CLAIMS

1. A method for determining non-uniform nucleic acid amplification potential among members of a set of oligonucleotide primers that is capable of amplifying rearranged nucleic acid molecules encoding one or more adaptive immune receptors in a biological sample that comprises rearranged nucleic acid molecules from lymphoid cells of a mammalian subject, the method comprising:

(a) amplifying a composition comprising a plurality of template oligonucleotides using the set of oligonucleotide primers in a multiplex PCR reaction to obtain a plurality of amplified template oligonucleotides, wherein each template oligonucleotide comprises in the 5'-3' direction

(i) a V oligonucleotide sequence comprising at least 20 and not more than 1000 contiguous nucleotides of an adaptive immune receptor variable (V) region encoding gene sequence, or the complement thereof, and each V oligonucleotide sequence comprising a unique V-region oligonucleotide sequence, and

(ii) a J oligonucleotide sequence comprising at least 15 and not more than 600 contiguous nucleotides of an adaptive immune receptor joining (J) region encoding gene sequence, or the complement thereof, and each J oligonucleotide sequence comprising a unique J-region oligonucleotide sequence,

and wherein each template oligonucleotide further comprises one or more random oligonucleotide sequences, and wherein the plurality of template oligonucleotides comprises at least a or at least b unique oligonucleotide sequences, whichever is larger, wherein a is the number of unique adaptive immune receptor V region-encoding gene segments in the subject and b is the number of unique adaptive immune receptor J region-encoding gene segments in the subject, and the composition comprises at least one template oligonucleotide for each unique V-region oligonucleotide sequence and at least one template oligonucleotide for each unique J-region oligonucleotide sequence;

(b) sequencing said plurality of amplified template oligonucleotides to determine, for each unique template oligonucleotide comprising said plurality,

(i) a template oligonucleotide sequence and

(ii) a frequency of occurrence of said template oligonucleotide sequence; and

(c) comparing a frequency of occurrence of each of said template oligonucleotide sequences to an expected distribution, wherein said expected distribution is based on predetermined molar ratios of said plurality of template oligonucleotides comprising said composition, and wherein a deviation between said frequency of occurrence of said template oligonucleotide sequences and said expected distribution indicates a non-uniform nucleic acid amplification potential among members of the set of oligonucleotide amplification primers.

2. The method of claim 1, wherein said predetermined molar ratios are equimolar.

3. The method of claim 1, wherein said expected distribution comprises a uniform amplification level for said plurality of template oligonucleotides amplified by said set of oligonucleotide primers.

4. The method of any one of claims 1-3, further comprising adjusting the relative representation of the oligonucleotide primer member in the set of oligonucleotide amplification primers for each member of the set of oligonucleotide primers that exhibits non-uniform amplification potential relative to the expected distribution.

5. The method of claim 4, wherein adjusting comprises: (a) increasing the relative representation of the member in the set of oligonucleotide primers, thereby correcting non-uniform nucleic acid amplification potential among members of the set of oligonucleotide primers; or (b) decreasing the relative representation of the member in the set of oligonucleotide primers, thereby correcting non-uniform nucleic acid amplification potential among members of the set of oligonucleotide primers.

6. The method of any one of claims 1-5, further comprising calculating a proportionately increased or decreased frequency of occurrence of the amplified template nucleic acid molecules for each member of the set of oligonucleotide amplification primers that

exhibits non-uniform amplification potential relative to the expected distribution, thereby correcting for non-uniform nucleic acid amplification potential among members of the set of oligonucleotide primers.

7. The method of claim any one of claims 1-6, wherein each amplified template nucleic acid molecule is less than 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 90, 80 or 70 nucleotides in length .

8. The method of any one of claims 1-7, wherein said set of oligonucleotide primers does not include oligonucleotide primers that specifically hybridize to a V-region pseudogene or orphon or to a J-region pseudogene or orphon.

9. The method of any one of claims 1-8 wherein said plurality of template oligonucleotides each comprise an oligonucleotide sequence of the general formula:

5'-U1-B1-V-B2-R-B3-J-B4-U2-3' [I]

wherein:

(a) V is an oligonucleotide sequence comprising at least 20 and not more than 1000 contiguous nucleotides of an adaptive immune receptor variable (V) region encoding gene sequence, or the complement thereof, and each V comprising a unique V-region oligonucleotide sequence;

(b) J is an oligonucleotide sequence comprising at least 15 and not more than 600 contiguous nucleotides of an adaptive immune receptor joining (J) region encoding gene sequence, or the complement thereof, and each J comprising a unique J-region oligonucleotide sequence;

(c) U1 is either nothing or comprises an oligonucleotide sequence that is selected from (i) a first universal adaptor oligonucleotide sequence and (ii) a first sequencing platform-specific oligonucleotide sequence that is linked to and positioned 5' to a first universal adaptor oligonucleotide sequence;

(d) U2 is either nothing or comprises an oligonucleotide sequence that is selected from (i) a second universal adaptor oligonucleotide sequence, and (ii) a second sequencing platform-specific oligonucleotide sequence that is linked to and positioned 5' to a second universal adaptor oligonucleotide sequence;

(e) at least one of B1, B2, B3, and B4 is present and each of B1, B2, B3, and B4 comprises an oligonucleotide comprising a barcode sequence of 3-25 contiguous nucleotides that uniquely identifies, as a paired combination, (i) the unique V-region oligonucleotide sequence of (a) and (ii) the unique J-region oligonucleotide sequence of (b);

(f) R is either nothing or comprises a restriction enzyme recognition site that comprises an oligonucleotide sequence that is absent from (a)-(e); and

(g) one or more random oligonucleotide sequences positioned between or within components of general formula I.

10. The method of claim 9, wherein each template oligonucleotide further comprises a stop codon sequence between V and B2.

11. The method of any one of claims 1-10, wherein:

(a) a ranges from 1 to a number of maximum V gene segments in the mammalian genome of the subject;

(b) b ranges from 1 to a number of maximum J gene segments in the mammalian genome of the subject; or

(c) the plurality of template oligonucleotides comprises at least (a x b) unique oligonucleotide sequences, where a is the number of unique adaptive immune receptor V region-encoding gene segments in the mammalian subject and b is the number of unique adaptive immune receptor J region-encoding gene segments in the mammalian subject, and the composition comprises at least one template oligonucleotide for every possible combination of a V region-encoding gene segment and a J region-encoding gene segment.

12. The method of any of one claims 1-11, wherein:

(a) the J oligonucleotide sequence comprises a constant region of the adaptive immune receptor J region encoding gene sequence;

(b) the V oligonucleotide sequence comprises at least 30 contiguous nucleotides of the adaptive immune receptor V region encoding gene sequence, or the complement thereof;

(c) the V oligonucleotide sequence comprises not more than 900, 800, 700, 600 or 500 contiguous nucleotides of an adaptive immune receptor V region encoding gene sequence, or the complement thereof;

(d) the J oligonucleotide sequence comprises at least 16 contiguous nucleotides of an adaptive immune receptor J region encoding gene sequence, or the complement thereof; or

(e) the J oligonucleotide sequence comprises not more than 500 contiguous nucleotides of an adaptive immune receptor J region encoding gene sequence, or the complement thereof.

13. The method of any one of claims 1-12, wherein the one or more adaptive immune receptors is selected from the group consisting of TCRB, TCRG, TCRA, TCRD, IGH, IGK, and IGL.

14. The method of any one of claims 1-13, wherein: (a) the V oligonucleotide sequence encodes a TCRB, TCRG, TCRA, TCRD, IGH, IGK, or IGL receptor V-region polypeptide; or (b) the J oligonucleotide sequence encodes a TCRB, TCRG, TCRA, TCRD, IGH, IGK, or IGL receptor J-region polypeptide.

15. The method of any one of claims 1-14, wherein each template oligonucleotide is less than 1000, 900, 800, 700, 600, 500, 400, 300 or 200 nucleotides in length.