Harnessing CRISPR to boost NGS sensitivity with CRISPRclean™

The revolutionary power of the CRISPR system

The CRISPR gene editing system has revolutionized biological and medical research. CRISPR consists of the Cas9 enzyme that targets DNA for cleavage in a site-specific manner when complexed with CRISPR guide RNA (gRNA) (Figure 1). Originally evolved to degrade viral genomes, the CRISPR system was later modified by scientists to become an important molecular tool. The adaptability of the CRISPR system enabled researchers to precisely target locations in the genome in a broad range of applications, such as gene editing, gene therapy, functional library screening, and more.

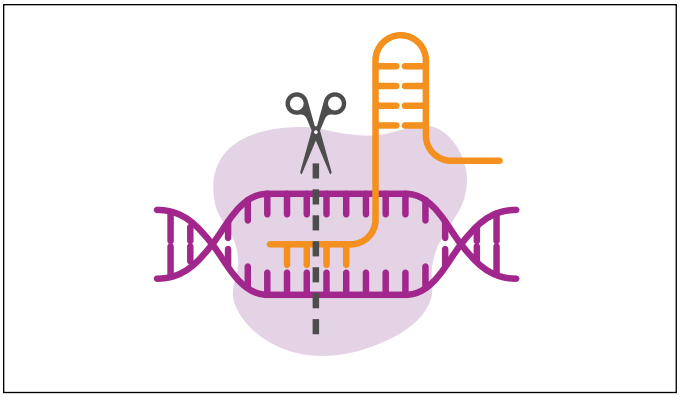

Figure 1: The CRISPR system. CRISPR guide RNA (gRNA, in orange) directs the Cas9 enzyme to a DNA sequence (purple) with complementarity to a 20 nucleotide target-specific sequence in the gRNA and Cas9 cleaves at that site.

CRISPRclean technology harnesses the specificity of CRISPR to refine NGS libraries

Like CRISPR, next-generation sequencing (NGS) technology has also revolutionized the research world. The ability to sequence genomes, transcriptomes, and epigenomes without *a priori* knowledge has yielded an unprecedented amount of discoveries about genes and genetic variation. As NGS technology continues to advance with innovations to increase sensitivity and throughput while lowering costs, analysis of previously elusive samples are possible with unprecedented depth, such as single cell transcriptomes, pathogen detection, or complex microbial communities.

Although NGS provides millions of reads from a given sample, much of the sequencing information can be biologically uninformative. This is particularly the case for RNA sequencing when ribosomal RNA (rRNA) comprises up to 90% of total RNA in both eukaryotes and prokaryotes. With CRISPRclean, >99% of rRNA is removed and thus, refining the sample to focus on more informative sequences can increase sensitivity by lowering background from abundantly expressed genes and may also save significantly on sequencing costs.

CRISPRclean technology harnesses the specificity of CRISPR to degrade abundant, uninformative sequences in prepared NGS libraries. This method, which can be completed in approximately 4 hours, is seamlessly integrated into NGS workflows (Figure 2) by adding Cas9/RNA complexes to the DNA of prepared libraries. The Cas9/RNA complexes consists of a pool of guide RNAs that target and deplete unwanted sequences in each application, but the CRISPR mechanism does not rely upon specific sequencing platforms.

Legacy targeted resequencing approaches trade off the risk of introducing bias with the benefit of improved sensitivity of desired genes or transcripts. CRISPRclean differs from these methods by performing negative selection of specific abundant, uninformative sequences, thus maintaining the ability to analyze informative sequences without *a priori* knowledge of which transcripts or variants will be relevant in a given study. Instead of fishing for a needle in a haystack, CRISPRclean removes the haystack, enabling analysis of the remaining "needles." This results in increased sequencing coverage across genes or transcripts of interest that remain in a library.

CRISPRclean technology is both specific and highly programmable towards unwanted sequences in any given application. Beyond ribosomal RNA depletion,

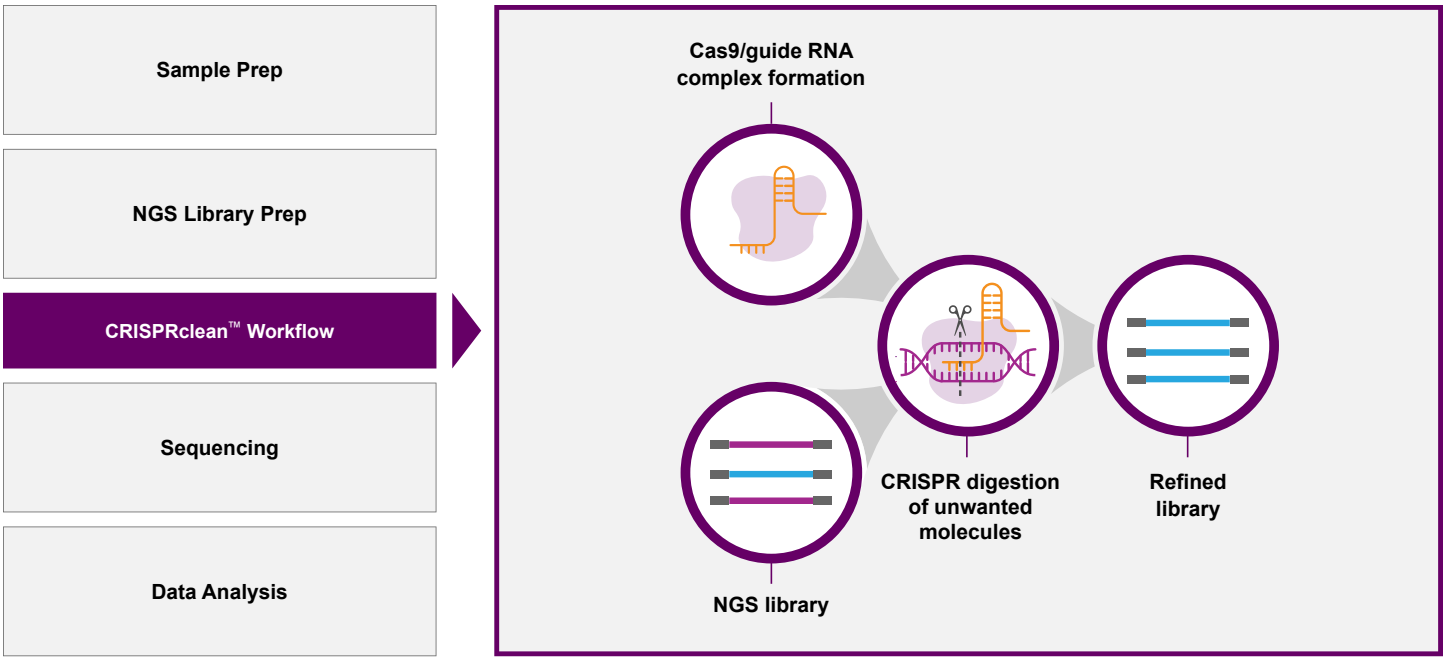

Figure 2: CRISPRclean mediated depletion fits seamlessly into NGS workflows. The CRISPRclean degradation step functions independently of sequencing platform. After degradation of unwanted sequences (purple), informative, lower abundance sequences (blue) are refined for sequencing. The entire workflow can be completed in about four hours.

CRISPRclean can be designed to deplete unwanted sequences from virtually any application. For example, a range of highly expressed genes that are relevant to specific tissues can be depleted during single-cell analysis. For DNA applications, CRISPRclean technology can target repetitive elements or other undesired wild-type loci.

Other key features of CRISPRclean technology add further flexibility towards a broad range of applications. By using negative selection, in which the targeted "haystack" removal approach only requires information about targeted sequences, CRISPRclean technology retains the ability to discover new variants of low abundance with its agnostic approach. Furthermore, the post-library depletion workflow has enabled CRISPRclean to access ultra low input samples with RNA inputs down to picogram amounts while maintaining performance, thus demonstrating its utility for samples from which nucleic acids are difficult to obtain.

The CRISPRclean workflow

CRISPRclean depletion (Figure 3) uses prepared NGS library as input. CRISPR complexes are formed with a pool of designed guide RNAs, and the complexes are mixed with the library DNA. After targeted sequences are cut, they cannot be substrates for PCR amplification and subsequent sequencing. The refined library (non-targeted sequences) is size-selected using magnetic beads, and then amplified a small number of rounds. The output is a refined, sequence-ready NGS library.

Several approaches to DNA analysis involve enrichment for selected genes or loci by positive target selection, either through primer-directed amplification or oligo-based hybrid capture. By performing negative selection of specific selected sequences, CRISPRclean technology maintains an unbiased approach to analysis of informative sequences that may include undiscovered variants.

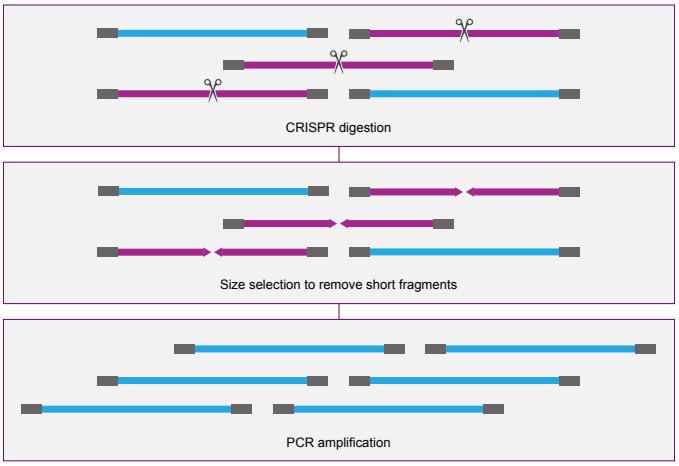

Figure 3: The CRISPRclean workflow.

Benefits of CRISPRclean technology

- Highly programmable
- Achieves efficient depletion
- Increased sensitivity
- Simple 4-hour workflow
- Access lower input amounts and sample types

Summary

CRISPRclean is a novel method for depletion of abundant or uninformative sequences in NGS libraries. It can increase the ability to detect rare transcripts in RNA applications, or analyze low-abundance variants in DNA applications, by increasing depth of coverage of informative sequences. The reduction of high background associated with abundant sequences further adds confidence to sequencing data for detection of rare sequences. Furthermore, focusing sequencing platforms on the most relevant information in genomic and transcriptomes can significantly reduce costs.

Examples of applications which CRISPRclean technology can be applied to are ribosomal removal, metatranscriptomics, infectious disease pathogen detection, host immune response profiling, single cell analysis, and more.

To learn more, visit jumpcodegenomics.com

Ordering information

Contact your sales representative to learn more

Catalog	Product	Designed to deplete
KIT1000	CRISPRclean Human rRNA Depletion Kit	Human 5S, 5.8S, 18S, 28S, 45S (precursor), mito 12S and 16S rRNA
KIT1001	CRISPRclean Pan Bacterial rRNA Depletion Kit	Over 200 bacteria covering all phyla: 5S, 16S, and 23S rRNA
KIT1005	CRISPRclean Metatranscriptomic rRNA Depletion Kit	Human 5S, 5.8S, 18S, 28S, 45S, mito 12S and 16S rRNA, and bacteria 5S, 16S, and 23S rRNA
KIT1006	CRISPRclean Housekeeping Genes 100 Depletion Kit	10 mitochondrial and 90 ribosomal protein coding transcripts (mRNA with poly A tails)
KIT1012	CRISPRclean Globin Depletion Kit	*HBA1, HBA2, HBB, HBD* genes