Single-Tube Total Nucleic Acid NGS Sample Preparation for FFPE

A combined method for DNA and RNA

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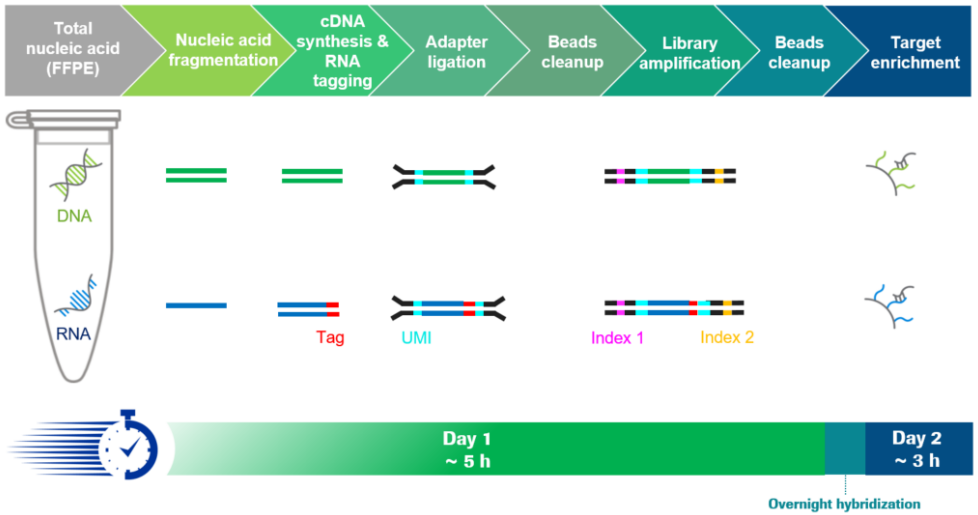
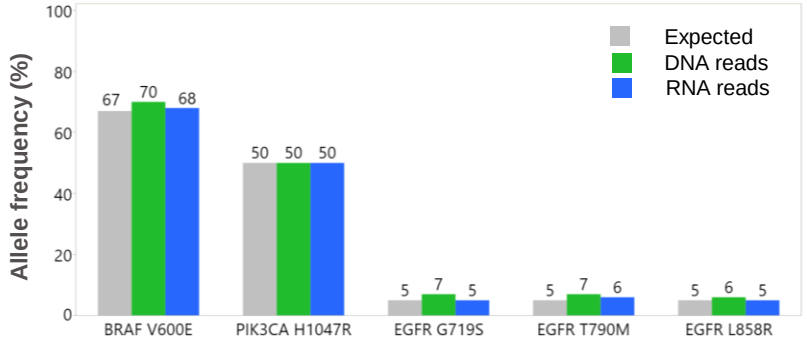


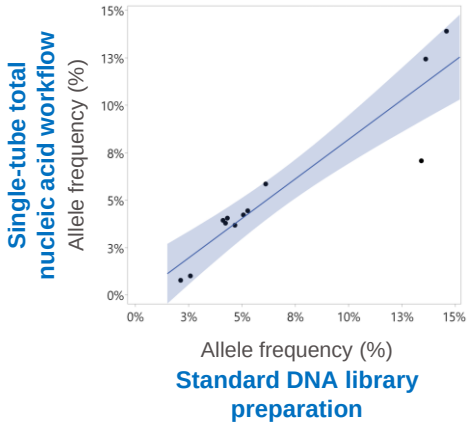
Single-tube total nucleic acid NGS sample prep workflow. DNA and RNA are simultaneously converted to libraries and then enriched for targeted sequencing. A tag is added to specifically mark the molecules derived from RNA, allowing the discrimination of reads originated from RNA and DNA in the sequencing data. The entire workflow, from library preparation to target enrichment, can be completed in less than 1.5 days.

	DNA-seq		RNA-seq	
	High-quality FFPE	Compromised FFPE	High-quality FFPE	Compromised FFPE
Mean coverage	1274 X	1033 X	166 X	554 X
On-target rate	86%	88.5%	96.7%	98.5%
Fold-80 base penalty	2.9	2.8	NA	NA
rRNA alignment	NA	NA	0%	0%

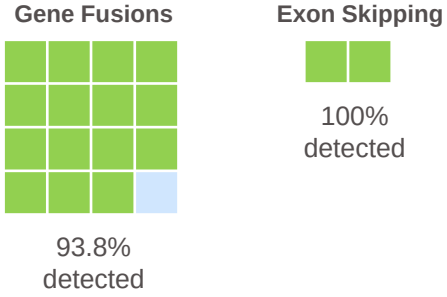
Sequencing metrics are not impacted by FFPE quality. High-quality and compromised wild-type FFPE TNA were each input into the single-tube total nucleic acid library preparation and enriched with the KAPA HyperCap Oncology Panel.



SNV allele frequency is consistent between DNA and RNA reads. The allele frequencies of the SNVs were concordant with the expected frequencies and consistent between DNA reads and RNA reads when using the single-tube total nucleic acid workflow.



SNV and InDel allele frequency in the DNA reads is concordant between library preparation workflows. The allele frequency of several SNVs and InDels was determined with both the single-tube total nucleic acid workflow and a standard DNA library preparation performed in parallel, followed by target enrichment with the KAPA HyperCap Oncology Panel.



The single-tube total nucleic acid workflow enables the detection of RNA fusions. Detection of 16 known gene fusions and 2 exon-skipping events was analyzed with the single-tube total nucleic acid workflow.

Disclaimer: While the results of this study are promising, this single-tube total nucleic acid protocol is still in development and has not been completely validated by Roche.