# Identification and validation of transcripts by RNA-sequencing lung tissue from patients with Idiopathic Pulmonary Fibrosis

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## Abstract

**Transcriptomic profiling in IPF has largely been performed by microarrays using RNA obtained from whole lung lysates obtained from fresh frozen tissues. These studies have provided significant mechanistic insights regarding IPF pathogenesis, and have largely impacted the field of lung fibrosis. However, these studies are limited because acquisition of fresh frozen tissues is only available in highly specialized academic centers with tissue banking facilities. Thus, the majority of studies contain mostly tissues explanted from patients with IPF at the time of biopsy, and studies containing tissues obtained from diagnostic biopsies are limited. Additionally, it is nearly impossible to assess the lung morphology on frozen tissue, thus the studies utilizing fresh frozen samples depend on histological assessment of adjacent tissue which may or may not contain the exact same pathology. While transcriptomic data generated from different dissections within a single lobe of the lung are highly correlated, and that transcriptomic data correlates well with UIP pattern itself the lack of visual confirmation of the histology of the region profiled is still considered a limitation RNA isolated from Formalin-Fixed Paraffin Embedded FFPE tissues is partially degraded, thus transcriptomic analysis of FFPE tissues was considered challenging. Several recent studies demonstrated that transcriptomic analysis of FFPE tissues using microarrays was possible, was nearly comparable to fresh frozen tissues but still had significant limitations. In contrast to microarrays, next generation RNA Sequencing (RNA-Seq) allows for relatively unbiased measurements of expression levels across the entire length of a transcript and its level of expression, and therefore may be more suitable for sequencing of partially degraded FFPE RNA. Transcriptomic analysis of FFPE tissues by RNA-Seq demonstrates high concordance to RNA-Seq data produced from matching fresh frozen tissues. Because formalin fixation and paraffin embedding are routinely done on all samples from clinically indicated lung biopsies, optimization of a method to perform genome scale transcript profiling of archived FFPE tissues will greatly enhance the access to IPF lungs. In this study, we sought to determine whether whole transcriptomic analysis of RNA isolated from FFPE biopsies by RNA-Seq was feasible in IPF, and whether the results are comparable to those obtained from gene expression microarrays. To test our hypothesis, we isolated RNA from FFPE lung biopsies of IPF individuals and controls, generated RNA-Seq expression data and compared it to publically available microarray array data previously generated by us from fresh frozen IPF lung tissues Our study demonstrates high concordance in IPF relevant genes and pathways between RNA-Seq and microarrays of un-paired tissues from patients evaluated in different cohorts, suggesting that RNA-Seq from FFPE tissues could be considered an acceptable technique for transcriptomic profiling in IPF.**