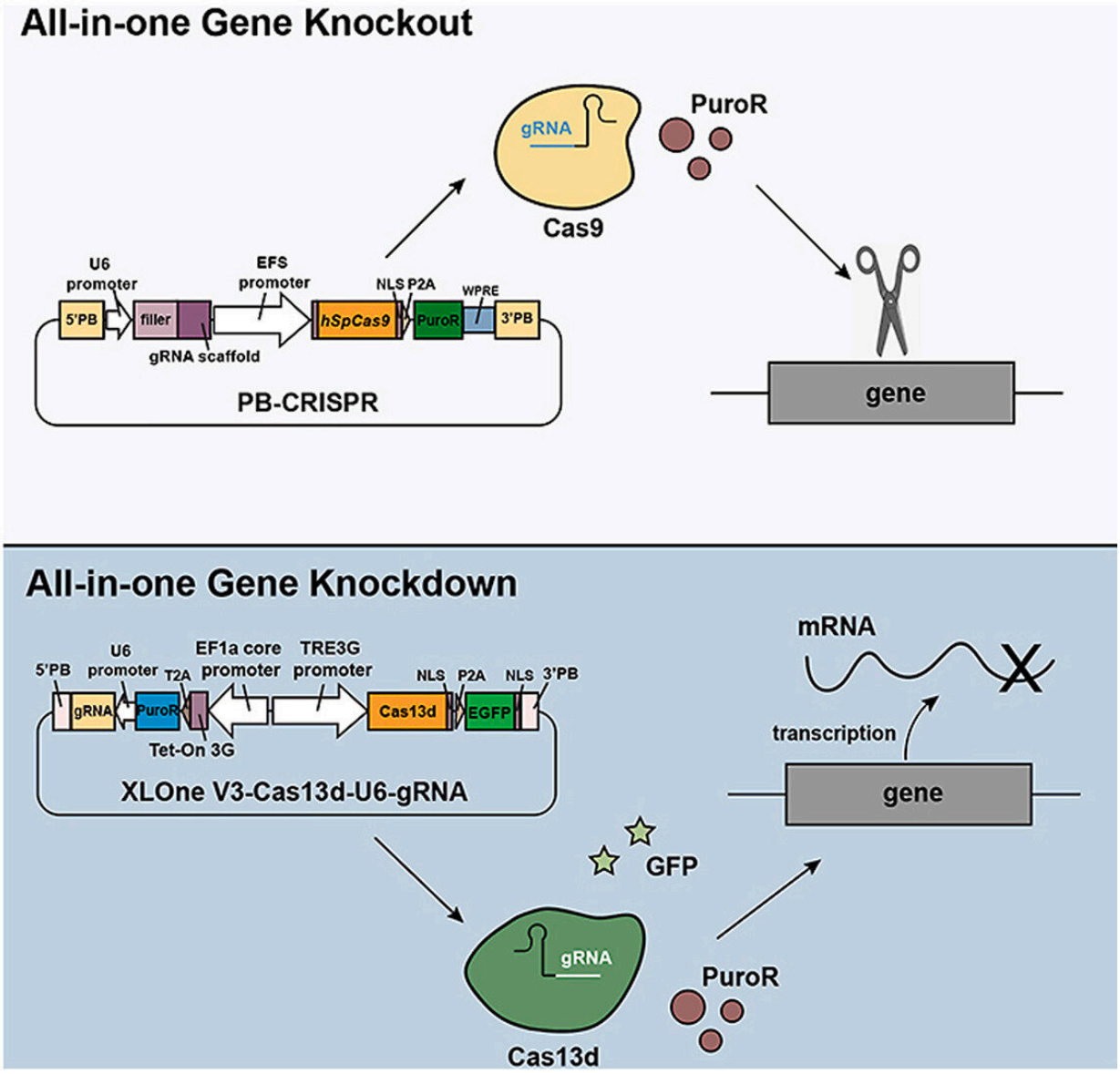
Studying diseases with better delivery of gene-editing tools

March 8 2022, by Gabrielle Stewart



Graphical abstract. Credit: *Bioactive Materials* (2022). DOI:

10.1016/j.bioactmat.2022.01.046

Stem cells can be used to study the effects of disease on numerous tissue types due to their pluripotency, or ability to produce cells of any type. But using gene-editing tools with them can be challenging, according to Xiaojun (Lance) Lian, associate professor of biomedical engineering, because delivery of gene-editing tools into stem cells can be inefficient, time-consuming or expensive.

A Penn State–led team of interdisciplinary researchers developed a method that improved the lifespan and efficiency of CRISPR gene-editing tools after delivery into stem cells. Their findings were made available online ahead of official publication in *Bioactive Materials*.

"We want to study diseases that arise from an inherited trait, but examining the diseased cells can be challenging," Lian said. "A patient can't donate a sample for a biopsy of cardiovascular or neurological disease as easily as they can for a skin disease. By reprogramming patient's skin cells into stem cells, we can address this to create diseased heart or neuron cells that can then be used to study a variety of conditions."

To design their new delivery system, Lian's team leveraged an enzyme that would allow for long-term integration of the gene-editing tools. With a conventional method that relies on DNA components to deliver the tools into cells, they can survive and edit genes for about three days before being destroyed, Lian said. But the use of the enzyme in the system, called PiggyBac, would enable a permanent establishment of the editing tools by implementing the gene-editing tools into the cell's genetic code.

The researchers evaluated the method using human embryonic stem cells from federally approved stem cell lines, which are naturally pluripotent, and stem cells with induced pluripotency. PiggyBac acted as packaging for the CRISPR tools, which were chosen to disable a specific protein and inserted into the stem cells using an electric pulse.

After a seven-day waiting period, the researchers assessed the results. They found that the PiggyBac system had successfully—and permanently—integrated the tools into the cells' genome, and that 99% of the cells tested had mutated to block the expression of the targeted protein. This PiggyBac delivery method led to a much higher number of mutated cells than traditional DNA delivery methods, which resulted in around 10% of cells blocking the protein, Lian said. Unlike other delivery methods, the PiggyBac method allowed for removal of gene-editing tools from the genome after successful creation of mutations in stem cells.

With the delivery of CRISPR tools through the PiggyBac system, the researchers can further explore the relationships between genes and the development of diseases in vital tissues, such as neural tissue and cardiac muscle.

"If we couple together stem cell technology and gene-editing tools as we have in this research, we can quickly study diseases without asking a patient to donate their cells," Lian said. "We can study human disease in a dish in a lab at a fast pace with nearly 100% efficiency of creating disease-related mutations in stem cells."

More information: Yuqian Jiang et al, Robust genome and RNA editing via CRISPR nucleases in PiggyBac systems, *Bioactive Materials* (2022). DOI: 10.1016/j.bioactmat.2022.01.046

Provided by Pennsylvania State University