

What is claimed is:

1. A method of determining carry over contamination in a patient being monitored for a minimal residual disease, the method comprising the steps of:
monitoring a patient for minimal residual disease by periodically measuring a clonotype profile of the patient by the following steps:
 - (i) obtaining a sample from an individual comprising T-cells and/or B-cells;
 - (ii) attaching sequence tags to molecules of recombined nucleic acids of T-cell receptor genes or immunoglobulin genes of the T-cells and/or B-cells to form tag-molecule conjugates, wherein substantially every molecule of the tag-molecule conjugates has a unique sequence tag;
 - (iii) amplifying the tag-molecule conjugates;
 - (iv) sequencing the tag-molecule conjugates;
 - (v) determining clonotypes from sequence reads aligned by like sequence tags and generating a clonotype profile from such clonotypes; andrecording nucleotide sequences of the sequence tags of each measurement of a clonotype profile; and
determining carry over contamination by the presence, absence and/or level sequence tags from any prior clonotype profile in a subsequent clonotype profile.
2. The method of claim 1 wherein said step of aligning further includes determining a nucleotide sequence of each of said clonotype of each of said tag-molecule conjugate by determining a majority nucleotide at each nucleotide position of said clonotypes of said like sequence tags.
3. The method of claim 1 wherein said step of attaching includes labeling by sampling said molecules of recombined nucleic acids.
4. The method of claim 3 wherein said step of attaching is implemented in a reaction mixture such that said sequence tags are present in the reaction mixture in a concentration at least 100 times that of said molecules of recombined nucleic acid.
5. The method of claim 4 wherein said sequence tags are incorporated into primers specific for said molecules of recombined nucleic acids.
6. The method of claim 1 wherein said clonotypes are each 25 to 400 nucleotide sequences encoding a segment of an immune receptor or immune receptor component selected from the group consisting of a VDJ rearrangement of IgH, a DJ rearrangement of IgH, a VJ rearrangement of IgK, a VJ rearrangement of IgL, a VDJ rearrangement of TCR β , a DJ

rearrangement of TCR β , a VJ rearrangement of TCR α , a VJ rearrangement of TCR γ , a VDJ rearrangement of TCR δ , and a VD rearrangement of TCR δ .

7. A method for determining a level of contamination in a clonotype profile of a tissue sample comprising T cells and/or B cells of an individual, the method comprising the steps of:

obtaining a tissue sample from an individual, the tissue sample comprising nucleic acids from the individual and possibly from one or more other individuals, the nucleic acids including recombined nucleic acids from T cells and/or B cells and non-recombined nucleic acids;

generating a clonotype profile from nucleic acid of the tissue sample;

sequencing genetic markers at one or more genetic loci of the nucleic acids from the tissue sample to obtain proportions of the nucleic acid from the one or more other individuals and from the individual based on the genetic identification of the individual and the one or more other individuals from the genetic markers, wherein the genetic markers occur at loci present in both the recombined nucleic acids and the non-recombined nucleic acids; and

determining the level of contaminating nucleic acid in the clonotype profile as the proportion of the nucleic acid from the one or more other individuals present in the nucleic acid of the tissue sample.

8. The method of claim 7 wherein said proportions are determined from numbers of sequence reads of one or more of said genetic markers identifying said individual and sequence reads of one or more of said genetic markers of said one or more other individuals.

9. The method of claim 7 wherein said genetic markers at genetic loci having a highest number of sequence reads are identified with the individual and remaining genetic markers at each genetic loci are identified with nucleic acids from said one or more other individuals.

10. The method of claim 9 wherein said level of contamination is proportional to a highest number of sequence reads of a genetic marker identified with cross-contaminating nucleic acids at a locus divided by a lowest number of sequence reads of a genetic marker identified with the individual at another locus.

11. The method of claim 7 further including the steps of:
measuring total amount of said nucleic acids in said tissue sample;
measuring total amount of said recombined nucleic acids in said tissue sample;
sequencing genetic markers at one or more genetic loci in an excisable segment of nucleic acid present in said non-recombined nucleic acids and absent from said recombined

nucleic acids to obtain proportions of said non-recombined nucleic acid from said individual and from said one or more other individuals; and

determining said level of contaminating nucleic acid in said clonotype profile from a level of contaminating recombined nucleic acids in said nucleic acid of said tissue sample, the level of contaminating recombined nucleic acids being determined from the total amount of nucleic acids, the total amount of said recombined nucleic acids, said proportions of said nucleic acid from said individual and said one or more other individuals, and the proportions of said non-recombined nucleic acid of said individual and said one or more other individuals.

12. The method of claim 11 wherein said excisable segment is a segment of nucleic acid excised during V(D)J recombination.

13. The method of claim 12 wherein said nucleic acids comprises recombined nucleic acids from human B cells and said excisable segment comprises a region between D region-encoding genes and V region-encoding genes on chromosome 14.

14. The method of claim 12 wherein said nucleic acids comprises recombined nucleic acids from human T cells and said excisable segment comprises a region between D region-encoding genes and V region-encoding genes on chromosome 11.

15. The method of claim 11 wherein said step of measuring said total amount of said nucleic acids includes combining known amounts of one or more internal standards for total nucleic acids with said nucleic acids from said tissue sample so that numbers of a common segment of said nucleic acids can be compared to numbers of the one or more internal standards.

16. The method of claim 15 wherein said common segment includes a portion of a gene selected from the group consisting of GAPDH, β_2 -microglobulin, 18S ribosomal RNA, and β -actin.

17. The method of claim 11 wherein said step of measuring said total amount of said recombined nucleic acids includes combining known amounts of one or more internal standards for recombined nucleic acids with said nucleic acids from said tissue sample so that numbers of clonotypes of said clonotype profile can be compared to numbers of the one or more internal standards for recombined nucleic acids.

18. The method of claim 17 wherein each of said clonotypes comprises a sequence of a portion of a V(D)J-encoding region and wherein said step of measuring includes amplifying in a PCR said nucleic acids from said tissue sample and said one or more internal standards for recombined nucleic acids using primers from the same set.

19. The method of claim 7 further including the steps of:
measuring total amount of said nucleic acids in said tissue sample;

measuring total amount of said recombined nucleic acids in said tissue sample;
measuring total amount of said nonrecombined nucleic acids in said tissue
sample; and

determining said level of contaminating nucleic acid in said clonotype profile
from a level of contaminating recombined nucleic acids in said nucleic acid of said tissue
sample, the level of contaminating recombined nucleic acids being determined from the total
amount of nucleic acids, the total amount of said recombined nucleic acids, said proportions of
said nucleic acid from said individual and said one or more other individuals, and the total
amount of said non-recombined nucleic acid.

20. The method of claim 19 wherein said total amount of said non-recombined
nucleic acids includes combining known amounts of one or more internal standards for non-
recombined nucleic acids with said nucleic acids from said tissue sample so that numbers of an
excisable segment present in non-recombined nucleic acids and absent in recombined nucleic
acids can be compared to numbers of the one or more internal standards.

21. The method of claim 20 wherein said nucleic acids comprise recombined nucleic
acids from human B cells and said excisable segment comprises a region between D region-
encoding genes and V region-encoding genes on chromosome 14.

22. The method of claim 20 wherein said nucleic acids comprise recombined nucleic
acids from human T cells and said excisable segment comprises a region between D region-
encoding genes and V region-encoding genes on chromosome 11.

Claims:

1. A method of determining carry over contamination in a sample containing T cells and/or B cells in vitro, comprising:
determining a clonotype profile for each of a plurality of samples by the following steps:
 - (i) attaching sequence tags to molecules of recombined nucleic acids of T-cell receptor genes or immunoglobulin genes of the T-cells and/or B-cells to form tag-molecule conjugates, wherein substantially every molecule of the tag-molecule conjugates has a unique sequence tag;
 - (ii) amplifying the tag-molecule conjugates;
 - (iii) sequencing the tag-molecule conjugates to produce sequence reads that each comprise a sequence tag portion and a clonotype portion; and
 - (iv) aligning like sequence tag portions of the sequence reads to determine a clonotype sequence from corresponding clonotype portions of the aligned sequence reads and generating a clonotype profile from the clonotype portions; andrecording nucleotide sequences of the sequence tags of each measurement of a clonotype profile; and
determining carry over contamination in the sample by the presence, absence and/or level of sequence tags from any clonotype profile of said plurality of samples.
2. The method of claim 1, wherein said step of aligning further includes determining the nucleotide sequence of each of said clonotype of each of said tag-molecule conjugate by determining a majority nucleotide at each nucleotide position of said clonotypes of said like sequence tag portions.
3. The method of claim 1, wherein said step of attaching includes labeling by sampling said molecules of recombined nucleic acids.
4. The method of claim 3, wherein said step of attaching is implemented in a reaction

mixture such that said sequence tags are present in the reaction mixture in a concentration at least 100 times that of said molecules of recombined nucleic acids.

5. The method of claim 4, wherein said sequence tags are incorporated into primers specific for said molecules of recombined nucleic acids.

6. The method of claim 1, wherein said clonotypes are each 25 to 400 nucleotides in length encoding a segment of an immune receptor or immune receptor component selected from the group consisting of a VDJ rearrangement of IgH, a DJ rearrangement of IgH, a VJ rearrangement of IgK, a VJ rearrangement of IgL, a VDJ rearrangement of TCR β , a DJ rearrangement of TCR β , a VJ rearrangement of TCR α , a VJ rearrangement of TCR γ , a VDJ rearrangement of TCR δ , and a VD rearrangement of TCR δ .

7. The method of claim 1, wherein determining carry over contamination comprises measuring the presence, absence, and/or level of sequence tags from any prior clonotype profile and comparing such against a subsequent clonotype profile.

8. The method of claim 1, wherein the presence of a unique sequence tag shared amongst said any clonotype profile of said plurality of samples indicates a carry over contamination event.