Sequencing platform provides powerful tool for identifying subtle changes in gene expression

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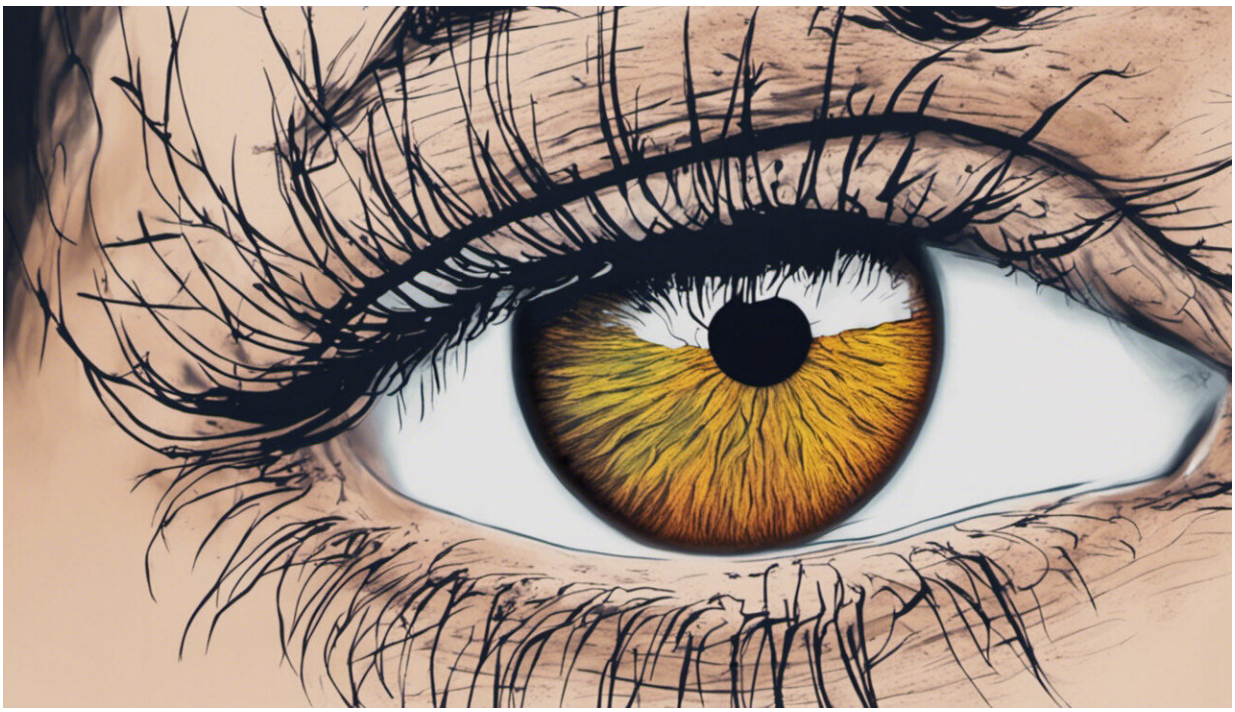

Credit: AI-generated image (disclaimer)

In a truly challenging task, the FANTOM5 Consortium, an international collaboration headed by scientists at the RIKEN Omics Science Center in Yokohama, Japan, is striving to profile the regulation of gene expression in every known human cell type. “We expect to generate on

the order of 3,000 or more [datasets] for this project," says Masayoshi Itoh, a RIKEN scientist involved in the effort. "This will capture the majority of human cell types, tissues and cancer subtypes."

The work will benefit greatly from HeliScopeCAGE, a sensitive expression analysis technique developed recently by FANTOM5 researchers1. Previous FANTOM studies used techniques based on 'cap analysis of gene expression' (CAGE), which enables quantification of messenger RNAs transcribed from active genes by generating short DNA 'tags' that can be analyzed by sequencing. Conventional CAGE relies on the polymerase chain reaction (PCR), a method for amplifying target nucleic acid molecules. However, PCR can also introduce biases into libraries by preferentially amplifying some molecules—a serious impediment to the accurate measurement of gene expression.

A solution arrived in the form of the HeliScope, an instrument developed by Helicos Biosciences for the analysis of individual molecules of DNA (Fig. 1). "We realized that we could use this system to apply a much-simplified protocol that completely avoids PCR," says Itoh. To test their streamlined HeliScopeCAGE technique, he and his colleagues generated tag libraries from different cell types, which they analyzed with no intervening amplification step.

Remarkably, the researchers could obtain accurate results from as little as 100 nanograms of material (the mRNA content of approximately 20–100,000 cells), and chart differences in gene expression levels ranging across five orders of magnitude. The researchers noted that the observed differences between different cell types correlated with previous findings. HeliScopeCAGE even revealed changes in thousands of genes that had been overlooked by older platforms.

Itoh points out that their technique proved highly quantitative, based on trial experiments with different concentrations of control templates, and

yielded consistent data in run after run. “The results were highly correlated,” he says. “They almost looked identical, to the extent that I could not find any differences by eye.”

FANTOM5 is already a third of the way to its 3,000-library goal, and an automated HeliScopeCAGE platform should accelerate their progress. However, this method is also being made available to the greater community through the RIKEN Yokohama Institute. “We believe HeliScopeCAGE could be useful not only to us, but to every scientist in Japan,” says Itoh.

More information: Kanamori-Katayama, M., et al. Unamplified cap analysis of gene expression on a single-molecule sequencer. Genome Research 21, 1150–1159 (2011).

Provided by RIKEN

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