OVERFRAG: an Overlapping DNA Fragments Generator for Molecular Cloning and Synthetic Biology

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Introduction: DNA synthesis and homologous recombination can be used to simplify molecular cloning and to make synthetic biology easily accessible. However, the design of overlapping DNA fragments to construct large molecules is time-consuming and requires verification of several parameters to ensure that fragment synthesis is attainable, given the restrictions found in chemical synthesis of DNA. OVERFRAG is a web-based tool that generates overlapping DNA fragments to assemble either in yeast cells by Gap Repair or in vitro by Gibson and In-Fusion methods. Materials and methods: The tool was developed using Perl programming language and the CGI (Common Gateway Interface) library. It receives as input the coding sequence (without introns), the promoter sequence, the terminator sequence, maximum fragment size, overlap size, and the maximum number of extra fragments allowed. Results: The fragments generated are suitable for chemical synthesis and molecular assembly. Some conditions are evaluated by the tool, in order to identify if the input sequences satisfy restrictions imposed by companies which sell synthetic DNA fragments. These restrictions are: a DNA sequence must not have homopolymeric runs larger than (a) five cytosine or guanine nucleotides; (b) or nine adenine or thymine nucleotides; and (c) must not contain GAC trinucleotide repeats. Conclusions: Some possible applications of OVERFRAG include gene cloning, cDNA cloning (to assemble exons), chromosome assembly, design of chimeric antibodies, and synthetic biology applications to construct genes, genetic pathways and genomes.

Keywords: DNA overlapping fragments, homologous recombination, yeast, gap repair synthetic biology.