Creative Biogene
BIOTECHNOLOGY
CRISPR/Cas9 Platform

CRISPR/Cas9 Applications

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Animal/Cell Models

Develop animal/cell models to promote understanding of gene functions and even disease processes.

Gene Expression and Epigenetic Regulation

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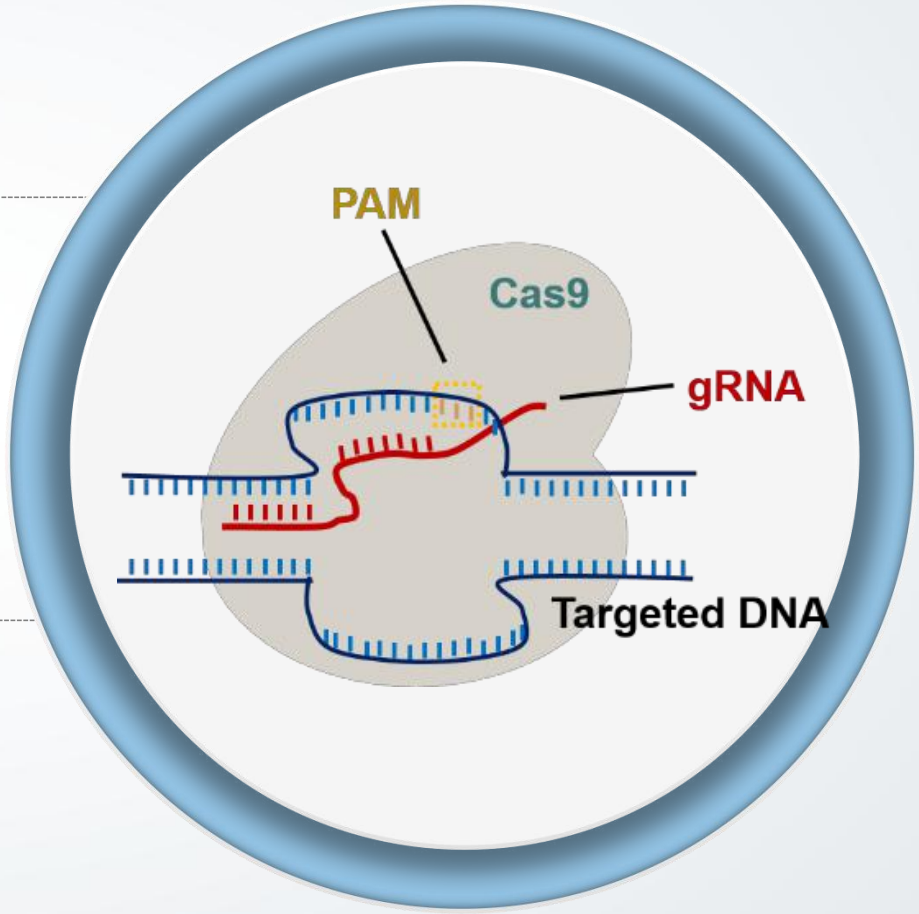
Epigenetic modification



1.1

Animal/Cell Models

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01 Gene conventional knock out by NHEJ

02 Conditional knockout/knockin

03 Knockin/Point mutation

04 Chromosome fusion

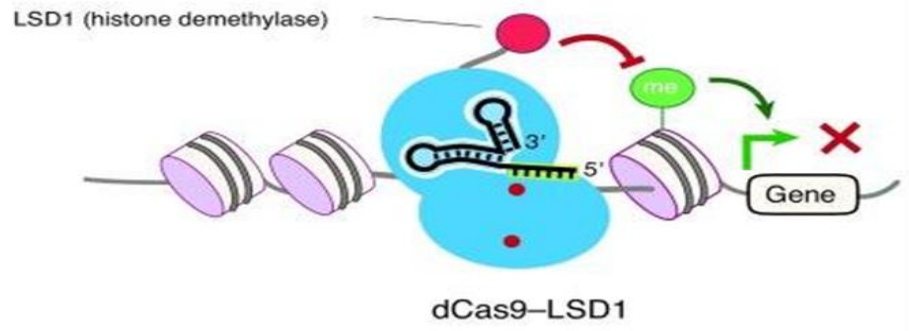
The CRISPR/Cas system simplifies the functional study of genes in cells and animals (such as yeast, fish, mice and many other animals) in an unprecedented way;

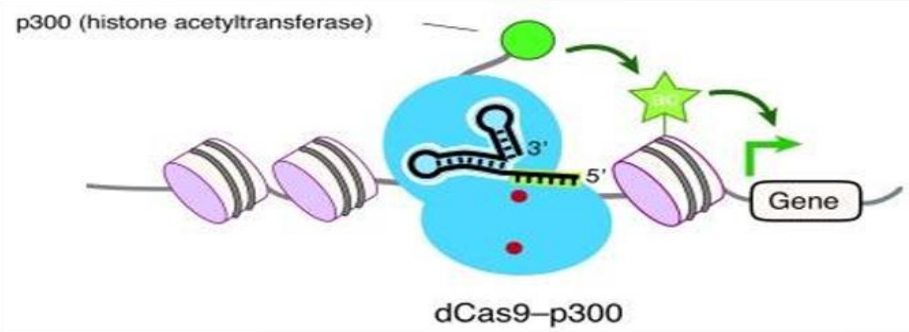
Develop more accurate models (especially hereditary diseases, neurodegenerative diseases, cardiovascular diseases, and cancer) to promote understanding of human disease processes and to provide directions for developing treatments to address these deficiencies.

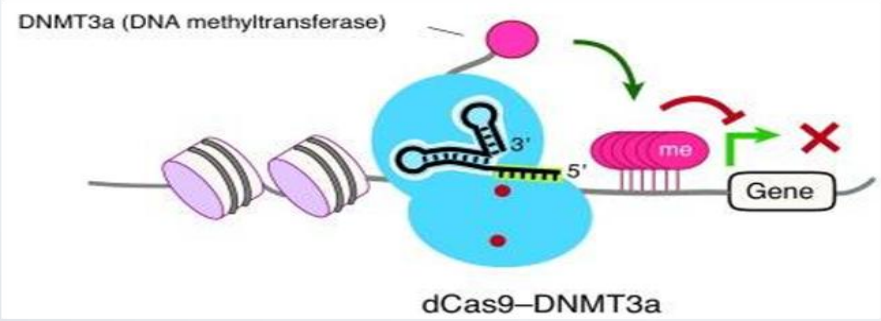
Gene Expression and Epigenetic Regulation

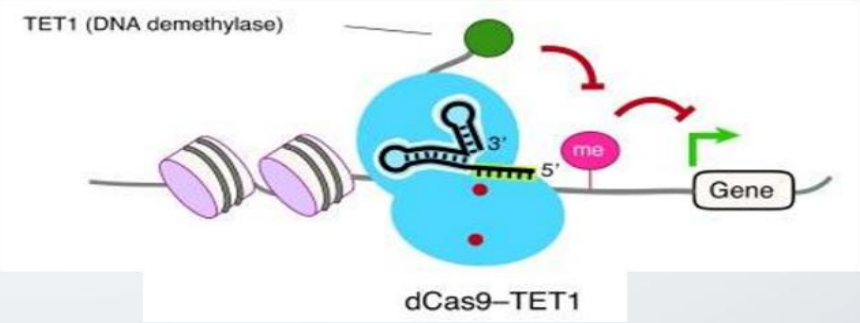
Epigenetic modification

Using non-active dCas9 (another type of Cas9 lacking nuclease activity but retaining DNA binding activity) fused enzymes such as DNA methylase, histone acetyltransferase, and deacetylase can be targeted to alter the epigenetic state of precise locations within the genome.







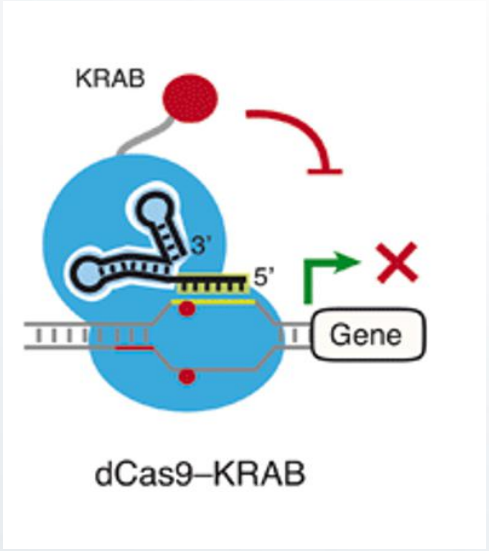


Gene Expression and Epigenetic Regulation

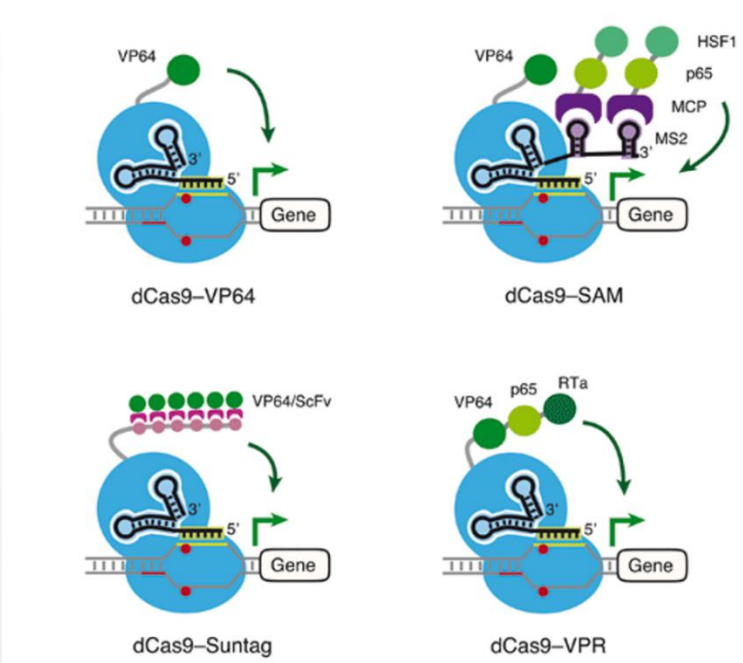
CRISPR-mediated transcriptional modulation

dCas9 can also be fused to transcriptional repressors or activators targeting the promoter region, and these dCas9 fusion proteins can lead to strong transcriptional repression (CRISPR interference or CRISPRi) or activation (CRISPRa) of downstream target genes.

CRISPRi



CRISPRa



02/ CRISPR/Cas9 in Medical research

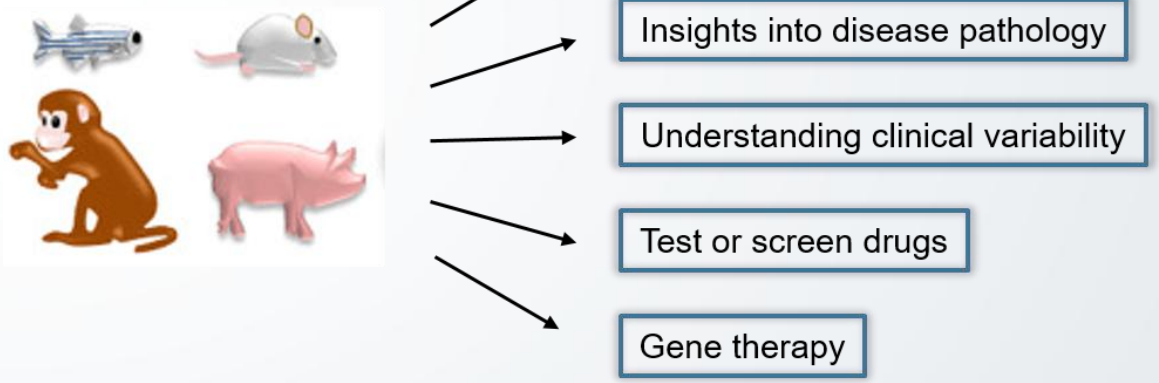
01 Human disease models

02 Drug discovery

03 Treatment of disease

2.1 CRISPR/Cas9-mediated human disease models

The CRISPR/Cas9 system has been used to generate many disease-based models for many important human diseases, including neurological diseases, cardiovascular diseases and cancer, as well as other Mendelian or complex genetic human diseases, which make it possible to study the molecular mechanisms of the underlying pathogenesis, drug screening and discovery, high-throughput research, and gene therapy. CRISPR/Cas9 can also efficiently target genes in somatic tissues, a method that is particularly useful for studying age-related disease physics.



2.2 Drug discovery

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Screening for target sites

The CRISPR library can detect living cells with specific conditions, such as drug therapy. By using the system, researchers can identify genes and proteins that cause or prevent disease, thereby identifying potential drug targets.

Drug discovery

CRISPR/Cas9 animal models allow scientists to discovery new drugs more accurately and verify the safety and efficacy of the drugs, ensuring that these models better predict what will happen in clinical trials. Upregulating or downregulating gene activity using the CRISPR/Cas9 system is a subtle way of studying the importance of genes and proteins that can be activated or inhibited by drugs to treat disease.

2.3 Treatment of disease

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Well-known pharmaceutical companies and emerging biotech companies are racing to develop CRISPR-based therapies. Compared to other gene therapy strategies, CRISPR genome editing is considered faster, cheaper, and potentially safer.

The CRISPR genome editing is especially useful for diseases that can be resolved by modifying cells. Autologous CRISPR cell therapy is promising in bypassing the exclusion problems that exist in donor-matched transplantation therapies.

03 CRISPR System in Biotechnology Research

01 CRISPR/Cas9-mediated ChIP

02 Live Imaging of the Cellular Genome via CRISPR System

03 RNA Editing

3.1 CRISPR/Cas9-mediated ChIP

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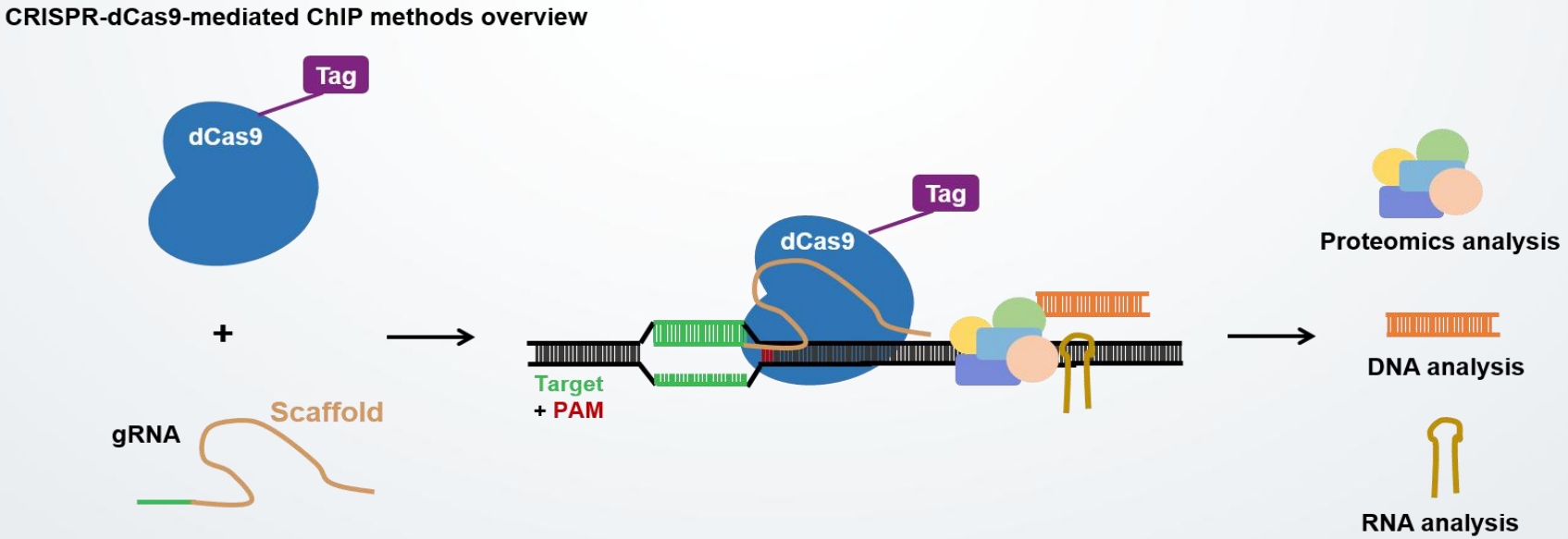
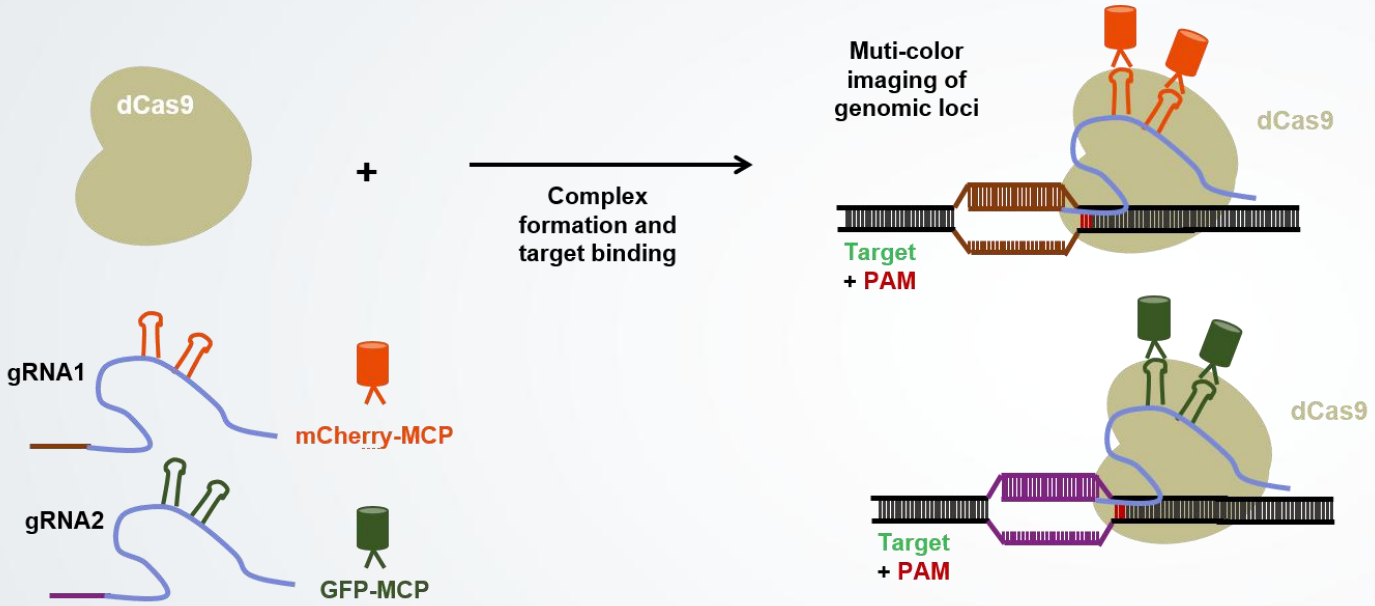
Epitope tags can be fused to dCas9 for efficient purification, including 3xFLAG-tags, biotin tags, *etc*. The locus is then isola ted by affinity purification against the epitope tag. After purification of the locus, the locus-associated molecules can be id entified by mass spectrometry (protein), RNA sequencing (RNA) and NGS (other genomic regions).



3.2 Live Imaging of the Cellular Genome via CRISPR System



01

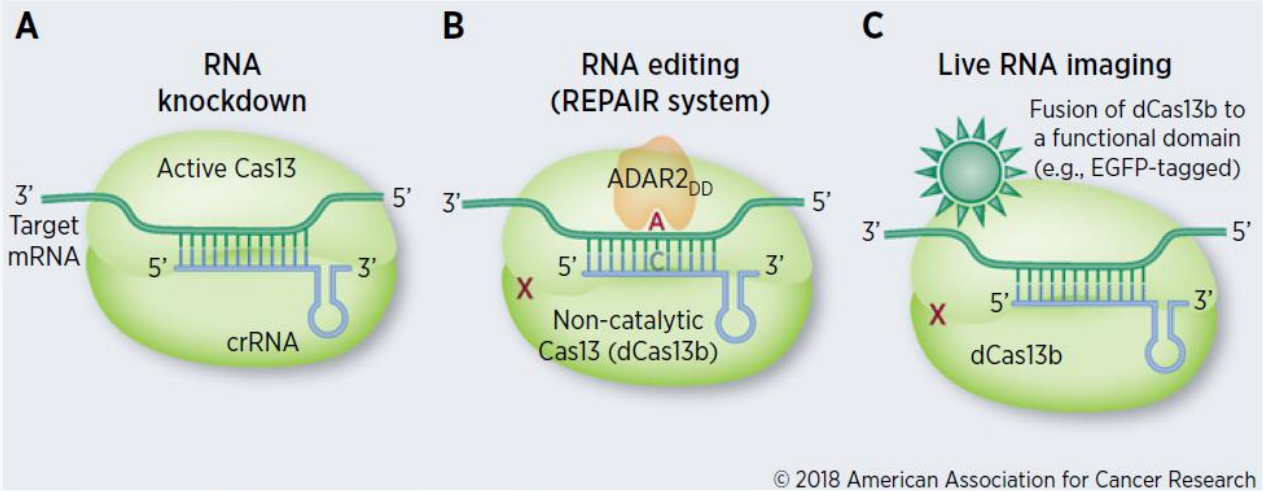
One method uses orthogonal dCas9 labeled with different fluorescent proteins.

02

Another method gRNA that interacts with orthologous proteins that recruit specific orthogonal RBPs labeled with different fluorescent proteins.

CRISPR imaging provides a unique method to detect chromatin dynamics in living cells and has many advantages, including the simplicity of gRNA design, ease of implementation, programmable for different genomic loci, the ability to detect multiple genomic loci, and compatibility with live-cell imaging.

3.3 RNA Editing with CRISPR/Cas13



01 CRISPR/Cas13 provides a transient gene expression suppression tool in mammalian cells

02 RNA-targeting CRISPR/Cas13 can be used for RNA editing as a catalytically dead Cas13 (dCas13) variant fused to the deaminase domain of human ADAR (ADARDD).

03 dCas13b can be coupled to fluorescent proteins (e.g. EGFP) to tracking the translocation of endogenous transcripts from the nucleus to the cytoplasm. In addition, dCas13 can be fused to functional effector domains for posttranscriptional manipulations of RNA substrates in living mammalian cells (e.g. splicing, RNA translation, and others).

THANK YOU

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