CLAIMS:

[Claim 1]. A sample-indexed nucleic acid library preparation, comprising:

a first nucleic acid library prepared from a first sample, wherein the first nucleic acid library comprises a first plurality of nucleic acid fragments, wherein each nucleic acid fragment of the first plurality comprises at least two different index sequences selected from a first set of index sequences, the first set of index sequences comprising a first 5' subset and a first 3' subset, wherein the the first set of index sequences are arranged on individual nucleic acid fragments of the first plurality such that index sequences of the first 5' subset are located 5' of a target sequence and not 3' of the target sequence, and index sequences of the first 3' subset are located 3' of the target sequence and not 5' of the target sequence, wherein the first plurality comprises more than one combination of the two different index sequences, wherein more than one fragment of the first plurality has a same combination of the two different index sequences and different target sequence, and wherein the same combination comprises an index sequence of the first 5' subset and an index sequence of the first 3' subset; and

a second nucleic acid library prepared from a second sample, wherein the second nucleic acid library comprises a plurality of nucleic acid fragments, wherein each nucleic acid fragment of the second plurality comprises at least two different index sequences selected from a second set of index sequences that are distinguishable from the first set of index sequences, the second set of index sequences comprising a second 5' subset and a second 3' subset, wherein the second set of index sequences are arranged on individual nucleic acid fragments of the second plurality such that index sequences of the second 5' subset are located 5' of a target sequence and not 3' of the target sequence and index sequences of the second 3' subset are located 3' of the target sequence and not 5' of the target sequence, wherein the first set of index sequences are associated with the first sample and not the second sample, wherein the second set of index sequences are associated with the second sample and not the first sample, wherein the second plurality comprises more than one combination of the two different index sequences, wherein more than one fragment of the second plurality has a same combination of the two different index sequences and different target sequence, and wherein the same combination comprises an index sequence of the second 5' subset and an index sequence of the second 3' subset.

[Claim 2]. The library preparation of claim 1, wherein:

(i) the first plurality of nucleic acid fragments comprises individual nucleic acid fragments having different combinations of the two different index sequences from the first set of index sequences relative to other individual nucleic acid fragments within the first plurality of nucleic acid fragments; and/or

(ii) the second plurality of nucleic acid fragments comprises individual nucleic acid fragments having different combinations of the two different index sequences from the second set of index sequences relative to other individual nucleic acid fragments within the second plurality of nucleic acid fragments.

[Claim 3]. The library preparation of claim 1 or claim 2, wherein each index sequence of the first set of index sequences is present in the first plurality of nucleic acid fragments and at a ratio relative to the other index sequences in the first set of index sequences between 0.9 to 1.1.

[Claim 4]. The library preparation of any one of the preceding claims, wherein each index sequence of the second set of index sequences is present in the second plurality of nucleic acid fragments and at a ratio relative to the other index sequences in the second set of index sequences between 0.9 to 1.1.

[Claim 5]. The library preparation of any one of the preceding claims, wherein the second 5' subset are only located 5' of the target sequence and are not located 3' of the target sequence in the second plurality of nucleic acid fragments.

[Claim 6]. The library preparation of claim 5, wherein the second 3' subset are only located 3' of the target sequence and are not located 5' of the target sequence in the second plurality of nucleic acid fragments.

[Claim 7]. The library preparation of any one of the preceding claims, wherein the first sample and the second sample are nucleic acid samples from different individuals.

[Claim 8]. The library preparation of claim 1, wherein an individual nucleic acid fragment of the first plurality of nucleic acid fragments comprises only one index sequence of the first 5' subset and only one index sequence of the first 3' subset.

[Claim 9]. The library preparation of claim 1, wherein the individual nucleic acid fragments of the first plurality of nucleic acid fragments comprise different combinations of the at least two different index sequences relative to one another.

[Claim 10]. The library preparation of claim 1, wherein the second 5' subset comprises four or more unique index sequences.

[Claim 11]. The library preparation of claim 10, wherein an individual nucleic acid fragment of the second plurality of nucleic acid fragments comprises only one index sequence of the second 5' subset and only one index sequence of the second 3' subset.

[Claim 12]. The library preparation of claim 10, wherein the second plurality of nucleic acid fragments comprises different combinations of the at least two different index sequences.

[Claim 13]. The library preparation of claim 1, wherein at each nucleotide position within the first 5' subset, at least three different nucleotides are represented.

[Claim 14]. The library preparation of claim 1, wherein for the first 5' subset, at at least half of nucleotide positions across the first 5' subset, four different nucleotides are present across the plurality.

[Claim 15]. The library preparation of claim 1, wherein the first 5' subset comprises four or more different index sequences and the first 3' subset comprises four or more

different index sequences and wherein the second 5’ subset comprises four or more different index sequences and the second 3’ subset comprises four or more different index sequences.

[Claim 16]. The library preparation of claim 1, wherein first plurality of nucleic acid fragments and the second plurality of nucleic acid fragments are single-stranded nucleic acids.