CLAIMS:

1. A nucleic acid probe comprising:

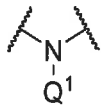
an oligonucleotide having the structure 5'-Y^1-L^1-L^{Q1}-L^2-Y^2-3', wherein

Y^1 comprises a sequence of two or more DNA or RNA nucleotides;

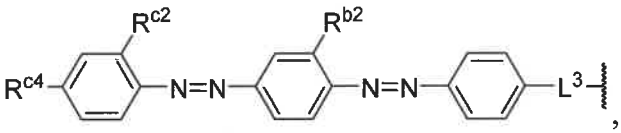
Y^2 comprises a sequence of two or more DNA or RNA nucleotides;

wherein one of Y^1 and Y^2 has a fluorophore covalently attached (directly or through a linker) to its nucleotide sequence, and the other of Y^1 and Y^2 has a second quencher covalently attached (directly or through a linker) to its nucleotide sequence;

L^1 and L^2 are independently selected from a bond, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heteroalkyl, and substituted or unsubstituted heterocycloalkyl; and

L^{Q1} is ,

wherein Q^1 is a first quencher having a structure:

,

wherein:

R^{b2} and R^{c2} are independently methyl or ethyl or n-propyl or isopropyl or n-butyl or isobutyl or *sec*-butyl or *tert*-butyl;

R^{c4} is H; and L^3 is a bond.

2. The nucleic acid probe of claim **1**, wherein

the nucleotide sequence of Y^1 includes:

a first nucleotide N^1 having a 5' phosphate covalently attached (directly or through a linker) to said fluorophore or said second quencher, and

a second nucleotide N^2 having a 3' phosphate covalently attached to L^1; and

the nucleotide sequence of Y^2 includes:

a third nucleotide N^3 having a 5' phosphate covalently attached to L^2, and

a fourth nucleotide N^4 having a 3' phosphate covalently attached (directly or through a linker) to said second quencher or said fluorophore;

wherein when the 5' phosphate of said first nucleotide N^1 is covalently attached (directly or through a linker) to said fluorophore, the 3' phosphate of said fourth nucleotide N^4 is

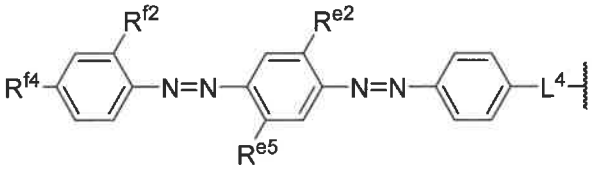
covalently attached (directly or through a linker) to said second quencher; and when the 5' phosphate of said first nucleotide N^1 is covalently attached (directly or through a linker) to said second quencher, the 3' phosphate of said fourth nucleotide N^4 is covalently attached (directly or through a linker) to said fluorophore.

3. The nucleic acid probe of any preceding claim, wherein said oligonucleotide comprises from 15 to 45 nucleotides.

4. The nucleic acid probe of any preceding claim, wherein:

(a) L^1 and L^2 are each unsubstituted C_1-C_7 alkyl; and/or

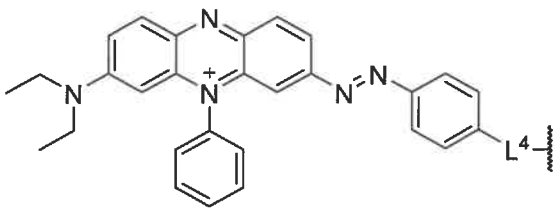
(b)(i) the second quencher has the structure:



wherein R^{e2}, R^{e5}, R^{f2}, and R^{f4} are independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted alkoxy, and nitro; and

L^4 is selected from a bond, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heteroalkyl, and substituted or unsubstituted heterocycloalkyl; or

(ii) the second quencher has the structure:



wherein L^4 is selected from a bond, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heteroalkyl, and substituted or unsubstituted heterocycloalkyl; or

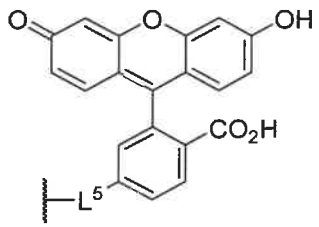
(iii) the second quencher is derived from BBQ-650®, Dabcyl, Dabsyl, Eclipse® quencher, Iowa Black® FQ, Iowa Black® RQ-nl, or Iowa Black® RQ-n2.

5. The nucleic acid probe of any preceding claim, wherein:

(a) the fluorophore is derived from 6-carboxyfluorescein (FAM), 2'7'-dimethoxy-4'5'-dichloro-6-carboxyfluorescein (JOE), tetrachlorofluorescein (TET), 6-carboxyrhodamine

(R6G), N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA), 6-carboxy-X-rhodamine (ROX), 1-dimethylaminonaphthyl-5-sulfonate, 1-anilino-8-naphthalene sulfonate, 2-p-toluidinyl-6-naphthalene sulfonate, 5-(2'-aminoethyl)aminonaphthalene-1-sulfonic acid (EDANS), a coumarin dye, an acridine dye, indodicarbocyanine 3 (Cy3), indodicarbocyanine 5 (Cy5), indodicarbocyanine 5.5 (Cy5.5), 3-(1-carboxy-pentyl)-3'-ethyl-5,5'-dimethyloxacarbocyanine (CyA), 1H,5H,11H,15H-Xantheno[2,3,4-ij:5,6,7-i'j']diquinolizin-18-ium, 9-[2(or 4)-[[[6-[2,5-dioxo-1-pyrrolidinyl)oxy]-6-oxohexyl]amino]sulfonyl]-4(or 2)-sulfophenyl]-2,3,6,7,12,13,16,17-octahydro-inner salt (TR or Texas Red), a BODIPY dye, benzoxaazole, stilbene, or pyrene; or

(b) the fluorophore has the structure:



wherein L^5 is selected from a bond, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heteroalkyl, and substituted or unsubstituted heterocycloalkyl.

6. The nucleic acid probe of claim **1**, wherein

the nucleotide sequence of Y^1 includes:

a first nucleotide N^1 having a 5' phosphate covalently attached (directly or through a linker) to said fluorophore, and

a second nucleotide N^2 having a 3' phosphate covalently attached to L^1; and

the nucleotide sequence of Y^2 includes:

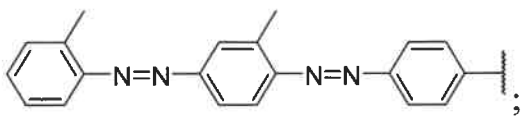
a third nucleotide N^3 having a 5' phosphate covalently attached to L^2, and

a fourth nucleotide N^4 having a 3' phosphate covalently attached (directly or through a linker) to said second quencher;

L^1 is unsubstituted C_2 alkyl;

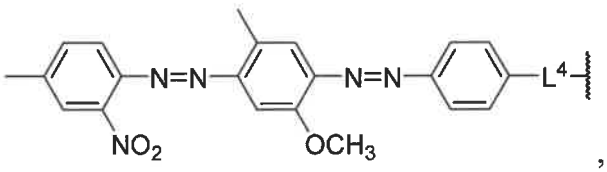
L^2 is unsubstituted C_2 alkyl;

Q^1 has the structure:



;

said second quencher has the structure:



wherein L^4 is selected from a bond, substituted or unsubstituted alkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heteroalkyl, and substituted or unsubstituted heterocycloalkyl; and

said fluorophore comprises a fluorescein moiety.

7. The nucleic acid probe of claim **2**, wherein the oligonucleotide is a first oligonucleotide, and the first oligonucleotide is adapted to hybridize to a second oligonucleotide having the structure 3'-Y^3-Y^4-5', wherein:

Y^3 comprises a sequence of four or more DNA or RNA nucleotides, including a fifth nucleotide N^5; and

Y^4 comprises a sequence of four or more DNA or RNA nucleotides, including a sixth nucleotide N^6 that is directly attached to nucleotide N^5;

wherein if the first oligonucleotide hybridizes to the second oligonucleotide, N^2 base pairs with N^5 and N^3 base pairs with N^6 to form a duplex having a T_m that is (a) higher than the T_m of a duplex formed between the second oligonuleotide and a third oligonucleotide having the structure 5'-Y^1-Y^2-3' or (b) lower than the T_m of a duplex formed between the second oligonuleotide and a third oligonucleotide having the structure 5'-Y^1-Y^2-3'.

8. The nucleic acid probe according to any preceding claim, wherein Q^1 is attached between the 9th and 10th nucleotides towards the 5'-terminus from the 3'-terminus of the oligonucleotide.

9. A method of detecting a second oligonucleotide in a sample, comprising:

contacting the sample with the nucleic acid probe of claim **7**, wherein the fluorescence of the fluorophore is reduced by donor-acceptor energy transfer to one or both of the first quencher and the second quencher when the first oligonucleotide is not hybridized to the second oligonucleotide; and

detecting an increase in fluorescence indicating the presence of the second oligonucleotide in the sample.

10. A method of detecting a second oligonucleotide in a sample, comprising:

contacting the sample with the nucleic acid probe of any one of claims **1-6**, wherein the oligonucleotide is a first oligonucleotide, and the first oligonucleotide is adapted to

hybridize to a second oligonucleotide, and fluorescence of the fluorophore is reduced by donor-acceptor energy transfer to one or both of the first quencher and the second quencher when the first oligonucleotide is not hybridized to the second oligonucleotide; and

detecting an increase in fluorescence indicating the presence of the second oligonucleotide in the sample.

11. The method of claim **9** or **10**, wherein (a) fluorescence is reduced by fluorescence resonance energy transfer when the first oligonucleotide is not hybridized to the second oligonucleotide, (b) fluorescence is reduced by ground state quenching when the first oligonucleotide is not hybridized to the second oligonucleotide, or (c) fluorescence is reduced by both fluorescence resonance energy transfer and ground state quenching when the first oligonucleotide is not hybridized to the second oligonucleotide.

12. The method of any one of claims **9-11**, wherein the increase in fluorescence arises from cleavage of the fluorophore from the first oligonucleotide.

13. The method of any one of claims **9-12**, wherein (a) the first oligonucleotide forms a random-coil conformation when the first oligonucleotide is unhybridized, such that the fluorescence of the fluorophore is reduced, or (b) wherein the first oligonucleotide comprises a self-complimentary sequence and wherein the quencher and the fluorophore are attached to the first oligonucleotide such that the fluorescence of the fluorophore is quenched by the quencher when the nucleic acid polymer undergoes intramoleeular base pairing.

14. The method of any one of claims **9-13**, wherein the method is used in a PCR reaction wherein synthesis of PCR product results in an increase in fluorescence.

15. A method for detecting a nucleic acid target sequence, said method comprising:

(a) contacting said target sequence with a detector nucleic acid comprising a single-stranded target binding sequence,

wherein said detector nucleic acid is a nucleic acid probe according to any one of claims 1-8, and said detector nucleic acid is in a conformation allowing donor- acceptor energy transfer between said fluorophore and one or both of said first quencher and said second quencher when said fluorophore is excited;

(b) hybridizing said target binding sequence to said target sequence, thereby altering said conformation of said detector nucleic acid, causing a change in a fluorescence parameter; and

(c) detecting said change in said fluorescence parameter, thereby detecting said nucleic acid target sequence.

16. A method of ascertaining whether a first nucleic acid and a second nucleic acid hybridize, wherein said first nucleic acid is a nucleic acid probe according to any one of claims 1-8, said method comprising:

(a) contacting said first nucleic acid with said second nucleic acid; and

(b) detecting an alteration in a fluorescent property of a member selected from said first nucleic acid, said second nucleic acid and a combination thereof, thereby ascertaining whether said hybridization occurs.

17. A method of monitoring a nucleic acid amplification reaction, said method comprising:

(a) preparing an amplification reaction mixture comprising a detector nucleic acid, wherein said detector nucleic acid is a nucleic acid probe according to any one of claims 1-8;

(b) subjecting the amplification reaction mixture to amplification conditions;

(c) monitoring the reaction mixture for a fluorescent signal from the detector nucleic acid to obtain an assay result; and

(d) employing the assay result to monitor the nucleic acid amplification reaction.

18. A method of detecting amplification of a target sequence, said method comprising:

(a) hybridizing a sample nucleic acid comprising the target sequence with PCR primers that flank the target sequence;

(b) extending the hybridized primers with a polymerase to produce the PCR product, and separating the two strands of the PCR product to make accessible the sense and antisense strands of the target sequence;

(c) hybridizing a detector nucleic acid to the sense or antisense strand of the target sequence in the PCR product, wherein the detector nucleic acid is a nucleic acid probe according to any one of claims 1-8;

wherein prior to its hybridization to the target sequence, the detector nucleic acid is in a conformation allowing donor-acceptor energy transfer between the fluorophore and one or both of the first quencher and the second quencher when the fluorophore is excited;

thereby altering the conformation of the detector nucleic acid, causing a change in a fluorescence parameter; and

(d) measuring the change in the fluorescence parameter to detect the target sequence and its amplification.