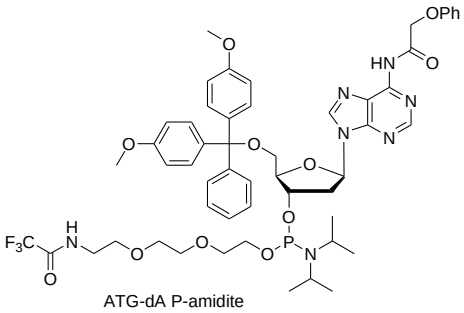
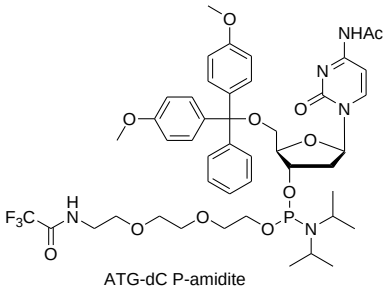


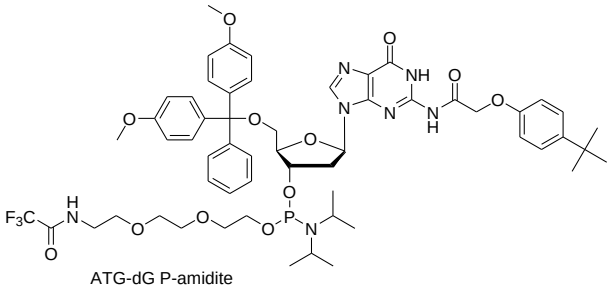
ATG-dA P-amidite

5'-O-dimethoxytrityl-N^6-phenoxyacetyl-2'-deoxyadenosine 3'-(2-[2-(2-trifluoroacetamidoethoxy)ethoxy]ethyl-N,N-diisopropyl)phosphoramidite



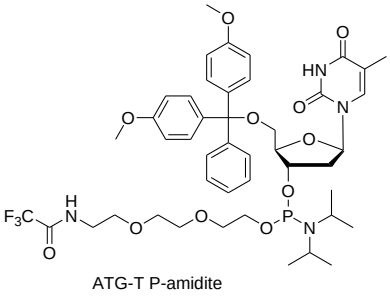
ATG-dC P-amidite

5'-O-dimethoxytrityl-N^4-acetyl-2'-deoxycytidine 3'-(2-[2-(2-trifluoroacetamidoethoxy)ethoxy]ethyl-N,N-diisopropyl)phosphoramidite



ATG-dG P-amidite

5'-O-dimethoxytrityl-N^2-(4-t-butylphenoxy)acetyl-2'-deoxyguanosine 3'-(2-[2-(2-trifluoroacetamidoethoxy)ethoxy]ethyl-N,N-diisopropyl)phosphoramidite



ATG-T P-amidite

5'-O-dimethoxytritylthymidine 3'-(2-[2-(2-trifluoroacetamidoethoxy)-ethoxy]ethyl-N,N-diisopropyl)phosphoramidite

Figure S1. Phosphoramidite synthons used for introduction of internucleoside amino linkers into ODNs.

Table S1. Cyanine-linked ODN duplexes with contact mode of quenching.

N	Duplex [1)]	b.p. distance, nucleotides	Absorbance spectra changes [2)]	Cy5.5 donor fluorescence intensity decrease, % [3)]	Acceptor fluorescence intensity change, % [4)]
1	5'**CGTA**TG⊗AGTGACTG-CAG**AGCT**3' 3'**GCAT**AC-TCACTGAC⊗GTC**TCGA**5'	8 G,G	√√	92.6	-81.3
2	5'**AGCC**TG⊗GAAAGTC-CCAC**ATCG**3' 3'**TCGG**AC-CTTTCAG⊗GGTG**TAGC**5'	7 G,G	√√	93.5	-86.7
3	5'**AGCC**TG⊗GAAAGTCC-CAC**ATCG**3' 3'**TCGG**AC-CTTTCAGG⊗GTG**TAGC**5'	8 G,G	√√	86.4	-71.1
4	5'**AGCC**TGGAAA⊗GTCCCAC**A-TCG**3' 3'**TCGG**ACCTTT-CAGGGTGT⊗**AGC**5'	8 A,A	√√	90.6	-20.8
5	5'**AGCC**T⊗GGAAAGTC-CCAC**ATCG**3' 3'**TCGG**A-CCTTTCAG⊗GGTG**TAGC**5'	8 T,G	√√	94.9	-42.7
6	5'**AGCC**TG⊗GAATTTCC-CAC**ATCG**3' 3'**TCGG**AC-CTTAAAGG⊗GTG**TAGC**5'	8 G,G	√√	93.8	-58.8
7	5'**AGCC**AT⊗TTCCCGTA-AAT**CTCG**3' 3'**TCGG**TA-AAGGGCAT⊗TTA**GAGC**5'	8 T,T	√√	86.1	-63.9
8	5'**AGCC**TGGAAAG⊗TCCCAC**AT-CG**3' 3'**TCGG**ACCTTTC-AGGGTGTA⊗**GC**5'	8 G,G	√√	89.8	17.8

1) ⊗ $OCH_2CH_2OCH_2CH_2OCH_2CH_2NH$-CW800, ⊗ $OCH_2CH_2OCH_2CH_2OCH_2CH_2NH$-Cy5.5

2) √√: new peak at 645 nm, with the concomitant decrease of absorbance at 675 nm.
3) Fluorescence intensity change at 700nm (excited at 675nm),
4) Fluorescence intensity change at 800 nm (excited at 675 nm).

Table S2. Cyanine-linked ODN duplexes exhibiting radiative FRET

N	Duplex [1)]	b.p. distance, nucleotides	Absorbance spectra changes	Cy5.5 donor fluorescence intensity decrease, % [2)]	Acceptor fluorescence intensity change, % [3)]
9	5′ **AGCC**TG-GAATTTCC⊗CAC**ATCG**3′ 3′ **TCGG**AC⊗CTTAAAGG-GTG**TAGC**5′	8 C,C	–	59.4	115.8
10	5′ **AGCC**TGGAAT-TTCCCACA⊗**TCG**3′ 3′ **TCGG**ACCTTA⊗AAGGGTG**T-AGC**5′	8 A,A	–	80.5	65.2
11	5′ **AGCC**TGGAA-TTTCCCACA⊗**TCG**3′ 3′ **TCGG**ACCTT⊗AAAGGGTG**T-AGC**5′	9 A,A	–	78.5	70.7
12	5′ **AGCC**TG-GAAAGTC⊗CCAC**ATCG**3′ 3′ **TCGG**AC⊗CTTTCAG-GGTG**TAGC**5′	7 C,C	–	73.2	118.1
13	5′ **AGCC**TG-GAAAGTCC⊗CAC**ATCG**3′ 3′ **TCGG**AC⊗CTTTCAGG-GTG**TAGC**5′	8 C,C	–	71.5	125.0
14	5' **AGC-C**TGGAAAG⊗TCCCAC**ATCG**3' 3' **TCG**⊗GACCTTTC-AGGGTG**TAGC**5'	8 G,G	–	67.7	127.4
15	5′ **AGCC**T⊗GGAAAGTCCC-AC**ATCG**3′ 3′ **TCGG**A-CCTTTCAGGG⊗TG**TAGC**5′	10 T,T	–	69.2	93.9
16	5′ **AGCC**T⊗GGAAAGTCCC-AC**ATCG**3′ 3′ **TCGG**A-CCTTTCAGGG⊗TG**TAGC**5′	10 T,T	–	70.6	103.6
17	5′ **AGCC**TG⊗GAAAGTCCCAC**ATCG** 3′ 3′ **TCGG**AC⊗CTTTCAGGGTG**TAGC** 5′	0 G,C	–	86.3	95.9

1) ⊗ $OCH_2CH_2OCH_2CH_2OCH_2CH_2NH$-CW800, ⊗ $OCH_2CH_2OCH_2CH_2OCH_2CH_2NH$-Cy5.5
2) Fluorescence intensity change at 700nm (excited at 675nm) (%)
3) Fluorescence intensity change at 800 nm (excited at 675 nm).

Table S3. Both modes of quenching/energy transfer, intermediate change in visible absorbance spectrum.

N	Duplex [1)]	b.p. distance, nucleotides	Absorbance spectra changes[2)]	Cy5.5 donor fluorescence intensity decrease, % [3)]	Acceptor fluorescence intensity change, % [4)]
18	5′ **AGCC**TG⊗GAAAGTCCC-AC**ATCG** 3′ 3′ **TCGG**AC-CTTTCAGGG⊗TG**TAGC** 5′	9 T,G	√	65.9	65.2
19	5′ **AGCC**TG⊗GAAAGTCCC-AC**ATCG** 3′ 3′ **TCGG**AC-CTTTCAGGG⊗TG**TAGC** 5′	9 T,G	√	73.2	65.2
20	5′ **AGCC**T⊗GGAAAGTCC-CAC**ATCG** 3′ 3′ **TCGG**A-CCTTTCAGG⊗GTG**TAGC** 5′	9 T,G	√	78.4	57.1
21	5′ **AGCC**T⊗GGAAAGTCC-CAC**ATCG** 3′ 3′ **TCGG**A-CCTTTCAGG⊗GTG**TAGC** 5′	9 T,G	√	81.4	63.4
22	5′ **AGCC**TGGAAAGTCC⊗CAC**ATCG** 3′ 3′ **TCGG**ACCTTTCAGG⊗GTG**TAGC** 5′	0 C,G	√	81.2	91.3
23	5′ **AGCC**TG⊗GAAAGTCCC-AC**ATCG** 3′ 3′ **TCGG**AC-CTTTCAGG⊗GTG**TAGC** 5′	8 G, G	√	81.7	0.9
24	5' **CGTA**TG⊗AGTGACTG-CAG**AGCT** 3' 3′ **GCAT**AC-TCACTGAC⊗GTC**TCGA** 5′	8 G, G	√	86.7	22.6

1) ⊗ $OCH_2CH_2OCH_2CH_2OCH_2CH_2NH$-CW800, ⊗ $OCH_2CH_2OCH_2CH_2OCH_2CH_2NH$-Cy5.5
⊗ $OCH_2CH_2OCH_2CH_2OCH_2CH_2NH$-Cy7
2) √: indicates a blue-shifted peak of Cy5.5 absorbance at 675 nm.
3) Fluorescence intensity change at 700nm (excited at 675nm) (%)
4) Fluorescence intensity change at 800 nm (excited at 675 nm).