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A Modified CE-SELEX Approach to Screen Aptamers for Small-Molecule Targets

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Abstract

Coupling capillary electrophoresis with traditional systematic evolution of ligands by exponential enrichment (CE-SELEX) is an improved SELEX technique that emerged in the 2000s, and is mainly used to screen aptamers for large molecular targets such as proteins. For small molecular targets, CE-SELEX only allows partial isolation of target-bound aptamers from the unbound library due to non-observable mobility shifts induced by small-molecule targets. To address this issue, the author proposes a modified CE-SELEX approach that first splits a DNA/RNA library into two or three subgroups to reduce the size/molecular weight differences among sequences in the library. Each sub-library then interacts with the target molecule; the target-aptamer complexes are then collected using the procedure described by previous authors. The modified CE-SELEX method would allow the isolation of aptamers with high affinity and selectivity that are otherwise buried in the original unbound library peak, thus increasing the suitability of CE-SELEX for screening aptamers for small-molecule targets.

Keywords: Systematic evolution of ligands by exponential enrichment (SELEX); Capillary electrophoresis (CE); Small-molecule target; DNA/RNA oligonucleotide library; Mobility shift

Developed in the 1990s, systematic evolution of ligands by exponential enrichment (SELEX) is an important technique for screening aptamers (i.e., single-stranded DNA or RNA oligonucleotides) that bind to a target with high affinity and selectivity [1-3]. The conventional SELEX approach uses nitrocellulose filtration or affinity chromatography to separate target-bound aptamers from unbound ones, which is time-consuming and labor-intensive due to its multiple rounds of positive and negative screening [4,5]. In the 2000s, Bowser et al. replaced nitrocellulose filtration/affinity chromatography with capillary electrophoresis for SELEX aptamer screening, creating CE-SELEX [6,7]. Compared to the traditional approach, CE-SELEX is much simpler and more efficient because: 1) CE has high resolving power, and 2) the aptamer candidates and target molecules are allowed to interact in a free solution rather than on a solid-phase support, which eliminates the steric hindrance between aptamers and targets, thus also eliminating the need for rounds of negative screening. Previous studies have demonstrated that aptamers with high affinity and selectivity for a target molecule can be isolated after only two to four rounds of screening using CE-SELEX [6,8,9].

Since the CE separation of target-bound aptamers from unbound ones is based on the mobility shifts induced by the changes of charge and/or size/mass upon target binding, CE-SELEX has mainly been used to screen aptamers for macromolecule targets such as proteins, as those large/heavy targets induce an observable mobility shift [6,9-12]. The target-aptamer complexes produce a distinct peak on the CE electropherogram that is completely separate from the unbound library peak, allowing researchers to determine the correct time to collect only the target-bound aptamers at the capillary outlet, based on the migration time of the complexes' peak. In 2013, the Bowser group used CE-SELEX to screen aptamers for porphyrin, a small-molecule target with a molecular weight of 580 g/mol [13]. Because the mobility of porphyrin-aptamer complexes is similar to those of the nonbinding sequences, a distinct peak for the complexes cannot be observed. Bowser et al. chose to set up the collection window for the aptamer complex so as to occur immediately before the leading edge of the unbound library peak (see Figure 1a). (Note: The porphyrin-aptamer complexes should migrate out of the capillary tube a little earlier than the unbound library when an uncoated fused-silica capillary is used under the normal polarity conditions.) They were able to isolate several aptamers with dissociation constants (K_d) in the low micromolar range [13].

The DNA or RNA oligonucleotides in a SELEX library usually contain a randomized central region of 30-60 nucleotides, and a fixed primer region of ~20 nucleotides at each end for amplifying target-bound central sequences [14-16]. The oligonucleotides in a DNA/RNA library are all the same length, but their size/molecular weight varies due to the composition of their central regions. For instance, the largest difference in mass between guanine, adenine, thymine, uracil, and cytosine exists between guanine and cytosine (i.e., 40.03 g/mol). Thus, one DNA/RNA molecule containing 60 guanine bases in its central region will be 2,402 g/mol heavier than one with 60 cytosine bases in the central region. Because of this range, CE produces a broad peak for a DNA/RNA library of 10^{12} sequences or more. In the author's opinion, it is likely that some small target-bound aptamers are buried in the unbound library peak, and cannot be identified using the Bowser group's collection strategy (Figure 1a). The author thus proposes a modified CE-SELEX approach to address this issue (Figure 1b).

In the first step of the new procedure, a high concentration of the DNA/RNA library solution will be injected onto the CE system to identify the location of the broad library peak. When the CE-SELEX is subsequently run, two or three separate vials of material are sequentially collected from the previously-determined area of the library's peak. The

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purpose of this step is to generate subgroups of the DNA/RNA oligonucleotides in a library so that the sequences in each subgroup are closer in mass and more similar in size. Each sub-library will then separately be exposed to the small-molecule target, with the subsequent collection procedure as in Bowser [13]. Splitting the broad DNA/RNA library peak into two or three narrower peaks reduces the chance of missing target-bound aptamers buried in the unbound library peak, even when a distinct peak for the target-aptamer complex cannot be observed on the electropherogram.

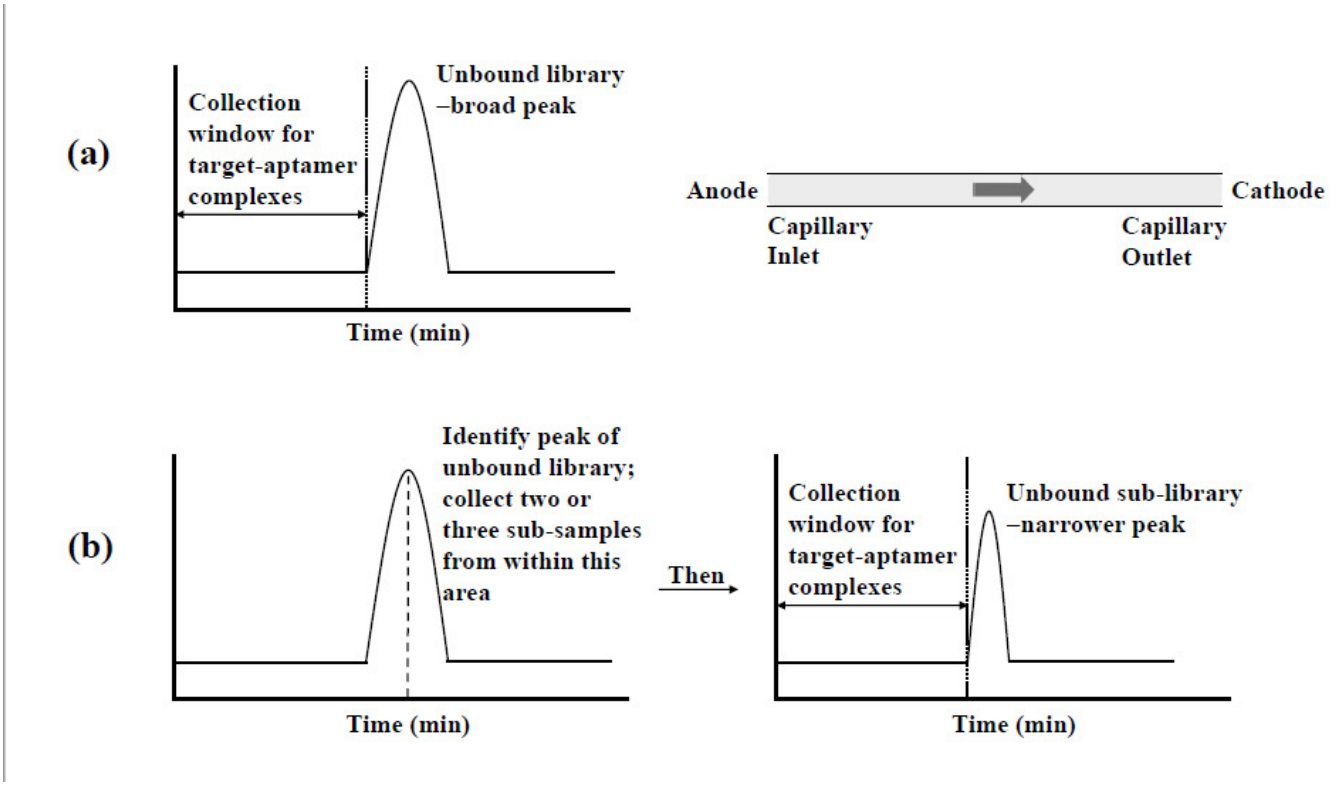

Figure 1: (a) Schematic illustration of the CE-SELEX collection strategy from the Bowser group [13]. When an uncoated fused-silica capillary is used under normal polarity (i.e., an anode is placed at the capillary inlet, and a cathode is placed at the capillary outlet), all species migrate toward the cathode. The small target-aptamer complexes should migrate out of the capillary tube a little earlier than the unbound DNA/RNA library. (b) Schematic illustration of the modified CE-SELEX approach proposed by the author. First, the broad DNA/RNA library peak is split into two or three subgroups by sequential collection at the capillary outlet. The sub-libraries are then separately exposed to the small-molecule target, after which the target-bound complexes are collected as per Bowser [13]. Note that the unbound DNA/RNA sub-library produces a much narrower peak compared to the original library.

In summary, the author proposes a modified CE-SELEX method that adds an additional step to the Bowser group's approach: First split the broad peak of a DNA/RNA library into two or three subgroups, then allow each sub-library to interact with the target molecule, after which the target-aptamer complexes are collected. This modified method would allow aptamers that are buried in the original unbound library peak to be isolated, thus increasing the suitability of CE-SELEX for screening aptamers for small-molecule targets.

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Conflict of Interest Statement

The author declares that she has no competing financial interests.

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