GeneCopoeia CRISPR Stable Cell Line Services

GeneCopoeia, Inc.

Presenter:

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Senior Application Scientist
GeneCopoeia, Inc.

May 27, 2015

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Goals of this presentation

CRISPR stable cell line services

- Save time & $$$ by letting us do the cell line construction work for you
- Focus more on planning new experiments & products

Outline

- Introduction to GeneCopoeia CRISPR services
- CRISPR technology & applications
- GeneCopoeia genome editing stable cell line service offerings
- GeneCopoeia genome editing stable cell line service: Case study

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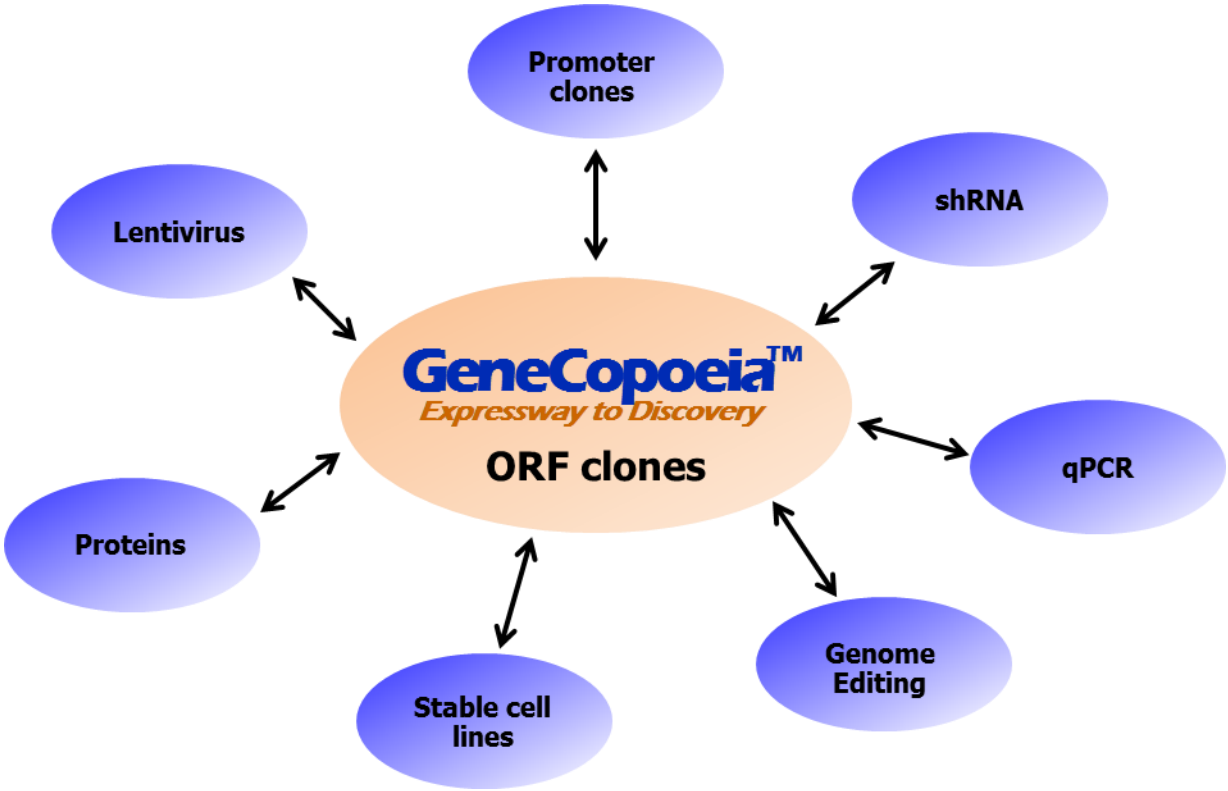
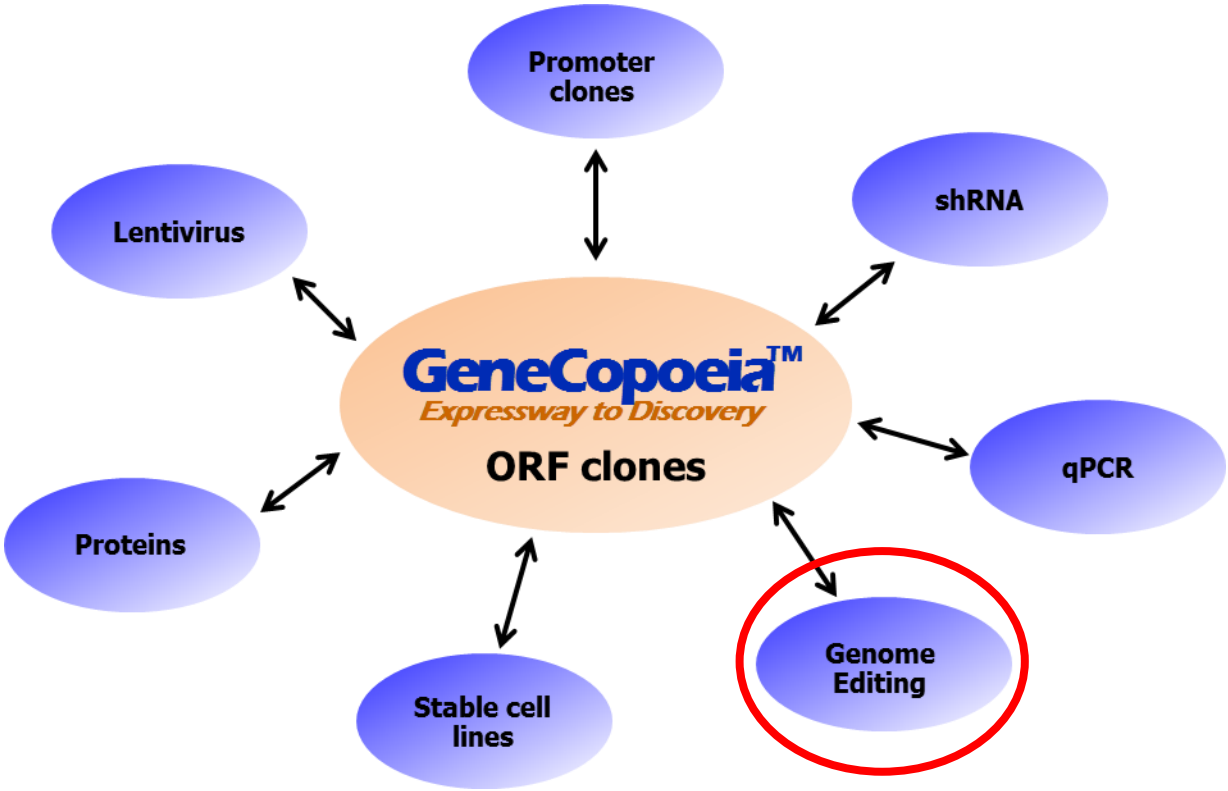
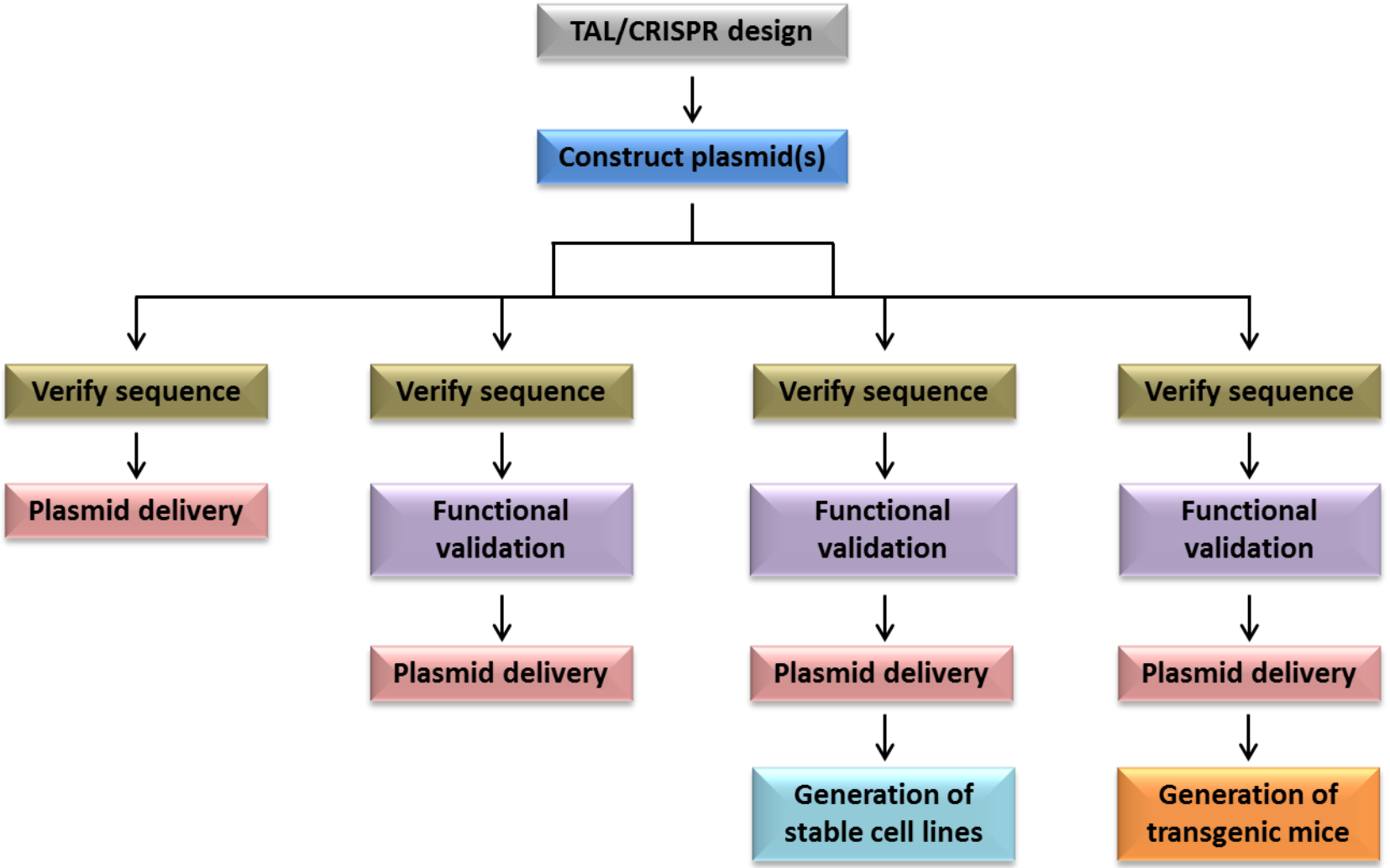
GeneCopoeia products & services



GeneCopoeia products & services



GeneCopoeia genome editing services



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GeneCopoeia CRISPR services

Mammalian CRISPR-editing stable cell line service

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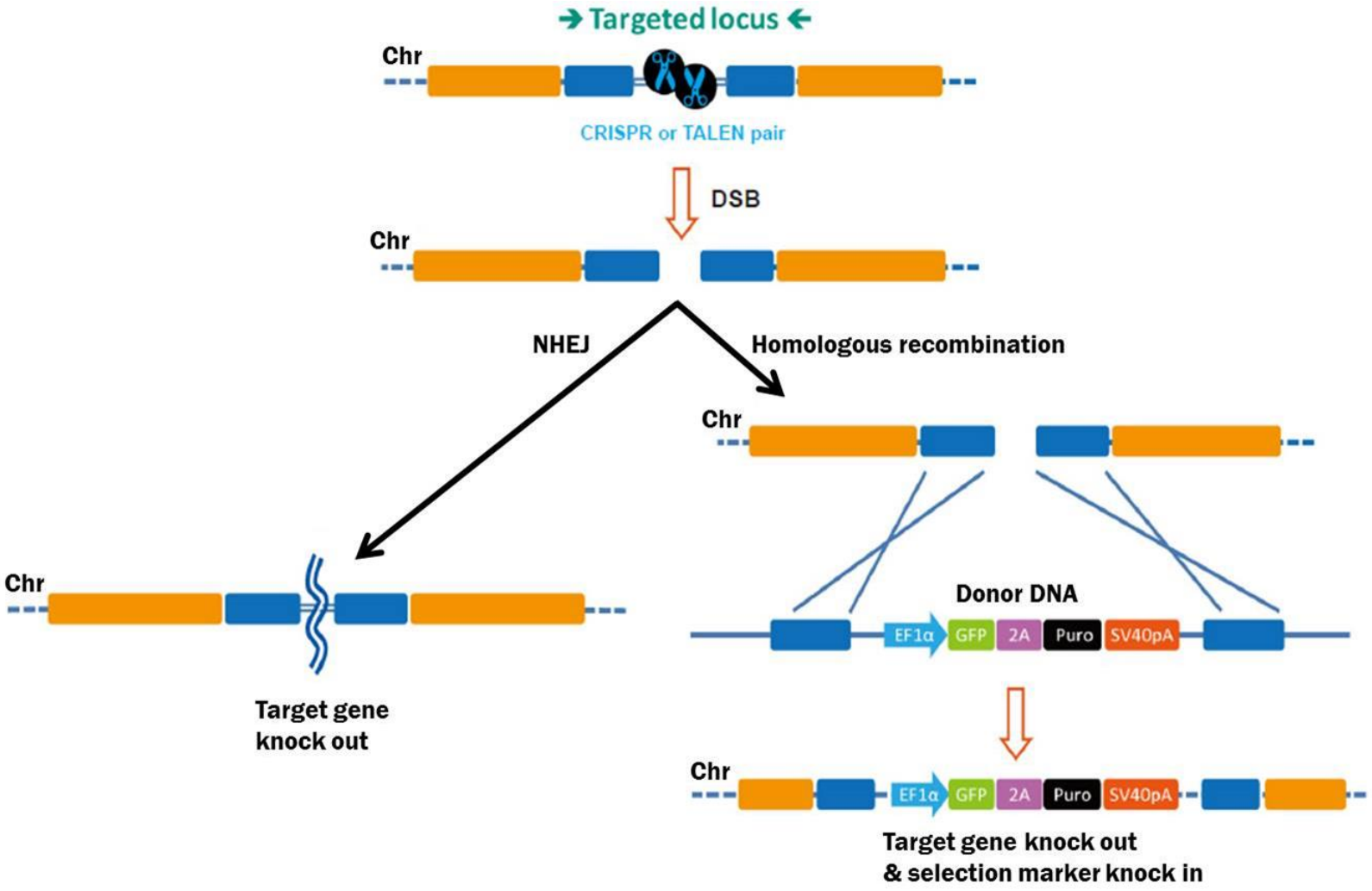
CRISPR or RNAi?

Knock out vs. Knock down

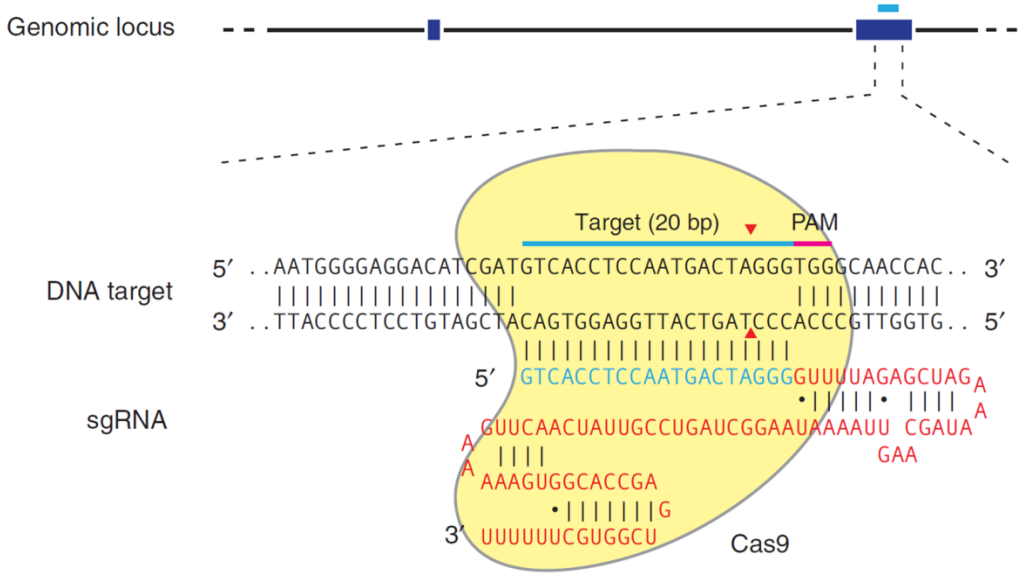
Method	Change genetic code	Change expression level	Knock down	Knock out
CRISPR	✔	✔		✔
RNAi		✔	✔	

Targeted DNA editing by DSB induction



CRISPR genome editing technology

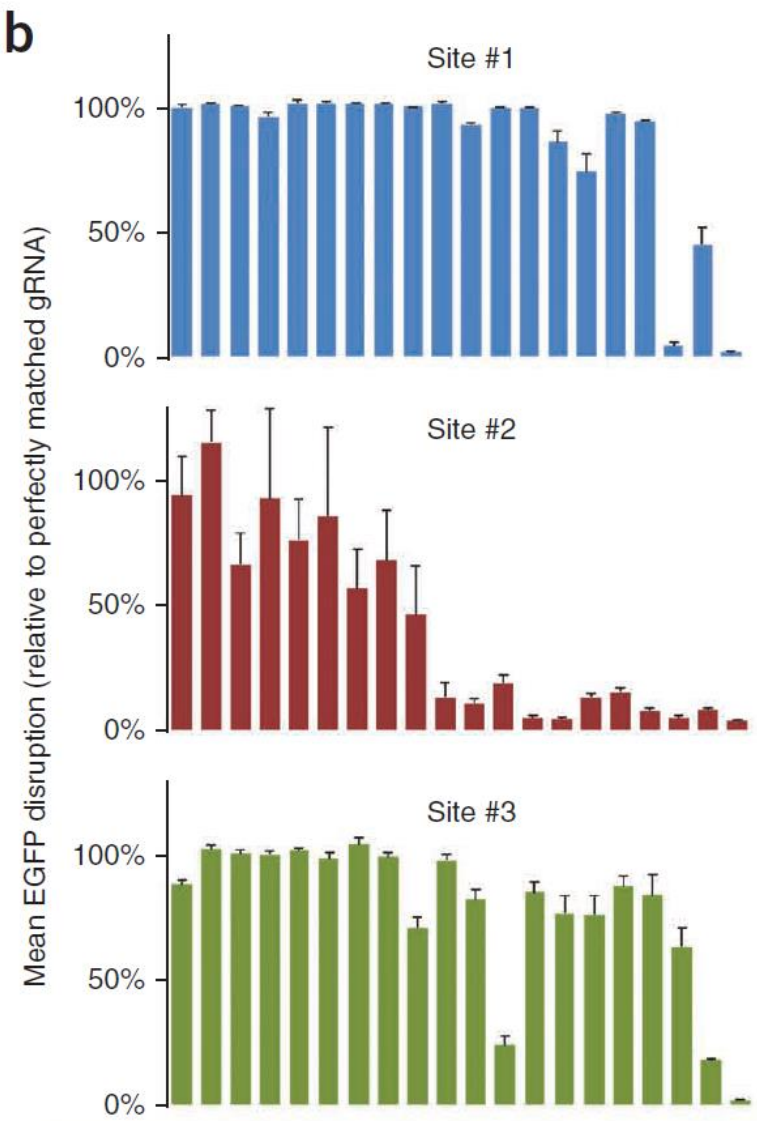
CRISPR-Cas9: RNA-guided endonuclease



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-Cas: Specificity



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

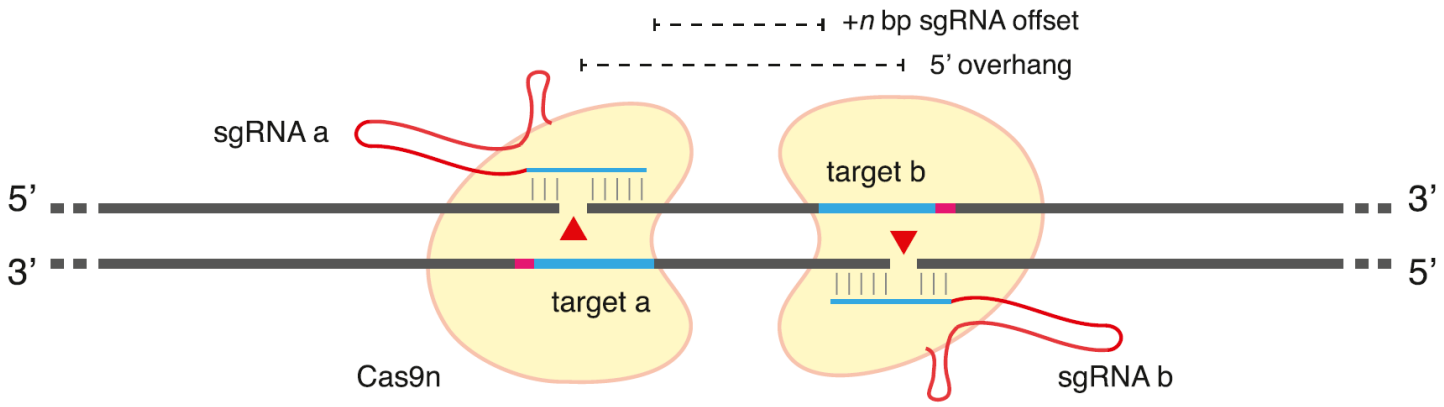
Yanfang Fu[1–4], Jennifer A Foden[1–3], Cyd Khayter[1–3], Morgan L Maeder[1–3,5], Deepak Reyon[1–4], J Keith Joung[1–5] & Jeffry D Sander[1–4]

- 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-Cas: Specificity

Double Nicking by RNA-Guided CRISPR Cas9 for Enhanced Genome Editing Specificity

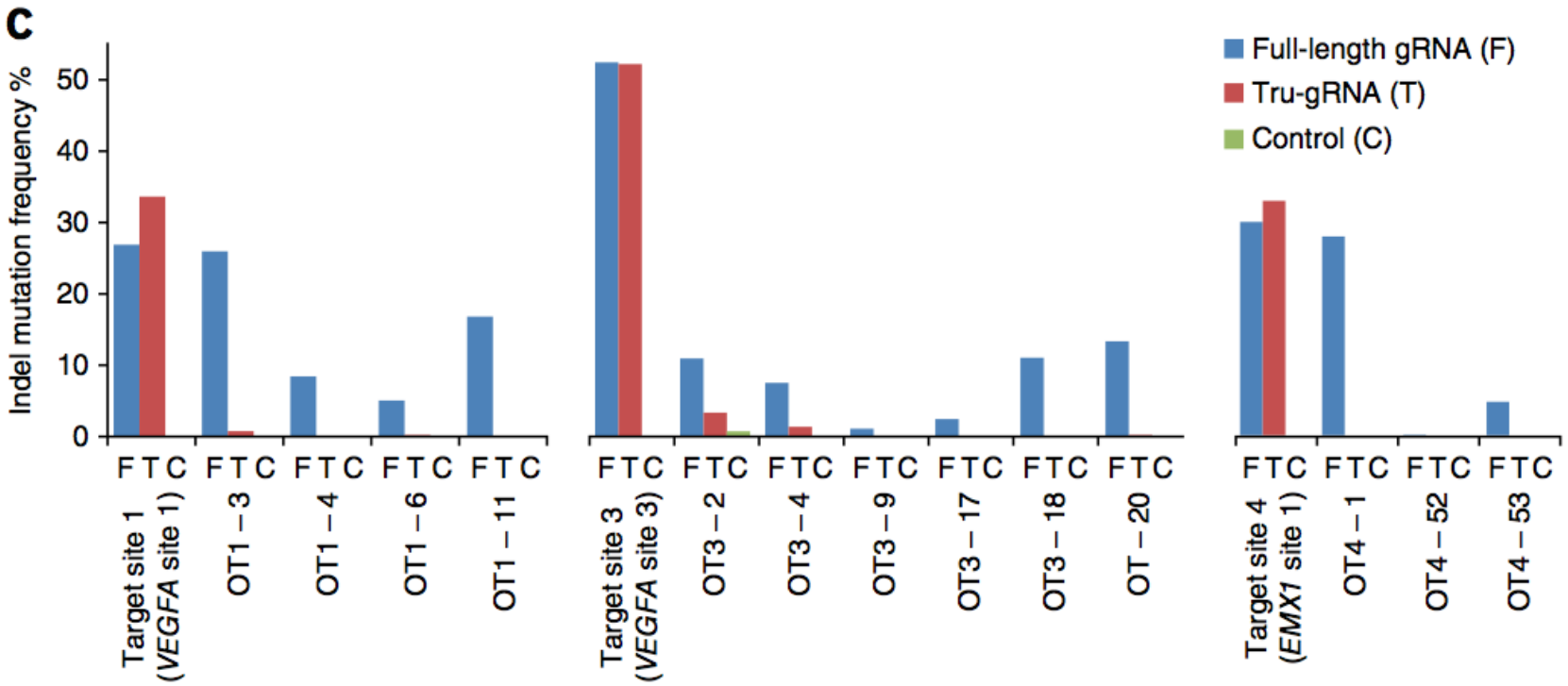


Ran, et al. (2013). Cell 154, 1380

- 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-Cas: Specificity

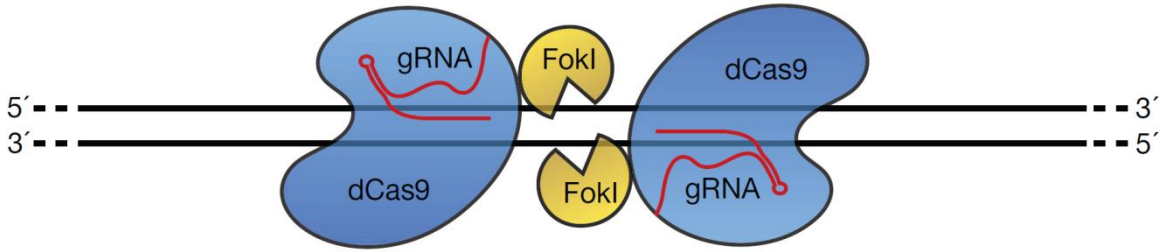
Truncated sgRNAs: 17-18 nt

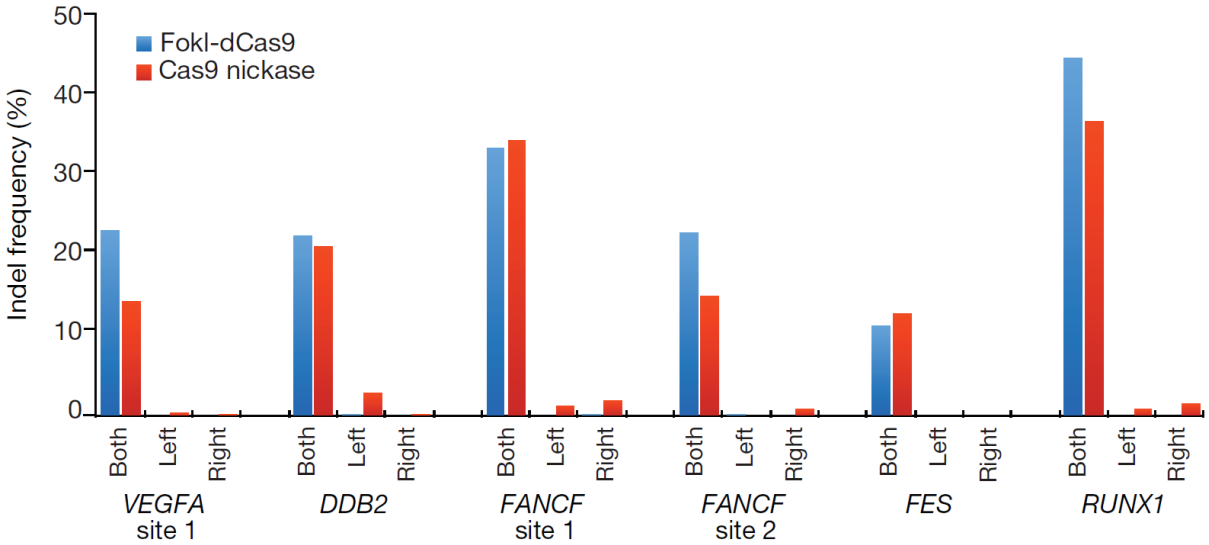


Fu, et al. (2014). Nature Biotechnology 32, 279

CRISPR-Cas: Specificity

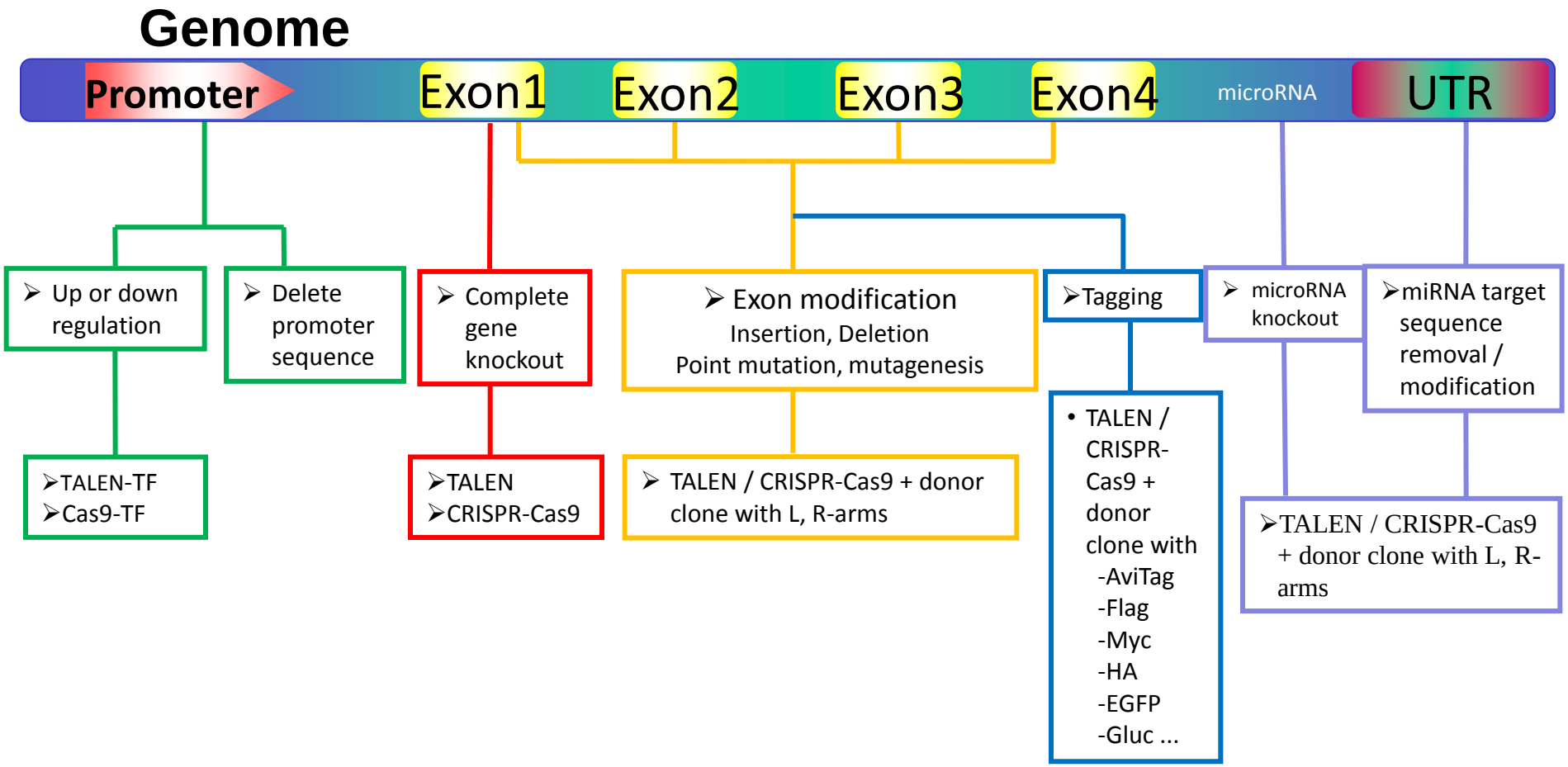
Cas9d-FokI fusion





Tsai, et al. (2014). Nature Biotechnology

GeneCopoeia Genome Editing Services



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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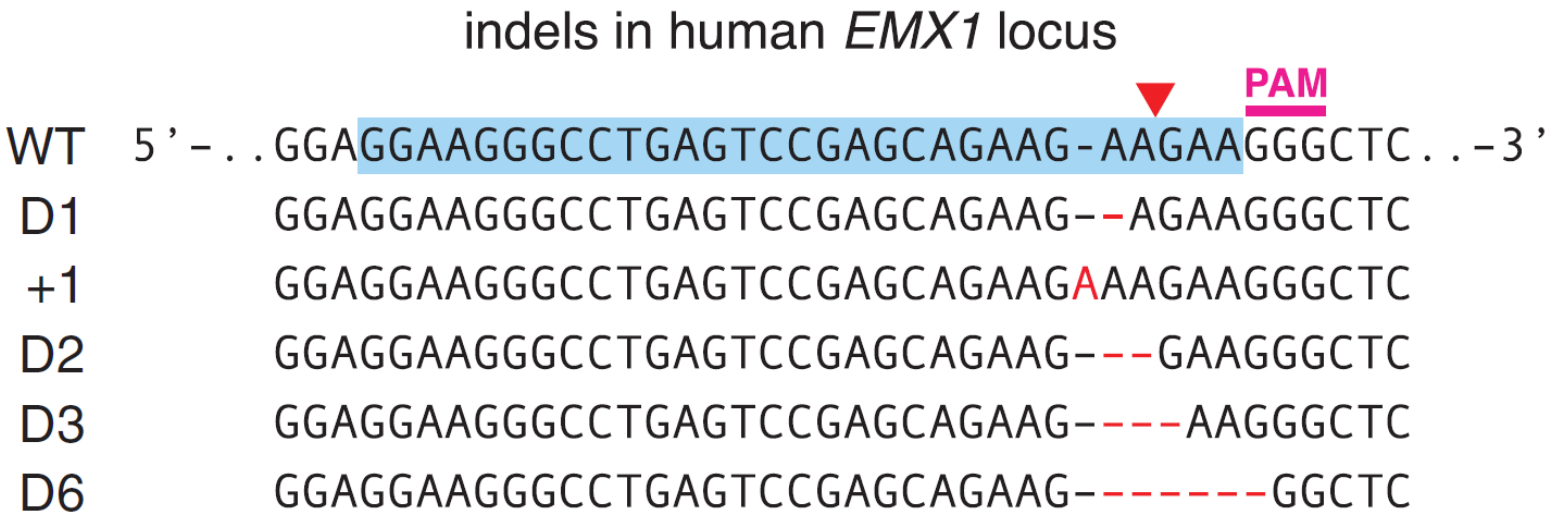
Applications for genome editing

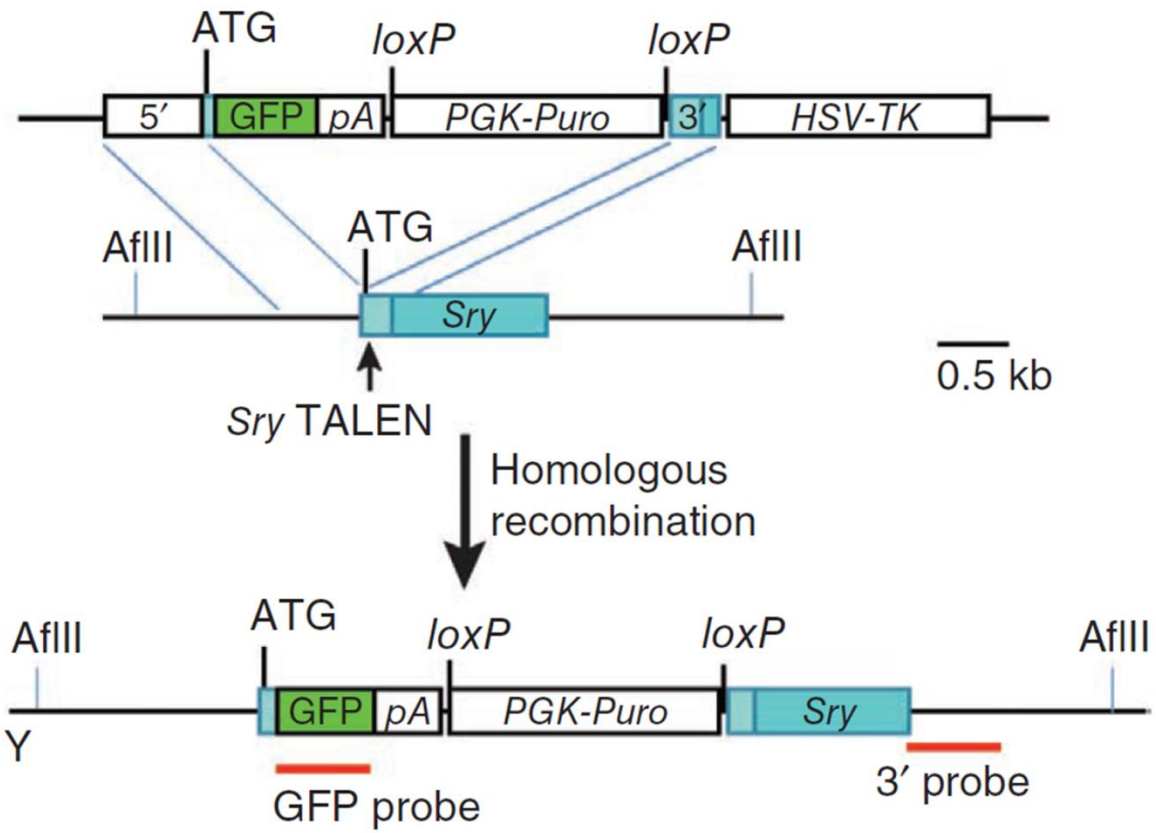
Knockout *via* NHEJ



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

Applications for genome editing

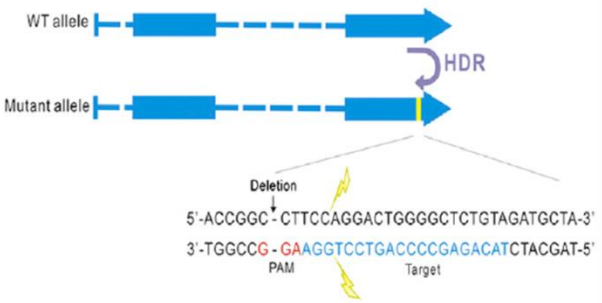
Knockout *via* HR: Donor plasmid

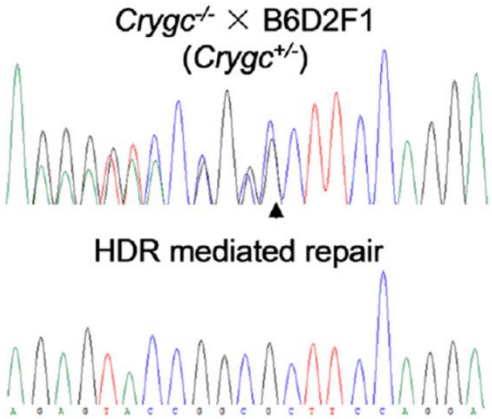


Wang, et al. (2013). Nature Biotech. 31, 530

Applications for genome editing

Mutagenesis *via* HR: Single strand oligonucleotide



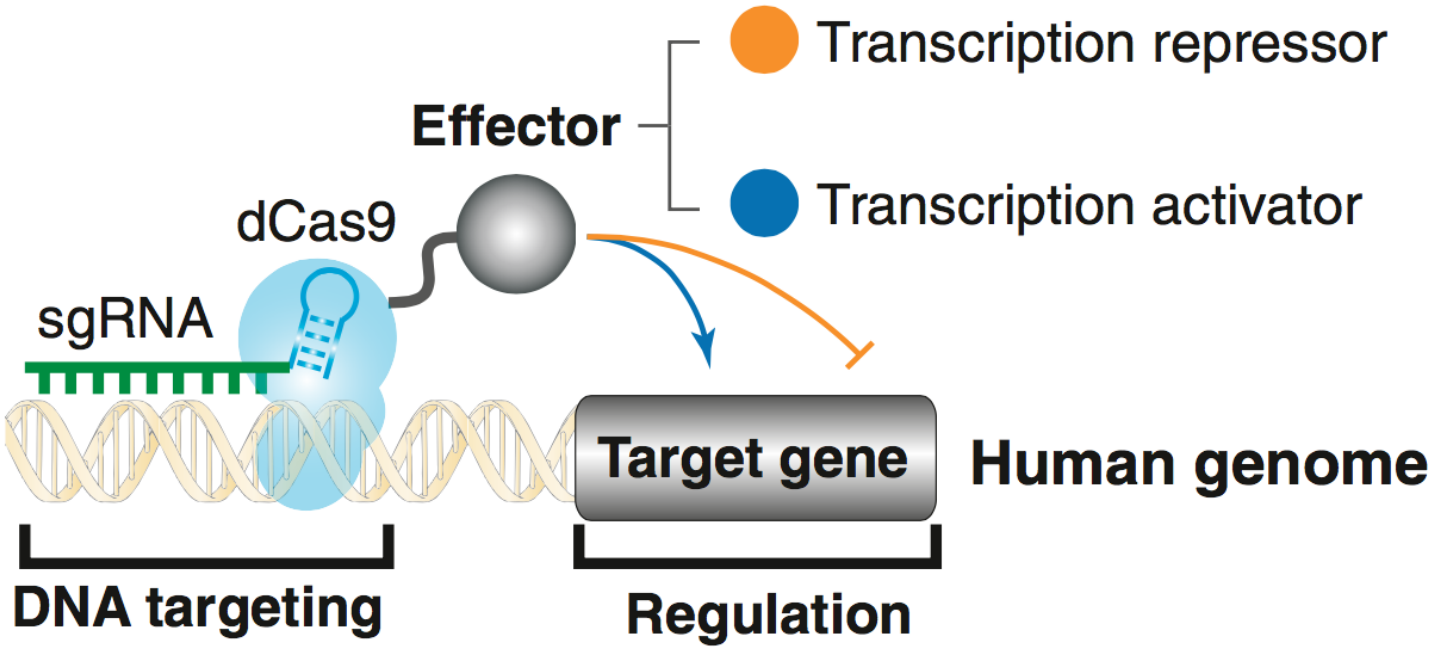


- Use single strand oligonucleotide (ssODN) to introduce base changes or small deletions.
- Use for mutagenesis or disease correction.
- Wu, et al.: Used CRISPR + ssODN to cure heritable cataract disease in mice

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

Applications for genome editing

Targeted gene activation/repression



Gilbert, et al. (2013). Cell 154, 442

Applications for genome editing

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 knockin	Sox2, Oct4 ORFs KI into humab AAVS1 “Safe Harbor”	TALEN via plasmid donor	GeneCopoeia internal data

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GeneCopoeia CRISPR services

CRISPR Off-target prediction report (optional)

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Genome-CRISP™ CRISPR sgRNA Off-target site Prediction and PCR Primer Design Report

Customer name:

Product name: sgRNA clones targeting human CASP8

Catalog number: HCP000455-CG02-1-B
Target Site: TCGAATTGACATCGCATCTG

Catalog number: HCP000456-CG02-1-B
Target Site: CTTCATAGTTCACTTCAGTC

Report date: March 12, 2015

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Section A: sgRNA HCP000455-CG02-1-B

Part 1: Off-target site prediction

Using the MIT CRISPR sgRNA design tool (http://crispr.mit.edu/), we obtained a list of all potential predicted off target sites for sgRNA HCP000455-CG02. The complete results can be found at http://crispr.mit.edu/job/9042636318577490. Below is a summary of the results, including a list of the 5 loci most likely to occur as off-sites:

Target site: TCGAATTGACATCGCATCTG (chromosomal PAM is CGG)

Quality score: 92 (high quality).

List of the 5 most likely off-target sites for HCP000455-CG02:

Off-target site	Sequence	Score	Number of mismatches	UCSC gene	Locus (hg38)
1	TGGAATTGACTCCGCATCTGGAG	0.8	3MMs [2:11:12]	None	chr1:46503855-46503877
2	TGAAATTAACATGGCATCTGGAG	0.6	4MMs [2:3:8:13]	None	chr9:84958537-84958559
3	TCATGTTGACATTGCATCTGGGG	0.6	4MMs [3:4:5:13]	None	chr15:39199982-39200004
4	TCCAATAGAAAACGCATCTGGGG	0.4	4MMs [3:7:10:12]	None	chr16:24956000-24956022
5	TCCAATTCTCATCGCATCTTCAG	0.4	4MMs [3:8:9:20]	None	chr14:46725370-46725392

These results indicate that none of these predicted off-target sites occur in known protein-coding genes.

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Primers to use for PCR:

Forward: CAAGAATGTCTGCGTACGTG

Reverse: ACTGGATCCCTTCCTTACAC

Predicted amplicon size: 798 bp

Predicted T7 Endonuclease cleavage bands if indels occur: ~243 bp, ~555 bp

Part 2: Off-target primer design for T7 Endonuclease I assay

Off-target site # 1

Region to be amplified:

hg38 chr1:46,503,105-46,504,627

Reverse-complement of sgRNA target site (with PAM) is in green. Forward PCR primer is in pink. Reverse-complement of reverse PCR primer is in yellow.

CACCCTGGTTTTCTTGCTGTTCTACACACACAAGCTTGTCTATGCCTCAGGGCCTTTGTCCTTGCTGTTACCTCTG
CCTGGAAAGCCTTTCCTGAGAGCTCCACATGGCTGACTTCAAGTTTCTGCTCAAATGTCGGAGAGCCCTTTCCTGA
CCACATGTTCTAAAACAATCTCTCCTTTCCCTCTTGTCATTCTCCTCCTCTGCCTTATTTTTCTTCAGAGCCCATA
TTCCAGTCCGACAGTAGTACATATATTTTTATTCATTGTTATACATCCCTCTATCCAGGAATGTAAATTCCATGAA
GTTAGGGACTTTGCTATTTAGTAAGTGGGTGCTTAATAAATATGTGTTGGATGAATATGTGTAATATACAAATGGA
TGTGAGTGTAATGTTTGTGTGTAGATGTCTCTGCTGCATGAGTAGATATCTGTGAGCATGTCTGTGTGTGGAACTG
TGGGAAATGACATTCAGTTTGTGTCTGTGATTAAGGAGGCTTCTGTGAATGCCTTGCCAAGAATGTCTGCGTACGT
GTATTTTTGCACGAAACGTTCATGTGCTTGCCTGCAACTGTGTGTGTTTGTGTGTAAGCTGTGGTTGTTTGATTAT
ATATAGCCTGTGTCTGTTTCAGTGAGGAGCTGAGATACCTGCATATGTCTTCCTTTTATCAAATGGACCTGCCATT
GCCCCAGCCTTCCCTTTTCACCTCCCACACTTGGGAAATAGGAAACACCTTTGTGTTTTGGGGAGACTCCAGATGC
GGAGTCAATTCCAAAAATGACATTTCACACTGCTGCAGACTGGGTGAGGGTCCGGATGGGACCTTGGCAGCCTCTT
GTTGAGGCTGGAGCCGTCTCAGTAGGATGGATGTCTTCTCTGATGAATGGAATATTTCCTTGGAACAATTGGCCGT
GAAAGAATAAGGCTCATGTCCTTCGCCCACTTTTTGATGGGGTTGTTTGTTTTTTTCTTGTAAATTTGTTTGAGTT
CGTTGTAGATTCTGGATATTAGCCCTTTGTCAGATGAGTAGGTTGCAAAAATTTTCTCCCATTTTGTAGGTTGCCT
GTTCACTCTGATGGTAGTTTCTTTTGCTGTGCAGAAGCTCTTTAGTTTAATTAGATCCCATTTGTCAATTTTGGCT
TTTGTTGCCATTGCTTTTGGTGTTTTAGACATGAAGTCCTTGCCCATGCCTATGTCCTGAATGGTAATGCCTAGGT
TTTCTTCTAGGGTTTTTATGGTTTTAGGTCTAACGTTTAAGTCTTTAATCCATCTTGAATTGATTTTTGTATAAGG
TGTAAGGAAGGGATCCAGTTTCAGCTTTCTACGTATGGCTAGCCAGTTTTCCCAGCACCATTTATTAAATAGGGAA
TCCTTTCCCCATTTCTTGTTTTTCTCAGGTTTGTCAAAGATCAGATAGTTGTAGATATGCAGCGTTATTTCTGAGG
GCTCTGTTCTGTTCCATTGATCTATATCTCTGTTTTGGTACCAGTACCATGCTGTTTTGGTTACTGTAGCCTTGTA
GTA

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We will sequence these 5 targets for you in up to 3 stable lines

These results indicate that none of these predicted off-target sites occur in known protein-coding genes.

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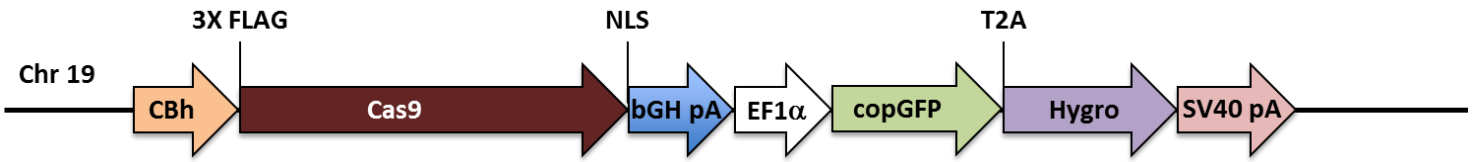
GeneCopoeia genome editing services

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 clone 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

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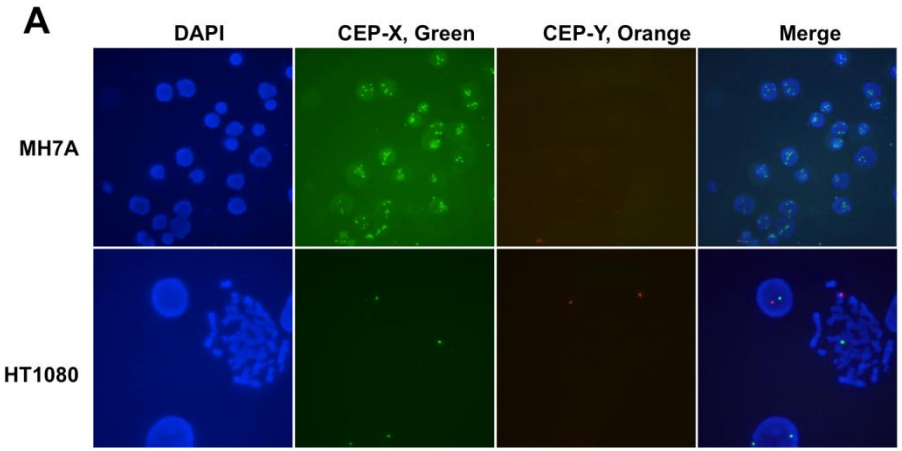

- Used CRISPR to KO HDAC6 gene in human cell line
- FISH showed that strain has 5 alleles of HDAC6
- Picked 80 single clones after transfection
- 3 clones had all 5 alleles of HDAC6 knocked out, each with a different deletion

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Summary

- GeneCopoeia provides a comprehensive suite of CRISPR products and services, from clones and kits to premium offerings such as sgRNA libraries and stable cell lines

- GeneCopoeia's CRISPR-edited cell line service provides the advantages of highly-experienced scientists, high-quality CRISPR reagents, and outstanding success

Upcoming webinar!

CRISPR & TALEN In Mammalian Cells: What Do I Do Next?

Wednesday, June 25, 2015 12:00 pm EDT

(GMT-0:400)

Register here:

https://attendee.gotowebinar.com/register/8619020342373657 61

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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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