Another example described herein provides for improved hybridization capture compositions. Another example described herein provides for improved hybridization capture buffers.

Another example described herein is a method for hybridization capture. The hybridization capture method is a Hyb-Capture-Melt workflow. In a first step a probe panel is contacted with a nucleic acid sample under conditions to promote hybridization. Optionally, the probe panel may contain a capture moiety, for example, but not limited to biotinylation. The biotinylated probes are complementary to target nucleic acids or nucleic acids to be enriched for. In the first step the goal is to increase the hybridization capture yield. Specificity of the nucleic acid capture is secondary to yield. In a second step the biotinylated probes are immobilized to streptavidin beads. Probes with and without hybridized targets are immobilized. In a third step a melt is performed to dissociate all non-specific hybridization interactions. Without being bound to a single theory it is also expected that after the melt is performed re-hybridization of captured probes with target sequence occurs, further improving both yield and specificity. In a fourth step a wash step is performed to further remove un-hybridized and weakly hybridized non-targeted nucleic acids.

Another example described herein is improved compositions for hybridization capture. In one example, hybridization capture buffers are provided that improve the hybridization capture efficiency. In another example, T_m modifying reagents may be added. In another example, additives that decrease T_m dependence on double stranded target GC base content may be added to improve capture.

Another example described herein is a method for hybridization capture. In one step, a hybridization at low stringency is provided. In another step, capture (immobilization) is performed at a temperature sufficient to promote immobilization. In another step, a melt is performed at an elevated temperature to reduce off-target hybridization. In another step, a wash is performed to reduce off-target hybridization and optionally this wash step may be in the absence of a hot-wash.

One aspect described herein is a method for enriching a population of nucleic acid target sequences in a sample comprising: (a) hybridizing a sample of nucleic acid molecules comprising a plurality of target nucleic acid sequences and a plurality of off-target nucleic acid sequences 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; (b) selectively immobilizing the probe/target complex to form an immobilized probe/target complex and adding a melt buffer; (c) heating the immobilized probe/target complex and the melt buffer to a

temperature 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 (d) 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; wherein step (c) is performed prior to step (d). In one example, the panel of nucleic acid probes that are complementary to the plurality of target nucleic acid sequences further comprises a capture moiety. In another example, the capture moiety is biotin. In another example, 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 phenoxypolyethoxylethanol (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). In another example, the hybridization buffer contains formamide. In another example, the hybridization buffer does not contain formamide.

In one example, the hybridization conditions comprise an incubation time ranging from 10 minutes to 48 hours. In one example, the hybridization conditions comprise an incubation time ranging from 2 hours to overnight. In another example, equivalent specificity and equivalent yield are achieved with a 2-hour incubation time as compared to an overnight incubation time. In another example, the hybridization conditions comprise an incubation temperature ranging from

CLAIMS

What is claimed:

1. A method for enriching a population of nucleic acid target sequences in a sample comprising:
   (a) hybridizing a sample of nucleic acid molecules comprising a plurality of target nucleic acid sequences and a plurality of off-target nucleic acid sequences 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;
   (b) selectively immobilizing the probe/target complex to form an immobilized probe/target complex and adding a melt buffer;
   (c) heating the immobilized probe/target complex and the melt buffer to a temperature 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
   (d) 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;

   wherein step (c) is performed prior to step (d).

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 phenoxypolyethoxylethanol (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 10 minutes to 48 hours.

8. The method of claim 1, wherein the hybridization conditions comprise an incubation time ranging from 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 55 °C to 75 °C.

11. The method of claim 1, wherein the hybridization conditions comprise an incubation temperature ranging from 60 °C to 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 10 minutes to 48 hours.

15. The method of claim 1, wherein the probe/target complex is selectively immobilized under conditions comprising an incubation temperature ranging from 20 °C to 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 melt buffer 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 phenoxypolyethoxylethanol (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 5 minutes to 1 hour.

19. The method of claim 1, wherein the heating of the immobilized probe/target complex comprises an incubation temperature ranging from 50 °C to 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 phenoxypolyethoxylethanol (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 5 minutes to 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.