

Optimization of Quantitative PCR for Amplification of Single Stranded DNA Library

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Aptamers are a class of oligonucleotide based synthetic antibodies showing remarkable advantages over its conventional proteinaceous counterpart in many aspects. Ability to generate aptamers for almost any target, their high thermal stability and relatively high resistance to both biological as well as chemical degradation, and low cost of production are the main features among many superior qualities which make aptamers preferred to traditional antibodies. The process of development of aptamers by systematic evolution of ligands by exponential enrichment (SELEX) is a universal process characterized by repetition of five main steps namely binding, partition, elution, amplification, and conditioning. The paper presents the optimization procedure for quantitative PCR based amplification approach used in the fourth stage (amplification) of this SELEX process. The template dilution of 1×10^{-5} μM showed good amplification under the thermocycling conditions of denaturation at 95°C for 10 sec, annealing at 63°C for 10 sec and extension at 72°C for 10 sec. Successful amplification of the library was confirmed by both the amplification plots and gel electrophoresis. In conclusion, the concentration of ssDNA was used as initial template served a significant factor in determining the success of amplification.

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