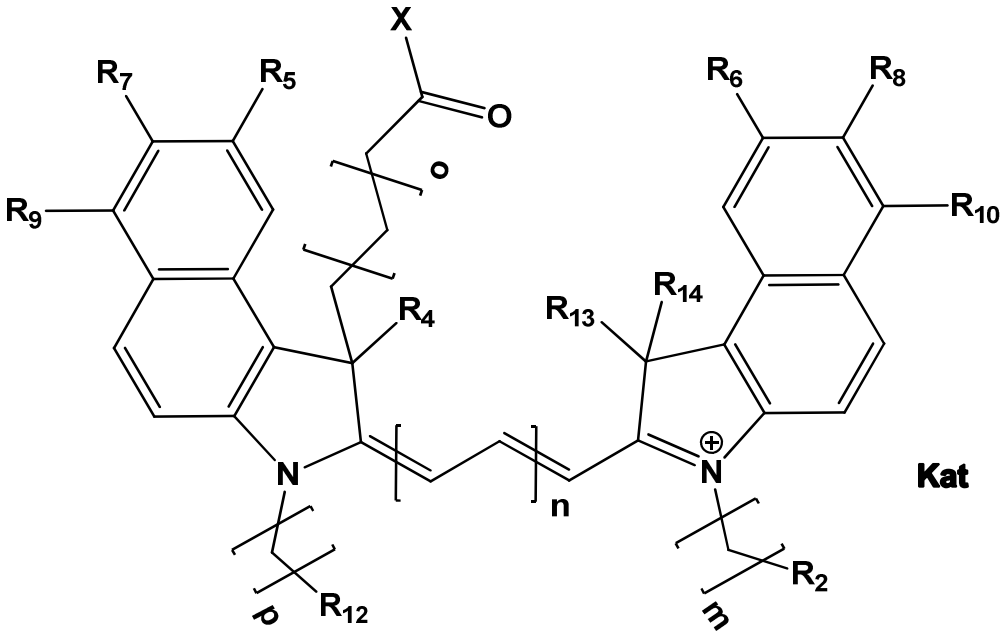
the use of only four spectral channels (i.e., 4-plex), such as for use in 5-plex and 6-plex multiplex PCR assays.

Summary

[0017] In one example, provided herein is a probe that comprises a product of covalent conjugation of: a dye having a general Formula Ia, Formula Ib, Formula Ic, or Formula Id; a quencher having general Formula II; and a linker joining the dye and the quencher. In one example, the linker is or includes an oligonucleotide.

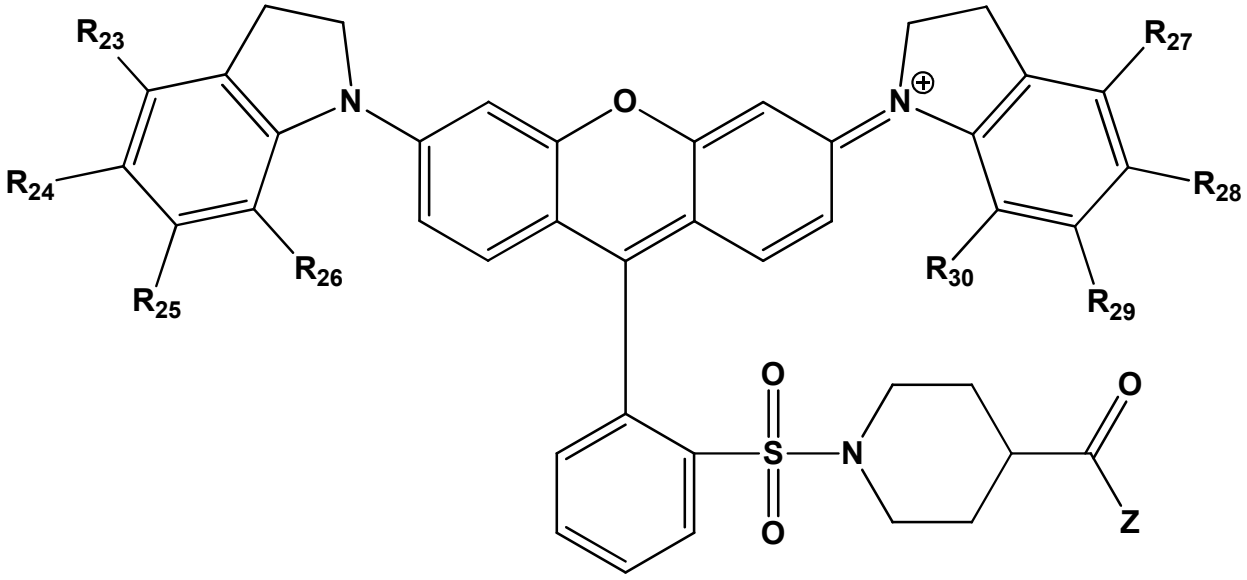
[0018] Formula Ia, Ib, Ic, and Id are as follows:



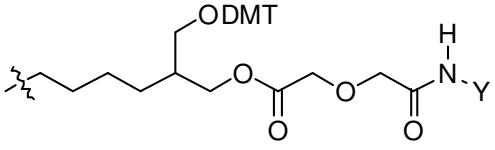
Formula Ia

ammonia, or other cation(s) needed to compensate the negative charge brought by the cyanine. In the Formulas, m is an integer from 0 to 5 inclusive; n is an integer from 1 to 3 inclusive; o is an integer from 0 to 12 inclusive; and p is an integer from 0 to 5 inclusive.

[0019] Formula II is as follows:



Formula II

[0020] Each of R_{23}, R_{24}, R_{25}, R_{26}, R_{27}, R_{28}, R_{29}, and R_{30} is the same or different and is independently selected from either H or SO_3. Z is OR, where R is H or alkyl, or NH-L, where L is  and Y is either H or a linkage to a solid support.

[0021] Another example provided herein is a method for conjugating or labeling a biomolecule with a dye and/or quencher using an azide-derivatized dye or quencher to label the biomolecule having a cyclooctyne moiety via a Cu-free "Click Reaction". In one embodiment, the biomolecule is an oligonucleotide. In one example, the cyclooctyne moiety is dibenzocyclooctyne (DIBO). In one example, the method for conjugating or labeling results in the probe as described herein.

[0022] Another example provided herein is a method of detecting or quantifying a target nucleic acid molecule in a sample by polymerase chain reaction (PCR), such as by

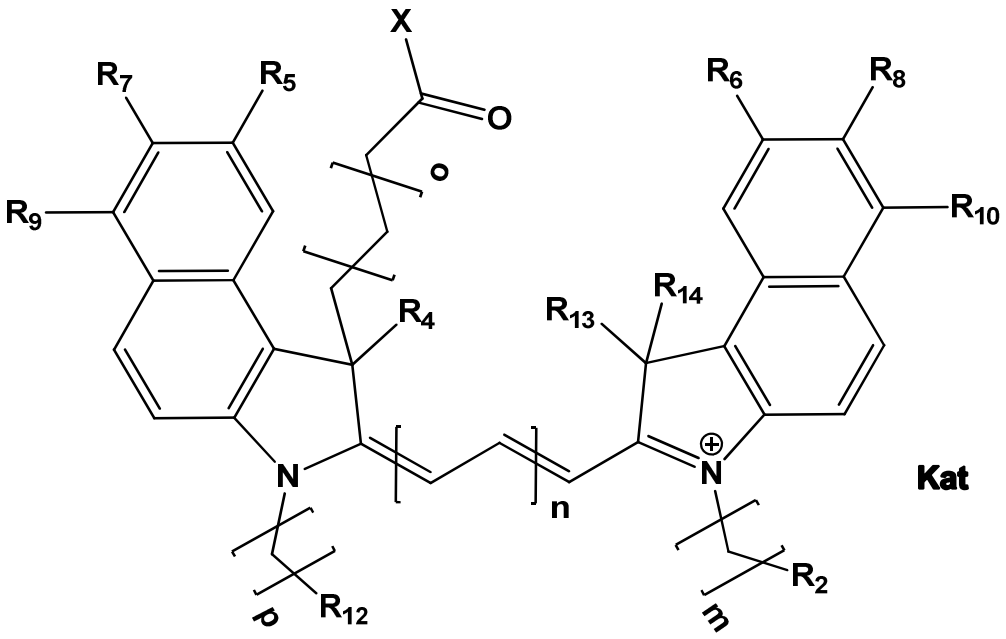
quantitative real-time polymerase chain reaction (qPCR). In one example, the method includes: (i) contacting a sample comprising one or more target nucleic acid molecules with a) at least one probe, such as those described herein, being sequence specific for the target nucleic acid molecule, where the at least one probe undergoes a detectable change in fluorescence upon amplification of the one or more target nucleic acid molecules; and with b) at least one oligonucleotide primer pair; (ii) incubating the mixture of step (i) with a DNA polymerase under conditions sufficient to amplify one or more target nucleic acid molecules; and (iii) detecting the presence or absence or quantifying the amount of the amplified target nucleic acid molecules by measuring fluorescence of the probe. In some examples, the DNA polymerase comprises 5' exonuclease activity. In some other examples, the DNA polymerase is a Thermus aquaticus (Taq) DNA polymerase. In some examples, the probe is a hydrolysis probe, such as a TaqMan probe.

[0023] Another example provided herein is a kit for PCR, such as quantitative real-time polymerase chain reaction (qPCR) and reverse transcription polymerase chain reaction (RT-PCR). In some examples the kit includes a probe, such as those described herein, instructions for conducting the PCR, and one or more of the following: a buffering agent, deoxynucleotide triphosphates (dNTPs), an organic solvent, an enzyme, enzyme cofactors, and an enzyme inhibitor. In another example, the kit for PCR comprises the described dye and/or quencher moiety, instructions for conjugating or labeling the dye and/or quencher moiety to a biomolecule, such as an oligonucleotide, instructions for conducting the PCR, and one or more of the following: a buffering agent, deoxynucleotide triphosphates (dNTPs), an organic solvent, an enzyme, enzyme cofactors, and an enzyme inhibitor.

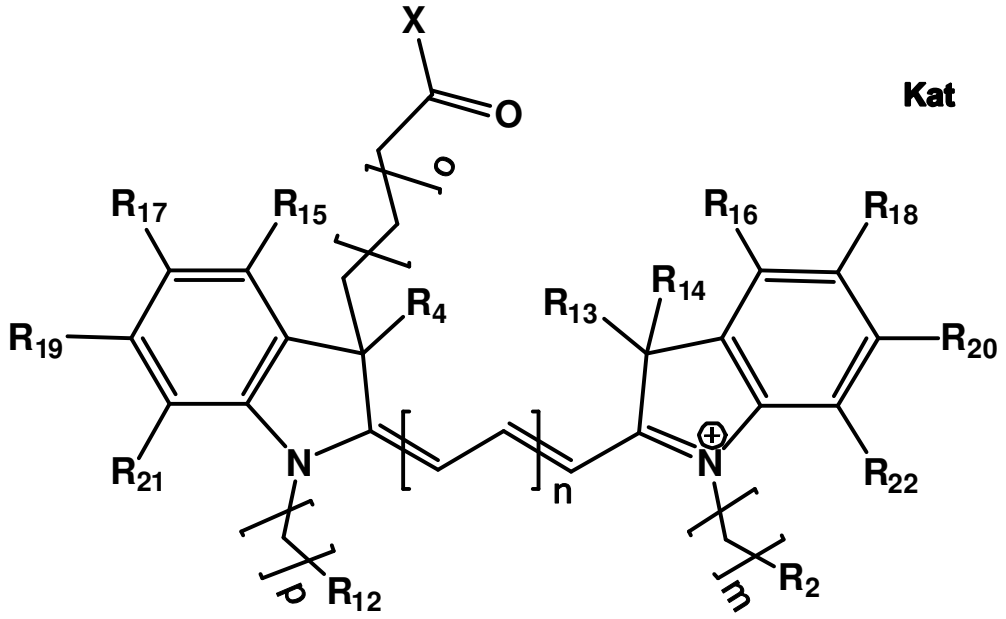
[0024] In yet further examples provided herein are compositions, such as a "master mix" for PCR comprising the described probe along with other components that are used in PCR. In some examples, the master mix is prepared such that it requires less than a 3X dilution prior to use in PCR, e.g., 2X dilution, 1.5X dilution, 1.2X dilution, etc.

[0024a] In one aspect, provided herein is a probe comprising a product of conjugation of:

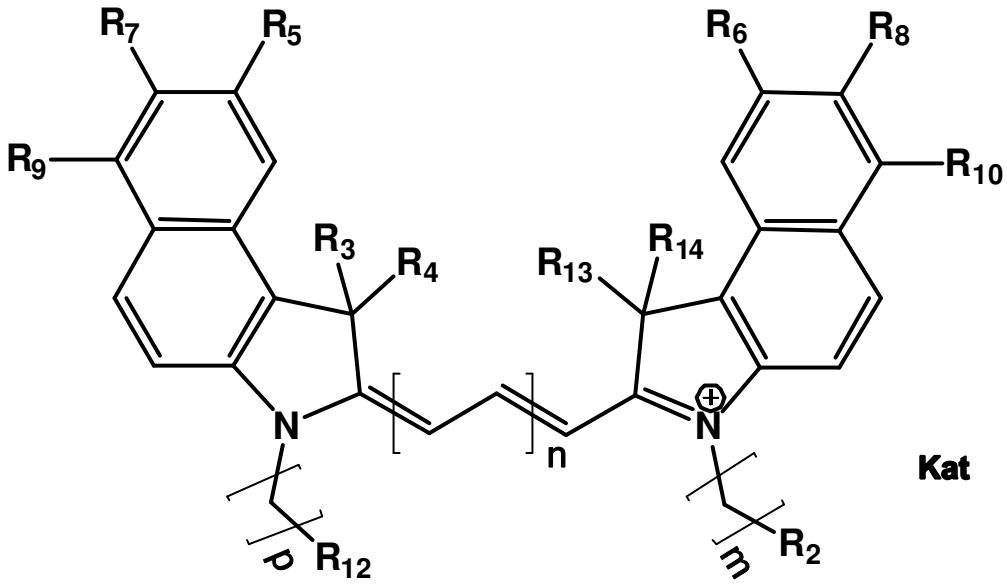
a) a dye having a general Formula Ia, Formula Ib, Formula Ic, or Formula Id



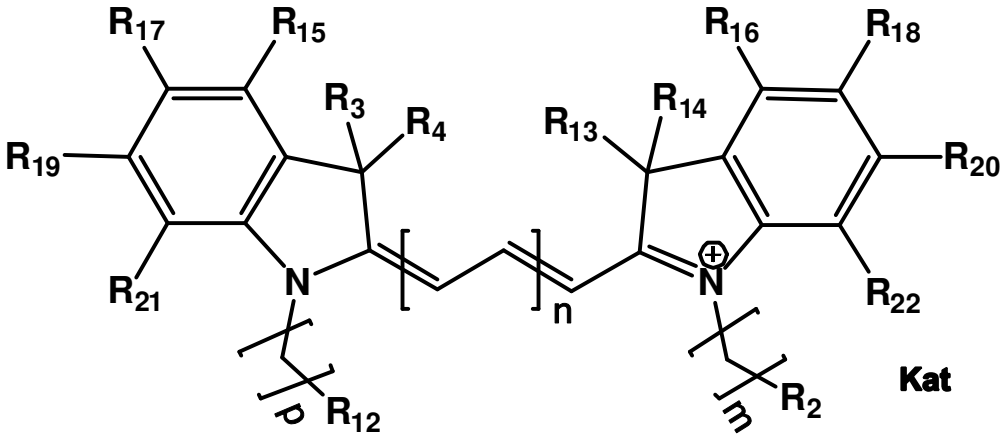
Formula Ia



Formula Ib



Formula Ic



Formula Id

wherein

each of R_4, R_{13}, and R_{14}, and R_3 when present, is the same or different and is selected from the group consisting of H, an aliphatic, a heteroaliphatic, a sulfoalkyl, a heteroaliphatic with terminal SO_3, a benzyl, and a substituted benzyl, where the substituted benzyl comprises at least one carboxy group, at least one sulfonate group, -F, -Cl, -Br, or a combination thereof;

each of R_5, R_6, R_7, R_8, R_9, R_{10}, R_{15}, R_{16}, R_{17}, R_{18}, R_{19}, R_{20}, R_{21}, and R_{22} is the same or different and is selected from the group consisting of H and SO_3;

each of R_2 and R_{12} is the same or different and is selected from the group consisting of H and SO_3;

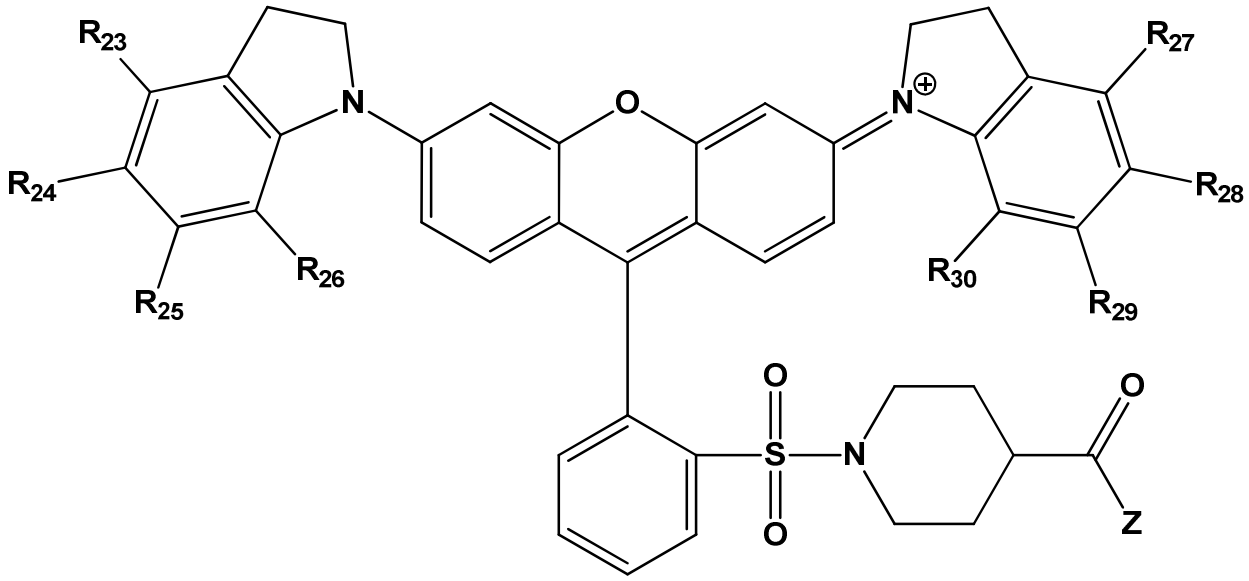
X is selected from the group consisting of -OH, -SH, -NH_2, -NH-NH_2, -F, -Cl, -Br, I, -O-NHS (hydroxysuccinimidyl/sulfosuccinimidyl), -O-TFP (2,3,5,6-tetrafluorophenoxy), -O-STP (4-sulfo-2,3,5,6-

tetrafluorophenoxy), -O-benzotriazole, -benzotriazole, -NR-L-OH, -NR-L-O-phosphoramidite, -NR-L-SH, -NR-L-NH_2, -NR-L-NH-NH_2, -NR-L-CO_2H, -NR-L-CO-NHS, -NR-L-CO-STP, -NR-L-CO-TFP, -NR-L-CO-benzotriazole, -NR-L-CHO, -NR-L-maleimide, -$NH(CH_2CH_2O)_zCH_2CH_2N_3$, -NR-L-NH-CO-$CH_2$-I, and an azide ($N_3$)-containing group, where R is -H or an aliphatic or heteroaliphatic group, z is an integer from 1 to 5 inclusive, and L is selected from the group consisting of a divalent linear, crossed-coupled, or cyclic alkyl group optionally substituted by at least one oxygen atom and/or sulfur atom;

Kat is a number of Na^+, K^+, Ca^{2+}, ammonia, or other cation(s) needed to compensate the negative charge brought by the cyanine;

m is an integer from 0 to 5 inclusive; n is an integer from 1 to 3 inclusive; o is an integer from 0 to 12 inclusive; and p is an integer from 0 to 5 inclusive;

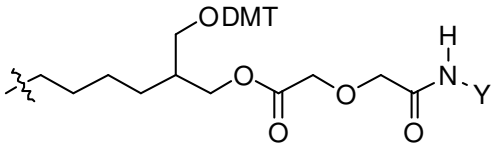
b) a quencher having general Formula II



Formula II

wherein

each of R_{23}, R_{24}, R_{25}, R_{26}, R_{27}, R_{28}, R_{29}, and R_{30} is the same or different and is independently selected from either H or SO_3;



Z is OR, where R is alkyl, or Z is NH-L, where L is and Y is either H or a linkage to a solid support;

and

c) an oligonucleotide linker joining the dye and the quencher, wherein the dye is covalently joined to a first nucleotide of the oligonucleotide and the quencher is covalently joined to a second, different nucleotide of the oligonucleotide.

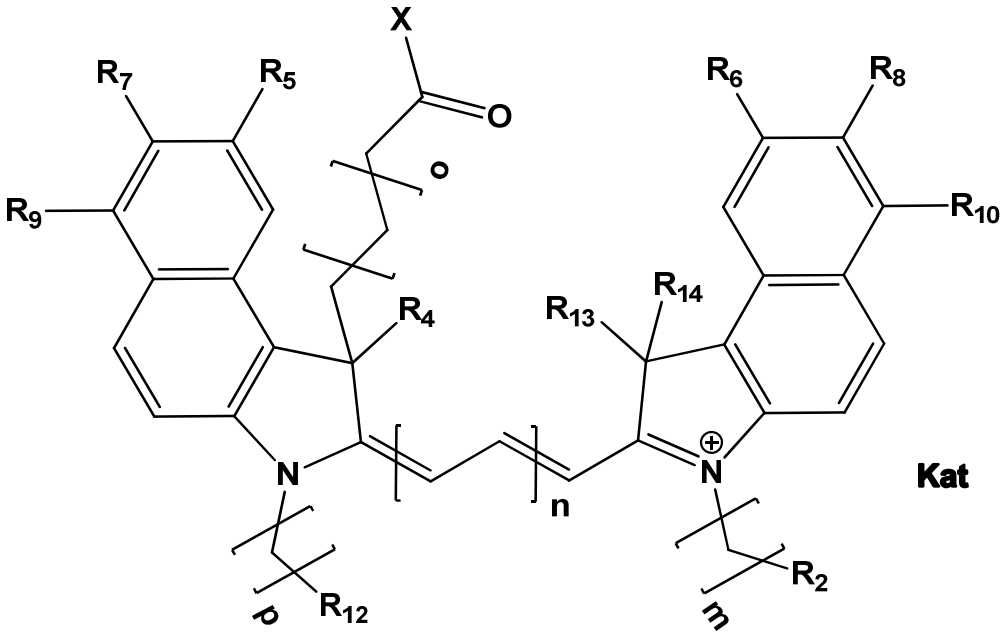
In another aspect, provided herein is a method of detecting or quantifying a target nucleic acid molecule in a sample by polymerase chain reaction (PCR), the method comprising:

(i) contacting the sample comprising one or more target nucleic acid molecules with a) at least one probe having a sequence that is at least partially complementary to the target nucleic acid molecule, where the at least one probe undergoes a detectable change in fluorescence upon amplification of the one or more target nucleic acid molecules; and with b) at least one oligonucleotide primer pair;

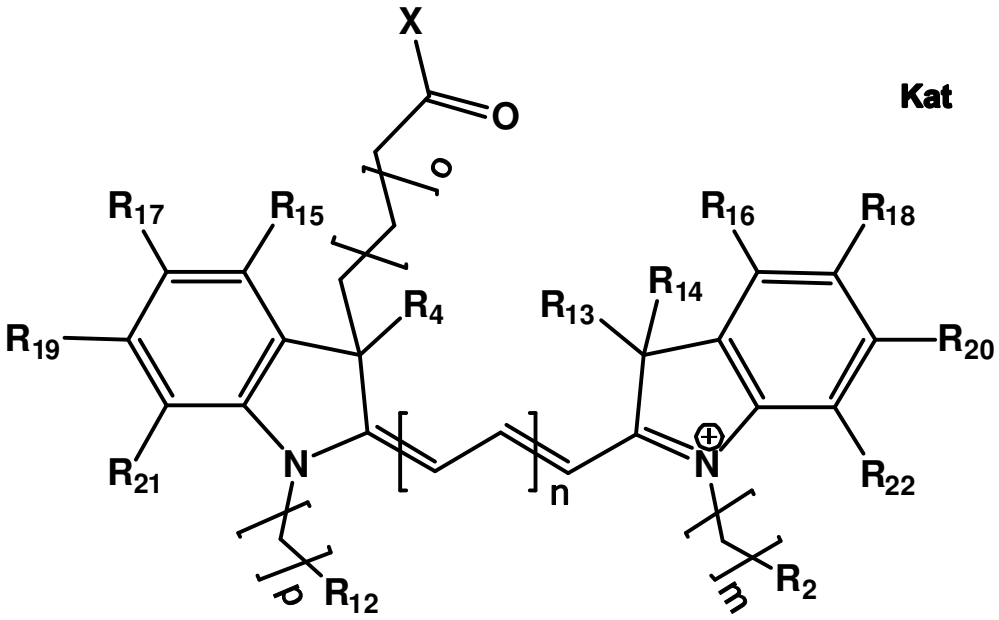
(ii) incubating the mixture of step (i) with a DNA polymerase under conditions sufficient to amplify one or more target nucleic acid molecules; and

(iii) detecting the presence or absence or quantifying the amount of the amplified target nucleic acid molecules by measuring fluorescence of the probe, wherein the probe comprises a product of conjugation of:

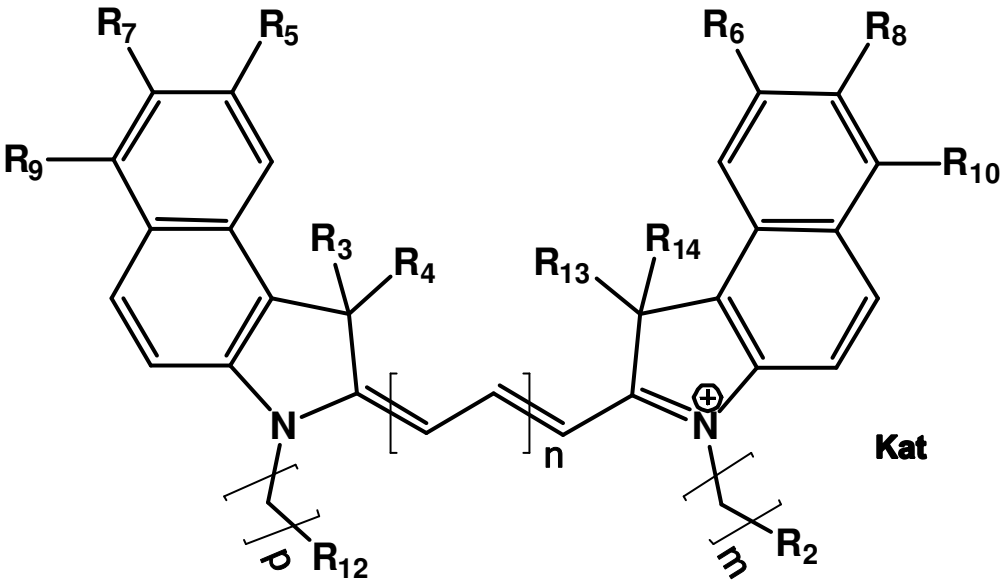
a) a dye having a general Formula Ia, Formula Ib, Formula Ic, or Formula Id



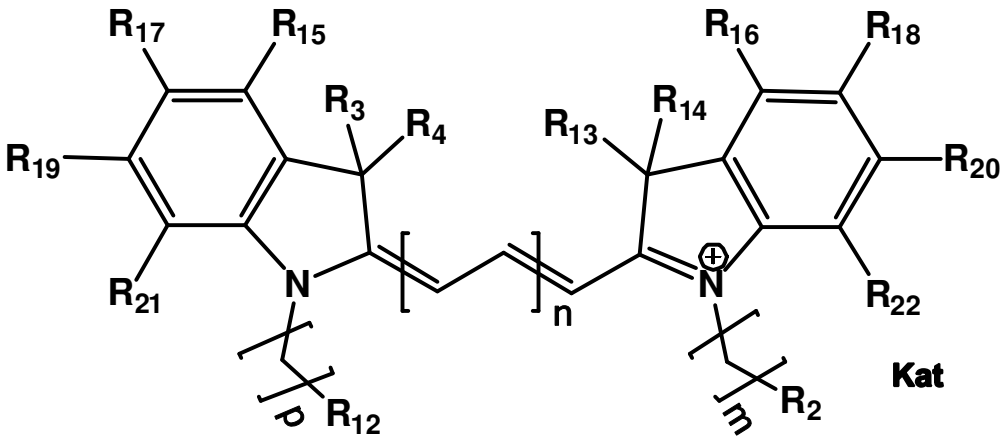
Formula Ia



Formula Ib



Formula Ic



Formula Id

wherein

each of R_4, R_{13}, and R_{14}, and R_3 when present, is the same or different and is selected from the group consisting of H, an aliphatic, a heteroaliphatic, a sulfoalkyl, a heteroaliphatic with terminal SO_3, a benzyl, and a substituted benzyl, where the substituted benzyl comprises at least one carboxy group, at least one sulfonate group, -F, -Cl, -Br, or a combination thereof;

each of R_5, R_6, R_7, R_8, R_9, R_{10}, R_{15}, R_{16}, R_{17}, R_{18}, R_{19}, R_{20}, R_{21}, and R_{22} is the same or different and is selected from the group consisting of H and SO_3;

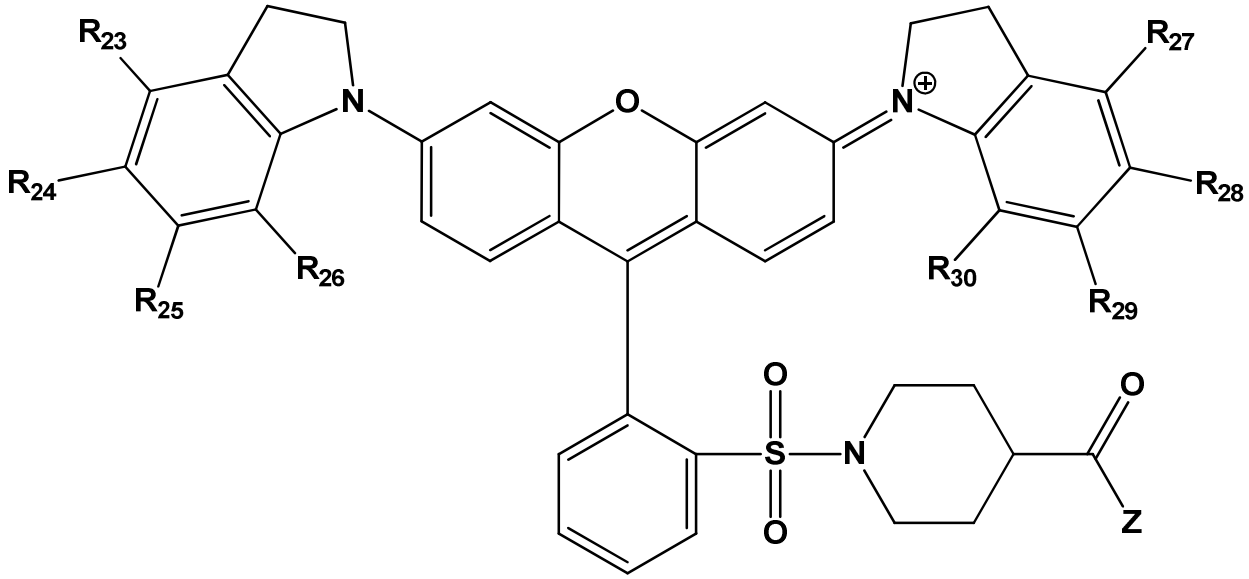
each of R_2 and R_{12} is the same or different and is selected from the group consisting of H and SO_3;

X is selected from the group consisting of -OH, -SH, $-NH_2$, $-NH-NH_2$, -F, -Cl, -Br, I, -O-NHS (hydroxysuccinimidyl/sulfosuccinimidyl), -O-TFP (2,3,5,6-tetrafluorophenoxy), -O-STP (4-sulfo-2,3,5,6-tetrafluorophenoxy), -O-benzotriazole, -benzotriazole, -NR-L-OH, -NR-L-O-phosphoramidite, -NR-L-SH, $-NR-L-NH_2$, $-NR-L-NH-NH_2$, $-NR-L-CO_2H$, -NR-L-CO-NHS, -NR-L-CO-STP, -NR-L-CO-TFP, -NR-L-CO-benzotriazole, -NR-L-CHO, -NR-L-maleimide, $NH(CH_2CH_2O)_zCH_2CH_2N_3$, $-NR-L-NH-CO-CH_2-I$, and an azide (N_3)-containing group, where R is -H or an aliphatic or heteroaliphatic group, z is an integer from 1 to 5 inclusive, and L is selected from the group consisting of a divalent linear, crossed-coupled, or cyclic alkyl group optionally substituted by at least one oxygen atom and/or sulfur atom;

Kat is a number of Na^+, K^+, Ca^{2+}, ammonia, or other cation(s) needed to compensate the negative charge brought by the cyanine;

m is an integer from 0 to 5 inclusive; n is an integer from 1 to 3 inclusive; o is an integer from 0 to 12 inclusive; and p is an integer from 0 to 5 inclusive;

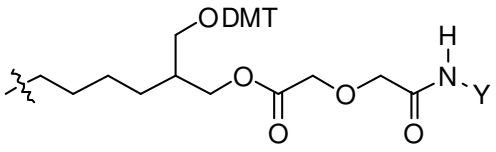
b) a quencher having general Formula II



Formula II

wherein

each of R_{23}, R_{24}, R_{25}, R_{26}, R_{27}, R_{28}, R_{29}, and R_{30} is the same or different and is independently selected from either H or SO_3;

Z is OR, where R is alkyl, or NH-L, where L is 

and Y is either H or a linkage to a solid support;

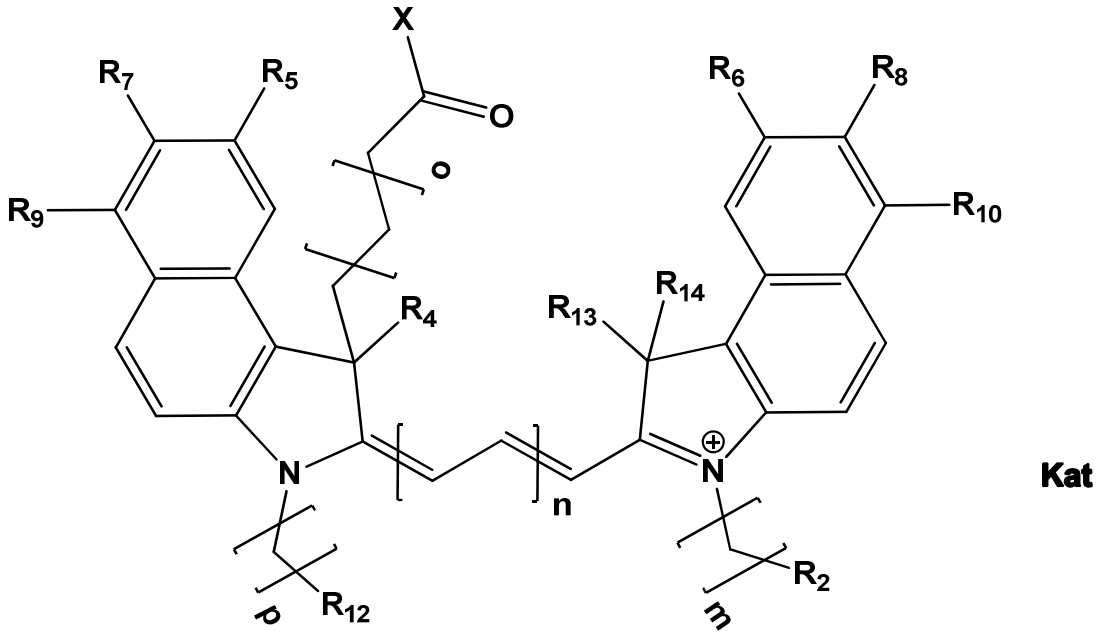
and

c) at least one oligonucleotide linker joining the dye and the quencher, wherein the dye is covalently joined to a first nucleotide of the oligonucleotide and the quencher is covalently joined to a second, different nucleotide of the oligonucleotide.

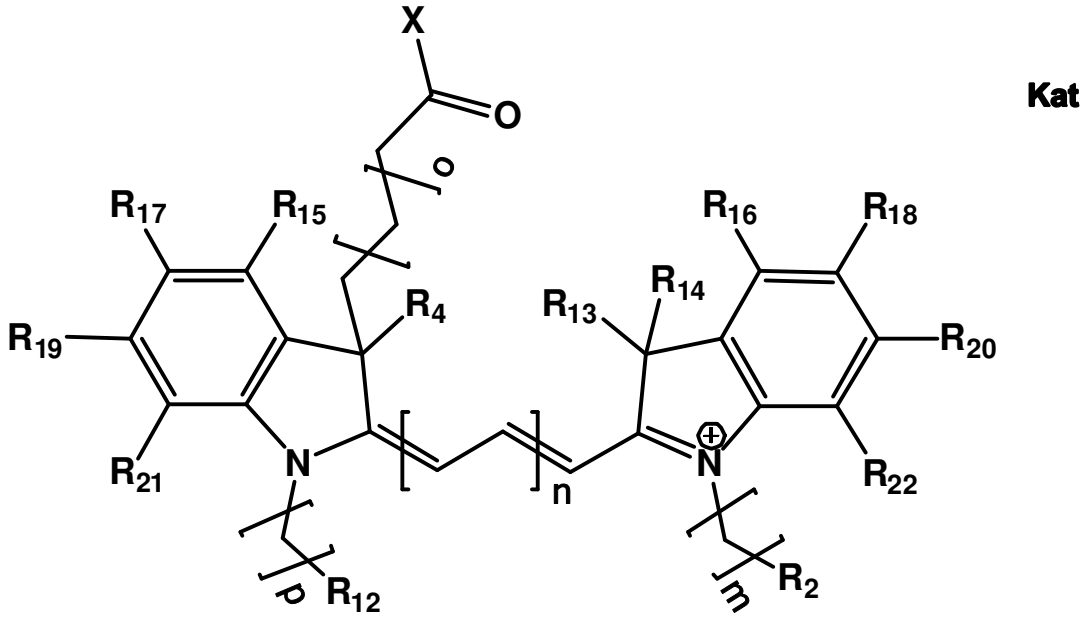
In another aspect, provided herein is a kit for polymerase chain reaction (PCR), the kit comprising:

a probe comprising a product of conjugation of:

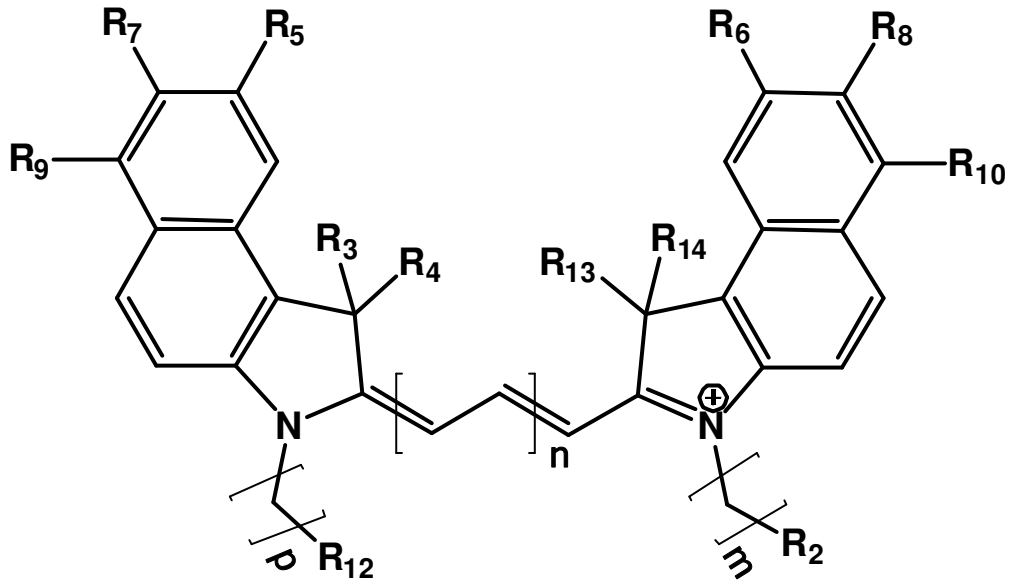
a) a dye having a general Formula Ia, Formula Ib, Formula Ic, or Formula Id



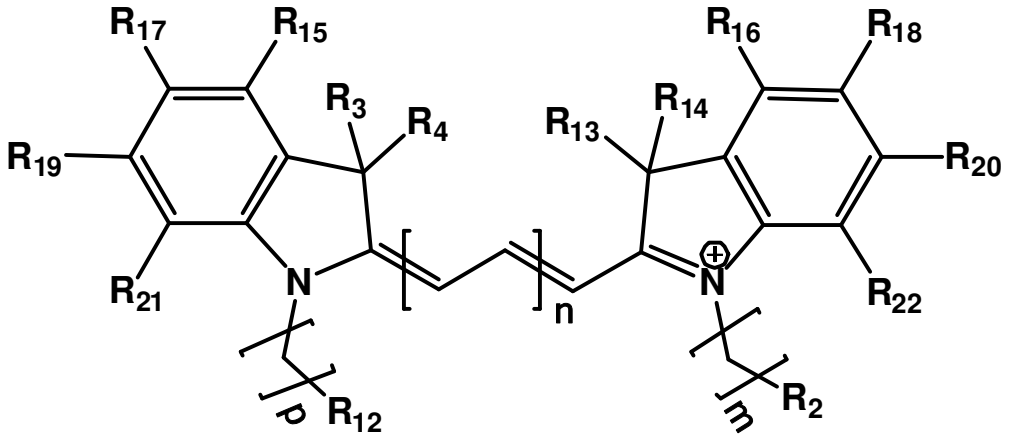
Formula Ia



Formula Ib



Formula Ic



Formula Id

wherein

each of R_4, R_{13}, and R_{14}, and R_3 when present, is the same or different and is selected from the group consisting of H, an aliphatic, a heteroaliphatic, a sulfoalkyl, a heteroaliphatic with terminal SO_3, a benzyl, and a substituted benzyl, where the substituted benzyl comprises at least one carboxy group, at least one sulfonate group, -F, -Cl, -Br, or a combination thereof;

each of R_5, R_6, R_7, R_8, R_9, R_{10}, R_{15}, R_{16}, R_{17}, R_{18}, R_{19}, R_{20}, R_{21}, and R_{22} is the same or different and is selected from the group consisting of H and SO_3;

each of R_2 and R_{12} is the same or different and is selected from the group consisting of H and SO_3;

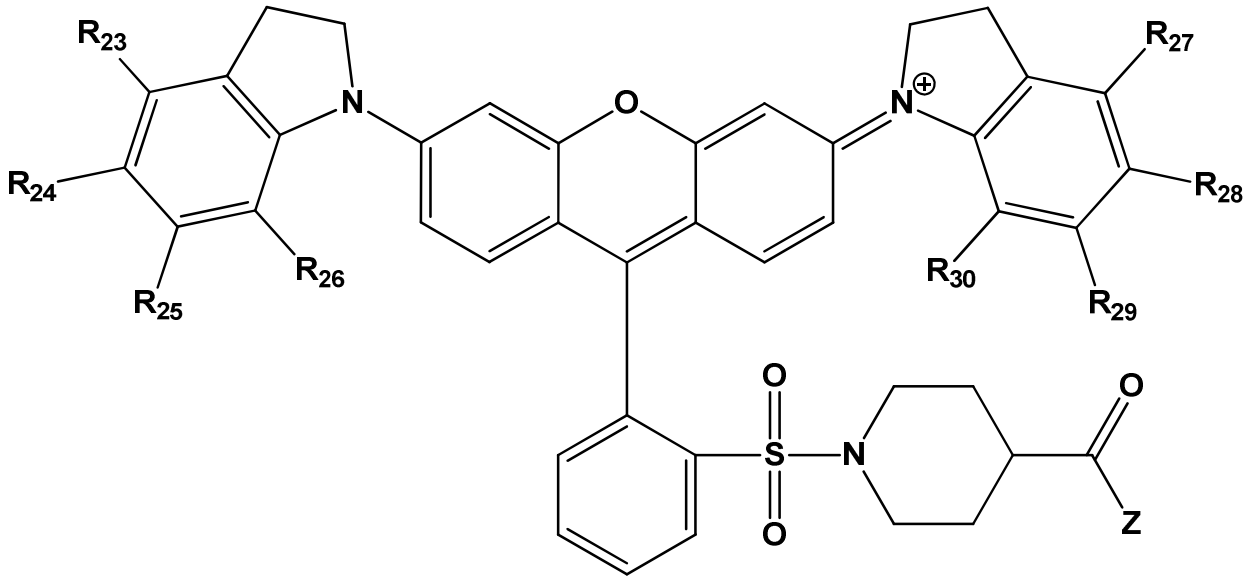
X is selected from the group consisting of -OH, -SH, -NH_2, -NH-NH_2, -F, -Cl, -Br, I, -O-NHS (hydroxysuccinimidyl/sulfosuccinimidyl), -O-TFP (2,3,5,6-tetrafluorophenoxy), -O-STP (4-sulfo-2,3,5,6-

tetrafluorophenoxy), -O-benzotriazole, -benzotriazole, -NR-L-OH, -NR-L-O-phosphoramidite, -NR-L-SH, -NR-L-NH_2, -NR-L-NH-NH_2, -NR-L-CO_2H, -NR-L-CO-NHS,
-NR-L-CO-STP, -NR-L-CO-TFP, -NR-L-CO-benzotriazole, -NR-L-CHO, -NR-L-maleimide, $NH(CH_2CH_2O)_zCH_2CH_2N_3$, -NR-L-NH-CO-$CH_2$-I, and an azide ($N_3$)-containing group, where R is -H or an aliphatic or heteroaliphatic group, z is an integer from 1 to 5 inclusive, and L is selected from the group consisting of a divalent linear, crossed-coupled, or cyclic alkyl group optionally substituted by at least one oxygen atom and/or sulfur atom;

Kat is a number of Na^+, K^+, Ca^{2+}, ammonia, or other cation(s) needed to compensate the negative charge brought by the cyanine;

m is an integer from 0 to 5 inclusive; n is an integer from 1 to 3 inclusive; o is an integer from 0 to 12 inclusive; and p is an integer from 0 to 5 inclusive;

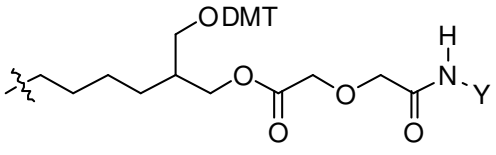
b) a quencher having general Formula II



Formula II

wherein

each of R_{23}, R_{24}, R_{25}, R_{26}, R_{27}, R_{28}, R_{29}, and R_{30} is the same or different and is independently selected from either H or SO_3;

Z is OR, where R is alkyl, or NH-L, where L is 

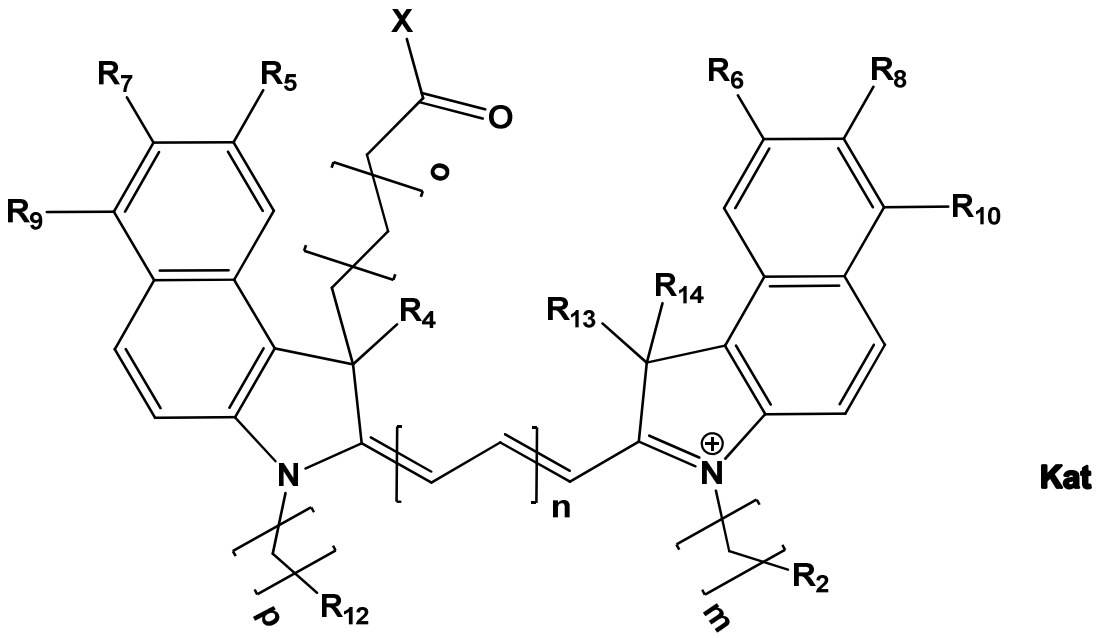
and Y is either H or a linkage to a solid support;

and

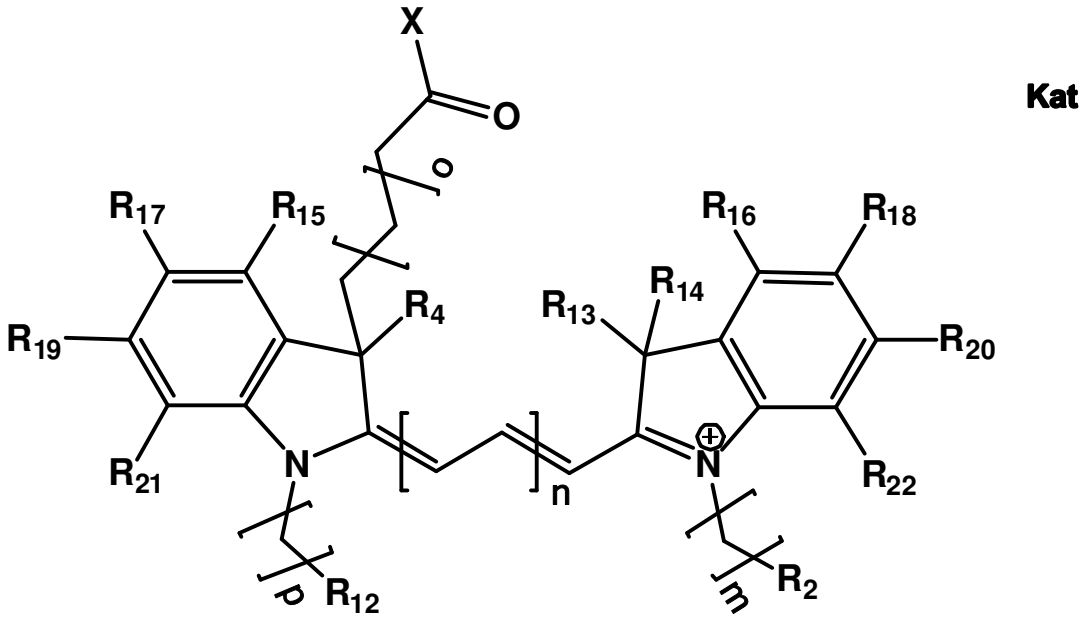
c) at least one oligonucleotide linker joining the dye and the quencher, wherein the dye is covalently joined to a first nucleotide of the oligonucleotide and the quencher is covalently joined to a second, different nucleotide of the oligonucleotide; and

one or more of the following: a buffering agent, a purification medium, the target, an organic solvent, an enzyme, and an enzyme inhibitor.

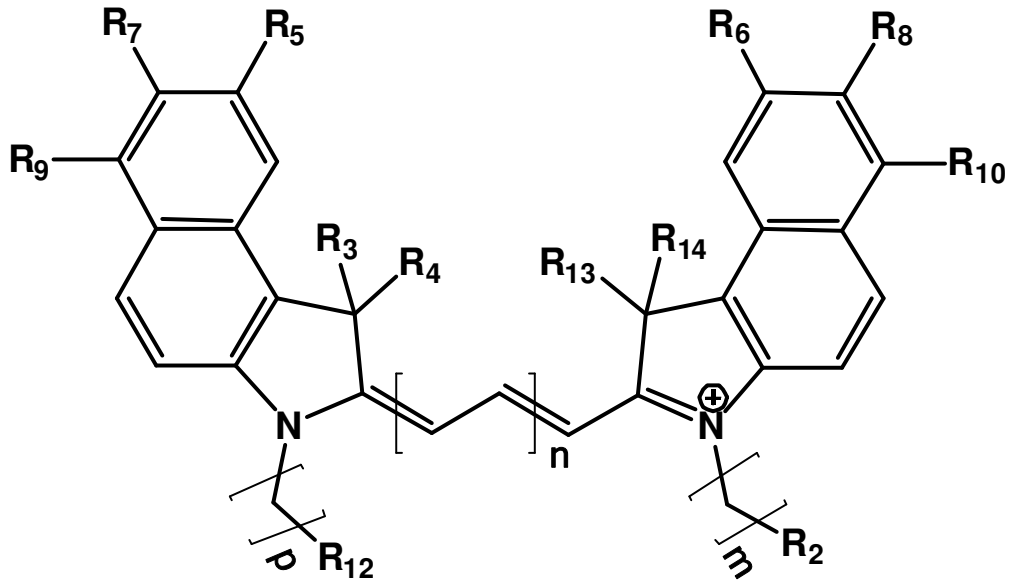
In another aspect, provided herein is a kit comprising at least one a dye having a general Formula Ia, Formula Ib, Formula Ic, or Formula Id



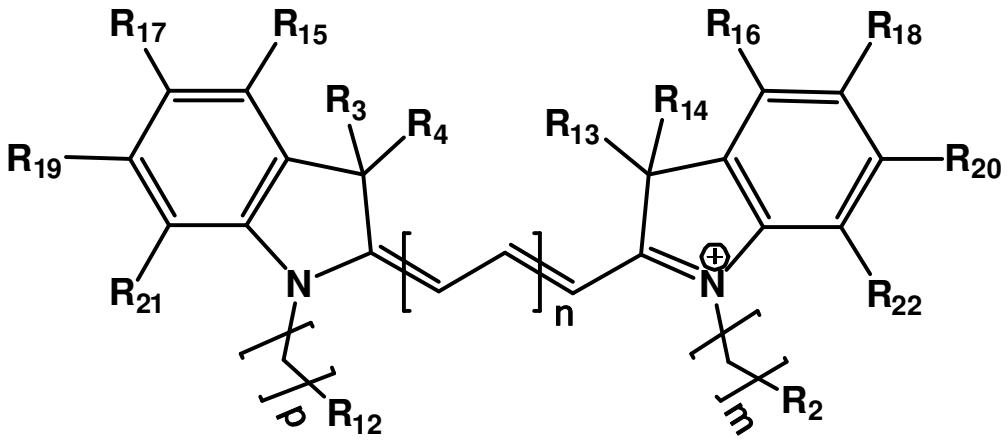
Formula Ia



Formula Ib



Formula Ic



Formula Id

wherein

each of R_4, R_{13}, and R_{14}, and R_3 when present, is the same or different and is selected from the group consisting of H, an aliphatic, a heteroaliphatic, a sulfoalkyl, a heteroaliphatic with terminal SO_3, a benzyl, and a substituted benzyl, where the substituted benzyl comprises at least one carboxy group, at least one sulfonate group, -F, -Cl, -Br, or a combination thereof;

each of R_5, R_6, R_7, R_8, R_9, R_{10}, R_{15}, R_{16}, R_{17}, R_{18}, R_{19}, R_{20}, R_{21}, and R_{22} is the same or different and is selected from the group consisting of H and SO_3;

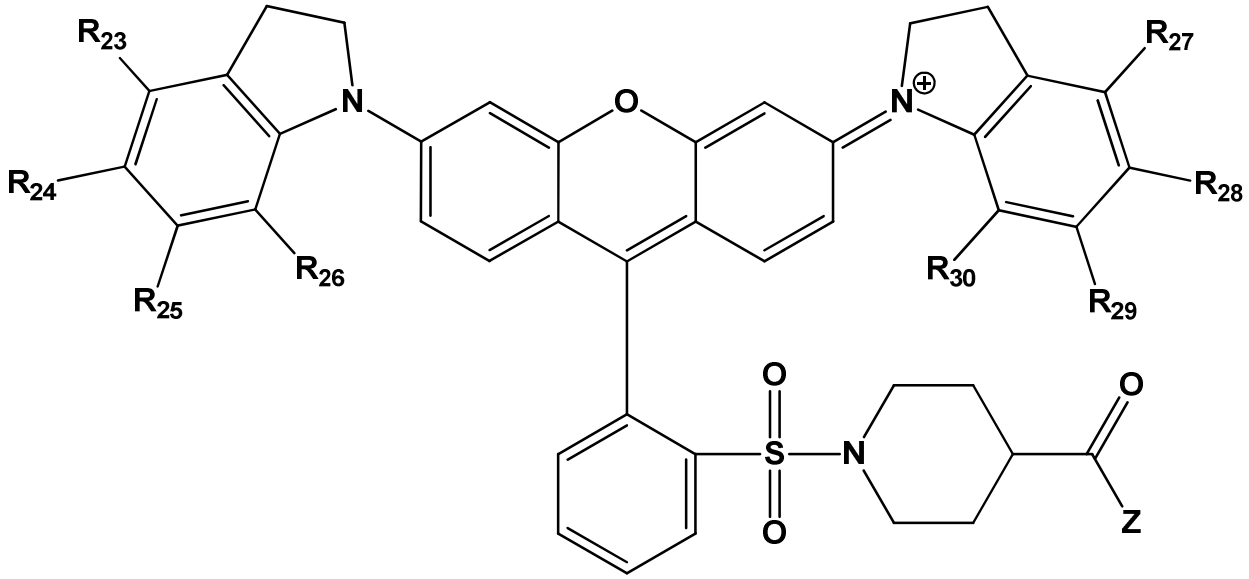
each of R_2 and R_{12} is the same or different and is selected from the group consisting of H and SO_3;

X is selected from the group consisting of -OH, -SH, -NH_2, -NH-NH_2, -F, -Cl, -Br, I, -O-NHS (hydroxysuccinimidyl/sulfosuccinimidyl), -O-TFP (2,3,5,6-tetrafluorophenoxy), -O-STP (4-sulfo-2,3,5,6-tetrafluorophenoxy), -O-benzotriazole, -benzotriazole, -NR-L-OH, -NR-L-O-phosphoramidite, -NR-L-SH, -NR-L-NH_2, -NR-L-NH-NH_2, -NR-L-CO_2H, -NR-L-CO-NHS, -NR-L-CO-STP, -NR-L-CO-TFP, -NR-L-CO-benzotriazole, -NR-L-CHO, -NR-L-maleimide, $NH(CH_2CH_2O)_zCH_2CH_2N_3$, -NR-L-NH-CO-$CH_2$-I, and an azide ($N_3$)-containing group, where R is -H or an aliphatic or heteroaliphatic group, z is an integer from 1 to 5 inclusive, and L is selected from the group consisting of a divalent linear, crossed-coupled, or cyclic alkyl group optionally substituted by at least one oxygen atom and/or sulfur atom;

Kat is a number of Na^+, K^+, Ca^{2+}, ammonia, or other cation(s) needed to compensate the negative charge brought by the cyanine;

m is an integer from 0 to 5 inclusive; n is an integer from 1 to 3 inclusive; o is an integer from 0 to 12 inclusive; and p is an integer from 0 to 5 inclusive; and

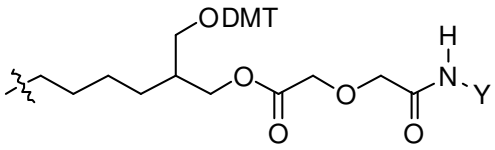
at least one quencher having general Formula II



Formula II

wherein

each of R_{23}, R_{24}, R_{25}, R_{26}, R_{27}, R_{28}, R_{29}, and R_{30} is the same or different and is independently selected from either H or SO_3;



Z is OR, where R is alkyl, or Z is NH-L, where L is

and Y is either H or a linkage to a solid support.

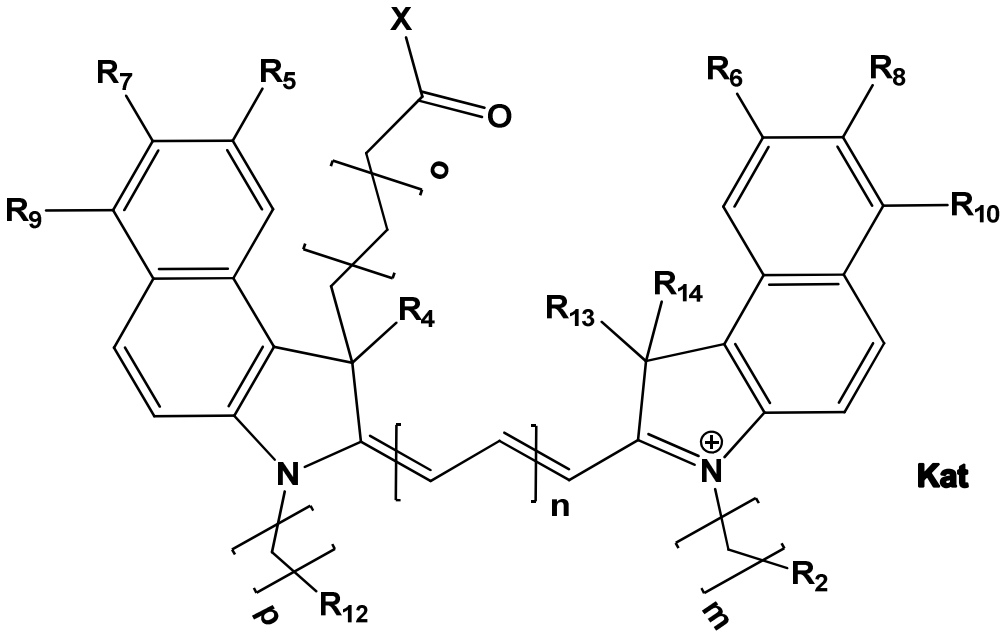
Detailed Description

[0025] The hydrolysis probe assay exploits the 5′ exonuclease activity of certain DNA polymerases, such as Taq, to cleave a labeled probe during PCR. One specific example

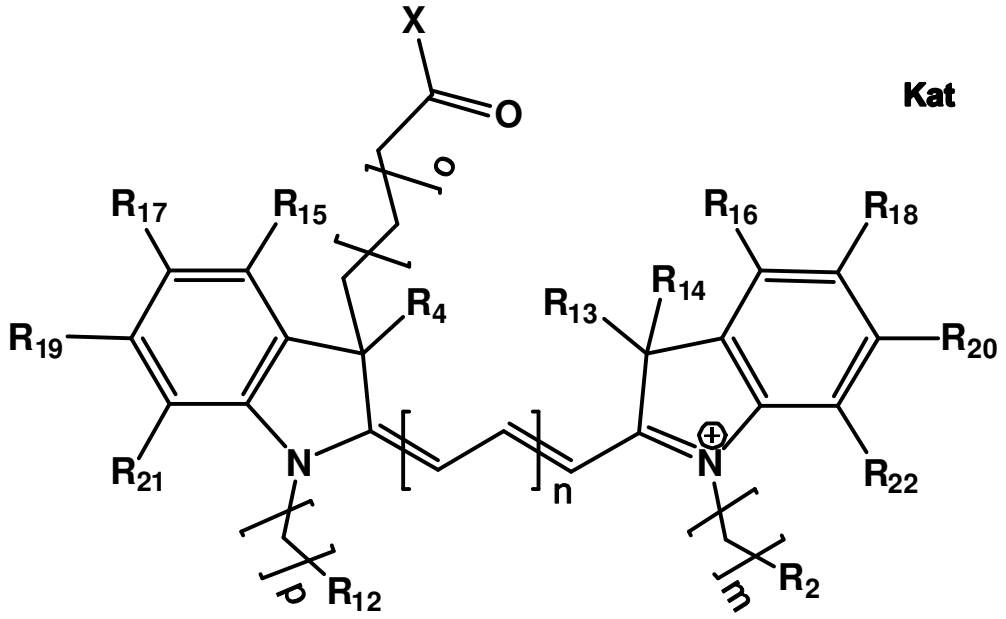
We Claim:

1. A probe comprising a product of conjugation of:

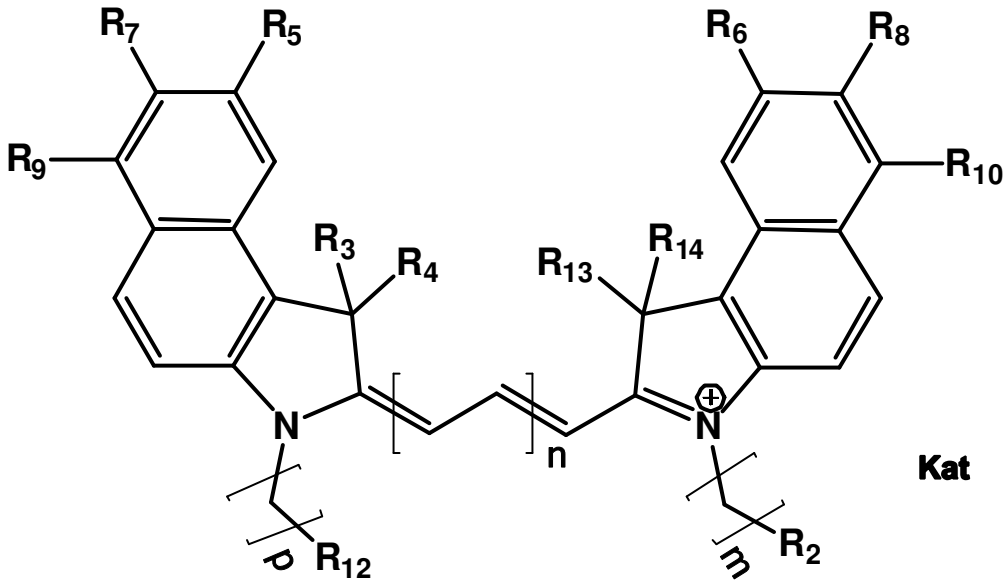
a) a dye having a general Formula Ia, Formula Ib, Formula Ic, or Formula Id



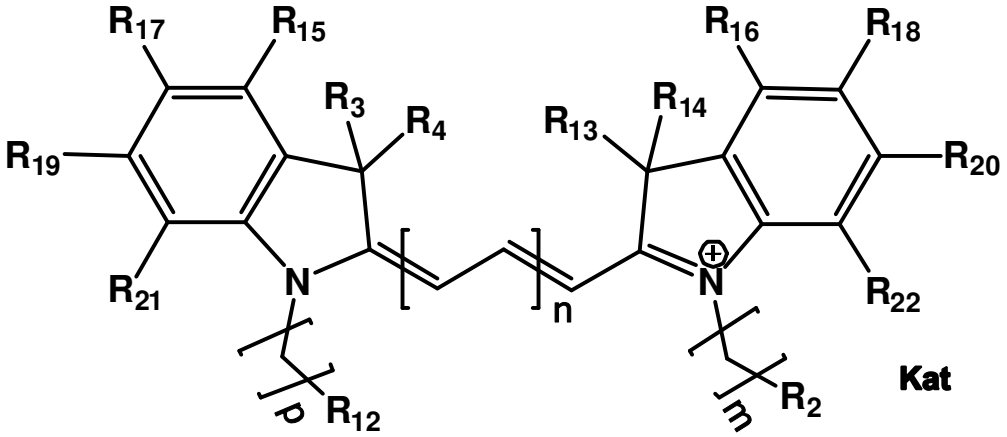
Formula Ia



Formula Ib



Formula Ic



Formula Id

wherein

each of R_4, R_{13}, and R_{14}, and R_3 when present, is the same or different and is selected from the group consisting of H, an aliphatic, a heteroaliphatic, a sulfoalkyl, a heteroaliphatic with terminal SO_3, a benzyl, and a substituted benzyl, where the substituted benzyl comprises at least one carboxy group, at least one sulfonate group, -F, -Cl, -Br, or a combination thereof;

each of R_5, R_6, R_7, R_8, R_9, R_{10}, R_{15}, R_{16}, R_{17}, R_{18}, R_{19}, R_{20}, R_{21}, and R_{22} is the same or different and is selected from the group consisting of H and SO_3;

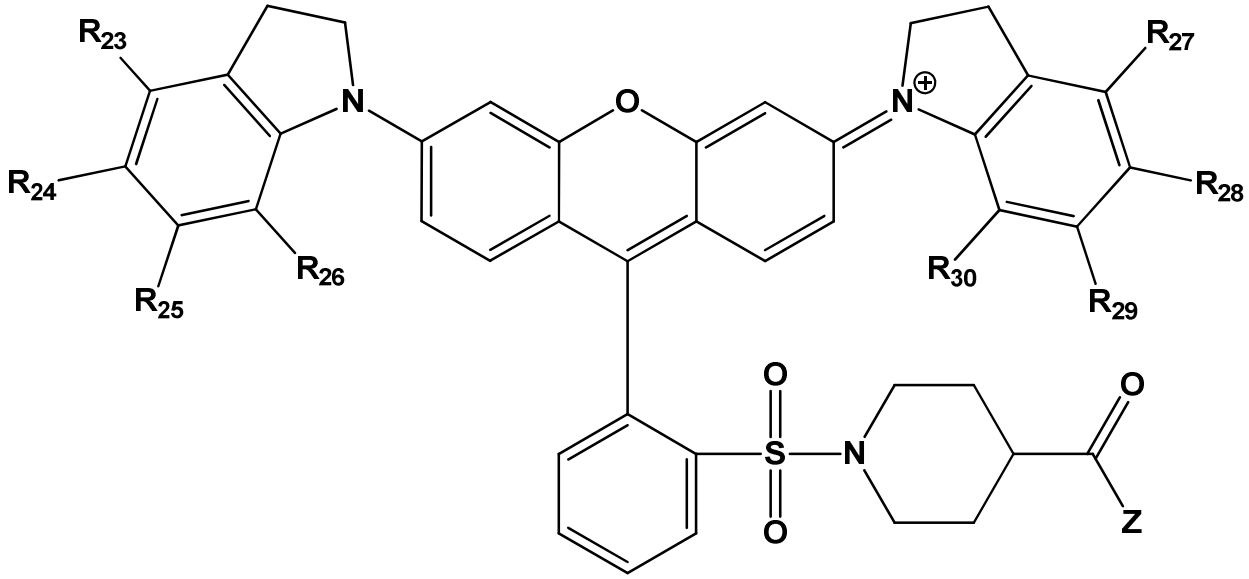
each of R_2 and R_{12} is the same or different and is selected from the group consisting of H and SO_3;

X is selected from the group consisting of -OH, -SH, -NH_2, -NH-NH_2, -F, -Cl, -Br, I, -O-NHS (hydroxysuccinimidyl/sulfosuccinimidyl), -O-TFP (2,3,5,6-tetrafluorophenoxy), -O-STP (4-sulfo-2,3,5,6-tetrafluorophenoxy), -O-benzotriazole, -benzotriazole, -NR-L-OH, -NR-L-O-phosphoramidite, -NR-L-SH, -NR-L-NH_2, -NR-L-NH-NH_2, -NR-L-CO_2H, -NR-L-CO-NHS, -NR-L-CO-STP, -NR-L-CO-TFP, -NR-L-CO-benzotriazole, -NR-L-CHO, -NR-L-maleimide, -NH$(CH_2CH_2O)_zCH_2CH_2N_3$, -NR-L-NH-CO-CH_2-I, and an azide (N_3)-containing group, where R is -H or an aliphatic or heteroaliphatic group, z is an integer from 1 to 5 inclusive, and L is selected from the group consisting of a divalent linear, crossed-coupled, or cyclic alkyl group optionally substituted by at least one oxygen atom and/or sulfur atom;

Kat is a number of Na^+, K^+, Ca^{2+}, ammonia, or other cation(s) needed to compensate the negative charge brought by the cyanine;

m is an integer from 0 to 5 inclusive; n is an integer from 1 to 3 inclusive; o is an integer from 0 to 12 inclusive; and p is an integer from 0 to 5 inclusive;

b) a quencher having general Formula II

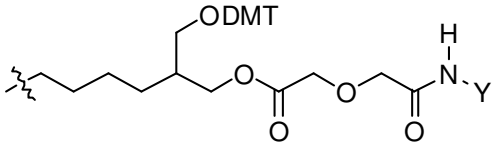


Formula II

wherein

each of R_{23}, R_{24}, R_{25}, R_{26}, R_{27}, R_{28}, R_{29}, and R_{30} is the same or different and is independently selected from either H or SO_3;

Z is OR, where R is alkyl, or Z is NH-L, where L is



and Y is either H or a linkage to a solid support; and

c) an oligonucleotide linker joining the dye and the quencher, wherein the dye is covalently joined to a first nucleotide of the oligonucleotide and the quencher is covalently joined to a second, different nucleotide of the oligonucleotide.

2. The probe of claim 1, wherein:

R_5, R_6, R_9, and R_{10} are SO_3;

R_7 and R_8 are H;

R_4, R_{13}, and R_{14} are methyl;

R_2 and R_{12} are SO_3;

m, o, and p are 3; and n is 2.

3. The probe of claim 1, wherein:

R_{10} is SO_3;

R_5, R_6, R_7, R_8, and R_9 are H;

R_4, R_{13}, and R_{14} are methyl;

R_{12} is H;

R_2 is SO_3;

m and o are 3; and n and p are 2.

4. The probe of claim 1, wherein:

R_{10} is SO_3;

R_5, R_6, R_7, R_8, and R_9 are H;

R_4, R_{13}, and R_{14} are methyl;

R_2 and R_{12} are SO_3;

m, o, and p are 3; and n is 2.

5. The probe of claim 1, wherein:

R_9 and R_{10} are SO_3;

R_5, R_6, R_7, and R_8 are H;

R_4, R_{13}, and R_{14} are methyl;

R_{12} is H;

R_2 is SO_3;

m and o are 3; and n and p are 2.

6. The probe of claim 1, wherein:

R_9 and R_{10} are SO_3;

R_5, R_6, R_7, and R_8 are H;

R_4, R_{13}, and R_{14} are methyl;

R_2 and R_{12} are SO_3;

m, o, and p are 3; and n is 2.

7. The probe of claim 1, wherein:

R_5, R_6, R_7, R_8, R_9, and R_{10} are H;

R_4, R_{13}, and R_{14} are methyl;

R_2 and R_{12} are H;

m and p are 1; n is 2; and o is 3.

8. The probe of claim 1, wherein:

R_{15}, R_{16}, R_{17}, R_{18}, R_{19}, R_{20}, R_{21}, and R_{22} are H;

R_4, R_{13}, and R_{14} are methyl;

R_2 and R_{12} are H;

m and p are 1; n is 2; and o is 3.

9. The probe of claim 1, wherein:

R_{17} and R_{18} are SO_3;

R_{15}, R_{16}, R_{19}, R_{20}, R_{21}, and R_{22} are H;

R_4, R_{13}, and R_{14} are methyl;

R_2 and R_{12} are SO_3;

m, o, and p are 3; and n is 2.

10. The probe of claim 1, wherein:

R_5, R_6, R_9, and R_{10} are SO_3;

R_7 and R_8 are H;

R_4, R_{13}, and R_{14} are methyl;

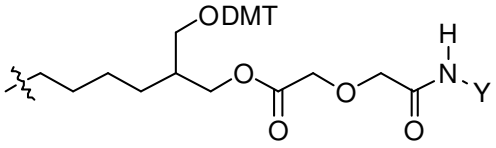
R_3 is -C-benzoate;

R_2 is SO_3 and R_{12} is H;

m is 3; p is 1; and n is 2.

11. The probe of claim 1, wherein each of R_{23}, R_{25}, R_{26}, R_{27}, R_{29}, and R_{30} is H and each of R_{24} and R_{28} is SO_3.

12. The probe of claim 1, wherein each of R_{23}, R_{24}, R_{25}, R_{26}, R_{27}, R_{28}, R_{29}, and R_{30} is H.

13. The probe of claim 1, wherein Z is NH-L, L being 

and Y being the linkage to the solid support.

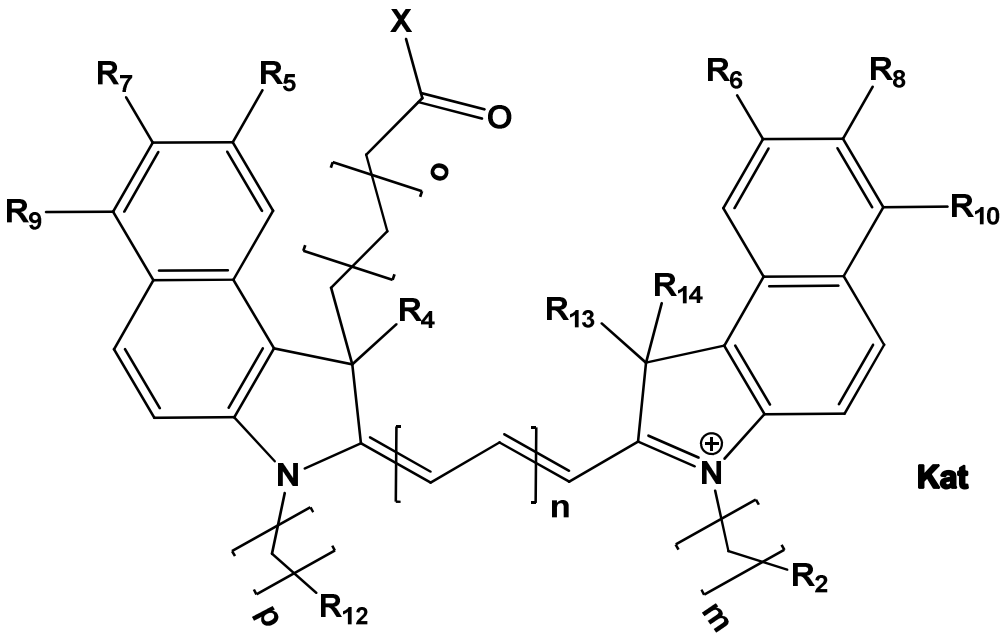
14. A method of detecting or quantifying a target nucleic acid molecule in a sample by polymerase chain reaction (PCR), the method comprising:

(i) contacting the sample comprising one or more target nucleic acid molecules with a) at least one probe having a sequence that is at least partially complementary to the target nucleic acid molecule, where the at least one probe undergoes a detectable change in fluorescence upon amplification of the one or more target nucleic acid molecules; and with b) at least one oligonucleotide primer pair;

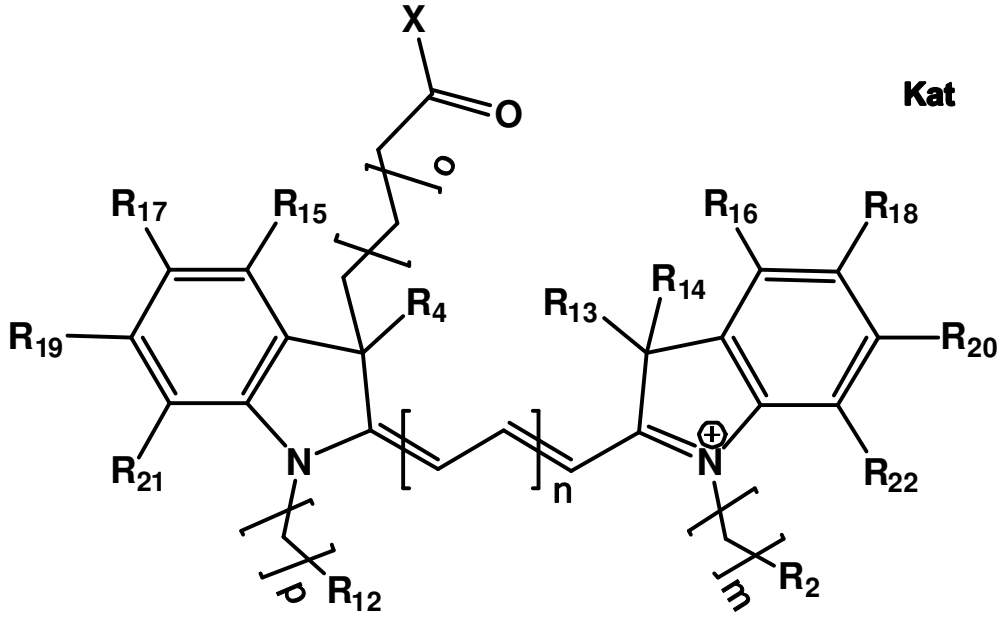
(ii) incubating the mixture of step (i) with a DNA polymerase under conditions sufficient to amplify one or more target nucleic acid molecules; and

(iii) detecting the presence or absence or quantifying the amount of the amplified target nucleic acid molecules by measuring fluorescence of the probe, wherein the probe comprises a product of conjugation of:

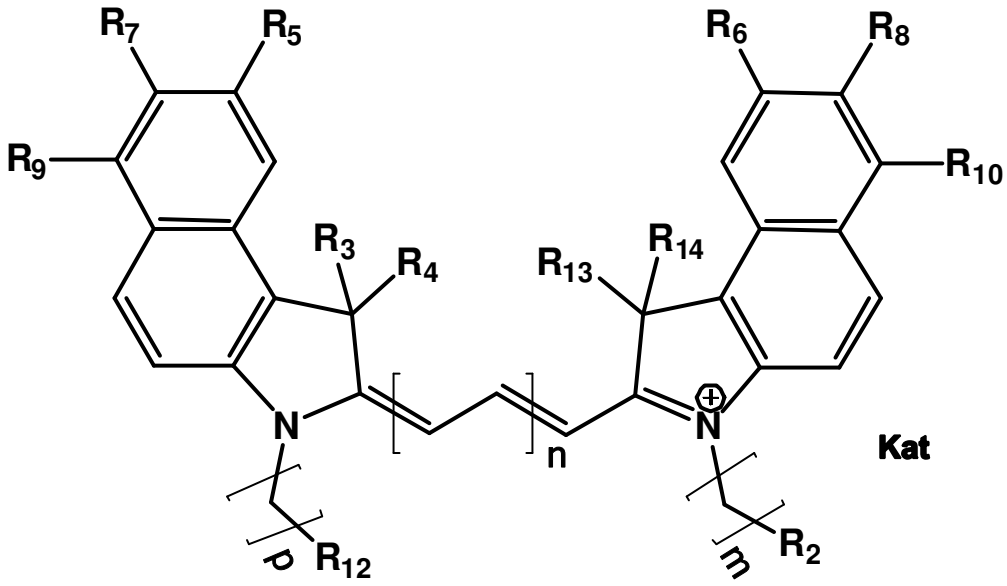
a) a dye having a general Formula Ia, Formula Ib, Formula Ic, or Formula Id



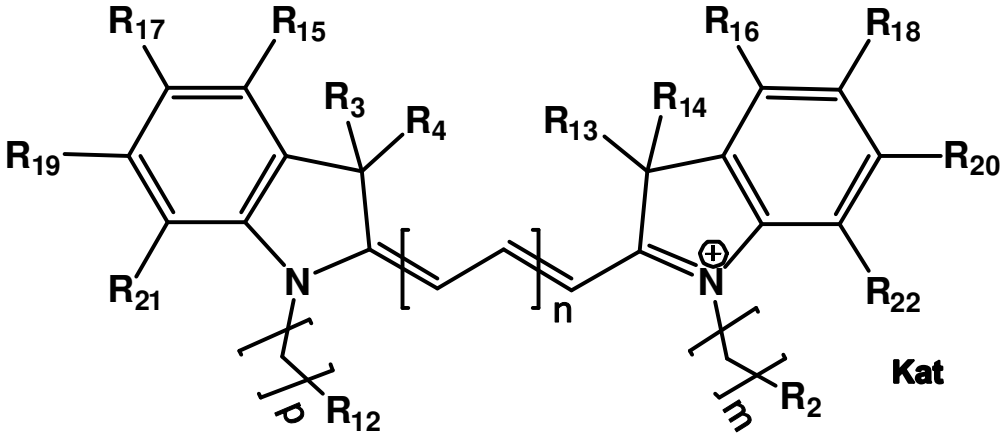
Formula Ia



Formula Ib



Formula Ic



Formula Id

wherein

each of R_4, R_{13}, and R_{14}, and R_3 when present, is the same or different and is selected from the group consisting of H, an aliphatic, a heteroaliphatic, a sulfoalkyl, a heteroaliphatic with terminal SO_3, a benzyl, and a substituted benzyl, where the substituted benzyl comprises at least one carboxy group, at least one sulfonate group, -F, -Cl, -Br, or a combination thereof;

each of R_5, R_6, R_7, R_8, R_9, R_{10}, R_{15}, R_{16}, R_{17}, R_{18}, R_{19}, R_{20}, R_{21}, and R_{22} is the same or different and is selected from the group consisting of H and SO_3;

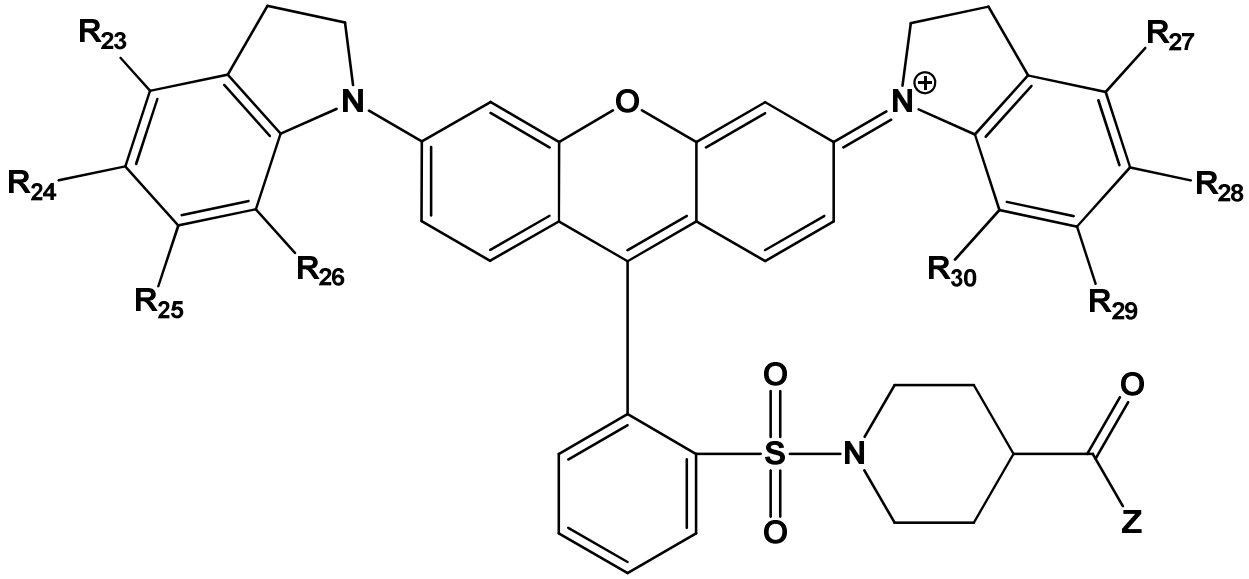
each of R_2 and R_{12} is the same or different and is selected from the group consisting of H and SO_3;

X is selected from the group consisting of -OH, -SH, -NH_2, -NH-NH_2, -F, -Cl, -Br, I, -O-NHS (hydroxysuccinimidyl/sulfosuccinimidyl), -O-TFP (2,3,5,6-tetrafluorophenoxy), -O-STP (4-sulfo-2,3,5,6-tetrafluorophenoxy), -O-benzotriazole, -benzotriazole, -NR-L-OH, -NR-L-O-phosphoramidite, -NR-L-SH, -NR-L-NH_2, -NR-L-NH-NH_2, -NR-L-CO_2H, -NR-L-CO-NHS, -NR-L-CO-STP, -NR-L-CO-TFP, -NR-L-CO-benzotriazole, -NR-L-CHO, -NR-L-maleimide, $NH(CH_2CH_2O)_zCH_2CH_2N_3$, -NR-L-NH-CO-$CH_2$-I, and an azide ($N_3$)-containing group, where R is -H or an aliphatic or heteroaliphatic group, z is an integer from 1 to 5 inclusive, and L is selected from the group consisting of a divalent linear, crossed-coupled, or cyclic alkyl group optionally substituted by at least one oxygen atom and/or sulfur atom;

Kat is a number of Na^+, K^+, Ca^{2+}, ammonia, or other cation(s) needed to compensate the negative charge brought by the cyanine;

m is an integer from 0 to 5 inclusive; n is an integer from 1 to 3 inclusive; o is an integer from 0 to 12 inclusive; and p is an integer from 0 to 5 inclusive;

b) a quencher having general Formula II

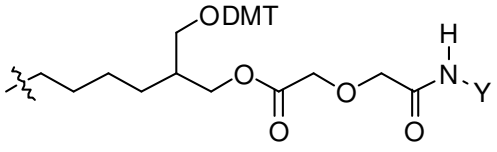


Formula II

wherein

each of R_{23}, R_{24}, R_{25}, R_{26}, R_{27}, R_{28}, R_{29}, and R_{30} is the same or different and is independently selected from either H or SO_3;

Z is OR, where R is alkyl, or Z is NH-L, where L is



and Y is either H or a linkage to a solid support;

and

c) at least one oligonucleotide linker joining the dye and the quencher, wherein the dye is covalently joined to a first nucleotide of the oligonucleotide and the quencher is covalently joined to a second, different nucleotide of the oligonucleotide.

15. The method of claim 14, wherein the PCR is real-time or quantitative PCR (qPCR).

16. The method of claim 14, wherein the polymerase is a Taq polymerase.

17. The method of claim 14, wherein the probe is a hydrolysis probe.

18. The method of claim 14, wherein the probe is a TaqMan probe.

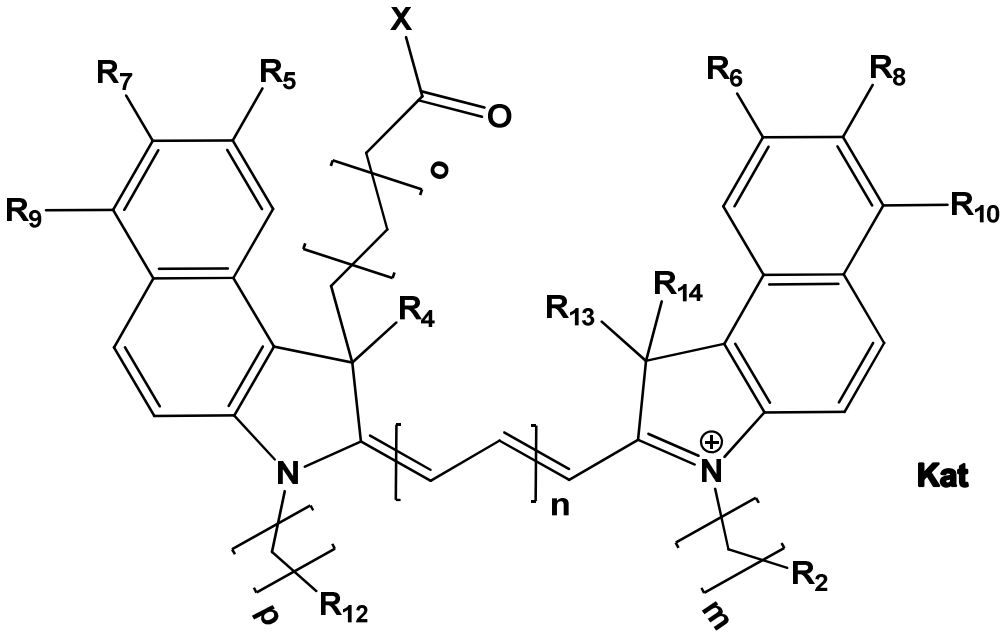
19. The method of claim 14, wherein the target nucleic acid comprises a mutation.

20. The method of claim 14, wherein the method is used for detection of a rare allele or SNP.

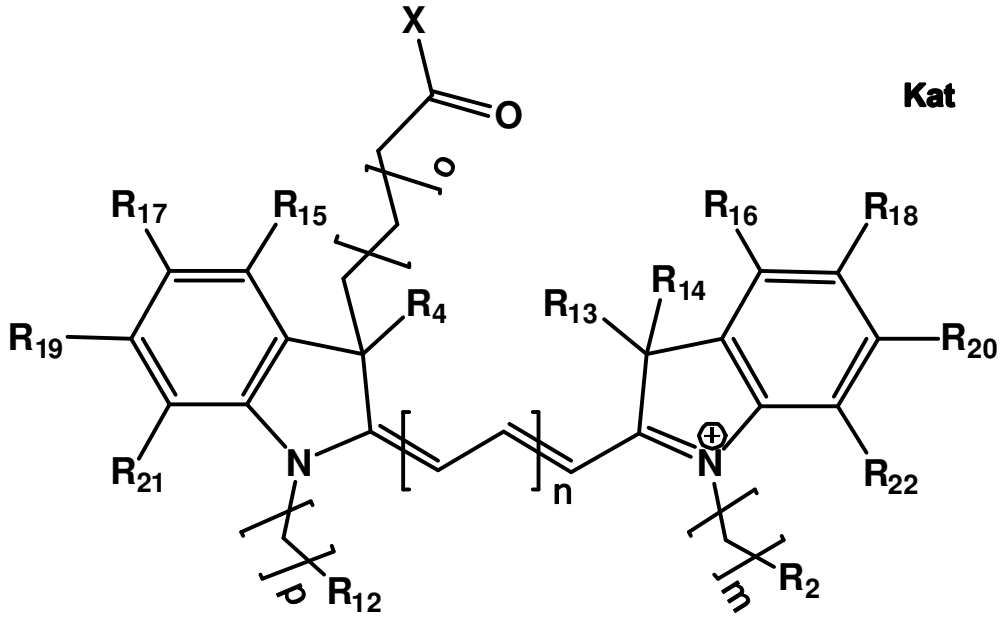
21. A kit for polymerase chain reaction (PCR), the kit comprising:

a probe comprising a product of conjugation of:

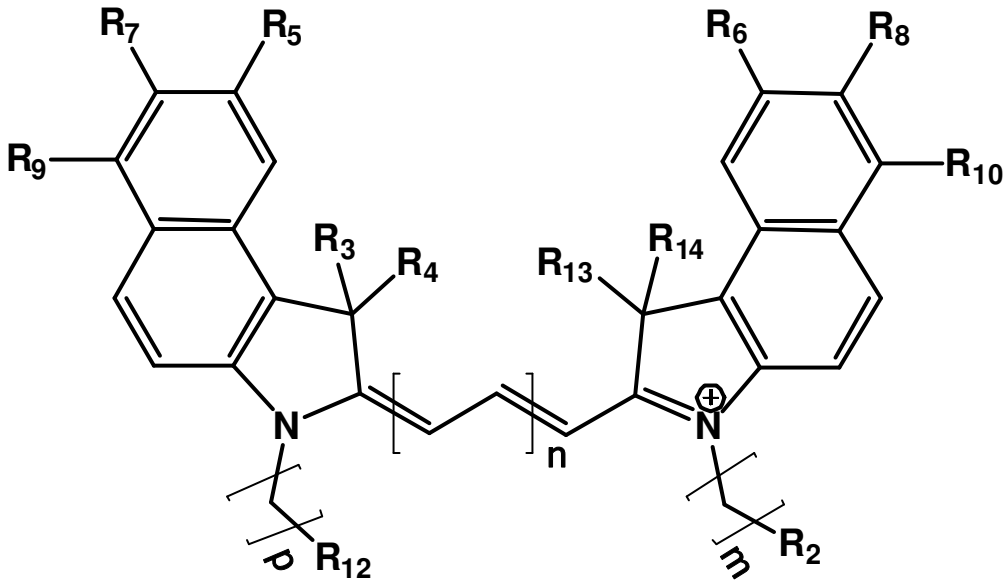
a) a dye having a general Formula Ia, Formula Ib, Formula Ic, or Formula Id



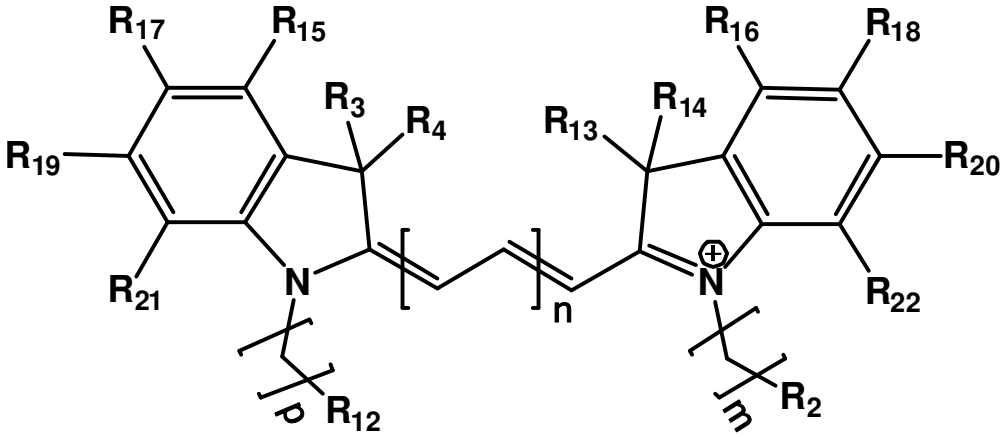
Formula Ia



Formula Ib



Formula Ic



Formula Id

wherein

each of R_4, R_{13}, and R_{14}, and R_3 when present, is the same or different and is selected from the group consisting of H, an aliphatic, a heteroaliphatic, a sulfoalkyl, a heteroaliphatic with terminal SO_3, a benzyl, and a substituted benzyl, where the substituted benzyl comprises at least one carboxy group, at least one sulfonate group, -F, -Cl, -Br, or a combination thereof;

each of R_5, R_6, R_7, R_8, R_9, R_{10}, R_{15}, R_{16}, R_{17}, R_{18}, R_{19}, R_{20}, R_{21}, and R_{22} is the same or different and is selected from the group consisting of H and SO_3;

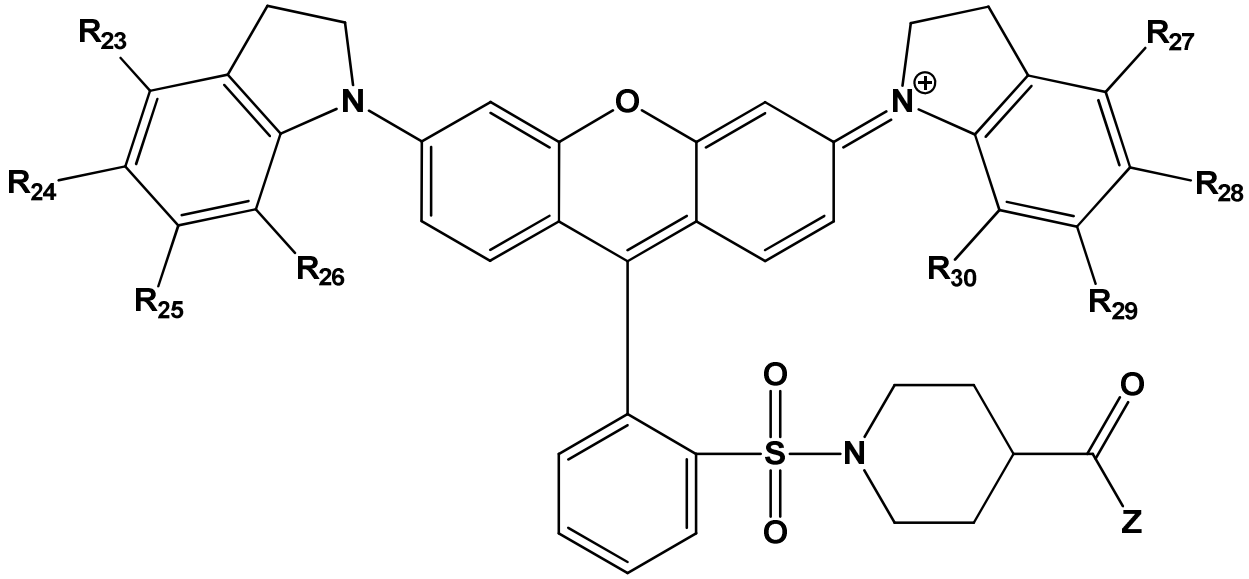
each of R_2 and R_{12} is the same or different and is selected from the group consisting of H and SO_3;

X is selected from the group consisting of -OH, -SH, -NH_2, -NH-NH_2, -F, -Cl, -Br, I, -O-NHS (hydroxysuccinimidyl/sulfosuccinimidyl), -O-TFP (2,3,5,6-tetrafluorophenoxy), -O-STP (4-sulfo-2,3,5,6-tetrafluorophenoxy), -O-benzotriazole, -benzotriazole, -NR-L-OH, -NR-L-O-phosphoramidite, -NR-L-SH, -NR-L-NH_2, -NR-L-NH-NH_2, -NR-L-CO_2H, -NR-L-CO-NHS, -NR-L-CO-STP, -NR-L-CO-TFP, -NR-L-CO-benzotriazole, -NR-L-CHO, -NR-L-maleimide, $NH(CH_2CH_2O)_zCH_2CH_2N_3$, -NR-L-NH-CO-$CH_2$-I, and an azide ($N_3$)-containing group, where R is -H or an aliphatic or heteroaliphatic group, z is an integer from 1 to 5 inclusive, and L is selected from the group consisting of a divalent linear, crossed-coupled, or cyclic alkyl group optionally substituted by at least one oxygen atom and/or sulfur atom;

Kat is a number of Na^+, K^+, Ca^{2+}, ammonia, or other cation(s) needed to compensate the negative charge brought by the cyanine;

m is an integer from 0 to 5 inclusive; n is an integer from 1 to 3 inclusive; o is an integer from 0 to 12 inclusive; and p is an integer from 0 to 5 inclusive;

b) a quencher having general Formula II

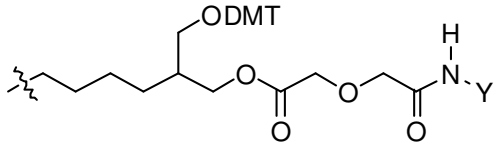


Formula II

wherein

each of R_{23}, R_{24}, R_{25}, R_{26}, R_{27}, R_{28}, R_{29}, and R_{30} is the same or different and is independently selected from either H or SO_3;

Z is OR, where R is alkyl, or Z is NH-L, where L is



and Y is either H or a linkage to a solid support;

and

c) at least one oligonucleotide linker joining the dye and the quencher, wherein the dye is covalently joined to a first nucleotide of the oligonucleotide and the quencher is covalently joined to a second, different nucleotide of the oligonucleotide; and

one or more of the following: a buffering agent, a purification medium, the target, an organic solvent, an enzyme, and an enzyme inhibitor.

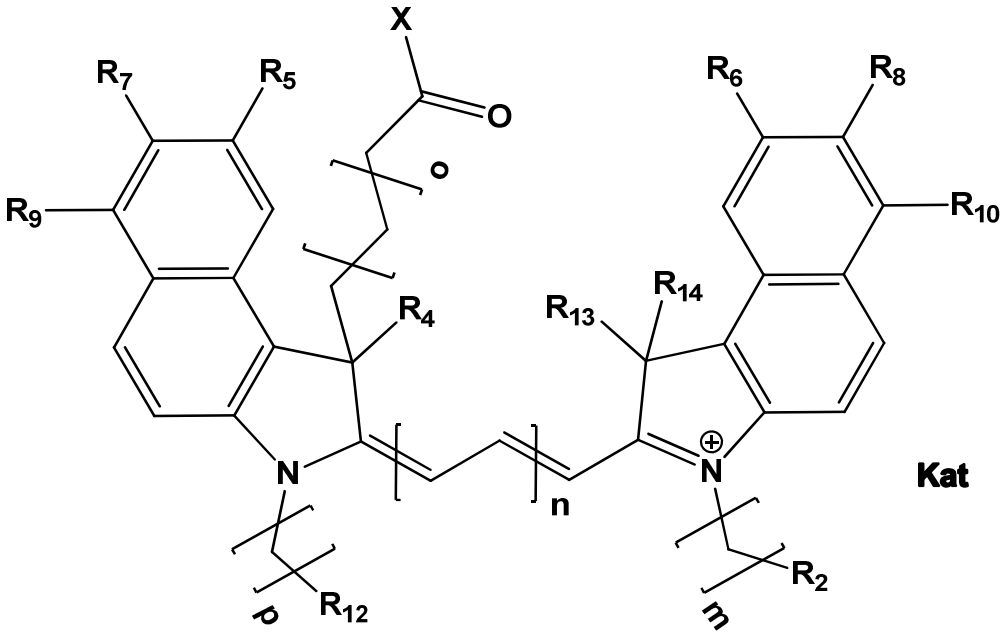
22. The kit of claim 21, wherein the PCR is real-time or quantitative PCR (qPCR).

23. The kit of claim 21, further comprising instructions for conducting the real-time or quantitative PCR (qPCR).

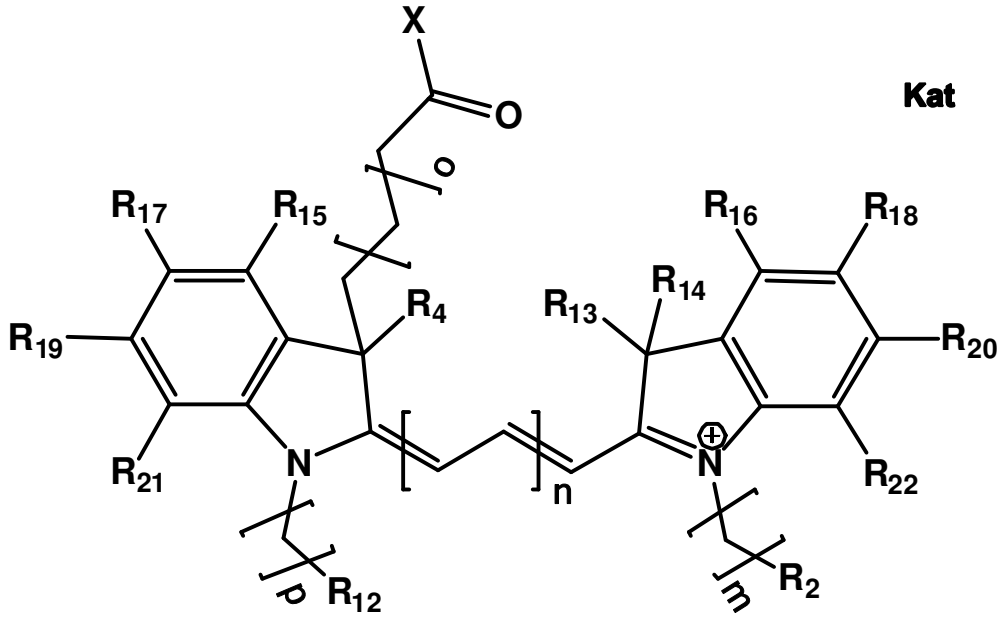
24. The kit of claim 21, wherein the components are packaged in separate containers.

25. The kit of claim 21, wherein two or more components are packaged together as a master mix.

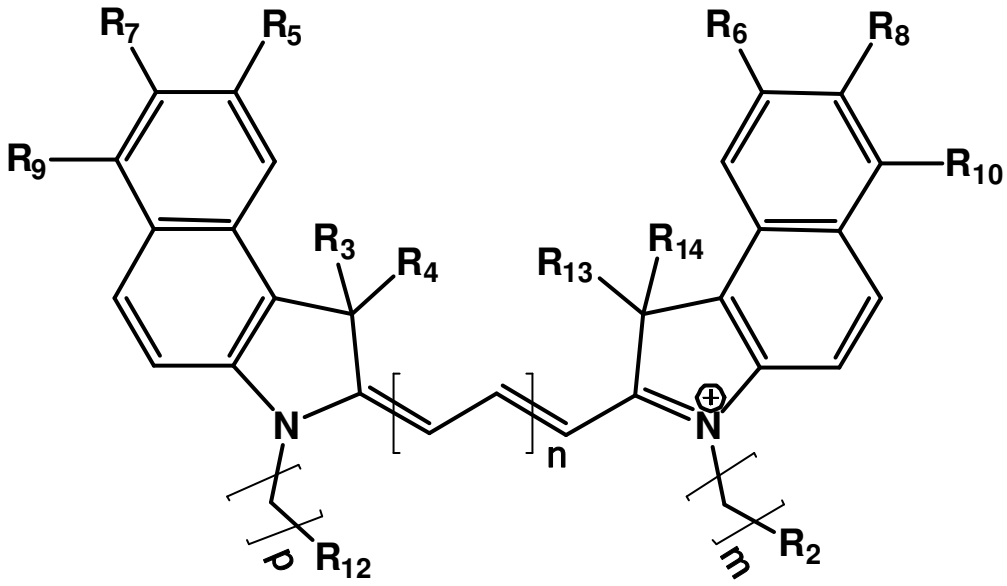
26. A kit comprising at least one a dye having a general Formula Ia, Formula Ib, Formula Ic, or Formula Id



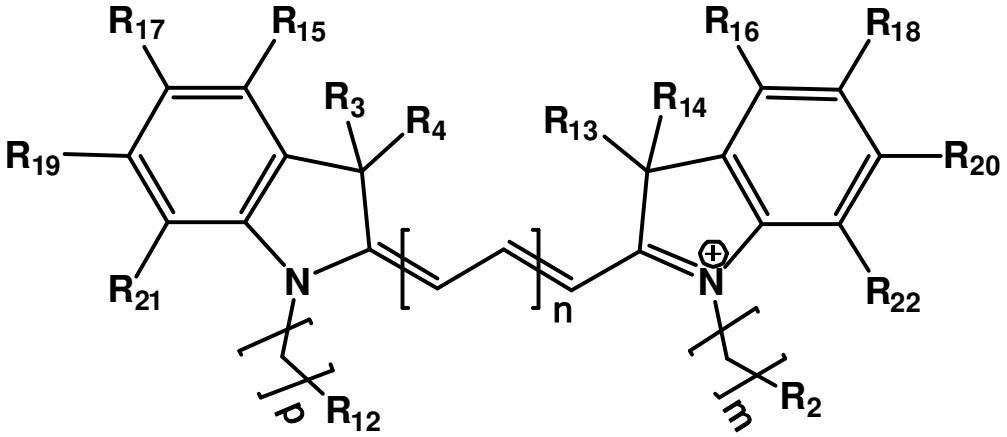
Formula Ia



Formula Ib



Formula Ic



Formula Id

wherein

each of R_4, R_{13}, and R_{14}, and R_3 when present, is the same or different and is selected from the group consisting of H, an aliphatic, a heteroaliphatic, a sulfoalkyl, a heteroaliphatic with terminal SO_3, a benzyl, and a substituted benzyl, where the substituted benzyl comprises at least one carboxy group, at least one sulfonate group, -F, -Cl, -Br, or a combination thereof;

each of R_5, R_6, R_7, R_8, R_9, R_{10}, R_{15}, R_{16}, R_{17}, R_{18}, R_{19}, R_{20}, R_{21}, and R_{22} is the same or different and is selected from the group consisting of H and SO_3;

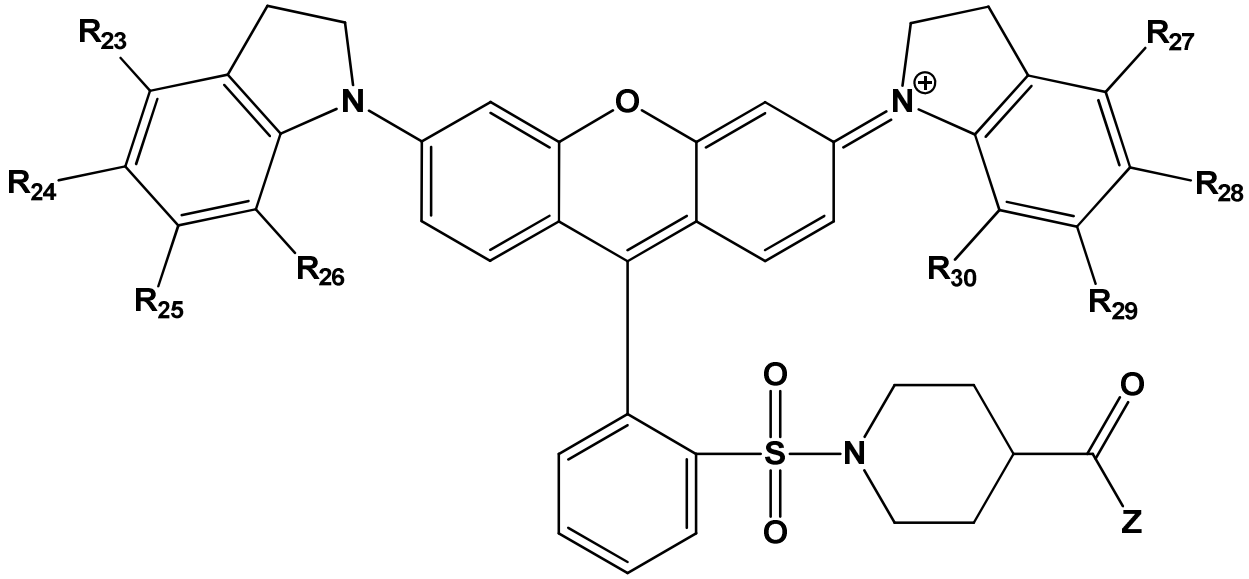
each of R_2 and R_{12} is the same or different and is selected from the group consisting of H and SO_3;

X is selected from the group consisting of -OH, -SH, -NH_2, -NH-NH_2, -F, -Cl, -Br, I, -O-NHS (hydroxysuccinimidyl/sulfosuccinimidyl), -O-TFP (2,3,5,6-tetrafluorophenoxy), -O-STP (4-sulfo-2,3,5,6-tetrafluorophenoxy), -O-benzotriazole, -benzotriazole, -NR-L-OH, -NR-L-O-phosphoramidite, -NR-L-SH, -NR-L-NH_2, -NR-L-NH-NH_2, -NR-L-CO_2H, -NR-L-CO-NHS, -NR-L-CO-STP, -NR-L-CO-TFP, -NR-L-CO-benzotriazole, -NR-L-CHO, -NR-L-maleimide, $NH(CH_2CH_2O)_zCH_2CH_2N_3$, -NR-L-NH-CO-$CH_2$-I, and an azide ($N_3$)-containing group, where R is -H or an aliphatic or heteroaliphatic group, z is an integer from 1 to 5 inclusive, and L is selected from the group consisting of a divalent linear, crossed-coupled, or cyclic alkyl group optionally substituted by at least one oxygen atom and/or sulfur atom;

Kat is a number of Na^+, K^+, Ca^{2+}, ammonia, or other cation(s) needed to compensate the negative charge brought by the cyanine;

m is an integer from 0 to 5 inclusive; n is an integer from 1 to 3 inclusive; o is an integer from 0 to 12 inclusive; and p is an integer from 0 to 5 inclusive; and

at least one quencher having general Formula II

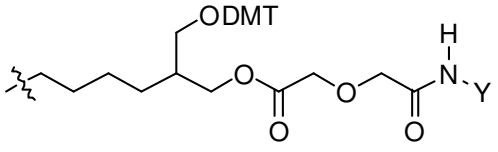


Formula II

wherein

each of R_{23}, R_{24}, R_{25}, R_{26}, R_{27}, R_{28}, R_{29}, and R_{30} is the same or different and is independently selected from either H or SO_3;

Z is OR, where R is alkyl, or Z is NH-L, where L is



and Y is either H or a linkage to a solid support.

27. The kit of claim 26, further comprising additional components for conjugating the at least one dye and the at least one quencher to an oligonucleotide such that the dye is covalently joined to a first nucleotide of the oligonucleotide and the quencher is covalently joined to a second, different nucleotide of the oligonucleotide.

28. The kit of claim 27, further comprising instructions for conjugating the at least one dye and the at least one quencher to an oligonucleotide such that the dye is covalently joined to a first nucleotide of the oligonucleotide and the quencher is covalently joined to a second, different nucleotide of the oligonucleotide.

29. The kit of claim 26, wherein the components are packaged in separate containers.

30. The probe of claim 1, wherein the azide-containing group comprises an aliphatic linker with a terminal azide.

31. The probe of claim 30, wherein the aliphatic linker with a terminal azide is selected from NH-CH_2-CH_2-CH_2-N_3 or NH_2-CH_2-CH_2-O-CH_2-CH_2-O-CH_2-CH_2-O-CH_2-CH_2-N_3.

32. The method of claim 14, wherein the azide-containing group comprises an aliphatic linker with a terminal azide.

33. The method of claim 32, wherein the aliphatic linker with a terminal azide is selected from NH-CH_2-CH_2-CH_2-N_3 or NH_2-CH_2-CH_2-O-CH_2-CH_2-O-CH_2-CH_2-O-CH_2-CH_2-N_3.

34. The kit of claim 21, wherein the azide-containing group comprises an aliphatic linker with a terminal azide.

35. The kit of claim 34, wherein the aliphatic linker with a terminal azide is selected from $NH\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}N_3$ or $NH_2\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CH_2\text{-}N_3$.

36. The kit of claim 26, wherein the azide-containing group comprises an aliphatic linker with a terminal azide.

37. The kit of claim 36, wherein the aliphatic linker with a terminal azide is selected from $NH\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}N_3$ or $NH_2\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CH_2\text{-}N_3$.