

CLAIMS

What is claimed:

1. A method for enrichment of a population of nucleic acid target sequences in a sample comprising:
 - (a) providing a sample of nucleic acid molecules comprising a plurality of target nucleic acid sequences and a plurality of off-target nucleic acid sequences;
 - (b) hybridizing the sample to a panel of nucleic acid probes that are complementary to the plurality of target nucleic acid sequences under hybridization conditions to generate a probe/target complex;
 - (c) selectively immobilizing the probe/target complex to form an immobilized probe/target complex;
 - (d) heating the immobilized probe/target complex to a temperature at or below the aggregate T_m of the probe/target complex for a period of time sufficient to disassociate the off-target nucleic acid sequences; and
 - (e) washing the immobilized probe/target complex to remove non-hybridized nucleic acid sequences and off-target nucleic acid sequences from the hybridized plurality of target nucleic acid sequences, thereby enriching for the plurality of target nucleic acid sequences in the sample.
2. The method of claim 1, wherein the panel of nucleic acid probes that are complementary to the plurality of target nucleic acid sequences further comprises a capture moiety.
3. The method of claim 2, wherein the capture moiety is biotin.
4. The method of claim 1, wherein the hybridization conditions comprise a hybridization buffer comprising one or more of:
 - (a) salts selected from one or more of monovalent, divalent, sodium, ammonium, cesium, or manganese, chlorides, citrates, sulfates, perchlorates, isothiocyanates, or guanidinium;
 - (b) chelating agents selected from one or more of ethylenediaminetetraacetic acid (EDTA), ethylene glycol-bis(β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), or (1,2-bis(o-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid (BAPTA);

- (c) buffering agents selected from one or more of tris(hydroxymethyl)aminomethane (Tris); 2-(bis(2-hydroxyethyl)amino)acetic acid (Bicine); (N-[tris(hydroxymethyl)methyl]glycine (Tricine); [tris(hydroxymethyl)methylamino]propanesulfonic acid (TAPS); 3-[N-tris(hydroxymethyl)methylamino]-2-hydroxypropanesulfonic acid (TAPSO); 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES); 2-[[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]amino]ethanesulfonic acid (TES); 3-(N-morpholino)propanesulfonic acid (MOPS); piperazine-*N,N'*-bis(2-ethanesulfonic acid) (PIPES); 2-(*N*-morpholino)ethanesulfonic acid (MES); or dimethylarsenic acid (Cacodylate);
 - (d) detergents selected from one or more of sodium dodecylsulfate (SDS), polysorbate 20 (Tween® 20), octyl phenol ethoxylate (Triton X-100), octylphenoxypolyethoxyethano (IGEPAL®-CA 630), nonyl phenoxypolyethoxyethanol (NP-40), or cetrimonium bromide (CTAB); or
 - (e) additives selected from one or more of glycine, betaine, glycine-betaine, 7-deaza-2'-deoxyguanosine, dimethyl sulfoxide (DMSO), polyethylene glycols 400–1,000,000, glycerol, magnesium, tetramethyl ammonium chloride (TMAC), tetraethylammonium chloride (TEAC), triethylamine hydrochloride, ethylene carbonate, dextran sulfate, or bovine serum albumin (BSA).
5. The method of claim 4, wherein the hybridization buffer contains formamide.
 6. The method of claim 4, wherein the hybridization buffer does not contain formamide.
 7. The method of claim 1, wherein the hybridization conditions comprise an incubation time ranging from about 10 minutes to about 48 hours.
 8. The method of claim 1, wherein the hybridization conditions comprise an incubation time ranging from about 2 hours to overnight.
 9. The method of claim 8, wherein equivalent specificity and equivalent yield are achieved with a 2-hour incubation time as compared to an overnight incubation time.

10. The method of claim 1, wherein the hybridization conditions comprise an incubation temperature ranging from about 55 °C to about 75 °C.
11. The method of claim 1, wherein the hybridization conditions comprise an incubation temperature ranging from about 60 °C to about 70 °C.
12. The method of claim 1, wherein the hybridization conditions comprise an incubation time of about 2 hours and an incubation temperature of about 65 °C.
13. The method of claim 1, wherein the probe/target complex is selectively immobilized using streptavidin beads.
14. The method of claim 1, wherein the probe/target complex is selectively immobilized under conditions comprising an incubation time ranging from about 10 minutes to about 48 hours.
15. The method of claim 1, wherein the probe/target complex is selectively immobilized under conditions comprising an incubation temperature ranging from about 20 °C to about 40 °C.
16. The method of claim 1, wherein the probe/target complex is selectively immobilized under conditions comprising an incubation time of about 30 minutes and an incubation temperature of room temperature.
17. The method of claim 1, wherein the heating of the immobilized probe/target complex comprises a melt buffer comprising one or more of:
 - (a) salts selected from one or more of monovalent, divalent, sodium, ammonium, cesium, or manganese, chlorides, citrates, sulfates, perchlorates, isothiocyanates, or guanidinium;
 - (b) chelating agents selected from one or more of ethylenediaminetetraacetic acid (EDTA), ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), or (1,2-bis(o-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid (BAPTA);
 - (c) buffering agents selected from one or more of tris(hydroxymethyl)aminomethane (Tris); 2-(bis(2-hydroxyethyl)amino)acetic acid (Bicine); (*N*-

[tris(hydroxymethyl)methyl]glycine (Tricine); [tris(hydroxymethyl)methylamino]propanesulfonic acid (TAPS); 3-[*N*-tris(hydroxymethyl)methylamino]-2-hydroxypropanesulfonic acid (TAPSO); 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES); 2-[[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]amino]ethanesulfonic acid (TES); 3-(*N*-morpholino)propanesulfonic acid (MOPS); piperazine-*N,N'*-bis(2-ethanesulfonic acid) (PIPES); 2-(*N*-morpholino)ethanesulfonic acid (MES); or dimethylarsenic acid (Cacodylate);

- (d) detergents selected from one or more of sodium dodecylsulfate (SDS), polysorbate 20 (Tween® 20), octyl phenol ethoxylate (Triton X-100), octylphenoxypolyethoxyethano (IGEPAL®-CA 630), nonyl phenoxypolyethoxyethanol (NP-40), or cetrimonium bromide (CTAB); or
 - (e) additives selected from one or more of glycine, betaine, glycine-betaine, 7-deaza-2'-deoxyguanosine, dimethyl sulfoxide (DMSO), polyethylene glycols 400–1,000,000, glycerol, magnesium, tetramethyl ammonium chloride (TMAC), tetraethylammonium chloride (TEAC), triethylamine hydrochloride, ethylene carbonate, dextran sulfate, or bovine serum albumin (BSA).
18. The method of claim 1, wherein the heating of the immobilized probe/target complex comprises an incubation time ranging from about 5 minutes to about 1 hour.
 19. The method of claim 1, wherein the heating of the immobilized probe/target complex comprises an incubation temperature ranging from about 50 °C to about 70 °C.
 20. The method of claim 1, wherein the heating of the immobilized probe/target complex comprises an incubation time of about 20 minutes and an incubation temperature of about 55 °C.
 21. The method of claim 1, wherein the washing of the immobilized probe/target complex comprises two distinct wash buffers, wherein each wash buffer independently comprises one or more of:
 - (a) salts selected from one or more of monovalent, divalent, sodium, ammonium, cesium, or manganese, chlorides, citrates, sulfates, perchlorates, isothiocyanates, or guanidinium;

- (b) chelating agents selected from one or more of ethylenediaminetetraacetic acid (EDTA), ethylene glycol-bis(β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), or (1,2-bis(o-aminophenoxy)ethane)-*N,N,N',N'*-tetraacetic acid (BAPTA);
 - (c) buffering agents selected from one or more of tris(hydroxymethyl)aminomethane (Tris); 2-(bis(2-hydroxyethyl)amino)acetic acid (Bicine); (*N*-[tris(hydroxymethyl)methyl]glycine (Tricine); [tris(hydroxymethyl)methylamino]propanesulfonic acid (TAPS); 3-[*N*-tris(hydroxymethyl)methylamino]-2-hydroxypropanesulfonic acid (TAPSO); 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES); 2-[[1,3-dihydroxy-2-(hydroxymethyl)propan-2-yl]amino]ethanesulfonic acid (TES); 3-(*N*-morpholino)propanesulfonic acid (MOPS); piperazine-*N,N'*-bis(2-ethanesulfonic acid) (PIPES); 2-(*N*-morpholino)ethanesulfonic acid (MES); or dimethylarsenic acid (Cacodylate);
 - (d) detergents selected from one or more of sodium dodecylsulfate (SDS), polysorbate 20 (Tween® 20), octyl phenol ethoxylate (Triton X-100), octylphenoxypolyethoxyethano (IGEPAL®-CA 630), nonyl phenoxypolyethoxyethanol (NP-40), or cetrimonium bromide (CTAB); or
 - (e) additives selected from one or more of glycine, betaine, glycine-betaine, 7-deaza-2'-deoxyguanosine, dimethyl sulfoxide (DMSO), polyethylene glycols 400–1,000,000, glycerol, magnesium, tetramethyl ammonium chloride (TMAC), tetraethylammonium chloride (TEAC), triethylamine hydrochloride, ethylene carbonate, dextran sulfate, or bovine serum albumin (BSA).
22. The method of claim 1, wherein the washing of the immobilized probe/target complex comprises an incubation time of about 5 minutes to about 15 minutes and an incubation temperature of about 60 °C.
23. The method of claim 1, wherein the method improves specificity and enhances yield as compared to a conventional method.
24. The method of claim 23, wherein the conventional method does not comprise a step of heating prior to washing.

25. The method of claim 1, wherein the method improves on-target percentage as compared to a conventional method.
26. The method of claim 25, wherein the conventional method does not comprise a step of heating prior to washing.
27. The method of claim 1, wherein the method reduces handling time and throughput time as compared to a conventional method.
28. The method of claim 27, wherein the conventional method does not comprise a step of heating prior to washing.
29. The method of claim 1, wherein the method is suitable for automation and reduces complexity as compared to a conventional method.
30. The method of claim 29, wherein the conventional method does not comprise a step of heating prior to washing.
31. A plurality of target nucleic acid sequences isolated using the methods of claim 1.
32. A means for isolating a plurality of target nucleic acid sequences comprising the steps of claim 1.
33. Use of the method of claim 1 to isolate a plurality of target nucleic acid sequences.