Gene-specific In Vivo Hypermutation for Continuous Directed Evolution

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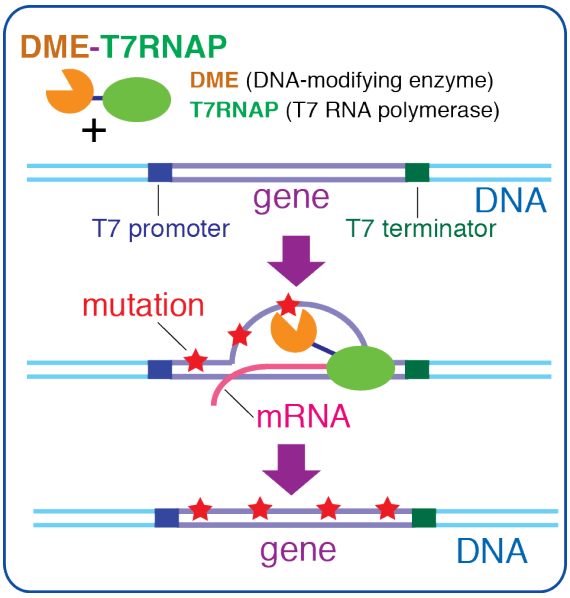
Directed evolution uses Nature's logic to drive biomolecular evolution in the laboratory. Despite its broad applications, traditional directed evolution methods usually rely on *in vitro* DNA diversification and subsequent insertion of DNA library into cells, suffering from labour-intensive protocols and limited sequence diversity. To overcome these limitations, various targeted *in vivo* mutagensis methods have recently been developed to introduce mutations directly into a defined region of DNA in living cells[1]. Here we describe our recent efforts to develop gene-specific *in vivo* mutagensis methods, eMutaT7 and eMutaT7^{transition}, which can greatly enhance the scale and depth of directed evolution experiments[2,3]. We show that chimeric mutator enzymes, composed of a nucleotide deaminase and an orthogonal RNA polymerase, enable rapid generation of transition mutations in a target gene. Furthermore, simple passaging of these cells in selection conditions promotes continuous directed evolution of proteins within cells. With their simplicity and high efficiency, these methods can accelerate protein directed evolution in research, biotechnology, and medicine.



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