Constructing multi-gene systems for simplified reconstitution of translation

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The word ‘synthetic’ is derived from ‘*sunthesis’*, which in Greek means ‘to put together’ and the recently emerging field of synthetic biology can be described as a combination of science and engineering which involves putting together many biological parts or devices to build novel systems. This approach not only allows us to design, construct and understand modular biological processes but also use the knowledge gained to tweak existing natural systems for useful purposes. But, can this principle be extended to build a whole ‘cell’, part by part?

The answer to such an ambitious question seems more plausible with the recently proposed outline for a synthetic ‘minimal cell’, a system capable of evolving and replicating in presence of small nutrient molecules and possessing machinery sufficient to synthesize DNA, RNA and proteins. It would carry all the genes necessary to reconstitute these processes on a circular genome that is 113 kb long with 151 genes, with each gene being tagged to enable subsequent purification of its protein and flanked by a promoter and a terminator for RNA polymerase (a protein that transcribes the gene into RNA).

The part of the minimal cell we have first chosen to build is the translation or protein synthesis module, as the genes that code for translation factors constitute 96% of our minimal genome. We aim to express and purify all the listed thirty translation factors from a single construct and use it to reconstitute protein synthesis.
In this project we have used two constructs (available in our lab) that encode all thirty translation factors between them and we have been successful in expressing and purifying all the thirteen encoded translation factors from one of the constructs in *E. coli*. The other construct encoding seventeen factors was found to be unstable, while its precursor constructs (encoding eight and nine factors respectively) were stable and were found to over-express most of the proteins. Thus we have three stable, modular, multigene constructs that could be used to express all thirty translation factors to reconstitute translation and also can be combined to form a single construct which would carry the ‘translatome’ of our minimal genome.

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