The KAPA Total Prep Workflow Facilitates Integrated Multi-Omics Data Analysis

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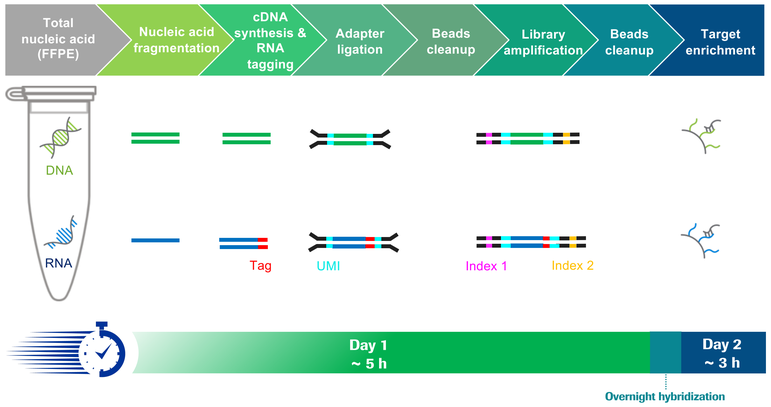
INTRODUCTION

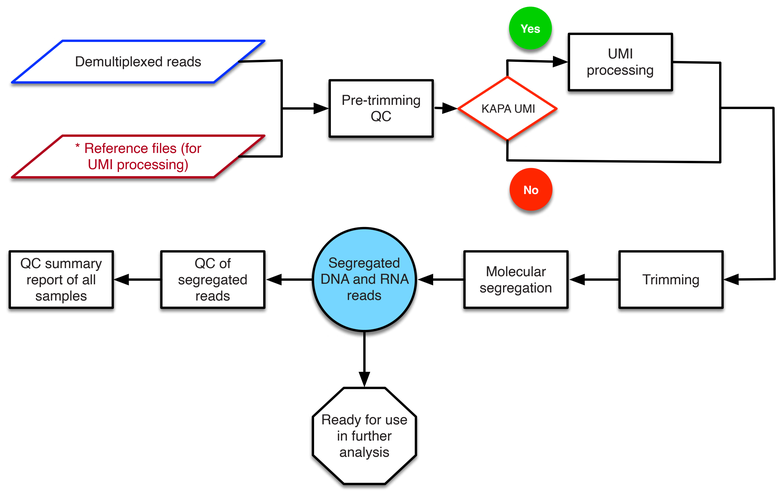
The KAPA Total Prep workflow facilitates simultaneous sequencing of both DNA and RNA from a single sample within a single tube, optimizing time and resource utilization. The workflow takes under 1.5 days to generate sequencing-ready, target-enriched libraries from samples containing total nucleic acid as initial input.

KAPA Total Prep FFPE workflow



We implemented a bioinformatics pipeline tailored specifically for the KAPA Total Prep workflow, streamlining data processing and ensuring robust quality control. The pipeline efficiently segregates RNA and DNA reads within the input data, enabling focused downstream analysis targeting distinct molecular types.

KAPA Total Prep Bioinformatics pipeline



*Note: GRCh38 reference files are provided as default in the available package of the KAPA Total Prep Bioinformatics pipeline.

LIBRARY QUALITY ASSESSMENT

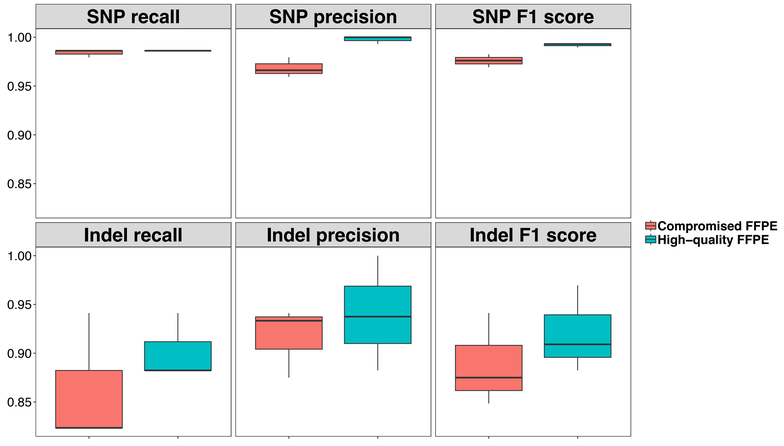
Libraries produced using low-quality Formalin-Fixed Paraffin-Embedded (FFPE) input material exhibit comparable sequencing quality compared to those from high-quality FFPE input material.

	DNA		RNA	
	High-quality FFPE	Compromised FFPE	High-quality FFPE	Compromised FFPE
Duplication rate after UMI processing	1.80%	3.40%	2.30%	8.40%
Genome mapping rate	100%	100%	99.40%	99.30%
Median coverage depth	998 X	691 X	45 X	76 X
On-target rate	86.00%	88.50%	96.70%	98.50%
Error rate	0.47%%	0.57%	NA	NA
Artifact rate	0.07%	0.04%	NA	NA
Fraction of targets with at least 30X coverage depth	99.20%	99.20%	NA	NA
Fold-80 base penalty	2.90	2.80	NA	NA
Transcript mapping rate	NA	NA	82.90%	94.20%
Exonic rate	NA	NA	77.27%	88.57%
rRNA rate	NA	NA	0.09%	0.02%
Number of detected genes within target regions	NA	NA	82	82

The data represent the mean of three replicates for each sample, processed using the KAPA Total Prep FFPE workflow and enriched with the KAPA HyperCap Oncology panel. Each library was subsampled to 10 million read pairs before undergoing processing using the KAPA Total Prep Bioinformatics pipeline.

DNA DATA EVALUATION

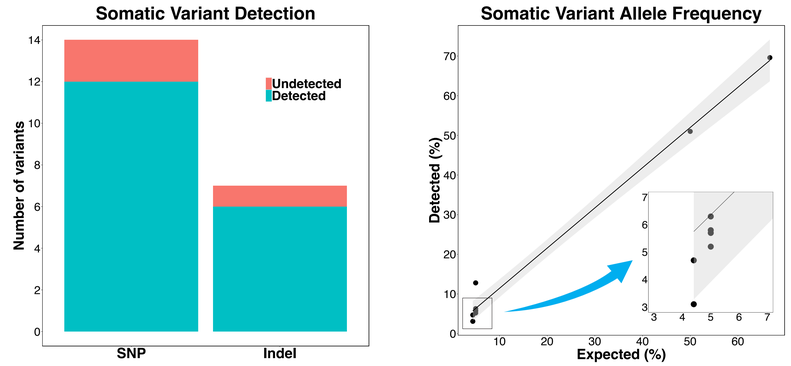
DNA data derived from both low-quality and high-quality FFPE input material demonstrate comparable performance in germline variant calling.



Detected variants were compared to the NA24385 benchmark variants within the target regions of the KAPA HyperCap Oncology panel.

DNA DATA EVALUATION (Continued)

DNA data obtained from somatic variant reference samples show reliable performance in somatic variant calling.



Benchmark variants within the target regions of the KAPA HyperCap Oncology panel were used for the evaluation.

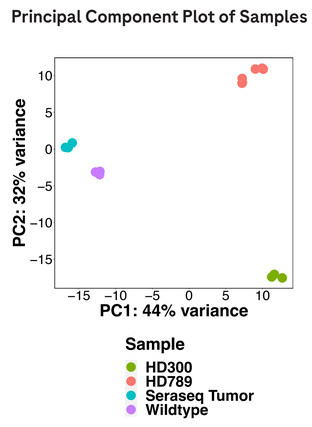
RNA DATA INTEGRATION

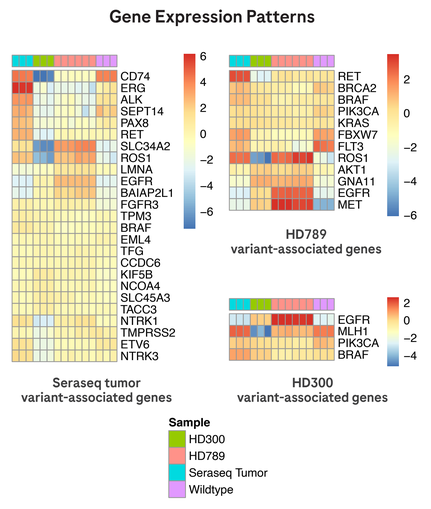
RNA data generated from reference samples show steady performance in detection of fusion genes and alternative splicing events.

Fusion genes
100% detected

Alternative splicing events
100% detected

Expression patterns of variant-associated genes altered in reference samples.





Data on file.
Project: Single-Tube Total Nucleic Acid Library Preparation for FFPE, Wilmington, MA 2023

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