The CRISPR/Cas9 system for targeted genome editing

A powerful method for disrupting your gene of interest

Although recently-developed programmable editing tools, such as zinc finger nucleases and transcription activator-like effector nucleases, have significantly improved the capacity for precise genome modification, these techniques have limitations. CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 technology represents a significant improvement over these other next-generation genome editing tools, reaching a new level of targeting, efficiency, and ease of use. The CRISPR/Cas9 system allows for site-specific genomic targeting in virtually any organism.

The type II CRISPR/Cas system is a prokaryotic adaptive immune response system that uses non-coding RNAs to guide the Cas9 nuclease to induce site-specific DNA cleavage. This DNA damage is repaired by cellular DNA repair mechanisms, either via the non-homologous end joining DNA repair pathway (NHEJ) or the homology directed repair (HDR) pathway.

The CRISPR/Cas9 system has been harnessed to create a simple, RNA-programmable method to mediate genome editing in mammalian cells, and can be used to generate gene knockouts (via insertion/deletion) or knockins (via HDR). To create gene disruptions (Figure 1), a single guide RNA (sgRNA) is generated to direct the Cas9 nuclease to a specific genomic location. Cas9-induced double strand breaks are repaired via the NHEJ DNA repair pathway. The repair is error prone, and thus insertions and deletions (INDELs) may be introduced that can disrupt gene function.

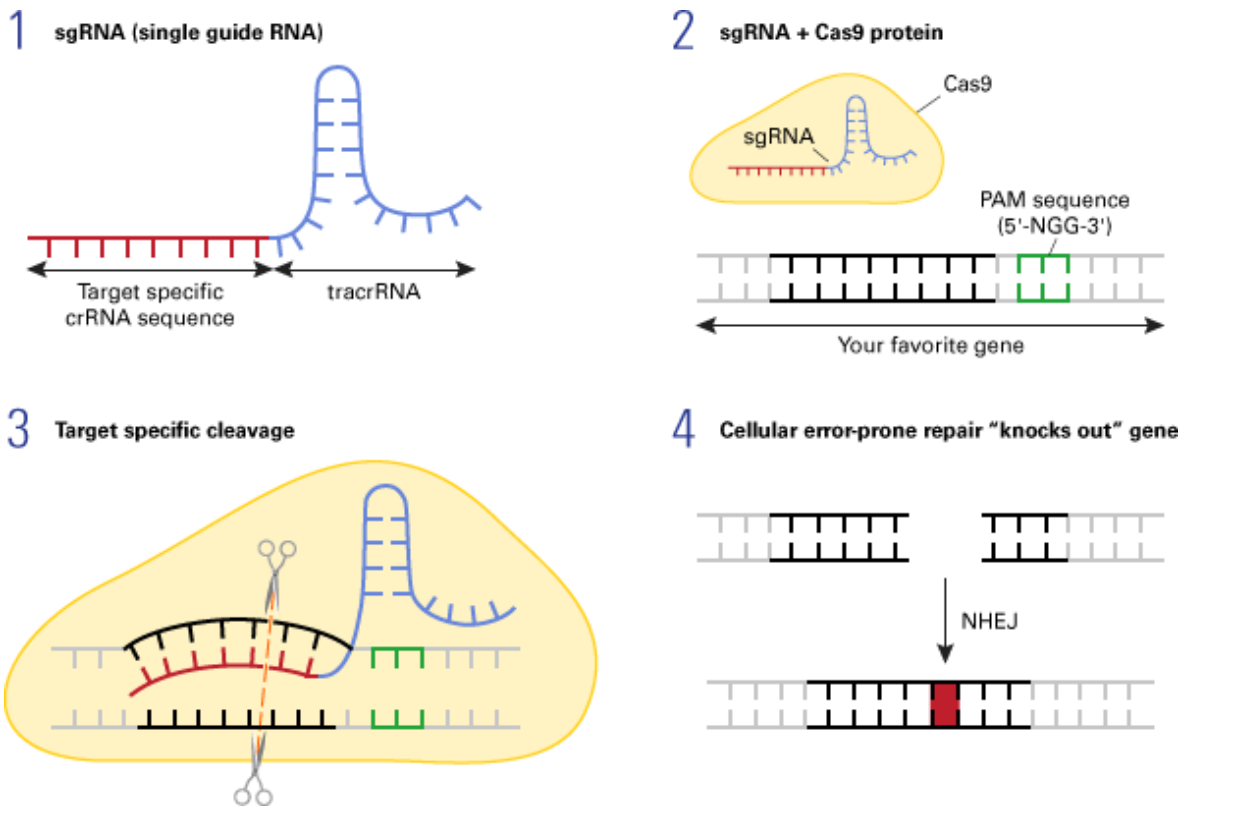

Figure 1. The principle of CRISPR/Cas9-mediated gene disruption. A single guide RNA (sgRNA), consisting of a crRNA sequence that is specific to the DNA target, and a tracrRNA sequence that interacts with the Cas9 protein (1), binds to a recombinant form of Cas9 protein that has DNA endonuclease activity (2). The resulting complex will cause target-specific double-stranded DNA cleavage (3). The cleavage site will be repaired by the non-homologous end joining (NHEJ) DNA repair pathway, an error-prone process that may result in insertions/deletions (INDELs) that may disrupt gene function (4).

CRISPR/Cas9 technology has revolutionized genome editing, allowing a previously unattainable level of genomic targeting, efficiency, and simplicity. Guide-it products further improve the usability of the CRISPR/Cas9 system by providing a streamlined method for:

- Producing sgRNAs *in vitro*
- Testing the cleavage efficiency of sgRNAs
- Monitoring the efficiency of genome editing in cultured cells

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