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Abstract

Validation and amplification of random DNA libraries with modified nucleobases for Click-SELEX †

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Abstract: Aptamers are nucleic acid ligands which display specific binding to a desired target. Oligonucleotides are limited in their ability to form hydrophobic interactions. The affinity of aptamers can be improved with the introduction of hydrophobic modifiers into the structure of nitrogenous bases. Click-SELEX is a powerful tool for the development of the aptamers containing modified nucleobases. This technology assumes adding of azide-bearing modifier of choice to alkyne-modified random DNA library using click-chemistry. The synthesis and amplification of modified libraries is a limitation for performing click-SELEX. This research is aimed to validate the modified DNA libraries using NGS and to study specific aspects of their amplification. Commercially available library with alkyne-modified uridine (5-ethynyl-deoxyuridine (EdU) instead of thymidine was obtained in four variants with different distribution of nucleobases. Alkyne-containing (before clicking of a hydrophobic moiety) and azide-modified (after clicking) libraries were amplified with four different DNA polymerases. All the enzymes screened were able to amplify non-natural DNA template, the best amplification efficiency was shown for Taq DNA polymerase. NGS of alkyne-containing libraries confirmed the correct length, high diversity of the libraries and the uniformity of nucleotide distribution per position. No significant difference between the sequenced samples amplified with two different DNA polymerases was recognized. In all four libraries the content of EdU was lower than it was assumed during the chemical synthesis; 14, 13.0, 16.4 and 18.5% EdU was detected in the random core instead of 20, 20.4, 23.1 and 25% of theoretical EdU content. To reach the equimolar distribution of nucleobases, a higher proportion (more than 25%) of EdU should be used during the chemical synthesis of the library. We believe these results provide an experimental basis for the expansion of click-SELEX technology to the routine aptamer research.

Keywords: aptamer; click-SELEX; modified random DNA library; amplification; next generation sequencing

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