New method brings increased efficiency, precision and reliability in DNA editing

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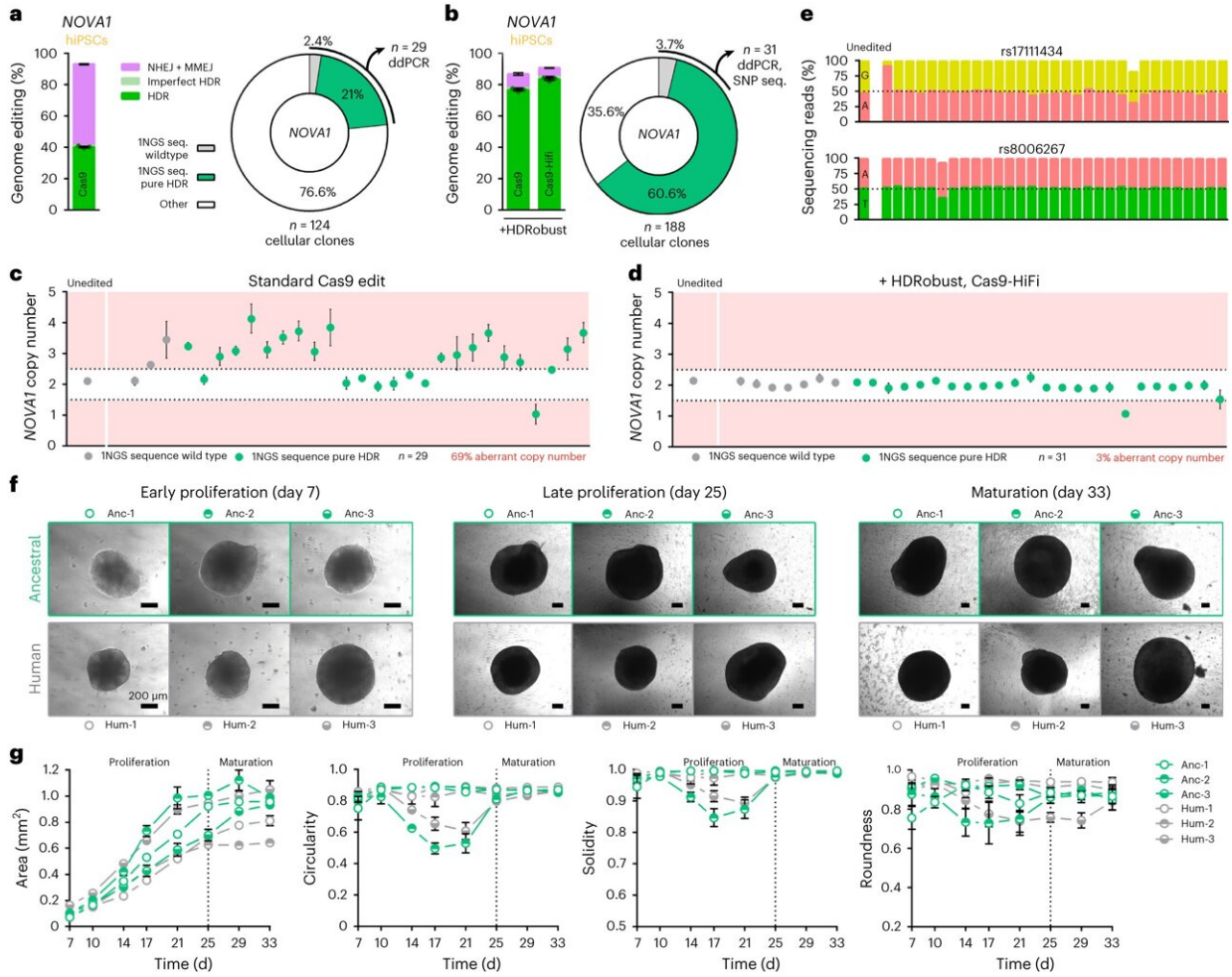

Brain organoid morphology after editing of NOVA1 to the Neandertal state. **a**, Standard genome editing of *NOVA1* as described in[51]. Independent biological replicates were performed ($n = 3$) and error bars show the s.e.m. For HDR, replicates are depicted by dots. The right panel shows the percentage of single cell-derived colonies that have one predominant wild-type DNA sequence

(apparent homozygous wild type, gray), one predominant 'pure' HDR sequence (apparent homozygous exclusive ancestral edit, green) or any other genotype (white). The total number of analyzed cellular clones is given. **b**, Genome editing efficiencies and genotypes of single cell-derived colonies after editing of *NOVA1* as done in **a**, but with HDRobust and Cas9-HiFi RNP. Independent biological replicates were performed ($n = 3$) and error bars show the s.e.m. **c**, Copy number (relative to the *FOXP2* gene) of the target site of the single cell-derived cellular clones from **a** that appear homozygous at the target site for wild type and 'pure' HDR on the basis of sequencing of the target site. Copy number estimates are plotted as gray and green circles for wild type and 'pure' HDR clones, respectively. The measure of center for the error bars represents the ratio of the Poisson-corrected number of target to reference molecules multiplied by two for the diploid state of the reference gene. The error bars represent the 95% confidence interval of this measurement. The number of analyzed cellular clones and percentage of clones with aberrant copy numbers of the target site are given. **d**, Copy number of the target site of the cellular clones from **b** that appear homozygous at the target site for wild type and 'pure' HDR on the basis of sequencing of the target site. The measure of center for the error bars represents the ratio of the Poisson-corrected number of target to reference molecules multiplied by two for the diploid state of the reference gene. The error bars represent the 95% confidence interval of this measurement. **e**, Genotypes of SNPs upstream (rs17111434) and downstream (rs8006267) of the target site from the cellular clones in **d**. **f**, Phase-contrast images of three typical cellular clones with the modern human (Hum-1–3, gray circles) or ancestral (Anc-1–3, green circles) *NOVA1* during early proliferation (day 7), late proliferation (day 25) and maturation (day 33). **g**, Organoid size and shape descriptors of circularity, solidity and roundness during brain organoid development from proliferation to maturation (days 7–33). Data for three different cellular clones for human (gray circles) and ancestral (green circles) are given. Circles show the mean and error bars show the s.e.m. of measurements of four different organoids for each day and clone. Credit: *Nature Methods*, (2023). DOI: 10.1038/s41592-023-01949-1

In a new study published in *Nature Methods*, researchers at the Max

Planck Institute for Evolutionary Anthropology in Leipzig, Germany, describe improvements in the methods with which mutations can be introduced in human and other genomes—making these methods much more efficient and less error prone.

In the field of genome editing, scientists often need to change one letter—corresponding to one of the DNA bases Adenine, Guanine, Cytosine or Thymine—to another letter at one specific position in the genome. To do this, they use reagents that cut both strands of the DNA close to the position they want to change.

They then provide the cell with DNA molecules that contain the desired new letter in the hope that the cell's repair systems will use these molecules to introduce the desired mutation when the DNA break is repaired. Since different repair systems in the cells compete with each other and only one of these systems is able to introduce the desired new mutation, applications of genome editing of single letters have so far been limited by low efficiency and unintended byproducts.

Through the combined inhibition of two repair pathways that compete with the desired one, the team achieved the induction of point mutations in up to 93 percent of chromosomes in populations of cells. Importantly, this method largely abolishes unwanted insertions, deletions, rearrangements in the area where the desired mutation is located as well as unintended changes at other sites in the genome. It therefore greatly increases the precision and reliability of the DNA editing process.

Studying the effects of mutations

The researchers demonstrated the efficiency of the novel method in the laboratory by changing 58 different target sites in human cells. This method is likely to have many applications in basic research. "We look forward to using this method to more easily introduce genetic changes

into cells to study the effects of mutations that set modern humans apart from Neandertals," says Svante Pääbo, who helped facilitate the work.

The scientists also corrected pathogenic mutations in cells derived from patients suffering from three genetic diseases: anemia, sickle cell disease , and thrombophilia.

"The implications for helping cure human diseases in the future are potentially vast. One could imagine removing cells from patients, editing them with the help of this method, and then giving them back to the patients," says Stephan Riesenberg, who led the work. "Nevertheless, the road from demonstrating that the method works in the laboratory to applying it to patients is still a long one," he cautions.

More information: Stephan Riesenberg et al, Efficient high-precision homology-directed repair-dependent genome editing by HDRobust, *Nature Methods* (2023). DOI: 10.1038/s41592-023-01949-1
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