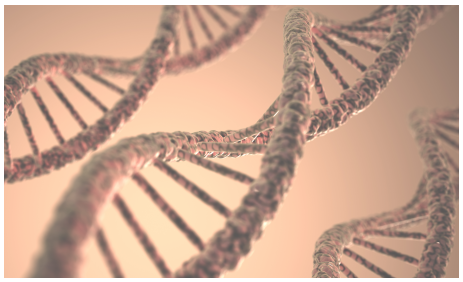

Duke File (IDF) Number

IDF #:T-005334

Meet the Inventors

Soderling, Scott
Gao, Yudong

Contact For More Info

Ferguson, Christy
919-681-7581
christy.ferguson@duke.edu

Department

Cell Biology

Publication(s)

•

External Link(s)

• From the lab of Dr. Scott Soderling
• Video demonstrating HiUGE system

Toolkit to edit any gene with universal CRISPR/Cas9 vectors

Unmet Need

Genetic editing with the CRISPR/Cas9 system has quickly become ubiquitous in biotechnology research. One common application is studying proteins by editing the genes that encode them. Proteins have numerous functions, acting as receptors, enzymes, hormones, and antibodies. Of all FDA approved drugs, proteins account for over 95% (856/893) of drug targets. By using CRISPR/Cas9 to insert a desired DNA sequence into a gene, the protein encoded by that gene can be manipulated in several useful ways. The addition of a fluorescent tag enables visualization of the protein's quantity and spatial location or its interaction with a therapeutic. An epitope tag improves protein purification and makes it possible to detect proteins when no antibody is available. Finally, editing the protein's structure can generate cell lines mimicking certain disease states. Though incredibly powerful, CRISPR/Cas9 editing is limited by a requirement for gene-specific reagents. The DNA sequence to be inserted – termed the donor vector – must be customized in a process that is technically challenging, time and labor intensive, and prevents commercialization of off-the-shelf reagents. There is a need for universal donor vectors, which would offer broad commercial potential and enable wider adoption of CRISPR/Cas9 technology.

Technology

Duke inventors have developed a toolkit of universal donor vectors, enabling efficient and accessible CRISPR/Cas9 editing of any gene of interest. The system builds upon homology independent targeted insertion (HITI), providing efficient DNA integration in both dividing and non-dividing cells. Crucially, the universal toolkit simplifies HITI by eliminating the costly and difficult process of generating gene-specific donor vectors. This is intended to make genetic editing with CRISPR/Cas9 more efficient, accessible, and commercially viable. The toolkit includes donor vectors that encode fluorescent tags, epitope tags, trafficking tags, enzymes and more. Specifically, this system leverages a proprietary synthetic guide RNA (gRNA) that recognizes only the universal donor vectors of the toolkit. This has been demonstrated in cell culture and live mice with many targeted genes and inserted sequences, efficiently labeling and manipulating a wide variety of proteins in dividing and non-dividing tissues.

Other Applications

This technology could also be used to manipulate the structure of a protein to identify functional domains, unveil protein interactions, and delineate structure-function relationships. Additionally, this system would increase the throughput of Gene Trap experiments, in which cells harboring single gene mutations are selected by a marker. Gene trap experiments are indispensable in the process of identifying genes, the proteins they produce, and their functions in healthy and diseased states.

Advantages

- Faster, simpler, and more effective CRISPR/Cas9 genome editing
- Universal donor vectors eliminate need for gene-specific reagents
- HiUGE vectors contain built-in cas9 coding sequences – minimizing workflow while retaining compatibility with AAV delivery