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CRISPR-Cas9 genome editingâ€”leading in engineering, inspiring design, and empowering discovery

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With increasing expansion into research areas of more biological relevance, existing molecular and cellular techniques need to be improved. The area of genome editing is rapidly growing and requires more advanced techniques to maximize its potential applications. Transcriptional activator-like effector nucleases (TALENs) and technology derived from clustered regularly interspaced short palindromic repeats (CRISPRs) allow precise cleavage of DNA at specific loci. However, the effectiveness of these tools is contingent upon the intrinsic properties of the locus of interest, efficient delivery, and the painstaking downstream processes of generating stable cell lines and knockout models to study the phenotypic effects of such genetic modifications.

CRISPRs and CRISPR-associated (Cas) proteins are found in many bacteria and most archaea. The CRISPR-Cas systems use sequences derived from plasmids and phages to activate Cas endonucleases to neutralize those plasmids and phages via RNA-guided sequence-specific DNA cleavage, thus blocking their transmission and creating a simple acquired immunity.

With their highly flexible but specific targeting, CRISPR-Cas systems can be manipulated and redirected to become powerful tools for genome editing. CRISPR-Cas technology permits targeted gene cleavage and gene editing in a variety of eukaryotic cells, and because the endonuclease cleavage specificity in CRISPR-Cas systems is guided by RNA sequences, editing can be directed to virtually any genomic locus by engineering the guide RNA sequence and delivering it along with the Cas endonuclease to a target cell.

Thermo Fisher Scientific provides an easy-to-use, optimized and validated genome editing solution, designed to expedite life science research. The Thermo Fisher Scientific solution spans the entire cell engineering workflow, making genome editing accessible to anyone at any level. And Thermo Fisher will continue to expand its suite of genome editing products, from cell culture and delivery reagents and sample preparation, to genome modification, detection and analysis of known genetic

variants.

An online CRISPR search and design tool along with CRISPR-Cas9 is available to researchers in four formats: an all-in-one expression vector, Cas9 mRNA, Cas9 protein, and CRISPR libraries services. These gene editing solutions are paired with the optimal cell culture reagents, delivery method, and analysis tools based on required application and cell type.

CRISPR Search & Design tool allows scientists to search a database of >600,000 predesigned CRISPR gRNAs in human and mouse genes or analyze their sequence of interest for de novo gRNA designs using algorithms. The tool is designed to analyze genes of interest, identify gRNA sequences adjacent to protospacer adjacent motif (PAM) sites, and rank order resulting gRNAs based on potential off-target effects. Up to 25 gRNA sequences per gene are provided with recommendations based on potential off-target effects for each CRISPR sequence.

Researchers may improve the cleavage efficiency up to 85 percent by pairing Cas9 Nuclease and Transfection Reagent. Cas9 protein and guide RNA (gRNA) form a very stable ribonucleoprotein (RNP) complex that provides the next level of cleavage efficiency over CRISPR-Cas9 vector and mRNA-based systems when paired with Transfection Reagent. The Cas9 RNP complex can act immediately after it enters the cell, since transcription and translation are not required.

Moreover, the complex is rapidly cleared from the cell, minimizing the chance for off-target cleavage events when compared to vector-based systems while eliminating time-consuming cloning steps.

Transfection reagent for CRISPR-Cas9 protein delivery provides the cleavage efficiency of electroporation with the simplicity and scalability of a reagent. It is an ideal alternative to electroporation as it is gentler on cells and more cost effective overall features and benefits include:
Low cell toxicity- fewer cells needed to initiate your experiment
Cost savings-including both cost-per-reaction and initial investment
Easy scalability-an ideal delivery solution for high-throughput experiments

Advances in genome modulation and editing have the potential to change the way we create energy, produce food, optimize industrial processing, and detect, prevent and cure diseases-improving the human condition and the world around us. Thermo Fisher's trusted products harness the power of science to transform lives. Its instruments, routine tools and services offer high-quality, innovative life science solutions for every lab.