Engineering phages and their hosts for the directed evolution of biomolecules

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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 inside the organism. Viruses with fast replicative cycles and able to support high mutagenesis rates allow implementing a faster evolution, where the host cell is re-engineered according to the desired selection. Phages are specially suited due to their small size, fast replication and the ease of engineering of their genomes and their hosts. We have developed directed evolution systems based on filamentous (M13) and lytic (T7) phages. We have engineered their genomes and hosts by removing from the phages genes required for their replication to later complement them within the host. The implementation of negative selections allowed the engineering of specificity. We demonstrate the usefulness of our system by engineering the largest known set of orthogonal transcription factors able to activate and/or repress cognate or combinatorial promoters in E. coli. We also show how to evolve riboswitches using cycles of positive and negative selections. Our methodology for directed evolution can be implemented in many phage systems to evolve proteins, nucleic acids and phage tropism determinants.

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