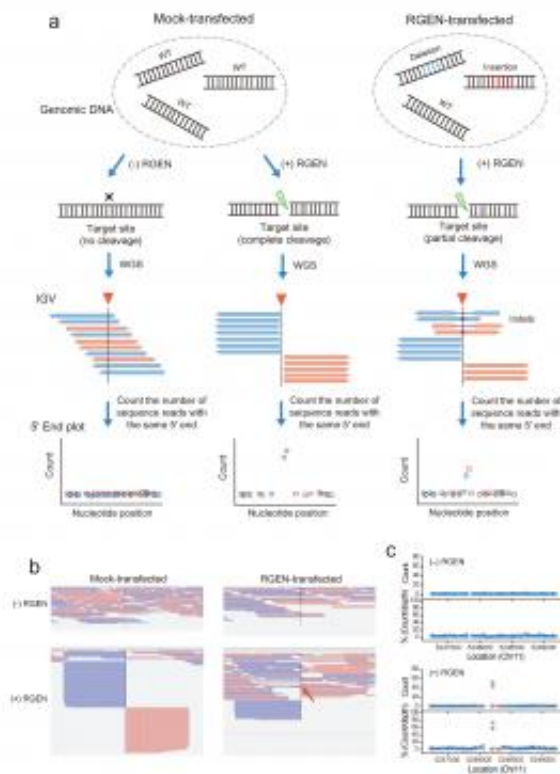


End of CRISPR-CAS9 controversy

February 10 2015



RGEN-induced ‘digenome’ sequencing to capture off-target sites. (a) Overview of Digenome-seq. Forward and reverse sequence reads are shown in pink and blue, respectively. Red triangles and vertical lines indicate cleavage positions. WT, wild type. (b) Representative IGV images obtained using the HBB-specific RGEN at the on-target site.

The IBS research team (Center for Genome Engineering) has

successfully confirmed that CRISPR-Cas9 has accurate on-target effects in human cells, through joint research with the Seoul National University College of Medicine and ToolGen, Inc.

There has been great interest in CRISPR-Cas9 as a tool to develop anticancer cell therapies or to correct genetic defects that cause hereditary in stem and [somatic cells](#). However, since there has been no reliable and sensitive method to measure the accuracy of CRISPR-Cas9 [genome](#)-wide, its safety has remained in question. Consequently, it has been difficult to eliminate the possibility that CRISPR-Cas9 may induce mutations in off-target [sequences](#) that are similar to on-target sequences. Off-target mutations in [tumor suppressor genes](#), for example, can cause cancer.

The researchers have developed a technique termed Digenome-seq to locate both on-target and off-target sequences that can be mutated by CRISPR-Cas9 via genome sequencing. They digested human genomic DNA using Cas9 nucleases in a test tube, which was then subjected by whole [genome sequencing](#). This in vitro digest yielded a unique pattern at both on-target and off-target sequences that can be computationally identified. Furthermore, by adding guanine nucleotides at the end of sgRNA(single guided RNA) that composes CRISPR-Cas9, they have successfully created this highly-developed programmable nuclease, which has no measurable off-target effects in the [human genome](#).

Jin-Soo Kim, the director of the Center for Genome Engineering at IBS, as well as the professor of the Department of Chemistry at Seoul National University says, "If CRISPR-Cas9 truncates off-target DNA sequences, it might induce unwanted mutations. Since we have succeeded in confirming the accuracy of CRISPR-Cas9, we anticipate that there will be a great progress in the development of gene or cell therapies," emphasizing the significance of this research achievement.

Nature Methods has also highlighted this achievement as one of the "2015 Methods to Watch" in its January issue.

More information: Daesik Kim, Sangsu Bae, Jeongbin Park, Eunji Kim, Seokjoong Kim, Hye Ryeong Yu, Jinha Hwang, Jong-Il Kim & Jin-Soo Kim.(2015) *Nature Methods*. [DOI: 10.1038/nmeth.3284](https://doi.org/10.1038/nmeth.3284)

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