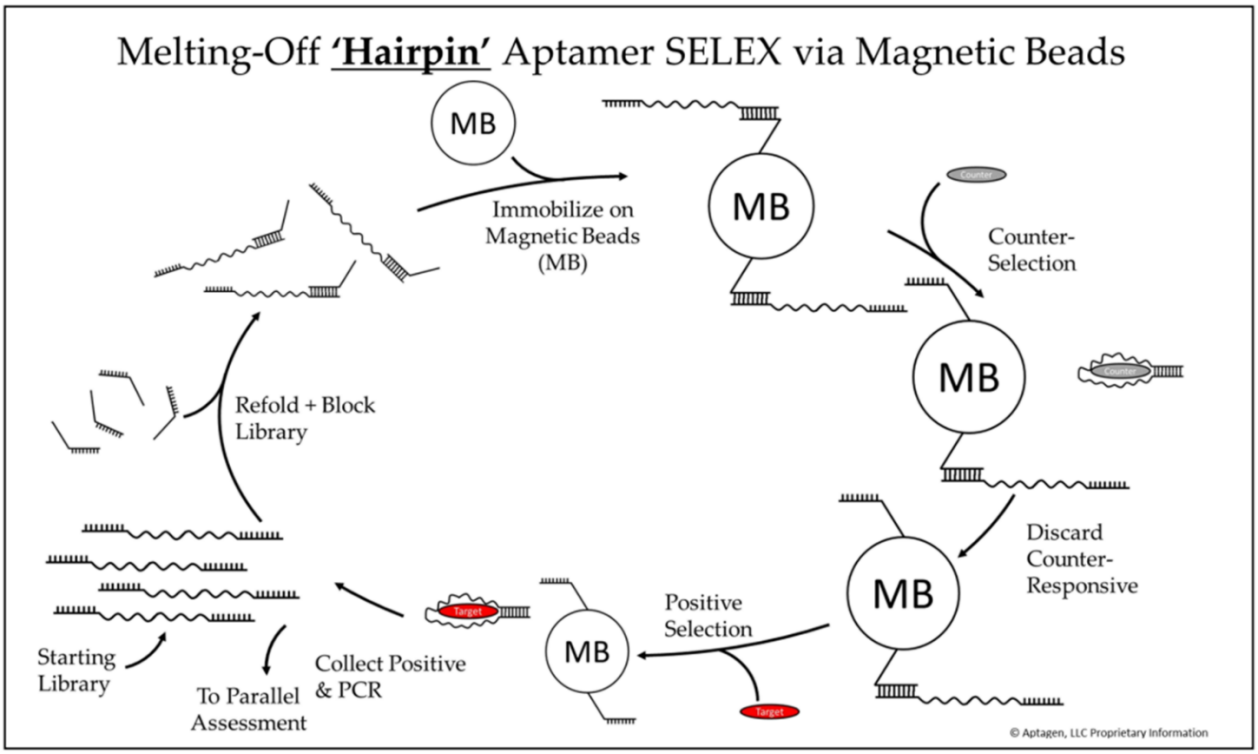
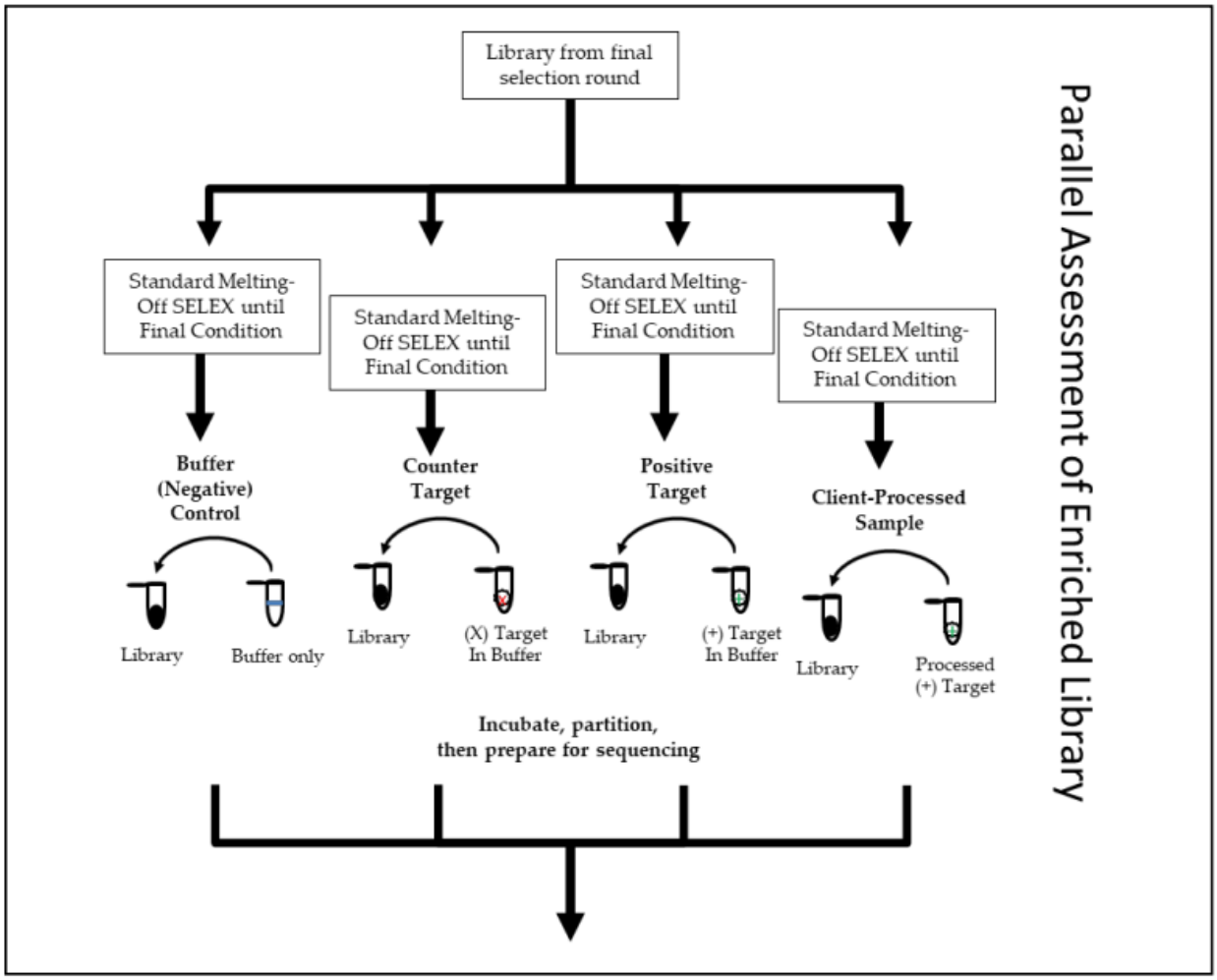
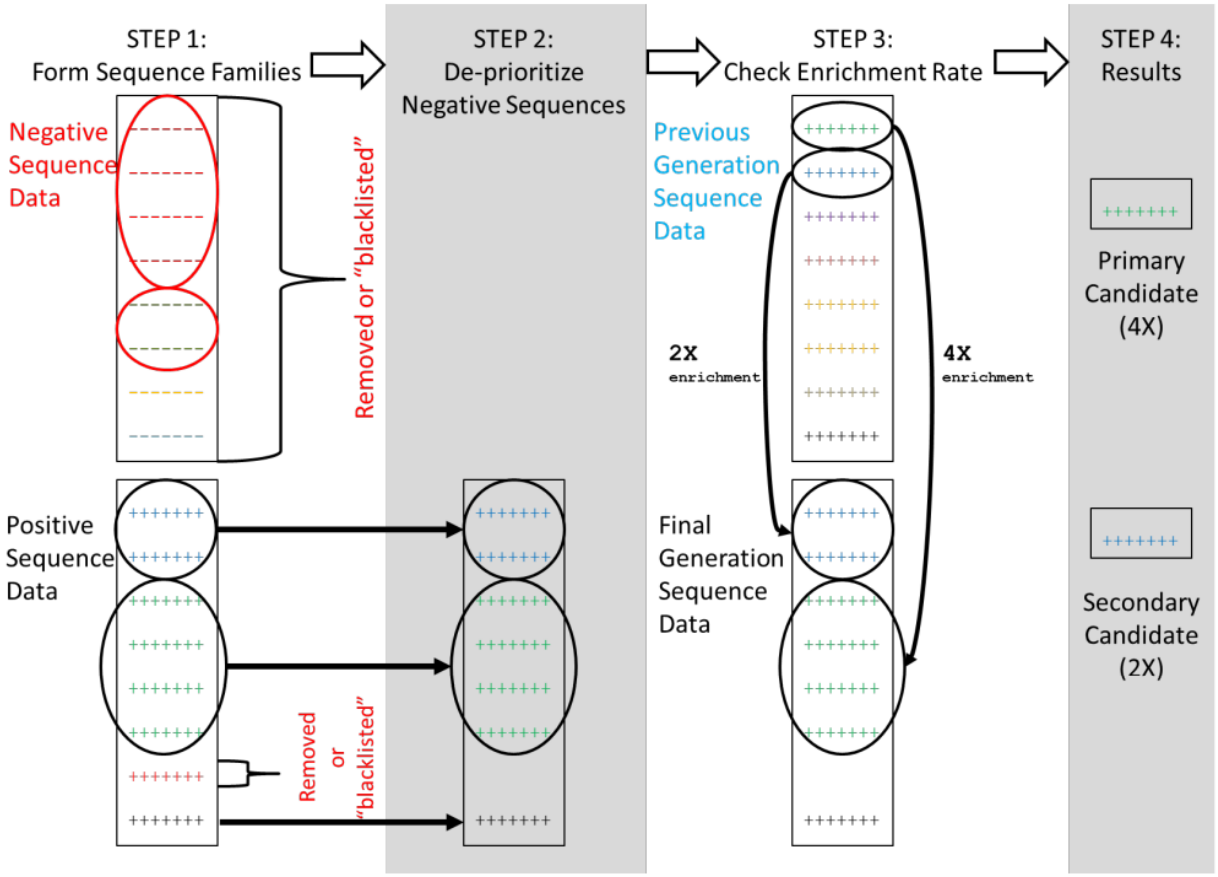
Supplemental Figures



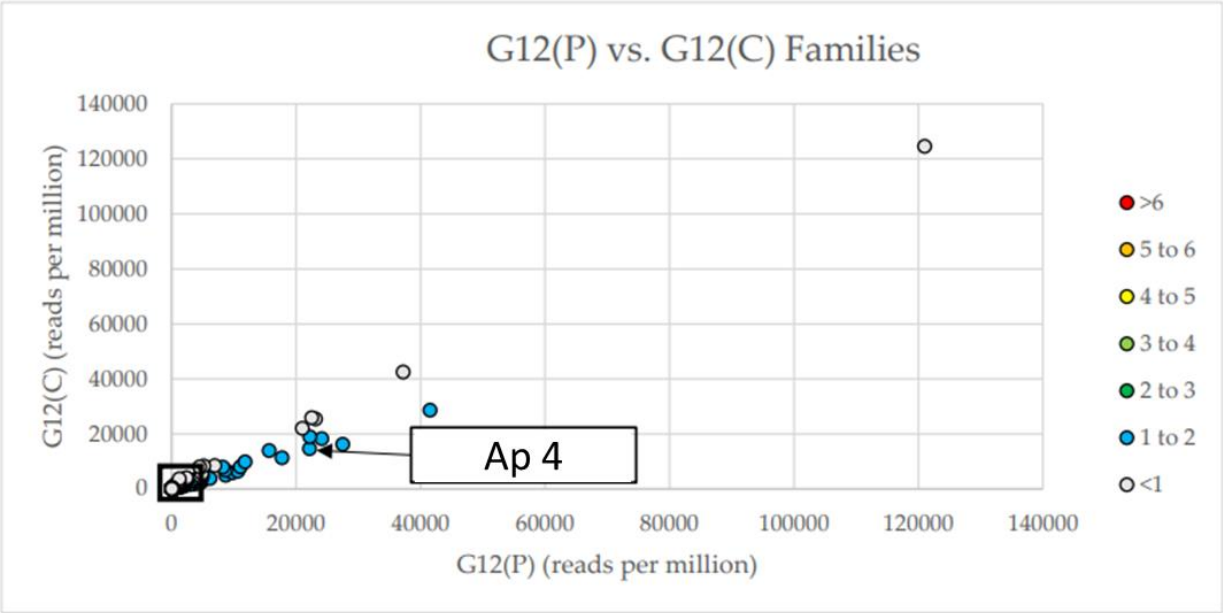
Supplemental Figure 1. Illustration of Melting-Off Aptamer Selection. Selection begins by refolding and blocking the library (left). After refolding is complete, the library is captured on magnetic beads (top center). The beads are washed with 1X SELEX buffer or incubated with counter-targets (counter-selection, top right). Species that change their conformation in response to counter-target are discarded (middle right). The beads were washed again to ensure the sample is clear of residual counter-targets before positive selection (bottom center). Species that change their conformation in response to the positive target are collected to undergo further experiments (bottom left).

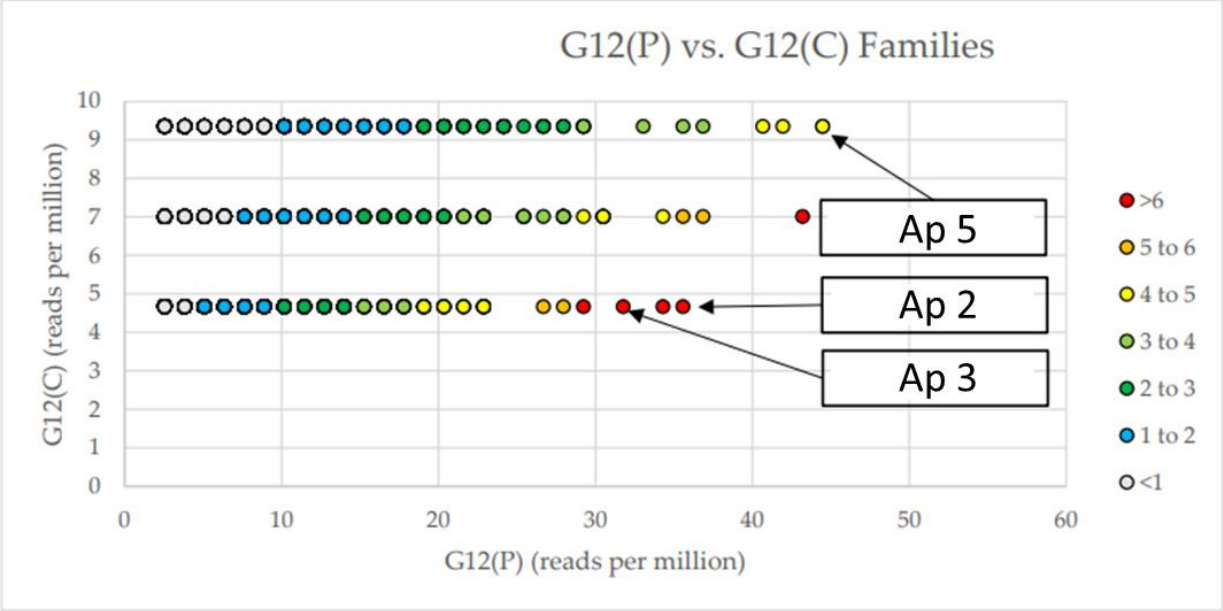


Supplemental Figure 2. Illustration of Parallel Assessment. The enriched library was divided into four equal portions for incubation with the following final conditions: buffer-only negative incubation (-), counter targets of common cold corona virus N proteins (x), SARS-CoV-2 Recombinant N protein (+), and SARS-CoV-2 inactivated virus (S). Based on the strategy described in Supplemental Figure 1, condition-responsive species were released for collection during the final incubation. Bead-bound nonresponsive sequences were then separated from responsive sequences by magnetic separation, and the supernatant containing responsive sequences was collected and prepared for sequencing. Only the positive samples will release responsive sequences while the countertarget and negative would have minimal material. A library that has significantly more recovery in the positive samples over counter-target and negative is considered sufficiently enriched to identify candidate aptamer sequences (see Supplemental Figure 1 for details).

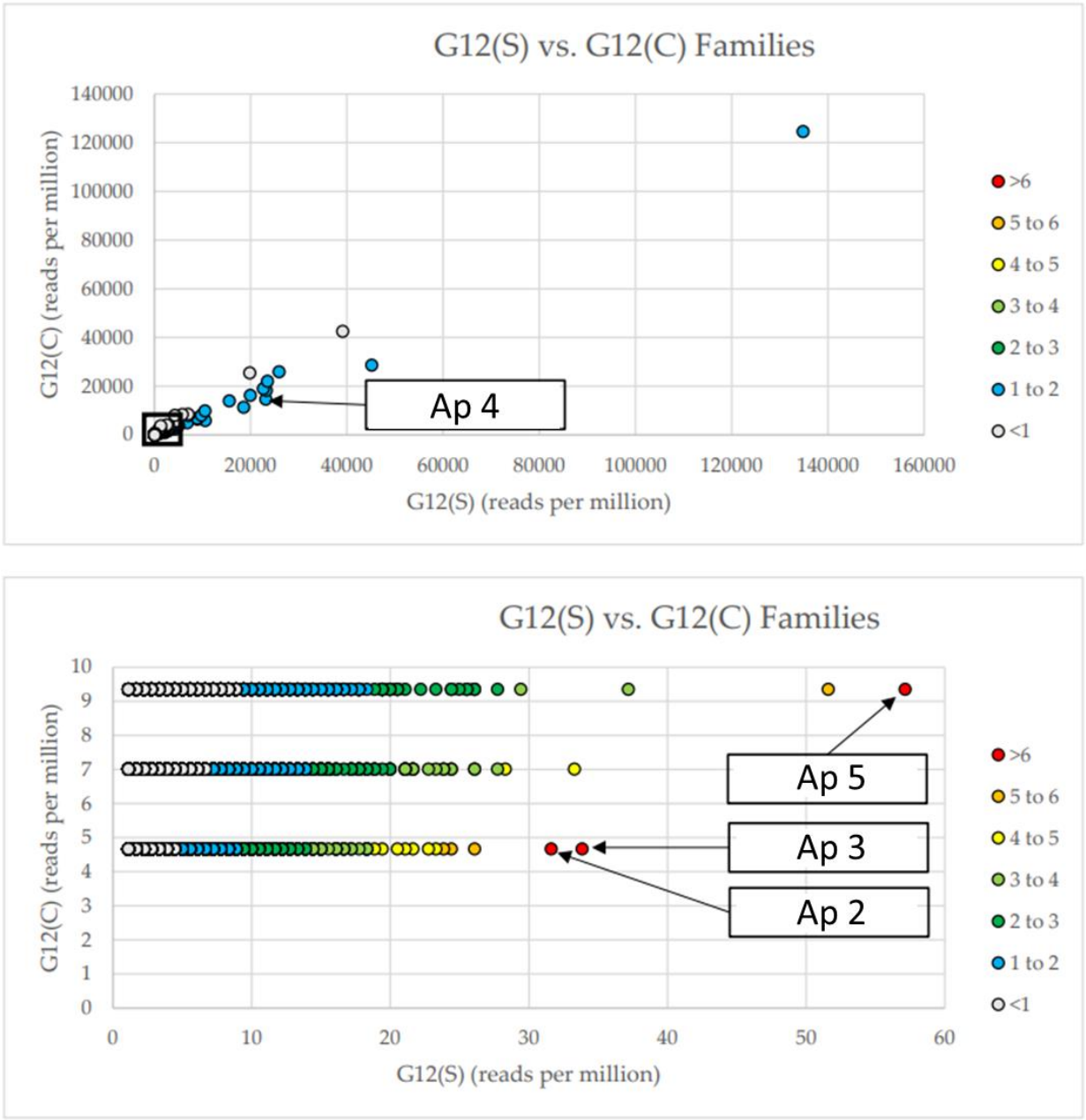


Supplemental Figure 3. Bioinformatics & Candidate Selection. (Step 1) Sequence data from the ultimate (i.e. post-parallel) round of selection are constructed into families with > 90% homology using the FASTAptamer algorithm. Characters with the same color in this figure represent members of the same sequence family. (Step 2) Families that are common to both the negative and positive final generation data sets are removed from the candidate pool. (Step 3-4) Remaining sequence families are compared against the penultimate (i.e. parallel) generation families to determine enrichment rate – families with the highest enrichment rates are the candidates for further analysis.

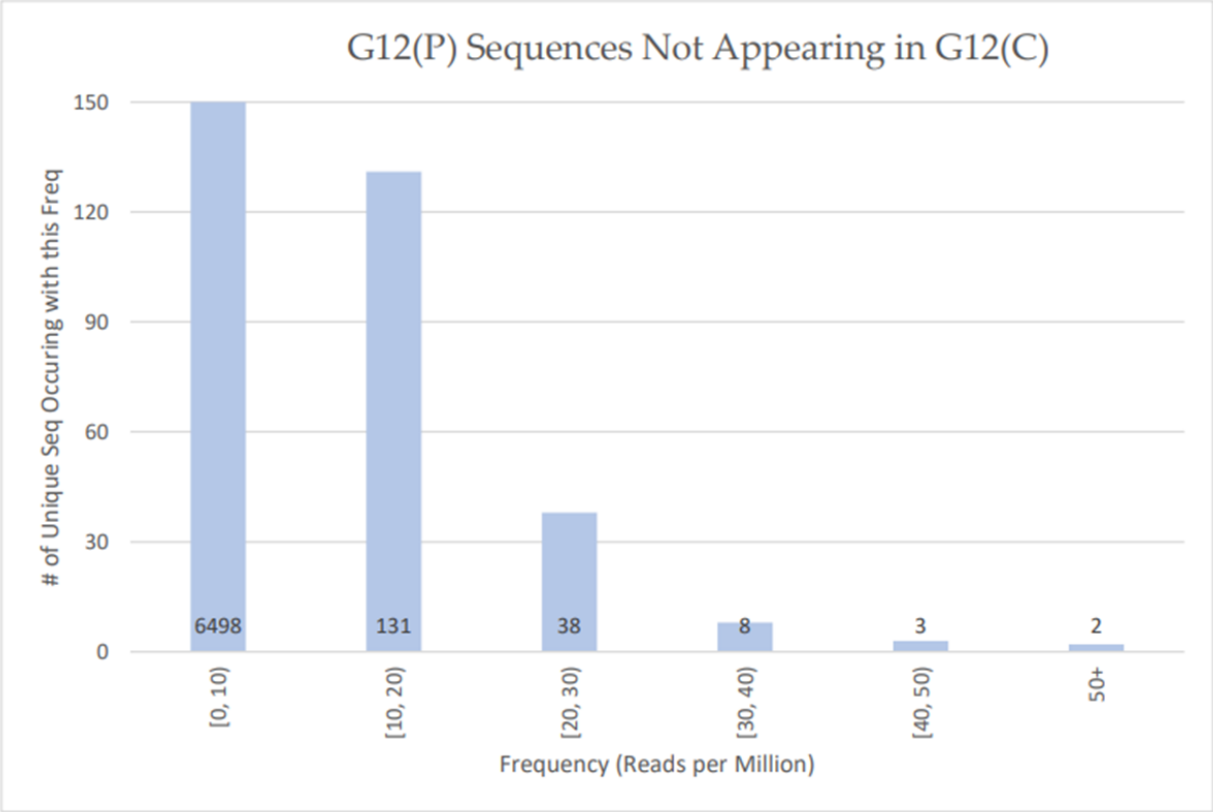




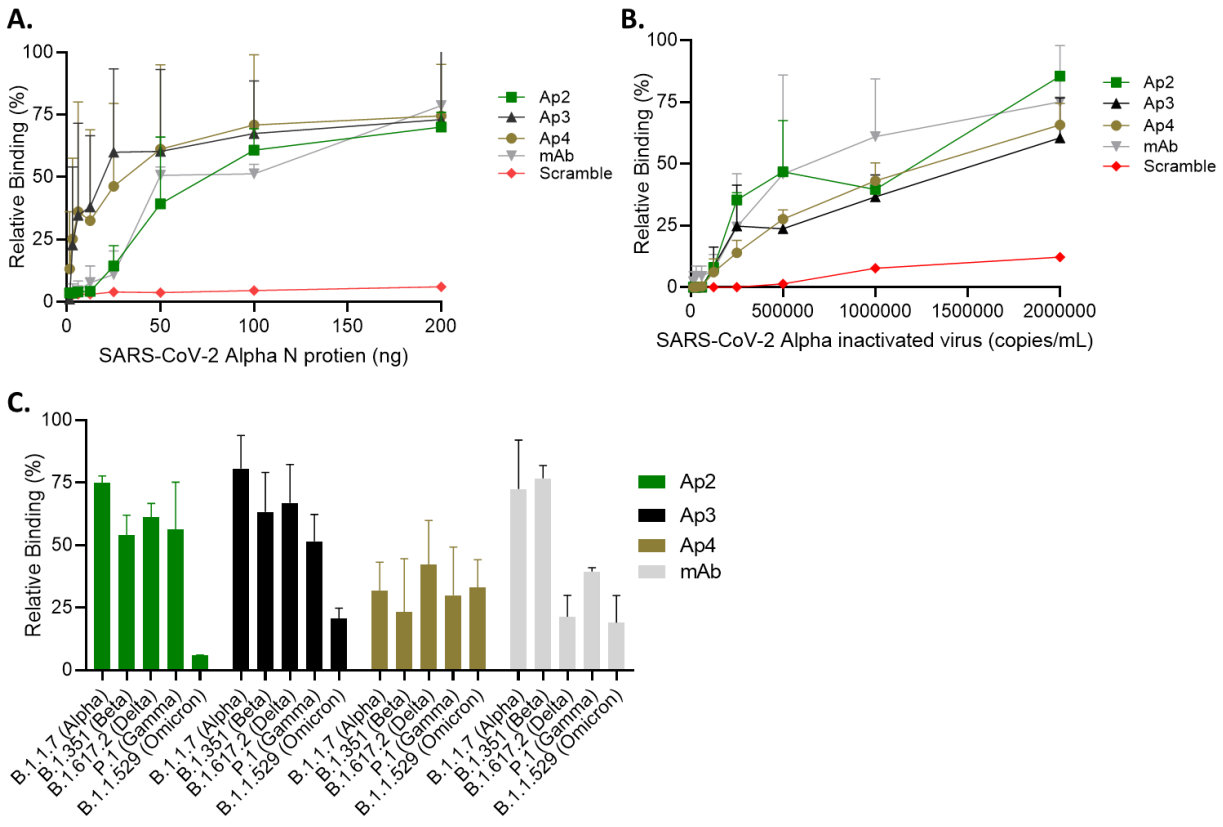
Supplemental Figure 4. Scatter Plot of Family Frequencies of G12(P) vs. G12(C). (Top) Scatter plot of the frequency of the sequence families discovered in the purified positive target- (G12(P)) and counter- (G12(C)) assessed libraries. G12(P) family frequencies are on the x-axis; G12(C) family frequencies are on the y-axis. Legend on right represents the ratio of a family's frequency between the two populations. Sequences represented with darker red colors demonstrate a high G12(P):G12(C) frequency ratio, indicating specificity for positive target in the final assessment round. Candidates not detected in a population do not appear.



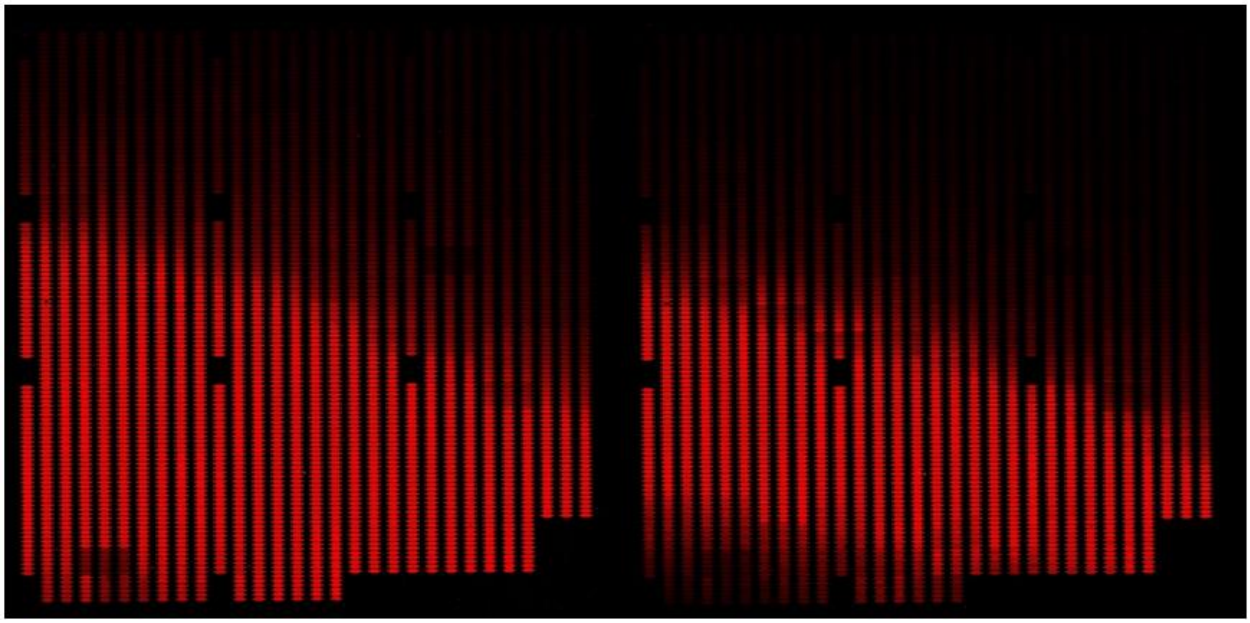
Supplemental Figure 5. Scatter Plot of Family Frequencies of G12(s) vs. G12(C). (Top) Scatter plot of the frequency of the sequence families discovered in the purified positive target- (G12(S)) and counter- (G12(C)) assessed libraries. G12(S) family frequencies are on the x-axis; G12(C) family frequencies are on the y-axis. Legend on right represents the ratio of a family's frequency between the two populations. Sequences represented with darker red colors demonstrate a high G12(S):G12(C) frequency ratio, potentially indicating specificity for positive target in the final assessment round. Candidates not detected in a population do not appear.



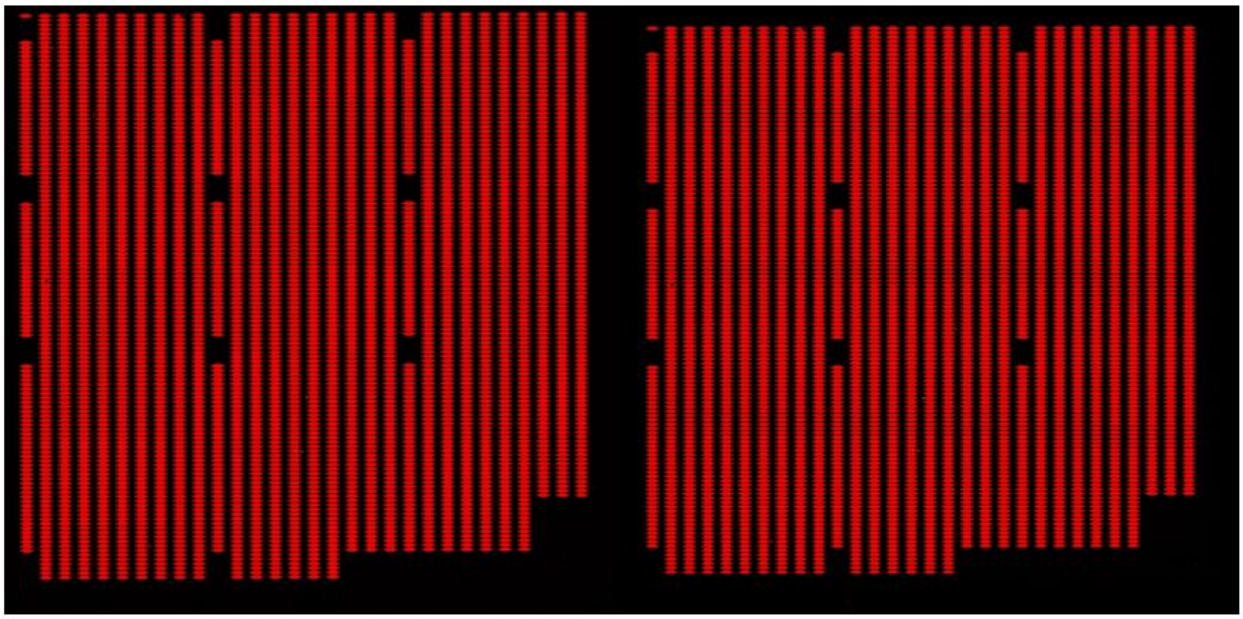
Supplemental Figure 6. Distribution of Family Frequencies from Library that do not Appear in G12(C). Plot of the frequency of sequences discovered in the final generation positive target- (G12(P)) that do not appear in the counter-target- (G12(C)) exposed libraries. G12(P) normalized sequence frequencies are on the x-axis; number of unique sequences with the given frequencies are on the y-axis.



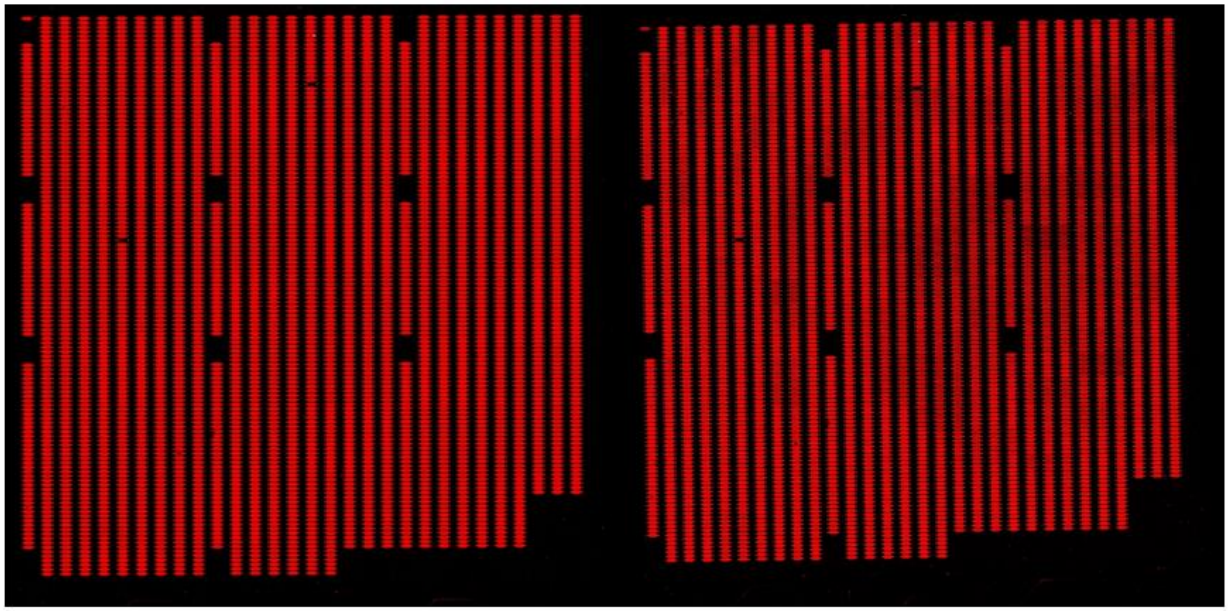
Supplemental Figure 7. Additional aptamers as detection reagents for SARS-CoV-2 N protein and inactivated virus in a hybrid ELASA format. Aptamers 2-4 were evaluated as detection reagents in a hybrid ELASA format where an antibody was used for capture and aptamers were used as detection reagents. **A.** Detection of SARS-CoV-2 Alpha recombinant nucleocapsid protein or **B.** inactivated virus. **C.** Aptamers were evaluated for their ability to detect N protein from SARS-CoV-2 viral strains of interest (200 ng). The error bars indicate standard deviation and statistical significance was calculated on relevant comparisons, but none reached a value below $p<0.05$.



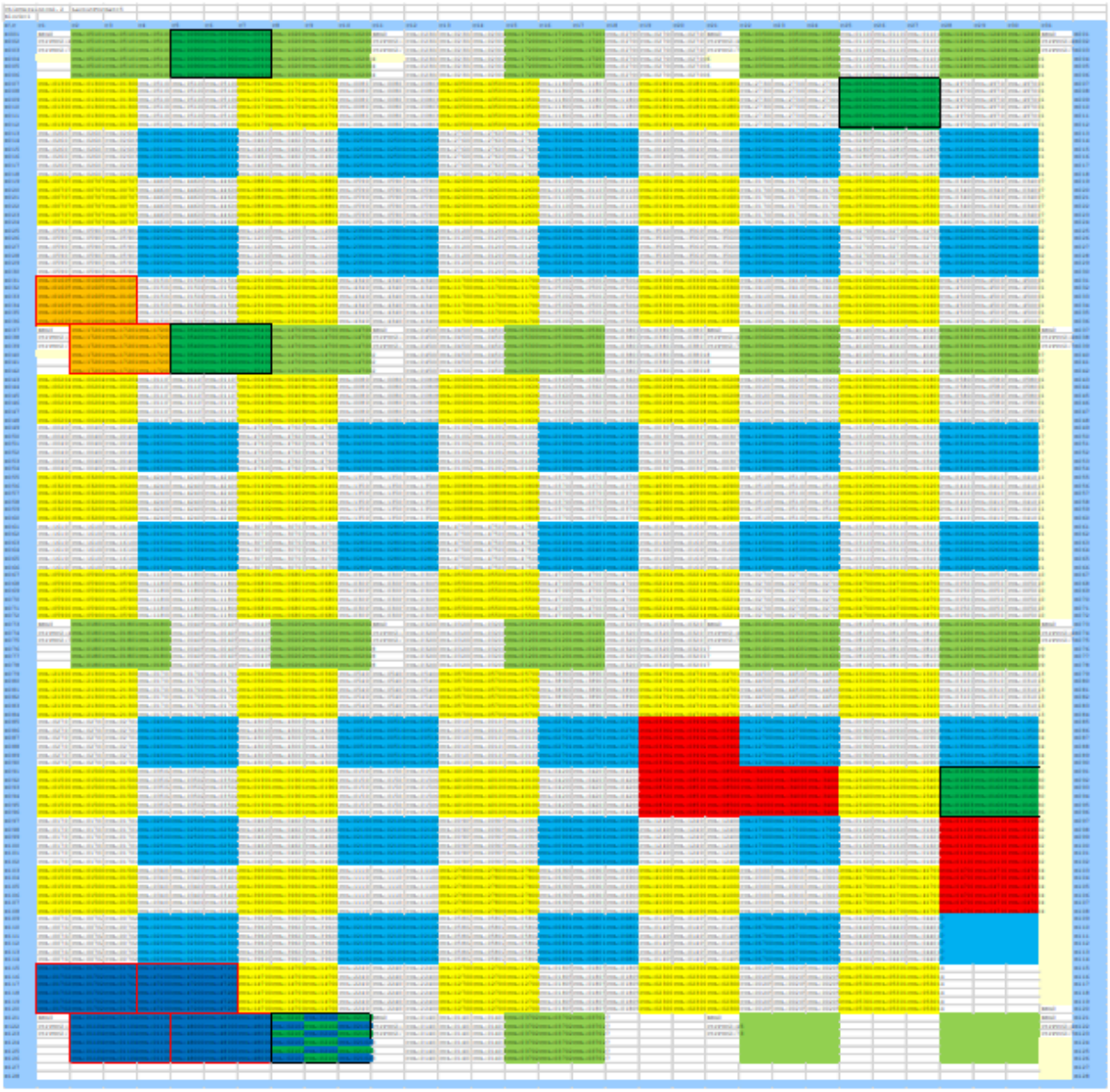
Supplemental Figure 8. Microarray Image of Candidate Fluorescence Responses to Omicron Target Sample. Replicates (clusters of 3 x 6 colonies) of aptamer candidates are synthesized in a microarray format covalently bound at the 3'-end. Candidates are then refolded (solution and chip were heated to 60°C for 20 minutes and then cooled to 23°C for 20 minutes) in the presence of a fluorescently-labeled reporting oligo. As seen in both images (lower right), some clusters were blocked from Cy5 probe exposure. (Left) Image taken after blocking candidates before incubation with Omicron Protein. Differences in blocking efficiency likely a result of secondary structures arising from specific sequences. (Right) Image taken after 16-hour incubation with Omicron Protein. Percent fluorescence loss (Equation 1) from the pre-target sample chip to the post-target solution chip was compared to the percent fluorescence loss from the pre-counter sample chip to the post-counter sample chip to rank aptamer strength (Equation 2). Control sequences are scattered throughout chip to monitor synthesis quality.



Supplemental Figure 9. Microarray Image of Candidate Fluorescence Responses to Delta Target Sample. Replicates (clusters of 3 x 6 colonies) of aptamer candidates are synthesized in a microarray format covalently bound at the 3'-end. Candidates are then refolded (solution and chip were heated to 60°C for 20 minutes and then cooled to 23°C for 20 minutes) in the presence of a fluorescently-labeled reporting oligo. (Left) Image taken after blocking candidates, before incubation with Delta Protein. Differences in blocking efficiency likely a result of secondary structures arising from specific sequences. (Right) Image taken after 16-hour incubation with target sample. Dark spots in colonies due to clogging of fluidics channels trapping air. Percent fluorescence loss (Equation 1) from the pre-target sample chip to the post-target solution chip was compared to the percent fluorescence loss from the pre-counter sample chip to the post-counter sample chip to rank aptamer strength (Equation 2). Control sequences are scattered throughout chip to monitor synthesis quality



Supplemental Figure 10. Microarray Image of Candidate Fluorescence Responses to Pooled Counter-Target Sample. Replicates (clusters of 3 x 6 colonies) of aptamer candidates are synthesized in a microarray format covalently bound at the 3'-end. Candidates are then refolded (solution and chip were heated to 60°C for 20 minutes and then cooled to 23°C for 20 minutes) in the presence of a fluorescently-labeled reporting oligo. (Left) Image taken after blocking candidates, before incubation with counter-target sample. Differences in blocking efficiency likely a result of secondary structures arising from specific sequences. (Right) Image taken after 16-hour incubation with counter-target sample. Dark spots in colonies due to clogging of fluidics channels trapping air. Percent fluorescence loss (Equation 1) from the pre-target sample chip to the post-target solution chip was compared to the percent fluorescence loss from the pre-counter sample chip to the postcounter sample chip to rank aptamer strength (Equation 2). Control sequences are scattered throughout chip to monitor synthesis quality



Supplemental Figure 11. Microarray Synthesis Layout. 200 aptamer candidates identified in Phase II were synthesized in replicates of 18 (3 x 6 colonies) on LC Science's 4k chip (Houston, TX). Replicates are needed to facilitate accurate detection of candidate response to test condition. Each orange, white, blue, yellow, or green block indicates a monoclonal cluster of 3 x 6 colonies. The best candidates suggested by bioinformatics analysis are indicated in forest green. The highest-performing candidates based on microarray analysis are marked in dark blue (lower left corner), with runner-up sequences indicated in orange. The worst-performing candidates by microarray analysis are indicated in red. Based on this meta-analysis, it is important to consider not just the strength of the measured candidate response but also the location on the microarray to determine if the response may have been affected by accessibility to the analyte.

Aptamer Name	Sequence
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128	CGAGGCTCTCGGGACGACGAAGCAGGCAGTGATTCGAAATAATTATTGGTCGTCCCGCCTTTAGGATTTACAG
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130	CGAGGCTCTCGGGACGACCTTTATAAATCTCGCAGCGATTCTCCCTATGTCGTCCCGCCTTTAGGATTTACAG
131	CGAGGCTCTCGGGACGACAAGCCAGCGATTCTTGTGAAAGCAATTTTTGTCGTCCCGCCTTTAGGATTTACAG
132	CGAGGCTCTCGGGACGACAGTTGCAGCGATTCCTGTTTATTCCCAGCTGTCGTCCCGCCTTTAGGATTTACAG
133	CGAGGCTCTCGGGACGACTTCCGAAGCGATTCTATCTTTTACGAGTAAGTCGTCCCGCCTTTAGGATTTACAG
134	CGAGGCTCTCGGGACGACGTATGCAGTGATTCAAAGATCATAATATGAGTCGTCCCGCCTTTAGGATTTACAG
135	CGAGGCTCTCGGGACGACTTTGCAGCGAATCGTCAAGAGATAAGAACAGTCGTCCCGCCTTTAGGATTTACAG
136	CGAGGCTCTCGGGACGACAGTCTGCAGCGATTCTTTAGTGAGTCCACGGTCGTCCCGCCTTTAGGATTTACAG
137	CGAGGCTCTCGGGACGACTGTTCTGCAGCGGTTCTCCTCAGATTTTGAGTCGTCCCGCCTTTAGGATTTACAG

138	CGAGGCTCTCGGGACGACTGTCTGTTCAGCAGCGATTCCGTAATCTAAGTCGTCCCGCCTTTAGGATTTACAG
139	CGAGGCTCTCGGGACGACACTCATGCAGTGATTCTTAAAACTTCTAGTGTCGTCCCGCCTTTAGGATTTACAG
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142	CGAGGCTCTCGGGACGACTGCTGCAGCGATTTTAAGATCGTAAGTCTGTCGTCCCGCCTTTAGGATTTACAG
143	CGAGGCTCTCGGGACGACTATATTGCAGCGATTCACTAATAGTCTATAGTCGTCCCGCCTTTAGGATTTACAG
144	CGAGGCTCTCGGGACGACAAAGCAGCGATTCTCTCACAGAAGTATTTGTCGTCCCGCCTTTAGGATTTACAG
145	CGAGGCTCTCGGGACGACGATGGTTTGCAGCGATTCGAAGTTCCAGTAGTCGTCCCGCCTTTAGGATTTACAG
146	CGAGGCTCTCGGGACGACTATCTGCAGGGATTCATTGCTCAATATATAGTCGTCCCGCCTTTAGGATTTACAG
147	CGAGGCTCTCGGGACGACTTTGGTGCAGCGATTCTGGCAAGATAAAAAGTCGTCCCGCCTTTAGGATTTACAG
148	CGAGGCTCTCGGGACGACATGGTTGCAGCGATTCCTGATGTGTTTTATGTCGTCCCGCCTTTAGGATTTACAG
149	CGAGGCTCTCGGGACGACTGATGTAGCGATTCTCAATTAAGTTTTAAAGTCGTCCCGCCTTTAGGATTTACAG
150	CGAGGCTCTCGGGACGACTTTTGAAGCGATTCAATAGGAATTATCATAGTCGTCCCGCCTTTAGGATTTACAG
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152	CGAGGCTCTCGGGACGACCTTGATGGTAGCCCTGCAGCGATTCTATTGGTCGTCCCGCCTTTAGGATTTACAG
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163	CGAGGCTCTCGGGACGACTGTGTGCAGCGGTTCTTGTTATGGATTACAGTCGTCCCGCCTTTAGGATTTACAG
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174	CGAGGCTCTCGGGACGACTGTAGTGATTCTTGCGTTTATAAATTACGAGTCGTCCCGCCTTTAGGATTTACAG
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176	CGAGGCTCTCGGGACGACTGTGTGCAGCGGTTCTTGTTATAGATTACAGTCGTCCCGCCTTTAGGATTTACAG
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178	CGAGGCTCTCGGGACGACTGGTGCAGCGATTCATTCTATATATAAGCAGTCGTCCCGCCTTTAGGATTTACAG
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182	CGAGGCTCTCGGGACGACTCTGCAGCGATTCGAATGAACCGATATACAGTCGTCCCGCCTTTAGGATTTACAG
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184	CGAGGCTCTCGGGACGACCTTTGGCAGCGATTCTACATGACTGTTACGGTCGTCCCGCCTTTAGGATTTACAG
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188	CGAGGCTCTCGGGACGACAAGACGCAGCGATTCTGGATCTATGTCTCTGTCGTCCCGCCTTTAGGATTTACAG
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192	CGAGGCTCTCGGGACGACATTGGGCAGCGATTCTCATGATATCCATACGTCGTCCCGCCTTTAGGATTTACAG
193	CGAGGCTCTCGGGACGACCTTTGGCAATAAGCAGCGATTCTTATTTTGGTCGTCCCGCCTTTAGGATTTACAG
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195	CGAGGCTCTCGGGACGACGAATGCAGCGATTCCAGGCTTATTTCAGTAGTCGTCCCGCCTTTAGGATTTACAG
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197	CGAGGCTCTCGGGACGACTATAGTTGCAGCGATTCAAGATTAACATACGTCGTCCCGCCTTTAGGATTTACAG
198	CGAGGCTCTCGGGACGACTGATTTGCAGCGATTCTAAATGTTAAGCTAGTCGTCCCGCCTTTAGGATTTACAG
199	CGAGGCTCTCGGGACGACAGTACGTGGCAGCGATTCTAGTCTAGTGTTGTCGTCCCGCCTTTAGGATTTACAG
200	CGAGGCTCTCGGGACGACTTCTGAAGCGATTCTATCTTTTACGAGTAAGTCGTCCCGCCTTTAGGATTTACAG

Supplemental Table 1: Top 200 Aptamer Sequences