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System For Continuous Mutagenesis In Vivo To Facilitate Directed Evolution

Tech ID: 33183 / UC Case 2015-656-0

BACKGROUND

This invention overcomes a limitation of in vivo mutagenesis systems. Some methods of mutagenesis involve treatment of plasmids with mutagenic chemicals or UV light prior to transformation, but these result in biased mutation spectra. Use of error prone DNA polymerases produces a more random set of mutations, but the rate of mutagenesis rapidly declines with continuous culture. As a result, using such polymerases separates mutagenesis and selection into multiple steps. Mutant genes in plasmids need to be generated by the error prone polymerase, then the plasmids isolated into libraries and selected in a separate step.

What is needed, then is an error prone DNA polymerase that does not result in a decline in the rate of mutagenesis in culture.

TECHNOLOGY DESCRIPTION

A typical error-prone Pol I mutant in bacteria is one that includes mutations in I709N, A759R, and D424A. The invention involves an error prone PolI mutant that also includes a K54 mutation. Such a mutant not only results in error prone replication, its mutation rate does not decline even in continuous culture. This allows for simultaneous mutation and selection, greatly enhancing the efficiency of directed evolution.

APPLICATIONS

Directed evolution of genes of interest that can be selected in E. coli culture.

ADVANTAGES

One step process, no plasmid isolation, ligation, amplification, iteration.

Cheaper, less labor intensive.

Scalable - limited only by the size of the culture.

INTELLECTUAL PROPERTY INFORMATION

Country	Туре	Number	Dated	Case
United States Of America	Issued Patent	10,760,071	09/01/2020	2015-656

RELATED MATERIALS

ADDITIONAL TECHNOLOGIES BY THESE INVENTORS

▶ GFP-Amplification Mutagenesis Assay (GMA): Quantitative, Scalable Detection of Chemical Mutagenesis

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Permalink

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OTHER INFORMATION

KEYWORDS

directed evolution, error prone
polymerase, continuous
mutagenesis, E. coli, mutation,
bacterial culture, plasmid

CATEGORIZED AS

- Biotechnology
 - Other
- **►** Environment
 - Sensing
- Research Tools
 - Expression System
 - Nucleic Acids/DNA/RNA
 - ▶ Screening Assays

RELATED CASES

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