EVOLUTION OF HIGLY EFFICIENT T7 RNA POLYMERASE FOR MRNA PRODUCTION USING APTAMER-BASED FLUORESCENCE-ACTIVATED DROPLET SORTING

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Messenger RNA (mRNA) therapies have recently gained tremendous attention since the approval of SARS-CoV-2 mRNA vaccines. In vitro transcription (IVT) production of mRNA is typically using T7 RNA polymerase (T7 RNAP) owing to its robust activity and strict promoter specificity. However, wild-type T7 RNAP suffers from the generation of undesirable byproducts such as abortive fragments and double-stranded RNA (dsRNA) that elicit adverse host immune responses and are difficult to remove at large scale. Here, we developed a highly efficient aptamer-based fluorescence-activated droplet sorting (AB-FADS) method for the directed evolution of T7 RNAPs. We applied our method to improve activity of T7 RNAP at elevated temperatures through four rounds of directed evolution. The half-life of the best variant is 270-fold higher than that of wild-type T7 RNAP and the RNA yield increased at least 1582 times at 50 °C. We also demonstrated that IVT products generated with the variant have reduced dsRNA content and higher purity full-length mRNA products. The mutant enzyme exhibits excellent catalytic features in the synthesis of mRNA with various lengths and different GC contents, such as high yield, high purity, and low dsRNA demonstrating its excellent industrial potential in mRNA drug synthesis.
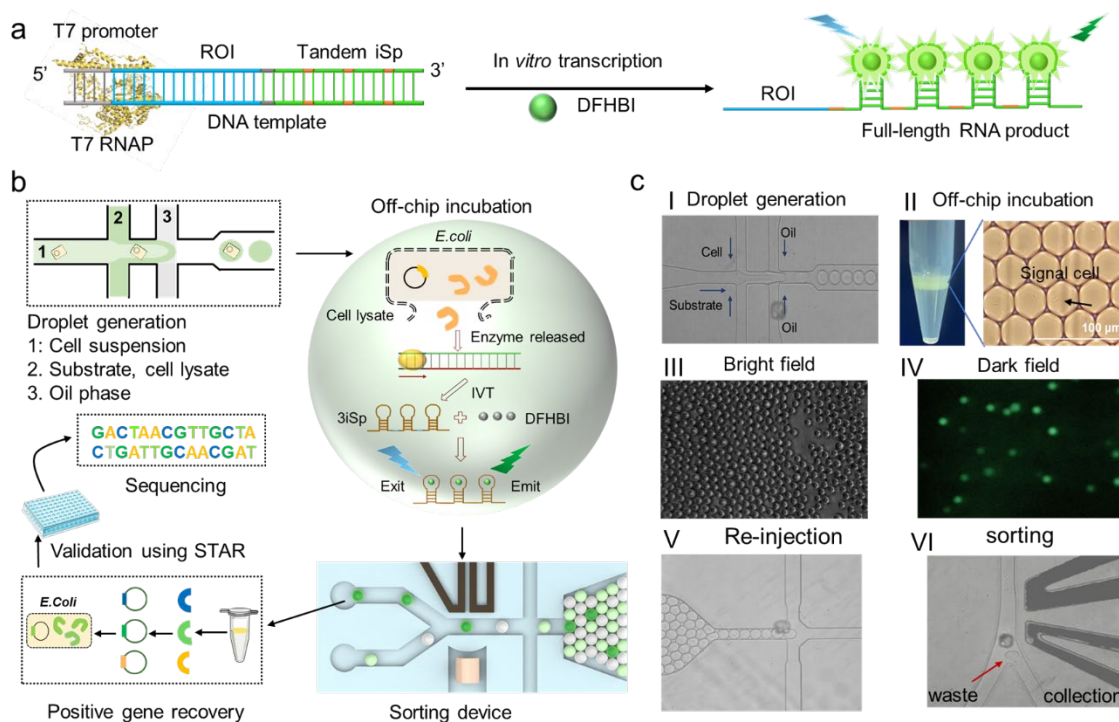
Figure 1 The scheme of aptamer-based FADS for the evolution of T7 RNAP