A single tube universal method for production of DNA aptamers

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Introduction: The development of aptamers has proved to be a promising alternative to the pharmaceutical and diagnostic industries, since is possible to select aptamers for a wide variety of applications, such as drugs, diagnostic tests and cosmetics. However, the large scale production of high purity aptamers has been a great challenge, mainly due to high production costs. Otherwise, enzymatic synthesis has been reported as an efficient and low cost strategy to produce some biopharmaceutical molecules. **Objectives:** The purpose of this work was to develop a simply enzymatic method to produce aptamers, using the rolling circle amplification followed by a single restriction step. **Material and Methods:** An ssDNA template, containing the aptamer sequence, was obtained by chemical synthesis and phosphorylated using the T4 Polynucleotide Kinase. Then, the template was circularized using T4 DNA Ligase. The rolling circle reaction was performed using the phi29 DNA polymerase and a specific primer. The digestion step was performed using the restriction enzyme SchI. The fragments were separated using agarose or denaturing polyacrilamide gel electrophoresis (PAGE), and visualized by ethidium bromide or silver staining, respectively. **Results and Discussion:** To validate the method, the 31-TBA aptamer was produced. An ssDNA template containing the aptamer reverse-complementary sequence, two restriction sites and a primer annealing site was used in the rolling circle replication reaction. All steps were standardized to be performed sequentially in a single tube, without the necessity of purify the product of each reaction before the next step. The expected amplicons (the aptamer and the primer linked to the restriction site) were detected using PAGE and silver staining. However, intriguingly, the aptamer sequence was less stained by silver staining than the other fragment, and we observe that this is related to the %G of the oligonucleotide sequence. **Conclusions:** We developed a single tube universal method for the enzymatic production of DNA aptamers.

Keywords: RCA, Enzymatic synthesis, Silver staining