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(54) Title: METHODS FOR PREPARING NUCLEIC ACID MOLECULES FOR SEQUENCING

(57) Abstract: The invention relates to means and methods for preparing double stranded target DNA molecules for sequencing. In embodiments double stranded backbone DNA molecules comprising 5' and 3' ends are provided that are: ligation compatible with 5' and 3' ends of said target DNA; form a first restriction enzyme recognition site when self-ligated; in a form that enables self-ligation. Methods may comprise providing, if not already present, said target DNA with 5' and 3' ends that are in a form that prevents self-ligation and that are ligation compatible with said backbone DNA 5' and 3' ends. Methods may further comprise ligating said target DNA to said backbone DNA in the presence of a ligase and a first restriction enzyme that cuts said first restriction enzyme recognition site, thereby producing at least one DNA circle comprising a backbone DNA molecule and a target DNA molecule. Linear DNA may be removed at this time and subsequently a concatemer DNA molecule comprising an ordered array of copies of said at least one DNA circle through rolling circle amplification is produced that can be sequenced.



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