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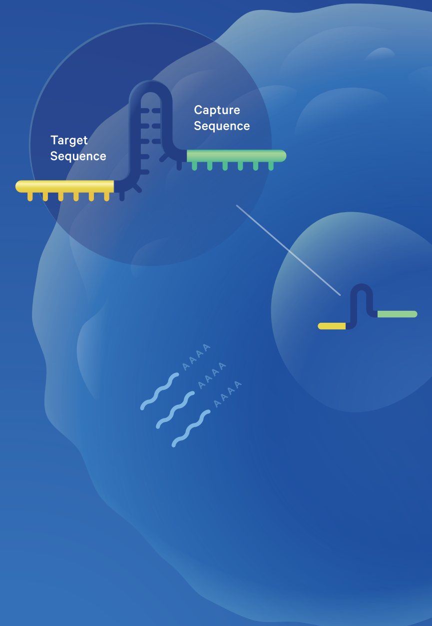
Functional Genomics at Single Cell Resolution

Chromium Single Cell CRISPR Screening



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- Link CRISPR edits to single cell phenotypes with direct capture of guide RNAs
- Scale CRISPR screens by simultaneously assessing hundreds of edits in tens of thousands of single cells
- Reduce time to results from weeks to days with streamlined workflows

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10x Genomics builds solutions to interrogate biological systems at a resolution and scale that matches the complexity of biology. Our rapidly expanding suite of products, which include instruments, consumables, and software, have enabled customers to make fundamental discoveries across multiple research areas, including cancer, immunology, and neuroscience.

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CRISPR-Cas: *The Next Generation*

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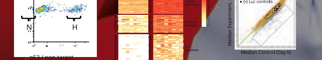

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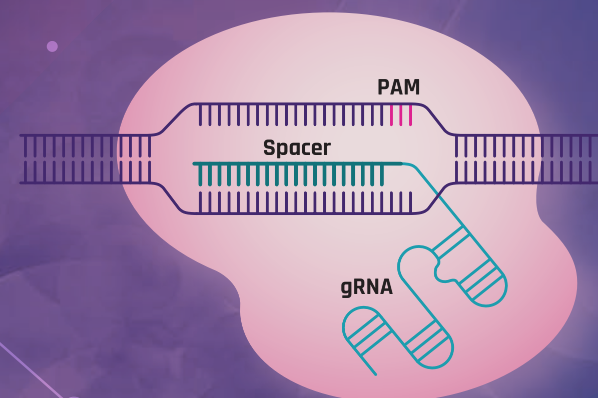
CRISPR-Cas: *The Next Generation*

The development of CRISPR-Cas systems transformed genome engineering. Driven by nucleic acid sequences, CRISPR-Cas targeting made genetic manipulation much more accessible, leading to a wide array of breakthroughs in basic, translational, and medical science.[1]

The CRISPR-Cas success story has inspired scientists to discover and create new CRISPR-Cas systems, including those that can target RNA, epigenetic modifications, or chromatin interactions. The next generation of CRISPR-Cas systems expands the power and potential of CRISPR-Cas, improving biological understanding and inching closer to the ultimate goal of clinical use.[2]

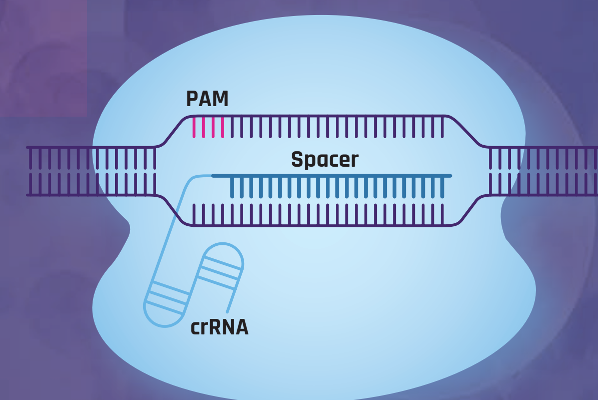
Cas9 (formerly known as Cas5, Csn1, or Csx12) — Class 2 Type II



Cas9 was the first CRISPR associated protein researchers used outside of prokaryotic cells, and it is still the most commonly used genome editing tool today.[2,3] It uses a 20-nucleotide spacer and targets the 5’-NGG (where N represents any nucleotide) protospacer adjacent motif (PAM).[3,4] As a type II system, Cas9 generates double-stranded DNA (dsDNA) cuts with blunt ends.

Researchers improved targeting by engineering Cas9 variants. The 5’-NGG PAM limits target site availability to roughly one per eight base pairs.[1] Cas9 variants or orthologues that recognize different or multiple PAMs—such as xCas9, which recognizes 5’-NG, 5’-GAA, and 5’-GAT—overcome this limitation.[5,6] Engineering secondary structures in guide RNA spacer regions also improves targeting specificity, thereby creating a barrier to strand invasion at off-target sites without overly affecting on-target activity.[7]

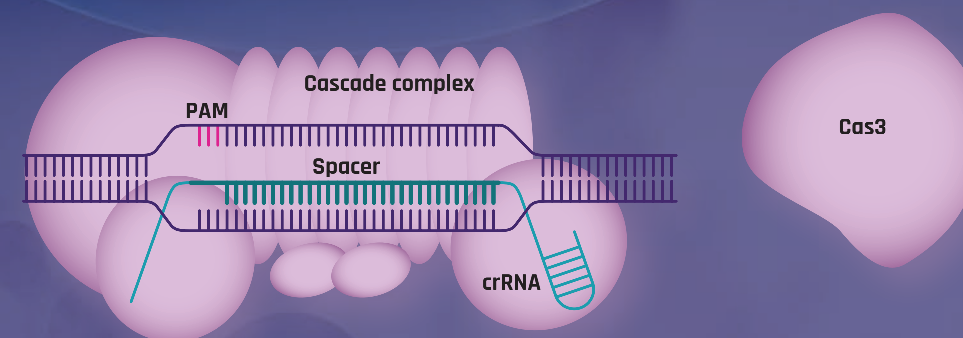
Cas12 (formerly known as Cpf1) — Class 2 Type V



Cas12a is a type V system, which means that it generates a staggered dsDNA cut with a 5’ overhang and does not use a transactivating CRISPR RNA (crRNA). This provides advantages in certain situations, such as integrating DNA sequences in a specific orientation. Cas12 can also generate its own crRNAs by cleaving crRNA arrays, enabling scientists to perform multiplex gene editing using only a single crRNA array.[8]

The first endogenous Cas12a orthologues with activity in mammalian cells recognize the PAM sequence 5’-TTTV. Newer engineered variants not only have higher editing activity for this canonical TTTV sequence, but also recognize and act on other PAMs including 5′-TYCV, 5′-VTTV, 5′-TTTT, 5′-TTCN, and 5′-TATV.[9,10]

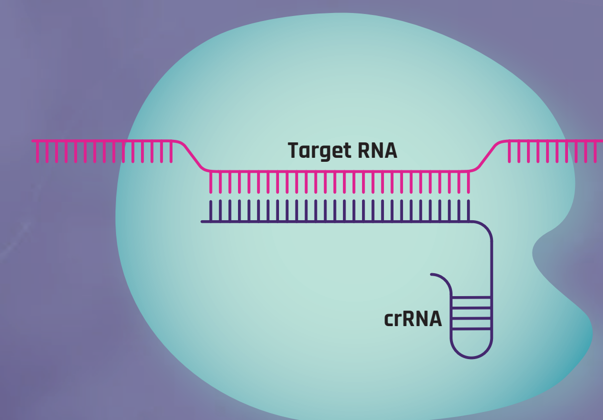
Cascade-Cas3 — Class 1 Type 1



Cascade is a multimeric DNA-targeting complex that binds DNA via PAM and spacer recognition and then recruits Cas3 to generate a single-strand nick, followed by 3′ to 5′ degradation of the targeted DNA.[11,12] Cascade recognizes more PAM sequences than other Cas proteins, giving the Cascade-Cas3 system greater target site flexibility.[13] Researchers are looking to Cas3’s unique cutting mechanism as a antimicrobial tool, given that Cas3 is endogenously essential for the degradation of foreign DNA in prokaryotes.[14]

TARGETING RNA

Cas13 (formerly known as C2c2) — Class 2 Type VI



Unlike most other Cas proteins, Cas13a is an RNA-guided RNA-targeting nuclease that activates upon recognition of ssRNA target sequences.[15] After target binding, Cas13a cuts at uracil bases anywhere in the local vicinity, potentially collaterally cleaving nearby, untargeted RNAs. Researchers used this to create a molecular detection platform aptly named SHERLOCK, where collateral RNA cleavage releases a reporter signal.[16] SHERLOCK detects viral and bacterial pathogens, discriminates between single-nucleotide polymorphisms in the human genome, and identifies cell-free, mutated tumor DNA.[16,17]

Beyond imaging, Cas13 has also been adapted for single-base RNA editing. Consisting of a catalytically deficient Cas13 (dCas13) fused to adenosine deaminase, the REPAIR system makes directed adenosine-to-inosine edits in eukaryotic cells.[18] dCas13 can also be fused with other RNA editing domains to enable cytidine-to-uridine editing.[2]

Modifying Cas9

Cas9 normally targets dsDNA, but it can also target single-stranded (ss) nucleic acids if PAM-presenting oligonucleotides (PAMmers) are used. PAMmers anneal to single stranded DNA or RNA, thereby directing Cas9 to single-stranded targets.[19] Termed ‘RCas9’ (RNA-targeting Cas9), this system allows researchers to detect endogenous RNA without genetically encoded tags and to control cellular processes at the transcript level through site-specific cleavage of ssRNA.[19,20]

A number of Cas9 orthologues, such as *Campylobacter jejuni* Cas9, also target RNA. *C. jejuni* Cas9 binds and cleaves endogenous RNAs without PAM guidance, while *Francisella novicida* Cas9 targets bacterial mRNA and alters gene expression.[21,22] Researchers continue to study any potential physiological consequences of Cas9 RNA targeting in eukaryotic cells.[2]

Beyond On/Off: Dynamic Genetic and Epigenetic Regulation

CRISPR-Cas9 regulates gene function by serving as a DNA recognition complex rather than as a targeted nuclease.[23] For example, binding catalytically deficient Cas9 (dCas9) to DNA elements creates gene silencing steric CRISPR interference (CRISPRi) that hinders RNA polymerases.[24] Additionally tethering dCas9 to transcription repressor domains enhances this effect.[25] The reverse is also possible: fusing dCas9 to activator effectors results in programmed transcription activation, or CRISPR activation (CRISPRa).[26] This enables researchers to direct synergistic gene activation by using CRISPRa with synthetic transcription factors or combining different activator domains, an important feature for cellular reprograming.[27-29] dCas9-based tools also enable targeted epigenetic modifications such as the acetylation and methylation of histones and methylation of DNA.[23]

Cas9 function can be dynamically controlled. Chemical compounds or light, for example, can activate Cas9 expression through inducible promoters. Scientists use this approach to generate animal models for research where timed gene knockout is desired or necessary.[30] Inducible Cas9 function gives researchers efficient, tunable, and reversible disease modeling capability and helps shed light on stem cell differentiation and development mechanisms.[31,32]

An Eye on the Clinic

How CRISPR-Cas technology shapes the future of disease research and medicine

Rather than gene insertion/deletion, gene editing is now the main focus for the CRISPR-Cas system.[2] This has obvious implications for genetic diseases caused by mutations, but editing may be a valid strategy for restoring physiological states in more common, complex diseases. For example, CRISPR-Cas9 disruption of the cholesterol homeostasis gene *Pcsk9* in mice reduced levels of low-density lipoprotein cholesterol.[33] CRISPR-Cas also modulates cells ex vivo to create candidates for cell-based therapeutics. Gene editing approaches have enhanced the properties of autologous T cells for immunotherapy and immunoncology.[34,35]

Before CRISPR-Cas can fully transition into the clinic, scientists need to overcome a number of obstacles. The biggest challenge lies in potential off-target effects and immunogenicity. Optimizing guide RNA selection and screening with greater sensitivity can address the former, while identifying and re-engineering immunogenic epitopes may ameliorate the latter.[2] Finally, adeno-associated viruses, the most popular delivery vector for CRISPR-Cas machinery, have limited capacity. Faced with this, researchers are investigating smaller Cas protein orthologues as well as non-viral delivery methods such as lipid nanoparticles.[36]