Supporting Information

Neutralizing Aptamers Block S/RBD-ACE2 Interactions and Prevent Host Cell Infection

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Methods

1. **Aptamer development**

   For aptamer development, S/RBD-virus mimics were generated as aptamer targets. Commercially available purified His-tagged S/RBD proteins (SPD-C5H3, ACRO Biosystems Inc., DE, USA) were conjugated to Ni-Sepharose beads (GE Healthcare, USA). His-tag proteins alone were used to form control His-tag beads. An ssDNA library (5'-Cy3-ATCCAGAGTGACGCAGCA-[N]40-TGGACACGGTGGCTTAGT-3' (N = A, T, G, or C)) consisting of 40-mer random core sequence and constant 18-mer arm sequences at both ends was used for target-based SELEX. Briefly, the synthetic ssDNA library was incubated with virus mimics for SELEX enrichment. The enriched ssDNA sequences were then subjected to counter-selection with control His-tag beads. At the end of each round, ssDNA sequences of interest were PCR-amplified and used for the next round of SELEX, which totaled six rounds of combined enrichment and counter-selection steps.

2. **sequencing analysis of aptamers**

   After target-based SELEX, the final products of aptamer pools R4 and R6 were amplified and submitted for next-generation sequencing (NGS) on an Illumina MiSeq system (Illumina, San Diego, CA) at GENEWIZ (South Plainfield, NJ). Over 100,000 total sequence reads were obtained in each aptamer pool and ranked by copy number predominance. Phylogenetic trees were generated from the top 50 predominant sequences using DNAman 9.0 software. For each cluster of aptamer phylogenetic trees, potential motif sequences were determined using MEME Suite v4.12.0 software. Representative aptamers derived from Motif sequences 1 and 2 were selected for functional analysis. Minimum free energy secondary structures of the selected aptamers (full length) and their core sequences (40 bases) were calculated using the NUPACK program.

3. **Flow cytometry analysis of aptamer binding to virus mimics**

   Selected aptamer sequences were synthesized and labeled with a Cy3 fluorescent reporter at the 5'-end. S/RBD or S protein-coated virus mimics were treated with serial dilutions of Cy3-labeled aptamers in Dulbecco's phosphate-buffered saline (DPBS) containing 1 mM MgCl₂ (DPBS/Mg) and incubated at RT for 25 min. His-tag Ni-Sepharose beads (GE Healthcare, USA) and random ssDNA sequences (ssDNA library) were used as baseline controls of virus mimics or aptamers, respectively. After washing twice, resultant binding of ssDNA sequences to virus mimics was quantified by flow cytometry. In addition, core sequences of aptamers 1 and 2 were synthesized and labeled with a Cy3 reporter, and their capacity to bind S/RBD-coated virus mimics was examined under identical conditions for comparison. To determine potential effects of reaction environments, aptamer binding capacity was tested in the presence of different concentrations of Mg and at different temperatures as indicated.

4. **Competition binding assays**

   Microplates (Nunc immobilizer amino plates) were pre-coated with purified SARS-CoV-2 S/RBD proteins (50 ng/well) in DPBS at 4 °C overnight. After two washes, blocking solution (2% BSA in DPBS) was added at 37 °C for 1.5 h. Individual competitive aptamers in DPBS/Mg were added into wells at 500 nM final concentration and incubated at RT for 45 min with gentle shaking (350 rpm). Each biotinylated aptamer of interest was then added at 500 nM final concentration and further incubated at RT for 45 min with gentle shaking in DPBS/Mg. After two washes, streptavidin-horseradish peroxidase (HRP) and its substrates were sequentially added into reactions. Color development was carried out using 3,3',5,5'-Tetramethylbenzidine (TMB) substrate and stop solution of 2 mol/L H₂SO₄ and measured using a microplate reader at 450 nm absorbance.
5. SPR studies of aptamer binding kinetics

Binding kinetic features of selected aptamers were characterized using a Biacore X100 instrument (Cytiva Sweden AB, Uppsala, Sweden). CM5 sensor chips were pre-activated using 400 mM of EDC (3-(N,N-dimethylamino) propyl-N-ethylcarboxydiimide) and 100 mM of NHS for 7 min at a flow of 10 μL·min⁻¹. Purified SARS-CoV-2 S proteins were dissolved in 10 mM sodium acetate (pH 4.5) to a concentration of 30 μg·mL⁻¹ and immobilized by amine coupling with a target level model at 5500–6000 RU (1 RU ≈ 1 pg·mm⁻²), whereas flow cell 1 served as a dextran surface control. The remaining unreacted functional groups on CM5 chips were blocked with 1 M ethanolamine hydrochloride (pH 8.5) solution for 7 min at a flow of 10 μL·min⁻¹. Sensor chips were primed three times and equilibrated with DPBS/Mg until a stable baseline was achieved. After immobilization of S proteins, the basal SPR response was measured and used as an assay baseline. For the SPR study, serial dilutions of individual aptamers and random ssDNA sequences at final concentrations of 0–500 nM were sequentially injected, starting with the lowest concentration. The flow rate (30 μL·min⁻¹) was maintained constant during the whole process, the association time was kept at 180 s, and the dissociation time was kept at 360 s, respectively. Regeneration was carried out with 5 M NaCl for 60 s. Data analysis was carried out using X100 evaluation software, and the data was fitted to the 1:1 binding model.

6. Blocking assay of S/RBD-ACE2 interaction

Microplates were pre-coated with purified ACE2 proteins (AC2-H82E6, ACRO Biosystems Inc., DE, USA) to mimic host cell surface. For blocking purposes, biotinylated S/RBD proteins were mixed with serial dilutions of aptamers in DPBS/Mg for 30 min at RT. S/RBD and aptamer mixtures were then added into microplates pre-coated with ACE2 and incubated at RT for 30 min with gentle shaking. Microplates were washed twice to remove free S/RBD and aptamers. For reporting purposes, streptavidin-HRP and its substrates were sequentially added into microplates. Color development was then measured at 450 nm absorbance using a microplate reader. Random ssDNA sequences/ssDNA library were used as baseline control.

For further validation, microplates were pre-coated with purified viral S/RBD to mimic the virus surface. For blocking purposes, microplates were treated with serial dilutions of aptamers in DPBS/Mg at RT for 30 min. Subsequently, biotinylated ACE2 proteins were added into microplates and incubated at RT for 1 h. Microplates were then washed twice to remove free ACE2 proteins and aptamers. For reporting purposes, streptavidin-HRP and its substrates were sequentially added, and color development was quantified at 450 nm absorbance using a microplate reader.

7. Generation of ACE2-expressing host cells

HEK 293T cells in 6-well plates were transfected with packaged pSIN-ACE2 viruses. The transfected cells were sorted by flow cytometry after they were labeled with PE conjugated mouse anti-HA tag IgG (BioLegend, San Diego, CA, USA) followed by washing with PBS. The obtained cells were designated ACE-293T host cells.

8. Blocking assays of S/RBD binding to host cells

For blocking purposes, biotinylated S/RBD proteins were mixed with serially diluted aptamers in DPBS/Mg at RT for 30 min. Subsequently, host cells were incubated with mixtures of S/RBD and aptamers at RT for 1 h. Treated cells were washed twice and stained with a streptavidin-Cy3 reporter. Resultant S/RBD-host cell binding was quantified by flow cytometry. Random ssDNA sequences/ssDNA library were used as baseline control. In addition, fluorescence microscopy was used to confirm S/RBD-host cell binding in treated cells.

9. Biostability assays of aptamers

Synthetic aptamers (2 µM) were incubated in 100% human serum for 24 h at 37°C. All the human serum procedures under IRB protocols (Pro00007175). Samples at were collected at different time points (0, 1, 4, 8, 24 h) and analyzed on a
2% agarose gel. Residual intact aptamer products were quantified (%). In addition, abilities of residual aptamer products to bind to virus mimics and block S/RBD-host cell interaction were also quantified (%) as described above.

10. Generation of SARS-CoV-2 pseudovirus

HEK 293T cells were co-transfected with 6 μg of plasmid encoding Env-defective, mCherry-expressing lentiviral vector (plasmid#104833, Addgene), 3 μg of psPAX2 (plasmid#12260), and 2 μg of pCDNA-S-ct (containing the full-length S with truncation of 19 amino acids at the C terminus, designated as S-ct) in 10 cm tissue culture plates using Lipofectamine™ 2000 Transfection Reagent (Invitrogen). At 16 h post-transfection, culture plate medium was replaced with fresh DMEM containing 10% FBS, and cells were cultured at 37 °C for 3 days. Supernatants of transfected cells were harvested and concentrated 50-fold using Retro-X concentrator at 1500 g for 45 mins at 4 °C. Pseudoviruses were aliquoted and stored at −80 °C until use.

11. Neutralization assays of viral particle-host cell binding

Viral S protein-mediated cell entry causes intracellular delivery of the firefly luciferase reporter gene and subsequent luciferase expression within infected host cells. Thus, luciferase activity is proportional to the number of infected cells and copies of viral particles per host cell. To validate S protein expression, serial dilutions of viral particles were coated on microplates (Nunc immobilizer amino plate) in DPBS. After blocking with 2% bovine serum albumin (BSA) in DPBS, individual biotinylated aptamers were added into microplates at 250 nM final concentration and incubated at 25 °C for 1 h with gentle shaking. In a control experiment, biotinylated ACE2 was used as a binding control probe and tested under identical conditions. For reporting purposes, streptavidin-HRP and its substrates were sequentially added into microplates. Color development was quantified at 450 nm absorbance using a microplate reader.

For cell infection, ACE-293T host cells were pre-seeded into microplates overnight (10⁴ cells/well). For virus neutralization, pseudoviruses were treated with serially diluted aptamers in DPBS/Mg at 4 °C for 2 h. Subsequently, mixtures of viral particles and aptamers were added into cultures of ACE-293T host cells pre-seeded in microplates. Cultures were centrifuged at 4000 × g for 30 min for cell spinoculation and then incubated at 37 °C for 2 h to allow virus infection. Microplate media was then replaced with fresh Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) and treated cells were further cultured at 37 °C for 72 h. To evaluate viral infection rates, cells were lysed in microplates using the Luciferase Assay System (Promega). Cellular luminescence activity was then quantified using a microplate luminometer. Image scanning was performed and IC₅₀ values were calculated using nonlinear regression in SigmaPlot 14.0.

For imaging measurements, ACE-293T host cells were seeded in black, clear-bottom 96-well microplates (Corning, Kennebunk). Virus neutralization and virus infection were carried out as earlier described. Cells were cultured for 48 h to allow virus infection and then washed twice. To detect virus infection, D-luciferin (Gold Biotechnology, Olivette) was added into culture microplates at a final concentration of 10 mM. Bioluminescent images were immediately acquired using an IVIS 200 Imaging System (Perkin Elmer, Waltham) with the Living Image software (version 4.5.2). Luminescent signals derived from intact host cells in microplates were correlated with virus infection rates and aptamer-induced virus neutralization efficacy.

12. Microneutralization assay of primary SARS-CoV-2 virus

The ability of aptamers to neutralize SARS-CoV-2 was determined using traditional virus microneutralization (VN) assay system composed of primary SARS-CoV-2 strain USA-WA1/2020 (NR-52281-BEI resources) and host Vero E6 cells as reported previously [34]. Briefly, primary SARS-CoV-2 (100 tissue culture infective dose 50 (TCID50) units) was mixed with 2-fold dilutions of synthetic aptamers or random ss DNA sequences at 37°C for 1 h. The virus and aptamer mixtures were then added to Vero E6 cells (ATCC CRL-1586) in a 96-well microplate and triplicate wells were used for each aptamer dilution. After 3 days of culture at 37°C, cells were stained with crystal violet–formaldehyde stain (0.013% crystal violet, 2.5% ethanol, and 10% formaldehyde in 0.01 M PBS). The neutralization effect (%) of each dilution was calculated based on the number of wells protected from virus infection: resultant 3, 2, 1, 0 of 3 wells protected was expressed as 100%, 66.6%, 33.3% or 0%.
neutralization, respectively. All experiments with SARS-CoV-2 were approved by the Penn State Institutional Biosafety Committee (IBC # 48625) and were performed in the Eva J Pell BSL-3 laboratory at Department of Veterinary and Biomedical Sciences, Pennsylvania State University.
Supplemental Figure S1. Development of aptamers specific for S/RBD of SARS-CoV-2. 

a. Virus mimics were generated by conjugating purified His-tagged S/RBD to Ni-Sepharose beads. His-tag proteins alone were used to form control His-tag beads. 

b. For target-based SELEX, an ssDNA library consisting of 40-mer random core sequences (Figure 1b) was used in combination with virus mimic-based aptamer enrichment and control bead-based counter-selection steps. 

c. Aptamer pools derived from each SELEX round were amplified and evolution of binding capacity to virus mimics was monitored by flow cytometry. 

d. Changes (fold) in binding capacity of aptamer pools to virus mimics was calculated. 

e. Fluorescent microscope examination of virus mimics post treatment with individual aptamer pools. Cy3-labeled ACE2 receptor protein was used as a positive binding control.
Supplemental Figure S2. Sequence analysis of developed S/RBD aptamers. Positions of motif sequences within aptamer central cores were located.
Supplemental Figure S3. Biocompatibility assays of aptamers. a, To validate binding specificity, mixed culture cells of leukemia/lymphoma cell lines (Maver-1, Jeko-1, and L428) were treated with aptamers at 200 nM final concentration in DPBS/Mg at RT for 30 min. Flow cytometry analysis revealed that no non-specific binding to mixed culture cells occurred for aptamers 1, 2, 6, or ACE2 protein. b, Fresh peripheral blood mononuclear cells were treated with aptamers at 400 nM final concentration in DPBS/Mg at RT for 30 min. Flow cytometry analysis confirmed that aptamers did not react with primary blood cells. c, Additional blocking assays of S/RBD-ACE2 binding (Figure 3a) confirmed that aptamers-1 and -2 were fully functioning at temperatures ranging from 4 °C to 37 °C.
Supplemental Figure S4. Development and functional characterization of S aptamers specifically targeting viral S protein. a, S aptamers were developed using S protein-coated virus mimics for target-based SELEX. For functional characterization, two representative sequences derived from S aptamers-2 and -6 were synthesized. b, Predicted secondary structures of S aptamers-2 and -6. c, Binding assays demonstrated that S aptamers-2 and -6 bound to spike protein-coated virus mimics with high affinity, $K_D = 4.5 \pm 1.3 \text{ nM}$ and $27.6 \pm 5.3 \text{ nM}$, respectively. d, S aptamers bound S/RBD-coated virus mimics in a pattern similar to that observed with ACE2 receptor proteins. e, However, S aptamers could not neutralize S/RBD and thus, failed to prevent its interaction with ACE2-expressing host cells. In contrast, treatment with neutralizing aptamer-1 completely blocked RBD interaction with host cells under the same experimental conditions.
Supplemental Figure S5. Biostability analysis of S/RBD-specific aptamers. Biostability of the aptamers in 100% human serum. 

a, Flow cytometry binding assay show minimal loss of binding ability of Aptamer-1 (5.4%) and Aptamer-2 (12.2%) after 24 h. 

b, Flow cytometry blocking assay show minimal loss of binding ability of the Aptamer-1 (0.9%) and Aptamer-2 (4.3%) after 24 h. 

c, Agarose gels showing stability of Aptamer-1 and Aptamer-2 over the time course of 24 h.
Supplemental Figure S6. Pilot study of virus microneutralization (VN) assay. Two-fold dilutions of synthetic aptamer and control ssDNA sequences were assessed for ability to neutralize primary SARS-CoV-2 strain USA-WA1/2020. Each aptamer dilution was tested in triplicate. Resultant neutralization effects are expressed as 100%, 66.67%, 33.3% or 0% where 3, 2, 1, 0 of 3 wells of host Vero E6 cells were protected from virus infection.
Supporting Table S1. Enrichment of top 50 Round 6 (R6) S/RBD aptamer sequences between rounds of S/RBD selection. Fold enrichment is calculated by dividing the percentage of the sequence from Round 6 (R6) by the percentage of the sequence from the Round 4 (R4).

| R6 Rank | % Representation | Fold Enrichment (R6/R4) | Sequence | Motif |
|---------|------------------|-------------------------|----------|-------|
| 1       | 41.94719         | 10.8539931              | TCGAGTGCGTTGTGTTGAATGTAGGGTTCGGTCGTGGGT | 1     |
| 2       | 24.2792          | 4.20812677              | ATACCAGTACGTGTTGTGTAATGTAGGGTGTCGGATG | 1     |
| 3       | 16.11665         | 26.40859892             | TTCGATGCTGGTTGTTGAATGTAGGGTTCGGTCGTGG | 1     |
| 4       | 2.396152         | 133.7563842             | ACTTAAAAGGCGCCTCGACTCGACTCGACTCGACTCG | 1     |
| 5       | 1.910107         | 14.67312447             | ATCGATGCGCTGTTGTGTAATGTAGGGTTCGGTCGG | 1     |
| 6       | 1.679611         | 25.11378607             | GCCGATGCTGGTTGTTGAATGTAGGGTTCGGTCGTA | 2     |
| 7       | 1.063286         | 16.48723456             | AGAAGATAGGGTCATTATTGGGGTTAGGAGGTCTTT | 1     |
| 8       | 0.926993         | 4.912593722             | GTGCAAGCGACGTCAAAAGAGTGCCATCGACGTGCTTA | 1     |
| 9       | 0.815754         | 5.02719952              | TCGAGTGCTGGTTGTTGAATGTAGGGTGTCGGATG | 1     |
| 10      | 0.715538         | 1.50948213              | GCTTAGATGCGCTGTTGTGTAATGTAGGGTTCGGTCG | 3     |
| 11      | 0.526131         | 133.7563842             | ACTTTAGGCA GCGCTTTTTGCAAGTTACCGGACACTATCC | 2     |
| 12      | 0.500997         | 0.2719952              | GCTAGATGCGCTGTTGTGTAATGTAGGGTTCGGTCG | 3     |
| 13      | 0.474019         | 133.7563842             | GCTTAGATGCGCTGTTGTGTAATGTAGGGTTCGGTCG | 3     |
| 14      | 0.422909         | 5.90183622              | CTCGCAAGGCTGGTTGTTGAATGTAGGGTGTCGGATG | 2     |
| 15      | 0.373384         | 12.94017717             | TGGTATGACCTGTTGTTAATGTAGGGTGTCGGATG | 1     |
| 16      | 0.290625         | 6.403841944             | CGACGAGGGGTGTCGATCGAGGTGTTCGGCGTGGCT | 2     |
| 17      | 0.26958          | 17.36341442             | CGACGAGGGGTGTCGATCGAGGTGTTCGGCGTGGCT | 2     |
| 18      | 0.258856         | 12.0737268              | CGACGAGGGGTGTCGATCGAGGTGTTCGGCGTGGCT | 2     |
| 19      | 0.173373         | 6.789276045             | CTCGCAAGGCTGGTTGTTGAATGTAGGGTGTCGGATG | 2     |
| 20      | 0.163351         | 12.0737268              | CGACGAGGGGTGTCGATCGAGGTGTTCGGCGTGGCT | 2     |
| 21      | 0.146315         | 12.0737268              | CGACGAGGGGTGTCGATCGAGGTGTTCGGCGTGGCT | 2     |
| 22      | 0.140302         | 16.48723456             | AGAAGATAGGGTCATTATTGGGGTTAGGAGGTCTTT | 1     |
| 23      | 0.134289         | 16.48723456             | AGAAGATAGGGTCATTATTGGGGTTAGGAGGTCTTT | 1     |
| 24      | 0.121261         | 16.48723456             | AGAAGATAGGGTCATTATTGGGGTTAGGAGGTCTTT | 1     |
| 25      | 0.120259         | 16.48723456             | AGAAGATAGGGTCATTATTGGGGTTAGGAGGTCTTT | 1     |
| 26      | 0.105226         | 16.48723456             | AGAAGATAGGGTCATTATTGGGGTTAGGAGGTCTTT | 1     |
| 27      | 0.090194         | 16.48723456             | AGAAGATAGGGTCATTATTGGGGTTAGGAGGTCTTT | 1     |
| 28      | 0.089192         | 6.789276045             | CTCGCAAGGCTGGTTGTTGAATGTAGGGTGTCGGATG | 2     |
| 29      | 0.081891         | 6.789276045             | CTCGCAAGGCTGGTTGTTGAATGTAGGGTGTCGGATG | 2     |
| 30      | 0.087187         | 0.20798029              | GGGGATCGTCGCTGTTGTTGAATGTAGGGTGTCGGATG | 2     |
| 31      | 0.083179         | 0.20798029              | GGGGATCGTCGCTGTTGTTGAATGTAGGGTGTCGGATG | 2     |
| 32      | 0.078168         | 0.20798029              | GGGGATCGTCGCTGTTGTTGAATGTAGGGTGTCGGATG | 2     |
| 33      | 0.071157         | 0.20798029              | GGGGATCGTCGCTGTTGTTGAATGTAGGGTGTCGGATG | 2     |
| 34      | 0.068147         | 0.20798029              | GGGGATCGTCGCTGTTGTTGAATGTAGGGTGTCGGATG | 2     |
| 35      | 0.066142         | 0.054725486             | TTGGTTTGTGGTTGTTGTTGTTGTTGTTGTTGTTG | 2     |
| 36      | 0.066142         | 0.054725486             | TTGGTTTGTGGTTGTTGTTGTTGTTGTTGTTG | 2     |
| 37      | 0.06514          | 0.054725486             | TTGGTTTGTGGTTGTTGTTGTTGTTGTTG | 2     |
| 38      | 0.060129         | 0.054725486             | TTGGTTTGTGGTTGTTGTTGTTGTTGTTG | 2     |
| 39      | 0.058125         | 0.054725486             | TTGGTTTGTGGTTGTTGTTGTTGTTGTTG | 2     |
| 40      | 0.054116         | 0.054725486             | TTGGTTTGTGGTTGTTGTTGTTGTTGTTG | 2     |
| 41      | 0.052112         | 0.054725486             | TTGGTTTGTGGTTGTTGTTGTTGTTGTTG | 2     |
| 42      | 0.052112         | 0.054725486             | TTGGTTTGTGGTTGTTGTTGTTGTTGTTG | 2     |
| 43      | 0.049106         | 0.054725486             | TTGGTTTGTGGTTGTTGTTGTTGTTGTTG | 2     |
| 44      | 0.03708          | 0.054725486             | TTGGTTTGTGGTTGTTGTTGTTGTTGTTG | 2     |
| 45      | 0.036078         | 0.054725486             | TTGGTTTGTGGTTGTTGTTGTTGTTGTTG | 2     |
| 46      | 0.032069         | 0.054725486             | TTGGTTTGTGGTTGTTGTTGTTGTTGTTG | 2     |
| 47      | 0.027058         | 0.054725486             | TTGGTTTGTGGTTGTTGTTGTTGTTGTTG | 2     |
| 48      | 0.025054         | 0.054725486             | TTGGTTTGTGGTTGTTGTTGTTGTTGTTG | 2     |
| 49      | 0.024052         | 0.054725486             | TTGGTTTGTGGTTGTTGTTGTTGTTGTTG | 2     |
| 50      | 0.024052         | 0.054725486             | TTGGTTTGTGGTTGTTGTTGTTGTTGTTG | 2     |
Supporting Table S2. Enrichment of top 50 Round 5 (R5) S1 aptamer sequences between rounds of protein-SELEX. Fold enrichment is calculated by dividing the percentage of the sequence from Round 5 (R5) by the percentage of the sequence from the Round 4 (R4).

| R5 Rank | % Representation | Fold Enrichment (R5/R4) | Sequence | Motif |
|---------|------------------|-------------------------|----------|-------|
| 1       | 69.1439801       | 9.381736501            | GTGCCACGCGCTAAAGAGGTCCGATCAGACTGCAGCTA         | 1(5) |
| 2       | 6.31803125       | 7.704916141            | GCTCAGGGGTCAGGGTTGGGCAGAGGATGTGGGAGAGCTG      | 2    |
| 3       | 2.65260293       | 5.76143542             | GGGCTTGGGTTGGGAATCAGGGATGTGGGAGAGCTG         | 2    |
| 4       | 2.42151759       | 7.33792089             | ACATTAGGCAGCGTCTTGTTTGAATCAGGGACACATTTCC    | 3    |
| 5       | 1.83782931       | 13.12735222            | GTCAGGGCTAGGGTTGGGCAGAGGATGTGGGAGAGCTA      | 2    |
| 6       | 1.23047799       | 12.30477993            | CGAGCAGGGGTCAGGGTTGGGCAGAGGATGTGGGAGAGCTG  | 2    |
| 7       | 0.74144186       | 1.95116281             | CGGGGCTTGGGTTTGGGAGTCGAGGATGTGGGAGGATG     | 2    |
| 8       | 0.57580059       | 0.053020313            | GCTGAGATTGCCCGCCTCAGCTCTTGAACACTTTGACC     | 4    |
| 9       | 0.45748541       | 0.93364122             | CAGTAAAACCAACGGCCGACACGCTGAGACCCGAGCTG     | 2    |
| 10      | 0.37072094       | 0.75657334             | TACCATCGGCGGCGCTTATATCTAAATTTACCTACTCC     | 3    |
| 11      | 0.32683325       | 2.791025034            | CGGCCGTTCAGGCTGTTAAGAAGGATGTGGGAGAGCTG    | 2    |
| 12      | 0.29973181       | 0.93666193             | TCGGGCTAACTCAAGACCCGTTATGGAAATCTTGGCTGA    | 2    |
| 13      | 0.28356464       | 4.73260767             | ACATGCTTCTTGTTAGTCGTGGACTCCGCGGCGATGTG     | 2    |
| 14      | 0.26818110       | 5.36322022             | TTGGAGGCAGTCTGTGGAGGGGATGTGGGAGGATG       | 2    |
| 15      | 0.25360383       | 7.39469948             | ATGGCAGCTTCTTGTTAAGAAGGATGTGGGAGGATG      | 2    |
| 16      | 0.25360383       | 1.57753589             | CGGGGCTTGGGTTTGGGAGTCGAGGATGTGGGAGGATG    | 2    |
| 17      | 0.20507966       | 5.12699163             | TGGGGAAGACGCTGTTAAGAAGGATGTGGGAGGATG      | 2    |
| 18      | 0.20507966       | 1.70899721             | TCAAAGATTAAGCCCAGGGGAGTCTGCTCTGCCGTG     | 2    |
| 19      | 0.19719199       | 3.94383972             | CGCCTTCGACTACGTGTTGCTGACGTTGCTGCTG        | 2    |
| 20      | 0.17352894       | 9.34383972             | GCCTTATGCTGAGCTGTTGCTGACGTTGCTGCTG       | 2    |
| 21      | 0.16561268       | 1.80458537             | ACCGAAAGGATGTAACAGACCTTGCTATTGGTCTTTC    | 3    |
| 22      | 0.15775359       | 3.94383972             | GGGTTCGGTATCGGCTGTTAGGGGATGTGGGAGGATG    | 2    |
| 23      | 0.15486599       | 0.15610322             | TTTGGGAAATTCAGGAACCTGGCGGCGGATCCTCCGCGC  | 3    |
| 24      | 0.14197823       | 2.8395646              | GGGGTCCAGGGGGTAAAGGGTTGGGATGTGGGAGGATG   | 2    |
| 25      | 0.13409055       | 1.17421255             | TTAATTATGCTGAGCGGATAGCTGAGCTGCTGAGCTG    | 2    |
| 26      | 0.13409055       | 1.17421255             | TTAATTATGCTGAGCGGATAGCTGAGCTGCTGAGCTG    | 2    |
| 27      | 0.11831519       | 1.34162421             | TCAAGGCTATTGCTTATTGCTTTTGGTCCTTCTC     | 3    |
| 28      | 0.11831519       | 1.34162421             | TCAAGGCTATTGCTTATTGCTTTTGGTCCTTCTC     | 3    |
| 29      | 0.10253983       | 1.46485745             | CAATTGCTGAGCGGATAGCTGAGCTGCTGAGCTG    | 2    |
| 30      | 0.09452153       | 0.94621533             | CTGAGGCTATTGCTTATTGCTTTTGGTCCTTCTC     | 3    |
| 31      | 0.08676474       | 0.882165328            | CAGTGAATTTGCCGGCGCCTACGTCTTGACTCTTTGACCC  | 4    |
| 32      | 0.07887679       | 7.87867945             | CAGCAGGTTCGCTTCTCAGTTGAACTCTTTGACCC      | 4    |
| 33      | 0.07887679       | 1.971919861            | GAGAAGTTGTTCGCTGTTAGCTGAGCTGCTTCTCC    | 3    |
| 34      | 0.070989115      | 3.78697941             | GAGAAGTTGTTCGCTGTTAGCTGAGCTGCTTCTCC    | 3    |
| 35      | 0.063101436      | 6.31043556             | GTGCCACGCGCTAAAGAGGTCCGATCAGACTGCTAGCTG    | 1(5) |
| 36      | 0.05521375       | 9.381736501            | GTGCACGCGCTAAAGAGGTCCGATCAGACTGCTAGCTA     | 1(5) |
