Aptamers Analysis

Application note Ref : AN 2.005-V1

Aptamers are DNA (or RNA) oligonucleotides selected from random libraries of DNA sequences. They fold into a three dimensional structure to bind different classes of targets with high affinity and selectivity. Both the chances for the aptamer to be selected and the quality of the selected aptamer are largely dependent on the method selection.
We present in this note an alternative method of selection of aptamers using CE-UV-LEDIF detection/non-SELEX. Non-SELEX involves repeated rounds of partitioning using CE without the need for amplification and strand separation after each subsequent round.

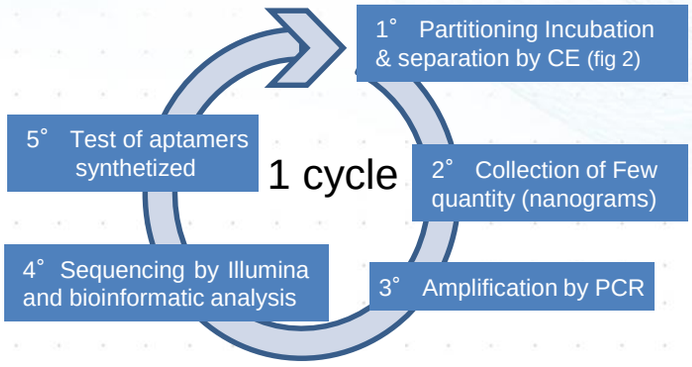

Figure 1: *CE-UV-LEDIF aptamers analysis*

Instruments:
Capillary Electrophoresis: Agilent Technologies 7100CE
Detector: Picometrics ZETALIF LED 480nm/30nm

Sample:
T29 aptamers labelled with FAM (6-carboxyfluorescein) (fig 4)
Thrombin protein is detected by UV absorbance (210nm) (fig 3)

Preparation:
DNA sample were denatured by heating at 95° C during 5min and let come to room temperature. Thrombin protein is then mixed with the T29 aptamers and injected into the capillary. All samples are diluted in a mixture of Tris-HCl 50mM + NaCl 100mM + $MgCl_2$ 1mM.

Method:
- Capillary: 90cm x 50 µm ID (effective length: 19cm)
- Migration buffer: Na_2HPO_4
- Voltage: +20kV
- Injection: 50mbars, 30s
- Cassette temperature: 25°C

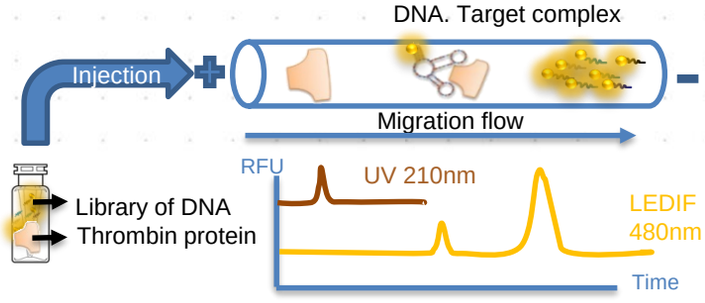

Figure 2*: Method of selection of aptamers by CE coupled to the double detection by UV-absorbance and LEDIF detection*

The double detection allows to determine the migration time of thrombin (T_mUV=9 minutes, Fig3) and of T29 aptamer (T_mLEDIF=18 minutes, Fig 4). The complex DNA target has an intermediate migration time (Fig 5).

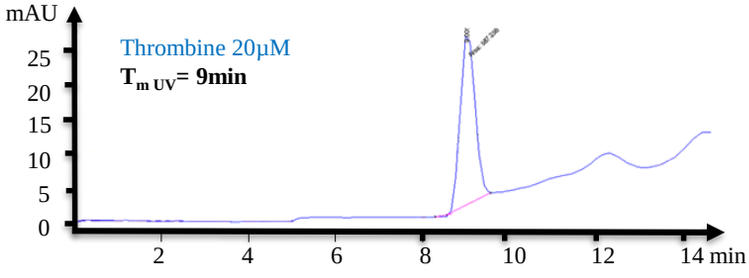

Figure 3*: Electropherogram with UV absorbance detection (210 nm) of thrombin protein at 20µM*

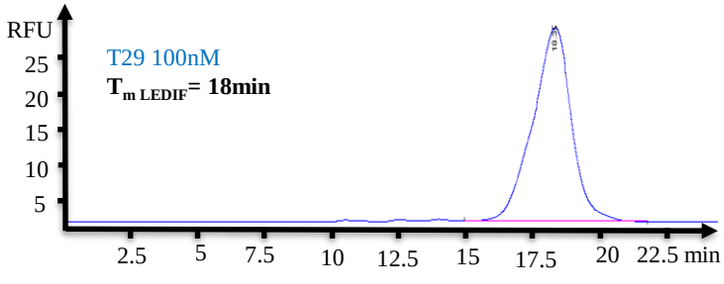

Figure 4*: Electropherogram with LEDIF detection (480nm) of T29 aptamer at 100nM*

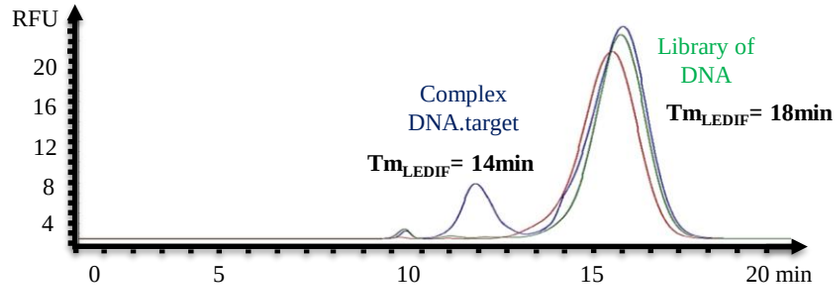

Figure 5: *Analysis of T29 aptamer at 100nM (red egram), Library of DNA (in green), and Thrombin+Library of DNA+T29 aptamer (in blue)*

Conclusion:

This application note shows the separation method of protein, complex DNA protein and DNA library.

Double detection gives migration time of each component.

The collection method by capillary electrophoresis is described in the application note AN 2.006.

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