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Structuralization and Incubation V.1

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We use this protocol and it's working

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Abstract

The interaction between an aptamer and his target depends on the tertiary structure of the aptamer, to attempt to always have the same structure for all the rounds, we forced the aptamer population to achieve the most thermodynamic stable structure.
Once all the sequences are structuralized, we can incubate the library with our target.

Materials

- Aptamer library (order to IDT).

5`- G TTG CTC GTA TTT AGG GAA TG $N_{40}$ ACA CCA GTC TTC ATC CGC $TTT_6$ – 3`

-Thermoblock with agitation.
- Eppendorf tubes 1.5 mL.
- Ice
- Bacteria strand (DHalfa5).
- Binding Buffer (pH=7,6):
    - 10 mM Tris-HCl
    - 150 mM NaCl
    - 5 mM $MgCl_2$

Safety warnings

Be careful when using the thermoblock as the working temperature id dangerous.

Before start

Turn on the thermoblock at 95ºC 15 min before start.
Check all working surfaces and materials are clean before start.

Day 0

1 **Preparation of the inoculum**

1.1 In 1mL of LB liquid medium, resuspend the *E. coli* cells.

Incubate it at 37 °C overnight with constant shaking at 180 rpm in a Incubator-shaker

Day 1

2 **Growing and harvesting cells**

2.1 With the spectrophotometer set at λ = 600 nm, measure the growth of the inoculum. It should be ≥ 1. If it's lower, it should be left growing until it reaches this value.

2.2 Take 250 µL of bacteria and inoculate it in 25 ml LB media.
(use 1% O.D. as an inoculum for subculturing)

2.3 Incubate at 37ºC, 180 rpm for 1.5h (until the culture reaches an O.D. between 0.5 – 0.6, which will indicate that the culture is in log-phase)

2.4 Centrifuge the tube at 3000 *X g* for 10 minutes

2.5 Remove supernatant and then wash the cells x3 with PBS (10 mM, pH 7.4). This will remove residual components from the previous step.

2.6 Measure the O.D. at λ=600 nm using a spectrophotometer at λ = 600 nm. The optical density (O.D.) should
be ≥ 1. This corresponds approximately to 1×10^8 *E. coli* cells (diluted in PBS). Further dilutions can be done by adding PBS to tubes.

3 Add 10 µL of 100 uM ssDNA library to 190 ul of binding buffer.

Incubate it at 95 °C for 10 min. After that, transfer immediately to ice-bath to prevent rehybridization of
the single stranded DNA library. If the transfer is poor performed, you can reheat again for another 10 min and repeat.

4 **Strcuturalization**

4.1 Add 10 µL of 100 uM ssDNA library to 190 ul of binding buffer.

Incubate it at 95 °C for 10 min. After that, transfer immediately to ice-bath to prevent rehybridization of
the single-stranded DNA library. If the transfer is poor performed, you can reheat again for another 10 min and repeat.

Incubated at 4ºC for 10 min. Then you attempered the library at room temperature for 10 min.

5

5.1 Resuspend 100 µL of 1 × 10^3 cells and add your library aptamer into a 1.5 ml Eppendorf tube. Place the tube into the thermoblock set and 40 ºC and incubate with agitation for 1h.