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(54) Title: COMPOSITIONS AND METHODS FOR PAIRWISE SEQUENCING

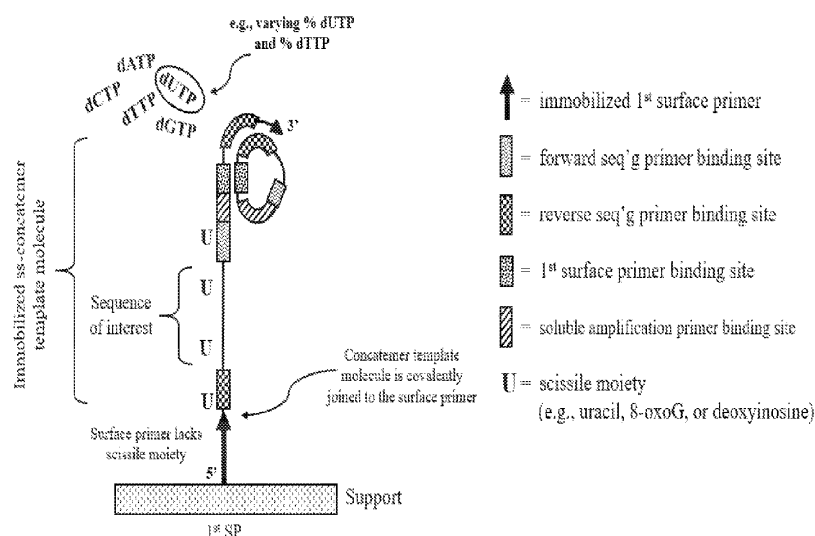


FIG. 26

(57) Abstract: The present disclosure provides compositions and methods that employ the compositions for conducting pairwise sequencing and for generating concatemer template molecules for pairwise sequencing. The concatemers can be generated using a rolling circle amplification reaction which is conducted either on-support, or conducted in-solution and then distributed onto a support. The rolling circle amplification reaction generates concatemers containing tandem copies of a sequence of interest and at least one universal adaptor sequence. An increase in the number of tandem copies in a given concatemer increases the number of sites along the concatemer for hybridizing to multiple sequencing primers which serve as multiple initiation sites for polymerase-catalyzed sequencing reactions. When the sequencing reaction employs detectably labeled nucleotides and/or detectably labeled multivalent molecules (e.g., having nucleotide units), the signals emitted by the nucleotides or nucleotide units that participate in the parallel sequencing reactions along the concatemer yields an increased signal intensity for each concatemer.

[Continued on next page]

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