Research Square

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Protocol to generate DNA aptamer coated particles and utilization for affinity-based screening with particle display

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Method Article

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Abstract

Aptamers are single-stranded nucleic acid ligands that bind to target molecules with high affinity and specificity. They are typically discovered by searching large libraries for sequences with desirable binding properties. These libraries, however, are practically constrained to a fraction of the theoretical sequence space. Machine learning provides an opportunity to intelligently navigate this space to identify high-performing aptamers. Here, we present a step-by-step protocol for utilizing particle display to select DNA aptamers for a 25 kDa protein biomarker neutrophil gelatinase-associated lipocalin (NGAL).

Introduction

Reagents

Dynabeads™ MyOne™ Carboxylic Acid beads (ThermoFisher, cat. no. 65011)

Sulfo-NHS (N-hydroxysulfosuccinimide) (ThermoFisher, cat. no. 24510)

EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride) (ThermoFisher, cat. no. 22980)

MA(PEG)12 Methyl-PEG-Amine Compound (ThermoFisher, cat. no. 26114)

Span 80 (Sigma-Aldrich. cat. no. 85548)

Tween 80 (Sigma-Aldrich. cat. no. P1754)

Triton X-100 (Sigma-Aldrich. cat. no. X100)

Mineral oil (Sigma-Aldrich. cat. no. M3516)

Gotaq Master Mix (Promega. cat. no. M7133)

Gotaq enzyme (Promega. cat. no. M5006)

Equipment

DynaMag™-2 Magnet (ThermoFisher, cat. no. 12321D)

Biologix Vortex Mixer

ECCO - Digital Ultrasonic Cleaner

Thermo Scientific™ Tube Revolver

ULTRA-TURRAX® Tube Drive (IKA, cat. no. 0003646000)

Eppendorf 6325 Mastercycler Pro S PCR

SONY SH800 cell sorter

Procedure

Coupling forward primers (FP) to particles:

1. Transfer 500 uL of Dynabeads™ MyOne™ Carboxylic Acid beads (ThermoFisher) to a 1.5mL Eppendorf tube.

2. Leave the tube on the DynaMag™-2 Magnet rack for 1 minute to pellet the beads.

3. Aspirate the liquid and add 1mL PCR water to the tube.

4. Remove the tube from the magnet rack, vortex for > 15 seconds, and sonicate for >30 seconds to wash the beads thoroughly with the PCR water.

5. Repeat the wash two times.

5. Next, prepare the 500 mM EDC (191.7 g/mole) in DMSO (95.85 mg/mL).

6. Aspirate the PCR water from the beads, and add the following reagents to the beads pellet.

1) 15 mL of coupling buffer (2 M NaCl and 10 mM of imidazole).

2) 75 mL of 500 mM EDC in DMSO.

3) 60 mL of amino-modified forward primer (500 mM stock).

6. Vortex for > 15 seconds and sonicate for >30 seconds.

7. Incubate for 2 hours at 37 °C with rotation.

8. Wash beads twice with 500ul PCR water.

9. Immediately before use, prepare the following:

1) 40ul 160mg/ml (~ 1M) EDC in DMSO (Incubate at 37deg can help dissolve EDC);

2) 40ul 90mg/ml (~0.41M) sulfo-NHS in PBS buffer

3) 75ul 120mM aminoPEG in PBS buffer.

10. Add 37.5ul NHS, 75ul aminoPEG, and 37.5 uL EDC to the beads, vortex, and sonicate to resuspend beads, gently spin to collect all beads at bottom of the tube.

11. Incubate at room temperature overnight with rotation.

12. Wash beads with 500ul TT (0.25M Tris pH8 + 0.01% Tween 20) buffer for 3 times, 10min each.

13. Wash beads with 500uL TET buffer (10 mM Tris, pH 8.0, 0.1 mM EDTA+ 0.01% Tween 20) 2 times.

14. Resuspend the forward primer conjugated beads (FP beads) in 500ul TET buffer to a concentration of 10^7/µl and store at 4 °C.

Monoclonal aptamer particles synthesis

15. Prepare 10 ml of the oil phase by adding Span 80, Tween 80, and Triton X-100 to a final v/v 4.5%, 0.40%, and 0.05% respectively in mineral oil.

16. Prepare 1ml aqueous phase consisted of GoTaq PCR Master Mix (Promega), 5 mM $MgCl_2$, 3 µM reverse primer, additional GoTaq Hot Start Polymerase (Promega, 0.5 U/µl), 2 pM template DNA, and $3x10^8$ FP-coated particles.

17. Add 7 ml oil phase to a DT-20 tube (IKA) locked into the Ultra-Turrax Device (IKA).

18. Add 1ml of the aqueous phase to the oil phase drop-wise over 30 seconds while the mixture was being stirred at 650 RPM in the Ultra-Turrax.

19. Pipette transfer 100 µl aliquots of the emulsion into ~80 wells of a 96-well PCR plate.

20. Perform the PCR under the following cycling conditions: 95 °C for 3 min, followed by 50 cycles of 95 °C for 15 s, 60 °C for 30 s and 72 °C for 75 s.

21. After PCR, the emulsion was broken by mixing 50 µl of 2-butanol with 100 µl of the PCR reagent mixture in each well.

22. The broken emulsions were combined into a 50 ml tube, vortexed, and centrifuged at 2,500 x *g* for 5 min.

23. Carefully remove the oil phase to avoid disturbing the particle pellet at the bottom of the tube.

24. Resuspend the particle pellet with 1 ml of single-strand generation (SSG) buffer (100mM NaOH, 100 mM NaCl, 1% Triton X-100, 10 mM Tris-HCl, pH 7.5, and 1 mM EDTA), transferred to a new 1.5 ml tube and incubated at 50 °C for 2 min.

25. Pellet the particles via a magnetic separator and remove the supernatant.

26. Wash the particles two more times with SSG buffer using magnetic separation, then resuspended in 300 µl TE and stored at 4 °C.

Characterize the monoclonality and the density of the aptamer particles

27. Add 10^6 aptamer particles to 100uL of 1uM AlexaFluor 488-labeled RP in TE buffer in a 1.5ml tube.

28. Incubate the tube at 55 °C for 10 min and leave on ice for 2 min.

29. The particles were then washed twice with 100 µl TE buffer.

30. Resuspend the particles in 100uL TE buffer and analyze by flow cytometry.

Particle display screening

31. Add 10^8 aptamer particles to 64nM NGAL protein diluted in 1 ml of PBSMCT buffer (DPBS with 2.5 mM MgCl2, 0.9 mM CaCl2, 0.01% Tween 20) in a 1.5ml Eppendorff tube.

32. Incubate at room temperature for 1hr with rotation.

33. Pellet the particles via a magnetic separator and remove the supernatant, which contains the unbound NGAL protein.

34. Dilute iFluor 647 labeled His-Tag antibody to 6 nM with PBSMCT buffer.

35. Add 1ml 6nM iFluor 647 labeled His-Tag antibody to the particle pellet.

36. Vortex the tube and incubated for 30 min at room temperature.

37. Magnetically wash the particles with PBSMCT to remove the excess antibody and resuspended in 1ml PBSMCT buffer.

38. Analyze the particles via FACS (SONY SH800) with the sorting threshold set to 1/3 of the maximum fluorescent intensity.

39. Collect the population with intensity greater than Fmax/3 as the positive population and intensity less than Fmax/3 as the negative population.

40. Amplify the isolated aptamers were amplified by 400uL GoTaq DNA polymerase, which is ready to serve as the template for the next round of particle display screening.

Troubleshooting

Step 30: If less than 5% of beads contain PCR products, then increase DNA template amount in the emulsion PCR. If higher than 50% of the beads contain PCR products, then decrease DNA template.

Time Taken

Day 1: Coupling forward primers (FP) to particles (4 h)

Day 2: Prepare oil and liquid phase reagents, and setup emulsion PCR (3 h)

Day 3: Break emulsion and generate single-strand DNA aptamer particles (3 h)

Day 4: Prepare NGAL bound aptamer particles (2 h)

Day 4: Screen with FACS (4 h)

Day 4: PCR amplify the collected aptamer particles with desired property (2 h)

Anticipated Results

Step 30: 20±15% of the beads should contain PCR products. Based on the Poisson distribution, most beads are monoclonal.

References

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