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# Workflow Summary

- 01 Using gloved hands, remove the Master Mixes from the freezer. Allow the 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 EagleTaq™ 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:  
Standardized 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. Add 90 µL of particles to each 50 µL reaction; elute DNA in 40 µL of eluant.
  - 08 Quantify amplicons with an appropriate method (e.g. Bioanalyzer® 2100 or LabChip® GX).
  - 09 Create the library by combining 4 nM of each amplicon in a single tube (do not include the no template control).
  - 10 Dilute the library.
  - 11 Prepare templates using the OT2 or Ion Chef system.  
\*The OT2 system is used to perform an emulsion PCR to create template-positive ion sphere particles (ISPs) that must be further enriched.
- | Ion PGM  |  | Ion S5                                 |
|----------|--|--|
| OT2      | Ion PGM Hi-Q OT2 Kit or Ion PGM Hi-Q view OT2 Kit or OT2 200 Kit if only testing TRG | Ion 520 & Ion 530 Kit – OT2            |
| Ion Chef | Not applicable   | Ion 510 & Ion 520 & Ion 530 Kit – Chef |
- 12 \*OT2 templates: Enrich template-positive ISPs with the Ion OneTouch(tm) ES.
  - 13 Initialize the S5 or PGM. Load S5 [Ion 520, Ion 530] or PGM chip [316 v2 BC, 318 v2 BC] with prepared templates.
  - 14 Create a Planned Run using the Torrent Browser.
  - 15 Start the S5/PGM run.
  - 16 Analyze and visualize the acquired data using the LymphoTrack Software for the S5/PGM.

## Ordering Information

CATALOG #	PRODUCTS	QUANTITY
7-121-0057	LymphoTrack® IGH FR1/2/3 Assay – S5/PGM™	12 indices – 5 sequencing reactions each
7-121-0007	LymphoTrack® IGH FR1 Assay – S5/PGM™	12 indices – 5 sequencing reactions each
7-121-0037	LymphoTrack® IGH FR2 Assay – S5/PGM™	12 indices – 5 sequencing reactions each
7-121-0047	LymphoTrack® IGH FR3 Assay – S5/PGM™	12 indices – 5 sequencing reactions each
7-122-0007	LymphoTrack® IGK Assay – S5/PGM™	12 indices – 5 sequencing reactions each
7-227-0007	LymphoTrack® TRG Assay – S5/PGM™	12 indices – 5 sequencing reactions each
7-500-0007	LymphoTrack® Software – S5/PGM™	1 CD complimentary with purchase
7-500-0008	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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