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Workflow Summary

- 01 Allow the Master Mix tubes to thaw; then gently vortex to mix.
- 02 In a containment hood or dead air box, pipette 45 µL of Master Mix into individual wells of a PCR plate (use a different indexed Master Mix for each sample and control).
- 03 Add 0.2 µL FalconTaq™ DNA polymerase to each Master Mix.
- 04 Add 5 µL of DNA (at a minimum of 10 ng/µL) from the unknown samples and controls to wells containing the respective Master Mix reactions then pipette up and down 5-10 times to mix.
- 05 Add 5 µL of molecular biology grade water to the well containing the Master Mix for the no template control then pipette up and down 5-10 times to mix.
- 06 Seal the plate and amplify target DNA using the following thermal cycler program:

| Step | Temperature | Time | Cycle |
|------|-------------|------------|-------|
| 1 | 95 °C | 7 minutes | 1 |
| 2 | 95 °C | 45 seconds | 29x* |
| 3 | 60 °C | 45 seconds | |
| 4 | 72 °C | 90 seconds | |
| 5 | 72 °C | 10 minutes | 1 |
| 6 | 15 °C | ∞ | 1 |
- 07 Purify the PCR products using the Agencourt® AMPure® XP PCR Purification system. When using the LymphoTrack TRB Assay add 35 µL of particles to each 50 µL reaction, for all other LymphoTrack Assays add 50 µL of particles to each 50 µL reaction; elute DNA in 25 µL of eluant.
- 08 Quantify amplicons using the KAPA™ library quantification kit according to the kit instructions. Dilute amplicons 1:4,000 before proceeding to qPCR.
- 09 Pool equal amounts of amplicons from samples and positive and negative controls (do not include the no template control), dilute 1:1,000 then quantify the library using the KAPA library quantification kit.
- 10 Denature and dilute the library.
- 11 Load 600 µL of denatured and diluted library on the MiSeq® Reagent Cartridge.
- 12 Set up a MiSeq® sample sheet using the Illumina® Experiment Manager or provided Sample Sheet *.csv file.
- 13 Start the MiSeq® run.
- 14 Analyze and visualize the acquired data using the LymphoTrack Software for the MiSeq®.

*LymphoTrack IGHV Leader Somatic Hypermutation Assay (32 cycles).

| CATALOG # | PRODUCTS | QUANTITY |
|-----------|---|--|
| 71210129 | LymphoTrack® IGH FR1/2/3 Assay Kit A - MiSeq® | indices 1-8 (5 sequencing reactions each) |
| 71210139 | LymphoTrack® IGH FR1/2/3 Assay Panel - MiSeq® | indices 1-24 (5 sequencing reactions each) |
| 71210009 | LymphoTrack® IGH FR1 Assay Kit A - MiSeq® | indices 1-8 (5 sequencing reactions each) |
| 71210039 | LymphoTrack® IGH FR1 Assay Panel - MiSeq® | indices 1-24 (5 sequencing reactions each) |
| 71210149 | LymphoTrack® IGH FR1 Assay Panel B - MiSeq® | indices 25-48 (5 sequencing reaction each) |
| 71210089 | LymphoTrack® IGH FR2 Assay Kit A - MiSeq® | indices 1-8 (5 sequencing reactions each) |
| 71210099 | LymphoTrack® IGH FR2 Assay Panel - MiSeq® | indices 1-24 (5 sequencing reactions each) |
| 71210109 | LymphoTrack® IGH FR3 Assay Kit A - MiSeq® | indices 1-8 (5 sequencing reactions each) |
| 71210119 | LymphoTrack® IGH FR3 Assay Panel - MiSeq® | indices 1-24 (5 sequencing reactions each) |
| 71210059 | LymphoTrack® IGHV Leader Somatic Hypermutation Assay Kit A - MiSeq® | indices 1-8 (5 sequencing reactions each) |
| 71210069 | LymphoTrack® IGHV Leader Somatic Hypermutation Assay Panel - MiSeq® | indices 1-24 (5 sequencing reactions each) |
| 71220009 | LymphoTrack® IGK Assay Kit A - MiSeq® | indices 1-8 (5 sequencing reactions each) |
| 71220019 | LymphoTrack® IGK Assay Panel - MiSeq® | indices 1-24 (5 sequencing reactions each) |
| 72250009 | LymphoTrack® TRB Assay Kit A - MiSeq® | indices 1-8 (5 sequencing reactions each) |
| 72250019 | LymphoTrack® TRB Assay Panel - MiSeq® | indices 1-24 (5 sequencing reactions each) |
| 72270019 | LymphoTrack® TRG Assay Kit A - MiSeq® | indices 1-8 (5 sequencing reactions each) |
| 72270009 | LymphoTrack® TRG Assay Panel - MiSeq® | indices 1-24 (5 sequencing reactions each) |
| 75000009 | LymphoTrack® Software - MiSeq® | 1 CD complimentary with purchase |
| 75000008 | LymphoTrack® MRD Software | 1 CD complimentary with purchase |

Storage Conditions: -85 °C to -65 °C (DNA controls may be separated from kits and stored at 2 °C to 8 °C)

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