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Abstract

The Validation and Amplification of Random DNA Libraries with Modified Nucleobases for Click-SELEX †

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Abstract: Aptamers are nucleic acid ligands which exhibit 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 used for the development of aptamers containing modified nucleobases. This technology assumes the addition of an azide-bearing modifier of choice to the alkyne-modified random DNA library using click chemistry. The synthesis and amplification of modified libraries can limit the performance of click-SELEX. This research aims to validate the modified DNA libraries using NGS and to study specific aspects of their amplification. Commercially available libraries with alkyne-modified uridine (5-ethynyl-deoxyuridine (EdU) instead of thymidine were obtained in four variants with different nucleobase distributions. 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 the non-natural DNA template, and the best amplification efficiency was shown for the Taq DNA polymerase. The NGS of alkyne-containing libraries confirmed the correct length and high diversity of the libraries, as well as 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 values were detected in the random core instead of 20, 20.4, 23.1, and 25% in the theoretical EdU content. To achieve 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 can provide an experimental basis for the expansion of click-SELEX technology within routine aptamer research.

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

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