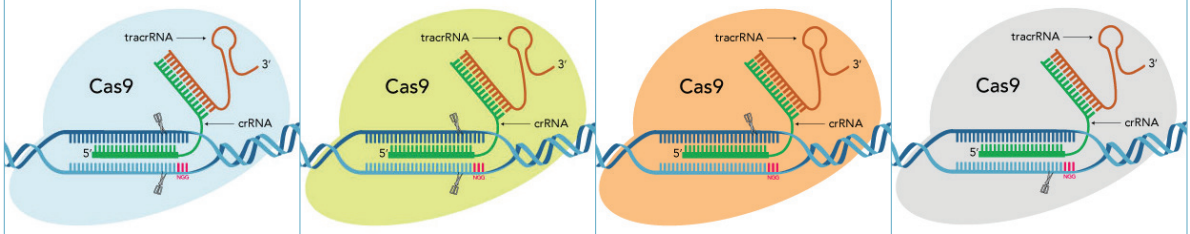
Comparison of Alt-R S.p. Cas9 nuclease with its variants

	Alt-R S.p. Cas9 Nuclease	Alt-R S.p. HiFi Cas9 Nuclease	Alt-R S.p. Cas9 D10A Nickase	Alt-R S.p. Cas9 H840A Nickase
				
Description	Wild-type Cas9 with high genome editing potency that is simple to use and economical	Cas9 variant with improved specificity based on reduced off-target effects, while preserving high on-target activity	Cas9 variant with a mutation in the RuvC domain that disables cleavage of the non-target strand	Cas9 variant with a mutation in the HNH domain that disables cleavage of the target strand
DNA cleavage	Both strands	Both strands	Target strand	Non-target strand
Suggested use	First choice for most CRISPR genome editing projects	Ideal for experiments that are sensitive to off-target events and require a high level of editing efficiency	May be beneficial for homology-directed repair (HDR) experiments, but requires two suitable cutting sites within an optimal distance of each other	
Molecular weight	162,200 g/mol			
Amount provided	100 µg or 500 µg			
Concentration	10 mg/mL (62 µM) in 50% glycerol			
Shipping conditions	Dry ice			
Storage conditions	–20°C at stock concentration			
Dilution	Dilute in Opti-MEM® medium (Thermo Fisher) or PBS before use			

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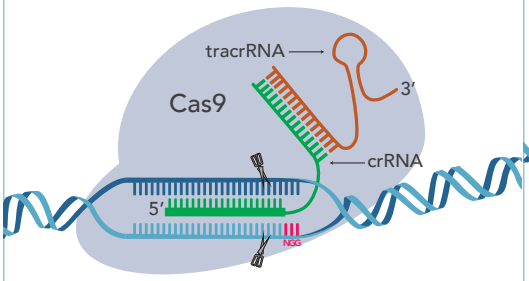
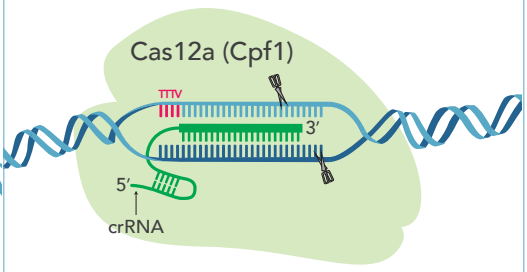
Comparison of CRISPR genome editing using Cas9 vs. Cas12a (Cpf1)

	Cas9 system	Cas12a system
		
Applications	General genome editing	• For species with AT-rich genomes • For regions with limiting design space for use of the CRISPR-Cas9 system
Ribonucleoprotein components	• gRNA options: 1. crRNA and tracrRNA 2. sgRNA • Cas9 endonuclease	• crRNA • Cas12a endonuclease
Alt-R CRISPR enzymes	• Wild-type • HiFi • Nickases (D10A and H840A)	Wild-type
Cas9 crRNA:tracrRNA (option 1)	crRNA • Native: 42 nt • Alt-R: 35–36 nt (36 nt recommended) tracrRNA • Native: 89 nt • Alt-R: 67 nt	—
Cas9 sgRNA (option 2)	• Alt-R: 99–100 nt (100 nt recommended)	—
Cas12a crRNA	—	• Native: 42–44 nt • Alt-R: 40–44 nt (41 nt recommended)
CRISPR enzyme	• Class 2, Cas type II • M.W.*: 162,200 g/mol • Endonuclease domains: RuvC-like and HNH	• Class 2, Cas type V • M.W.*: 156,400 g/mol • Endonuclease domain: RuvC-like only
Double-stranded DNA cleavage	• Wild-type and HiFi: blunt-ended cut 3 bases upstream of the protospacer sequence • D10A nickase with paired crRNAs: 5′ overhang • H840A nickase with paired crRNAs: 3′ overhang • PAM site often destroyed during genome editing	• 5′ overhanging cut on the 5′ side of the protospacer sequence • PAM site may be preserved after genome editing
PAM sequence†	NGG	TTTV
Current recommendations for Alt-R RNP delivery	• Lipid-mediated transfection • Electroporation ± Alt-R enhancer • Microinjection	• Electroporation with Alt-R enhancer • Microinjection

* Molecular weight of Alt-R nuclease

† N = any base; V = A, C, or G