Harnessing CRISPR For Activation of Gene Expression

November 14, 2018

Presenter:

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

GeneCopoeia Products and Services

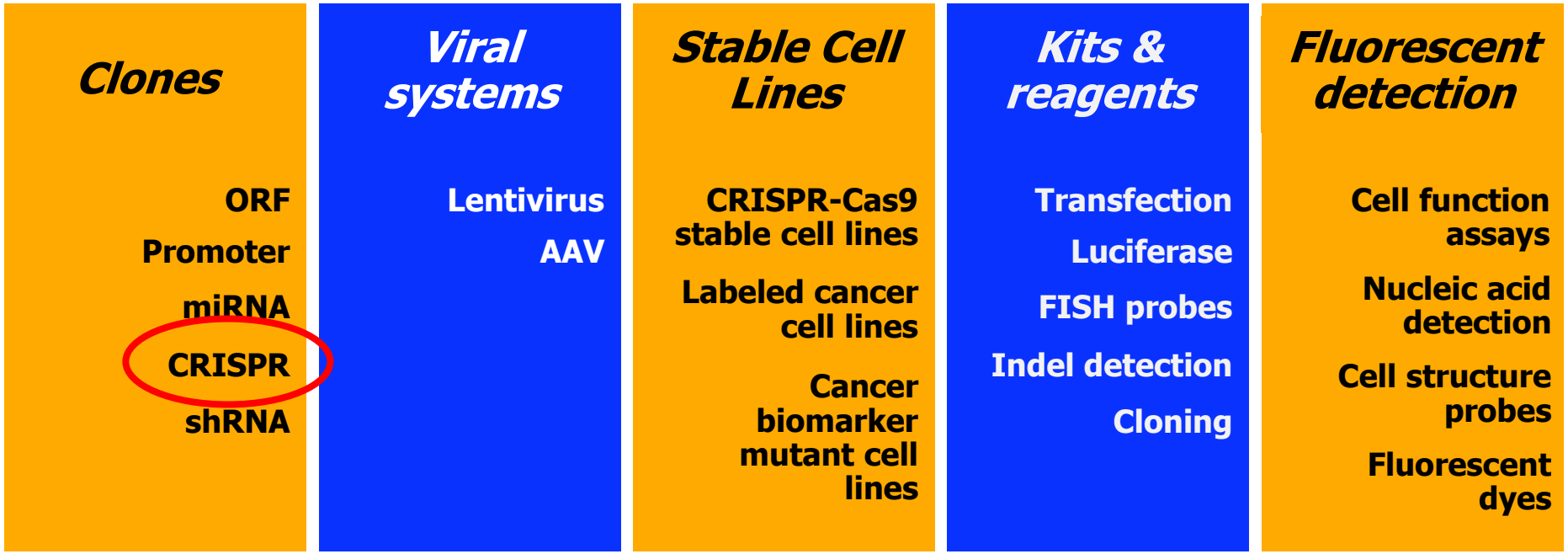
Functional Genomics & Cell Biology

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



GeneCopoeia™
Expressway to Discovery

Outline

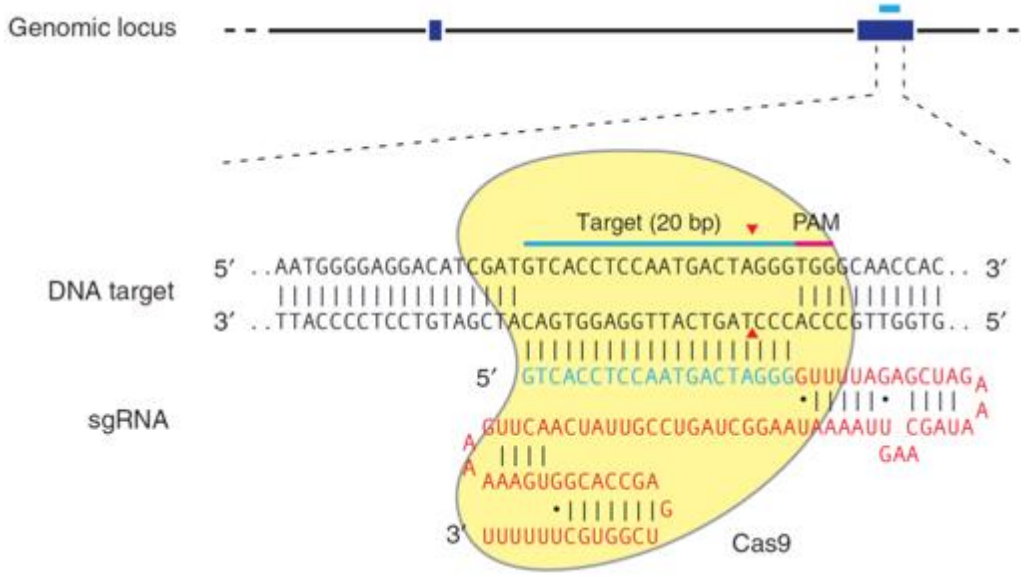
- CRISPR technologies & applications
- Introduction to CRISPR activation
- Delivery methods
- Preparation
- Screening

Outline

- CRISPR technologies & applications
- Introduction to CRISPR activation
- Delivery methods
- Preparation
- Screening

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

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

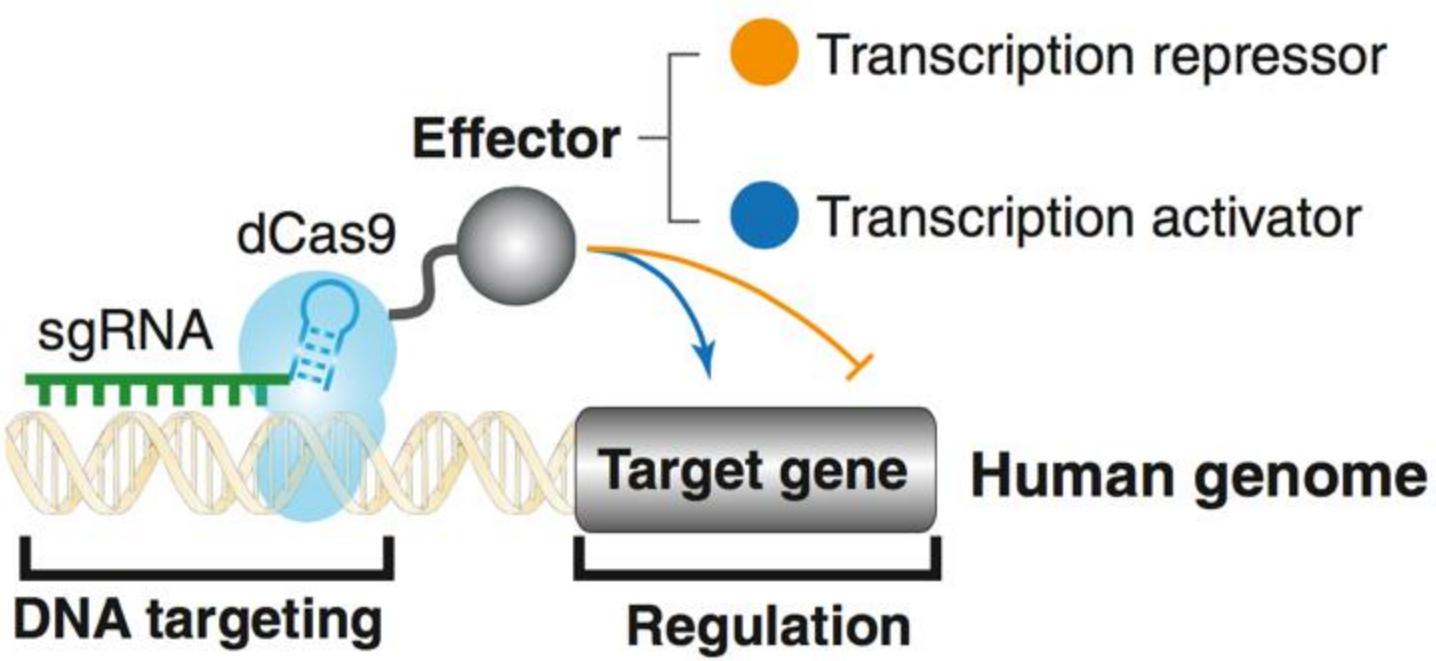
Outline

- CRISPR technologies & applications
- Introduction to CRISPR activation
- Delivery methods
- Preparation
- Screening

GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 applications

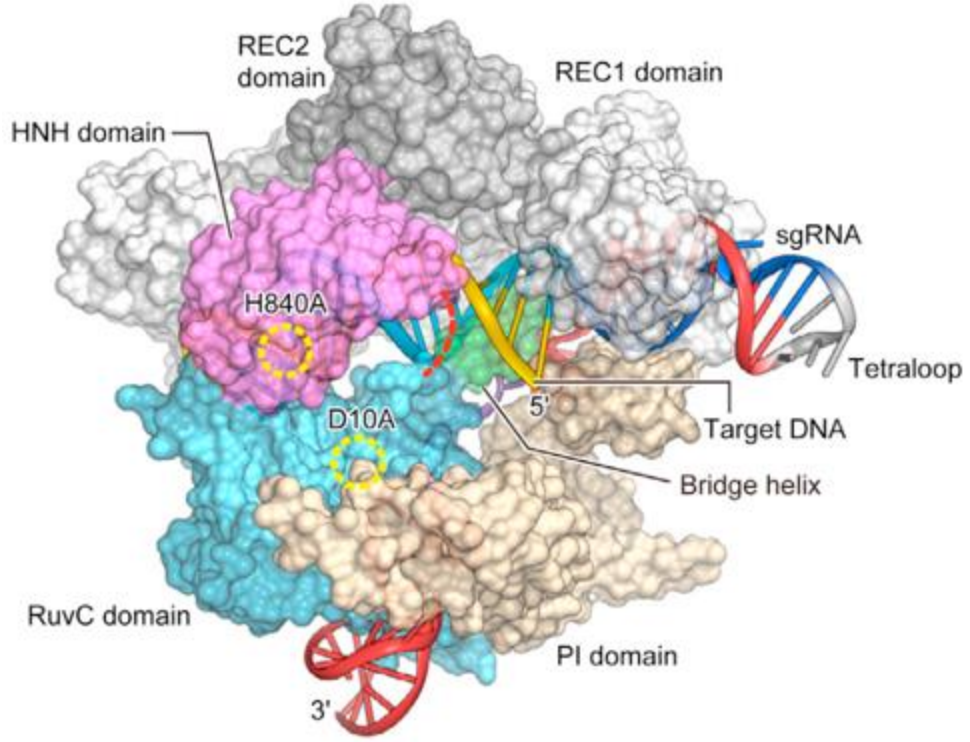
Gene activation or repression



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

CRISPR-Cas9 applications

Nuclease-dead Cas9



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

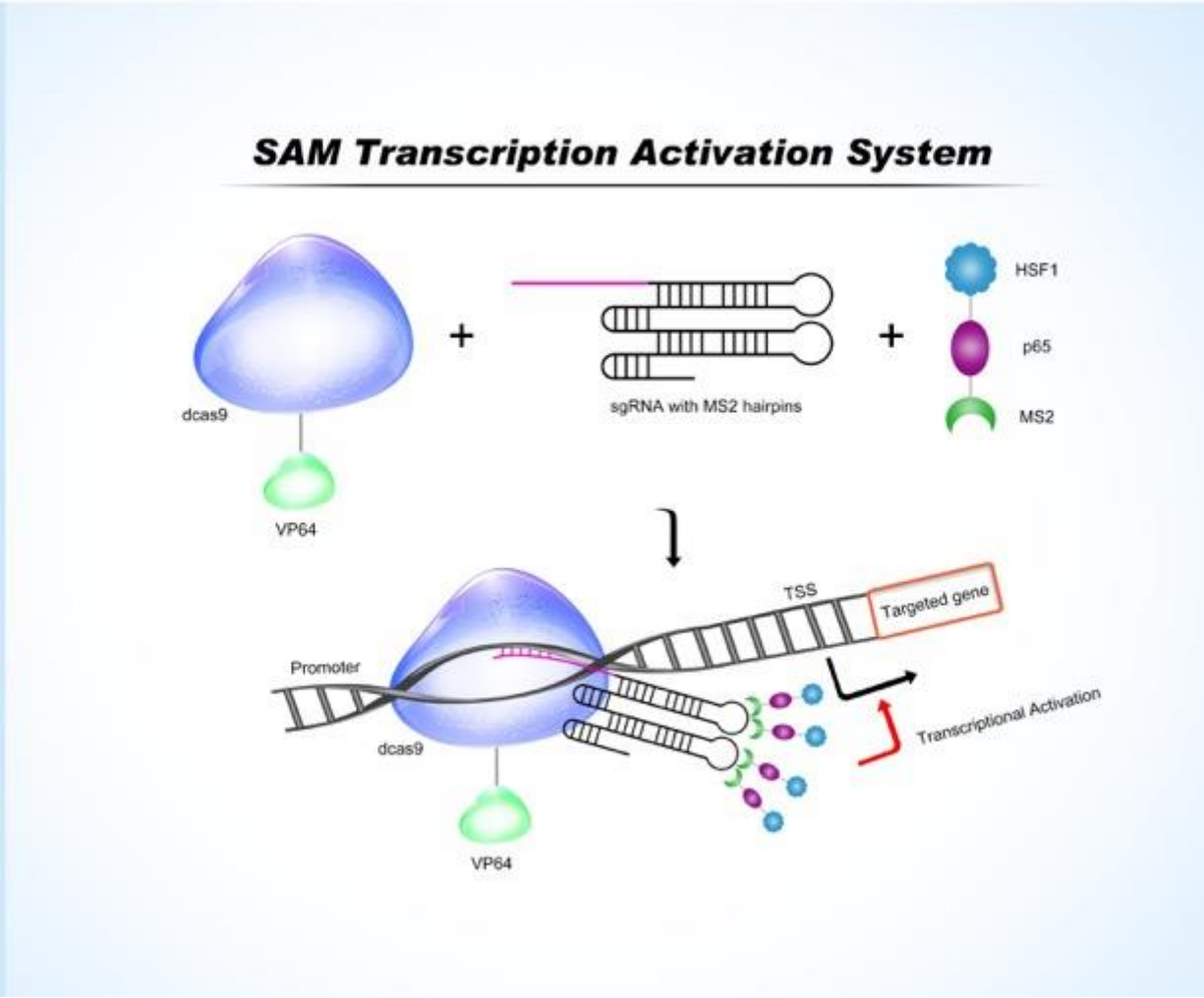
Cas9-mediated gene activation

Why use CRISPR for this?

- Can overexpress genes using open reading frame (ORF) clones, but…
- ORF clones use artificial promoters
- Not all protein isoforms are always covered
- Large ORFs sometimes too difficult to clone

dCas9-SAM system

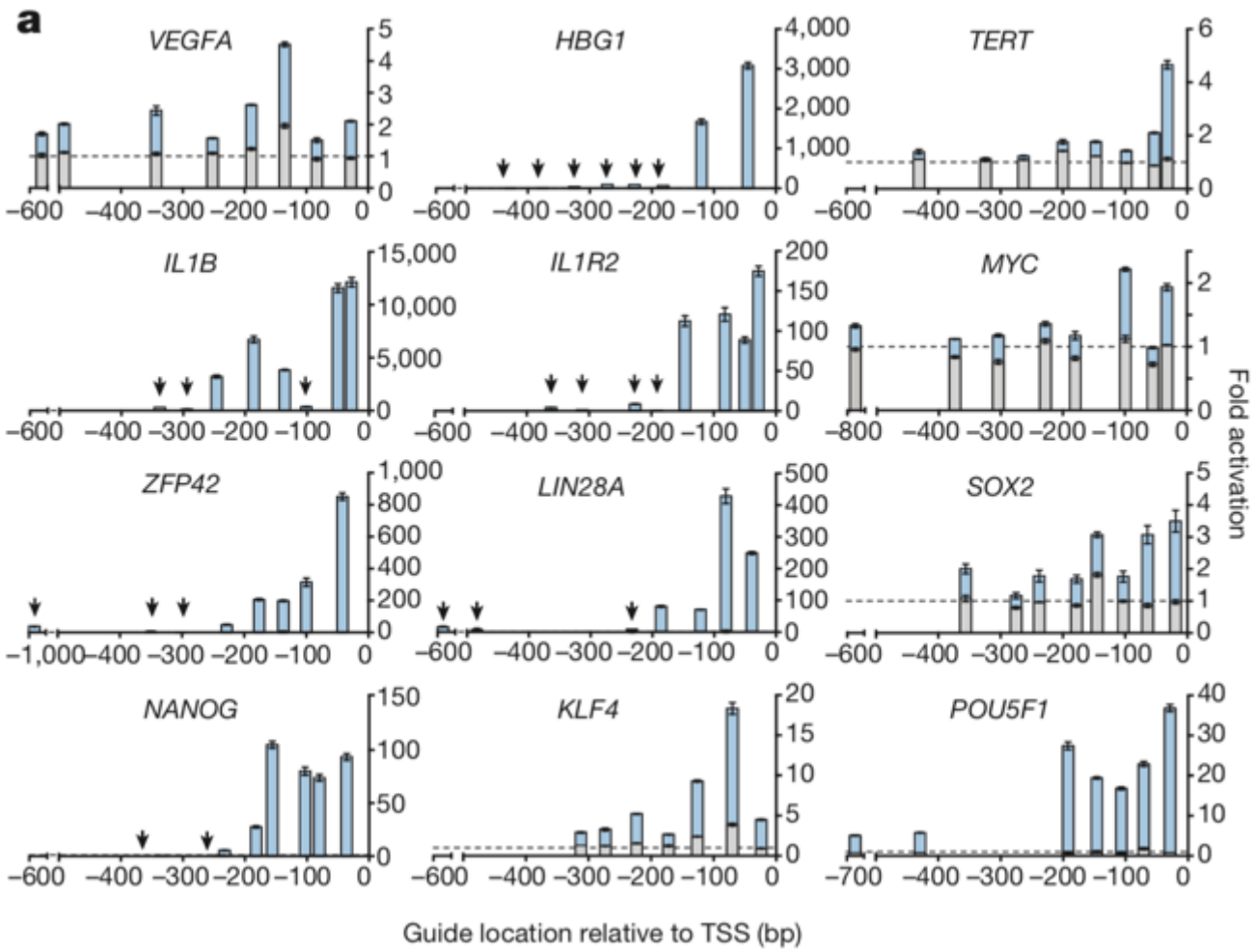
Konermann, *et al.*, (2015). Nature 517, 583



- 3-component system: 1) dCas9-VP64 fusion; 2) HSF1-p65-MS2 fusion; 3) Hybrid sgRNA-apatamer
- Uses same type of 20 nt sgRNA sequence that recognizes NGG PAM as Cas9 nuclease for DSBs
- Optimal activation occurs with sgRNAs up to 200 bp 5' of transcription start site (TSS)

dCas9-SAM system

Konermann, *et al.*, (2015). Nature 517, 583



GeneCopoeia™
Expressway to Discovery

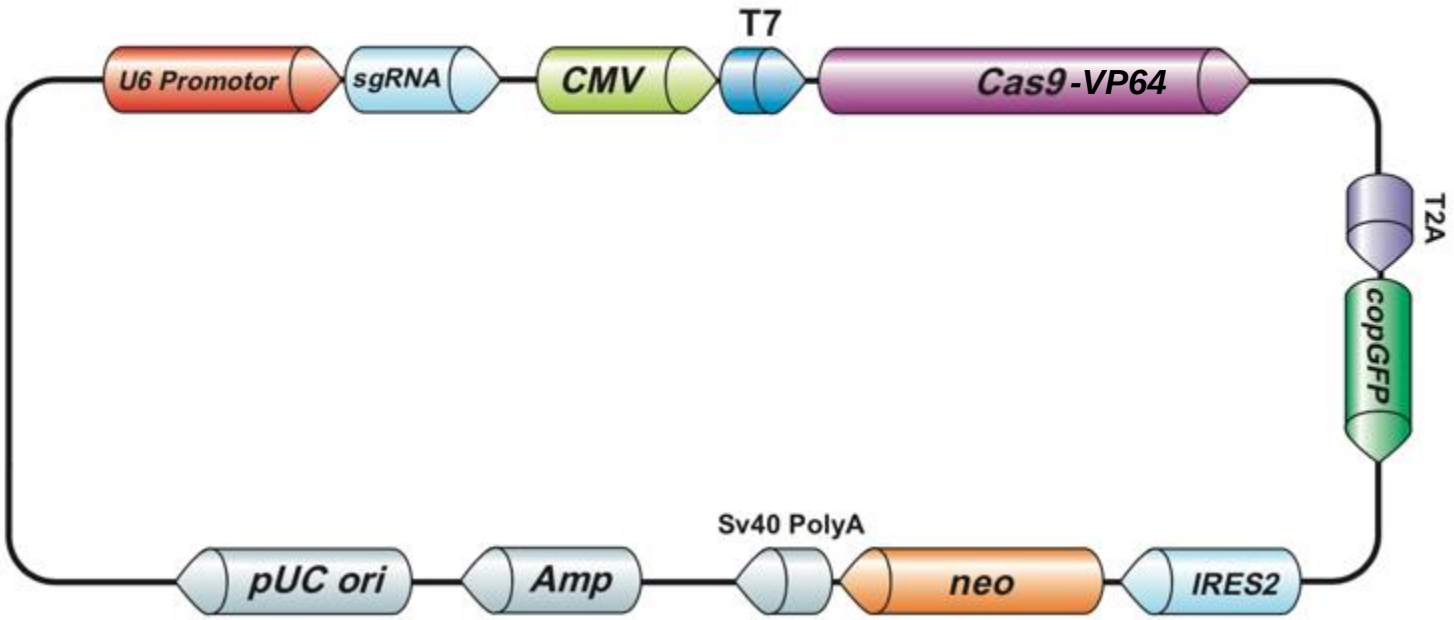
Outline

- CRISPR technologies & applications
- Introduction to CRISPR activation
- **Delivery methods**
- Preparation
- Screening

GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 gene activation technology

Delivery method 1: Plasmid DNA transfection



- GeneCopoeia Cas9 + sgRNA “All-in-one” plasmid
- Would also need separate plasmid expressing HSF1-p65-MS2 fusion
- 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 gene activation technology

Delivery method 2: RNA transfection

- Co-transfect *in vitro* transcribed dCas9-VP64 mRNA, HSF1-p65-MS2 fusion, and sgRNA (aptamer modified)
- sgRNA can either be encoded on plasmid, *in vitro* transcribed, or synthetic
- Advantage: Plasmid-free, so cannot integrate
- dCas9-VP64 still needs to be translated

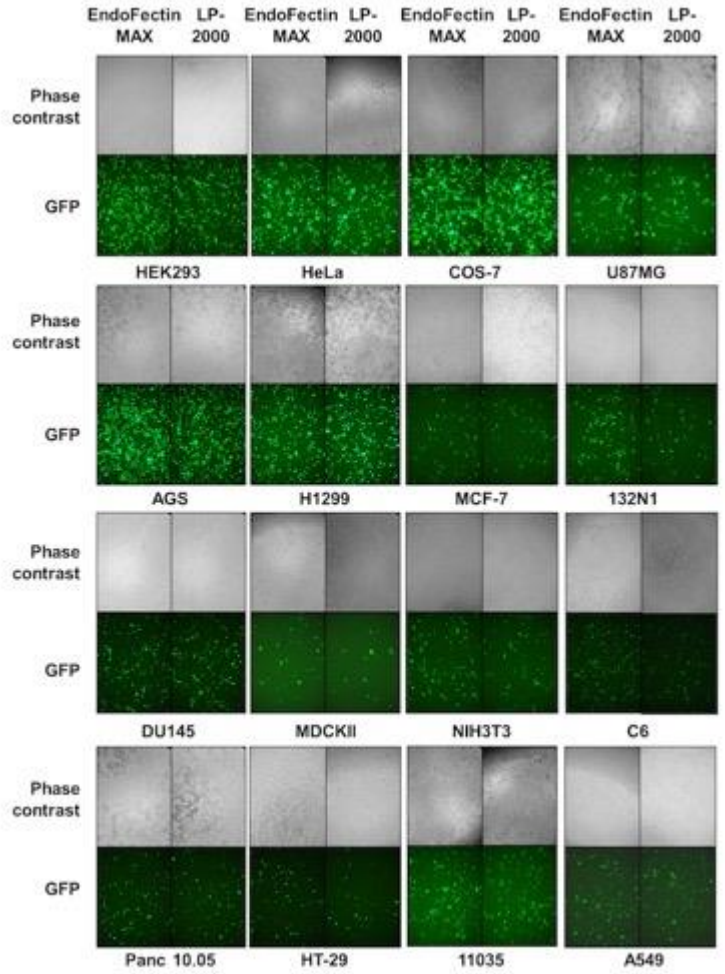
CRISPR-Cas9 gene activation technology

Delivery method 3: Protein transfection

- Pre-form complex of dCas9-VP64 protein and aptamer-modified sgRNA in vitro to form RNP
- Aptamer-modified sgRNA can be either chemically synthesized or *in vitro* transcribed
- Transfect cells with RNP complex using either lipid transfection reagent or electroporation. Co-transfect with HSF1-p65-MS2 mRNA
- Does not integrate, unlike plasmids

CRISPR-Cas9 gene activation technology

EndoFectin™ Max: Robust transfection reagent

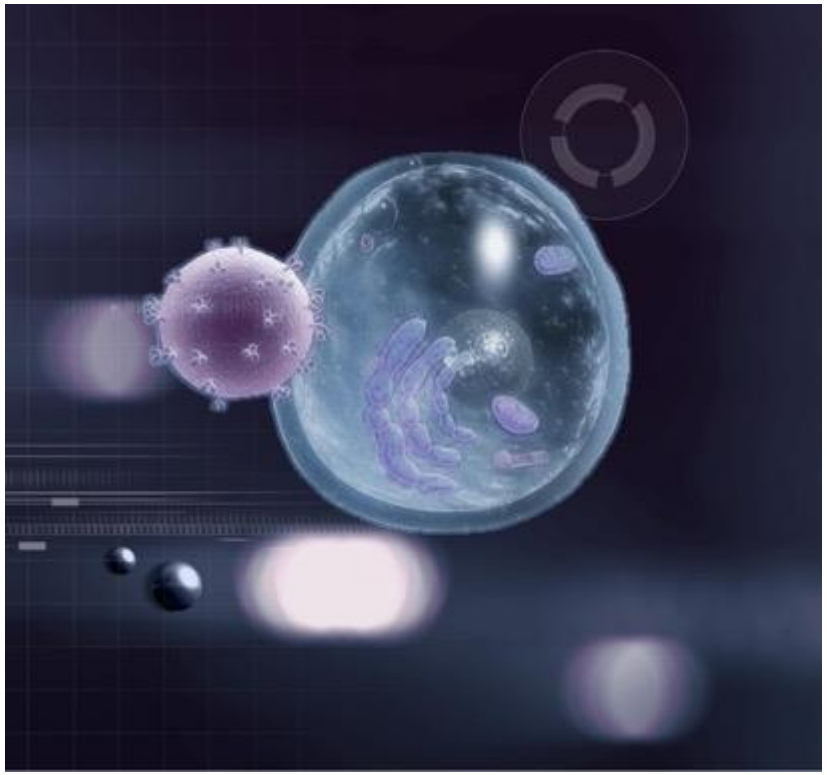


- Equal to or greater transfection efficiency compared with Lipofetcamine™ 2000 or Lipofetcamine™ 3000
- Especially robust with difficult-to-tranfect cell lines
- Capable of efficient transfection of DNA, RNA, and protein

GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 gene activation technology

Delivery method 4: Lentivirus



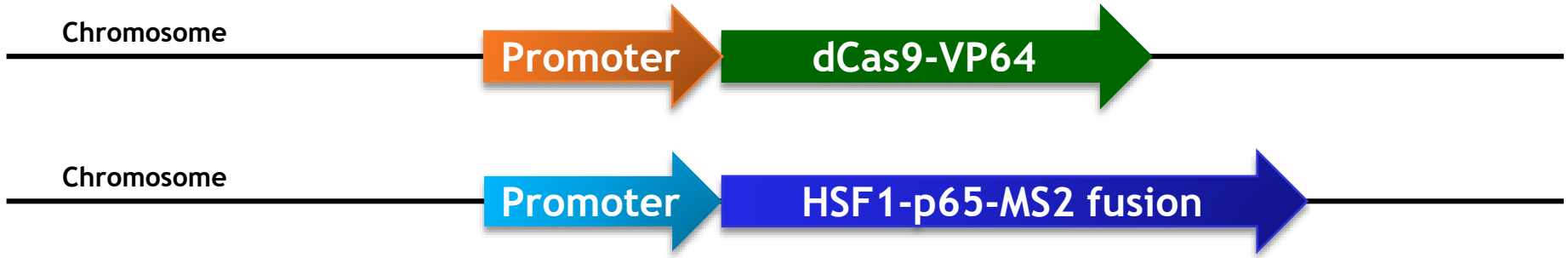
Why lentivirus?

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

GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 gene activation technology

GeneHero™ Cas9a stable cell lines



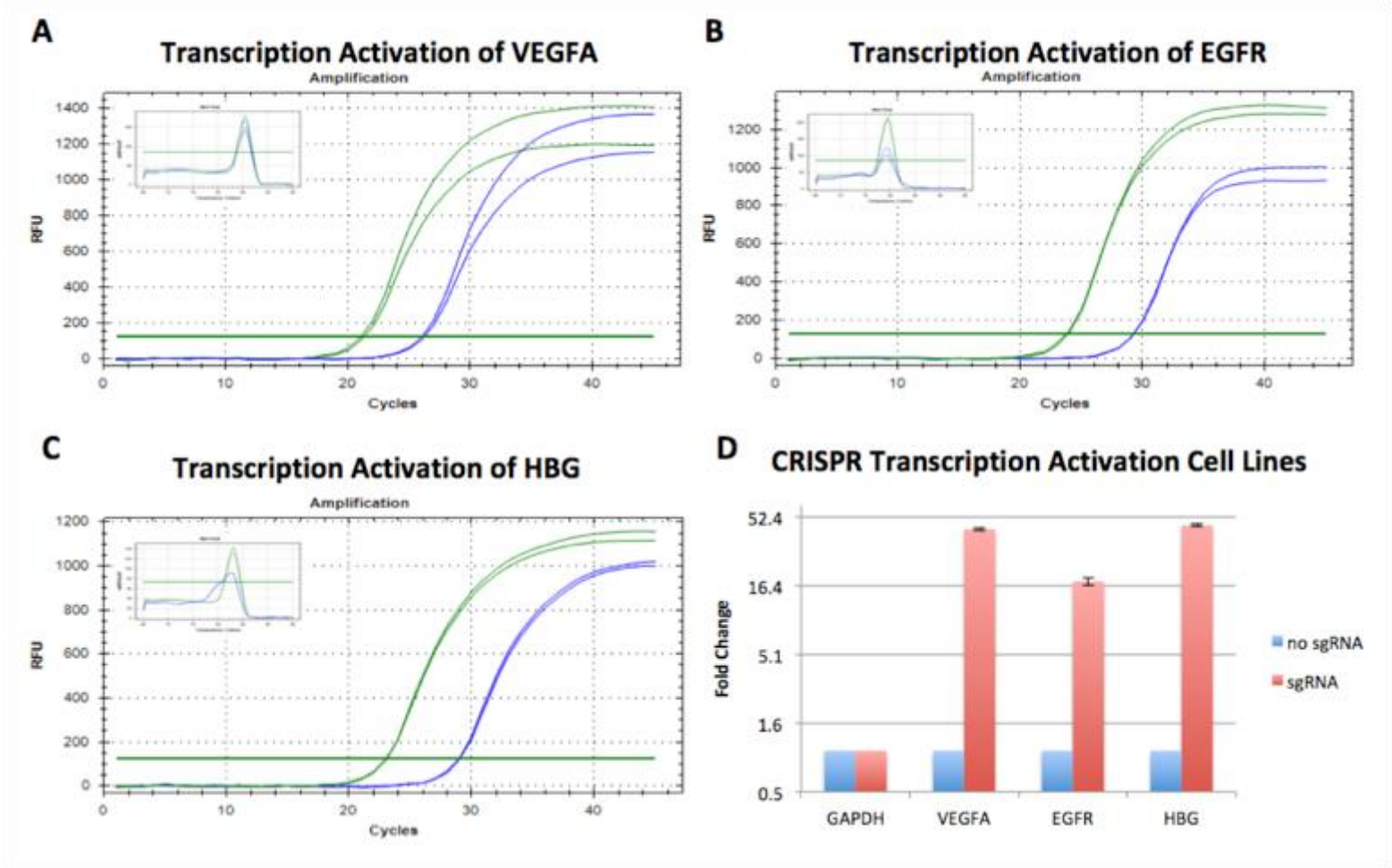
Features

- Cell lines with dCas9-VP64 and HSF1-p65-MS2 stably integrated in the genome
- 2 pre-made cell lines available: HEK293 and MCF-7
- Functionally validated for CRISPR-mediated activation
- Ideal for lentiviral CRISPRa applications
- Need to provide sgRNA with aptamer

GeneCopoeia™
Expressway to Discovery

GeneHero™ Cas9a stable cell lines

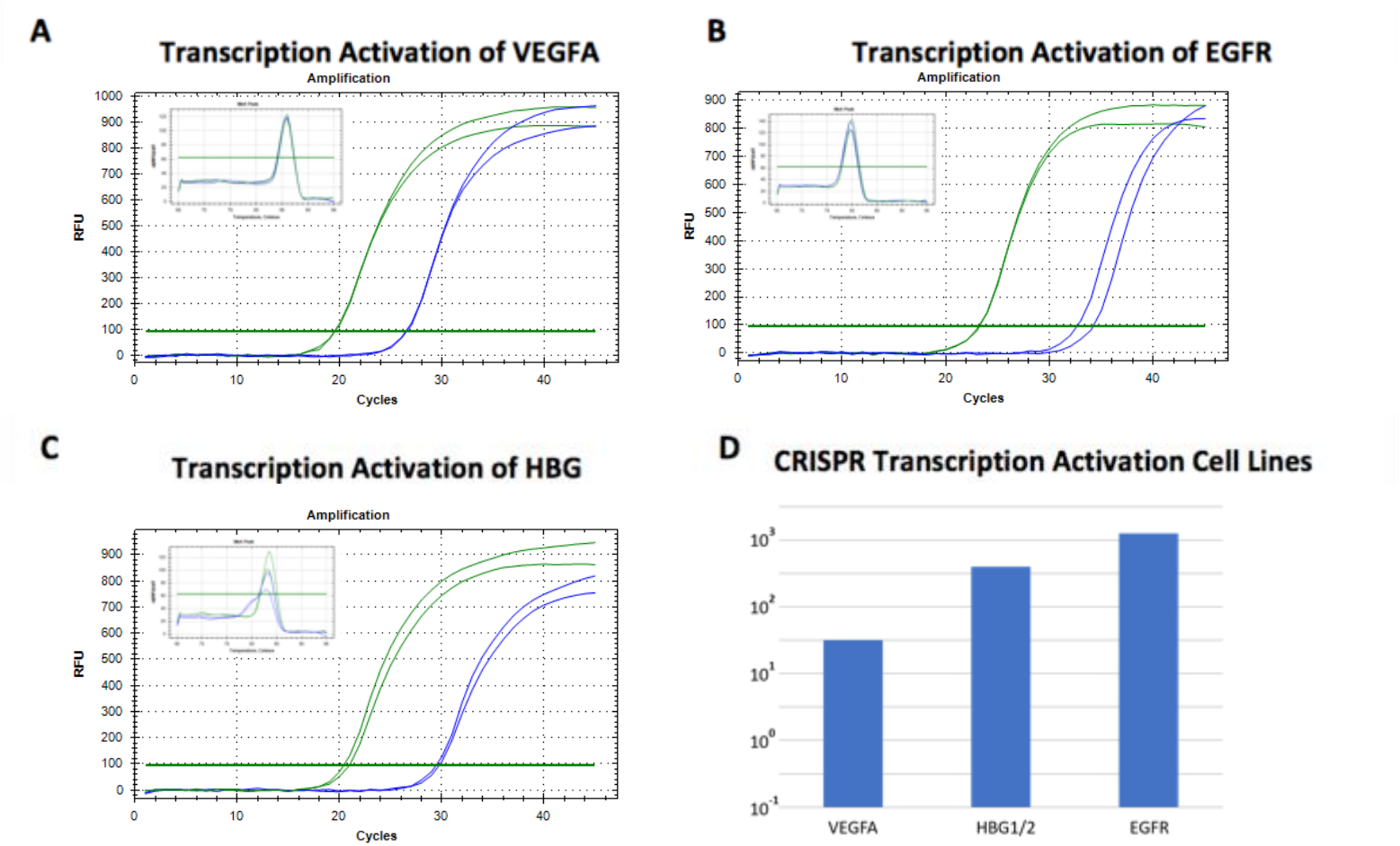
SL302-HEK293



GeneCopoeia™
Expressway to Discovery

GeneHero™ Cas9a stable cell lines

SL504-MCF-7



GeneCopoeia™
Expressway to Discovery

Outline

- CRISPR technologies & applications
- Strategy considerations
- Delivery methods
- Preparation
- Screening

CRISPR-Cas9 gene activation technology

Workflow: Preparation

Step 1. Determine the DNA sequence of the sgRNA target site in your cell line

- Necessary to ensure highest possible nucleotide match before sgRNA synthesis

GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 gene activation technology

Smart-Join™ Blunt-end PCR Cloning Kit

Features

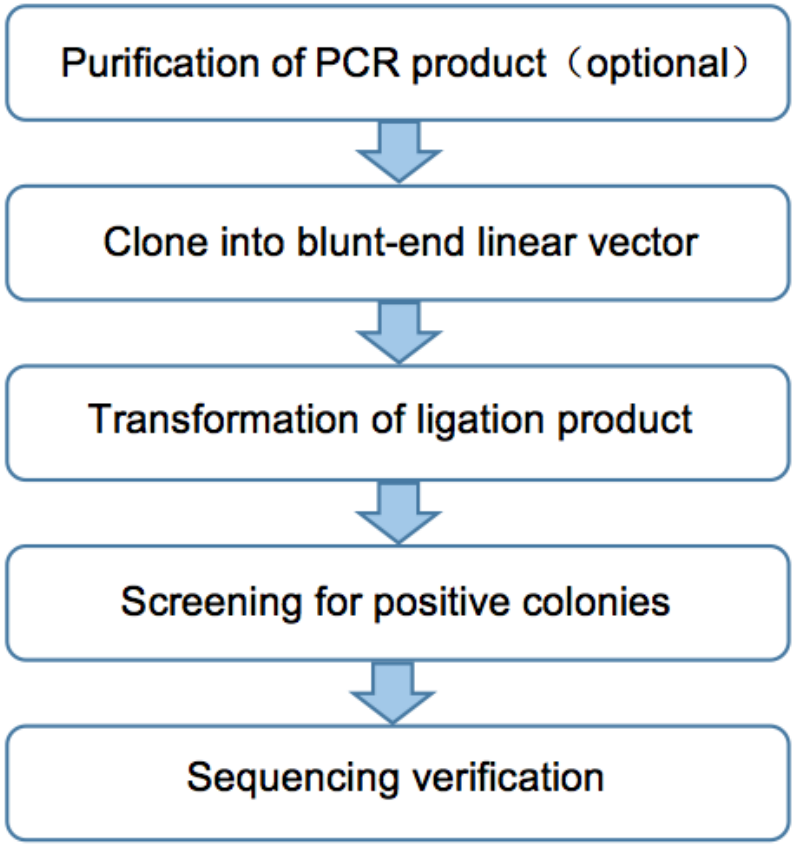
- Convenient method for high efficiency cloning of blunt-end PCR products
- Higher positive ligation rate and true positive colonies by the elimination of self-ligating products
- Cloning of blunt-end PCR products without additional sequences or restriction sites
- Ideal for sequencing regions of genomic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

GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 gene activation technology

Smart-Join™ Blunt-end PCR Cloning Kit

Workflow



GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 gene activation technology

Workflow: Preparation

Step 2: 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

- Validate via qPCR

CRISPR-Cas9 gene activation technology

BlazeTaq™ SYBR green qPCR system

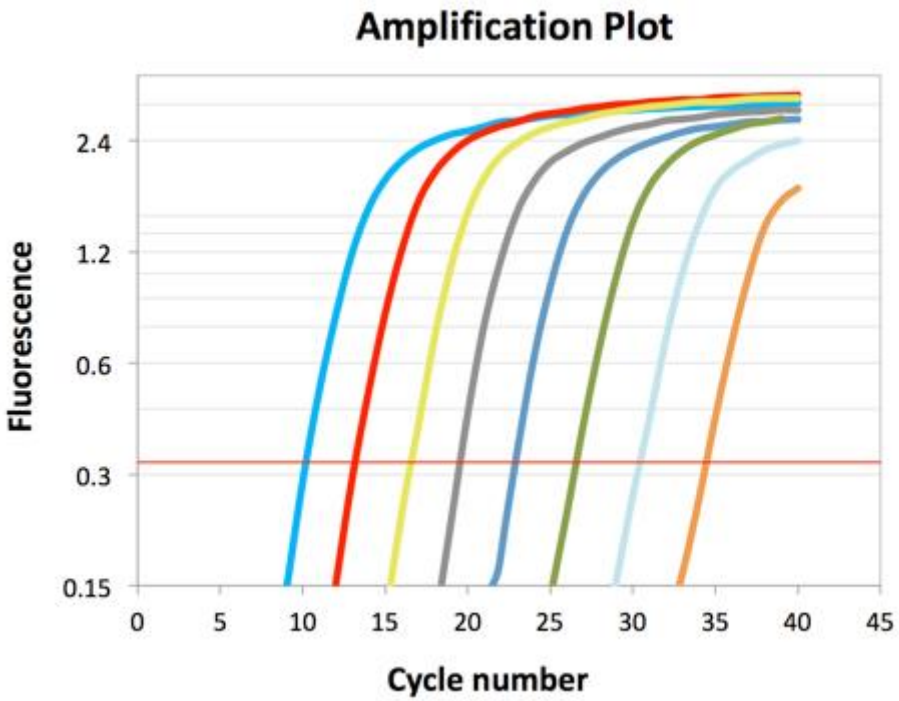
Features

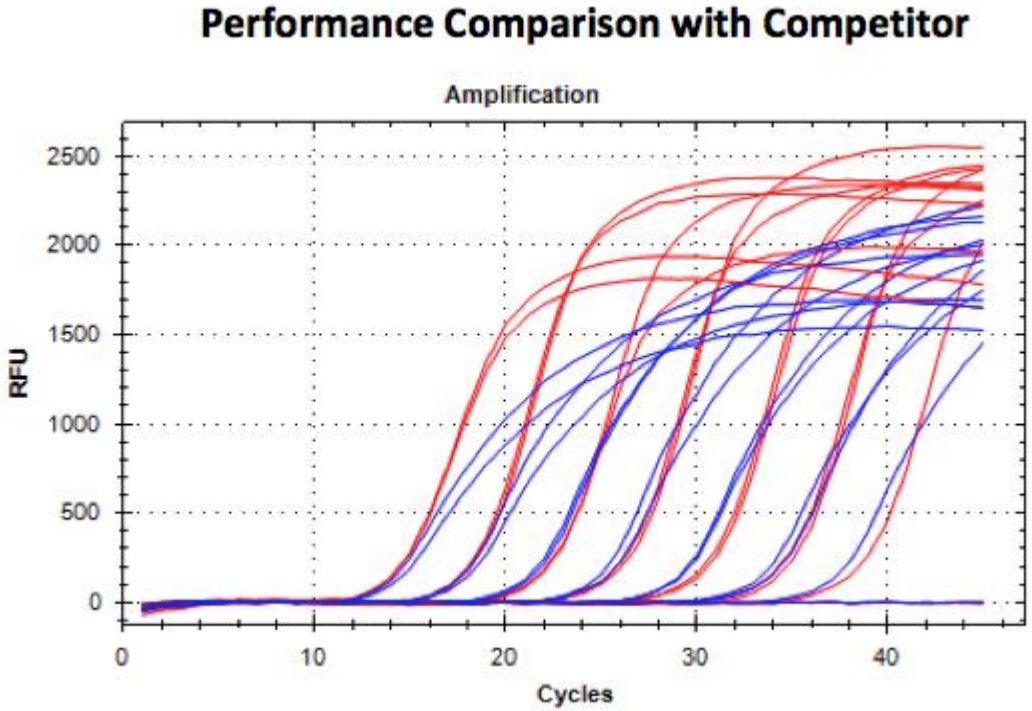
- Available as either 2-step (RT + qPCR separately) or 1-step (RT and qPCR in the same reaction) options
- Highly sensitive. Detects as little as 5 copies of DNA template or 0.1 pg of RNA template
- High specificity with minimal level of primer-dimer and non-specific product formation
- Antibody-modified polymerase provides faster heat activation than chemically modified enzyme (30 sec. vs. 15 min.)

GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 gene activation technology

BlazeTaq™ SYBR green qPCR system

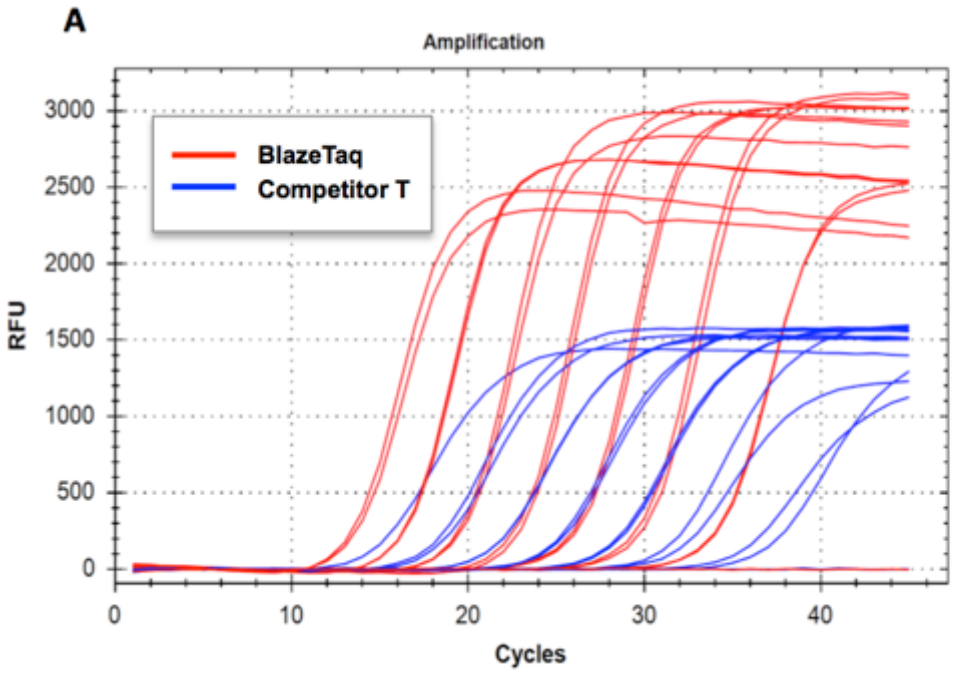


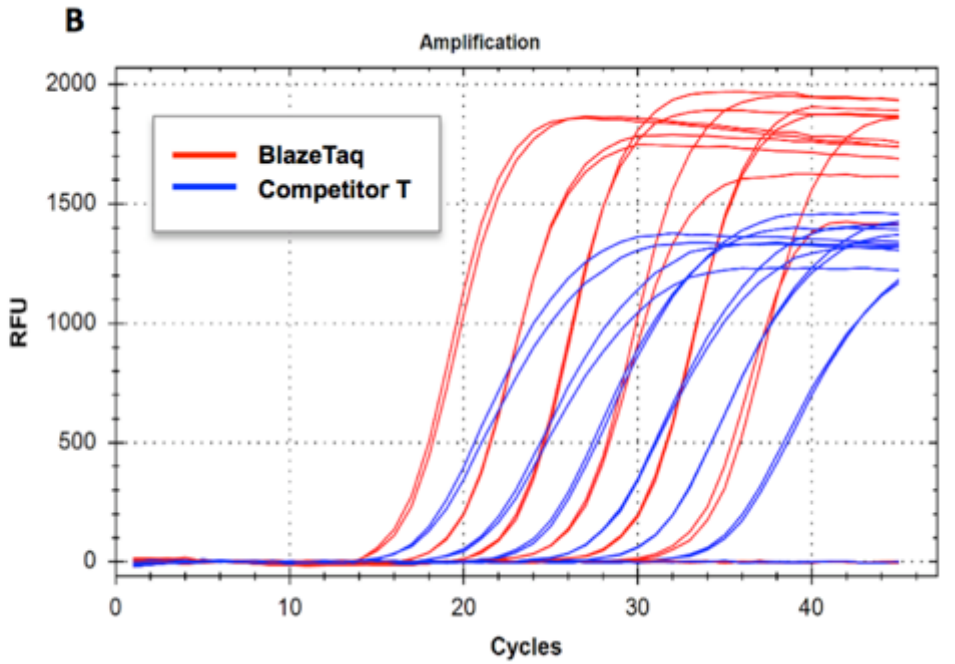


GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 gene activation technology

BlazeTaq™ SYBR green one-step qPCR system

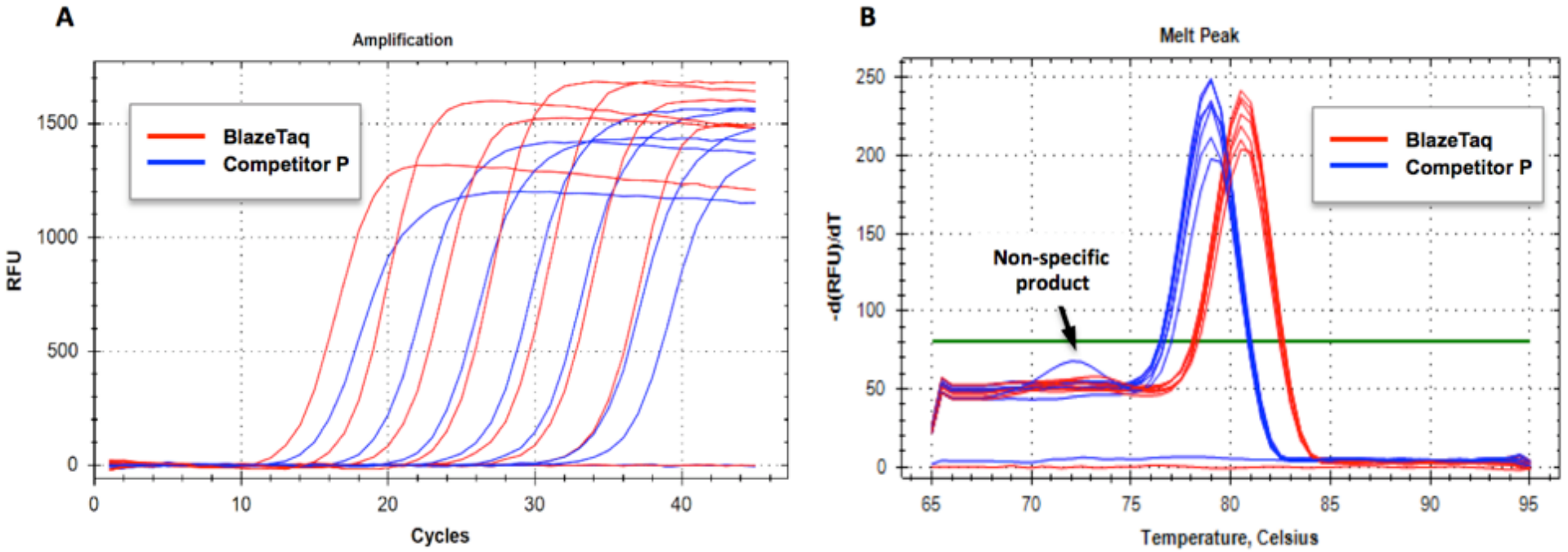




GeneCopoeia™
Expressway to Discovery

CRISPR-Cas9 gene activation technology

BlazeTaq™ SYBR green one-step qPCR system



GeneCopoeia™
Expressway to Discovery

Outline

- CRISPR technologies & applications
- Introduction to CRISPR activation
- Preparation
- Delivery methods
- Screening

GeneCopoeia™
Expressway to Discovery

Screening

Isolate single clones post-transfection/transduction

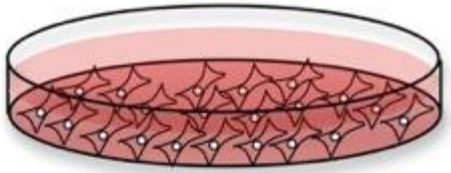
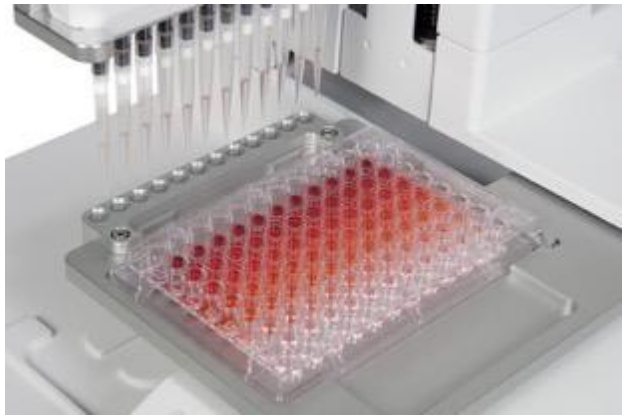


Plate for single colonies and pick off dish

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

- Screen single clones using the BlazeTaq™ SYBR green qPCR kits to analyze for transcriptional activation

Summary

- CRISPR-Cas9-mediated gene activation is a useful system for potentially overexpressing genes, and has distinct advantages over the use of ORF clones, especially for high-throughput screening

- Currently, the most robust CRISPR activation system in use is the dCas9 system of Konermann, et al.

- GeneCopoeia offers many products and services, including plasmid DNA clones, Cas9 activation cell lines, and robust qPCR reagents, to help you meet your CRISPR gene activation 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