Development of Phage-assisted Evolution and Riboregulation Strategies

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Riboswitches are short RNA sequences that modify their 3D structure in the presence of a specific molecule, regulating gene expression in the process. A few riboswitches have been described in eukaryotes, but they are mostly present in bacterial species. They are of great interest in synthetic biology for their inductive regulatory properties, which could be used in metabolic studies, or as biosensors in medical, industrial or environmental cases. However, riboswitches are very substrate-specific, creating a problem for their use with novel compounds; and current development methods suffer from issues such as being too laborious and not using *in vivo* conditions. By developing strategies based on Phage Assisted Continuous Evolution (PACE) and using T7 phage along a double selection system, we developed a way of obtaining riboswitches that show improvements compared to a control sequence. Once the method is tuned, it could be used to develop novel riboswitches not present in nature, and not only for the directed evolution of riboswitches, but also other types of sensors, such as protein or RNA receptors. A second inducible RNA system based on the phage Qβ was also tested. These have yielded a randomised library of riboswitch sequences in phages and an inducible RNA plasmid, respectively. In the first case, using a double selection process to achieve evolution, we have shown sequence variation in phage populations decrease over time, until a single sequence was prominently represented. The sequence showed higher activation folds at a lower concentration of the activating molecule than in initial generations, indicative of evolution. As for the RNA-based plasmid system, we have shown it to be an inducible, transient expression system that could be used as a novel way to regulate gene expression and bypass CRISPR systems. These results speak of the possibilities held by these strategies, not just for their specific areas of research, but for synthetic biology at large.

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