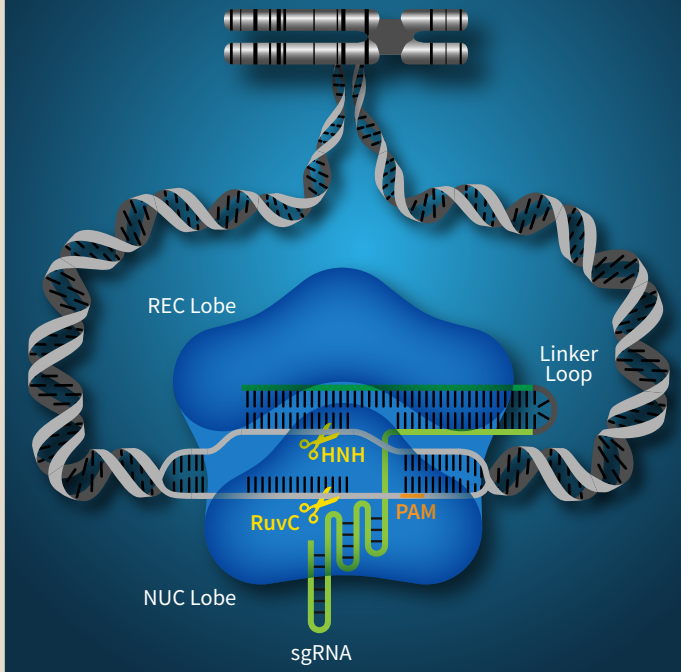
# Cas9 Introduces Targeted DNA Breaks



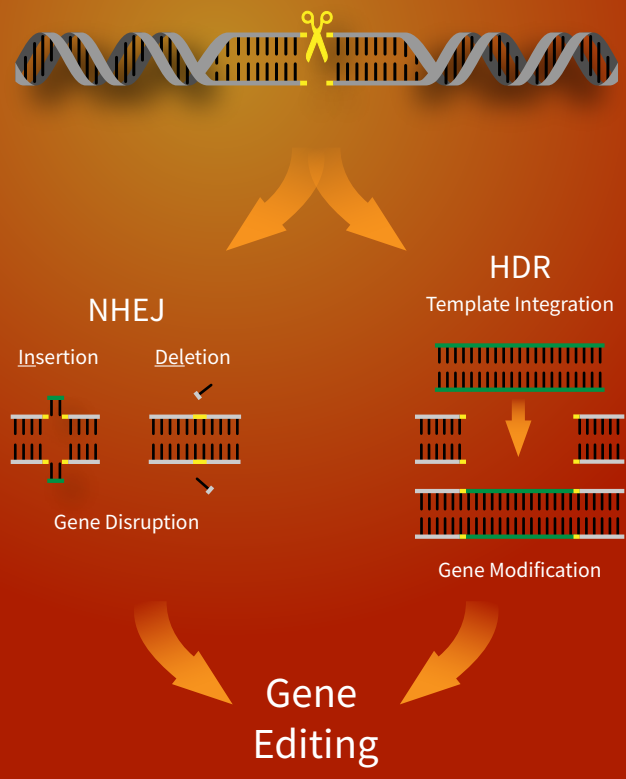

By joining **tracrRNA** and **crRNA** derived sequences with a **linker loop,** a simplified programmable tool for DNA manipulation is created. The **tracrRNA**-**crRNA**-chimera is called a single guide RNA (**sgRNA**). sgRNA can be easily synthesized. The complex, consisting of Cas9 and sgRNA, scans DNA for the presence of a protospacer adjacent motif (**PAM**). For Cas9 from *Streptococcus pyogenes*, this is a 5′-NGG-3′ sequence. When a PAM sequence is detected, the complementary DNA strand is compared to the crRNA derived guide region. If these sequences match, the DNA double strand is cleaved ~3 bp away of the PAM. This introduces a double-strand break (DSB).

With both domains located in the **NUC** lobe of Cas9, the **HNH** domain cuts the strand complementary to the guide sequence (target strand) while the **RuvC** domain cuts the opposite strand. A DSB introduced precisely at a desired genetic site provides a tool for targeted genetic manipulations.

*References:* Nishimasu H et al. *CELL* 2014, Hsu P et al. *CELL* 2014



# Double-Strand DNA Breaks Allow Gene Editing




When a double-strand break (**DSB**) is introduced in a cell's DNA, there are two major repair pathways. In one pathway the blunt DNA ends are joined in a process called non-homologous end joining (**NHEJ**). This mechanism is error-prone and may produce **insertions** or **deletions** of bases. These alterations are collectively called Indel mutations. Indel mutations may result in a loss of function of the affected gene, e.g., by introducing a frameshift and/ or a premature stop codon.

Another pathway is called homology directed repair (**HDR**). This pathway uses a DNA template to repair the site where the DSB occurred. When exogenous DNA is added, the sequence may serve as a template and is then integrated into the repair process. This may lead to the introduction of a new genetic sequence. Both repair pathways may result in a genetic alteration. Thus, targeted introduction of DSBs opens the door for gene editing.

*Reference:* Doudna JA and Charpentier E *SCIENCE* 2014.



# Fields of Application for Cas9 Technology


By introducing double-strand breaks into a living cell's genomic DNA, CRISPR-Cas9 technology provides a potential molecular tool. This technology enables precise targeting of genes in live organisms to render them accessible to modifications. This technique has already been shown to work successfully in a variety of species and seems to be applicable to virtually any organism. In maize plants, CRISPR-Cas9 technology has been employed to modify genes to increase herbicide resistance. In mice, a multitude of mutants have been produced, often serving as models to analyze human diseases. Genetic modifications in animals are feasible in humans too. Data have already been published from experiments on human embryos.

For this reason, a group of scientists, led by those who have invented the technique, have called for a public discussion on the responsible use of CRISPR-Cas9 technology.

*Reference:* Baltimore D et al. *SCIENCE* 2015.