

# Safe Harbor Transgenesis in Human & Mouse Genome Editing

**Presenter:**

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

***April 19, 2017***

# GeneCopoeia products & services

## Functional Genomics & Cell Biology

<i><b>Clones</b></i>	<i><b>Viral systems</b></i>	<i><b>Kits &amp; reagents</b></i>	<i><b>Fluorescent detection</b></i>
<b>ORF</b>	<b>Lentivirus</b>	<b>Transfection</b>	<b>Cell function assays</b>
<b>Promoter</b>	<b>AAV</b>	<b>Luciferase</b>	<b>Nucleic acid detection</b>
<b>miRNA</b>		<b>FISH probes</b>	<b>Cell structure probes</b>
<b>CRISPR</b>		<b>Indel detection</b>	<b>Fluorescent dyes</b>
<b>siRNA</b>		<b>Cloning</b>	

# Safe Harbor transgenesis

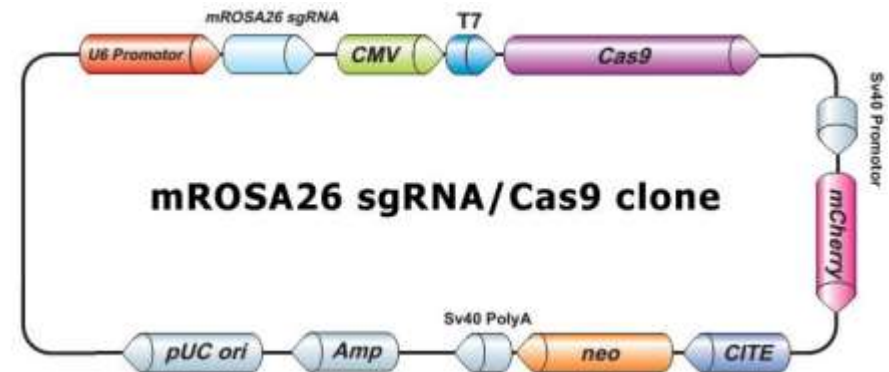
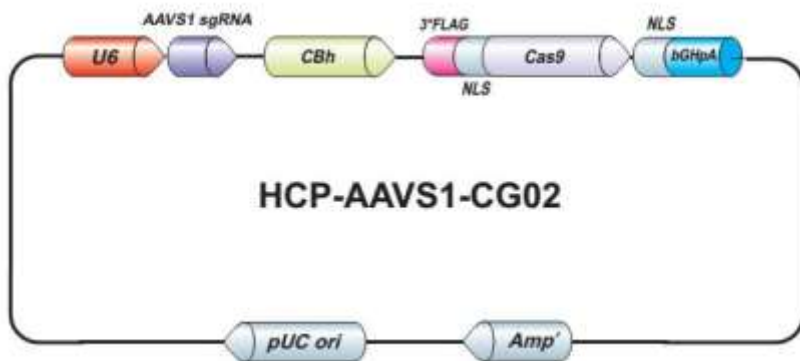
## What is Safe Harbor?

- ❖ Genome sites that permit transgene insertion with no known adverse consequences on cell fitness or viability
- ❖ Open chromatin structure, allowing for consistent, stable transgene expression

# Safe Harbor transgenesis

## GeneCopoeia Safe Harbor products

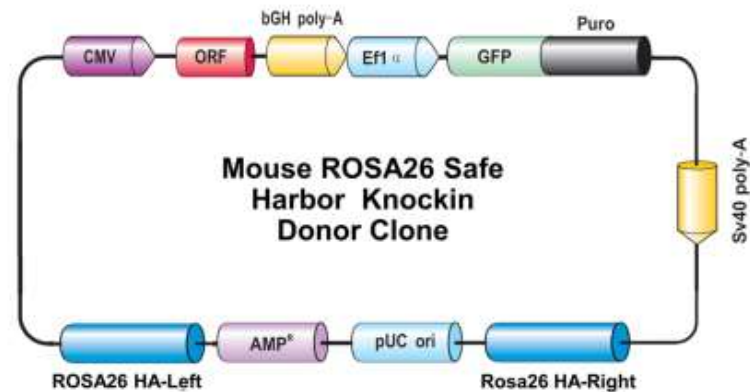
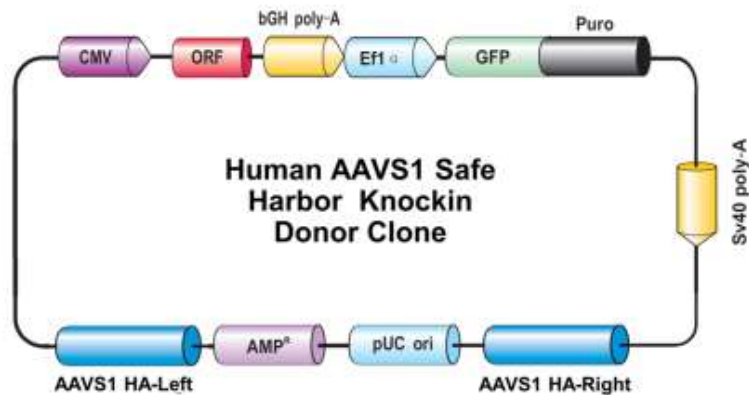
- ❖ **Safe Harbor Knock-in kits.** Kits with validated CRISPR- or TALEN-based plasmid clones, knock-in verification PCR primers, and knock-in control donor clone. Can also come with or without either knock-in donor cloning vectors or pre-made clones for knocking in genes of interest (e.g. CRISPR-Cas9 nuclease), as well as knock-in verification PCR primers



# Safe Harbor transgenesis

## GeneCopoeia Safe Harbor products

- ❖ **Safe Harbor Knock-in ORF clones.** More than 20,000 human and more than 15,000 mouse sequence-verified ORFs in custom-built knock-in donor clones



# Outline

- ❖ Transgenesis: Applications
- ❖ Transgenesis: Considerations
- ❖ Introduction to Safe Harbor
- ❖ Introduction to CRISPR
- ❖ GeneCopoeia Safe Harbor solutions

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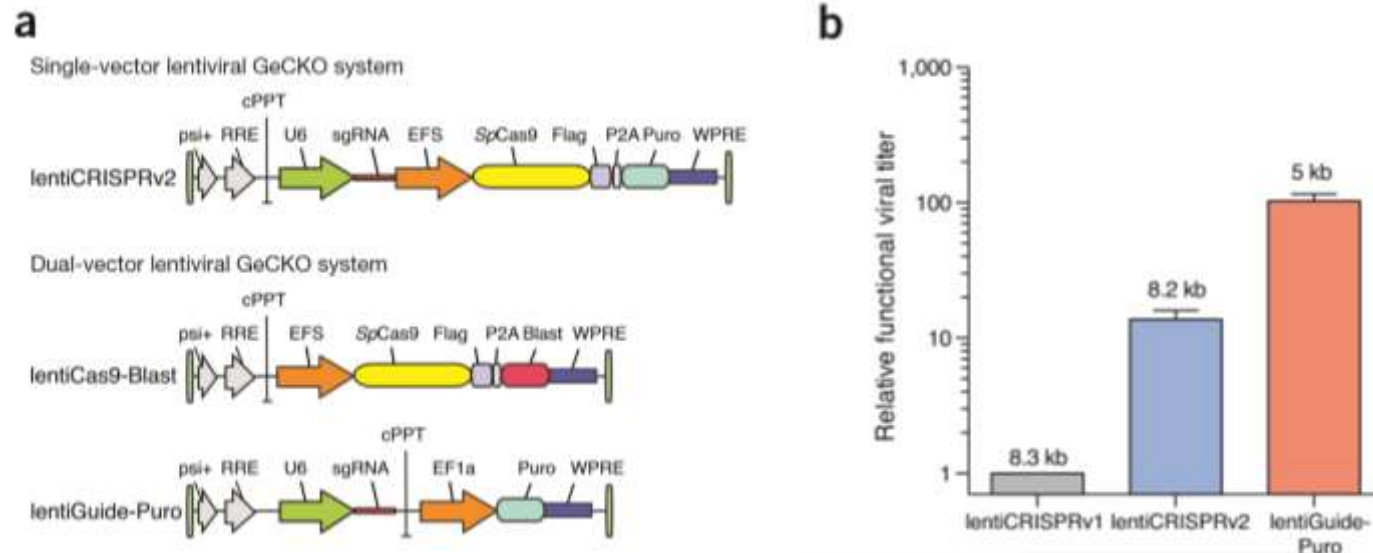
# Applications for transgene insertion

- ❖ Cross-species expression (example: Express a human gene in a mouse)
- ❖ Rescue a mutant phenotype
- ❖ Gene overexpression
- ❖ Gene tagging



# CRISPR sgRNA libraries

## Application: CRISPR library screening

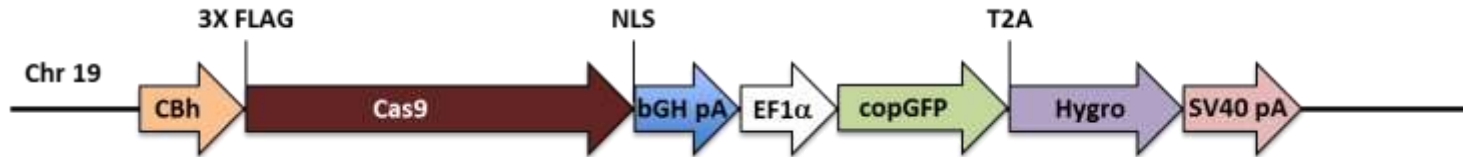


Sanjana, et al. (2014). Nature Methods 11, 783

- ❖ CRISPR libraries often used for high-throughput knockout or expression screening
- ❖ Viral titers of sgRNA-alone constructs is much higher than “all-in-one” (Cas9 + sgRNA) constructs

# Applications for transgene insertion

## Application: CRISPR library screening



- ❖ Cell lines with stably expressing Cas9
- ❖ Have >40 pre-made lines, or can have us integrate Cas9 in your cell line
- ❖ Plasmids are available for DIY stable cell line creation

# Outline

- ❖ Transgenesis: Applications
- ❖ **Transgenesis: Considerations**
- ❖ Introduction to Safe Harbor
- ❖ Introduction to CRISPR
- ❖ GeneCopoeia Safe Harbor solutions

# Considerations for transgene insertion

- ❖ Should not disrupt a gene important for cell growth or other function
- ❖ Should not cause tumorigenesis by either disrupting a tumor suppressor gene or activating an oncogene
- ❖ Insertion should allow genes to be expressed in all cell types
- ❖ Insertions should be stable
- ❖ Ideally, insertions should allow creation of isogenic lines (same insertion site)

# Traditional transgenesis approaches

- ❖ Viral integration. Usually lentiviral. Very efficient, but integration is random, favors transcription units.
- ❖ Random integration (non-viral).
- ❖ Transient plasmid transfection: Efficient, but usually not stable

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- ❖ Transgenesis: Considerations
- ❖ **Introduction to Safe Harbor**
- ❖ Introduction to CRISPR
- ❖ GeneCopoeia Safe Harbor solutions

# Safe Harbor transgenesis

## What is Safe Harbor?

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# Safe Harbor transgenesis

Mouse ROSA26 (Zambrowicz, et al., 1997)

- ❖ Found that one strain of mice (ROSA $\beta$ geo26) expressed  $\beta$  galactosidase from a randomly inserted transgene at high levels uniformly in nearly all tissues examined
- ❖ Located on chromosome 6
- ❖ Locus expresses one coding transcript and two noncoding transcripts, and only the non-coding transcripts are disrupted by the insertion.
- ❖ Slightly fewer mouse pups are born from homozygous mothers than from heterozygotes, but pups develop normally and are fertile
- ❖ Standard locus for transgene insertion in mouse, *in vitro* and *in vivo*.





# Safe Harbor transgenesis

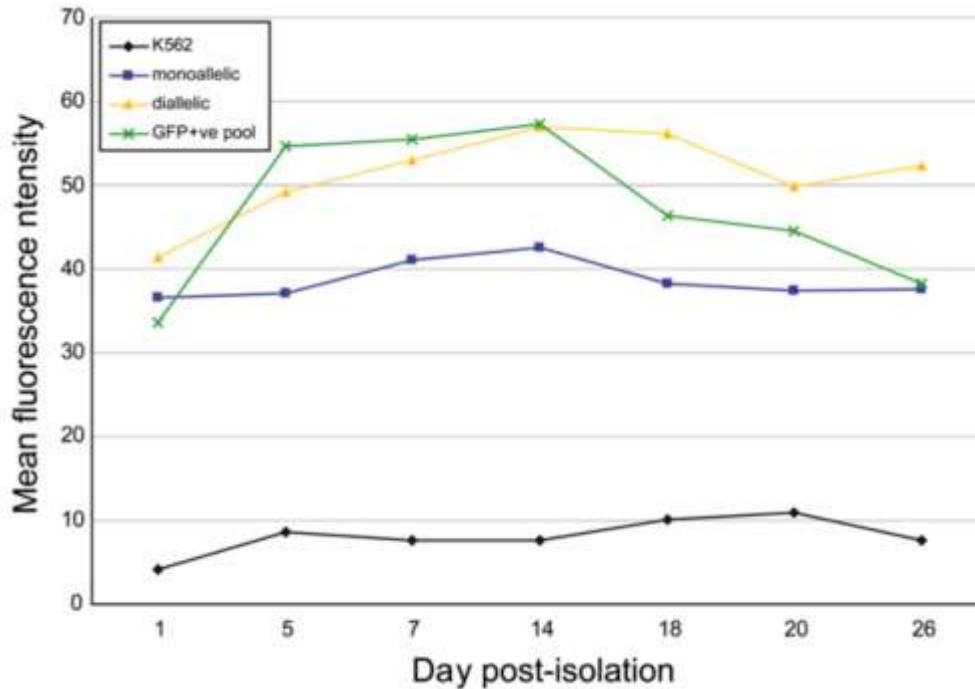
Human AAVS1 (DeKolver, et al., 2010)

- ❖ The PPP1R12C gene is the preferred site of insertion for Adeno-associated virus (AAV). This locus is also known as “AAVS1”
- ❖ Located on chromosome 19
- ❖ Showed that insertion of transgenes at this locus has no visible effect on the growth or fitness of many cell types, including primary and immortalized cells, induced pluripotent stem cells (iPSC), and embryonic stem cells
- ❖ Transgenes displayed consistent levels of expression over many cell divisions



# Safe Harbor transgenesis

## Expression stability



- ❖ Inserted GFP at AAVS1 in K562 cells
- ❖ Followed GFP expression over time (approx. 24 generations)

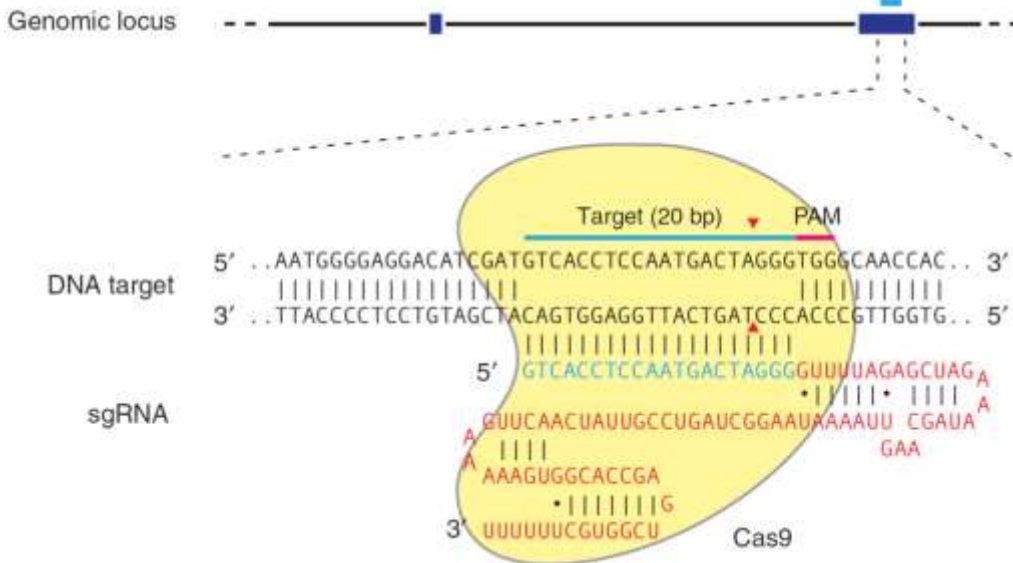


# Outline

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- ❖ Transgenesis: Considerations
- ❖ Introduction to Safe Harbor
- ❖ **Introduction to CRISPR**
- ❖ GeneCopoeia Safe Harbor solutions

# CRISPR genome editing technology

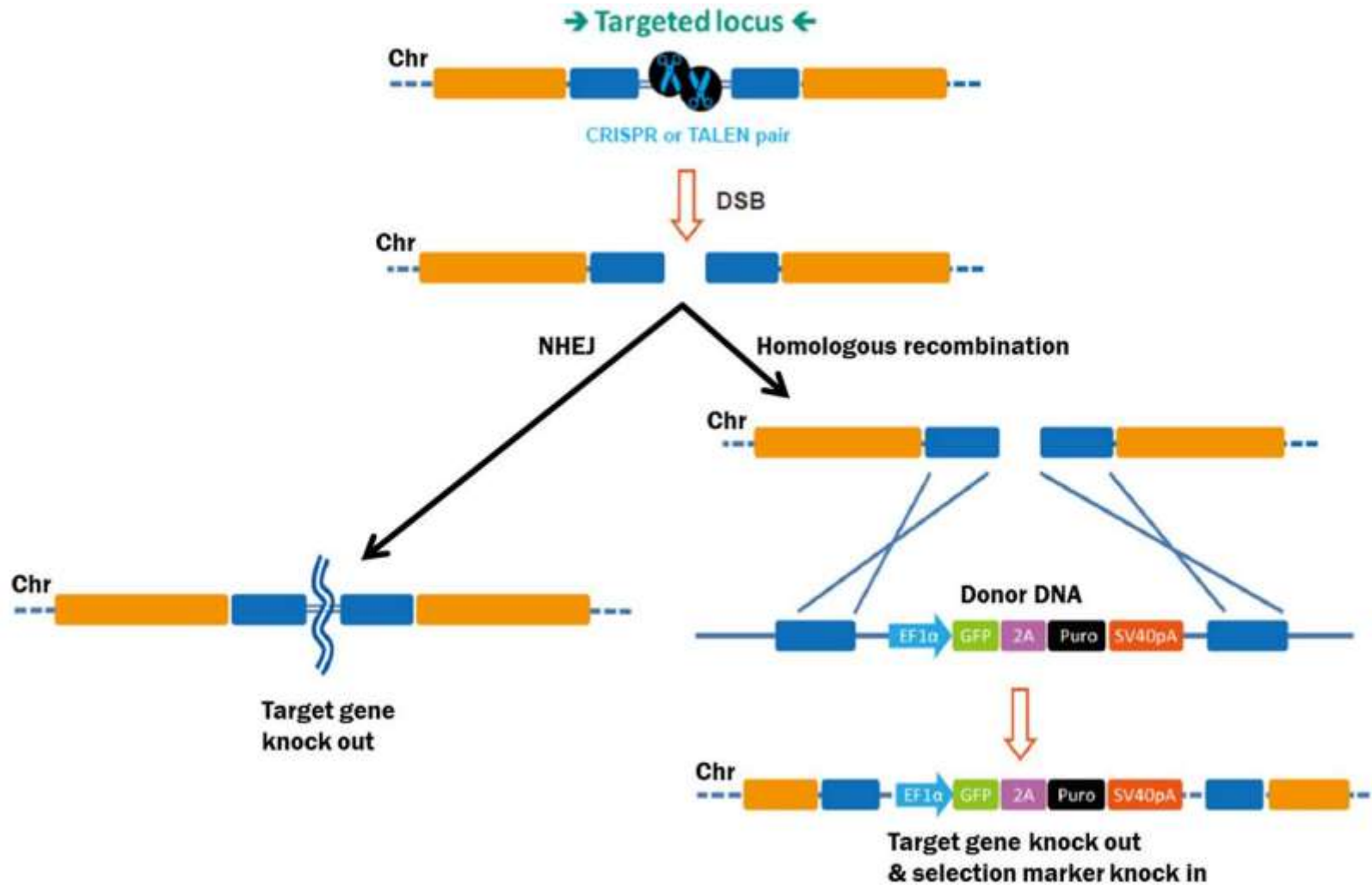
## CRISPR-Cas9: RNA-guided endonuclease



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



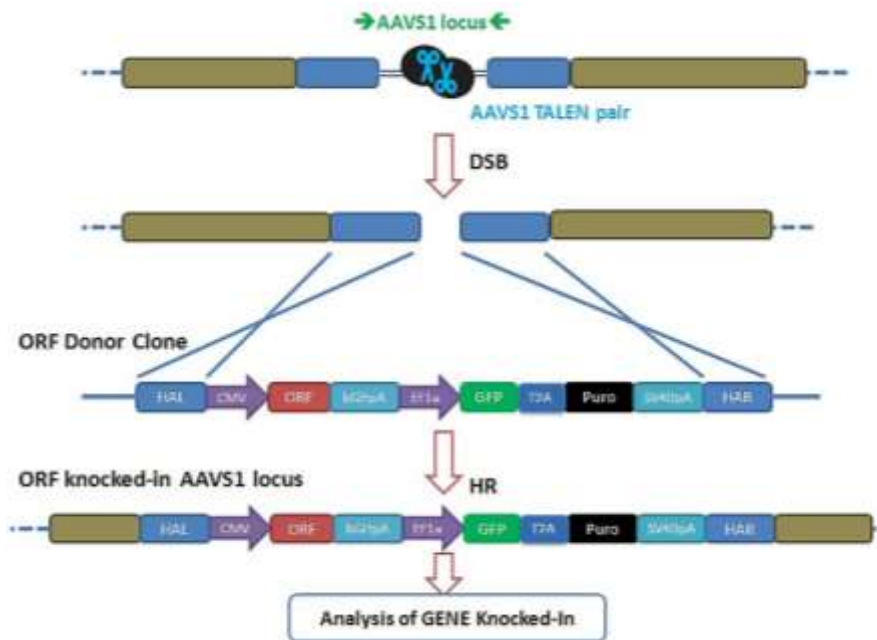
# Outline

- ❖ Transgenesis: Applications
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- ❖ **GeneCopoeia Safe Harbor solutions**

# GeneCopoeia Safe Harbor products

## Features

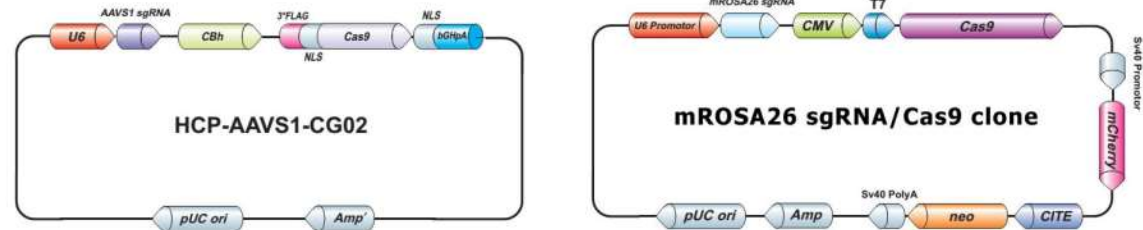
- ❖ Human AAVS1 & mouse Rosa26 sites ensure transcription-competency of the transgenes & present no known adverse effects on cells
- ❖ Safe Harbor integration provides low copy number of transgene & close to physiological-level expression.



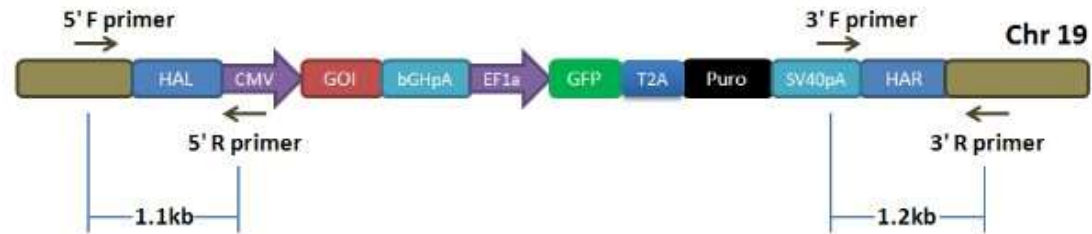
# GeneCopoeia Safe Harbor products

## Safe Harbor Knock-in kit components

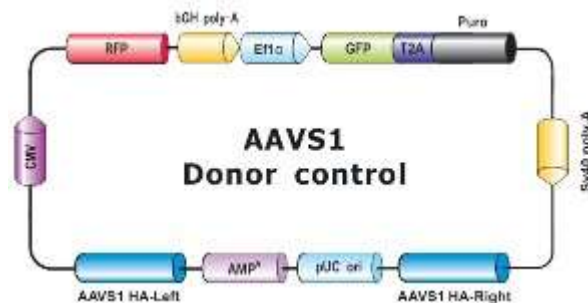
1. Plasmid clones expressing Cas9 and validated sgRNA



2. Knock-in verification PCR primer pairs



3. Knock-in control donor clone

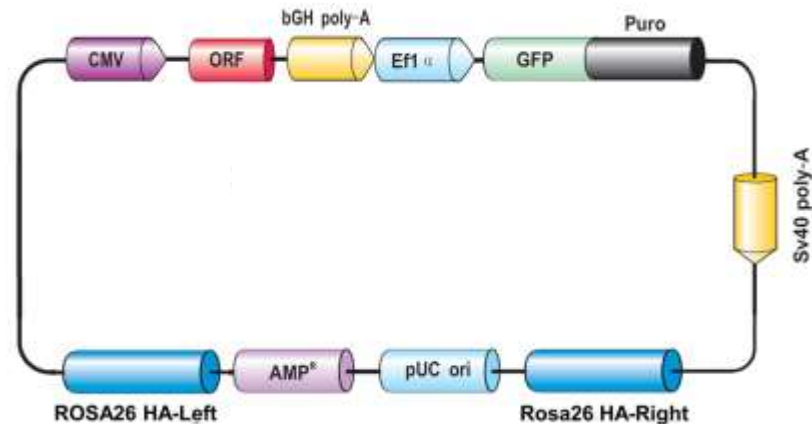
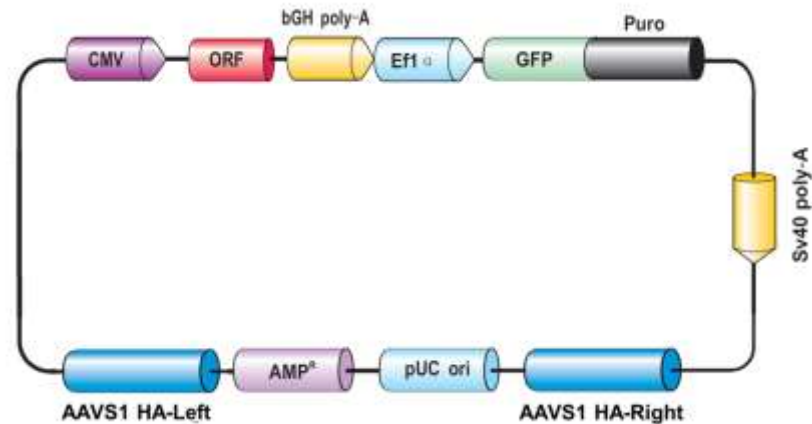




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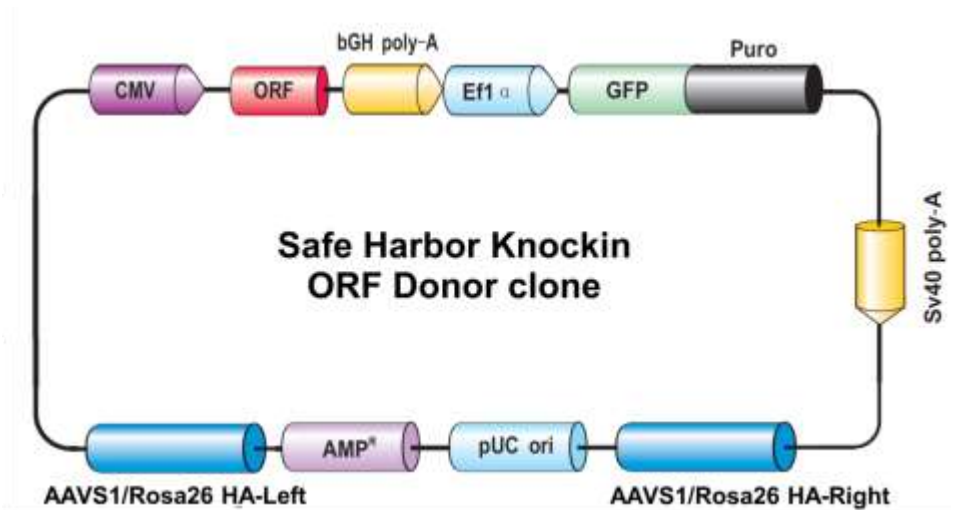
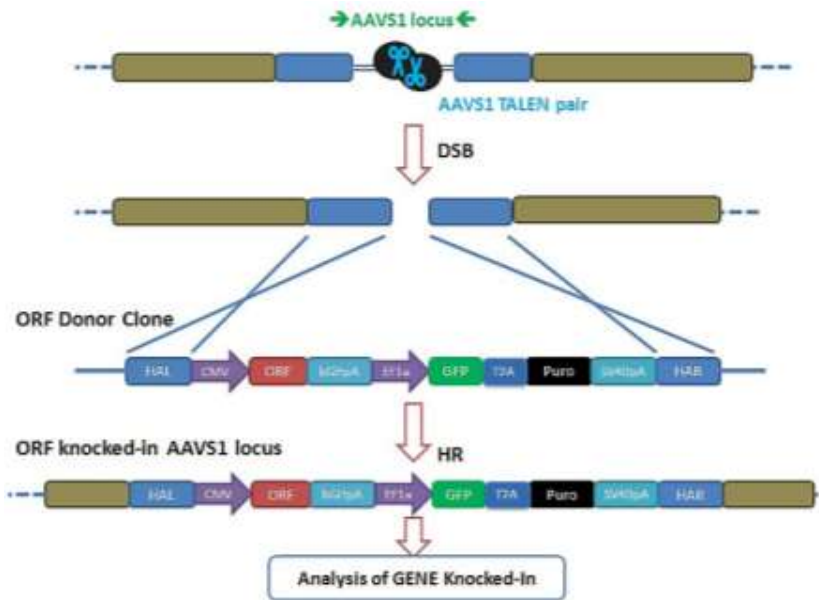
## Safe Harbor Knock-in kit components

4. Optional: Safe Harbor donor cloning vector, pre-made donor clone, or custom donor clone



# GeneCopoeia Safe Harbor products

## Safe Harbor knock-in ORF clones

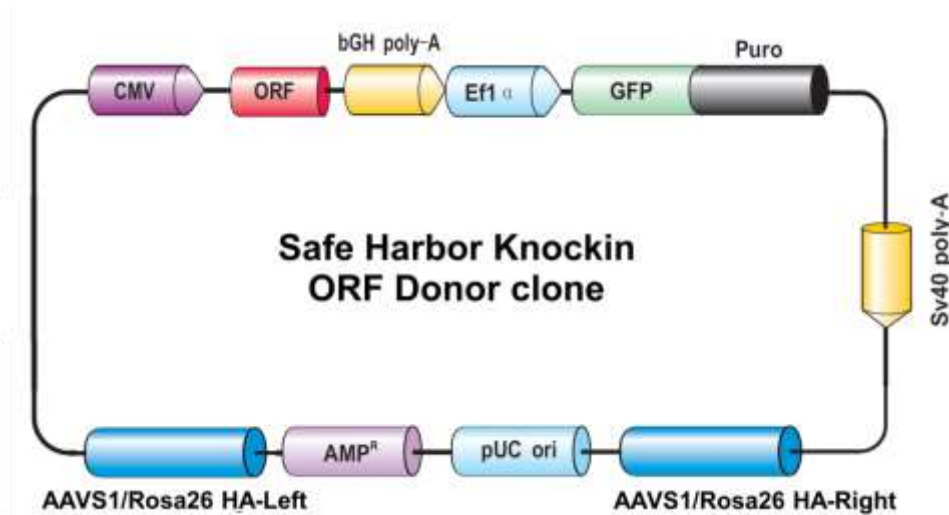


## Features

- ❖ Over 40,000 sequence-verified human & mouse ORFs available
- ❖ Inserted between AAVS1 or Rosa26 sites for ready safe harbor integration

# GeneCopoeia Safe Harbor products

## Safe Harbor knock-in ORF clones

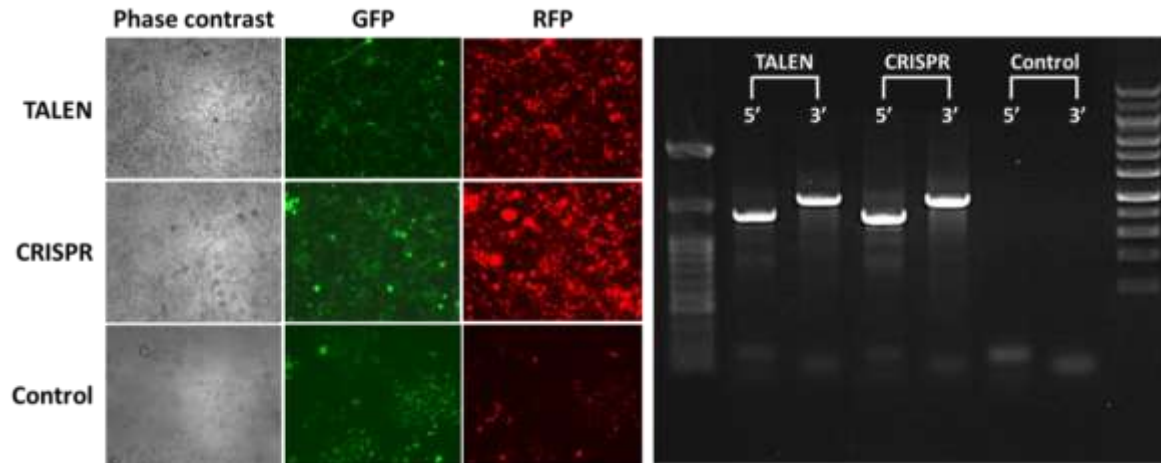


### Some applications for Safe Harbor knockin ORF clones:

- ❖ 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)

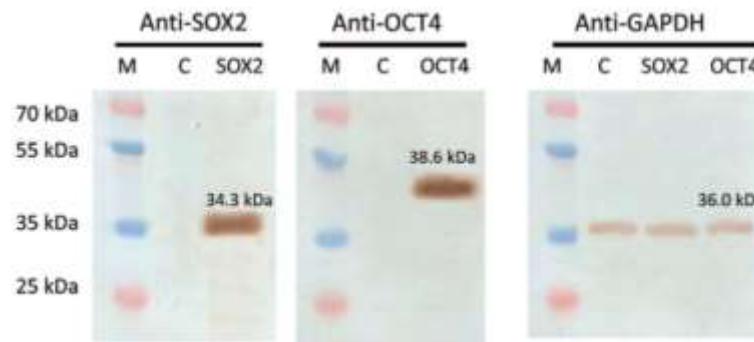
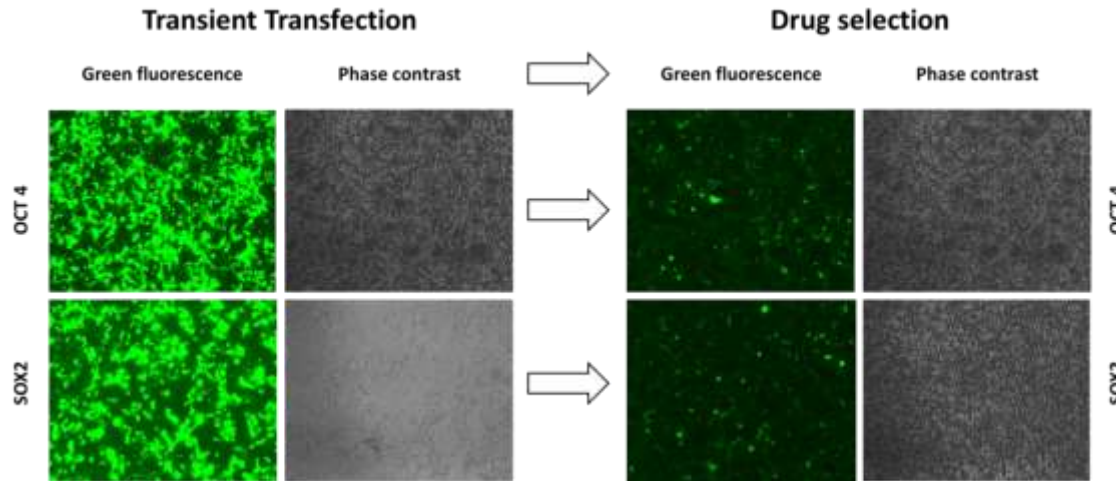
# GeneCopoeia Safe Harbor products

ROSA26



# GeneCopoeia Safe Harbor products

## AAVS1



# GeneCopoeia Safe Harbor products

## Ordering Safe Harbor kits and knock-in clones

The screenshot shows the GeneCopoeia website homepage. At the top, there is a navigation bar with the following links: Products and Services, Technical Resources, Order Support, Contact Us, and About Us. Below this is a search bar with the text "Please enter the keyword" and a "Search" button. A "Search by Gene" link is also present. The main banner features the text "Luciferase Assays and Reporters" over a background image of a glowing cell. Below the banner, there are two main sections: "Popular product areas and services" and "Latest Promotions".

**Popular product areas and services**

- Clone collections**: ORF cDNA, CRISPR, shRNA, miRNA, promoter, TRE, and more
- Genome editing**: CRISPR, indel detection, donors, cell lines, mouse lines, Safe Harbor, FISH, TALEN
- Lentiviral system**: Purified particles, clones, packaging system
- Fluorescent labeling and detection**: Nucleic acid gel stains and quantitation
- Cell biology**: Apoptosis, cell proliferation and viability assays, cell structure probes, and more
- microRNA solutions**: qPCR arrays and primers, qPCR kits, 3'UTR clones, precursor clones, inhibitor clones
- qPCR products**: Arrays, primers, RT-PCR kits
- Reagents and kits**: Luciferase kits, transfection

**Latest Promotions**

- 30% off untagged ORF clones
- 30% off lentiviral particles express...
- \$119 / CRISPR sgRNA done
- 30% off Secrete Pair luciferase assay...
- qPCR master mixes - buy 1 get 1 free

**Events and Exhibitions**

- Experimental Biology 2017
- AACR Annual Meeting 2017

Additional elements include a "My Account" and "Shopping Cart" link, a phone number "1-866-380-9531", and a "Chat live now!" button.

# GeneCopoeia Safe Harbor products

## Ordering Safe Harbor kits and knock-in clones

The screenshot shows a web browser window with the URL [www.genecopoeia.com/product/safe-harbor/](http://www.genecopoeia.com/product/safe-harbor/). The page features the GeneCopoeia logo and navigation menu. The main content area is titled "Safe Harbor Gene Knock-in Kits and Clones" and includes a sub-menu with "Introduction", "How it works", "Related Products", and "Resources". The "How it works" tab is selected. The text describes the system as an ideal solution for expressing transgenes in cells and animals, with consistent and stable expression. A callout box promotes a webinar on Wednesday, April 19, 2017, at 12:00 PM ET. The "Advantages" section lists three key features: safe integration, a comprehensive system, and compatibility with ORFs. A chat bubble is visible in the bottom right corner.

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Please enter the keyword  All  Search by Gene

You are here: [Home](#) > [Products](#) >

### Safe Harbor Gene Knock-in Kits and Clones

[Introduction](#) | [How it works](#) | [Related Products](#) | [Resources](#)

GeneCopoeia's Safe Harbor system is the ideal solution for expressing transgenes in cells and animals. Transgene insertion at the AAVS1 and ROSA26 sites in humans and mice, respectively, permits consistent and stable expression with no adverse effects on fitness.

GeneCopoeia offers safe harbor knockin kits and clones as well as safe harbor premade cell lines.

**Register for our latest CRISPR webinar:**  
Wednesday, April 19, 2017  
12:00 PM ET

#### Advantages

- **Safe integration.** Transgene integration at well-characterized safe harbor sites ensures consistent, stable expression with no adverse effects on cell viability or fitness.
- **Comprehensive system.** CRISPR or TALEN-based kits come with a choice of donor cloning vectors, premade knockin clones, or made-to-order knockin clones, as well as verification PCR primers.
- **Compatible knock-in ORFs.** Choose from more than 20,000 human and more than 15,000 mouse sequence-verified ORFs in custom-built knock-in donor clones.

Chat live now!

Online

# GeneCopoeia Safe Harbor products

## Ordering Safe Harbor kits and knock-in clones

The screenshot displays the GeneCopoeia website interface. The browser address bar shows the URL [www.genecopoeia.com/product/safe-harbor/](http://www.genecopoeia.com/product/safe-harbor/). The left sidebar contains a navigation menu with categories such as qPCR Products, Reagent Kits & Proteins, mRNA Solutions, Fluorescent Labeling and Detection, Cell Biology Reagents, and Premade Stable Cell Lines. The main content area is titled "Order Safe Harbor Knockin kits" and provides information about the components of these kits: (1) AAVS1 or ROSA26 site-specific CRISPR or TALEN pair, (2) knockin junction verification primers, and (3) a donor cloning vector or a donor clone. Three donor options are listed: Do-it-yourself cloning vectors, Pre-made Cas9 donor clones, and ORF knockin clones. Below this text is a selection form with three dropdown menus labeled "Species", "CRISPR or TALEN", and "Donor", each with a "Select" option. A green "Add to cart to view price" button is located below the form. The section "Order pre-made Cas9 stable cell lines" follows, describing the availability of these cell lines in various human and mouse cell lines. At the bottom, a table is partially visible with columns for "Buy", "Catalog #", "Cell line", "Description", "Cell type", "Selection marker", "Price, Academic", and "Price, Industry". A "Chat live now!" button is also present in the bottom right corner.



# GeneCopoeia Safe Harbor products

## Ordering Safe Harbor kits and knock-in clones

**Order Safe Harbor Knockin kits**

Safe harbor kits contain the following major components: (1) AAVS1 or ROSA26 site-specific CRISPR or TALEN pair, (2) knockin junction verification primers and (3) a donor cloning vector or a donor clone. We currently offer three donor options:

- **Do-it-yourself cloning vectors.** For creating safe harbor knockin donor clones carrying any transgene you want.
- **Pre-made Cas9 donor clones.** Express CRISPR-Cas9 nuclease from the safe harbor site.
- **ORF knockin clones.** Made-to-order from GeneCopoeia's vast, searchable collections of more than 20,000 human and more than 15,000 mouse safe harbor knockin ORFs.

To display your kits of interest, select species, genome editing technology, and donor options from the table below.

Species	CRISPR or TALEN	Donor
Select	Select	Select

**Order pre-made Cas9 stable cell lines**

GeneCopoeia also offers stable cell lines constitutively expressing the CRISPR Cas9 nuclease, enabling you to carry out CRISPR genome editing applications with high efficiency.

The Genome-CRISPR™ Cas9 stable cell lines are available pre-made in many human cell lines, and in mouse cell line Neuro2a. The CRISPR Cas9 nuclease is stably integrated into either the human AAVS1 or mouse ROSA26 "Safe Harbor" sites (Figure 1), or randomly via lentiviral-mediated transduction.

Choose your human or mouse Cas9-expressing stable cell line from the list below. For more information, please visit our Cas9 stable cell lines page.

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Species	CRISPR or TALEN	Donor
Human AAVS1	CRISPR	Cloning vector

Buy	Catalog #	Product	Description	Price	User Manual
<input type="checkbox"/>	SH004	Genome-CRISPR™ human AAVS1 safe harbor gene knock-in kit	CRISPR-based cloning kit for human AAVS1 Safe Harbor knockin. Includes CRISPR-Cas9 clone targeting AAVS1, donor cloning vector, control RFP knockin clone, and verification PCR primer pairs.	\$1395	<a href="#">User Manual</a>

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### Safe Harbor Gene Knock-in Kits and Clones

Introduction How it works Related Products Resources

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**Budget Friendly ORF Clones**  
Mammalian, lentiviral and Gateway clones at next day shipping!

**Search full-length ORF cDNA clones**

1. Simple search by gene symbol, aliases, description, nucleotide accession, Entrez gene ID, catalog or product ID  
Select species:  
 Human+Mouse  Human  Mouse  Zebrafish  
Enter Keyword(s):  [GO](#)

2. Search by a specific field  
Select species:  
 Human+Mouse  Human  Mouse  Zebrafish  
Select field:  
 Description  Symbols/Aliases  
 Nucleotide Accession  UniGene ID  
 Entrez Gene ID  Catalog/product ID  
Enter Keyword(s):  [GO](#)

3. Browse genes by signal pathway  
Over 50 signaling pathways  
Easy to search and quick to order

4. Search by sequence homology (BLAST)  
Select species:

ORF clone sets  
Available in mammalian, lentiviral and Gateway vectors

ORF  
OmicLink™ ORF Expression Clones  
ORF  
ORF  
OmicLink™ ORF Expression Clones  
ORF  
ORF  
OmicLink™ ORF Expression Clones

Chat live now!

Online

# GeneCopoeia Safe Harbor products

## Ordering Safe Harbor kits and knock-in clones

The screenshot shows the GeneCopoeia website's search interface. The browser address bar displays 'www.geneCopoeia.com/product/search/index.php?&prt=25'. The website header includes the GeneCopoeia logo with the tagline 'Expressway to Discovery', navigation links for 'Products and Services', 'Technical Resources', 'Order Support', 'Contact Us', and 'About Us', and a search bar with the placeholder text 'Please enter the keyword'. A secondary search bar labeled 'Search by Gene' is also present. Below the navigation, there are tabs for various product categories: ORF cDNA clones, CRISPR / TALEN, Lentivirus, Promoter clones, qPCR primers, shRNA clones, miRNA products, siRNA target clones, TALE-TP, and ORF knockin clones. The main content area is titled 'Search human AAVS1 and mouse ROSA26 knock-in ORF clones'. It features three search methods: 1. Simple Search (Description, Nucleotide Accession, Entrez Gene ID, Symbols/Aliases or Catalog/product ID), 2. Search by Keywords or Descriptions, and 3. Search by sequence homology (BLAST). Each method includes options to select species (Human+Mouse, Human, Mouse) and fields for entering search criteria. A 'Search' button is provided for each method. On the right side, there are two yellow callout boxes: 'Not finding what you need? Check out AAVS1 & ROSA26 Donor DNA Customer Service' and 'Learn more about Safe Harbor gene knock-in'. A chat window is visible in the bottom right corner with the text 'Chat live now!' and an 'Online' indicator.

# GeneCopoeia Safe Harbor products

## Ordering Safe Harbor kits and knock-in clones

The screenshot shows the GeneCopoeia website's search interface. The browser address bar displays [www.genecopoeia.com/product/search/index.php?&prt=25](http://www.genecopoeia.com/product/search/index.php?&prt=25). The website header includes the GeneCopoeia logo with the tagline "Expressway to Discovery", navigation menus for "Products and Services", "Technical Resources", "Order Support", "Contact Us", and "About Us", and a search bar with the placeholder text "Please enter the keyword".

Below the navigation is a horizontal menu of product categories: ORF cDNA clones, CRISPR / TALEN, Lentivirus, Promoter clones, qPCR primers, shRNA clones, miRNA products, siRNA target clones, TALE-TP, and ORF knockin clones. The "ORF knockin clones" category is currently selected.

The main content area is titled "Search human AAVS1 and mouse ROSA26 knock-in ORF clones". It features three search methods:

- 1. Simple Search (Description, Nucleotide Accession, Entrez Gene ID, Symbols/Aliases or Catalog/product ID):**
  - Select species:  Human+Mouse  Human  Mouse
  - Search input: "sox2" (with a search button)
  - Results list:
    - SOX2
    - SOX20
    - SOX21
    - SOX22
    - SOX25
    - SOX26
    - SOX27
    - SOX28
  - Search criteria:  Accession,  InterPro ID,  UniProt
  - Search input: "Enter Keyword(s)" (with a search button)
- 2. Search by sequence homology (BLAST):**
  - Select species:  Human  Mouse
  - Select sequence type:  Protein  Nucleotide
  - Search input: "Enter query sequence:" (with a text area)

On the right side of the search area, there are two yellow callout boxes:

- Not finding what you need? Check out AAVS1 & ROSA26 Donor DNA Customer Service**
- Learn more about Safe Harbor gene knock-in**

At the bottom right, there is a "Chat live now!" button and an "Online" indicator.



# GeneCopoeia Safe Harbor products

## Ordering Safe Harbor kits and knock-in clones

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# GeneCopoeia Safe Harbor products

## Ordering Safe Harbor kits and knock-in clones

The screenshot shows the GeneCopoeia website's search interface. The browser address bar displays the URL [www.genecopoeia.com/product/search/index.php?&prt=25](http://www.genecopoeia.com/product/search/index.php?&prt=25). The website header includes the GeneCopoeia logo with the tagline "Expressway to Discovery", navigation menus for "Products and Services", "Technical Resources", "Order Support", "Contact Us", and "About Us", and a search bar with the placeholder text "Please enter the keyword".

Below the search bar, there are several product category tabs: ORF cDNA clones, CRISPR / TALEN, Lentivirus, Promoter clones, qPCR primers, shRNA clones, miRNA products, siRNA target clones, TALE-TP, and ORF knockin clones. The "ORF knockin clones" tab is currently selected.

The main content area is titled "Search human AAVS1 and mouse ROSA26 knock-in ORF clones". It features three search methods:

- 1. Simple Search (Description, Nucleotide Accession, Entrez Gene ID, Symbols/Aliases or Catalog/product ID):**
  - Select species:  Human+Mouse  Human  Mouse
  - Enter Keyword(s):
- 2. Search by Keywords or Descriptions:**
  - Select species:  Human+Mouse  Human  Mouse
  - Select Field:
    - Description
    - Nucleotide Accession
    - Entrez Gene ID
    - Symbols/Aliases
    - UniGene ID
    - Catalog/product ID
    - Protein Accession
    - InterPro ID
    - UniProt
  - Enter Keyword(s):
- 3. Search by sequence homology (BLAST):**
  - Select species:  Human  Mouse
  - Select sequence type:  Protein  Nucleotide
  - Enter query sequence:

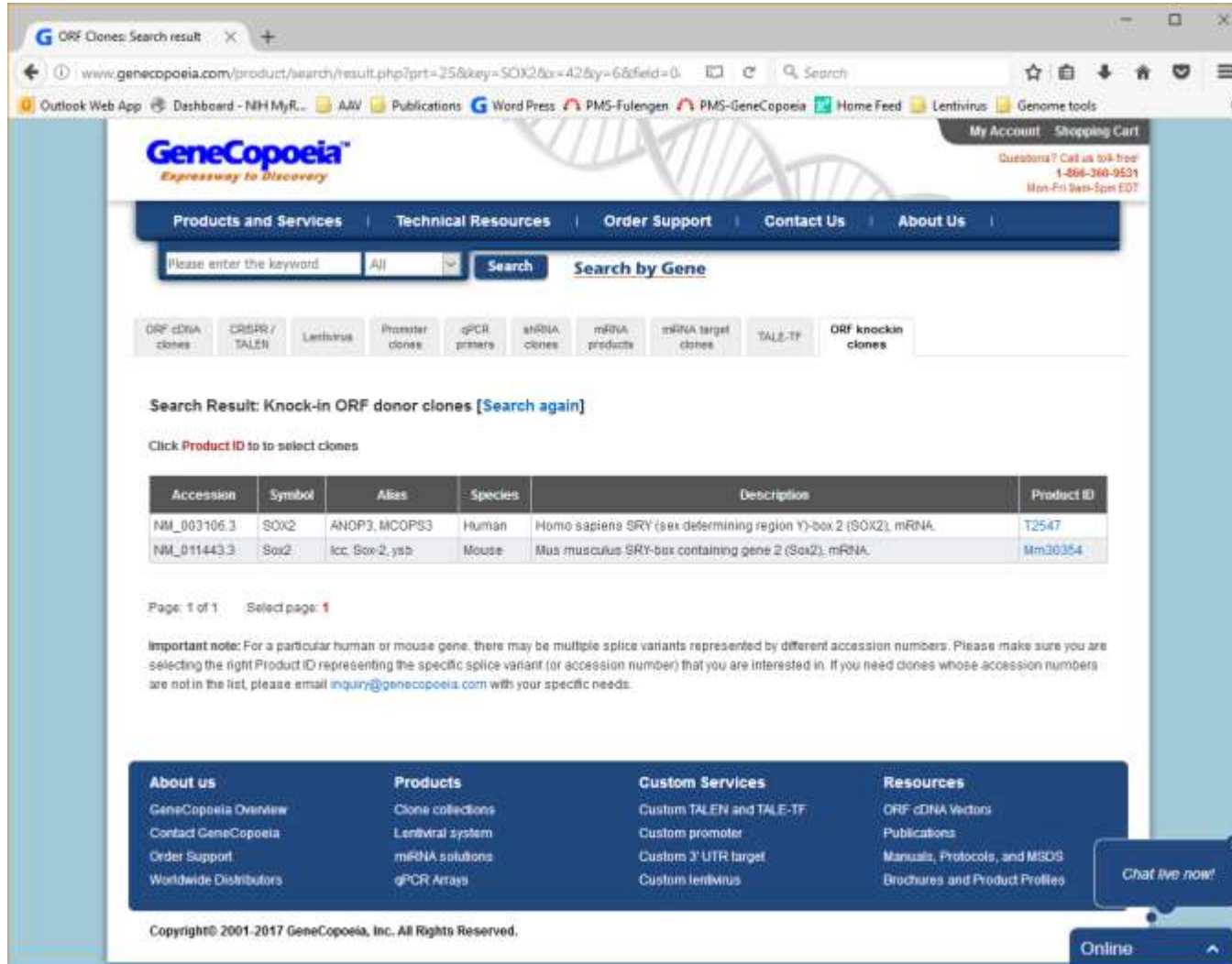
Two yellow callout boxes are present on the right side of the search area:

- Not finding what you need? Check out AAVS1 & ROSA26 Donor DNA Customer Service**
- Learn more about Safe Harbor gene knock-in**

In the bottom right corner, there is a "Chat live now!" button and an "Online" indicator.

# GeneCopoeia Safe Harbor products

## Ordering Safe Harbor kits and knock-in clones



The screenshot shows the GeneCopoeia website interface. At the top, there is a navigation bar with links for 'Products and Services', 'Technical Resources', 'Order Support', 'Contact Us', and 'About Us'. Below this is a search bar with the text 'Please enter the keyword' and a 'Search' button. A secondary search option 'Search by Gene' is also present. The main content area displays search results for 'Knock-in ORF donor clones'. A table lists two entries: one for Human (SOX2) and one for Mouse (Sox2). Below the table, there is an 'Important note' regarding multiple splice variants. The footer contains sections for 'About us', 'Products', 'Custom Services', and 'Resources', along with a 'Chat live now!' button and a copyright notice.

GeneCopoeia™  
Expressway to Discovery

Products and Services | Technical Resources | Order Support | Contact Us | About Us

Please enter the keyword   [Search by Gene](#)

ORF cDNA clones | CRISPR / TALEN | Lentivirus | Promoter clones | qPCR primers | shRNA clones | miRNA products | mRNA target clones | TALEN | **ORF knock-in clones**

**Search Result: Knock-in ORF donor clones** [\[Search again\]](#)

Click **Product ID** to select clones

Accession	Symbol	Alias	Species	Description	Product ID
NM_003106.3	SOX2	ANOP3, MCOPB3	Human	Homo sapiens SRY (sex determining region Y)-box 2 (SOX2), mRNA.	T2547
NM_011443.3	Sox2	lcc, Sox-2, ysb	Mouse	Mus musculus SRY-box containing gene 2 (Sox2), mRNA.	Mm36354

Page: 1 of 1    Select page: **1**

**Important note:** For a particular human or mouse gene, there may be multiple splice variants represented by different accession numbers. Please make sure you are selecting the right Product ID representing the specific splice variant (or accession number) that you are interested in. If you need clones whose accession numbers are not in the list, please email [inquiry@genecopoeia.com](mailto:inquiry@genecopoeia.com) with your specific needs.

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Brochures and Product Profiles

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Online

Chat live now!

# GeneCopoeia Safe Harbor products

## Ordering Safe Harbor kits and knock-in clones

The screenshot shows a web browser window displaying the GeneCopoeia website. The page title is "Clones: Detail information". The URL is [www.genecopoeia.com/product/search/detail.php?prt=25&cid=&key=Mm30354](http://www.genecopoeia.com/product/search/detail.php?prt=25&cid=&key=Mm30354). The browser's address bar shows several tabs: Outlook Web App, Dashboard - NIH MyR..., AW, Publications, Word Press, PMS-Fulengen, PMS-Genecopoeia, Home Feed, Lentivirus, and Genome tools. The GeneCopoeia logo is at the top left, with the tagline "Expressway to Discovery". A navigation menu includes "Products and Services", "Technical Resources", "Order Support", "Contact Us", and "About Us". A search bar is present with the text "Please enter the keyword" and a "Search" button. Below the search bar, there are several category buttons: ORF cDNA clones, CRISPR / TALEN, Lentivirus, Promoter clones, qPCR primers, shRNA clones, mRNA products, siRNA target clones, TALE-TP, and ORF knockin clones. The main content area displays product information for a knock-in ORF donor clone. It includes the product ID "Mm30354", symbol "Sox2", accession "NM\_011443.3", and alias "lcc: Sox-2, ysb". The description is "Mus musculus SRY-box containing gene 2 (Sox2), mRNA." The delivery format is "purified plasmid". There are two yellow callout boxes: "Learn more about Mouse ROSA26 safe harbor gene targeting" and "Not finding what need? Check out ROSA26 donor clone custom services". A blue callout box says "30% off Safe Harbor Kits". A "Read Warranty Statement" link is visible. At the bottom, there is a table with columns: Buy, Catalog#, Promoter, Reporter gene, Selection Marker, Tag, Integration Site, and Vector. The "Vector" column contains the text "Online". A "Chat live now!" button is in the bottom right corner.

GeneCopoeia™  
Expressway to Discovery

Products and Services | Technical Resources | Order Support | Contact Us | About Us

Please enter the keyword  All  Search by Gene

ORF cDNA clones | CRISPR / TALEN | Lentivirus | Promoter clones | qPCR primers | shRNA clones | mRNA products | siRNA target clones | TALE-TP | ORF knockin clones

Select knock-in ORF donor clones (Add products to shopping cart to view prices)

Product ID: Mm30354 (click here to view gene annotation page)  
Symbol: Sox2  
Accession: NM\_011443.3  
Alias: lcc: Sox-2, ysb  
Description: Mus musculus SRY-box containing gene 2 (Sox2), mRNA.  
Delivery format: purified plasmid  
Download:  
• Product datasheet  
• AWS1 and ROSA26 knock-in ORF donor clones warranty and other policy

Learn more about Mouse ROSA26 safe harbor gene targeting

Not finding what need? Check out ROSA26 donor clone custom services

30% off Safe Harbor Kits

Read Warranty Statement

To view clone prices and proceed with order, click on buy check box next to catalog#, and then click on 'Add to Shopping Cart' button

Add to cart to view price

Buy	Catalog#	Promoter	Reporter gene	Selection Marker	Tag	Integration Site	Vector
							Online

knock-in ORF donor clones (1 type)

Chat live now!

# GeneCopoeia Safe Harbor products

## Ordering Safe Harbor kits and knock-in clones

The screenshot shows a web browser window with the URL [www.genecopoeia.com/product/search/detail.php?prt=25&add=&key=Mm30354](http://www.genecopoeia.com/product/search/detail.php?prt=25&add=&key=Mm30354). The page title is "Clones: Detail information".

At the top, there is a "Read Warranty Statement" link and a green button labeled "Add to cart to view price". Below this, a section titled "knock-in ORF donor clones (1 type)" contains a table with the following data:

Buy	Catalog#	Promoter	Reporter gene	Selection Marker	Tag	Integration Site	Vector
<input type="checkbox"/>	DC-Mm30354-SH02	CMV	eGFP	Puromycin	copGFP	ROSA26, Ch. 6 (Mouse)	pDonor-SH02

Below this table is a section titled "ROSA26 targeting kits (2 types)" with another table:

Buy	Catalog#	Product	Description
<input type="checkbox"/>	SH050	Genome-CRISP™ mouse ROSA26 safe harbor gene knock-in kit (without donor)	CRISPR-based kit for mouse ROSA26 Safe Harbor knockin. Includes CRISPR-Cas9 clone targeting ROSA26 and verification PCR primer pairs
<input type="checkbox"/>	SH075	Genome-TALER™ mouse ROSA26 safe harbor gene knock-in kit (without donor)	TALEN-based kit for mouse ROSA26 Safe Harbor knockin. Includes TALEN clone targeting ROSA26 and verification PCR primer pairs

Below the second table is another green button labeled "Add to cart to view price".

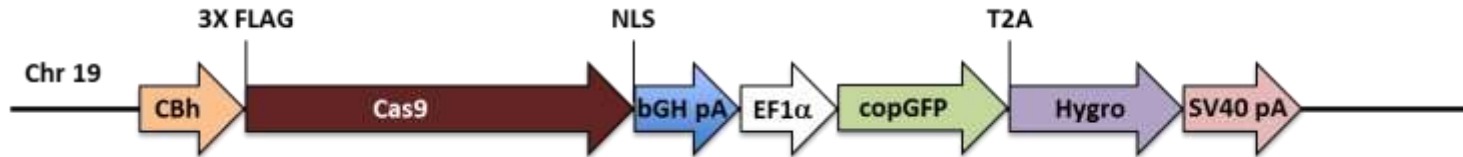
At the bottom left, there is a section titled "Related Kits and Antibodies:" with a list of links:

- Anti-tag antibodies
- Cell Free Protein Synthesis Kits
- EndoFectin™ Transfection Reagents

At the bottom right, there is a blue chat bubble that says "Chat live now!" and a button labeled "Online".

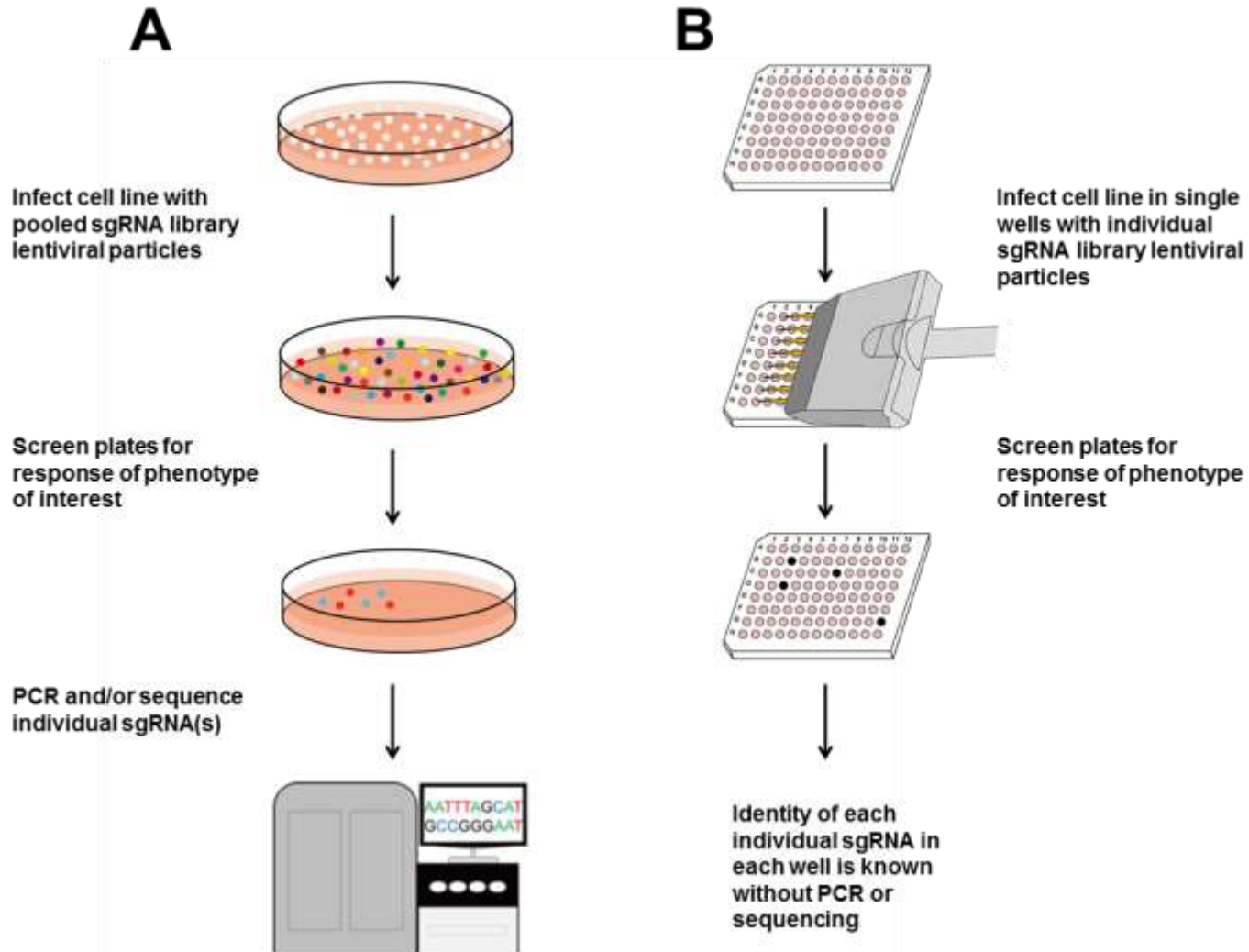
# GeneCopoeia genome editing services

## Cas9-expressing stable cell lines

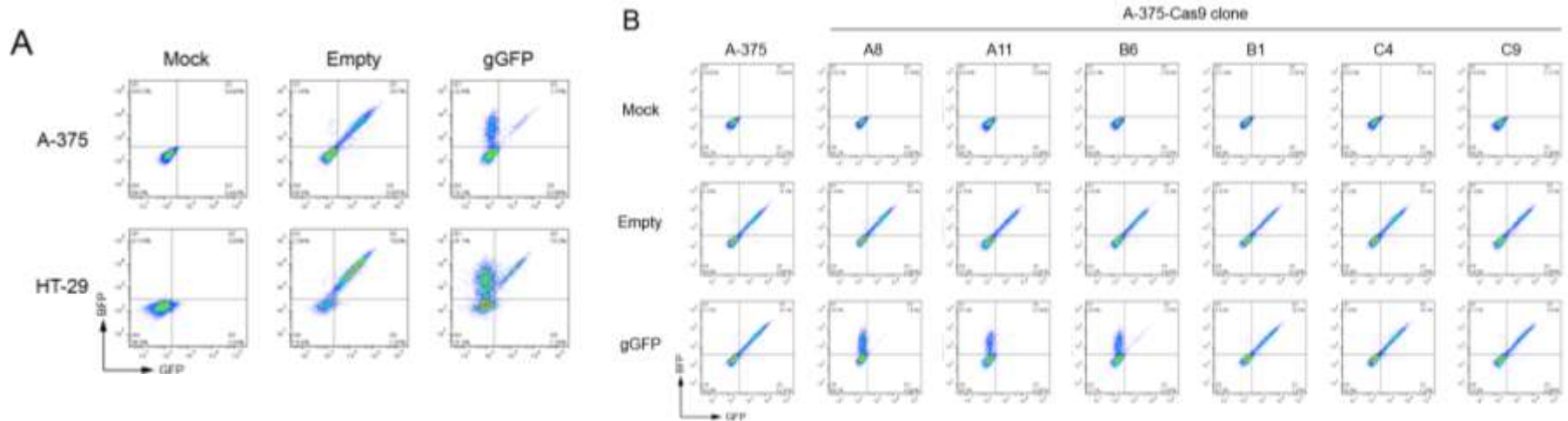


- ❖ Cell lines with stably expressing Cas9
- ❖ Have >40 pre-made lines, or can have us integrate Cas9 in your cell line
- ❖ Plasmids are available for DIY stable cell line creation
- ❖ Ideal for sgRNA library screening, validation, inducible CRISPR, and more

# CRISPR sgRNA libraries



# CRISPR sgRNA libraries



Tzelepis, et al. (2016). Cell Reports 17, 1193

- ❖ Transduced cells with Cas9-expressing lentivirus
- ❖ Found that bulk population had some cells that did not express Cas9
- ❖ Subcloned bulk cells to get clones with uniformly-expressing Cas9



# Summary

- ❖ Transgenesis is an important approach in molecular biology, with many applications, such as cross-species gene expression and mutant gene rescue
- ❖ Some methods for transgene insertion, such as lentiviral-mediated integration, are efficient but can harm cells and are not always stable
- ❖ “Safe Harbor” loci in human and mouse provide genomic sites for transgene insertion that permit consistent, stable expression with no known adverse effects on cell fitness or viability
- ❖ GeneCopoeia’s Safe Harbor knock-in kits and knock-in ORF clones for human and mouse provide you with powerful and comprehensive tools for efficient and safe transgene insertion

# Thank you!

If you have any additional  
questions, please call

1-866-360-9531 x227

Email: [edavis@genecopoeia.com](mailto:edavis@genecopoeia.com)

Or visit us on the web:

[www.genecopoeia.com](http://www.genecopoeia.com)

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