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Plate cell – SELEX V.2

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Protocol status: In development
We are still developing and optimizing this protocol. When optimized, we might add changes in the future. If you use this protocol and find a different way to perform it in a better way, let us know!

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Abstract

Aptamers are short single-stranded oligonucleotide (DNA or RNA) molecules with the ability to bind to other molecules with high affinity and specificity. Nowadays, aptamers are used as biosensing molecules with broader applications, such as ribozymes (RNA enzyme molecules with catalytic activities); riboswitches (modulating translational activities) and ligands, recognizing specific target binding. Aptamers are folded, directed by their sequences, acquiring specific 3D conformations. This 3D conformation property has been widely applied in order to recognize cell structures.

Aptamers evolve from random oligonucleotide pools by a process called **Systematic Evolution of Ligands by Exponential enrichment (SELEX)**. Conceptually, the SELEX process is controlled by the ability of these small oligonucleotides to fold into unique 3D structures that can interact with a specific target with high specificity and affinity [1]. This is a dynamic process, repeating exposures and elutions, allowing to screen in a heterogenic pool in low concentration ranges.

A standard SELEX method has four major steps:

1.- Exposure of random sequence single stranded nucleotide oligo library to a target.
2.- Binding of oligos to the target molecule.
3.- Selection of binders and removal of non-binding oligos.
4.- Amplification of the binder fraction and portioning of the amplicon to single strand.

These steps are iteratively performed till a pool of high binding aptamer is screened out from the library. The oligonucleotide library consists of a random base-sequence flanked on both ends by primer binding sites, which aids in amplification and enrichment (Safeh et al., 2010; Kim et al., 2013).
Here is described how SELEX have been applied to develop aptamers, recognizing specific outer membrane structures, as LamB.

Guidelines

It is important in this protocol to be strict with the timing. If the time windows are missed, you will probably have to repeat the whole protocol to make sure it is well performed.

Materials

MATERIALS

DMSO

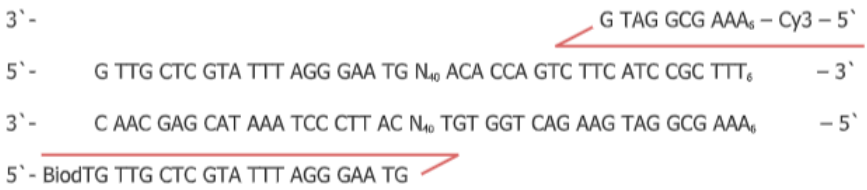
Agarose

PBS

LB Broth

Materials and reagents:

- Pipette tips: 10 µl, 200 µl and 1 ml.
- Microtitre plates.
- Centrifuge tubes 50 ml and microcentrifuge tubes 1.5 ml.
- Bacterial strains (E. coli)
- ssDNA library and primers: 100uM prepared on Binding buffer and 10 uM Primers



ssDNA library and primers (IDT custom synthesis). Forward primer on top; reverse primer on the bottom of the image. Library in between

Buffers and solutions

- Binding buffer (2X)
    - o Sodium phosphate buffer 100mM
    - o NaCl 600 mM
    - o Magnesium chloride
    - o Tris-HCl

- Glycine
- Sodium carbonate and sodium bicarbonate.
- Tris-base
- Carbonate buffer
- Tris-HCl binding buffer
- Glycine-HCl elution buffer
- Tris neutralization solution
- Phenylboronic acid (PBA) coating solution

EQUIPMENT

- Pipettes

- Incubator-Shaker
- Refrigerator (4 °C)
- Freezer (-20 °C)
- pH meter
- Spectrophotometer
- Centrifuge

Safety warnings

Lab coat and gloves should be worn throughout the whole experiment. All working surfaces must be clean and all the reactives should be treated following manufacturer's instructions.
Careful when touching the incubator, it can burn your fingers.

Before start

Whenever using the incubator, make sure to turn it on in advance (it can take up to 15 minutes to get to the desired temperature)

DAY 1

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

2 **Microtiter plate functionalization**

2.1 Take a sterile microtiter plate and coat it with 100 µl of 2.5 mM PBA prepared in carbonate buffer (pH 9.2).

Incubate the plate overnight at 4ºC. This will make the well surface to functionalize with PBA.

3 **Strcuturalization**

3.1 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.

Incubate the tube at 4 °C overnight (in the refrigerator). This will allow the single stranded DNA oligos to take
their respective 3D conformations.

DAY 2

4 **Growing and harvesting cells**

4.1 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.

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

4.3 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)

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

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

4.6 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.

5 **SELEX INITIAL ROUND: Exposure and Screening**

5.1 Wash the PBA coated plate x2 with carbonate buffer. Then, resuspend 100 µL of 1×10^3 cells in well #1.

5.2 Incubate at 25 ºC for 1h. This will allow the cells to coat the surface of the well

5.3 Decant the suspension by inversion and wash the well with 200 µL of Binding buffer (x3). The washing will remove the cells that didn't bind to the surface.

5.4 Incubate 200 µL of naïve library (prepared the previous day) at 37ºC for 15 minutes

5.5 Add 200 µL of library to the cell coated well (gently!) and then incubate at 37ºC for 1h.

5.6 Decant the solution from the plate by inverting.

5.7 Wash x3 with 200 ul of Binding buffer to separate loosely bound sequences

5.8 Add 200 µL of elution buffer to the well and then incubate at room temperature for 5 min.

5.9 Remove the solution by using micropipette (be careful not to touch the walls) and neutralize inmediately by adding 10 µL of neutralization buffer. This will bring the pH to ~7.

5.10 Quantify the obtained sequences with the spectrophotometer and label as Round #1. To avoid bacterial contamination due to detachment, the DNA elutes can be centrifuged at 3,000 *x g* for 10 min and take the supernatant prior to quantification. This can be stored at -20 °C till further use.