Establishing Broad Generality of DNA Catalysts for Site-Specific Hydrolysis of Single-Stranded DNA

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Sequences of additional deoxyribozymes

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                1         10        20        30        40
                |         |         |         |         |
17PA1    TAGTGCTTGT  AT-TCAGAAC  GCAATTCTTC  TGAGCCGCCT  A
17PA19   TAAGGTGCCT  CC-ATTTTCC  GTTTAGGAAC  TGCCCGGTGT  G
11PC1    CAGCGTCGCC  AA-TTCGTAC  CTTCGATATT  GAATCTCTCT  G
11PC5    CAGTGTCGCC  CA-CGCGTAA  TTAGCTTGTG  GAATATTTCC  G
13PD1    TATACCGGGC  AACTATTGCC  TCGTCATCGC  TATTTTCTGC  G
13PD2    TAACCCGGGT  GC-TAGCCTC  GTCATGGCCA  TAGTTTTTGC  C
13PD34   TAACCCGGAT  CA-TATCTCG  TCATGGTCAT  CTATTTTTGC  C
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Figure S1. Sequences of the initially random (N_{40}) catalytic regions of the additional new deoxyribozymes described in this Supplementary Material (Figures S2, S4, and S5). See Figure 3 for details. Note that 13PD1 has 41 nt in its catalytic region, presumably due to an insertion by Taq polymerase during a selection round.

Establishing optimal number of unpaired DNA substrate nucleotides

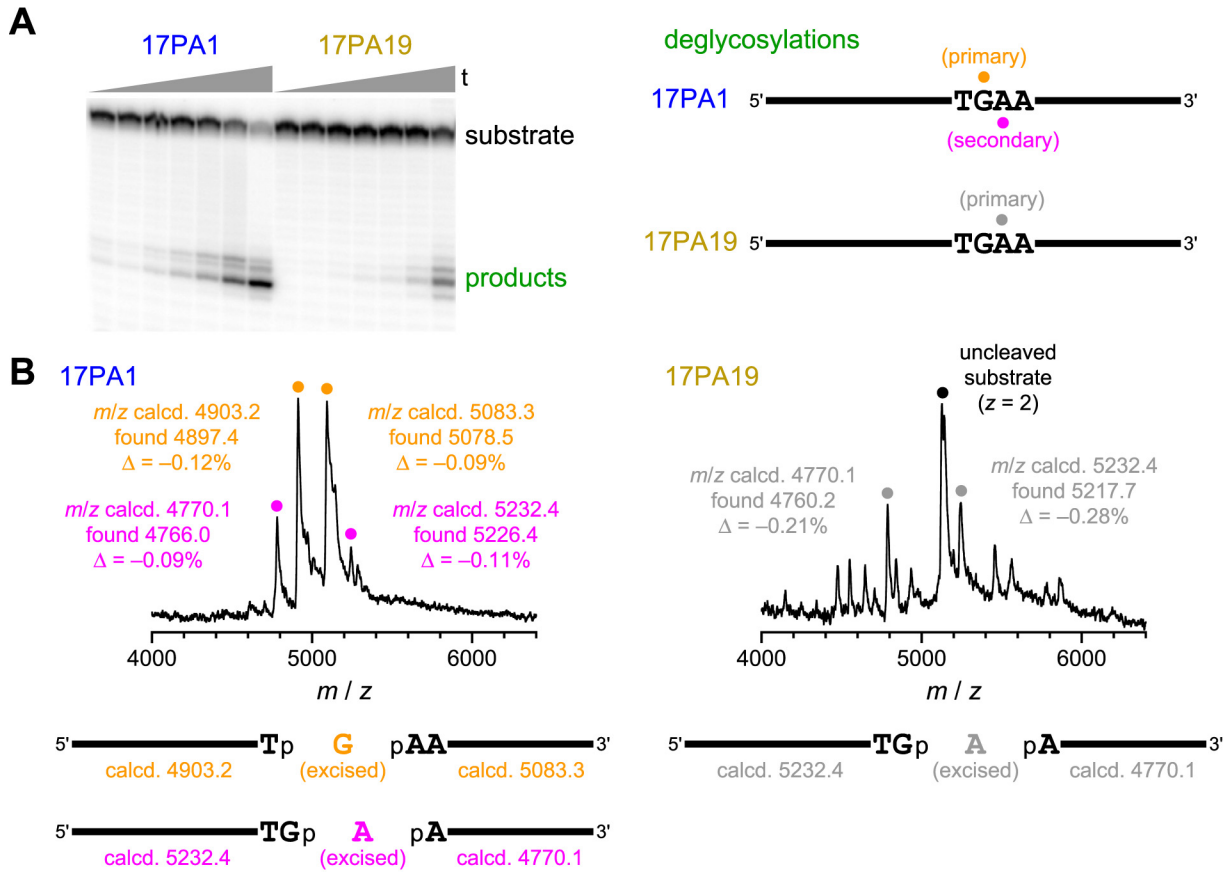

Figure S2. Deoxyribozymes identified by selection with 0 unpaired nucleotides in the DNA substrate. (A) PAGE images for single-turnover in trans assays with 17PA1 and 17PA19 using the parent DNA substrate sequence (round 17 in cis uncloned pool yield 22%). See Figure S1 for deoxyribozyme sequences. Assigned deglycosylation sites are marked on the accompanying substrate sequences (TG = same two nucleotides as for 0 unpaired nucleotides substrate in Figure 2). (B) MALDI mass spectrometry data for the reaction products for each deoxyribozyme. The indicated peaks correspond to deglycosylation followed by two β-elimination reactions, which leads to net excision of a single nucleoside and formation of 3′-phosphate + 5′-phosphate termini on the oligonucleotide products (ref. 30).

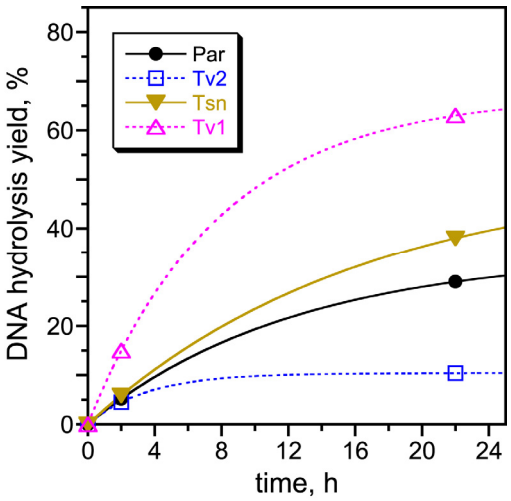

Figure S3. Single-turnover in trans assays of 13PB2 (see Figure 3 for sequence) with substrates that have G^G at the cleavage site and systematic sequence changes at all other nucleotides as in Figure 2. In contrast, only trace cleavage activity was observed with T^G and A^G substrates, as well as the C^C substrate (data not shown). The round 13 in cis uncloned pool yield was 37%.

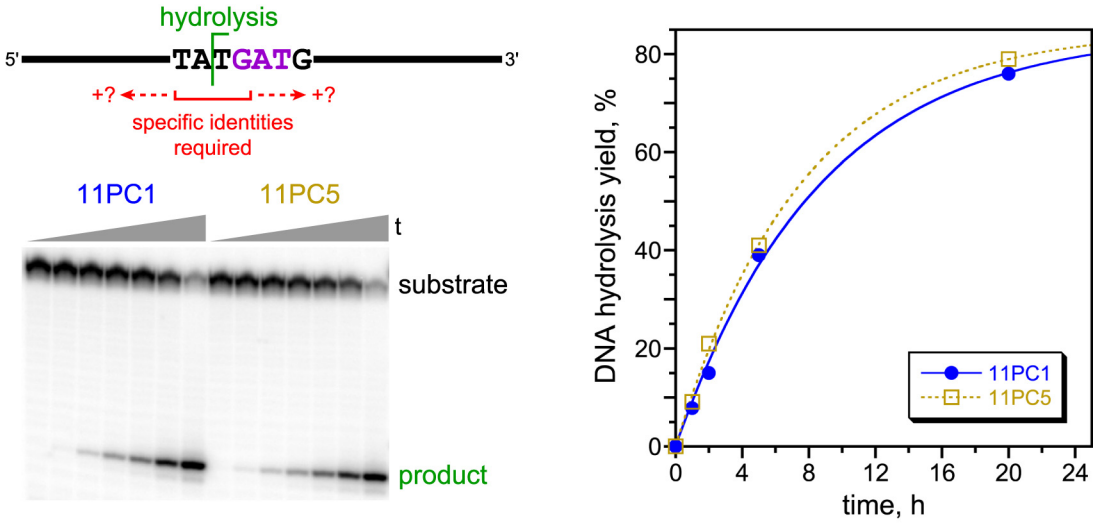

Figure S4. Deoxyribozymes identified by selection with 3 unpaired nucleotides in the DNA substrate. Shown are single-turnover in trans assays of 11PC1 and 11PC5 (see Figure S1 for sequences; round 11 in cis uncloned pool yield 45%). Specific nucleotide identities were found to be required beyond the four indicated nucleotides to an undetermined extent (data not shown). k_{obs} values (h^{-1}): 11PC1 0.11, 11PC5 0.13. MALDI mass spectrometry confirmed that 11PC1 and 11PC5 each hydrolyze their DNA substrate at the indicated site, leaving 3′-hydroxyl + 5′-phosphate termini (data not shown).

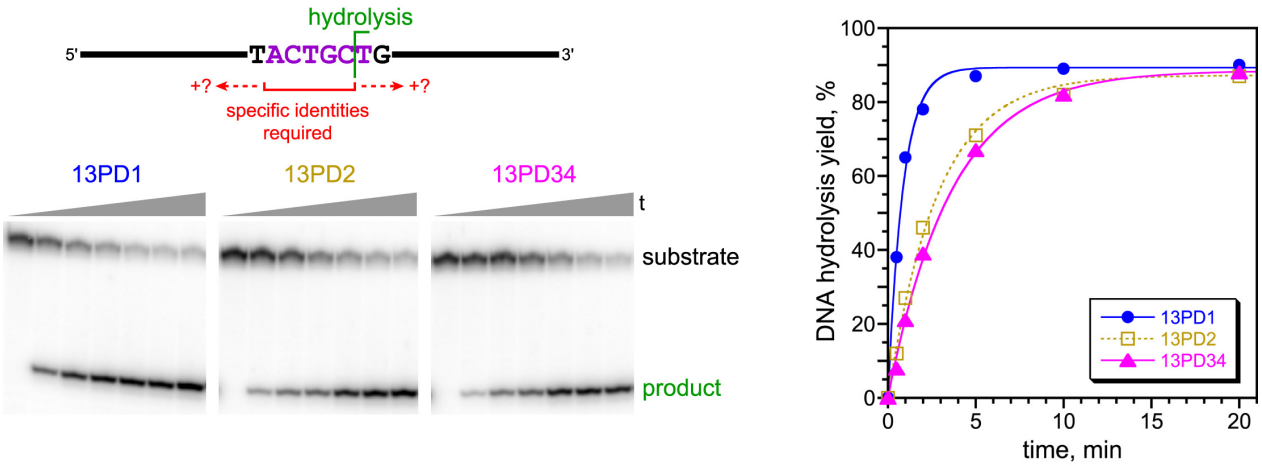

Figure S5. Deoxyribozymes identified by selection with 6 unpaired nucleotides in the DNA substrate. Shown are single-turnover in trans assays of 13PD1, 13PD2, and 13PD34 (see Figure S1 for sequences; round 13 in cis uncloned pool yield 18%; rounds 1–8 with 14 h incubation time; rounds 9–13 with 1 min incubation time). Specific nucleotide identities were found to be required beyond the five indicated nucleotides to an undetermined extent (data not shown). k_{obs} values (min^{-1}): 13PD1 1.2, 13PD2 0.35, 13PD34 0.28. MALDI mass spectrometry confirmed that 13PD1, 13PD2, and 13PD34 each hydrolyze their DNA substrate at the indicated site, leaving 3′-hydroxyl + 5′-phosphate termini (data not shown). One additional deoxyribozyme identified during the initial PD selection experiment cleaved the substrate within the unpaired nucleotides at AC^TGCT, leaving 3′-phosphate + 5′-hydroxyl termini (data not shown).

Observation of deglycosylation-induced DNA cleavage (to accompany Figure 4)

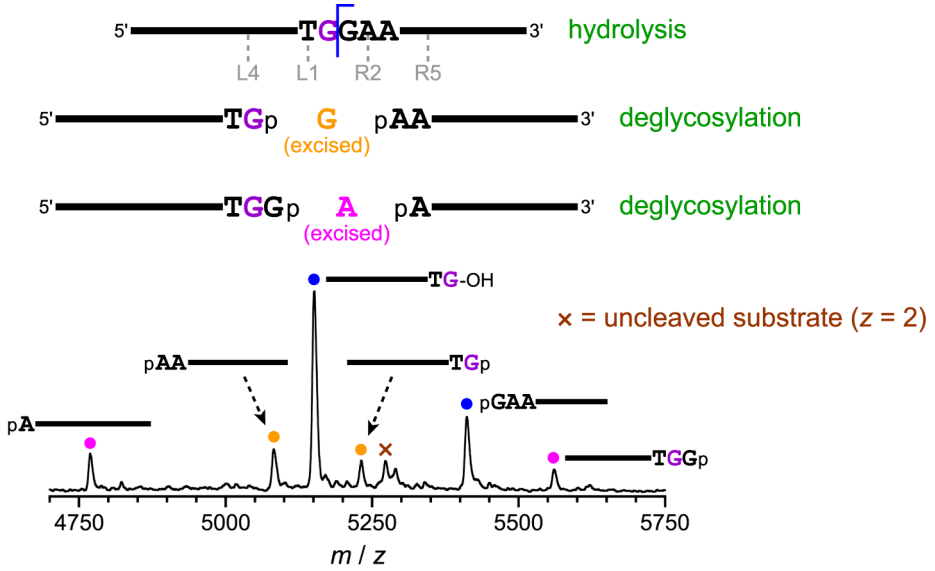

Figure S6. MALDI mass spectrometry data for the Figure 4 assay of the uncloned round 7 pool of the selection with a single unpaired G nucleotide in the substrate. Each assigned peak has observed mass within 0.05% of the calculated value (data not shown).

PAGE images of DNA-catalyzed DNA hydrolysis (to accompany Figure 6)

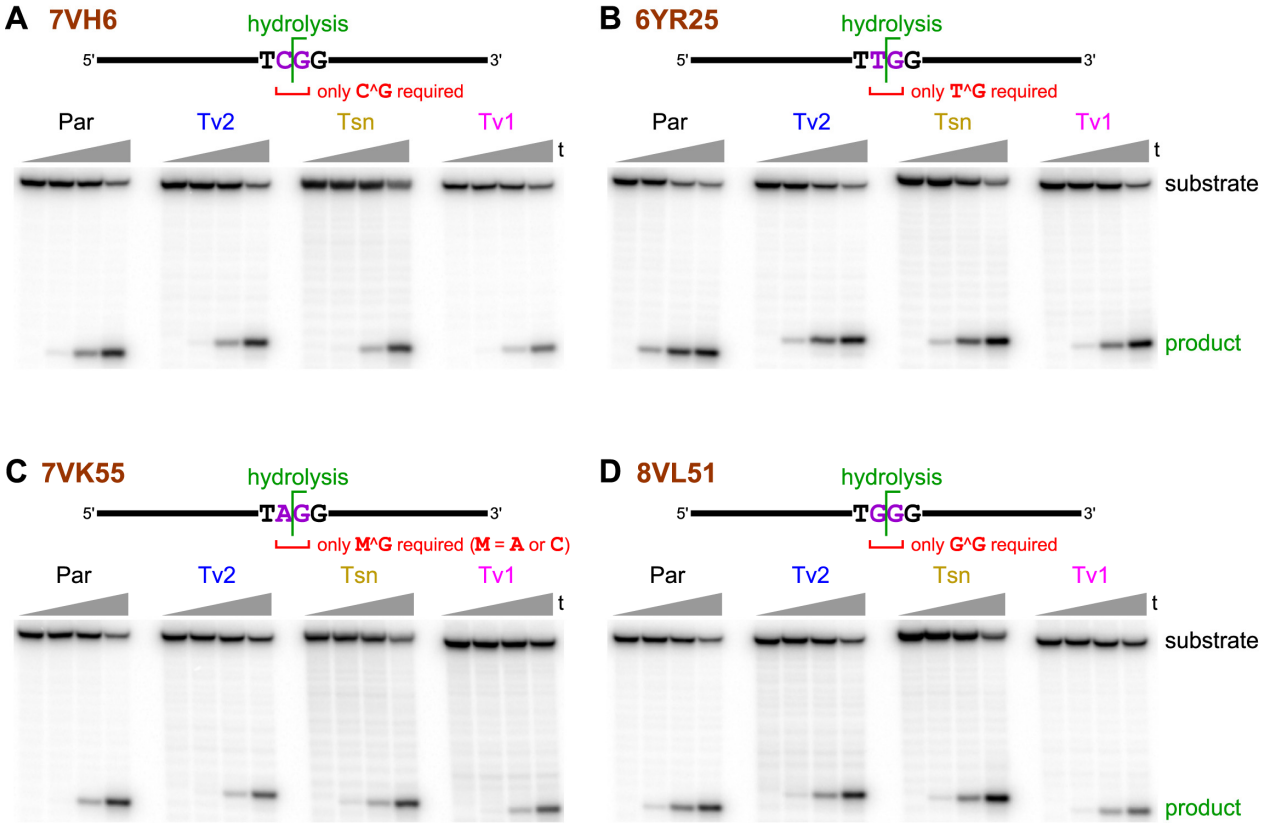

Figure S7. PAGE images to accompany Figure 6. Shown are representative time points at t = 30 sec, 15 min, 2 h, and 22 h.

Assays of deoxyribozymes for tolerance of changes at the cleavage-site nucleotides

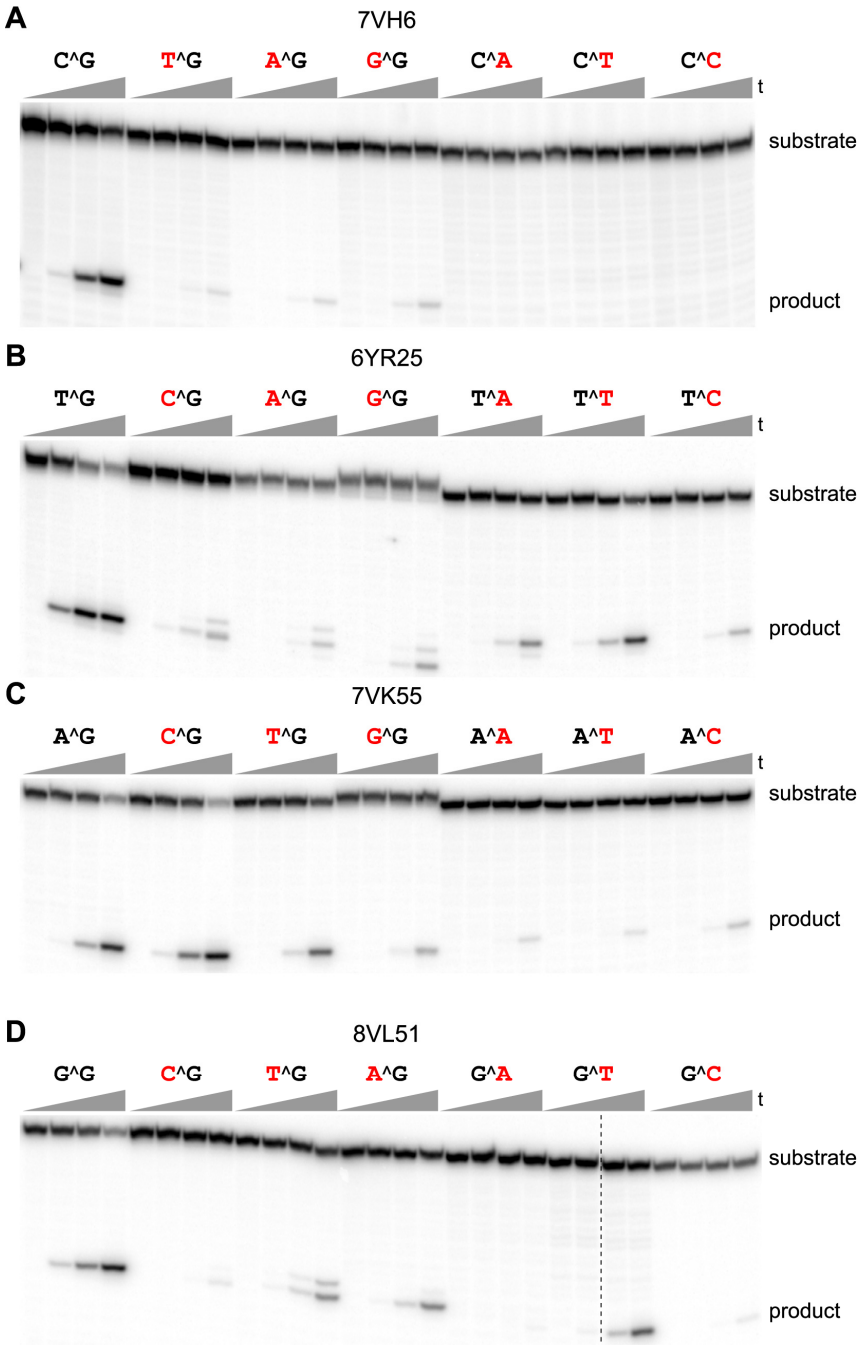

Figure S8. Assays of deoxyribozymes for generality at the two nucleotides immediately at the cleavage site, N^N. (A) 7VH6. (B) 6YR25 (data for 6YR11 was similar). (C) 7VK55. (D) 8VL51. For each set of lanes, t = 30 s, 15 min, 2 h, and 22 h. Above each set of lanes are the N^N nucleotides at the cleavage site in the assay (black = nucleotide present in substrate as used during selection; red = nucleotide changed from selection substrate). Note that activity was observed in some cases even when one of the two nucleotides was changed, although this activity was generally modest in rate or yield (e.g., T^T for 6YR25) or accompanied by obvious relaxation of the cleavage site specificity (e.g., C^G for 6YR25 and T^G for 8VL51). Substantial activity for 7VK55 was maintained with C^G rather than A^G, as shown more completely in Figure S9. Analogous assays for 13PB2—for which changing the G of C^G was accompanied by Watson-Crick covariation of the corresponding binding arm nucleotide—revealed that C^G is required for full activity (Figure 2), but substantial activity is also observed with G^G substrates (Figure S3).

Assays of the 7VK55 deoxyribozyme with the C^G substrate

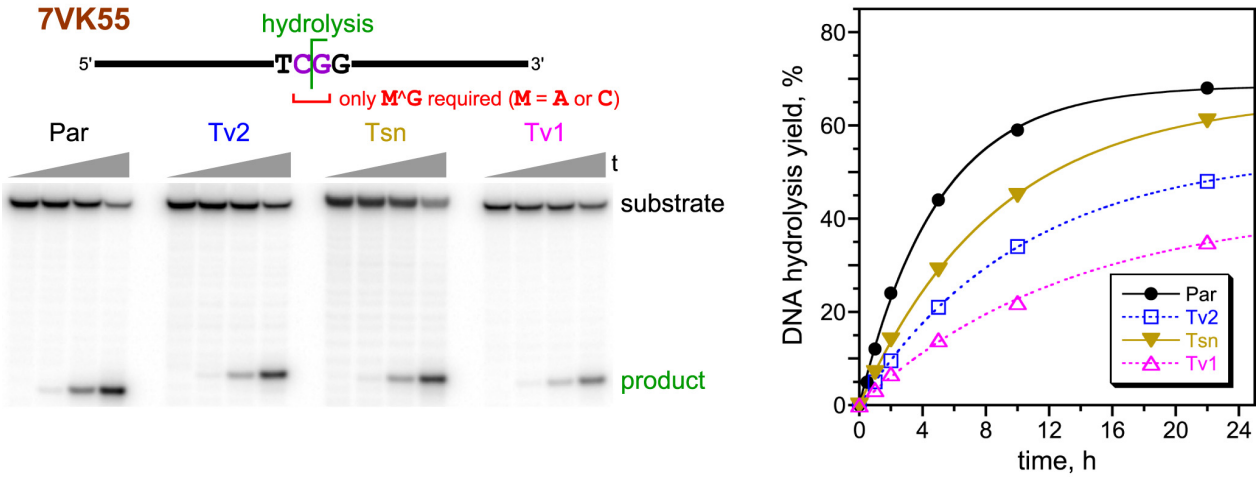

Figure S9. Assays of the 7VK55 deoxyribozyme with the C^G substrate, analogous to the assays with A^G substrate as shown in Figure 6 and Figure S7. On the PAGE image are shown representative time points at t = 30 sec, 15 min, 2 h, and 22 h. k_{obs} values (h^{-1}): Par 0.20, Tv2 0.098, Tsn 0.12, Tv1 0.076.

Mass spectrometry assays to validate DNA hydrolysis

deoxyribozyme	substrates [a]	mass L calcd.	mass L found	L error, % (found – calcd.)	mass R calcd.	mass R found	R error, % (found – calcd.)
13PB2	Par C^G [b]	5112.4	5112.7	+0.006	5412.5	5411.8	–0.01
	Tv2 C^G [b]	5223.5	5222.8	–0.01	5176.3	5175.3	–0.02
7VH6	Par C^G	5112.4	5111.8	–0.01	5741.7	5739.8	–0.03
	Tv2 C^G	5223.5	5222.4	–0.02	5465.5	5464.4	–0.02
6YR25	Par T^G	5127.4	5126.2	–0.02	5741.7	5740.2	–0.03
	Tv2 T^G	5238.5	5237.4	–0.02	5465.5	5464.4	–0.02
7VK55	Par A^G	5136.4	5136.5	+0.002	5741.7	5738.9	–0.05
	Tv2 A^G	5247.5	5246.7	–0.02	5465.5	5464.9	–0.01
	Par C^G	5112.4	5112.1	–0.006	5741.7	5741.4	–0.005
	Tv2 C^G	5223.5	5222.9	–0.01	5465.5	5463.7	–0.03
8VL51	Par G^G	5152.4	5152.1	–0.006	5741.7	5739.4	–0.04
	Tv2 G^G	5263.5	5262.5	–0.02	5465.5	5464.3	–0.02

Table S1. Mass spectrometry assays to validate DNA-catalyzed DNA hydrolysis by site-specific and sequence-general deoxyribozymes. Each listed deoxyribozyme was used to cleave the substrate with the indicated unpaired nucleotides, where the binding arms (all nucleotides except those unpaired and, in the case of 13PB2 only, the first G to the 3′-side of the cleavage site) were either the parent nucleotide sequences (Par) or systematically changed by transversions (Tv2; A↔T, G↔C).

[a] Substrate sequences were either parent (Par) or transversions-2 (Tv2) as indicated in the caption. In addition, the two unpaired nucleotides immediately at the cleavage site (X^G) are listed.

[b] The G directly to the 3′-side of the cleavage site was base-paired with the corresponding deoxyribozyme nucleotide.