Supporting Information

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SI Text

SI Materials and Methods. ***1. Cloning and sequencing of aptamers from the R3 pool.*** After 3 rounds of selection, the selected pool was PCR amplified with unlabeled forward and reverse primers at the optimized PCR cycle number determined by the pilot PCR. PCR products were purified with the MinElute PCR Purification Kit (Qiagen) and cloned into *Escherichia coli* using the TOPO TA cloning kit (Invitrogen). 98 colonies were randomly picked and sequenced at the GENEWIZ San Diego Laboratory.

2. Nitrocellulose filter binding assay. T4 polynucleotide kinase (NEB, Ipswich, MA) was used to 5′ end label 10 μM concentrations of ssDNA aptamers with γ [^{32}P]-ATP. Following a 40 min, 37 °C incubation step, the reaction was heat killed by taking the end labeled samples to a temperature of 70 °C for 10 min. The end labeled oligonucleotides were purified using Sephadex G-50 microspin columns (GE Healthcare).

Following purification, a 100 pM solution of γ [^{32}P] 5′ end labeled aptamers was incubated with 1 nM of target protein (PDGF-BB) for 1.5 h at room temperature. 50 μL of each sample was subsequently applied to a mixed ester filter (Millipore) that had been prewetted with 1 mL of 0.1 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, and 1 mM $MgCl_2$ (PBSM) (pH = 7.4) buffer. The filters were immediately washed with 1 mL of PBSM buffer, and then removed from the filter holders and placed in scintillation liquid. Scintillation counts were generated, and the fraction of aptamers bound to the protein, q, was calculated from the following equation

$$q = [(A + P + F) - (A + F)]/(A + P)$$

where A stands for aptamer (either the library or the #1 sequence from Quantitative Selection of Aptamers through Sequencing (QSAS) R3/R2 set), P stands for PDGF-BB protein, and F stands for filtering. In other words, the fraction of aptamers in bound form q, is equal to the signal (in cpm) generated by filters treated with a mixture of protein and aptamer minus the signal generated by aptamer nonspecifically bound to the filter in the absence of protein, divided by the total signal generated from an unfiltered mixture of aptamer plus protein.

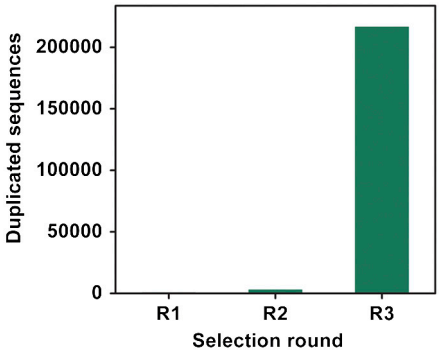


Fig. S1. Representation of the ten most frequently observed sequences from each selection pool. These had an average copy number of 594, 2,789, and 216,488 for R1, R2, and R3 pools, respectively.

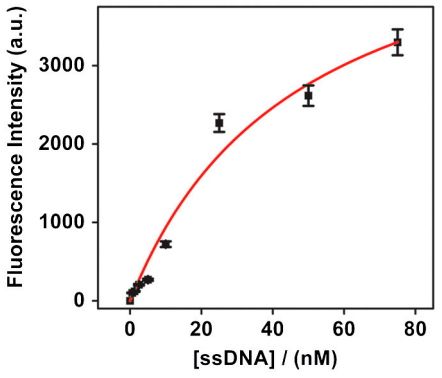


Fig. S2. The most frequently appeared sequence in the three pools was tested for its affinity for the PDGF-BB target via a fluorescence binding assay. Based on these measurements, we calculated a K_d of 47.58 ± 16.1 nM, which is considerably higher (i.e., lower affinity) than other aptamers sorted by QSAS.

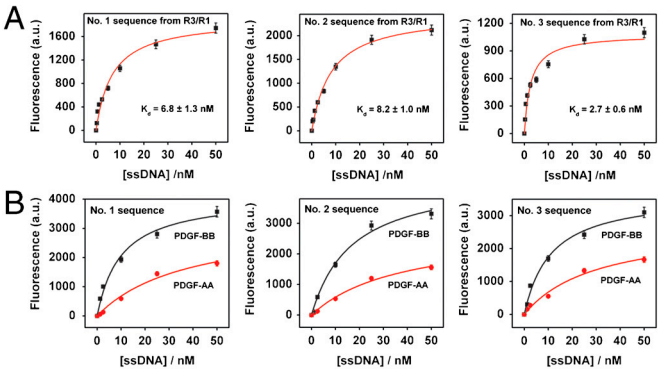


Fig. S3. The three most highly enriched sequences from QSAS R3/R1 were tested for their affinity (*A*) and specificity (*B*) for the PDGF-BB target via a fluorescence binding assay.

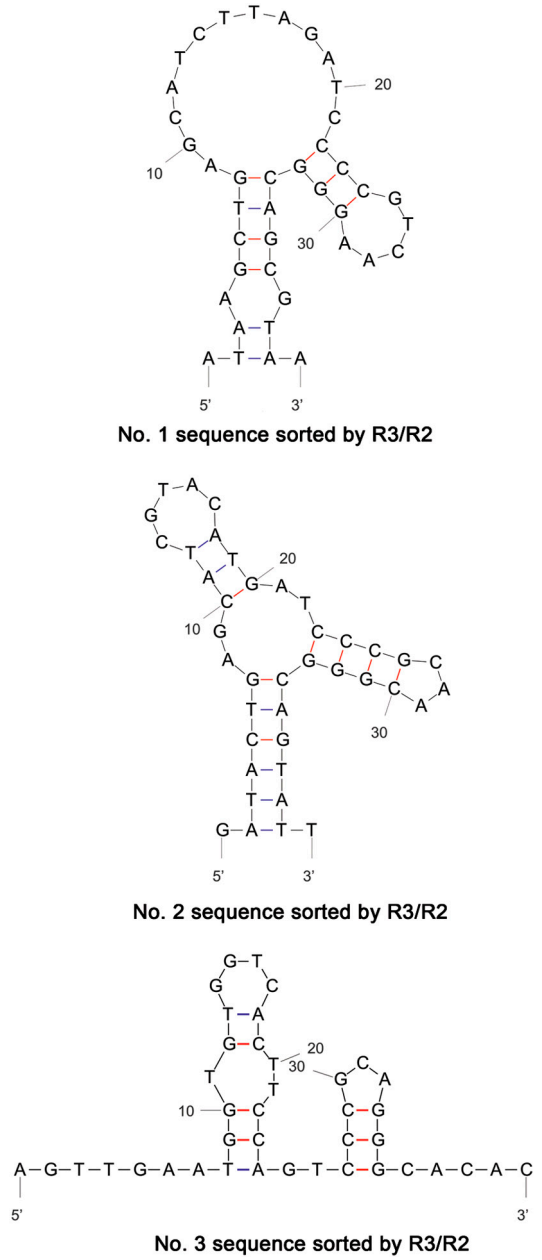
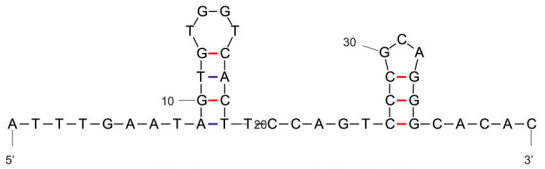
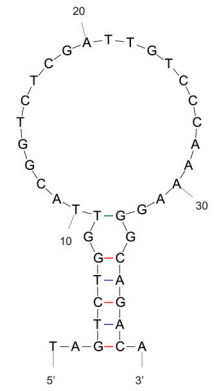


Fig. S4. Secondary structures of the three most highly enriched aptamer sequences obtained from QSAS R3/R2.





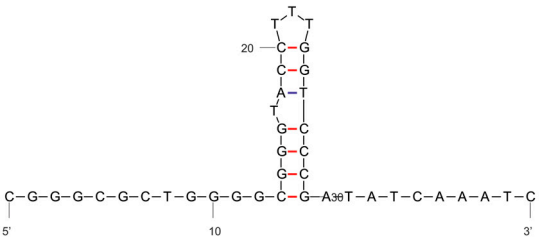


Fig. S5. Secondary structures of the three most highly enriched aptamer sequences obtained from QSAS R3/R1.

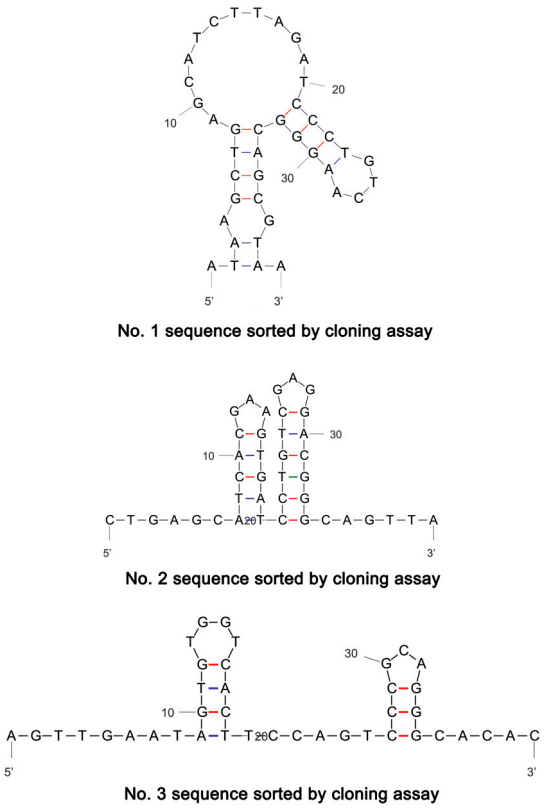


Fig. S6. Secondary structures of the top three enriched aptamer sequences obtained from randomly picked clones from the R3 pool.

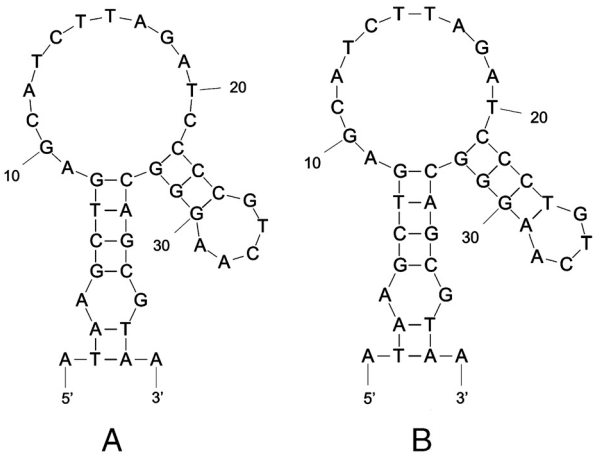


Fig. S7. The secondary structure of highly enriched PDGF-BB aptamers. (*A*) Sequence #1 identified by the QSAS R3/R2 method. (*B*) Sequence #3 identified by standard cloning and sequencing of the R3 pool. Although these two aptamers varied by only a single base, they exhibited markedly different binding affinities and specificities: the QSAS-derived sequence exhibited 6.4-fold higher binding affinity and 2.2-fold higher specificity for PDGF-BB in comparison with the cloning-derived sequence.

Table S1. Top 36 enriched aptamer sequences from R1 to R3

Aptamer ID	Sequences of selected aptamers (5′ → 3′)	Enrichment fold (R3/R1)
1	ATTTGAATAGTGTGGTCACTTCCAGTCCCGCAGGGCACAC	2347
2	TAGTCTGGTTACGGTCTCGATTGTCCCAAAAGGGCAGACA	1929
3	CGGGCGCTGGGGCGGGTACCTTTGGTCCCGATATCAAATC	1884
4	TACTTGAGCATCGTTTTATGATCCTTTTGGGCAAGTCGGT	1882
5	GCTGGTCACTCTAGAGTCCTTGCAGGCAGCAAAACATAAT	1742
6	ATTCAGATTAAGGGCTGCCTGAACTCGTTCCCGGTATCAT	1728
7	TCCATCGAGGCATCCCTGTAAAGAGGATCATGAGTCACGT	1720
8	TGCGGGTTACTTTTAAGTCCCAAGGGGCCGTAGATCACCA	1705
9	ATGCACGGGCACCATGTATGGTCCTGATTATAGGGCGTGG	1593
10	AATACCGTCACTTTTAGTCCTGTTGTTAAGAGCAGGGGGT	1535
11	GGAGCGTCACTAATTTAGTCCCAATAGGGGGATCCTTATC	1504
12	TTACGGTGTTGGTCACTATTTAGTCCCTACTGGGCACACC	1501
13	TAGGCATGTAATGAGCATCTCTGATCCTATGGTTCTTCAT	1466
14	CTCTCGTCACTTCTAGTCCCTTTTAAAGGGGAGAGCTTCG	1465
15	GAGAGCTGATGCAATCCTAGTATAGGCAGCATAACATTTC	1460
16	GGCTCGGAACCGCATAGTGGTCCTGGTCGCCAGGGGAGCC	1408
17	TATGATCAAATCCTCGTTGTACGGGGCATAAACTGATTTG	1402
18	GGTGGTCACTAGCTTAGTCCCATTACTTGGGCACCAGGGT	1346
19	TCAAGTCCTTTTTAGGCAACCATATGTTGGTCATTTGATA	1343
20	TGTCGAGCATCAGTGCGGTGATCCTGAGGGACCAGGCGAC	1316
21	GGGACGGAGCATCGTTTACGATCCCCGCGGGCCGTCGGCA	1314
22	GCGTTCCAAGTTAGCGGAACTTCCTCACTAGGCGCAAGGA	1303
23	ACGTACGTGGGCACCTCTGGTCCAGAAGGGCACGGACCTG	1280
24	TAAAGGGTGTGGGGCACCCCTCGGGTCCCTGTGGGCGCAC	1277
25	TTGGATCATTGGCACCTCAGGTCCCTACGGGGGAATGATT	1252
26	CGAGGGGTACCCTGCTGGGTCCCCGGTAGGGGCGAGTTCT	1244
27	CCGTTACAGTCTAAGACTCCTATGAACATGGGCGGAAGTA	1225
28	GTCGCTCGTTGTACGGTAGGTCCCTGAAGGGGGAGCGATG	1218
29	CGTCTCAGTAGATATTACTCCTAGCTAAACTAGGCGTGAG	1218
30	CAGTGGATGGTCACTCCCAGTCCTTAACAGGCATCAACGG	1184
31	TAGGTTATGTAATCCTGTCTTCAGGCCCAGACGGGTAACC	1163
32	GTTTCCATACGGCACCTATGGTCCTTGTAAAGGGTATGGT	1161
33	CTGACGAGGCACGCAAGTGGGCACCTTTAGGTCCTCCTTT	1157
34	AACACGTGGGCACCTTCAGGTCCCCCGCGGGGGGCGCGTG	1149
35	ATAATATCATGGTTGGGCACCTAAGGTCACGCAGTGCAAC	1144
36	CTGCGGTCACTGCTCAGTCCTGCAATAGCAGGCGCAGACT	1142

Table S2. Top 1 sequence with the highest copy number in the three pools

Aptamer ID	Sequences of selected aptamers (5′ → 3′)
1	CCGTTACAGTCTAAGACTCCTATGAACATAGGCGGAAGTA

Table S3. Aligned aptamer sequences obtained by traditional cloning from the selected R3 pool

Seq. ID	Sequences (5′ → 3′)	
21	A—A——GTGGGGCACCAGGT————TTGGTCCCCGGATAGGGGCCACTT	Group I (16.50%)
48	GGGTCAGCGGCGAGTATCGTGTGGATCCCGTAGGGCGCCG	
32	T———CGGTGAGGTATCCATTTGGATCCTCAGTCCTCAGGCACC	
3	GC–G———CCGGGTACCCTTAAGG—GTCCCGTCAGGGGGGCGTCA	
58	GGCGTCACTTCTAGT———CCCGCTCCACGGGGGCCAGGGTTCT	
17	T———CGCGGTCACTGCAATTAGATAGGAGTCCCGGAGGGCGCG	
26	GCTTGTCACTATA————ACATAGTCCTGGAAGGGAAGCGTAC	
12	TCCTGCAGTTGGTCACTATCATTAGTCCCACAGGGCAACT	
35	CGCTAAAGCTGTCAC——TCTCAGTCCCGAAGGGGAGCTTTTT	
42	CGCTAAAGCTGTCAC——TCTCAGTCCCGAAGGGGAGCTTTTT	
94	A———GTTGAATAGTGTGGTCACTTCCAGTCCCGCAGGGCACAC	
83	A———GTTGAATAGTGTGGTCACTTCCAGTCCCGCAGGGCACAC	
1	A———GTTGAATAGTGTGGTCACTTCCAGTCCCGCAGGGCACAC	
4	A———GTTGAATAGTGTGGTCACTTCCAGTCCCGCAGGGCACAC	
53	G——AGGAGTATCGGGGC–ACGATCCTATTTAAGGCCGATAACC	Group II (12.90%)
62	C———GGAGTATACATTCGT—ATCCTATATAGGTAGGCCGAAACC	
81	C———GGAGTATACATTCGT—ATCCTATATAGGTAGGCCGAAACC	
85	T———CGACTATGTTGCTAC—ATCCTGTCACGGTCAGGCGGAAAG	
25	CTGATTATATAACGTG—GTGATATCCGGACAAGGCAGAAAA	
67	AA–G——CTGATTACCAAAACTGC–GTGCCGCCCGGACCCGGTGA	
14	GCTGAGCATCAATTCGCAT–TGATCCCACAGTTGGGCAGCTA	
92	CTGAGCATCACGAAGT–GATCCTGTCGAGGACGGGCAGTTA	
52	CTGAGCATCACGAAGT–GATCCTGTCGAGGACGGGCAGTTA	
13	CTGAGCATCACGAAGT–GATCCTGTCGAGGACGGGCAGTTA	
16	CTGAGCATCACGAAGT–GATCCTGTCGAGGACGGGCAGTTA	
71	CG–C———ACGAGCATCGTACTTCC–GTTATGATCATGAGACACAG	Group III (10.60%)
77	C————GTGGTTCCTGCGGGCAGGTTAT—CAACATGGGCAATGCGT	
27	AT—AA–GCTGAGCATCTTAG————GTCCCTGTCA–AGGGGCAGCGTAA	
10	ATAAGCTGAGCATCTTAG————ATCC—CTGTCAAGGGCAGCGTAA	
11	ATAAGCTGAGCATCTTAG————ATCC—CTGTCAAGGGCAGCGTAA	
31	ATAAGCTGAGCATCTTAG————ATCC—CTGTCAAGGGCAGCGTAA	
24	ATAAGCTGAGCATCTTAG————ATCC—CTGTCAAGGGCAGCGTAA	
60	ATAAGCTGAGCATCTTAG————ATCC—CTGTCAAGGGCAGCGTAA	
78	ATAAGCTGAGCATCTTAG————ATCC—CTGTCAAGGGCAGCGTAA	
28	A——A——TTGGATTACTTCT————CAAGTCCTGATGAGGGCAATTTAAA	
56	T————TGGGTACCTCGTAGG–TCCTAATGGAGGTGAGGCAACTCC	

Table S4. Alignment of the top 36 aptamer sequences obtained from QSAS R3/R2

Seq. ID	Sequences (5′ → 3′)	
11	CTGAGCATCACGAAGTGATCCTGTCGGGGACGGGCAGTTA	Group I (47.20)%
32	CTGAGCATCACGAAGTGGTCCTGTCGAGGACGGGCAGTTA	
33	CGAATGCAAGTCCTGACGGAGGCCAGGCGCTGAAACTTGC	
21	ATAAGCTGAGCATCTTA——GATCC—CTGTCAAGGGCAGTGTAA	
1	ATAAGCTGAGCATCTTA——GATCC—CCGTCAAGGGCAGCGTAA	
23	GATACTGAGAATCGTA-CATGATCC——CGCAACGGGCAGTATC	
2	GATACTGAGCATCGTA-CATGATCC——CGCAACGGGCAGTATT	
12	CCGGGAACATTGTCTAAACAATGTCATTTATGTCGGAAGC	
14	TAAGGGCACTATTGCATGGTGAGCATCCTTGGATCCCGCT	
9	ATAAGCTGAGCATCTTA——GATCC—CTGTTAAGGGCAGCGTAA	
13	ATAAGCTGAGCACCTTA——GATCC—CTGTCAAGGGCAGCGTAA	
18	AATGGATGGGCACCGCTATAGTTGGTCCCGAAGGGCATGC	
22	GATACTGAGCATCGTACA-TGATCC——CGCAACGAGCAGTATC	
29	CATACTGAGCATCGTACATGATCCC———GCAACGGGCAGTATC	
25	CGGGGCAACAACGTTGAGCATCATTCATGATCGCCGCTAT	
24	AGGTGAGCATCTTTAGATCCCATTTGGCGCCTTTTCTTTC	
8	TTGGGGCGGTCCTGTGAAAGGCAAAAATCTATTATACCGC	
16	CCGTTACAGTCTAAGACTCCCATGAACATAGGCGGAAGTA	Group II (22.20%)
6	CCGTTACAGTCTAAGACTTCTATGAACATAGGCGGAAGTA	
30	CCGTTACAGTCTAAGACTCCTATGAACATAGGCGGAACTA	
34	CTGTTACAGTCTAAGACTCCTATGAACATAGGCGGAAGTA	
4	CCGTTACAGTCTAAGACTCCTATCAACATAGGCGGAAGTA	
26	GCTCCGTACCATTCTTATGGTCCCGGCACGGGCGCAAACG	
20	AGTTGAATAGTGTGGTCACTTCCAGTCCCGCGGGGCACAC	
3	AGTTGAATGGTGTGGTCACTTCCAGTCCCGCAGGGCACAC	
5	TGTGGGTATGGTCCTAATTTTTTAGGCACGGAGGTACCAT	Group III (13.90%)
31	GCTGAGTTAGATCCCTTTCGTAAGGGCAGCCGGGTATCTA	
7	TACGAGTTTGATCCTTTCCATTAGGCGTACAGCTCATCAA	
15	TACGAGGTTGATCCTTTTTATTAGGCGTACAGCTCATCAA	
19	TACGAGTTTGATCCTTTTTATTATGCGTACAGCTCATCAA	
36	GAGGGCTGATGCAATCCTAGTATAGGCAGCATAACATTGC	GroupIV (16.70%)
10	GCTGAGTTAGATCCCTTTC————GTAAGGGCAGCCGGGCATCGA	
17	GCTGAGTTAGATCCATTTC————GTAAGGGCAGCCGGGCATCTA	
35	TAAGGTGGGGATAAGGACGTCGGGGATCGGGGGGGGGGAT	
27	CTTATT————————————GTGCGGGCACCTCAGGTCCTAAAGTTAGGCGCAC	
28	TTTATT————————————GTGCGGGCACCTCAGTTCCTAAAGTTAGGCGCAC	

Table S5. Alignment of the top 36 aptamer sequences obtained from QSAS R3/R1

Seq. ID	Sequences (5′ → 3′)	
8	TGCGGGTTACTTTTAAGTCCCAAGGGGCCGTAGATCACCA	Group I (38.90%)
26	CGAGGGGTACCCTGCTGGGTCCCCGGTAGGGGCGAGTTCT	
12	TTACGGTGTTGGTCACTATTTAGTCCCTACTGGGCACACC	
5	GCTGGTCACTCTAGAGTCCTTGCAGGCAGCAAAACATAAT	
24	TAAAGGGTGTGGGGCACCCCTCGGGTCCCTGTGGGCGCAC	
25	TTGGATCATTGGCACCTCAGGTCCCTACGGGGGAATGATT	
30	CAGTGGATGGTCACTCCCAGTCCTTAACAGGCATCAACGG	
19	TCAAGTCCTTTTTAGGCAACCATATGTTGGTCATTTGATA	
33	CTGACGAGGCACGCAAGTGGGCACCTTTAGGTCCTCCTTT	
1	ATTTGAATAGTGTGGTCACTTCCAGTCCCGCAGGGCACAC	
34	AACACGTGGGCACCTTCAGGTCCCCCGCGGGGGGCGCGTG	
35	ATAATATCATGGTTGGGCACCTAAGGTCACGCAGTGCAAC	
23	ACGTACGTGGGCACCTCTGGTCCAGAAGGGCACGGACCTG	
9	ATGCACGGGCACCATGTATGGTCCTGATTATAGGGCGTGG	
31	TAGGTTATGTAATCCTGTCTTCAGGCCCAGACGGGTAACC	Group II (11.10%)
18	GGTGGTCACTAGCTTAGTCCCATTACTTGGGCACCAGGGT	
11	GGAGCGTCACTAATTTAGTCCCAATAGGGGGATCCTTATC	
20	TGTCGAGCATCAGTGCGGTGATCCTGAGGGACCAGGCGAC	
15	GAGAGCTGATGCAATCCTAGTATAGGCAGCATAACATTTC	
21	GGGACGGAGCATCGTTTACGATCCCCGCGGGCCGTCGGCA	
27	CCGTTACAGTCTAAGACTCCTATGAACATGGGCGGAAGTA	
36	CTGCGGTCACTGCTCAGTCCTGCAATAGCAGGCGCAGACT	
3	CGGGCGCTGGGGCGGGTACCTTTGGTCCCGATATCAAATC	
6	ATTCAGATTAAGGGCTGCCTGAACTCGTTCCCGGTATCAT	
32	GTTTCCATACGGCACCTATGGTCCTTGTAAAGGGTATGGT	
17	TATGATCAAATCCTCGTTGTACGGGGCATAAACTGATTTG	
7	TCCATCGAGGCATCCCTGTAAAGAGGATCATGAGTCACGT	
29	CGTCTCAGTAGATATTACTCCTAGCTAAACTAGGCGTGAG	
4	TACTTGAGCATCGTTTTATGATCCTTTTGGGCAAGTCGGT	
10	AATACCGTCACTTTTAGTCCTGTTGTTAAGAGCAGGGGGT	
14	CTCTCGTCACTTCTAGTCCCTTTTAAAGGGGAGAGCTTCG	
28	GTCGCTCGTTGTACGGTAGGTCCCTGAAGGGGGAGCGATG	
13	TAGGCATGTAATGAGCATCTCTGATCCTATGGTTCTTCAT	
22	GCGTTCCAAGTTAGCGGAACTTCCTCACTAGGCGCAAGGA	
16	GGCTCGGAACCGCATAGTGGTCCTGGTCGCCAGGGGAGCC	
2	TAGTCTGGTTACGGTCTCGATTGTCCCAAAAGGGCAGACA	