Translation-Coupling Cassette for Quickly and Reliably Monitoring Protein Translation in Host Cells

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WARF: P090401US02

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The Wisconsin Alumni Research Foundation (WARF) is seeking commercial partners interested in developing a system and method for quickly and reliably monitoring protein translation in bacteria or other host cells.

Overview

Bacterial production of recombinant proteins is used commonly by researchers and commercial entities to manufacture a large variety of proteins. However, many proteins are not produced efficiently in bacterial cells and must be produced through a different expression system. In these cases the researcher generally does not realize that protein expression failed until the bacteria are destroyed, the protein isolated and a gel run to detect the presence of the protein, a time-consuming process.

Monitoring translation in real time would allow scientists to know more quickly if they should use a different expression system, but the ability to monitor protein translation directly in bacterial cells is limited. One method of monitoring protein expression in cells is to fuse the protein of interest to a fluorescent protein. Protein translation then is measured through detection of fluorescence in the cells. However, this method is limited because the fusion can affect the activity of both the protein of interest and the fluorescent protein and also makes purification and isolation difficult.

The Invention

UW–Madison researchers have developed a method of using translation coupling to quickly and reliably determine whether a given host is capable of expressing the gene product of any given gene. This method could be used to monitor protein translation efficiency in bacterial cells.

The method involves a cassette that couples the full translation of a desired target gene to that of a detectable response gene. If the target gene is fully translated, so is the response gene. If the target gene is not translated, the response gene product is not detectable.

For example, one embodiment utilizes a DNA plasmid with a cloning site upstream of a DNA hairpin that masks the ribosome binding site required for translation of an antibiotic resistance protein. Scientists clone the protein of interest into the plasmid without a stop codon in frame and with a tag for purification purposes. Expression of the desired protein leads to unwinding of the DNA hairpin, unmasking the ribosome binding site and promoting translation of the antibiotic resistance protein. If the protein of interest is expressed in *E. coli*, the bacteria will survive in the presence of the antibiotic. If the protein is not expressed, the bacteria will die when exposed to the antibiotic.

Applications

- Bacterial production of proteins for research or commercial use
- Determining culture conditions that enable a host to efficiently express a target gene

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- Screening a DNA library for factors that facilitate expression of a target gene

Key Benefits

- Provides a quick and easy means of determining protein translation in bacterial or other host cells
- Does not affect the activity of the protein of interest
- Because the protein of interest is not fused to another protein and is tagged with a purification tag, the protein can be isolated and purified using traditional means.

Stage of Development

The vector and method have been developed and tested and are ready to be used in a commercial setting.

Additional Information

For More Information About the Inventors

- Brian Pfleger

Related Intellectual Property

- View Continuation Patent in PDF format.

Tech Fields

- Research Tools : Other research tools
- Research Tools : Synthesis & purification

For current licensing status, please contact Jennifer Gottwald at jennifer@warf.org or 608-960-9854

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