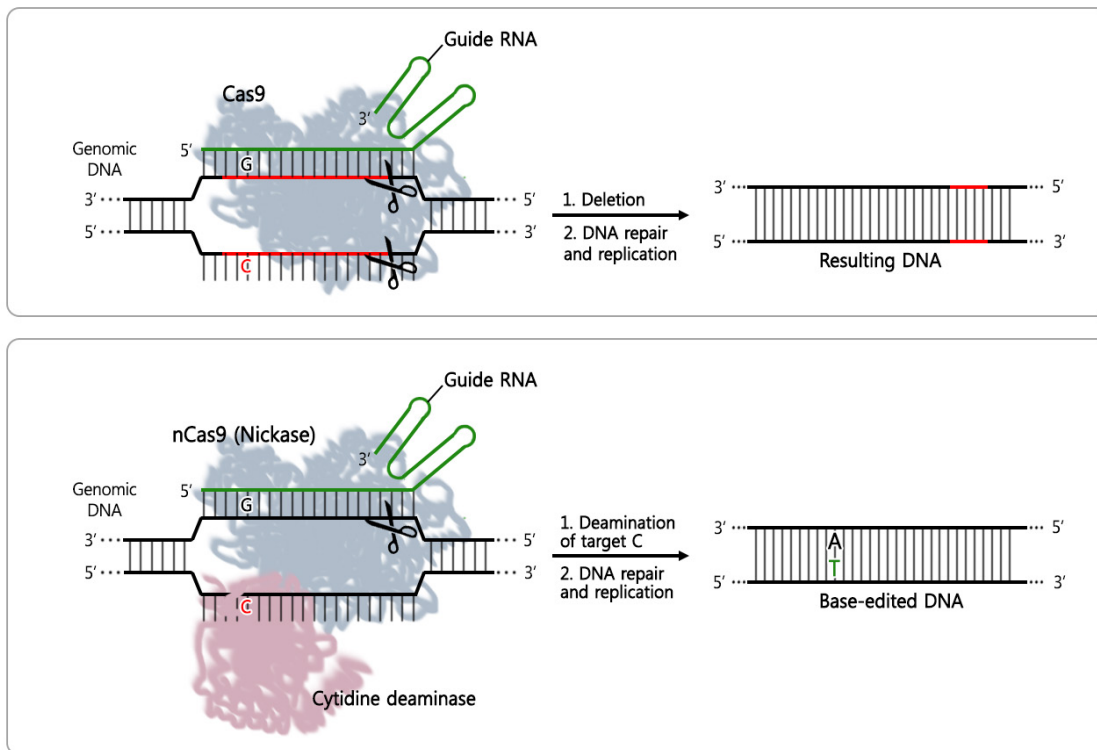


Accurate DNA misspelling correction method

April 11 2017

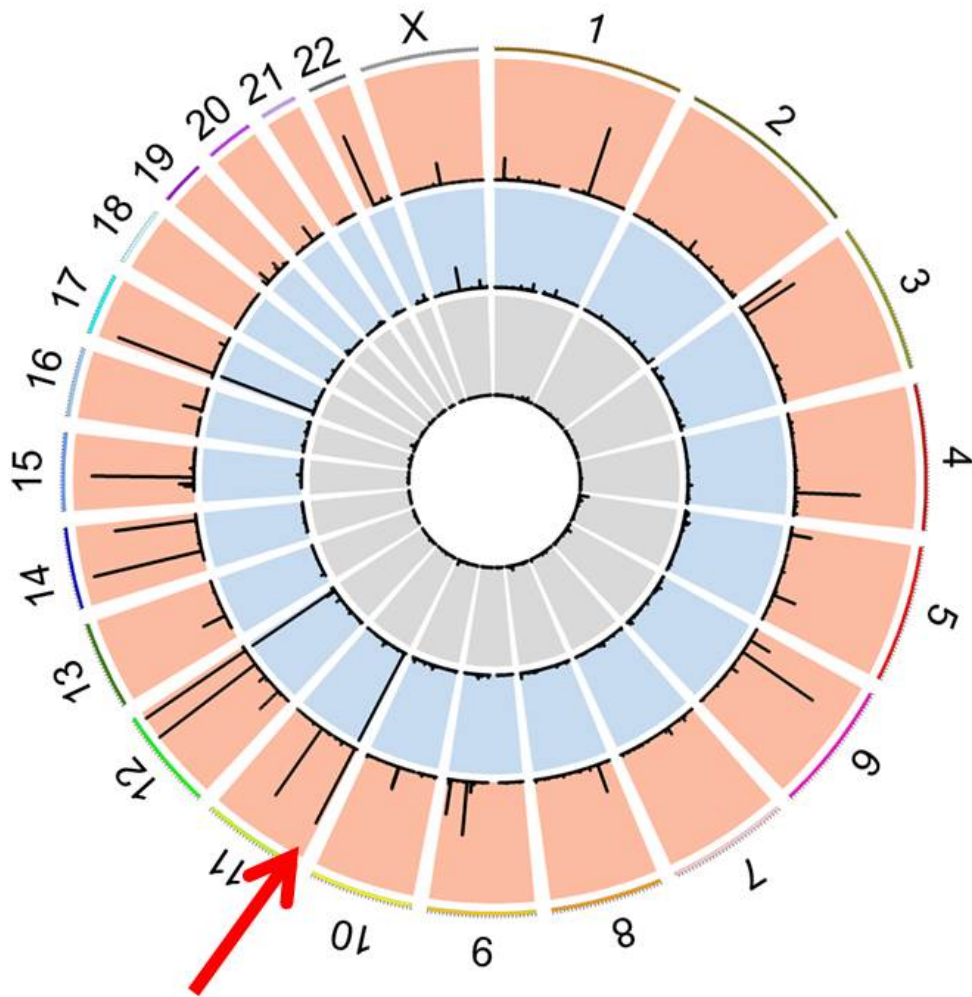


In the original CRISPR-Cas9 technique (top), the guide RNA (green) binds to the target DNA and the cleavage enzyme Cas9 (scissors) cuts out a small DNA sequence (red). A modified version of Cas9 called nCas9 (bottom) is different as it cuts off only one strand of DNA and the cytidine deaminase (pink) transforms a single cytosine (C) into uracil (U). Uracil (U) is then converted to thymine (T) by DNA replication. Credit: IBS

Researchers at the Institute of Basic Science (IBS) proved the accuracy of a recently developed gene editing method. This works as "DNA scissors" designed to identify and substitute just one nucleotide among the 3 billion. "It is the first time that the accuracy of this base editor has been verified at the whole genome level," explains KIM Jin-Soo, leading author of the study. Published in *Nature Biotechnology*, this validation will help to expand the use of this method in agriculture, livestock, and gene therapy.

Rapid advances in gene editing tools have created excitement in the biology community. The primary third-generation DNA editing technology is CRISPR—a tool that is quicker and cheaper than its predecessors. By cutting out a small DNA sequence, CRISPR-Cas9 and CRISPR-Cpf1 are used to silence or reduce the expression of faulty [genes](#). However, last year, biologists discovered a new base editor method that does not cause random DNA deletions and insertions, but instead replaces only one DNA base. These types of gene corrections are critical, as several diseases are caused by the misspelling of one of the four basic components of DNA; adenine (A), cytosine (C), guanine (G), and thymine (T). Single-nucleotide errors in DNA are referred to as point mutations. Examples of conditions caused by point mutations include cystic fibrosis, sickle cell anemia and color blindness.

Unlike the existing technologies, the base editor method consists of a variation of CRISPR-Cas9 (nCas9, nickase) fused with another enzyme called cytosine deaminase, which replaces the DNA component C with T. The scissors are directed to the correct position on the DNA by a guide RNA. However, until recently, it was not known whether the base editor was working only in the area of the faulty gene or if it was unnecessarily substituting Cs in off-target areas.



■ Untreated ■ Base Editor ■ Cas9

The vertical black bars represent C-to-T substitutions made in the genome, by the base editor (blue) and the leading third-generation technique CRISPR-Cas9 (red). The inner circle (gray) is a control where no gene editing tools were used. The red arrow shows the targeted C-to-T substitution, while all the other bars are off-target conversions. Since the black bars seen in the red zone are markedly higher than in the blue zone, the result suggests that the base editor acts more accurately on the target position than CRISPR-Cas9. The data is based on a previously-validated technique called Digenome-seq. Credit: IBS

Just one month after reporting the first successful base editing in animals in *Nature Biotechnology* to modify a single nucleotide in dystrophin and tyrosinase genes, the same team demonstrated the accuracy of this method at the genome scale.

In order to identify the soundness of the method, IBS researchers modified the error-checking technique known as Digenome-seq, in order to adapt it to the base editor [method](#). Digenome-seq was used and validated last year, when the team analyzed the accuracy of CRISPR-Cpf1 and Cas9. IBS researchers also improved the computer program Digenome 2.0 to identify off-targets more comprehensively and compared different guide RNAs to find the one that reduces malfunctions and increases specificity.

Using this technique, the team found the base editing technique to be even more accurate than the current third-generation CRISPR-Cas9. The base editing technique induced C-to-T conversions in multiple sites in the human genome, while CRISPR-Cas9 caused cleavages in multiple sites, meaning that the new base editor technique makes fewer off-target changes. "Therefore, it is expected that these base [editors](#) will be used as widely as the popular CRISPR technology," says KIM.

More information: Genome-wide target specificities of CRISPR RNA-guided programmable deaminases, *Nature Biotechnology* (2017). [nature.com/articles/doi:10.1038/nbt.3852](https://doi.org/10.1038/nbt.3852)

Provided by Institute for Basic Science

Citation: Accurate DNA misspelling correction method (2017, April 11) retrieved 10 September

2024 from <https://phys.org/news/2017-04-accurate-dna-method.html>

This document is subject to copyright. Apart from any fair dealing for the purpose of private study or research, no part may be reproduced without the written permission. The content is provided for information purposes only.