Supplementary Material

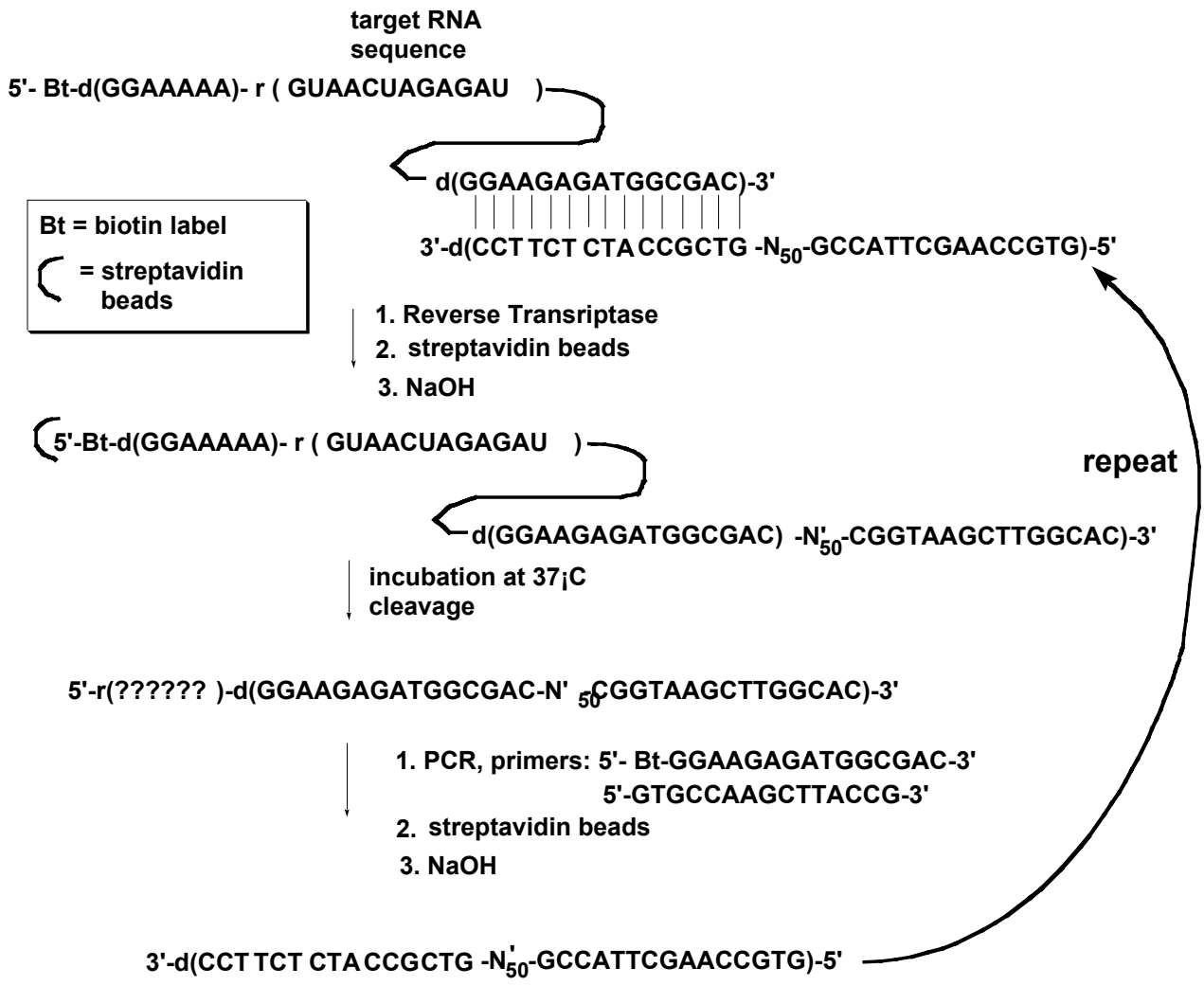

Figure S1. Selection protocol showing primer and template sequences used.

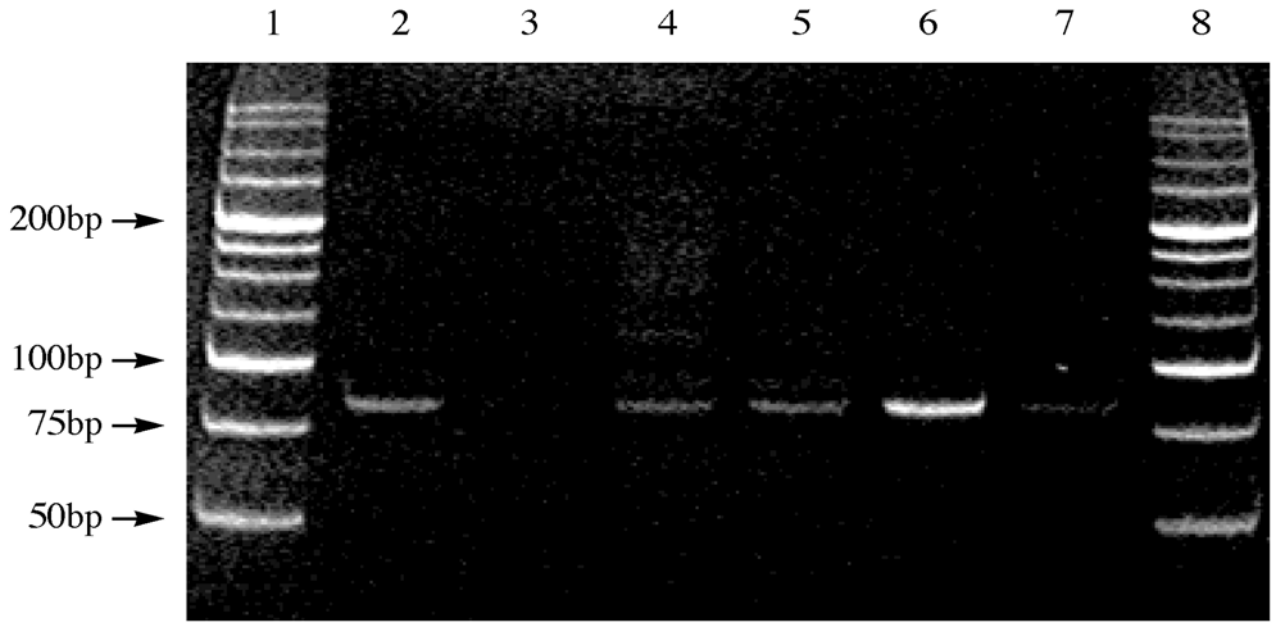

Figure S2. Denaturing 14% PAGE gel image of the ethidium bromide stained and UV visualized cleavage products amplified by PCR at round 10 of selection using different dilutions of the DNA template. *Lanes 1 and 8* – molecular mass markers; bands corresponding to different dsDNA fragments are marked by arrows on the left; *Lane 2* – PCR at round 1 of selection (positive control); *Lane 3* – PCR minus DNA template (negative control); *Lanes 4-7* – PCR following successive dilution of the DNA template - 100 times, 1000 times, 10^4 times and 10^5 times respectively. In round 10 of the selection, the 10^4 dilution (lane 6) was used for preparative PCR; in the other rounds working dilutions were from 10^4 to 10^6.

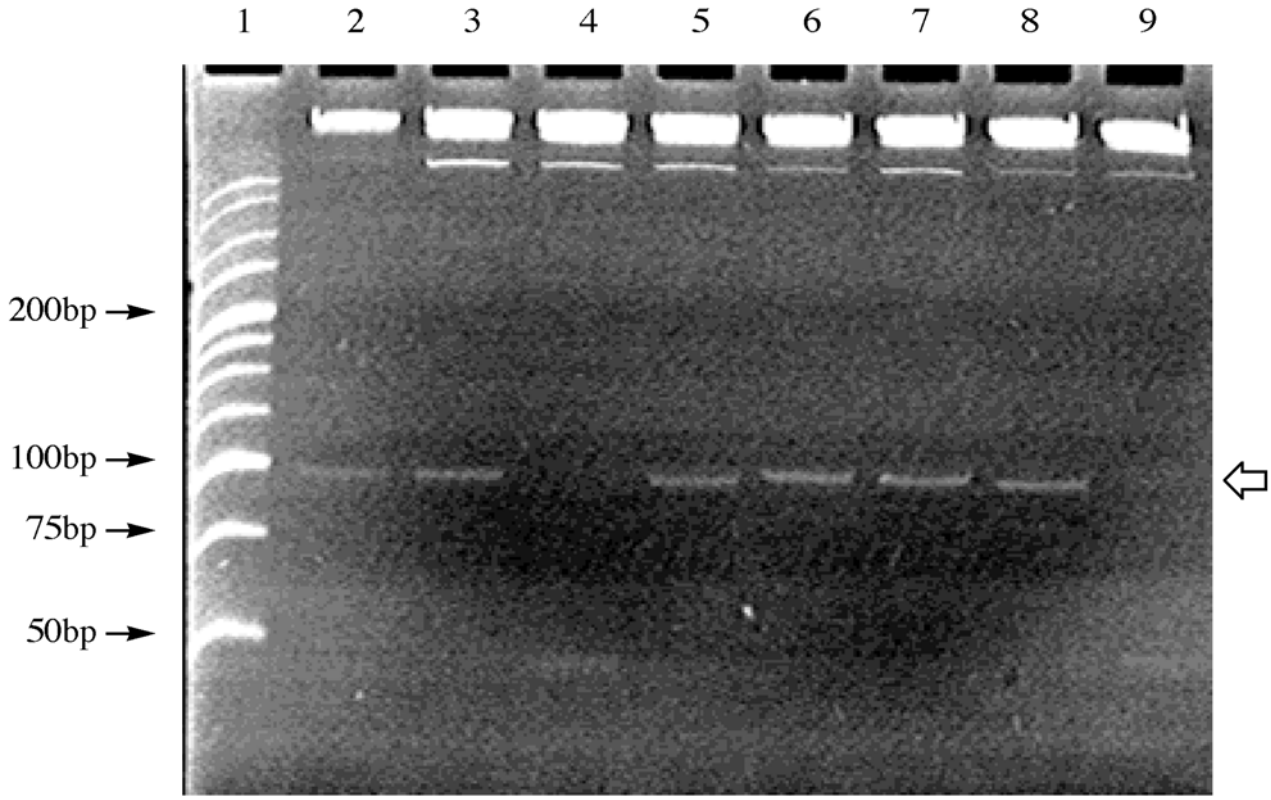

Figure S3. Denaturing 14% PAGE gel image of the ethidium bromide stained and UV visualized *Eco*RI digested plasmids purified from different clones picked up after cloning procedure. Lane 1– molecular mass markers; bands correspond to different dsDNA fragments are marked by arrows on the left; bp – base pairs. Lanes 2-9 – different plasmids digested by EcoRI. Arrow on the right indicates the insertions of interest.

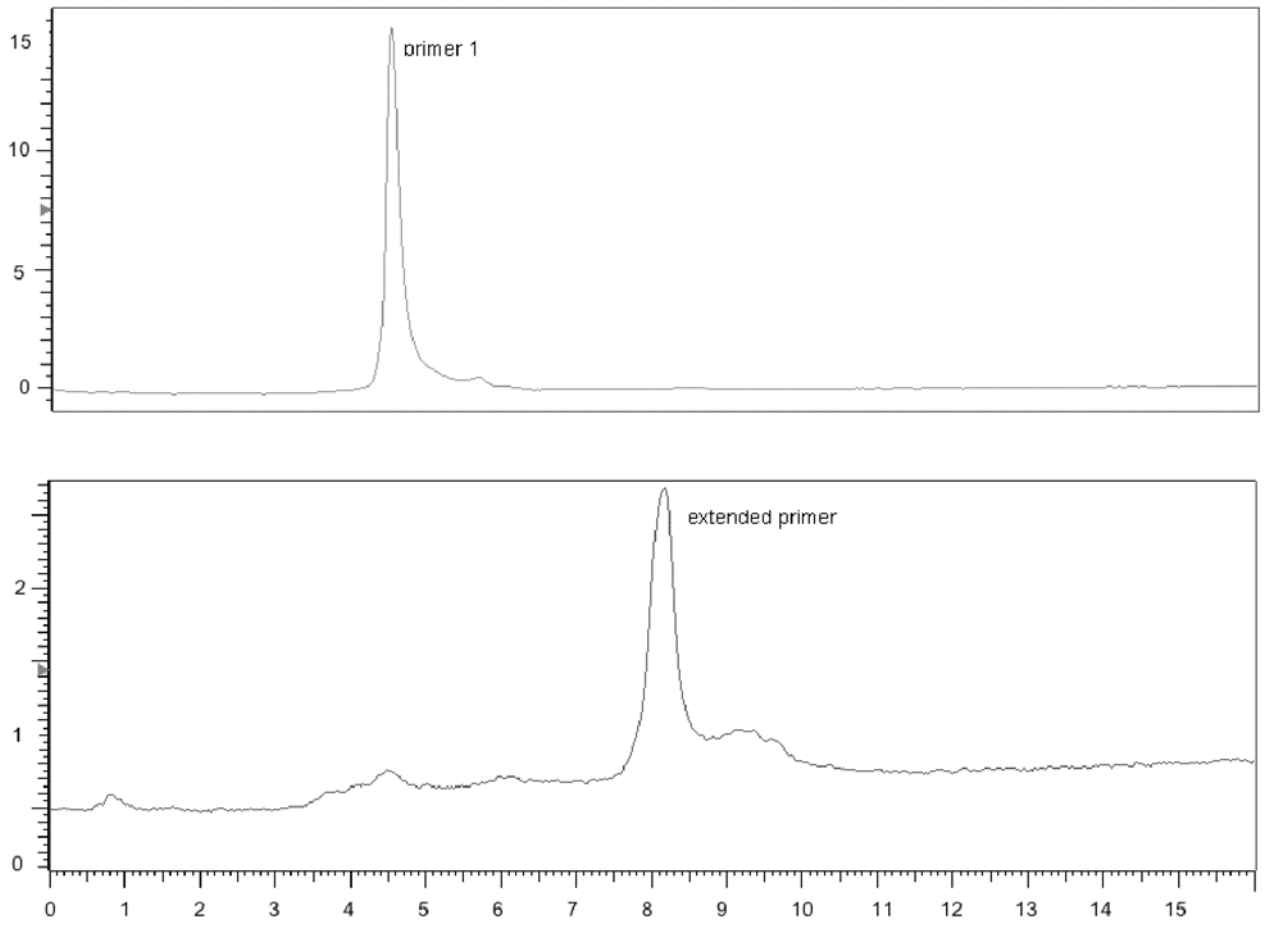
primer 1
extended primer
15
10
5
0
2
1
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Figure S4. WAVE system DHPLC traces of fluorescein-labelled primer 1 Fl-d(GGAAAAA)-r(GUAACUAGAGAU)-d(GGAAGAGATGGCGAC) (upper panel) and the reaction mixture after template-directed synthesis of random pool template d(GTGCCAAGCTTACCG-N_{50}-GTCGCCATCTCTTCC) using dCTP, dGTP, **1** and **2** and superscript II reverse transcriptase (lower panel). For full details see experimental section.

Family 1

```
                10        20        30        40
                *         *         *         *
5       CCTTTCATCTCTCGCTTTTGGCCTTTCTGCCTTTTTATTTTTCTTTGGC
14      CCTTTCATCCCTCGCTTTTCG-CTTTTTGTCTTTTTATTTTTCTTTGGC
24      CCTTTCATCTCTCGCTTTTCGCCTTTTTGTCTTTTTATTTTTCTTTGGC
42      CCTTTCATCTCTCGC-TTTCGCCTTTTTGTCTTTTTATTTTTCTTTGGC
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Family 2

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               10        20        30        40
               *         *         *         *
22      CCATTCTCCTCTGCTGGACTATTCGGGTCTTTGTTCTCTCATGGTTGTGT
32      CCATTCTCCTCTGCTGGACTATTCGGGTCTTTGTTCTCTCATGGTTGTGT
35      CCATTCTCCTCTGCTGGACTATTCGGGTCTTTGTTCTCTCATGGTTGTGT
36      CCATTCTCCTCTGCTGTACTATTCGGGTCTTTGTTCTCTCATGGTTGTGT
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Figure S5. The results of multiple alignment of sequences. Two families were defined from the pool of 49 sequences. The clones are numbered on the left. T-rich family 1 has about 95% structural homology whereas in case of family 2 only one substitution was found in clone 36 in comparison with 3 others. In the DNAzyme structures all As and Ts in the above sequences are modified.

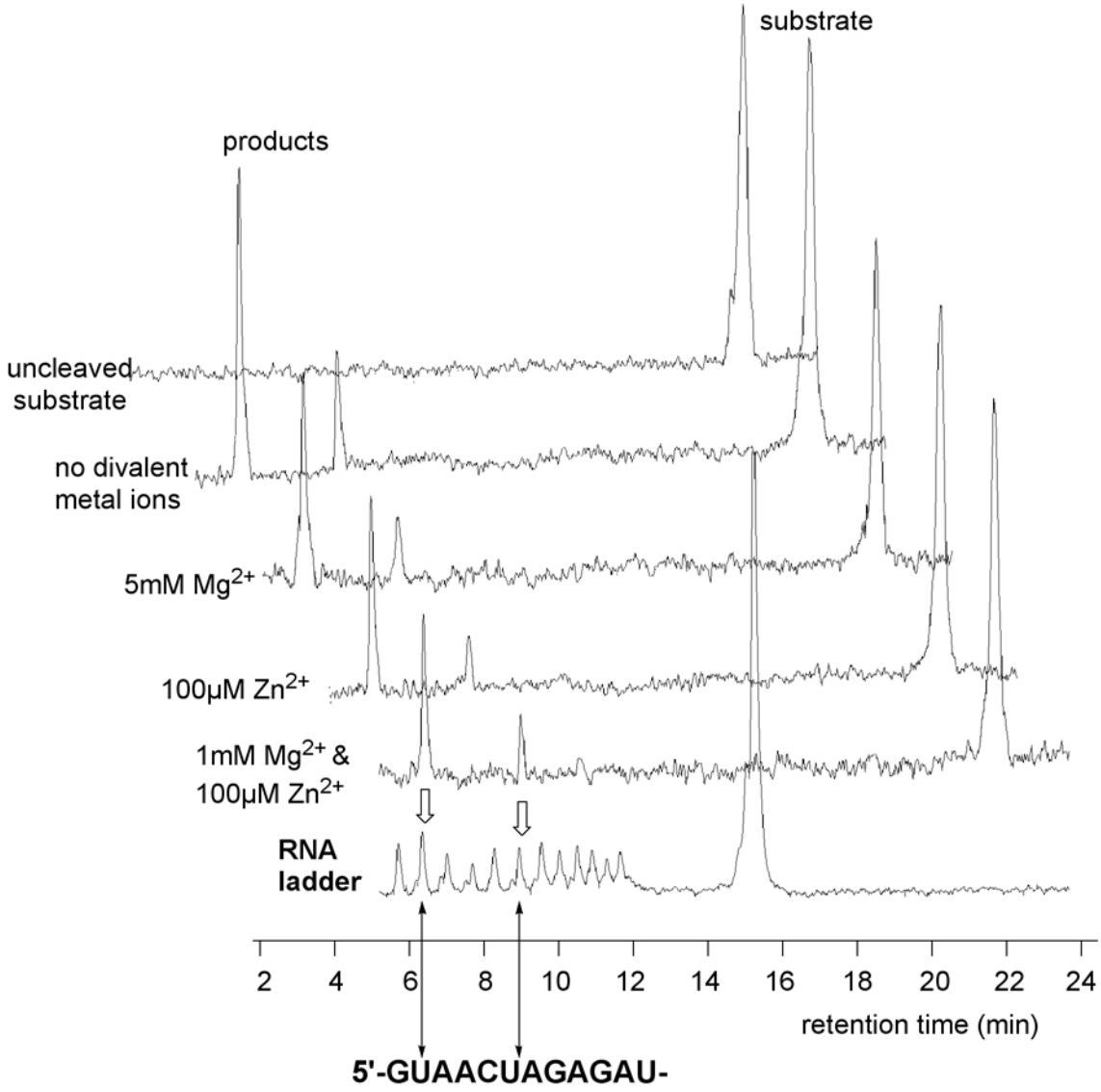

Figure S6. WAVE system DHPLC traces of self-cleavage of 5'-fluorescein-labelled clone-32 sequence in the presence of divalent metal ions. Self-cleavage reactions of clone-32 sequence after 20 min. at 37°C in 50mM Tris-HCl, pH7.5, 200 mM sodium chloride. The uppermost trace was obtained in the presence of 10 mM EDTA and the absence of NaCl (no cleavage), whilst the trace in the absence of divalent metal ions contained 10 mM EDTA and 200 mM NaCl. The lower traces contained no EDTA but divalent metal ions at the concentrations indicated. Arrows indicate positions of cleavage in comparison with an RNA ladder (sequence inset).

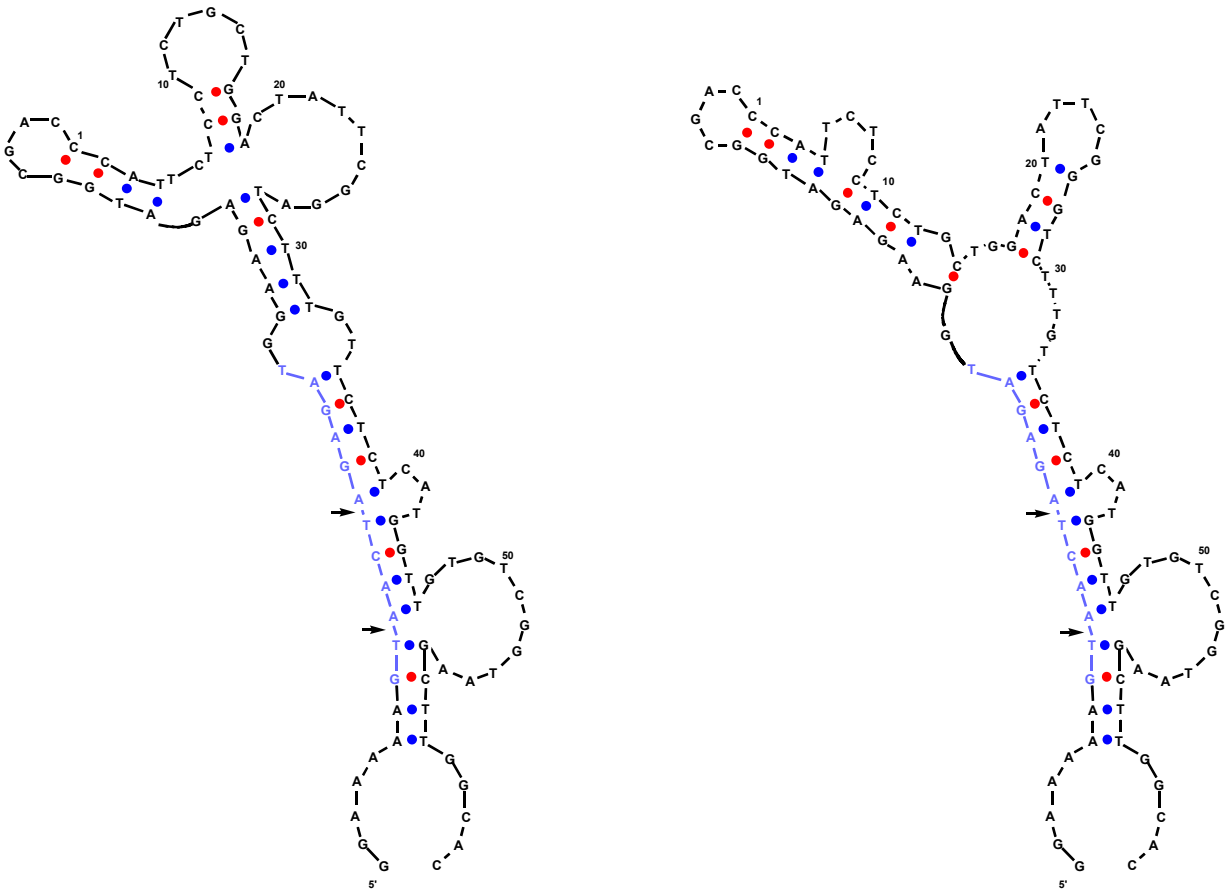

Figure S7. Secondary structure prediction (M-fold (48)) of the clone-32 sequence which was found to be the most active catalyst during self-cleavage. Sites of cleavage are shown by arrows, the RNA region is shown in grey. Numbering refers to the 50 nucleotide region of the DNAzyme that was selected from the initial random pool of sequences. (In the self-cleaving DNAzyme, Ts in the RNA target sequence are replaced by Us, and all Ts and As in the enzyme (from nucleotide are substituted by nucleotides **1** and **2** respectively). A 6-8 nucleotide triplet AAG near the 5'-end was forced to interact with a 3'-TTC-5' triplet at the 3'-end to form double helix prior to the folding calculation. See also Figure 7.