

# Strategies For Effective CRISPR-Mediated Gene Modification

*October 17, 2018*

**Presenter:**

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

# Goals of this presentation

- ❖ Help guide you through basic considerations, steps, and potential problems for using CRISPR to modify genes in cell lines
- ❖ Present CRISPR-based tools from GeneCopoeia for modifying genes in cell lines
- ❖ Q & A

# GeneCopoeia Products and Services

## Functional Genomics & Cell Biology

<b><i>Clones</i></b>	<b><i>Viral systems</i></b>	<b><i>Stable Cell Lines</i></b>	<b><i>Kits &amp; reagents</i></b>	<b><i>Fluorescent detection</i></b>
<b>ORF</b> <b>Promoter</b> <b>miRNA</b> <b>CRISPR</b> <b>shRNA</b>	<b>Lentivirus</b> <b>AAV</b>	<b>CRISPR-Cas9</b> <b>stable cell lines</b> <b>Labeled cancer</b> <b>cell lines</b> <b>Cancer</b> <b>biomarker</b> <b>mutant cell</b> <b>lines</b>	<b>Transfection</b> <b>Luciferase</b> <b>FISH probes</b> <b>Indel detection</b> <b>Cloning</b>	<b>Cell function</b> <b>assays</b> <b>Nucleic acid</b> <b>detection</b> <b>Cell structure</b> <b>probes</b> <b>Fluorescent</b> <b>dyes</b>

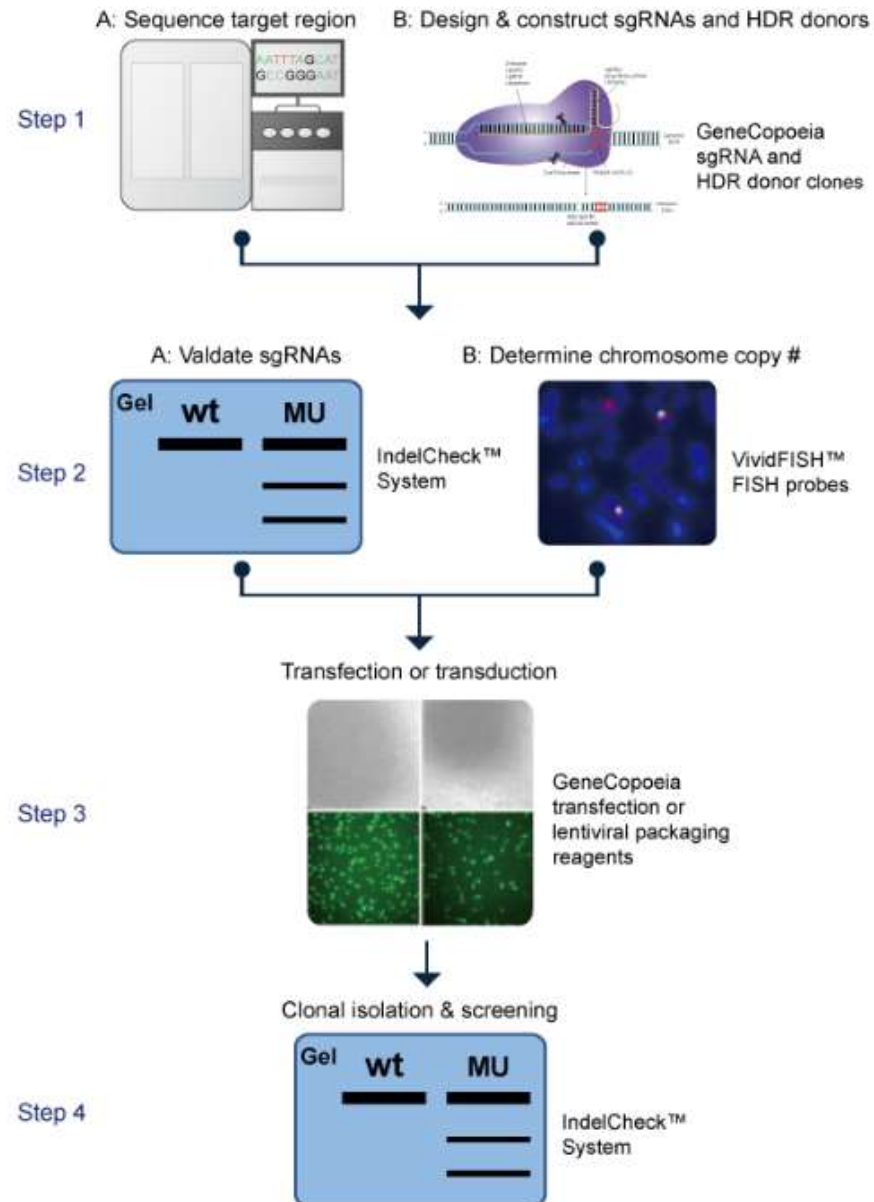


# GeneCopoeia Products and Services

## Functional Genomics & Cell Biology

<i><b>Clones</b></i>	<i><b>Viral systems</b></i>	<i><b>Stable Cell Lines</b></i>	<i><b>Kits &amp; reagents</b></i>	<i><b>Fluorescent detection</b></i>
ORF Promoter miRNA <b>CRISPR</b> shRNA	Lentivirus AAV	CRISPR-Cas9 stable cell lines Labeled cancer cell lines Cancer biomarker mutant cell lines	Transfection Luciferase FISH probes Indel detection Cloning	Cell function assays Nucleic acid detection Cell structure probes Fluorescent dyes

# GeneCopoeia CRISPR products for cell lines



# Outline

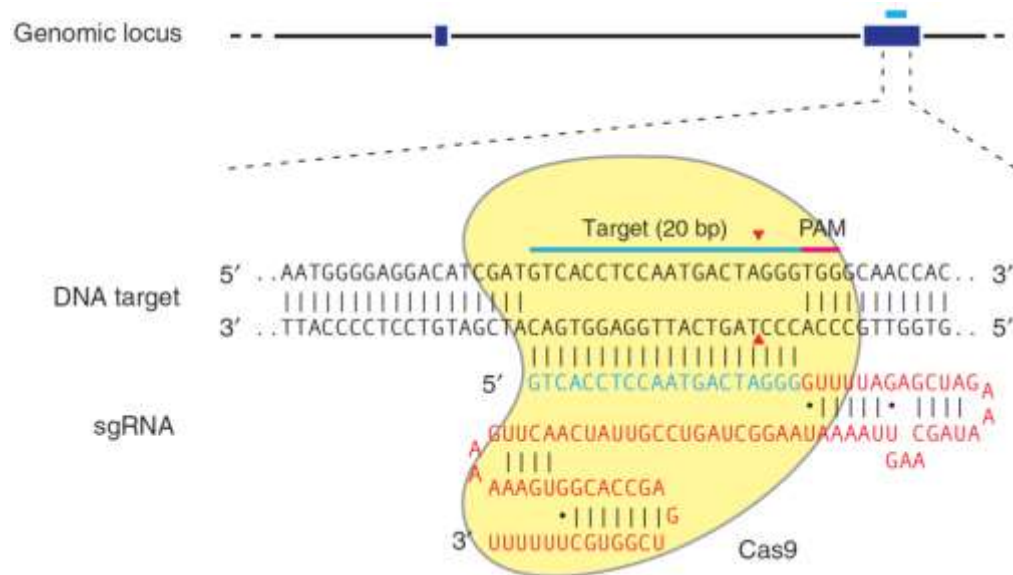
- ❖ CRISPR technologies & applications
- ❖ Strategy considerations
- ❖ Delivery methods
- ❖ Preparation
- ❖ Screening
- ❖ Things to look out for

# Outline

- ❖ CRISPR technologies & applications
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# CRISPR-Cas9 genome editing technology

## CRISPR-Cas9: RNA-guided endonuclease

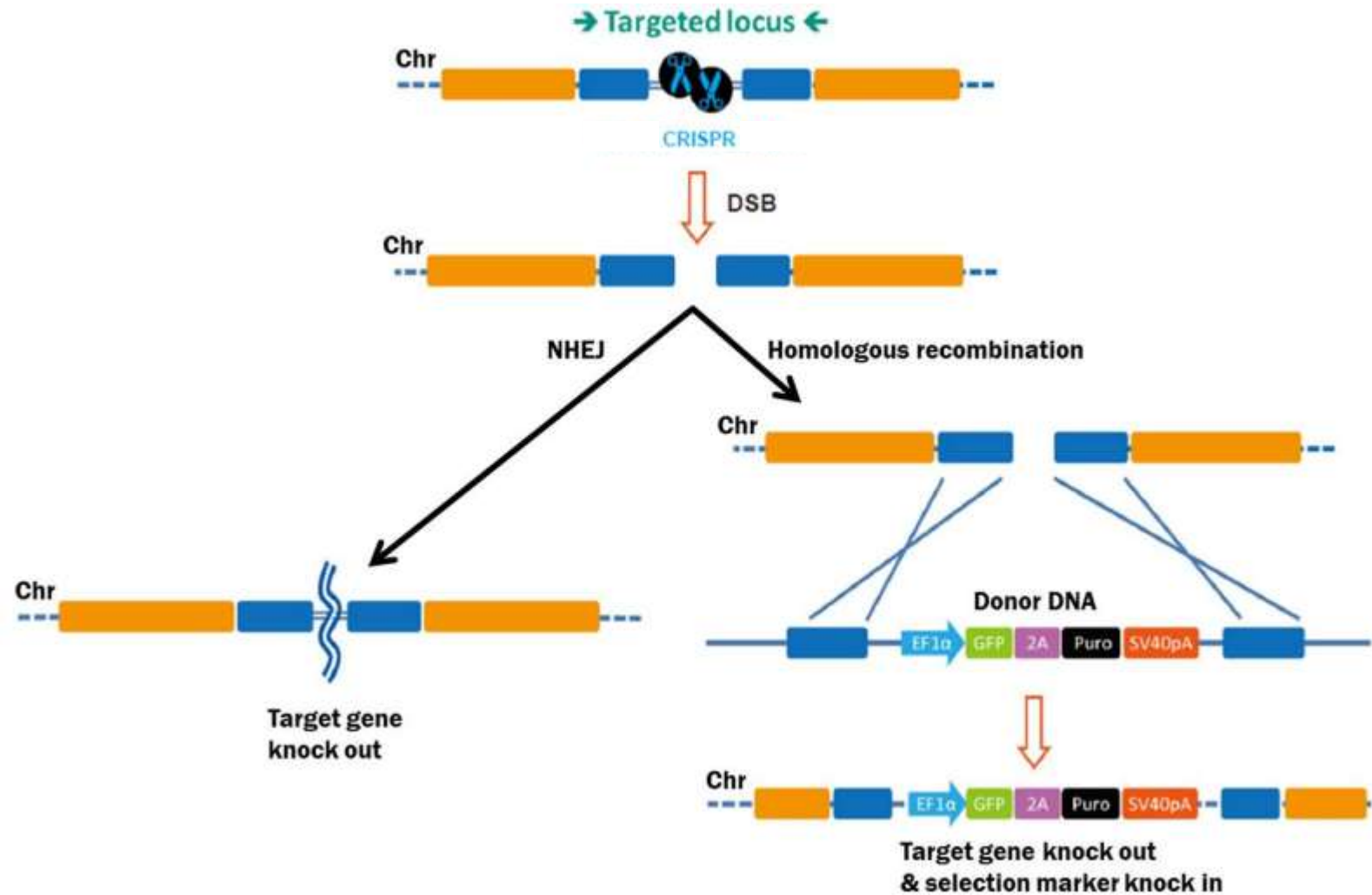


- ❖ 17-20 nt single guide RNA (sgRNA) guides Cas9 nuclease to target site.
- ❖ Requires NGG “PAM” site immediately downstream of sgRNA target sequence.
- ❖ Cas9-sgRNA complex makes DSB 3 nt upstream of PAM.

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



# Targeted DNA editing by DSB induction



# CRISPR-Cas9 genome editing technology

## Technological variants

Technology	Description	PAM	Applications
SpCas9	Unmodified Cas9 from <i>Streptococcus pyogenes</i>	NGG	Gene knockout, knock-in, tagging, mutagenesis
SpCas9-NG	Mutagenized SpCas9 with relaxed (NG) PAM requirement	NG	Gene knockout, knock-in, tagging, mutagenesis
Cas9-HF, eSpCas9	Mutagenized, high-fidelity SpCas9 for reduced off-targeting	NGG	Gene knockout, knock-in, tagging, mutagenesis
HiFi Cas9	Mutagenized, high-fidelity SpCas9 for reduced off-targeting	NGG	Gene knockout, knock-in, tagging, mutagenesis
Cpf1 (Cas12a)	Nucleases from <i>Acidaminococcus</i> sp. BV3L6 or <i>Lachnospiraceae</i> bacterium ND2006. Smaller than SpCas9, no requirement for tracrRNA, staggered DSB, lower off-targeting.	T/CTTN (5')	Gene knockout, knock-in, tagging, mutagenesis
SaCas9	Cas9 from <i>Staphylococcus aureus</i> . Smaller than SpCas9, usually used with adenoassociated virus (AAV)	NNGRRT	Gene knockout, knock-in, tagging, mutagenesis
dCas9	Nuclease-dead Cas9 for tethering to effectors	NGG	Gene mutagenesis, chromatin modification, transcriptional activation/repression
Cas13	Nuclease from <i>Leptotrichia wadei</i> . Targets RNA. 20-28 nt spacer	None	RNA knockdown or mutagenesis



# CRISPR-Cas9 applications

## Gene knockout-frameshift



ATGACCTCAACCGGCCAGGATTCCACCACAACCAGGCAGCGAAGAAGTAGGCAGAACCCCCAGTCGCCCCCTCAGGACTCCAGTGTCACCTTCGgtgaggccctagacccgcctgatgaggg



ATGACCTCAACCGGCCAGGATTCCACCACAACCAGGCAGCGAAGAAGTAGGCAGAACCCCCAGTCGCCCCCTCAGGACTCCAGTGTCACCTTCGgtgaggccctagacccgcctgatgaggg

└─ (Multiple of 3) +/- 1 indel-frameshift

ATGACCTCAACCGGCCAGGATTCCACCACAACCAGGCAGCGAAGAAGTAGGCAGAACCCCCAGTCGCCCCCTCAGGACTCCAGTGTCACCTTCGgtgaggccctagacccgcctgatgaggg

└─ (Multiple of 3) +/- 2 indel-frameshift

ATGACCTCAACCGGCCAGGATTCCACCACAACCAGGCAGCGAAGAAGTAGGCAGAACCCCCAGTCGCCCCCTCAGGACTCCAGTGTCACCTTCGgtgaggccctagacccgcctgatgaggg

└─ Multiple of 3 indel-in frame



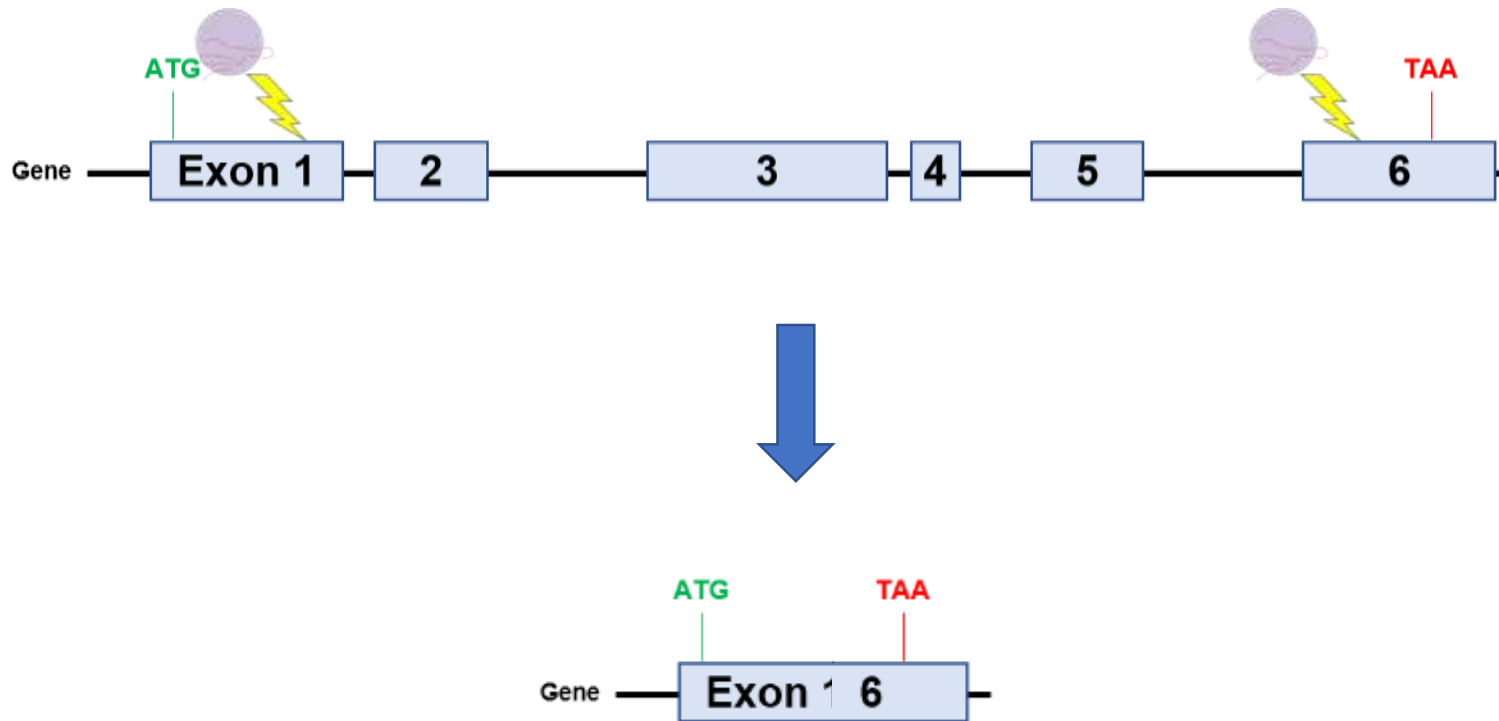
# CRISPR-Cas9 applications

## Gene knockout-frameshift

- ❖ NHEJ-mediated indels occur stochastically, with unpredictable lengths
- ❖ In general, 2/3 of indels will cause a frameshift. In most cases this will knock the gene out.
- ❖ In general, 1/3 of indels will be in-frame. This may or may not knock the gene out. It could also lead to the production of a protein with a new or altered function.
- ❖ Typically, each chromosome will contain a different indel (or no indel). So, only 4/9 of diploid cells with 2 indels will contain 2 frameshift indels.

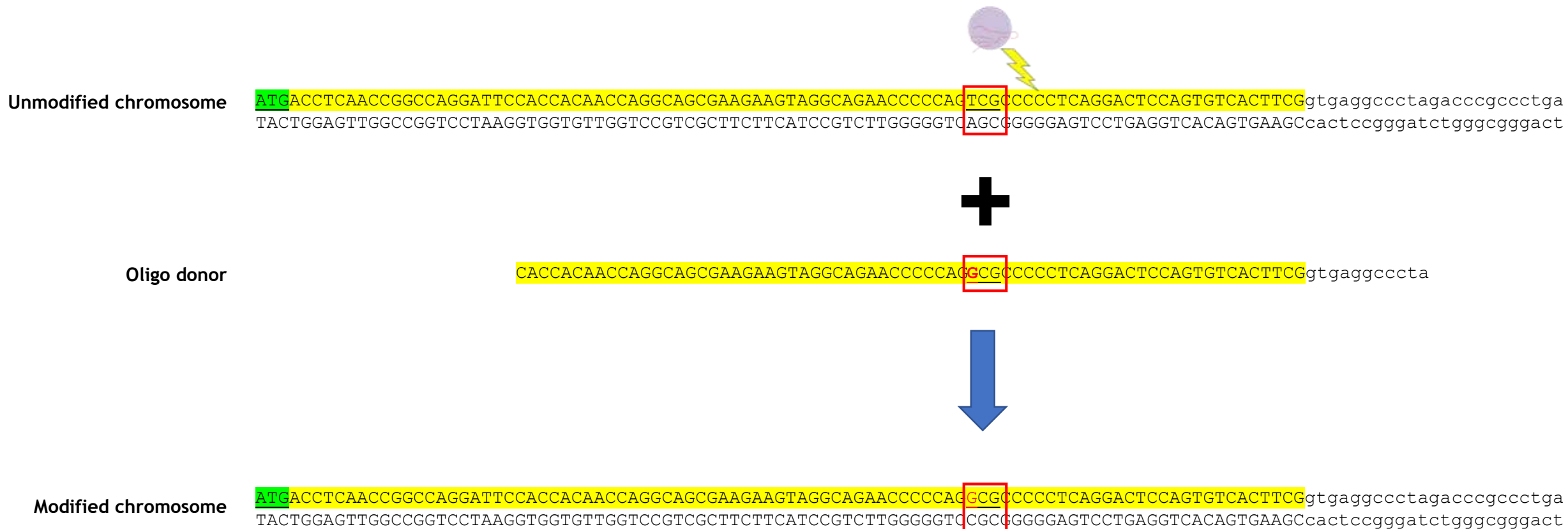
# CRISPR-Cas9 applications

## Gene knockout-large deletion



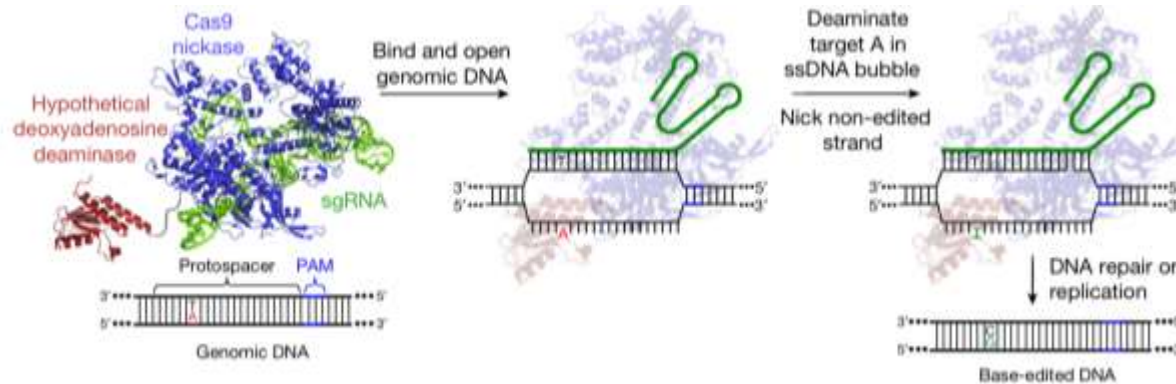
# CRISPR-Cas9 applications

## Gene mutagenesis-HDR



# CRISPR-Cas9 applications

## Gene mutagenesis-Base editors

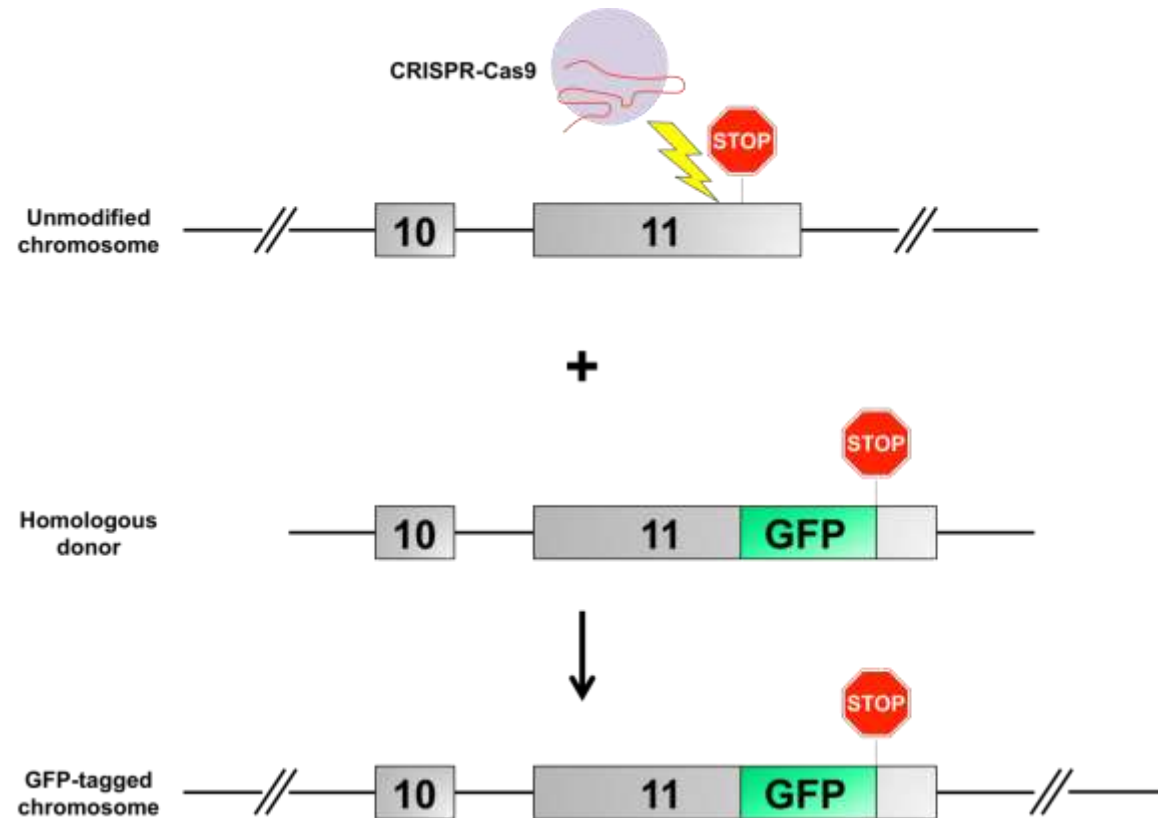


Gaudelli, et al. (2017). Nature 551, 464

- ❖ Cas9 nickase fused to cytidine and adenine deaminases
- ❖ Permit changing of bases without DSBs
- ❖ Can change C to T, G to A, A to G, and T to C

# CRISPR-Cas9 applications

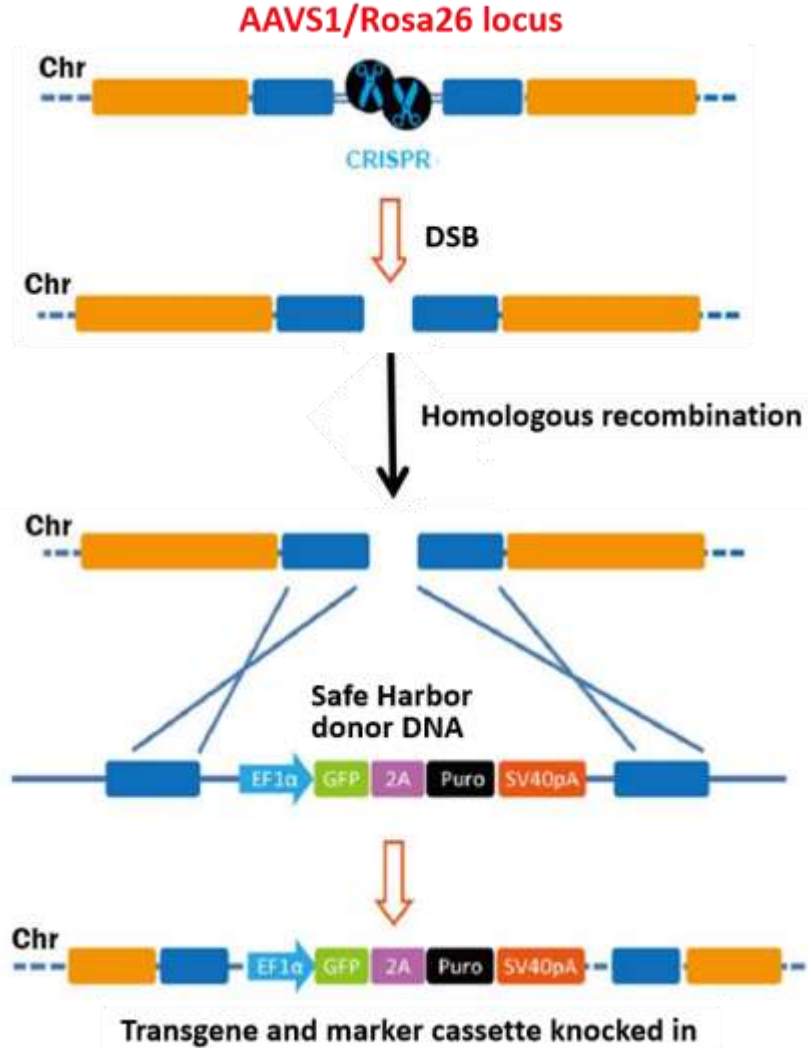
## Gene tagging





# CRISPR-Cas9 applications

## Gene knock-in (transgenesis)

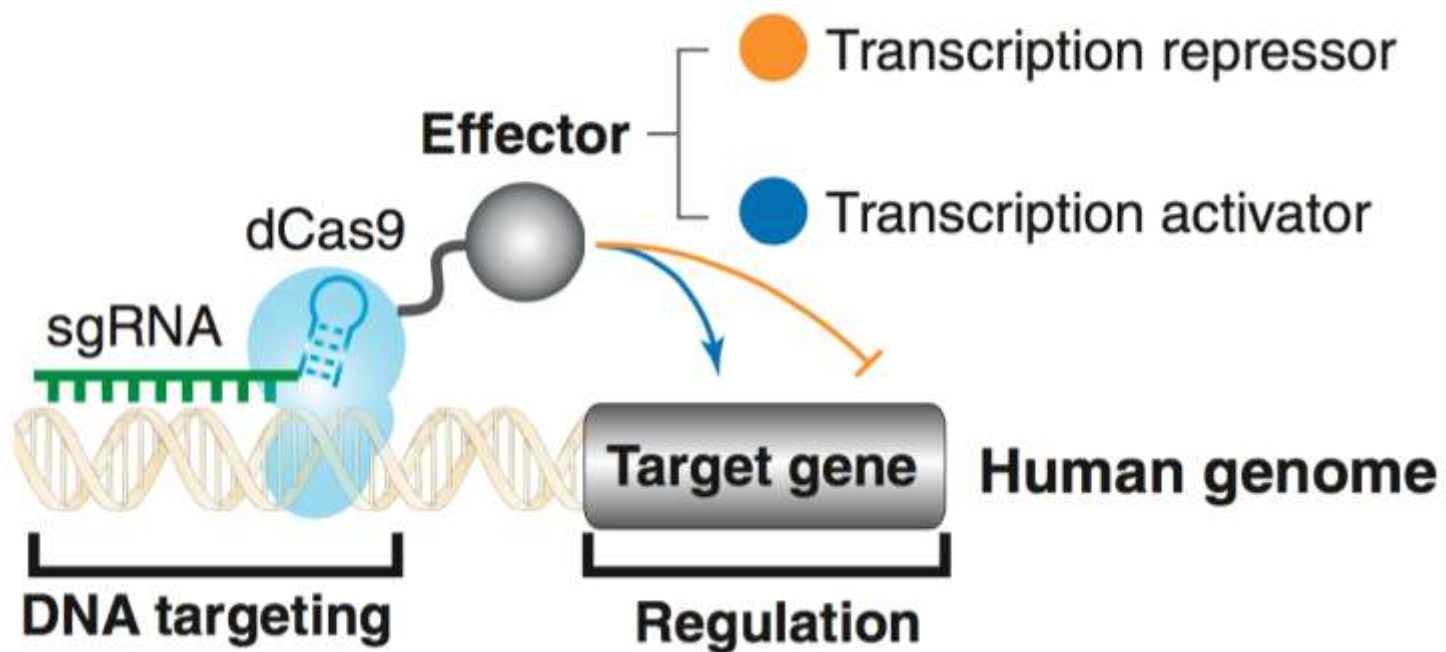


### Features

- ❖ 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.

# CRISPR-Cas9 applications

## Gene activation or repression



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

# Outline

- ❖ CRISPR technologies & applications
- ❖ **Strategy considerations**
- ❖ Delivery methods
- ❖ Preparation
- ❖ Screening
- ❖ Things to look out for

# CRISPR-Cas9 genome editing technology

## Considerations before you start

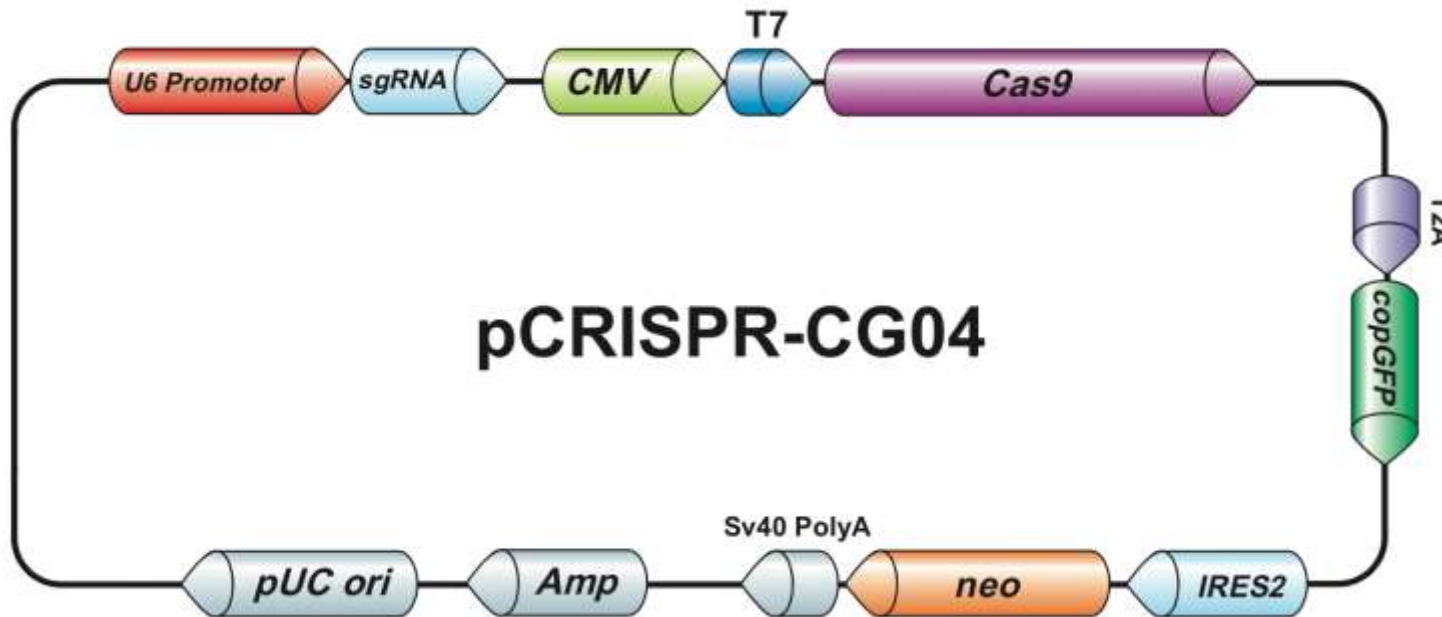
- ❖ What type of application are you doing (gene knockout, mutagenesis, etc.)? This decision can effect the efficiency and the degree of difficulty screening for the modification.
- ❖ What type of cell line are you using (primary, immortalized)?
- ❖ Can the cell line be transfected? If it does not transfect well, or not at all, then you might need to use lentivirus for delivery. However, lentivirus cannot be used for HDR applications.
- ❖ Other cell line considerations: Growth rate, growth characteristics (adherent vs. suspension growth), target gene copy number.

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# CRISPR-Cas9 genome editing technology

## Delivery method 1: Cas9/sgRNA plasmid DNA



- ❖ GeneCopoeia Cas9 + sgRNA “All-in-one” plasmid
- ❖ Fluorescent reporter allows for sorting
- ❖ Selection marker can be used for stable integration
- ❖ Cas9 needs to be transcribed and translated

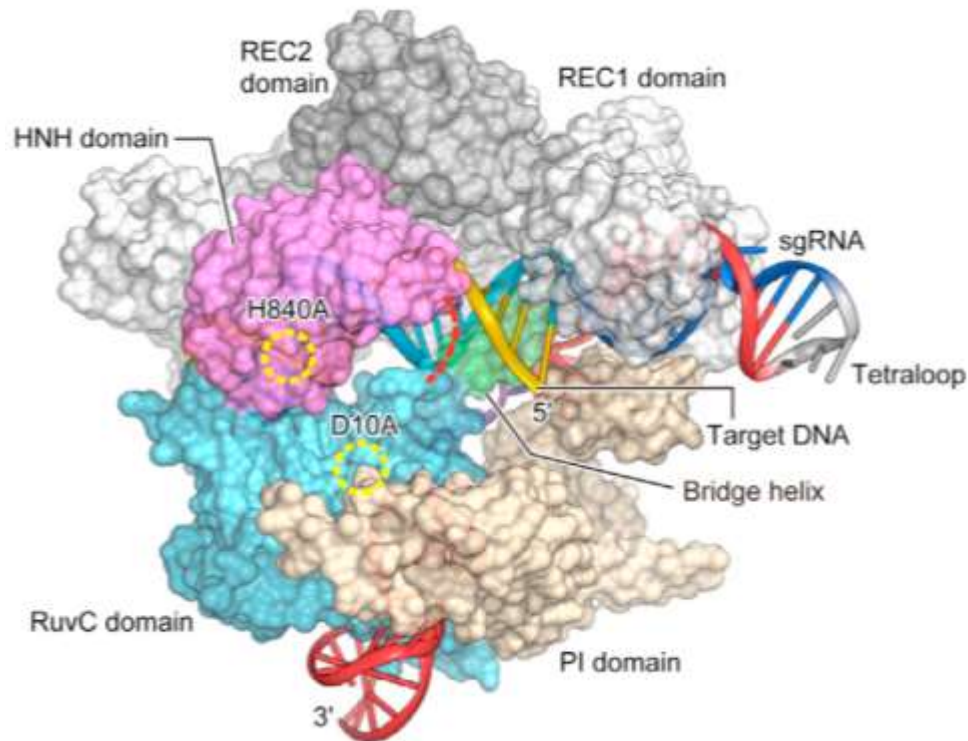
# CRISPR-Cas9 genome editing technology

## Delivery method 2: Cas9 mRNA + sgRNA

- ❖ Co-transfect *in vitro* transcribed Cas9 mRNA with sgRNA
- ❖ sgRNA can either be encoded on plasmid, *in vitro* transcribed, or synthetic
- ❖ Advantage: Plasmid-free, so cannot integrate
- ❖ Cas9 still needs to be translated

# CRISPR-Cas9 genome editing technology

## Delivery method 3: Cas9 ribonucleoprotein (RNP)



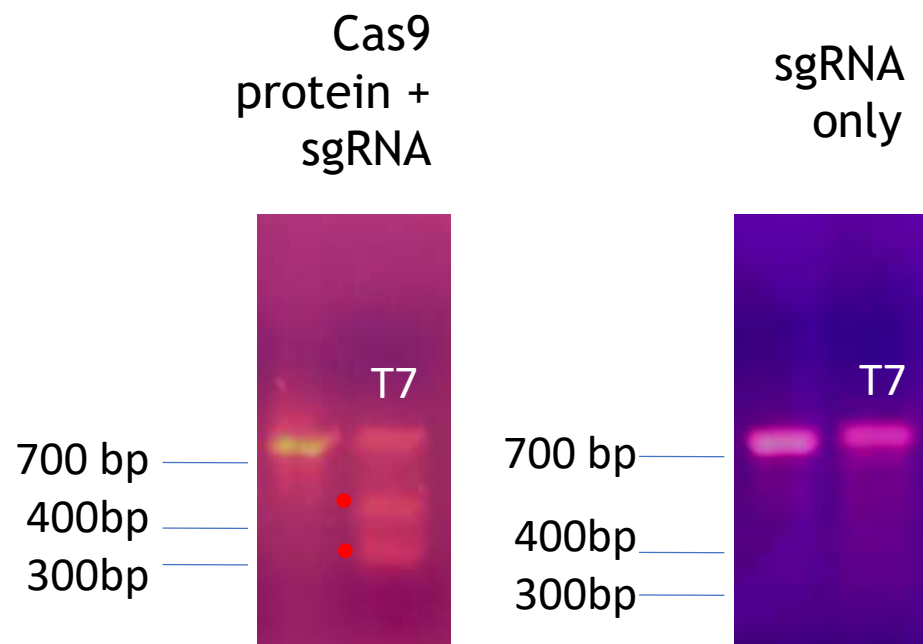
Nishimasu, et al. (2014). Cell 156, 935

- ❖ Pre-form complex of Cas9 protein and sgRNA *in vitro* to form RNP
- ❖ sgRNA can be either chemically synthesized or *in vitro* transcribed
- ❖ Transfect cells with RNP complex using either lipid transfection reagent or electroporation
- ❖ Efficiency of indel formation is generally similar to that of plasmid transfection (Kim, et al., 2014. Genome Research)
- ❖ Lower off-targeting compared with plasmid transfection (Kim, et al., 2014. Genome Research)
- ❖ Does not integrate, unlike plasmids



# CRISPR-Cas9 genome editing technology

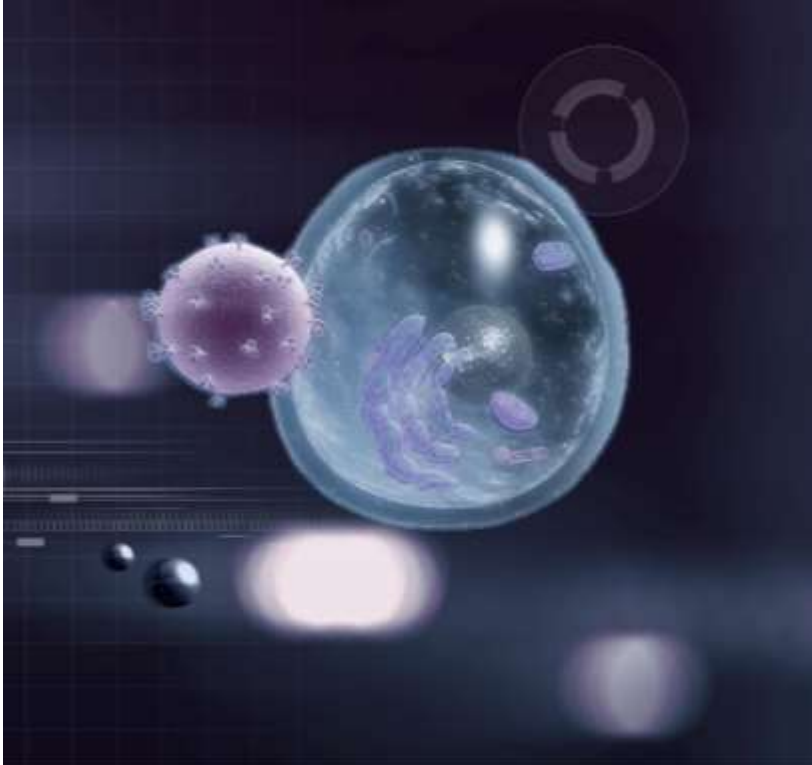
Coming soon! GeneHero™ Cas9 nuclease



❖ Transfection of HEK293 cells using EndoFectin™ Max

# CRISPR-Cas9 genome editing technology

## Delivery method 4: Lentivirus

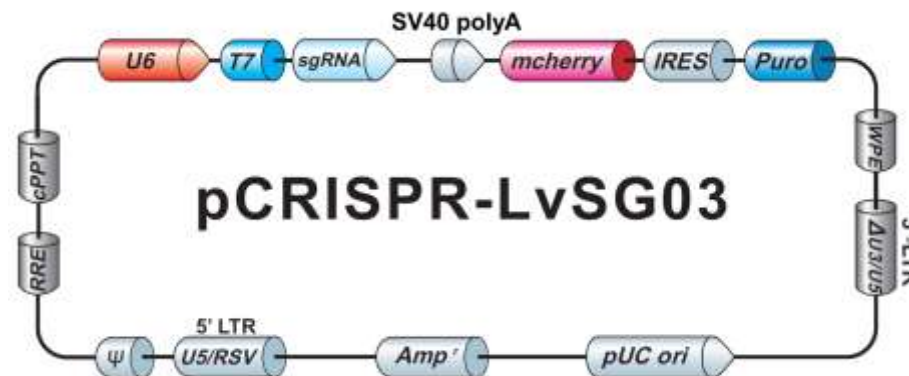
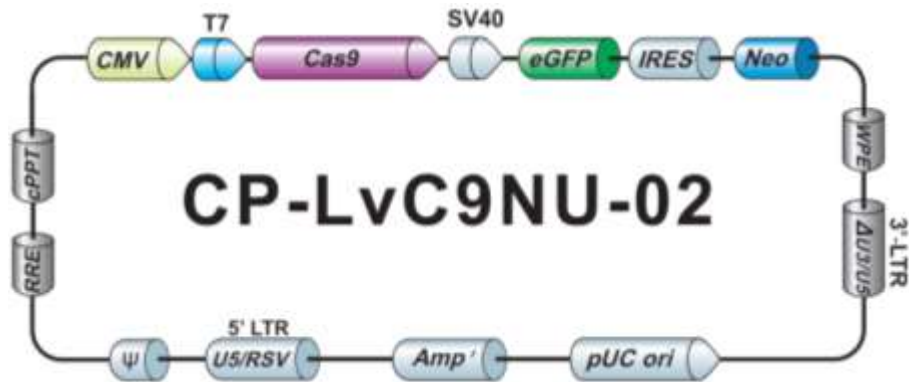


### Why lentivirus?

- ❖ DNA transfection not always possible or practical. Some cell lines difficult or impossible to transfect.
- ❖ Most mammalian cells support infection by engineered lentivirus

# CRISPR-Cas9 genome editing technology

## Delivery method 4: Lentivirus



### Features

- ❖ 2-component system: 1) Cas9 lentivirus + sgRNA lentivirus
- ❖ Cannot be used for HDR
- ❖ Leads to stable selection
- ❖ GeneCopoeia provides either plasmids for do-it-yourself packaging or ready-to-use particles
- ❖ Recommend that you first establish or obtain Cas9-stable cell line

# CRISPR-Cas9 genome editing technology

## GeneHero™ Cas9 stable cell lines

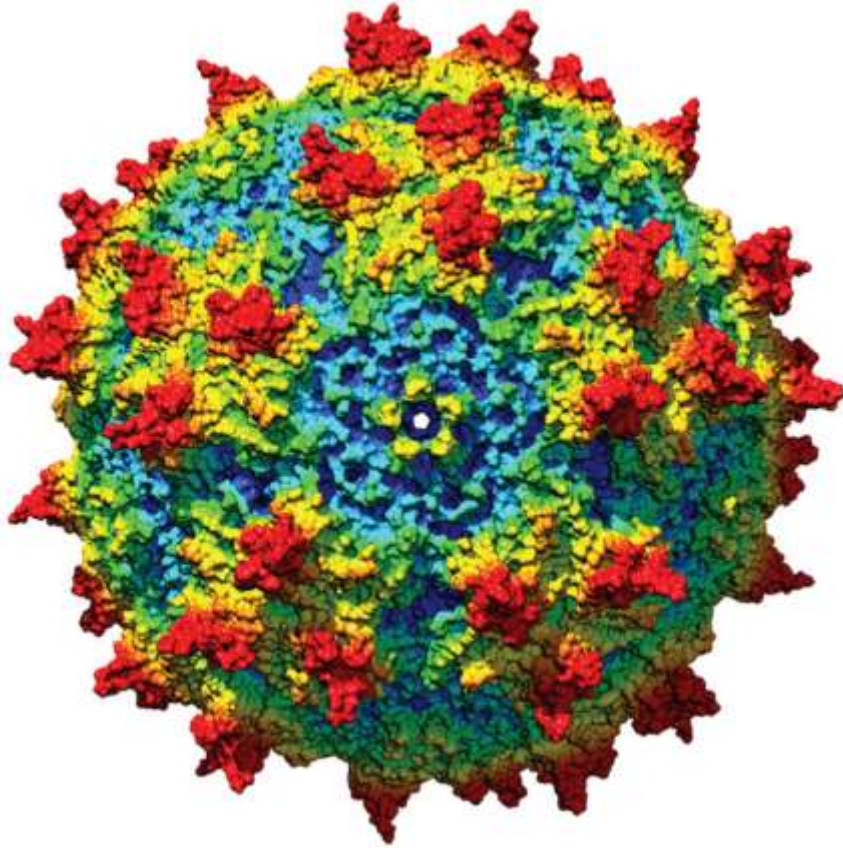


### Features

- ❖ Cell lines with Cas9 stably integrated in the genome
- ❖ >70 pre-made cell lines available in human, mouse, and rat cell lines
- ❖ Functionally validated for Cas9 activity
- ❖ Ideal for lentiviral CRISPR applications

# CRISPR-Cas9 genome editing technology

## Delivery method 5: Adenoassociated virus (AAV)



- ❖ Identified as co-isolate of adenovirus (Ad)
- ❖ Single stranded DNA genome of 4.7 kb
- ❖ Limited genome size favors use of smaller Cas9 (e.g. SaCas9)
- ❖ Engineered AAV does not integrate into genomic DNA
- ❖ Infects dividing & non-dividing cells
- ❖ More frequently used *in vivo*



# Outline

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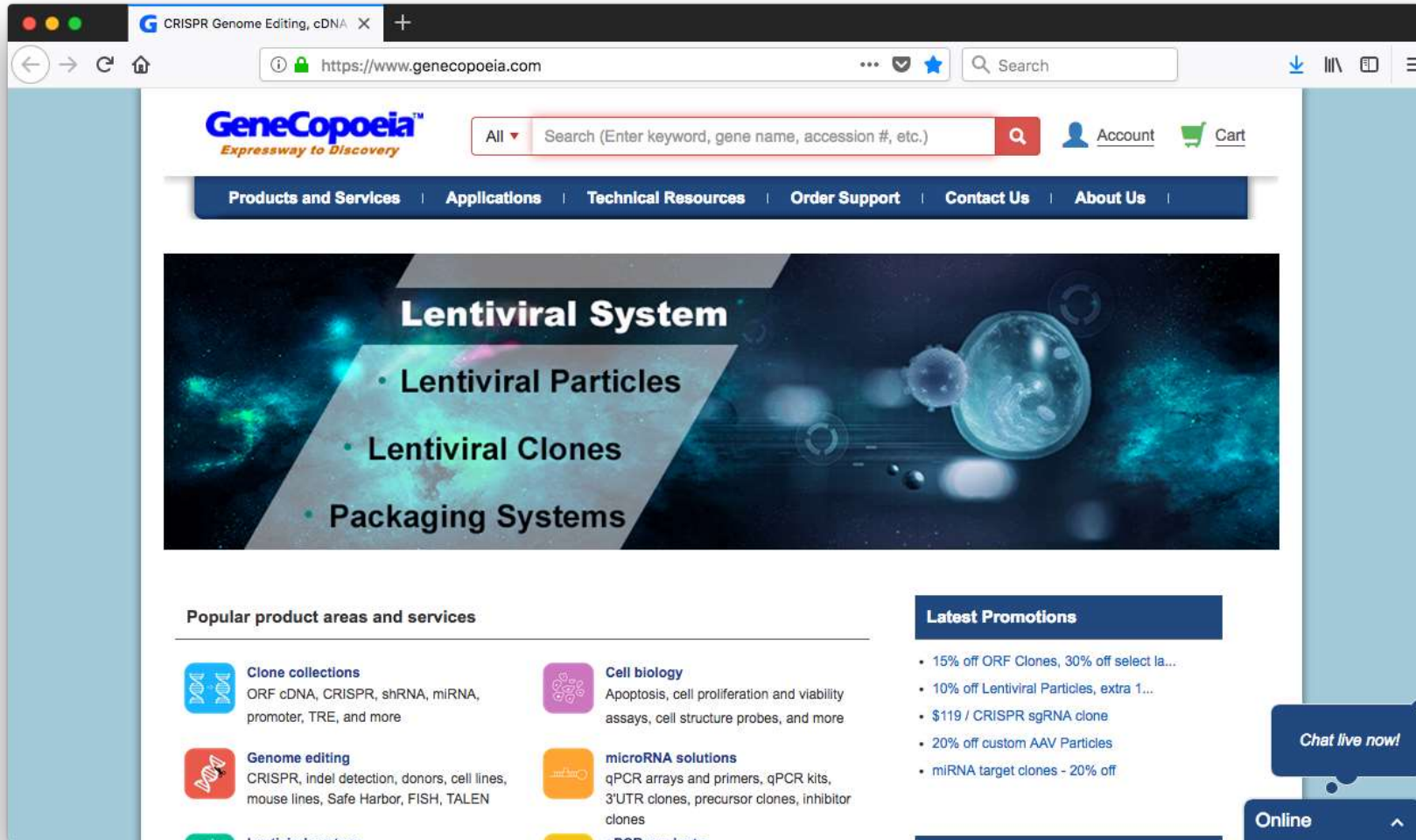
# CRISPR-Cas9 genome editing technology

## Workflow: Preparation

- ❖ Step 1: Choose your application (gene knockout, mutagenesis, etc.) This decision can affect the efficiency and the degree of difficulty in screening for modification.
- ❖ Use GeneCopoeia's website to choose targets for knockout, or contact us for custom sgRNA design and donor design, if applicable

# CRISPR-Cas9 genome editing technology

## Workflow: Preparation



The screenshot displays the GeneCopoeia website interface. The browser's address bar shows the URL <https://www.genecopoeia.com>. The website header includes the GeneCopoeia logo with the tagline "Expressway to Discovery", a search bar, and links for "Account" and "Cart". A navigation menu lists "Products and Services", "Applications", "Technical Resources", "Order Support", "Contact Us", and "About Us".

The main content area features a large banner for the "Lentiviral System" with a list of services:

- Lentiviral Particles
- Lentiviral Clones
- Packaging Systems

Below the banner, the "Popular product areas and services" section is divided into four categories:

- 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.
- 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.

The "Latest Promotions" section on the right lists several offers:

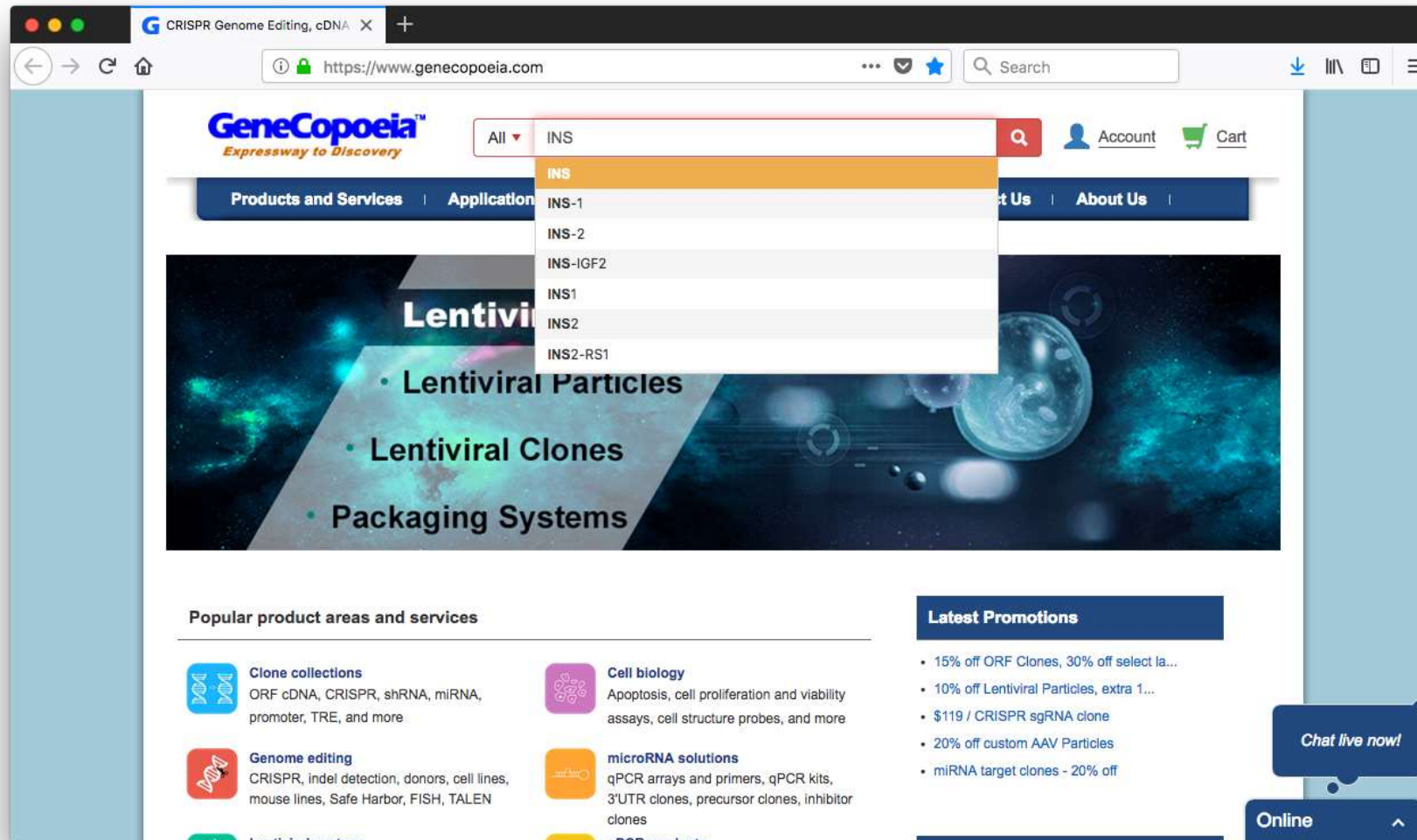
- 15% off ORF Clones, 30% off select la...
- 10% off Lentiviral Particles, extra 1...
- \$119 / CRISPR sgRNA clone
- 20% off custom AAV Particles
- miRNA target clones - 20% off

A "Chat live now!" button is visible in the bottom right corner, along with an "Online" status indicator.



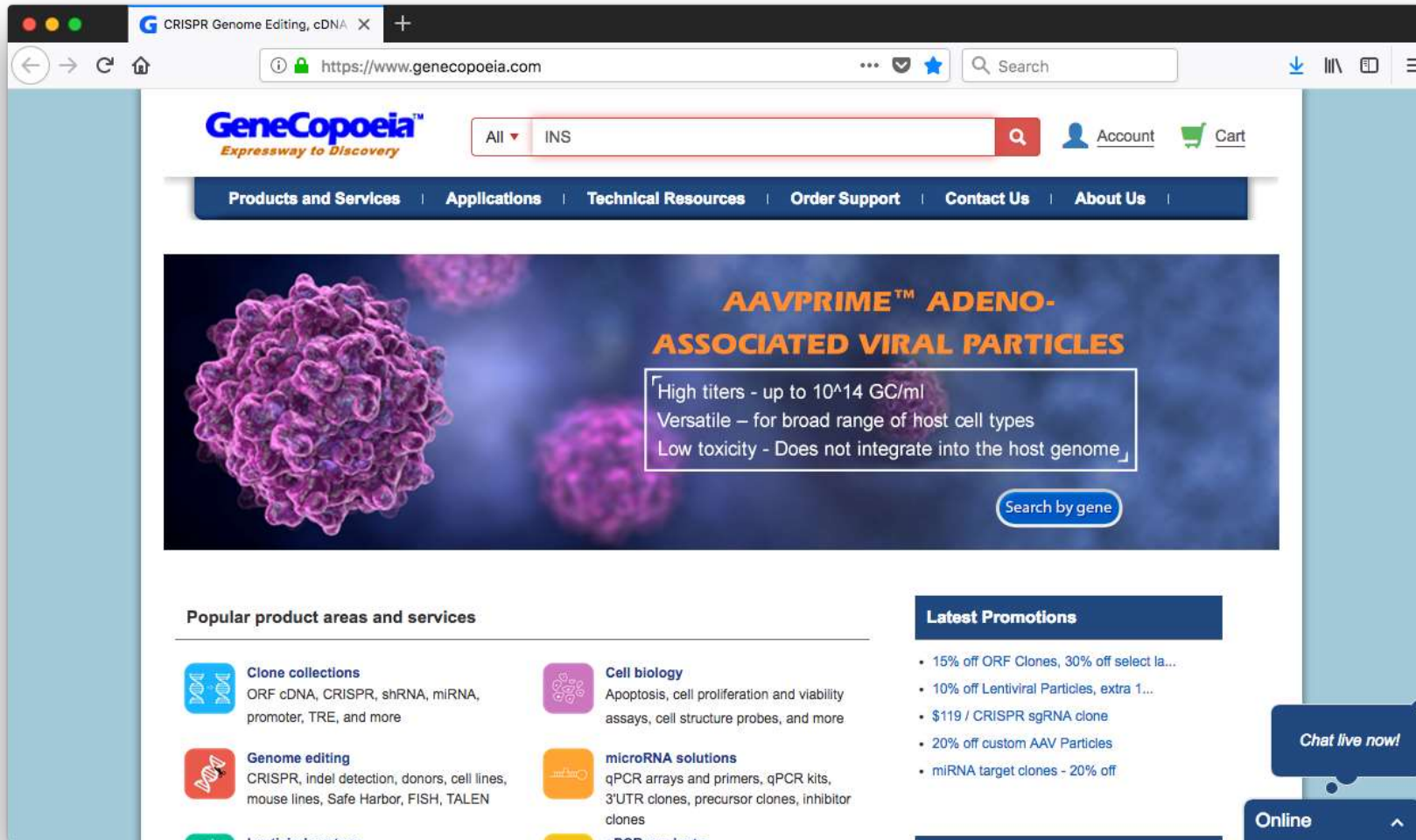
# CRISPR-Cas9 genome editing technology

## Workflow: Preparation



# CRISPR-Cas9 genome editing technology

## Workflow: Preparation



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The main content area features a large banner for "AAVPRIME™ ADENO-ASSOCIATED VIRAL PARTICLES". The banner includes a 3D model of a viral particle and the following text:

- High titers - up to  $10^{14}$  GC/ml
- Versatile – for broad range of host cell types
- Low toxicity - Does not integrate into the host genome

A "Search by gene" button is located at the bottom right of the banner.

Below the banner, the "Popular product areas and services" section is divided into four categories:

- 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
- 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

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# CRISPR-Cas9 genome editing technology

## Workflow: Preparation

The screenshot displays the GeneCopoeia website interface. At the top, there is a navigation bar with the GeneCopoeia logo and the tagline "Expressway to Discovery". Below the navigation bar, a search bar is visible with the text "Search (Enter keyword, gene name, accession #, etc.)". To the right of the search bar are links for "Account" and "Cart".

A prominent banner advertisement is displayed, featuring the text "ORF cDNA Clones-15% Off\*" and "30% off select larger genes". To the right of the banner, a list of features is shown:

- >140,000 ORF Clones
- 200+ Expression Vectors
- Next-Day Shipping

Below the banner, the search results are displayed. The search criteria are "ORF cDNA, CRISPR, shRNA, miRNA, Promoter or qPCR primer". The results are shown in a table with the following columns: Product, Product ID, Accession, Symbol, Alias, Species, and Description.

Product	Product ID	Accession	Symbol	Alias	Species	Description
ORF cDNA clones	A0446	NM_001291897.1	INS	IDDM, IDDM1, IDDM2, ILPR, IRDN, MODY10	Human	Homo sapiens insulin (INS), transcript variant 4, mRNA.
ORF cDNA clones	H5101	NM_202003.2	FOXO1	FKHL16, FOXO1B, HFH-11, HFH11, HNF-3, INS-1, MPHOSPH2, MPP-2, MPP2, PIG29, TRIDENT	Human	Homo sapiens forkhead box M1 (FOXO1), transcript variant 3, mRNA.
ORF cDNA clones	H9190	NM_021953.3	FOXO1	FKHL16, FOXO1B, HFH-11, HFH11, HNF-3, INS-1, MPHOSPH2, MPP-2, MPP2, PIG29, TRIDENT	Human	Homo sapiens forkhead box M1 (FOXO1), transcript variant 2, mRNA.

On the left side of the search results, there is a "Product Type" filter with a dropdown menu. The filter is currently set to "Product Type: -". Below the filter, a list of checkboxes is shown, including "ORF cDNA", "CRISPR", "shRNA", "miRNA", "Gene qPCR Primers", "miRNA target", "Promoter", "ORF Knock-in", and "TALE-TF".

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

# CRISPR-Cas9 genome editing technology

## Workflow: Preparation

The screenshot displays the GeneCopoeia website's search results for the term 'INS'. The interface includes a sidebar on the left with filters for Product Type, Format, and Species, and a main table listing search results. A 'Chat live now!' bubble is visible in the bottom right corner.

**Product Type:**

- ☐ ORF cDNA
- ☐ CRISPR
- ☐ shRNA
- ☐ miRNA
- ☐ Gene qPCR Primers
- ☐ miRNA target
- ☐ Promoter
- ☐ ORF Knock-in
- ☐ TALE-TF

**Format:**

- ☐ Clone
- ☐ Lentiviral Particle
- ☐ AAV Particle

**Species:**

- ☐ Human
- ☐ Mouse
- ☐ Rat

**Reset**

Product	Product ID	Accession	Symbol	Alias	Species	Description
ORF cDNA clones	A0446	NM_001291897.1	INS	IDDM, IDDM1, IDDM2, ILPR, IRDN, MODY10	Human	Homo sapiens insulin (INS), transcript variant 4, mRNA.
ORF cDNA clones	H5101	NM_202003.2	FOXM1	FKHL16, FOXM1B, HFH-11, HFH11, HNF-3, INS-1, MPHOSPH2, MPP-2, MPP2, PIG29, TRIDENT	Human	Homo sapiens forkhead box M1 (FOXM1), transcript variant 3, mRNA.
ORF cDNA clones	H9190	NM_021953.3	FOXM1	FKHL16, FOXM1B, HFH-11, HFH11, HNF-3, INS-1, MPHOSPH2, MPP-2, MPP2, PIG29, TRIDENT	Human	Homo sapiens forkhead box M1 (FOXM1), transcript variant 2, mRNA.
ORF cDNA clones	I0607	NM_001243088.1	FOXM1	FKHL16, FOXM1B, HFH-11, HFH11, HNF-3, INS-1, MPHOSPH2, MPP-2, MPP2, PIG29, TRIDENT	Human	Homo sapiens forkhead box M1 (FOXM1), transcript variant 4, mRNA.
ORF cDNA clones	I0608	NM_001243089.1	FOXM1	FKHL16, FOXM1B, HFH-11, HFH11, HNF-3, INS-1, MPHOSPH2, MPP-2, MPP2, PIG29, TRIDENT	Human	Homo sapiens forkhead box M1 (FOXM1), transcript variant 5, mRNA.
ORF cDNA clones	T8218	BC006192.2	FOXM1	FKHL16, FOXM1B, HFH-11, HFH11, HNF-3, INS-1, MPHOSPH2, MPP-2, MPP2, PIG29, TGT3, TRIDENT	Human	Homo sapiens forkhead box M1, mRNA (cDNA clone MGC:10704 IMAGE:3833837).
ORF cDNA clones	U1376	U83113.1	FOXM1	FKHL16, FOXM1B, HFH-11, HFH11, HNF-3, INS-1, MPHOSPH2, MPP-2, MPP2, PIG29, TGT3, TRIDENT	Human	Human INS-1 winged-helix homolog mRNA.
ORF cDNA clones	Z3073	NM_001042376.2	INS-IGF2	INSIGF	Human	Homo sapiens INS-IGF2 readthrough (INS-IGF2 transcript variant 2, mRNA).

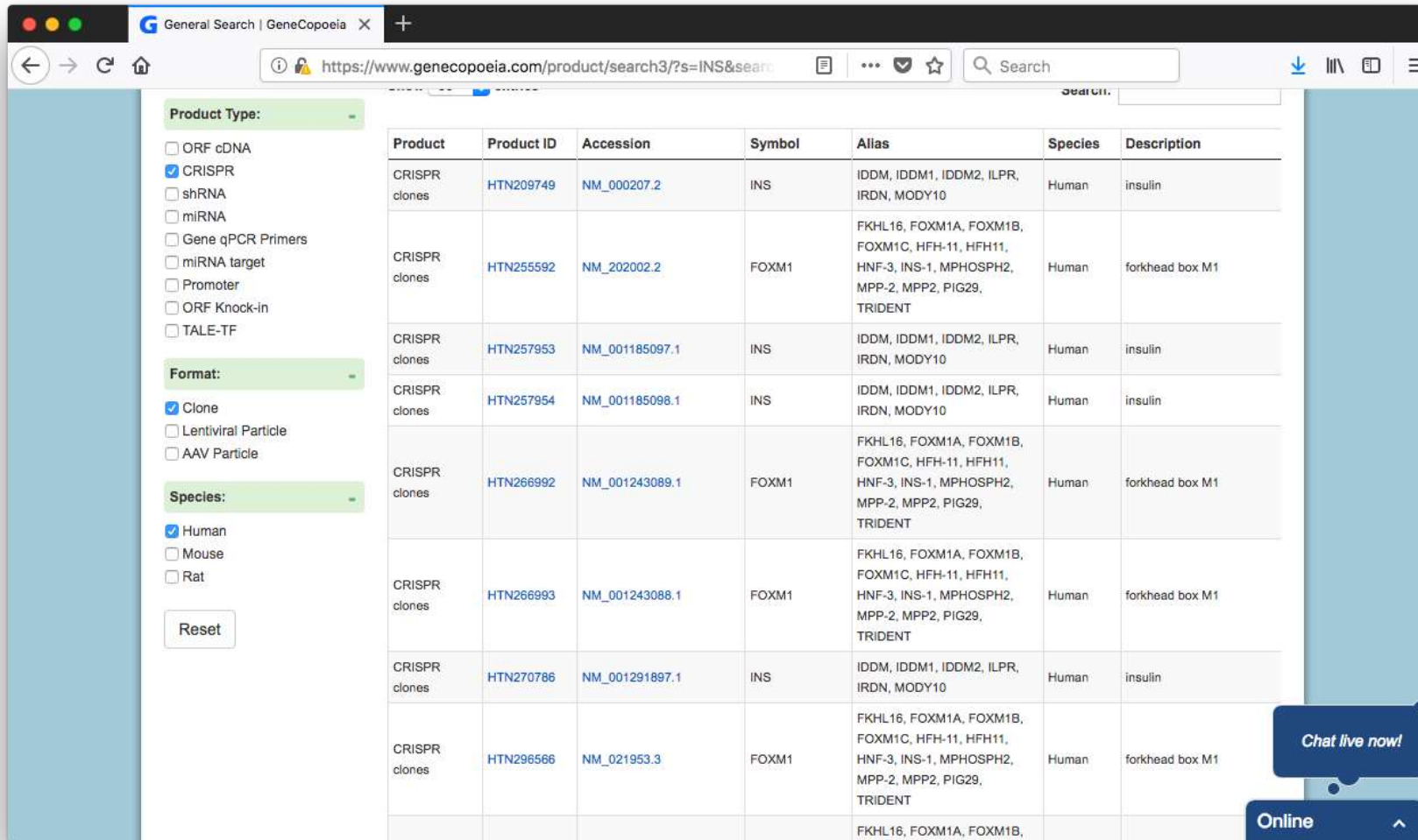
**Chat live now!**

**Online**



# CRISPR-Cas9 genome editing technology

## Workflow: Preparation



The screenshot displays the GeneCopoeia website's search results page. On the left, there are filters for Product Type, Format, and Species. The main table lists various CRISPR clones with their respective IDs, accessions, symbols, aliases, species, and descriptions. A chat bubble is visible in the bottom right corner.

**Product Type:**

- ☐ ORF cDNA
- ☒ CRISPR
- ☐ shRNA
- ☐ miRNA
- ☐ Gene qPCR Primers
- ☐ miRNA target
- ☐ Promoter
- ☐ ORF Knock-in
- ☐ TALE-TF

**Format:**

- ☒ Clone
- ☐ Lentiviral Particle
- ☐ AAV Particle

**Species:**

- ☒ Human
- ☐ Mouse
- ☐ Rat

**Reset**

Product	Product ID	Accession	Symbol	Alias	Species	Description
CRISPR clones	HTN209749	NM_000207.2	INS	IDDM, IDDM1, IDDM2, ILPR, IRDN, MODY10	Human	insulin
CRISPR clones	HTN255592	NM_202002.2	FOXM1	FKHL16, FOXM1A, FOXM1B, FOXM1C, HFH-11, HFH11, HNF-3, INS-1, MPHOSPH2, MPP-2, MPP2, PIG29, TRIDENT	Human	forkhead box M1
CRISPR clones	HTN257953	NM_001185097.1	INS	IDDM, IDDM1, IDDM2, ILPR, IRDN, MODY10	Human	insulin
CRISPR clones	HTN257954	NM_001185098.1	INS	IDDM, IDDM1, IDDM2, ILPR, IRDN, MODY10	Human	insulin
CRISPR clones	HTN266992	NM_001243089.1	FOXM1	FKHL16, FOXM1A, FOXM1B, FOXM1C, HFH-11, HFH11, HNF-3, INS-1, MPHOSPH2, MPP-2, MPP2, PIG29, TRIDENT	Human	forkhead box M1
CRISPR clones	HTN266993	NM_001243088.1	FOXM1	FKHL16, FOXM1A, FOXM1B, FOXM1C, HFH-11, HFH11, HNF-3, INS-1, MPHOSPH2, MPP-2, MPP2, PIG29, TRIDENT	Human	forkhead box M1
CRISPR clones	HTN270786	NM_001291897.1	INS	IDDM, IDDM1, IDDM2, ILPR, IRDN, MODY10	Human	insulin
CRISPR clones	HTN296566	NM_021953.3	FOXM1	FKHL16, FOXM1A, FOXM1B, FOXM1C, HFH-11, HFH11, HNF-3, INS-1, MPHOSPH2, MPP-2, MPP2, PIG29, TRIDENT	Human	forkhead box M1
				FKHL16, FOXM1A, FOXM1B,		

**Chat live now!**

**Online**

# CRISPR-Cas9 genome editing technology

## Workflow: Preparation

The screenshot shows the GeneCopoeia website interface. At the top, there's a navigation bar with the GeneCopoeia logo and a search bar. Below the navigation bar, there's a banner for CRISPR sgRNA/Cas9 clones, highlighting features like 'Knockout, mutagenesis, tagging, & more', 'Ready-to-express', and 'Expert design'. The banner also mentions 'Only \$119' and 'Complete solutions for precision gene editing'. Below the banner, there's a row of buttons for different products: ORF cDNA clones, CRISPR / TALEN, Lentivirus, AAV, Promoter clones, qPCR primers, shRNA clones, miRNA products, miRNA target clones, TALE-TF, and ORF knockin clones. The 'CRISPR / TALEN' button is selected. Below this, there's a section for 'Select TALEN / CRISPR-Cas9 Expression Clones' with a search bar. The selected product is 'Product ID: HTN209749', which is a 'gene annotation page' (click here to view gene annotation page). The product details include: Symbol: INS, Species: Human, Target Gene Accession: NM\_000207.2, Alias: IDDM, IDDM1, IDDM2, ILPR, IRDN, MODY10, Gene Description: insulin, Delivery format: 10 µg purified plasmid, and Estimated Delivery: Approximately 2-3 weeks, but may vary. Please email sales@genecopoeia.com or call 301-762-0888 to confirm ETA. There's a 'Download Datasheet' button. At the bottom, there's an 'Important Note' about the design strategy. A chat bubble is visible in the bottom right corner.

General Search | GeneCopoeia X Human TALEN or CRISPR-Cas9 X

https://www.genecopoeia.com/product/search/detail.php?prt=

GeneCopoeia™  
Expressway to Discovery

All Search (Enter keyword, gene name, accession #, etc.) Account Cart

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•Knockout, mutagenesis, tagging, & more  
•Ready-to-express  
•Expert design

CRISPR sgRNA/Cas9 clones  
Only \$119

Complete solutions for precision gene editing

ORF cDNA clones CRISPR / TALEN Lentivirus AAV Promoter clones qPCR primers shRNA clones miRNA products miRNA target clones TALE-TF ORF knockin clones

Select TALEN / CRISPR-Cas9 Expression Clones [Search again]

Product ID: HTN209749 (click here to view gene annotation page) (click here to view gene annotation page)

Symbol: INS

Species: Human

Target Gene Accession: NM\_000207.2

Alias: IDDM, IDDM1, IDDM2, ILPR, IRDN, MODY10

Gene Description: insulin

Delivery format: 10 µg purified plasmid

Estimated Delivery: Approximately 2-3 weeks, but may vary. Please email sales@genecopoeia.com or call 301-762-0888 to confirm ETA.

Download:  
Download Datasheet

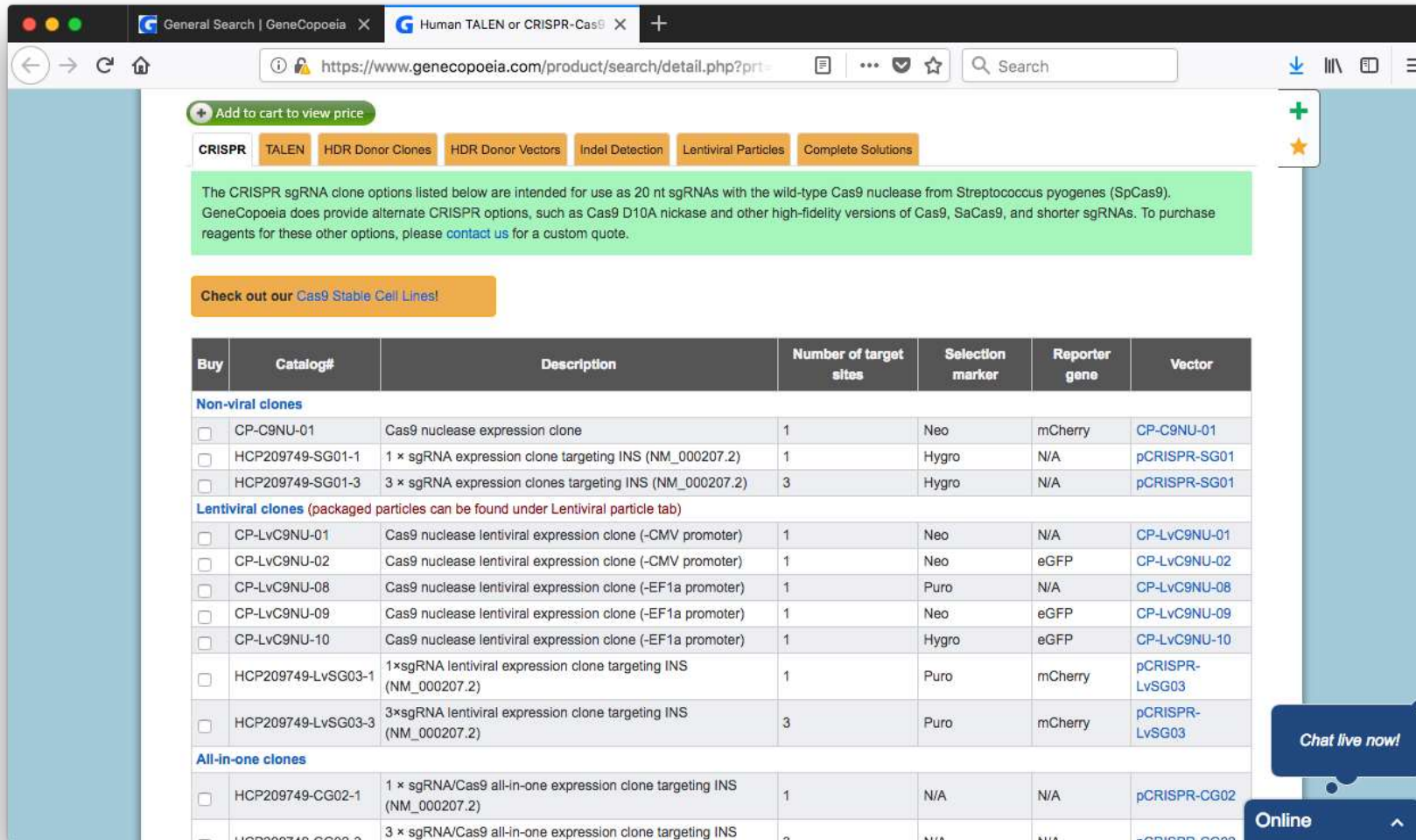
Important Note: By default, the TALEN/CRISPR constructs are designed to target the protein coding-region as closely as possible to the initiator ATG of the splice

Chat live now!

Online

# CRISPR-Cas9 genome editing technology

## Workflow: Preparation



The screenshot shows the GeneCopoeia website's product search page for CRISPR-Cas9. The page includes a navigation bar with tabs for CRISPR, TALEN, HDR Donor Clones, HDR Donor Vectors, Indel Detection, Lentiviral Particles, and Complete Solutions. A green box contains text about CRISPR sgRNA clone options. Below this is an orange button labeled 'Check out our Cas9 Stable Cell Lines!'. The main content is a table of clones, categorized into Non-viral clones, Lentiviral clones, and All-in-one clones. The table has columns for Buy, Catalog#, Description, Number of target sites, Selection marker, Reporter gene, and Vector. A chat bubble in the bottom right corner says 'Chat live now!' and 'Online'.

[Add to cart to view price](#)

**CRISPR** | TALEN | HDR Donor Clones | HDR Donor Vectors | Indel Detection | Lentiviral Particles | Complete Solutions

The CRISPR sgRNA clone options listed below are intended for use as 20 nt sgRNAs with the wild-type Cas9 nuclease from *Streptococcus pyogenes* (SpCas9). GeneCopoeia does provide alternate CRISPR options, such as Cas9 D10A nickase and other high-fidelity versions of Cas9, SaCas9, and shorter sgRNAs. To purchase reagents for these other options, please [contact us](#) for a custom quote.

[Check out our Cas9 Stable Cell Lines!](#)

Buy	Catalog#	Description	Number of target sites	Selection marker	Reporter gene	Vector
<b>Non-viral clones</b>						
<input type="checkbox"/>	CP-C9NU-01	Cas9 nuclease expression clone	1	Neo	mCherry	CP-C9NU-01
<input type="checkbox"/>	HCP209749-SG01-1	1 × sgRNA expression clone targeting INS (NM_000207.2)	1	Hygro	N/A	pCRISPR-SG01
<input type="checkbox"/>	HCP209749-SG01-3	3 × sgRNA expression clones targeting INS (NM_000207.2)	3	Hygro	N/A	pCRISPR-SG01
<b>Lentiviral clones</b> (packaged particles can be found under Lentiviral particle tab)						
<input type="checkbox"/>	CP-LvC9NU-01	Cas9 nuclease lentiviral expression clone (-CMV promoter)	1	Neo	N/A	CP-LvC9NU-01
<input type="checkbox"/>	CP-LvC9NU-02	Cas9 nuclease lentiviral expression clone (-CMV promoter)	1	Neo	eGFP	CP-LvC9NU-02
<input type="checkbox"/>	CP-LvC9NU-08	Cas9 nuclease lentiviral expression clone (-EF1a promoter)	1	Puro	N/A	CP-LvC9NU-08
<input type="checkbox"/>	CP-LvC9NU-09	Cas9 nuclease lentiviral expression clone (-EF1a promoter)	1	Neo	eGFP	CP-LvC9NU-09
<input type="checkbox"/>	CP-LvC9NU-10	Cas9 nuclease lentiviral expression clone (-EF1a promoter)	1	Hygro	eGFP	CP-LvC9NU-10
<input type="checkbox"/>	HCP209749-LvSG03-1	1×sgRNA lentiviral expression clone targeting INS (NM_000207.2)	1	Puro	mCherry	pCRISPR-LvSG03
<input type="checkbox"/>	HCP209749-LvSG03-3	3×sgRNA lentiviral expression clone targeting INS (NM_000207.2)	3	Puro	mCherry	pCRISPR-LvSG03
<b>All-in-one clones</b>						
<input type="checkbox"/>	HCP209749-CG02-1	1 × sgRNA/Cas9 all-in-one expression clone targeting INS (NM_000207.2)	1	N/A	N/A	pCRISPR-CG02
<input type="checkbox"/>	HCP209749-CG02-3	3 × sgRNA/Cas9 all-in-one expression clone targeting INS	3	N/A	N/A	pCRISPR-CG02

[Chat live now!](#)

[Online](#)

# CRISPR-Cas9 genome editing technology

## Workflow: Preparation

### Step 2: Determine the DNA sequence of the sgRNA target site in your cell line

- ❖ Necessary to ensure highest possible nucleotide match. Might need to do custom sgRNA synthesis



# CRISPR-Cas9 genome editing technology

## Smart-Join™ Blunt-end PCR Cloning Kit

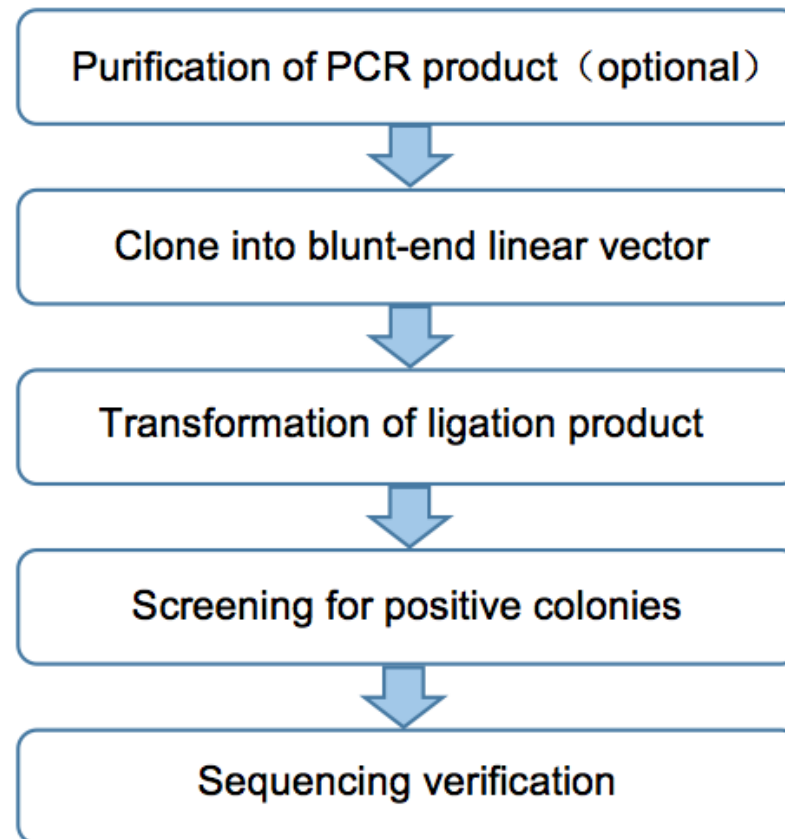
### Features

- ❖ Convenient method for high efficiency cloning of blunt-end PCR products
- ❖ Higher positive ligation rate and true positive colonies by the elimination of self-ligating products
- ❖ Cloning of blunt-end PCR products without additional sequences or restriction sites
- ❖ Ideal for sequencing regions of genomic DNA

# CRISPR-Cas9 genome editing technology

## Smart-Join™ Blunt-end PCR Cloning Kit

### Workflow



# CRISPR-Cas9 genome editing technology

## Workflow: Preparation

### Step 3: Validate sgRNA function in your cell line

- ❖ Not all sgRNAs are created equal. It is the best practice to try multiple target sites and pre-validate the best one

# CRISPR-Cas9 genome editing technology

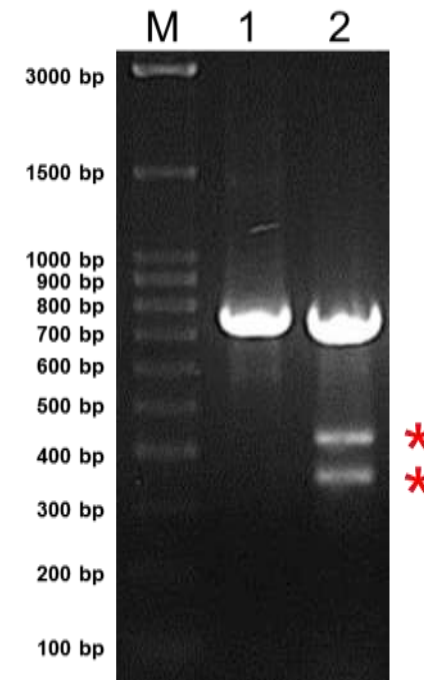
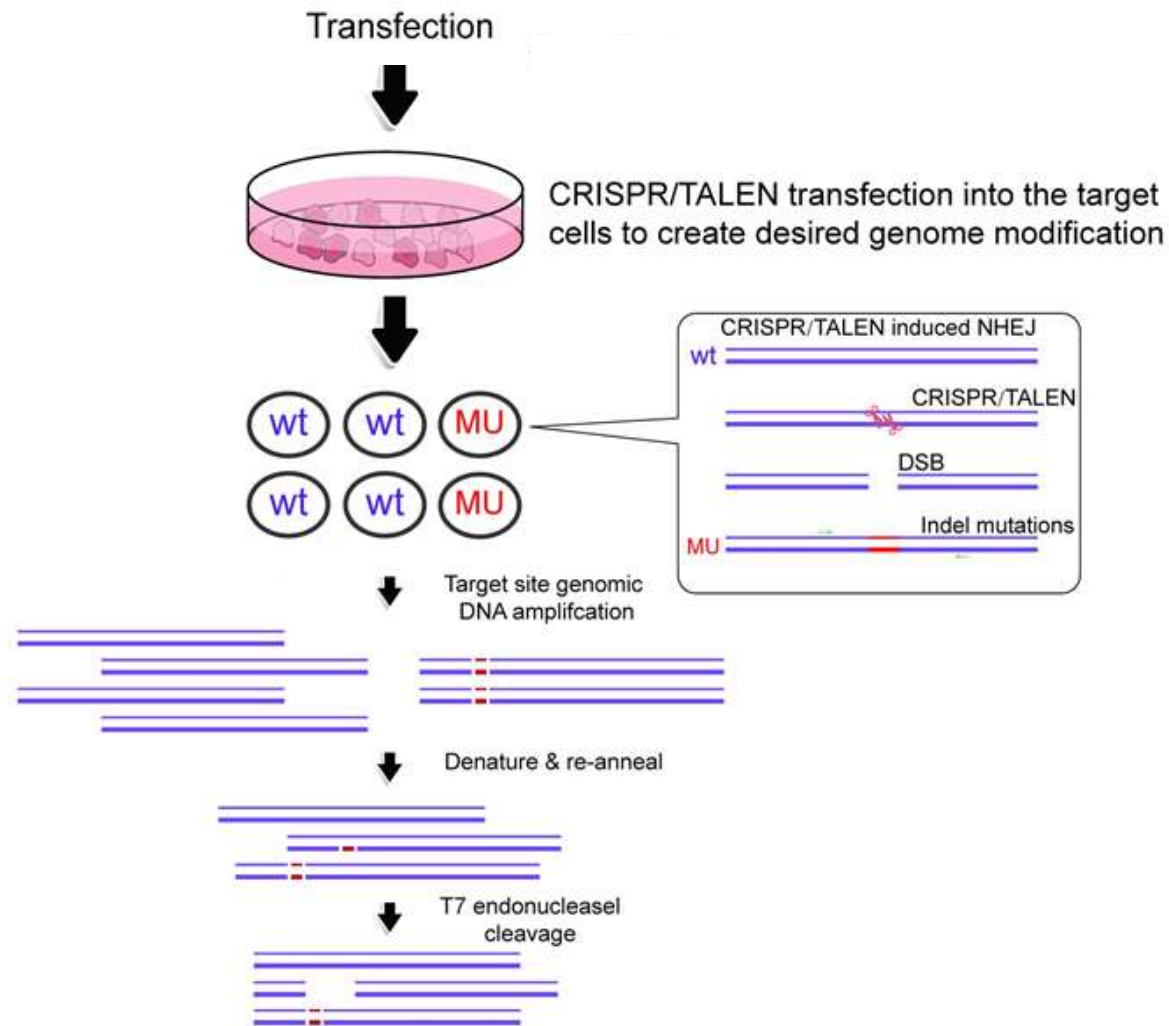
## IndelCheck™ CRISPR indel detection system

### Features

- ❖ Useful for 1) CRISPR sgRNA functional validation; 2) Screening for positive clones
- ❖ 3-component system: 1) Target site PCR kit; 2) T7 Endonuclease I kit; 3) Blunt-end PCR cloning kit
- ❖ No genomic DNA isolation is required

# CRISPR-Cas9 genome editing technology

## IndelCheck™ CRISPR indel detection system



# CRISPR-Cas9 genome editing technology

## Workflow: Preparation

### Step 4: Copy number determination

- ❖ For complete knockouts or mutagenesis, might need to modify all alleles
- ❖ Not all cell lines are diploid. Some lines, like HeLa, have 3, 4, or more genomes
- ❖ Would expect multi-allele modification to be more difficult to achieve than single allele
- ❖ However, double allele occurs more frequently than single allele (Gonzalez, et al., 2014. Cell Stem Cell 15, 1)

# CRISPR-Cas9 genome editing technology

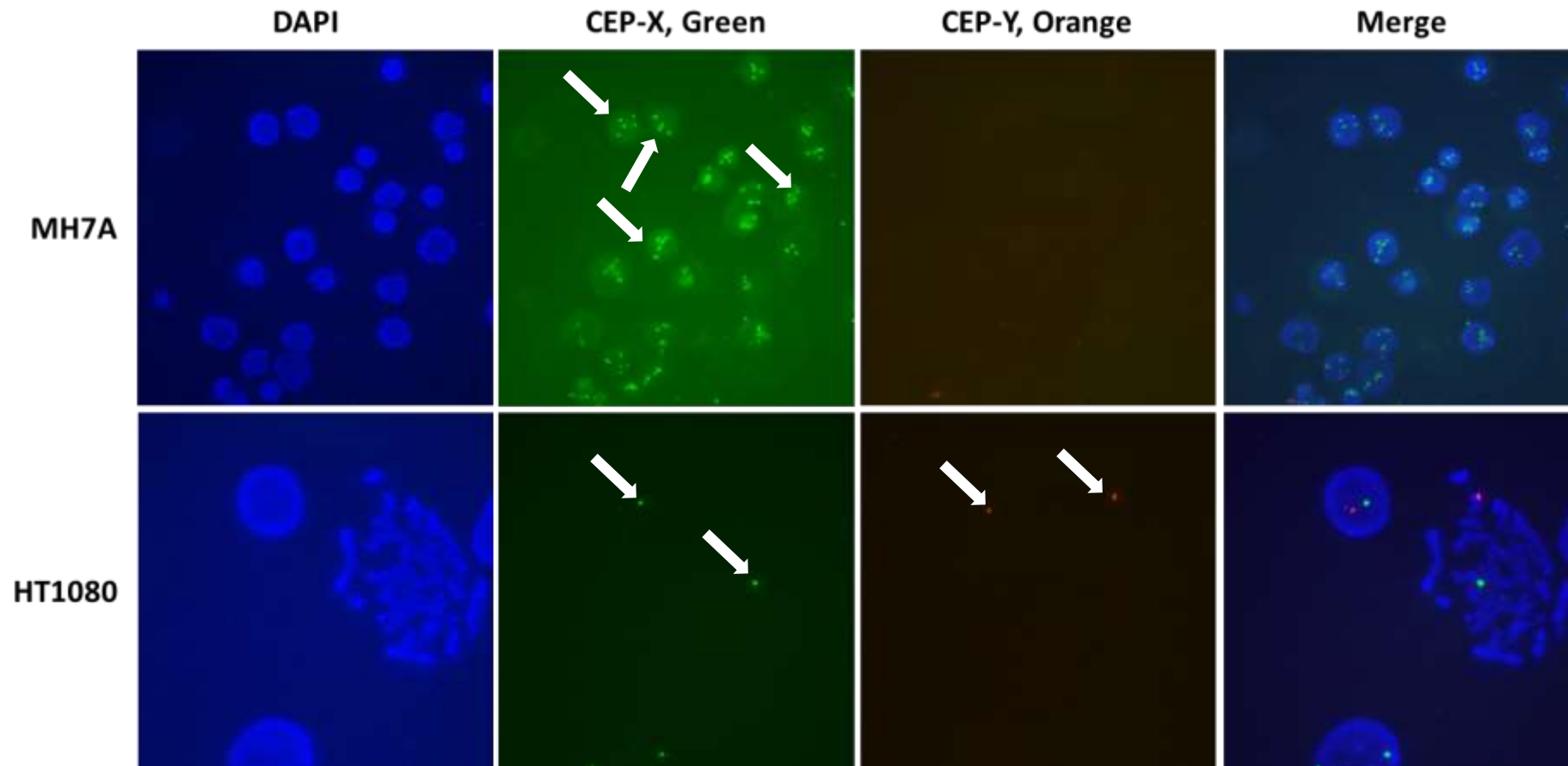
## Application: Using FISH with a CRISPR knockout

Project: Knock out HDAC6 gene in human MH7A cells

- ❖ HDAC6 (NCBI geneID:10013): Located on X chromosome (Xp11.23)
- ❖ MH7A cells: Human immortalized synovial fibroblast line
- ❖ HDAC6 copy number: Unknown

# CRISPR-Cas9 genome editing technology

Application: Using FISH with a CRISPR knockout





# CRISPR-Cas9 genome editing technology

## Application: Using FISH with a CRISPR knockout

### Clone 1

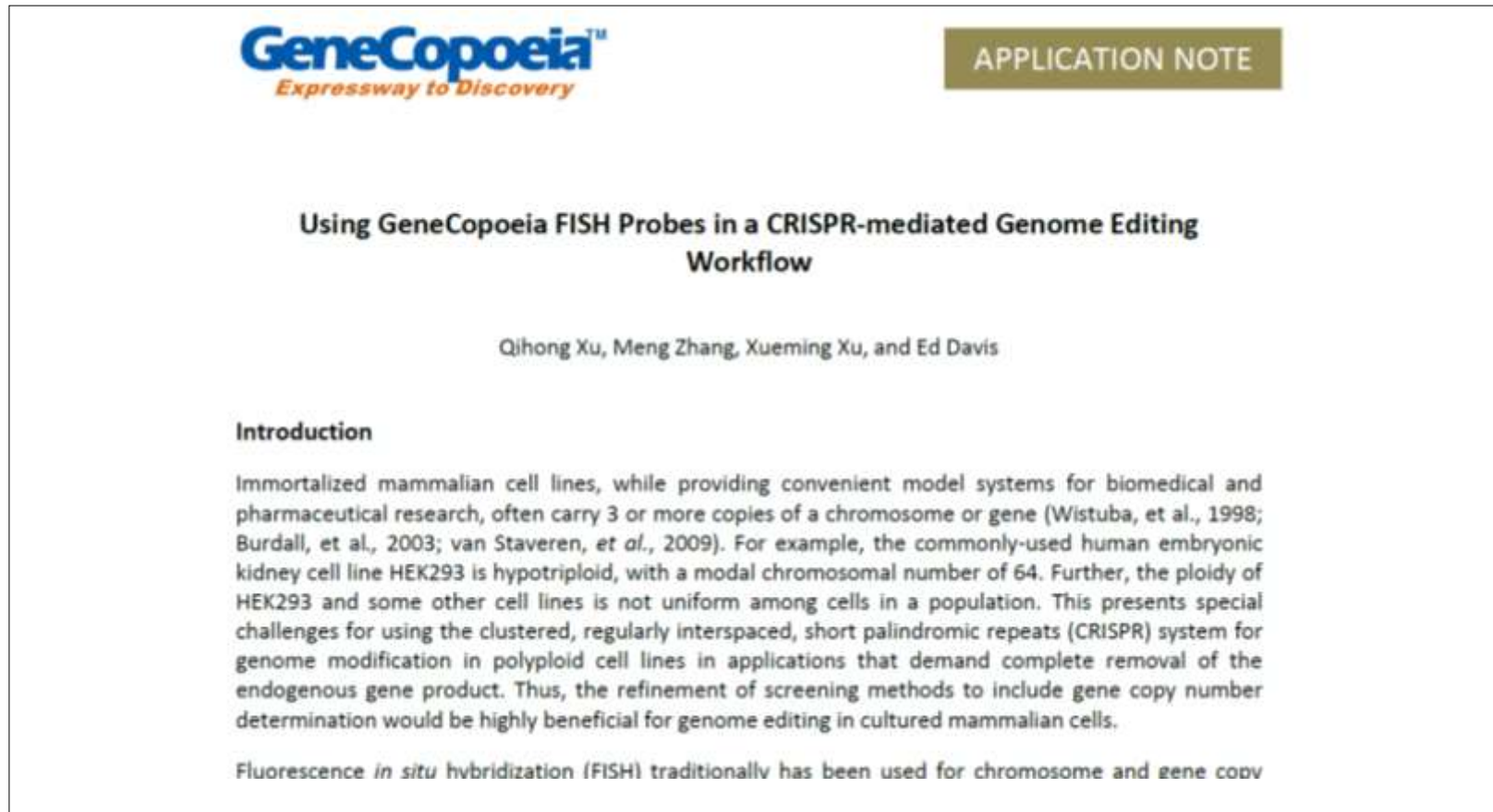
		(500)	500	510	520	530	540	550	560	570	580	590	600	610	620	635
HDAC6 Targeting region NC_018934	C2-4_PREMIX (471)	TTCACAGAGCGAATATTAAAGG	GGAGCGGTCCCGCTCTATCCCAATCTAG	AGGCTCTCTCCGAGCGGATGTACCCGAGGACGGCGGCC	CGAGTAAAG	AAGAAAGCCAAATGAGAGCTGGGCCAA										
Allele a	C2-6_PREMIX (470)	TTCACAGAGCGAATATTAAAGG														AAGAAAGCCAAATGAGAGCTGGGCCAA
Allele b	C2-11_PREMIX (469)	TTCACAGAGCGAATATTAAAGG	GGAGCGGTCCCG													AAGAAAGCCAAATGAGAGCTGGGCCAA
Allele c	C2-7_PREMIX (472)	TTCACAGAGCGAATATTAAAGG	GGAGCGGTCCCGCTCTATCCCAATCTAG													CGAGTAAAG
Allele c	2A-F_PREMIX (470)	TTCACAGAGCGAATATTAAAGG	GGAGCGGTCCCGCTCTATCCCAATCTAG													CGAGTAAAG
Allele d	C2-5_PREMIX (470)	TTCACAGAGCGAATATTAAAGG	GGAGCGGTCCCGCTCTATCCCAATCTAG													CGAGTAAAG
Allele d	C2-8_PREMIX (474)	TTCACAGAGCGAATATTAAAGG	GGAGCGGTCCCGCTCTATCCCAATCTAG													CGAGTAAAG
Allele d	2B-F_PREMIX (472)	TTCACAGAGCGAATATTAAAGG	GGAGCGGTCCCGCTCTATCCCAATCTAG													CGAGTAAAG
Allele d	2C-F_PREMIX (470)	TTCACAGAGCGAATATTAAAGG	GGAGCGGTCCCGCTCTATCCCAATCTAG													CGAGTAAAG
Allele e	HDAC6 Targeting Region NC_018934 (500)	TTCACAGAGCGAATATTAAAGG	GGAGCGGTCCCGCTCTATCCCAATCTAG													CGAGTAAAG
Consensus	Consensus (500)	TTCACAGAGCGAATATTAAAGG	GGAGCGGTCCCGCTCTATCCCAATCTAG													CGAGTAAAG

### 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)	TAAAGGGAGCGGTCCCGCTCTATCCCAATCTAGAGGCTCTCCGAGCGGATGTACCCGAGGACGGCGCCCGGAGGT	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG
Allele f	C3-8_PREMIX (516)	GCATTATGAATCGCCAAAGCGCGGAGAGGCGGTTCG														AAAGAAAGG
Allele g	C3-6_PREMIX (487)	TAAAGGGAGCGGTCCCGCTCTATCCCAATCTAGAGGCTCTCCGAGCGGATGTACCCGAGGACGGCGCCCGGAGGT	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG
Allele g	3C-F_PREMIX (487)	TAAAGGGAGCGGTCCCGCTCTATCCCAATCTAGAGGCTCTCCGAGCGGATGTACCCGAGGACGGCGCCCGGAGGT	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG
Allele h	C3-10_PREMIX (487)	TAAAGGGAGCGGTCCCGCTCTATCCCAATCTAGAGGCTCTCCGAGCGGATGTACCCGAGGACGGCGCCCGGAGGT	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG
Allele i	3A-F_PREMIX (487)	TAAAGGGAGCGGTCCCGCTCTATCCCAATCTAGAGGCTCTCCGAGCGGATGTACCCGAGGACGGCGCCCGGAGGT	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG
Allele i	3B-F_PREMIX (492)	TAAAGGGAGCGGTCCCGCTCTATCCCAATCTAGAGGCTCTCCGAGCGGATGTACCCGAGGACGGCGCCCGGAGGT	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG
Allele i	C3-4_PREMIX (488)	TAAAGGGAGCGGTCCCGCTCTATCCCAATCTAGAGGCTCTCCGAGCGGATGTACCCGAGGACGGCGCCCGGAGGT	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG
Allele i	C3-5_PREMIX (487)	TAAAGGGAGCGGTCCCGCTCTATCCCAATCTAGAGGCTCTCCGAGCGGATGTACCCGAGGACGGCGCCCGGAGGT	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG
Allele i	C3-9_PREMIX (488)	TAAAGGGAGCGGTCCCGCTCTATCCCAATCTAGAGGCTCTCCGAGCGGATGTACCCGAGGACGGCGCCCGGAGGT	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG
Allele i	HDAC6 Targeting Region NC_018934 (518)	TAAAGGGAGCGGTCCCGCTCTATCCCAATCTAGAGGCTCTCCGAGCGGATGTACCCGAGGACGGCGCCCGGAGGT	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG
Consensus	Consensus (553)	TAAAGGGAGCGGTCCCGCTCTATCCCAATCTAGAGGCTCTCCGAGCGGATGTACCCGAGGACGGCGCCCGGAGGT	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG	AAAGAAAGG

# CRISPR-Cas9 genome editing technology

## GeneCopoeia Application Note: FISH with CRISPR



Download from:

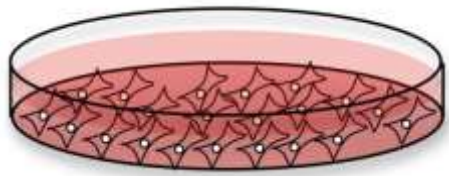
<http://www.genecopoeia.com/wp-content/uploads/2016/02/FISH-for-CRISPR.pdf>

# Outline

- ❖ CRISPR technologies & applications
- ❖ Strategy considerations
- ❖ Preparation
- ❖ Delivery methods
- ❖ Screening**
- ❖ Things to look out for

# Screening

Isolate single clones post-transfection/transduction



**OR**



**OR**



Plate for single colonies  
and pick off dish

Fluorescence sorting

Do serial dilutions in  
multi-well plates

- ❖ Minimizes potential effects of unwanted modifications resulting from random insertion, cell division or off-targeting



# Screening

Isolate single clones post-transfection/transduction

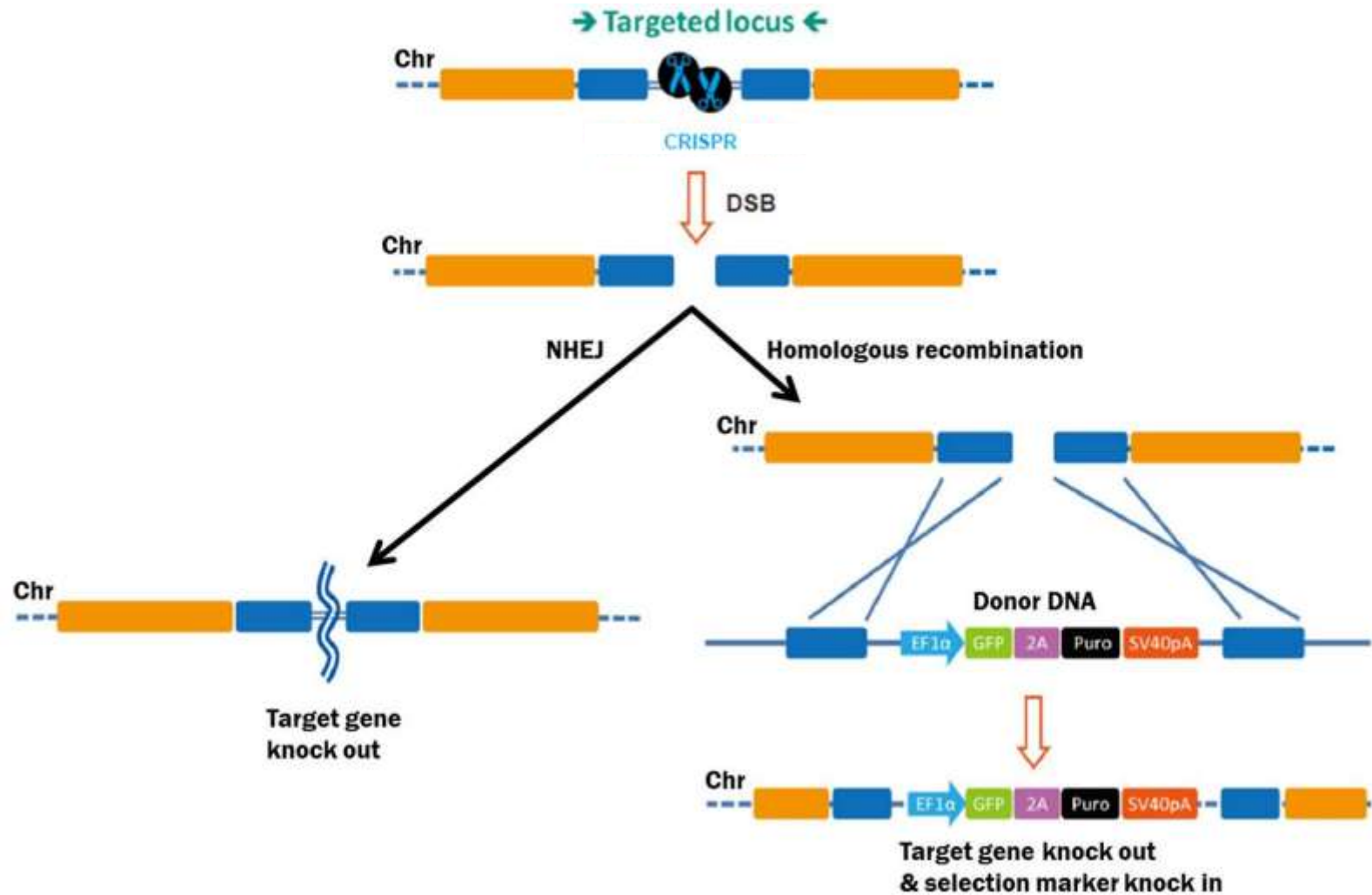
## IMPORTANT:

Connect genotype to phenotype!

(or, check the chromosome first before doing  
functional assays for your target)

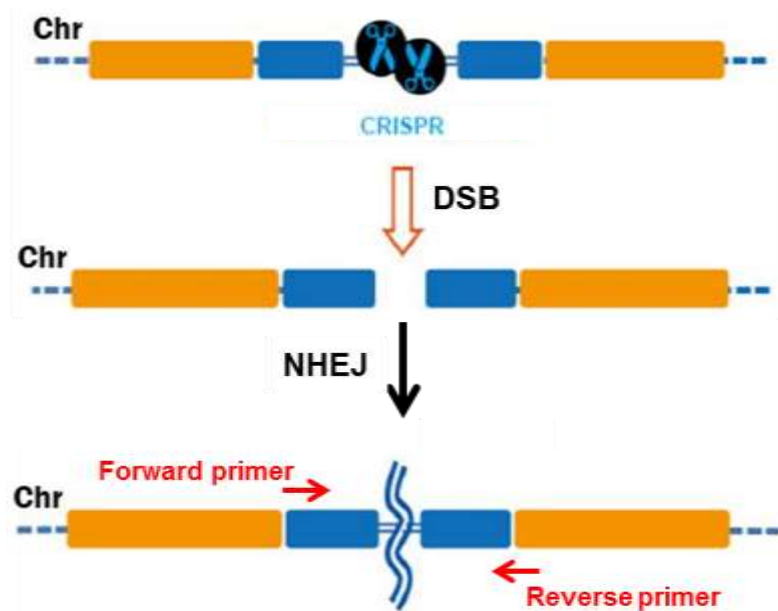
# Screening

Isolate single clones post-transfection/transduction



# Screening

## NHEJ-mediated mutations (knockouts)

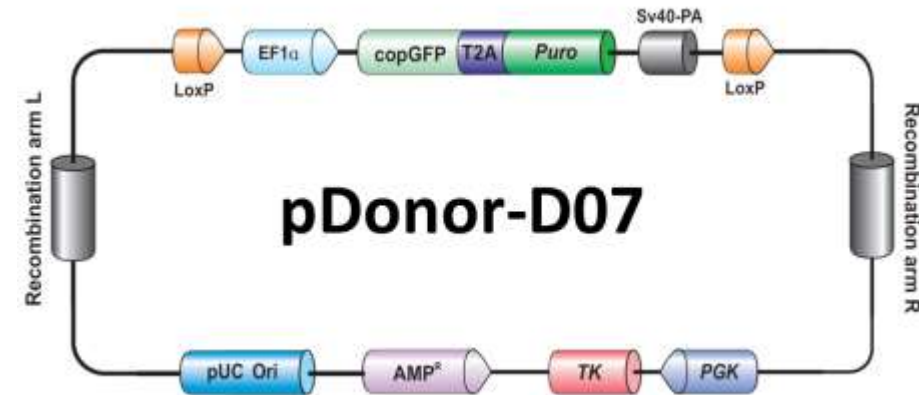
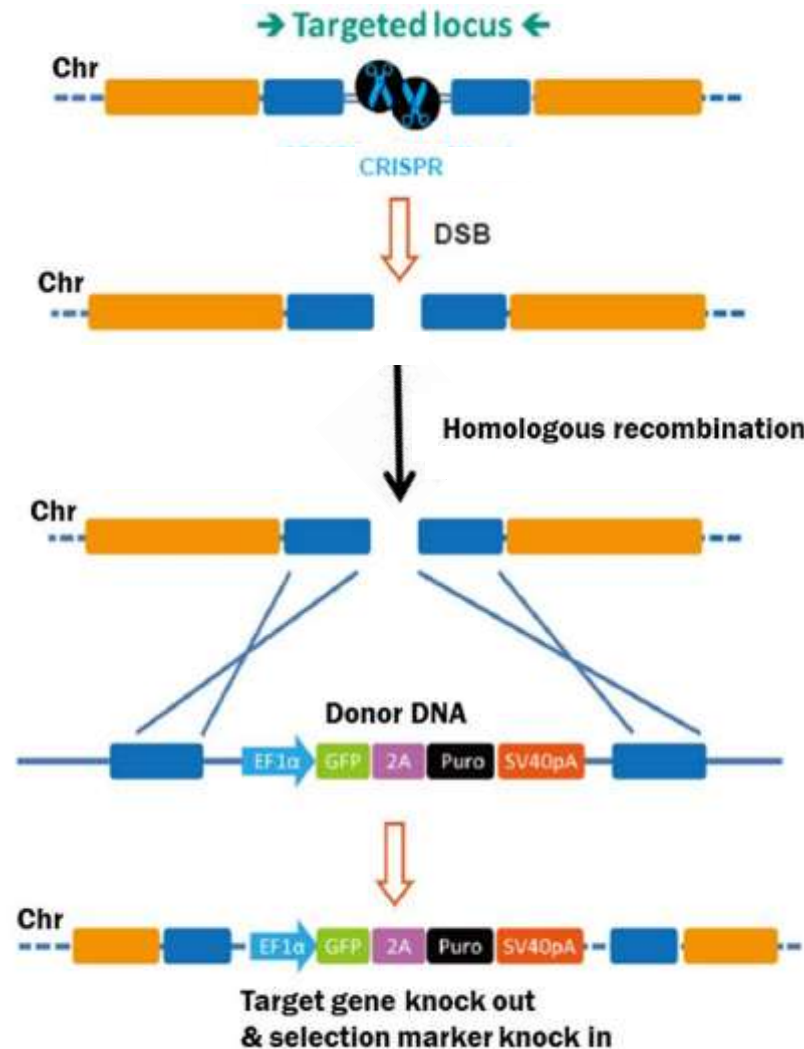


- ❖ Isolate clones without selection 2-3 days post transfection/transduction
- ❖ Generate PCR products using primers flanking the DSB site (can use same primers as used for validation)
- ❖ Screen PCR products by T7 Endonuclease I assay OR direct sequencing. Use GeneCopoeia's IndelCheck system
- ❖ Re-screen positive clones by direct sequencing



# Screening

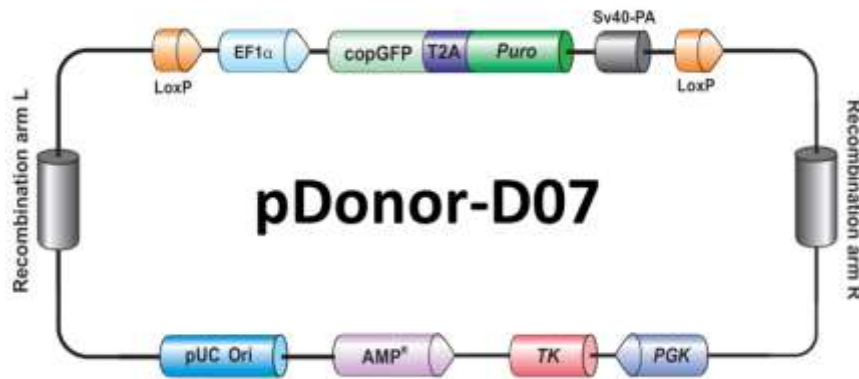
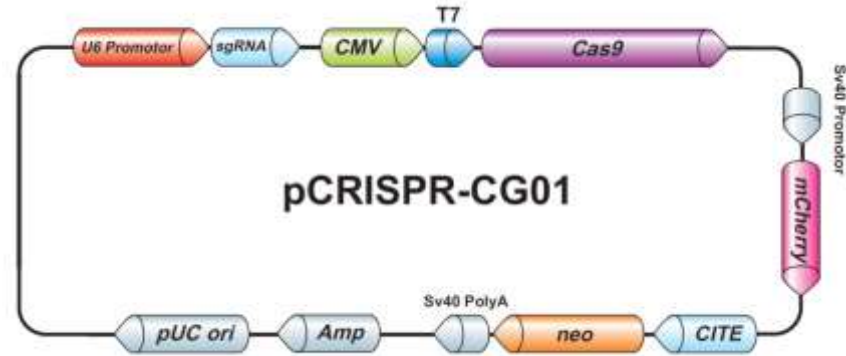
## HDR-mediated applications using donor plasmids





# Screening

## HDR-mediated applications using donor plasmids

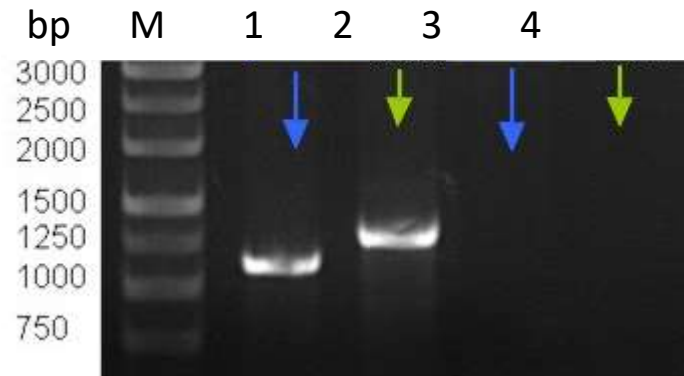
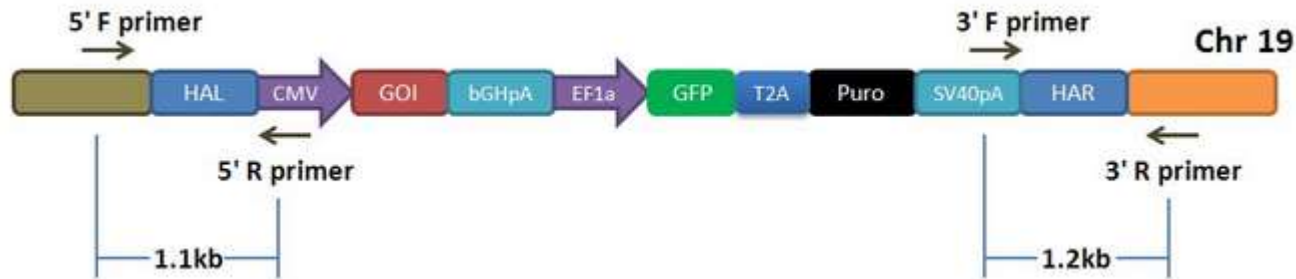


- ❖ Co-transfect Cas9, sgRNA, and donor plasmids
- ❖ 2-3 days post transfection, apply selection for donor selection cassette, or sort cells expressing fluorescent reporter
- ❖ Isolate single clones
- ❖ Screen single clones for correct integration by PCR

# Screening

HDR-mediated applications using donor plasmids

## Junction PCR



**Will only get PCR products if donor has integrated at the correct site**

# Outline

- ❖ CRISPR technologies & applications
- ❖ Strategy considerations
- ❖ Preparation
- ❖ Delivery methods
- ❖ Screening
- ❖ Things to look out for

# Things to look out for!

## Gene copy number

- ❖ For complete knockouts or mutagenesis, might need to modify all alleles
- ❖ Not all cell lines are diploid. Some lines, like HeLa, have 3, 4, or more genomes!
- ❖ Would expect multi-allele modification to be more difficult to achieve than single allele
- ❖ However, one publication showed that double allele occurs more frequently than single allele (Gonzalez, et al., 2014. Cell Stem Cell 15, 1)

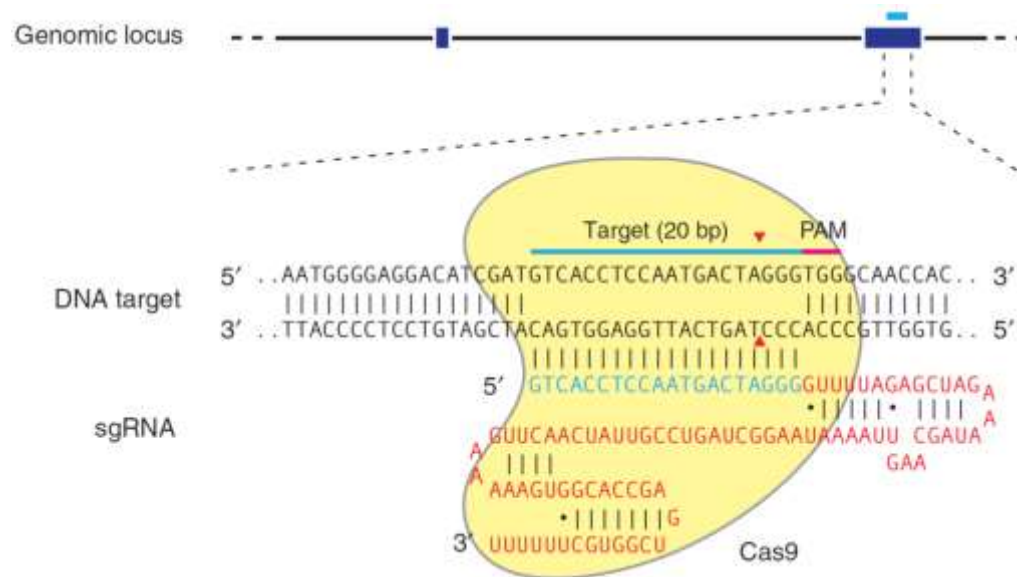
# Things to look out for!

## Cleavage efficiency

- ❖ Efficiencies of indel formation by CRISPR usually 5%-70%
- ❖ Expect to screen 2x as many clones for a CRISPR sgRNA with 40% cutting efficiency as you would for one with 80% cutting efficiency
- ❖ Selection for modifications using a donor can help screening

# Things to look out for!

## Off-targeting



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

- ❖ CRISPR sgRNAs can tolerate up to 5 mismatches with chromosomal target
- ❖ CRISPR sgRNAs can also recognize non-canonical NAG PAM
- ❖ Mismatch tolerance and non-canonical PAM recognition can lead to cleavage at other, non-target, or “off-target” sites

- ❖ Off-target modifications can have adverse consequences on research results or patient safety!

# Things to look out for!

## Off-targeting

- ❖ Off-targeting should be addressed. GeneCopoeia designs sgRNAs with the lowest possible predicted off-target potential. However, this is a prediction, not experimentally determined.
- ❖ Whole genome next-generation sequencing (NGS) is the most comprehensive, unbiased approach. However, this is expensive.
- ❖ Can also use targeted, NGS methods such as Digenome-seq and VIVO
- ❖ Alternatively, can sequence predicted off-target sites. This is an inexpensive method but is biased.

# Summary

- ❖ The workflow for CRISPR genome editing experiments must be carefully considered, for aspects such as type of modification you are using, the delivery method, the cell line you are using, etc.
- ❖ Other considerations must be taken into account for successful genome editing experiments, such as transfection efficiency, copy number variation, and cleavage efficiency
- ❖ GeneCopoeia offers many products and services, including plasmid design & construction, functional validation, validation and screening kits, and transgenic mice, to meet your genome editing needs



# 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)

GeneCopoeia, Inc.

9260 Medical Center Drive Suite 101

Rockville, Maryland USA 20850

