

TABLE S1. Effects of different P2 oligos on polymerization efficiency.^a

P2 oligo	DNA nt/primer	RNA nt/primer	DNA % full length	RNA % full length
5'-GGCACCA	7.13 ± 0.13	5.73 ± 0.08	3.0 ± 0.3	4.1 ± 0.5
5'-GGCGCCA	7.89 ± 0.18	7.37 ± 0.14	23 ± 2	11.8 ± 0.4
5'-GGCACC	7.17 ± 0.05	8.62 ± 0.18	2.7 ± 0.2	40.4 ± 1.0
5'-GGCGCC	8.49 ± 0.04	8.1 ± 0.2	32.6 ± 1.3	40 ± 3
5'-GCGCC	8.07 ± 0.11	6.41 ± 0.06	20.4 ± 1.1	5.5 ± 0.2
5'-GGCGC	9.01 ± 0.08	4.6 ± 0.2	36.8 ± 1.1	19.1 ± 1.4
5'-CGCC	5.80 ± 0.04	5.98 ± 0.09	0.11 ± 0.07	0.20 ± 0.13
5'-GCGC	6.79 ± 0.07	8.19 ± 0.03	0.11 ± 0.14	6.70 ± 0.06
5'-GGCG	7.14 ± 0.07	7.31 ± 0.10	0.21 ± 0.19	37 ± 1
5'-GCC	5.81 ± 0.08	5.74 ± 0.04	< 0.1	< 0.1
5'-CGC	5.77 ± 0.06	5.77 ± 0.05	< 0.1	< 0.1
5'-GCG	5.84 ± 0.05	5.68 ± 0.04	< 0.1	< 0.1
5'-GGC	6.28 ± 0.01	7.6 ± 0.2	0.18 ± 0.08	36 ± 2
5'-CC	5.72 ± 0.04	5.70 ± 0.09	< 0.1	0.15 ± 0.09
5'-GC	5.74 ± 0.05	5.72 ± 0.03	< 0.1	< 0.1
5'-CG	5.73 ± 0.11	5.77 ± 0.04	< 0.1	< 0.1
5'-GG	5.70 ± 0.10	5.76 ± 0.04	< 0.1	< 0.1

^a All polymerization reactions used the template T21. Without P2 oligo, the primer was extended with an average of 5.78 ± 0.07 nt/primer and less than 0.1 % of the primer were extended to full-length. Note that the P2 oligo 5'-GC can basepair in two registers to the P2 stem site. All errors are standard deviations from three independent experiments.

TABLE S2. Effects of different P2 oligos on different polymerase ribozymes.^a

P2 oligo	Pol1e nt/primer	B6.61 nt/primer	Pol1e % full length	B6.61 % full length
No P2 oligo	5.78 ± 0.07	6.05 ± 0.04	< 0.1	< 0.1
5'-GGCACCA	5.73 ± 0.08	5.50 ± 0.09	4.1 ± 0.5	3.9 ± 0.3
5'-GGCACC	8.62 ± 0.18	7.3 ± 0.2	40.4 ± 1.0	16.5 ± 0.6
5'-GGCGCC	8.1 ± 0.2	6.7 ± 0.4	40 ± 3	21.2 ± 1.4
5'-GGCGCC (DNA)	8.49 ± 0.04	6.7 ± 0.2	32.6 ± 1.3	11.2 ± 0.9
5'-GGCGC	4.6 ± 0.2	5.34 ± 0.13	19.1 ± 1.4	19.7 ± 1.1
5'-GGCGC (DNA)	9.20 ± 0.04	8.44 ± 0.14	36.8 ± 1.1	9.3 ± 1.1
5'-GGCG	7.31 ± 0.10	7.07 ± 0.06	36.7 ± 1.0	6.9 ± 0.3
5'-GGC	7.6 ± 0.2	7.47 ± 0.09	36 ± 2	6.6 ± 0.4

^a All polymerization reactions used the template T21. Note that the P2 oligo 5'-GC can basepair in two registers to the P2 stem site. All errors are standard deviations from three independent experiments.

TABLE S3. Effects of the nucleotide composition in the 3'-extensions of P2 oligos on polymerization efficiency.^a

3'-extension	T21	T50a	T50b	T50c	mean
(A) ₁₅	7.4 ± 0.3	0.2 ± 0.04	1.05 ± 0.02	0.3 ± 0.04	0.80 ± 0.06
(T) ₁₅	1.0 ± 0.2	< 0.1	0.15 ± 0.02	< 0.1	0.10 ± 0.01
(C) ₁₅	4.6 ± 0.6	< 0.1	0.56 ± 0.07	< 0.1	0.26 ± 0.03
(G) ₁₅	7.1 ± 0.2	1.7 ± 0.09	0.96 ± 0.05	0.38 ± 0.05	1.44 ± 0.05
(A/T) ₁₅	5.5 ± 0.2	0.1 ± 0.03	0.79 ± 0.06	0.10 ± 0.03	0.44 ± 0.06
(A/C) ₁₅	4.7 ± 0.4	0.2 ± 0.12	0.84 ± 0.05	0.16 ± 0.03	0.55 ± 0.10
(A/G) ₁₅	7.7 ± 0.3	1.8 ± 0.16	1.14 ± 0.01	1.12 ± 0.08	2.04 ± 0.00
(T/C) ₁₅	2.1 ± 1.1	0.1 ± 0.09	0.21 ± 0.03	0.05 ± 0.00	0.17 ± 0.02
(T/G)₁₅	6.3 ± 7.5	1.8 ± 0.5	1.2 ± 0.3	1.44 ± 0.5	2.6 ± 0.5
(C/G) ₁₅	7.5 ± 0.18	1.6 ± 0.09	1.01 ± 0.02	0.28 ± 0.07	1.34 ± 0.10
(A/T/C) ₁₅	3.7 ± 0.8	< 0.1	0.55 ± 0.09	0.07 ± 0.02	0.25 ± 0.02
(A/T/G)₁₅	6.8 ± 0.2	1.9 ± 0.18	1.65 ± 0.10	2.07 ± 0.16	2.58 ± 0.08
(A/C/G) ₁₅	7.4 ± 0.2	1.9 ± 0.14	1.16 ± 0.05	0.51 ± 0.09	1.68 ± 0.11
(T/C/G)₁₅	5.7 ± 0.3	2.2 ± 0.2	1.75 ± 0.15	2.1 ± 0.4	2.6 ± 0.3
(A/T/C/G)₁₅	6.9 ± 0.3	2.4 ± 0.07	2.00 ± 0.03	2.8 ± 0.3	3.08 ± 0.07

^a The polymerization efficiency was measured as the average number of nucleotides added per primer (nt/primer). Note that the four highest polymerization efficiencies result from the only four 3'-extensions that contain both G and T (bold). The mean was calculated as the geometric mean to optimize for relative improvements and not absolute improvements. Errors are standard deviations from triplicate experiments.

TABLE S4. Effects of the nucleotide bias in (G/T)₁₅ 3'-extensions of P2 oligos on polymerization efficiency.^a

% G in extension	P2 oligo sequence	polymerization efficiency
100	5'-GGCGCCGGGGGGGGGGGGGGGG	1.37 ± 0.06
67	5'-GGCGCCGGTGGTGGTGGTGGT	2.50 ± 0.11
67	5'-GGCGCCTGGTGGTGGTGGTGG	2.05 ± 0.06
53	5'-GGCGCCGTGTGTGTGTGTGTG	2.27 ± 0.11
47	5'-GGCGCCTGTGTGTGTGTGTGT	1.81 ± 0.12
33	5'-GGCGCCGTTGTTGTTGTTGTT	1.01 ± 0.09
33	5'-GGCGCCTTGTTGTTGTTGTTG	0.98 ± 0.06
0	5'-GGCGCCTTTTTTTTTTTTTTTT	0.10 ± 0.02
87	5'-GGCGCCGGGTGGTGGGGGGGG	1.90 ± 0.05
87	5'-GGCGCCGGGGTGGGTGGGGGG	1.72 ± 0.06
80	5'-GGCGCCGGGGGGGTGGTTGGG	2.38 ± 0.04
80	5'-GGCGCCGTGGGGGTGGGGGTG	1.98 ± 0.02
80	5'-GGCGCCGGTGGGGGTGGGTGG	1.94 ± 0.01
73	5'-GGCGCCGGTGGTGGGGGGTTG	2.70 ± 0.06
73	5'-GGCGCCGGGGTTGGTGGTGGG	2.41 ± 0.06
67	5'-GGCGCCGTGTGGGTGGGTTGG	3.35 ± 0.04
67	5'-GGCGCCGTGTTGGGTGTGGGG	2.04 ± 0.05
60	5'-GGCGCCGGGTGTGGGGTTGTT	3.46 ± 0.03
60	5'-GGCGCCGTGTTGGGGTTGGTG	2.68 ± 0.06
60	5'-GGCGCCGTTGGGGGTGTTGT	2.52 ± 0.01
53	5'-GGCGCCGTGTTGTGGGTTGGT	3.35 ± 0.05
53	5'-GGCGCCGTGGGGTTGTTTGT	2.50 ± 0.13
47	5'-GGCGCCGTGGTTGTTGTGTT	3.01 ± 0.04
47	5'-GGCGCCGTGTTTGTGGGTT	2.03 ± 0.04
47	5'-GGCGCCTGTGGTTTGGTGTG	1.84 ± 0.01
40	5'-GGCGCCGTTTTGTGTTGGTGT	1.62 ± 0.05
40	5'-GGCGCCGTGTGTTTTTGTGGT	0.46 ± 0.02
33	5'-GGCGCCGTTTTGGTTGTTTGT	1.85 ± 0.05
33	5'-GGCGCCGTGTTGTTTGTGTT	0.80 ± 0.01
27	5'-GGCGCCGTGTTTGGTTTTTT	0.25 ± 0.04
27	5'-GGCGCCGTTTTTTTGTGGTTT	0.16 ± 0.01
20	5'-GGCGCCGTTTGTTTTTTGT	0.09 ± 0.01
20	5'-GGCGCCTGTTTGTTTTTTGT	0.08 ± 0.01
13	5'-GGCGCCTTTTTTGT	0.26 ± 0.00
13	5'-GGCGCCGTTTTTTTGT	0.07 ± 0.00
6.7	5'-GGCGCCTTTTTTGT	0.09 ± 0.01
6.7	5'-GGCGCCTTTGTTTTTTTTTT	0.04 ± 0.01
50	5'-GGCGCC(G/T) ₁₅	2.81 ± 0.06

^a The polymerization efficiency is given as the geometric mean for the polymerization with the four templates T21, T50a, T50b, and T50c. The extensions with repeated sequence motifs are shown at the top, the extensions without repeated sequence motifs are shown in the middle, and the extension with a randomized sequence is shown at the bottom. Polymerization efficiency was measured as the average number of nucleotides added per primer (nt/primer). The mean is the geometric mean between the four templates. Errors are standard deviations from triplicate experiments.