

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;

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.

2. The method of claim 1, further comprising 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.
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. The method of claim 1, wherein the first strand cDNA comprises a first strand cDNA:RNA complex.
9. 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 of claim 1 on the nucleic acids in the sample;
 - sequencing the amplified DNA strands; and
 - detecting a target sequence from the disease agent.
10. 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;
 - 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.
11. The method of claim 1, wherein the mRNA is obtained from a single cell.

12. 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;

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;

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.

13. The method of claim 12,

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.

14. The method of claim 12,

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.

15. The method of claim 12,

wherein the first primer mix is a primer mix comprising at least one forward and at least one reverse primer, each first primer is a forward or a reverse primer, each first common primer is a forward or reverse common primer and each first common primer binding site is a forward or reverse common primer binding site; and

wherein the second primer mix comprises at least one forward and at least one reverse primer, wherein no forward or reverse primer in the second primer mix is included in the first primer mix, each second common primer is a forward or reverse common primer and each second common primer binding site is a forward or reverse common primer binding site.

16. The method of claim 12, further comprising the step 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.
17. The method of claim 12, wherein primers in the first primer mix comprise additional nucleotides which incorporate into each first DNA strand as an identifying marker.
18. The method of claim 12, wherein primers in the second primer mix comprise additional nucleotides which incorporate into each second DNA strand as an identifying marker.
19. The method of claim 12, wherein each selection comprises separation of DNA strands from primer mix using magnetic beads.
20. The method of claim 12, wherein each selection comprises separation of DNA strands from primer mix by column purification.
21. The method of claim 12, wherein each selection comprises enzymatic cleavage of primer mix.
22. The method of claim 12, wherein the genomic DNA is obtained from a single cell.

23. 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 of claim 12 on the nucleic acids in the sample;
 - sequencing the amplified DNA strands; and
 - detecting a target sequence from the disease agent.
24. A method for producing an immune status profile for a subject, the method comprising:
- performing the method of claim 12 on the nucleic acids from a sample of white blood cells from the subject;
 - 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.