

# Lung Precancer Study

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# Overview

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3 Methods

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# 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

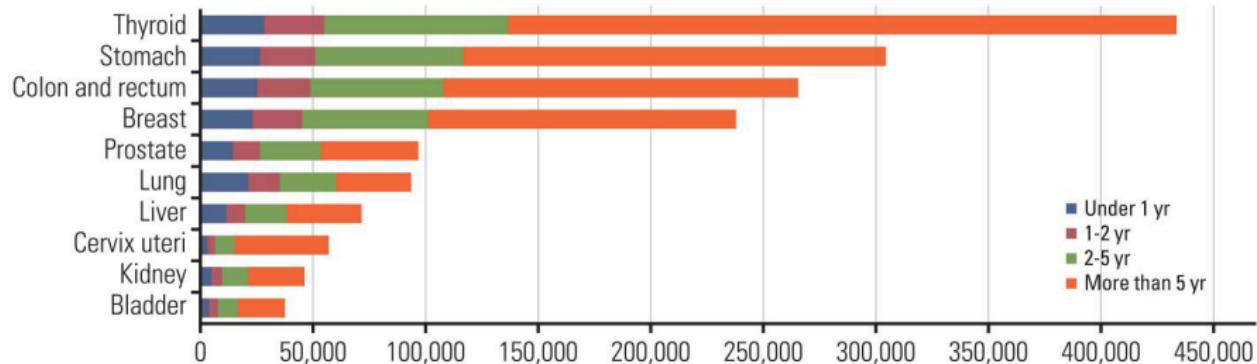


Figure: Common cancer survival rates (Hong et al., 2021)

## 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)

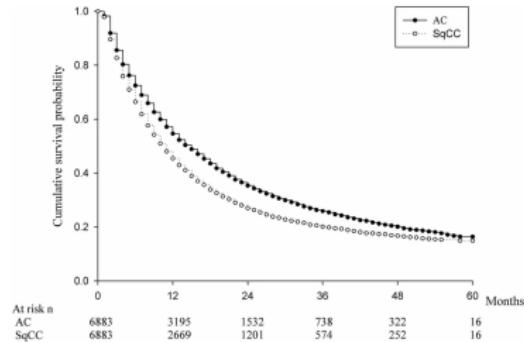
(Vincent et al., 1977; Collins, Haines, Perkel, & Enck, 2007)

# ADC vs. SQC I

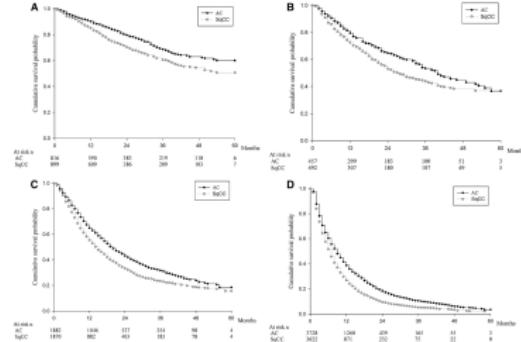


Figure: ADC and SQC histology in Lung cancer (Travis, 2002)

# ADC vs. SQC II



(a) All patients



(b) By cancer stages

Figure: Kaplan-Meier survival curves for ADC & SQC (B.-Y. 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

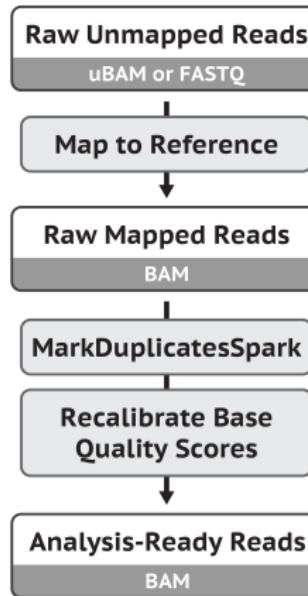
- WES (n=289) + Transcriptome (n=166)
- Normal + {Primary, CIS + AIS, AAH, Dysplasia, MIA}
  - Carcinoma in situ
  - Adenocarcinoma in situ
  - Atypical adenomatous hyperplasia
  - Dysplasia
  - Minimally invasive adenocarcinoma
- Squamous cell carcinoma (SQC) & Adenocarcinoma (ADC)
  - ① Normal → Dysplasia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

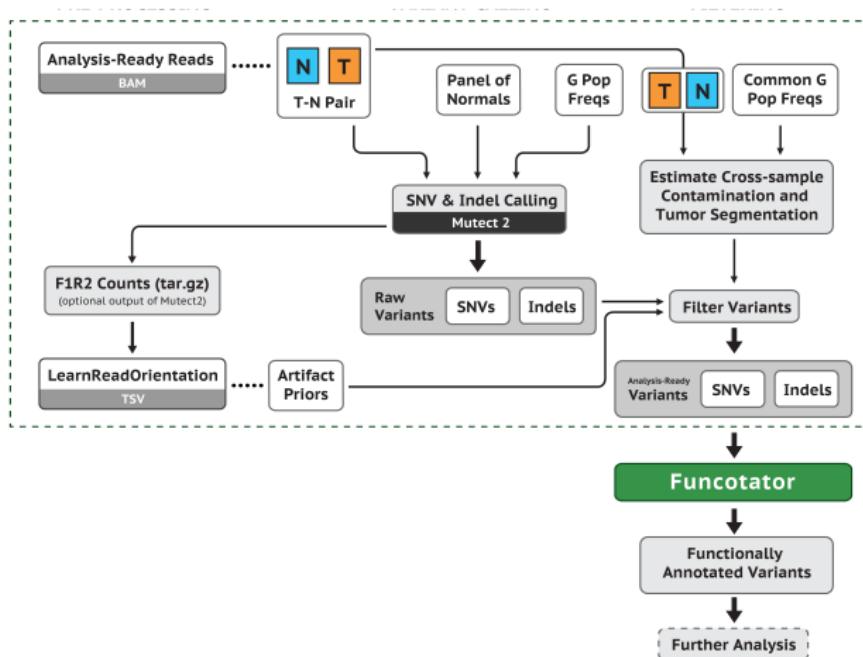
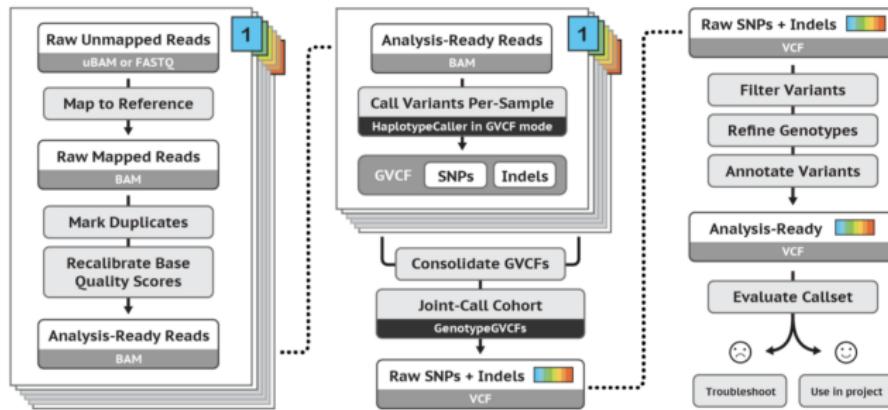


Figure: Somatic short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

# 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)

# Results

# Results

## Quality Checks

# 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

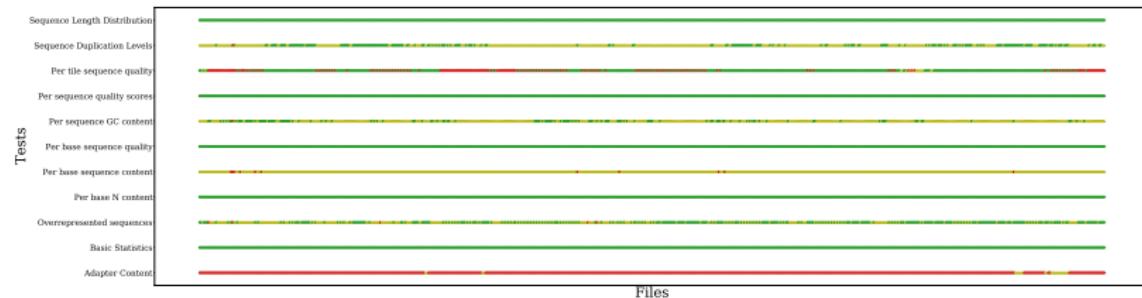


Figure: FastQC with WES data

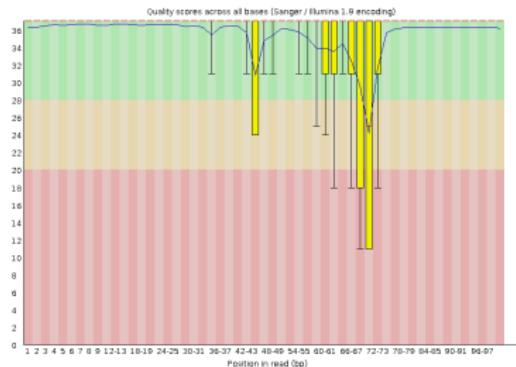
Failure on 33P1 sample

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

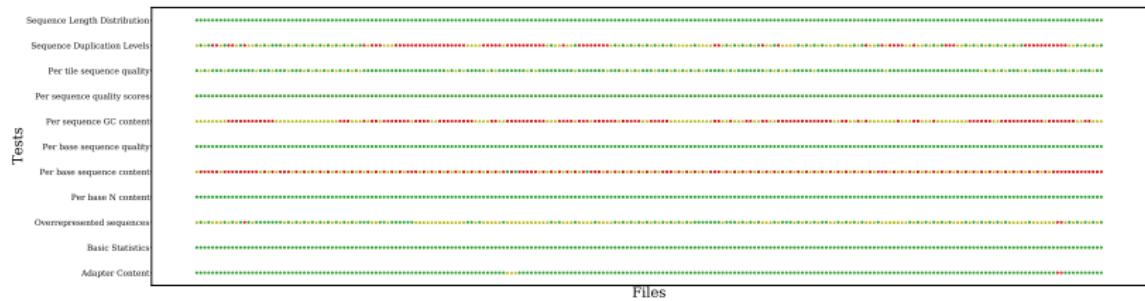


Figure: FastQC with WTS data

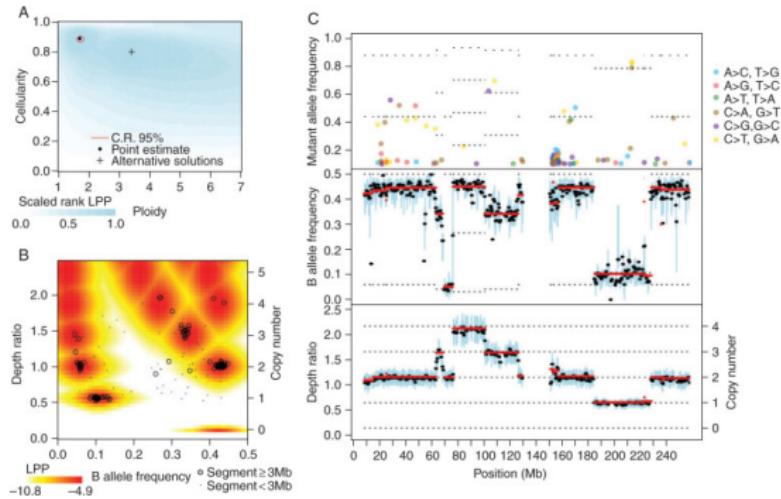
All sample are good to analysis

∴ No sample has more than 5 failures.

# Results

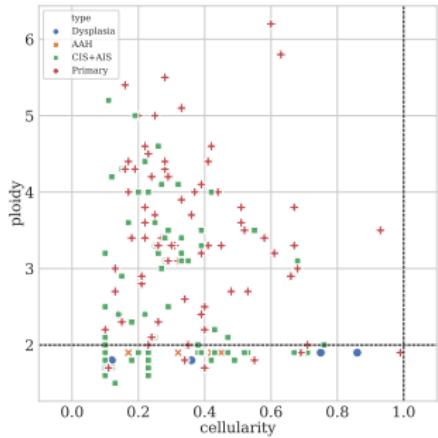
## Copy Number Variations

# Sequenza?

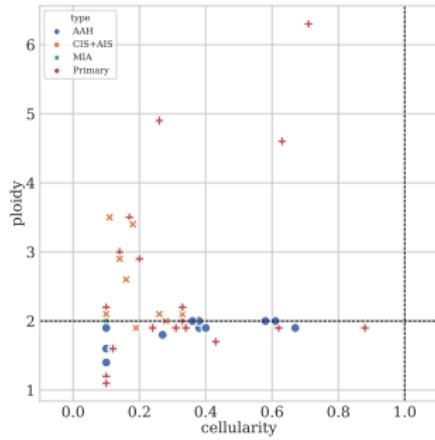


**Figure:** Representative Output of the Sequenza (Favero et al., 2015)

# Cellularity & Ploidy on WES



(a) SQC Samples



(b) ADC Samples

Figure: Cellularity and Ploidy from Sequenza

# Genome View on Patient #57

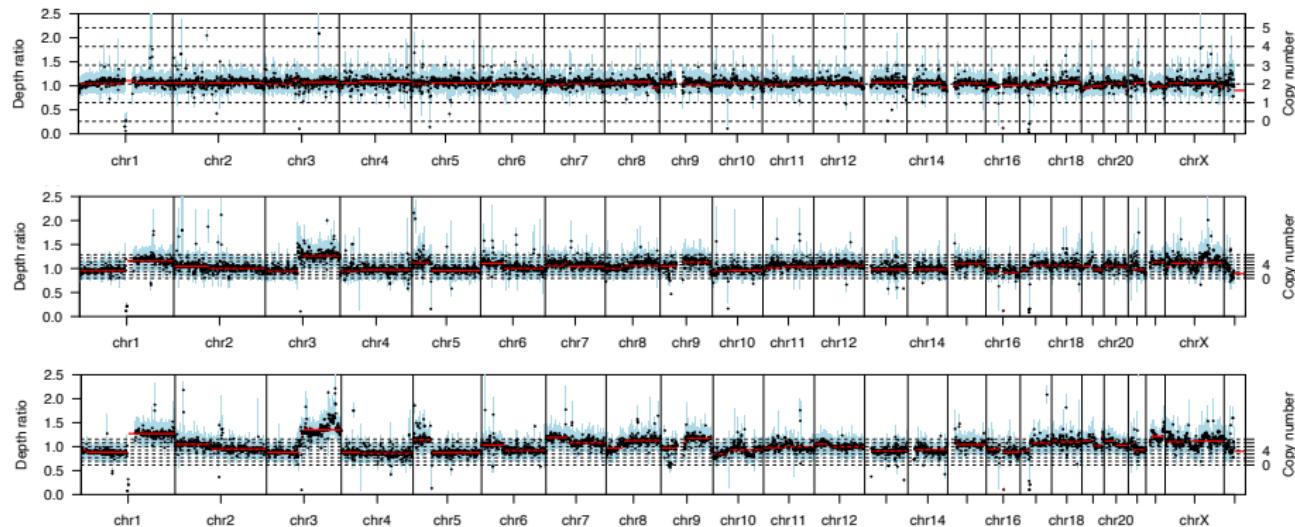


Figure: Dysplasia-CIS-Primary Tumor on Patient #57

# CNVs of SQC

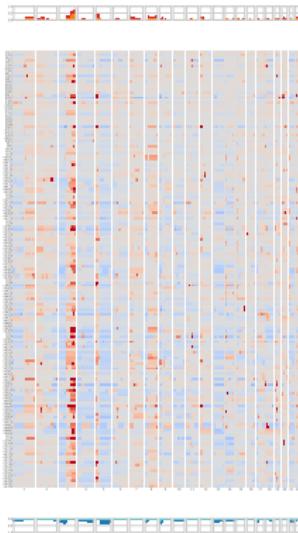


Figure: CNV Plot with SQC Patients

# CNVs of ADC

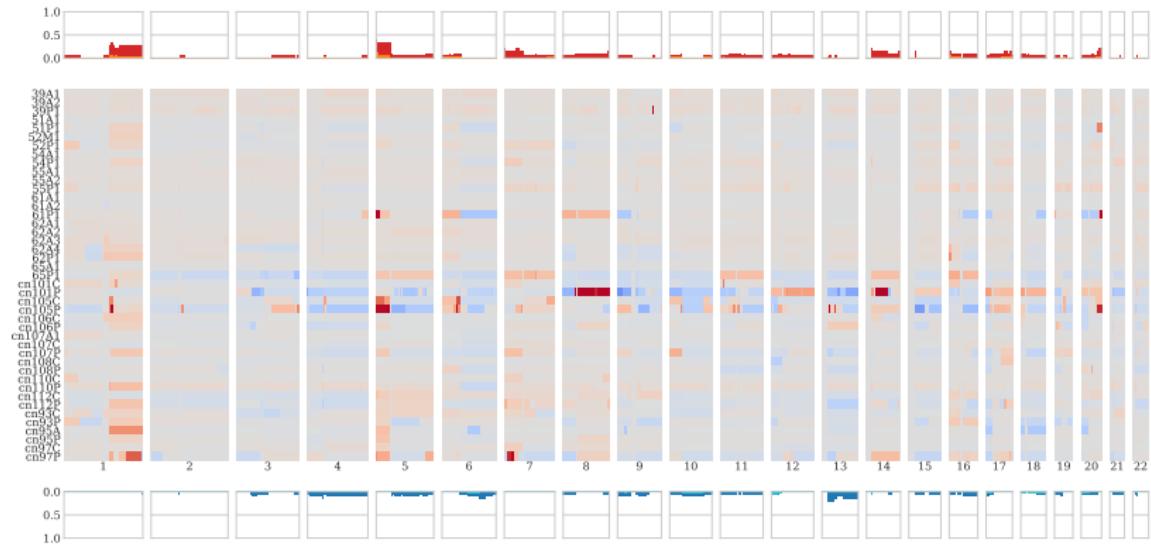


Figure: CNV Plot with ADC Patients

# SQC vs. ADC

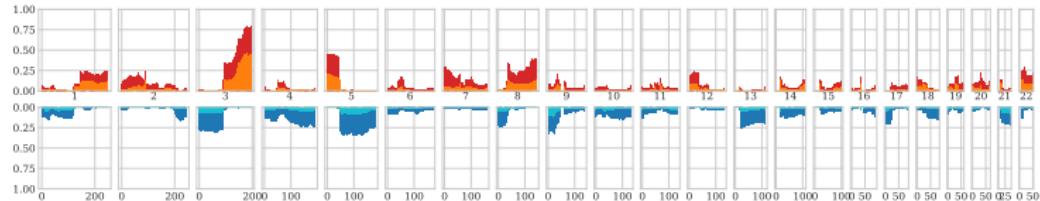


Figure: Simple CNV Plot with SQC Patients

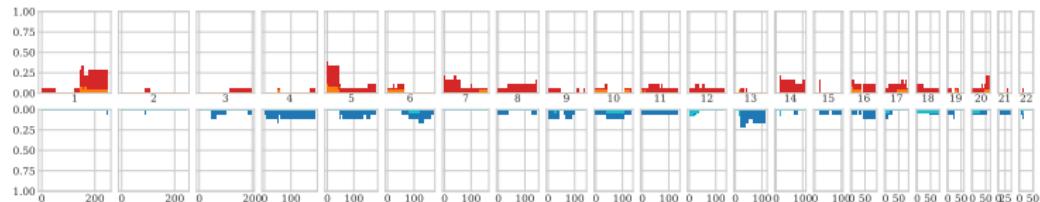


Figure: Simple CNV Plot with ADC Patients

# Findings in Sequenza

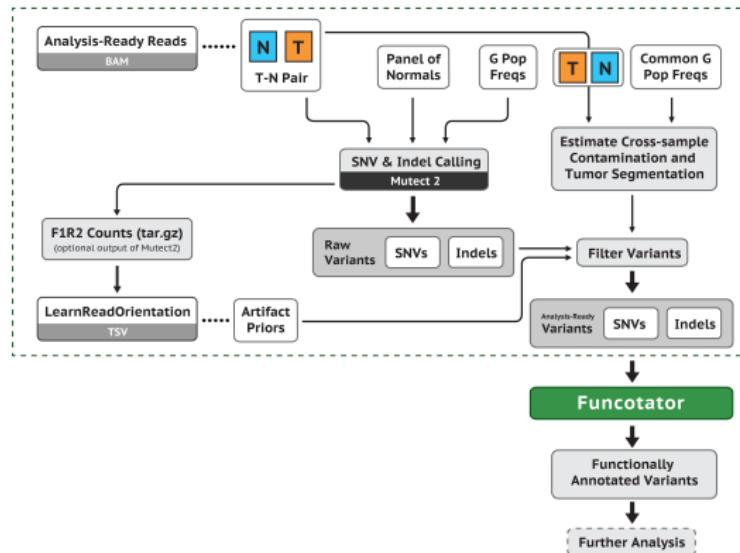
## Sequenza Findings

- SQC have more CNVs than ADC.
- SQC have aggressive CNVs on chromosome #3 and #5.
- ADC have aggressive CNVs on chromosome #5.

# Results

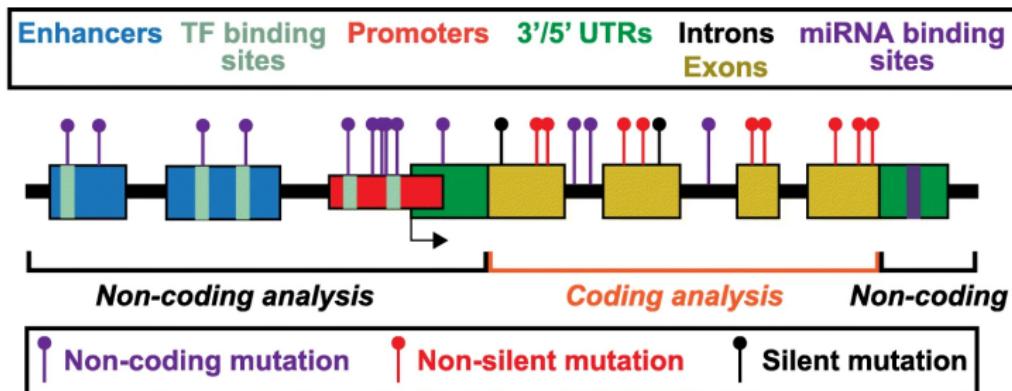
## SNVs Analysis

# Mutect2?



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

# MutEnricher?



## Analysis summary:

### Inputs:

- Somatic mutations
- Features of interest:
  - Coding genes
  - Non-coding regions
- Genomic covariates (optional)

### Analyses:

- Background calculations:
  - global, local, or covariate clustered
- Mutation enrichments:
  - coding/non-coding modules

### Outputs:

- Gene or non-coding region enrichments:
  - Overall genes/regions
  - Hotspots
  - Combined

**Figure:** Schematic representation of MunEnricher's analysis procedures (Soltis et al., 2020)

# Somatic Variant in SQC

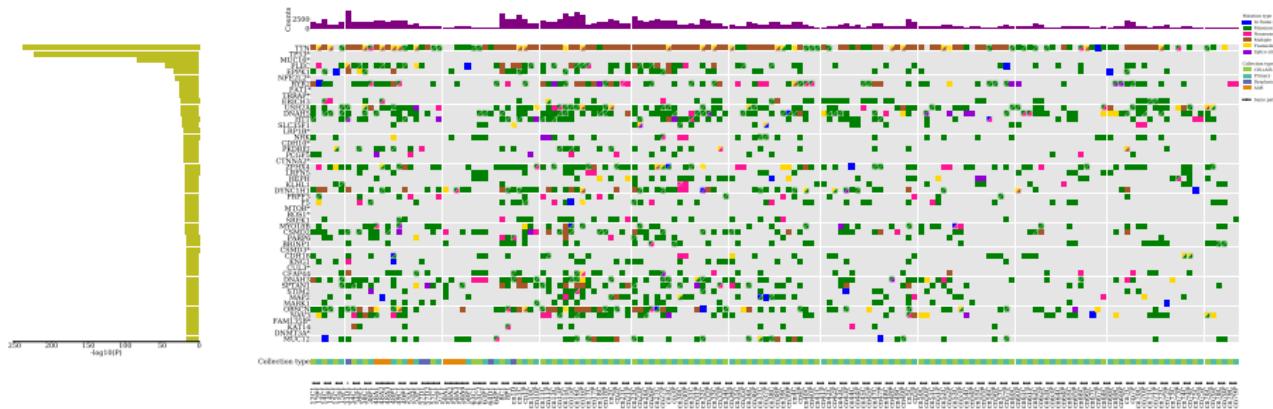


Figure: CoMut Plot with SQC Patients

# Somatic Variant in ADC

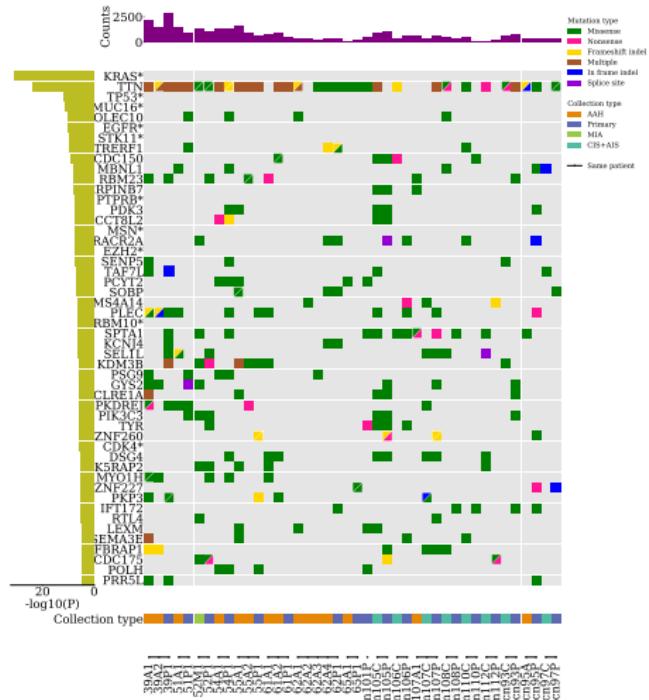


Figure: CoMut Plot with ADC Patients

# Findings in SNVs Analysis

## TTN

TTN is the most mutated gene both in SQC and ADC.

# Results

## VAF Analysis

# VAF?

- Variant allele frequency
- VAF = Alternative allele read count/Total read count
- To find tumor evolution

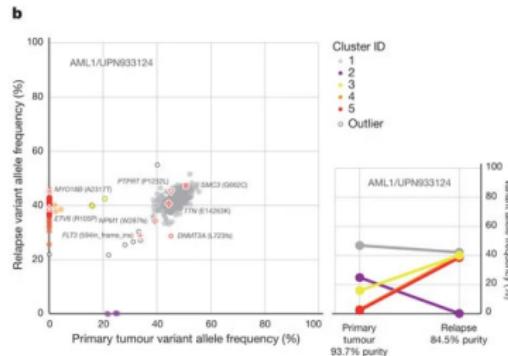


Figure: VAF distribution of validated mutations (Ding et al., 2012)

# VAF Plots I

# PyClone?

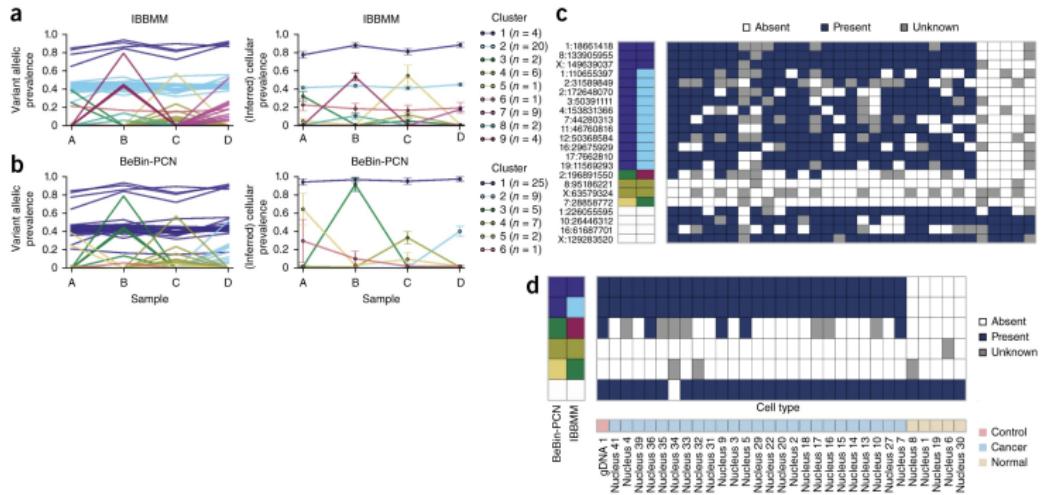


Figure: Analysis of multiple samples by PyClone (Roth et al., 2014)

# PyClone Plots I

# Findings in VAF Analysis

# Results

Differences in Gene Expression Levels

# RSEM?

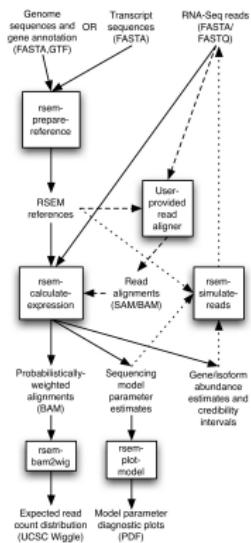


Figure: RSEM workflow (Li & Dewey, 2011)

# DESeq2?

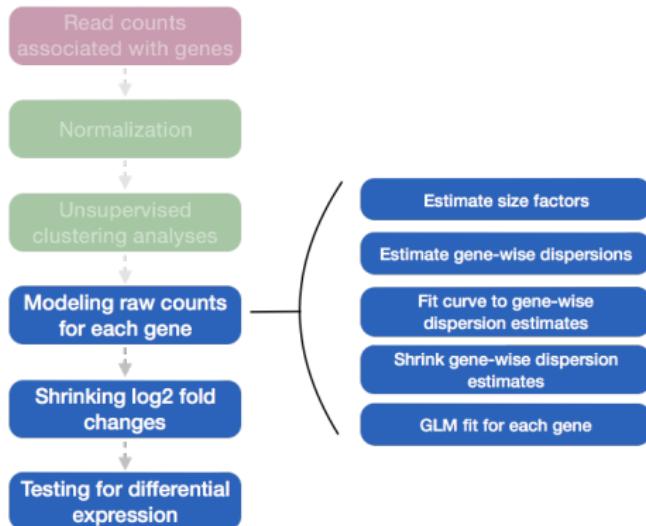


Figure: DESeq2 workflow (Love, Huber, & Anders, 2014)

# Enrichr?

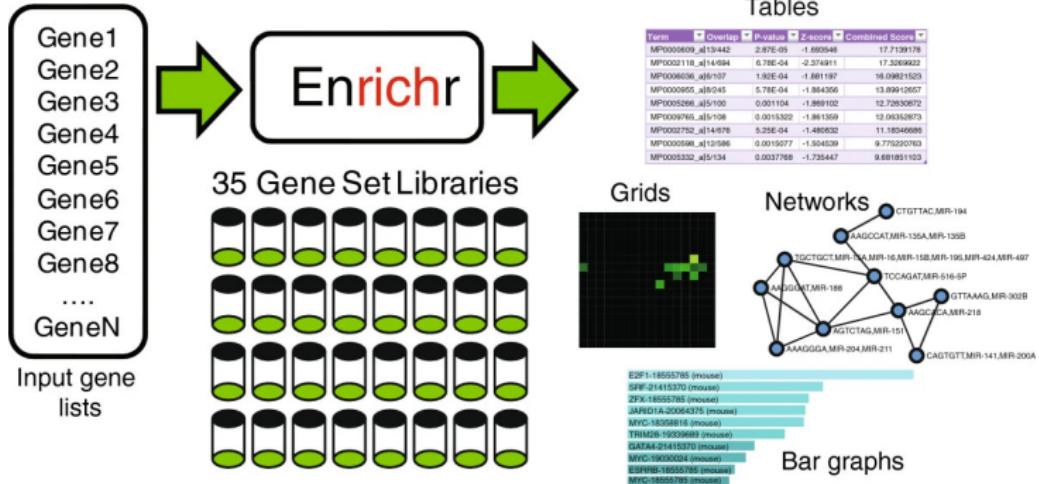


Figure: Enrichr workflow (Chen et al., 2013; Kuleshov et al., 2016)

# DEG Selection Strategy

DEG: differentially expressed genes

Fold Change

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

P-value

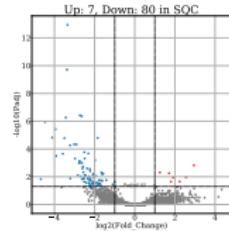
$$P\text{-value} < 0.05$$

Adjusted P-value

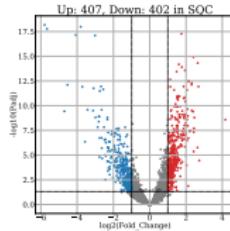
$$P_{adj} < 0.05$$

# DEG Volcano Plots in SQC

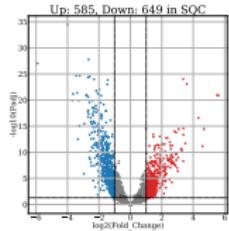
Normal → Dysplasia → CIS → Primary (SQC)



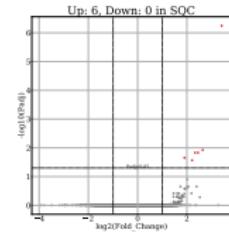
(a) Normal-Dysplasia



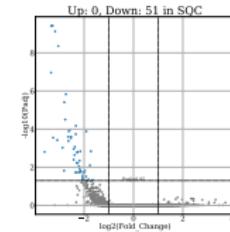
(b) Normal-CIS



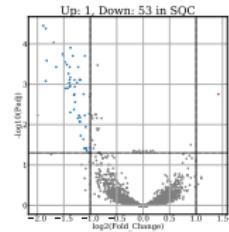
(c) Normal-Primary



(d) Dysplasia-CIS



(e) Dysplasia-Primary

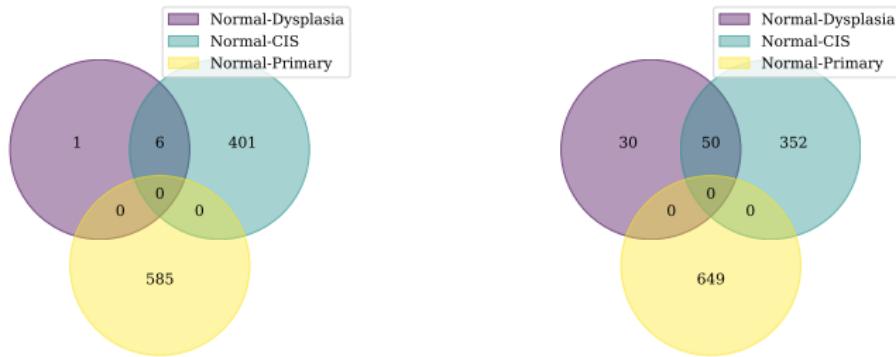


(f) CIS-Primary

Figure: DEG Volcano Plots in SQC

# DEG Venn Diagram with Bowtie2 in SQC

Normal → Dysplasia → CIS → Primary (SQC)



(a) Up-regulated

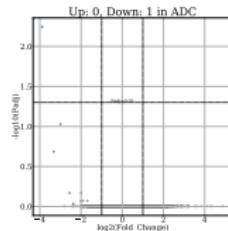
(b) Down-regulated

Figure: DEG Venn Diagram in SQC

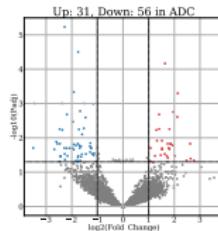
# Enrichment test with Enrichr in SQC

# DEG Volcano Plots with Bowtie2 in ADC

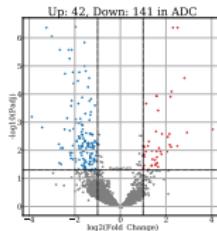
Normal → AAH → AIS → MIA → Primary (ADC)



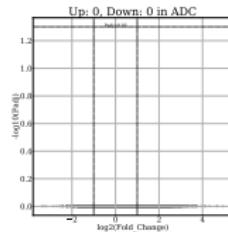
(a) Normal-AAH



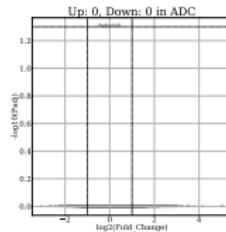
(b) Normal-AIS



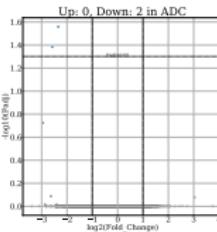
(c) Normal-Primary



(d) AAH-AIS



(e) AAH-Primary



(f) AIS-Primary

Figure: DEG Volcano Plots 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 in ADC

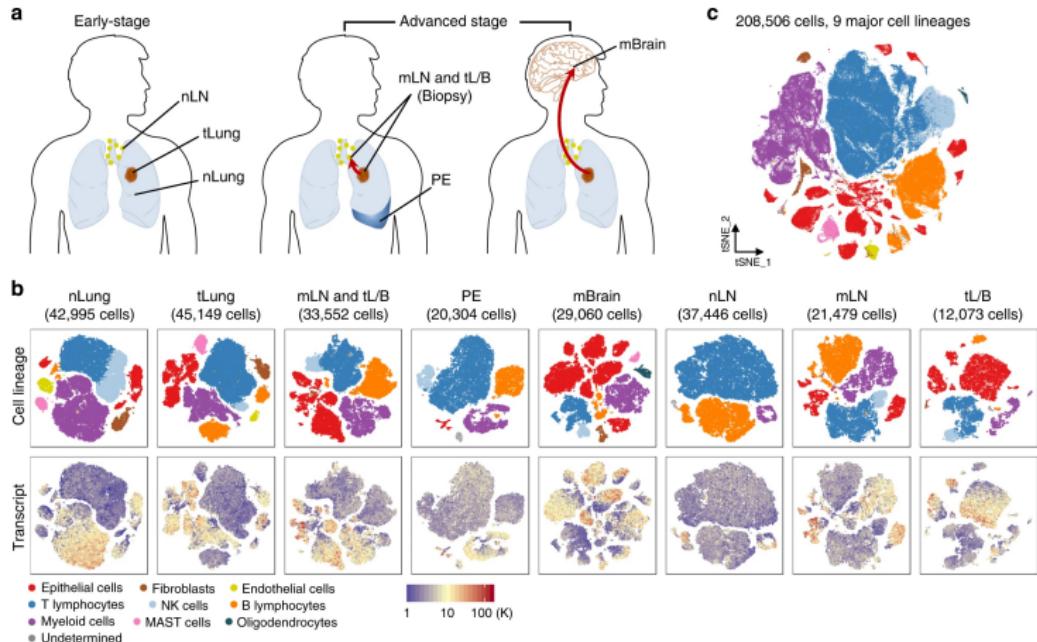
# Enrichment test with Enrichr in ADC

# Findings in DEG Analysis

# Results

## Bulk Cell Deconvolution

# Single-cell data as Reference



**Figure:** Comprehensive dissection and clustering of 208,506 single cells from LUAD patients (Kim et al., 2020)

# CIBERSORTx

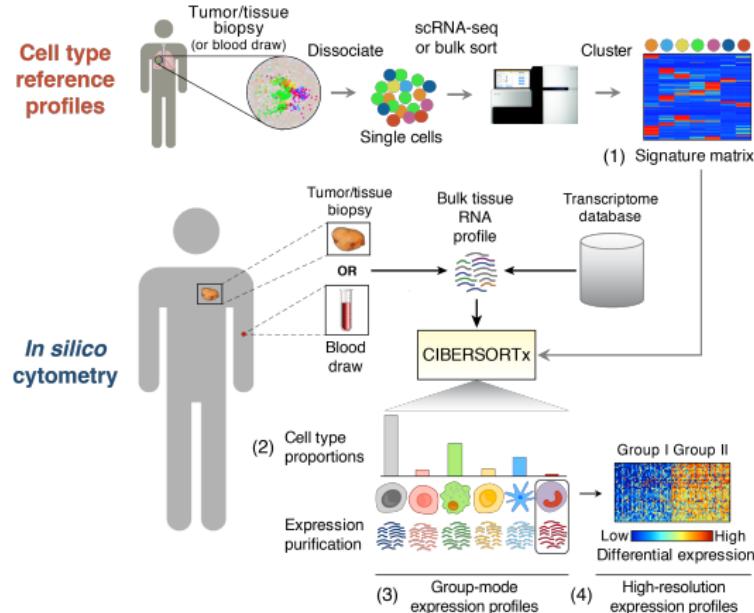


Figure: Workflow for CIBERSORTx (Steen et al., 2020; Newman et al., 2019)

# Cluster Plot with Bowtie2 in ADC

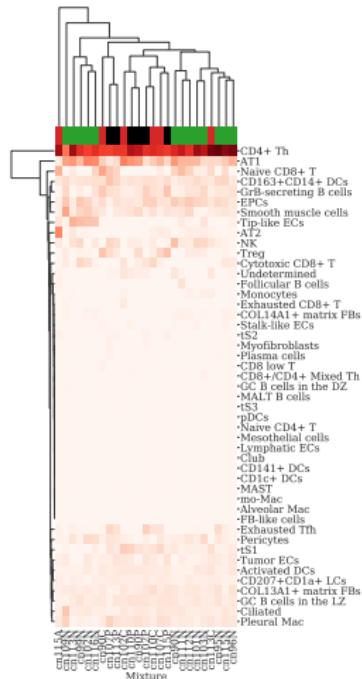


Figure: Cluster Plot in ADC

# Cluster Plot with Bowtie2 in SQC

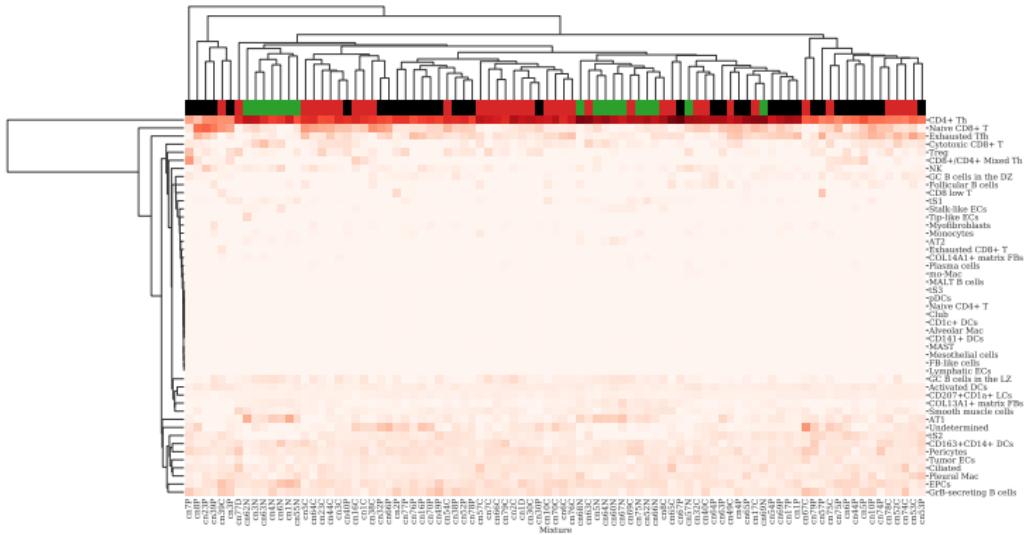


Figure: Cluster Plot in SQC

# Benchmarking of Cell Deconvolution Tools

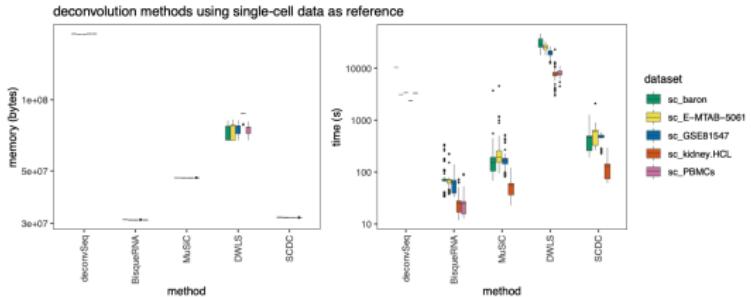
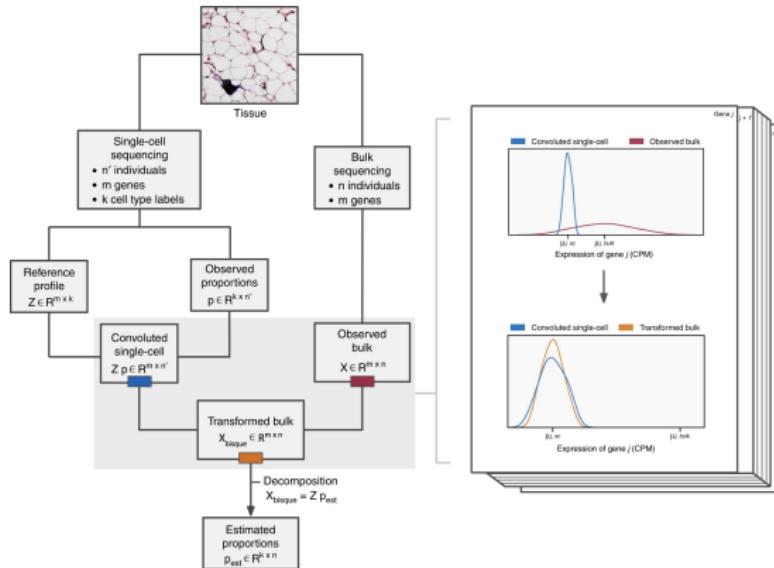


Figure: Memory and time requirements for the cell deconvolution methods (Cobos et al., 2020)

## Top 3 Methods

- ① BisqueRNA (Jew et al., 2020)
- ② MuSiC (X. Wang, Park, Susztak, Zhang, & Li, 2019)
- ③ SCDC (Dong et al., 2021)

# BisqueRNA?



**Figure:** Graphical overview of the Bisque decomposition methods (Jew et al., 2020)

# MuSiC?

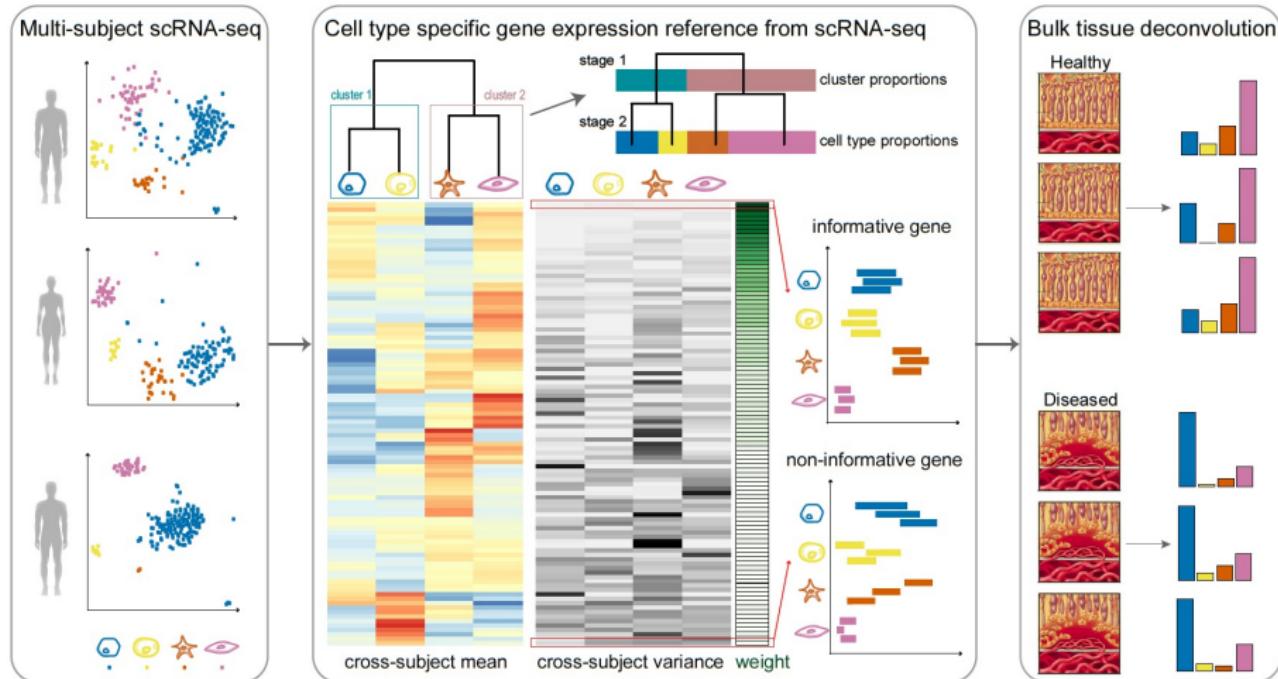
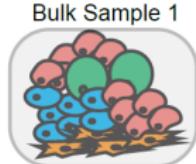


Figure: Overview of MuSiC framework (X. Wang et al., 2019)

# SCDC?

Bulk RNA-seq



Bulk Sample 2

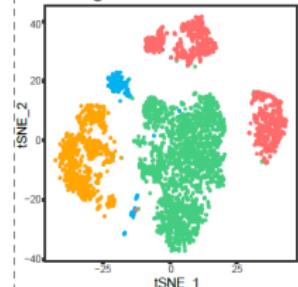


Bulk Sample 3

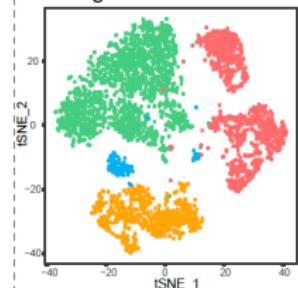


scRNA-seq

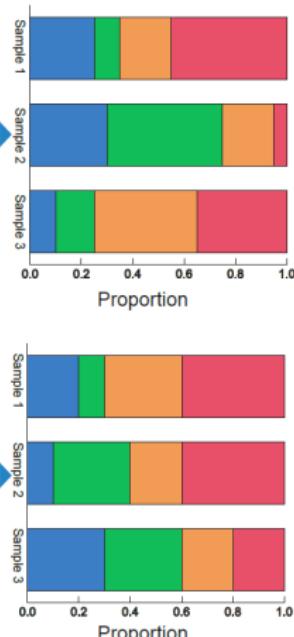
Single-Cell Reference 1



Single-Cell Reference 2



Deconvolution



ENSEMBLE

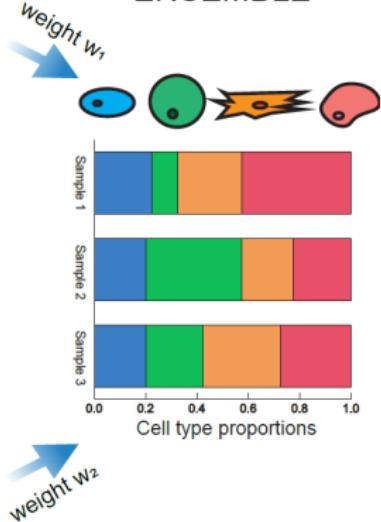


Figure: Overview of deconvolution by SCDC (Dong et al., 2021)

# Findings in Bulk Cell Deconvolution

# Results

## Tumor Evolution Trajectories Analysis

# Revolver?

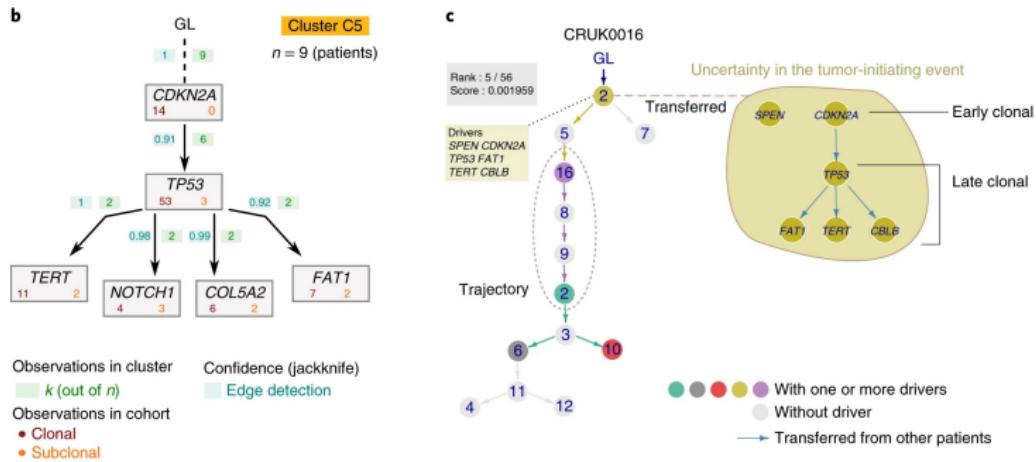


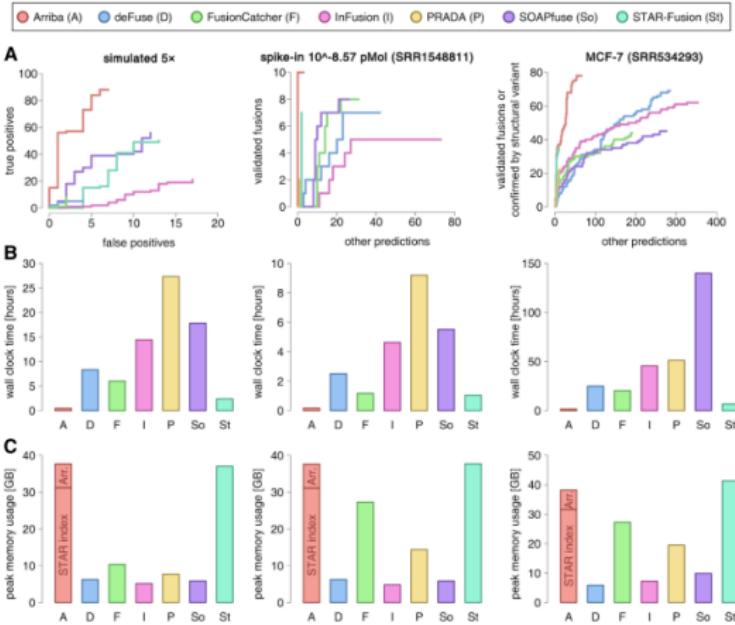
Figure: Repeated Evolutionary Trajectories (Caravagna et al., 2018)

# Findings in Tumor Evolution Trajectories Analysis

# Results

## Discovery of Gene Fusion

# Arriba?



**Figure:** Benchmark of Arriba versus alternative methods (Uhrig et al., 2021)

# FusionCatcher?

**FusionCatcher** – a tool for finding somatic fusion genes in paired-end RNA-sequencing data

Daniel Nicorici<sup>\*1</sup>, Mihaela Şatalan<sup>2</sup>, Henrik Edgren<sup>3</sup>, Sara Kangaspeska<sup>3</sup>, Astrid Murumägi<sup>3</sup>, Olli Kallioniemi<sup>3</sup>, Sami Virtanen<sup>1</sup>, and Olavi Kilkku<sup>1</sup>

<sup>1</sup>Orion Corporation, P.O. Box 65, FI-02101 Espoo, Finland

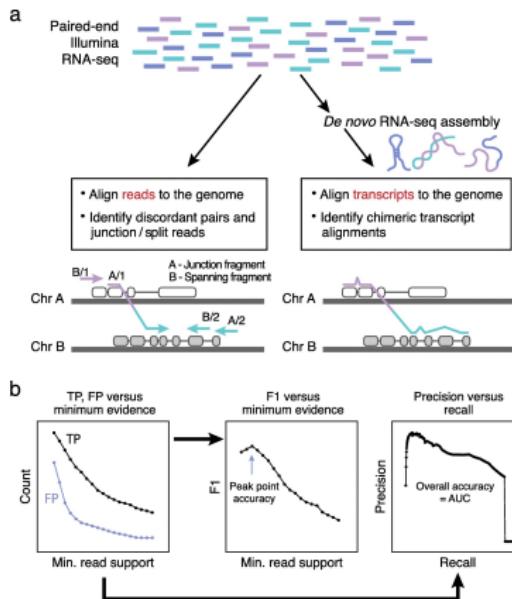
<sup>2</sup>Florida Hospital Heartland Medical Center, P.O. Box 9400,  
Sebring, Florida 33871-9400, USA

<sup>3</sup>Institute for Molecular Medicine Finland, P.O. Box 20,  
FI-00014 Helsinki, Finland

November 19, 2014

**Figure: FusionCatcher (Nicorici et al., 2014)**

# STAR-fusion?



**Figure:** Methods for fusion transcript prediction and accuracy evaluation (Haas et al., 2019)

# Findings in Gene Fusion Discovery

# Discussion

## References

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