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 though 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

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

GeneCopoeia™
Expressway to Discovery

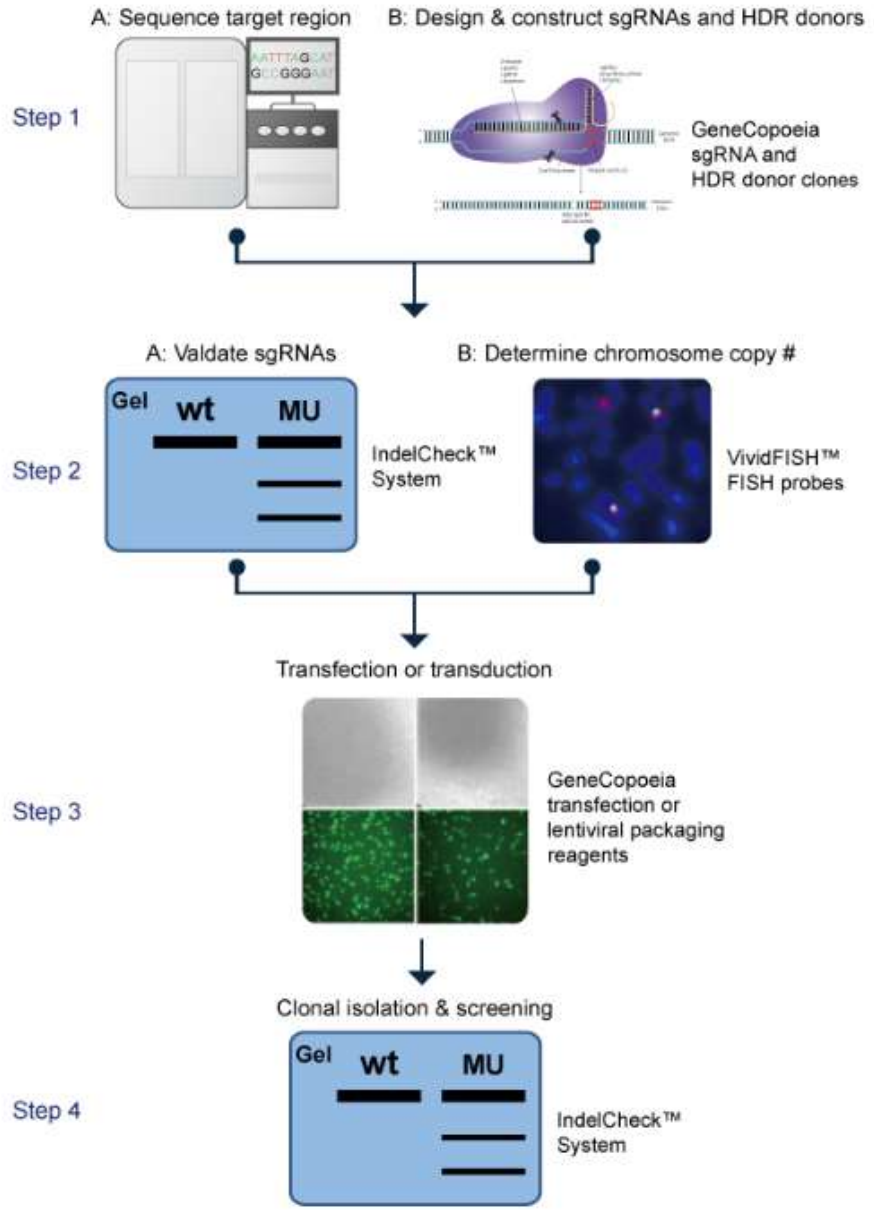
GeneCopoeia Products and Services

Functional Genomics & Cell Biology

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

GeneCopoeia™
Expressway to Discovery

GeneCopoeia CRISPR products for cell lines



GeneCopoeia™
Expressway to Discovery

Outline

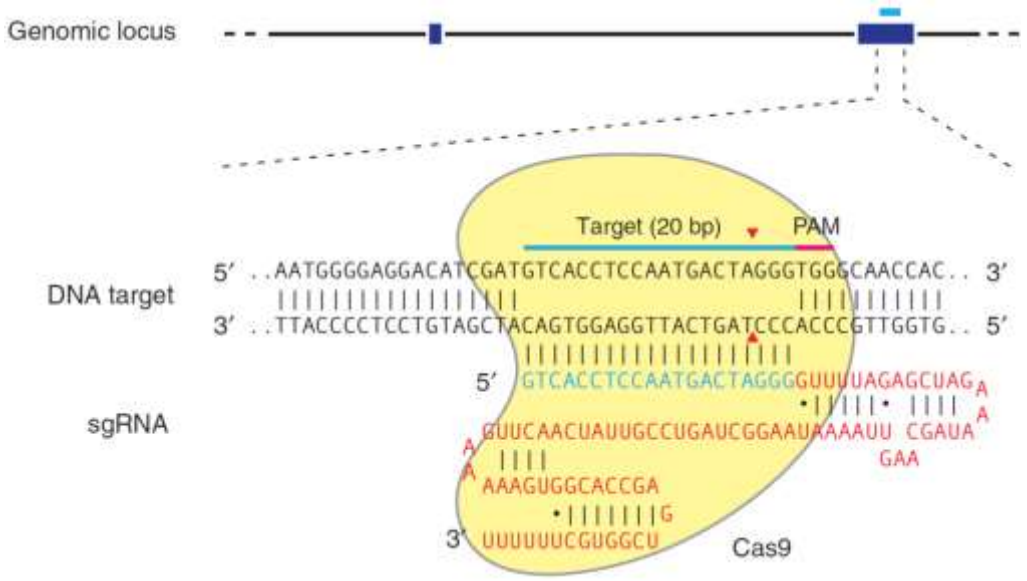
- CRISPR technologies & applications
- Strategy considerations
- Delivery methods
- Preparation
- Screening
- Things to look out for

Outline

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

CRISPR-Cas9 genome editing technology

CRISPR-Cas9: RNA-guided endonuclease

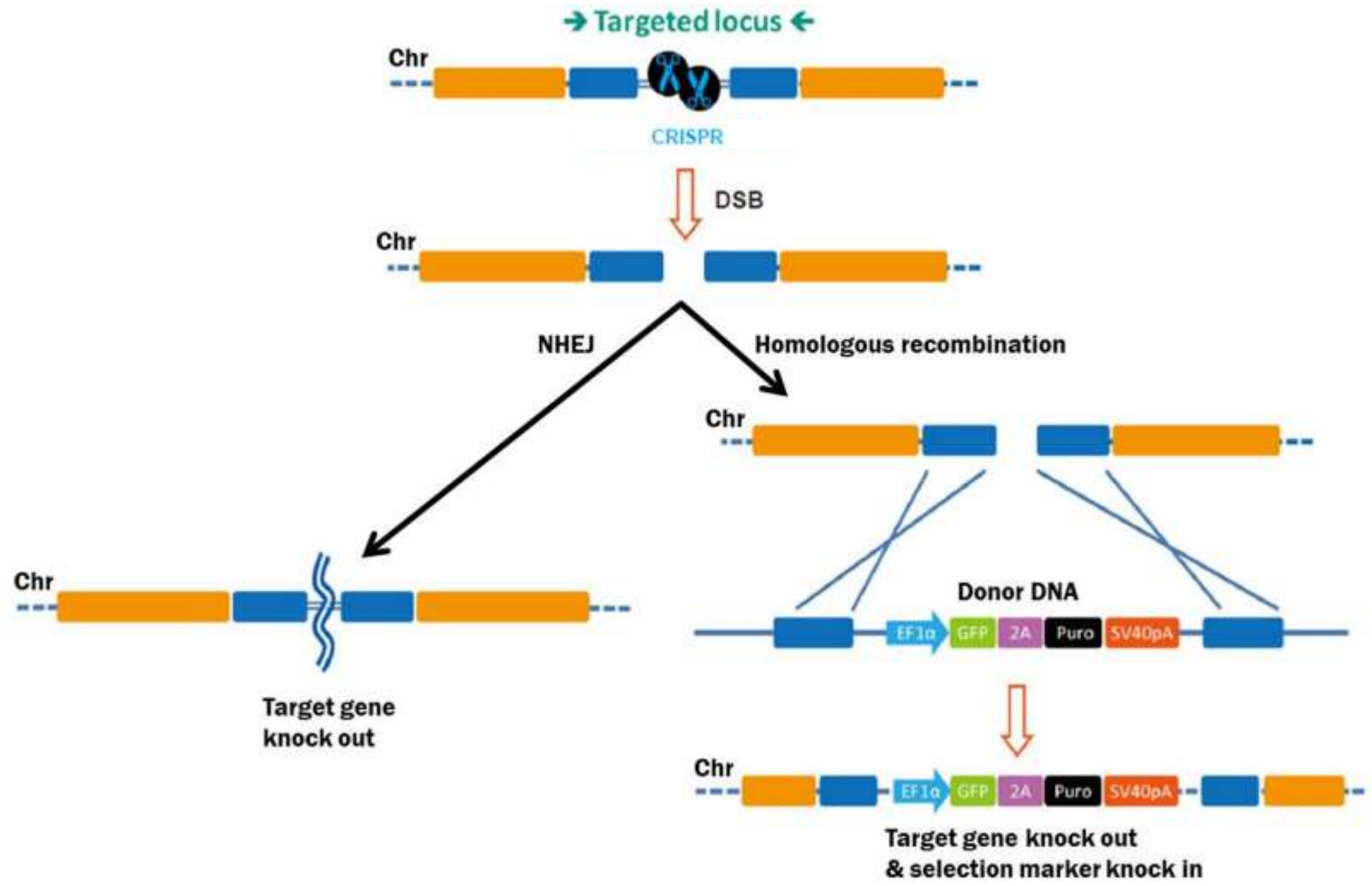


Ran, et al. (2013). Nature Protocols 8, 2281

- 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.

GeneCopoeia™
Expressway to Discovery

Targeted DNA editing by DSB induction



GeneCopoeia™
Expressway to Discovery

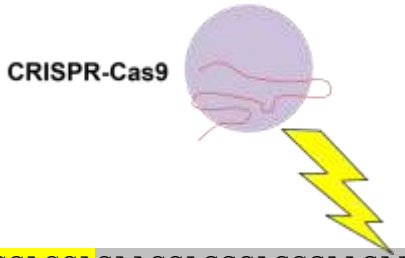
CRISPR-Cas9 genome editing technology

Technological variants

Technology	Description	PAM	Applications
SpCas9	Unmodified Cas9 from *Streptococcus pyogenes*	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 *Acidaminococcus sp. BV3L6* or *Lachnospiraceae 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 *Staphylococcus aureus*. 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 *Leptotrichia wadei*. Targets RNA. 20-28 nt spacer	None	RNA knockdown or mutagenesis

CRISPR-Cas9 applications

Gene knockout-frameshift



ATGACCTCAACCGGCCAGGATTCCACCACAACCAGGCAGCGAAGAAGTAGGCAGAACCCCCAGTCGCCCCCTCAGGACTCCAGTGTCACTTCGgtgaggccctagacccgccctgatgaggg

ATGACCTCAACCGGCCAGGATTCCACCACAACCAGGCAGCGAAGAAGTAGGCAGAACCCCCAGTCGCCCCCTCAGGACTCCAGTGTCACTTCGgtgaggccctagacccgccctgatgaggg

→ (Multiple of 3) +/- 1 indel-frameshift

ATGACCTCAACCGGCCAGGATTCCACCACAACCAGGCAGCGAAGAAGTAGGCAGAACCCCCAGTCGCCCCCTCAGGACTCCAGTGTCACTTCGgtgaggccctagacccgccctgatgaggg

→ (Multiple of 3) +/- 2 indel-frameshift

ATGACCTCAACCGGCCAGGATTCCACCACAACCAGGCAGCGAAGAAGTAGGCAGAACCCCCAGTCGCCCCCTCAGGACTCCAGTGTCACTTCGgtgaggccctagacccgccctgatgaggg

→ Multiple of 3 indel-in frame

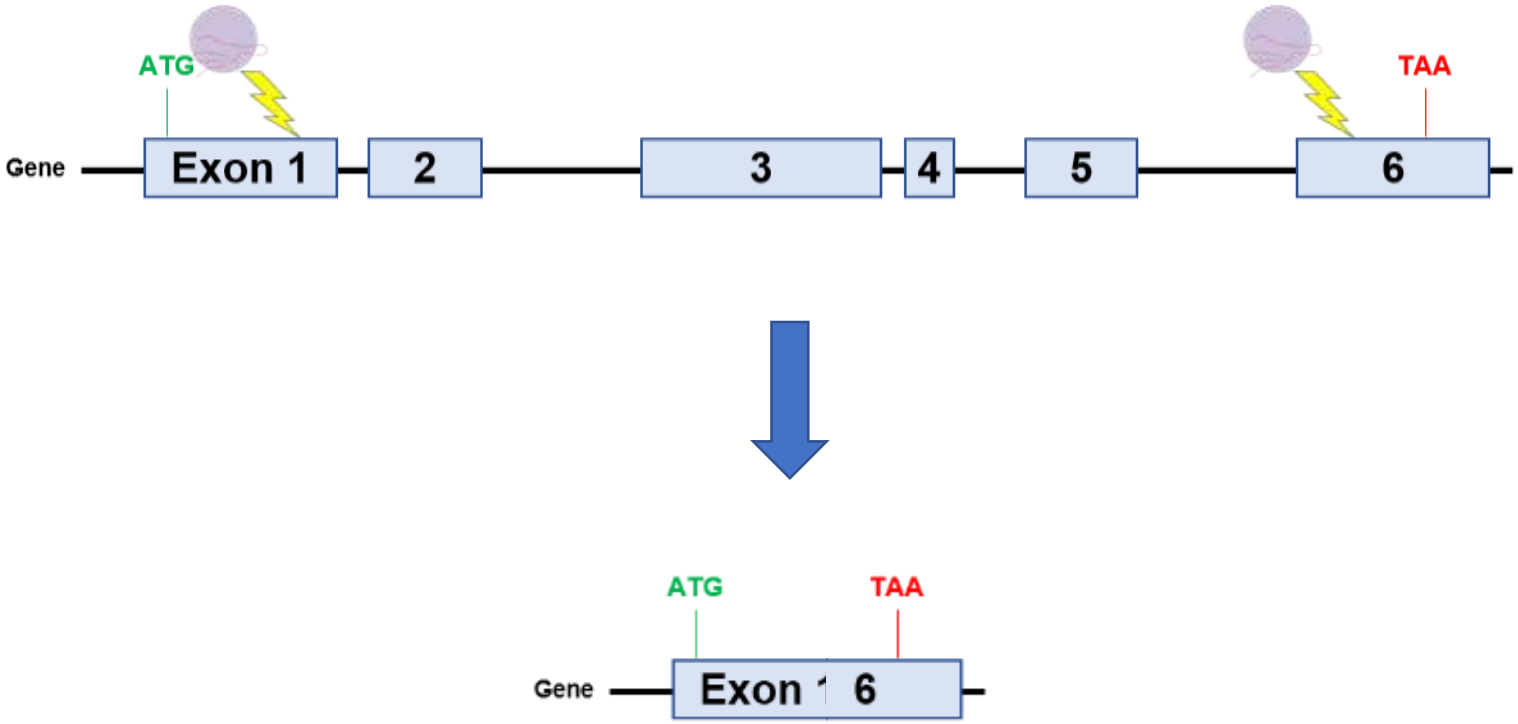
CRISPR-Cas9 applications

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.

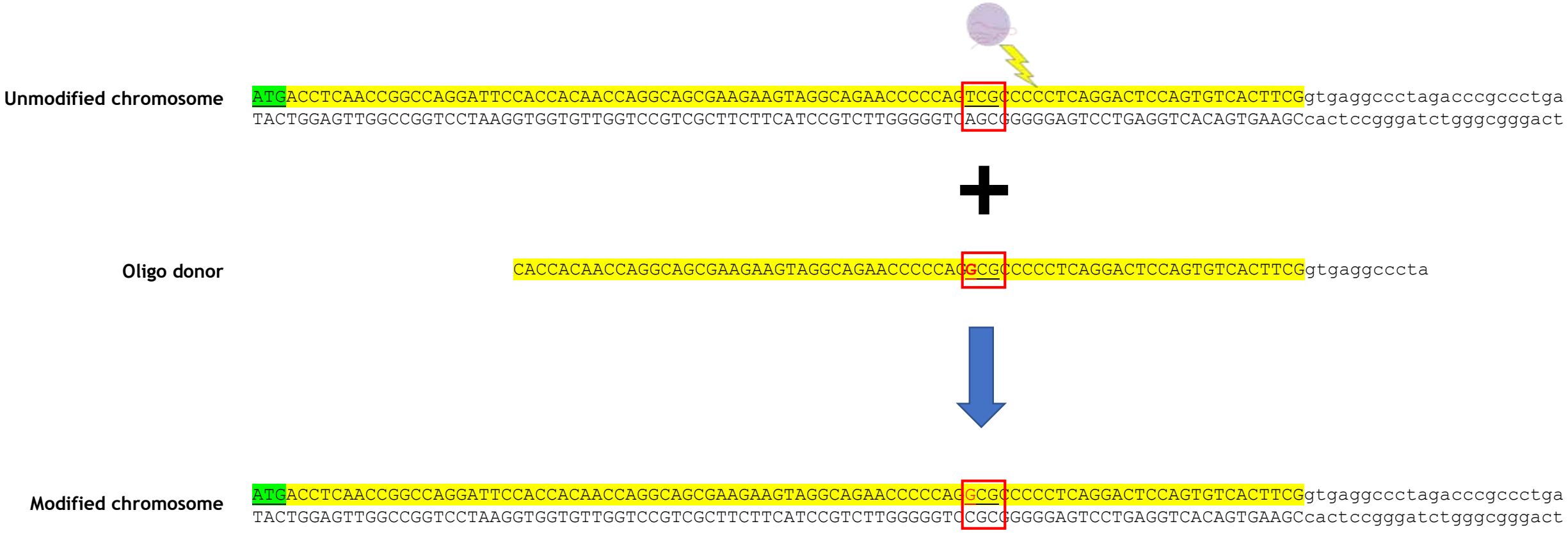
CRISPR-Cas9 applications

Gene knockout-large deletion



CRISPR-Cas9 applications

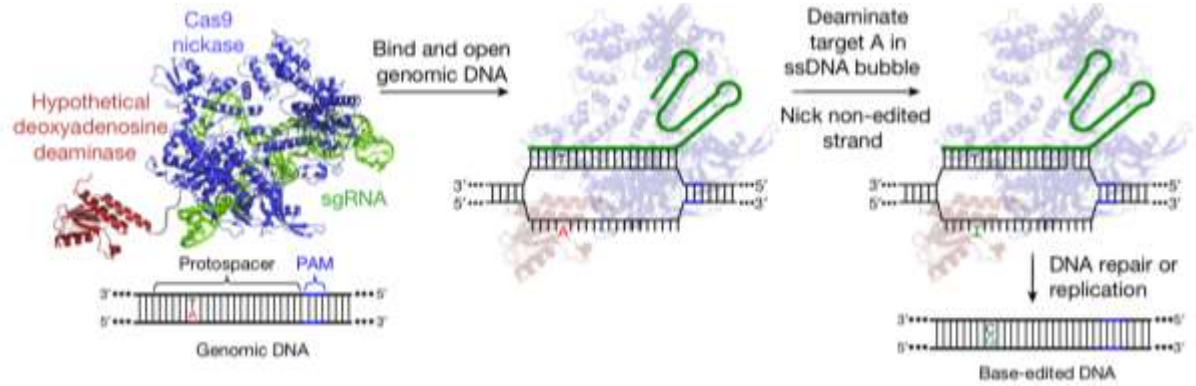
Gene mutagenesis-HDR



GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 applications

Gene mutagenesis-Base editors



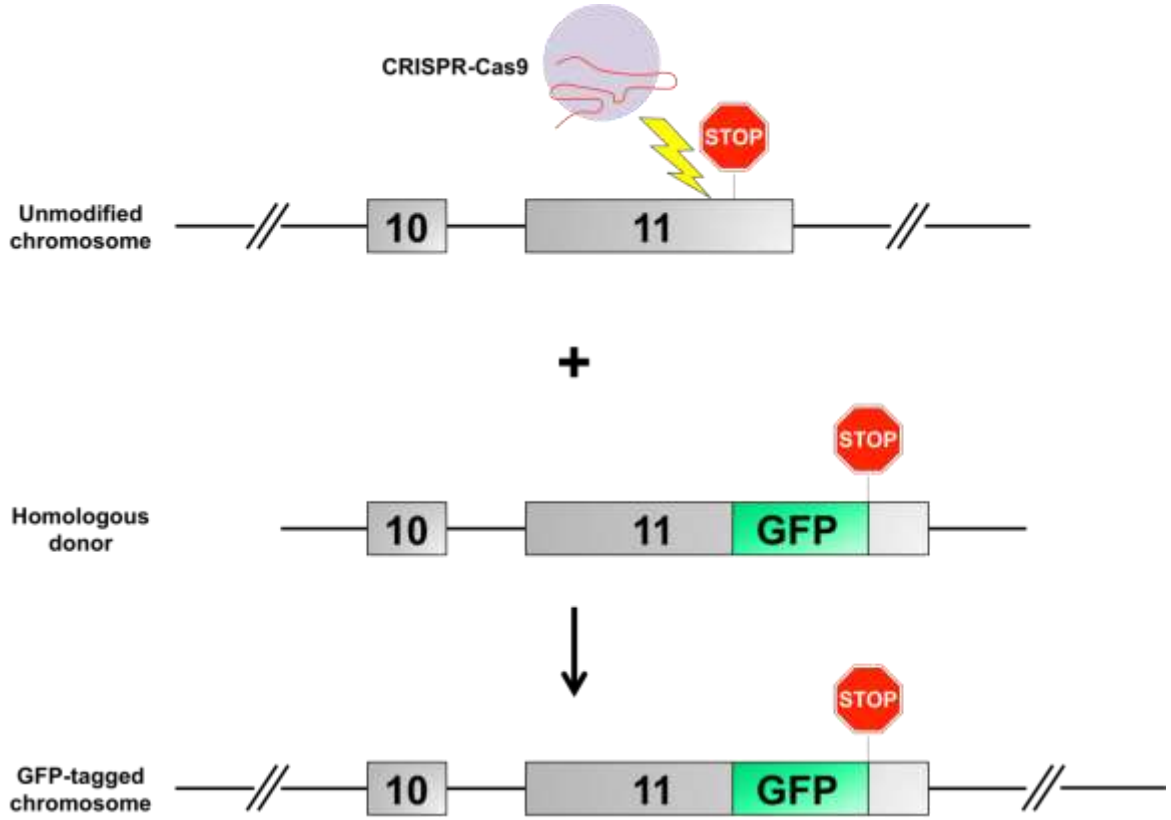
Gaudelli, et al. (2017). Nature 551, 464

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

GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 applications

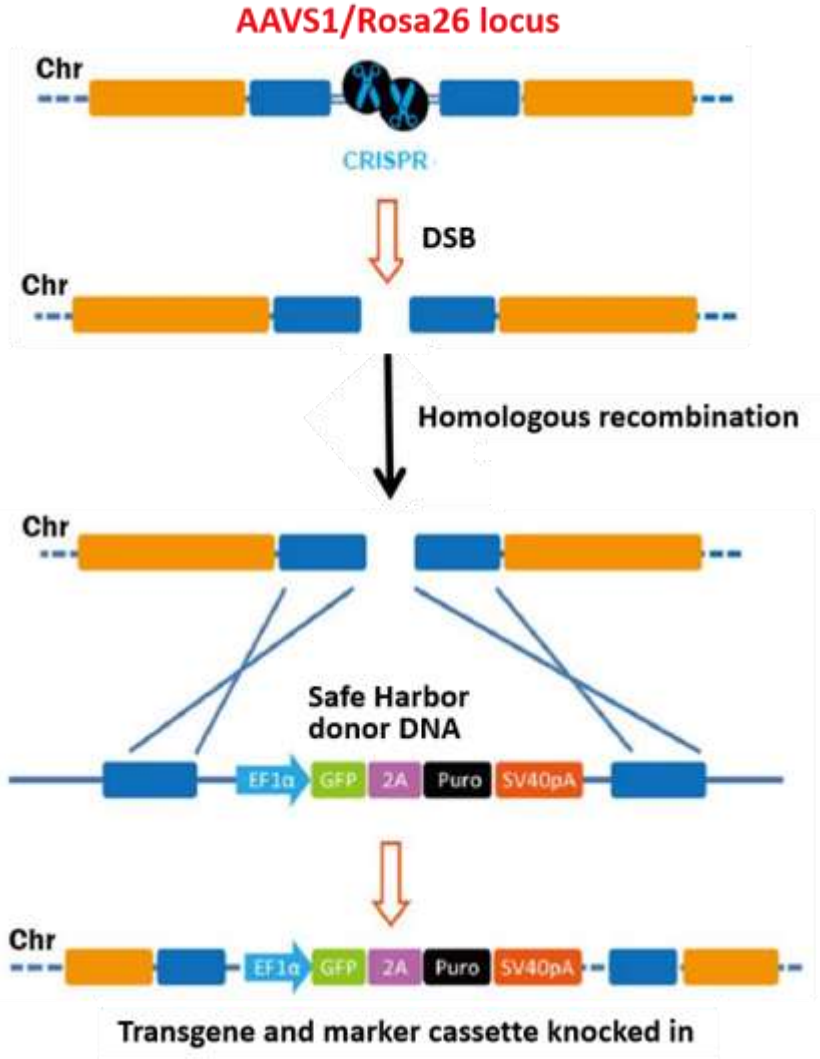
Gene tagging



GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 applications

Gene knock-in (transgenesis)



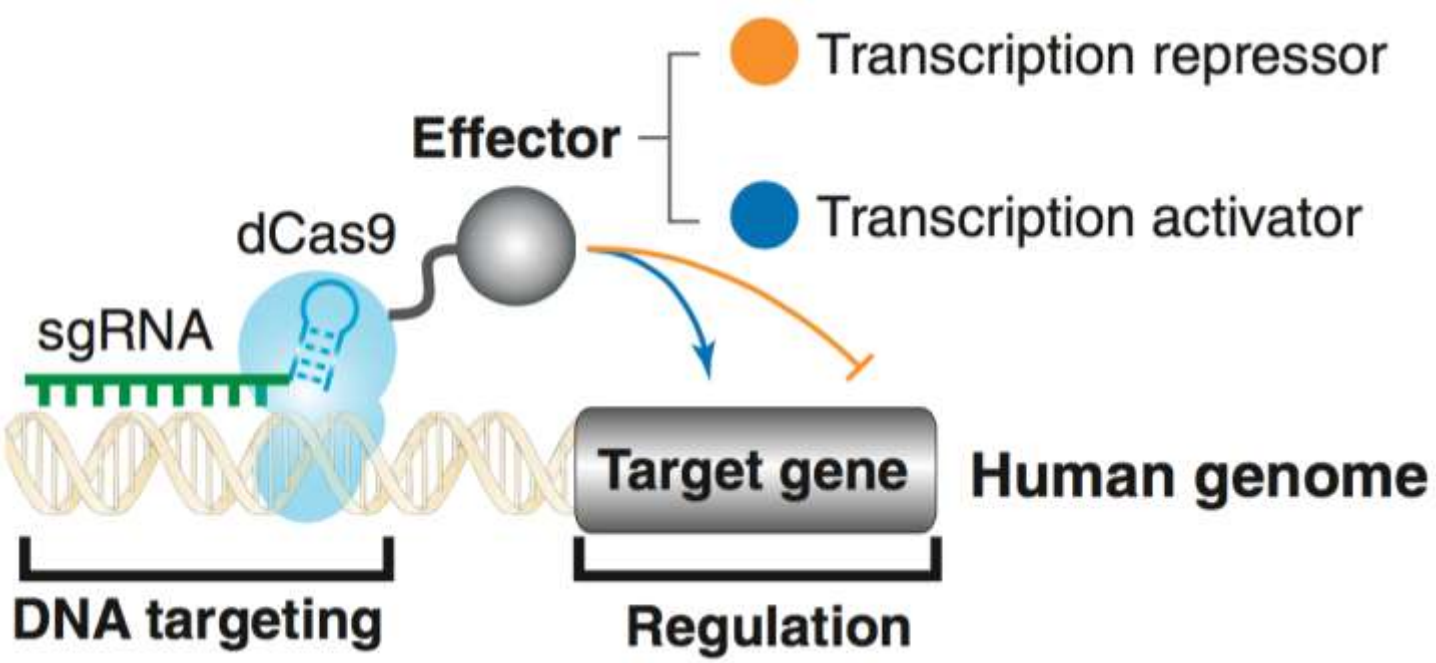
Features

- 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.

GeneCopoeia™
Expressway to Discovery

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

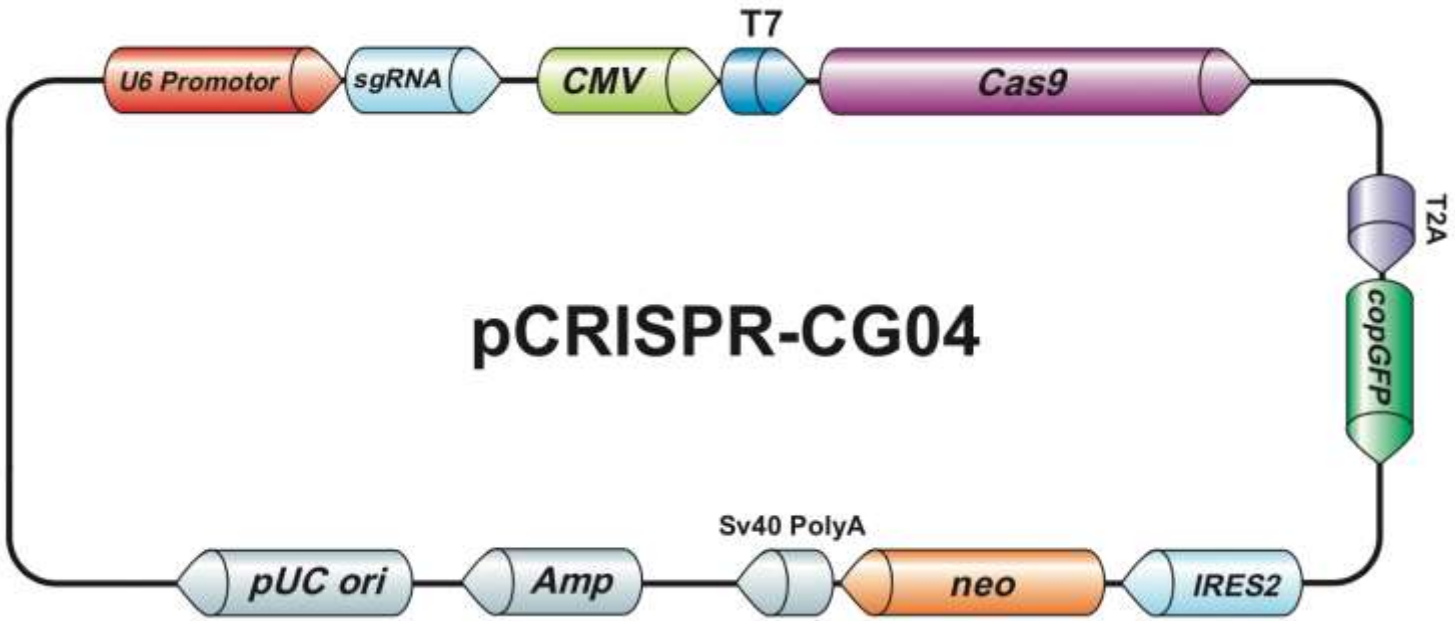
- What type of application are you doing (gene knockout, mutagenesis, etc.)? This decision can effect the efficiency and the degree of difficulty screening for the modification.
- What type of cell line are you using (primary, immortalized)?
- Can the cell line be transfected? If it does not transfect 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.

Outline

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

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

GeneCopoeia™
Expressway to Discovery

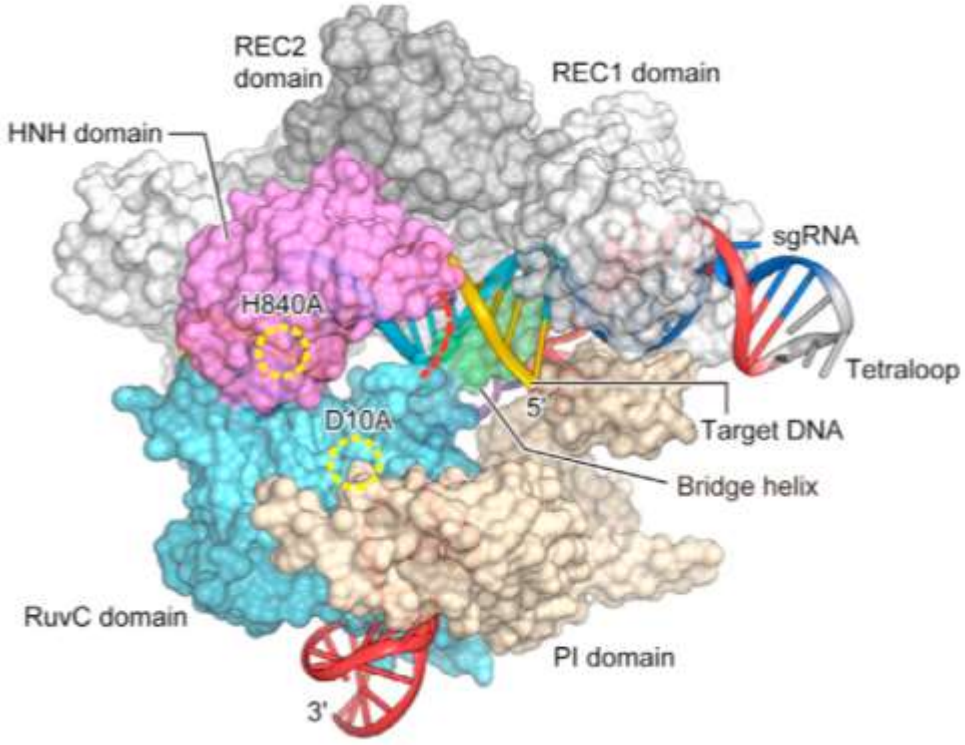
CRISPR-Cas9 genome editing technology

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)



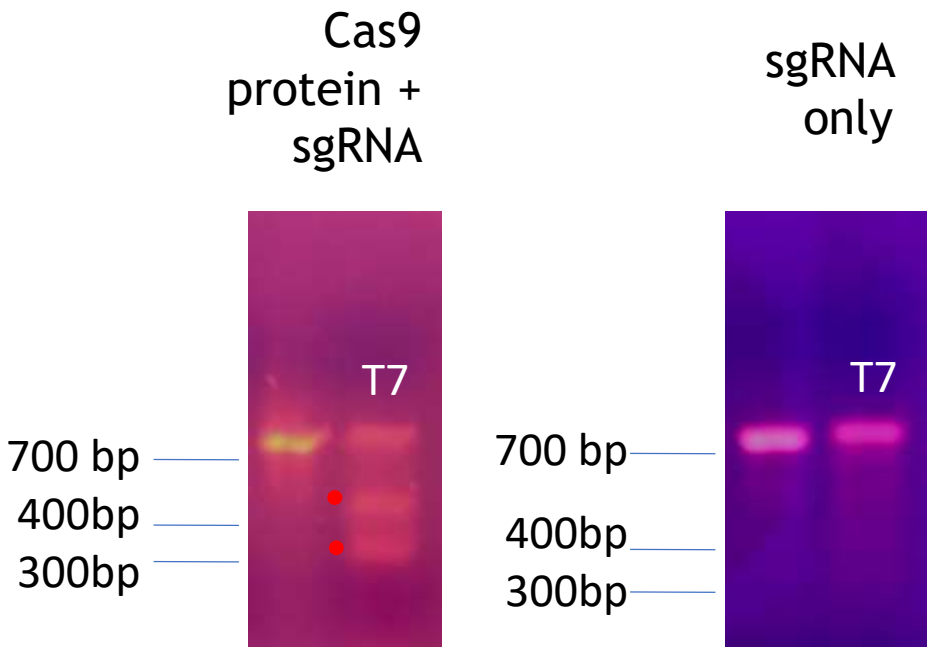
Nishimasu, et al. (2014). Cell 156, 935

- 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

GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 genome editing technology

Coming soon! GeneHero™ Cas9 nuclease

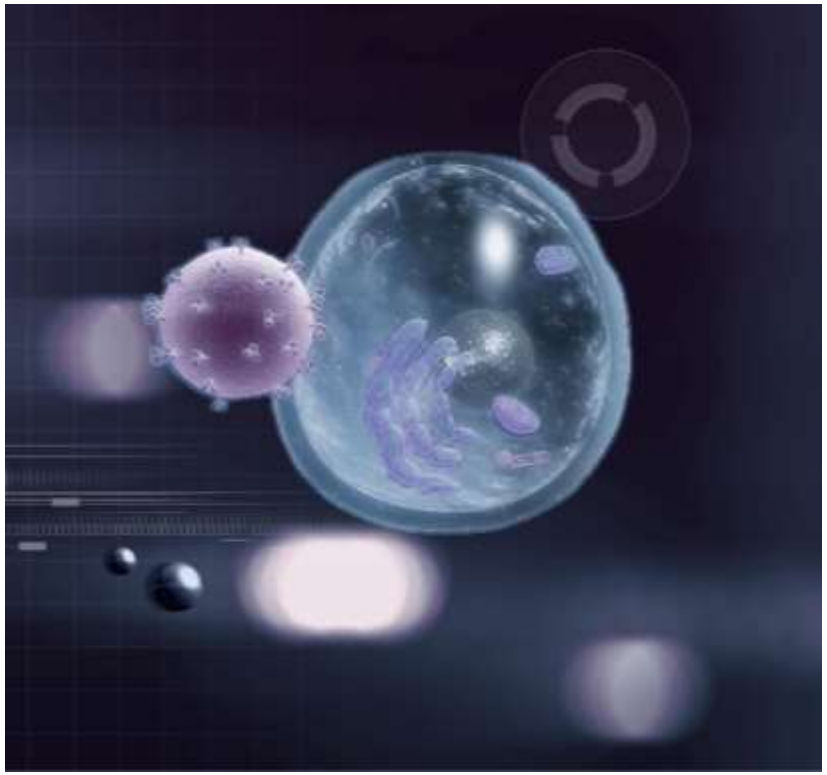


- Transfection of HEK293 cells using EndoFectin™ Max

GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 genome editing technology

Delivery method 4: Lentivirus

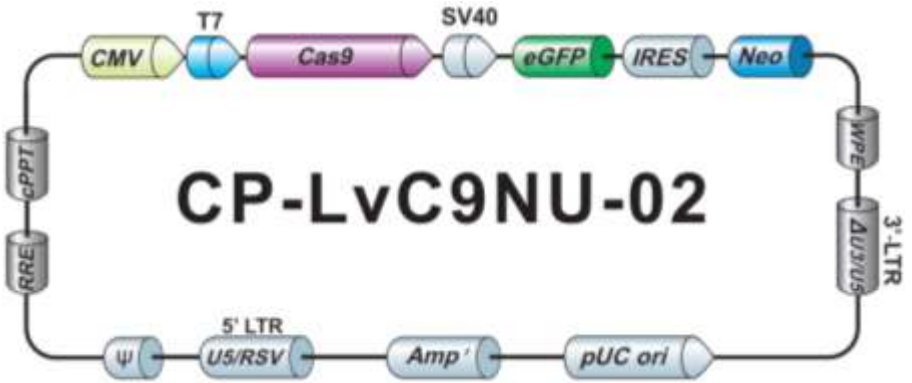


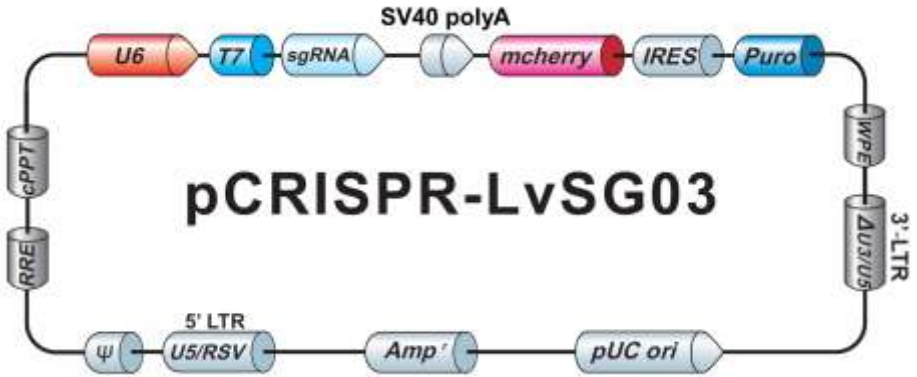
Why lentivirus?

- 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

GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 genome editing technology

GeneHero™ Cas9 stable cell lines

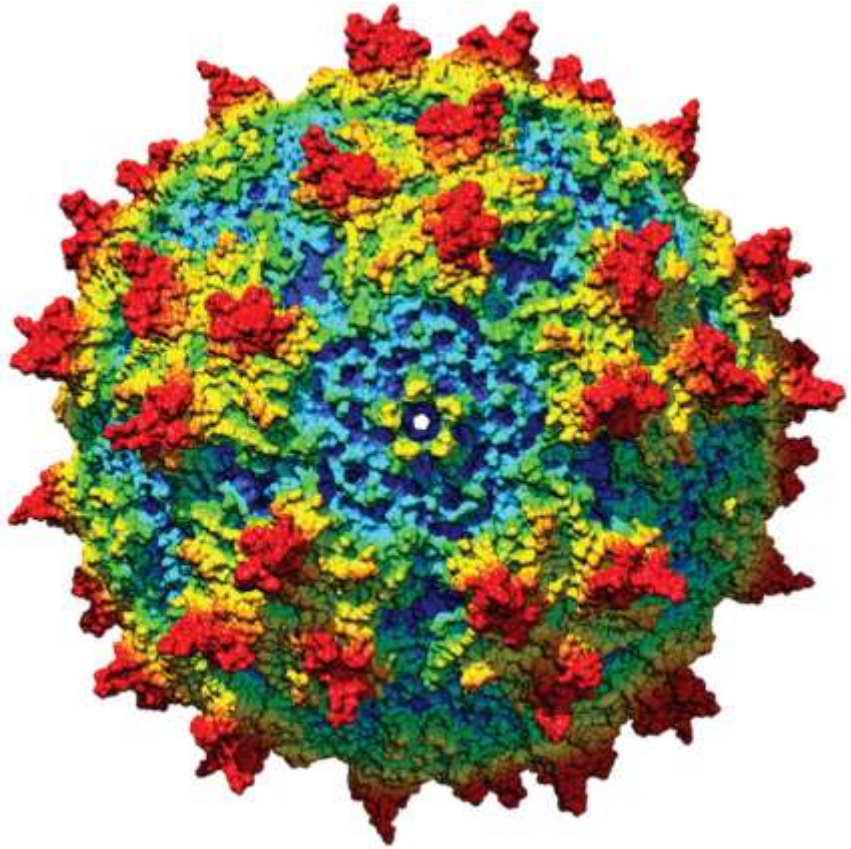
Features

- 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

GeneCopoeia™
Expressway to Discovery

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

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

GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 genome editing technology

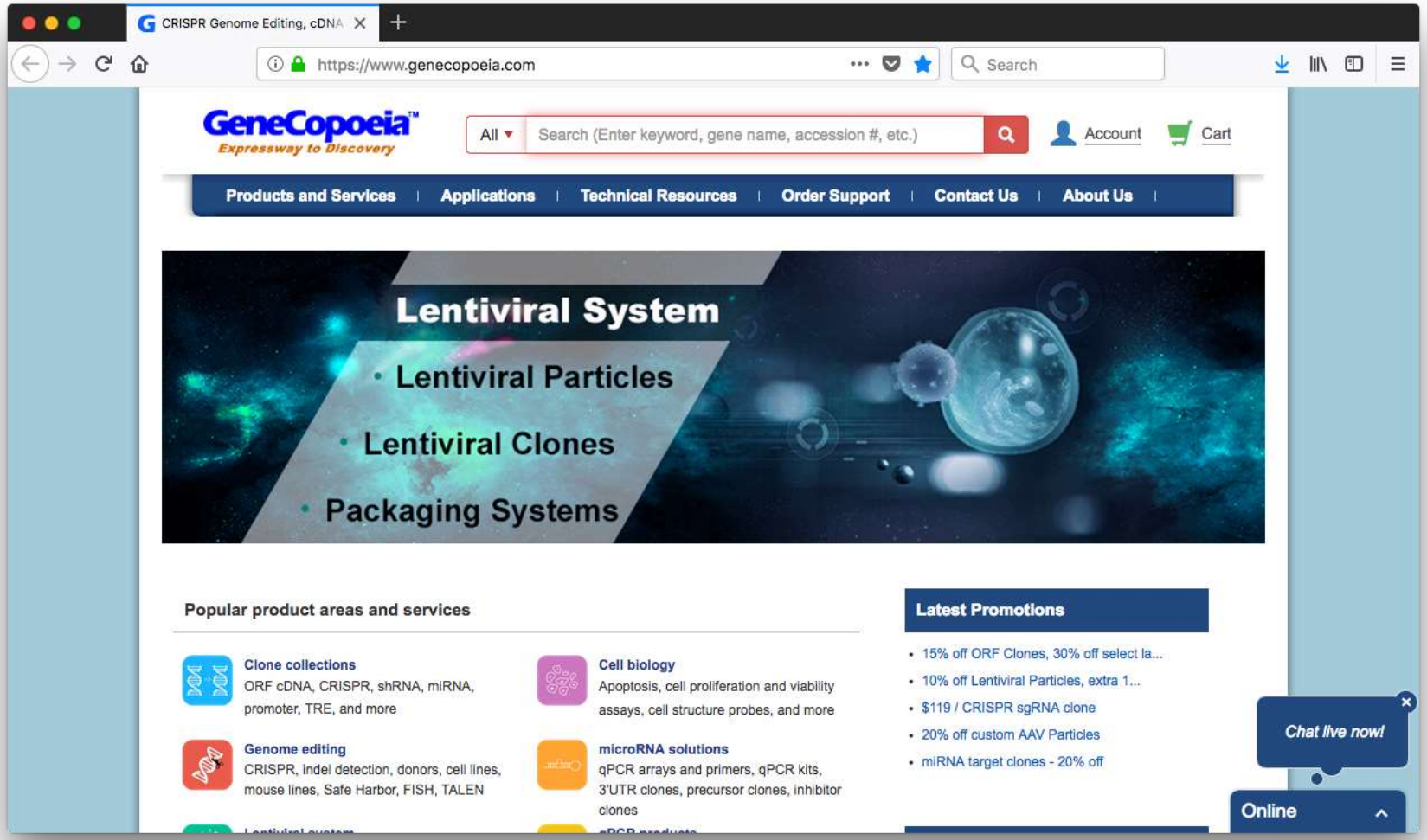
Workflow: Preparation

- 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

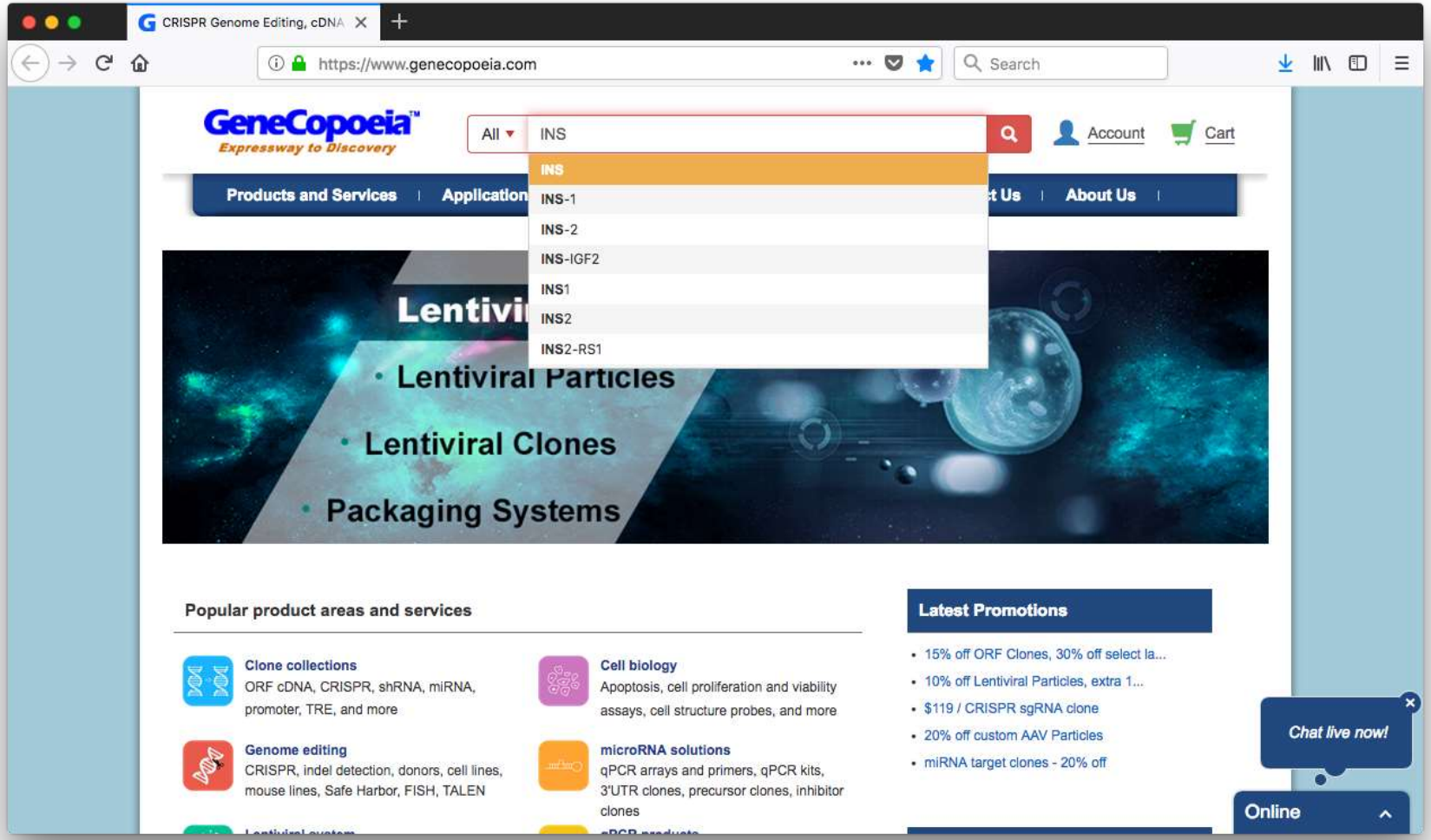
Workflow: Preparation



GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 genome editing technology

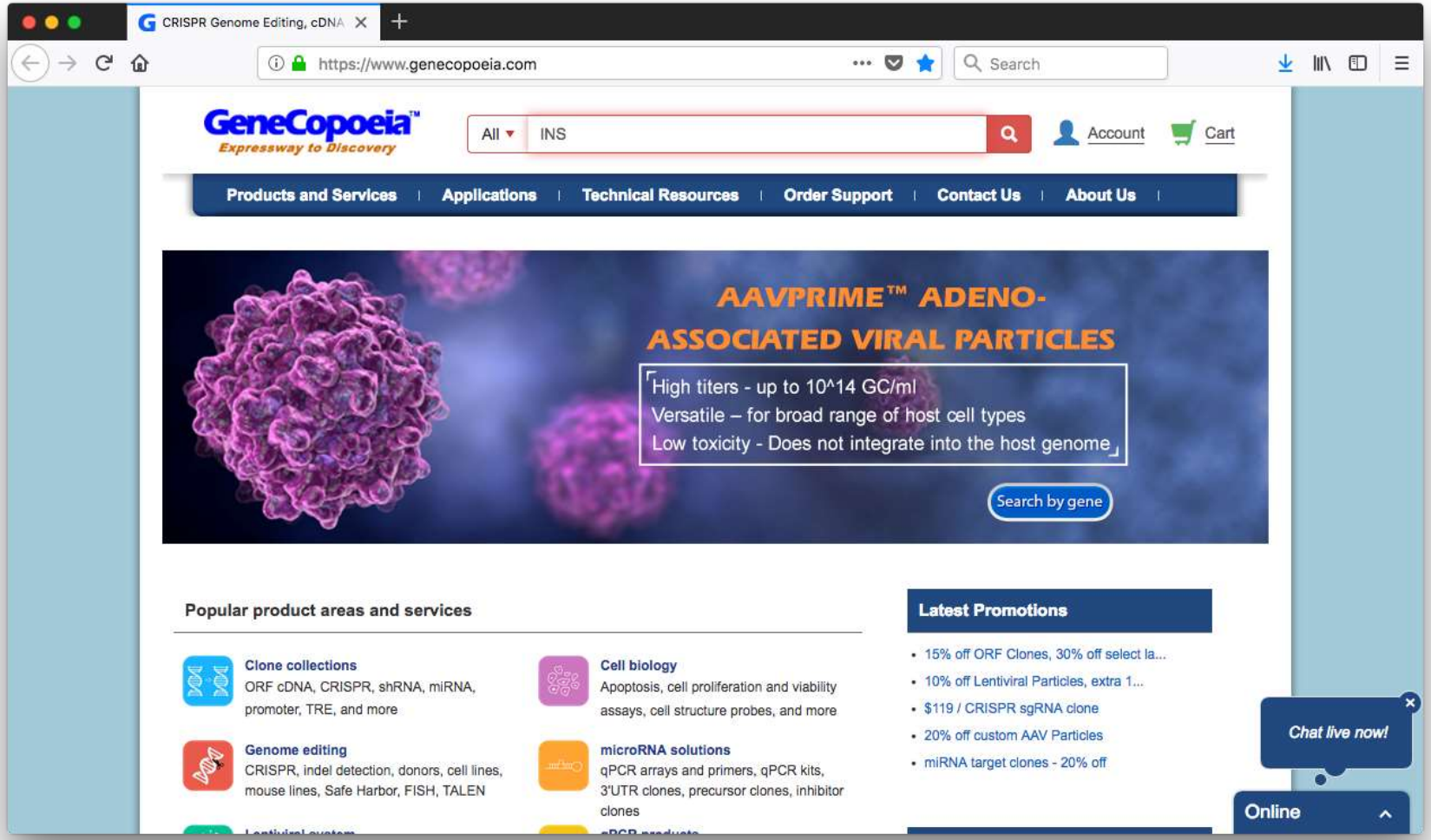
Workflow: Preparation



GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 genome editing technology

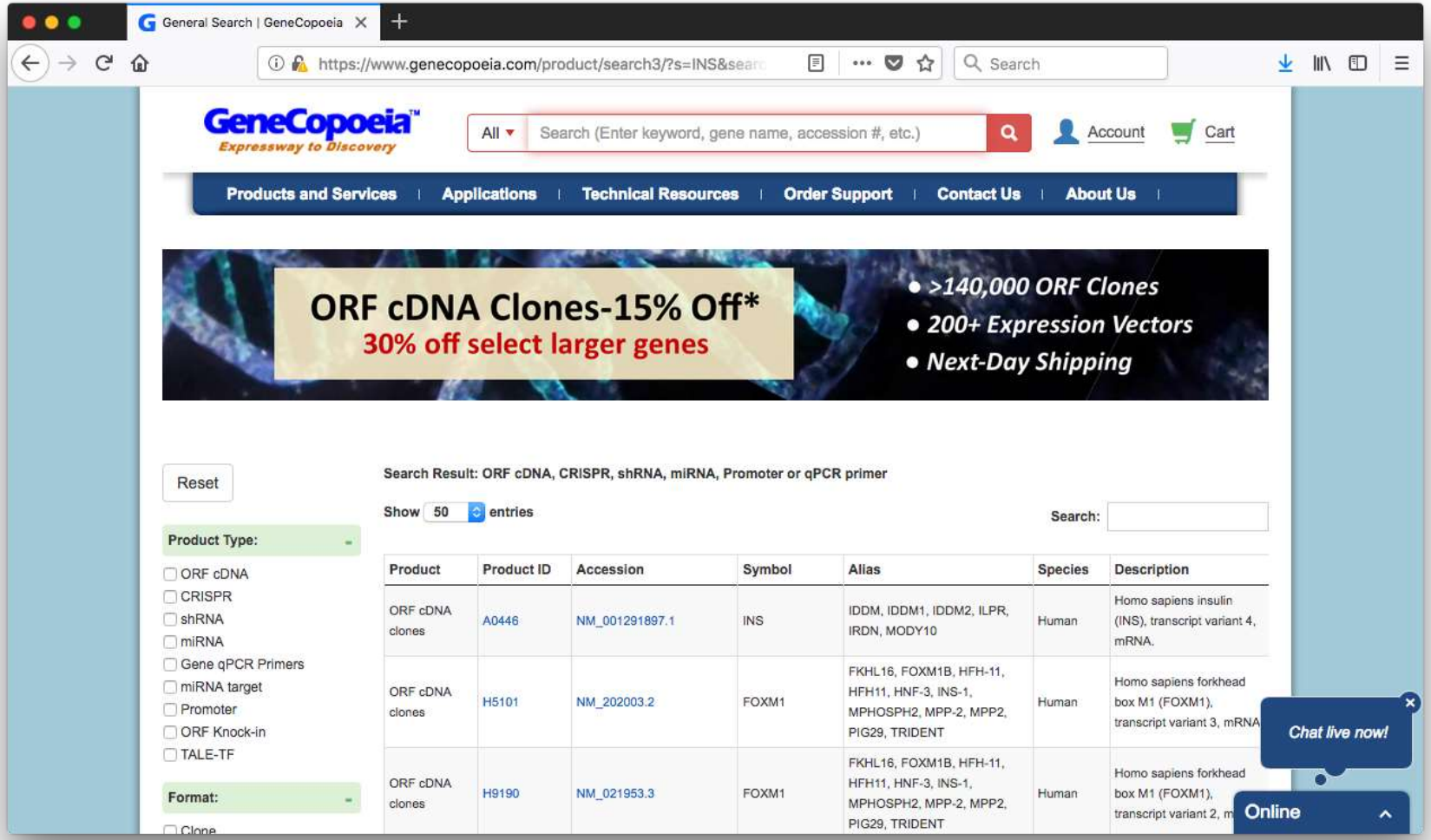
Workflow: Preparation



GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 genome editing technology

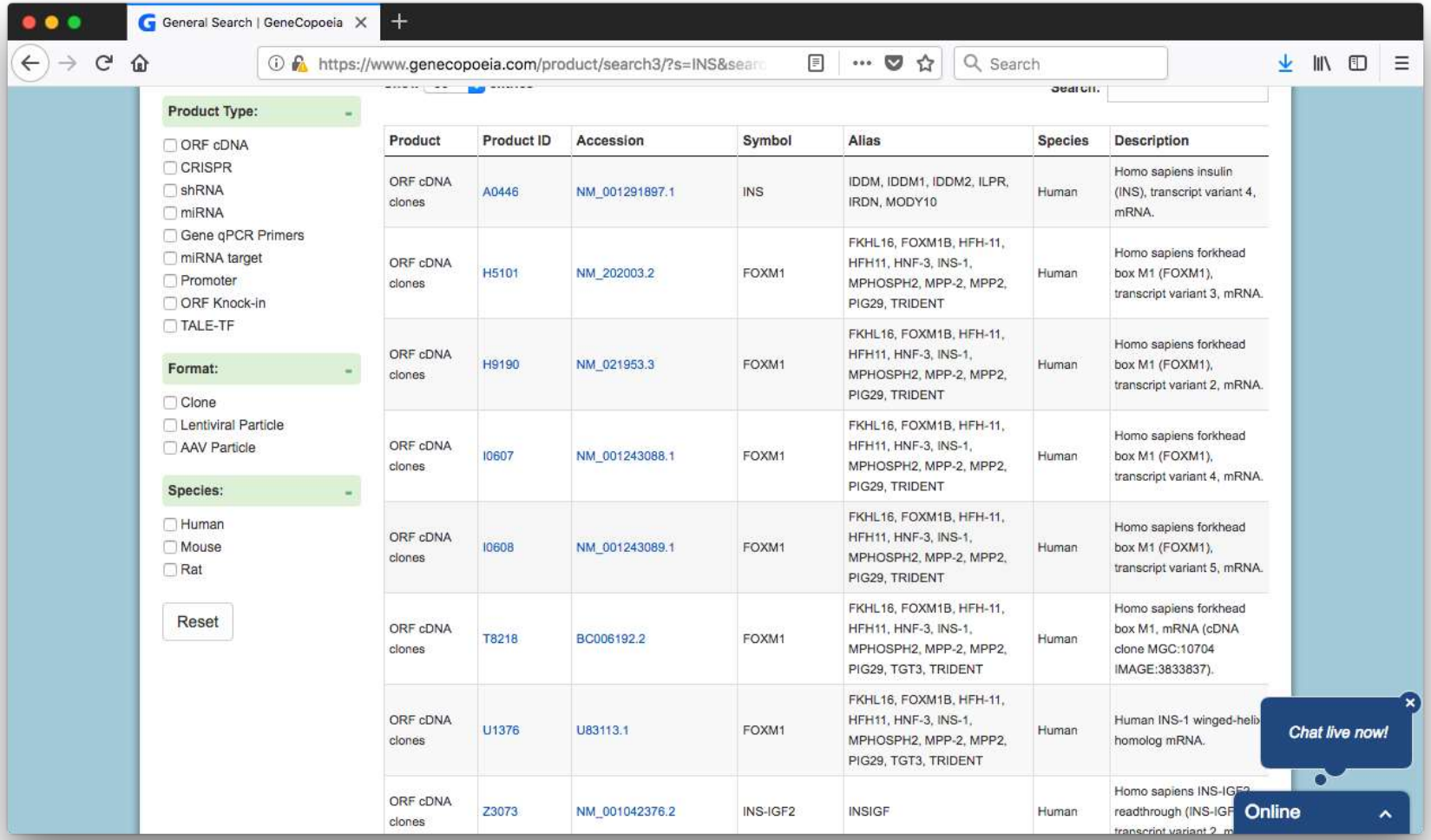
Workflow: Preparation



GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 genome editing technology

Workflow: Preparation

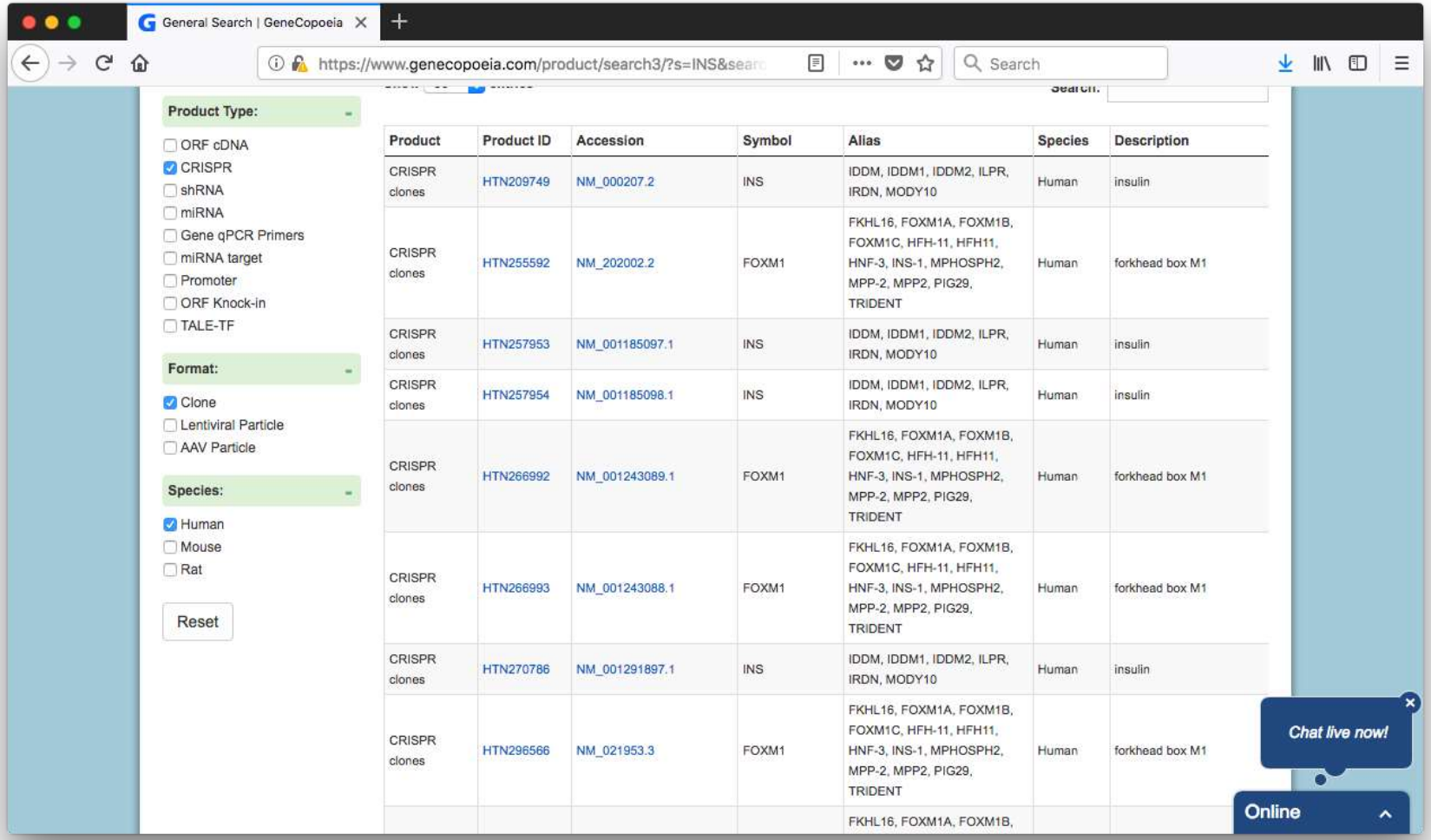


Product	Product ID	Accession	Symbol	Alias	Species	Description
ORF cDNA clones	A0446	NM_001291897.1	INS	IDDM, IDDM1, IDDM2, ILPR, IRDN, MODY10	Human	Homo sapiens insulin (INS), transcript variant 4, mRNA.
ORF cDNA clones	H5101	NM_202003.2	FOXM1	FKHL16, FOXM1B, HFH-11, HFH11, HNF-3, INS-1, MPHOSPH2, MPP-2, 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-heli homolog mRNA.
ORF cDNA clones	Z3073	NM_001042376.2	INS-IGF2	INSIGF	Human	Homo sapiens INS-IGF2 readthrough (INS-IGF

GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 genome editing technology

Workflow: Preparation

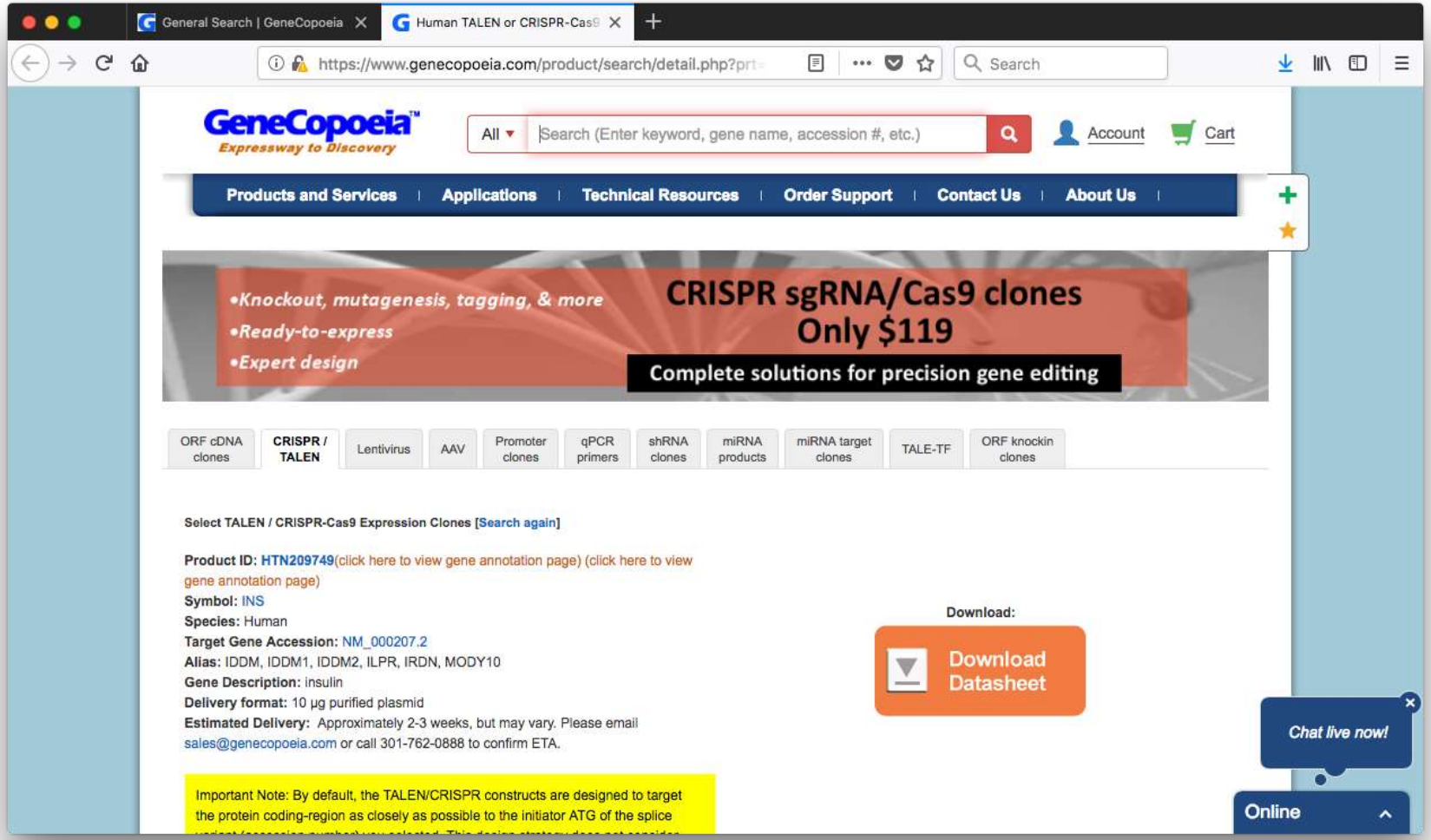


Product	Product ID	Accession	Symbol	Alias	Species	Description
CRISPR clones	HTN209749	NM_000207.2	INS	IDDM, IDDM1, IDDM2, ILPR, IRDN, MODY10	Human	insulin
CRISPR clones	HTN255592	NM_202002.2	FOXM1	FKHL16, FOXM1A, FOXM1B, FOXM1C, HFH-11, HFH11, HNF-3, INS-1, MPHOSPH2, MPP-2, MPP2, PIG29, TRIDENT	Human	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,		

GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 genome editing technology

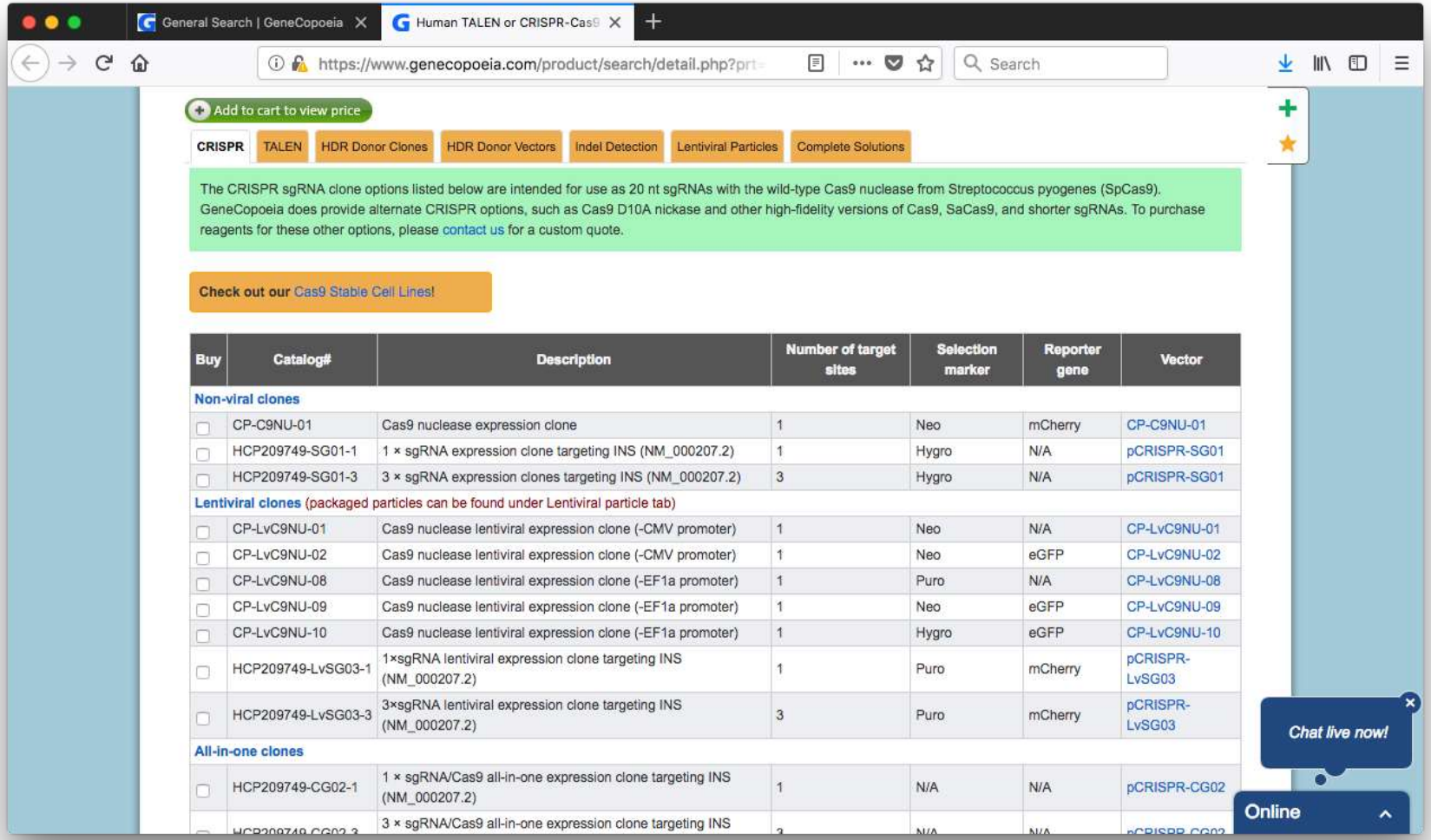
Workflow: Preparation



GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 genome editing technology

Workflow: Preparation



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

GeneCopoeia™
Expressway to Discovery

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

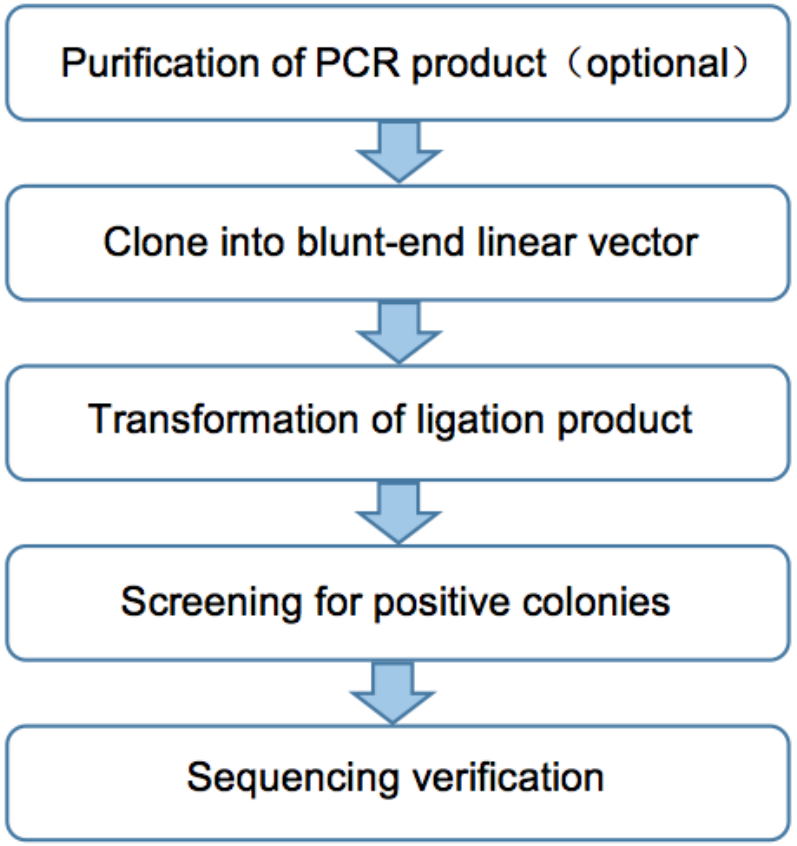
- Convenient method for high efficiency cloning of blunt-end PCR products
- Higher positive ligation rate and true positive colonies by the elimination of self-ligating products
- Cloning of blunt-end PCR products without additional sequences or restriction sites
- Ideal for sequencing regions of genomic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

GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 genome editing technology

Smart-Join™ Blunt-end PCR Cloning Kit

Workflow



GeneCopoeia™
Expressway to Discovery

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

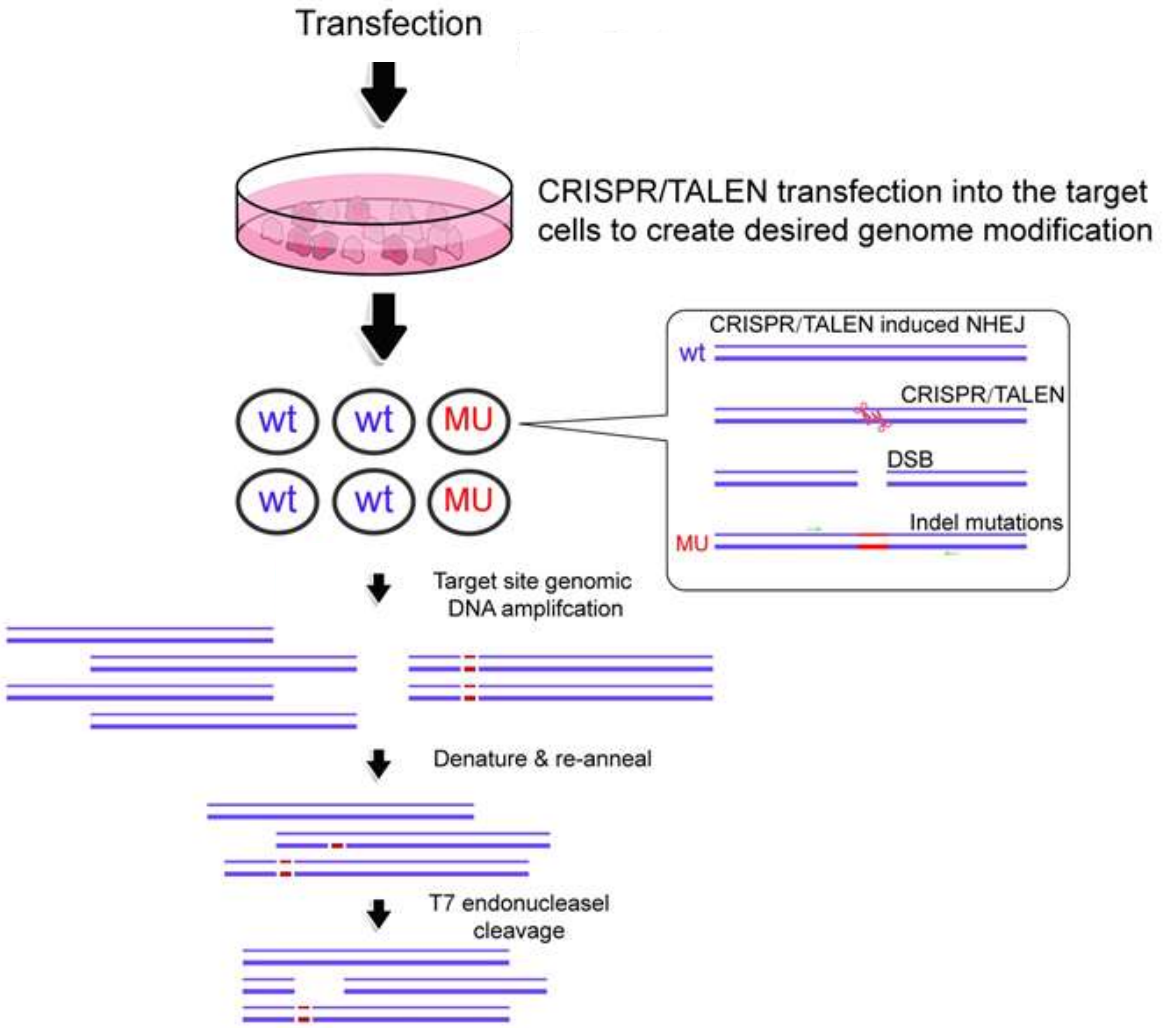
Features

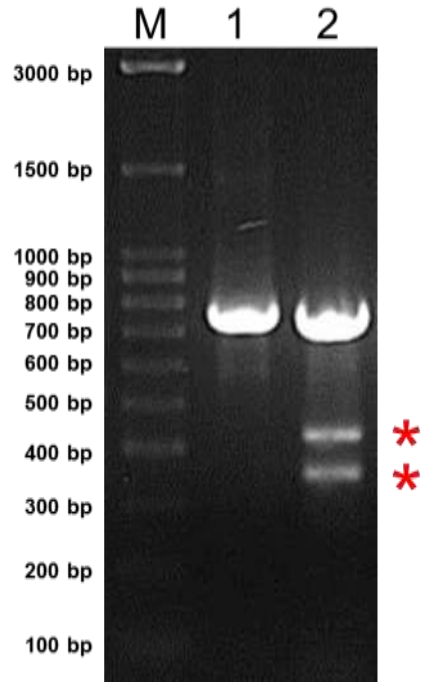
- Useful for 1) CRISPR sgRNA functional validation; 2) Screening for positive clones
- 3-component system: 1) Target site PCR kit; 2) T7 Endonuclease I kit; 3) Blunt-end PCR cloning kit
- No genomic DNA isolation is required

GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 genome editing technology

IndelCheck™ CRISPR indel detection system





GeneCopoeia™
Expressway to Discovery

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

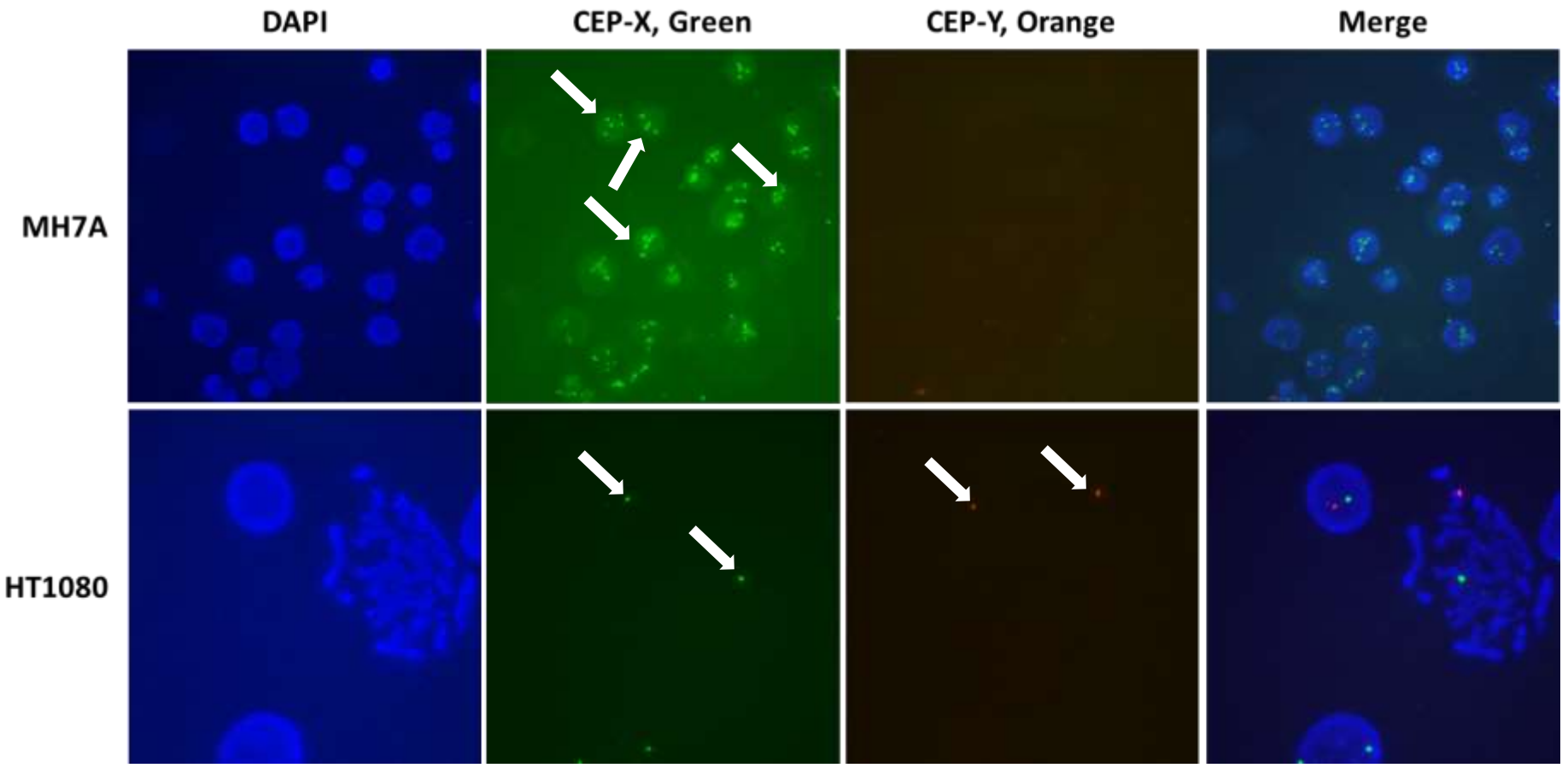
Application: Using FISH with a CRISPR knockout

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

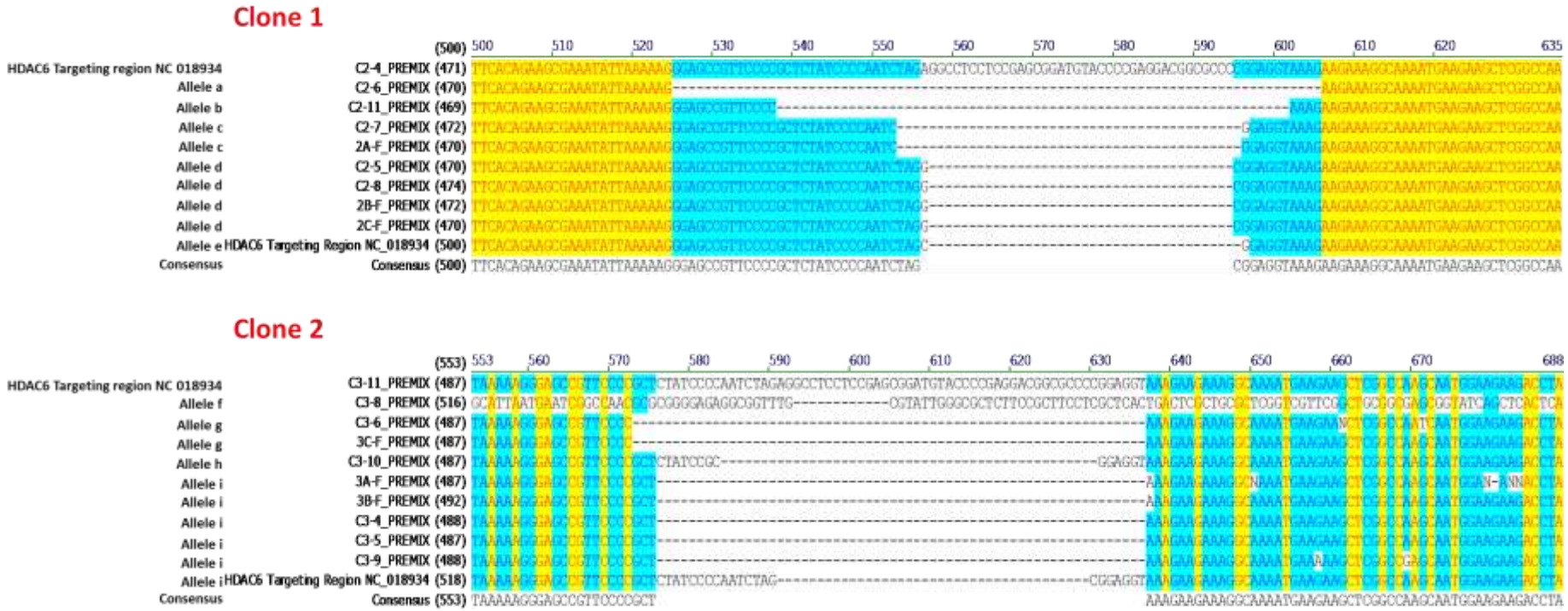
Application: Using FISH with a CRISPR knockout



GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 genome editing technology

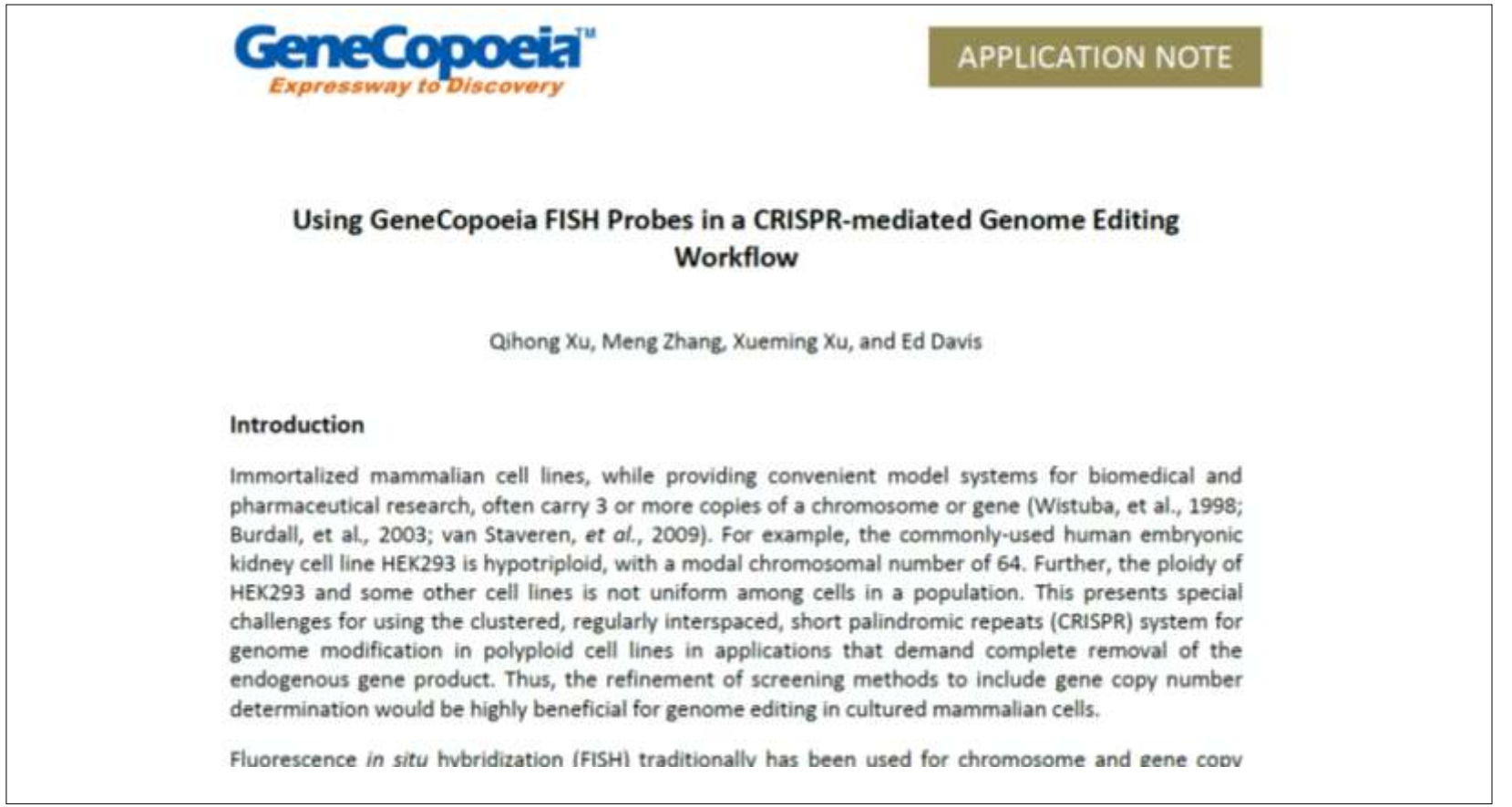
Application: Using FISH with a CRISPR knockout



GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 genome editing technology

GeneCopoeia Application Note: FISH with CRISPR



GeneCopoeia™
Expressway to Discovery

APPLICATION NOTE

Using GeneCopoeia FISH Probes in a CRISPR-mediated Genome Editing Workflow

Qihong Xu, Meng Zhang, Xueming Xu, and Ed Davis

Introduction

Immortalized mammalian cell lines, while providing convenient model systems for biomedical and pharmaceutical research, often carry 3 or more copies of a chromosome or gene (Wistuba, et al., 1998; Burdall, et al., 2003; van Staveren, *et al.*, 2009). For example, the commonly-used human embryonic kidney cell line HEK293 is hypotriploid, with a modal chromosomal number of 64. Further, the ploidy of HEK293 and some other cell lines is not uniform among cells in a population. This presents special challenges for using the clustered, regularly interspaced, short palindromic repeats (CRISPR) system for genome modification in polyploid cell lines in applications that demand complete removal of the endogenous gene product. Thus, the refinement of screening methods to include gene copy number determination would be highly beneficial for genome editing in cultured mammalian cells.

Fluorescence *in situ* hybridization (FISH) traditionally has been used for chromosome and gene copy

Download from:

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

GeneCopoeia™
Expressway to Discovery

Outline

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

GeneCopoeia™
Expressway to Discovery

Screening

Isolate single clones post-transfection/transduction

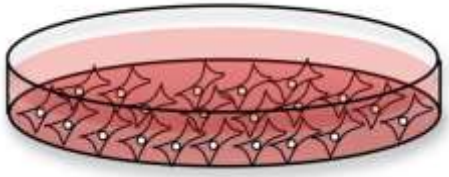


Plate for single colonies and pick off dish

OR

Fluorescence sorting

OR

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

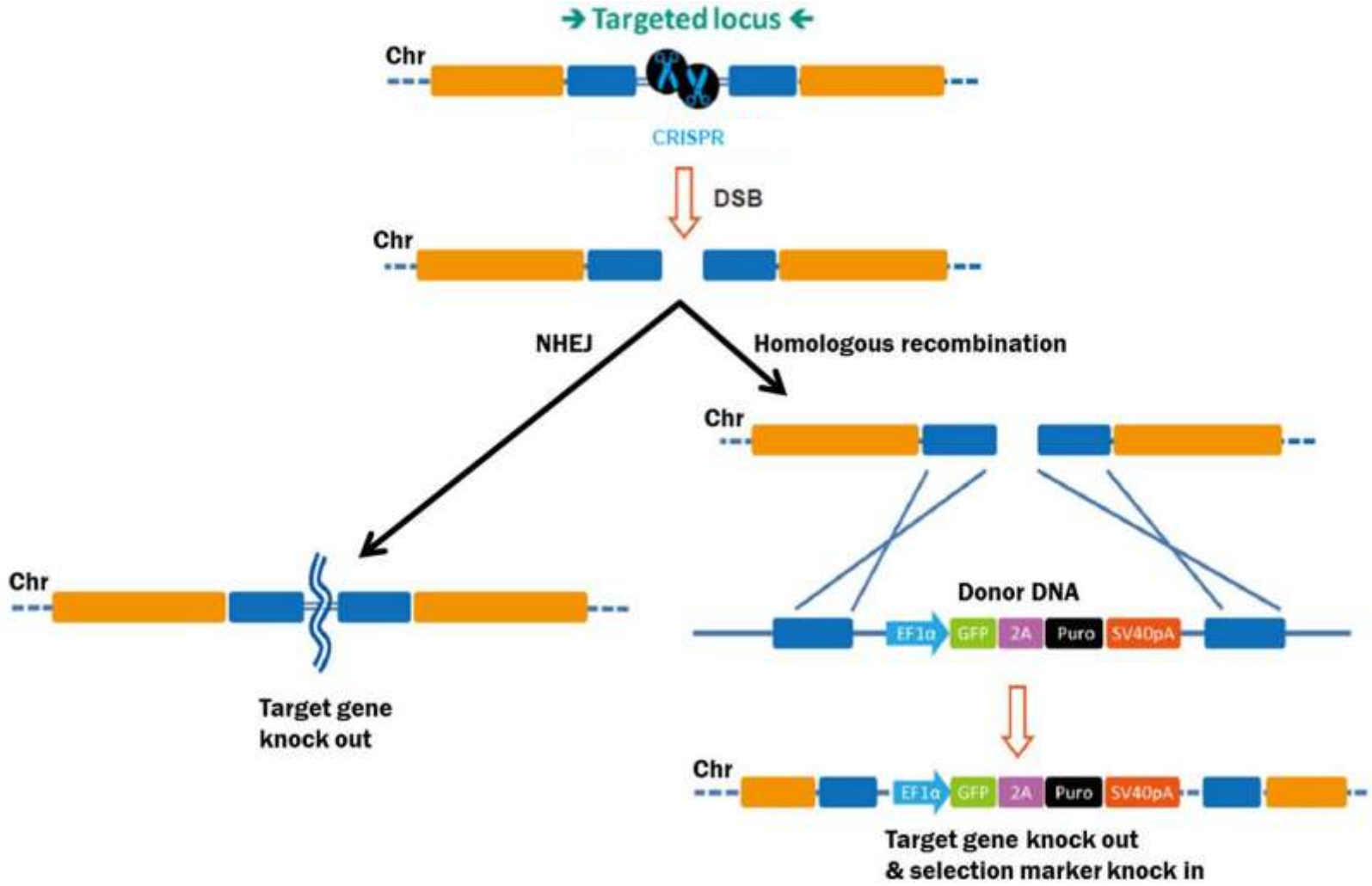
IMPORTANT:

Connect genotype to phenotype!

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

Screening

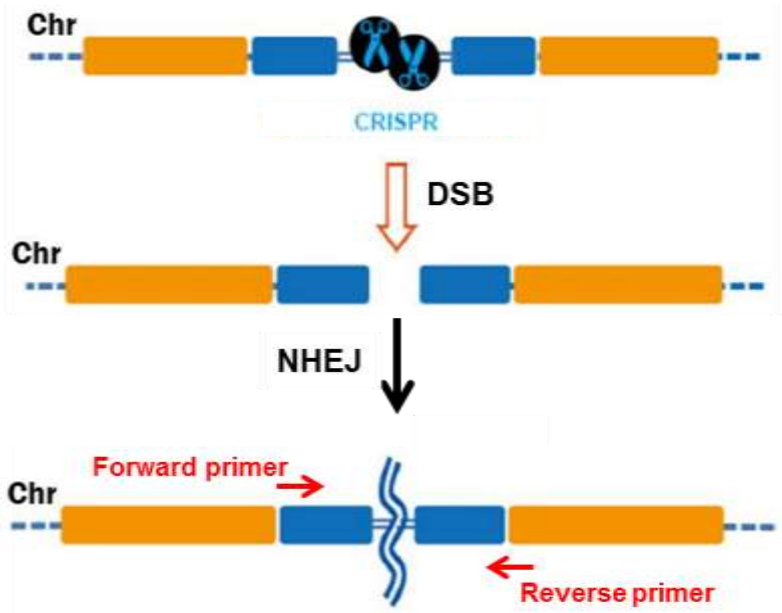
Isolate single clones post-transfection/transduction



GeneCopoeia™
Expressway to Discovery

Screening

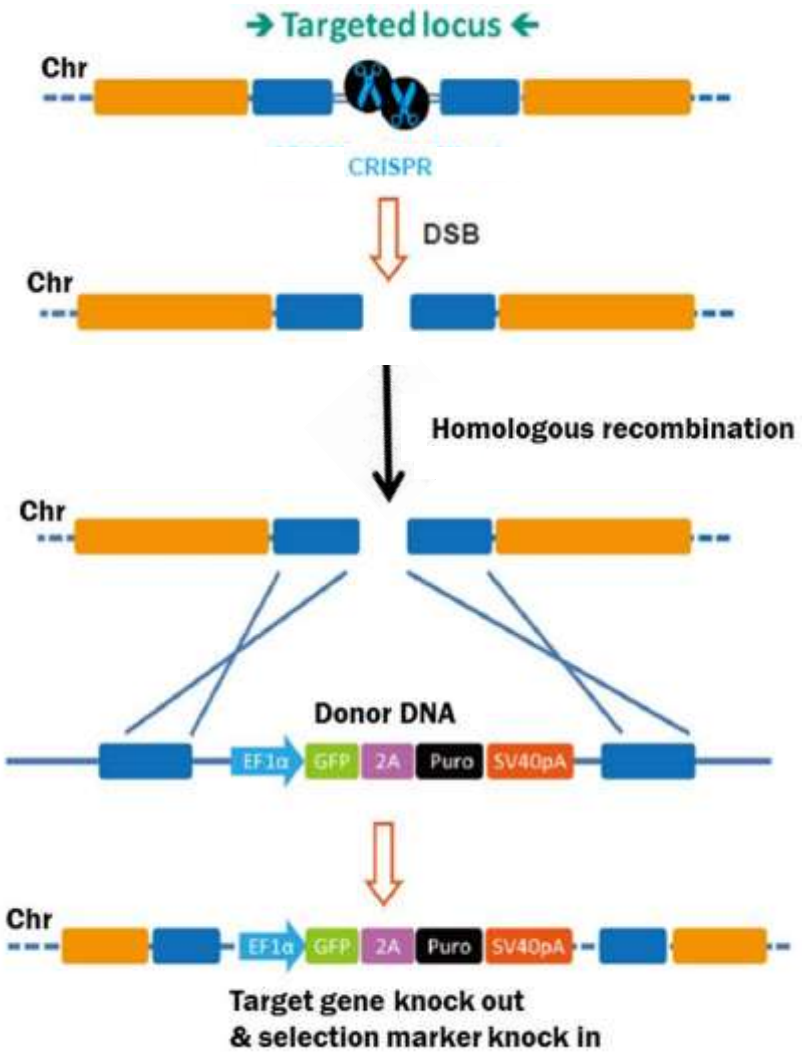
NHEJ-mediated mutations (knockouts)

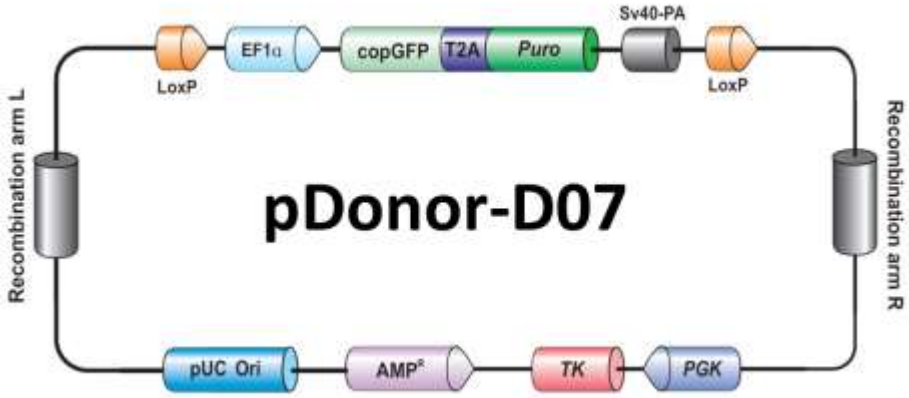


- Isolate clones without selection 2-3 days post transfection/transduction
- Generate PCR products using primers flanking the DSB site (can use same primers as used for validation)
- Screen PCR products by T7 Endonuclease I assay OR direct sequencing. Use GeneCopoeia's IndelCheck system
- Re-screen positive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

Screening

HDR-mediated applications using donor plasmids

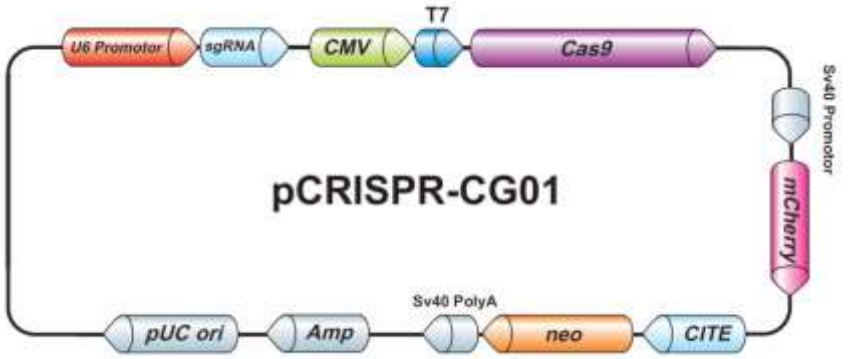


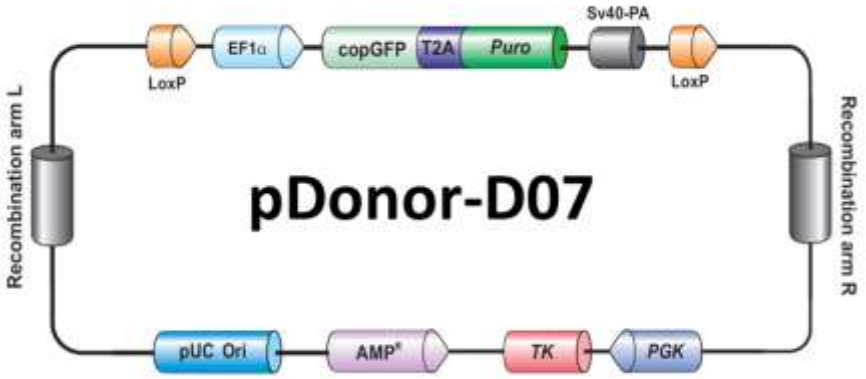


GeneCopoeia™
Expressway to Discovery

Screening

HDR-mediated applications using donor plasmids





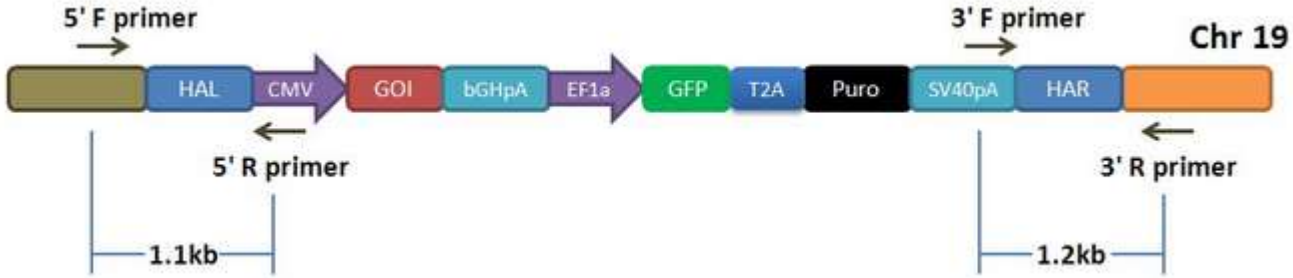
- Co-transfect Cas9, sgRNA, and donor plasmids
- 2-3 days post transfection, apply selection for donor selection cassette, or sort cells expressing fluorescent reporter
- Isolate single clones
- Screen single clones for correct integration by PCR

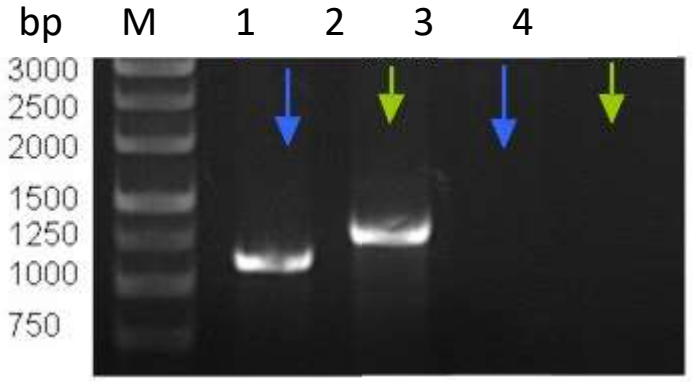
GeneCopoeia™
Expressway to Discovery

Screening

HDR-mediated applications using donor plasmids

Junction PCR





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

GeneCopoeia™
Expressway to Discovery

Outline

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

GeneCopoeia™
Expressway to Discovery

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)

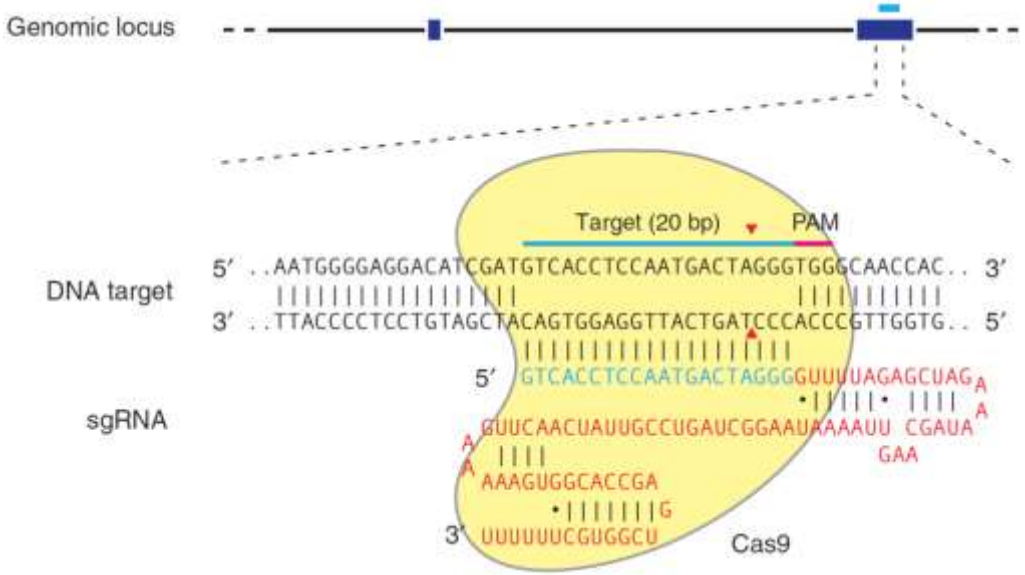
Things to look out for!

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

Or visit us on the web:

www.genecopoeia.com

GeneCopoeia, Inc.

9260 Medical Center Drive Suite 101

Rockville, Maryland USA 20850

GeneCopoeia™ Expressway to Discovery