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Structuralization and Incubation V.3

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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 1

1 **Strcuturalization**

1.1 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.

2 **Incubation**

2.1 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.