ABSTRACT

METHODS FOR THE PRODUCTION OF LIBRARIES FOR DIRECTED EVOLUTION

Disclosed herein is an efficient method of generating a library of variants of a sequence of interest, such as may be used in directed evolution. In one embodiment, the method includes an amplification reaction, e.g., error-prone PCR, to generate double-stranded DNA (dsDNA) variants of a sequence of interest, after which one strand of the dsDNA variants may be selectively degraded to produce single-stranded DNA (ssDNA) variants. The ssDNA variants may be hybridized to ssDNA intermediaries, e.g., uracilated circular ssDNA intermediaries, to form heteroduplex DNA, which may be transformed into cells, such as *E. coli* cells, yielding a library of variants. This method eliminates the inefficient sub-cloning steps and the need for costly primer sets required by many prior methods.

No Suitable Figure.