

METHODS FOR *IN VITRO* JOINING AND COMBINATIONAL ASSEMBLY OF NUCLEIC ACID MOLECULES

Abstract of the Disclosure

The present invention relates to methods of joining two or more double-stranded (ds) or single-stranded (ss) DNA molecules of interest *in vitro*, wherein the distal region of the first DNA molecule and the proximal region of the second DNA molecule of each pair share a region of sequence identity. The method allows the joining of a large number of DNA fragments, in a predetermined order and orientation, without the use of restriction enzymes. It can be used, *e.g.*, to join synthetically produced sub-fragments of a gene or genome of interest. Kits for performing the method are also disclosed. The methods of joining DNA molecules may be used to generate combinatorial libraries useful to generate, for example, optimal protein expression through codon optimization, gene optimization, and pathway optimization.

No suitable figure