PIONEER SCIENTIST INTERVIEW

Multicopy Chromosomal Integration Using CRISPR-associated Transposases

On 17 June 2020, Dr. Sheng Yang's research on "Multicopy Chromosomal Integration Using CRISPR- Associated Transposases" got published on *ACS Synthetic Biology*. In this interview, we'll talk with Dr. Yang to learn more about their recent studies.

Dr. Sheng Yang

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Dr. Yang received his doctoral degree in 2000 from the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (CAS). After received his doctorate, he began his faculty career at CAS. Dr. Yang is currently leading a research group at the CAS Center for Excellence in Molecular Plant Sciences, China.

Dr. Yang's research focuses on metabolic engineering and genomic editing in bacteria and yeast. His lab has developed several high efficiency CRISPR systems for genome editing in bacteria and yeast. Through these research, Dr. Yang aims to develop and optimize these findings into genetically based bio products and bioprocesses.

Congratulations to your group's recent paper on ACS Synthetic Biology. Could you briefly introduce this study?

The integration of expression cassettes into a bacterial chromosome has advantages for high-efficiency biocatalysts, but iterative integration is laborious.
Our research demonstrated a new method for multicopy chromosomal integration by CRISPR-associated transposases (MUCICAT).

What has prompted your team to develop this technology?

Our lab has been focusing on genetic manipulation tools especially multiplexed genome editing for years.

In *E. coli*, the multiplexed genome editing efficiency of CRISPR-Cas decreased sharply as the number of targets increased, especially for gene insertions. Because the low frequency of homologous recombination can't repair multiple double strand break generated by Cas effector nuclease.

The CRISPR-transposase systems reported in 2019 are ideal for multiplexed genome editing because they do not depend on homologous recombination at all.

MUCICAT can insert up to 8 copies of Gene of Interest (GOI) into the chromosome within 3 days, which not only speeds up the chromosome integration, but also forms a library of gene cassettes with different copies on chromosomes. It also can help to screen the optimal dose more accurately. We believe that MUCICAT will be a convenient tool to accelerate strain construction and optimization in synthetic biology and metabolic engineering.

Any difficulties or interesting things happened during this research project?

The editor of *ACS Synthetic Biology* was very friendly and the overall reviewing process was very smooth and efficient. We did encounter some difficulties and interesting things in MUCICAT project and will describe them in our next manuscript.

What are your team's main research areas currently and in the near future? Any plans you'd like to share with us?

Our team mainly focuses on metabolic engineering in microorganism and enzyme engineering field, as metabolic engineering has been developed into a very effective way to optimize industrial fermentation processes, by introducing directed genetic modifications using recombinant DNA technology.

For sure, we will continue to develop and improve the MUCICAT technology, including the improvement of its efficiency and the application in more strains and metabolic engineering cases.

It is reported that China's industrial fermentation ranks the first in the world. However, most of the producer strains are sub-optimal. We believe MUCICAT is a powerful tool for the strain improvement.

You have deposited 65 plasmids onto MolecularCloud and received more than 160 requests for your plasmids so far. Is there anything you'd like to say about MolecularCloud?

Plasmids used in this research have been deposited onto MolecularCloud.

For more information of Dr. Sheng Yang's research and plasmids, please visit

https://www.molecularcloud.org/s/sheng-yang

"MolecularCloud makes research more efficient. For example, it provides an open and free platform to share and request biological materials like plasmids to save researchers' time. Both deposit and ordering process are very convenient. I'd like to share more plasmids and ideas onto MolecularCloud."