CLAIMS

1. Method of detecting a mutation in a target nucleic acid molecule in sample, comprising the steps of:
(i) contacting a first conjugate comprising a first nanoparticle and a first oligonucleotide analog, wherein the first oligonucleotide analog is a phosphorodiamidate morpholino oligo (PMO) or a derivative thereof that is covalently coupled to the first nanoparticle with a first aliquot of the sample containing the target nucleic acid sequence, wherein the first oligonucleotide analog comprises a base sequence that is complementary to the wildtype sequence of the target nucleic acid, under conditions which allow the first conjugate and the target nucleic acid molecule to hybridize to each other, to form a first conjugate:target nucleic acid molecule complex;
(ii) contacting a second conjugate comprising a second nanoparticle and a second oligonucleotide analog, wherein the second oligonucleotide analog is a phosphorodiamidate morpholino oligo (PMO) or a derivative thereof that is covalently coupled to the second nanoparticle with a second aliquot of the sample containing the target nucleic acid sequence, wherein the second oligonucleotide analog comprises a base sequence that is complementary to the mutated sequence of the target nucleic acid and differs from the base sequence of the first oligonucleotide analog by at least one nucleobase and wherein the first oligonucleotide analog and the second oligonucleotide analog have a sequence identity of at least 85%, under conditions which allow the second conjugate and the target nucleic acid molecule to hybridize to each other, to form a second conjugate:target nucleic acid molecule complex, and
(iii) determining the melting temperatures T_m of the complexes formed in the separate aliquots of the sample in steps (i) and (ii) and detecting whether said target nucleic acid molecule contains said mutation by comparing the T_m of said complexes.

2. The method according to claim 1 further comprising the step of amplifying the target nucleic acid molecule by PCR previous to step (i).

3. The method according to claim 1 or 2, wherein said sample is a heterogeneous sample.

4. The method according to any one claims 1 to 3, wherein the target nucleic acid molecule comprises the nucleotide sequence set forth in SEQ ID Nos. 3, 4, 13, 14, 15, 16, 17 or 18.

5. The method according to any one of claims 1 to 4, wherein

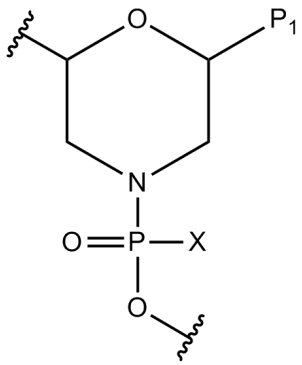
(1) the first oligonucleotide comprises the base sequence set forth in SEQ ID NO:1 and the second oligonucleotide comprises the base sequence set forth in SEQ ID NO:2;

(2) the first oligonucleotide comprises the base sequence set forth in SEQ ID NO:7 and the second oligonucleotide comprises the base sequence set forth in SEQ ID NO:8;

(3) the first oligonucleotide comprises the base sequence set forth in SEQ ID NO:9 and the second oligonucleotide comprises the base sequence set forth in SEQ ID NO:10; or

(4) the first oligonucleotide comprises the base sequence set forth in SEQ ID NO:11 and the second oligonucleotide comprises the base sequence set forth in SEQ ID NO:12.

6. The method according to any one of claims 1 to 5, wherein a monomeric unit of the phosphorodiamidate morpholino oligo or derivative thereof is represented by formula (I)



(I),

wherein

P_1 is a purine or pyrimidine base that forms Watson-Crick base pairs with another purine or pyrimidine base; and

X is NH_2, NHR, or NR_2, wherein R is C1-C6 alkyl.

7. The method according to claim 6, wherein

(1) the other purine or pyrimidine base that forms Watson-Crick base pairs with the purine or pyrimidine base of P_1 is a nucleobase;

(2) X is NR_2 and R is methyl;

(3) the purine or pyrimidine base is a nucleobase selected from the group consisting of adenine (A), cytosine (C), guanine (G), thymine (T) and uracil (U); and/or

(4) the purine or pyrimidine base is a nucleobase selected from the group consisting of 5-methylcytosine (m5C), pseudouridine (Ψ), dihydrouridine (D), inosine (I), and 7-methylguanosine (m7G), xanthine and hypoxanthine.

8. Use of a kit for the method of any one of claims 1 to 7, wherein the kit consists of a first and second conjugate and, optionally, PCR primers,
the first conjugate comprising a first nanoparticle and a first oligonucleotide analog, wherein the first oligonucleotide analog is a phosphorodiamidate morpholino oligo (PMO) or a derivative thereof that is covalently coupled to the first nanoparticle, and
the second conjugate comprising a second nanoparticle and a second oligonucleotide analog, wherein the second oligonucleotide analog is a phosphorodiamidate morpholino oligo (PMO) or a derivative thereof that is covalently coupled to the second nanoparticle,
wherein the first oligonucleotide analog differs from the second oligonucleotide analog by at least one nucleobase and wherein the first oligonucleotide analog has a sequence identity to the second oligonucleotide analog of at least 85%.

9. The use of a kit according to claim 8, wherein the first and/or the second nanoparticle is a metal nanoparticle.

10. The use of a kit according to claim 9, wherein

(1) the metal is a noble metal;

(2) the metal is selected from the group consisting of gold, silver, platinum, palladium, ruthenium, osmium, iridium and mixtures thereof; or

(3) the metal is gold.

11. The use of a kit according to any one of claims 8 to 10, wherein the first and second oligonucleotide analogs comprise about 15 to about 35 monomeric units.

12. The use of a kit according to any one of claims 8 to 11 for the detection of mutation in a target nucleic acid molecule or for assigning a genotype to a target nucleic acid.