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Design of an ssDNA oligodeoxynucleotide library with diverse random sequences and its amplification optimization

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Background and Aims: It has been clear for some time that the single-stranded state of nucleic acids allows them to adopt a variety of 3-dimensional structures. In these situations, they are able to act as catalytic or affinity agents. In vitro evolution of single-stranded oligonucleotide pools has been applied to discover the catalytic and binding functional nucleic acids (FNAs), which have the potential to be used as pharmaceuticals or the affinity ligands for targeted drug delivery. Random pools of single stranded oligonucleotides are critical agents for FNAs in vitro selection. For the design of a proper library, some fundamental points should be considered that have major influences on its effectiveness. Amplification of these oligodeoxynucleotides is also an important challenge and the most critical step of amplification is the conversion of the double-stranded DNA (dsDNA) into single-stranded ones (ssDNA).
Methods: Among the several computational or online softwares that are available for random pool design, Generuner® was selected and applied for the present study. Different parameters such as the expectancy for the formation of different secondary structures, Tm of primers and random sequence length were considered and optimized. The amplification of the deigned library into ssDNA molecules was performed with PCR and Asymmetric PCR and agarose gel electrophoresis on PCR products was applied for method development and optimization.
Results: The designed library includes sequences with 70 nucleotides in random region surrounded by 30 nucleotides as two fixed primer regions. After synthesis of the random library, amplification was performed by PCR. Various important parameters such as magnesium concentration, annealing temperature and cycle numbers of amplification were evaluated and adjusted. Finally, after optimization, the asymmetric PCR was performed to find the optimal primer ratio.
Conclusions: The designed library can be applied for further in vitro evolution studies in our laboratory.

Keywords: Functional nucleic acids; In-vitro selection; Random single stranded oligonucleotide pool; Asymmetric PCR