Development of CRISTOFF: A bioinformatics pipeline for detecting CRISPR-Cas9 on- and off-target insertions and deletions

MNEXT MATERIALS & ENERGY X TRANSITION

Background

There is a growing interest in the production of sustainable bio-based chemicals and foods using **precision fermentation** (PF) (Crandall *et al.*, 2023). This methodology employs evolutionarily optimized or genetically engineered **microorganisms** to ferment/digest organic feedstocks into specific **high-value products** (e.g., proteins, vitamins, pigments, biofuels, etc.) with minimal or no by-products.

PF frequently utilizes **genetic engineering** to enhance or introduce novel functionalities in microorganisms though the targeted **insertions, deletions**, or modification of specific genes and metabolic pathways (Eastham *et al.*, 2024). **CRISPR-Cas9** (Figure 1) is currently the most widely utilized method of genetic engineering in PF (Chai *et al.*, 2022).

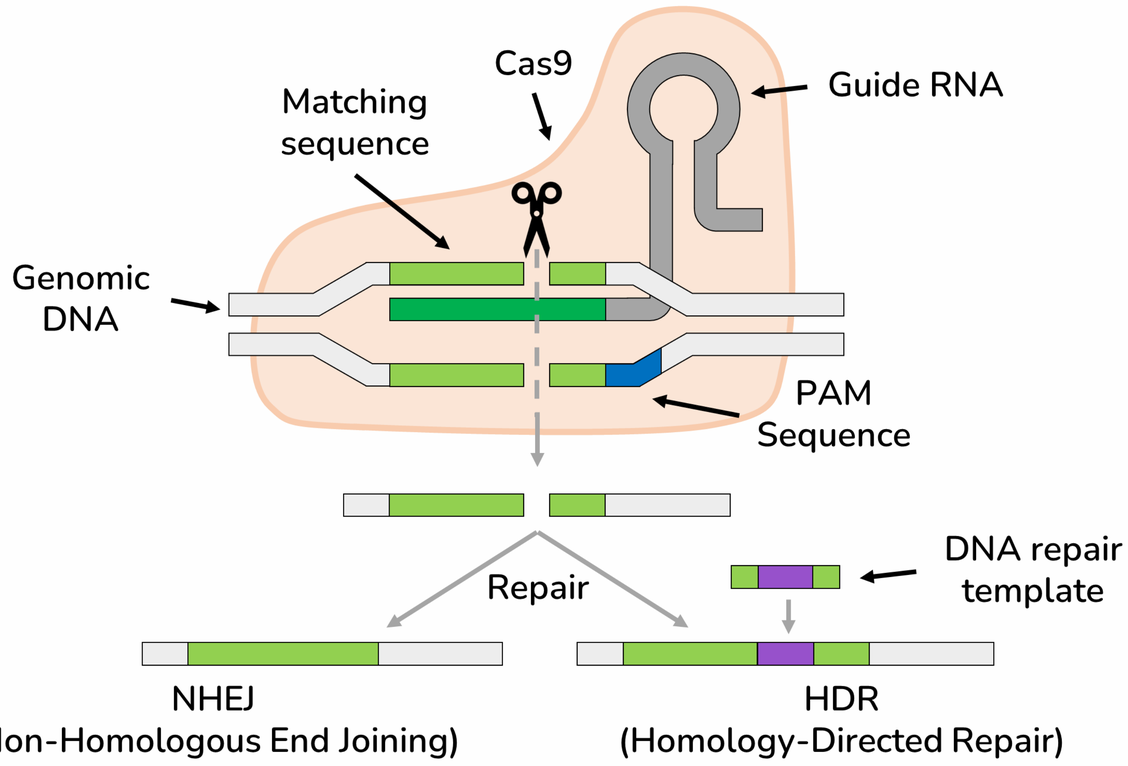

Figure 1. Schematic overview of CRISPR-Cas9 gene editing. The Cas9 proteins contains a "programmable" guide RNA capable of binding to a specific genomic sequence into which it creates a double stranded break.

Problem

Despite its advantages, **CRISPR-Cas9** has one significant **limitation**, namely the introduction of double stranded breaks at unintended genomic loci resulting in **off-target effects** (Figure 2), which could lead to loss of gene function.

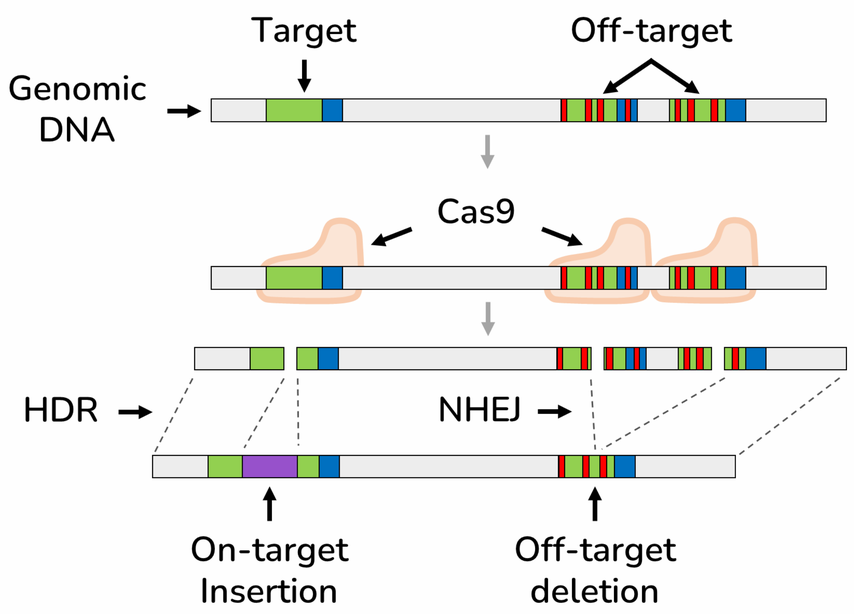

Figure 2. Example of an CRISPR-Cas9 on- and off-target effects.

Solution

To **validate CRISPR-Cas9** experiments we are developing **CRISTOFF**: A bioinformatics pipeline (Figure 3) for detecting CRISPR-Cas9 on- and off-target insertions and deletions, utilizing **long-read whole genome sequencing** data.

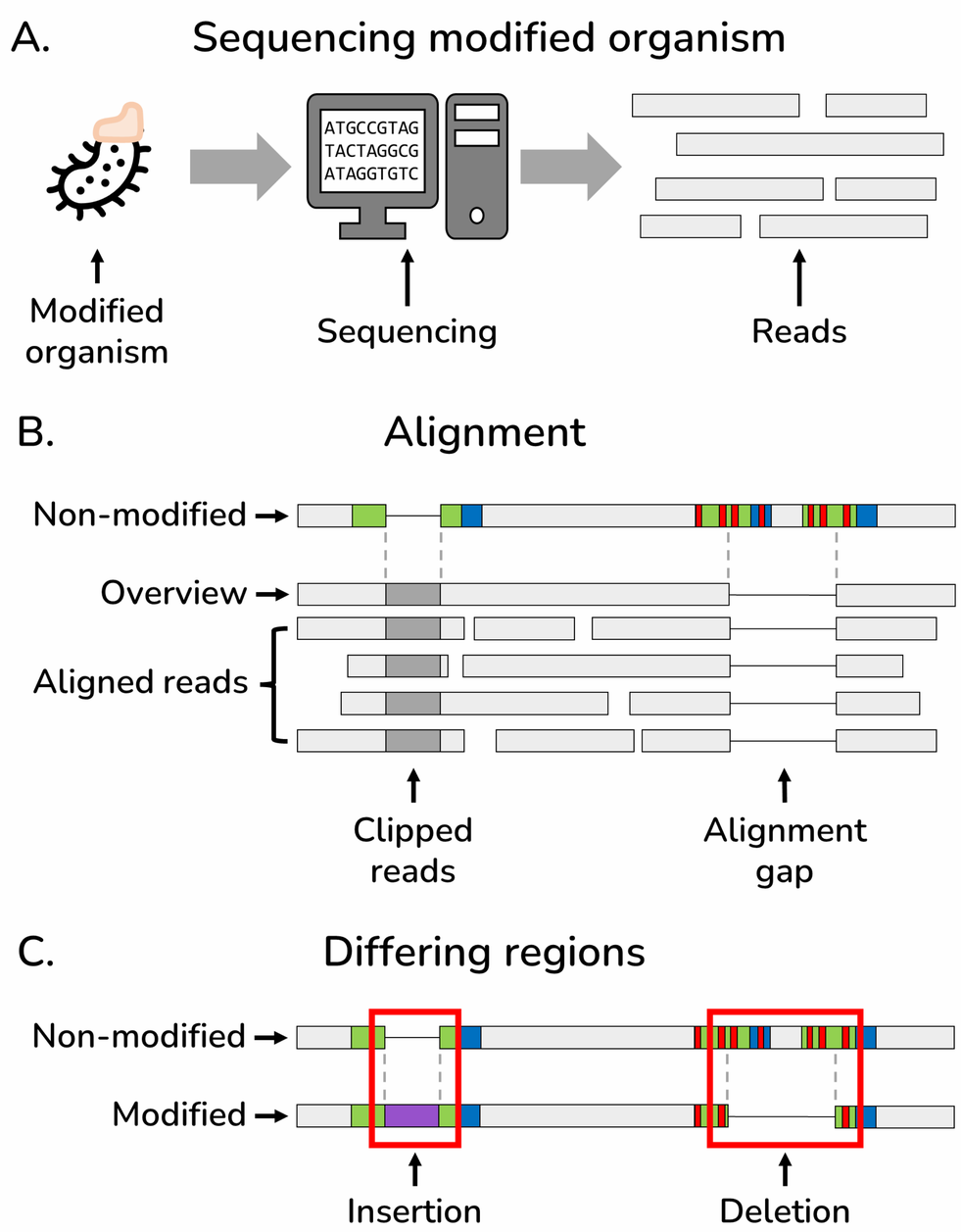

Figure 3. Simplified overview of the CRISTOFF pipeline. **A.** Whole genome sequencing of modified organism. **B.** Aligning reads from the modified organism against the genome of the non-modified organism. **C.** Detect and classify differing regions between the two organisms.

Applications

The CRISTOFF pipeline is beneficial to all fields that can utilize CRISPR, which include but are not limited to: **biofuel production** (engineering yeast to enhance bioethanol production), **agriculture** (inserting a vitamin A-producing pathway into rice to create golden rice), **pharmaceutics** (editing *E. coli* to produce insulin or antibiotics), and **gene therapy** (editing blood stem cells to treat sickle cell disease).

References

1. Crandall B. S. *et all.* (2023). *Accounts of Chemical Research* 56(12), 1505-1516
2. Eastham J. L. and A. R. Leman (2024). *Current Opinion in Food Science* 58, 101194
3. Chai K. F. *et all.* (2022). *Current Opinion in Food Science* 47, 200881

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Researcher: Martijn Prevoo
Supervisors: Tim Verschuren & Bazante Sanders
Contact information: t.verschuren3@avans.nl

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