



Oct 20, 2019

Plate cell – SELEX

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In Development

dx.doi.org/10.17504/protocols.io.8g6htze

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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

NAME ▾

CATALOG # ▾

VENDOR ▾

DMSO

Agarose

PBS

LB Broth

MATERIALS TEXT

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: 100µM prepared on Binding buffer and 10 µM Primers

3' - G TAG GCG AAA₆ - Cy3 - 5'
 5' - G TTG CTC GTA TTT AGG GAA TG N₄₀ ACA CCA GTC TTC ATC CGC TTT₆ - 3'
 3' - C AAC GAG CAT AAA TCC CTT AC N₄₀ TGT GGT CAG AAG TAG GCG AAA₆ - 5'
 5' - BiodTG TTG CTC GTA TTT AGG GAA TG

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 STARTING

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 μ L of 2.5 mM PBA prepared in carbonate buffer (pH 9.2).

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

3 Strcuturalization

- 3.1 Add 10 μ L of 100 μ M ssDNA library to 190 μ L 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 $\lambda = 600$ 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 μ L of bacteria and inoculate it in 25 ml LB media.
(use 1% O.D. as an inoculum for subculturing)
- 4.3 Incubate at 37°C, 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 $\times g$ 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 $\lambda = 600$ nm using a spectrophotometer at $\lambda = 600$ 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 μ L of 1×10^3 cells in well #1.
- 5.2 Incubate at 25 °C 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 μ L of Binding buffer (x3). The washing will remove the cells that didn't bind to the surface.
- 5.4 Incubate 200 μ L of naïve library (prepared the previous day) at 37°C for 15 minutes
- 5.5 Add 200 μ L of library to the cell coated well (gently!) and then incubate at 37°C for 1h.
- 5.6 Decant the solution from the plate by inverting.
- 5.7 Wash x3 with 200 μ L of Binding buffer to separate loosely bound sequences
- 5.8 Add 200 μ L 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 immediately by adding 10 μ L 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 $\times g$ for 10 min and take the supernatant prior to quantification. This can be stored at -20 °C till further use.



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