

CRISTOFF; a control system for the modification of industrial micro-organisms

Finding (off-target) deletions in CRISPR/Cas9 modified genomes using Nanopore long-read sequencing

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BACKGROUND

The genetic engineering process to boost desired properties in industrial micro-organisms is commonly performed using the Nobel prize-winning CRISPR/Cas9 technique. This modification method shows high on-target successes, but is simultaneously prone to introduce unintended cuts/breaks in the DNA, that directly affect the micro-organism's health: **OFF-TARGETS**

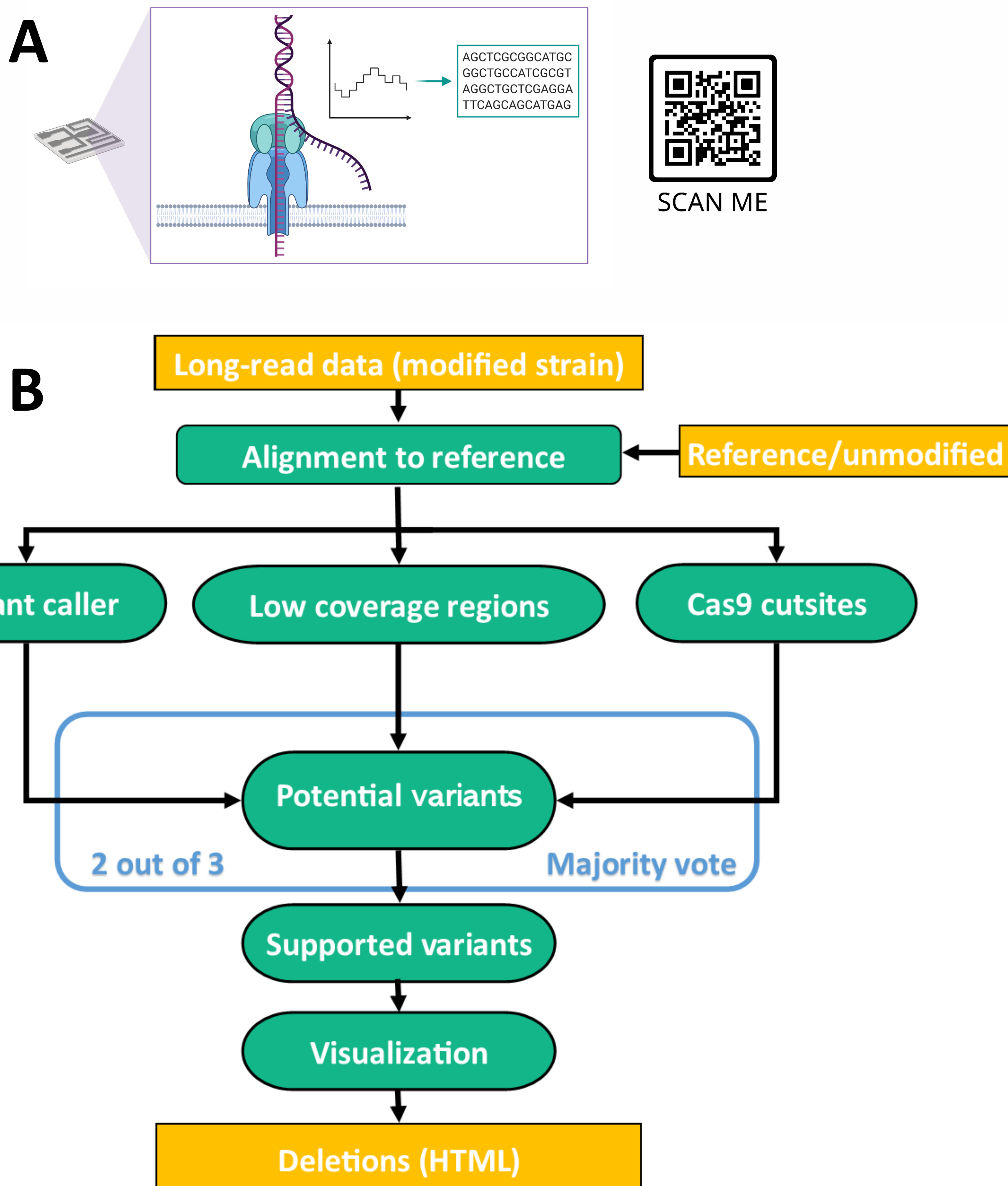
PROBLEM

Even though the prediction methods are increasingly improving, these limitations remain:

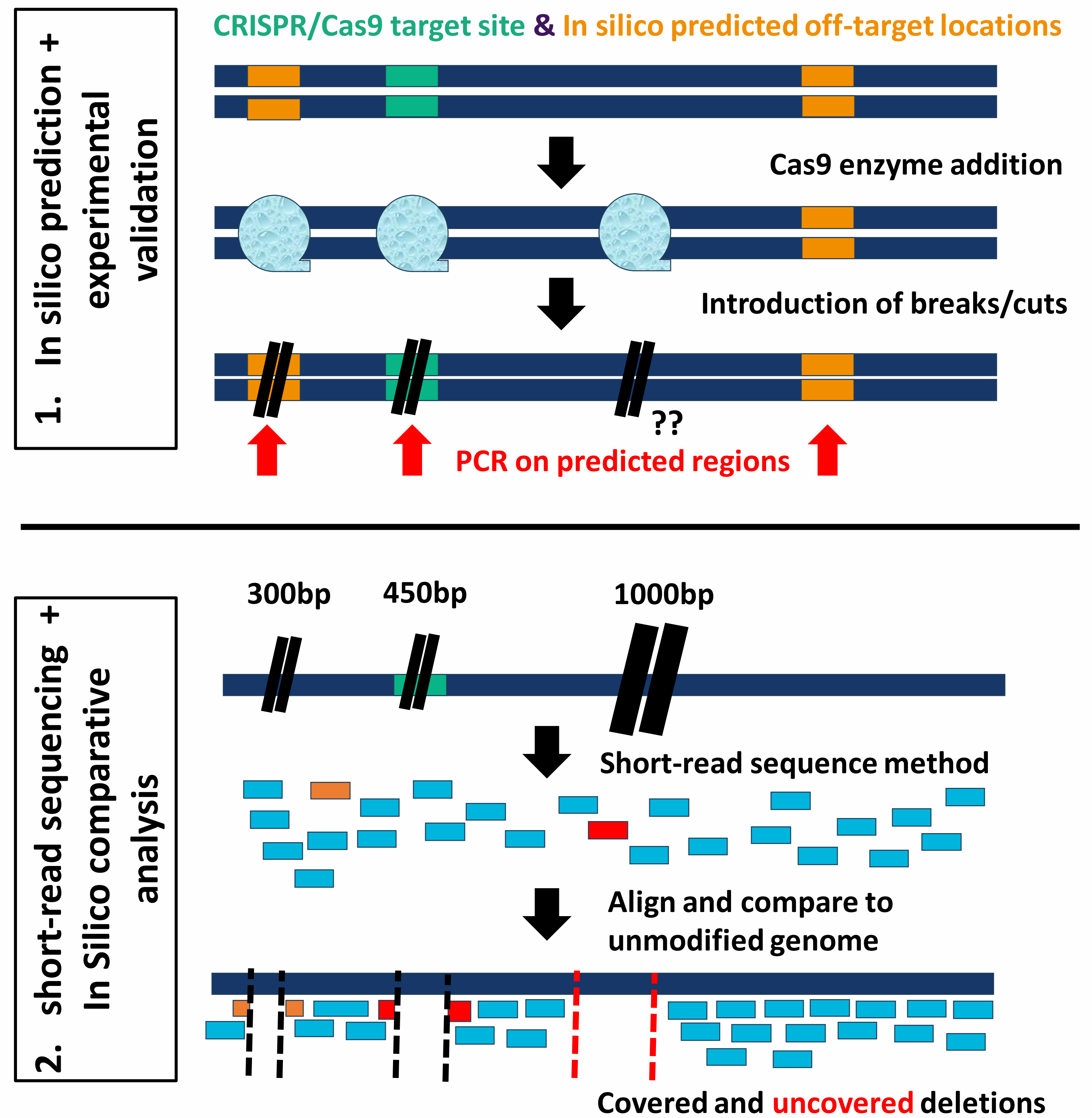
- Targeted validation approaches tend to miss unpredicted off-targets (1)
- Validation methods utilizing short-read sequencing don't cover large modifications (2)

CRISTOFF

CRISTOFF uses (Oxford Nanopore Technologies) long-read sequence data (A) as input in the analysis pipeline (B) and returns the detected deletions



STATE-OF-THE-ART DETECTION



PERFORMANCE REVIEW

Table: (un)modified *A. niger* strains used to benchmark the detection capacities of CRISTOFF

	Strain name	Expected deletions	Predicted deletions	Recall (%)
Aspergillus niger strains	CP1	0	7	100
	CP2	6	14	100
	CP3	10	22	100
	CP4	20	45	100
	CP5	42	55	100
	CP13	52	61	96.2
	CP14	61	71	96.7

CONCLUSIVE POINTS

- CRISTOFF captures all on-target deletions, as long as the input data is complete and of standard quality
- The user is provided with the on- and potential off-targets to manually follow-up on
- CRISTOFF works great on haploid genomes, but was not tested on diploid genomes
- Platform will be extended to detect other variant types, such as insertions, as well

REFERENCES

1. Vicente MM, Chaves-Ferreira M, Jorge JMP, Proença JT and Barreto VM (2021) The Off-Targets of Clustered Regularly Interspaced Short Palindromic Repeats Gene Editing.
2. <https://www.bcgsc.ca/news/new-study-illustrates-benefits-long-read-sequencing-technology-precision-oncology>