Biomedical engineers teach bacteria to count

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Biomedical engineers at Boston University have taught bacteria how to count. Professor James J. Collins and colleagues have wired a new sequence of genes that allow the microbes to count discrete events, opening the door for a host of potential applications, which could include drug delivery and sensing environmental hazards.

"This was probably the major application still to be addressed within synthetic biology: Can you count discrete events?" said Collins, who is also a Howard Hughes Medical Institute investigator and a Boston University William Fairfield Warren Distinguished Professor. "And now we've come up with two different designs to do this."

The research is detailed in an article, "Synthetic Gene Networks That Count," that appears in the May 29 issue of *Science*.

The young but burgeoning field of synthetic biology addresses biological research questions with an engineering approach. Researchers design and build networks of genes, splicing them into bacterial genomes to run specific tasks or manufacture desired molecules - a process akin to installing biological computer software. Though the field is rapidly advancing, the gene-based tools available to synthetic biologists remain limited.

Gene networks that give bacteria the ability to count could become powerful devices in the synthetic biology toolkit because they can be coupled to almost any other bacterial function or environmental cue that bacteria can sense, such as presence of a toxin or sunlight. In the future,

bacteria might be set to self-destruct after a certain number of cell divisions or after a specified period of time.

"The fundamental application is as a safety mechanism," said Collins. "If you've engineered an organism to be released into the environment as a biosensor, or you've engineered an organism to go into your body to deliver a therapeutic, in many cases you want to ensure after a certain period of time that the organism is no longer in the environment or your body."

Collins' team designed two separate synthetic gene networks not found naturally in E. coli bacteria. Each uses a different method to make the bacteria count.

The first, the Riboregulated Transcriptional Cascade (RTC) synthetic gene network, counts by starting and stopping transcription and translation - the process by which a gene's instructions are executed - of a series of genes every time an event occurs. The researchers programmed the system so that after the third interruption, the network translates and transcribes the gene for a fluorescing protein, which is visible to researchers.

The second synthetic gene network, called DNA Invertase Cascade (DIC), works in an entirely different way. At the first event, such as the presence of a chemical, the first gene manufactures a protein that cannibalistically snips its own gene out of the network, flips it over and sticks it back in. Once the gene is backwards, it can no longer be transcribed - but an extra snippet of DNA the researchers attached to its tail acts as a bookmark, showing protein-making machinery where to resume work. Each successive flip-over counts another event, and the fluorescing protein is activated after the third one.

In both methods, researchers can move the genetic parts in these

counters around to fit their needs. The network might be extended to count to higher numbers, or additional genes for fluorescing proteins might be added, for example, to glow red when the bacteria have counted to two, and green at three. The network's counting can be linked to any periodic signal from the outside world the bacteria can detect, or to an internal event, such as a protein only expressed at one point during each cell division.

Each counter has strengths. The RTC can count quickly and works best when events happen every 20 to 30 minutes. The DIC takes longer to execute its flipping action, so works best when the events being counted are long and have long gaps between them, such as periods of sunlight and darkness, to count days. The options for using the counters are nearly endless, say the researchers.

"These are such basic tools that it's really hard to say what thousand things they might be used for in the future," said Ari Friedland, a graduate student in Collins' lab and a co-author of the paper. "Consider computing - what does one transistor do for you? Not that much, but if you pack a few thousand onto a chip, then you really have some power. These are fundamental biocomputing devices."

Source: Boston University Medical Center