Lung Precancer Study

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Overview

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

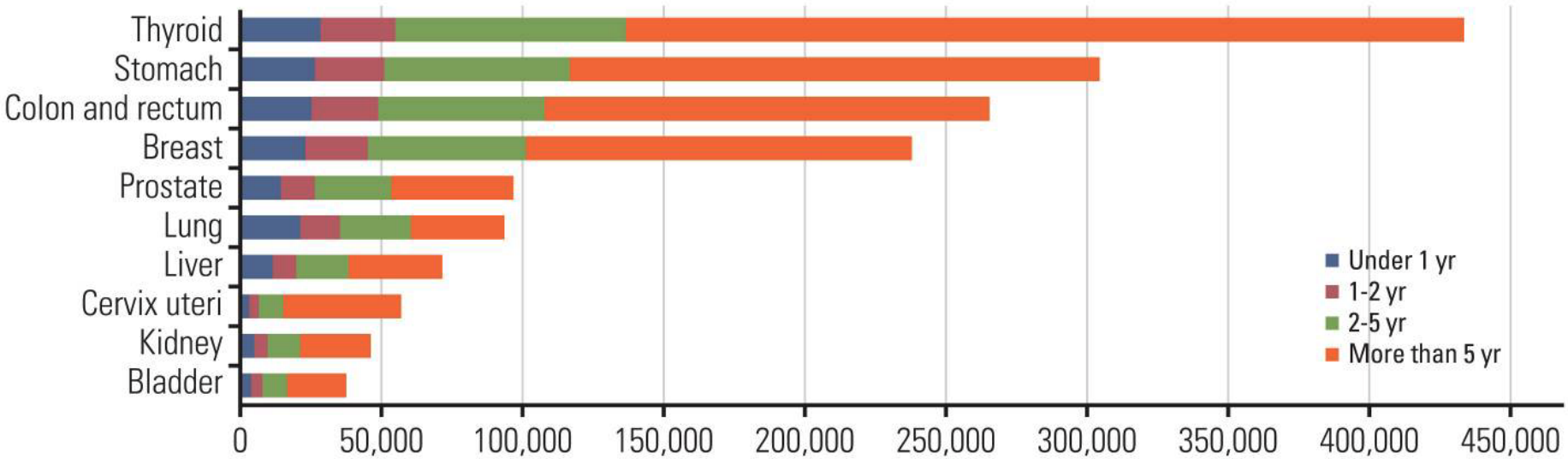


Figure: Common cancer survival rates (Hong et al., 2021)

Survival rate (More than 5 yr)

- Tyroid: 68.4 %
- Lung: 35.4 %

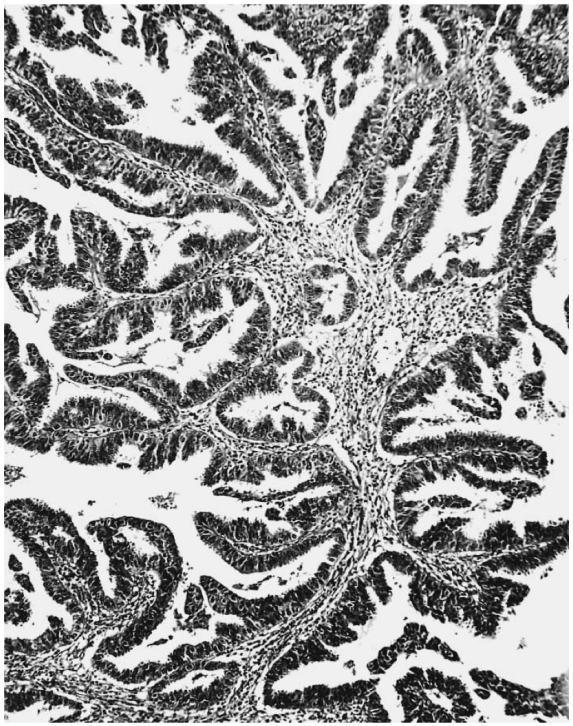
Type of Lung Cancer

Types of lung cancer:

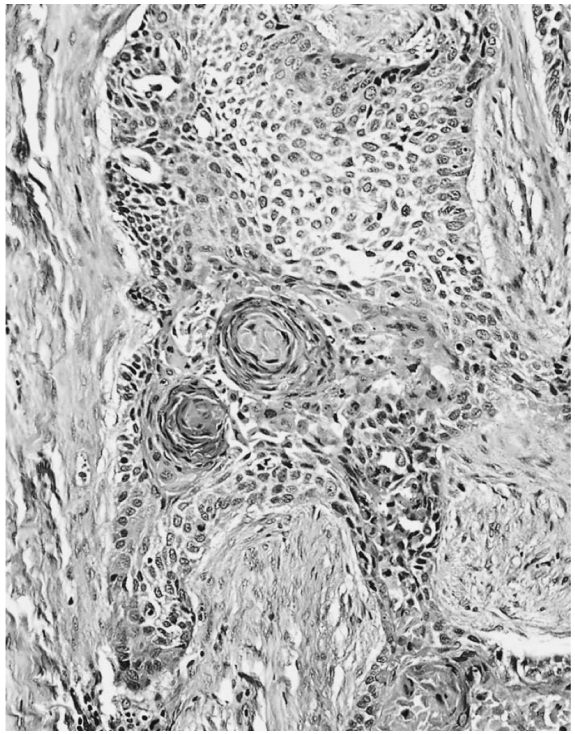
1. Adenocarcinoma (ADC) (40 %) ★
2. Squamous cell carcinoma (SQC) (25 %) ★
3. Small cell carcinoma (20 %)
4. Large cell carcinoma (10 %)
5. Adenosquamous carcinoma (< 5 %)
6. Carcinoid (< 5 %)
7. Bronchioalveolar (Bronchial gland carcinoma)

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

ADC vs. SQC I



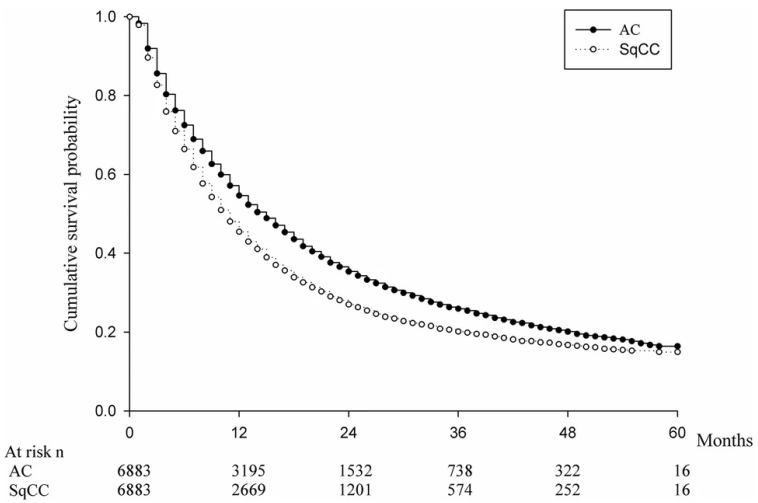
(a) ADC



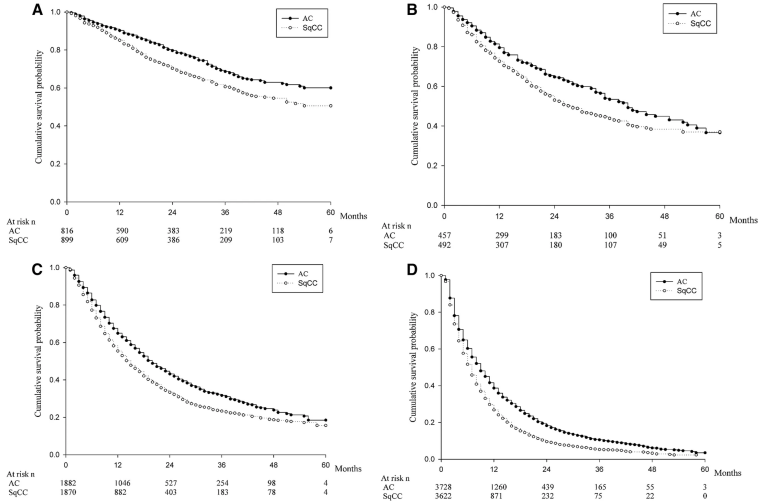
(b) SQC

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

ADC vs. SQC II



(a) All patients



(b) By cancer stages

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

Findings

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

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
- Squamouse cell carcinoma (SQC) & Adenocarcinoma (ADC)
  1. Normal $\rightarrow$ Dysplasia $\rightarrow$ CIS $\rightarrow$ SQC (n=80)
  2. Normal $\rightarrow$ AAH $\rightarrow$ AIS $\rightarrow$ MIA $\rightarrow$ ADC (n=28)

Methods

Methods

Workflows

Data pre-processing for variant discovery

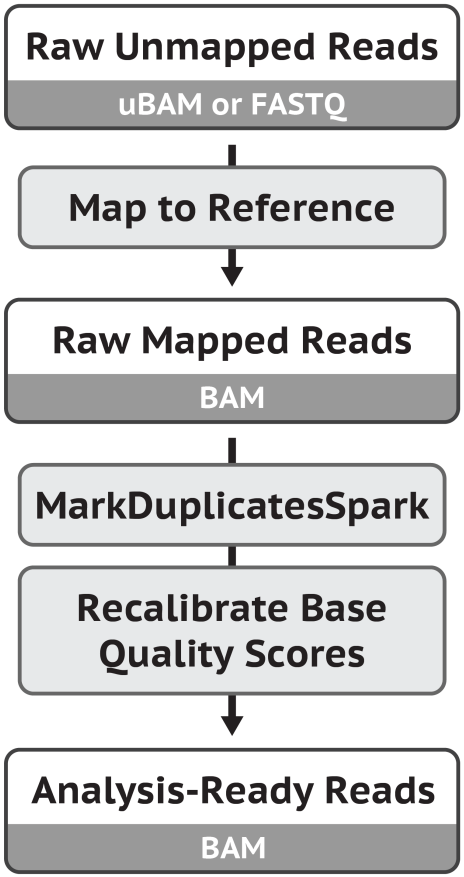


Figure: Data pre-processing for variant discovery (Van der Auwera et al., 2013; DePristo et al., 2011)

Somatic short variant discovery

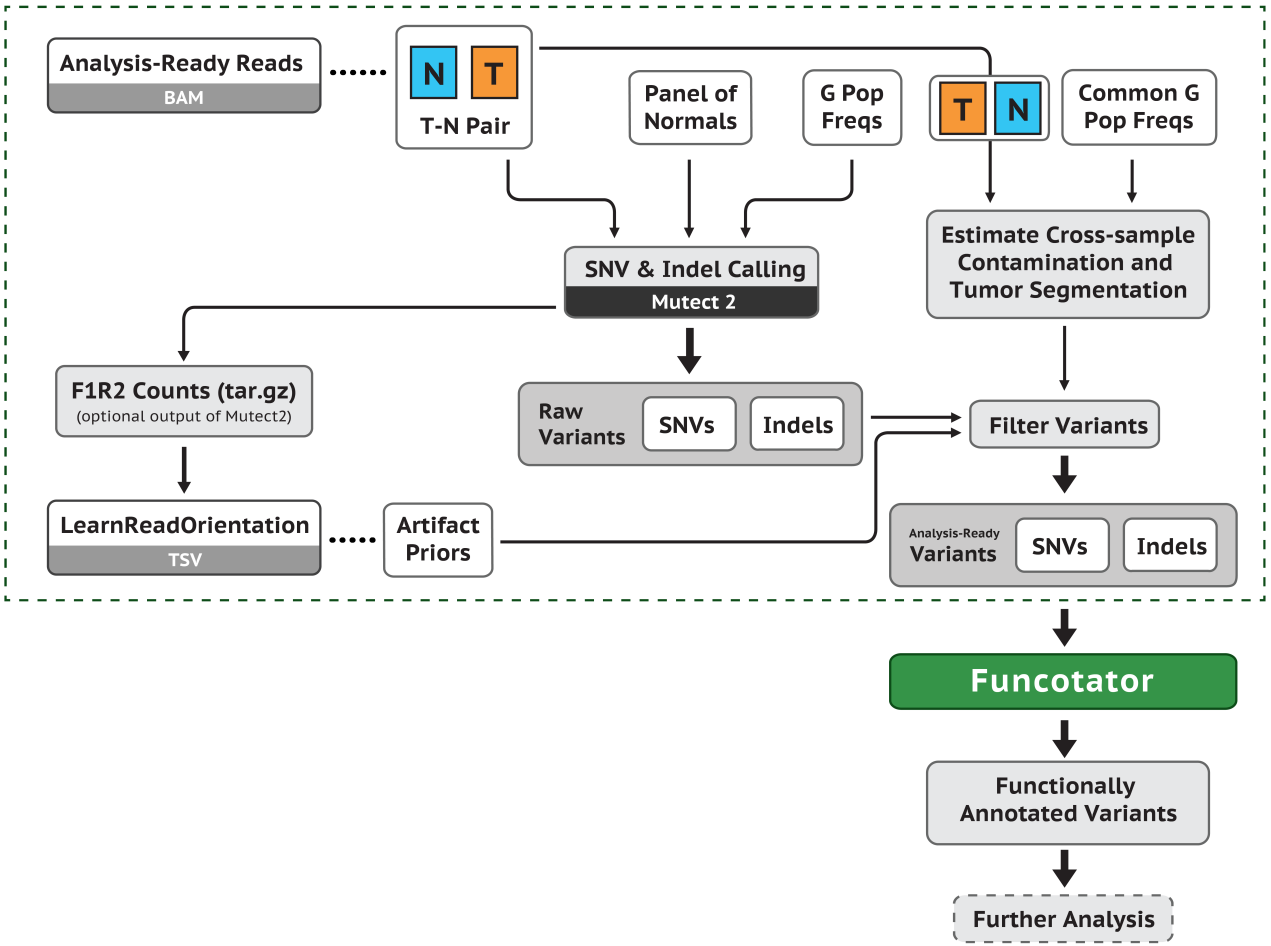


Figure: Somatic short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

Germline short variant discovery

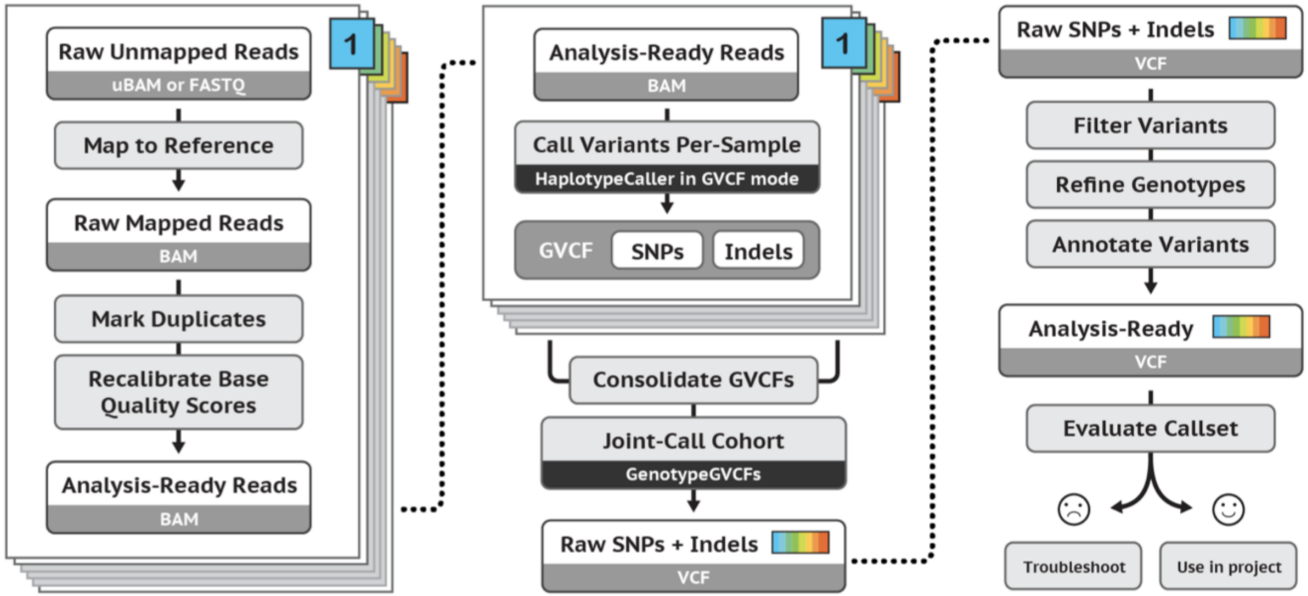


Figure: Germline short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

RNA-seq short variant discovery

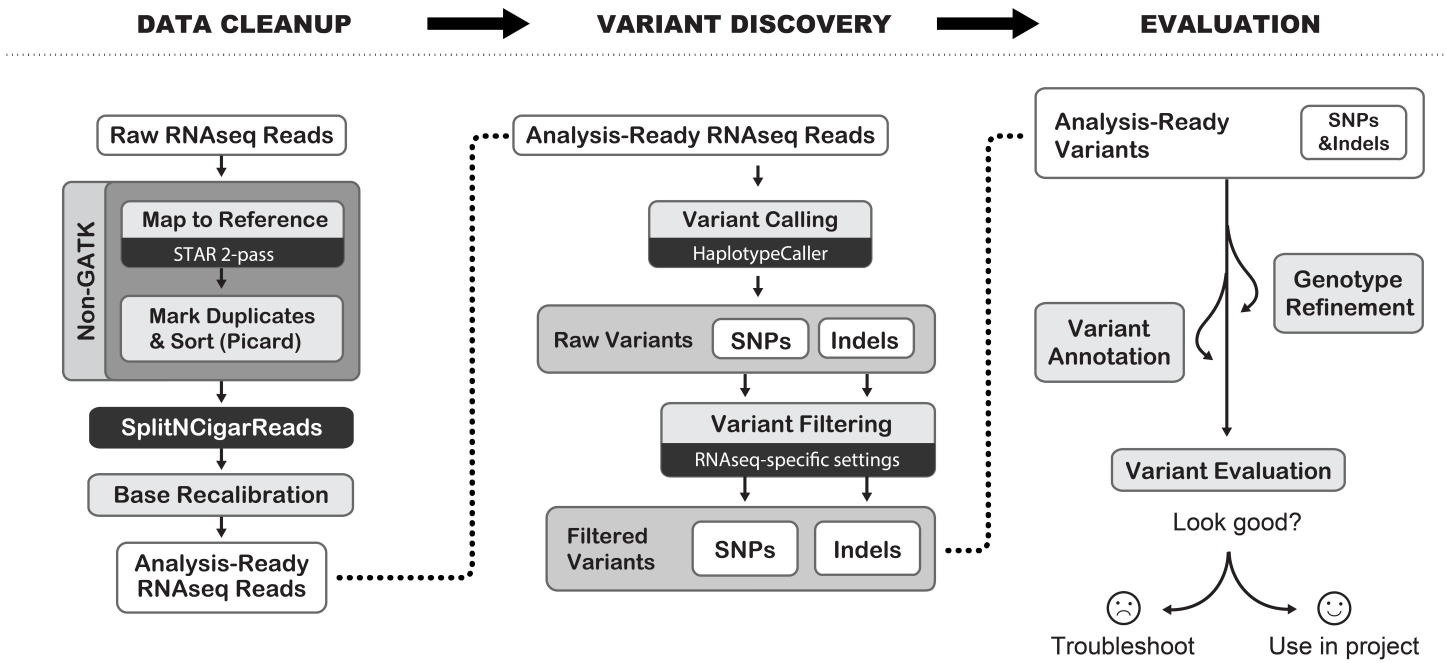


Figure: RNA-seq short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

Methods

Miscellaneous

Used Bioinformatics Tools

- 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)

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?

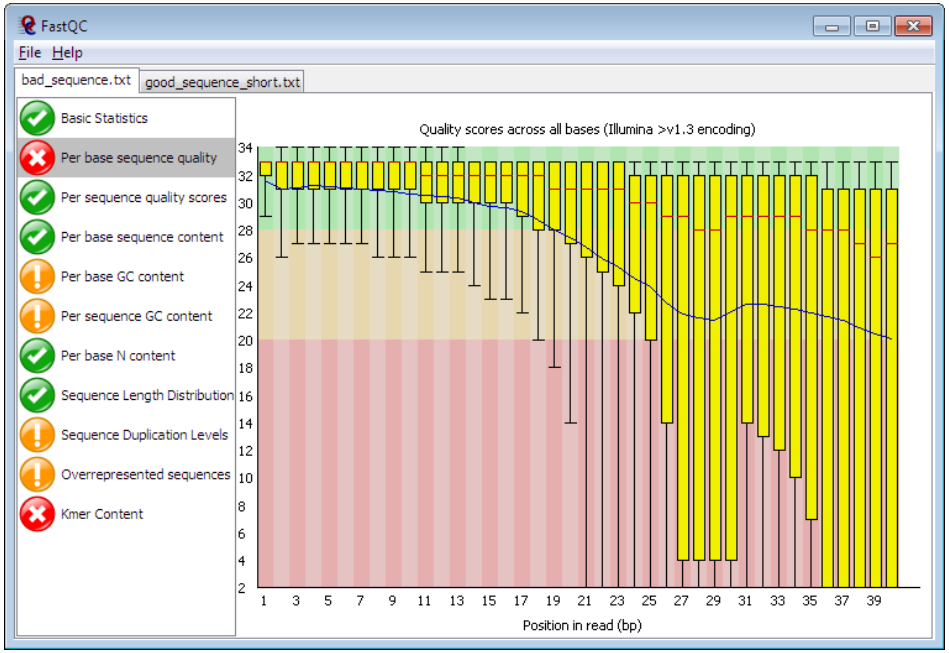


Figure: Example of FastQC Result (Andrews et al., 2012)

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

FastQC on WES

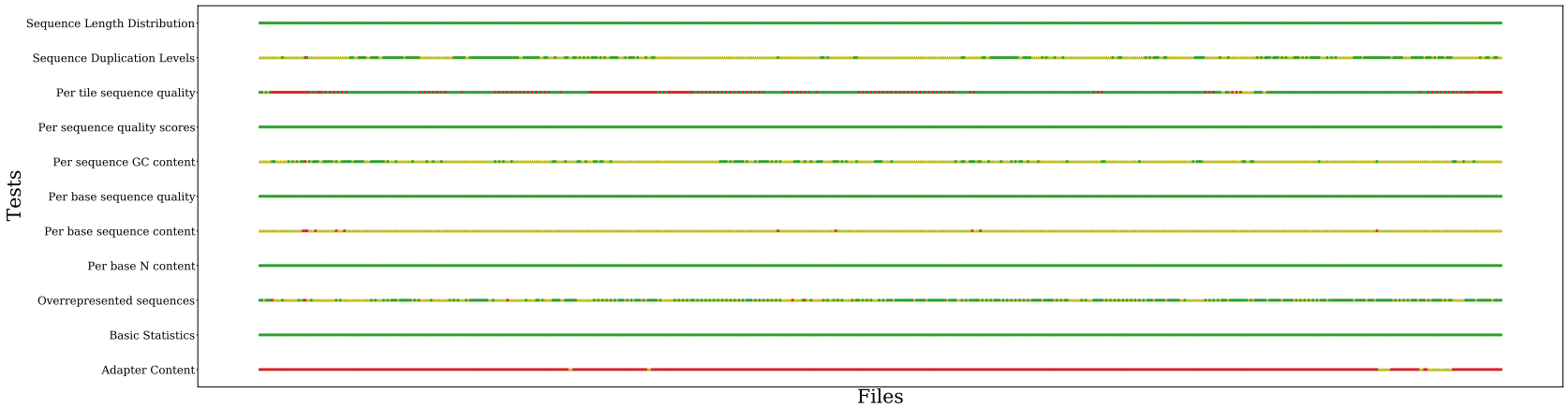
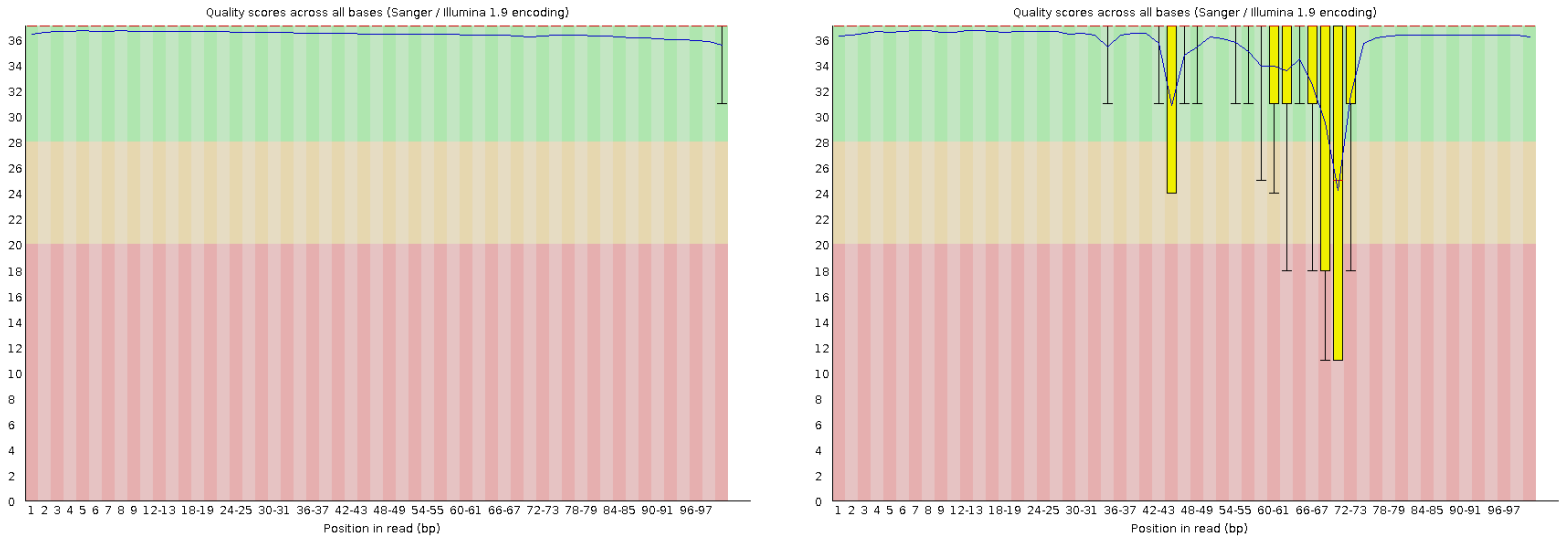


Figure: FastQC with WES data

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

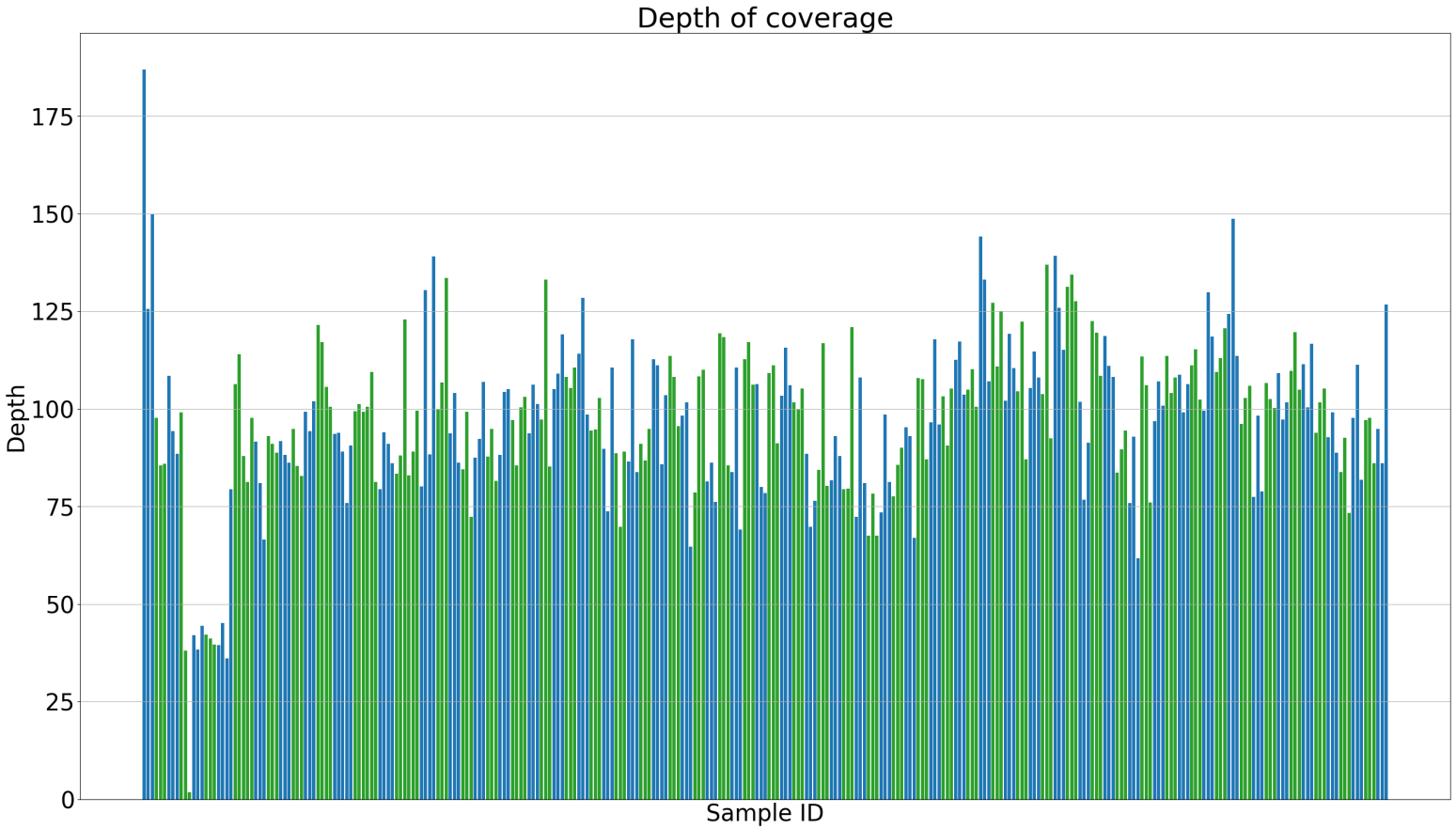


Figure: Coverage Depth Plot

FastQC on WTS

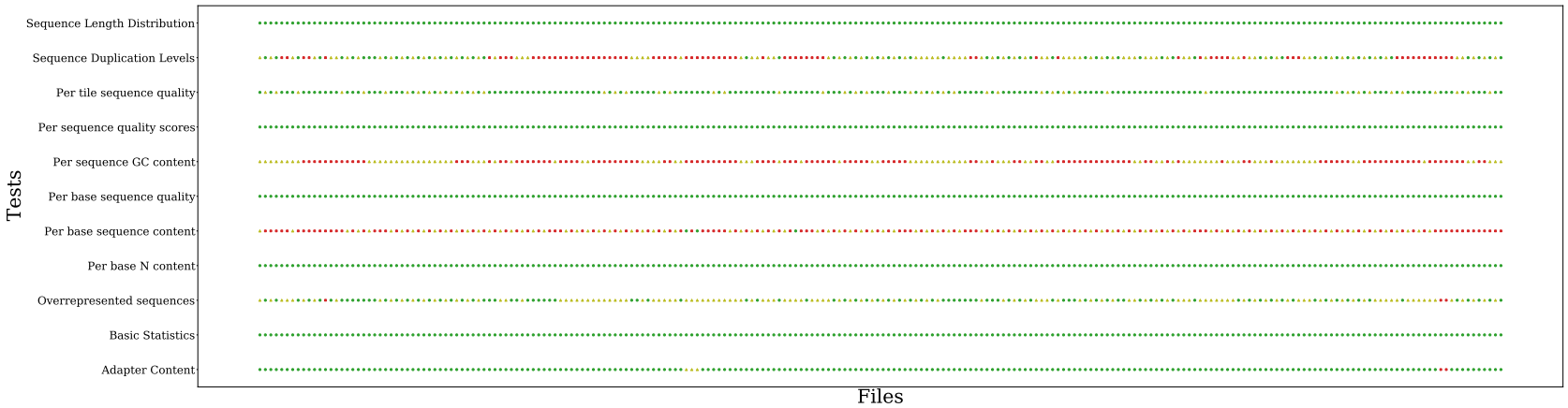


Figure: FastQC with WTS data

All sample are good to analysis

∵ No sample has more than 5 failures.

Results

Copy Number Variations (CNVs) with Sequenza

Sequenza?

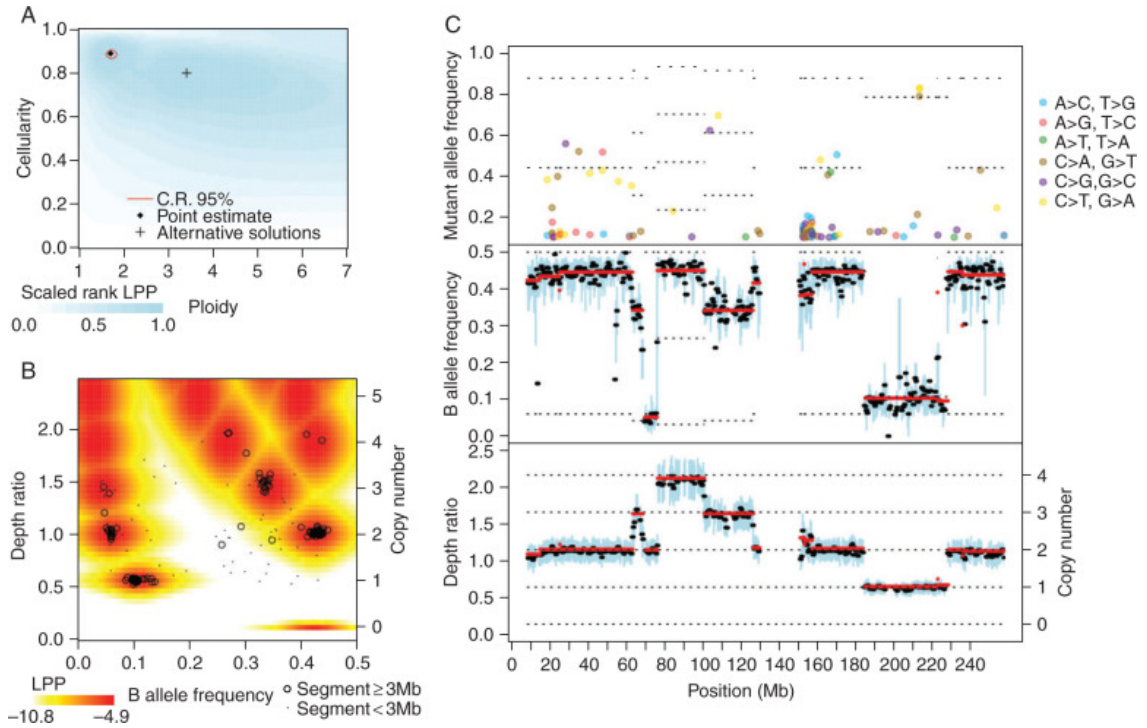
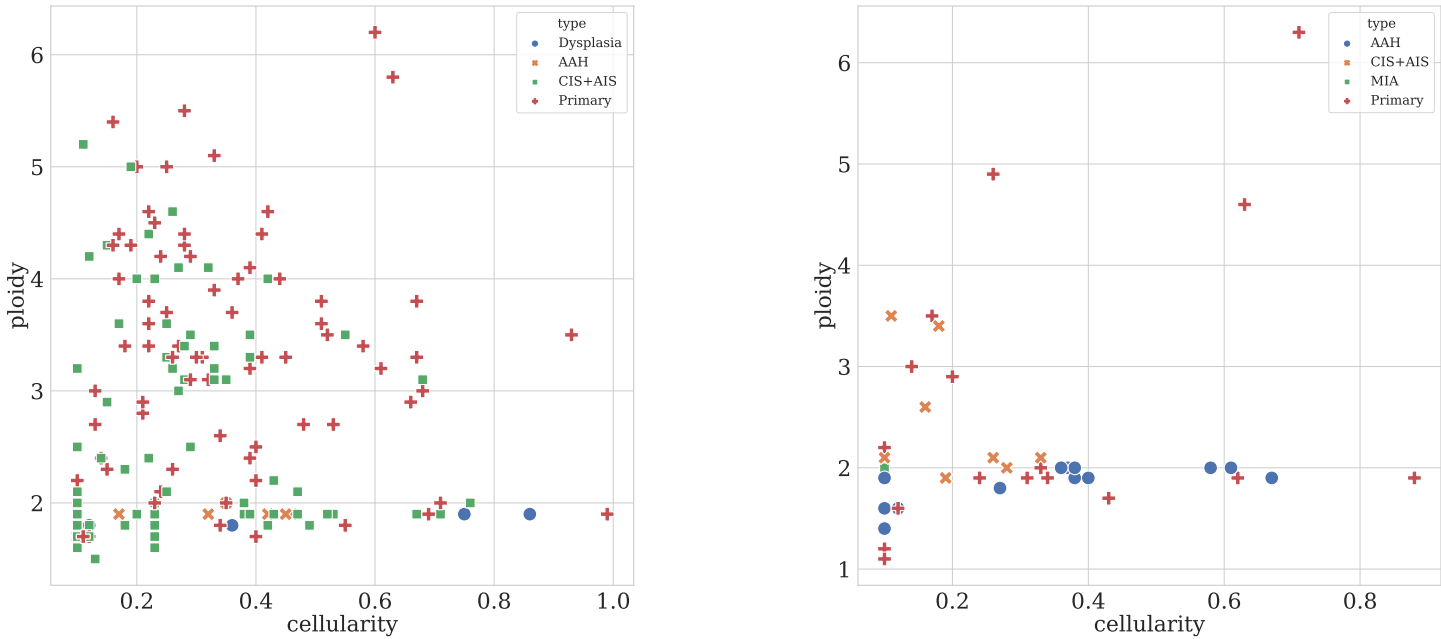


Figure: Representative Output of the Sequenza (Favero et al., 2015)

Cellularity & Ploidy on WES



(a) SQC Samples

(b) ADC Samples

Figure: Cellularity and Ploidy from Sequenza

Genome View on Patient #57

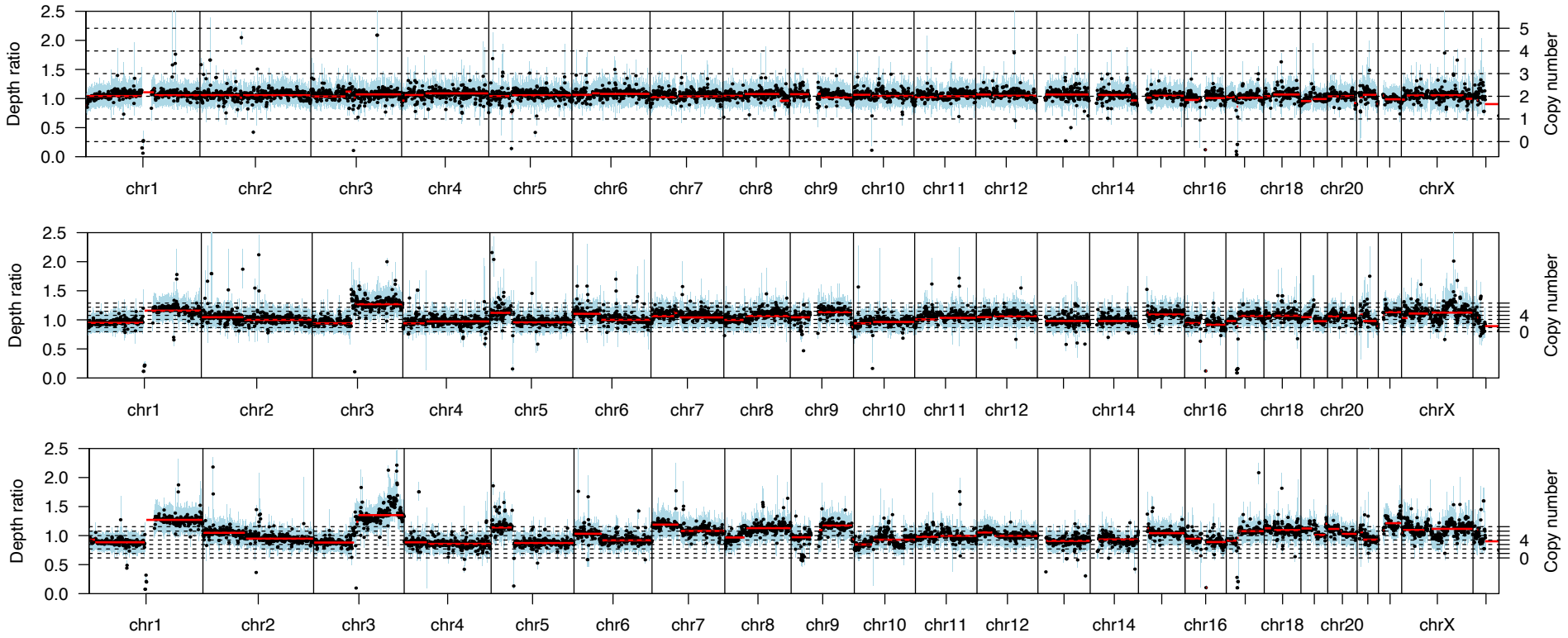


Figure: Dysplasia-CIS-Primary Tumor on Patient #57

CNVs of SQC

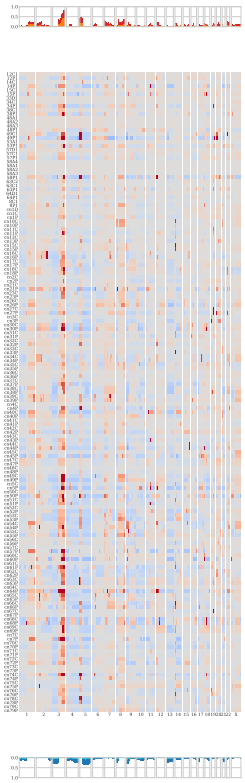


Figure: CNV Plot with SQC Patients

CNVs of ADC

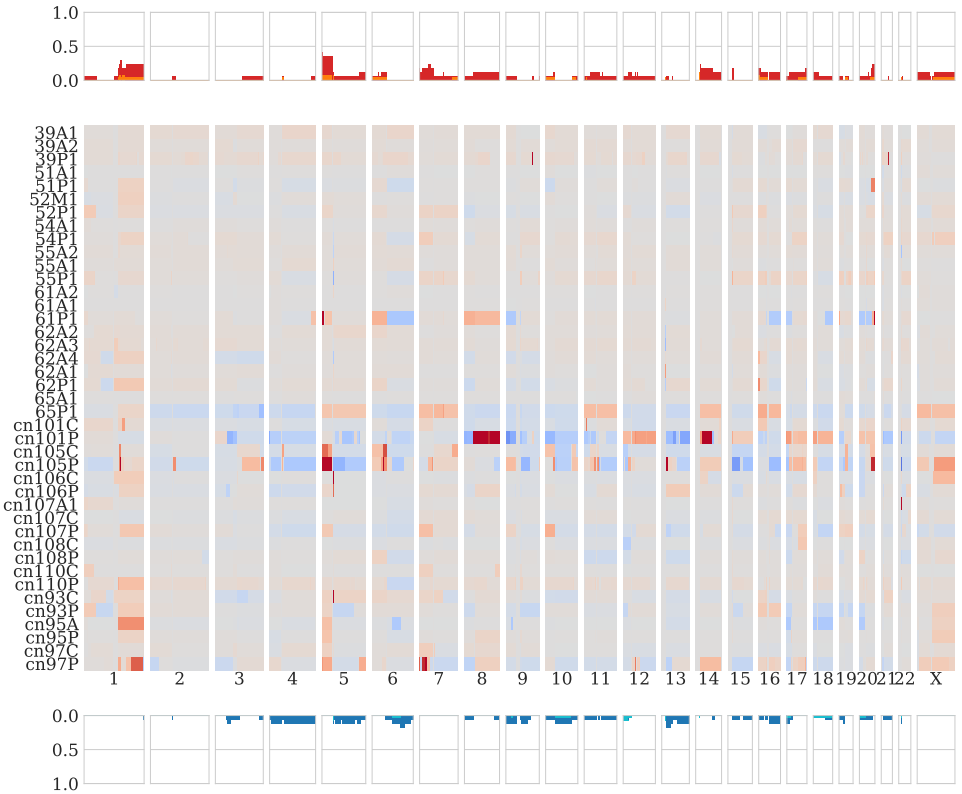


Figure: CNV Plot with ADC Patients

SQC vs. ADC

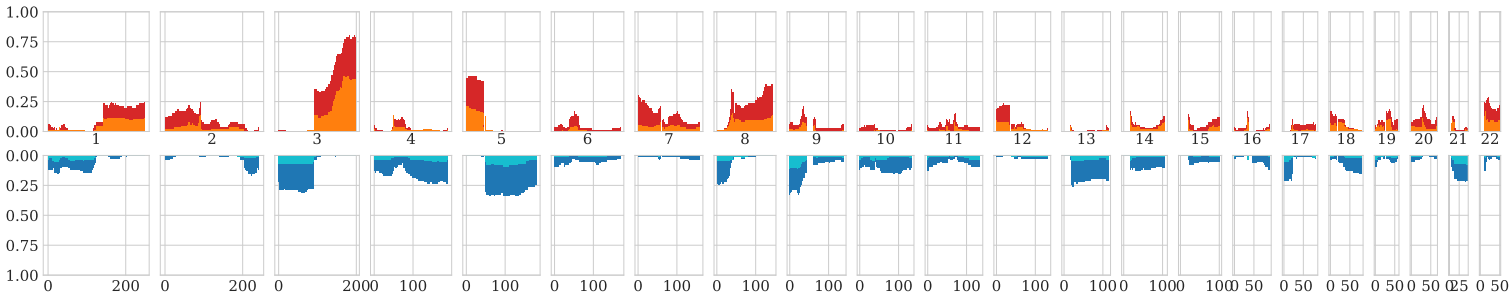


Figure: Simple CNV Plot with SQC Patients

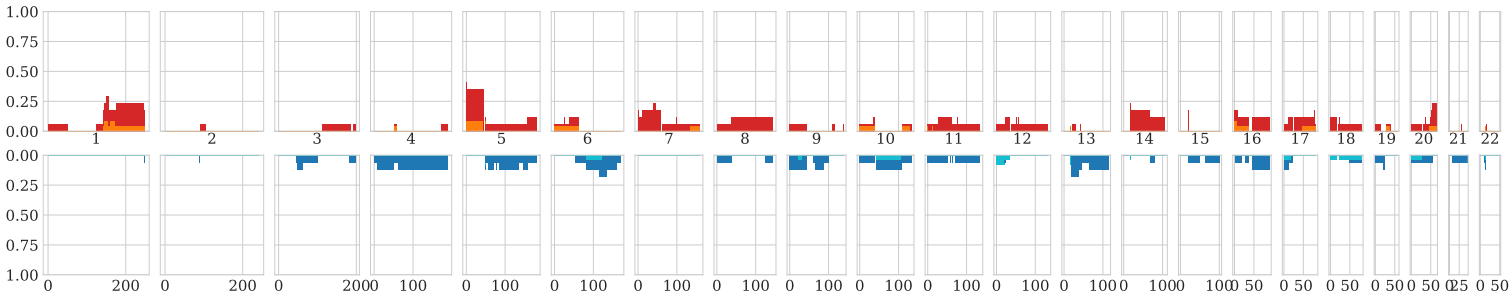


Figure: Simple CNV Plot with ADC Patients

Findings in Sequenza

Results

SNVs Analysis

Mutect2?

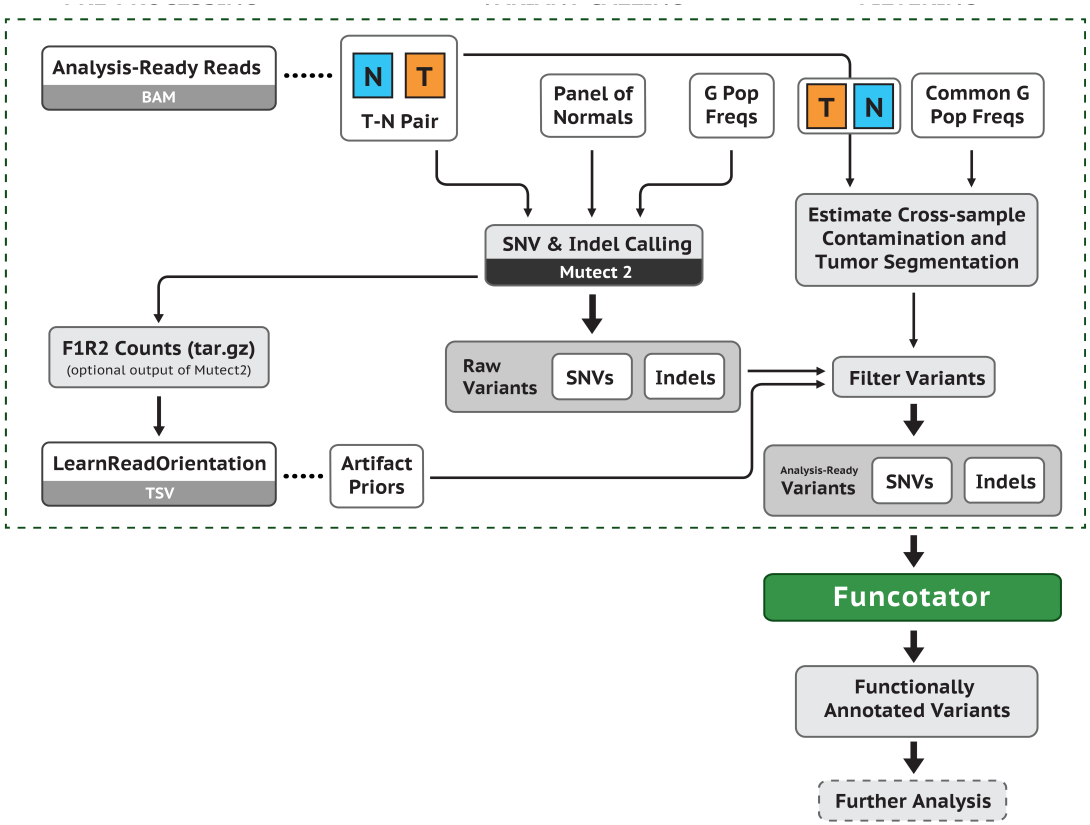


Figure: Somatic short variant discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

Witer?

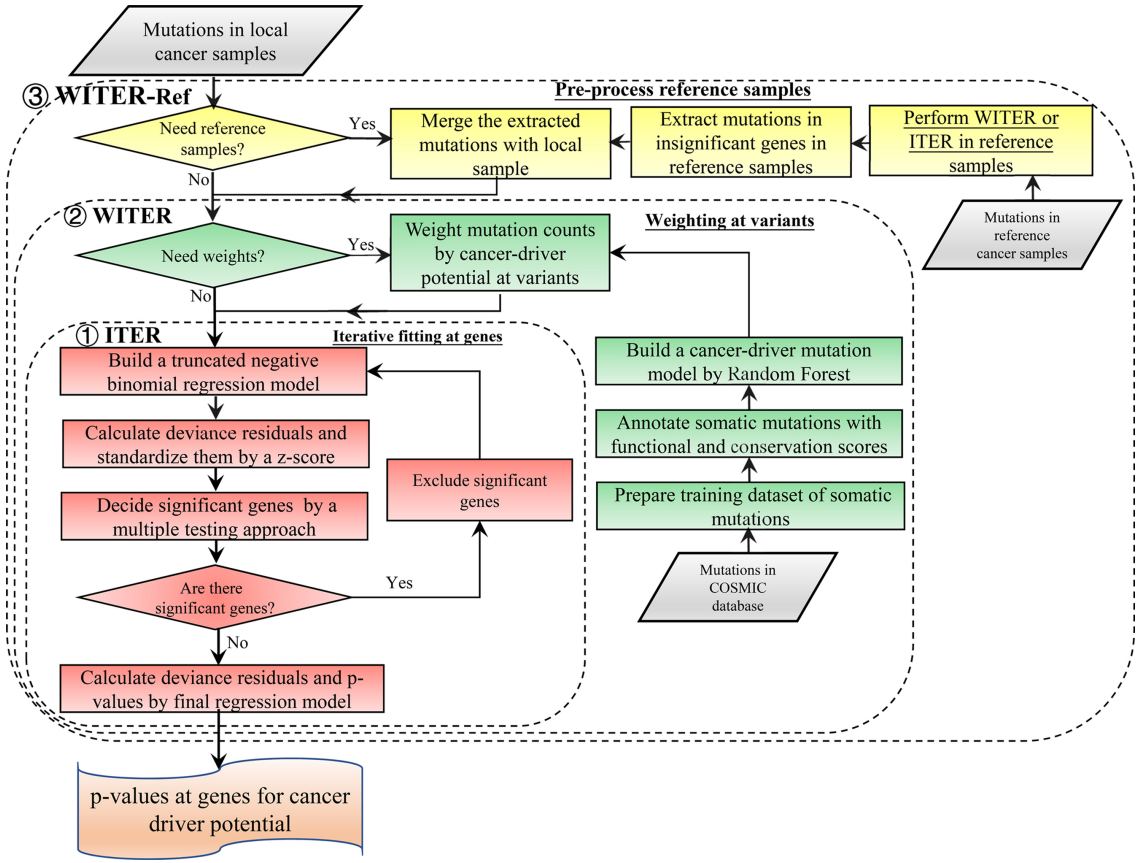


Figure: Witer diagram for detecting cancer-drive genes (Jiang et al., 2019)

Somatic Variant with BWA in SQC

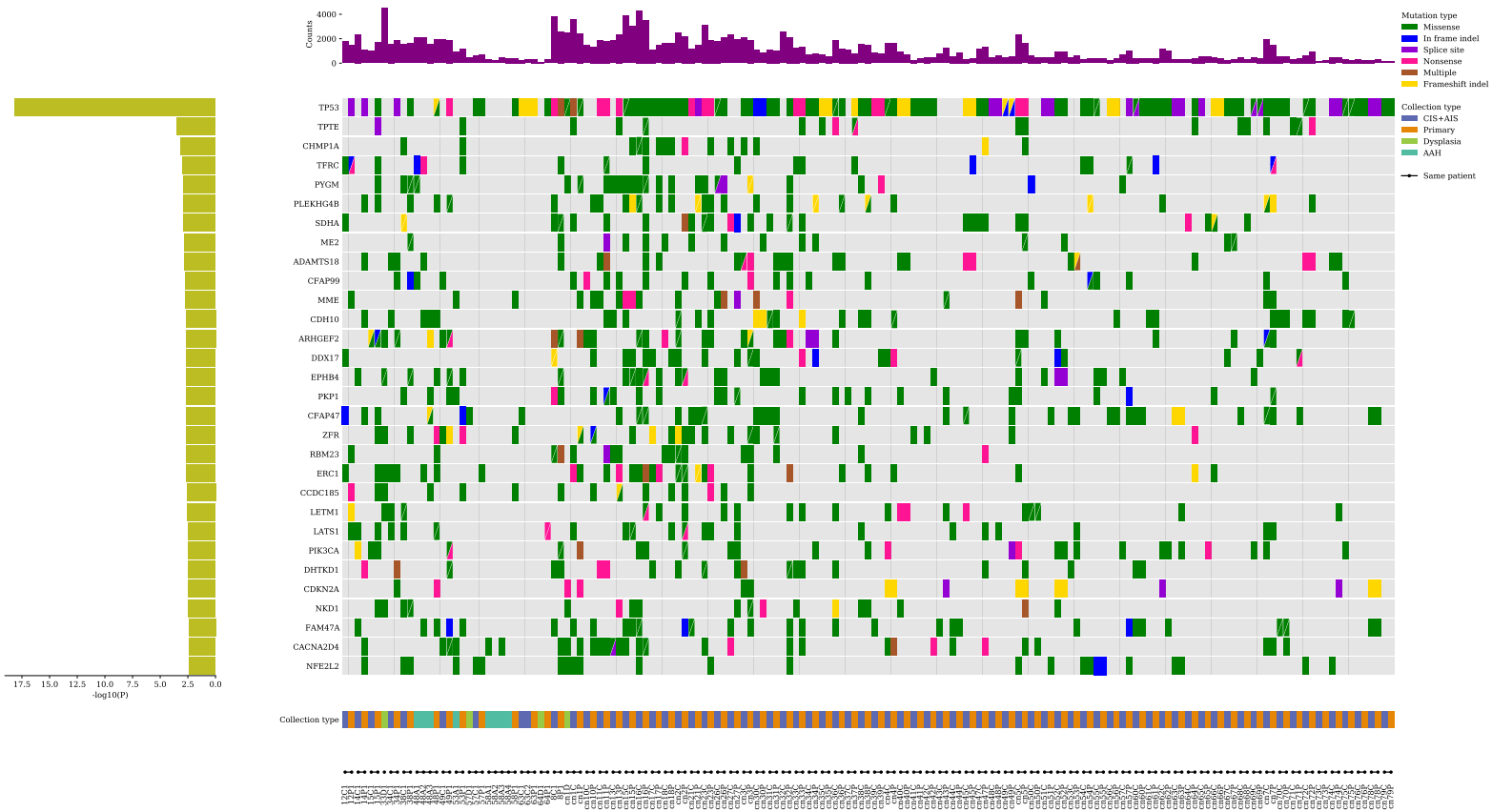


Figure: CoMut Plot with SQC Patients

Somatic Variant with BWA in ADC

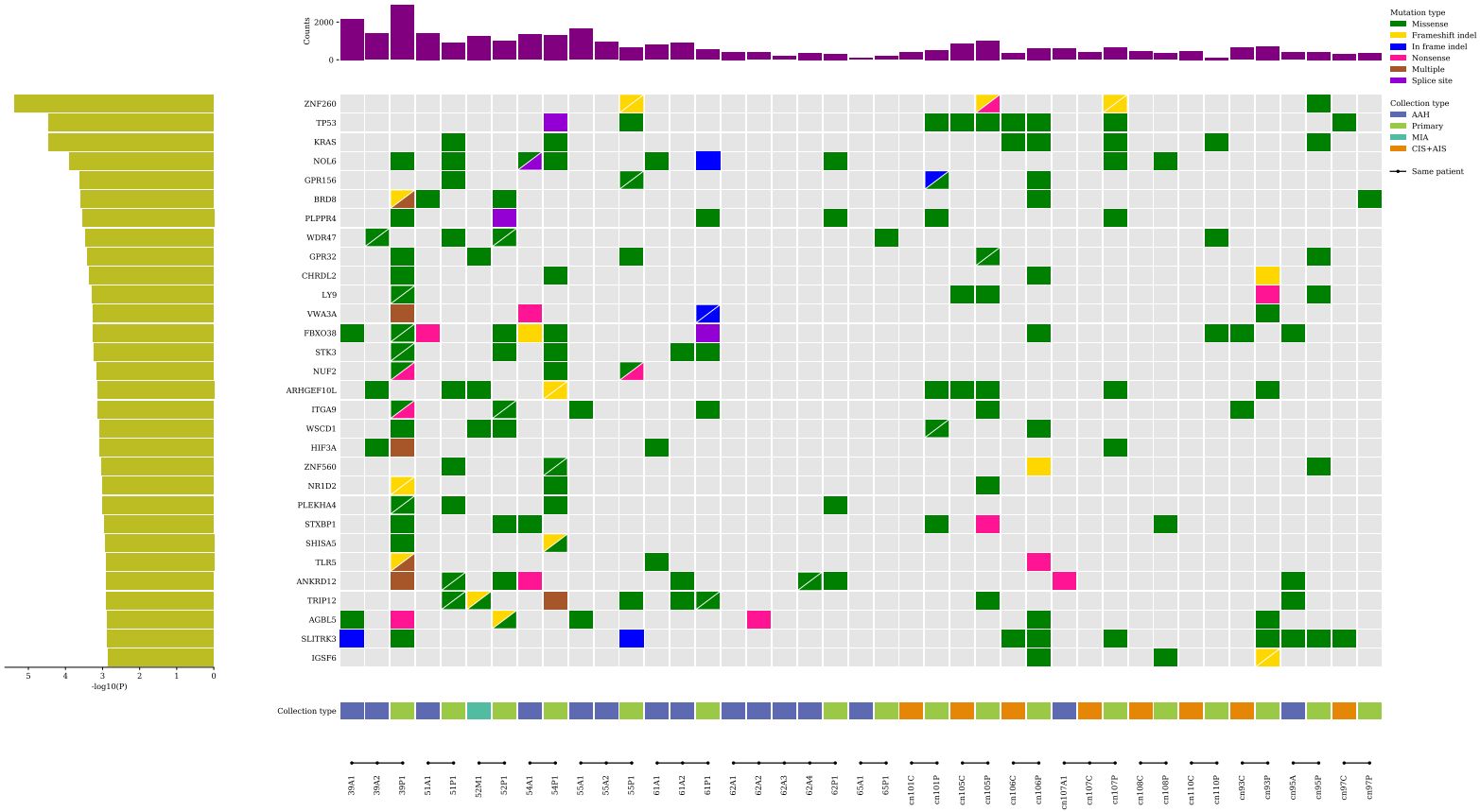


Figure: CoMut Plot with ADC Patients

Findings in SNVs Analysis

Results

VAF Analysis

VAF?

Findings in VAF Analysis

Results

Differences in Gene Expression Levels

RSEM?

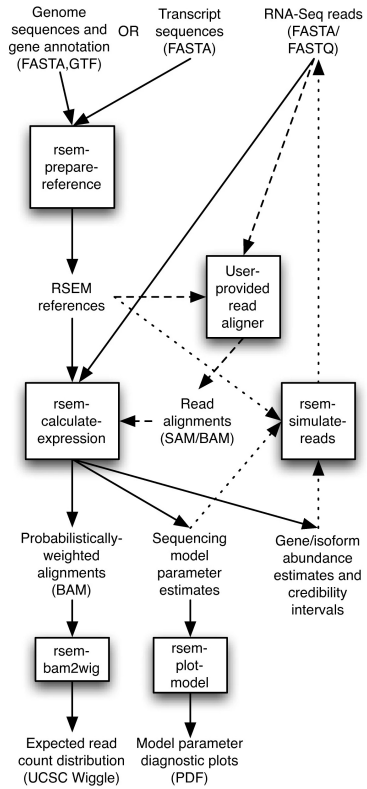


Figure: RSEM workflow (B. Li & Dewey, 2011)

DESeq2

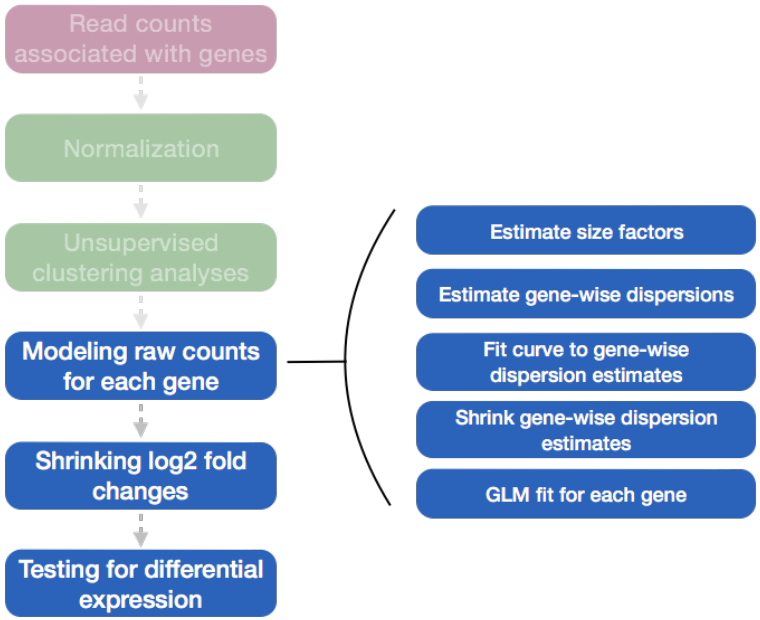


Figure: DESeq2 workflow (Love et al., 2014)

DEG Selection Strategy

DEG: differentially expressed genes

Fold Change

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

P-value

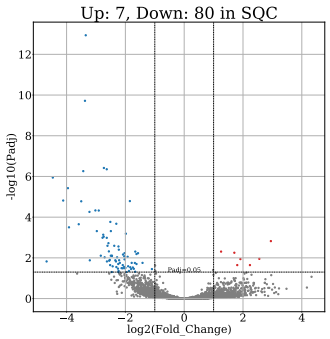
P-value < 0.05

Adjusted P-value

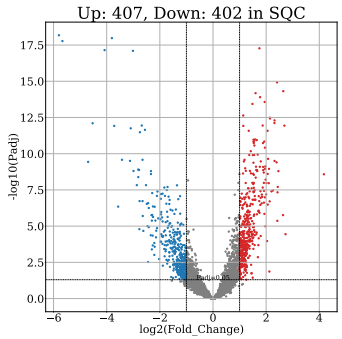
Padj < 0.05

DEG Volcano Plots in SQC

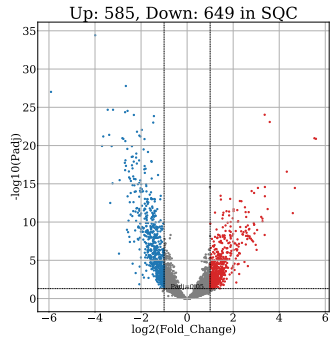
Normal → Dysplasia → CIS → Primary (SQC)



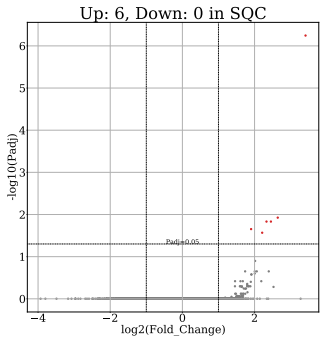
(a) Normal-Dysplasia



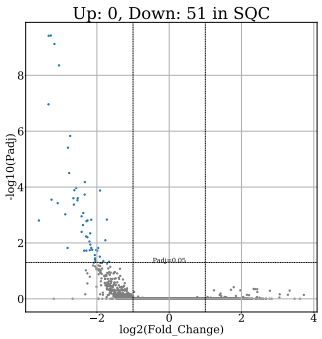
(b) Normal-CIS



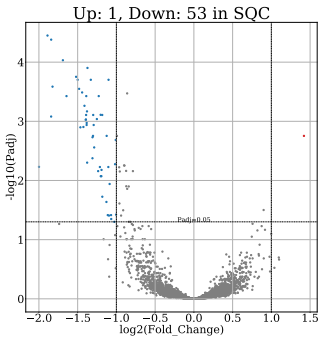
(c) Normal-Primary



(d) Dysplasia-CIS



(e) Dysplasia-Primary

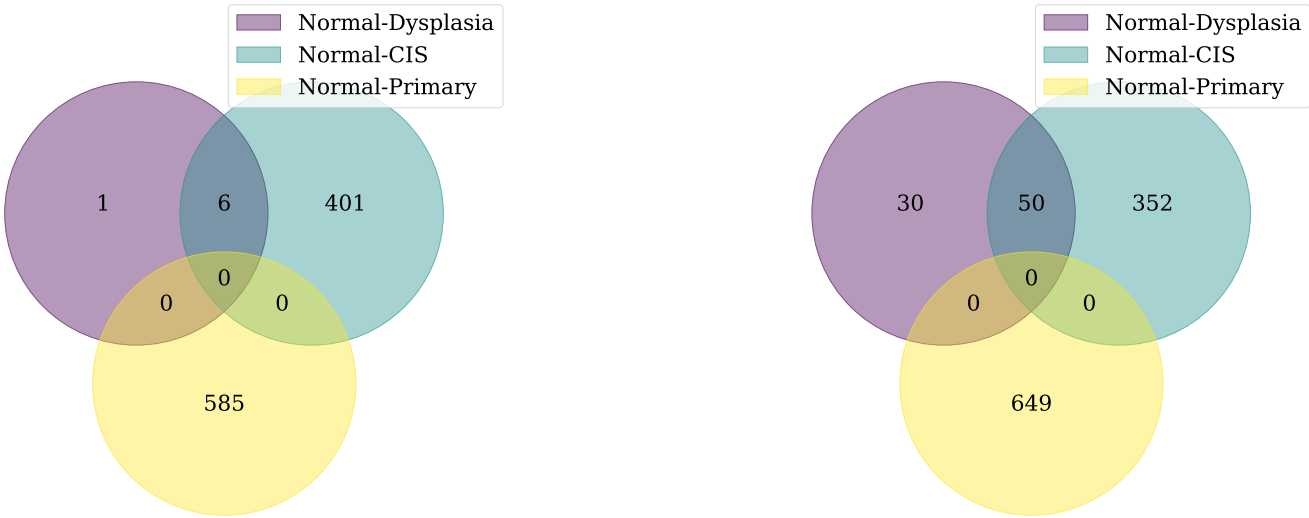


(f) CIS-Primary

Figure: DEG Volcano Plots in SQC

DEG Venn Diagram with Bowtie2 in SQC

Normal → Dysplasia → CIS → Primary (SQC)

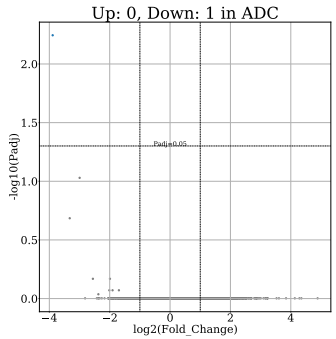


(a) Up-regulated (b) Down-regulated

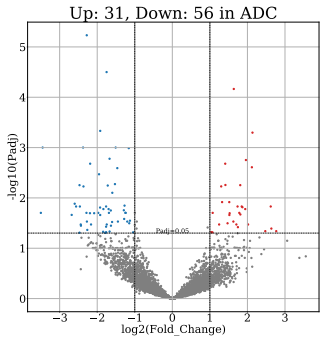
Figure: DEG Venn Diagram in SQC

DEG Volcano Plots with Bowtie2 in ADC

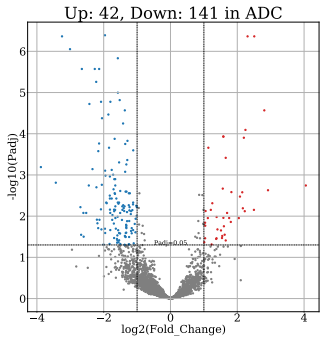
Normal → AAH → AIS → MIA → Primary (ADC)



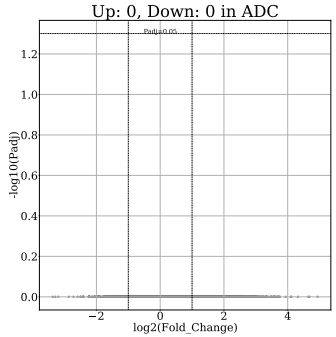
(a) Normal-AAH



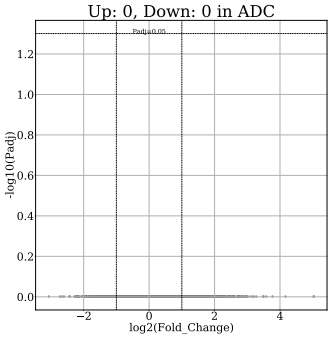
(b) Normal-AIS



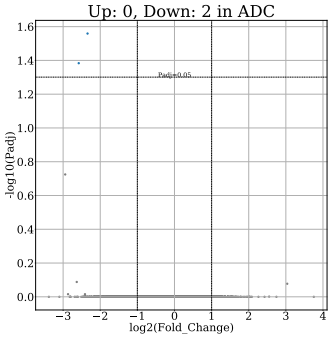
(c) Normal-Primary



(d) AAH-AIS



(e) AAH-Primary

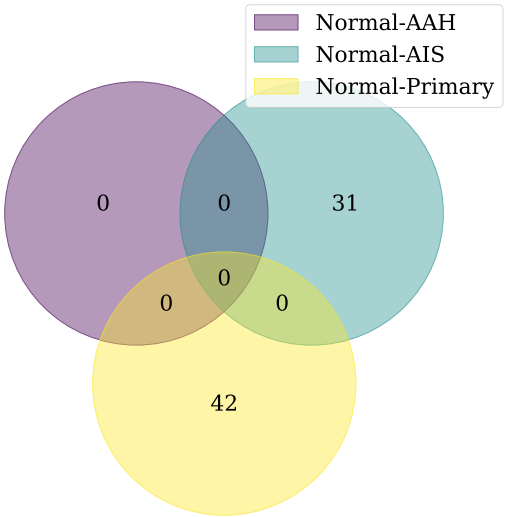


(f) AIS-Primary

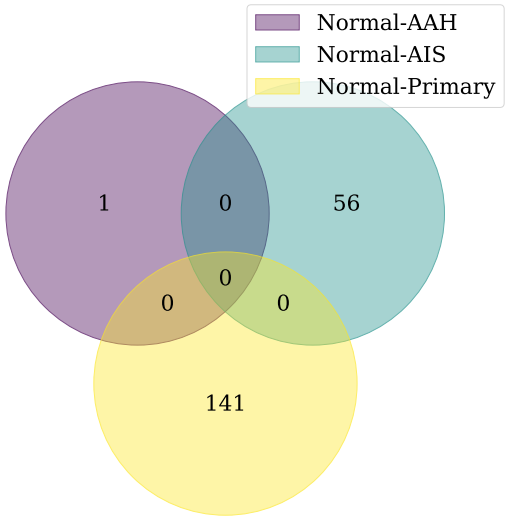
Figure: DEG Volcano Plots in ADC

DEG Venn Diagram with Bowtie2 in ADC

Normal $\rightarrow$ AAH $\rightarrow$ AIS $\rightarrow$ MIA $\rightarrow$ Primary (ADC)



(a) Up-regulated



(b) Down-regulated

Figure: DEG Venn Diagram in ADC

Findings in DEG Analysis

Results

Bulk Cell Deconvolution

CIBERSORTx

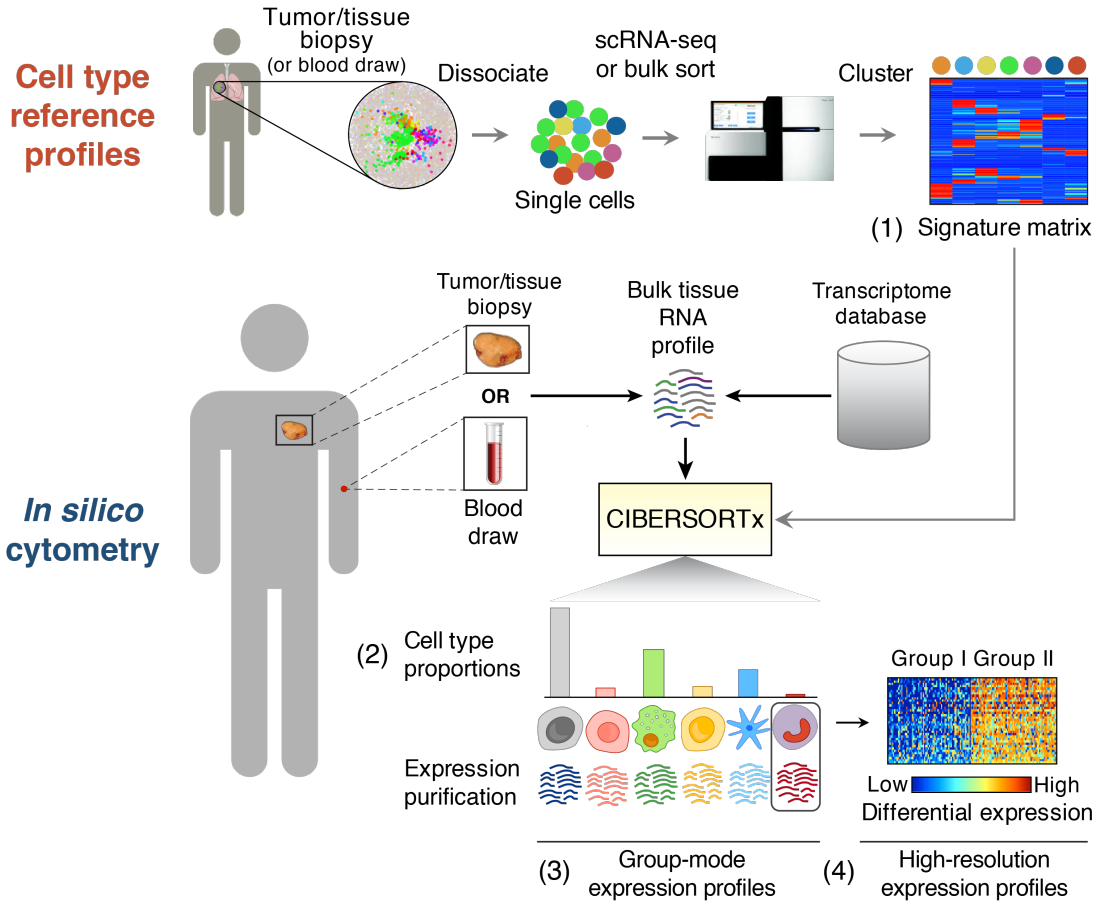


Figure: Workflow for CIBERSORTx (Steen et al., 2020; Newman et al., 2019)

Benchmarking of Cell Deconvolution Tools

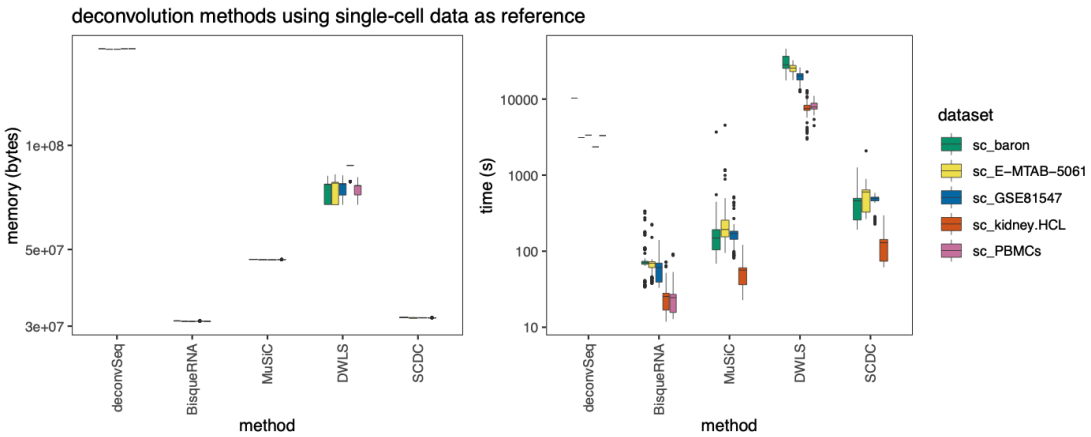


Figure: Memory and time requirements for the cell deconvolution methods (Cobos et al., 2020)

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?

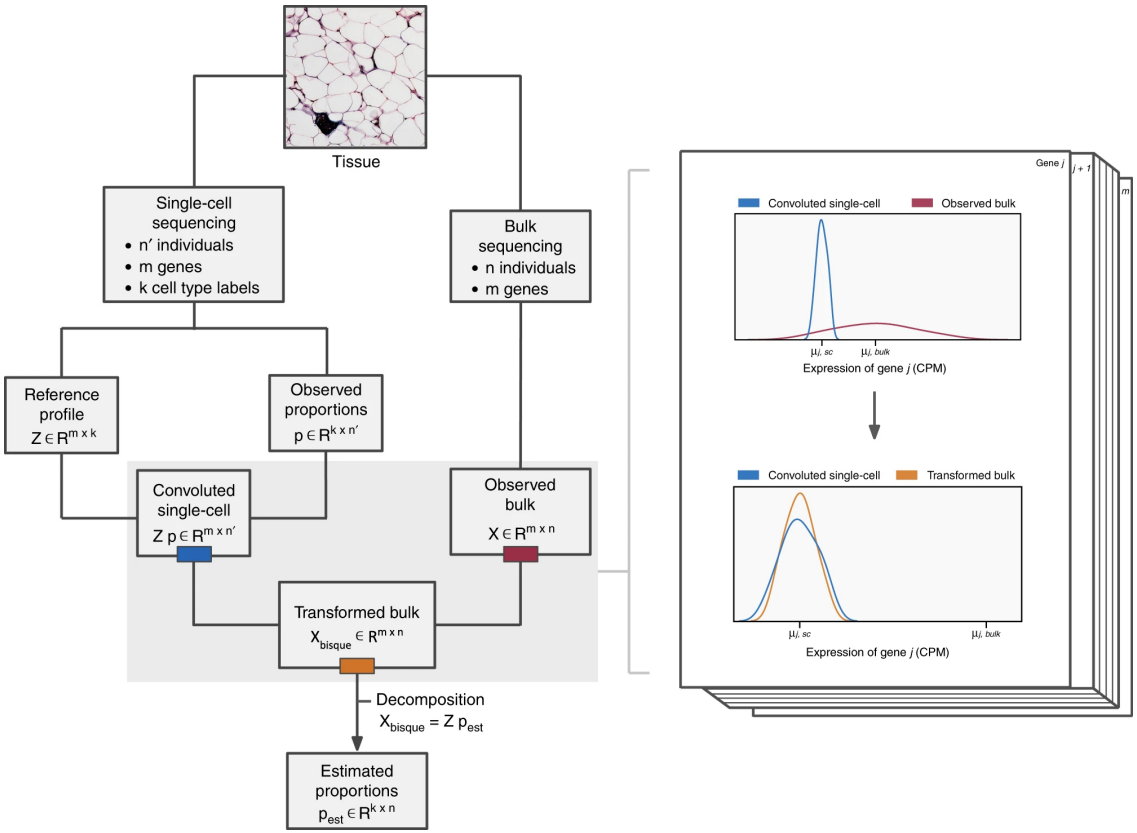


Figure: Graphical overview of the Bisque decomposition methods (Jew et al., 2020)

MuSiC?

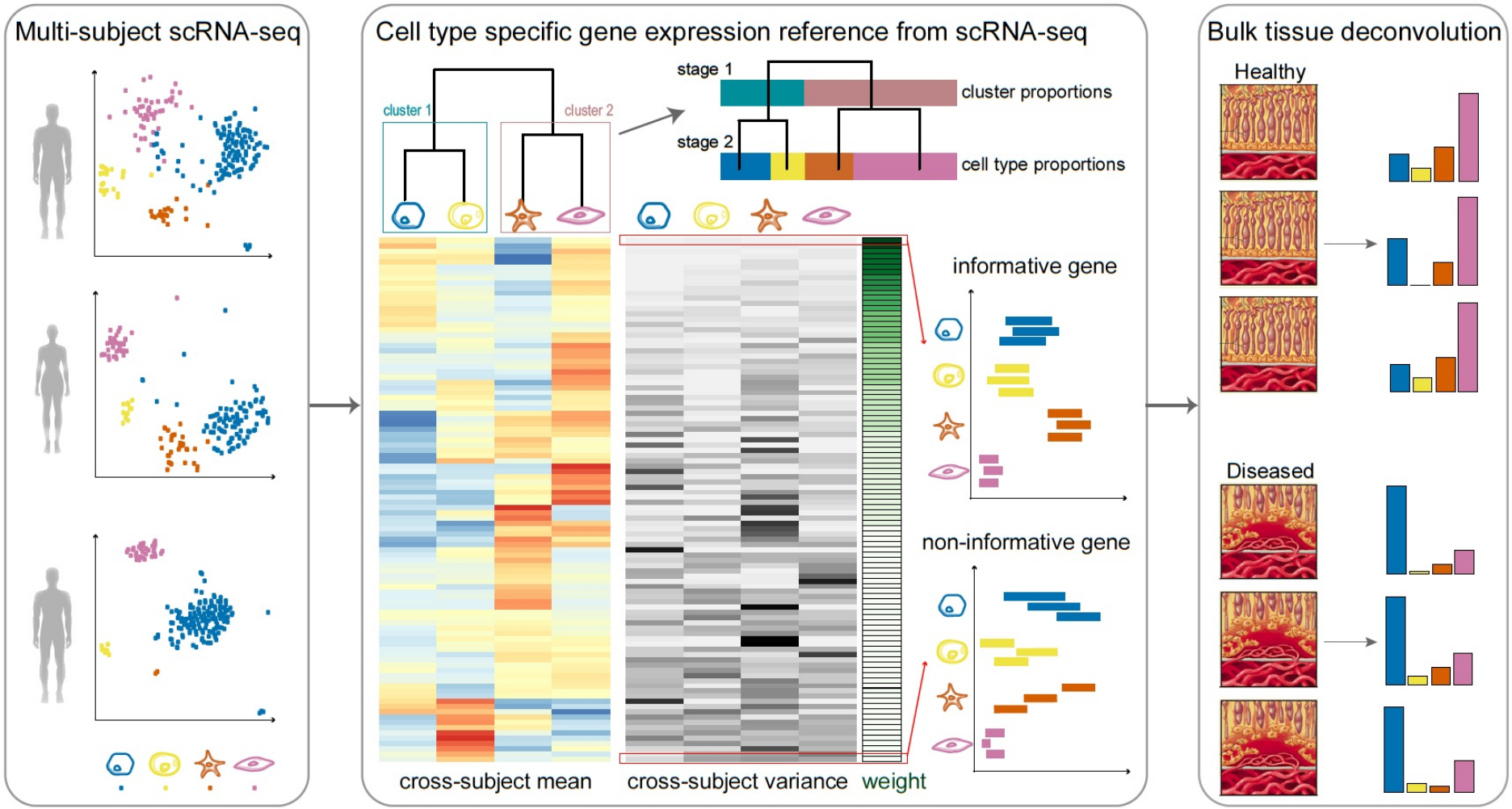


Figure: Overview of MuSiC framework (X. Wang et al., 2019)

SCDC?

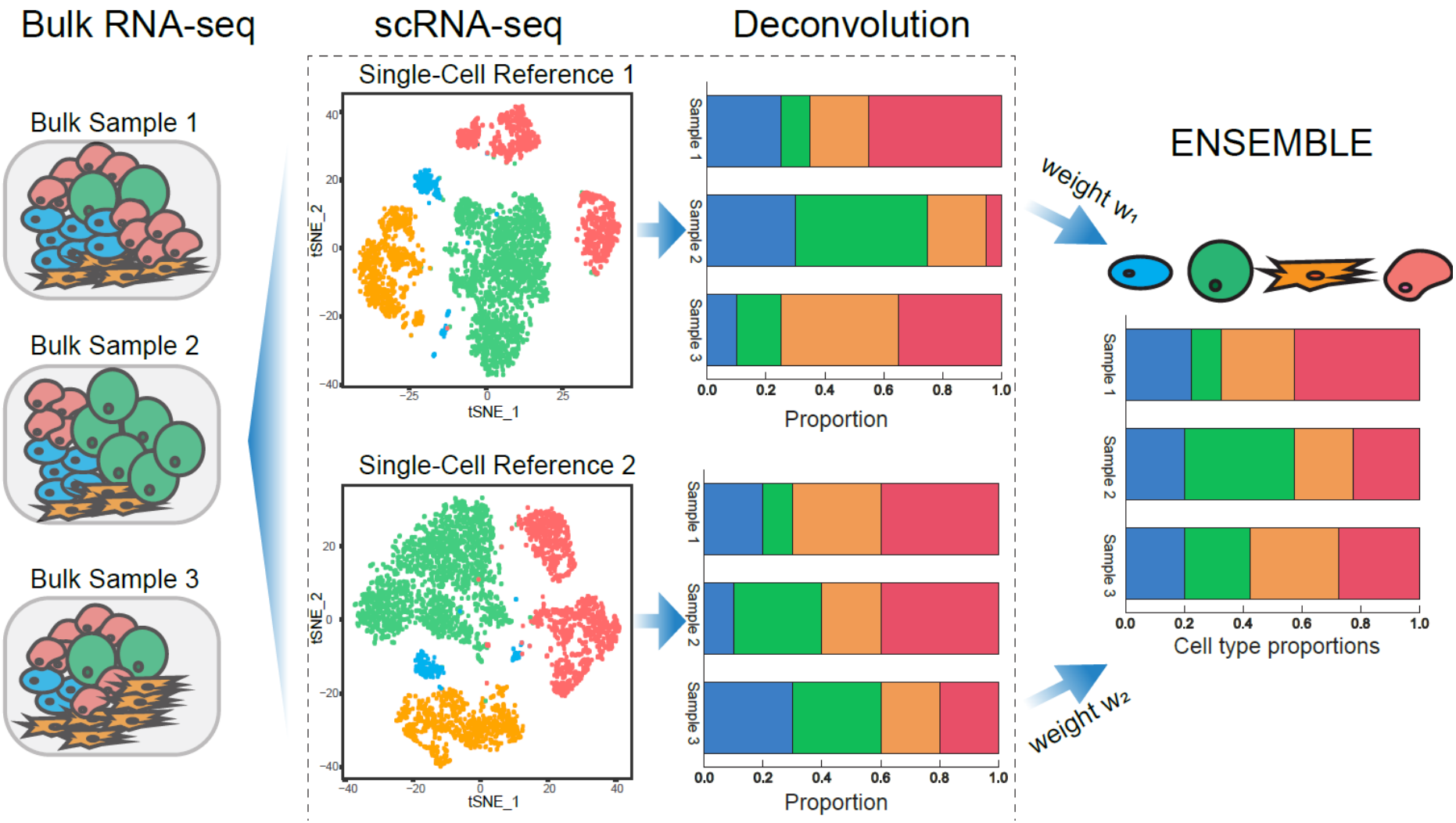


Figure: Overview of deconvolution by SCDC (Dong et al., 2021)

Findings in Bulk Cell Deconvolution

Results

Tumor Evolution Trajectories Analysis

Revolver?

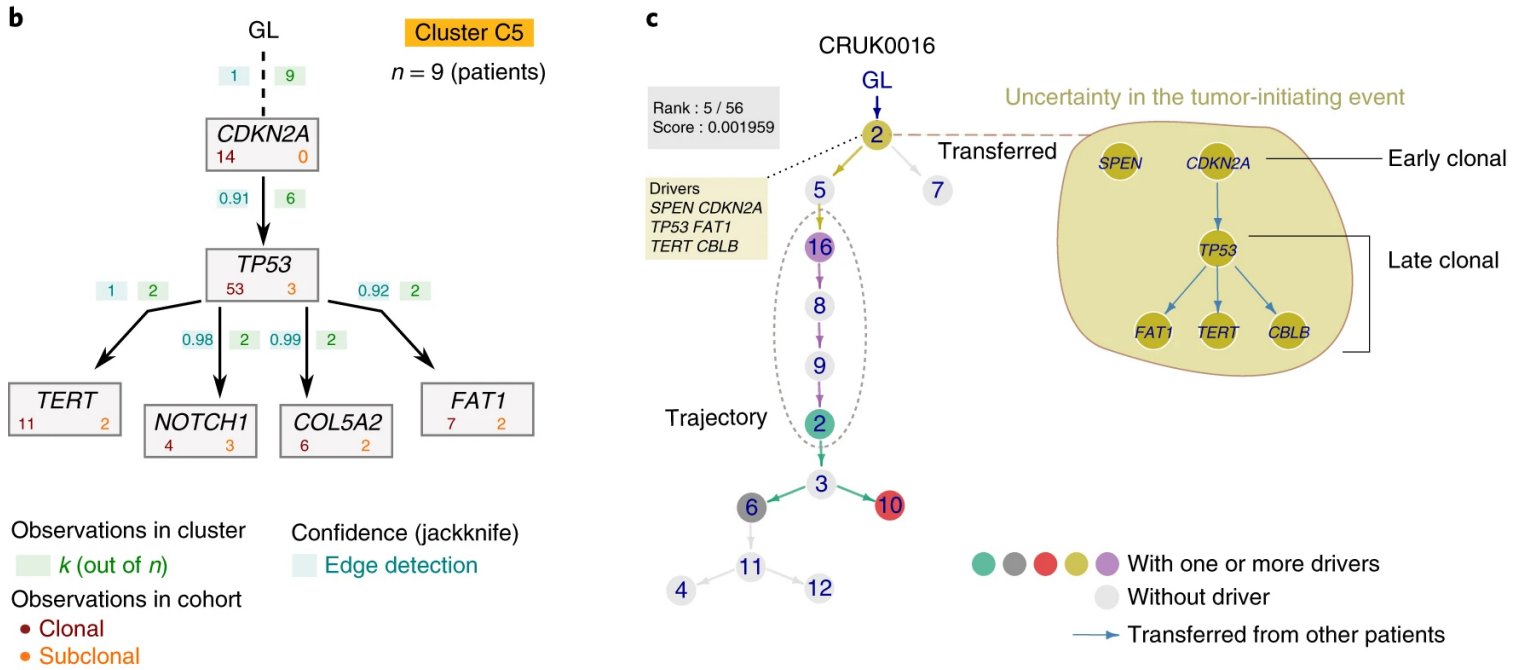


Figure: Repeated Evolutionary Trajectories (Caravagna et al., 2018)

Findings in Tumor Evolution Trajectories Analysis

Discussion

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