

ABSTRACT

METHODS AND ARRAYS FOR PRODUCING AND SEQUENCING MONOCLONAL CLUSTERS OF NUCLEIC ACID

Methods for capturing and amplifying target polynucleotides on a solid surface, in particular in a well in a microarray, wherein the microarray may comprise a) a substrate comprising at least one well, a surface surrounding the well and an inner well surface; b) a first layer covering the inner well surface and comprising at least one first capture primer pair; and c) a second layer covering the first layer and the surface surrounding the well. Alternatively, the microarray may comprise a) a substrate comprising at least one well, a surface surrounding the well and an inner well surface; and b) a layer covering the inner well surface and comprising at least one first capture primer pair and at least one second capture primer pair. In particular kinetic exclusion amplification is used in creating monoclonal populations of the nucleic acids in the wells. The application also discloses a method for modifying an immobilized capture primer comprising: a) contacting a substrate comprising a plurality of immobilized capture primers with a plurality of template nucleic acids to produce one or more immobilized template nucleic acids, wherein the plurality of immobilized capture primers comprises a first plurality of primers comprising a 3'-terminal universal capture region Y, e.g. primer P5, and a second plurality of primers comprising a 3'-terminal universal capture region Z, e.g. primer P7; and wherein each template nucleic acid is flanked by a 5'-terminal and a 3'-terminal universal capture region Y or Z and comprises one or more, e.g. SapI, restriction sites and a target-specific capture region between the one or more restriction sites and the 3'-terminal universal capture region; and b) extending one or more immobilized capture primer. Finally, the application discloses a method for modifying an immobilized capture primer comprising: a) contacting a substrate comprising a plurality of immobilized capture primers with a plurality of different seed nucleic acids to produce a plurality of different immobilized seed nucleic acids; b) extending two or more of the immobilized capture primers to produce a plurality of different immobilized extension products complementary to two or more of the plurality of different immobilized seed nucleic acids; and c) activating one immobilized extension product of the plurality of different immobilized extension products, to form an activated capture primer.

[Figure 1]