

Substudy 082516 RNAseq

This data set contains RNAseq analysis of 81 samples derived from 12 LungMAP donor subjects ranging in age from 1 day of life to adult. For further clinical details see the associated metadata file.

Briefly, RNA samples were generated* for the 12 subjects studied, from live(7-AAD⁻), CD45⁺ mixed immune cells (**MIC**), CD45⁻PECAM⁻VECadherin⁻EpCAM⁺ mixed epithelial cells (**EPI**), CD45⁻PECAM⁺VECadherin⁺ mixed endothelial cells (**END**) and CD45⁻PECAM⁻VECadherin⁻EpCAM⁻ mixed mesenchymal cells (**MES**) isolated by fluorescence activated sorting of cells obtained from digesting (using our standard protocol available on BREATH web site) the combined right upper and right middle lobes (**CBL**) or, in the case of the adult lung, the right middle lobe (**RML**). Antibody stained, unsorted cells (presort mixed cells; **PMX**) were also studied. Finally, RNA samples were obtained from the periphery of the right middle lobe as parenchymal lung tissue biopsies (**RML-BPS**) as well as from stained, unsorted cells derived from digestion of dissected airway (bronchiole-bronchiolar) tissues (**BRR-PMX**).

RNA was extracted using the Qiagen RNeasy kit (Qiagen, Cat# 74104) and was judged to be of high quality (RIN \geq 7; see metadata). Approximately 1ng of total RNA was amplified using the SMARTer Ultra Low amplification kit (Clontech, Mountain View, CA). Libraries were constructed using the NexteraXT library kit (Illumina, San Diego, CA). Library sequencing was performed on a HiSeq2500, using 100 bp SER (1x100), at a target depth of 10-20M reads. Sequenced reads were cleaned using Trimmomatic-0.32 and mapped to human reference genome GRCh38.p2 with STAR-2.4.2a. Raw read counts were obtained using HTSeq with GenCode 23 human gene annotations.

The data presented in graphic form are CPM-normalized counts summary. A non-normalized counts summary dataset is also available. Raw data can be obtained through dbGAP upon request.

*Bandyopadhyay G, Huyck HL, Misra RS, Bhattacharya S, Wang Q, Mereness J, Lillis J, Myers JR, Ashton J, Bushnell T, Cochran M, Holden-Wiltse J, Katzman P, Deutsch G, Whitsett JA, Xu Y, Mariani TJ, Pryhuber GS. Dissociation, cellular isolation, and initial molecular characterization of neonatal and pediatric human lung tissues. Am J Physiol Lung Cell Mol Physiol. 2018;315(4):L576-L83. Epub 2018/07/06. doi: 10.1152/ajplung.00041.2018. PubMed PMID: 29975103.

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