Table S1

splice site	# sequences
G3	2
U33	3
C43	1
A50	1
U54	1
U84	1
U87	1
U97	5
U99	1
U110	1
U177	1
U179	1
U448	1

Table S2

selection round \ sub-pool	-9	-6	-3	0	+3	+6
0	7	14	7	10	6	5
1	1	2	1	1	9	5
2	0	3	5	0	4	4
3	0	1	1	0	7	11
4	0	1	4	0	5	10
5	0	1	0	0	10	9

Table S3

Primer 1: 5’-GCGTAATACGACTCACTATAGNNNNNAAAAGTTATCAGGCATGCACC
Primer 2: 5’-GAAGCCTCTGAGCCGCGCCAG
Primer 3: 5’- CAGGAGCTAAGGAAGCTAAAATG .
Primer 4: 5’-CGCCCCGCCCTGCCACTCATC
Primer 5: 5’-GCGTAATACGACTCACTATAGCAGGAGCTAAGGAAGCTAAAATG
Primer 6: 5’-CGCCCCGCCCTGCCACTCATC
Primer 7: 5’- GAAGCCTCTGAGCCGCG
Primer 8: 5’- CGAATTCCAGGAGCTAAGGAAGCTAAAATG
Primer 9: 5’- GCGGATCCGAAGCCTCTGAGCCGCGCCAGC
Primer 10: 5’- GATCCTCTAGAGTCGACCTGCAGGCATGCAGCTCGTACTGA
Primer 11: 5’- AGCTTCAGTACGAGCTGCATGCCTGCAGGTCGACTCTAGAG
Sequence 12: 5’-
GCATGCGCACGTAAGAGGTTCCAACT…ATGAGTGGCAGGGCGGGGCGTAGCATAACCCCTT
GGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGAAGCTT
Primer 13: 5’- CGCTCTGGAGTAATACCACGACG
Primer 14: 5’- CGTCGTGGTATTACTCCAGAGCG
Primer 15: 5’-
GCGGATCCTGTTGACAATTAATCATCCGGCTCGTATAATGTGTGGAAGAAGGCAAAAGTTAT
CAGGCATGC
Primer 16: 5’-
ACGGAATTCCGGGTGTGCGTTCATTAAGCGTGCTAAGATATGGATCGAGTACTCCAAAACTA
ATC (underlined positions denote silent mutations made in the ribozyme 3’-exon)
Primer 17: 5’- GCGGATCCTGTTTACAATTAATCATC
Primer 18: 5’- ACGGAATTCCGGGTGTGCGTTC
Primer 19: 5’- CCGGAATTCCGTATGGCAATGAAAG
Primer 20: 5’- GCGAGCTCCAAAAAACCCCTCAAGACCCG
Primer 21: 5’-
CGGCTCGTATAATGTGTGGAAXXXXXXXXNNNNNNNNNAANGAAGGCAAAAGTTATCAGG
(the Xs represent the antisense nucleotides setting the bulge register as denoted in Fig. 3 and the Ns represent the randomized nucleotides)
Primer 22: 5'-ACGGCCAGTGAATTCGAGCTC
Primer 23: 5’-GCGGATCCTGTTTACAATTAATCATCCGGCTCGTATAATGTGTGG
Primer 24: 5'-ACGGCCAGTGAATTCGAGCTC
Primer 25: 5’-GCGGATCCTGTTTACAATTAATCATCCGGCTCGTATAATGTGTGG
Primer 26: 5'-ACGGCCAGTGAATTCGAGCTC
Sequence 27: 5’- GAGGATCCTAATACGACTCACTATTAAN$_x$GAAGGCAAAAGTTATCAGG where N_x represents the EGS region for the ribozyme being created
Primer 28: 5’-ACGGCCAGTGAATTCGAGCTC
Primer 29: 5’-GAGGATCCTAATACGACTCAC
Primer 30: 5’-GGTATTCACTCCAGAGCGATG
Primer 31: 5’- GCGTAATACGACTCACTATAGTACCTATAACCAGACCGTTC
Primer 32: 5’-GGATGAGCATTCATCAGGCG
Primer 33: 5’- AATTTAATACGACTCACTATAGAGCTAGCGGATGAAGTGATG
Primer 34: 5’-GGTATTCACTCCAGAGCGATG
Primer 35: 5’-GCGTAATACGACTCACTATAGTACCTATAACCAGACCGTTC
Primer 36: 5’-GCCAGTCCACGGCTTAGGAGG
Primer 37: 5'-GCGTAATACGACTCACTATAG
Primer 38: 5’-GGTATTCACTCCAGAGCGATG

Primer 39: 5’- CACCGTCTTTCATTGC
Primer 40: 5’-GTATTACCGCGGCTGCTG
Primer 41: 5’-GAGCTAGCGGATGAAGTGATG
Primer 42: 5’-TTAAGCGTGCTAAGATATGG
Primer 43: 5’-GTACCTATAACCAGACCGTTC
Primer 44: 5’-ATCAGGCGGGCAAGAATGTG
Primer 45: 5’-GTACCTATAACCAGACCGTTC
Primer 46: 5’-TTAAGCGTGCTAAGATATGG
Primer 47: 5'-CTCCTACGGGAGGCAGCAG
Primer 48: 5’- GTATTACCGCGGCTGCTG

Figure S1

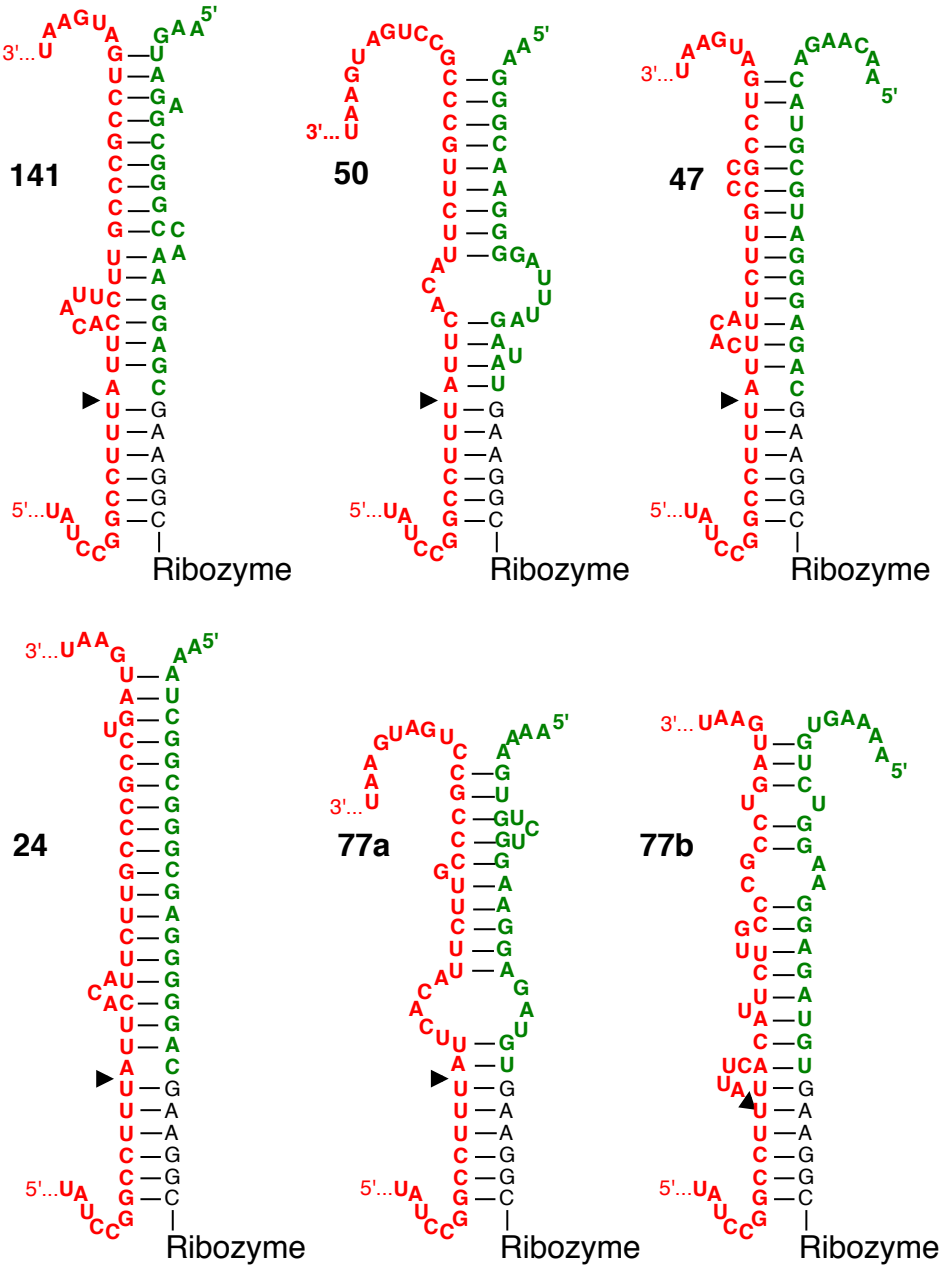

FIGURE S1. Predicted secondary structures between EGSs identified in an earlier selection, and the mRNA target site. The EGSs were selected from a single pool that contained 20 randomized nucleotides as the EGS, after two 5'-terminal As. Note that three of the four most efficient isolates (clone 141, clone 50, clone 24) show a C:A pair as the first pair of the P1 extension. The EGS of clone 77 was predicted to fold into two distinct secondary structures.

Figure S2

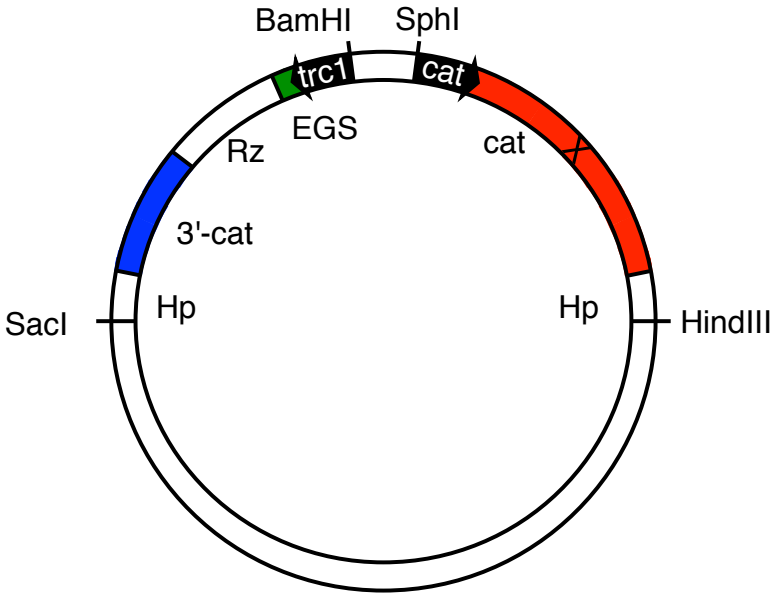

FIGURE S2. Library plasmid for the in vivo selection of ribozymes that can repair the mRNA of the chloramphenicol acetyl transferase gene (cat). The plasmid contains the cat gene (red) under the control of the cat promoter (black; cat), inactivated with a frame shift (X), and terminated by a hairpin terminator (Hp; white). The ribozyme gene (white) is under control of a modified trc promoter (black; trc1), and is linked to a 5'-terminal, randomized EGS (green). The 3'-exon (blue) of the ribozyme is also terminated by a hairpin terminator (Hp; white). Restriction sites for cloning the cat cassette and the ribozyme cassette are indicated.