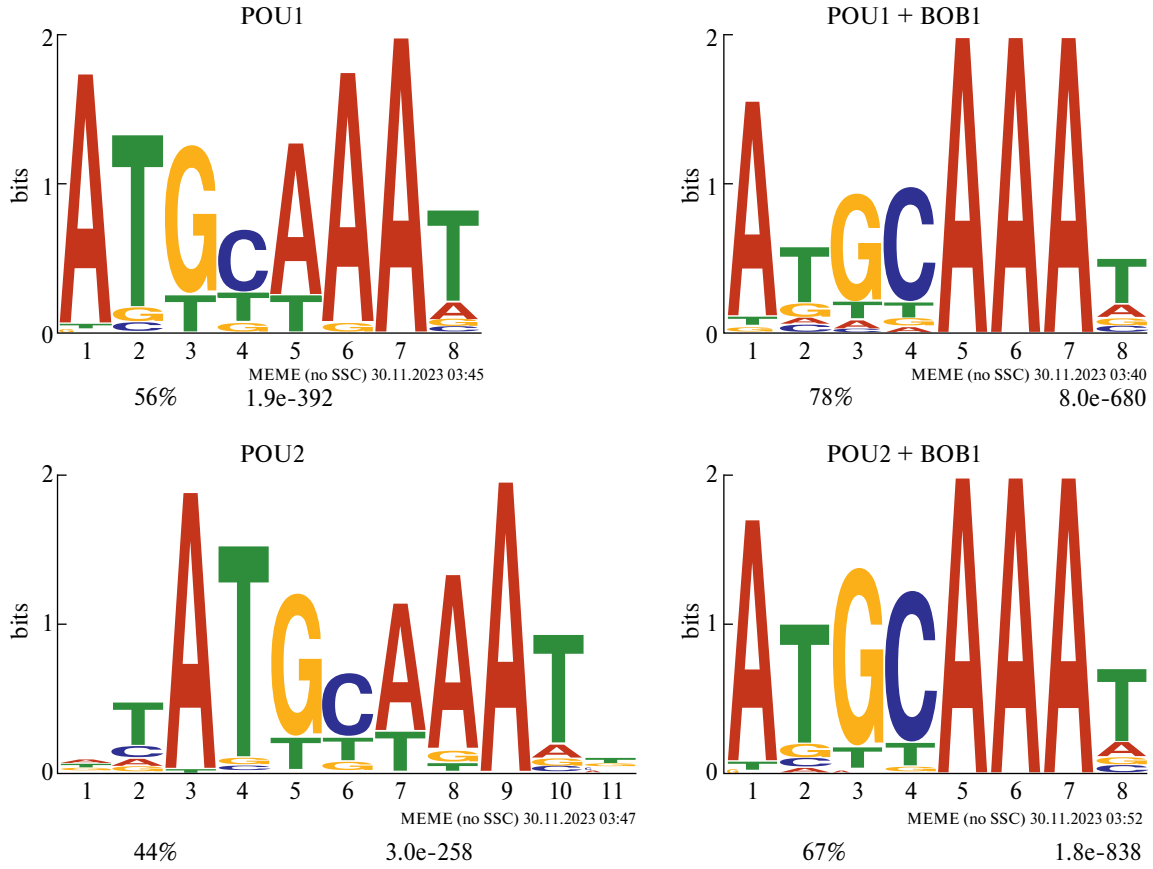

Рис. 4. Основные мотивы, выявленные с помощью онлайн-инструмента MEME (Bailey, Elkan, 1994) после обогащения с помощью EMSA-SELEX и NGS секвенирования. Под изображениями мотивов указана частота (%, слева) встречаемости данных мотивов в последовательностях и статистическая значимость мотива (E-value, справа).

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СОБЛЮДЕНИЕ ЭТИЧЕСКИХ СТАНДАРТОВ

Настоящая статья не содержит исследований с использованием животных или людей в качестве объектов.

КОНФЛИКТ ИНТЕРЕСОВ

Авторы заявляют об отсутствии конфликта интересов.

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EMSA-SELEX-SEQ METHOD FOR ANALYSIS OF BINDING SITE SEQUENCES IN DNA-PROTEIN COMPLEXES

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The BOB1 protein (OBF1, OCA-B) is a transcriptional coactivator of two POU domain proteins — OCT1, expressed in all cells, and lymphoid-specific OCT2. The interaction of BOB1 with OCT1/2 plays an important role in the regulation of immune responses in both physiological and pathological contexts. BOB1 is known to form a ternary complex with OCT1/2 bound to DNA in monomeric and certain dimeric configurations, changing the sequence specificity of the binding. To analyze DNA sequences from these complexes, in this work we proposed the EMSA-SELEX-seq method, based on the separation of OCT/BOB1 complexes of various compositions in a non-denaturing polyacrylamide gel (EMSA) followed by the isolation and amplification of the oligonucleotides that they contain (SELEX). Based on several rounds of the enrichment followed by the NGS sequencing and bioinformatics analysis, the DNA sequences were determined and the relevance of this approach was confirmed. Thus, the proposed EMSA-SELEX-seq method allows the analysis of DNA sequences in DNA-protein complexes with varying dimensions of its protein components.

Keywords: EMSA, SELEX, NGS sequencing, OCT1, OCT2, BOB1, OBF1, OCA-B