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(54) Title: COMPOSITIONS AND METHODS FOR THE ASSEMBLY OF POLYNUCLEOTIDES

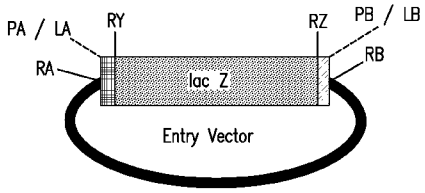


FIG.1A

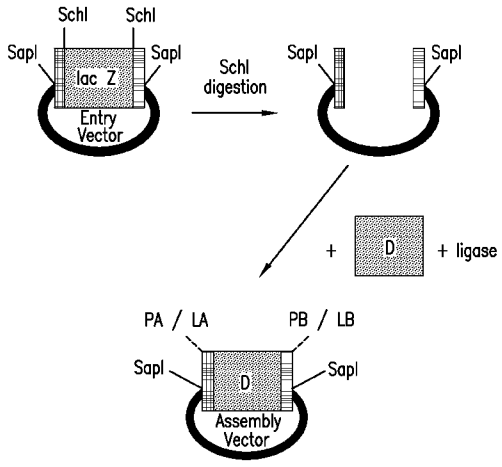


FIG.1B

(57) Abstract: The present invention provides compositions and methods for rapid assembly of one or more assembled polynucleotides from a plurality of component polynucleotides. The methods of the invention utilize circular nucleic acid vectors that comprise a DNA segment D flanked by an annealable linker sequence, annealable linker sequence pairs LA and LB, or annealable linker sequence / primer binding segment pairs LA and PB or PA and LB. Restriction endonuclease digestion of a plurality of vectors containing the DNA segments to be assembled generates a plurality of DNA fragments comprising the elements PA-D-LB, LA-D-LB, and LA-D-PB or D-LB, LA-D-LB, and LA-D. The sequences of annealable linker sequences LA and LB provide complementary termini to the DNA fragments, which are utilized in host cell mediated homologous recombination or together with promer binding segments PA and PB in a polymerase cycling assembly reaction for the ordered assembly of the various DNA segments into one or more assembled polynucleotides.

WO 2010/059763 A1

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