

# Lung Precancer Study

Jaewoong Lee    Y. Choi    I. Yun    S. Park    Semin Lee

Department of Biomedical Engineering  
Ulsan National Institute of Science and Technology

*jwlee230@unist.ac.kr*

2021-06-18

# Overview

1 Introduction

2 Materials

3 Methods

4 Results

5 Discussion

# Introduction

# Introduction

## Lung Cancer

# Lung Cancer? I

The most common cancer

The most common form of cancer:

12.3 % of all cancers (Minna, Roth, & Gazdar, 2002)

The most important factor

Tobacco

# Cancer Survival Rate in Korea



Figure: Common cancer survival rates (Hong et al., 2021)

## Survival rate (More than 5 yr)

- Thyroid: 68.4 %
- Lung: 35.4 %

# Type of Lung Cancer

Types of lung cancer:

- ① Adenocarcinoma (ADC) (40 %) ★
- ② Squamous cell carcinoma (SQC) (25 %) ★
- ③ Small cell carcinoma (20 %)
- ④ Large cell carcinoma (10 %)
- ⑤ Adenosquamous carcinoma (< 5 %)
- ⑥ Carcinoid (< 5 %)
- ⑦ Bronchioalveolar (Bronchial gland carcinoma)

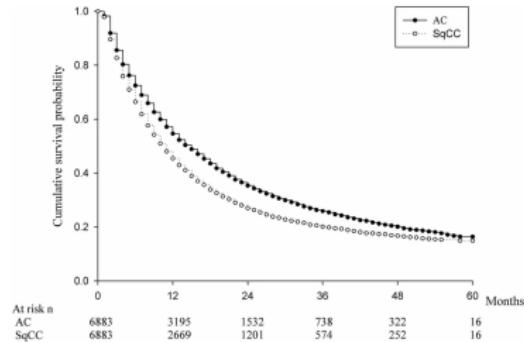
(Vincent et al., 1977; Collins, Haines, Perkel, & Enck, 2007)

# ADC vs. SQC I

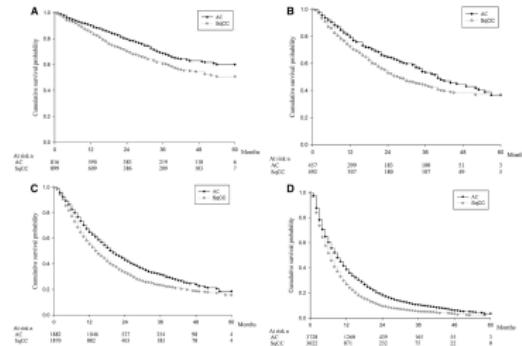


Figure: ADC and SQC histology in Lung cancer (Travis, 2002)

# ADC vs. SQC II



(a) All patients



(b) By cancer stages

Figure: Kaplan-Meier survival curves for ADC & SQC (Wang et al., 2020)

## Findings

SQC is more dangerous than ADC.  $\therefore p < 0.001$

## Introduction

## Study Objectives

# Study Objectives

## Find different mutations

- between WES vs. WTS
- from cancer vs. precancer

## Pathway examine

- with the mutation of WES & RNA-seq
- with immune-depleted animal models

## Ultra-deep sequencing

to find an *infinitesimal* quantity of Non-Circulating Tumor DNA

- from blood
- from urine
- from bronchus

# Materials

# Lung Cancer Data

- WES (n=289) + Transcriptome (n=166)
- Normal + {Primary, CIS + AIS, AAH, Dysplasia, MIA}
  - Carcinoma in situ
  - Adenocarcinoma in situ
  - Atypical adenomatous hyperplasia
  - Dysplasia
  - Minimally invasive adenocarcinoma
- Squamous cell carcinoma (SQC) & Adenocarcinoma (ADC)
  - ① Normal → Dysplasia → CIS → SQC (n=80)
  - ② Normal → AAH → AIS → MIA → ADC (n=28)

# Methods

## Methods

## Workflows

# Data pre-processing for variant discovery



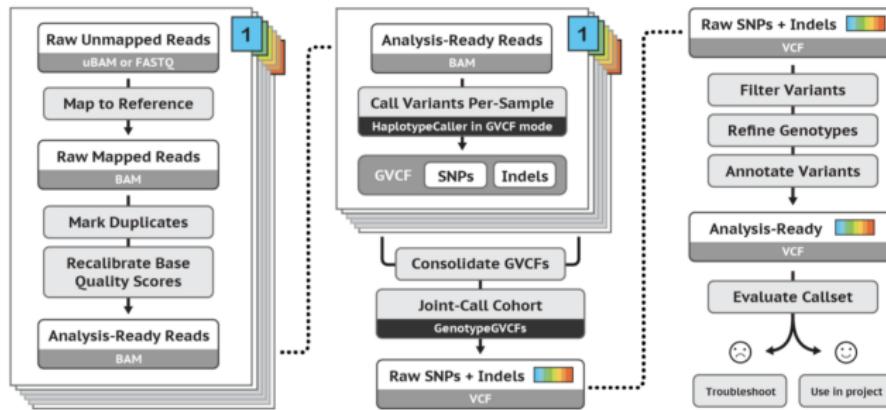
**Figure:** Data pre-processing for variant discovery (Van der Auwera et al., 2013; DePristo et al., 2011)

# Somatic short variant discovery



Figure: Somatic short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

# Germline short variant discovery



**Figure:** Germline short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

# RNA-seq short variant discovery



**Figure:** RNA-seq short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

## Methods

## Miscellaneous

# Used Bioinformatics Tools

- FastQC (Andrews et al., 2012)
- BWA (H. Li & Durbin, 2009; H. Li, 2013)
- STAR (Dobin et al., 2013)
- Bowtie2 (Langmead & Salzberg, 2012)
- Samtools (H. Li et al., 2009)
- GATK (Van der Auwera et al., 2013; DePristo et al., 2011)
- Picard (*Picard toolkit*, 2019)
- VCF2MAF (Kandoth et al., 2018)
- BCFtools (Danecek et al., 2021)
- VEP (McLaren et al., 2016)
- RSEM (B. Li & Dewey, 2011)
- CIBERSORTx (Steen, Liu, Alizadeh, & Newman, 2020)

# R Packages

- Sequenza (Favero et al., 2015)
- Copynumber (Nilsen, Liestol, & Lingjaerde, 2013; Nilsen et al., 2012)
- DESeq2 (Love, Huber, & Anders, 2014)

# Python Packages

- Pandas (pandas development team, 2020; Wes McKinney, 2010)
- Sequenza-utils (Favero et al., 2015)
- Matplotlib (Hunter, 2007)
- Seaborn (Waskom & the seaborn development team, 2020)
- CoMut (Crowdis, He, Reardon, & Van Allen, 2020)
- PyClone (Roth et al., 2014)
- Statannot

# Results

# Results

## Quality Checks with FastQC

# FastQC?

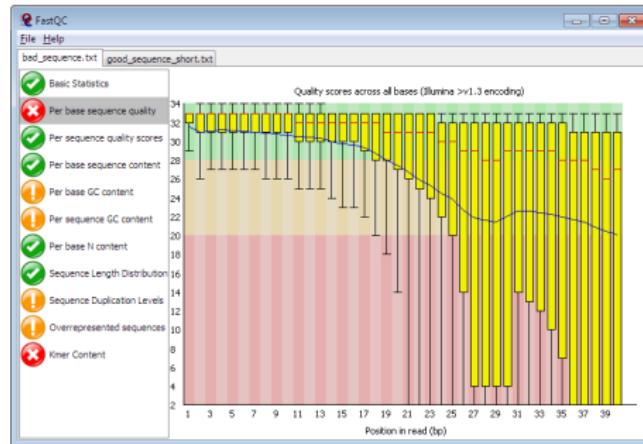


Figure: Example of FastQC Result (Andrews et al., 2012)

- A quality check tool for sequence data
- Give an overview that which test may be problems

# FastQC on WES

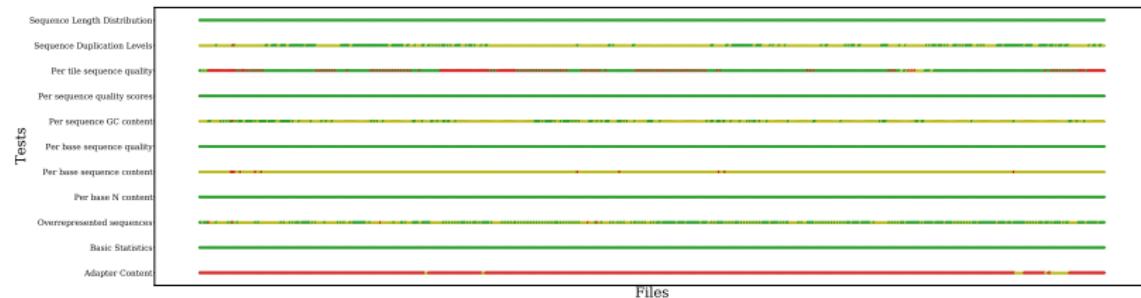
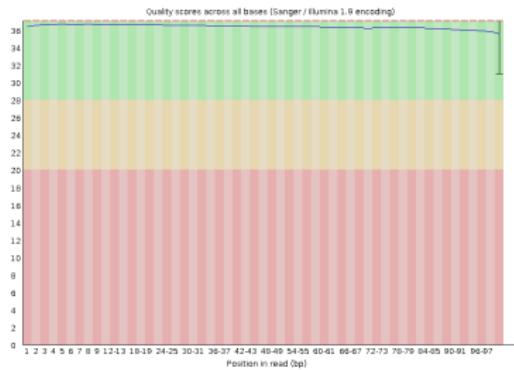


Figure: FastQC with WES data

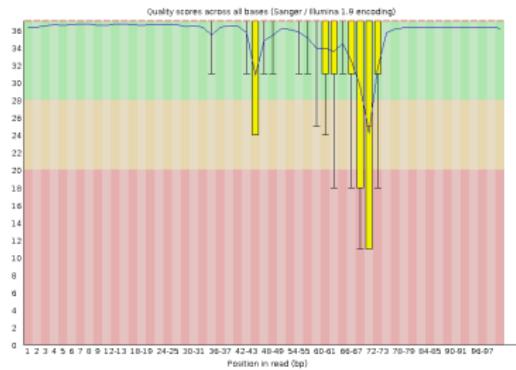
Failure on 33P1 sample

33P1 is excluded at further analysis.

# Failure on 33P1 I



(a) 33N



(b) 33P1

Figure: Per Base Sequence Quality Results

# Failure on 33P1 II

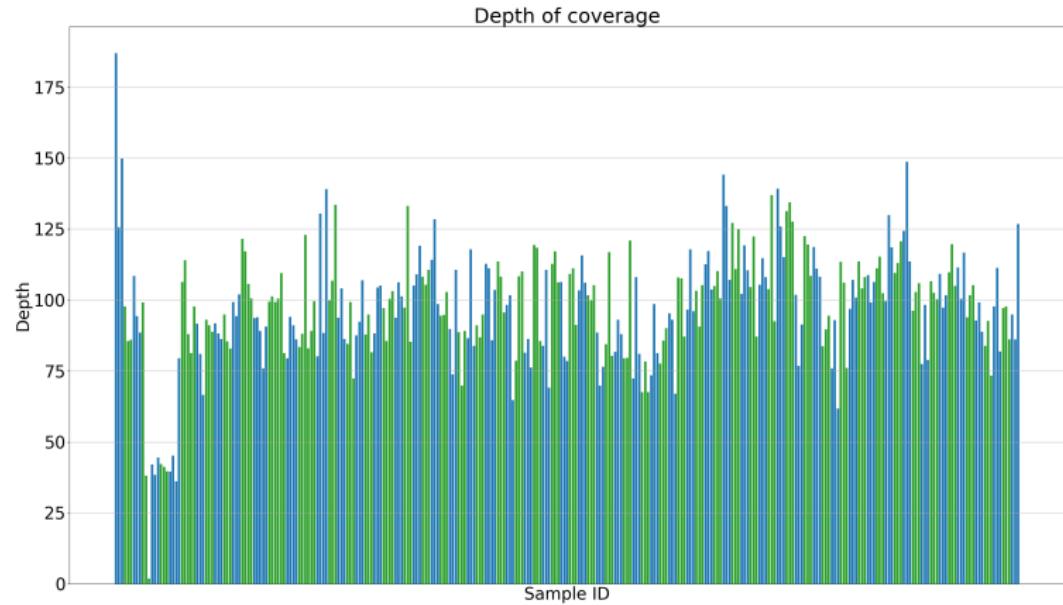


Figure: Coverage Depth Plot

# FastQC on WTS

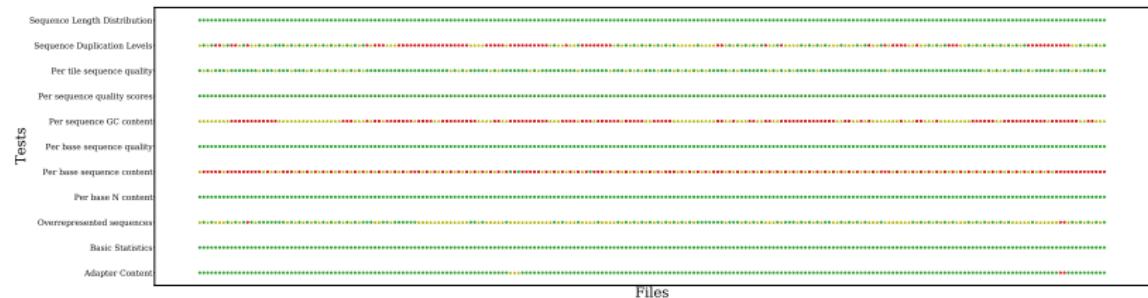


Figure: FastQC with WTS data

All sample are good to analysis

∴ No sample has more than 5 failures.

# Results

Copy Number Variations (CNVs) with Sequenza

# Sequenza?

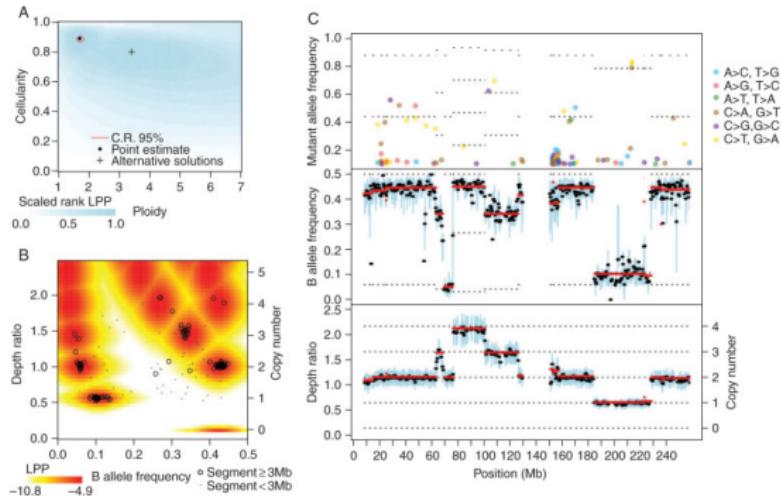
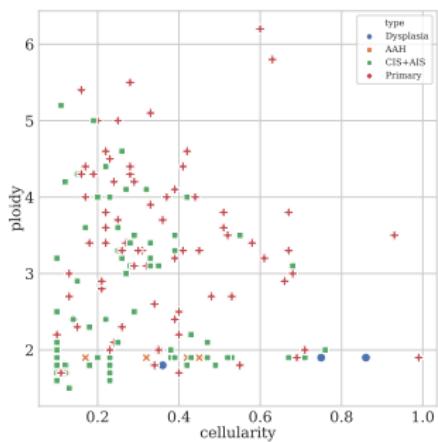
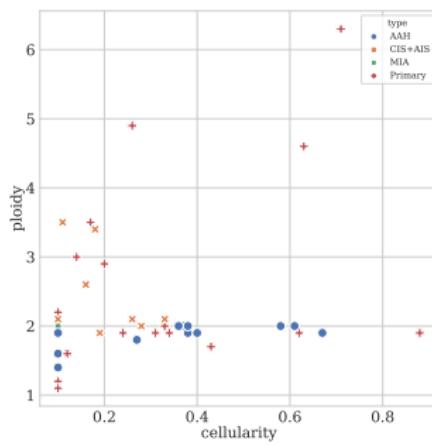


Figure: Representative Output of the Sequenza (Favero et al., 2015)

# Cellularity & Ploidy on WES



(a) SQC Samples



(b) ADC Samples

Figure: Cellularity and Ploidy from Sequenza

# Genome View on Patient #57

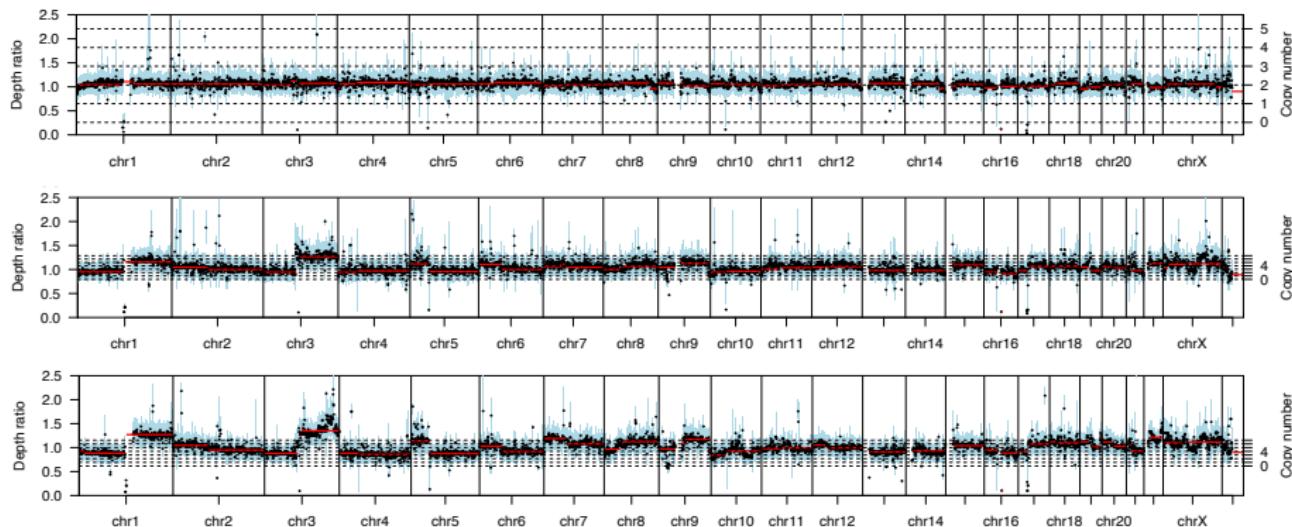


Figure: Dysplasia-CIS-Primary Tumor on Patient #57

# CNVs of SQC

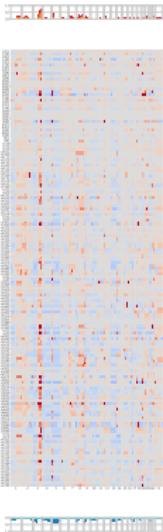


Figure: CNV Plot with SQC Patients

# CNVs of ADC

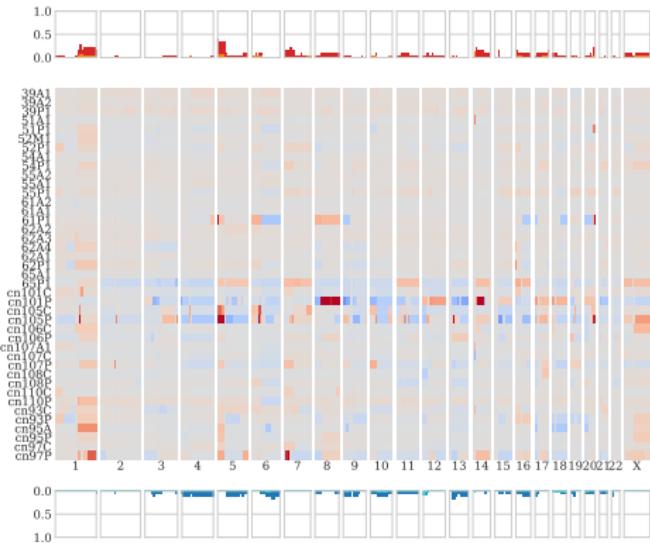


Figure:

CNV Plot with ADC Patients

# SQC vs. ADC

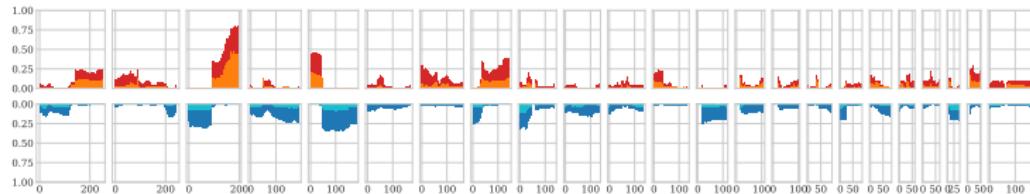


Figure: Simple CNV Plot with SQC Patients

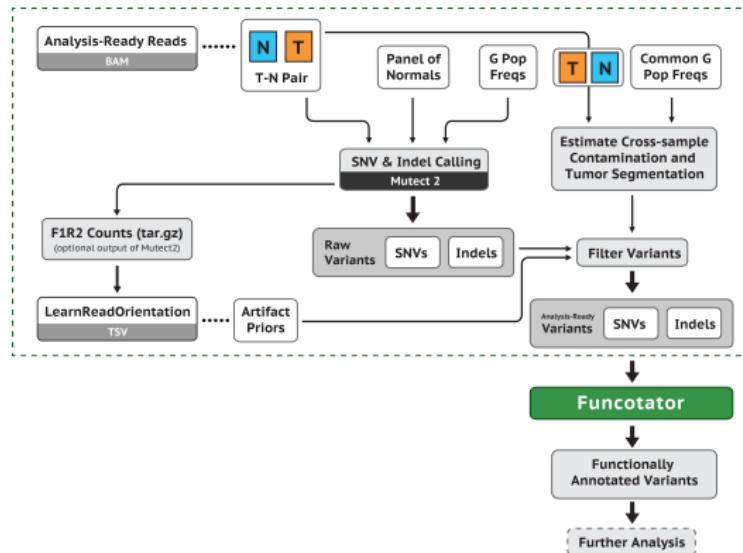
Figure: Simple CNV Plot with ADC Patients

# Findings in Sequenza

# Results

Find SNVs with Mutect2

# Mutect2?



**Figure:** Somatic short variant discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

# Witer?

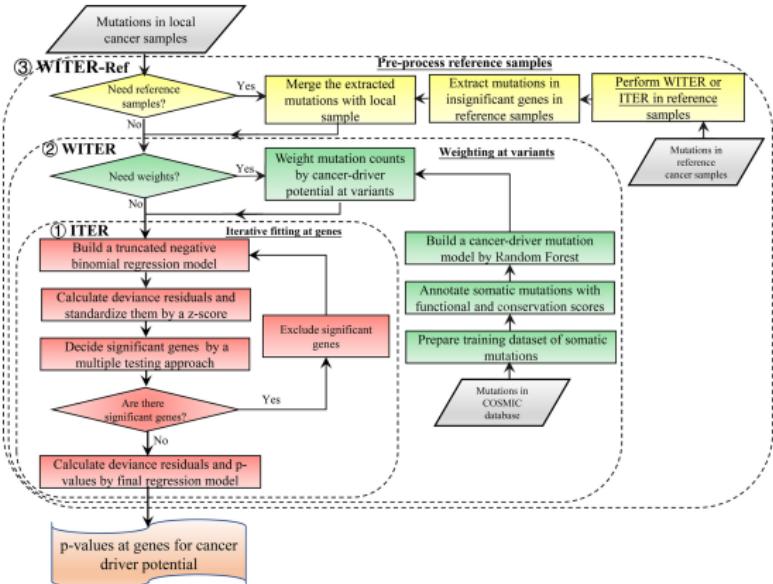


Figure: Witer diagram for detecting cancer-drive genes (Jiang et al., 2019)

# Somatic Variant with BWA in SQC

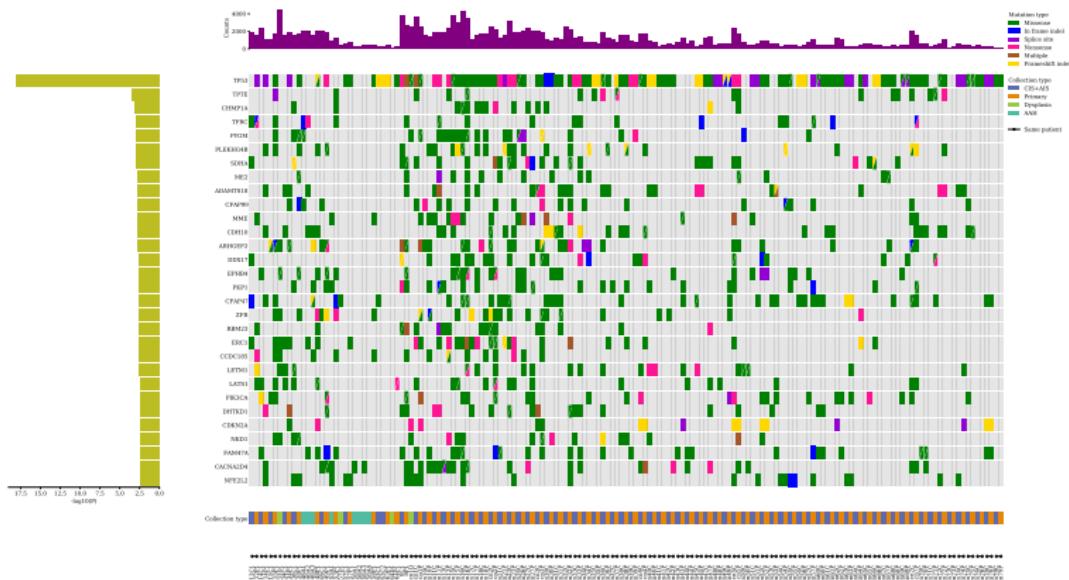
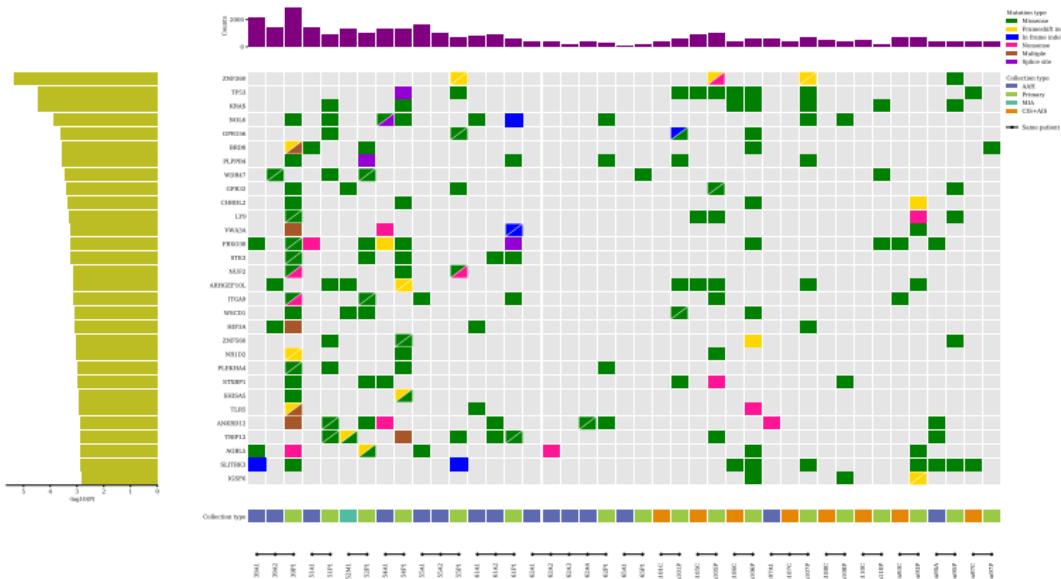


Figure: CoMut Plot with SQC Patients

## Somatic Variant with BWA in ADC



## Figure: CoMut Plot with ADC Patients

# Findings in Mutect2

# Results

Gene Expression Levels from RSEM & DESeq2

# RSEM?

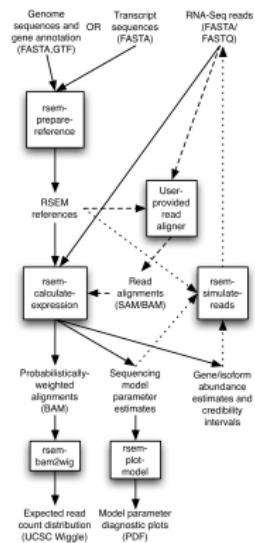


Figure: RSEM workflow (B. Li & Dewey, 2011)

# DESeq2

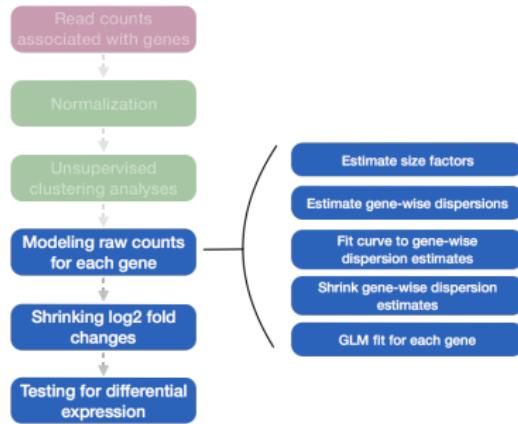


Figure: DESeq2 workflow (Love et al., 2014)

# DEG Selection Strategy

DEG: differentially expressed genes

Fold Change

$$\log_2(\text{Fold Change}) > 1 \vee \log_2(\text{Fold Change}) < -1$$

P-value

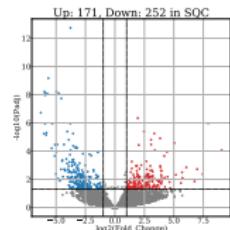
$$P\text{-value} < 0.05$$

Adjusted P-value

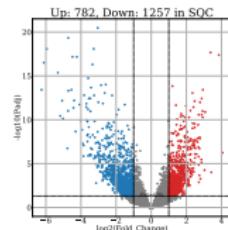
$$P_{adj} < 0.05$$

# DEG Volcano Plots with Bowtie2 in SQC

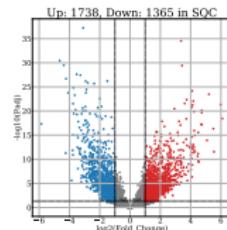
Normal → Dysplasia → CIS → Primary (SQC)



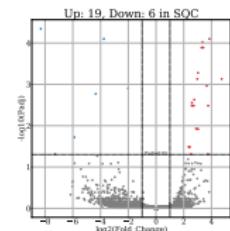
(a) Normal-Dysplasia



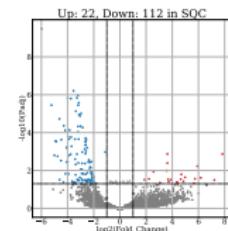
(b) Normal-CIS



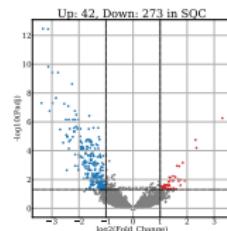
(c) Normal-Primary



(d) Dysplasia-CIS



(e) Dysplasia-Primary

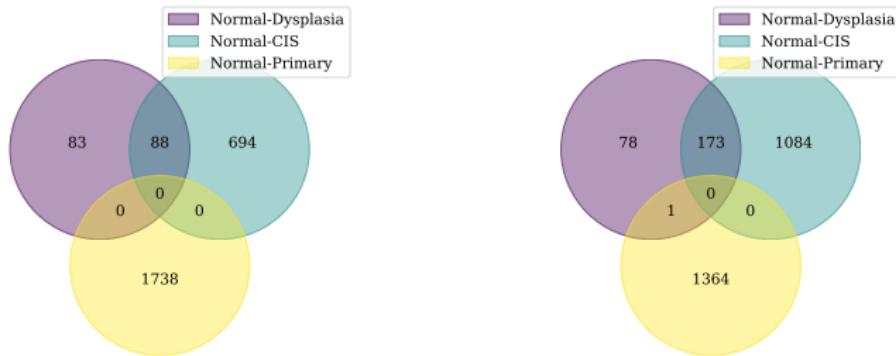


(f) CIS-Primary

Figure: DEG Volcano Plots with Bowtie2 in SQC

# DEG Venn Diagram with Bowtie2 in SQC

Normal → Dysplasia → CIS → Primary (SQC)



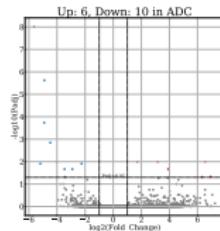
(a) Up-regulated

(b) Down-regulated

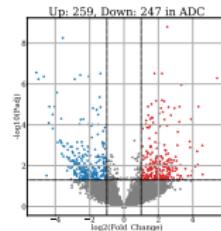
Figure: DEG Venn Diagram with Bowtie2 in SQC

# DEG Volcano Plots with Bowtie2 in ADC

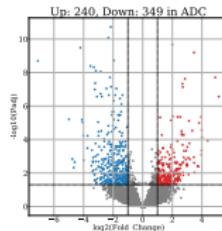
Normal → AAH → AIS → MIA → Primary (ADC)



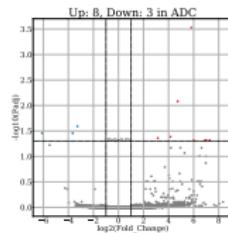
(a) Normal-AAH



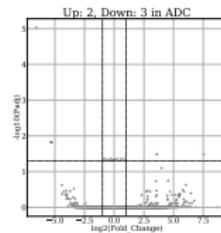
(b) Normal-AIS



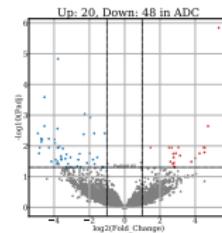
(c) Normal-Primary



(d) AAH-AIS



(e) AAH-Primary

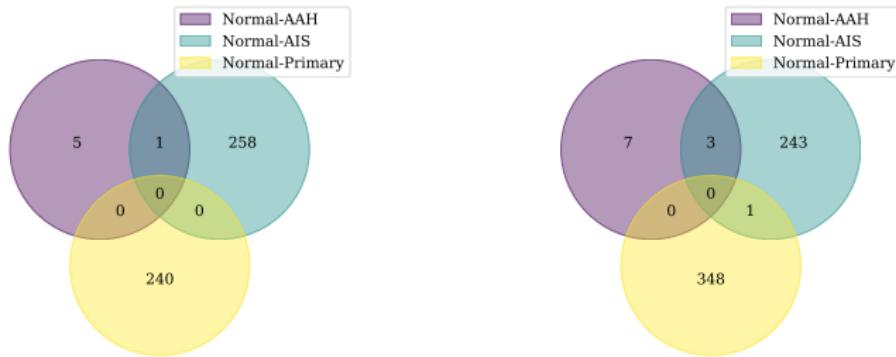


(f) AIS-Primary

Figure: DEG Volcano Plots with Bowtie2 in ADC

# DEG Venn Diagram with Bowtie2 in ADC

Normal → AAH → AIS → MIA → Primary (ADC)



(a) Up-regulated

(b) Down-regulated

Figure: DEG Venn Diagram with Bowtie2 in ADC

# Finding in DEG Analysis

# Results

## Tumor Evolution with MesKit

# MesKit?

# Results

## Estimated Cell Types with CIBERSORTx

# CIBERSORTx?

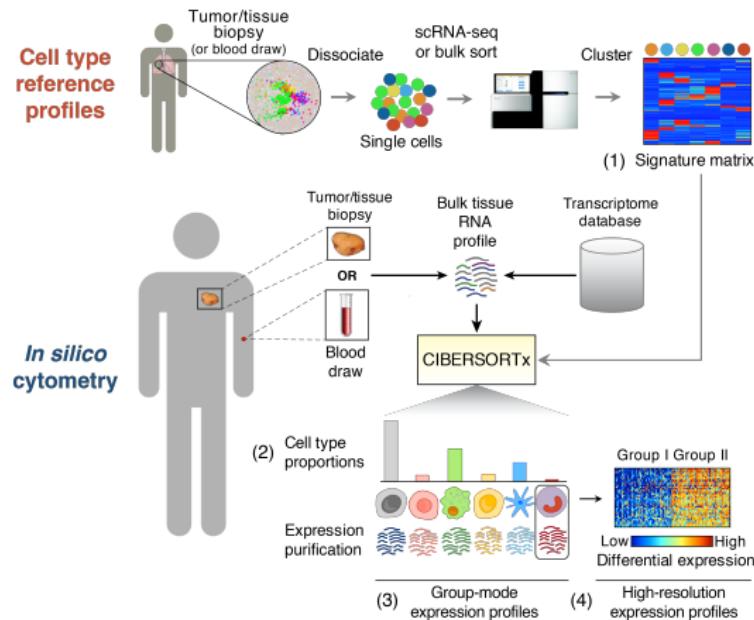
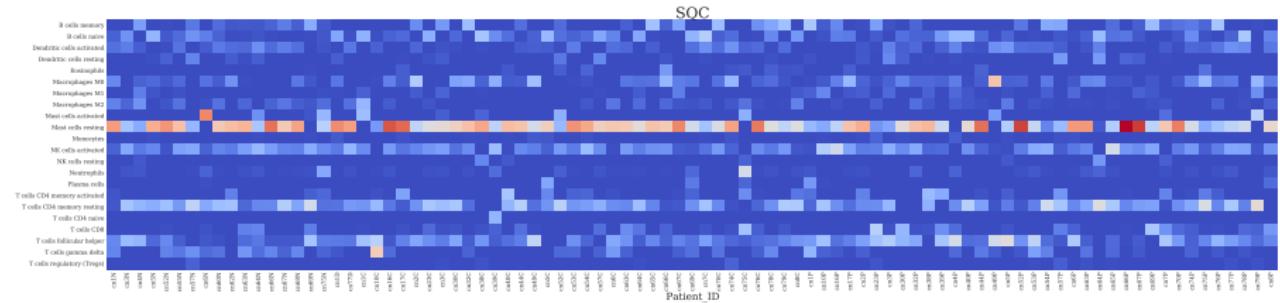


Figure: CIBERSORTx workflow (Steen et al., 2020)

CIBERSORTx with SQC



## Figure: Estimated Cell Types with SQC Samples

# CIBERSORTx with ADC

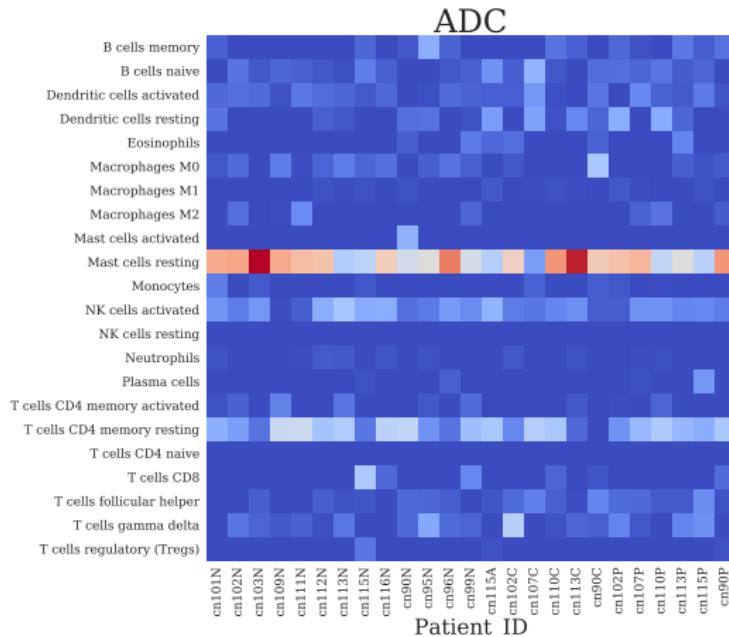


Figure: Estimated Cell Types with ADC Samples

# Discussion

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