Accurate evaluation of CRISPR genome editing

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CRISPR technology allows researchers to edit genomes by altering DNA sequences and by thus modifying gene function. Its many potential applications include correcting genetic defects, treating and preventing

the spread of diseases and improving crops.

Genome editing tools, such as the CRISPR-Cas9 technology, can be engineered to make extremely well-defined alterations to the intended target on a chromosome where a particular gene or functional element is located. However, one potential complication is that CRISPR editing may lead to other, unintended, genomic changes. These are known as off-target activity. When targeting several sites in the genome, off-target activity can lead to translocations, unusual rearrangement of chromosomes, as well as other unintended genomic modifications.

Controlling off-target editing activity is one of the central challenges in making CRISPR-Cas9 technology accurate and applicable in medical practice. Current measurement assays and data analysis methods for quantifying off-target activity do not provide statistical evaluation, are not sufficiently sensitive in separating signal from noise in experiments with low editing rates, and require cumbersome efforts to address the detection of translocations.

In the May 24th issue of the journal *Nature Communications*, a multidisciplinary team of researchers from the Interdisciplinary Center Herzliya and Bar-Ilan University report the development of a new software tool to detect, evaluate and quantify off-target editing activity, including adverse translocation events that can cause cancer. The software is based on input taken from a standard measurement assay, involving multiplexed PCR amplification and Next-Generation Sequencing (NGS).

Known as CRISPECTOR, the tool analyzes next generation sequencing data obtained from CRISPR-Cas9 experiments, and applies statistical modeling to determine and quantify editing activity. CRISPECTOR accurately measures off-target activity at every interrogated locus. It further enables better false-negative rates in sites with weak, yet

significant off-target activity. Importantly, one of the novel features of CRISPECTOR is its ability to detect adverse translocation events occurring in an editing experiment.

"In genome editing, especially for clinical applications, it is critical to identify low level off-target activity and adverse translocation events. Even a small number of cells with carcinogenic potential, when transplanted into a patient in the context of gene therapy, can have detrimental consequences in terms of cancer pathogenesis. As part of treatment protocols, it is therefore important to detect these potential events in advance," says Dr. Ayal Hendel, of Bar-Ilan University's Mina and Everard Goodman Faculty of Life Sciences.

Dr. Hendel led the study, together with Prof. Zohar Yakhini of the Arazi School of Computer Science at Interdisciplinary Center (IDC) Herzliya. "CRISPECTOR provides an effective method to characterize and quantify potential CRISPR-induced errors, thereby significantly improving the safety of future clinical use of genome editing." Hendel's team used CRISPR-Cas9 technology to edit genes in stem cells relevant to disorders of the blood and the immune system. In the process of analyzing the data, they became aware of the shortcomings of the existing tools for quantifying off-target activity and of gaps that should be bridged to improve applicability. This experience led to the collaboration with Prof Yakhini's leading computational biology and bioinformatics group.

Prof. Zohar Yakhini, of IDC Herzliya and the Technion, says, "In experiments utilizing deep-sequencing techniques that have significant levels of background noise, low levels of true off-target activity can get lost under the noise. The need for a measurement approach and related data analysis that are capable of seeing beyond the noise, as well as of detecting adverse translocation events occurring in an editing experiment, is evident to genome editing scientists and practitioners.

CRISPECTOR is a tool that can sift through the background noise to identify and quantify true off-target signals. Moreover, using statistical modeling and careful analysis of the data, CRISPECTOR can also identify a wider spectrum of genomic aberrations. By characterizing and quantifying potential CRISPR-induced errors our methods will support the safer clinical use of genome editing therapeutic approaches."