R2s comprise R2s that comprise a functional first member of the binding pair and R2s that lack a functional first member of the binding pair;

wherein the first member of the binding pair is capable of binding to a second member of the binding pair disposed on substrate, and

wherein the proportion of R1s that comprises a functional first member of the binding pair is greater than the proportion of R2s that comprise a functional first member of the binding pair.

In one aspect, the present disclosure refers to a plurality of target capture reagents, comprising first target capture reagents (R1s), wherein each R1comprises the same first nucleic acid sequence configured to hybridize to a first target nucleic acid sequence, and second target capture reagents (R2s), wherein each R2 comprises the same second nucleic acid sequence configured to hybridize to a second target nucleic acid sequence, wherein:

~~R1s comprise R1s that~~at least a portion of the R1s comprise a ~~functional~~ first member of a binding pair~~, and optionally, R1s that lack a functional first member of the binding pair~~; and

a first portion of the R2s comprise ~~R2s that comprise a~~ the~~functional~~ first member of the binding pair and

a second portion of the R2s ~~that~~ lack ~~a~~the ~~functional~~ first member of the binding pair;

wherein the first member of the binding pair is capable of binding to a second member of the binding pair disposed on substrate, and

wherein the proportion of R1s that comprises a ~~functional~~ first member of the binding pair is greater than the proportion of R2s that comprise a ~~functional~~ first member of the binding pair.

In some examples of the plurality of target capture reagents, the proportion of R1s that comprise a functional first member of the binding pair is at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1,000-fold, greater than the proportion of R2s that comprise a functional first member of the binding pair.

In some examples of the plurality of target capture reagents, each of the R1s is capable of forming a first fragment/first target capture reagent (F1/R1) hybrid, and each of the R2s is capable of forming a second fragment/second target capture reagent (F2/R2) hybrid, and wherein F1, F2, or both, comprises a subject interval from a gene described in **Tables 1A-5A**.

In some examples, F1 comprises a high sequencing depth event.

In some examples, F2 comprises a low sequencing depth event. In other examples, the level of the low sequencing depth event is associated with determination of one or more biomarkers, e.g., tumor mutational burden (TMB), or microsatellite instability (MSI).

In some examples, the plurality of target capture reagents further comprises third target capture reagents (R3s),

wherein R3s comprise R3s that comprise a functional first member of the binding pair and R3s that lack a functional first member of the binding pair;

wherein the first member of the binding pair is capable of binding to a second member of the binding pair disposed on substrate, and

wherein the proportion of R2s that comprises a functional first member of the binding pair is greater (e.g., at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400,

In another example~~aspect~~, disclosed herein is a method of analyzing a sample, comprising:

contacting a plurality of first fragment/first target capture reagent (F1/R1) hybrids with substrate to form F1/R1 hybrid/substrate complexes; and

contacting a plurality of second fragment/second target capture reagent (F2/R2) hybrids with substrate to form F2/R2 hybrid/substrate complexes,

wherein the proportion of F1/R1 hybrids which bind to substrate is greater than the proportion of F2/R2 hybrids which bind to substrate,

thereby analyzing the sample.

In one aspect, the present disclosure refers to a method of analyzing a sample, comprising:

contacting a plurality of first fragments (F1s) and a plurality of second fragments (F2s) with the plurality of target capture reagents as disclosed herein;

contacting a plurality of first fragment/first target capture reagent (F1/R1) hybrids with substrate to form F1/R1 hybrid/substrate complexes; and

contacting a plurality of second fragment/second target capture reagent (F2/R2) hybrids with substrate to form F2/R2 hybrid/substrate complexes,

wherein the proportion of F1/R1 hybrids which bind to substrate is greater than the proportion of F2/R2 hybrids which bind to substrate,

thereby analyzing the sample.

In some examples, a portion of the R1s and a portion of the R2s comprise a functional first member of a binding pair. In some examples, the first member of the binding pair is capable of binding to a second member of the binding pair disposed on substrate.

In some examples, a portion of the R1s, a portion of the R2s, or both, lack a functional first member of a binding pair, e.g., an altered or blocked first member that is not capable of binding, or has reduced binding affinity, to a second member of the binding pair disposed on substrate.

In some examples, the R1s comprise R1s that comprise a functional first member of a binding pair and R1s that lack a functional first member of the binding pair; and the R2s comprise R2s that comprise a functional first member of a binding pair and R2s that lack a functional first member of the binding pair.

In some examples, the proportion of R1s that comprise a functional first member of the binding pair is greater than the proportion of R2s that comprise a functional first member of the binding pair.

In some examples, the proportion of R1s that comprise a functional first member of the binding pair is at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1,000-fold, greater than the proportion of R2s that comprise a functional first member of the binding pair.

In some examples, F1 comprises a high sequencing depth event.

In some examples, F2 comprises a low sequencing depth event. In other examples, the level of the low sequencing depth event is associated with determination of one or more biomarkers, e.g., tumor mutational burden (TMB), or microsatellite instability (MSI).

In some examples, the proportion of R1s that comprise a functional first member of the binding pair and the proportion of R2s that comprise a functional first member of the binding pair are such that, upon formation of the F1/R1 hybrid/substrate complexes and the F2/R2 hybrid/substrate complexes, the number of F1s in the F1/R1 hybrid/substrate complexes and the number of F2s in the F2/R2 hybrid/substrate complexes have one or both of the following relationships:

(i) the number of F1s is greater than, or is substantially the same as, the number of F2s; and/or

In some examples, any of the methods disclosed herein further comprises contacting a plurality of F1s to R1 to provide a plurality of F1/R1 hybrids, and contacting a plurality of F2s to R2 to provide a plurality of F2/R2 hybrids.

In some examples, any of the methods disclosed herein further comprises contacting a plurality of AF1s to R1 to provide a plurality of AF1/R1 hybrids, and contacting a plurality of AF2s to R2 to provide a plurality of AF2/R2 hybrids.

In some examples, a method disclosed herein comprises contacting a plurality of F1/R1 hybrids with substrate to form F1/R1 hybrid/substrate complexes comprises contacting a plurality of AF1/R1 hybrids with substrate to form AF1/R1 hybrid/substrate complexes; and contacting a plurality of F2/R2 hybrids with substrate to form F2/R2 hybrid/substrate complexes comprises contacting a plurality of AF2/R2 hybrids with substrate to form AF2/R2 hybrid/substrate complexes.

In some examples, the contacting occurs in solution or on a solid surface.

In some examples of any of the methods disclosed herein, the first member of the binding pair comprises a biotin moiety, and wherein the second member of the binding pair comprises a streptavidin or avidin (or a modified version, e.g., NeutrAvidin or CaptAvidin) moiety.

In some examples of any of the methods disclosed herein, the first member of the binding pair comprises a digoxigenin moiety, and wherein the second member of the binding pair comprises an anti-digoxigenin antibody moiety.

In some examples of any of the methods disclosed herein, the first member of the binding pair comprises an FITC moiety, and wherein the second member of the binding pair comprises an anti-FITC antibody moiety.

In some examples of any of the methods disclosed herein, the first member of the binding pair in R1 is coupled to a moiety (e.g., a nucleotide sequence) in R1 that captures (e.g., hybridizes to) F1 via a linker. In some examples, the first member of the binding pair in R2 is coupled to a moiety (e.g., a nucleotide sequence) in R2 that captures (e.g., hybridizes to) F2 via a linker. In some examples, the linker is a cleavable linker.

In some examples, any of the methods disclosed herein further comprises sequencing F1 from the plurality of F1/R1 hybrid/substrate complexes, and sequencing F2 from the plurality of F2/R2 hybrid/substrate complexes. In some examples, F1 is sequenced to a greater depth than F2, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10-fold greater.

In yet another example~~aspect~~, the disclosure provides a method of analyzing a sample, comprising:

a) providing a plurality of first fragment/first target capture reagent (F1/R1) hybrids and a plurality of second fragment/second target capture reagent (F2/R2) hybrids,

wherein the proportion of R1s that comprise a functional first member of the binding pair is greater than the proportion of R2s that comprise a functional first member of the binding pair, and

wherein the first member of the binding pair is capable of binding to a second member of the binding pair disposed on substrate;

b) contacting the plurality of F1/R1 hybrids with substrate to form F1/R1 hybrid/substrate complexes, and contacting the plurality of F2/R2 hybrids with substrate to form F2/R2 hybrid/substrate complexes,

wherein the proportion of F1/R1 hybrids which bind to the substrate is greater than the proportion of F2/R2 hybrids which bind to the substrate; and

c) sequencing F1 from the plurality of F1/R1 hybrid/substrate complexes, and sequencing F2 from the plurality of F2/R2 hybrid/substrate complexes,

wherein F1 is sequenced to a greater depth than F2,

thereby analyzing the sample.

In one aspect, the present disclosure refers to a method of analyzing a sample, comprising:

a) contacting a plurality of first fragments (F1s) and a plurality of second fragments (F2s) with the plurality of target capture reagents as disclosed herein to provide a plurality of first fragment/first target capture reagent (F1/R1) hybrids and a plurality of second fragment/second target capture reagent (F2/R2) hybrids;

b) contacting the plurality of F1/R1 hybrids with substrate to form F1/R1 hybrid/substrate complexes, and contacting the plurality of F2/R2 hybrids with substrate to form F2/R2 hybrid/substrate complexes,

wherein the proportion of F1/R1 hybrids which bind to the substrate is greater than the proportion of F2/R2 hybrids which bind to the substrate; and

c) sequencing F1 from the plurality of F1/R1 hybrid/substrate complexes, and sequencing F2 from the plurality of F2/R2 hybrid/substrate complexes,

wherein F1 is sequenced to a greater depth than F2,

thereby analyzing the sample.

In an example~~aspect~~, disclosed herein is a method of analyzing a sample, comprising:

1) providing a sample, e.g., a sample comprising genomic DNA, e.g., cell-free DNA (cfDNA) or circulating tumor DNA (ctDNA), from a subject;

2) obtaining, e.g., isolating, nucleic acids from the sample;

3) fragmenting the nucleic acids to provide a plurality of fragments (Fs);

4) attaching adapter sequences to the plurality of fragments (Fs) to provide a plurality of adapterized fragments (AFs);

5) amplifying a first AF (AF1) to provide a plurality of AF1, and amplifying a second AF (AF2) to provide a plurality of AF2;

6) contacting a plurality of AF1 with first target capture reagents (R1s), each comprising a nucleotide sequence that hybridizes to AF1, to provide a plurality of AF1/R1 hybrids, and contacting a plurality of AF2 with second target capture reagents (R2s), each comprising a nucleotide sequence that hybridizes to AF2, to provide a plurality of AF2/R2 hybrids,

wherein a portion of the R1s and a portion of the R2s comprise a functional first member of a binding pair, and wherein the first member of the binding pair is capable of binding to a second member of the binding pair disposed on substrate, and

wherein a portion of the R1s, a portion of the R2s, or both, lack a functional first member of a binding pair;

7) contacting the plurality of AF1/R1 hybrids with substrate to form AF1/R1 hybrid/substrate complexes, and contacting the plurality of AF2/R2 hybrids with substrate to form AF2/R2 hybrid/substrate complexes,

wherein the proportion of AF1/R1 hybrids which bind to the substrate is greater than the proportion of AF2/R2 hybrids which bind to the substrate; and

8) sequencing AF1 from the plurality of AF1/R1 hybrid/substrate complexes, and sequencing AF2 from the plurality of AF2/R2 hybrid/substrate complexes,

optionally, wherein AF1 is sequenced to a greater depth than AF2, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10-fold greater;

thereby analyzing the sample.

In one aspect, the present disclosure refers to a method of analyzing a sample, comprising:

1) providing a sample, e.g., a sample comprising genomic DNA, e.g., cell-free DNA (cfDNA) or circulating tumor DNA (ctDNA), from a subject;

2) obtaining, e.g., isolating, nucleic acids from the sample;

3) fragmenting the nucleic acids to provide a plurality of fragments (Fs);

4) attaching adapter sequences to the plurality of fragments (Fs) to provide a plurality of adapterized fragments (AFs);

5) amplifying a first AF (AF1) to provide a plurality of AF1, and amplifying a second AF (AF2) to provide a plurality of AF2;

6) contacting a plurality of AF1 and a plurality of AF2 with the plurality of target capture reagents as disclosed herein to provide a plurality of AF1/R1 hybrids and a plurality of AF2/R2 hybrids;

7) contacting the plurality of AF1/R1 hybrids with substrate to form AF1/R1 hybrid/substrate complexes, and contacting the plurality of AF2/R2 hybrids with substrate to form AF2/R2 hybrid/substrate complexes,

wherein the proportion of AF1/R1 hybrids which bind to the substrate is greater than the proportion of AF2/R2 hybrids which bind to the substrate; and

8) sequencing AF1 from the plurality of AF1/R1 hybrid/substrate complexes, and sequencing AF2 from the plurality of AF2/R2 hybrid/substrate complexes,

optionally, wherein AF1 is sequenced to a greater depth than AF2, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10-fold greater;

thereby analyzing the sample.

In some examples, any of the methods disclosed herein further comprises acquiring a library comprising a plurality of nucleic acid molecules from the sample.

In some examples, any of the methods disclosed herein further comprises contacting the library with target capture reagents to provide selected nucleic acid molecules, wherein said target capture reagents hybridize with the nucleic acid molecule, thereby providing a library catch.

In some examples, any of the methods disclosed herein further comprises acquiring a read for a subject interval comprising an alteration (e.g., a somatic alteration) from a nucleic acid molecule from said library or library catch, thereby acquiring a read for the subject interval, e.g., by a next-generation sequencing method.

In some examples, the method comprises acquiring reads for subject intervals in a plurality of genes.

In some examples, the plurality of genes comprises genes in mutant form, e.g., the mutant genes are associated with an effect on cell division, growth or survival, or are associated with cancer. In some examples, the plurality of genes comprises at least about 50 or more, about 100 or more, about 150 or more, about 200 or more, about 250 or more, about 300 or more, about 350 or more, about 400 or more, about 450 or more, about 500 or more genes, or about 1,000 or more genes, or all genes for whole exon sequencing (WES).

In some examples, the plurality of genes comprises at least about 50 or more, about 100 or more, about 150 or more, about 200 or more, about 250 or more, about 300 or more, or all of the genes described in **Tables 1A-5A**.

In some examples, acquiring reads for subject intervals comprises sequencing subject intervals from at least about 50 or more, about 100 or more, about 150 or more, about 200 or more, about 250 or more, about 300 or more, or all of the genes described in **Tables 1A-5A**.

In some examples, subject intervals are sequenced to greater than about 100X, greater than about 250X, greater than about 500X, greater than about 800X, greater than about 1,000X, greater than about 2,000X, greater than about 3,000X, greater than about 4,000X, or greater than about 5,000X, average depth.

In some examples, subject intervals are sequenced to greater than about 100X, greater than about 250X, greater than about 500X, greater than about 800X, greater than about 1,000X, greater than about 2,000X, greater than about 3,000X, greater than about 4,000X, or greater than about 5,000X, average depth, at greater than about 95%, greater than about 97%, or greater than about 99%, of the genes (e.g., exons) sequenced.

CLAIMS

What is claimed is:

1. A plurality of target capture reagents, comprising first target capture reagents (R1s), wherein each R1comprises the same first nucleic acid sequence configured to hybridize to a first target nucleic acid sequence, and second target capture reagents (R2s), wherein each R2 comprises the same second nucleic acid sequence configured to hybridize to a second target nucleic acid sequence,

wherein:

R1s comprise R1s thatat least a portion of the R1s comprise a functional first member of a binding pair, and optionally, R1s that lack a functional first member of the binding pair; and

a first portion of the R2s comprise R2s that comprise a thefunctional first member of the binding pair and

a second portion of the R2s that lack athe functional first member of the binding pair;

wherein the first member of the binding pair is capable of binding to a second member of the binding pair disposed on substrate, and

wherein the proportion of R1s that comprises a functional first member of the binding pair is greater than the proportion of R2s that comprise a functional first member of the binding pair.

2. The plurality of target capture reagents of claim 1, wherein the proportion of R1s that comprise a functional first member of the binding pair is at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1,000-fold, greater than the proportion of R2s that comprise a functional first member of the binding pair.

3. The plurality of target capture reagents of claim 1 or 2, wherein each of the R1s is capable of forming a first fragment/first target capture reagent (F1/R1) hybrid, and each of the R2s is capable of forming a second fragment/second target capture reagent (F2/R2) hybrid, and

wherein F1, F2, or both, comprises a subject interval from a gene described in Tables 1A-5A.

4. The plurality of target capture reagents of claim 3, wherein:

F1 comprises a high sequencing depth event; and

F2 comprises a low sequencing depth event, e.g., the level of which is associated with determination of one or more biomarkers, e.g., tumor mutational burden (TMB), or microsatellite instability (MSI).

5. The plurality of target capture reagents of any of the preceding claims, further comprising third target capture reagents (R3s),

wherein R3s comprise R3s that comprise a ~~functional~~ first member of the binding pair and R3s that lack a ~~functional~~ first member of the binding pair;

wherein the first member of the binding pair is capable of binding to a second member of the binding pair disposed on substrate, and

wherein the proportion of R2s that comprises a ~~functional~~ first member of the binding pair is greater than the proportion of R3s that comprise a ~~functional~~ first member of the binding pair.

6. The plurality of target capture reagents of ~~any of the preceding claims~~claim 5, wherein each of the R3s is capable of forming a third fragment/first target capture reagent (F3/R3) hybrid, and

wherein F3 comprises a subject interval from a gene described in Tables 1A-5A.

7. A method of analyzing a sample, comprising:

contacting a plurality of first fragments (F1s) and a plurality of second fragments (F2s) with the plurality of target capture reagents according to any one of claims 1-6;

contacting a plurality of first fragment/first target capture reagent (F1/R1) hybrids with substrate to form F1/R1 hybrid/substrate complexes; and

contacting a plurality of second fragment/second target capture reagent (F2/R2) hybrids with substrate to form F2/R2 hybrid/substrate complexes,

wherein the proportion of F1/R1 hybrids which bind to substrate is greater than the proportion of F2/R2 hybrids which bind to substrate,

thereby analyzing the sample.

8. The method of claim 7, wherein:

F1 comprises a high sequencing depth event; and

F2 comprises a low sequencing depth event, e.g., the level of which is associated with determination of one or more biomarkers, e.g., tumor mutational burden (TMB), or microsatellite instability (MSI).

9. The method of claim 7 or 8, wherein a portion of the R1s and a portion of the R2s comprise a ~~functional~~ first member of a binding pair, and wherein the first member of the binding pair is capable of binding to a second member of the binding pair disposed on substrate.

10. The method of any of claims 7-9, wherein a portion of the R1s, a portion of the R2s, or both, lack a ~~functional~~ first member of a binding pair, e.g., an altered or blocked first member that is not capable of binding, or has reduced binding affinity, to a second member of the binding pair disposed on substrate.

11. The method of any of claims 7-10, wherein:

the R1s comprise R1s that comprise a ~~functional~~ first member of a binding pair and R1s that lack a ~~functional~~ first member of the binding pair; and

the R2s comprise R2s that comprise a ~~functional~~ first member of a binding pair and R2s that lack a ~~functional~~ first member of the binding pair.

12. The method of any of claims 7-11, wherein the proportion of R1s that comprise a ~~functional~~ first member of the binding pair is greater than the proportion of R2s that comprise a ~~functional~~ first member of the binding pair.

13. The method of claim 11, wherein the proportion of R1s that comprise a ~~functional~~ first member of the binding pair is at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1,000-fold, greater than the proportion of R2s that comprise a ~~functional~~ first member of the binding pair.

14. The method of any of claims 7-13, wherein the proportion of R1s that comprise a ~~functional~~ first member of the binding pair and the proportion of R2s that comprise a ~~functional~~ first member of the binding pair are such that, upon formation of the F1/R1 hybrid/substrate complexes and the F2/R2 hybrid/substrate complexes, the number of F1s in the F1/R1 hybrid/substrate complexes and the number of F2s in the F2/R2 hybrid/substrate complexes have one or both of the following relationships:

(i) the number of F1s is greater than, or is substantially the same as, the number of F2s; and/or

(ii) the number of F1s comprising an alteration in a first subject interval is greater than, or is substantially the same as, the number of F2s comprising an alteration in a second subject interval.

15. The method of claim 14, wherein the number of F1s is at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1,000-fold, greater than the number of F2s.

16. The method of claim 14 or 15, wherein the number of F1s comprising an alteration in a first subject interval is at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1,000-fold, greater than the number of F2s comprising an alteration in a second subject interval.

17. The method of any of claims 14-16, wherein the first subject interval, the second subject interval, or both, is from a gene described in Tables 1A-5A.

18. The method of any of claims 14-17, wherein the alteration in the first subject interval is present at a mutant allele frequency of equal to or greater than ~~about~~ 0.1% (e.g., equal to or greater than ~~about~~ 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, or 0.9%, e.g., ~~about~~ 0.1% to 0.9%, 0.2% to 0.8%, 0.3% to 0.7%, or 0.4% to 0.6%) in the sample.

19. The method of any of claims 14-18, wherein the alteration in the second subject interval is present at a mutant allele frequency of equal to or greater than ~~about~~ 1% (e.g., equal to or greater than ~~about~~ 2%, 3%, 4%, 5%, 6%, 7%, 8%, or 9%, e.g., ~~about~~ 1% to 9%, 2% to 8%, 3% to 7%, or 4% to 6%) in the sample.

20. The method of any of claims 7-19, wherein F1, F2, or both, comprises a subject interval from a gene described in Tables 1A-5A.

21. The method of claim 20, wherein the subject interval in F1 is sequenced to a first depth, and the subject interval in F2 is sequenced to a second depth, wherein the first depth is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10-fold greater than the second depth.

22. The method of any of claims 14-21, wherein F1 comprises a subject interval from a gene described in Tables 1A-5A, and wherein the subject interval comprises an alteration, e.g., a somatic alteration, e.g., a functional alteration in cancer.

23. The method of claim 17, wherein the subject interval is sequenced to at least ~~about~~ 5,000X depth.

24. The method of any of claims 7-23, wherein F2 comprises a subject interval from a gene described in Tables 1A-5A, and wherein the subject interval comprises an alteration, e.g., a somatic

alteration, wherein the determination of the alteration is used for evaluating one or more genomic signatures, e.g., continuous/complex biomarkers.

25. The method of claim 24, wherein the subject interval is sequenced to at least ~~about~~ 800X but less than ~~about~~ 5,000X., e.g., for evaluating one or more genomic signatures, e.g., continuous/complex biomarkers.

26. The method of any of claims 7-25, further contacting a plurality of third fragment/third target capture reagent (F3/R3) hybrids with substrate to form F3/R3 hybrid/substrate complexes.

27. The method of claim 25 or 26, wherein R3s comprise R3s that comprise a ~~functional~~ first member of the binding pair and R3s that lack a ~~functional~~ first member of the binding pair.

28. The method of any of claims 26-27, wherein the proportion of R2s that comprise a ~~functional~~ first member of the binding pair is greater than the proportion of R3s that comprise a ~~functional~~ first member of the binding pair.

29. The method of any of claims 26-28, wherein the proportion of R2s that comprise a ~~functional~~ first member of the binding pair is at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1,000-fold, greater than the proportion of R3s that comprise a ~~functional~~ first member of the binding pair.

30. The method of any of claims 26-29, wherein the proportion of R2s that comprise a ~~functional~~ first member of the binding pair and the proportion of R3s that comprise a ~~functional~~ first member of the binding pair are such that, upon formation of the F2/R2 hybrid/substrate complexes and the F3/R3 hybrid/substrate complexes, the number of F2s in the F2/R2 hybrid/substrate complexes and the number of F3s in the F3/R3 hybrid/substrate complexes have one or both of the following relationships:

(i) the number of F2s is greater than the number of F3s; and/or

(ii) the number of F2s comprising an alteration in a second subject interval is greater than the number of F3s comprising an alteration in a third subject interval.

31. The method of claim 30, wherein the number of F2s is at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1,000-fold, greater than the number of F3s.

32. The method of claim 30 or 31, wherein the number of F2s comprising an alteration in a second subject interval is at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1,000-fold, greater than the number of F3s comprising an alteration in a third subject interval.

33. The method of any of claims 30-32, wherein the second subject interval, the third subject interval, or both, is from a gene described in Tables 1A-5A.

34. The method of any of claims 26-33, wherein one, two or all of F1, F2, or F3 comprises a subject interval from a gene described in Tables 1A-5A.

35. The method of claim 34, wherein the subject interval in F2 is sequenced to a second depth, and the subject interval in F3 is sequenced to a third depth, wherein the second depth is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10-fold greater than the third depth.

36. The method of any of claims 26-35, wherein F3 comprises a subject interval from a gene described in Tables 1A-5A, and wherein the subject interval comprises a germline alteration, e.g., a germline SNP.

37. The method of claim 36, wherein the subject interval is sequenced to at least ~~about~~ 100X depth but less than ~~about~~ 800X.

38. The method of any of claims 7-37, further comprising providing the sample from a subject.

39. The method of any of claims 7-38, wherein the sample comprises DNA, e.g., genomic DNA, e.g., cell-free DNA (cfDNA) or circulating tumor DNA (ctDNA).

40. The method of any of claims 7-39, wherein the sample comprises RNA, e.g., mRNA.

41. The method of claim 40, further comprising providing cDNA from RNA.

42. The method of any claims 7-41, further comprising obtaining, e.g., isolating, nucleic acids from the sample.

43. The method of any of claims 7-42, further comprising fragmenting nucleic acids in the sample to provide F1 and F2.

44. The method of any of claims 7-43, further comprising amplifying F1 to provide a plurality of F1s, and amplifying F2 to provide a plurality of F2s.

45. The method of any of claims 7-44, further comprising attaching adapter sequences to F1 and F2 to provide adapterized F1 (AF1) and adapterized F2 (AF2).

46. The method of any of claims 7-45, further comprising amplifying AF1 to provide a plurality of AF1s, and amplifying AF2 to provide a plurality of AF2s.

47. The method of any of claims 7-46, further comprising contacting a plurality of F1s to R1 to provide a plurality of F1/R1 hybrids, and contacting a plurality of F2s to R2 to provide a plurality of F2/R2 hybrids.

48. The method of any of claims 7-47, further comprising contacting a plurality of AF1s to R1 to provide a plurality of AF1/R1 hybrids, and contacting a plurality of AF2s to R2 to provide a plurality of AF2/R2 hybrids.

49. The method of any of claims 7-48, wherein:

contacting a plurality of F1/R1 hybrids with substrate to form F1/R1 hybrid/substrate complexes comprises contacting a plurality of AF1/R1 hybrids with substrate to form AF1/R1 hybrid/substrate complexes; and

contacting a plurality of F2/R2 hybrids with substrate to form F2/R2 hybrid/substrate complexes comprises contacting a plurality of AF2/R2 hybrids with substrate to form AF2/R2 hybrid/substrate complexes.

50. The method of any of claims 47-49, wherein the contacting occurs in solution.

51. The method of any of claims 47-49, wherein the contacting occurs on a solid surface.

52. The method of any of claims 8-51, wherein the first member of the binding pair comprises a biotin moiety, and wherein the second member of the binding pair comprises a streptavidin or avidin (or a modified version, e.g., NeutrAvidin or CaptAvidin) moiety.

53. The method of any of claims 8-51, wherein the first member of the binding pair comprises a digoxigenin moiety, and wherein the second member of the binding pair comprises an anti-digoxigenin antibody moiety.

54. The method of any of claims 8-51, wherein the first member of the binding pair comprises an FITC moiety, and wherein the second member of the binding pair comprises an anti-FITC antibody moiety.

55. The method of any of claims 8-51, wherein the first member of the binding pair in R1 is coupled to a moiety (e.g., a nucleotide sequence) in R1 that captures (e.g., hybridizes to) F1 via a linker, and wherein the first member of the binding pair in R2 is coupled to a moiety (e.g., a nucleotide sequence) in R2 that captures (e.g., hybridizes to) F2 via a linker,

optionally, wherein the linker is a cleavable linker.

56. The method of any of claims 7-55, further comprising sequencing F1 from the plurality of F1/R1 hybrid/substrate complexes, and sequencing F2 from the plurality of F2/R2 hybrid/substrate complexes.

57. The method of claim 56, wherein F1 is sequenced to a greater depth than F2, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10-fold greater.

58. A method of analyzing a sample, comprising:

a) contacting a plurality of first fragments (F1s) and a plurality of second fragments (F2s) with the plurality of target capture reagents according to any one of claims 1-6 to provide~~providing~~ a plurality of first fragment/first target capture reagent (F1/R1) hybrids and a plurality of second fragment/second target capture reagent (F2/R2) hybrids~~,~~;

~~wherein the proportion of R1s that comprise a functional first member of the binding pair is greater than the proportion of R2s that comprise a functional first member of the binding pair, and~~

~~wherein the first member of the binding pair is capable of binding to a second member of the binding pair disposed on substrate;~~

b) contacting the plurality of F1/R1 hybrids with substrate to form F1/R1 hybrid/substrate complexes, and contacting the plurality of F2/R2 hybrids with substrate to form F2/R2 hybrid/substrate complexes,

wherein the proportion of F1/R1 hybrids which bind to the substrate is greater than the proportion of F2/R2 hybrids which bind to the substrate; and

c) sequencing F1 from the plurality of F1/R1 hybrid/substrate complexes, and sequencing F2 from the plurality of F2/R2 hybrid/substrate complexes,

wherein F1 is sequenced to a greater depth than F2,

thereby analyzing the sample.

59. The method of claim 58, wherein:

F1 comprises a high sequencing depth event; and

F2 comprises a low sequencing depth event, e.g., the level of which is associated with determination of one or more biomarkers, e.g., tumor mutational burden (TMB), or microsatellite instability (MSI).

60. A method of analyzing a sample, comprising:

1) providing a sample, e.g., a sample comprising genomic DNA, e.g., cell-free DNA (cfDNA) or circulating tumor DNA (ctDNA), from a subject;

2) obtaining, e.g., isolating, nucleic acids from the sample;

3) fragmenting the nucleic acids to provide a plurality of fragments (Fs);

4) attaching adapter sequences to the plurality of fragments (Fs) to provide a plurality of adapterized fragments (AFs);

5) amplifying a first AF (AF1) to provide a plurality of AF1, and amplifying a second AF (AF2) to provide a plurality of AF2;

6) contacting a plurality of AF1 and a plurality of AF2 with the plurality of target capture reagents of any one of claims 1-6~~with first target capture reagents (R1s), each comprising a nucleotide sequence that hybridizes to AF1,~~ to provide a plurality of AF1/R1 hybrids~~, and contacting a plurality of AF2 with second target capture reagents (R2s), each comprising a nucleotide sequence that hybridizes to AF2, to provide~~ and a plurality of AF2/R2 hybrids~~,~~;

~~wherein a portion of the R1s and a portion of the R2s comprise a functional first member of a binding pair, and wherein the first member of the binding pair is capable of binding to a second member of the binding pair disposed on substrate, and~~

~~wherein a portion of the R1s, a portion of the R2s, or both, lack a functional first member of a binding pair;~~

7) contacting the plurality of AF1/R1 hybrids with substrate to form AF1/R1 hybrid/substrate complexes, and contacting the plurality of AF2/R2 hybrids with substrate to form AF2/R2 hybrid/substrate complexes,

wherein the proportion of AF1/R1 hybrids which bind to the substrate is greater than the proportion of AF2/R2 hybrids which bind to the substrate; and

8) sequencing AF1 from the plurality of AF1/R1 hybrid/substrate complexes, and sequencing AF2 from the plurality of AF2/R2 hybrid/substrate complexes,

optionally, wherein AF1 is sequenced to a greater depth than AF2, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10-fold greater;

thereby analyzing the sample.

61. The method of claim 60, wherein:

F1 comprises a high sequencing depth event; and

F2 comprises a low sequencing depth event, e.g., the level of which is associated with determination of one or more biomarkers, e.g., tumor mutational burden (TMB), or microsatellite instability (MSI).

62. The method of any of claims 7-61, further comprising acquiring a library comprising a plurality of nucleic acid molecules from the sample.

63. The method of claim 62, further comprising contacting the library with target capture reagents to provide selected nucleic acid molecules, wherein said target capture reagents hybridize with the nucleic acid molecule, thereby providing a library catch.

64. The method of claim 63, further comprising acquiring a read for a subject interval comprising an alteration (e.g., a somatic alteration) from a nucleic acid molecule from said library or library catch, thereby acquiring a read for the subject interval, *e.g.*, by a next-generation sequencing method.

65. The method of claim 64, comprising acquiring reads for subject intervals in a plurality of genes.

66. The method of claim 65, wherein the plurality of genes comprises genes in mutant form, e.g., the mutant genes are associated with an effect on cell division, growth or survival, or are associated with cancer.

67. The method of claim 65 or 66, wherein the plurality of genes comprises at least ~~about~~ 50 or more, ~~about~~ 100 or more, ~~about~~ 150 or more, ~~about~~ 200 or more, ~~about~~ 250 or more, ~~about~~ 300 or more, ~~about~~ 350 or more, ~~about~~ 400 or more, ~~about~~ 450 or more, ~~about~~ 500 or more genes, or ~~about~~ 1,000 or more genes, or all genes for whole exon sequencing (WES).

68. The method of any of claims 64-67, wherein the plurality of genes comprises at least ~~about~~ 50 or more, ~~about~~ 100 or more, ~~about~~ 150 or more, ~~about~~ 200 or more, ~~about~~ 250 or more, ~~about~~ 300 or more, or all of the genes described in Tables 1A-5A.

69. The method of any of claims 64-68, wherein acquiring reads for subject intervals comprises sequencing subject intervals from at least ~~about~~ 50 or more, ~~about~~ 100 or more, ~~about~~ 150 or more, ~~about~~ 200 or more, ~~about~~ 250 or more, ~~about~~ 300 or more, or all of the genes described in Tables 1A-5A.

70. The method of any of claims 64-69, wherein subject intervals are sequenced to greater than ~~about~~ 100X, greater than ~~about~~ 250X, greater than ~~about~~ 500X, greater than ~~about~~ 800X, greater than ~~about~~ 1,000X, greater than ~~about~~ 2,000X, greater than ~~about~~ 3,000X, greater than ~~about~~ 4,000X, or greater than ~~about~~ 5,000X, average depth.

71. The method of any of claims 64-70, wherein subject intervals are sequenced to greater than ~~about~~ 100X, greater than ~~about~~ 250X, greater than ~~about~~ 500X, greater than ~~about~~ 800X, greater than ~~about~~ 1,000X, greater than ~~about~~ 2,000X, greater than ~~about~~ 3,000X, greater than ~~about~~ 4,000X, or greater than ~~about~~ 5,000X, average depth, at greater than ~~about~~ 95%, greater than ~~about~~ 97%, or greater than ~~about~~ 99%, of the genes (*e.g.*, exons) sequenced.

72. The method of any of claims 64-71, further comprising aligning said read by an alignment method.

73. The method of claim 72, further comprising assigning a nucleotide value from said read for a nucleotide position.

74. The method of any of claims 7-73, further comprising evaluating one or more genomic signatures, e.g., continuous/ complex biomarkers in the sample.

75. The method of claim 74, wherein the sample is a blood sample.

76. The method of any of claims 7-75, further comprising characterizing an alteration in the sample as a somatic or germline alteration.

77. The method of any of claims 7-76, further comprising determining the zygosity of an alteration in the sample.

78. The method of any of claims 7-77, further comprising classifying the sample or a subject from which the sample was obtained responsive to the analysis of the sample.

79. The method of any of claims 7-78, further comprising providing a report, *e.g.*, an electronic, web-based, or paper report, to the subject from which the sample is obtained or to another person or entity, a caregiver, a physician, an oncologist, a hospital, clinic, third-party payor, insurance company or government office.