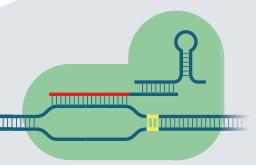
# **CRISPR Systems: What's the Difference?**

With all the various CRISPR-Cas systems being developed, how do you know which is right for your business? Cell editor April Pawluk breaks down the difference.

# **CRISPR-Cas9**



# Type II CRISPR-Cas systems (comprised of subtypes II-A, II-B, and II-C)

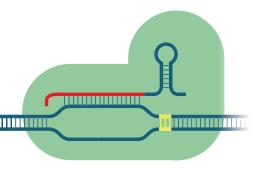
Organisms: Streptococcus pyogenes, Streptococcus thermophilus, Staphylococcus aureus, Neisseria meningitidis, Campylobacter jejuni Size: ~1,000-1,600 aa

Guide spacer length: 18-24 nt

Total guide length: ~100 nt (sgRNA)
PAM: 3'-NGG (SpCas9), 3'-NNGRRT (SaCas9), 3'-NNNNGATT (NmCas9)
Cut: Blunt-ended dsDNA break

The first and best-characterized single-protein CRISPR effector. Cas9 makes a blunt double-stranded DNA break, which can then be repaired by either non-homologous end joining or homologous recombination with a donor template DNA to create site-specific edits. Type II-A Cas9s generally have high genome editing efficiency, but off-target cleavage at unintended genome sites can be a disadvantage. Variants have been engineered to overcome these limitations, and type II-C Cas9s tend to have naturally higher fidelity.

#### CRISPR-Cas12



# Type V CRISPR-Cas systems (comprised of subtypes V-A and V-B) Also known as Cpf1 (type V-A) or C2c1 (type V-B)

Organisms: Francisella novicida, Acidaminococcus sp.,

Lachnospiraceae sp., Prevotella sp.

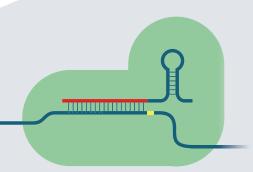
Size: ~1,100-1,300 aa

Guide spacer length: 18-25 nt Total guide length: 42–44 nt PAM: 5'-TTTN (FnCas12a)

Cut: 5 nt 5'overhang dsDNA break

Cas12 is a compact and efficient enzyme that creates staggered cuts in dsDNA. Cas12 processes its own guide RNAs, leading to increased multiplexing ability. Cas12 has also been engineered as a platform for epigenome editing, and it was recently discovered that Cas12a can indiscriminately chop up single-stranded DNA once activated by a target DNA molecule matching its spacer sequence. This property makes Cas12a a powerful tool for detecting tiny amounts of target DNA in a mixture.

# CRISPR-Cas13



## Type VI CRISPR-Cas systems (comprised of subtypes VI-A, VI-B, VI-C, and VI-D) Also known as C2c2 or CasRx (type VI-D)

Organisms: Leptotrichia buccalis, Leptotrichia shahii, Ruminococcus flavefaciens, Bergeyella zoohelcum, Prevotella buccae, Listeria seeligeri

Size: ~900-1,300 aa

Guide spacer length: 22-30 nt Total guide length: 52-66 nt

PAM: 3'-H (LshCas13a), 5'-D and 3'-NAN or NNA (BzCas13b), none (RfCas13d)

Cut: ssRNÀ

Cas13 is an outlier in the CRISPR world because it targets RNA, not DNA. Once it is activated by a ssRNA sequence bearing complementarity to its crRNA spacer, it unleashes a nonspecific RNase activity and destroys all nearby RNA regardless of their sequence. This property has been harnessed in vitro for precision diagnostics. These systems can also be used for efficient, multiplexable, and specific RNA knockdown or RNA sequence editing in mammalian cells. This makes Cas13 a potentially significant therapeutic for influencing gene expression without altering genome sequence.

