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## Workflow Summary

- Using gloved hands, remove the Master Mixes from the freezer. Allow the tubes to thaw; then gently vortex to mix.
- O2 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).
- Λ3 Add 0.2 μL Taq DNA polymerase to each Master Mix.
- O4 Add 5 μL of DNA (at a minimum concentration of 10 ng/μL) from the unknown and 5 μL of control samples to wells containing the respective Master Mix reactions, then pipette up and down 5-10 times to mix.
- O5 Add 5 μL of molecular biology grade water to the well containing the respective Master Mix for the no template control, then pipette up and down 5-10 times to mix.
- O6 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	
3	60 °C	45 seconds	29x
4	72 °C	90 seconds	
5	72 °C	10 minutes	1
6	15 °C	∞	1

- Purify the PCR products using the Agencourt® AMPure® XP PCR Purification system. Add 90  $\mu$ L of particles to each 50  $\mu$ L reaction; elute DNA in 40  $\mu$ L TE buffer.
- Quantify amplicons with an appropriate method (e.g. 2100 Bioanalyzer® or LabChip® GX).
- OP Create the library by combining an equal amount 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.
- 12 Initialize the S5 or PGM. Load S5 [lon 520, lon 530] or PGM chip [316 v2 BC, 318 v2 BC].
- 13 Create a Planned Run using the Torrent Browser.
- 14 Start the Ion S5 or Ion PGM run.
- Analyze and visualize the acquired data using the LymphoTrack Dx Software for the Ion S5/PGM.

NOTE: Always follow the applicable instructions for use (IFU) when testing samples.

## Ordering Information

CATALOG #	PRODUCTS	QUANTITY
9-121-0057	LymphoTrack® Dx <i>IGH</i> FR1/2/3 Assay - S5 <sup>™</sup> /PGM <sup>™</sup>	12 + 12 + 12 indices - 5 sequencing reactions each
9-121-0007	LymphoTrack® Dx <i>IGH</i> FR1 Assay – S5 <sup>TM</sup> /PGM <sup>TM</sup>	12 indices - 5 sequencing reactions each
9-121-0037	LymphoTrack® Dx <i>IGH</i> FR2 Assay - S5™/PGM™	12 indices - 5 sequencing reactions each
9-121-0047	LymphoTrack® Dx <i>IGH</i> FR3 Assay - S5™/PGM™	12 indices - 5 sequencing reactions each
9-122-0007	LymphoTrack® Dx <i>IGK</i> Assay – S5 <sup>TM</sup> /PGM <sup>TM</sup>	12 indices - 5 sequencing reactions each
9-227-0007	LymphoTrack® Dx <i>TRG</i> Assay - S5™/PGM™	12 indices - 5 sequencing reactions each
9-500-0007	LymphoTrack® Dx Software - S5™/PGM™	1 CD complimentary with purchase

**Storage Conditions:** -85  $^{\circ}$ C to -65  $^{\circ}$ C (DNA controls may be separated from kits and stored at 2  $^{\circ}$ C to 8  $^{\circ}$ C).

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