Safe Harbor Transgenesis in Human & Mouse Genome Editing

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GeneCopoeia products & services

Functional Genomics & Cell Biology

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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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- Transgenesis: Applications
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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

- 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

Sanjana, et al. (2014). Nature Methods 11, 783
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
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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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Mouse ROSA26 (Zambrowicz, et al., 1997)

- Found that one strain of mice (ROSAβgeo26) expressed β 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 (DeKelver, 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)
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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
Outline

- Transgenesis: Applications
- Transgenesis: Considerations
- Introduction to Safe Harbor
- Introduction to CRISPR

- 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.
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Safe Harbor Knock-in kit components

1. Plasmid clones expressing Cas9 and validated sgRNA

- HCP-AAVS1-CG02
- mROSA26 sgRNA/Cas9 clone

2. Knock-in verification PCR primer pairs

3. Knock-in control donor clone

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Expressway to Discovery
GeneCopoeia Safe Harbor products

Safe Harbor Knock-in kit components

4. Optional: Safe Harbor donor cloning vector, pre-made donor clone, or custom donor clone
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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
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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)
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ROSA26
GeneCopoeia Safe Harbor products

AAVS1
GeneCopoeia Safe Harbor products

Ordering Safe Harbor kits and knock-in clones
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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.

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<th>Donor</th>
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<td>Mouse ROSA26</td>
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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 Harbot” 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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Ordering Safe Harbor kits and knock-in clones
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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

- 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

Tzelepis, et al. (2016). Cell Reports 17, 1193
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
Or visit us on the web:
www.genecopoeia.com