New CRISPR-based technology to speed identification of genes involved in health and disease

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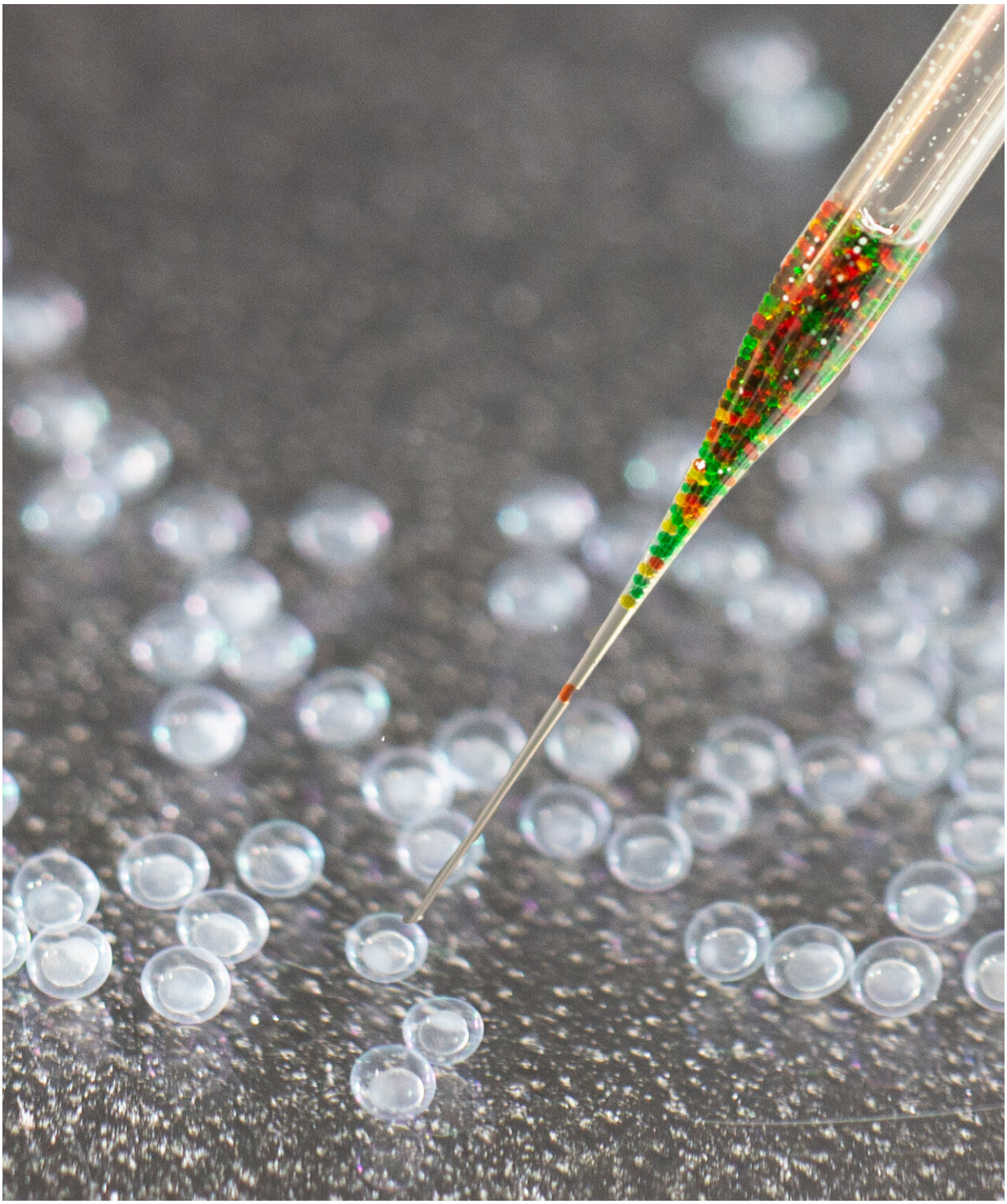


Which genes are MIC-Drop is a new CRISPR-based technology for rapidly and efficiently screening the functions of hundreds of genes in zebrafish to better understand human health and disease. A glass needle filled with MIC-Drops (colored droplets) is used to inject fertilized zebrafish eggs (translucent spheres).

The MIC-Drops are dyed different colors for easy visualization. MIC-Drops are one-billionth of a liter in volume, and each one contains the materials needed to barcode and disrupt a single gene. Credit: James Herron, Saba Parvez

Zebrafish—small, fast-growing creatures who share many of the same genes as humans—are instrumental to many biologists, who find them uniquely well suited for studying a wide range of questions, from how organisms develop to how the nervous system drives behavior. Now, with a new technology developed by University of Utah Health scientists called MIC-Drop, the fish will be even more powerful for large-scale genetic studies.

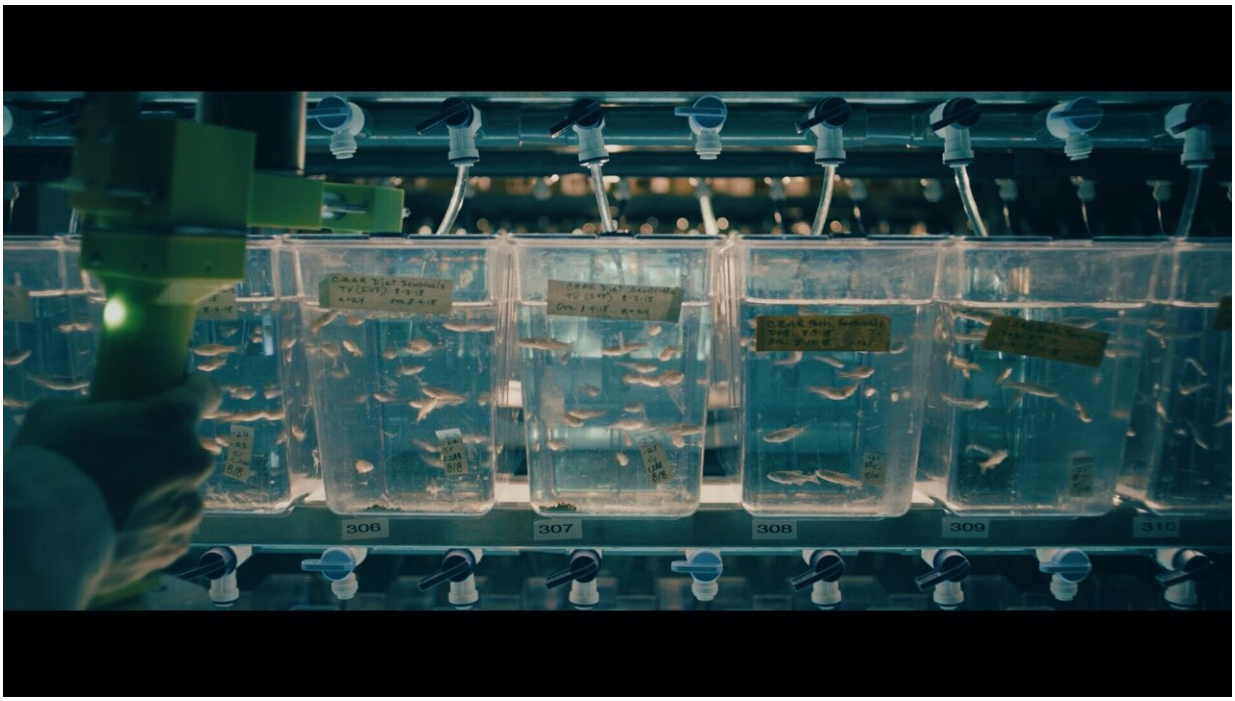
MIC-Drop, whose development was led by chemical biologist Randall Peterson, Ph.D., Dean of U of U Health's School of Pharmacy, enables researchers to efficiently deploy the CRISPR gene editing system into zebrafish to rapidly evaluate the functions of hundreds of genes in a single experiment. The advance marks the first time that screens using the robust, Nobel-prize winning CRISPR technology have been possible in any animal model. Already, Peterson's team has used MIC-Drop to identify several genes that are essential for healthy development and function of the heart. Their method and findings are reported August 19, 2021, in the journal *Science*.

The CRISPR system is a programmable method for modifying DNA. To use it, researchers introduce a DNA-cutting enzyme (usually an enzyme called Cas9) into cells, accompanied by an RNA guide that tells the enzyme where to cut. This can be the first step in modifying the gene's sequence, or simply shut the gene off.

The method has made gene editing in zebrafish and other laboratory organisms faster, cheaper, and more precise—but, Peterson says, it has

been difficult to scale up to study more than a few genes at a time. To inactivate a single gene in a zebrafish embryo, researchers prepare a guide RNA targeting that gene, then mix it with the Cas9 enzyme, load the solution into a needle, and inject a carefully calibrated volume of the solution into the embryo. If they want to inactivate a different gene in a different embryo, they must load a new needle with a new Cas9/guide RNA solution. "The process has always been focused on a single gene or a single modification at a time," Peterson says. "So if you want to do 100 genes, it's 100 times as much work."

MIC-Drop, which stands for Multiplexed Intermixed CRISPR Droplets, solves that problem by packaging the components of the CRISPR system into microscopic oil-encased droplets, which can mingle together without mixing up their contents. To set up a screen of many genes with MIC-Drop, researchers begin by creating a library of guide RNAs. Each guide RNA is packaged into its own droplet, along with the Cas9 enzyme. To keep track of target genes, every droplet also includes a DNA barcode identifying its contents.



The tropical fish, zebrafish, is a popular model for understanding human health and disease. 70% of their genes are the same as in humans and they have the same major organ and tissue systems. Credit: Charlie Ehlert, University of Utah Health

The team fine-tuned the chemistry of the droplets to ensure they would remain stable and discrete, so droplets designed to target different genes can be mixed together and loaded into the same needle. Under a microscope, the MIC-Drop user injects a single droplet into a zebrafish embryo, then moves on to the next embryo and injects the next droplet. The process can be repeated hundreds of times, delivering a single packet of CRISPR components to each embryo, so that in every embryo, the system inactivates a single gene. Then it's up to the researchers to monitor the animals for potential effects.

Previously, setting up a CRISPR screen of hundreds of genes in zebrafish would have taken a team of researchers many days and required hundreds of needles, says postdoctoral researcher Saba Parvez, Ph.D., who developed and optimized MIC-Drop's packaging technique and barcoding system. "Now you have streamlined that process into one user doing it in a span of a couple of hours," he says.

To demonstrate MIC-Drop's potential, Parvez and colleagues worked with U of U Health colleague H. Joseph Yost, Ph.D., Calum MacRae, M.D., Ph.D., at Harvard Medical School, and Jing-Ruey Joanna Yeh, Ph.D., at Massachusetts General Hospital to test 188 different zebrafish genes for a potential role in heart development. After creating guide RNAs targeting those genes and introducing the CRISPR system into hundreds of fish embryos, they identified several animals that developed

heart defects as they matured. Using the DNA barcodes in those fish, the team was able to trace the defects back to 13 different inactivated genes. Because of the similarities between zebrafish and human genes, the finding may point toward previously unknown aspects of heart development in humans.

Peterson and Parvez are eager to see MIC-Drop put to work in other labs, and they say a 188-gene screen is just a beginning. "Ultimately, people would like to be able to do genome-scale screening," Peterson says. "I think that scale actually becomes imaginable with this technology."

The research publishes as "MIC-Drop: A platform for large-scale in vivo CRISPR screens."

More information: MIC-Drop: A platform for large-scale in vivo CRISPR screens, *Science* (2021). DOI: 10.1126/science.abi8870

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