Supporting Information:

A Method for Selecting Modified DNAzymes Without PCR Amplification of Modified DNA

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1. Materials

All oligonucleotides were purchased from IDT (San Diego, CA, USA). $dU^{guan}TP$, $dU^{tyr}TP$ and $dA^{Im}TP$ (**1**, **2** and **3** from Figure 1B respectively) were synthesized as described previously.[1,2,3] $dC^{aa}TP$ (**4** from Figure 1B) was purchased from Trilink Biotechnologies (San Diego, CA, USA). Ultrapure dNTPs were obtained from Fermentas. Sequenase Version 2.0 T7 DNA polymerase was acquired from Affymetrix. T4 polynucleotide kinase, T4 DNA ligase, λ exonuclease, $Vent_R$® (exo-) DNA polymerase and yeast inorganic pyrophosphatase were purchased from New England Biolabs. PCR products were visualized on agarose gels (agarose D, Bio Basic) using in-gel ethidium bromide staining. Sephadex G-25 fine from GE Healthcare was used to freshly prepare G-25 columns. α-^{32}P-dGTP (10 mCi/ml) and γ-^{32}P-ATP (10 mCi/ml) were obtained from Perkin Elmer. Streptavidin magnetic particles (10mg/ml) were purchased from Pierce. Sheared Salmon Sperm DNA (10 mg/mL) was obtained from Eppendorf. Polyacrylamide gels were made using a 40 % 19:1 acrylamide/bisacrylamide solution (Accugel, National Diagnostics). The low molecular weight DNA ladder was purchased from New England Biolabs.

2. Methods

2.1 5′-end ^{32}P-labeling of oligonucleotides

400 pmol of the unlabelled oligonucleotide was added to a mixture containing 4 μl of a 10 mCi/ml solution of γ-^{32}P-ATP, 45 units of T4 polynucleotide kinase and T4 polynucleotide kinase buffer (10x). The mixture was incubated at 37 °C for two hours after which the kinase was inactivated at 65 °C for 20 min and the solution was desalted over a freshly prepared G-25 column. A 5′-end ^{32}P-labeled solution of the oligonucleotide (6.5 μM) in H_2O was obtained.

2.2 Ligation reactions

The template DNA (12.5 μM) was annealed to the selection primer (10 μM) in T4 DNA ligase buffer by heating to 100 °C and slowly cooling down to room temperature. T4 DNA ligase (2,000 units) was added and the mixture was incubated at 37 °C for one hour after which it was cooled to 15 °C and left to react overnight.

2.3 Elongation with Sequenase Version 2.0

15 pmol of the 5′-labelled construct was self-hybridized in the presence of Sequenase reaction buffer by heating up the mixture to 100 °C and slowly cooling down to room temperature. To this mixture, DTT was added to a final concentration of 5 mM. Natural

or modified nucleotides were added to a final concentration of 10 μM for the natural nucleotides, 20 μM for $dU^{guan}TP$, $dC^{aa}TP$ and dGTP, and 50 μM for $dA^{Im}TP$ and $dU^{tyr}TP$. α-^{32}P-dGTP was added to a final concentration of 0.375 mCi/ml for the internal labeling of the elongation product unless stated otherwise. Yeast inorganic pyrophosphatase (YIPP, 0.1 unit) was added to drive the reaction equilibrium towards the incorporation and 15 units of Sequenase Version 2.0 were added to initialize the reaction. The resulting 40 μl reaction solution was topped with mineral oil and kept at 32 °C for four hours, after which it was cooled to 4 °C.

2.4 Self-cleavage experiments

Elongation reactions were incubated for thirty minutes with 40 μl streptavidin magnetic particles prewashed three times with 100 μl TEN buffer (50 mM Tris HCl, 1 mM EDTA, 100 mM NaCl, pH 7) containing salmon sperm DNA (100 μM final). The first wash was left to incubate for 5 minutes, followed by two 30-second washes. The unbound fraction was removed by washing the particles with TEN buffer (3 x 100 μl). The non-ligated templates were removed and the constructs were denatured by washing the beads with NaOH 0.1 M containing 1 mM EDTA (4 x 100 μl). The samples were renatured by incubating with TEN buffer in the presence of 20 μM displacement primer for five minutes. After a final wash with 60 μl H_2O, cleavage buffer (100 μl; 50 mM sodium cacodylate pH 7.4, 200 mM NaCl, 1 mM EDTA) was added and the reaction was incubated at room temperature for one hour. After 30 seconds, 1 minute, 3 minutes, 10 minutes, 30 minutes, and 1 hour, 5 μl of the slurry was removed and mixed with 5 μl gel loading buffer (98% formamide, 10 mM Na_2EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol) containing 1 mM biotin. Cleavage control samples were prepared by removing 4 μl of the slurry from the reaction in the final wash, adding 1 μl of a 2 M NaOH solution and incubating the sample at 65 °C for twenty minutes before mixing with an equal volume of gel loading buffer.

2.6 Denaturing PAGE

Denaturing PAGE gels were run in the presence of 7 M urea, containing 100 mM Tris-borate pH 8.3 and 2.5 mM EDTA. The visualization of the cleavage reactions was accomplished using an Amersham Typhoon 9200 phosphorimager. The quantification of the radiographic data was carried out by selecting pixel volumes corresponding to the cleavage bands using the Imagequant software program. Data analysis was carried out using Graphpad Prism Ver. 6.0c.

2.7 Agarose gel analysis

5 pmol of the ligation product of the Dz10-66 template to the selection primer and of the elongation product of the ligated Dz10-66 construct with $dU^{guan}TP$, $dC^{aa}TP$, $dA^{Im}TP$ and dGTP (containing a fraction of α-^{32}P-dGTP) were heated at 95°C for 5 minutes and snap cooled on ice for 10 minutes. The products were analysed on a 1.5% agarose D gel containing 2 μg/mL ethidium bromide ran at 100 V for 25 minutes.

2.9 Oligonucleotide sequences

Oligonucleotide name	Oligonucleotide sequence
Dz10-66 template	5′ - TGT CTA CAC GCA AGC TTA CAG CGT GC A TAC ACG CAC GCA CAC TCA TAG CGC GCC TCA CTT GCG CTG CTA G TG TTG GTA GGG CCC AAC AGA CGG GCA CGC ACT ACG TAC CCA CAA CCT CGG CCG TAC CAC GGT ACG TAG TGC - 3′ (141)
Nx template	5′ - TGT CTA CAC GCA AGCTTA CAG CGT GC(N)nTGT TGG TAG GGC CCA ACA GAC GGG CAC GCA CTA CGT ACC CAC AAC CTC GGC CGT ACC ACG GTA CGT AGT GC - 3′ (117 or 137)
Selection Primer	5′ - GTGCCrCGTCTGTTGGGCCCTbioACCAACA - 3′ (27)
PCR primer forward	5′ - Bio - GC ACT ACG TAC CGT GGT ACG GCC GAG GTT G - 3′ (30)
PCR primer back	5′ - TGT CTA CAC GCA AGC TTA CA GCG TGC -3′ (26)
displacement primer	5'- /5Phos/GC ACT ACG TAC CGT GGT ACG GCC GAG GTT GTG GGT ACG TAG TGC GTG CCC GTC TGT TGG GCC CTA CCA ACA -3' (71)

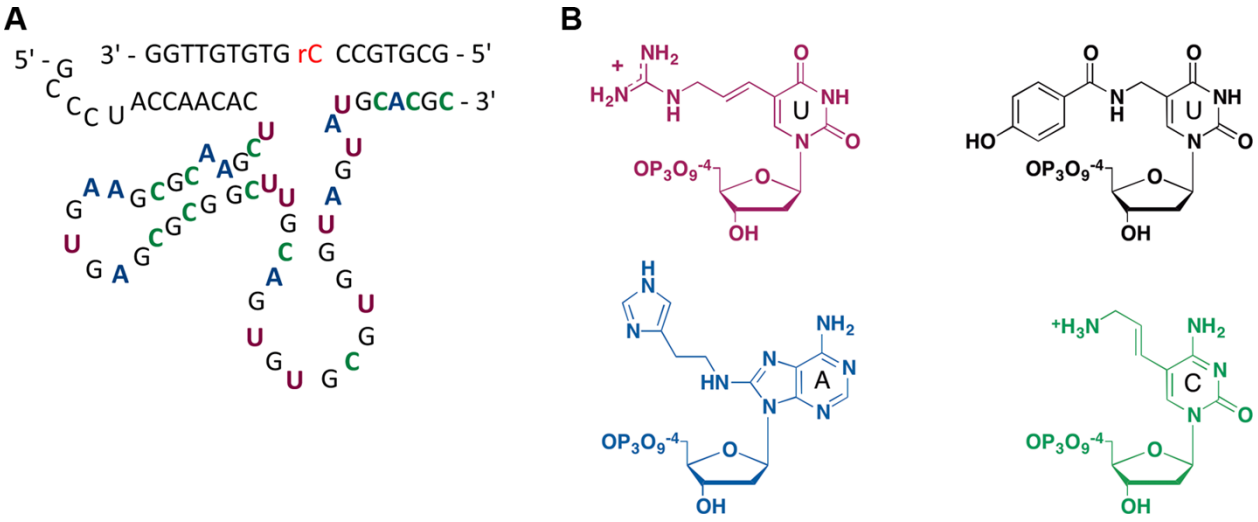


Figure 1 A) the Mfold predicted 2D structure of Dz10-66 prepared from B) dUTP^{guan} (magenta), dATP^{his} (blue), dCTP^{am} (green) and dGTP, with the modified bases in bold and the cleavage site highlighted in red.

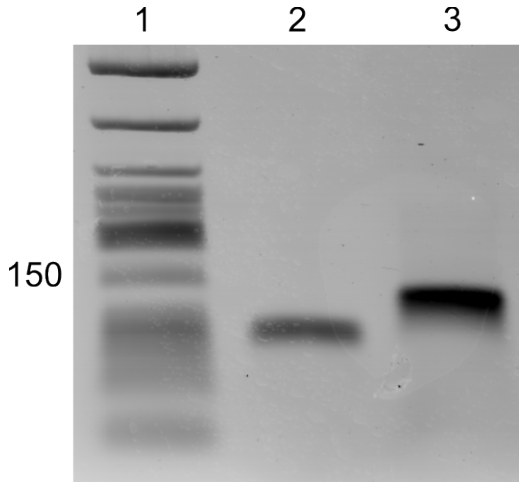
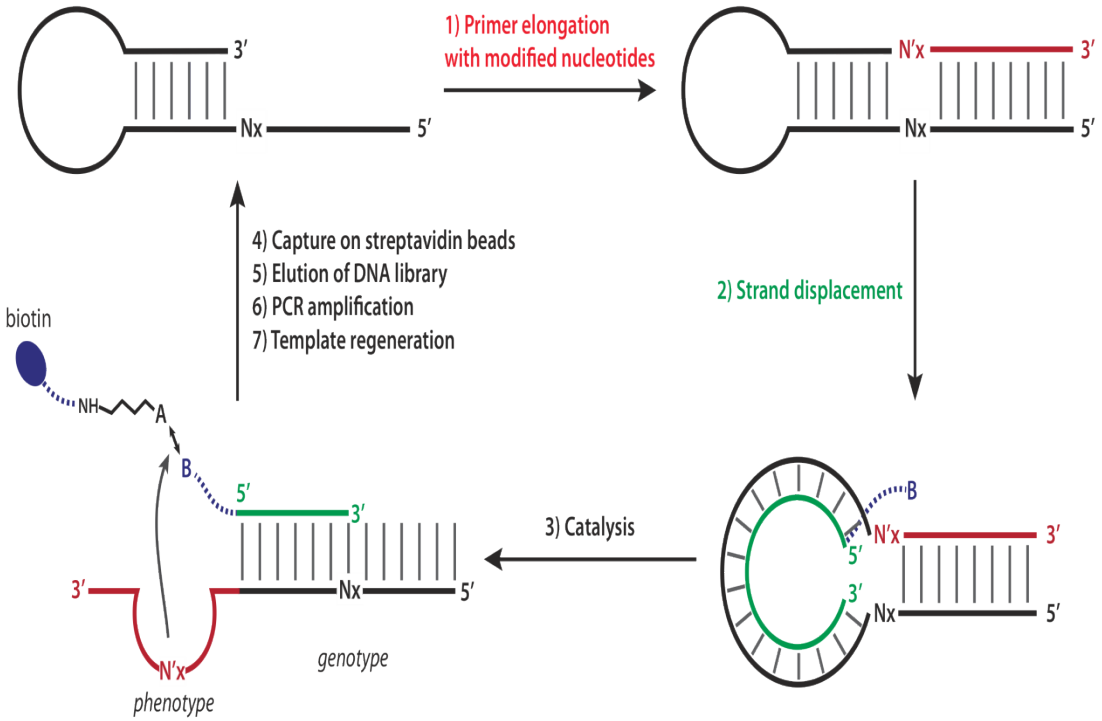


Figure 2SI The analysis of the ligation product of the Dz10-66 template with the selection primer elongated with $dU^{guan}TP$, $dC^{aa}TP$, $dA^{Im}TP$ and dGTP (containing a fraction of α-^{32}P-dGTP) on a 1.5 % agarose gel with the low MW ladder in lane 1, the ligated material in lane 2 and the elongated ligation product in lane 3.



Scheme 1SI The improved *in vitro* selection procedure for modified DNAzymes that can catalyze bond-breaking or –forming reactions. Nx represents the stretch of randomized nucleotides. The modified DNA library is indicated in red and the displacement primer is shown in green.

References

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