primers are used to enrich targets sequences during an initial PCR cycling prior to further amplification using a common primer. Nonetheless, the current paradigm is that the selection of primers must be optimized to achieve the ideal performance of multiplex PCR [e.g., Canzar, et al. *Bioinformatics*, 2016, 1-3 ("Canzar")].

[0004] Therefore, a need remains for a method that is capable of amplifying multiple target sequences using multiple primers while avoiding the negative effects of primer-dimer formation without requiring onerous and costly primer optimization.

SUMMARY

[0005] In one aspect, the present disclosure relates to a method comprising the steps of: reverse transcribing at least one first strand of cDNA from mRNA containing at least one target sequence, using a reverse primer mix, forming a first strand cDNA; wherein the reverse primer mix contains at least one reverse primer configured to incorporate a reverse common primer binding site into each first strand of cDNA; selecting each first strand Cdna by purifying the first strand Cdna, wherein the first strand cDNA comprises a first strand cDNA:RNA complex; synthesizing at least one second strand of cDNA from each of the at least one first strand of cDNA using a forward primer mix, forming at least one first strand:second strand complex; wherein the forward primer mix contains at least one forward primer, each forward primer configured to bind to a particular first strand of cDNA and to incorporate a forward common primer binding site into each second strand of cDNA; selecting each second strand of cDNA; selecting each first strand:second strand complex; amplifying the cDNA strands using a reverse common primer which binds to the at least one reverse common primer binding site and using a forward common primer which binds to the at least one forward common primer binding site; and selecting the amplified cDNA strands by purifying the amplified cDNA strands. In certain examples, the method further comprises the step of amplifying the amplified cDNA strands using a reverse common primer which binds to the at least one reverse common primer binding

site and using a forward common primer which binds to the at least one forward common primer binding site. In certain examples of the method, the reverse primer mix comprises at least one reverse primer, wherein the at least one reverse primer comprises additional nucleotides which incorporate into each first cDNA strand as an identifying marker. In certain examples of the method, the forward primer mix comprises at least one forward primer, wherein the at least one forward primer comprises additional nucleotides which incorporate into each second cDNA strand as an identifying marker. In certain examples of the method, each selection comprises separation of cDNA strands from primer mix using magnetic beads. In certain examples of the method, each selection comprises separation of cDNA strands from primer mix by column purification. In certain examples of the method, each selection comprises enzymatic cleavage of primer mix. In certain examples, the first strand cDNA comprises a first strand cDNA:RNA complex. In another aspect, the present disclosure refers to a method of diagnosing the presence of a disease in a subject, said method comprising: performing the method as disclosed herein on the nucleic acids in a sample obtained from the subject, wherein the sample is suspected of containing a disease agent, wherein the disease agent is characterized by a target sequence; sequencing the amplified DNA strands; and detecting a target sequence from the disease agent. In certain examples, the present disclosure relates to a method of diagnosing the presence of a disease in a subject, said method comprising: providing a sample from the subject, the sample suspected of containing a disease agent, wherein the disease agent is characterized by a target sequence; performing the method described in this paragraph on the nucleic acids in the sample; sequencing the amplified DNA strands; and detecting a target sequence from the disease agent. In another aspect, the present disclosure relates to a method for producing an immune status profile for a subject, the method comprising: performing the method described in this paragraph on the nucleic acids from a sample of white blood

cells from the subject wherein the at least one target sequence represents a T-cell receptor, antibody, or MHC rearrangement; sequencing the amplified DNA strands; and identifying and quantifying one or more DNA sequences representing T-cell receptor, antibody, and MHC rearrangements to create an immune status profile of the subject. In certain examples, the mRNA is obtained from a single cell.

[0006] In another aspect, the present disclosure relates to a method comprising the steps of: synthesizing at least one first strand of DNA from genomic DNA containing at least one target sequence using a first primer mix, forming a first strand:DNA complex; wherein the first primer mix contains at least one first primer, each first primer is configured to bind to a particular target sequence and to incorporate a first common primer binding site into each first strand of DNA; selecting each first strand:DNA complex by purifying each first strand:DNA complex; synthesizing at least one second strand of DNA from each of the at least one first strand of DNA using a second primer mix, forming a first strand:second strand complex; wherein the second primer mix contains at least one second primer, each second primer configured to bind to a particular first strand of DNA and to incorporate a second common primer binding site into each second strand of DNA; selecting each first strand:second strand complex by purifying each first strand:second strand complex; amplifying the DNA strands using a first common primer which binds to the at least one first common primer binding site and using a second common primer which binds to the at least one second common primer binding site; and selecting the amplified DNA strands by purifying the amplified DNA strands. In certain examples, the genomic DNA is obtained from a single cell. In another aspect, the present disclosure refers to a method of diagnosing the presence of a disease in a subject, said method comprising: performing the method as disclosed herein on the nucleic acids in a sample obtained from the subject, wherein the sample is suspected of containing a disease agent, wherein the disease agent is characterized by

a target sequence; sequencing the amplified DNA strands; and detecting a target sequence from the disease agent. In certain examples, the present disclosure relates to a method of diagnosing the presence of a disease in a subject, said method comprising: providing a sample from the subject, the sample suspected of containing a disease agent, wherein the disease agent is characterized by a target sequence; isolating nucleic acids from the sample; performing the method described in this paragraph on the isolated nucleic acids; sequencing the amplified DNA strands; and detecting a target sequence from the disease agent. In another aspect, the present disclosure relates to a method for producing an immune status profile for a subject, the method comprising: performing the method described in this paragraph on the nucleic acids from a sample of white blood cells from the subject wherein the at least one target sequence represents a T-cell receptor, antibody, or MHC rearrangement; sequencing the amplified DNA strands; and identifying and quantifying one or more DNA sequences representing T-cell receptor, antibody, and MHC rearrangements to create an immune status profile of the subject.

CLAIMS

Now, therefore, the following is claimed:

1. A method comprising the steps of:

reverse transcribing at least one first strand of cDNA from mRNA containing at least one target sequence, using a reverse primer mix, forming at least one first strand cDNA;

wherein the reverse primer mix contains at least one reverse primer configured to incorporate a reverse common primer binding site into each first strand of cDNA;

selecting each first strand cDNA by purifying the first strand cDNA, wherein the first strand cDNA comprises a first strand cDNA:RNA complex;

synthesizing at least one second strand of cDNA from each of the at least one first strand of cDNA using a forward primer mix, forming at least one first strand:second strand complex;

wherein the forward primer mix contains at least one forward primer, each forward primer configured to bind to a particular first strand of cDNA and to incorporate a forward common primer binding site into each second strand of cDNA;

selecting each first strand:second strand complex;

amplifying the cDNA strands using a reverse common primer which binds to the at least one reverse common primer binding site and using a forward common primer which binds to the at least one forward common primer binding site; and

selecting the amplified cDNA strands by purifying the amplified cDNA strands.

2. The method of claim 1, further comprising the step, prior to selecting the amplified cDNA strands, of amplifying the amplified cDNA strands using a reverse common primer which binds to the at least one reverse common primer binding site and using a forward common primer which binds to the at least one forward common primer binding site.

3. The method of claim 1, wherein the reverse primer mix comprises at least one reverse primer, wherein the at least one reverse primer comprises additional nucleotides which incorporate into each first cDNA strand as an identifying marker.

4. The method of claim 1, wherein the forward primer mix comprises at least one forward primer, wherein the at least one forward primer comprises additional nucleotides which incorporate into each second cDNA strand as an identifying marker.

5. The method of claim 1, wherein each selection comprises separation of cDNA strands from primer mix using magnetic beads.

6. The method of claim 1, wherein each selection comprises separation of cDNA strands from primer mix by column purification.

7. The method of claim 1, wherein each selection comprises enzymatic cleavage of primer mix.

8. A method of diagnosing the presence of a disease in a subject, said

method comprising:

performing the method of claim 1 on the nucleic acids in a sample obtained from the subject, wherein the sample is suspected of containing a disease agent, wherein the disease agent is characterized by a target sequence;

sequencing the amplified DNA strands; and

detecting a target sequence from the disease agent.

9. A method for producing an immune status profile for a subject, the method comprising:

performing the method of claim 1 on the nucleic acids from a sample of white blood cells from the subject, wherein the at least one target sequence represents a T-cell receptor, antibody, or MHC rearrangement;

sequencing the amplified DNA strands; and

identifying and quantifying one or more DNA sequences representing T-cell receptor, antibody, and MHC rearrangements to create an immune status profile of the subject.

10. The method of claim 1, wherein the mRNA is obtained from a single cell.

11. A method comprising the steps of:

synthesizing at least one first strand of DNA from genomic DNA containing at least one target sequence using a first primer mix, forming a first strand:DNA complex;

wherein the first primer mix contains at least one first primer, each first primer configured to bind to a particular target sequence and to incorporate a first common primer binding site into each first strand of DNA;

selecting each first strand:DNA complex by purifying each first strand:DNA complex;

synthesizing at least one second strand of DNA from each of the at least one first strand of DNA using a second primer mix, forming a first strand:second strand complex;

wherein the second primer mix contains at least one second primer, each second primer configured to bind to a particular first strand of DNA and to incorporate a second common primer binding site into each second strand of DNA;

selecting each first strand:second strand complex by purifying each first strand:second strand complex;

amplifying the DNA strands using a first common primer which binds to the at least one first common primer binding site and using a second common primer which binds to the at least one second common primer binding site; and

selecting the amplified DNA strands by purifying the amplified DNA strands.

12. The method of claim 11,

wherein the first primer mix is a reverse primer mix, wherein each first primer is a reverse primer, wherein each first common primer is a reverse common primer and wherein each first common primer binding site is a reverse common primer binding site; and

wherein the second primer mix is a forward primer mix, wherein each second primer is a forward primer, wherein each second common primer is a forward common primer and wherein each second common primer binding site is a forward common primer binding site.

13. The method of claim 11,

wherein the first primer mix is a forward primer mix, wherein each first primer is a forward primer, wherein each first common primer is a forward common primer and wherein each first common primer binding site is a forward common primer binding site; and

wherein the second primer mix is a reverse primer mix, wherein each second primer is a reverse primer, wherein each second common primer is a reverse common primer and wherein each second common primer binding site is a reverse common primer binding site.

14. The method of claim 11, further comprising the step, prior to selecting the amplified cDNA strands, of amplifying the amplified DNA strands using a reverse common primer which binds to the at least one reverse common primer binding site and using a forward common primer which binds to the at least one forward common primer binding site.

15. The method of claim 11, wherein primers in the first primer mix comprise additional nucleotides which incorporate into each first DNA strand as an identifying marker.

16. The method of claim 11, wherein primers in the second primer mix comprise additional nucleotides which incorporate into each second DNA strand as an identifying marker.

17. The method of claim 11, wherein each selection comprises separation of DNA strands from primer mix using magnetic beads.

18. The method of claim 11, wherein each selection comprises separation of DNA strands from primer mix by column purification.

19. The method of claim 11, wherein each selection comprises enzymatic cleavage of primer mix.

20. The method of claim 11, wherein the genomic DNA is obtained from a single cell.

21. A method of diagnosing the presence of a disease in a subject, said method comprising:

performing the method of claim 11 on the nucleic acids in a sample obtained from the subject, wherein the sample is suspected of containing a disease agent, wherein the disease agent is characterized by a target sequence;

sequencing the amplified DNA strands; and

detecting a target sequence from the disease agent.

22. A method for producing an immune status profile for a subject, the method comprising:

performing the method of claim 11 on the nucleic acids from a sample of white blood cells from the subject, wherein the at least one target sequence represents a T-cell receptor, antibody, or MHC rearrangement;

sequencing the amplified DNA strands; and

identifying and quantifying one or more DNA sequences representing T-cell receptor, antibody, and MHC rearrangements to create an immune status profile of the subject.