

Directing the Outcome of Deoxyribozyme Selections to Favor Native 3'–5' RNA Ligation

Yangming Wang and Scott K. Silverman*

*Department of Chemistry, University of Illinois at Urbana-Champaign, 600 S. Mathews Ave., Urbana, Illinois 61801*Sequences and preliminary ligation activities of deoxyribozymes

Numerous deoxyribozymes from the 6BX, 6CA, and 9BX pools were cloned and sequenced. The choice of which particular clones to sequence was based on preliminary surveys of ligation activity, using deoxyribozymes prepared by PCR amplification of miniprep DNA (see refs. 9 and 15 for details). In general, clones with >50% ligation yield at 60 min in the standard incubation conditions of 50 mM HEPES, pH 7.5, 150 mM NaCl, 2 mM KCl, and 20 mM MnCl₂ at 37 °C were sequenced, although some clones with lower yields were sequenced as well. The sequences and preliminary assessments of ligation activities are collected in Tables X1, X2, and X3 for the 6BX, 6CA, and 9BX deoxyribozymes, respectively. Note that most of these deoxyribozymes have not been synthesized independently by solid-phase synthesis, so their quantitative yield-versus-time characteristics can only be estimated from the tabulated data. However, the assigned linkage sites and types are firm; we have never observed a linkage determined from such assays to be incorrect when redetermined for the product from a deoxyribozyme subsequently prepared by solid-phase synthesis. The L substrate sequence for the BX selection was 5'-GGAAGUCUCAUGUACUAACA-3', and the L substrate sequence for the CA selection was 5'-GGAAGUCUCAUGUACUAUCG-3'. For both L substrates, the underlined nucleotides are the ones at which ligation was observed (in both cases, the first underlined U nucleotide is denoted U16 in Tables X1–X3, and so on for the remainder of the underlined nucleotides).

clone ^a	enzyme region, 5' to 3' ^b	linkage ^c	fraction ligation ^d			
			5 min	10 min	30 min	60 min
6BX6	CGAGGCGTAGGTTAATTACAAACGGCTCAGACAACGTATG	U16	0.06	–	0.32	0.48
6BX35	GGCGAAGGGACTACGTTGGCCATGCGGGTGGGCCGCTATG	U16	–	0.06	0.19	0.29
6BX39	GAACGTGAGGTGCGGGAATTAATCACAACCGGAAACGGA	A17	–	0.25	0.57	0.71
6BX43	CACCACAATGGTTGATGCCGGCCAAGTGGCGCAGCATA	A17	–	0.25	0.61	0.75
6BX45	CGCACGGAGCCCCTTAGGGGTCCACGGAGTGGGTGAACCT	A17	–	0.45	0.77	0.83
6BX47	CCGGGCATCAAGTGCAGAACGATGACGAAATCGGGTGGGT	A17	–	0.53	0.82	0.88
6BX20 (2)	CGCACGGTCTGGTGTGGAAGCCAGGTTGCCTCCCTGCAAA	A18	0.24	–	0.77	0.88
6BX22 (4)	CAGGGGGAGCGAGCACTAATACAAGCGGGTAGGAGGCCCT	A18	0.34	–	0.85	0.91
6BX23	CGGGAGGAGGCAAAGGCTAGTTTGTTCGATAGGAGGCCCT	A18	0.16	–	0.63	0.72
6BX34	CGTGGGAGCCATTGGGAGGGGCTGAGAACATAAGTCACGA	A18	–	0.21	0.57	0.74
6BX8	GCAGTGCAAATGAGGCATGGAGAACTACTCTATGCTGAA	C19	0.10	–	0.51	0.71
6BX16	GCAGTGCAAATGAGTAAGGACTGATATCAGTCACTACGAA	C19	0.13	–	0.59	0.74
6BX40	GCAGTGCTAATGAGGGTGTGGCAGAAGCTATACAGCCGAA	C19	–	0.49	0.76	0.84
6BX42	GCAGTGCTAATGAGGTATCGCAAATAAGTGCAGCCGAA	C19	–	0.26	0.59	0.74
6BX19	GACGGTGGGAAATCAGCCTGTATTGGTTTCAGAGCGGA	2'–5'	0.18	–	0.64	0.78
6BX21	GACGACAGCGGTTCCAGCTCAGTAGTGATAGTTTACTGC	2'–5'	0.03	–	0.17	0.26
6BX26	GACACCGAGCAGAGGACCGGACCTAGTTGGTAAAAGGTAA	2'–5'	0.10	–	0.47	0.83

Table X1. Sequences and preliminary ligation activities of the 6BX deoxyribozymes.

^a In parentheses is the number of clones with the identical sequence (or at most one different nucleotide).^b Only the enzyme region is shown. In all cases, to the 5'-side is the sequence CGAAGCGCTAGAACAT, and to the 3'-side is the sequence AGTACATGAGACTT.^c The linkage site and type are shown. For U16–C19, the entry means a 2',5'-branch at this nucleotide. For 2'–5' and 3'–5', the entry means linear RNA with the indicated connectivity.^d Fraction ligation at the indicated timepoint during the preliminary survey of ligation activities.

clone	enzyme region, 5' to 3' ^a	linkage ^b	fraction ligation ^c		
			10 min	30 min	60 min
6CA17	CACAGGCGCGAGGGGCTATGTCCGGTGGTGACAGCGGA	U16	0.14	0.26	0.30
6CA28	CGGAAGGATGACAAGGAGCGTAGCTGATGGGGACTCAGAC	A17	0.17	0.37	0.49
6CA2	GGGGCGTAGTGGAGACCGGGGACAGTGGAGTACGTCCAGC	U18	0.30	0.56	0.62
6CA20	GTTAACGCAGGGCGTCTACTACATCCTTGTGAGCTACG	C19	0.14	0.36	0.43
6CA1	TGAAGCGCTTATCCATGCAAAAAATGGATCCGGTACCAAC	2'-5'	0.24	0.53	0.60
6CA5	TGCAGCGGCTGCGCGGGTATCCGGTCTCCAGGGGACGCTT	2'-5'	0.11	0.35	0.49
6CA7	AGACCAGTGTCTCAGCCGCCCGCCGAGCGGGGAGGTA	2'-5'	0.04	0.14	0.21
6CA14	CGAAGACGAGGAGTAACCTTAGATGAAGGTGGGGACGATT	2'-5'	0.11	0.30	0.52
6CA24	GGGGCAGGACCGGGTACTAGGGGAACACGCCGACGCGGG	2'-5'	0.27	0.55	0.64
6CA25	AGACCGAGTGTGAGGGTACCACAGACATGTGGAGTGGCTG	2'-5'	0.03	0.06	0.09
6CA4	CACGTGGACTTGGCATGGTCCCAGCGCTAGTTTTAAGCGC	3'-5'	0.24	0.54	0.70
6CA6	CCCGTACACGACTGTGGTGTGAACGGCTGCTAGCCGGCA	3'-5'	0.31	0.59	0.70
6CA10	CACGTACACTTGGTATGGTGTGACACCTCAGCTCATACT	3'-5'	0.22	0.52	0.66
6CA15	CCCGTGACATTCATATGGGGTCCGGTCCAATCGCGAATTA	3'-5'	0.20	0.47	0.60
6CA16	CCCGTACACTCGACGTGGTGTGACGCTGTGGCGTGAGCCTGT	3'-5'	0.21	0.51	0.64
6CA18	CAGAGATGGGGTGTGATCTGGGTTGATAACACGGAGCGGTC	3'-5'	0.12	0.39	0.55
6CA21	CCCGTACACTTAGAATGGTGCAGCCAGCATGCTGTACAGTA	3'-5'	0.31	0.57	0.66
6CA23	CCCGTGCACTCTATGATGGTGTCCCTGATACCAACAGGG	3'-5'	0.08	0.20	0.31
6CA27	CACGTGACCTCAGGAATGGGTTGAGGTGGGTGCACCTGC	3'-5'	0.09	0.24	0.33
6CA30	CCCGTACGCTGCCATGGCGCCATTTGGCACGTTAGTGTG	3'-5'	0.19	0.44	0.55

Table X2. Sequences and preliminary ligation activities of the 6CA deoxyribozymes.

^a Only the enzyme region is shown. In all cases, to the 5'-side is the sequence CGAAGCGCTAGAACAT, and to the 3'-side is the sequence AGTACATGAGACTT. The sequences of 6CA1, 6CA18, and 6CA21 are one nucleotide longer than 40 nt, presumably due to an insertion during a PCR step of selection.

^b The linkage site and type are shown. For U16–C19, the entry means a 2',5'-branch at this nucleotide. For 2'-5' and 3'-5', the entry means linear RNA with the indicated connectivity.

^c Fraction ligation at the indicated timepoint during the preliminary survey of ligation activities.

clone ^a	enzyme region, 5' to 3' ^b	linkage ^c	fraction ligation ^d		
			10 min	30 min	60 min
9BX11	CATGCGTGTGGCGTGGATACGAGGCTATTCAGAGGGTAAA	C19	0.15	0.29	0.37
9BX1 (9)	GGAACGCAGTCGCACGTGACTAGGGAATCGTATCCTCATG	3'-5'	0.55	0.76	0.78
9BX3 (2)	CGTGGTTCCGGAATGCCTAGCGTTCAAGTGTGGATGCTT	3'-5'	0.18	0.42	0.59
9BX4 (2)	CCGCGAGGCCGTGGCCCGAGTTGGTCAAAGCACACGAGT	3'-5'	0.11	0.29	0.44
9BX10	GATGTGGTCCAGCTGATAGGGCAGCGTTAGGCATACGTTG	3'-5'	0.04	0.05	0.06
9BX15	GCGACGGATTTAGGTGTGTTAGACAGGGTCCGGGAATAGAT	3'-5'	0.15	0.30	0.45
9BX16	GCAGCGGGGAAGGCACTTCCAGGCAGGGGGAAAAACAA	3'-5'	0.26	0.44	0.54

Table X3. Sequences and preliminary ligation activities of the 9BX deoxyribozymes.

^a In parentheses is the number of clones with the identical sequence (or at most one different nucleotide).

^b Only the enzyme region is shown. In all cases, to the 5'-side is the sequence CGAAGCGCTAGAACAT, and to the 3'-side is the sequence AGTACATGAGACTT. The sequence of 9BX16 is one nucleotide shorter than 40 nt, presumably due to a deletion during a PCR step of selection.

^c The linkage site and type are shown. For C19, the entry means a 2',5'-branch at this nucleotide. For 3'-5', the entry means linear RNA with this connectivity.

^d Fraction ligation at the indicated timepoint during the preliminary survey of ligation activities.

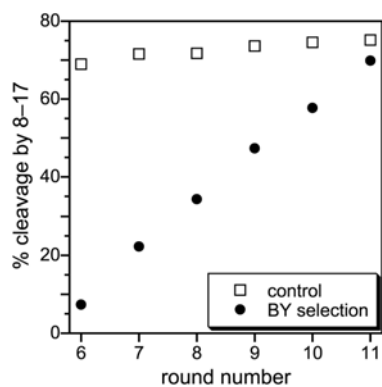
Redirecting the BY selection using the 8–17 deoxyribozyme

Figure X1. Plot for the BY selection analogous to the plot for the BX selection shown in Figure 6B.