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Bio-SELEX: A new strategy to identify new biomarkers from biological samples.

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Abstract

• Bio-SELEX allows the identification of new biomarkers from biological samples.
• Three steps are essential to perform Bio-SELEX; 1) Traditional SELEX, 2) Pull down, and 3) Mass spectrometry.
• Bio-SELEX strategy allows the identification of biomarkers for infectious and non-infectious diseases.

Keywords: Bio-SELEX, aptamers, biomarkers

The SELEX strategy was discovered in 1990 by two groups of independent researchers [Tuerk and Gold 1990; Ellington and Szostak 1990]. This technique makes it possible to obtain aptamers, which are ssDNA and ssRNA molecules, which adopt unique threedimensional (3D) structures allowing them to recognize specific targets with high affinity and specificity.

Since then, the creation of multiple variations of the SELEX technique has evolved. Negative-SELEX [Ellington and Szostak 1992], Counter-SELEX [Jenison et al. 1994], Capillary electrophoresis SELEX (CE-SELEX) [Mendosa and Bowser 2004], Microfluidic-SELEX [Lou et al. 2009], Cell-SELEX [Daniels et al. 2003] are some of the variants that, based on advances in molecular biology, biomedical engineering, and biotechnology have improved the time of acquisition and the ability to recognition of the aptamers by different types of targets.

Our research group has been developing a variant of the SELEX strategy that can be adaptable to multiple studies. Therefore, we report a new SELEX strategy modification to identify new biomarkers from biological samples called Bio-SELEX. The word Bio refers to the search for biomarkers and the biological nature of the samples used to obtain them.
1. **Traditional SELEX strategy.**

   The aptamer isolation starts by following the traditional SELEX strategy. Multiple previously published protocols described this strategy [Manley 2013; Tan et al. 2023]. For biomarker discovery is essential to consider some critical steps.

   **Nature of biological samples:** Initially, any biological sample is optionable for the Bio-SELEX strategy. Each sample composition has different challenges, but protein extraction is a simple procedure for each sample matrix using commercial kits available on the market.

   **Controls:** Using control samples where biomarkers are absent is important. Counter-selection is an essential step in any SELEX experiment.

   **NGS:** Next-generation sequencing offers essential data to choose aptamers based on abundance and representativeness. In addition, post-NGS results help to predict motives and tridimensional (3D) fundamentals for interacting with a specific target.

   **Aptamer synthesis:** Aptamers can be synthesized and coupled with many different molecules to interact with different matrices—for example, his (polyhistidine), GST, Myc tags, and biotin, among others.

   **NOTE:** An easy way to purify aptamers is the biotin integration in 5’ or 3’ extremes of the sequence for biotin-streptavidin interaction.

2. **Pull-down**

   Once the best aptamers were selected by NGS and synthesized with any tag, it is necessary to immobilize the aptamer to probe selection capacity directly from the original biological samples.
sample. If the matrix of the biological sample does not allow the identification of biomarkers, it would be necessary to incorporate a previous step of protein extraction.

**NOTE:** Incorporating artificial nucleotides improve aptamer affinity, preventing degradation in a biological environment.

**STEP 3. Mass spectrometry (MS)**

Once the best methodology for isolating or recovering the biomarker is determined, it is necessary to identify the molecules using mass spectrometry. A simple way to recover the biomarker is through electrophoresis in SDS-PAGE polyacrylamide gels to visualize the protein (biomarker) of interest. Then, the bands will be cut and subjected to digestion with trypsin to be analyzed by mass spectrometry and, consequently, by bioinformatic tools to identify the proteins and peptides in the sample of interest. After that, laboratory tests are necessary to check the aptamer-target interaction. Parameters such as a dissociation constant (Kd) and limit of detection (LOD) are crucial for evaluating biomarker utility.

**NOTE:** Site-directed mutagenesis could be conducted on the aptamer and the protein to identify binding sites and improve interaction.

**Conclusions**

We report Bio-SELEX, a new SELEX strategy variant that identifies new biomarkers from biological samples or their implementation in research for infectious and non-infectious diseases. The constant development in NGS and MS technologies permits the implementation of this technique with minimal need for resources; these originating technologies are constantly expanding the availability of their services and making them more accessible (<400 USD per run). Through Bio-SELEX, our research group has identified new biomarkers for leishmaniasis [Ospina-Villa et al. 2022], and we are currently working on identifying biomarkers for Chagas disease (unpublished).

**Competing interests:** Authors declare no conflict of interest.

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