

# Applications For CRISPR-Cas9 Stable Cell Lines

**Presenter:** 

March 22, 2017

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

#### GeneCopoeia products & services

**Functional Genomics & Cell Biology** 

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



#### GeneCopoeia genome editing services

#### Cas9-expressing stable cell lines



- Cell lines with stably expressing Cas9
- ✤ Have 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



#### Outline

- Genome editing: Technologies
- Applications for genome editing
- sgRNA validation
- Lentiviral CRISPR
- CRISPR library screening
- Inducible CRISPR



#### Outline

### Genome editing: Technologies

Applications for genome editing

sgRNA validation

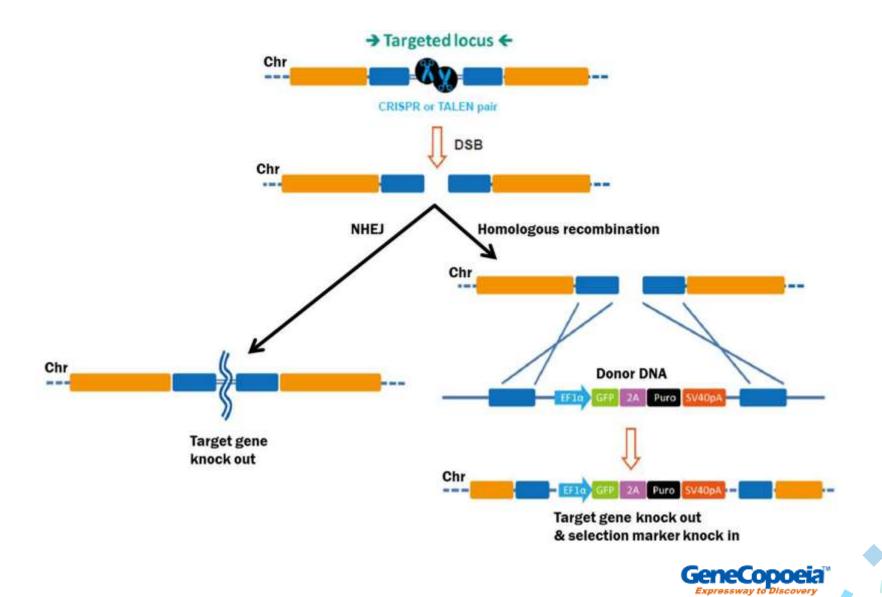
Lentiviral CRISPR

CRISPR library screening

Inducible CRISPR

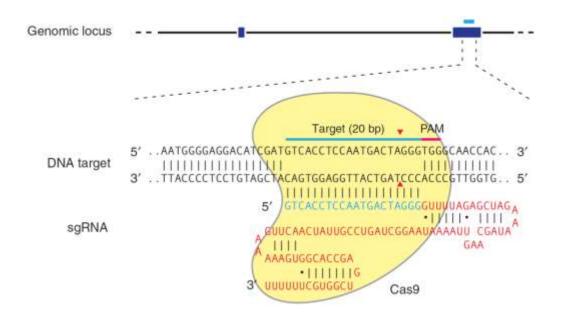


#### Targeted DNA editing by DSB induction



# CRISPR genome editing technology

CRISPR-Cas9: RNA-guided endonuclease

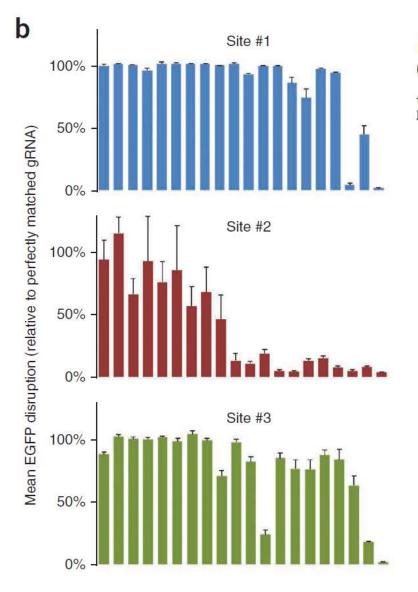


Ran, et al. (2013). Nature Protocols 8, 2281

- 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



### **CRISPR-Cas9: Specificity**



High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells

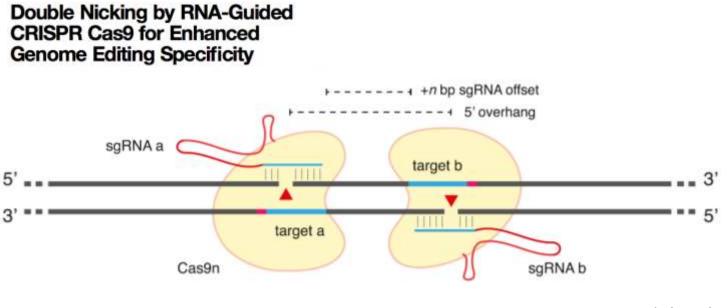
Yanfang Fu<sup>1-4</sup>, Jennifer A Foden<sup>1-3</sup>, Cyd Khayter<sup>1-3</sup>, Morgan L Maeder<sup>1-3,5</sup>, Deepak Reyon<sup>1-4</sup>, J Keith Joung<sup>1-5</sup> & Jeffry D Sander<sup>1-4</sup>

- Showed that some sgRNAs with single, double, and even up to 5 transversion mismatches could still direct Cas9 to mutate EGFP.
- Found that for 4 of 6 tested sgRNAs, evidence of off-target mutagenesis (5.6% -125% of the intended targets).



Fu, et al. (2013). Nature Biotech. 21, 822

#### **CRISPR-Cas9: Specificity**



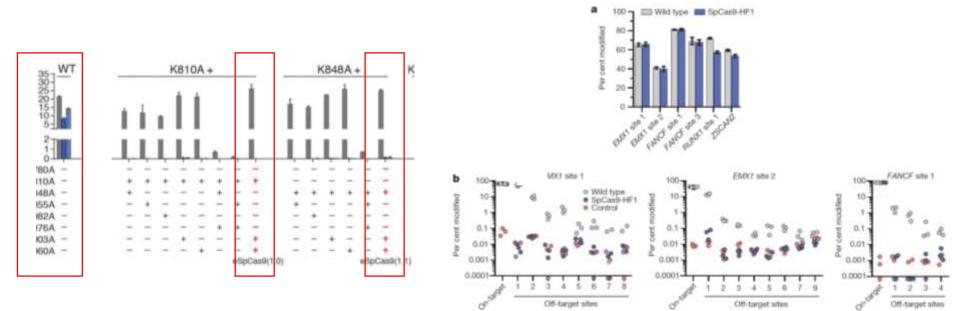
Ran, et al. (2013). Cell 154, 1380

- Cas9 D10A "nickase" mutant creates single-strand nicks instead of DSBs
- > Off-target nicks repaired by high-fidelity base excision repair
- Permits ability to generate dimer-like chimeric endonuclease, similar to TALEN. 2 nicks will create a DSB
- > Dramatically (50x-1,500x) reduces incidence of off-target effects



#### **CRISPR-Cas9: Specificity**

#### Cas9 point mutants



Slaymaker, et al. (2015). Science

Kleinstiver, et al. (2016). Nature 529, 490



#### Outline

Genome editing: Technologies

## Applications for genome editing

sgRNA validation

Lentiviral CRISPR

CRISPR library screening

Inducible CRISPR



Application	Example	Technology	Reference
Gene knockout	Knockout SRY, UTY genes in cultured dells	TALEN via NHEJ and plasmid donor	Wang, et al. (2013). Nature Biotech. 31, 530
Gene knockout	Knockout of tet genes in transgenic rats	CRISPR via NHEJ	Li, et al. (2013). Nature Biotech. 8, 684
Correction of disease mutations	Cured heritable cataracts in transgenic mice	CRISPR via oligonucleotide donor	Wu, et al. (2013). Cell Stem Cell 13, 659
Engineered disease resistance	Knockout CCR5 gene to cure patients of HIV	ZFN via NHEJ	Perez, et al. (2008). Nat Biotech 26, 808
Forward mutagenesis screens	Identified genes in mismatch repair pathway by selecting for 6- thioguanosine resistance	CRISPR via NHEJ	Wang, et al. (2014). Science 343, 80
In-frame fusion tagging	C-terminal tag Sox2p with V5	CRISPR via oligonucleotide donor	Yang, et al. (2013). Cell 154, 1370
Transgene knock-in	Sox2, Oct4 ORFs KI into human AAVS1 "Safe Harbor"	TALEN via plasmid donor	GeneCopoeia internal data



#### Applications for genome editing Knockout via NHEJ

#### Clone 1

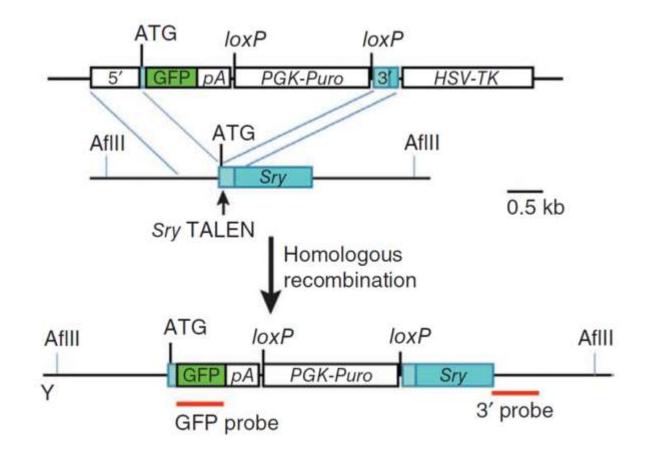
	(500)	500 510	520	530	540	550	560	570	580	590	600	610	620	635
HDAC6 Targeting region NC 018934	C2-4_PREMIX (471)	TTCACAGAAGCGAAA	ATTAAAAAAG	<b>GCAGCOGTICO</b>	CCCCT TATOO	CAATUTA	AGGCCTCCTCC	GAGOGGATGT	ACCCCCGAOGA	CGGDGCCC	CAGGTAAAI	AAGAAAGGCAI	AATGAAGAAGC	TOGGCCAA
Allele a	C2-6_PREMIX (470)	TTCACAGAAGCGAAA	FATTRAAAAG									AAGAAAGGCAI	VANTGANGANCO	TOGGECAA
Allele b	C2-11_PREMIX (469)	TTCACAGAAAGCGAAA	TATTAAAAAG	GGAGCCGTTCO	<u></u>					*********	AAA	AAGAAAGGCAI	MATGAAGAAGC	TOGGCCAR
Allele c	C2-7_PREMIX (472)	TTCACAGAAGCGAAA	TATTAAAAAG	<b>BGAGCCUTTCO</b>	CLIGETUTATOOL	CAAT				G	GAGGTAAAA	AAGAAAGGCAI	AATGAAGAAGE	TOGGCCAN
Allele c	2A-F_PREMIX (470)	TTCACAGAAGCGAAA	TRITTARAAAG	<b>GGAGCCGTTCO</b>	COSCILCTATION	CANTS					GAGGTAAAA	AAGAAAGGCN	MATGAAGAAGC	TOGGOCAA
Allele d	C2-5_PREMIX (470)	TTCACAGAAACCGAAA	FATT'AAAAAG	<b>BGAGCOGTTCO</b>	coord charcos	CAAD TA	G		*********		AGARA	AAGAAAGGCAI	MATGAAGAACE	TOGECCAR
Allele d	C2-8_PREMIX (474)	TTCMCNGAAGCGAAA	TATTAAAAAG	DEMOCOSTTCO	COGCTCTATOOD	CAATCEN	G	********		<mark>('6</mark>	GAGGTAAAO	AAGAAAGGCAI	MATGAAGAAGC	TOGGOCAA
Allele d	2B-F_PREMIX (472)	TTCACAGAAOCGAAA	TATTAAAAAG	GGAGCOUTTCO	CERCITCIATOOU	CARTCIPAC	G			<mark>UG</mark>	GAGGTAAA	AAGAAAGGEAI	AATGAAGAACC	TOGGOUAR
Allele d	2C-F_PREMIX (470)	TTCACAGAAGCGAAA	PATTABAAAS	GEAGCOSTTCO	COSC TOTATOOO	CANTITAC	G			<mark>CG</mark>	GAGGTAAAG	AAGAAAGGCAI	MATGAAGAAGC	TOGGOCAA
Allele e H	AC6 Targeting Region NC_018934 (500)	<b>TTCACAGAAGCGAA</b>	PATTAAAAAG	GGAGOOGTICO	COSCICIATOOO	CAAPCTA	C			G	GAGGTAAAA	AAGAAAGGCN	MATGAAGAAGC	TOGGOCAR
Consensus	Consensus (500)	TICACAGAAGCGAAA	TATTAAAAAG	GGAGCCGTTCC	CCOCTCTATCCC	CAATCTA	8			CG	GAGGTAAAA	IAAGAAAGGCAI	AATGAAGAAGC	TOGGOCAA

#### Clone 2

	(553)	553 560	570	580	590	600	610	620	630	640	650	660	670	688
HDAC6 Targeting region NC 018934	C3-11_PREMIX (487)	TANANAGGARC	ET COE	CTATOCOCAN	PCTAGAGGCCT	CCTCCGAGC	XGGATGTACCCO	GAGGACGGCG	CCCCGGAGGT	ALCINGAN	GCAMATCI	AGAAGCTC	GCCAAGCAATC	GAAGAAG <mark>ACCTA</mark>
Allele f	C3-8_PREMIX (516)	<b>CATTAATGAAT</b>	COGC CAAC	GOGGGGAGAGO	CGGTTTG	(	STATIGGCCCC	CTTOCGCTI	CCTCGCTCACT	GACICICICI	C <mark>CCTCCCT</mark> CC	TTCG CTG	C <mark>GCCCNOC</mark> CC <mark>T</mark> A	TCACTCNCTCA
Allele g	C3-6_PREMIX (487)	TANAAAGGAGC	CODUCTION							ALLAGAA	GCAMATC/	AGAANCTC	GOCHATCAATO	GANGAAG <mark>ACUTA</mark>
Allele g	3C-F_PREMIX (487)	TAAAAAOGACC	3077000C							A <mark>A</mark> TAA <mark>G</mark> AAA	G <mark>SCAAAA</mark> TG	AGAAGCTO	<b>OCAAOCAAT</b> U	GAAGAAG <mark>ACCT</mark> A
Allele h	C3-10_PREMIX (487)	TRAAAAGGGAGG	161110CC <mark>C</mark> CO	CTATCCGC					GGAGGT	A <mark>A</mark> GAA <mark>G</mark> AAA	G <mark>ZZNAMA</mark> BGI	NGANG <mark>CT</mark> CI	S <mark>GCCAAGCAAT</mark> C	GAAGAAG <mark>ACCT</mark> A
Allele i	3A-F_PREMIX (487)	TAAAAAGGAACC	COLOR COLOR	<b></b>					/	A <mark>a</mark> kaa <mark>k</mark> aaa	G <mark>GCNRAA</mark> TCI	AGAAG <mark>CT</mark> C	GOCABOCA TO	CAN-ANN <mark>ACCUA</mark>
Allele i	3B-F_PREMIX (492)	TANANGGANGG	agir reco <mark>r</mark> ect	CT					}	A <mark>h</mark> eaa <mark>g</mark> aaa	G <mark>əc</mark> hma <mark>t</mark> G	AGANGCTO	H <mark>GCCAACCAAT</mark> G	GAAGAAGACCTA
Allele i	C3-4_PREMIX (488)	TAARAAGGAAGG	GT QC QD	(T <mark></mark>					1	A <mark>A</mark> CAR <mark>C</mark> AAA	GSCAMATIG	AGAAG <mark>CT</mark> CI	G <mark>ugaageaat</mark> u	GAAGAAG <mark>ACCTA</mark>
Allele i	C3-5_PREMIX (487)	TAAAAAGCACC								A <mark>A</mark> GAA <mark>G</mark> AAA	G <mark>OCANAT</mark> GI	NIANO TO	BOCAADCAATC	GAAGRAG <mark>ACETA</mark>
Allele i	C3-9_PREMIX (488)	INANAGGAGO	CGT TOL COL	<b></b>					/	A <mark>A</mark> GAA <mark>G</mark> AAA	G <mark>SCRAIA1</mark> G7	AAAAO <mark>C1</mark> O	GOOGAGCAATO	gaagaag <mark>ac</mark> ut <mark>a</mark>
Allele i HDAC6 Targeting	) Region NC_018934 (518)	TAAAAAGGEAOC	26/70CCP	CTATOCOCAA	ICTAG				CGGAGGT	ANGAAGAAA	G <mark>əc</mark> ama <b>t</b> ra	A ANGCIC	IGCONACICANTO	erangare <mark>ac</mark> ct <mark>a</mark>
Consensus	Consensus (553)	TAAAAAGGGAGC	CGTTOCCOG	CT					- 1	AAGAAGAAA	GGERARATGI	MAAGCTO	GCCAAGCAATG	GAAGAAGACCTA



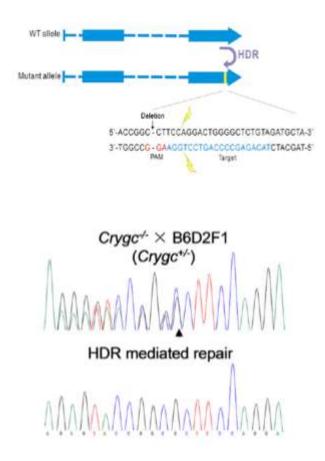
#### Knockout via HDR: Donor plasmid



Wang, et al. (2013). Nature Biotech. 31, 530



Mutagenesis via HDR: Oligo donor

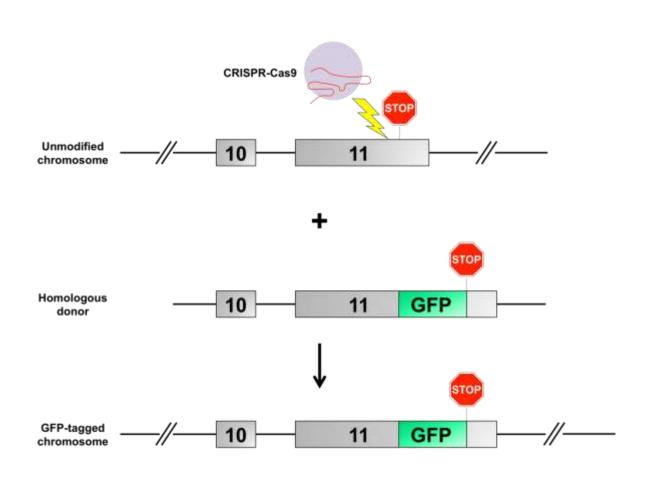


- Use single strand oligonucleotide (ssODN) to introduce base changes or small deletions.
- Use for mutagenesis or disease correction.
- Wu, et al.: Used CRISPR + ssODN to cure heritable cataract disease in mice



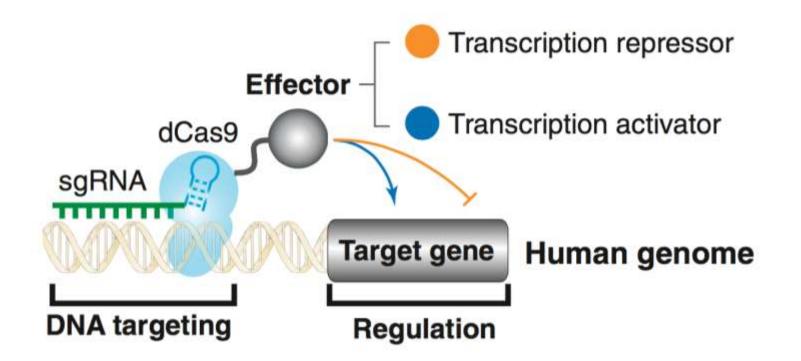
Wu, et al. (2013). Cell Stem Cell 13, 659

#### In-frame fusion tagging





Targeted gene activation/repression





#### GeneCopoeia genome editing services

#### Cas9-expressing stable cell lines



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

Genome editing: Technologies

Applications for genome editing

#### sgRNA validation

Lentiviral CRISPR

CRISPR library screening

Inducible CRISPR



### **Functional validation**

Why do functional validation?

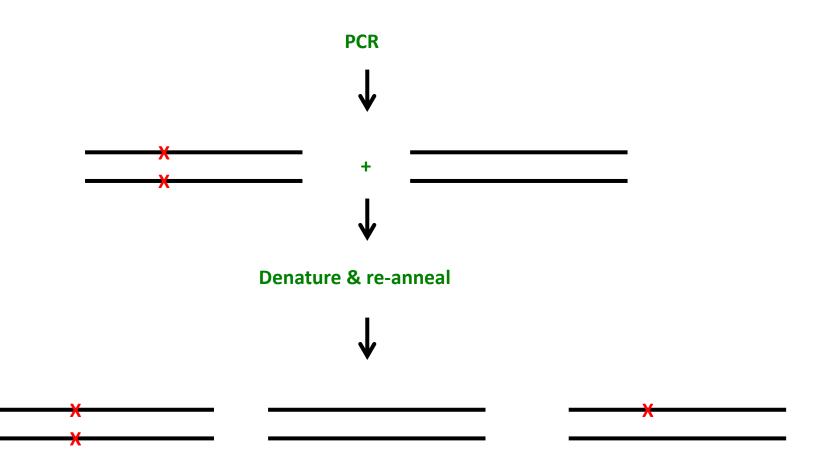
Cell culture-based genome editing projects can take 3 or more months to complete

Not all CRISPR sgRNAs are created equal! Test first before undergoing long genome editing project to avoid wasting time and expense.

Recommend cell culture based strategy using T7 Endonuclease I assay.



#### **Functional validation**

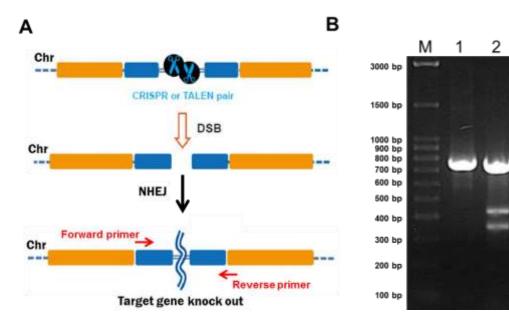




### Functional validation IndelCheck<sup>™</sup> T7 Endonuclease System

\*

\*



- Contains PCR and T7 endonuclease I reagents
- Purchase with or without target-specific primers



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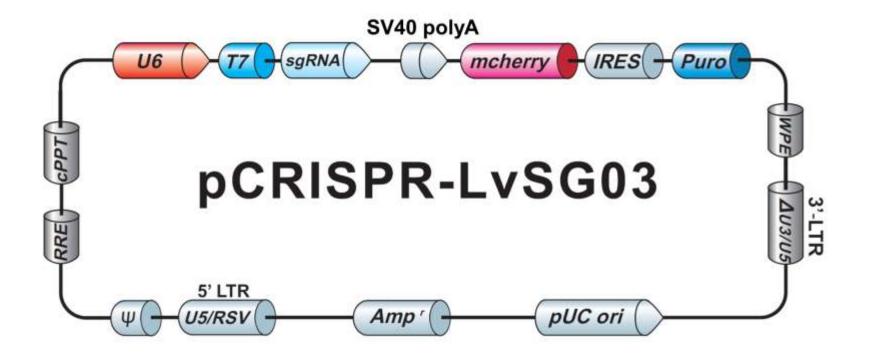
Lentiviral CRISPR

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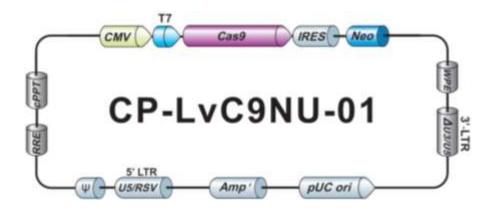


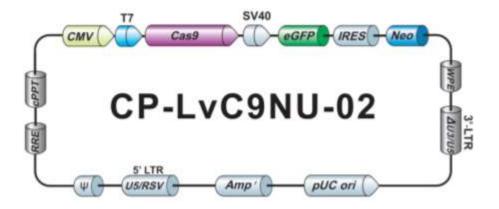
### Lentiviral CRISPR





#### Lentiviral CRISPR







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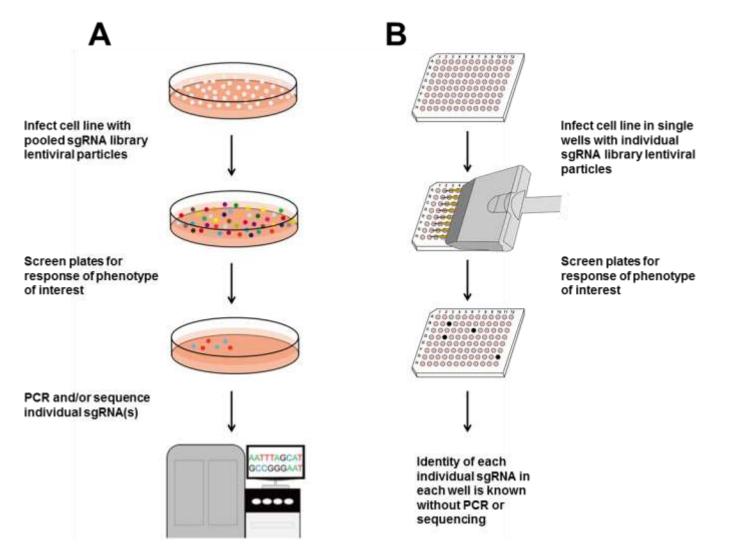
#### **CRISPR or RNAi?**

Knock <u>down</u> vs. Knock <u>out</u>

Method	Change expression level	Knock down	Knock out	Change genetic code
CRISPR	$\checkmark$		$\checkmark$	$\checkmark$
RNAi	$\checkmark$	$\checkmark$		

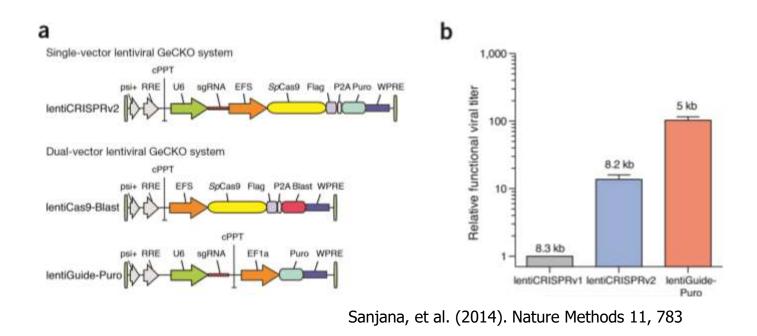


### **CRISPR** sgRNA libraries





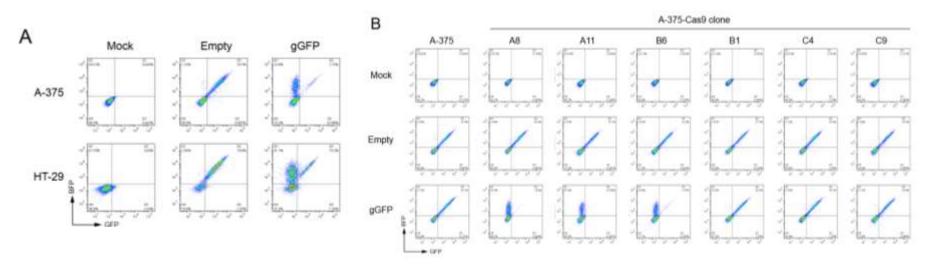
### **CRISPR sgRNA libraries**



- 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



### **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



### GeneCopoeia sgRNA libraries

#### 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



#### GeneCopoeia sgRNA libraries

#### **Advantages**

- 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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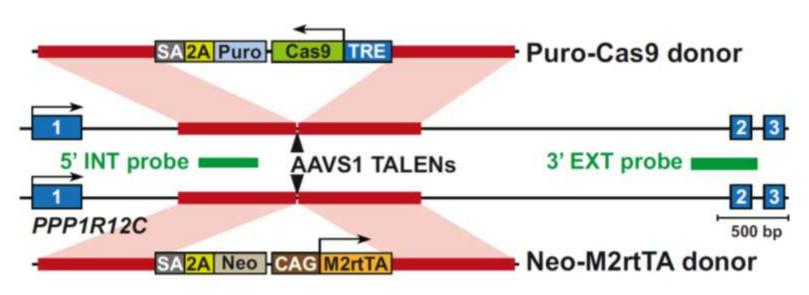
CRISPR library screening

Inducible CRISPR



### Inducible CRISPR

#### Tet system

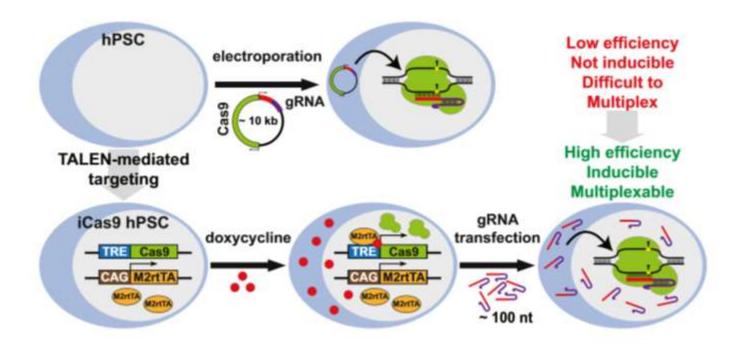


Gonxalez, et al. (2014). Cell Stem Cell 15, 1

- One chromosome: Construct donor with Tet-inducible promoter at AAVS1. Marked with puro.
- Other chromosome: Construct donor with CMV-driven tet transactivator (M2rtTA) at AAVS1. Marked with neo.
- Used TALEN with puro + G418 selection in one transfection for knockin at AAVS1.

#### Inducible CRISPR

#### Tet system

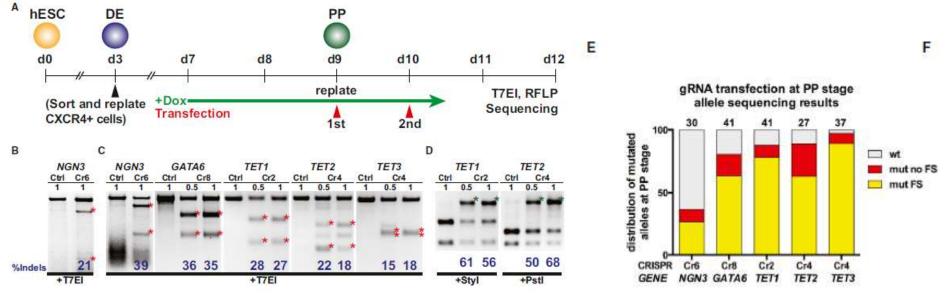


Gonxalez, et al. (2014). Cell Stem Cell 15, 1



### Inducible CRISPR





- 2 cell lines. Differentiated to pancreatic progenitor (PP) stage in absence of dox and sgRNA (A).
- ✤ At PP stage, transfected sgRNAs and added dox
- ✤ T7 (B, C, and D) shows stage-specific induction of indels at 5 genes.
- Sequencing (E) shows that mutation frequencies are very high and most are indels.

Gonxalez, et al. (2014). Cell Stem Cell 15, 1

#### Human lung cancer cell lines

Catalog #	Cell line	Selection marker	Cas9 integration site
SL501	NCI-1299	Puro	AAVS1
SL533	NCI-H1299	Hygro	Random
SL504	A549	Hygro	AAVS1
SL529	H1975	Hygro	Random
SL536	NCI-H1437	Hygro	Random



#### Human cervical cancer cell line

Catalog #	Cell line	Selection marker	Cas9 integration site
SL503	HeLa	Hygro	AAVS1



#### Human breast cancer cell lines

Catalog #	Cell line	Selection marker	Cas9 integration site
SL514	MCF-7	Hygro	AAVS1
SL524	MCF-7	Hygro	Random
SL515	MDA-MB-231	Hygro	Random
SL516	MDA-MB-468	Hygro	Random
SL517	T47D	Hygro	Random
SL526	SNU-1	Hygro	Random
SL535	SK-BR-3	Hygro	Random
SL544	HCC70	Hygro	Random
SL547	DU4475	Hygro	Random



#### Human liver cancer cell lines

Catalog #	Cell line	Selection marker	Cas9 integration site
SL518	HepG2	Hygro	AAVS1
SL522	SNU-475	Hygro	Random
SL530	SNU-449	Hygro	Random
SL531	PLC/PRF/5	Hygro	Random
SL537	SNU-387	Hygro	Random
SL538	SNU-423	Hygro	Random
SL543	СЗА	Hygro	Random



#### Human stomach/gastric cancer cell lines

Catalog #	Cell line	Selection marker	Cas9 integration site
SL520	AGS	Hygro	Random
SL527	KATO111	Hygro	Random
SL545	SNU-16	Hygro	Random



### Human pancreatic cancer cell lines

Catalog #	Cell line	Selection marker	Cas9 integration site
SL521	BXPC-3	Hygro	Random
SL540	CFPAC-1*	Hygro	Random

\*derived from metastatic liver



#### Human colon cancer cell lines

Catalog #	Cell line	Selection marker	Cas9 integration site
SL523	HT-29	Hygro	Random
SL525	LoVo	Hygro	Random
SL539	RKO	Hygro	Random
SL541	T84	Hygro	Random
SL542	COLO 205*	Hygro	Random

\*derived from metastatic site



#### Human caecum cancer cell line

Catalog #	Cell line	Selection marker	Cas9 integration site
SL532	LS411N	Hygro	Random



Human bone/bone marrow/blood cancer cell lines

Catalog #	Cell line	Selection marker	Cas9 integration site
SL546	U-2 OS	Hygro	Random
SL552	К562	Hygro	Random
SL555	Jurkat	Hygro	Random



#### Human embryonic kidney cell line

Catalog #	Cell line	Selection marker	Cas9 integration site
SL502	HEK293T	Puro	AAVS1
SL553	HEK293T*	Hygro	AAVS1

\*Expresses high-fidelity Cas9



#### Human neuroblastoma cancer cell line

Catalog #	Cell line	Selection marker	Cas9 integration site
SL550	SH-SY5Y	Hygro	Random



#### Mouse neuroblastoma cancer cell lines

Catalog #	Cell line	Selection marker	Cas9 integration site
SL509	Neuro2a	Hygro	ROSA26
SL510	Neuro2a	Puro	ROSA26
SL511	Neuro2a	Neo	ROSA26



#### Rat glioma cancer cell line

Catalog #	Cell line	Selection marker	Cas9 integration site
SL534	C6	Hygro	Random



### Summary

- CRISPR is a highly effective method for many applications, from knockout, knockin, activation, & more
- GeneCopoeia provides a large number of cell lines stably expressing the CRISPR-Cas9 nuclease
- GeneCopoeia's CRISPR-Cas9-expressing stable cell lines provide great utility for several genome editing applications, such as:
  - sgRNA functional validation
  - Lentiviral CRISPR
  - ✤ sgRNA library screening
  - ✤ Inducible CRISPR



### GeneCopoeia Technical Note: Cas9 stable cell lines



**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 easeof-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 pvoaenes* (SpCas9). SpCas9 is a DNA endonuclease that. in the presence of a chimeric

Download from:

http://www.genecopoeia.com/wp-content/uploads/2017/03/GeneCopoeia-Technical-Note-Cas9stable-cell-lines-03-2017.pdf



Upcoming webinar!

### How To Choose Between Adeno-associated Virus and Lentivirus For DNA Delivery

Wednesday, April 12, 2017 12:00 pm ET

**Register here:** 

https://attendee.gotowebinar.com/register/14390983 74222287363



Upcoming webinar!

### Applications For Safe Harbor Transgenesis in Genome Editing

Wednesday, April 19, 2017 12:00 pm ET

**Register here:** 

https://attendee.gotowebinar.com/register/73880445 19385274881



## 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

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