

Reference: Mosing, R.K.; Mendonsa, S.D.; Bowser, M.T. "Capillary Electrophoresis-SELEX Selection of Aptamers with Affinity for HIV-1 Reverse Transcriptase" *Analytical Chemistry*, **2005**, *77*, 6107-6112.

Bioanalysis and Sample: In this paper, the authors describe the use of capillary electrophoresis-SELEX (CE-SELEX) to select ssDNA aptamers with high affinity and specificity for HIV-1 reverse transcriptase (HIVRT) from a DNA library containing 1.8×10^{13} random sequences.

Importance: AIDS is caused by HIV, a retrovirus that utilizes HIVRT to convert viral RNA into dsDNA. This viral dsDNA is then incorporated into the cDNA of the host to be transcribed. Because of its importance to the replication of HIV, HIVRT is a major pharmacological target for the treatment of AIDS. Current HIVRT inhibitors are only effective for short periods of time and have significant patient side effects. Recently, RNA and DNA aptamers have become attractive as potential HIVRT inhibitors due to their low-nanomolar dissociation constants and high specificity. Previous SELEX (Systematic Evolution of Ligands by EXponential Enrichment) studies have shown that RNA aptamers have the highest affinity for HIVRT with dissociation constants as low as 40 pM after nine rounds of selection. The lowest dissociation constant reported for ssDNA aptamers prior to this paper was 1 nM after twelve rounds of selection. The CE-SELEX technique described in this paper not only reports the lowest ssDNA aptamer dissociation constants to date (180 ± 70 pM), but also takes only two to four rounds of selection as compared to twelve as stated above.

Technique: The CE-SELEX technique described starts by incubating HIVRT in solution with the random DNA library. The incubation mixture is then separated by CE under high voltage. Since electrophoretic mobility is dependent on the charge-to-mass ratio of the analyte, the ssDNA sequences bound to HIVRT will have a different mobility than the unbound sequences and can be collected in different fractions. The bound sequences are then purified and amplified by PCR for further rounds of selection by the same process (minus the original DNA library step). Experiments show that aptamer affinity actually worsens after more than four rounds of selection so all selections are terminated after a maximum of four rounds. Aptamer affinities are measured by creating ^{32}P -labeled clones of the ssDNA pool after the fourth round of selection and using an ultrafiltration assay which separates the bound sequences from the free sequences at varying HIVRT concentrations. The fraction of bound vs. unbound aptamer is determined using a scintillation counter. Binding curves (plots of fraction bound vs. HIVRT concentration) are then utilized to determine the dissociation constants of the high-affinity ssDNA aptamers.

Example: Figure 1 shows a typical binding curve of a ssDNA aptamer after four rounds of selection using CE-SELEX. The seven data points are used to fit a non-linear regression line in accordance with the following equation (Eq. 1 in paper):

$$\frac{I_0 - I}{I_0} = \frac{\text{const.}}{K_d + [\text{HIVRT}]}$$

where I is the measured scintillation counts of the unbound aptamers and I_0 is the measured counts of the aptamers in the absence of HIVRT.

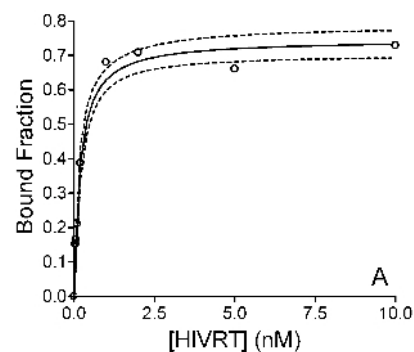


Figure 1. Binding curve of selected ssDNA aptamer clone.

Opinion: The usefulness of this technique is unprecedented, reporting lower dissociation constants in fewer rounds of selection for ssDNA aptamers that bind HIVRT than any previous publication. I think this technique will be relevant in finding many other inhibitors/binders for targets of interest in the fields of pharmacology, biology, and chemistry.