Evaluation of RNA Purification Protocols for Targeted Deep Sequencing of RNA from FFPE Tumors

Geoff Otto[1], Kristina Brennan[1], Jie He[1], Geneva Young[1], Doug Horejsh[2], Doron Lipson[1], Jeff S Ross[1,3], Phil Stephens[1], Alex Parker[1]

[1]Foundation Medicine Inc., Cambridge, MA (USA); [2]Promega Corporation, Madison WI; [3]Department of Pathology and Laboratory Medicine, Albany Medical College, Albany, NY (USA)

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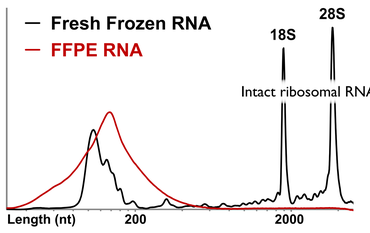
Abstract

A single assay capable of characterizing both the genome and the transcriptome of diverse tumor types using targeted, deep sequencing will improve the application of personalized medicine to cancer. Integrating RNA-seq into our clinical FFPE DNA-seq test, which is highly sensitive to many types of genomic alterations in over 200 cancer related genes, will enable a comparison of changes in gene copy number with digital gene expression levels, facilitate validation of both recurrent and novel gene rearrangements and reveal changes exclusive to the transcriptome such as alternative splicing. Tumor samples are commonly stored as formalin fixed paraffin embedded (FFPE) blocks which degrades nucleic acids and requires careful processing to extract sufficient quantity and quality of DNA and RNA to enable comprehensive molecular profiling. Quantitative recovery of high purity RNA from FFPE tissues is essential to implementation of a targeted RNA-seq cancer assay.

Background: Challenges Working with FFPE RNA

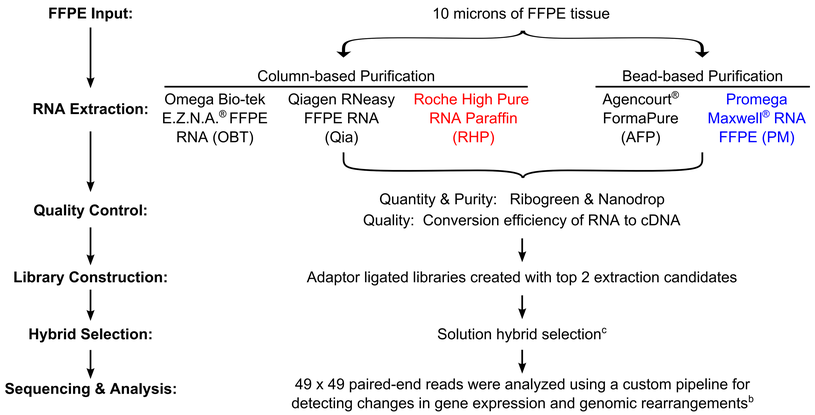
FFPE RNA is highly degraded[a] and can co-purify with inhibitors of downstream enzymatic reactions making comprehensive molecular profiling difficult.



Bioanalyzer RNA Pico analysis of a typical FFPE RNA and fresh frozen RNA.

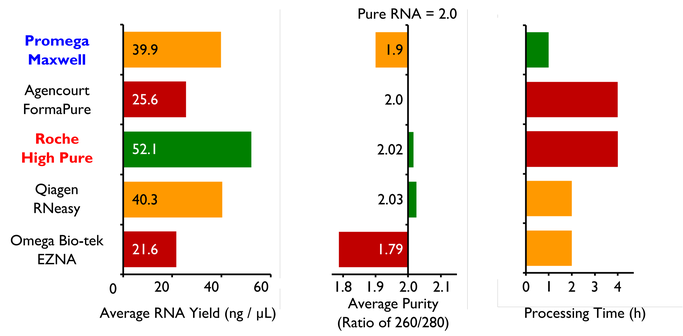
Materials & Methods: RNA-seq Sample Preparation Workflow

Five FFPE RNA extraction kits were evaluated for RNA yield, quality and laboratory workflow using sets of 8 tumor:normal pairs from four different tumor types. FFPE tissue digestion and RNA purification was performed using a prototype kit for the Promega Maxwell® and commercially available kits from Roche, Agencourt, Qiagen and Omega Bio-tek. The two best performing kits were further compared using targeted, deep sequencing on an Illumina HiSeq™ 2000.

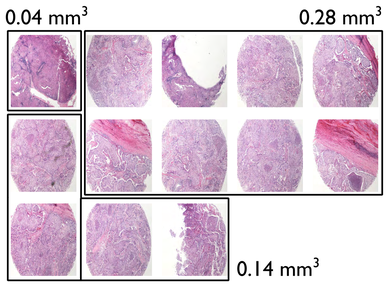


Results

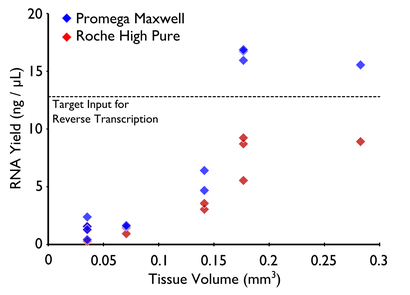
From the results of testing kit performance on 16 FFPE samples, RHP had the highest yield and good purity and PM had the easiest workflow and acceptable yield and purity.



The PM kit yielded more FFPE RNA than the RHP kit from small tissue inputs and the PM kit extracted sufficient RNA for molecular profiling from as little as 0.18 mm^3.

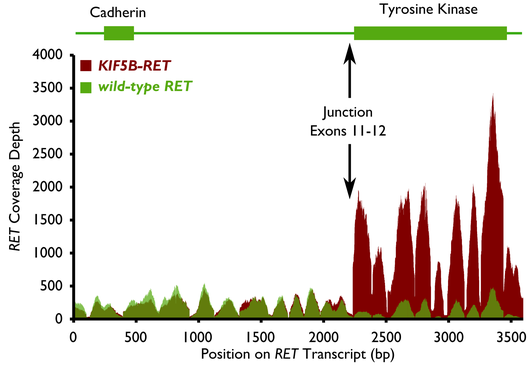


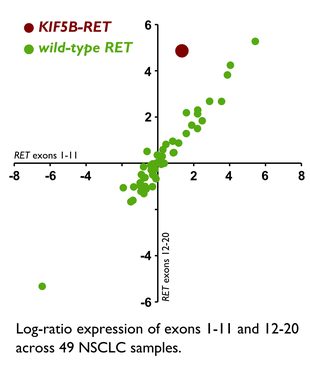
Hematoxylin and eosin stained slide of a 3 mm Tissue Microarray (TMA).



RNA yield vs. total tissue volume from pools of 1 to 8 3 mm TMA cores combined in a single extraction.

Targeted RNA-seq confirmed a novel gene fusion, *KIF5B-RET*, in a NSCLC FFPE tumor sample that was initially detected by DNA-seq and revealed a 30-fold over-expression of the tyrosine kinase domain of *RET*[b].

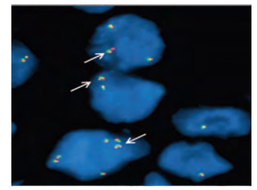




Log-ratio expression of exons 1-11 and 12-20 across 49 NSCLC samples.

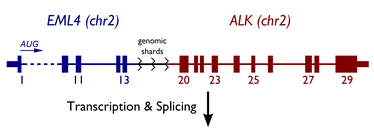
Targeted RNA-seq revealed that a complex genomic rearrangement involving *EML4* and *ALK*, fusion negative by *ALK* Break-Apart FISH, expressed an *EML4-ALK* fusion transcript[d].

A.



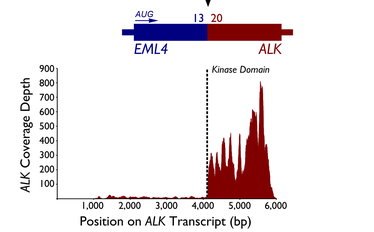
A. FFPE section from a 43 year-old never smoker was classified as *EML4-ALK* fusion negative based upon the results of a fluorescent *ALK* Break-Apart FISH assay (Abbott Molecular). The results of the fluorescent hybridization test displayed an atypical pattern of double 3' *ALK* signals (in red) fused with the 5' *ALK* (in green).

B.



B. Targeted DNA-seq detected novel inter- and intra-chromosomal chimeric reads between *ALK* intron 19 on chromosome 2, a canonical breakpoint, and 4 different regions of the genome distal to *EML4*.

C.

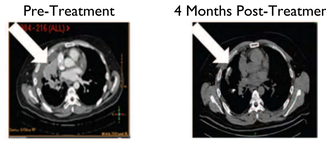


C. Targeted RNA-seq detected a canonical gene fusion between exon 13 of *EML4* and exon 20 of *ALK* by sequencing reads spanning the *EML4-ALK* junction and by increased expression of the *ALK* kinase domain.

D.

D. *ALK* immunohistochemistry confirmed the increased expression of the *ALK* kinase domain detected by targeted RNA-seq.

E.



E. Pelvic positron emission tomographic computed tomographic scans before and after 4 months of crizotinib therapy showed shrinkage of the primary tumor.

Summary

RNA compatible with targeted deep sequencing was successfully extracted from FFPE tissue sections with both the Roche High Pure Paraffin and Promega Maxwell kits.

Targeted RNA-seq on FFPE tissue confirmed a novel *KIF5B-RET* gene fusion observed by DNA-seq and revealed increased expression of the tyrosine kinase domain of *RET*.

Targeted RNA-seq detected a canonical *EML4-ALK* gene fusion that was not detected by the standard of care *ALK* Break-Apart FISH assay.

References

a) von Ahlfen S, et al, 2007. Determinants of RNA quality from FFPE samples. PLoS ONE 2(12): e1261.

b) Lipson D, et al, 2012. Identification of recurrent KIF5B-RET gene fusions from lung cancer biopsies. Nature Medicine 18(3): 382-384.

c) Levin J, et al, 2009. Targeted next-generation sequencing of a cancer transcriptome enhances detection of sequence variants and novel fusion transcripts. Genome Biology 10(10): R115.

d) Peled N, et al, 2012. Next generation sequencing identifies and immunohistochemistry confirms a novel crizotinib sensitive ALK rearrangement in a patient with metastatic non-small cell lung cancer. J. Thoracic Oncology 7(9): e14-e16.