Lac operator-based mitigation of heterologous DNA instability in plasmids propagated in *E. coli*

Technology Summary

Plasmid-based cloning and propagation of DNA in *E. coli* is common practice in laboratories and in the biopharmaceutical industry, however, unwanted expression of the heterologous DNA in *E. coli* can be toxic preventing the replication or resulting in the accumulation of unwanted mutations in the heterologous DNA. These mutations allow the *E. coli* to replicate the DNA by eliminating the toxicity, but it will result in an unwanted final protein product. This invention makes it possible to efficiently clone toxic heterologous DNA in *E. coli* by virtually eliminating its expression.

Gene expression in *E. coli* is silenced by sandwiching the heterologous DNA between 2 or more lac operators in the cloning plasmid and propagating the DNA in a strain that synthesizes the Lac repressor. Depending on the application, additional transcriptional terminator sequences can be included in the regions flanking the heterologous DNA to further increase its stability.

The use of transcriptional regulatory elements to increase the ability to clone and propagate heterologous DNA in *E. coli* was developed to address difficulties associated with the cloning and stability of influenza hemagglutinin (HA) and neuraminidase (NA) variants into a common reverse genetics vector. The result was a robust viral reverse genetic vector for viral vaccine and antigen production that used this simple approach which can be readily implemented for stabilizing heterologous DNA in any plasmid that is propagated in E. coli.

Potential Commercial Applications

- Stable cloning and propagation of otherwise unstable heterologous DNA
- Efficient production of desired antigens and viruses by reverse genetics
- Generation of tightly regulated expression vectors

Competitive Advantages

- Increases clonability of heterologous DNA in *E. coli*
- Enables stable amplification of otherwise toxic heterologous DNA in *E. coli*
- Facilitates the reproducible production of proteins and viruses for vaccines

Development Stage: Proof-of-concept; Research and Development Tool

Inventors: Robert Daniels (FDA), Tahir Malik (FDA), and Jan-Willem de Gier (Stockholm University)

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Intellectual Property: U.S. provisional application 63/346,568 was filed May 22, 2022

Product Area: Vaccine Development; Protein Expression; Cloning; Plasmid; Expression Vector

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FDA Technology Transfer Program
Email: FDAInventionLicensing@fda.hhs.gov