Phage-assisted directed evolution of proteins and RNAs

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Résumé

In vivo directed evolution techniques allow engineering protein and nucleic acids with targeted functions inside living cells. The efficiency of such techniques is determined by the evolution speed and sampling size. Transducing phage particles are able to support higher mutagenesis rates than any viral system, allowing for a faster evolution, where the host cell is re-engineered according to the desired selection. We will show how phages can be used to accelerate the directed evolution of proteins. We have engineered the genomes and hosts of phages M13, T7 and P2 to evolve proteins and RNA. For this, we have developed phage infection cycles implementing positive and negative selections. The implementation of positive and negative selections allowed the engineering of stronger activity and specificity respectively. We demonstrate the usefulness of our system by engineering the smallest transcription factor activator/repressor, a set of orthogonal transcription factors activator/repressor and a riboswitch in *E. coli*. Our methodology for accelerated directed evolution can be used to evolve any protein or RNA where its activity could be coupled to gene expression.

Mots-Clés: synthetic biology, directed evolution

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