Base Pairing and Miscoding Properties of 1,N^6-Ethenoadenine and 3,N^4-Ethenocytosine Containing Oligoribonucleotides

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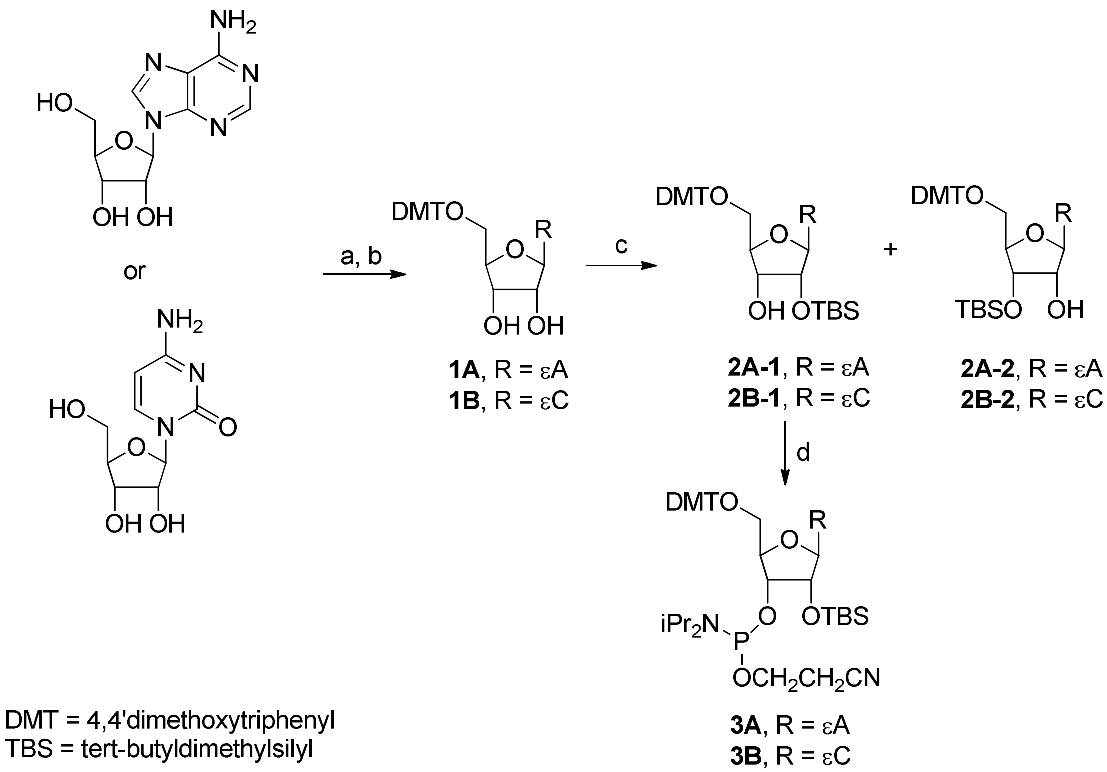
Synthesis of εA- and εC- phosphoramidite building blocks



Scheme S1. a) ClH_2C-CHO, H_2O, rt, 20-36h; b) 4,4'-dimethoxytriphenyl chloride, pyridine, rt, 2h, 61-66% over 2 steps; c) $AgNO_3$, pyridine, THF, TBSCl, rt, 6h, 40-54% of **2A-1** and **2B-1**; d) 2-cyanoethyl diisopropyl chlorophosphine, N-ethyl diisopropylamine, THF, rt, 20h, 81-89%.

5′-*O*-(4,4′-Dimethoxytriphenyl)-etheno-adenine (1A)

Adenosine (3gr, 11.22 mmol) was dissolved in water (90 ml). To the solution chloroacetaldehyde was added (35.4 ml of 50% solution in water, 20 eq.) and the pH was adjusted to 4.2 adding NaOH 1M. The pH was monitored for four hours until it became stable. After 20 more hours the solution was evaporated in high vacuum and the yellow gum obtained was precipitated with ethyl acetate /hexane, to get a yellowish precipitate. The solid was coevaporated with pyridine (2 x 30 ml) and dissolved in dry pyridine (140 ml). 4,4'-dimethoxytriphenyl chloride (2.10 gr, 6.15 mmol, 0.5 eq.) was added to the solution, and, after 1 hour a second batch of 4,4'-dimethoxytriphenyl chloride (2.10 gr, 6.15 mmol, 0.5 eq.) was added. The reaction was quenched after two more hours with ethanol (10 ml) and the solvents were evaporated. The residual oil was dissolved in ethyl acetate and extracted with sat. $NaHCO_3$ (2 times) and brine (2 times). The organic phase was dried over Na_2SO_4, filtered and evaporated.
The product was purified by flash chromatography (eluent: methylene chloride: methanol = 98:2 to 95:5 to 90:10 +0.1% triethylamine), obtaining 1A (4.04 gr, 6.8 mmol, 61%) as a pale yellow foam.
R_f: 0.59 (CH_2Cl_2: methanol= 9:1)
^{1}H-NMR (400 MHz, DMSO): δ 9.23 (s, 1H), 8.45 (s, 1H), 8.10 (d, J=1.5 Hz, 1H), 7.57 (d, J=1.5 Hz, 1H), 7.38-7.14 (m, 9H), 6.84-6.77 (m, 4H), 6.08 (d, J=4.5 Hz, 1H), 5.63 (d, J=5.6 Hz, 1H), 5.27 (d, J=5.8 Hz, 1H), 4.75-4.67 (m, 1H), 4.36-4.26 (m, 1H), 4.15-4.06 (m, 1H), 3.70 (s, 3H), 3.69 (s, 3H), 3.29-3.20 (m, 2H).
^{13}C NMR (101 MHz, DMSO): δ 158.0, 157.9, 144.8, 140.5, 140.2, 138.2, 136.8, 135.5, 132.8, 129.7, 129.6, 127.7, 127.6, 126.6, 123.3, 113.1, 113.0, 112.2, 88.5, 85.4, 83.2, 73.3, 70.3, 63.7, 55.0, 54.9.
HRMS (MH^+) for $C_{33}H_{32}N_5O_6$. Calculated: 594.2347. Found: 594.2345.

5′-*O*-(4,4′-Dimethoxytriphenyl)-etheno-cytidine (1B)

Cytidine (910 mg, 3.74 mmol) was dissolved in water (30 ml). To the solution chloroacetaldehyde was added (11.8 ml of 50% solution in water, 20 eq.), the pH was adjusted to 4.1 adding NaOH 1M, and the reaction was warmed up to 37°C. The pH was monitored for four hours until no more changes were observed.After 36 more hours, the solution was evaporated in high vacuum and the yellow gum obtained was precipitated with ethyl acetate /hexane, to get a yellowish precipitate. The solid was coevaporated with pyridine (2 x 15 ml) and dissolved in dry pyridine (40 ml). To the solution 4,4'-dimethoxytriphenyl chloride (610 mg, 1.93 mmol, 0.5 eq.) was added, and after 1 hour, a second batch was added too. After two more hours the reaction was quenched with ethanol (4 ml) and the solvents were evaporated. The residual oil was dissolved in ethyl acetate and extracted with sat. $NaHCO_3$ (2 times) and brine (2 times). The organic phase was dried over $NaSO_4$, filtered and evaporated.
The product was purified by flash chromatography (eluent: methylene chloride: methanol = 98:2 to 95:5 to 90:10 +0.1% triethylamine), obtaining 1B (1.33 gr, 2.3 mmol, 66%) as a pale yellow foam.
R_f: 0.56 (CH_2Cl_2: methanol= 95:5)
^{1}H-NMR (400 MHz, DMSO): δ 1H NMR (400 MHz, DMSO) δ 7.82 (d, J= 1 Hz, 1H), 7.60 (d, J = 8.0 Hz, 1H), 7.43 – 7.21 (m, 10H), 6.94-6.88 (m, 4H), 6.39 (d, J = 7.9 Hz, 1H), 6.04 (d, J = 3.7 Hz, 1H), 5.58 (d, J = 4.7 Hz, 1H), 5.23 (d, J = 5.5 Hz, 1H), 4.24 – 4.15 (m, 2H), 4.08-4.03 (m, 1H), 3.74 (s, 6H), 3.36-3.26 (m, 2H).
^{13}C NMR (101 MHz, DMSO): δ 158.1, 145.6, 144.7, 144.4, 135.4, 135.1, 132.6, 129.8, 128.1, 127.9, 127.7, 126.8, 113.3, 112.9, 98.2, 89.8, 85.9, 82.8, 74.0, 69.6, 62.9, 55.0, 54.9.
HRMS (MH^+) for $C_{32}H_{32}N_3O_7$. Calculated: 570.2235. Found: 570.2236.

5′-*O*-(4,4′-Dimethoxytriphenyl) 2',3'-O-tert-butyldimethylsilyl-etheno-adenine (2A-1, 2A-2)
5′-*O*-(4,4′-Dimethoxytriphenyl) 2',3'-O-tert-butyldimethylsilyl-etheno-cytidine (2B-1, 2B-2)

Compound 1A (1.25g, 2.1 mmol) or 1B (1.20g, 2.1 mmol) was dissolved in dry THF (20 ml). $AgNO_3$ (435.2 mg, 2.56 mmol, 1.2 eq.) and pyridine (568 µl, 7.84 mmol, 3.6 eq.) were added to the solution. In the case of compound 1A, when these reagents were added, the solution solidified to a thick white paste which was dissolved adding 60 ml more of dry THF. The solution obtained was stirred in the dark for 15 min, and tert-buthyldimethylsilyl chloride (416 mg, 2.8 mmol, 1.3 eq.) was added. After 4 hours, a second batch of $AgNO_3$(215 mg, 1.28 mmol), pyridine (284 µl, 3.92 mmol) and tert-buthyldimethylsilyl chloride (208 mg, 1.4 mmol) was added. The reaction was quenched after two more hours filtering the mixture over celite into 5% $NaHCO_3$. This suspension was extracted 2 times with CH_2Cl_2, and the organic phase was washed with sat. $NaHCO_3$ 2 times) and brine (2 times), dried over Na_2SO_4, filtrated and evaporated. The product was purified by flash chromatography (ethyl acetate: hexane = 7:3 to 8:2 to 9:1 for compound 1A and Ethyl acetate: hexane = 1:1 to 1.5:1 for compound 1B), obtaining a white solid (2A-1: 587.2 mg, 0.83 mmol, 40 %, 2A-2: 503.8 mg, 0.71 mmol, 33%, 2B-1:774.7 mg, 1.13 mmol, 54% 2B-2: 237.3 mg, 0.35 mmol, 17%).

5′-*O*-(4,4′-Dimethoxytriphenyl) 2'-O-tert-butyldimethylsilyl-etheno-adenine (2A-1)

R_f: 0.31 (ethyl acetate)
^{1}H-NMR (400 MHz, DMSO): δ 9.22 (s, 1H), 8.45 (s, 1H), 8.10 (d, J = 1.5 Hz, 1H), 7.57 (d, J = 1.4 Hz, 1H), 7.44 – 7.16 (m, 9H), 6.87 – 6.80 (m, 4H), 6.09 (d, J = 5.0 Hz, 1H), 5.19 (d, J = 5.9 Hz, 1H), 4.84-4.80 (m, 1H), 4.27 – 4.20 (m, 1H), 4.17 – 4.11 (m, 1H), 3.72 (s, 3H), 3.71 (s, 3H), 3.32-3.28 (m, 2H), 0.75 (s, 9H), -0.04 (s, 3H), -0.15 (s, 3H).
^{13}C NMR (101 MHz, DMSO): δ 158.1, 144.8, 140.4, 140.1, 138.1, 136.8, 135.5, 135.4, 132.8, 129.7, 127.7, 127.6, 126.6, 123.4, 113.1, 112.2, 88.4, 85.5, 83.6, 75.3, 70.2, 63.4, 55.0, 31.2, 29.0, 28.7, 25.5, 22.0, 17.8, 13.9, -4.9, -5.3.
HRMS (MH^+) for $C_{39}H_{46}N_5O_6Si$. Calculated: 708.3212. Found: 708.3210.

5′-*O*-(4,4′-Dimethoxytriphenyl) 3'-O-tert-butyldimethylsilyl-etheno-adenine (2A-2)

R_f: 0.17 (ethyl acetate)
^{1}H-NMR (400 MHz, DMSO): δ 9.25 (s, 1H), 8.51 (s, 1H), 8.11 (d, J = 1.5 Hz, 1H), 7.57 (d, J = 1.5 Hz, 1H), 7.37 – 7.14 (m, 9H), 6.88 – 6.75 (m, 4H), 6.04 (d, J = 4.9 Hz, 1H), 5.47 (d, J = 6.1 Hz, 1H), 4.86-4.80 (m, 1H), 4.55-4.50 (m, 1H), 4.09 – 4.07 (m, 1H), 3.70 (s, 3H), 3.69 (s, 3H), 3.39-3.32 (m, 1H), 3.19-3.12 (m, 1H), 0.85 (s, 9H), 0.08 (s, 3H), 0.04 (s, 3H).
^{13}C NMR (101 MHz, DMSO): δ 158.0, 144.7, 140.8, 140.4, 138.1, 136.7, 135.5, 135.4, 132.8, 129.6, 129.5, 127.7, 126.6, 123.5, 113.0, 112.2, 88.6, 85.6, 83.4, 72.5, 72.1, 63.0, 55.0, 31.3, 29.0, 28.7, 25.7, 22.1, 18.0, 13.9, -4.5, -5.1.

5′-*O*-(4,4′-Dimethoxytriphenyl) 2’-O-tert-butyldimethylsilyl-etheno-cytidine (2B-1)

R_f: 0.47 (ethyl acetate: hexane = 2:1)
^{1}H NMR (400 MHz, DMSO) δ 7.85 (s, 1H), 7.66 (d, J = 8.0 Hz, 1H), 7.44 – 7.23 (m, 10H), 6.94-6.89 (m, 4H), 6.36 (d, J = 7.9 Hz, 1H), 6.02 (d, J = 3.7 Hz, 1H), 5.20 (d, J = 6.1 Hz, 1H), 4.36-4.32 (m, 1H), 4.20 – 4.14 (m, 1H), 4.12-4.06 (m, 1H), 3.74 (s, 6H), 3.42 – 3.30 (m, 2H), 0.83 (s, 9H), 0.04 (s, 3H), 0.02 (s, 3H).
^{13}C NMR (101 MHz, DMSO) δ 158.2, 145.5, 144.7, 144.3, 135.2, 135.0, 132.6, 129.8, 127.9, 127.7, 127.6, 126.8, 113.2, 112.9, 98.1, 89.6, 86.0, 82.9, 76.1, 69.3, 62.5, 55.0, 25.6, 17.9, -4.8, -5.2.
HRMS (MH^+) for $C_{38}H_{46}N_3O_7Si$. Calculated: 684.3100. Found: 684.3098.

5′-*O*-(4,4′-Dimethoxytriphenyl) 3’-O-tert-butyldimethylsilyl-etheno-cytidine (2B-2)

R_f: 0.29 (ethyl acetate: hexane = 2:1)
^{1}H NMR (400 MHz, DMSO) δ 7.82 (s, J = 1.0 Hz, 1H), 7.67 (d, J = 8.0 Hz, 1H), 7.43 – 7.23 (m, 10H), 6.94-6.86 (m, 4H), 6.43 (d, J = 7.9 Hz, 1H), 6.01 (d, J = 3.5 Hz, 1H), 5.41 (d, J = 5.4 Hz, 1H), 4.28 – 4.19 (m, 2H), 4.07 – 3.97 (m, 1H), 3.74 (s, 6H), 3.46 – 3.38 (m, 1H), 3.28 – 3.17 (m, 1H), 0.79 (s, 9H), 0.03 (s, 3H), -0.02 (s, 3H).
^{13}C NMR (101 MHz, DMSO) δ 158.2, 145.6, 144.5, 135.2, 135.1, 132.60, 129.8, 128.1, 127.9, 127.7, 126.8, 113.2, 112.9, 98.2, 90.1, 86.1, 83.0, 73.4, 71.2, 62.5, 55.0, 25.7, 17.9, -4.6, -5.3.

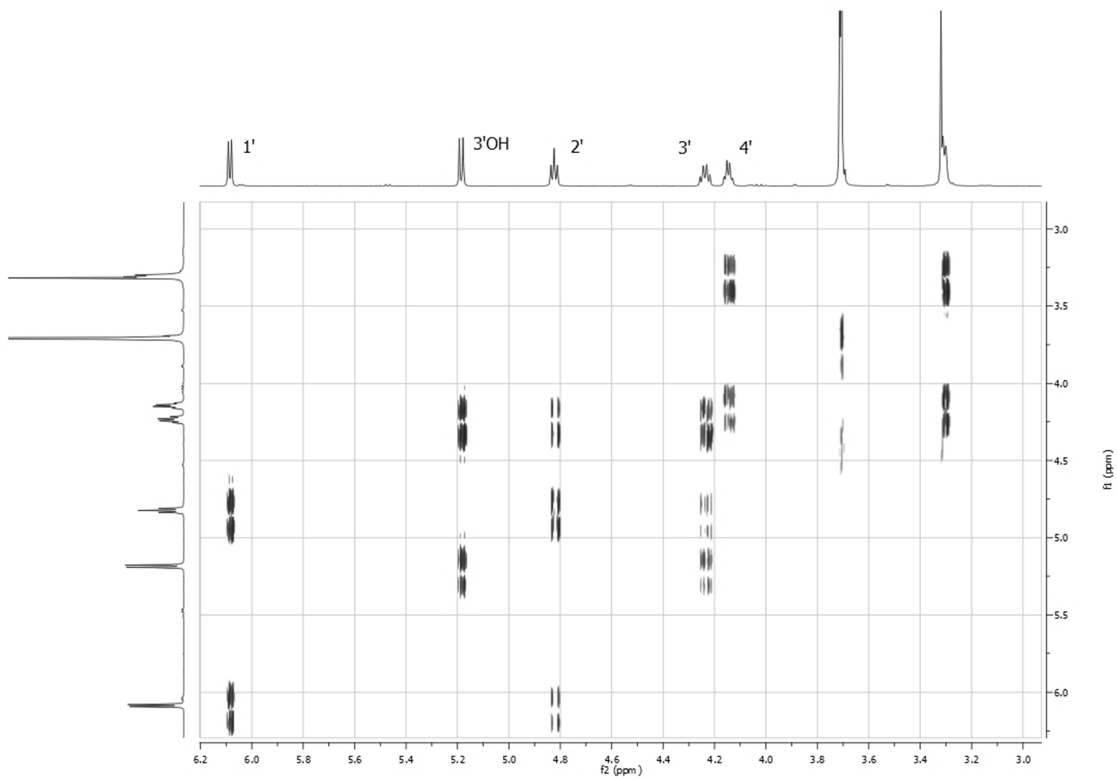


Figure S1. ^{1}H,^{1}H-COSY-NMR of 2A-1

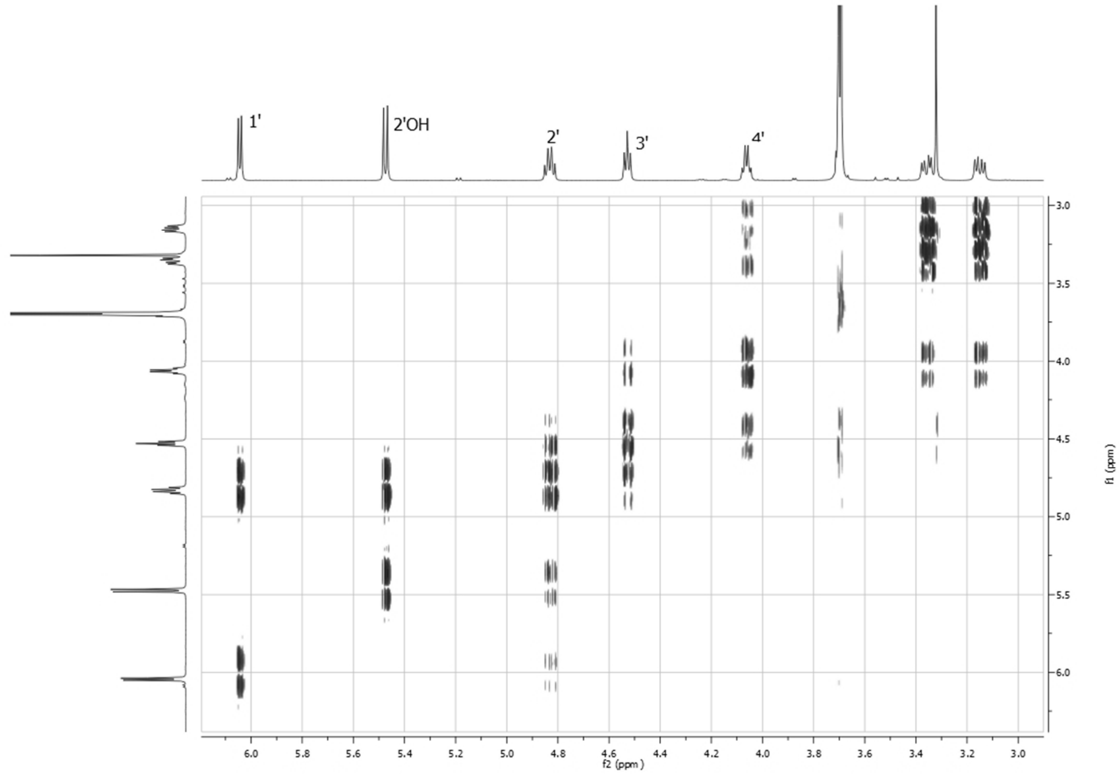


Figure S2. $^{1}H,^{1}H$-COSY-NMR of 2A-2

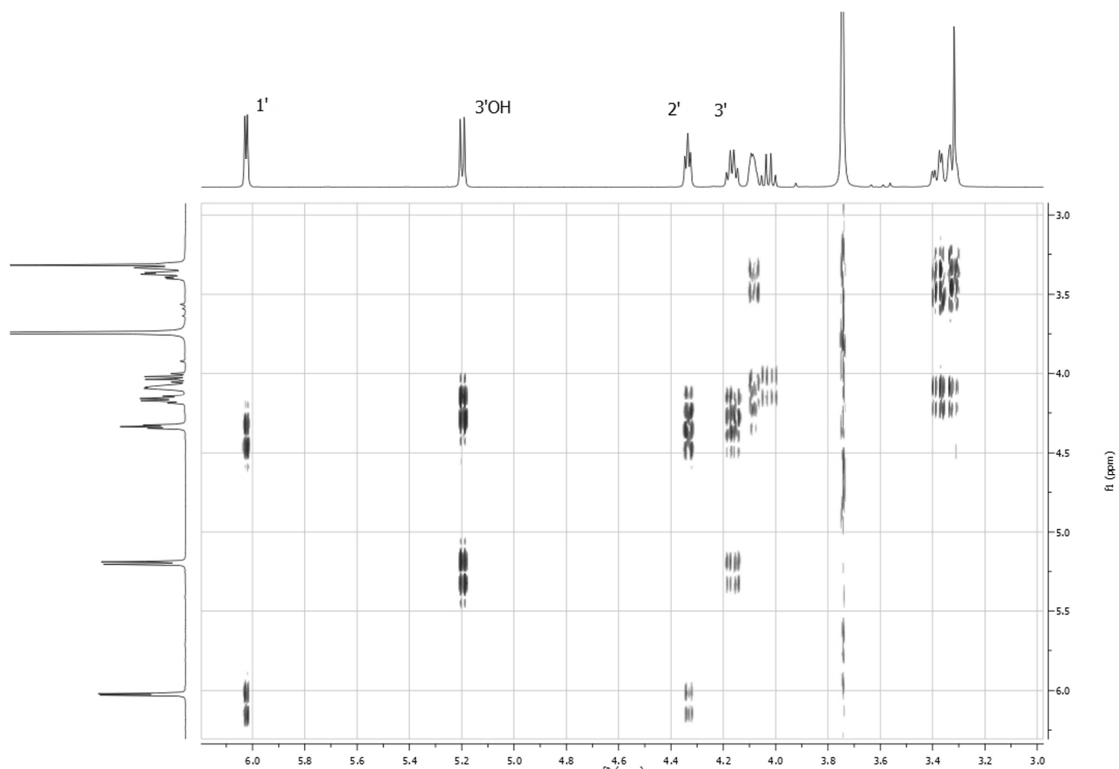


Figure S3. $^{1}H,^{1}H$-COSY-NMR of 2B-1

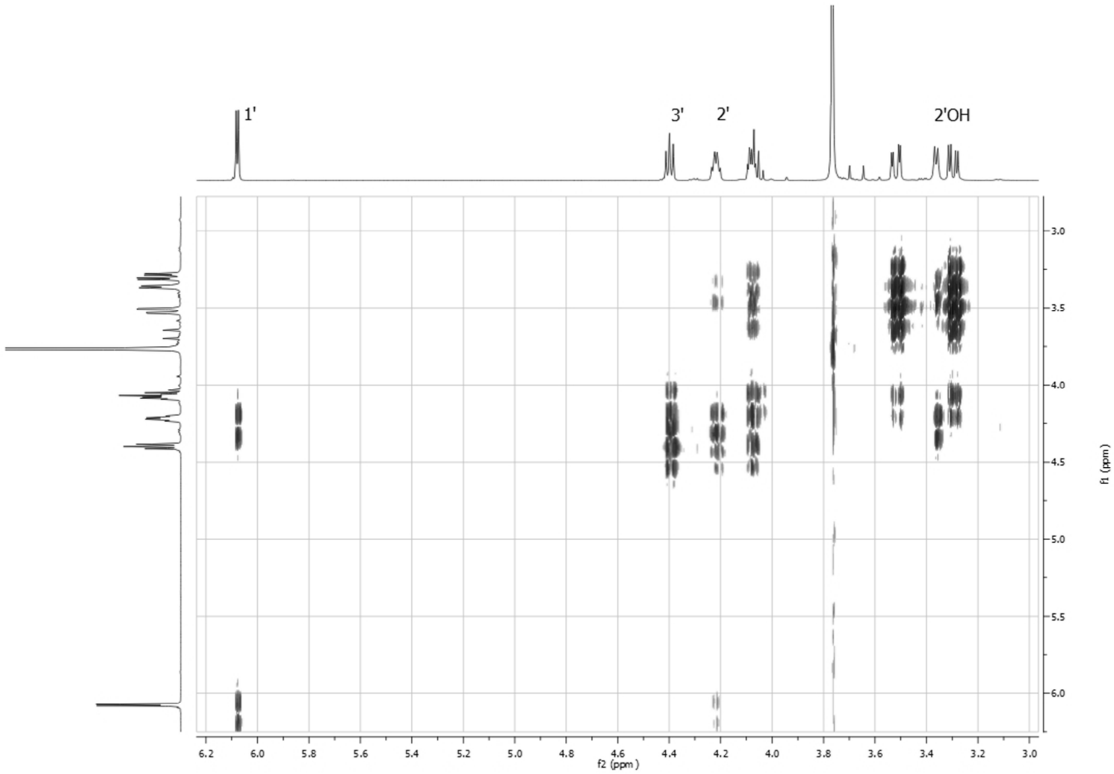


Figure S4. ^{1}H,^{1}H-COSY-NMR of 2B-2

5′-*O*-(4,4′-Dimethoxytriphenyl) 2’-O-tert-butyldimethylsilyl-3’-O-(diisopropylamino cyanoethoxy)phosphino-etheno-adenine (3A)
5′-*O*-(4,4′-Dimethoxytriphenyl) 2’-O-tert-butyldimethylsilyl-3’-O-(diisopropylamino cyanoethoxy)phosphino-etheno-cytidine (3B)

Compound 2A-1 (300 mg, 0.42 mmol) or 2B-1 (200 mg, 0.29 mmol) was dissolved in dry THF (9 or 4 ml) under argon. N-ethldiisopropylamine (208 µl, 1.20 mmol, or 168 µl, 0.96 mmol, 2.9 eq.) and 2-cyanoethyl diisopropylamino chlorophosphine (136 µl, 0.61 mmol, or 107 µl, 0.48 mmol, 1.45 eq.) were added to the solution and the system was stirred in argon atmosphere for 20 hours. The suspension was diluted with 30 ml of CH_2Cl_2 and extracted with sat. $NaHCO_3$ (2 times) and brine (brine). The organic phase was dried over Na_2SO_4, filtered and evaporated to dryness. The residue was purified by flash chromatography (ethyl acetate: hexane = 8:2 to 85:15 or 40:60 to 50:50) to obtain the product 3A (312 mg, 0.34 mmol, 81%) or 3B (230 mg, 0.26 mmol, 89%) as a white foam.

5′-*O*-(4,4′-Dimethoxytriphenyl) 2’-O-tert-butyldimethylsilyl-3’-O-(diisopropylamino cyanoethoxy)phosphino-etheno-adenine (3A)

R_f: 0.31 (ethyl acetate)
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.65 – 8.60 (m, 1H), 8.23 – 8.15 (m, 1H), 7.68 – 7.63 (m, 2H), 7.55 – 7.17 (m, 10H), 6.85 – 6.77 (m, 4H), 6.18 – 6.01 (m, 1H), 5.13 – 5.00 (m, 1H), 4.50 – 4.33 (m, 2H), 4.05 – 3.83 (m, 1H), 3.78 (s, 6H), 3.70 – 3.23 (m, 5H), 2.73 – 2.60 (m, 1H), 2.37 – 2.21 (m, 1H), 1.23 – 1.01 (m, 12H), 0.76 (s, 9H), 0.00 – -0.27 (m, 6H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 158.8, 144.9, 144.8, 141.7, 140.3, 140.0, 139.0, 136.0, 135.8, 135.2, 135.0, 134.1, 130.4, 130.3, 128.5, 128.3, 128.1, 127.2, 124.9, 124.8, 117.7, 117.4, 113.4, 113.3, 110.9, 88.9, 88.4, 87.0, 86.8, 84.5, 84.1, 75.8, 74.9, 73.7, 73.1, 72.9, 63.7, 63.6, 59.2, 59.0, 57.9, 57.7, 55.5, 47.5, 43.7, 43.6, 43.2, 43.1, 25.8, 25.7, 25.0, 24.9, 24.8, 20.7, 20.6, 20.3, 19.6, 18.2, -4.4, -4.9.
^{31}P NMR (122 MHz, $CDCl_3$) δ 151.28, 149.24.
HRMS (MH^+) for $C_{48}H_{63}N_7O_7PSi$. Calculated: 908.4290. Found: 908.4287.

5′-*O*-(4,4′-Dimethoxytriphenyl) 2’-O-tert-butyldimethylsilyl-3’-O-(diisopropylamino cyanoethoxy)phosphino-etheno-cytidine (3B)

R_f: 0.47 (ethyl acetate: hexane = 2:1)
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 – 7.69 (m, 2H), 7.51 – 7.20 (m, 11H), 6.92 – 6.79 (m, 4H), 6.33 – 6.16 (m, 2H), 4.58 – 4.24 (m, 3H), 4.05 – 3.83 (m, 1H), 3.80 (s, 6H), 3.75 – 3.35 (m, 5H), 2.71 – 2.60 (m, 1H), 2.43 – 2.33 (m, 1H), 1.22 – 0.99 (m, 12H), 0.86 (s, 9H), 0.12 – 0.00 (m, 6H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 159.0, 146.3, 146.2, 145.3, 145.2, 144.6, 144.5, 135.6, 135.5, 135.4, 135.3, 132.9, 130.5, 130.4, 128.5, 128.4, 128.2, 128.1, 127.5, 127.4, 117.8, 117.5, 113.6, 113.5, 113.4, 113.1, 113.0, 99.5, 99.3, 89.6, 89.0, 87.5, 87.4, 83.4, 83.2, 76.4, 75.8, 72.6, 72.4, 72.2, 63.1, 62.5, 59.0, 58.8, 58.1, 57.9, 55.5, 43.6, 43.5, 43.3, 43.1, 25.9, 25.0, 24.9, 24.8, 24.7, 20.7, 20.4, 18.2, -4.4, -4.5, -4.6.
^{31}P NMR (122 MHz, $CDCl_3$) δ 150.35, 149.61.
HRMS (MH^+) for $C_{47}H_{63}N_5O_8PSi$. Calculated: 884.4178. Found: 884.4175.

Mass Spectra of Oligoribonucleotides

All the oligonucleotide samples to be characterized by mass spectrometry were prepared in H_20:CH_3CN=1:1 + 1% of triethylamine. Masses were measured by ESI-MS (negative ion mode).

ON 1. Calculated for M: 3843,4; for M-H+Na: 3865.4. Obtained by mass spectrum deconvolution: 3843.0, 3866.0

ON 2. Calculated for M: 3819.4 for M-H+Na: 3841.4. Obtained by mass spectrum deconvolution: 3819.0, 3842.0

ON 5. Calculated for M-4H+Na+3K: 10054.3; for M-4H+4K: 10070.5; for M-5H+4K+Na: 10092.4; for M-6H+4K+2Na: 10114.4. Obtained by mass spectrum deconvolution: 10054.8; 10071.0; 10092.8; 10112.4

ON 6. Calculated for M-4H+Na+3K: 10030.3; for M-4H+4K: 10046.4; for M-5H+4K+Na: 10068.4. Obtained by mass spectrum deconvolution: 10030.5; 10048.6; 10067.8

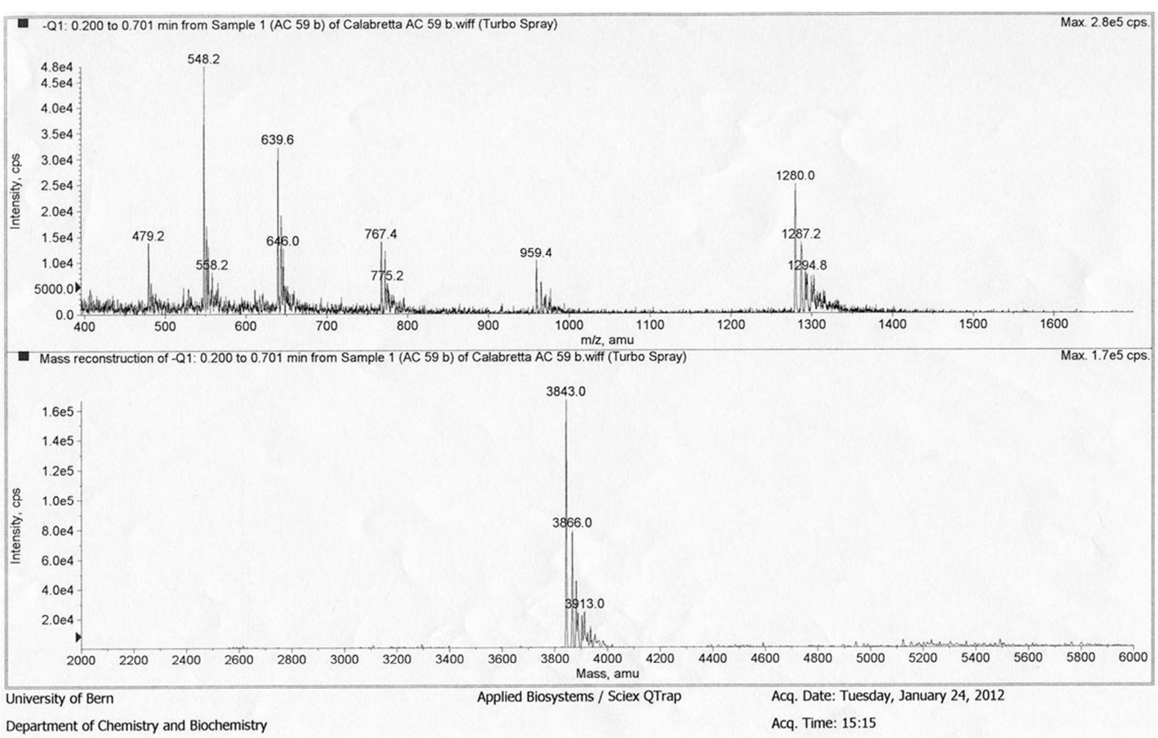


Figure S5. ESI-MS spectrum (negative mode) of ON 1

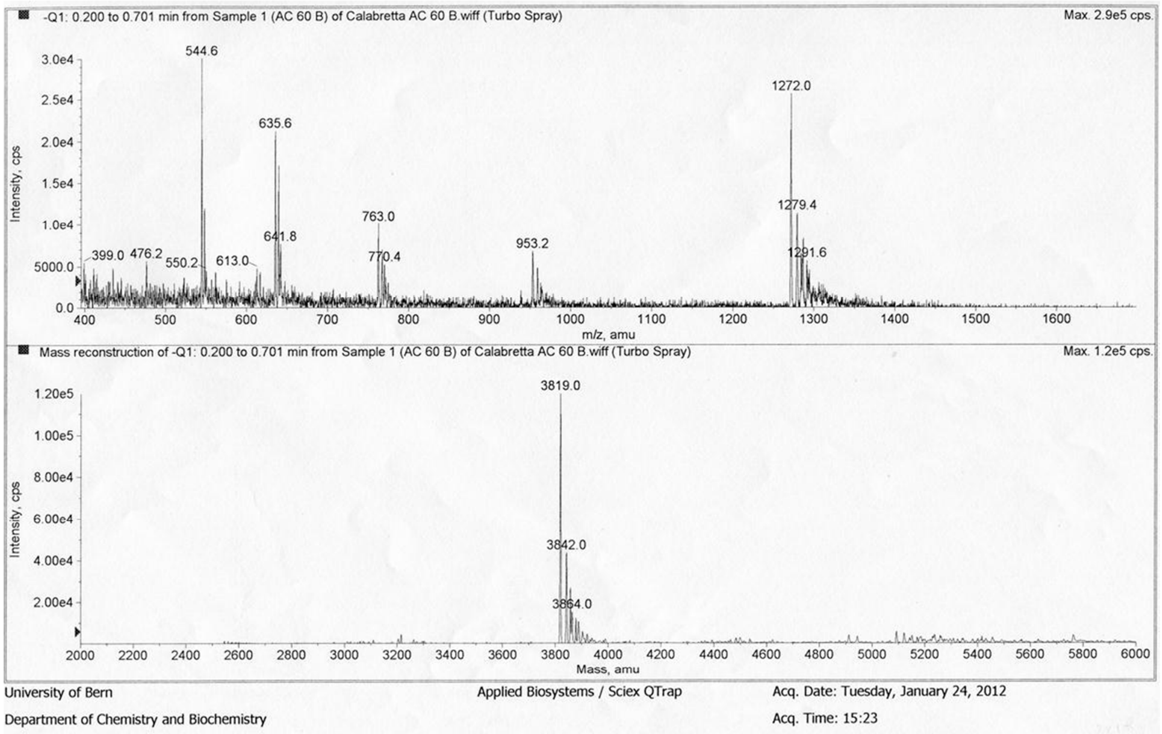


Figure S6. ESI-MS spectrum (negative mode) of ON 2

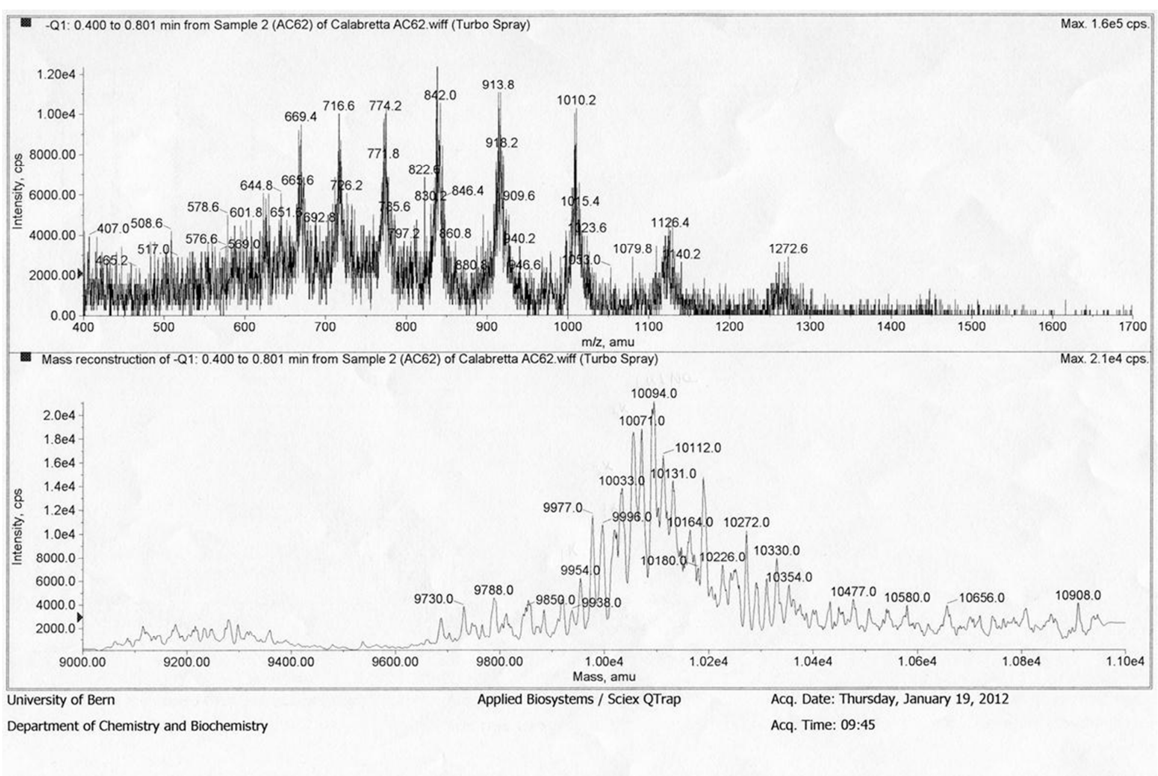


Figure S7. ESI-MS spectrum (negative mode) of ON 5

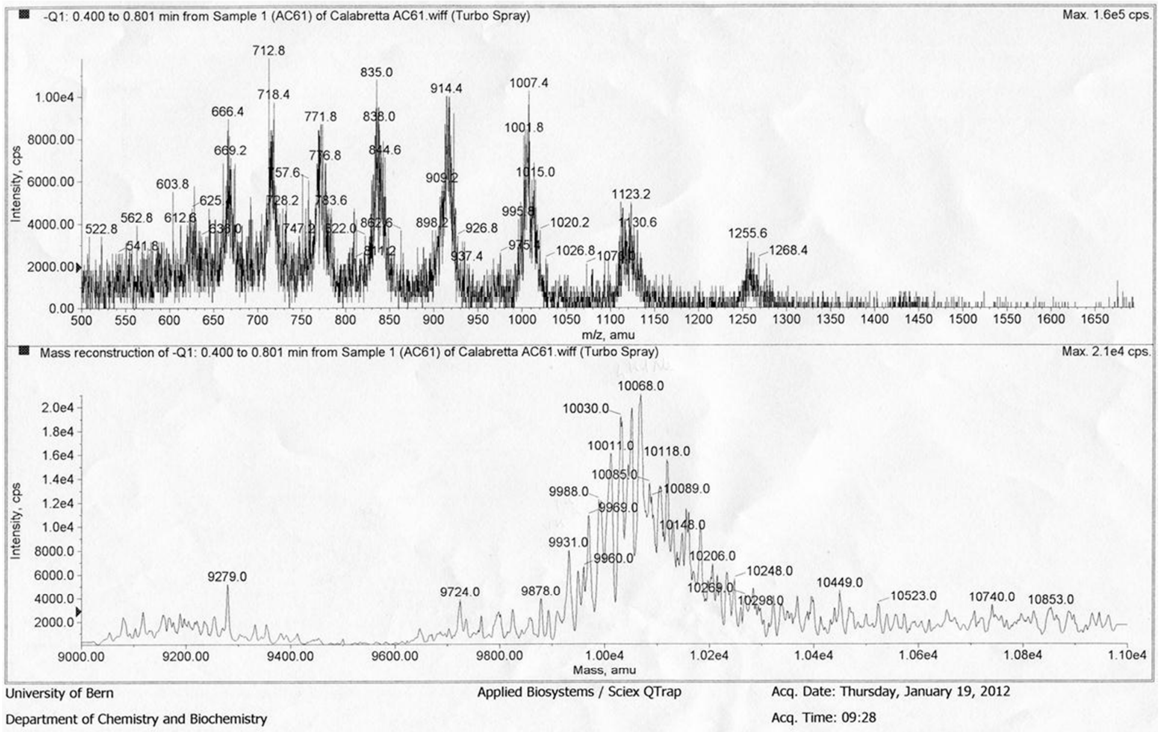


Figure S8. ESI-MS spectrum (negative mode) of ON 6

Quantitative data of dNTP incorporation (%)

Table 1. Quantitative data of dNMP incorporation in the primer extension reaction (normalized, in %).

	ON 5 AMV						ON 5 HIV						ON 5 MMLV					
	A	C	G	T	N	Nat	A	C	G	T	N	Nat	A	C	G	T	N	Nat
-2	0.0	0.0	3.2	2.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
-1	0.0	47.8	35.0	36.2	0.0	0.0	0.0	7.3	0.0	21.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0	100.0	52.2	61.8	61.2	100.0	21.4	17.8	78.3	71.8	57.4	20.3	4.4	100.0	100.0	100.0	91.2	98.8	11.2
+1	0.0	0.0	0.0	0.0	0.0	4.0	56.7	14.4	6.4	11.0	3.8	1.8	0.0	0.0	0.0	8.8	0.0	7.8
+2	0.0	0.0	0.0	0.0	0.0	0.0	25.4	0.0	19.3	1.2	1.8	4.7	0.0	0.0	0.0	0.0	0.0	0.0
+3	0.0	0.0	0.0	0.0	0.0	1.7	0.0	0.0	0.0	7.7	4.8	5.4	0.0	0.0	0.0	0.0	0.0	2.5
+4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5	1.5	5.9	1.8	0.0	0.0	0.0	0.0	0.0	1.9
+5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.2	0.0	0.0	0.0	0.0	0.0	1.2	1.7
+6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
+7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.8	0.0	0.0	0.0	0.0	0.0	1.0
+8	0.0	0.0	0.0	0.0	0.0	2.2	0.0	0.0	0.0	0.0	1.4	1.5	0.0	0.0	0.0	0.0	0.0	1.9
+9	0.0	0.0	0.0	0.0	0.0	4.5	0.0	0.0	0.0	0.0	3.3	4.3	0.0	0.0	0.0	0.0	0.0	3.7
+10	0.0	0.0	0.0	0.0	0.0	8.5	0.0	0.0	0.0	0.0	11.8	10.0	0.0	0.0	0.0	0.0	0.0	5.5
full	0.0	0.0	0.0	0.0	0.0	57.7	0.0	0.0	0.0	0.0	40.2	65.6	0.0	0.0	0.0	0.0	0.0	62.8

	ON 7 AMV						ON 7 HIV						ON 7 MMLV					
	A	C	G	T	N		A	C	G	T	N		A	C	G	T	N	
-2	0.0	0.0	4.1	0.0	0.0		0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	
-1	0.0	30.9	27.1	0.0	0.0		0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	
0	100.0	69.1	65.4	15.9	17.3		100.0	42.4	91.7	0.0	15.9		100.0	100.0	96.1	8.6	10.4	
+1	0.0	0.0	0.0	81.1	4.8		0.0	57.6	0.0	6.5	0.0		0.0	0.0	0.0	89.8	9.5	
+2	0.0	0.0	3.4	0.0	0.0		0.0	0.0	8.3	0.0	2.6		0.0	0.0	3.9	0.0	0.0	
+3	0.0	0.0	0.0	3.0	1.7		0.0	0.0	0.0	73.9	4.2		0.0	0.0	0.0	1.6	1.7	
+4	0.0	0.0	0.0	0.0	0.8		0.0	0.0	0.0	19.6	1.8		0.0	0.0	0.0	0.0	1.2	
+5	0.0	0.0	0.0	0.0	0.9		0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	1.2	
+6	0.0	0.0	0.0	0.0	0.7		0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.7	
+7	0.0	0.0	0.0	0.0	1.0		0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	1.0	
+8	0.0	0.0	0.0	0.0	2.2		0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	2.1	
+9	0.0	0.0	0.0	0.0	4.4		0.0	0.0	0.0	0.0	2.0		0.0	0.0	0.0	0.0	4.2	
+10	0.0	0.0	0.0	0.0	8.0		0.0	0.0	0.0	0.0	6.7		0.0	0.0	0.0	0.0	6.9	
full	0.0	0.0	0.0	0.0	58.3		0.0	0.0	0.0	0.0	66.8		0.0	0.0	0.0	0.0	61.2	

	ON 6 AMV					
	A	C	G	T	N	Nat
-2	0.0	0.0	4.5	3.6	0.0	0.0
-1	0.0	72.6	55.4	52.4	0.0	0.0
0	100.0	100.0	100.0	100.0	100.0	17.7
+1	0.0	0.0	0.0	0.0	0.0	0.0
+2	0.0	0.0	0.0	0.0	0.0	0.0
+3	0.0	0.0	0.0	0.0	0.0	1.1
+4	0.0	0.0	0.0	0.0	0.0	1.1
+5	0.0	0.0	0.0	0.0	0.0	1.1
+6	0.0	0.0	0.0	0.0	0.0	0.7
+7	0.0	0.0	0.0	0.0	0.0	1.0
+8	0.0	0.0	0.0	0.0	0.0	1.4
+9	0.0	0.0	0.0	0.0	0.0	0.0
+10	0.0	0.0	0.0	0.0	0.0	18.3
full	0.0	0.0	0.0	0.0	0.0	57.5

	ON 6 HIV					
	A	C	G	T	N	Nat
-2	0.0	0.0	0.0	0.0	0.0	0.0
-1	0.0	5.5	0.0	11.7	0.0	0.0
0	10.4	61.3	93.0	19.2	9.0	13.0
+1	79.2	33.1	0.0	61.8	0.0	0.0
+2	10.4	0.0	4.8	0.0	0.0	0.0
+3	0.0	0.0	2.2	5.4	2.3	0.9
+4	0.0	0.0	0.0	1.8	1.1	1.9
+5	0.0	0.0	0.0	0.0	0.0	0.0
+6	0.0	0.0	0.0	0.0	0.0	0.0
+7	0.0	0.0	0.0	0.0	0.0	0.0
+8	0.0	0.0	0.0	0.0	0.0	0.0
+9	0.0	0.0	0.0	0.0	2.4	0.0
+10	0.0	0.0	0.0	0.0	9.0	8.1
full	0.0	0.0	0.0	0.0	76.1	76.1

	ON 6 MMLV					
	A	C	G	T	N	Nat
-2	0.0	0.0	0.0	0.0	0.0	0.0
-1	0.0	0.0	0.0	0.0	0.0	0.0
0	100.0	100.0	100.0	100.0	100.0	10.3
+1	0.0	0.0	0.0	0.0	0.0	2.2
+2	0.0	0.0	0.0	0.0	0.0	1.1
+3	0.0	0.0	0.0	0.0	0.0	1.2
+4	0.0	0.0	0.0	0.0	0.0	2.5
+5	0.0	0.0	0.0	0.0	0.0	3.0
+6	0.0	0.0	0.0	0.0	0.0	1.4
+7	0.0	0.0	0.0	0.0	0.0	1.1
+8	0.0	0.0	0.0	0.0	0.0	1.6
+9	0.0	0.0	0.0	0.0	0.0	1.6
+10	0.0	0.0	0.0	0.0	0.0	5.3
full	0.0	0.0	0.0	0.0	0.0	68.9

	ON 8 AMV				
	A	C	G	T	N
-2	0.0	0.0	0.0	0.0	0.0
-1	0.0	28.2	0.0	7.7	0.0
0	100.0	71.8	18.0	92.3	11.7
+1	0.0	0.0	3.1	0.0	0.0
+2	0.0	0.0	76.6	0.0	1.4
+3	0.0	0.0	2.4	0.0	0.7
+4	0.0	0.0	0.0	0.0	1.0
+5	0.0	0.0	0.0	0.0	1.0
+6	0.0	0.0	0.0	0.0	0.6
+7	0.0	0.0	0.0	0.0	1.0
+8	0.0	0.0	0.0	0.0	1.6
+9	0.0	0.0	0.0	0.0	1.6
+10	0.0	0.0	0.0	0.0	8.9
full	0.0	0.0	0.0	0.0	70.5

	ON 8 HIV				
	A	C	G	T	N
-2	0.0	0.0	0.0	0.0	0.0
-1	0.0	12.1	0.0	12.5	0.0
0	16.9	87.9	0.0	15.2	4.0
+1	14.5	0.0	0.0	50.3	0.0
+2	58.5	0.0	56.7	7.6	0.0
+3	10.0	0.0	12.5	14.3	1.0
+4	0.0	0.0	19.7	0.0	1.5
+5	0.0	0.0	9.9	0.0	2.6
+6	0.0	0.0	1.3	0.0	0.0
+7	0.0	0.0	0.0	0.0	0.0
+8	0.0	0.0	0.0	0.0	0.0
+9	0.0	0.0	0.0	0.0	1.8
+10	0.0	0.0	0.0	0.0	8.4
full	0.0	0.0	0.0	0.0	80.6

	ON 8 MMLV				
	A	C	G	T	N
-2	0.0	0.0	0.0	0.0	0.0
-1	0.0	0.0	0.0	0.0	0.0
0	100.0	100.0	14.5	100.0	10.1
+1	0.0	0.0	4.2	0.0	1.7
+2	0.0	0.0	77.0	0.0	1.0
+3	0.0	0.0	4.4	0.0	1.2
+4	0.0	0.0	0.0	0.0	2.2
+5	0.0	0.0	0.0	0.0	3.0
+6	0.0	0.0	0.0	0.0	0.8
+7	0.0	0.0	0.0	0.0	1.1
+8	0.0	0.0	0.0	0.0	1.6
+9	0.0	0.0	0.0	0.0	0.0
+10	0.0	0.0	0.0	0.0	5.7
full	0.0	0.0	0.0	0.0	71.5