ANNEXURE 1

Targeted RNA sequencing (Next Generation Sequencing)

Formalin-Fixed Paraffin-Embedded (FFPE) and blood RNA samples of patients from this study were sequenced following the below protocols:

- The first libraries (from July 2020 to April 2021) were prepared from 100 ng of RNA using the KAPA RNA HyperPrep Kit (Roche®, Cat# KK8541/8098107702, 96 reactions) (Figure 1) and as per the manufacturer's protocol (KAPA® Biosystems, KAPA® Hyper Prep Kit Technical Sheet, KR1350 – v4.21). The subsequent libraries (from May 2021 to July 2022) were constructed from 85 ng of RNA using the Illumina RNA Prep with Enrichment, (L) Tagmentation (Illumina®; USA, Cat# 20040537, 96 samples) (Figure 2) following the manufacturer's protocol (Illumina®, Library construction, Document # 1000000124435 v03).

- Each library was quantified using the Qubit™ 3.0 Fluorometer. Samples with sufficient amount of library were processed.
  - The libraries prepared using the KAPA RNA HyperPrep Kit were enriched using Roche HyperCap v3.0 kit (Roche®, Cat# 8333114001, 96 reactions) that allows short hybridization step with KAPA HyperExome probes (Roche®, Cat# 09062572001, 96 reactions).
  - The libraries prepared using the Illumina RNA Prep with Enrichment, (L) Tagmentation Kit were enriched following Illumina's protocol using the KAPA HyperExome panel (Roche®, Cat# 09062572001, 96 reactions).

  Enriched libraries were quantified using Qubit™ 3.0 Fluorometer and the Qubit™ dsDNA BR assay kit (Invitrogen™, Q32850, 100 assays or Q32853, 500 assays).

- The validated barcoded libraries were normalized, pooled, denatured, and diluted to a final loading volume at recommended concentration either for KAPA RNA HyperPrep Kit or Illumina RNA Prep libraries.

  The sequencing was performed on the Illumina platform using NextSeq 500 or NextSeq 2000 following Illumina System Guide (NextSeq 500: #15046563 v06; 15048776 v09, or (NextSeq 2000: # 1000000109376 v01).

Figure 1 - Targeted RNA sequencing workflow

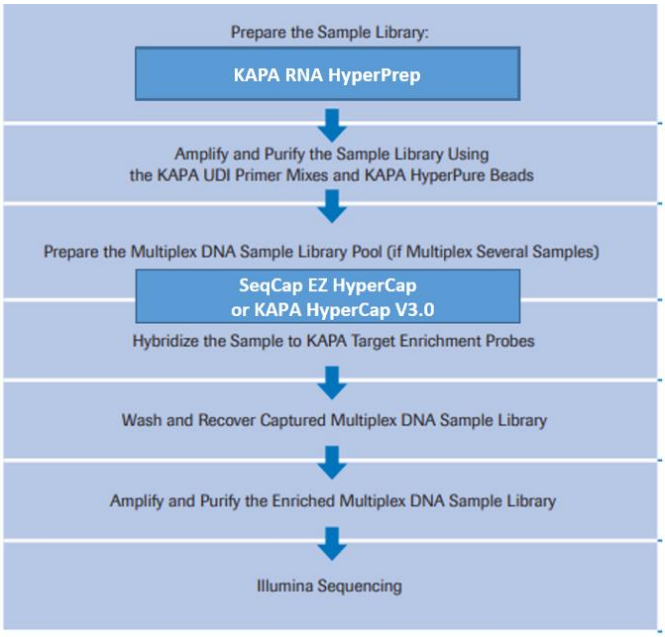


Figure 2 - Targeted RNA sequencing workflow

