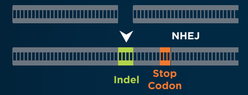
CRISPR TECHNOLOGY IS TRANSFORMING LIFE SCIENCE RESEARCH

sgRNA PAM Cas9

CAPITALIZING ON CRISPR

Genetic engineering has been boosted by the discovery and characterization of CRISPR-associated Cas9 RNA-guided endonuclease.[1] This technology enables rapid genetic perturbation and manipulation, as well as genome-wide functional screening. With broad basic science and translational applications, this technology is driving innovative life science and medical research.

1



Cas9-induced DSB. Indel (insertion/deletion) created by NHEJ of DSB introduces premature stop codon.

KNOCKOUTS

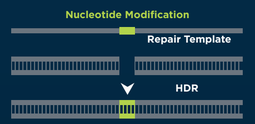
Use non-homologous end joining (NHEJ) to inactivate genes by frameshift mutation.[2,3]

- Rapidly generate knockout cell lines and animals.
- Analyze loss of function for single-gene knockout.
- Study combinatorial effects, pathway redundancy, and epistatic relationships via multigene knockout.
- Investigate the role of genes in cell processes, disease models, and drug response.

KNOCKINS

Introduce specific nucleotide modifications to genomic DNA via homology-directed repair (HDR).[4,5]

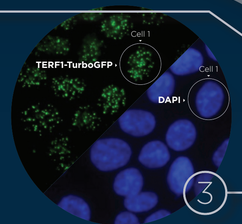
- Rapidly generate cell lines and animals.
- Target gene expression and noncoding DNA elements (enhancers, promoters, etc.).
- Introduce gain-of-function and loss-of-function mutations in endogenous genes to study the impact of SNPs or somatic mutations on gene function.
- Simultaneously target mutations to multiple genomic regions.



Cas9-induced DSB in presence of repair template. Targeted nucleotide modification is introduced to genomic DNA by HDR.

2

3



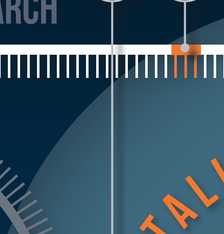
Use of CRISPR/Cas technology to tag TERF1 with TurboGFP. Endogenous TERF1 was tagged with TurboGFP at the N-terminus. Cells were fixed in paraformaldehyde and stained with DAPI. Microscopy images show a dotted TERF1 pattern, indicative of TERF1 binding to telomeres.

TAGGING

Use HDR to introduce an epitope tag or fluorescent marker at a targeted locus or visualize genomic DNA with a tagged-Cas9 mutant.

- Fuse endogenous genes or genomic loci to fluorescent proteins.[6]
- Introduce conditional alleles, e.g. LoxP or FRT.[7]
- Epitope tag (e.g. His or FLAG) targeted genomic sequence to enable downstream analysis.[8]
- Visualize genomic elements in living cells via sgRNA tiling.[9]

4



Use of CRISPR/Cas technology to engineer the CD74-ROS1 translocation. HEK 293T cells were treated with Cas9 along with two guide RNAs that introduce two DNA double-strand breaks on chromosomes 5 and 6. The resulting clonal cell line bears a balanced translocation that can be visualized by spectral karyotyping.[10]

CHROMOSOMAL REARRANGEMENTS

Use two double-stranded breaks to delete intervening genomic regions.[11]

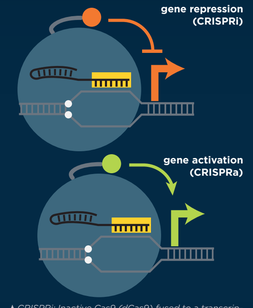
- Study complete gene knockouts.
- Investigate the function of noncoding regulatory elements.
- Analyze haploinsufficiency with monoallelic deletion clones.
- Rearrangements include deletions, translocations, and other modifications.

5

TRANSCRIPTIONAL INTERFERENCE OR ACTIVATION

Target genes to repress expression with CRISPR interference (CRISPRi) or activate expression with CRISPR activation (CRISPRa).[12–14]

- Study the effect of gene knockdown or activation in a reversible system.
- Target multiple genes simultaneously to study gene networks and combinatorial effects.



CRISPRi: Inactive Cas9 (dCas9) fused to a transcriptional repressor is targeted to inhibit gene expression.

CRISPRa: dCas9 fused to a transcriptional activator is targeted to activate gene expression.

6

GENETIC SCREENING

Systematically identify genes involved in biological processes via high-throughput CRISPR screens.

- Genome-wide gene activation and repression screening.[13,14]
- Systematically identify genes involved in biological processes.[15]
- Screen to identify genes involved in disease models and drug response.[16,17]

REFERENCES:

1. M. Jinek et al., "A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity," *Science,* 337(6096):816–821, 2012.
2. L. Cong et al., "Multiplex genome engineering using CRISPR/Cas systems," *Science,* 339(6121):819–823, 2013.
3. P. Mali et al., "RNA-guided human genome engineering via Cas9," *Science,* 339(6121):823–826, 2013.
4. A. Park et al., "CRISPR/Cas9 allows efficient and complete knock-in of a destabilization domain-tagged essential protein in a human cell line, allowing rapid knockdown of protein function," *PLOS ONE,* 9(4): e95101, 2014.
5. R.J. Platt et al., "CRISPR-Cas9 knockin mice for genome editing and cancer modeling," *Cell,* 159(2):440–455, 2014.
6. M. Ratz et al., "CRISPR/Cas9-mediated endogenous protein tagging for RESOLFT super-resolution microscopy of living human cells," *Sci Rep,* 5:9592, 2015.
7. H. Yang et al., "One-step generation of mice carrying reporter and conditional alleles by CRISPR/Cas-mediated genome engineering," *Cell,* 154(6): 1370–1379, 2013.
8. T. Fujita, H. Fujii, "Efficient isolation of specific genomic regions and identification of associated proteins by engineered DNA-binding molecule-mediated chromatin immunoprecipitation (enChIP) using CRISPR", *Biochem Biophys Res Commun,* 439(1):132–136, 2013.
9. B. Chen et al., "Dynamic imaging of genomic loci in living human cells by an optimized CRISPR/Cas system," *Cell,* 155(7):1479–1491, 2013.
10. P.S. Choi, M. Meyerson, "Targeted genomic rearrangements using CRISPR/Cas technology," *Nat Commun,* 5:3728, 2014
11. L.S. Qi et al., "Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression," *Cell,* 152(5):1173–1183, 2013.
12. S. Konermann et al., "Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex," *Nature,* 517(7536):583–588, 2015.
13. L.A. Gilbert et al., "Genome-scale CRISPR-mediated control of gene repression and activation," Cell, 159(3):647-661, 2014.
14. T. Wang et al., "Genetic screens in human cells using the CRISPR-Cas9 system," *Science,* 343(6166):80-84, 2014.
15. S. Chen et al., "Genome-wide CRISPR screen in a mouse model of tumor growth and metastasis," *Cell,* 160(6):1246-1260, 2015.
16. O. Shalem et al., "Genome-scale CRISPR-Cas9 knockout screening in human cells," *Science,* 343(6166):84-87, 2014.

CRISPR–Cas9 Screening Services

Knockout Thousands of Genes - All at Once

Why Horizon?

- Horizon's proprietary all-in one vector system provides improved performance in a rapid workflow
- Optimized screening platform
- Sophisticated bioinformatics analysis pipeline
- Multiple off-the-shelf and custom libraries

PATHFINDER RESEARCH SERVICES

To learn more about CRISPR–Cas9 screening service visit:
www.horizondiscovery.com

horizon

Horizon Discovery is a life science company that supplies research tools and services to organizations engaged in translational genomics research and the development of personalized medicines. Horizon's core capabilities are built around our proprietary translational genomics platform, a high-precision and flexible suite of gene editing tools that include CRISPR, rAAV and ZFNs, enabling us to alter almost any endogenous gene sequence of human or mammalian cell-lines.

We offer over 20,000 catalogue cell line and in vivo products, models that accurately recapitulate the disease-causing mutations found in genetically based diseases. These 'patients-in-a-test-tube' are being used by customers to identify the effect of individual or compound genetic mutations on drug activity, patient responsiveness, and resistance, which may lead to the successful prediction of which patient subgroups will respond to currently available and future drug treatments. Further, we offer a wide range of molecular reference standards, helping research and clinical laboratories reduce the sources of variability in their assays every day.

Finally, we also provide custom cell line and in vivo model generation services for research and bioproduction applications, and a wide selection of specialist contract research and custom screening services (including drug combination and CRISPR-based screening).

Our diverse and international customer base comprises over 1,200 organizations across more than 50 countries, including major pharmaceutical, biotechnology and diagnostic companies, as well as leading academic research centers and clinical labs.

Horizon is headquartered in Cambridge, UK, and is listed on the London Stock Exchange's AIM market under the ticker "HZD."
For further information please visit:
www.horizondiscovery.com

horizon

Contact Horizon to help with any of these CRISPR applications. www.horizondiscovery.com

CAPITALIZING ON CRISPR

Sponsored by

horizon

Custom publishing from

TheScientist
EXPLORING LIFE, INSPIRING INNOVATION

Because we are all unique.

Many diseases have complex genetics that can present similarly, but could have very different outcomes and consequently, different requirements for treatment. Our precisely defined isogenic cell lines and *in vivo* research models provide tools to help researchers understand these complex genetic diseases.

X-MAN® Cell Lines

X-MAN® Cell Lines can be used to study the role of any gene in a variety of cellular processes and thus provide a tractable model for the study of human gene function.

SAGE® *In Vivo* Models

Horizon partners with the leading minds in neurobiology, cardiobiology, immunology and toxicology to develop next-generation *in vivo* models for advanced disease research.

To learn more about Horizon's Engineered Models visit:
www.horizondiscovery.com

horizon