Bacteria-Free Approach for Expressing RNA and Proteins in Human Cells (2024-008)

Rapid expression of RNA and proteins through a novel DNA-cloning approach that eliminates the need for laborious E. coli-based work and traditional amplification of reaction products.

Market Overview

In the past 40 years, E. coli-based DNA cloning has been one of the major time- and labor-consuming steps in molecular biology. This approach is the first to eliminate E. coli from DNA cloning for protein and RNA expression in human cells. Rather than days spent on E. coli-based work, this method simply requires a 30-minute single-tube approach that employs the joining of two or more double-stranded DNA molecules in a single, brief, room temperature reaction, enabling the direct use of the resulting reaction products in human cells for efficient expression of RNAs and/or proteins. In addition to the time saved, a major advantage of this approach is its high yield, which eliminates the need for traditional amplification of the reaction products in bacteria prior to the use in human cells. There is a significant commercial opportunity as nearly every molecular biology lab in the world currently uses laborious E.coli-based DNA cloning.

Technical Summary

The Lucky7 approach combines two activities in a single 30-minute, room temperature reaction: the 5'-exonucleolytic activity of T7 DNA Exonuclease and the nick-sealing activity of T7 DNA ligase. In this reaction, precisely positioned phosphonothioate DNA modifications serve as stops for T7 DNA Exonuclease, producing fully defined perfectly matching singlestranded sticky ends for anneali ng of a DNA insert to nano-backbone. T7 DNA Ligase seals the newly formed insert-backbone nicks simultaneously with the process of sticky end formation and annealing. Following a 10-minute column clean-up, the reaction products are ready for use in human cells.

In comparison to conventional E. coli-based DNA cloning methods, the Lucky7 approach requires none of the following procedures: DNA restriction digest, utilization of E. coli competent cells, transformation of DNA into E. coli, plating of transformed bacteria on LB plates followed by their overnight incubation, bacterial colony picking, secondary overnight growth of bacterial cultures in liquid LB media, bacterial culture centrifugation, mini/midi/maxipreps of plasmid from pelleted bacteria, and endotoxin removal from the bacteria derived plasmid DNA.

Applications

DNA-cloning for expressing RNA and proteins in human cells.

Development Stage

Advantages

- Bacteria free method for DNA cloning
- Drastically faster than traditional cloning methods; 40 minutes instead of 2+ days
- Significantly reduces the cost burden of DNA cloning.
- High yield amplification with successful plasmid transfection into human cells
- Significantly less laborious (no restriction digests, agar plates, bacterial colonies, overnight cultures, and maxipreps required)

About the Inventors

Andrei Alexandrov, PhD.

Assistant Professor in Genetics and Biochemistry at Clemson University

Dr. Alexandrov received his PhD at the University of Rochester School of Medicine and had his post-doctoral training in the (i) Center for Human Genetics and Molecular Pediatric Disease at the University of Rochester and (ii) Department of Molecular Biochemistry and Biophysics at Yale University. His lab at the Clemson Center for Human Genetics uses forward genetics and ultra-high throughput CRISPR-based genome interrogation to identify components of disease-associated human RNA pathways.

Rui “Jerry” Che

PhD Student at Clemson University

Jerry Che received a Bachelor’s degree in Agriculture at Jinling Institute of Technology and Bachelor of Science in Biotechnology at the State University of New York College. In August of 2021, he joined Dr. Andrei Alexandrov’s as a PhD student. Jerry’s research focuses on identifying components of disease-associated human RNA pathways using a variety of high-throughput technologies.

Bhoomi Mirani

PhD Student at Clemson University

Bhoomi Mirani received her Bachelor’s degree in Biotechnology in summer of 2022 at the Biotechnology and Bioinformatics Institute in Pune, India. In fall 2022, Bhoomi joined Dr. Andrei Alexandrov’s lab to pursue her graduate studies in Genetics. Her research is focused on the roles of human long non-coding RNAs (lncRNAs) in cancer.

Monireh Mohammadpanah

PhD Student at Clemson University

Monireh obtained her Bachelor’s degree in Cellular and Molecular Biology and Master’s degree in Molecular Genetics. In January 2022, she joined the Clemson Center for Human Genetics as a doctoral student. Her research focuses on the validation of factors regulating human metastasis-associated long non-coding RNA MALAT1 and the development of ultra-high throughput functional screening technology to map potential druggable sites within multi-functional essential human proteins.

For more information on this technology contact:

Pushparajah Thavarajah
Business Development Associate
E: pthavar@clemson.edu
P: 864.656.5708