Aptamer selection protocol new

Library preparation

1. Let ssDNA pool denature by heating at 95°C for 5 min
2. Dissolve 20pmol naive ssDNA library in 500 µl binding buffer (BB)
3. Cool on ice for 10 min → Formation of stable tertiary structure

Negative selection → don't do in the first SELEX round

4. Wash "negative control" bacteria 2x 1 min with 500 µl Wash buffer (WB)
   (10 min, 1 200g for beads)
5. Wash bacteria 1x 1min with 500µl BB
6. Incubate ssDNA library with bacteria 10^7 CFU in 1mL BB for 30min on orbital shaker at 4ºC
   → Supernatant → unbound DNA sequences

Positive selection

7. Wash "positive" bacteria (C. acnes) 2x 1 min with 500µl Wash buffer (WB)
8. Wash "positive" bacteria 1x 1min in 500µl BB
9. Incubate ssDNA library (supernatant with unbound sequences) with bacteria 10^7 in 1mL BB for 1h on orbital shaker at 4ºC
10. Wash pellet 1 x 1min with 500µl WB

    → in later rounds: increase number of washing steps by one more wash and one more minute → until you reached 5 minutes of washing time

Elution of bound aptamers

11. Add 500µL BB
12. Heat at 95°C for 15min
13. Cool on ice for 5 min
14. Centrifuge at 14,000 rpm for 2 min → Supernatant contains aptamers
    (10 min for beads)

DNA precipitation

15. Add 0.1 x 3M NaCl/ Acetate → Na-Acetate
16. Add glycoblue to see the pellet.
17. Add 1V of isopropanol
18. Vortex
19. Centrifuge at 10000g for 30min at RT

    Alternative for step 16:
    Add 2.5 x Ethanol
    Keep 30min at -20° or overnight at - 80 °C
    Centrifuge at 10000g for 30 min at 4 °C
    Remove supernatant

20. Wash in ~10µl 70%ethanol (just to cover the pellet)
21. Take as much supernatant as possible and let dry (until you can't smell the alcohol anymore)
22. Resuspend in 30µl of nuclease free water (ambion as bottle of 100ml in a six-pack)
23. Measure with nanodrop.

Aptamer amplification by PCR

24. In 200 µl PCR tubes add:
    - 20 µl of primer (10 µM each)
    - 1 µl of dNTPs (50 µM).
    - 30 µl of DNA template (100 ng/µl).
    - 5µL 10X polymerase buffer
    - 1 µl of DNA polymerase (0.5 U/µl).

    Keep tubes on ice.
    Add polymerase last.

25. Amplification by PCR:
    - Denaturation: 95°C for 30 s
    - Annealing: 60°C for 30 s
    - Elongation: 72°C for 30 s

x6 cycles

→ Now we have a new library!!!

26. Amplification of 7 tubes

    Prepare 7x 50µl tubes with each + 1 non template control (1NTC):
    - 20 µl of primer (10 µM each)
    - 1 µl of dNTPs (50 µM).
    - 1 µl of DNA template (100 ng/µl). → Ask Dimitri: Do we need to measure the concentration before in the Nanodrop and then use 1µl (100ng/µl) ? No, only measure after elution, once past cycle 1, you typically take 1/10 of your pool (pool = pre-amplified library)
    - 1 µl of DNA polymerase (0.5 U/µl).

    Keep tubes on ice.
    Add polymerase last.

x14 cycles → Run PCR and take out one tube after 4, 6,8,10,12,14 cycles (20 cycles total)

27. Remove 2µl each and run on 2% agarose gel , with a 50bp DNA ladder → you do this to decide which cycle number is best
28. Repeat step 24 with the specific number of cycles → with all the library we have left

Separation of sense and antisense ssDNA

29. Add 50 µl of Dynabeads to bind antisense ssDNA (separation from sense ssDNA) → magnet
30. Elute the ssDNA from the beads by melting in a 0.1M NaOH solution
31. Desalting: use GE healthcare Cytiva illustra NAP column NAP-5, cat no 10218284 in fisher sci
    - Run the protocol as given in this kit, recover the flow through, and it will filter out the NaOH solution for you. Here you measure your yield

      → SELEX cycle 1 finished → repeat: binding + flow cytometry to assess enrichment + PCR

      *repeat until flow cytometry shows plateau → send "plateau" library for sequencing

Buffers

Wash Buffer (WB)

- PBS (1X ?)
- BSA (1 mg/mL)
- 5mM Mg^{2+}

Binding Buffer (BB)

- yeast tRNA (0.1mg/mL)
- in WB

Washing

- in WB
- 3000 RPM for 5 min