Lung Precancer Analysis

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2021-05-18

Overview

Introduction

Lung Cancer

- Squamous cell carcinoma
- Adenocarcinoma

Precancer

Introduction

Study Objectives

Study Objectives

- Find different mutations
  - between WES
  - between WTS
  - from cancer
  - from precancer
- Pathway examine from the mutations
  - of WES
  - of RNA-seq
- Ultra-deep sequencing to find an *infinitesimal* quantity of Non-Circulating Tumor DNA
  - from blood
  - from urine
  - frrom bronchus
- Diagnostic performace

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 - Dysplasia - CIS - SQC (n=80)
  2. Normal - AAH - AIS - MIA - ADC (n=28)

Methods

Methods

Workflows

Data pre-processing for variant discovery

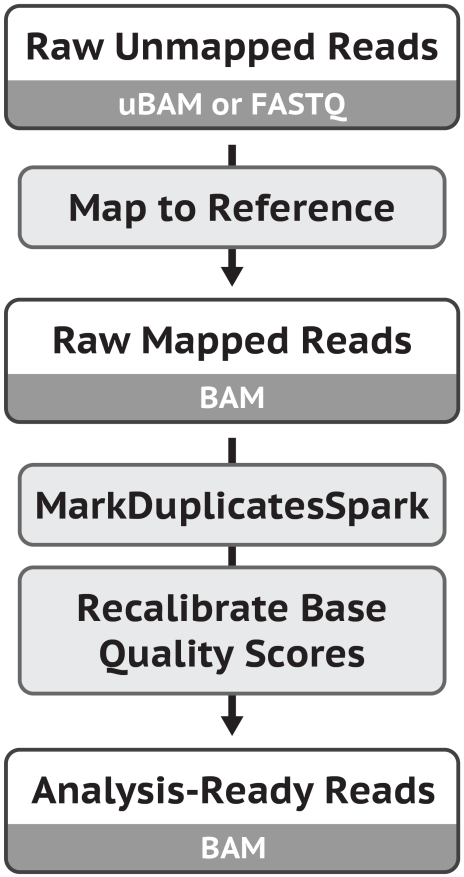


Figure: Data pre-processing for variant discovery (Van der Auwera et al., 2013; DePristo et al., 2011)

Somatic short variant discovery

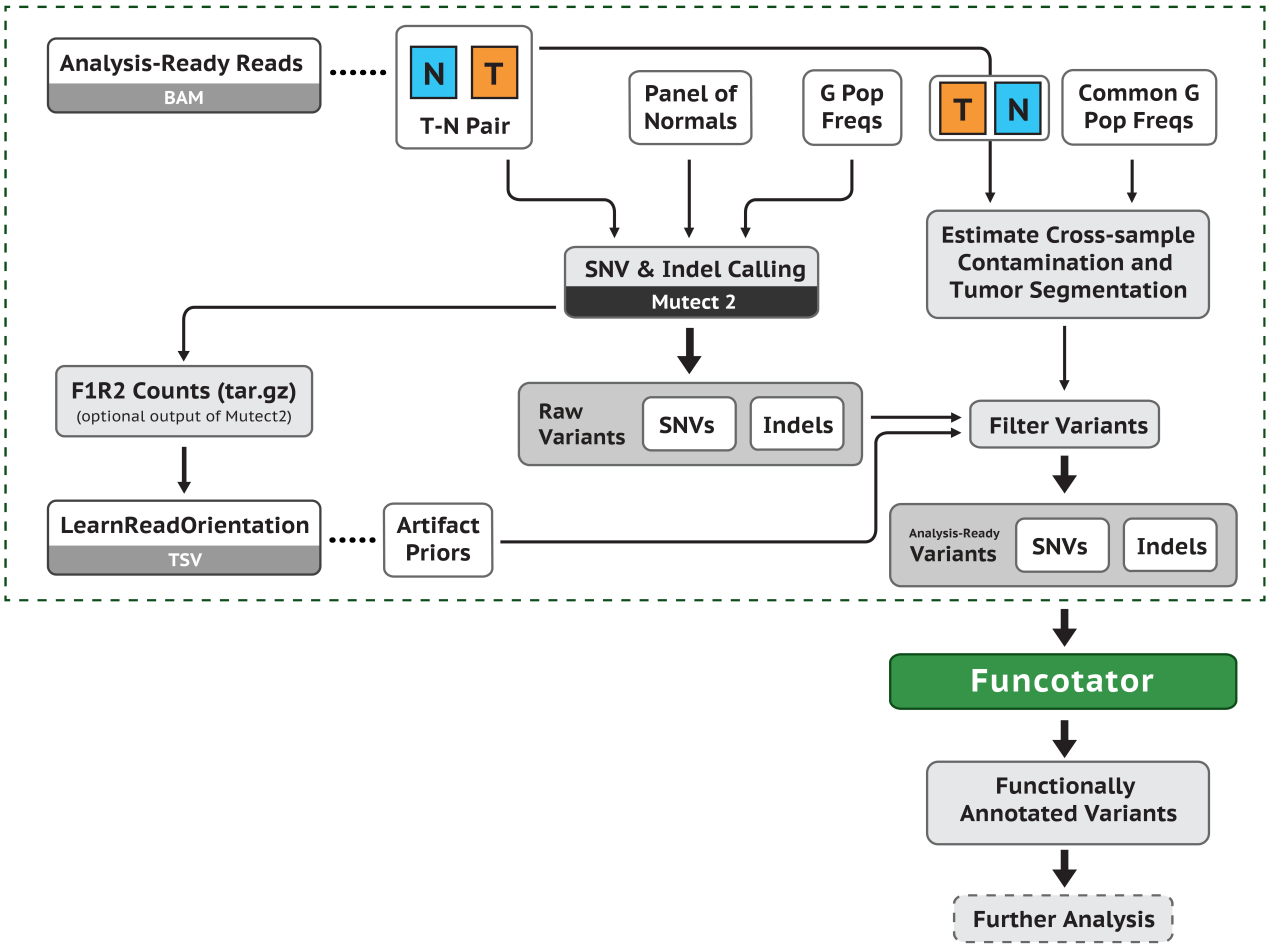


Figure: Somatic short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

Germline short variant discovery

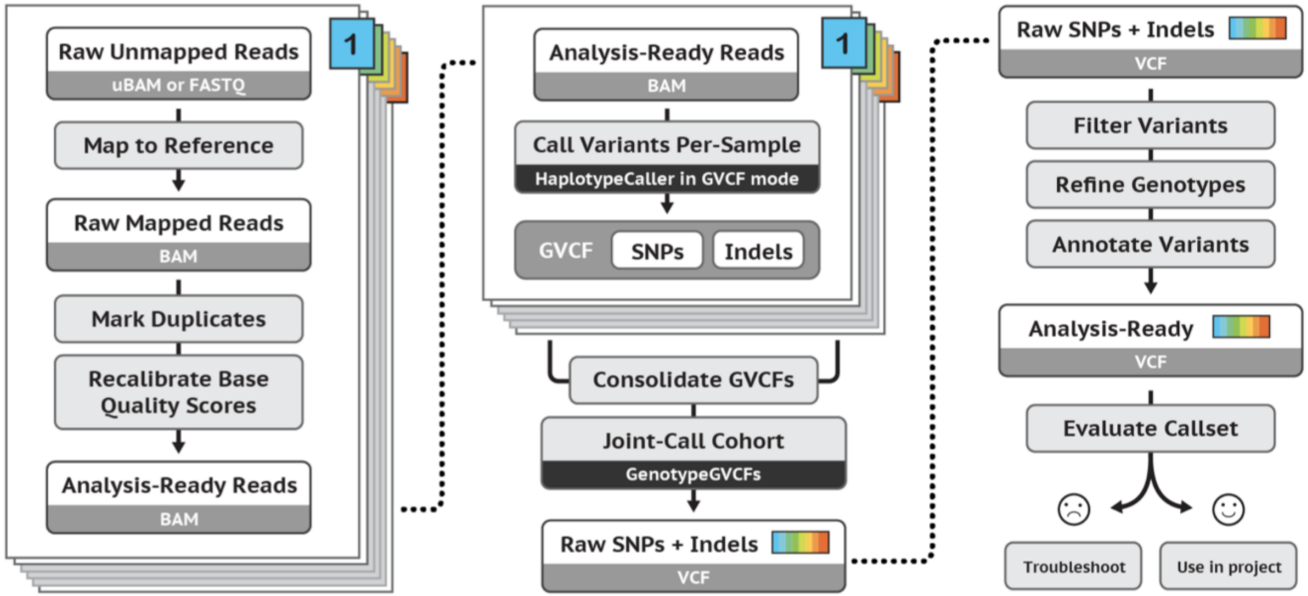


Figure: Germline short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

RNA-seq short variant discovery

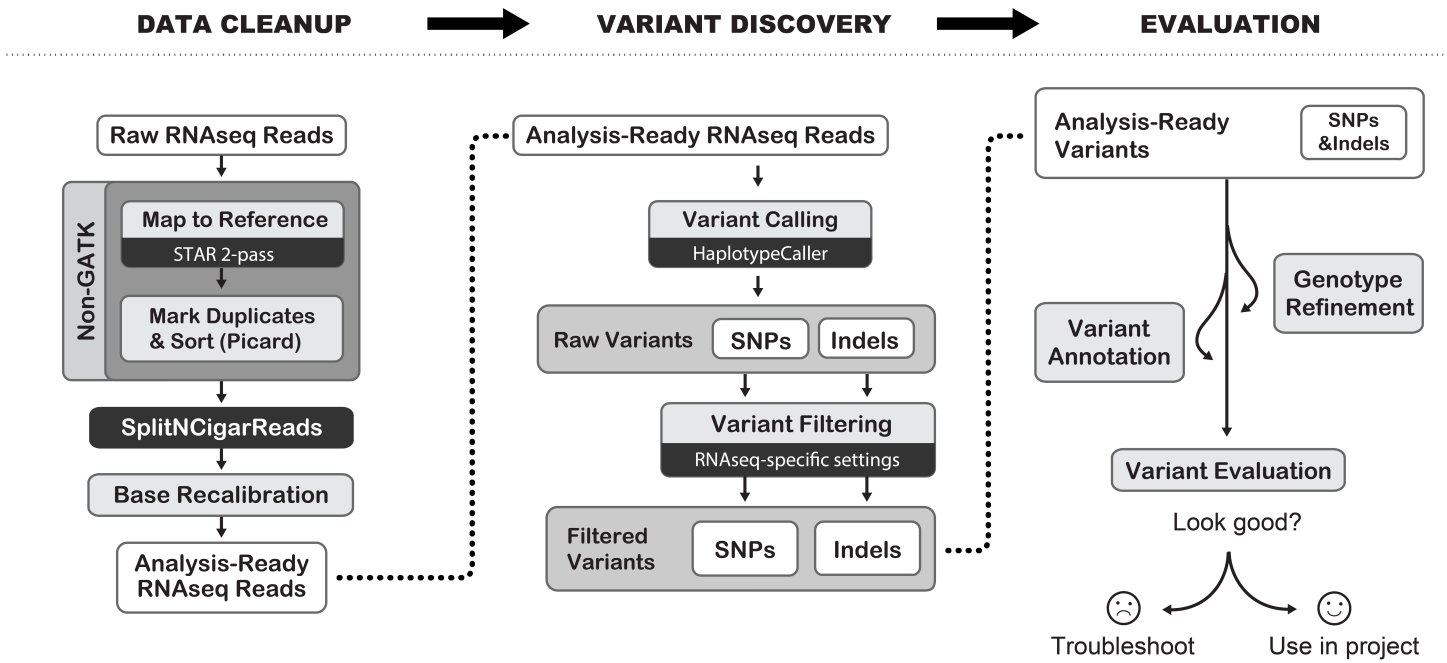


Figure: RNA-seq short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

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)

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)

Results

Results

Quality Checks with FastQC

FastQC?

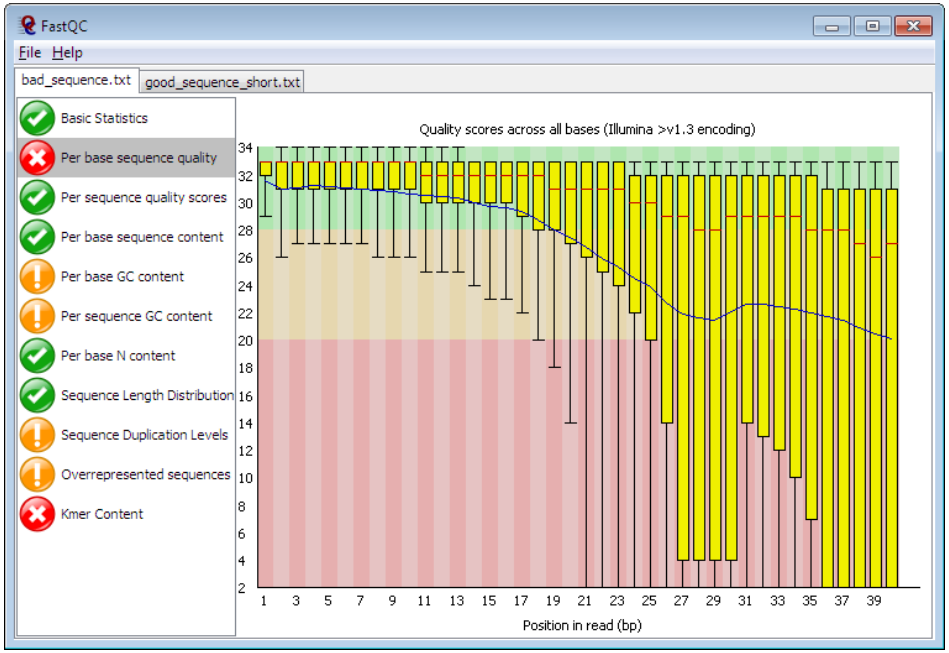


Figure: Example of FastQC Result (Andrews et al., 2012)

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

FastQC on WES

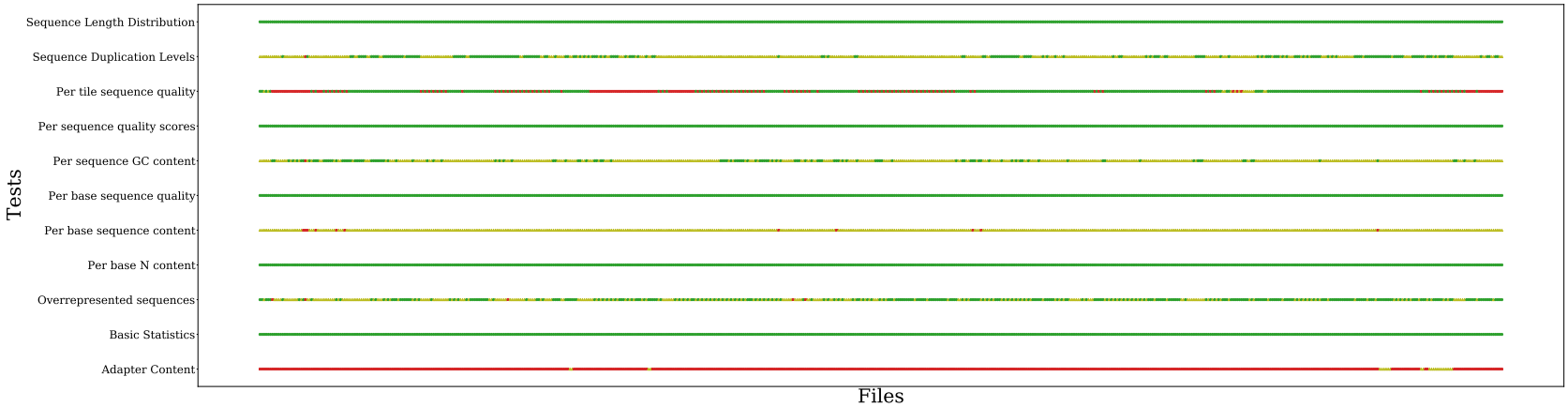
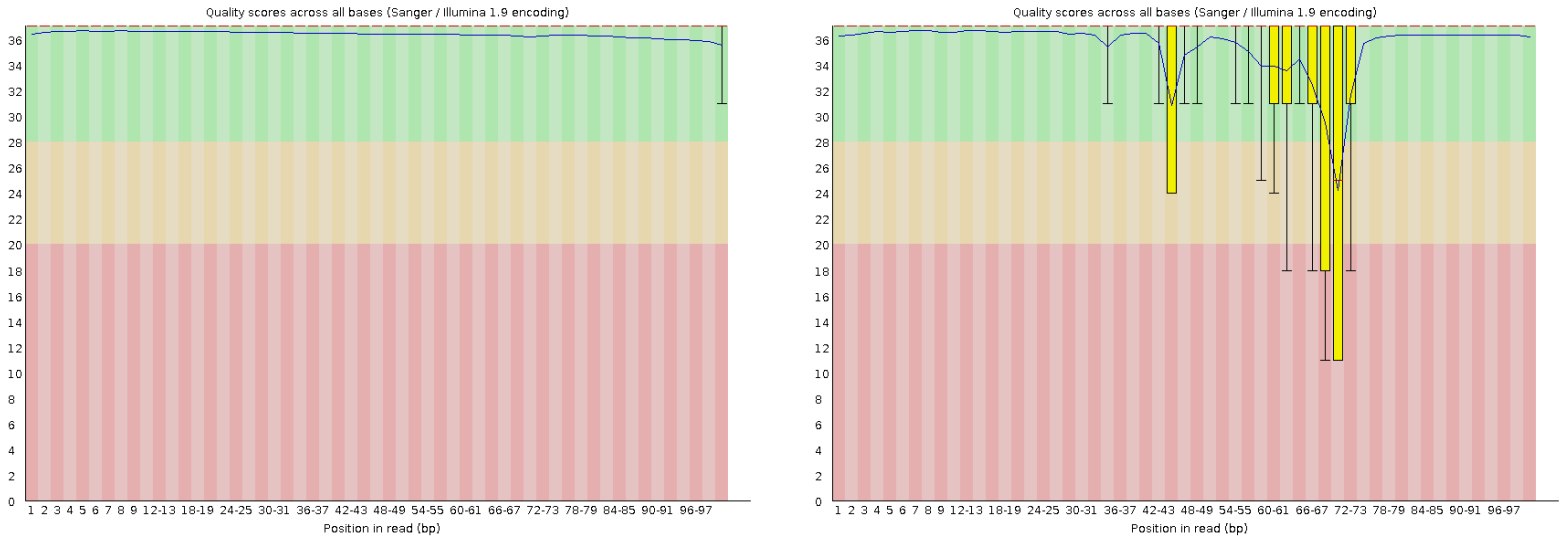


Figure: FastQC with WES data

∴ Only 33P1 has more than 3 failures: 6 FAILs.
∴ 33P1 is excluded at further analysis.

Failure on 33P1 I



(a) 33N (b) 33P1

Figure: Per Base Sequence Quality Results

Failure on 33P1 II

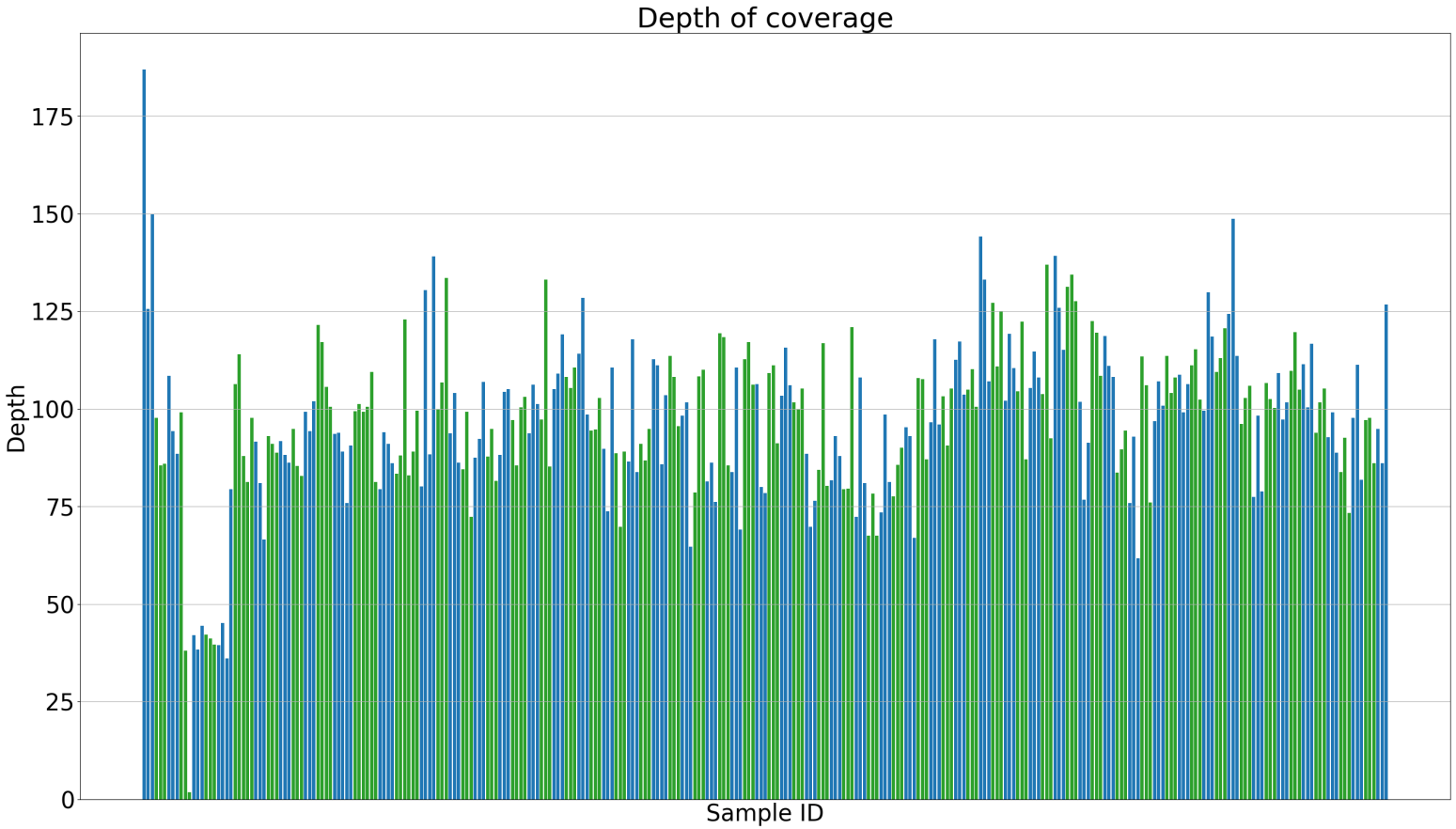


Figure: Coverage Depth Plot

FastQC on WTS

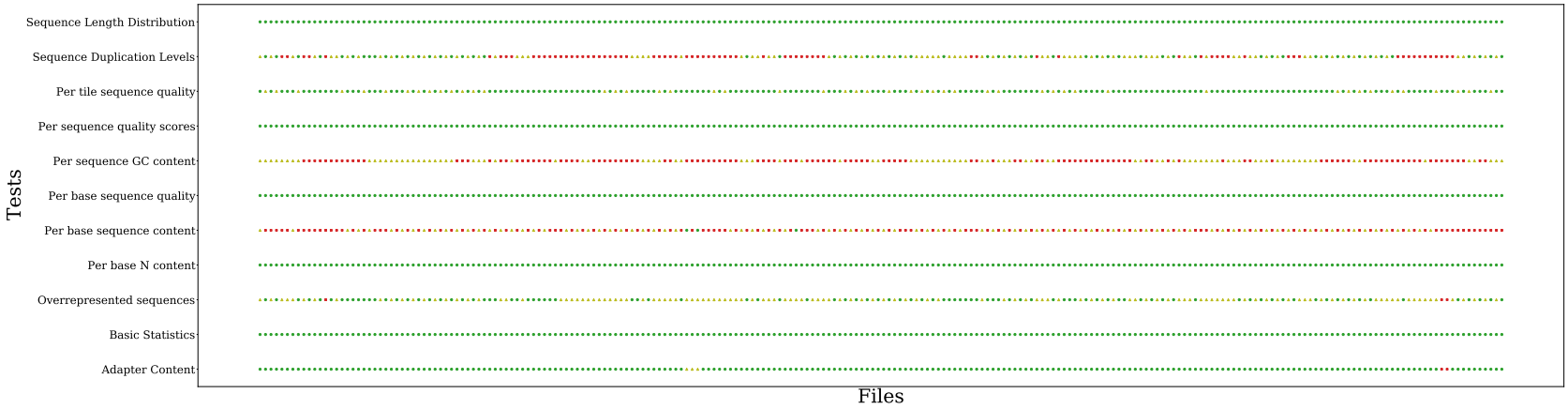


Figure: FastQC with WTS data

$\therefore$ No sample has more than 5 failures.

$\therefore$ All sample are good to analysis.

Results

Quality Checks with Sequenza

Sequenza?

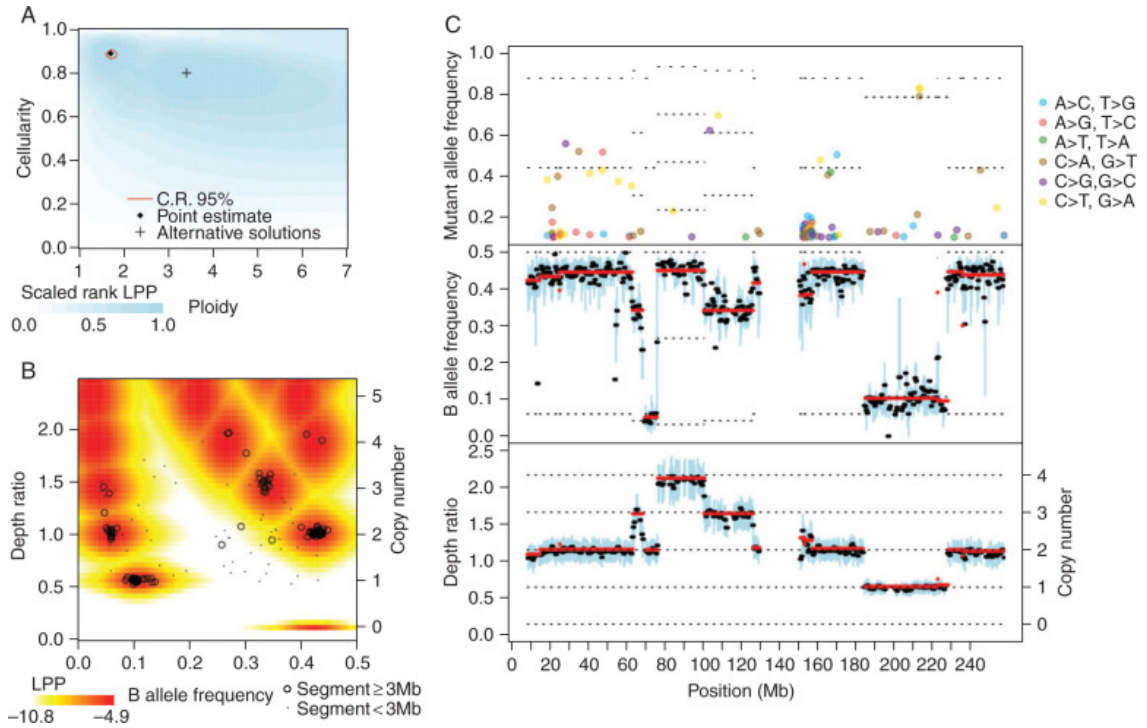
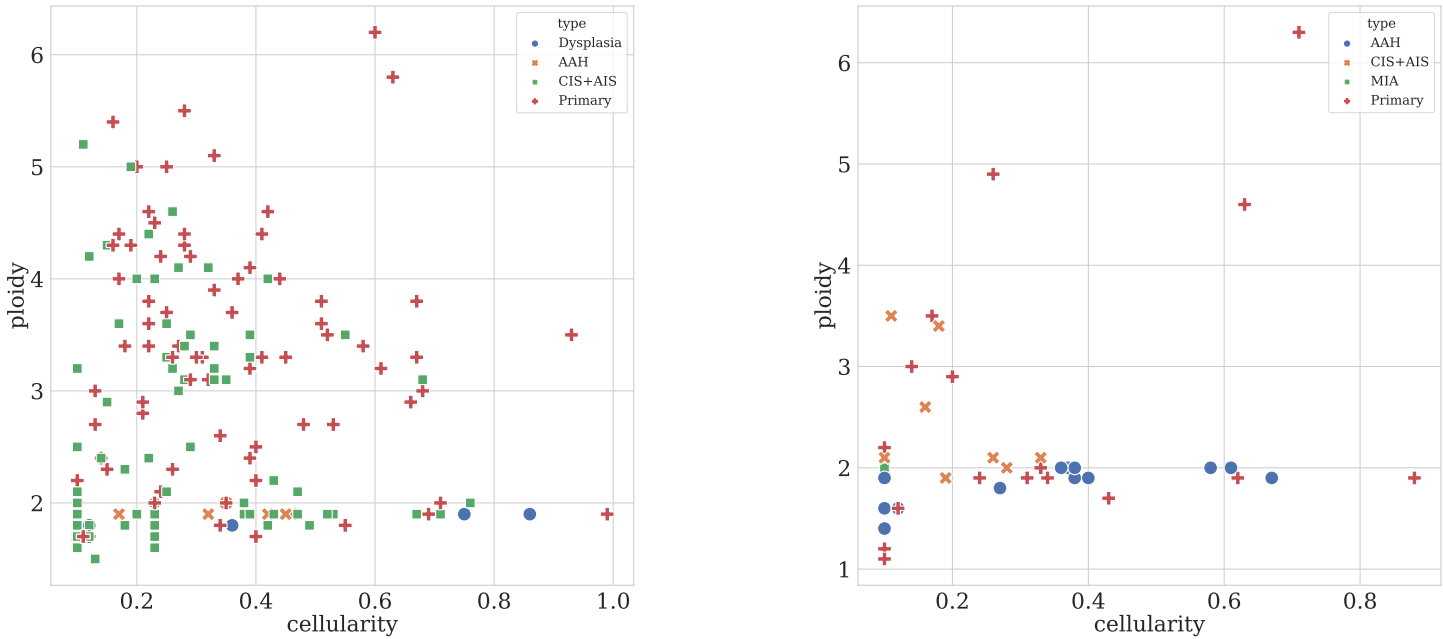


Figure: Representative Output of the Sequenza (Favero et al., 2015)

Cellularity & Ploidy on WES



(a) SQC Samples

(b) ADC Samples

Figure: Cellularity and Ploidy from Sequenza

Genome View on Patient #57

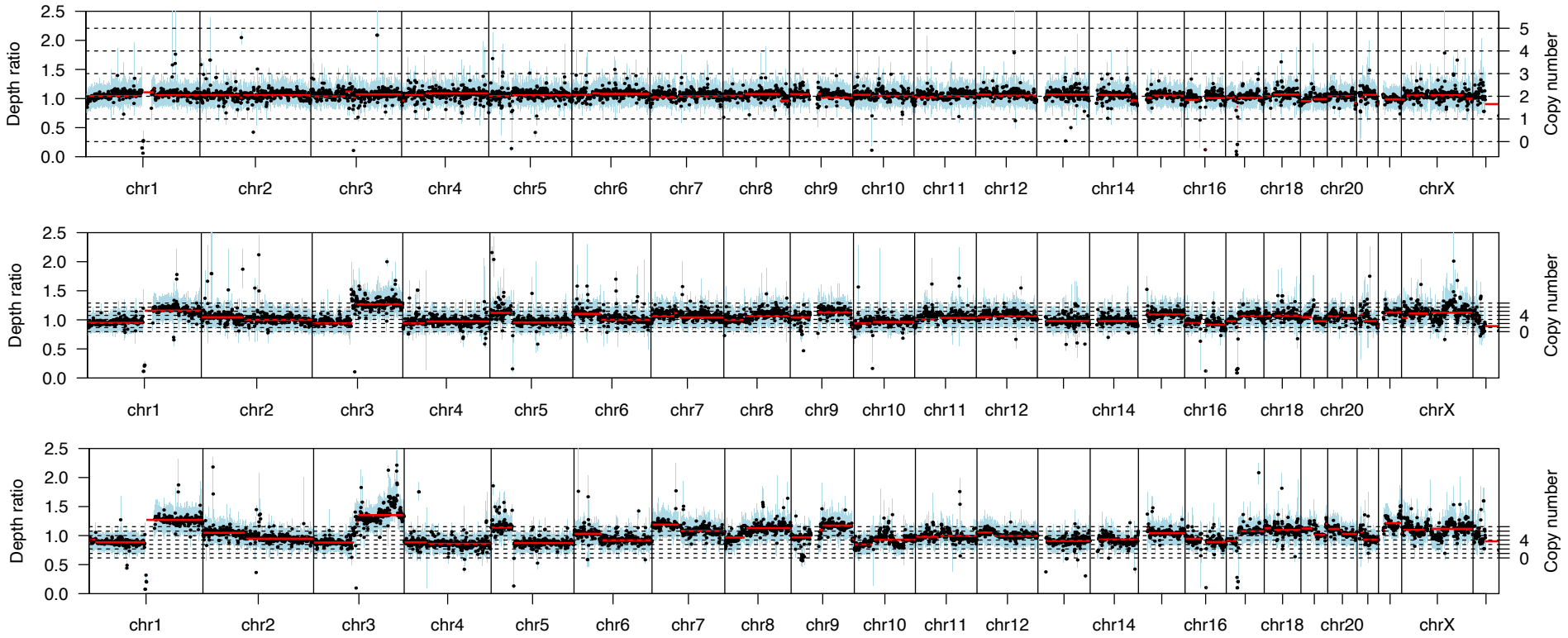


Figure: Dysplasia-CIS-Primary tumor

CNV of SQC

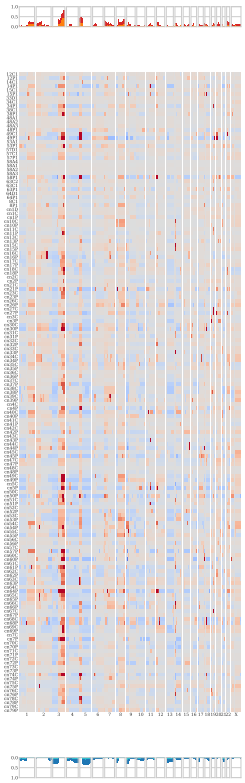


Figure: CNV Plot with SQC Patients

CNV of ADC

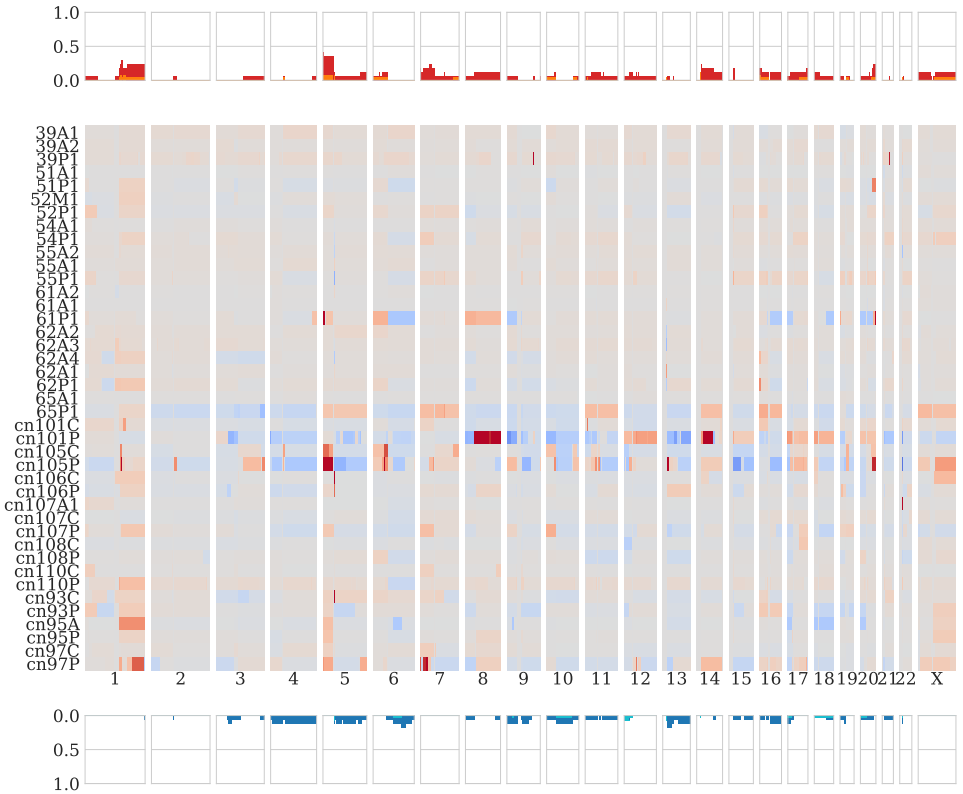


Figure: CNV Plot with ADC Patients

Results

Mutect2

Mutect2?

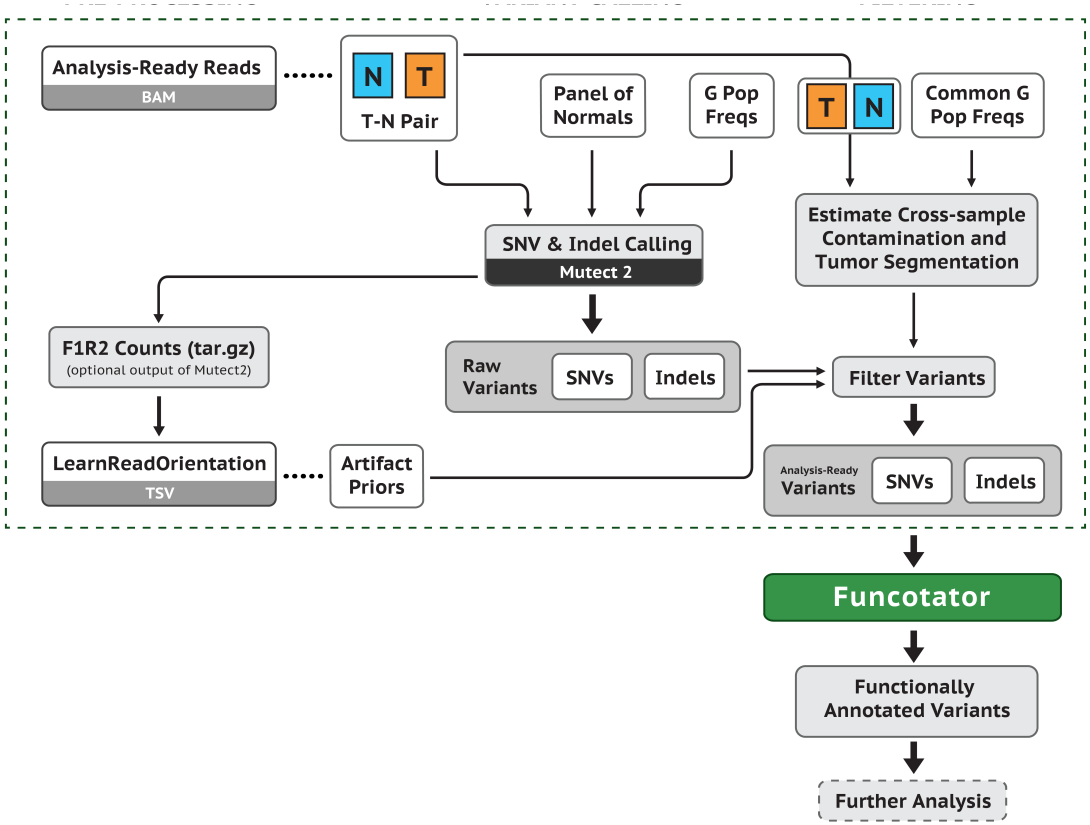


Figure: Somatic short variant discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

Witer?

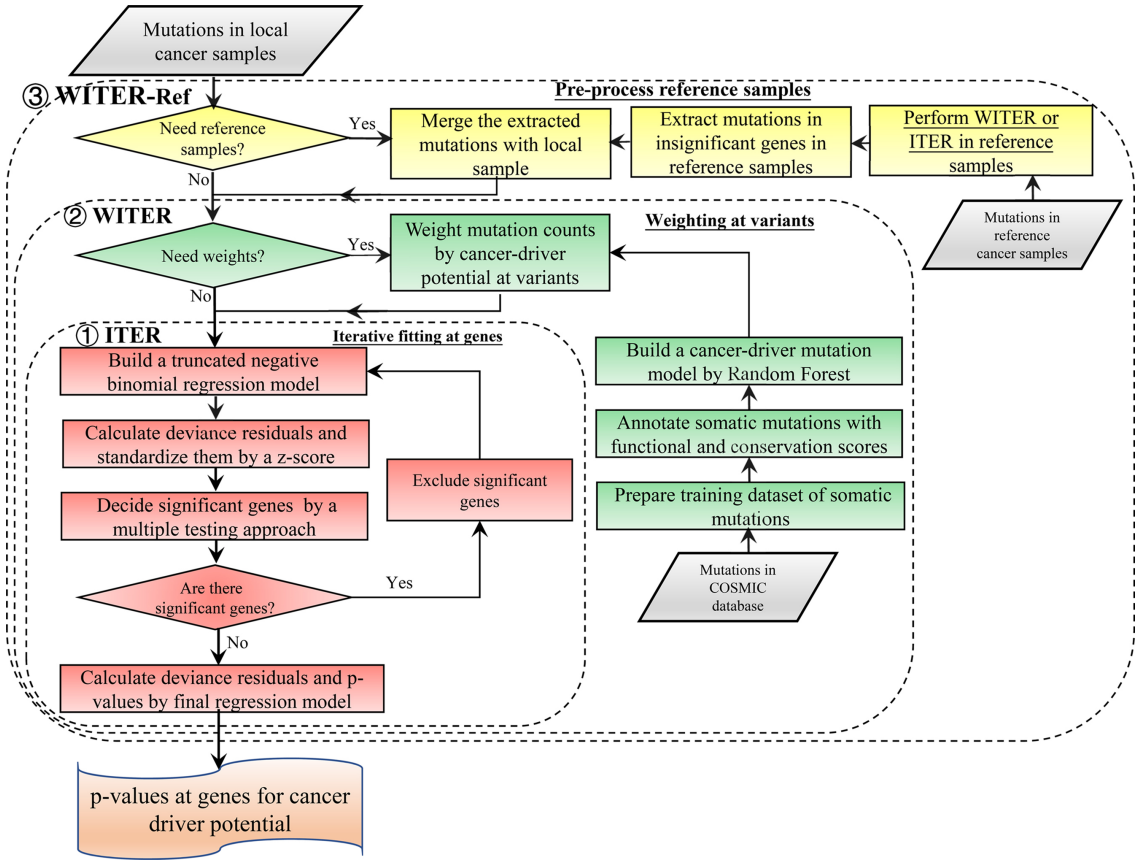


Figure: Witer diagram for detecting cancer-drive genes (Jiang et al., 2019)

Somatic Variant in SQC

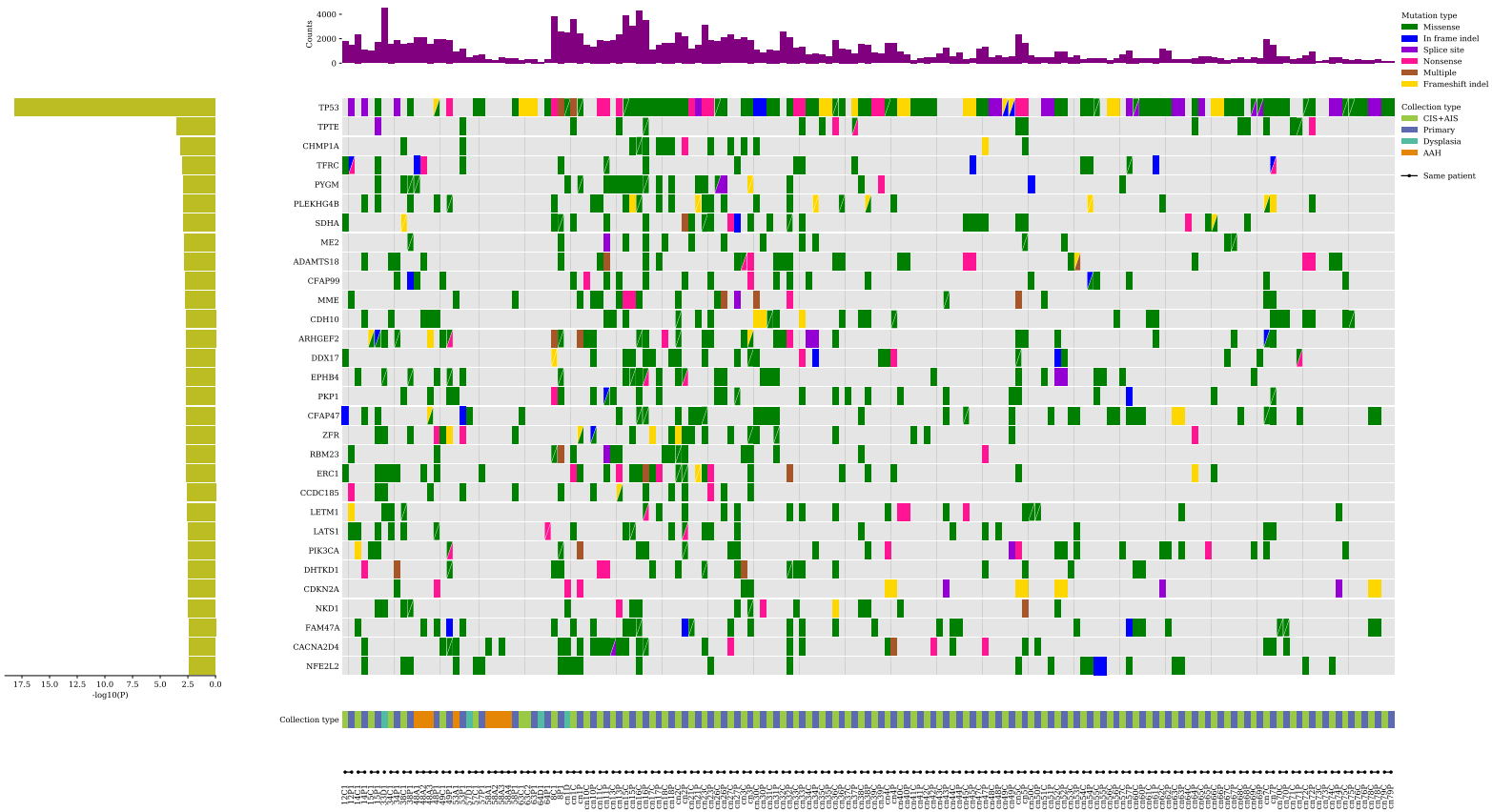


Figure: CoMut Plot with SQC Patients

Somatic Variant in ADC

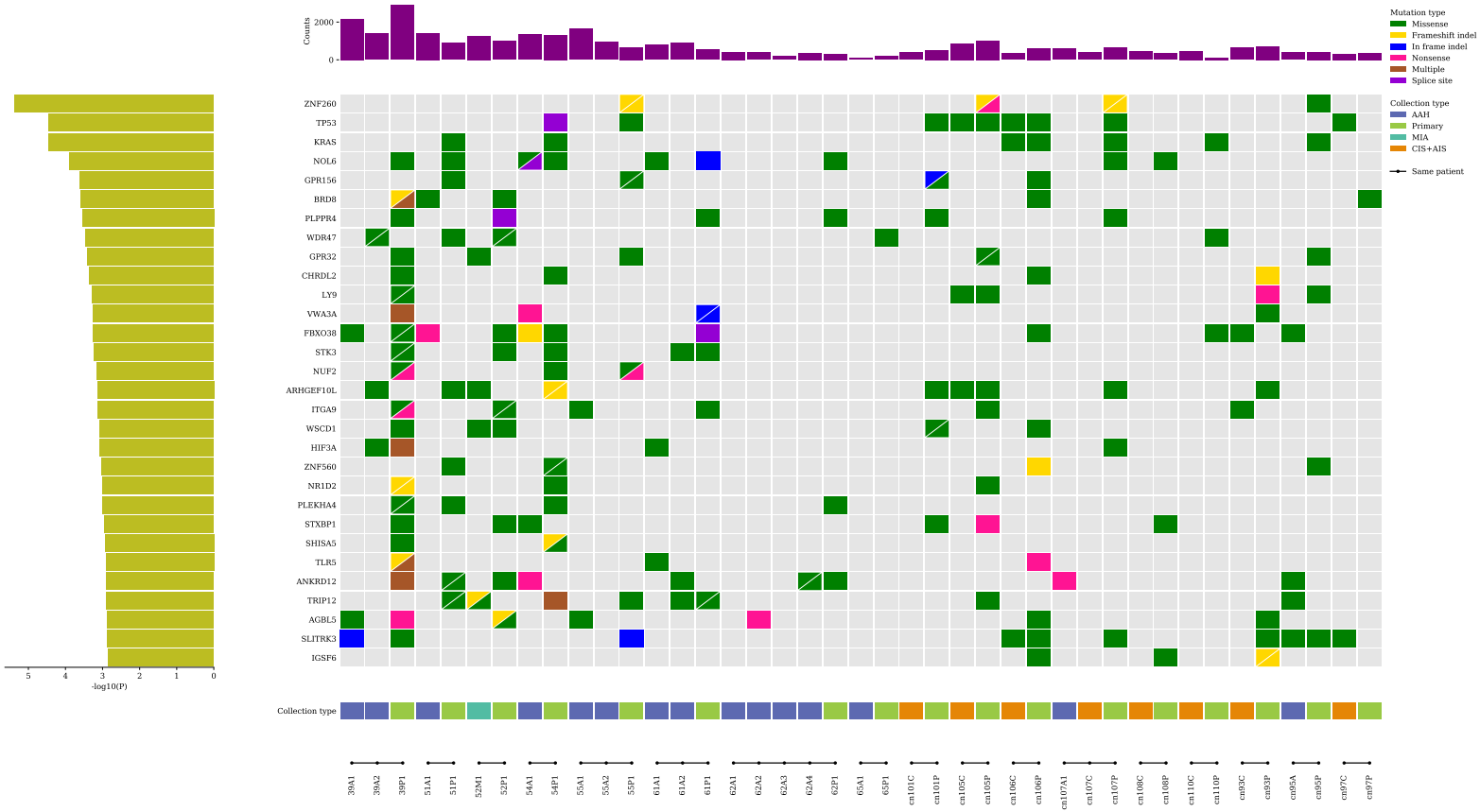


Figure: CoMut Plot with ADC Patients

Results

RSEM

RSEM?

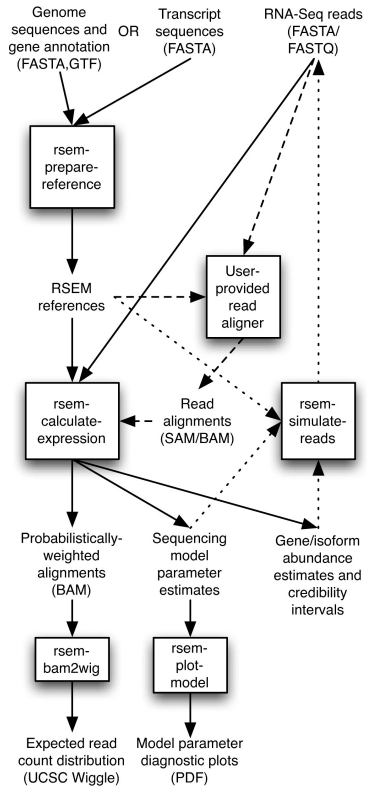


Figure: The RSEM workflow (B. Li & Dewey, 2011)

Volcano Plot in SQC

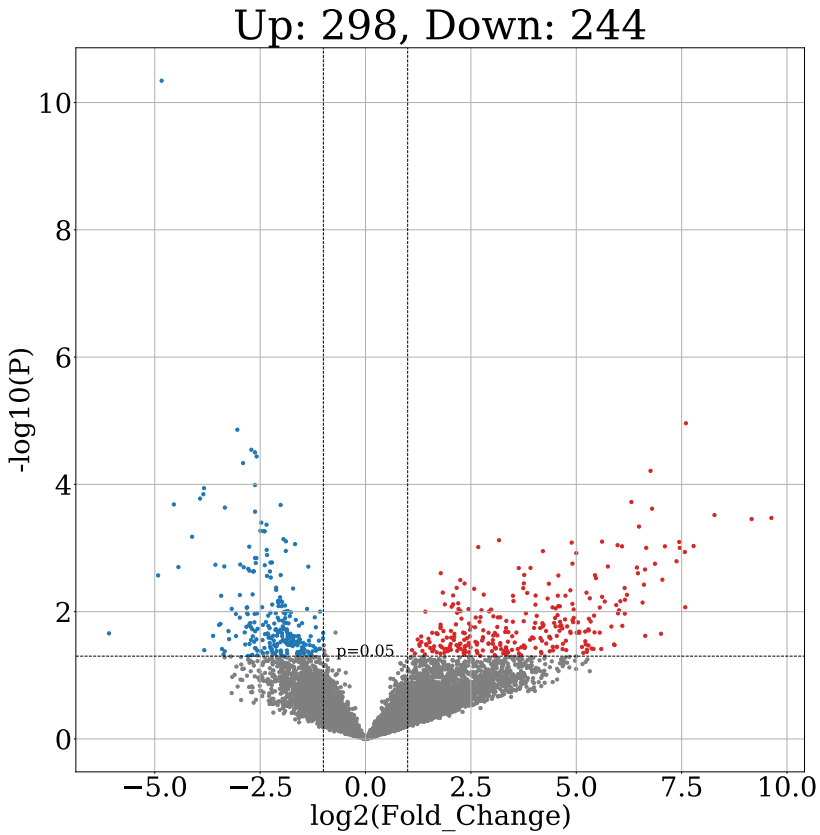


Figure: Volcano Plot in SQC

Volcano Plot in ADC

Discussion

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