BPS Bioscience

CRISPR-Cas9

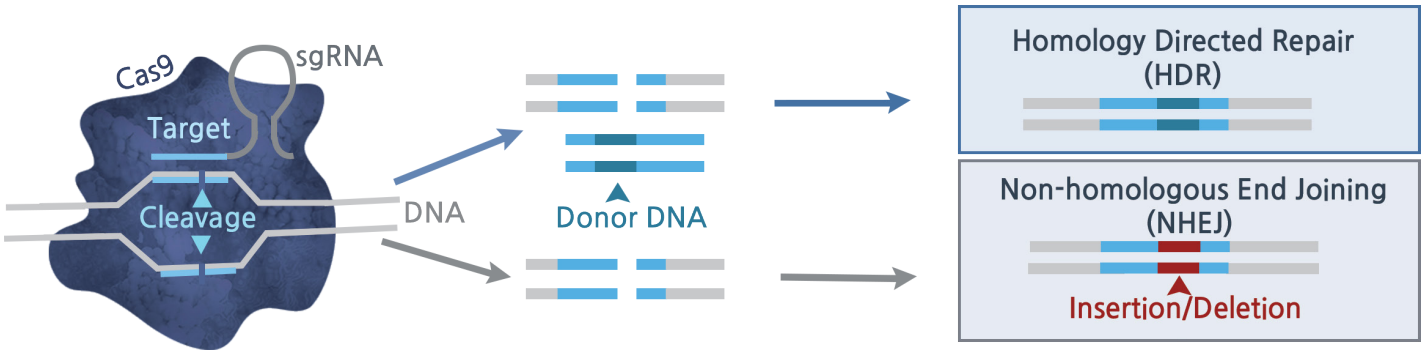
TOOLS & SERVICES FOR GENE EDITING

Screening | Knock-out | Knock-in | Cell Line Development

Simple and Effective Genome Editing

CRISPR-Cas9 is a technique used for genome editing that is adapted from bacterial antiviral immune mechanisms. Bacteria capture and store DNA fragments from invading viruses within a region of their genome, and these CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) guide sequences help detect and protect the bacteria from future infections. When the CRISPR guide sequences detect an invading virus or DNA whose sequence is complementary to the CRISPR guide, the Cas9 (CRISPR-associated protein 9) nuclease is recruited to specifically cleave the invading DNA, resulting in its degradation.

This CRISPR-Cas9 system has been modified for use in mammalian cells. By introducing a guide sequence (sgRNA) specific for our gene of interest, we can either knock-out specific genes through introducing frame shift mutations via Non-Homologous End Joining (NHEJ), or generate knock-in mutations by additionally providing a template for Homologous Recombination (HR).



Our Advantages

Conducted In-House

- All CRISPR products and services are produced and performed in the USA at our San Diego, CA laboratory
- Get customized, personal support directly from our CRISPR experts

Committed to Excellence

- ISO 9001:2015-certified Quality Management System

Customized For Your Research Needs

- Screening & Profiling: >200 optimized cell lines and cell-based assays
- Cell line development: choose from >30 cell types and >70 reporter systems
- Ready-to-use Lentiviruses: integrating and non-integrating options

Multiple CRISPR Editing Tools & Applications

- CRISPR knock-out
- CRISPR knock-in
- CRISPR activation
- Stable cell lines, cell pools, lentiviruses, & plasmids
- CRISPR screens

Knock-in Cell Lines

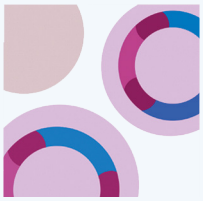
CRISPR-Cas9 can be used to introduce specific changes within the sequence of a gene, resulting in targeted protein alteration, a process known as gene knock-in. The knock-in can either be a single nucleotide substitution or an extended sequence encoding a full protein. This is an effective approach to study known protein mutations, to screen for mutations that affect protein function, or add tags to endogenous proteins in cell lines, in addition to other applications.

With BPS Bioscience's custom cell line development services, our team of highly experienced scientists can generate custom knock-in cell lines in more than 30 different cell types (or your preferred cell types) using CRISPR-Cas9 licensed technology, targeting your gene(s) of interest. The development process is comprised of distinct milestones where data is provided after each milestone completion. Each project can be fully customizable for your desired deliverables.

A Milestone-Measured Process Ensures Success

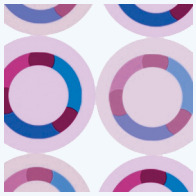
1 Molecular Biology

BPS Bioscience will design and construct the sgRNA and HDR template according to your experimental needs.



2 Stable Cell Line Generation

The cells will be transduced with the Cas9, sgRNA, and HDR template, followed by genome editing evaluation. Single clones will be selected and expanded.



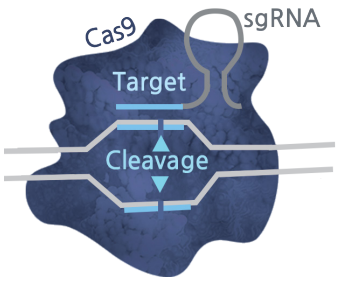
3 Genotyping & Confirmation

The knock-in mutations will be confirmed by genomic sequencing, and positive clones will be expanded for further confirmation.

Consider the Advantages and Limitations of CRISPR-Cas9 Technology

Advantages

- Precise gene editing
- Relatively high efficiency
- Permanent gene editing to generate stable knock-ins
- Relatively simple compared to other gene editing techniques
- Can target multiple genes simultaneously
- Effective across many cell types
- Potential for use in screening



Limitations

- Potential for off-target effects
- Variable effectiveness of sgRNAs
- Target selection may be limited by PAM (protospacer adjacent motif) sequence
- Potential for low homology directed repair efficiency in your target cell
- Large proteins may be difficult to introduce

The milestone-measured process ensures that any potential limitations are overcome before advancing in the project.

Knock-out Cell Lines & Cell Pools

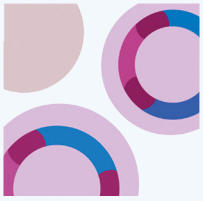
CRISPR-Cas9 is an ideal system for targeted gene knock-out. The achieved result can be a crude cell pool, in which the cell population is heterogenous with differing degrees of gene knock-out, or a stable cell line derived from a single clone. Stable cell lines are best for long-term or complex studies to provide experimental consistency over time. Cell pools are useful for lower cost, initial testing.

BPS Bioscience provides ready-to-use Cas9-expressing cell lines and cell pools that allow you to perform your own CRISPR knock-out assays. Alternatively, we can generate knock-outs for you with our milestone-measured process.

Project Milestones for Knock-out Results

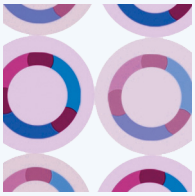
1 Molecular Biology

We will synthesize up to 5 sgRNA sequences and clone into a CRISPR expression vector.



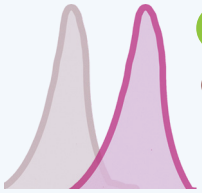
2 CRISPR Transfection

Depending on the cell type, cells can be transduced via electroporation, liposome-based transfection, or viral infection.



3 Limiting Dilution

Based on the results of the initial pool testing, the cell pool will be clonally diluted and single cell-derived clones will be expanded.



4 Confirmation of Expression

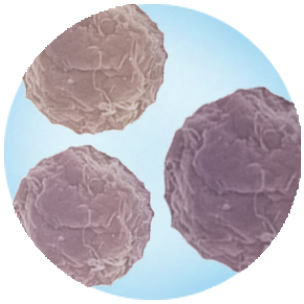
The expression level of the target protein will be analyzed via Western blot or flow cytometry.

5 Confirmation & Delivery

Gene knock-out will be confirmed through genomic sequencing. Confirmed clones will be expanded, frozen, and tested for mycoplasma contamination.

Why knock-out genes in cells?

- Study transcriptional regulation by knocking out transcription factors, repressors, or epigenetic enzymes
- Generate disease models
- Generate knock-out libraries for screening
- Generate unique immune phenotypes, such as MHC-deficient cells

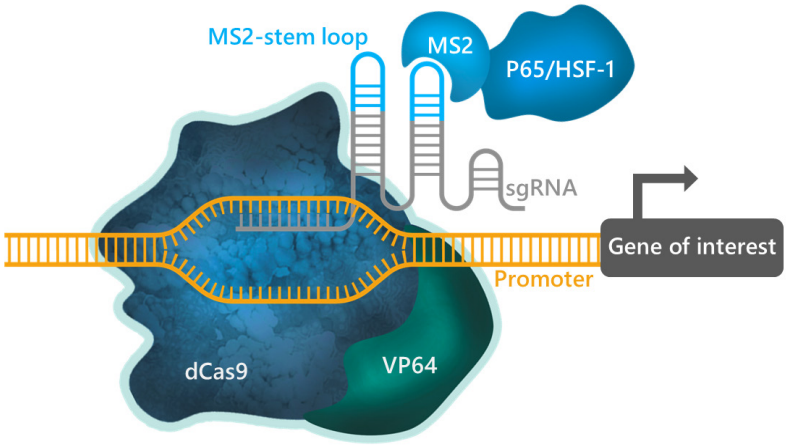


- Identify potential cancer targets
- Identify critical factors in signaling pathways
- Create systems for cell & gene therapy
- Optimize CAR-T cell function and limit toxicity
- Identify factors for viral entry
- And many more...

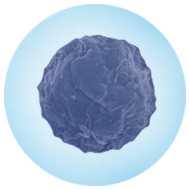
CRISPR Activation

The CRISPR (SAM) system (CRISPR-based Synergistic Activation Mediator) is a combination of dCas9 and other molecular biology tools designed to activate the transcription of any endogenous gene of interest.

The system comprises 3 components that form a DNA-binding complex upon transfection into the cells. The first component is dCas9 (dead Cas9 with a disabled endonuclease activity) fused to transcriptional activator VP64, typically composed of four tandem copies of VP16 (Herpes Simplex Viral Protein 16, amino acids 437-447). The other two components exploit the unique MS2 bacteriophage protein/RNA interaction system in which the coat protein of the bacteriophage binds tightly and specifically to a distinct 19-nucleotide RNA aptamer. In the second component of SAM, MS2 aptamers forming a characteristic stem loop structure are added to the sgRNA. The sgRNA-MS2 component forms a complex with dCas9 and directs it to the target DNA sequence next to the promoter region of the gene of interest. The sgRNA-MS2 aptamer recruits the third SAM component consisting of transcriptional activators P65 (Nuclear Factor NF-κB p65) and HSF1 (Heat Shock Factor 1) fused with an MS2-tag corresponding to the minimal aptamer-binding peptide of the MS2 coat protein. Once captured in the assembled complex at the gene promoter, P65 and HSF1 synergize with VP64 to robustly activate transcription of the downstream target gene, as much as a hundred-fold depending on the gene. Theoretically, the SAM system can be used to target one or several gene promoters in the same cell.

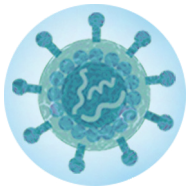


Accelerate your discoveries with our CRISPRa solutions



Cell Lines

- Stably expressing dCas9-VP64 and MS2-P65-HSF1
- Convenient and cost-effective platform for setting up your own activating experiments or screens



Lentiviruses

- Integrating lentiviruses containing dCas9-VP64 and MS2-P65-HSF1
- Integrating sgRNA-MS2 lentiviruses, containing 4 validated sgRNA targeting your gene of interest
- Ready for transduction into almost all types of mammalian cells, including primary and non-dividing cells

Plasmids

- sgRNA-MS2 plasmids can be transfected or electroporated into your cells for transient or stable (following drug selection) activation
- Virus-free option

Kinase Knock-out Library

Our CRISPR/Cas9 Kinase Knockout Lentivirus Library consists of ready-to-use, VSV-G pseudotyped integrating lentiviruses intended for use in generating kinase knockouts for screening purposes.

Array Format (#78487)

Targets 619 human kinases and pseudokinases

5 sgRNAs per gene

150 control sgRNAs included

Puromycin selection

- 649 vials come with 200 µl of lentivirus each at a titer ≥1 x 10^7 TU/ml; the exact titer is provided with each shipment.

Advantages

- All-in-One: each vial contains lentiviral particles to transduce the Cas9 gene, 5 sgRNAs targeting the kinase of interest, and a puromycin selection marker. There is no requirement for the target cell to already express Cas9.
- Safe: requires only Biosafety Level 2 (BSL-2).
- Each sgRNA lentiviral particle is individually constructed, sequence-verified, individually cultured, and titered to ensure high quality and representation across the entire library.
- VSV-G pseudoviruses transduce most mammalian cells, including primary and non-dividing cells.

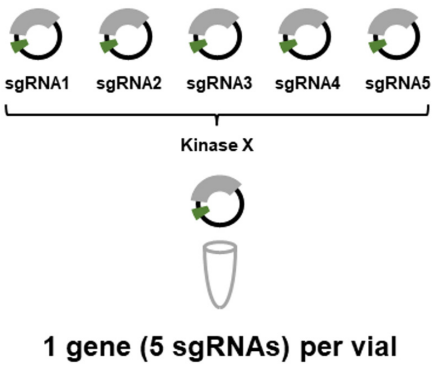
Custom Panels

- The Custom Panel option allows you to pick between 2-50 kinases out of the full human kinase library. One vial of 200 µl of lentivirus is provided for each target.
- Download the full target list and request a quote here: bpsbioscience.com/crispr-kinasepanel

Single Target (#78488)

- If you are only interested in a single kinase target to knock out, this option is available.
- Receive two vials, each with 200 µl of lentivirus at a titer ≥1 x 10^7 TU/ml.

All our CRISPR/Cas9 Kinase Knockout Lentiviruses include 5 sgRNAs to ensure effective target knock-out.



CRISPR Cell Lines & Cell Pools	Catalog#
Cas9 Expressing A549 Cell Pool	78072
Cas9 Expressing Daudi Cell Pool	78089
Cas9 Expressing HCT116 Cell Pool	78073
Cas9 Expressing Jurkat Cell Pool	78070
Cas9 Expressing MDA-MB-231 Cell Pool	78069
Cas9 Expressing Raji Cell Pool	78071
Cas9-Expressing A549 Cell Line (High Expression or Low Expression)	78134
Cas9-Expressing Daudi Cell Line	78157
Cas9-Expressing HCT116 Cell Line (High or Low Expression)	78135
Cas9-Expressing HEK293 Cell Line	78166
Cas9-Expressing HeLa Cell Pool	78161
Cas9-Expressing Jurkat Cell Line (High or Low Expression)	78136
Cas9-Expressing MCF7 Cell Pool	78179
Cas9-Expressing MDA-MB-231 Cell Line (High or Low Expression)	78150
Cas9-Expressing Neuro2A Cell Line (High or Low Expression)	78137
Cas9-Expressing Neuro2A Cell Pool	78087
Cas9-Expressing Raji Cell Line	78156
CRISPRa (SAM) HEK293 Cell Line	78192
CRISPRa (SAM) HeLa Cell Line	78193
CRISPRa (SAM) HepG2 Cell Line	78194
CRISPRa (SAM) Jurkat Cell Line	78080
CRISPRa (SAM) MCF7 Cell Line	78522
CRISPRa (SAM) MDA-MB-231 Cell Line	78521
RFP/GFP Safe-Harbor HEK293 Cell Line	78581

Proteins	Catalog#
Cas12a, His-Tag (Acidaminococcus sp.) Recombinant	101627
Cas12b (A. acidiphilus) Recombinant	101626
Cas13a (L. buccalis) Recombinant	101629
Cas13a, His-Tag (L. wadei) Recombinant	101630
Cas13b, His-Tag (Prevotella sp) Recombinant	101631
Cas9 (D10A), NLS, His-Tag (S. pyogenes) Recombinant	101632
Cas9 (D10A, H840A), NLS, His-Tag (S. pyogenes) Recombinant	101633
Cas9 (H840A), NLS, His-Tag (S. pyogenes) Recombinant	101634
Cas9, His-tag (S. pyogenes) Recombinant	100206
Cas9, NLS, His-Tag (S. pyogenes) Recombinant	101635
Csm6, His-Tag (E. italicus) Recombinant	101628
Csm6, His-Tag (T. thermophilius) Recombinant	101636

Plasmids	Catalog#
PD-1 sgRNA-MS2 for CRISPRa (Plasmid)	78091

Lentiviruses	Catalog#
B2M (Human) CRISPR/Cas9 Lentivirus (Integrating)	78340
B2M (Human) CRISPR/Cas9 Lentivirus (Non-Integrating)	78341
Cas9 Lentivirus (Hygromycin Selection)	78067
Cas9 Lentivirus (Neomycin Selection)	78432
Cas9 Lentivirus (Puromycin Selection)	78066
CBL-B (Human) CRISPR/Cas9 Lentivirus (Integrating)	78343
CBL-B (Human) CRISPR/Cas9 Lentivirus (Non-Integrating)	78344
CD47 CRISPR/Cas9 Lentivirus (Integrating)	78056
CD47 CRISPR/Cas9 Lentivirus (Non-Integrating)	78063
CD5 (Human) CRISPR/Cas9 Lentivirus (Integrating)	78119
CD5 (Human) CRISPR/Cas9 Lentivirus (Non-Integrating)	78198
CIITA (Human) CRISPR/Cas9 Lentivirus (Integrating)	78435
CIITA (Human) CRISPR/Cas9 Lentivirus (Non-integrating)	78434
CRBN CRISPR/Cas9 Lentivirus (Integrating)	78517
CRBN CRISPR/Cas9 Lentivirus (Non-Integrating)	78518
CRISPR/Cas9 Kinase Knockout Lentivirus Library (Array Format)	78487
CTLA4 CRISPR/Cas9 Lentivirus (Integrating)	78054
CTLA4 CRISPR/Cas9 Lentivirus (Non-Integrating)	78061
Kinase (Human) CRISPR/Cas9 Lentivirus (Integrating)	78488
LAG3 CRISPR/Cas9 Lentivirus (Integrating)	78053
LAG3 CRISPR/Cas9 Lentivirus (Non-Integrating)	78060
PD-1 (Human) sgRNA-MS2 Lentivirus (Integrating)	78190
PD-1 CRISPR/Cas9 Lentivirus (Integrating)	78052
PD-1 CRISPR/Cas9 Lentivirus (Non-Integrating)	78059
PD-L1 CRISPR/Cas9 Lentivirus (Integrating)	78057
PD-L1 CRISPR/Cas9 Lentivirus (Non-Integrating)	78064
TCR CRISPR/Cas9 Lentivirus (Integrating)	78055
TCR CRISPR/Cas9 Lentivirus (Non-Integrating)	78062
TGFBR2 CRISPR/Cas9 Lentivirus (Non-Integrating)	78536
TIGIT CRISPR/Cas9 Lentivirus (Integrating)	78058
TIGIT CRISPR/Cas9 Lentivirus (Non-Integrating)	78065

AAV	Catalog#
AAV2 SaCas9	78480

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