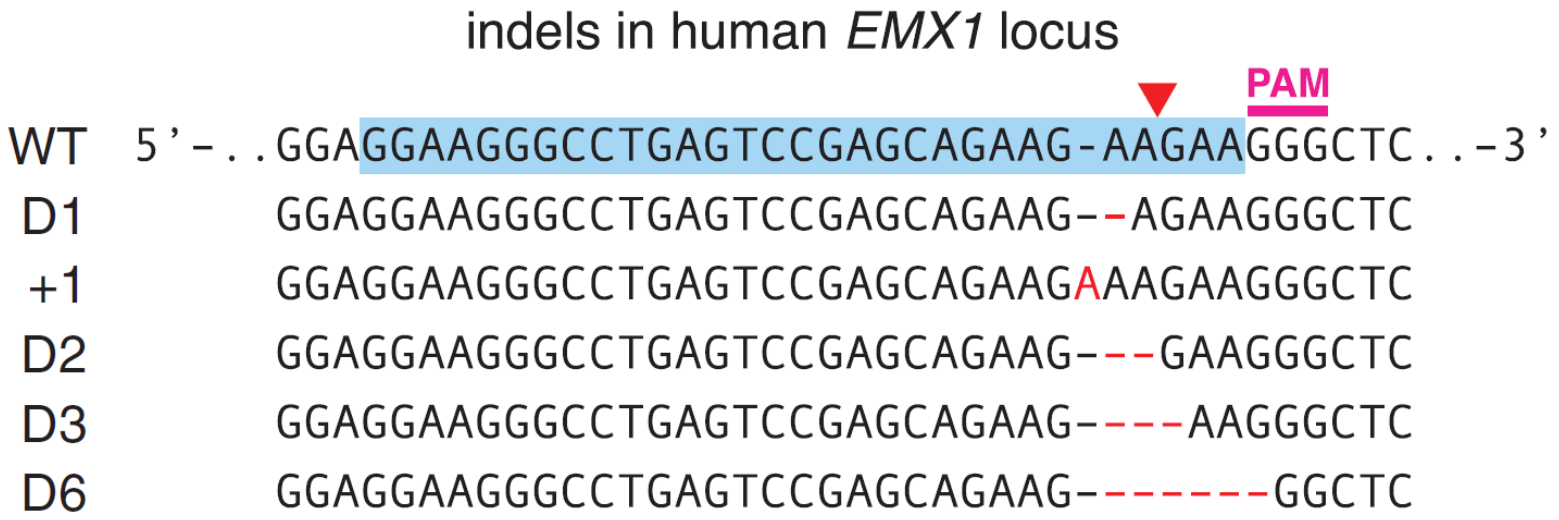
- 20 nt single guide RNA (sgRNA) guides Cas9 nuclease to target site.
- Requires NGG "PAM" site immediately downstream of sgRNA target sequence.
- Cas9-RNA complex makes DSB 3-4 nt upstream of PAM.
- Target almost any gene in any cell

Targeted DNA editing by DSB induction



Applications for genome editing

Knockout *via* NHEJ



Cong, et al. (2013). Science 339, 819

GeneCopoeia CRISPR services

Pathway & gene group sgRNA libraries

Library name	Number of genes
Innate kinases & ubiquitin ligases	239
Nuclear hormone receptors	118
Tumor metastasis genes	57
Oncogenes	288
Tumor suppressor genes	231
Protein kinases	658
Key genes in 50 pathways	139
Custom	Made-to-order

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. *PLoSONE* **7**.)
- Loss-of function phenotypic screening (*e.g.* Zhang, *et al.*, 2014. *Proc Natl Acad Sci U S A* **110**, 12361.
- Others (Drug target validation, *etc.*)

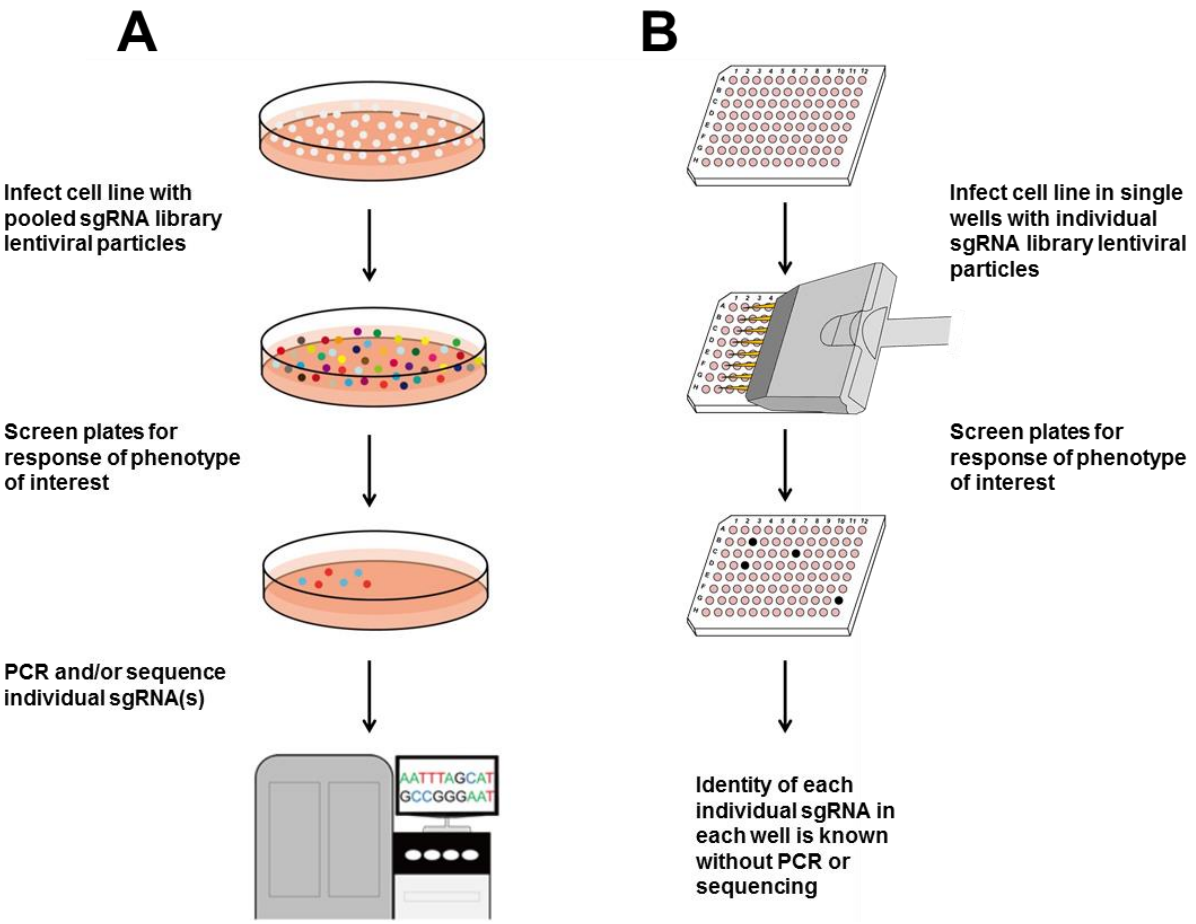
CRISPR or RNAi?

Knock out vs. Knock down

Method	Change genetic code	Change expression level	Knock down	Knock out
CRISPR	✔	✔		✔
RNAi		✔	✔	

How are CRISPR sgRNA libraries used?

Important: Need "readout" (phenotype or assay)



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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

OR

  - 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

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- 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

Tim Wang,[1,2,3,4] Jenny J. Wei,[1,2] David M. Sabatini,[1,2,3,4,5]*† Eric S. Lander[1,3,6]*†

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

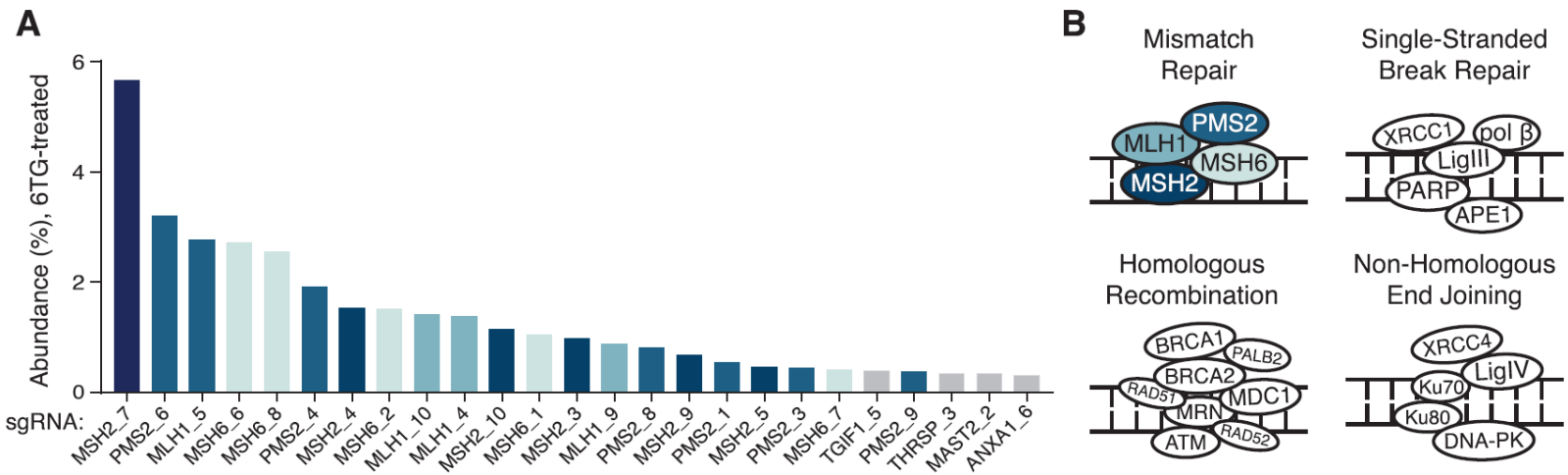
Genome-Scale CRISPR-Cas9 Knockout Screening in Human Cells

Ophir Shalem,[1,2]* Neville E. Sanjana,[1,2]* Ella Hartenian,[1] Xi Shi,[1,3]
David A. Scott,[1,2] Tarjei S. Mikkelsen,[1] Dirk Heckl,[4] Benjamin L. Ebert,[4] David E. Root,[1]
John G. Doench,[1] Feng Zhang[1,2]†

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



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

Pool of sgRNAs targeting 7,300 genes

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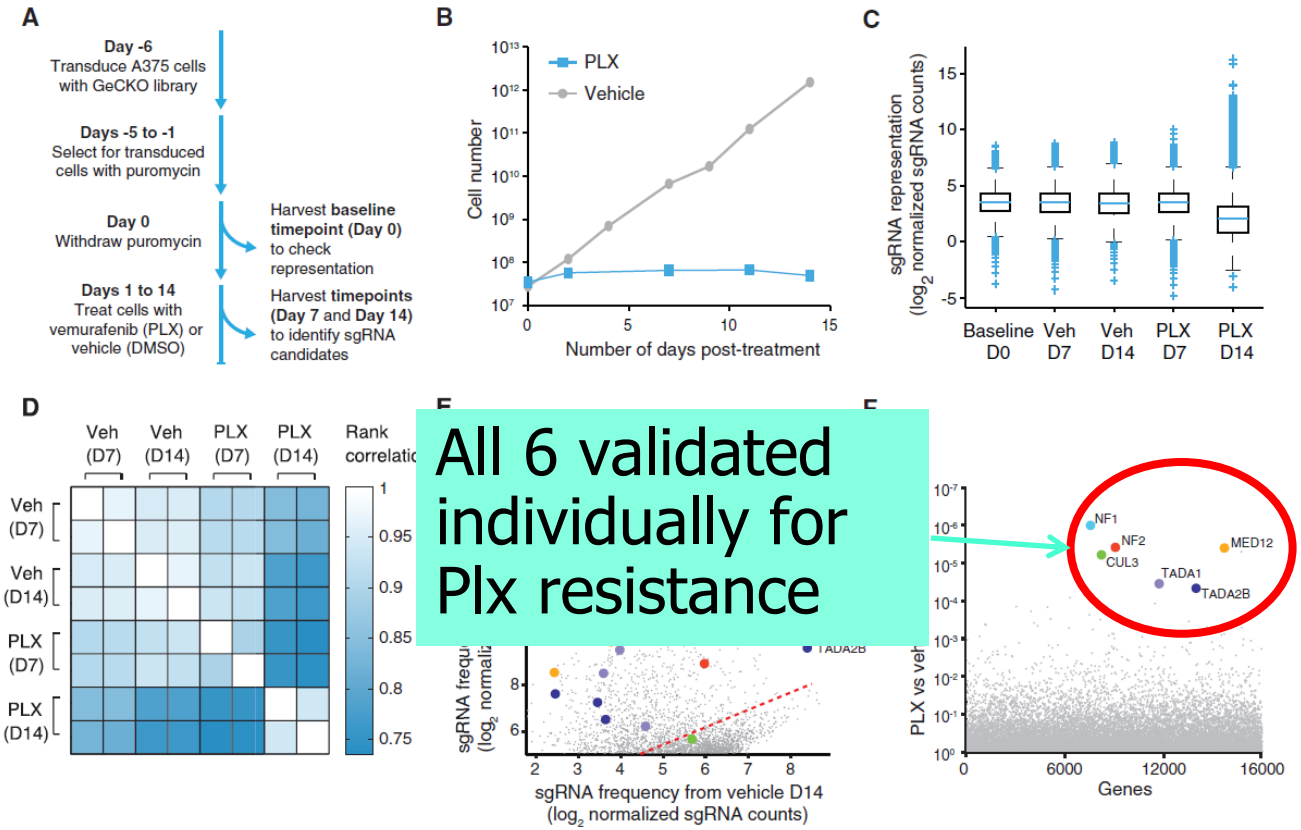
Previously-developed libraries

Plx drug resistance screen in A375 melanoma cells



All 6 validated individually for Plx resistance

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

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GeneCopoeia CRISPR services

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Available as bacteria, DNA, or lentiviral particles either:

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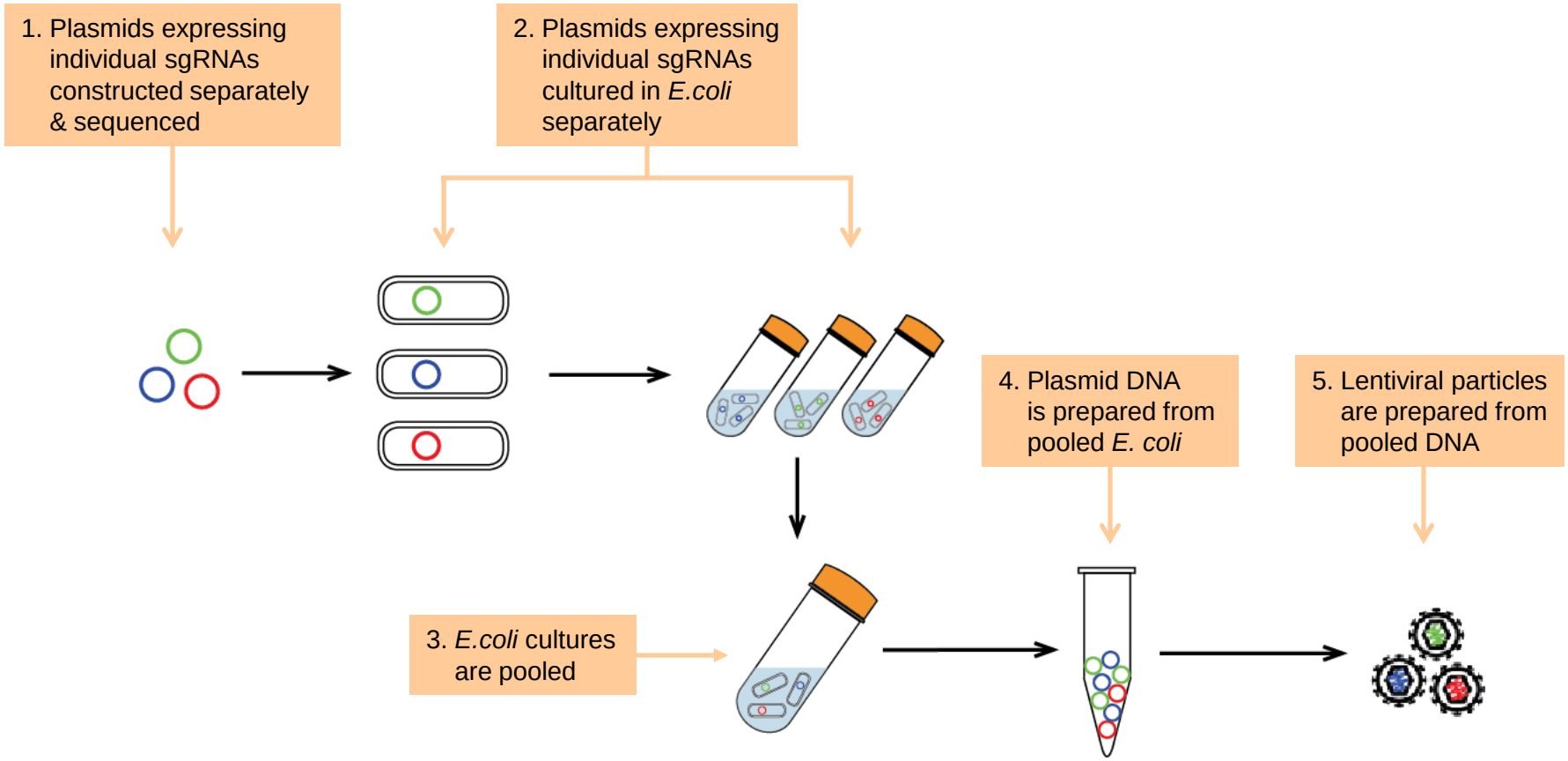
GeneCopoeia CRISPR services

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

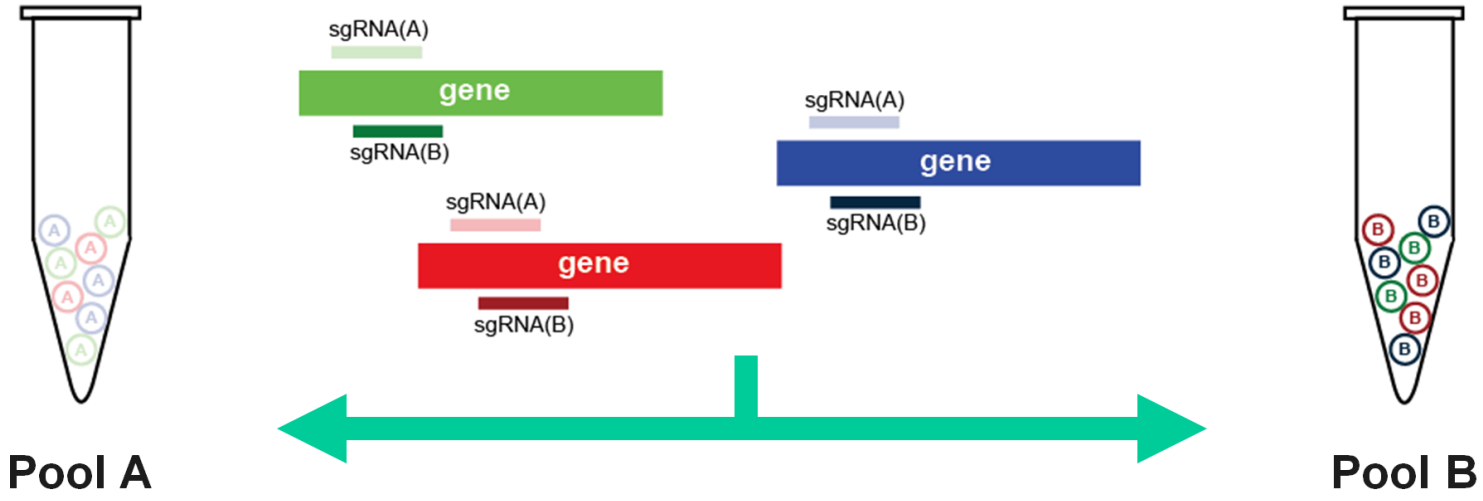
GeneCopoeia CRISPR services

Library pooling strategy



GeneCopoeia CRISPR services

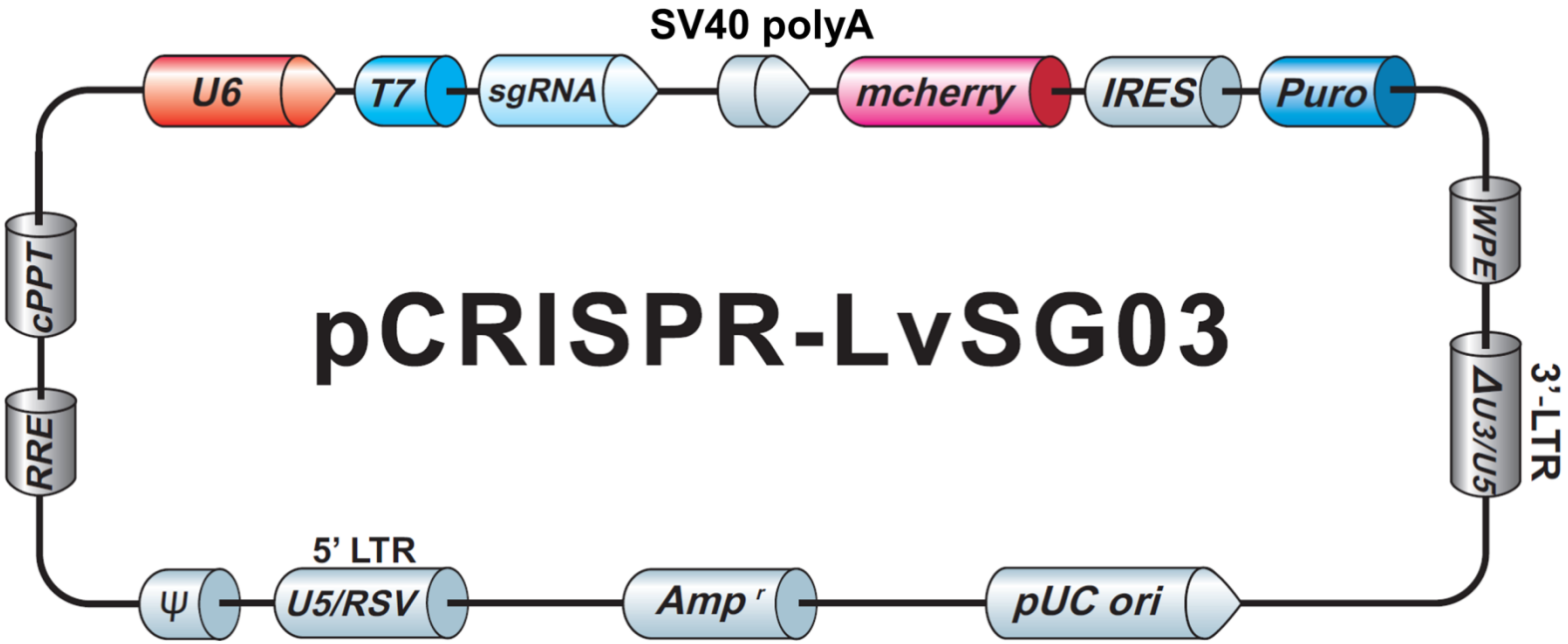
Library pooling strategy



Number of sgRNAs in each pool is limited to 150

GeneCopoeia sgRNA libraries

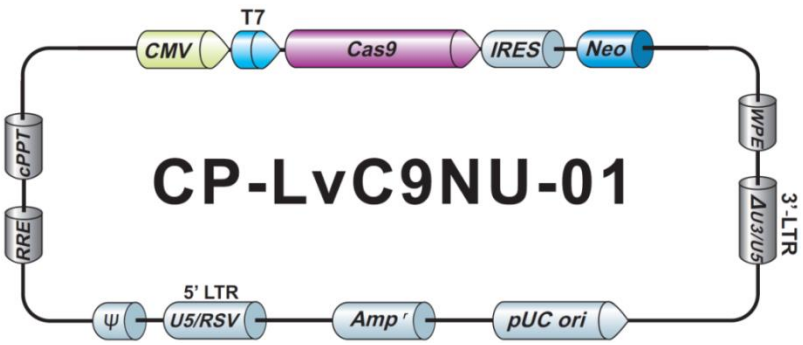
"Dual-use" lentiviral vectors

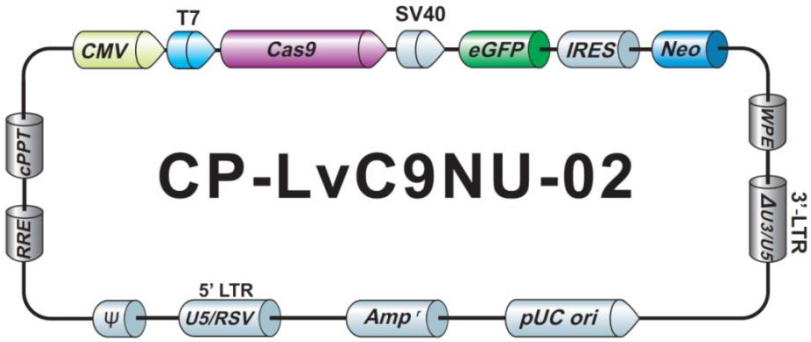


GeneCopoeia sgRNA libraries

sgRNA library considerations

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



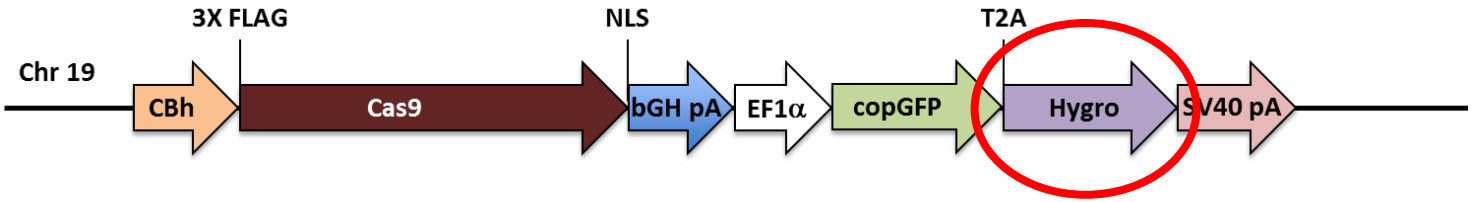


GeneCopoeia sgRNA libraries

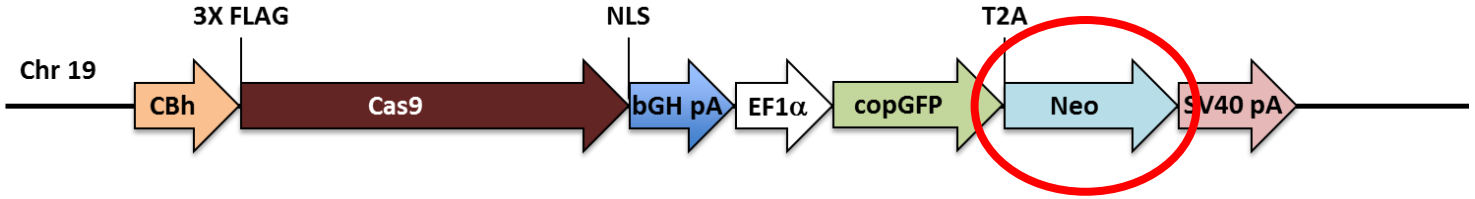
sgRNA library considerations

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

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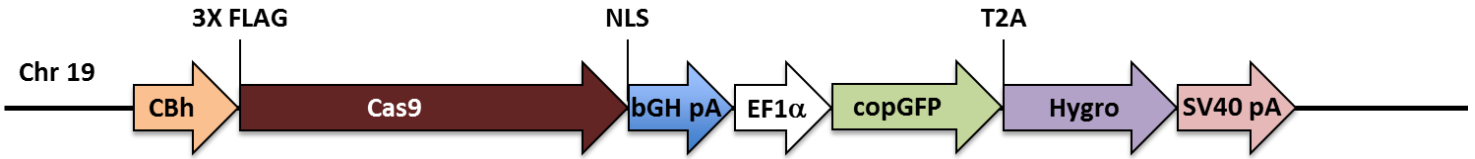
GeneCopoeia sgRNA libraries

How do I order sgRNA libraries from GeneCopoeia?

Related products & services

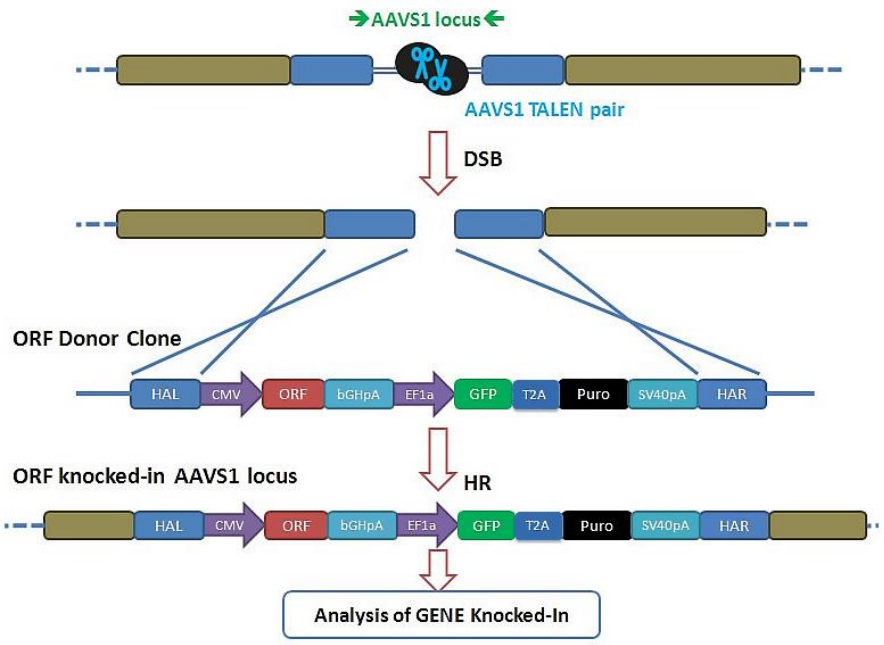
Premade Cas9-expressing stable cell lines



- 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

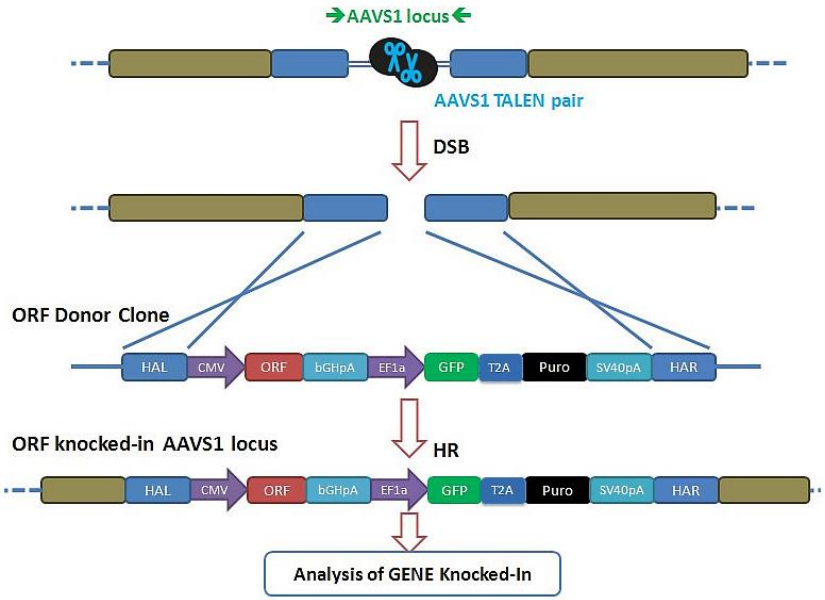


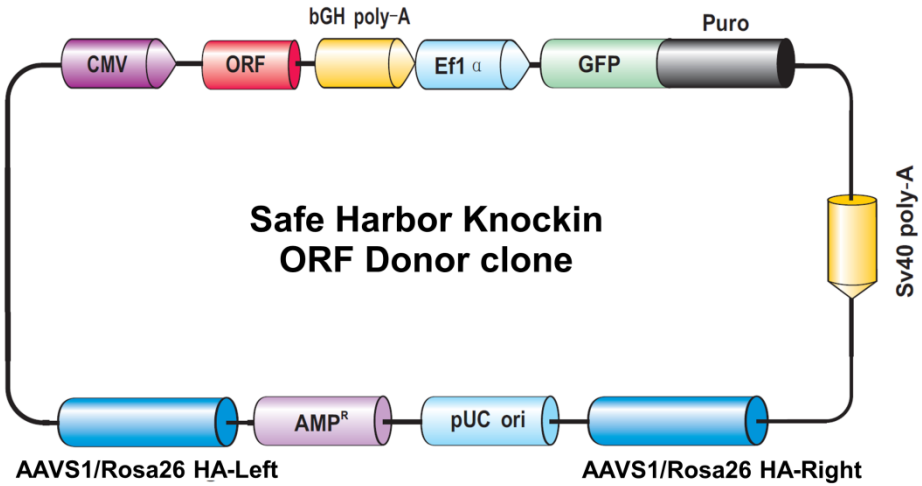
Features

- 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

Related products & services

Safe Harbor knockin ORF clones





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

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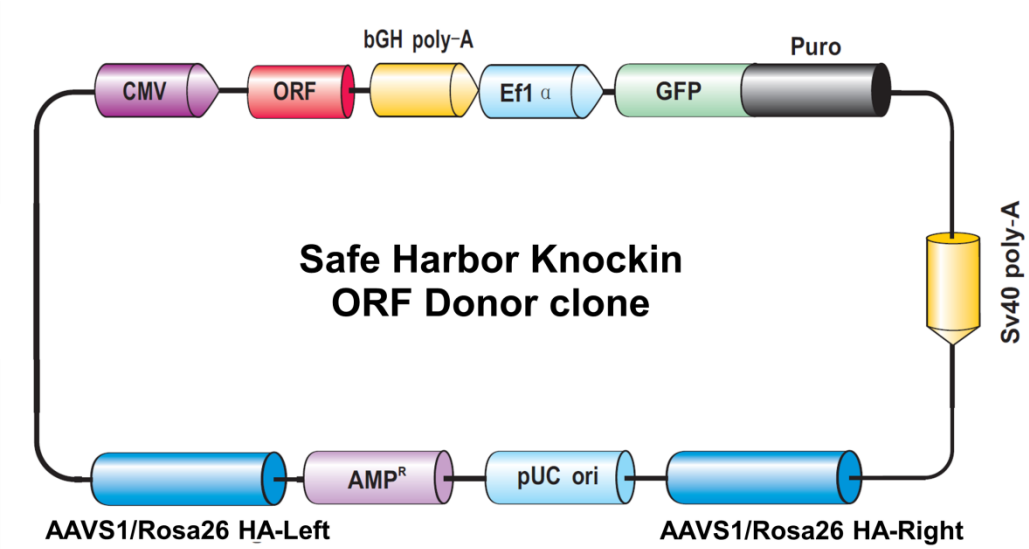
Related products & services

Safe Harbor knockin ORF clones



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)

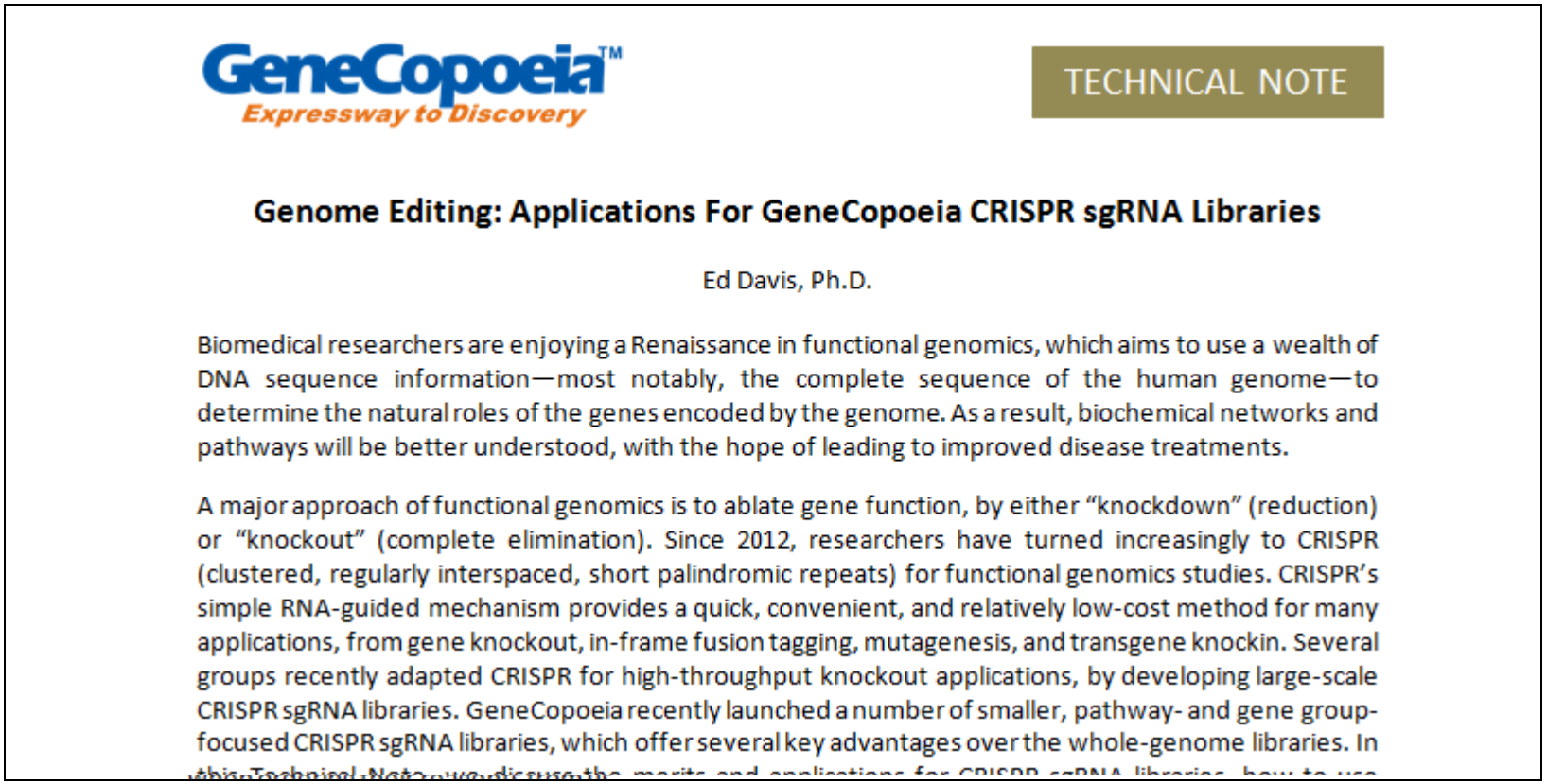
Related products & services

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



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TECHNICAL NOTE

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:

http://www.genecopoeia.com/wp-content/uploads/2015/04/GeneCopoeia-Technical-Note-CRISPR-sgRNA-Libraries-05-2015.pdf

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

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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

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Thank you!

Any questions?

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