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TECHNICAL NOTE

Genome Editing: Cas9 Stable Cell Lines for CRISPR sgRNA Validation, Library Screening, and More

Ed Davis, Ph.D.

Introduction

The CRISPR-Cas9 system has become greatly popular for genome editing in recent years, due to its ease-of-design, efficiency, specificity, and relatively low cost (Wang, et al., 2016). In mammalian cell culture systems, most genome editing is achieved using transient transfection or lentiviral transduction, which works well for routine, low-throughput applications. However, for other applications, it would be beneficial to have a system in which one component, namely the CRISPR-Cas9 nuclease, was stably integrated into the genome. In this Technical Note, we introduce GeneCopoeia's suite of Cas9 stable cell lines, and discuss the great utility that these cell lines provide for genome editing applications.

The CRISPR-Cas9 system for genome editing has been adapted from anti-viral immunity mechanisms present in many bacterial species. The most commonly used CRISPR-Cas9 system to date is from *Streptococcus pyogenes* (SpCas9). SpCas9 is a DNA endonuclease that, in the presence of a chimeric single guide RNA (sgRNA), makes a double strand break (DSB) in chromosomal DNA. Creation of a DSB is the necessary initiation event for most CRISPR-mediated genome editing applications.

Why use a Cas9-expressing stable cell line?

In cell line-based genome editing, Cas9 and sgRNA are typically introduced into cells lacking stably integrated Cas9 using transient transfection of either DNA, RNA, or (for Cas9) protein. Frequencies of DSB formation, as measured in insertion/deletion (indel) transfection assays, can exceed 60% (Fu, et al., 2013). Therefore, transfection of cell lines that do not have Cas9 previously integrated is usually sufficient for gene knockouts resulting from nonhomologous end joining (NHEJ), and for applications requiring donor-mediated, homology-directed repair (HDR), including gene knockout, gene mutagenesis, gene tagging, and Safe Harbor transgene knock-in.

There are additional applications that, while it is not required, are enhanced by having Cas9 stably integrated into the genome: 1) sgRNA functional validation; 2) gene knockout using lentiviral CRISPR; 3) sgRNA library screening; and 4) inducible genome editing. Each of these applications is discussed below.

Application 1: sgRNA functional validation

Cell line-based CRISPR genome editing projects typically require several (>3) months of work, including transfection, clone isolation, and screening. While CRISPR typically provides a highly efficient method for genome editing, the individual sgRNAs themselves do exhibit variations in efficiency due to the nature of individual target sites. Therefore, it would be helpful to determine which CRISPR sgRNAs have the highest potential for successful genome editing before doing lots of screening work.

We recommend the mismatch cleavage assay (Qiu, et al., 2004) for sgRNA functional validation in a Cas9 stable cell line. GeneCopoeia's IndelCheck™ mismatch cleavage assay

(http://www.genecopoeia.com/product/indelcheck-detection-system/) is a cell culture-based assay designed to efficiently detect indels resulting from DSB-mediated NHEJ. The basic workflow for the IndelCheck™ mismatch cleavage assay for sgRNA functional validation is illustrated in Figure 1.

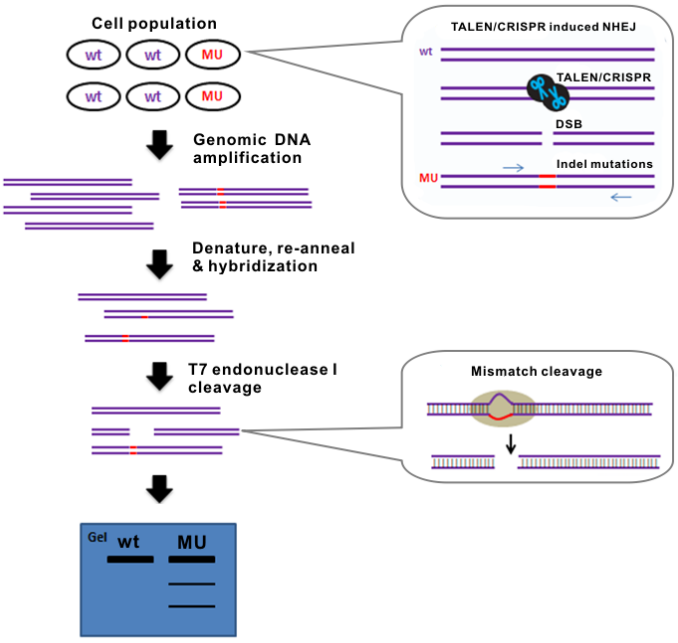

Figure 1. Workflow of the IndelCheck™ mismatch cleavage assay. A population of cells that has been exposed to CRISPR components is subjected to genomic DNA PCR using primers flanking the target site. Denaturation and reannealing of the PCR products creates a mixed population of homoduplex and heteroduplex molecules. The fragments are treated with T7 Endonuclease I, which cuts only the heteroduplex molecules. Cleavage products are detected by standard agarose gel electrophoresis.

Functional validation of CRISPR sgRNAs can be performed either in a commonly used, "model" cell line (*e.g.* HEK293), or in your own cell line. GeneCopoeia has several pre-made human and mouse Cas9-expressing stable cell lines in widely-used backgrounds (Table 1). Alternatively, you can purchase CRISPR reagents from us that allow you to readily build your own Cas9-expressing stable cell line.

Application 2: Gene knockout using lentiviral CRISPR

While transfection is a commonly used method for cell line-based genome editing, some cell lines do not transfect well. For these cell lines, researchers often turn to lentiviral-mediated CRISPR delivery, because lentiviruses efficiently infect a very large number of mammalian cell types. It is possible to express both Cas9 nuclease and an sgRNA from the same lentivirus, as has been shown previously (Sanjana, et al), or to co-transduce two different lentiviruses, one expressing Cas9 and the other expressing sgRNA. However, the titres of such "all in one" or Cas9-only lentiviral CRISPR viruses tend to be significantly lower than that achieved from sgRNA-only lentiviral vectors due to the large (>4.4 kb) size of the Cas9 ORF. It is for this reason that GeneCopoeia recommends, for genome editing using lentiviral CRISPR applications, that scientists transduce a Cas9-expressing stable cell line with sgRNA-only lentiviruses.

Application 3: sgRNA library screening

Another application for Cas9-expressing stable cell lines is sgRNA library screening. CRISPR sgRNA libraries are collections of hundreds-to-thousands of plasmids, each expressing a unique sgRNA, and are highly useful for forward mutagenesis screens (Sanjana, et al, 2014). GeneCopoeia carries pre-made CRISPR sgRNA libraries that cover several well-studied pathways and gene groups, and also offers custom sgRNA library construction (http://www.genecopoeia.com/product/sgrna_libraries/).

CRISPR sgRNA libraries enable researchers to knock out many genes simultaneously in mammalian cells, opening the door to drug target identification and validation, phenotypic changes, and reporter assays. Previously, these applications were achieved using RNA interference (RNAi). However, while shRNA libraries are useful for high-throughput loss-of-function screens, RNAi has a number of disadvantages compared with CRISPR: 1) RNAi causes knockdown of gene expression, not knockout, so false negatives resulting from residual gene expression can be missed; 2) RNAi acts only on cytoplasmic RNA, and so cannot silence nuclear RNAs like long non-coding RNAs. Conversely, CRISPR makes permanent changes to the genetic code. Thus, CRISPR can make a complete knockout of all alleles of a gene, whether their transcription products are localized to the nucleus or cytoplasm. So, CRISPR sgRNA library screening can be straightforward, provided that you have a good assay or phenotype for screening (Figure 2).

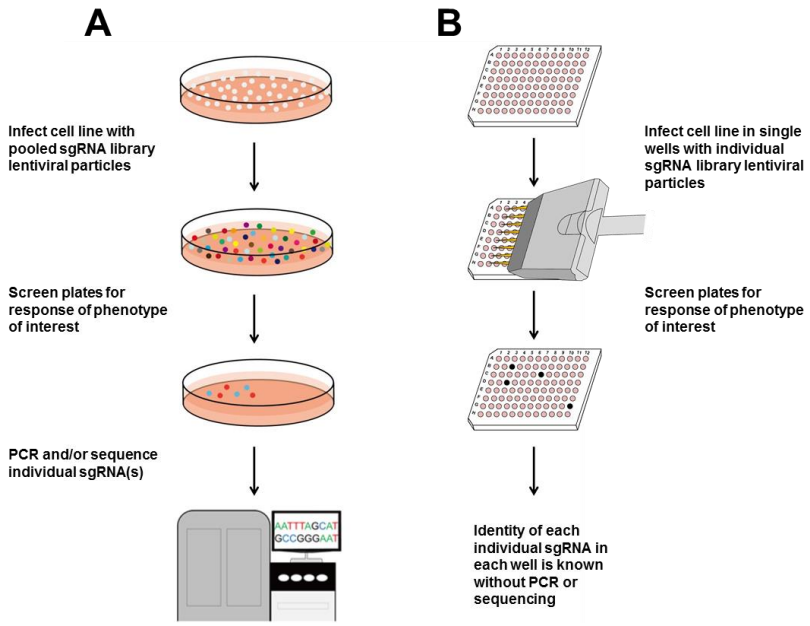

Figure 2. Workflow for CRISPR sgRNA libraries. A. Pooled screen. Cells infected with each sgRNA library pool are screened for the desired readout. Pooled cells are subjected to Sanger sequencing for individual sgRNAs, or deep sequencing to look for over- or under-representation of individual sgRNAs. B. GeneCopoeia sgRNA libraries are available either as pools or individual sgRNAs. Cells are infected with individual sgRNA lentiviruses. Wells are screened for the readout of interest. Individual sgRNAs corresponding to the phenotype of interest are already known without sequencing.

sgRNA libraries are best suited for lentiviral delivery rather than plasmid transfection, because it provides higher delivery efficiency. However, because the titer of Cas9-expressing lentiviruses is lower than sgRNA-expressing lentiviruses, GeneCopoeia sgRNA libraries are built without Cas9. We recommend using our sgRNA libraries in Cas9-expressing stable cell lines.

Application 4: Inducible genome editing

CRISPR is commonly used to generate genome modifications in a constitutive fashion, without regard to a specific time or stage in cell growth. Sometimes, though, it is useful to introduce the modification after a particular event has occurred, such as treatment of cells with a compound, or at a specific time during differentiation. Inducible CRISPR (iCRISPR) was first demonstrated by Gonzalez, et al. (2014), who built an induced pluripotent stem cell (iPSC) model cell line carrying Cas9 that can be expressed after addition of the tetracycline analog doxycycline.

GeneCopoeia Cas9 stable cell lines

GeneCopoeia carries a large suite of cell lines stably expressing the Cas9 nuclease (Table 1).

Catalog #	Cell line	Cell type	Selection marker	Cas9 integration site
Human Cas9-expressing stable cell lines				
SL502	HEK293	Embryonic kidney	Puro	AAVS1
SL501	NCI-H1299	Lung cancer	Puro	AAVS1
SL533	NCI-H1299	Lung cancer	Hygro	Random
SL504	A549	Lung cancer	Hygro	AAVS1
SL529	H1975	Lung cancer	Hygro	Random
SL536	NCI-H1437	Lung cancer	Hygro	Random
SL503	HeLa	Cervical cancer	Hygro	AAVS1
SL514	MCF-7	Breast cancer	Hygro	AAVS1
SL524	MCF-7	Breast cancer	Hygro	Random
SL515	MDA-MB-231	Breast cancer	Hygro	Random
SL516	MDA-MB-468	Breast cancer	Hygro	Random
SL517	T47D	Breast cancer	Hygro	Random
SL526	SNU-1	Breast cancer	Hygro	Random
SL535	SK-BR-3	Breast cancer	Hygro	Random
SL544	HCC70	Breast cancer	Hygro	Random
SL547	DU4475	Breast cancer	Hygro	Random
SL518	HepG2	Liver cancer	Hygro	AAVS1
SL522	SNU-475	Liver cancer	Hygro	Random
SL530	SNU-449	Liver cancer	Hygro	Random
SL531	PLC/PRF/5	Liver cancer	Hygro	Random
SL537	SNU-387	Liver cancer	Hygro	Random
SL538	SNU-423	Liver cancer	Hygro	Random
SL543	C3A	Liver cancer	Hygro	Random
SL520	AGS	Stomach/gastric cancer	Hygro	Random
SL527	KATO111	Stomach/ggastric cancer	Hygro	Random
SL545	SNU-16	Stomach cancer	Hygro	Random
SL521	BXPC-3	Pancreatic cancer	Hygro	Random
SL540	CFPAC-1	Pancreatic cancer (derived from metastatic liver)	Hygro	Random
SL523	HT-29	Colon cancer	Hygro	Random
SL525	LoVo	Colon cancer	Hygro	Random
SL539	RKO	Colon cancer	Hygro	Random
SL541	T84	Colon cancer	Hygro	Random
SL542	COLO 205	Colon cancer (derived from metastatic site)	Hygro	Random
SL532	LS411N	Caecum cancer	Hygro	Random
SL546	U-2 OS	Bone cancer	Hygro	Random
SL552	K562	Bone marrow cancer	Hygro	Random
SL555	Jurkat	Peripheral blood cancer	Hygro	Random
SL550	SH-SY5Y	Neuroblastoma	Hygro	Random
Human stable cell line expressing high-fidelity Cas9 (SpCas9-HF1)				
SL553	HEK293	Embryonic kidney	Hygro	AAVS1
Human Cas9-expressing stable cell lines				
SL509	Neuro2a	Neuroblastoma	Hygro	ROSA26
SL510	Neuro2a	Neuroblastoma	Puro	ROSA26
SL511	Neuro2a	Neuroblastoma	Neo	ROSA26
Rat Cas9-expressing stable cell line				
SL534	C6	Glioma	Hygro	Random

Table 1. List of GeneCopoeia pre-made human, mouse, and rat Cas9 stable cell lines.

These cell lines provide several benefits and advantages for genome editing work, including:

- **Built-in, stable Cas9 expression.** Minimizes need for co-transfection/transduction of sgRNAs. Ideal for sgRNA library screening, lentiviral CRISPR, sgRNA validation, and inducible CRISPR.
- **Single clone isolation.** GeneCopoeia's Cas9 stable cell lines are derived from single cell clones, providing a uniform genetic background, unlike stable pools, which can vary in transgene expression and carry unwanted background mutations.
- **Functionally validated.** The function of Cs9 is validated for cleavage ability using the IndelCheck™ T7 Endonuclease I assay, assuring high quality.
- **Compatibility.** Compatible with GeneCopoeia Genome-CRISP™ sgRNA clones, sgRNA libraries, and donor clones.

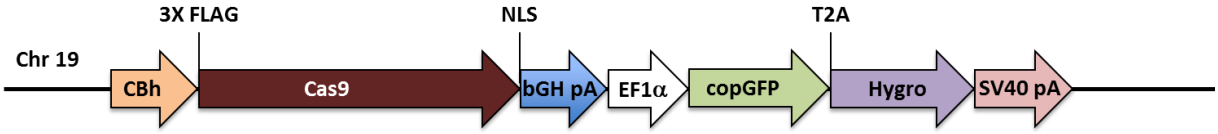

Figure 3. Structure of a stably integrated CRISPR Cas9 nuclease construct. Hygromycin (Hygro) selection is used for isolation of single clones. CBh: Hybrid CBA promoter. 3X FLAG: N-terminal 3X FLAG tag. NLS: nuclear localization signal. bGH pA: Bovine growth hormone polyadenylation signal. EF1a: Elongation factor 1a promoter. copGFP: Copepod green fluorescent protein. T2A: Element to allow translation of copGFP and Puro independently from the same transcript. SV40 pA: Simian virus 40 polyadenylation signal.

If you don't see your cell line of interest in Table 1, you can use either GeneCopoeia's Cas9 lentiviral clones or GeneCopoeia's Safe Harbor knock-in system to integrate the Cas9 gene yourself. GeneCopoeia's Safe Harbor knock-in system consists of kits containing CRISPR reagents targeting the human AAVS1 and mouse ROSA26 Safe Harbor sites, and can be ordered with many donors for HDR, such as those expressing Cas9 used for the cell lines in Table 1, or any of more than 45,000 human and mouse ORFs. For more information about GeneCopoeia's Safe Harbor kits and knock-in ORF clones, visit http://www.genecopoeia.com/product/safe-harbor/.

At GeneCopoeia, our Genome Editing team has a wealth of expertise with CRISPR applications in mammalian systems. We start at CRISPR design and deliver sequence-verified plasmid DNA. We also offer functional validation kits and services for your CRISPR constructs, construct stable cell lines or transgenic mice containing your CRISPR-mediated modification of interest, and provide scientific consulting services to help you plan your projects. For more information, visit our website: http://www.genecopoeia.com, call 1-866-360-9531, or email inquiry@genecopoeia.com

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