Automatic Natural Transformation (ANT) Cloning

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Automated cloning platforms are essential to achieve high throughput in synthetic biology studies. However, *E. coli*-based cloning is challenging to be fully automated in a high throughput way. Unlike *E.coli*, *Acinetobacter baylyi* ADP1 is able to take up DNA from the environment by themselves, a phenomenon called as natural transformation. Here, we explored this mechanism for automatic DNA cloning and engineering. Recombinant plasmids are efficiently generated from Gibson or overlap extension PCR (OE-PCR) products by simply adding the DNA into *Acinetobacter baylyi* ADP1 cultures. Up to 10,000 colonies were obtained per microgram of DNA, while the number of false positive colonies was low. We cloned and engineered 21 biosynthetic gene clusters (BGCs) of various types, with length from 1.5 to 19 kb and GC content from 35% to 72%. One of them, a nucleoside BGC, showed antibacterial activity. Furthermore, the method was easily transferred to a low-cost benchtop robot.