

[0003] This deficiency is due, in part, to the fact that the *de novo* VDJ-rearrangement is far from the poly-A tail of the RNA making sequencing on certain high throughput next-generation sequencing platforms a challenge, because the resulting products are 1-1.5 kb in length. This can be overcome by switching to longer read length NGS platforms or using commercially available Illumina sequencing kits in a unique manner. This switch, however, still does not overcome the deficiencies of conventional methods.

Summary

[0004] 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 using an oligo-dT primer, wherein the mRNA comprises at least one target sequence to produce a first strand cDNA and wherein the oligo-dT primer comprises an engineered sequence on the 5' end; producing a first set of amplicons by amplifying the first strand cDNA using a multiplex primer mix, wherein the multiplex primer mix comprises two or more primers configured to bind two or more sequences selected from the group consisting of: TCR alpha chain VDJ and TCR beta chain VDJ sequences, and a reverse primer configured to bind to the engineered sequence on the 5' end of the oligo-dT primer; using probe beads to capture amplicons comprising TCR alpha chain VDJ sequences and TCR beta chain VDJ sequences from the first set of amplicons, wherein the probe beads comprise primers configured to bind to the TCR alpha chain constant gene region and the TCR beta chain constant gene region; washing the sample to remove uncaptured amplicons from the first set of amplicons; eluting captured amplicons from the probe beads to produce a pool of eluted amplicons; and amplifying the eluted amplicons using PCR to produce a second set of amplicons.

[0005] In some examples, the method further comprises the step of, after amplifying the eluted amplicons, sequencing the second set of amplicons using next generation sequencing. In some examples, the method further comprises the step of, after

sequencing the second set of amplicons using next generation sequencing, evaluating the sequences to determine the frequency of TCR alpha chain VDJ sequences and TCR beta chain VDJ sequences.

[0006] In some examples of the method, the probe bead primers are configured to bind to at least one location within the TCR alpha chain constant gene region and at least one location within the TCR beta chain constant gene region. In some examples of the method, the probe bead primers are configured to bind to at least one location within the TCR alpha chain constant gene region. In some examples of the method, the probe bead primers are configured to bind to at least one location within the TCR beta chain constant gene region.

[0007] In some examples of the method, the oligo-dT primer comprises a molecular barcode. In some examples of the method, the multiplex primer mix comprises an engineered sequence on the 5' end of the primers and which is configured as a universal binding site.

[0008] In some examples of the method, a majority of the two or more primers of the multiplex primer mix are configured to favor amplification of the sense strand VDJ sequences. In some examples of the method, a majority of the two or more primers of the multiplex primer mix are configured to bind to one or more TCR alpha chain VDJ sequences. In some examples of the method, a majority of the two or more primers of the multiplex primer mix are configured to bind to one or more TCR beta chain VDJ sequences.

[0009] In some examples of the method, prior to using probe beads, the first set of amplicons is cleaned by SPRI bead selection and an additional round of PCR is performed.

Brief Description of the Drawings

[0010] The disclosure can be better understood with reference to the following drawings. The elements of the drawings are not necessarily to scale relative to each other, emphasis

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 using an oligo-dT primer, wherein the mRNA comprises at least one target sequence to produce a first strand cDNA and wherein the oligo-dT primer comprises an engineered sequence on the 5' end;

producing a first set of amplicons by amplifying the first strand cDNA using a multiplex primer mix, wherein the multiplex primer mix comprises two or more primers configured to bind two or more sequences selected from the group consisting of: TCR alpha chain VDJ and TCR beta chain VDJ sequences, and a reverse primer configured to bind to the engineered sequence on the 5' end of the oligo-dT primer;

using probe beads to capture amplicons comprising TCR alpha chain VDJ sequences and TCR beta chain VDJ sequences from the first set of amplicons, wherein the probe beads comprise primers configured to bind to the TCR alpha chain constant gene region and the TCR beta chain constant gene region;

washing the sample to remove uncaptured amplicons from the first set of amplicons;

eluting captured amplicons from the probe beads to produce a pool of eluted amplicons; and

amplifying the eluted amplicons using PCR to produce a second set of amplicons.

2. The method of claim 1, further comprising the step of, after amplifying the eluted amplicons, sequencing the second set of amplicons using next generation sequencing.

3. The method of claim 2, further comprising the step of, after sequencing the second set of amplicons using next generation sequencing, evaluating the sequences to determine the frequency of TCR alpha chain VDJ sequences and TCR beta chain VDJ sequences.
4. The method of claim 1, wherein the probe bead primers are configured to bind to at least one location within the TCR alpha chain constant gene region and at least one location within the TCR beta chain constant gene region.
5. The method of claim 1, wherein the probe bead primers are configured to bind to at least one location within the TCR alpha chain constant gene region.
6. The method of claim 1, wherein the probe bead primers are configured to bind to at least one location within the TCR beta chain constant gene region.
7. The method of claim 1, wherein the oligo-dT primer comprises a molecular barcode.
8. The method of claim 1, wherein the multiplex primer mix comprises an engineered sequence on the 5' end of the primers and which is configured as a universal binding site.
9. The method of claim 1, wherein, prior to using probe beads, the first set of amplicons is cleaned by SPRI bead selection and an additional round of PCR is performed.