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Two-step emulsion PCR to prevent formation of the chimeric molecules

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Motivation and Aim: Application of the conventional PCR method for the amplification of the random nucleotide sequence libraries often causes a formation of the undesired chimeric molecules. Invention of the water-in-oil emulsion PCR (ePCR) approach allowed to reduce the probability of the chimeric molecule formation compared to the conventional PCR. However, in the non-optimized conditions even ePCR causes the formation of the chimeric products.
Methods and Algorithms: We developed a step-by-step protocol for the ePCR consisting of two consequent rounds. We found that both an initial amount of the DNA template and number of amplification cycles play a critical role in the formation of the chimeric molecules. We suggest to use only 10^6 DNA molecule copies for the first round of ePCR.
Results: We analyzed a formation of the chimeric products during amplification of heterogeneous plasmid library in different conditions. To assess the percent of the formed chimers we used Illumina MiSeq platform. A proportion of chimeric molecules under optimal conditions was lower than 0.25 %. We suppose that this two-step ePCR approach may be useful for the preparation of heterogeneous sequence libraries for the next-generation sequencing and other issues which demand avoiding of the chimeric molecule formation.
Conclusion: ePCR approach allows to separate the DNA template molecules from each other using the water-in-oil emulsion. Our data suggest that ePCR approach is suitable for the preparation of random DNA libraries for the next-generation sequencing. However it requires additional adjustment to reduce a formation of chimeric molecules as much as possible.
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