Trendalert

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Trendalert - CRISPR-cas9 Targeted Enrichment Metagenomics

Summary

Ensuring safe drinking water requires the ability to detect pathogens, virulence genes, antimicrobial resistance genes (ARGs), and indicator organisms even at very low concentrations. However, commonly used molecular methods, such as 16S rRNA gene sequencing, metagenomics, and metatranscriptomics, often lack the sensitivity needed to identify rare or low-abundance targets, especially in low-biomass environments such as drinking water.

CRISPR-based targeted metagenomics offers a solution by enabling precise selection, enrichment, and sequencing of specific low-abundant DNA or RNA from microorganisms of interest.

CRISPR-Cas9-enriched metagenomics uses the Cas9 protein, guided by a custom-designed RNA, to cut out only the DNA sequences of interest from a complex mix. This "enriches" the amount of target DNA in the sample, making it easier to study specific microbes or genes.

Studies using CRISPR-Cas9 targeted enrichment metagenomics have successfully detected low-abundance microorganisms. Given its proven value in other fields, we recommend testing this technique on drinking water at the KWR lab within the exploring research program of Waterwijs.

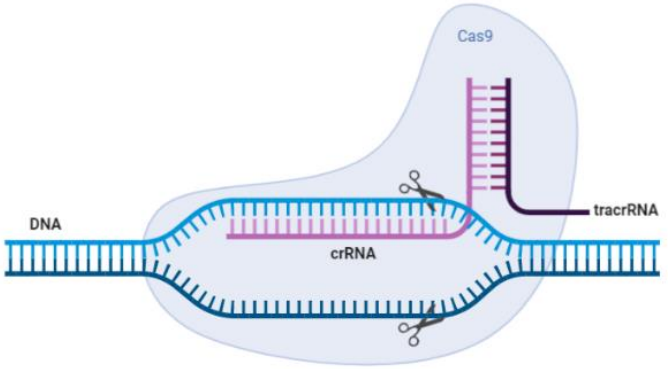

Figure 1. ***Components of the CRISPR-Cas9 System for directing Cas9 endonuclease to genomic targets. (BioRender)***

Consequences

	Low	Average	High	Comments
Impact			X	
Certainty		X		

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Trend description and background

Molecular microbiological methods

Molecular microbiology uses DNA- and RNA-based tools to detect microbes, assess functions, and monitor risks. Common methods include quantitative-PCR (qPCR) and NGS. qPCR is sensitive and specific for known targets but limited in detecting multiple targets. NGS methods like 16S rRNA gene sequencing provide community profiles but may introduce PCR bias. Shotgun metagenomics and metatranscriptomics avoid primer bias and offer broader insight into microbial diversity and function.

Current limitations

A limitation of applying NGS methods in drinking water is that it is often biased towards abundant species, making rare organisms harder to detect due to the even distribution of reads across all DNA. However, many pathogens or other nuisance organisms are often present at low concentrations in drinking water, making detection by NGS difficult.
In the past, hybridization-based probe capture has shown to be able to enrich specific targets, but the method remains limited by scalability and efficiency. More sensitive, targeted methods are, therefore, still needed. CRISPR (Clustered-Regularly-Interspaced-Short-Palindromic-Repeats)-cas (CRISPR-associated proteins)–based targeted metagenomics might offer a promising solution by precisely enriching DNA sequences of interest, enhancing sensitivity in low-abundance samples like drinking water. This approach can reduce background sequencing and might improve detection of targets often missed by untargeted metagenomic workflows.

CRISPR-cas9 targeted metagenomic

CRISPR-cas9, a bacterial immune system against bacterial viruses, has been adapted for gene editing and targeted enrichment of DNA or RNA in complex samples. In this system, the Cas9 DNA-cutting enzyme is directed to a specific DNA sequence by a ribonucleoprotein (RNP) complex made of CRISPR-RNA (crRNA) containing a sequence complementary to the target DNA, and trans-activating-CRISPR-RNA (tracrRNA) (Fig. 1).
Cas9 recognizes a short sequence next to the target, called the protospacer adjacent motif (PAM), and introduces a double-stranded break. The exposed DNA ends can then be ligated to sequencing adapters, enabling selective enrichment of regions of interest, even in samples with high background DNA (Malekshoar et al. 2023).
CRISPR-Cas9-based enrichment improves sensitivity and is compatible with both Illumina (short-read) and Oxford Nanopore Technologies (ONT) (long-read) sequencing platforms.

Illumina short reads

Quan et al. (2019) first combined CRISPR-cas9 with Illumina sequencing to enrich 127 antimicrobial resistance genes (ARGs) in *Staphylococcus aureus* and *Enterococcus faecium*, demonstrating high sensitivity and multiplexing. Mao et al. (2025) extended this by designing over 6,000 guide RNAs, detecting 1,189 additional ARGs in wastewater with a tenfold increase in sensitivity compared to the regular NGS method.
Both studies used multiplexed targeting, but synthesizing large crRNA and tracrRNA sets can be costly. Mao et al. (2025) addressed this through optimized design strategies.
A limitation of using Illumina to sequence the enriched targets, is the need for adapters at both ends of a DNA fragment (~100–300 bp), requiring at least two guide RNAs per target. Short reads also limit contextual information, structural variant detection, and epigenetic analysis. Additionally, PCR amplification is needed before sequencing, which can introduce bias.

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Despite these constraints, Illumina still offers high accuracy for small variant detection and remains widely used for targeted enrichment applications. The Illumina CRISPR-cas9 targeted metagenomic workflow is illustrated in Fig.2.

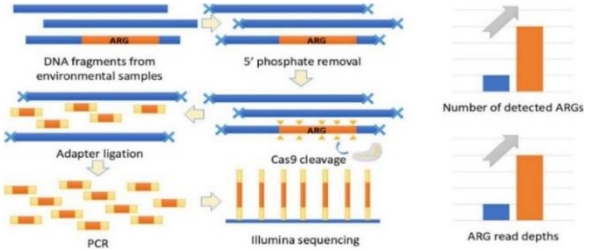

Figure 2 Illumina CRISPR-cas targeted metagenomic, enrichment with PCR before Illumina short reads sequencing (Mao et al. 2025).

Long read sequencing

CRISPR-Cas9 can be paired with ONT, is PCR-free and a direct analysis of DNA. Unlike Illumina, ONT does not require fragment amplification, each DNA strand is sequenced individually.

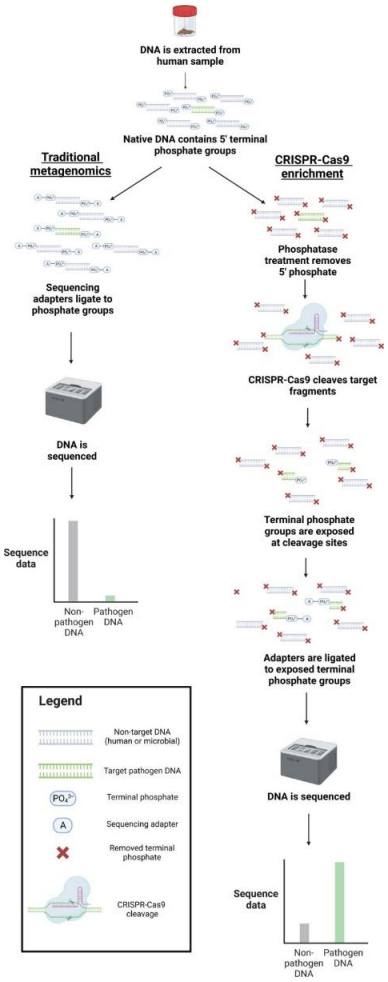

Figure 3 ONT library preparation differences between unenriched and CRISPR-Cas9 enriched sequencing. During unenriched sequencing, sequencing adapters are ligated to native terminal phosphate groups on DNA molecules to allow for sequencing. During CRISPR-Cas9 enrichment, native phosphate groups are removed from all DNA molecules so that adapters cannot ligate. CRISPR-Cas9 is then used to cleave molecules of interest, exposing their terminal phosphate groups and allowing for specific adapter ligation and sequencing (Cottingham et al. 2025).

Cas9 cuts at target sites, after which ONT-specific adapters are ligated, enabling direct sequencing from one or both ends. While up to five targets are supported by default, higher multiplexing (up to ~20) is possible with further validation.
ONT's long reads provide richer genomic context, structural variant detection, and epigenetic insights. Studies using CRISPR-ONT include antimicrobial resistance (Serpa et al. 2022, Sajuthi et al. 2020, Baltrus et al. 2019), cancer and epigenetics (Gilpatrick et al., 2020), plant genomics (López-Girona et al., 2020), and multiplexed microbial, nematodes, and fungi community profiling (Nikolaeva-Reynolds et al., 2025). Cottingham et al. (2025) showed enhanced detection of ARGs and MLST markers in *Klebsiella pneumoniae*, with more than 50 times read improvement and reduced data volume. ONT provides kits and guide design tools to support CRISPR-based targeted long-read workflows (link). The ONT CRISPR-cas9 targeted metagenomic workflow is illustrated in Fig.3.

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Relevance

Possible applications

The enhanced sensitivity of CRISPR-cas9-enriched metagenomic methods is likely to enable:

- Detection of low-abundance (nuisance) microorganisms: Traditional metagenomic approaches often fail to detect rare microbial taxa in low-biomass environments. CRISPR-cas9 enrichment can selectively amplify sequences of interest, enabling the detection of microorganisms that would otherwise go unnoticed. For instance, Quan et al. (2019) demonstrated that the FLASH method could detect specific genes of *S. aureus* from as little as 100 femtograms of input DNA (approximately 35 copies of the *S. aureus* genome in 30 µL), with all target genes detected at ≥10 reads per million.
- Targeted detection of functional genes of interest such as ARGs: CRISPR-cas9-enriched metagenomics enables the sensitive and specific detection of ARGs, even at extremely low concentrations. This facilitates a more complete understanding of the resistome in low-biomass environments such as drinking water. In wastewater-based surveillance (link), this method allows public health authorities to detect emerging resistance threats early and monitor the spread of ARGs across different bacterial hosts and environmental compartments.
- The combination of CRISPR-cas9 enrichment with metatranscriptomics offers potential for detecting actively expressed genes in low-biomass environments like drinking water. This approach, though underexplored, could minimize bias from stress-induced gene expression caused by filtration, enabling more accurate detection of functional microbial activity.

Potential limitations

- Insufficient starting material: If DNA or RNA levels fall below the detection threshold, even CRISPR-cas9 enrichment may not recover adequate target fragments.
- Risk of contamination: Low-input samples are highly susceptible to contamination, which can bias the results.
- Amplification bias: Steps like reverse transcription (for metatranscriptomics) may introduce biases or noise, particularly in low-abundance samples.

Conclusions and recommendations

It is concluded from this trend alert that CRISPR-cas9 Targeted Enrichment Metagenomics might be a promising tool to detect multiple targets present at low concentrations in low-biomass environments like drinking water. The method has several advantages compared to the currently applied qPCR and 16S rRNA gene amplicon sequencing methods in drinking water and might be a desirable addition to the current molecular toolbox applied in drinking water.

To investigate whether CRISPR-cas9 Targeted Enrichment Metagenomics is a useful tool, further validation is needed, particularly for coupling CRISPR-cas9 enrichment with metatranscriptomics in low-biomass samples. Controlled trials using Illumina and Nanopore sequencing, with known microorganisms or gene targets present at low abundance in the sample matrix, are recommended to evaluate sensitivity, accuracy, and reproducibility.

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More information

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