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SYNTHETIC BIOLOGY

UBER — a portable system for cross-species genetic engineering

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Genetic engineering approaches typically rely on the host's transcriptional machinery and, therefore, can only normally be used in the species for which they were designed. Kushwaha and Salis have now developed a genetic engineering system that they name the Universal Bacterial Expression Resource (UBER), which they report in *Nature Communications*. UBER uses cross-species regulatory sequences and feedback loops to produce a host-independent viral RNA polymerase that acts as a built-in 'power supply' to drive the expression of shared genes and pathways in different bacterial host systems. The proof-of-principle application of UBER shows that it is a versatile expression system that is portable across species.

In UBER, initial expression of the T7 RNA polymerase under the control of a cross-species priming promoter relies on the cell's native transcriptional machinery. Although the priming promoter has a low transcription rate, UBER uses a strong positive-feedback loop that relies on a T7 promoter to generate much higher levels of the polymerase. This process means that T7 RNA polymerase production is practically independent from the host's endogenous transcriptional system.

High levels of the T7 RNA polymerase are toxic to host cells and decrease their growth rate. Therefore, UBER also includes a negative-feedback loop that relies on the production of TetR, which represses T7 RNA polymerase expression. By placing the expression of TetR under the control of a T7 promoter, the researchers generated what they term a 'mixed feedback loop' system that is capable of both auto-activation and self-limitation.

This 'power supply' unit can be used to support transcription of heterologous genes or pathways of interest under the control of T7 promoters. Engineered translation signals ensure cross-species compatibility of the protein-production process.

The researchers used a GFP reporter and a three-enzyme pathway that controls the synthesis of the terpenoid neurosporene as outputs of their system in three bacterial strains: *Escherichia coli* DH10B, *Pseudomonas putida* KT2440 and *Bacillus subtilis* 168. The results highlight the portability of UBER in tuning gene expression in a similar way in diverse Gram-negative and Gram-positive bacteria. The applicability of the system beyond model species such as *E. coli* opens up metabolic engineering possibilities in environmentally and industrially relevant species.

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ORIGINAL RESEARCH PAPER Kushwaha, M. & Salis, H. M. A portable expression resource for engineering cross-species genetic circuits and pathways. *Nat. Commun.* **6**, 7832 (2015)