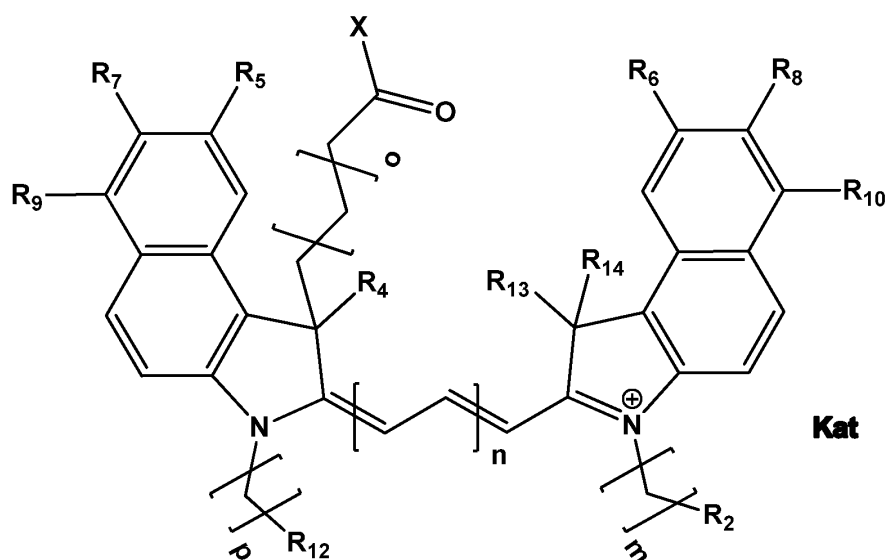
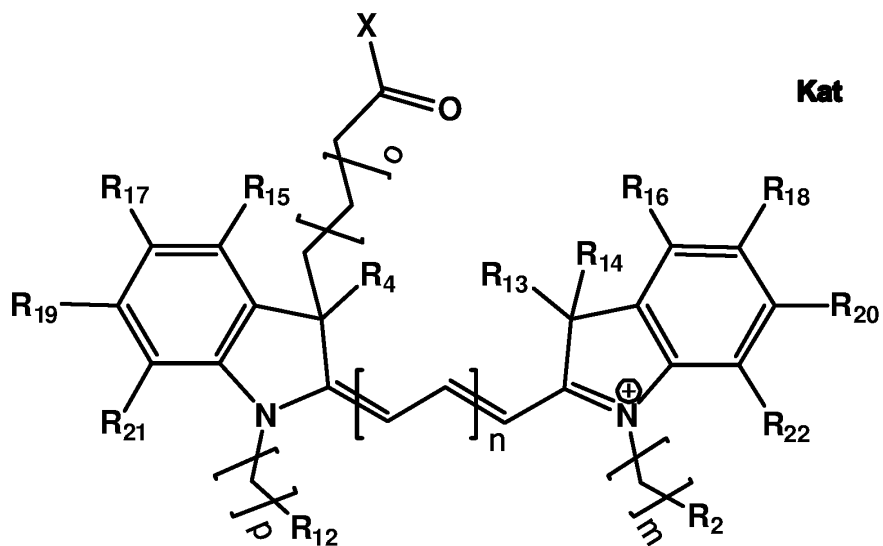


We Claim:

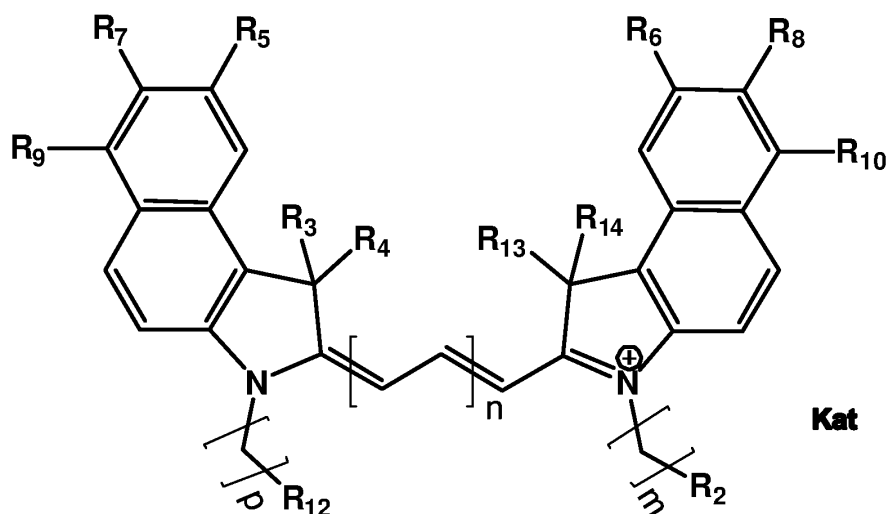
1. A probe comprising a product of conjugation of:
 - a) a dye having a general Formula Ia, Formula Ib, Formula Ic, or Formula Id



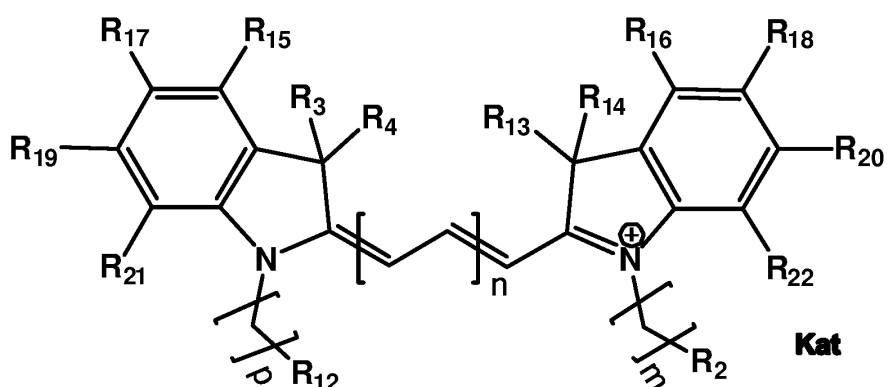
Formula Ia



Formula Ib



Formula Ic



Formula Id

wherein

each of R₄, R₁₃, and R₁₄, and R₃ 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 SO₃, 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 R₅, R₆, R₇, R₈, R₉, R₁₀, R₁₅, R₁₆, R₁₇, R₁₈, R₁₉, R₂₀, R₂₁, and R₂₂ is the same or different and is selected from the group consisting of H and SO₃;

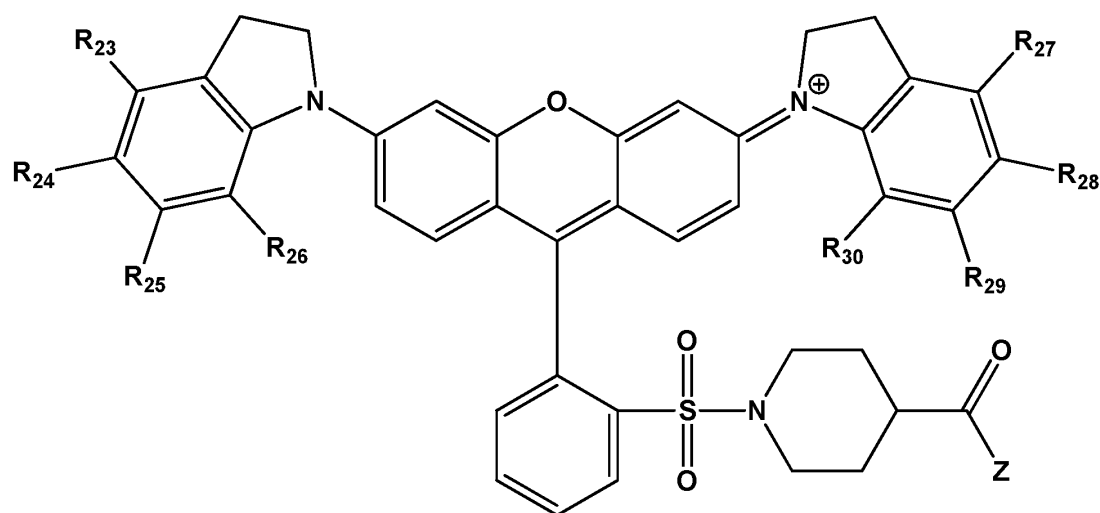
each of R₂ and R₁₂ is the same or different and is selected from the group consisting of H and SO₃;

X is selected from the group consisting of -OH, -SH, -NH₂, -NH-NH₂, -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-NH₂, -NR-L-NH-NH₂, -NR-L-CO₂H, -NR-L-CO-NHS, -NR-L-CO-STP, -NR-L-CO-TFP, -NR-L-CO-benzotriazole, -NR-L-CHO, -NR-L-maleimide, -NH(CH₂CH₂O)_zCH₂CH₂N₃, -NR-L-NH-CO-CH₂-I, and an azide (N₃)-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, or cyclic alkyl group optionally substituted by at least one oxygen atom and/or sulfur atom;

Kat is a number of Na⁺, K⁺, Ca²⁺, 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

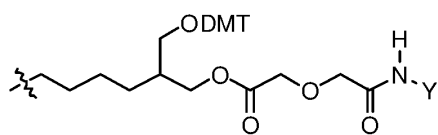


Formula II

wherein

each of R₂₃, R₂₄, R₂₅, R₂₆, R₂₇, R₂₈, R₂₉, and R₃₀ is the same or different and is independently selected from either H or SO₃;

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;

and

c) an oligonucleotide linker joining the dye and the quencher.

2. The probe of claim 1, wherein:

R₅, R₆, R₉, and R₁₀ are SO₃;

R₇ and R₈ are H;

R₄, R₁₃, and R₁₄ 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 ;

R₅, R₆, R₇, R₈, and R₉ are H;

R₄, R₁₃, and R₁₄ 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;

R₄, R₁₃, and R₁₄ are methyl;

R_2 and R_{12} are SO_3 ;

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

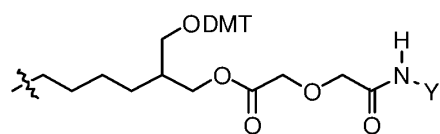
5. The probe of claim 1, wherein:

R_9 and R_{10} are SO_3 ;

R₅, R₆, R₇, and R₈ are H;
R₄, R₁₃, and R₁₄ are methyl;
R₁₂ is H;
R₂ is SO₃;
m and o are 3; and n and p are 2.

6. The probe of claim 1, wherein:
R₉ and R₁₀ are SO₃;
R₅, R₆, R₇, and R₈ are H;
R₄, R₁₃, and R₁₄ are methyl;
R₂ and R₁₂ are SO₃;
m, o, and p are 3; and n is 2.
7. The probe of claim 1, wherein:
R₅, R₆, R₇, R₈, R₉, and R₁₀ are H;
R₄, R₁₃, and R₁₄ are methyl;
R₂ and R₁₂ are H;
m and p are 1; n is 2; and o is 3.
8. The probe of claim 1, wherein:
R₁₅, R₁₆, R₁₇, R₁₈, R₁₉, R₂₀, R₂₁, and R₂₂ are H;
R₄, R₁₃, and R₁₄ are methyl;
R₂ and R₁₂ are H;
m and p are 1; n is 2; and o is 3.
9. The probe of claim 1, wherein:
R₁₇ and R₁₈ are SO₃;
R₁₅, R₁₆, R₁₉, R₂₀, R₂₁, and R₂₂ are H;
R₄, R₁₃, and R₁₄ are methyl;
R₂ and R₁₂ are SO₃;
m, o, and p are 3; and n is 2.

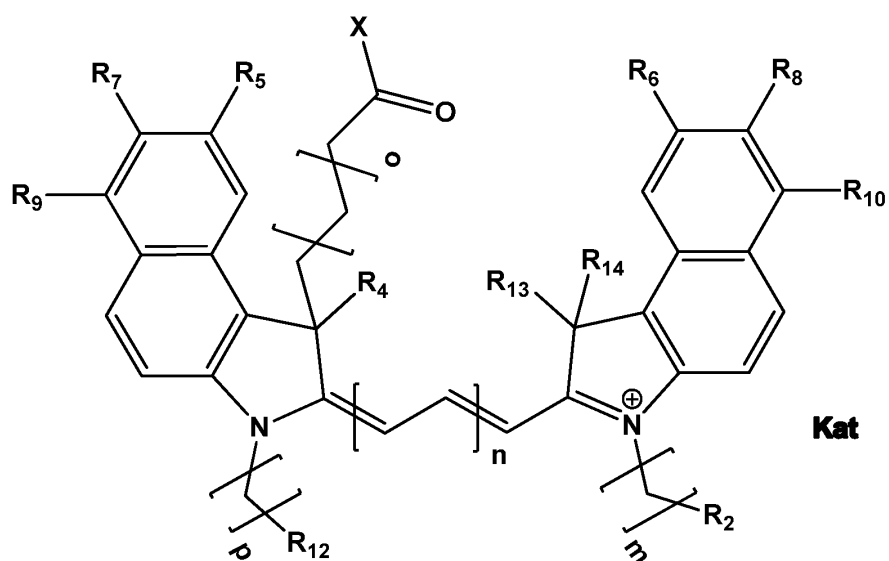
10. The probe of claim 1, wherein:
 R_5 , R_6 , R_9 , and R_{10} are SO_3 ;
 R_7 and R_8 are H;
 R_4 , R_{13} , and R_{14} are methyl;
 R_3 is -C-benzoate;
 R_2 is SO_3 and R_{12} is H;
 m is 3; p is 1; and n is 2.
11. The probe of claim 1, wherein each of R_{23} , R_{25} , R_{26} , R_{27} , R_{29} , 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 CO_2R , where R is NH-L, L being



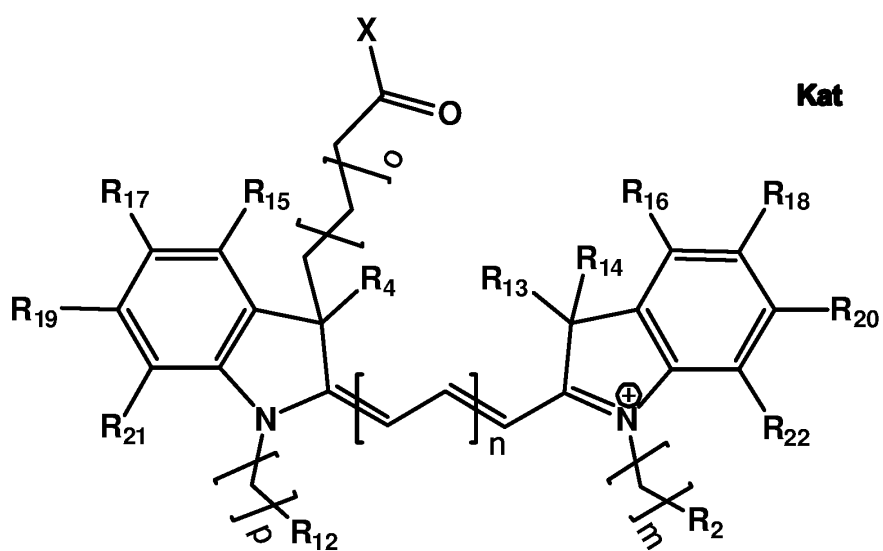
and Y being the linkage to the solid support.

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:

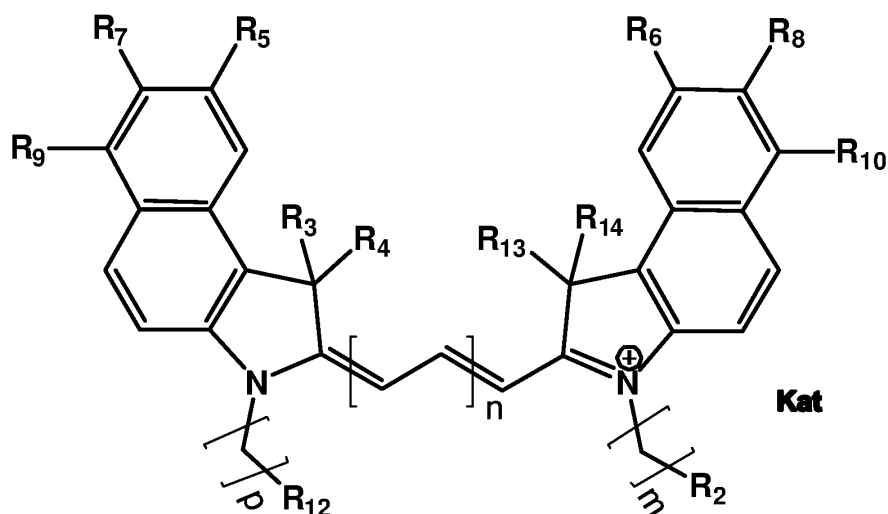
a) a dye having a general Formula Ia, Formula Ib, Formula Ic, or Formula Id



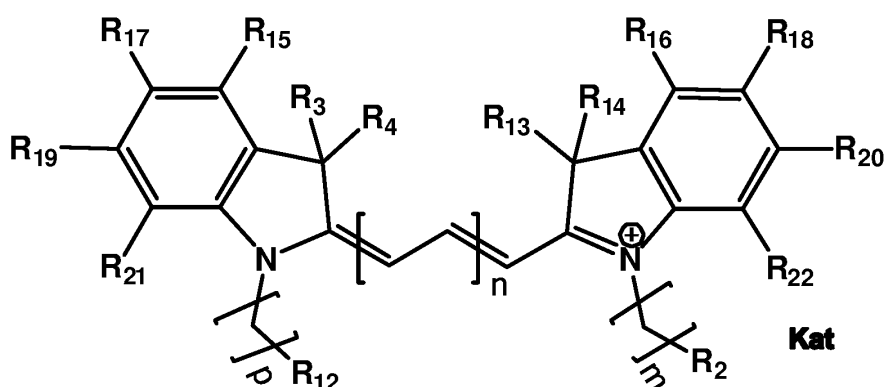
Formula Ia



Formula Ib



Formula Ic



Formula Id

wherein

each of R₄, R₁₃, and R₁₄, and R₃ 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 SO₃, 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 R₅, R₆, R₇, R₈, R₉, R₁₀, R₁₅, R₁₆, R₁₇, R₁₈, R₁₉, R₂₀, R₂₁, and R₂₂ is the same or different and is selected from the group consisting of H and SO₃;

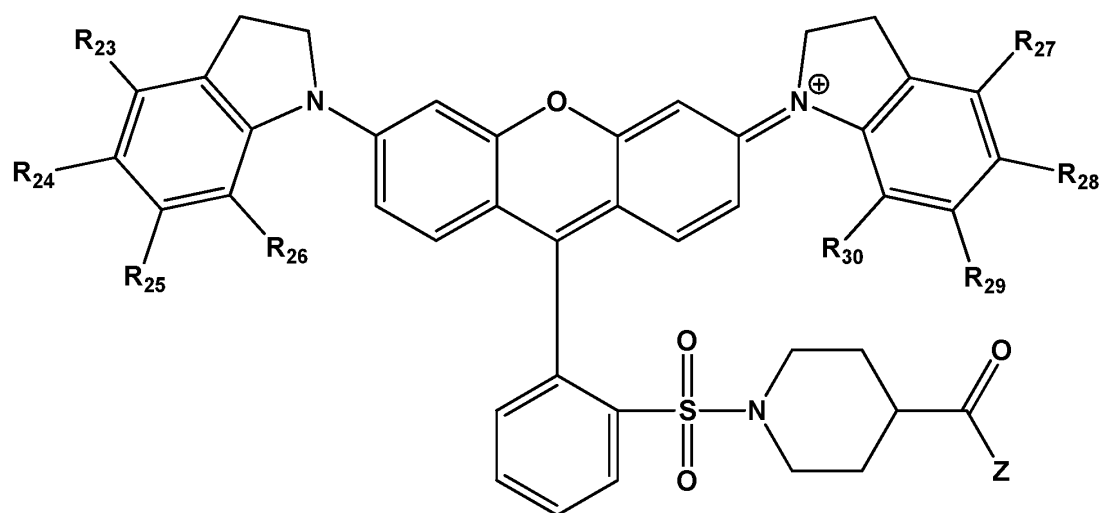
each of R₂ and R₁₂ is the same or different and is selected from the group consisting of H and SO₃;

X is selected from the group consisting of -OH, -SH, -NH₂, -NH-NH₂, -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-NH₂, -NR-L-NH-NH₂, -NR-L-CO₂H, -NR-L-CO-NHS, -NR-L-CO-STP, -NR-L-CO-TFP, -NR-L-CO-benzotriazole, -NR-L-CHO, -NR-L-maleimide, NH(CH₂CH₂O)_zCH₂CH₂N₃, -NR-L-NH-CO-CH₂-I, and an azide (N₃)-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, or cyclic alkyl group optionally substituted by at least one oxygen atom and/or sulfur atom;

Kat is a number of Na⁺, K⁺, Ca²⁺, 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

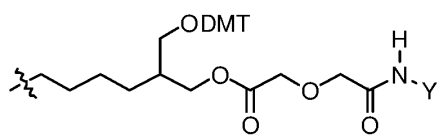


Formula II

wherein

each of R₂₃, R₂₄, R₂₅, R₂₆, R₂₇, R₂₈, R₂₉, and R₃₀ is the same or different and is independently selected from either H or SO₃;

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

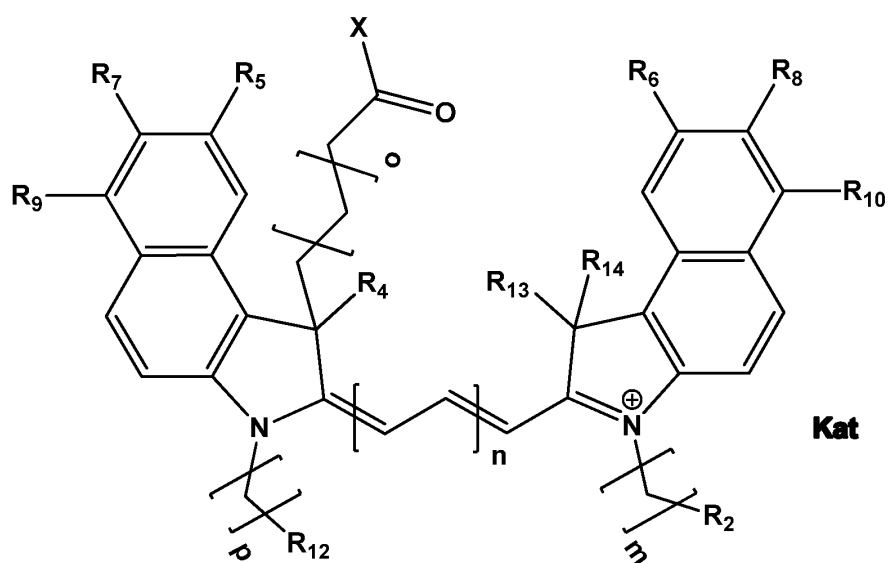


and Y is either H or a linkage to a solid support;

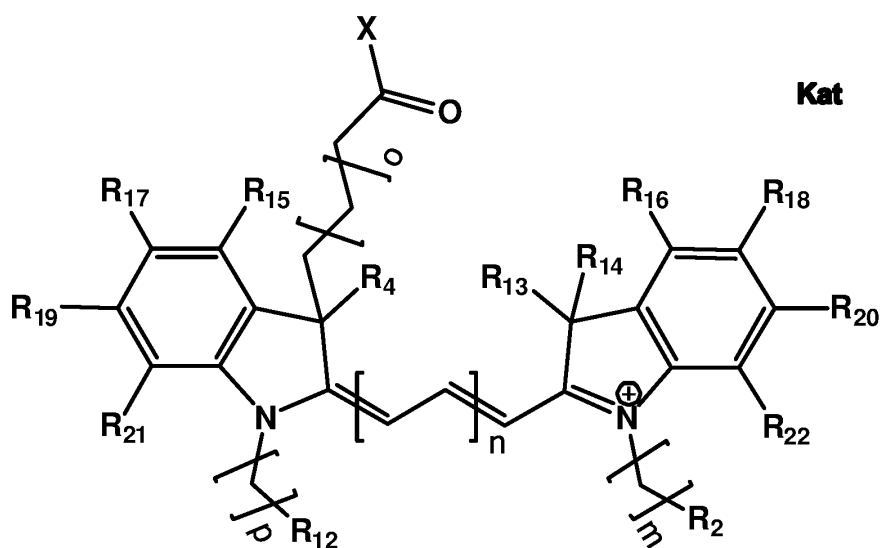
and

c) at least one oligonucleotide linker joining the dye and the quencher.

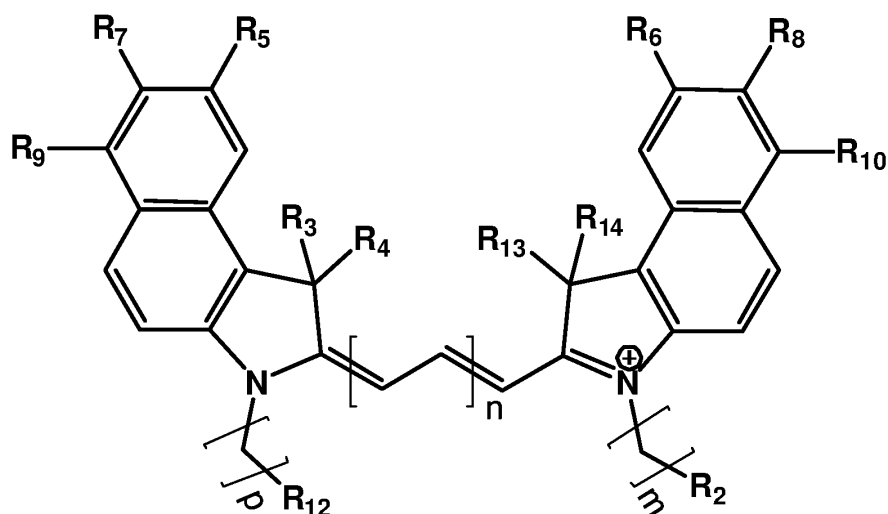
15. The method of claim 14, wherein the PCR is real-time or quantitative PCR (qPCR).
16. The method of claim 14, wherein the polymerase is a Taq polymerase.
17. The method of claim 14, wherein the probe is a hydrolysis probe.
18. The method of claim 14, wherein the probe is a TaqMan probe.
19. The method 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



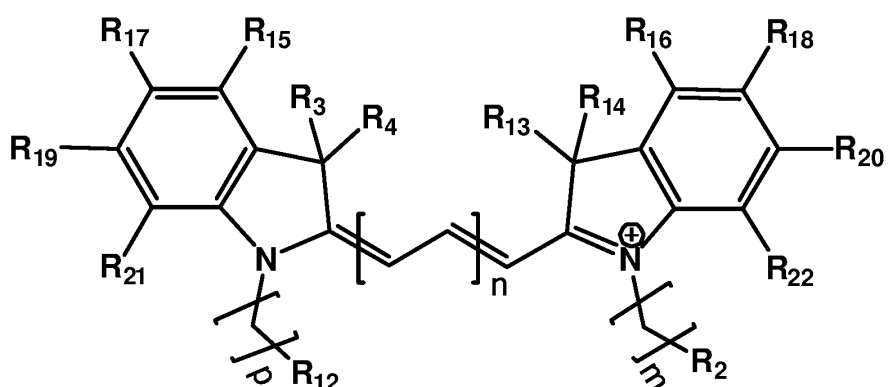
Formula Ia



Formula Ib



Formula Ic



Formula Id

wherein

each of R₄, R₁₃, and R₁₄, and R₃ 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 SO₃, 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 R₅, R₆, R₇, R₈, R₉, R₁₀, R₁₅, R₁₆, R₁₇, R₁₈, R₁₉, R₂₀, R₂₁, and R₂₂ is the same or different and is selected from the group consisting of H and SO₃;

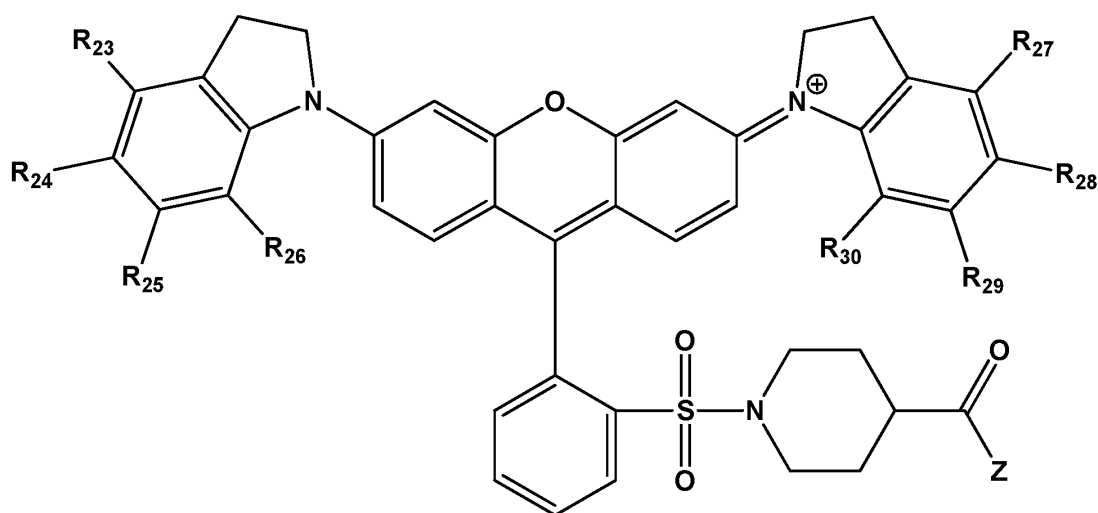
each of R₂ and R₁₂ is the same or different and is selected from the group consisting of H and SO₃;

X is selected from the group consisting of -OH, -SH, -NH₂, -NH-NH₂, -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-NH₂, -NR-L-NH-NH₂, -NR-L-CO₂H, -NR-L-CO-NHS, -NR-L-CO-STP, -NR-L-CO-TFP, -NR-L-CO-benzotriazole, -NR-L-CHO, -NR-L-maleimide, NH(CH₂CH₂O)_zCH₂CH₂N₃, -NR-L-NH-CO-CH₂-I, and an azide (N₃)-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, or cyclic alkyl group optionally substituted by at least one oxygen atom and/or sulfur atom;

Kat is a number of Na⁺, K⁺, Ca²⁺, 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

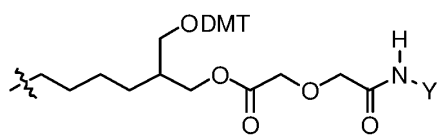


Formula II

wherein

each of R₂₃, R₂₄, R₂₅, R₂₆, R₂₇, R₂₈, R₂₉, and R₃₀ is the same or different and is independently selected from either H or SO₃;

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

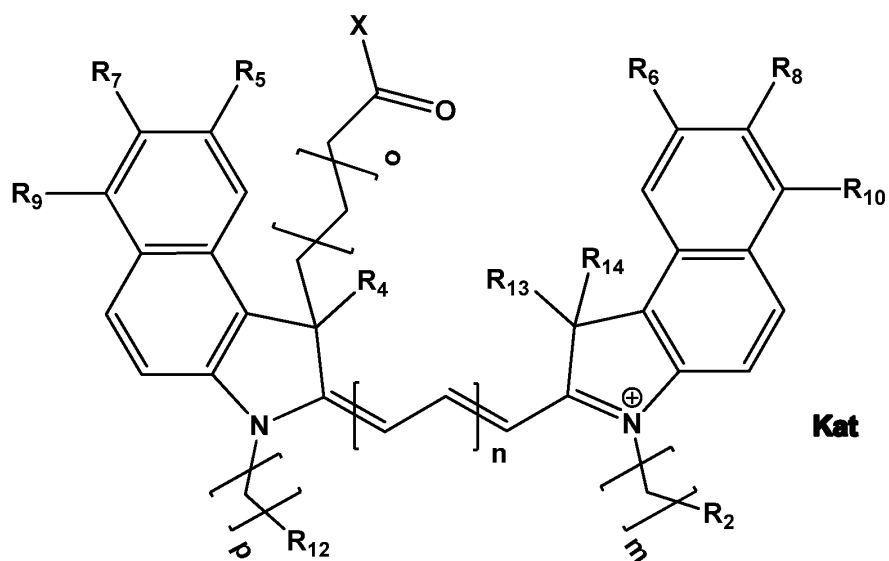


and Y is either H or a linkage to a solid support;

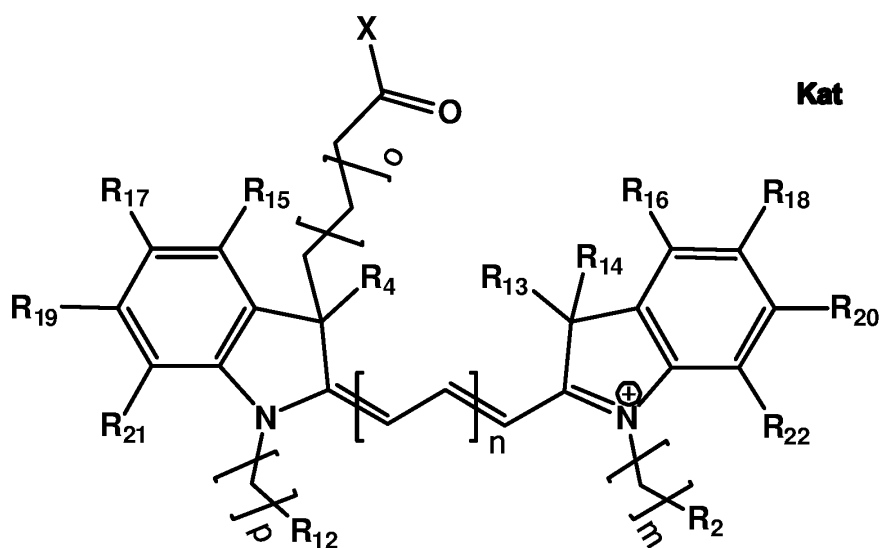
and

c) at least one oligonucleotide linker joining the dye and the quencher; and
one or more of the following: a buffering agent, a purification medium, the target, an organic solvent, an enzyme, and an enzyme inhibitor.

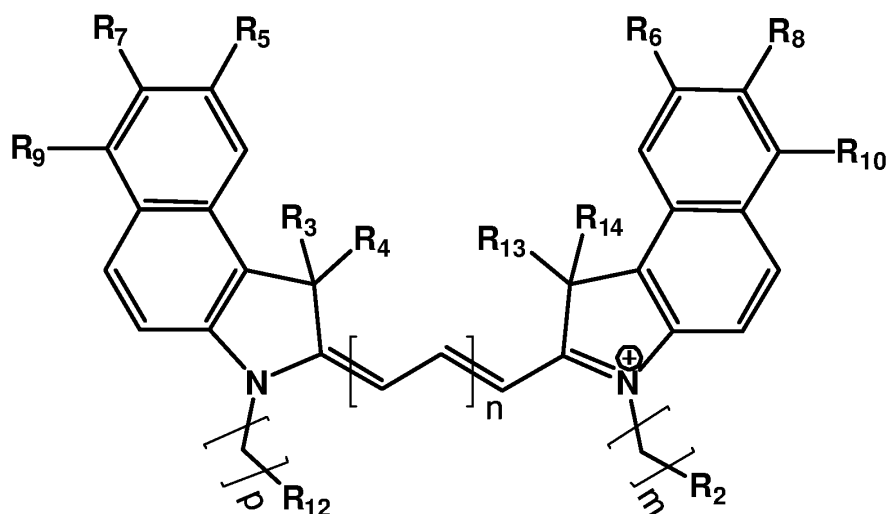
22. The kit of claim 21, wherein the PCR is real-time or quantitative PCR (qPCR).
23. The kit of claim 21, further comprising instructions for conducting the real-time or quantitative PCR (qPCR).
24. The kit of claim 21, wherein the components are packaged in separate containers.
25. The kit of claim 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



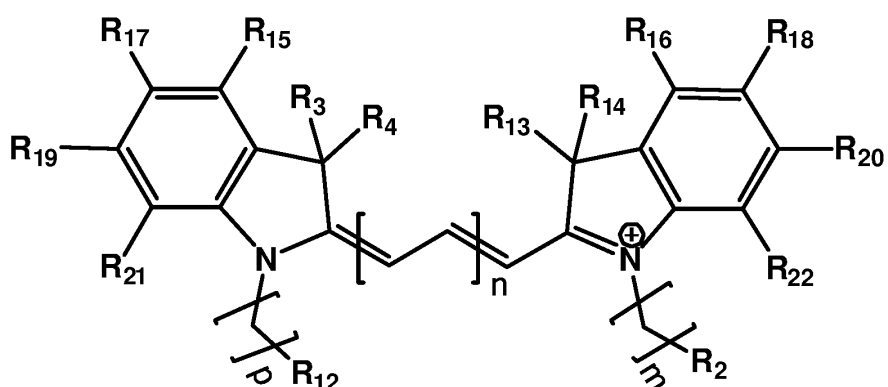
Formula Ia



Formula Ib



Formula Ic



Formula Id

wherein

each of R₄, R₁₃, and R₁₄, and R₃ 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 SO₃, 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 R₅, R₆, R₇, R₈, R₉, R₁₀, R₁₅, R₁₆, R₁₇, R₁₈, R₁₉, R₂₀, R₂₁, and R₂₂ is the same or different and is selected from the group consisting of H and SO₃;

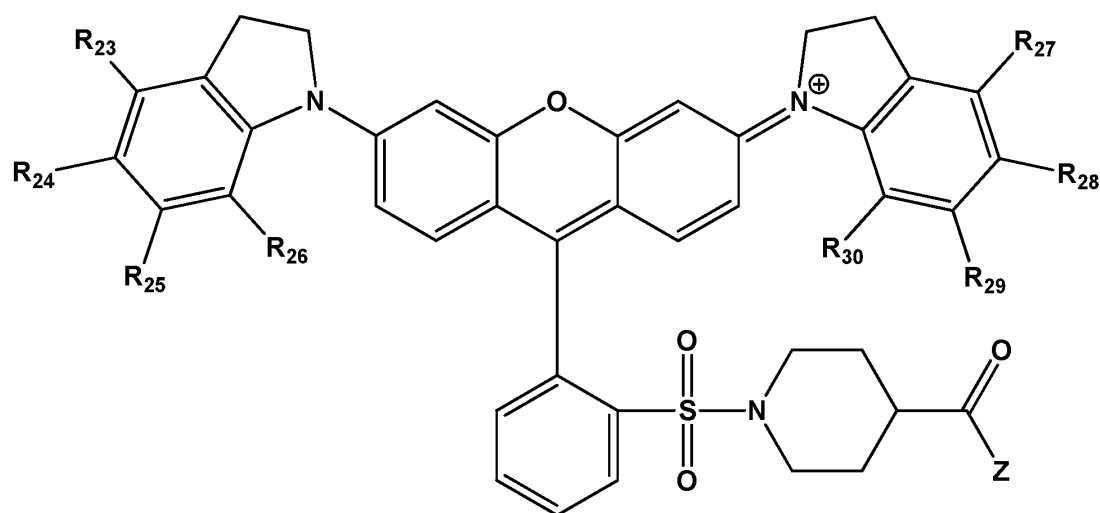
each of R₂ and R₁₂ is the same or different and is selected from the group consisting of H and SO₃;

X is selected from the group consisting of -OH, -SH, -NH₂, -NH-NH₂, -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-NH₂, -NR-L-NH-NH₂, -NR-L-CO₂H, -NR-L-CO-NHS, -NR-L-CO-STP, -NR-L-CO-TFP, -NR-L-CO-benzotriazole, -NR-L-CHO, -NR-L-maleimide, NH(CH₂CH₂O)_zCH₂CH₂N₃, -NR-L-NH-CO-CH₂-I, and an azide (N₃)-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, or cyclic alkyl group optionally substituted by at least one oxygen atom and/or sulfur atom;

Kat is a number of Na⁺, K⁺, Ca²⁺, 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

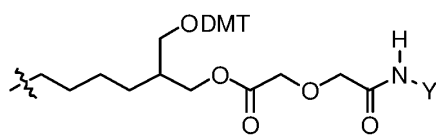


Formula II

wherein

each of R₂₃, R₂₄, R₂₅, R₂₆, R₂₇, R₂₈, R₂₉, and R₃₀ is the same or different and is independently selected from either H or SO₃;

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.

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.
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.
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 $\text{NH-CH}_2\text{-CH}_2\text{-CH}_2\text{-N}_3$ or $\text{NH}_2\text{-CH}_2\text{-CH}_2\text{-O-CH}_2\text{-CH}_2\text{-O-CH}_2\text{-CH}_2\text{-O-CH}_2\text{-CH}_2\text{-N}_3$.
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 $\text{NH-CH}_2\text{-CH}_2\text{-CH}_2\text{-N}_3$ or $\text{NH}_2\text{-CH}_2\text{-CH}_2\text{-O-CH}_2\text{-CH}_2\text{-O-CH}_2\text{-CH}_2\text{-O-CH}_2\text{-CH}_2\text{-N}_3$.
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 $\text{NH-CH}_2\text{-CH}_2\text{-CH}_2\text{-N}_3$ or $\text{NH}_2\text{-CH}_2\text{-CH}_2\text{-O-CH}_2\text{-CH}_2\text{-O-CH}_2\text{-CH}_2\text{-O-CH}_2\text{-CH}_2\text{-N}_3$.

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 $\text{NH-CH}_2\text{-CH}_2\text{-CH}_2\text{-N}_3$ or $\text{NH}_2\text{-CH}_2\text{-CH}_2\text{-O-CH}_2\text{-CH}_2\text{-O-CH}_2\text{-CH}_2\text{-O-CH}_2\text{-CH}_2\text{-N}_3$.