Accelerated aptamer selection via SELEX and Molecular simulations for Lipopolysaccharide detection

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Table S1: List of aptamers selected for simulation investigation in this study. For each sequence, the parts in bold are the primer parts for PCR and the rest part is the variable region.

No	Aptamer residue sequence consisting of 91 residues
1	**ATCCGTCACACCTGCTCT**ACGTAATCTGAGTTTCTTGGTATAAGGTCTCGGACGTTACTTAGCTGAACTGCAA**TGGTGTTGGCTCCCGTAT**
2	**ATCCGTCACACCTGCTCT**CAGGCTCGATGTAAGGCGCCTTCTACACAGTCTTACCGATCTGTATTCTTGTCGA**TGGTGTTGGCTCCCGTAT**
3	**ATCCGTCACACCTGCTCT**GGAGAAATGATTAATACCTCCTGATCGTTAGAGCTTCTTATAATATAGCTGATAA**TGGTGTTGGCTCCCGTAT**
4	**ATCCGTCACACCTGCTCT**AACAAATCGATGAGTGAATGTTGAACTGCTGTTTTCTTTTCAAAGAAAGGCACGA**TGGTGTTGGCTCCCGTAT**
5	**ATCCGTCACACCTGCTCT**ACAATGACGACCACAATGCTC

	CCCCAATAACTGTGTATAAGCCGTCTCCATTGTG**TGGTGT TGGCTCCCGTAT**
6	**ATCCGTCACACCTGCTCT**ACAATGACGACCACAATGCTC CCCCAATAACTGTGTATAAGCCGTCTCCATTGTG**TGGTGT TGGCTCCCGTAT**
7	**ATCCGTCACACCTGCTCT**CAGGGTTGTTTAATTCGTCTCA ACGTGACATTTGCTTGAAGGTTGAGTATGGCGG**TGGTGTT GGCTCCCGTAT**
8	**ATCCGTCACACCTGCTCT**TGGGAAGATCTATCGAGTTCT GGAGTGTACCGTACTGTGCATTGGCTGGTCCCAT**TGGTGT TGGCTCCCGTAT**
9	**ATCCGTCACACCTGCTCT**TTGGCGATCATCGGTATTTTCA ATTGAAAAAATTTGTTGGAAGGCTGGGCATGCT**TGGTGT TGGCTCCCGTAT**
10	**ATCCGTCACACCTGCTCT**GGTCAGGGACCTTTCGTCTGA AGTATAAGGTATCGCGATCCGAAGTAGTACGCAG **TGGTGTTGGCTCCCGTAT**
11	**ATCCGTCACACCTGCTCT**GGGCTCAGGGTTGAACGCACC AAACTACGTTATATATCACTCTGATCACTATTAA**TGGTGT TGGCTCCCGTAT**
12	**ATCCGTCACACCTGCTCT**ACATTTTACTGTCACCCCACC AAACGTCGACGACGCTCTAATCGTCCATGATCAG**TGGTG TTGGCTCCCGTAT**
13	**ATCCGTCACACCTGCTCT**GCGGGACTAATGTTCATTGCG TTTAAATATCGATGGAATAAAATGGCAGTACAAG**TGGTG TTGGCTCCCGTAT**
14	**ATCCGTCACACCTGCTCT**AAACTTCGATATTAAGAGCGA ACGATTCTGCGATTATTGTGAGTAATTATCTCCA**TGGTGT TGGCTCCCGTAT**
15	**ATCCGTCACACCTGCTCT**CGAGTAGCCAAGGGATGATCG GAAGGTGCATTAAGGTTACCGCGCCCCGTACGCA**TGGTG TTGGCTCCCGTAT**

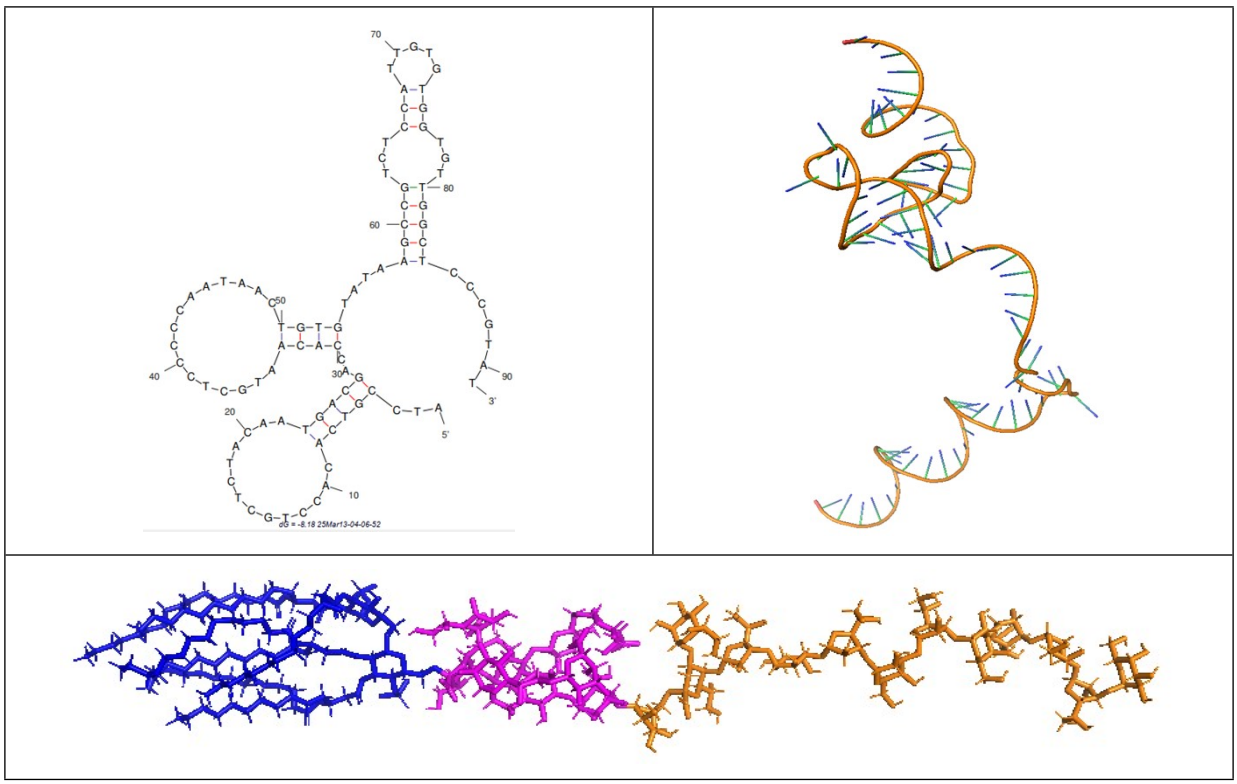

Figure S1. Representative secondary structure of an aptamer Seq 5 (top left) and its corresponding (3D) reconstruction (top right). The bottom panel shows the 3D structure of a representative LPS molecule, which comprises three main regions: the lipid A tail (Blue), the core oligosaccharide (Magenta), and the O–antigen head (Orange).

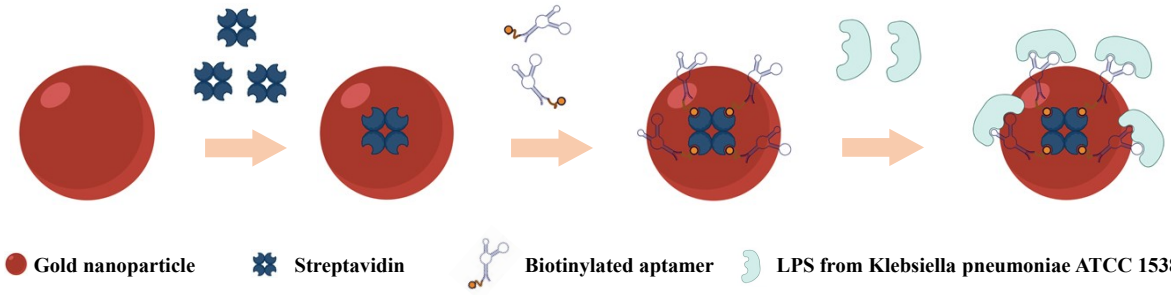

Figure S2. Schematic illustration of aptamer immobilization on AuNPs via streptavidin-biotin interaction.

Table S2: Summary of optimized conditions for symmetric PCR in the first and second SELEX rounds.

Annealing temperature	Number of PCR cycle	Template final concentration
50.0 °C 50.7 °C 52.0 °C 54.0 °C 56.3 °C 58.2 °C 59.5 °C	8 cycles	0.1 ng/µL
Optimized value	6 cycles 8 cycles 10 cycles 12 cycles 14 cycles 16 cycles 18 cycles	0.1 ng/µL
Optimized value	Optimized value	0.01 ng/µL 0.02 ng/µL 0.1 ng/µL 0.2 ng/µL 1 ng/µL 2 ng/µL

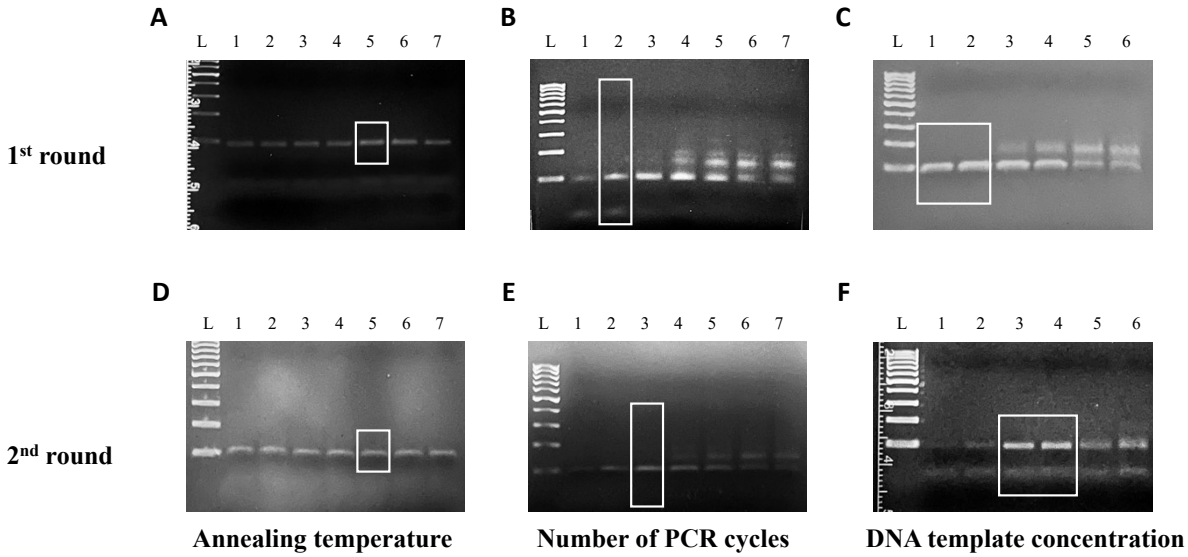

Figure S3. Effect of different variables on symmetric PCR products in the first and second SELEX rounds, visualized on 2% agarose gel electrophoresis. (A, D) Different annealing temperatures (°C), (B, E) different PCR cycle numbers, and (C, F) different DNA template concentrations (ng/µL) were evaluated. 'L' denotes the 100 bp DNA ladder.

Table S3: Summary of conditions for asymmetric PCR optimization.

Annealing temperature	Template final concentration	Primer ratio (Forward primer/ reverse primer	Number of PCR cycle
55 °C 56 °C 57 °C 58 °C 59 °C	0.1 ng/µL	20:1	25 cycles
Optimized value	0 ng/µL 0.05 ng/µL 0.1 ng/µL 0.2 ng/µL 1 ng/µL 2 ng/µL	20:1	25 cycles
Optimized value	Optimized value	20:1 40:1 60:1 80:1 100:1	25 cycless
Optimized value	Optimized value	Optimized value	15 cycles 20 cycles 25 cycles 30 cycles 35 cycles

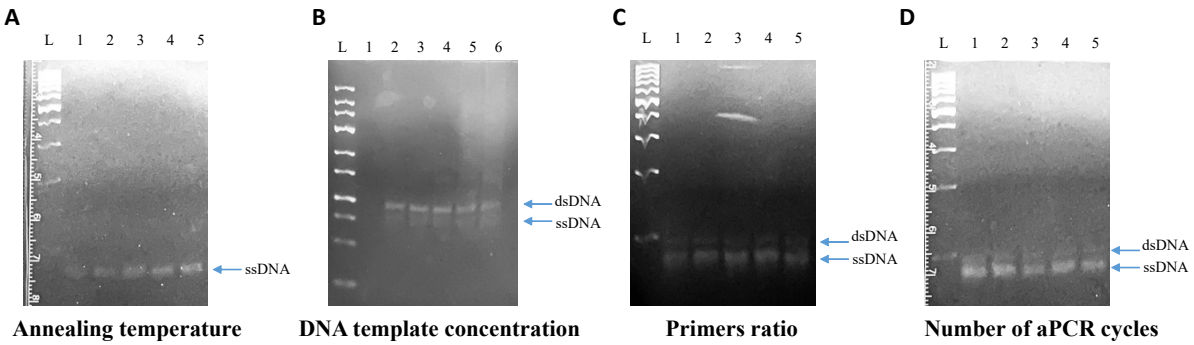

Figure S4. Optimization of asymmetric PCR in the first round of SELEX visualizing on 4% agarose gel electrophoresis. (A) Different annealing temperatures (°C), "L" is referred to 100 bp DNA ladder. (B) Different amount of template (ng/µL), "L" is referred to 25 bp DNA ladder. (C) Different ratio of forward primer and reverse primer, "L" is referred to 100 bp DNA ladder. (D) Different cycles of aPCR were investigated. "L" is referred to 100 bp DNA ladder.

Table S4. Parameters for SELEX targeting the LPS of Klebsiella pneumoniae ATCC 15380.

Round	LPS concentration (μg/μL)	Incubation time (min)
1	1.00	60
2	0.80	60
3	0.50	60
4	0.20	45
5	0.10	45
6	0.04	45
7	0.04	30
8	0.02	30
9	0.01	30
10	0.01	15

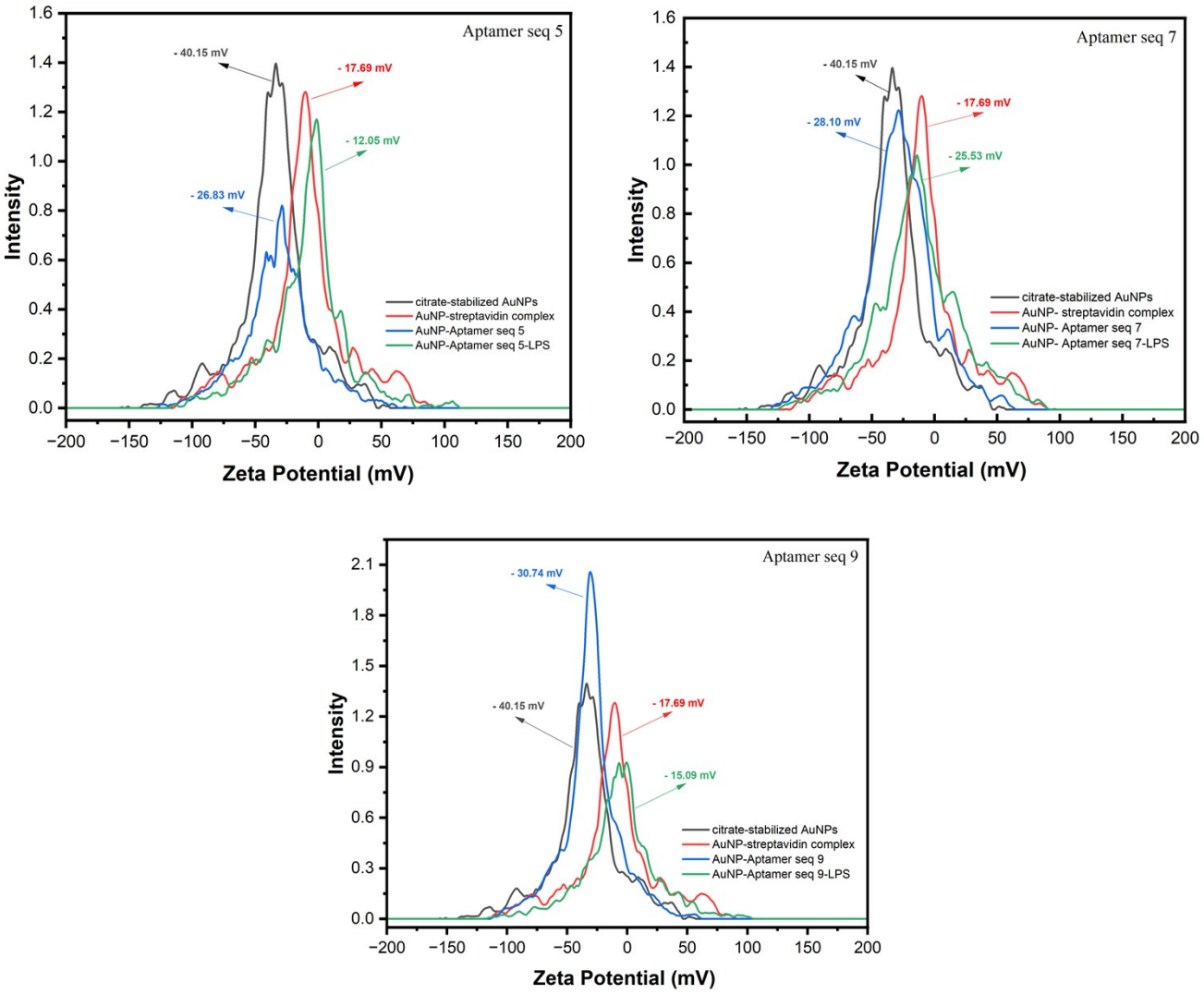

Figure S5: Zeta potential profiles of Au nanoparticles at different functionalization steps: citrate-stabilized AuNPs, AuNP-streptavidin complex, AuNP-streptavidin-biotin-aptamer (seq 5/seq 7/seq 9) conjugates, and AuNP-streptavidin-biotin-aptamer-LPS complexes.

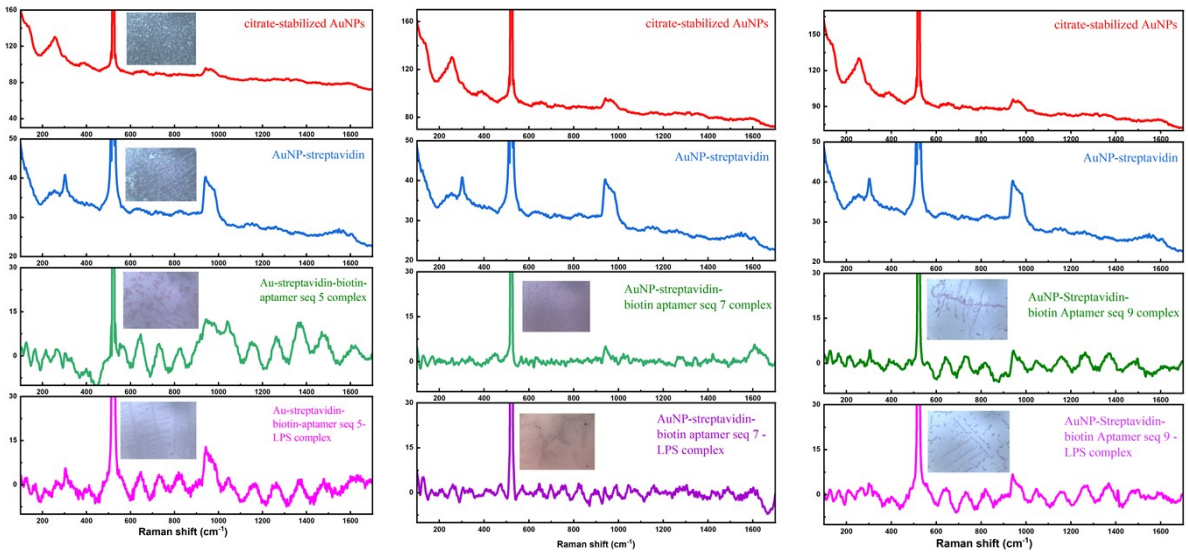

Figure S6: Surface-enhanced Raman scattering (SERS) spectra recorded at different functionalization steps of the gold nanoparticles: citrate-stabilized AuNPs, AuNP-streptavidin complex, AuNP-streptavidin-biotin aptamer complex, and AuNP-streptavidin-biotin aptamer-LPS complex. These spectra confirm the sequential surface modifications and serve as supporting data for the main SERS analysis presented in Figure 7.

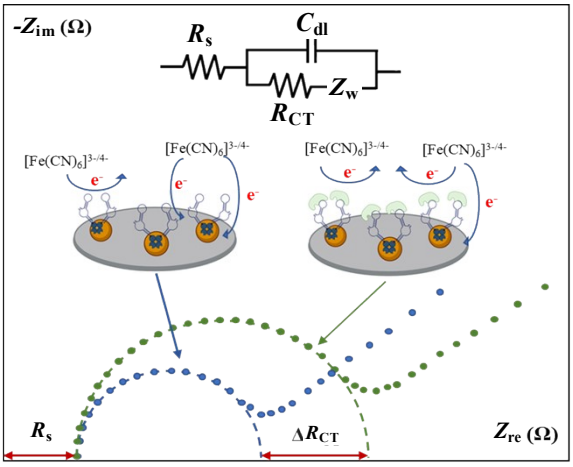

Figure S7: Electrochemical impedance spectroscopy (EIS) measurements. Nyquist plots of AuNPs/SPCE were recorded in 0.1 M KCl solution containing 5 mM $[Fe(CN)_6]^{-3/-4}$ at open-circuit potential (OCP) over the frequency range 50 mHz–100 kHz with an AC amplitude of 10 mV. The inset shows the equivalent Randles circuit used for fitting, highlighting the charge-transfer resistance (R_{CT}) paramater.

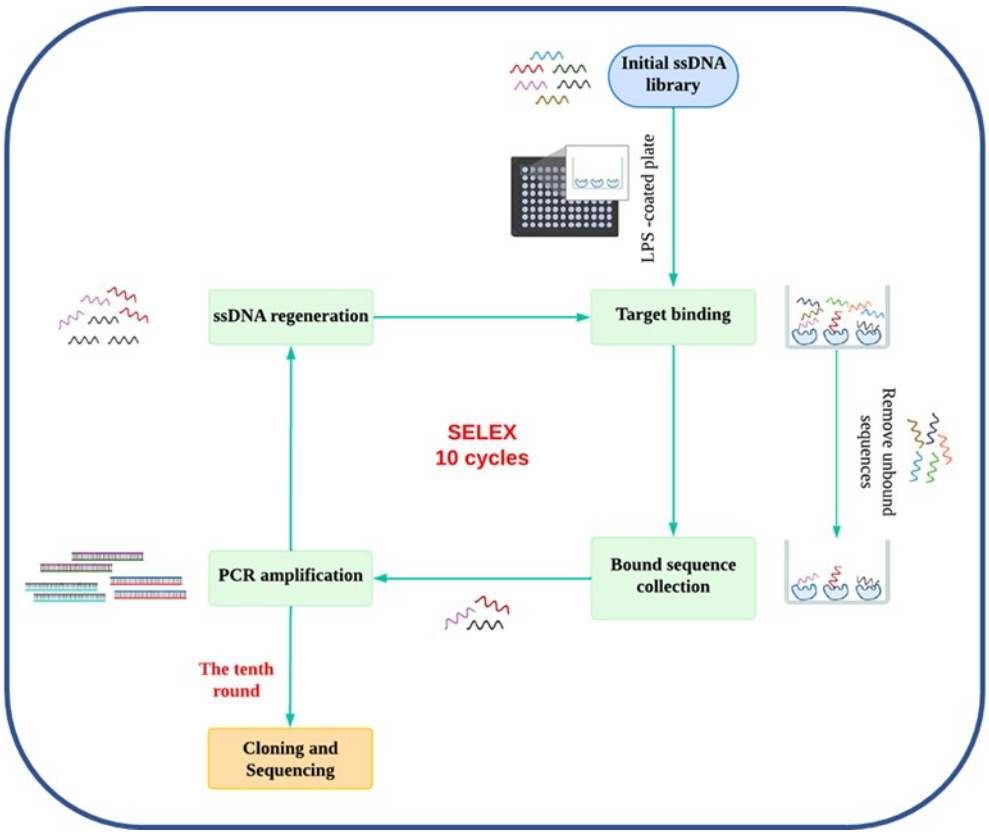

Figure S8: Schematic illustration of SELEX process for the selecion of aptamer candidates for LPS from Klebsiella pneumoniae ATCC 15380.