

# Lung Precancer Analysis

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*

2021-05-20

# Overview

1 Introduction

2 Materials

3 Methods

4 Results

5 Discussion

# 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
  - from bronchus
- Diagnostic performance

# Materials

# Lung Cancer Data

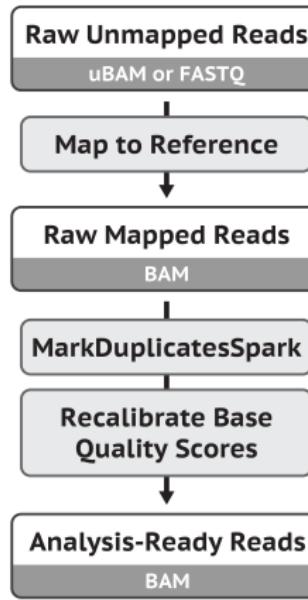
- WES (n=289) + Transcriptome (n=166)
- Normal + {Primary, CIS + AIS, AAH, Dysplasia, MIA}
  - Carcinoma in situ
  - Adenocarcinoma in situ
  - Atypical adenomatous hyperplasia
  - Dysplasia
  - Minimally invasive adenocarcinoma
- Squamous cell carcinoma (SQC) & Adenocarcinoma (ADC)
  - ① Normal - Dysplasia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

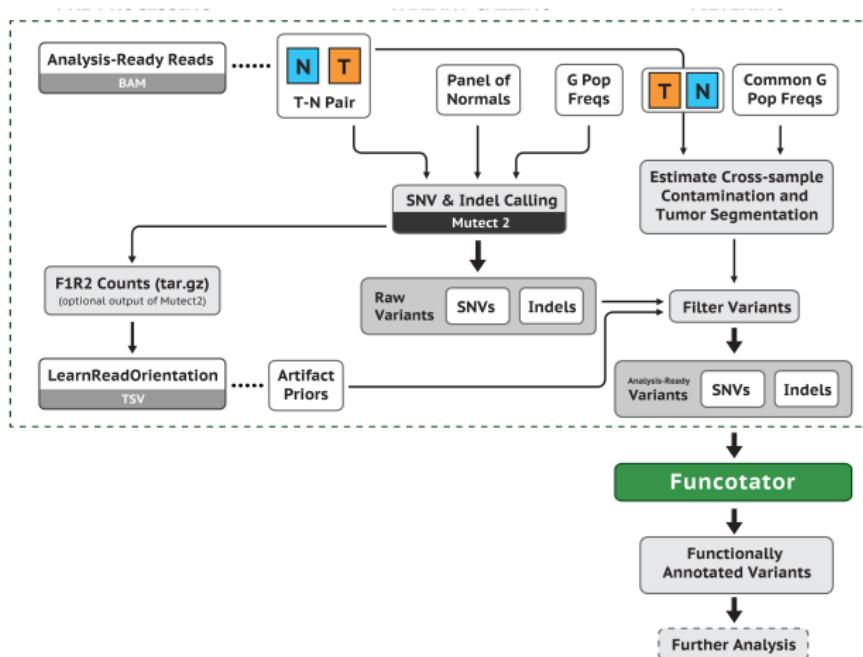
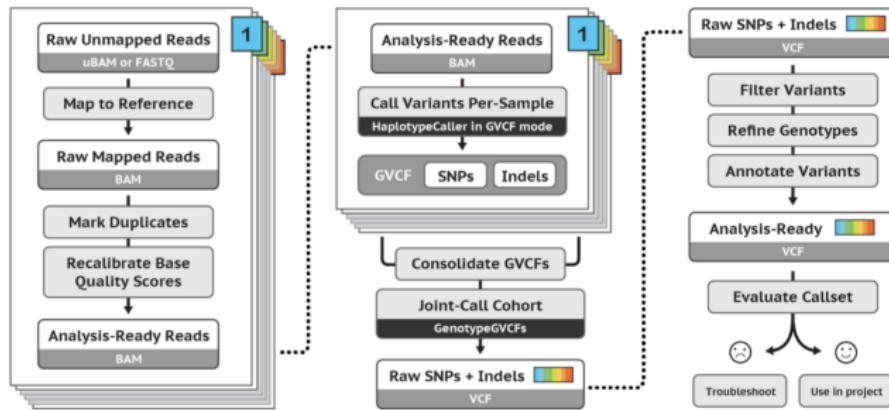


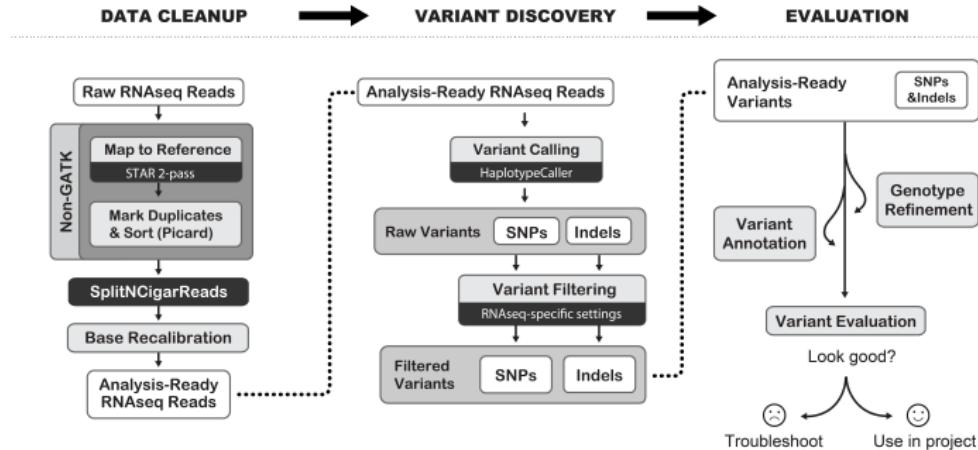
Figure: Somatic short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

# 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)

# 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

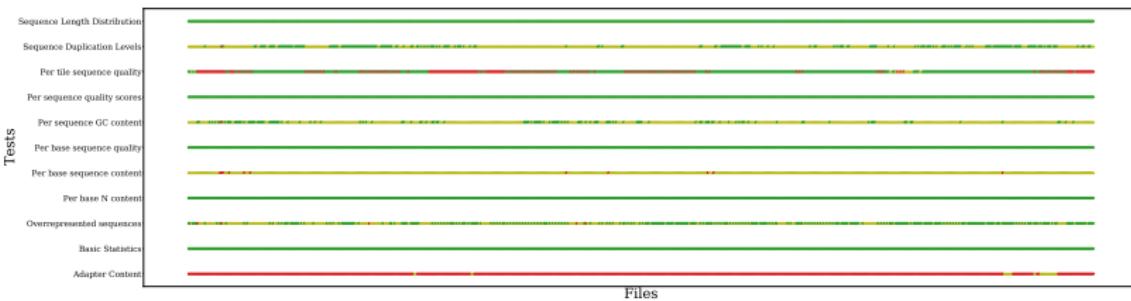
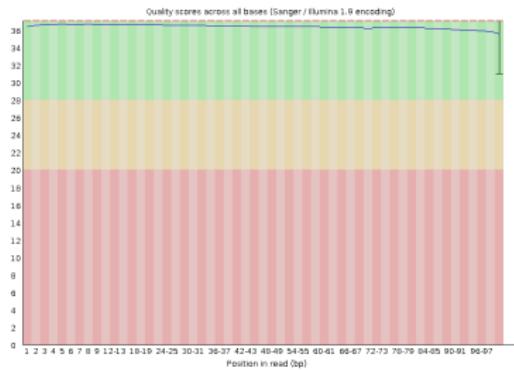


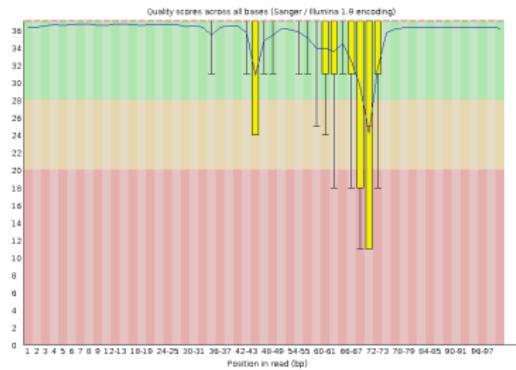
Figure: FastQC with WES data

∴ 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

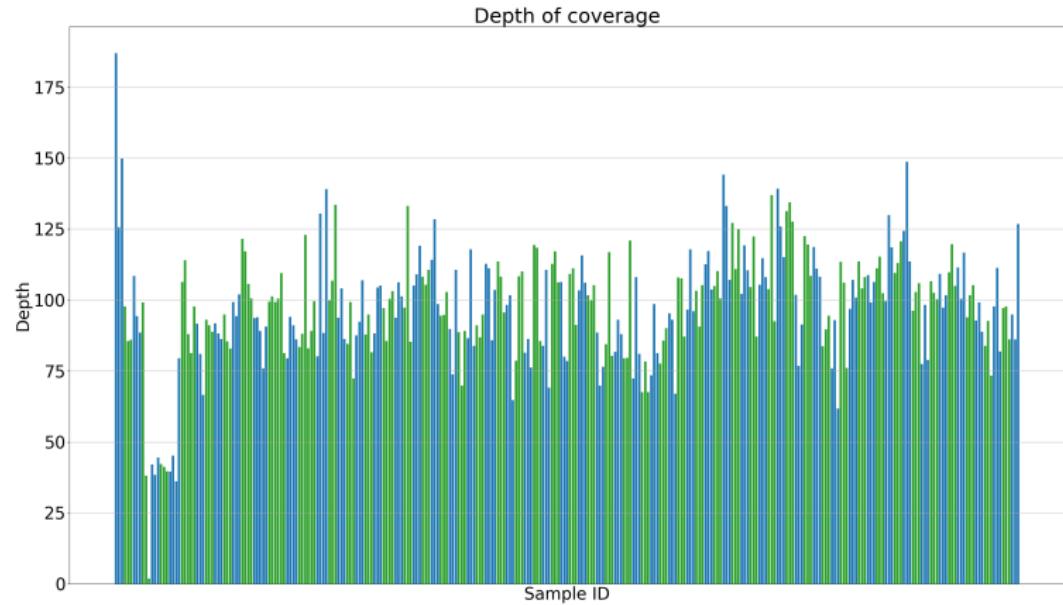


Figure: Coverage Depth Plot

# FastQC on WTS

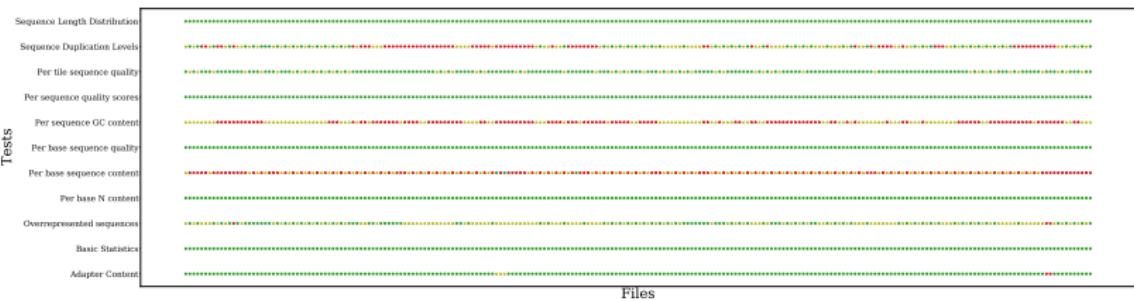


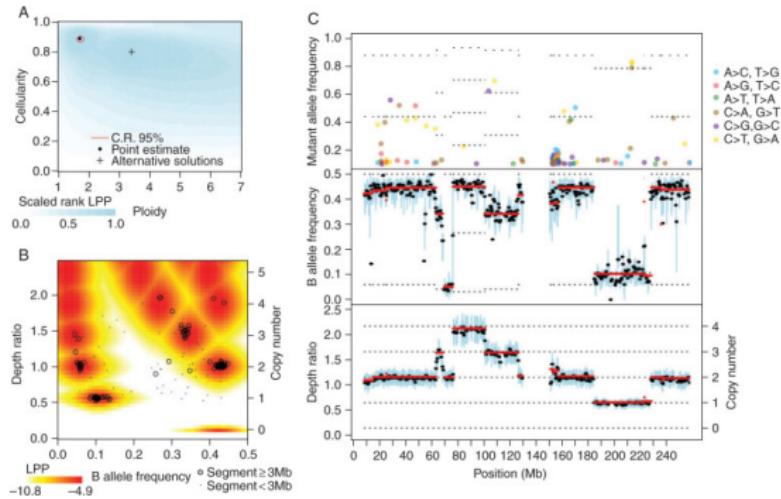
Figure: FastQC with WTS data

∴ No sample has more than 5 failures.  
∴ All sample are good to analysis.

# Results

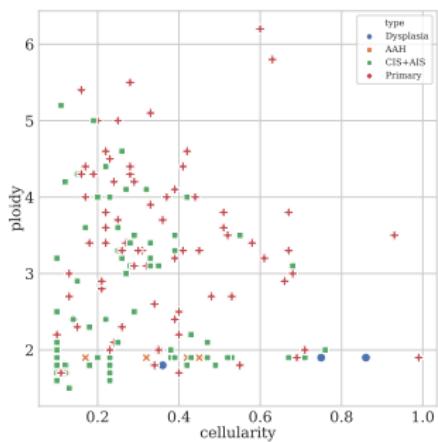
## Quality Checks with Sequenza

# Sequenza?

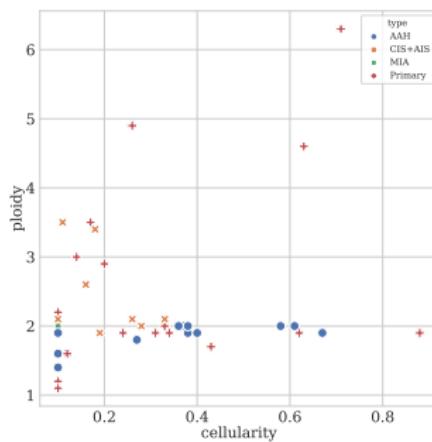


**Figure:** Representative Output of the Sequenza (Favero et al., 2015)

# Cellularity & Ploidy on WES



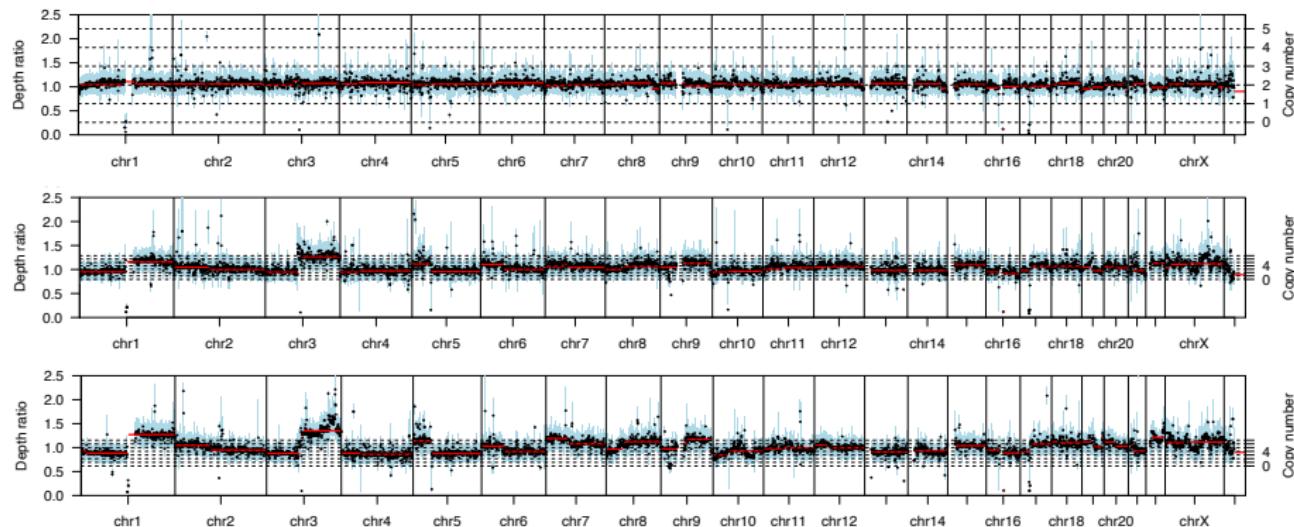
(a) SQC Samples



(b) ADC Samples

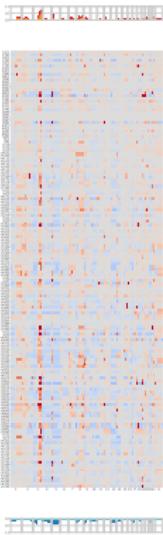
Figure: Cellularity and Ploidy from Sequenza

## Genome View on Patient #57



### Figure: Dysplasia-CIS-Primary tumor

CNV of SQC



## Figure: CNV Plot with SQC Patients

# CNV of ADC

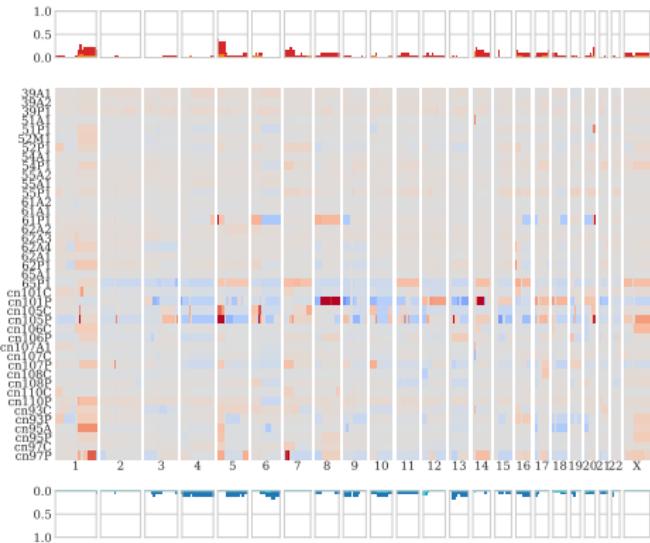
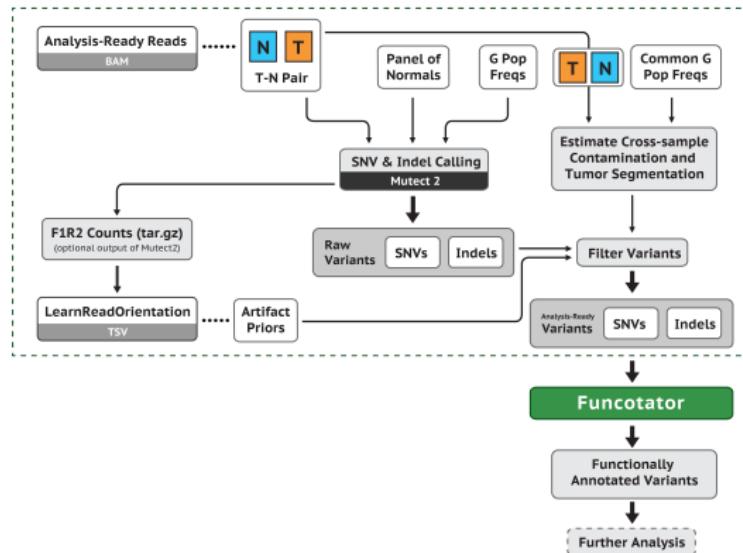


Figure: CNV Plot with ADC Patients

# Results

Find SNVs with Mutect2

# Mutect2?



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

# Witer?

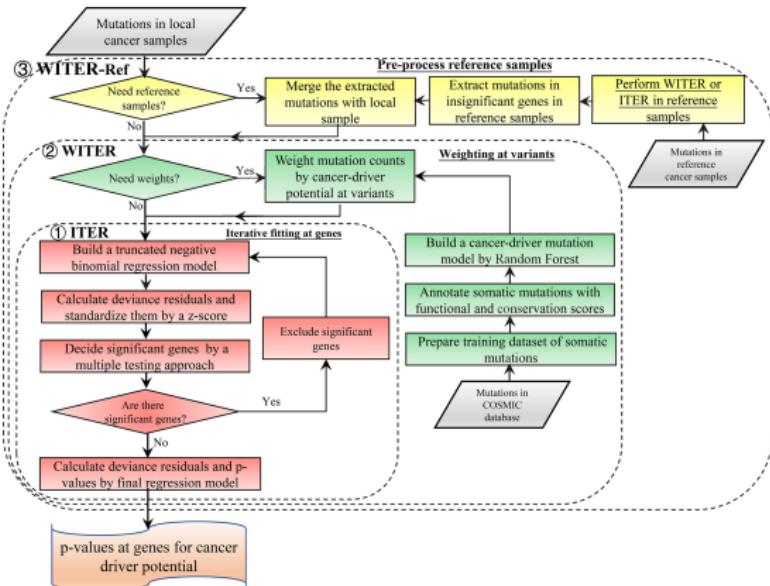


Figure: Witer diagram for detecting cancer-drive genes (Jiang et al., 2019)

# Somatic Variant in SQC

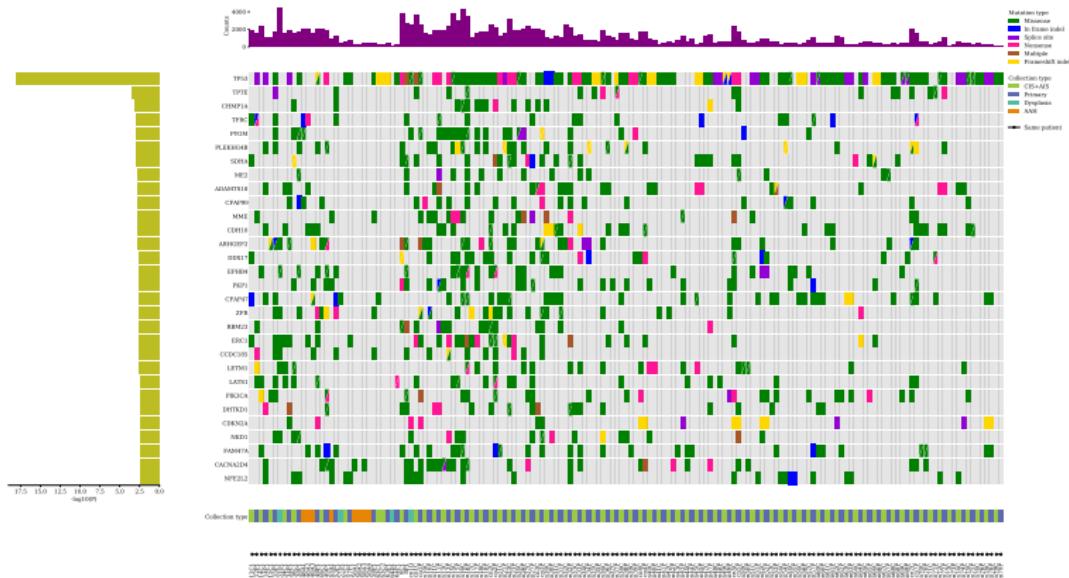
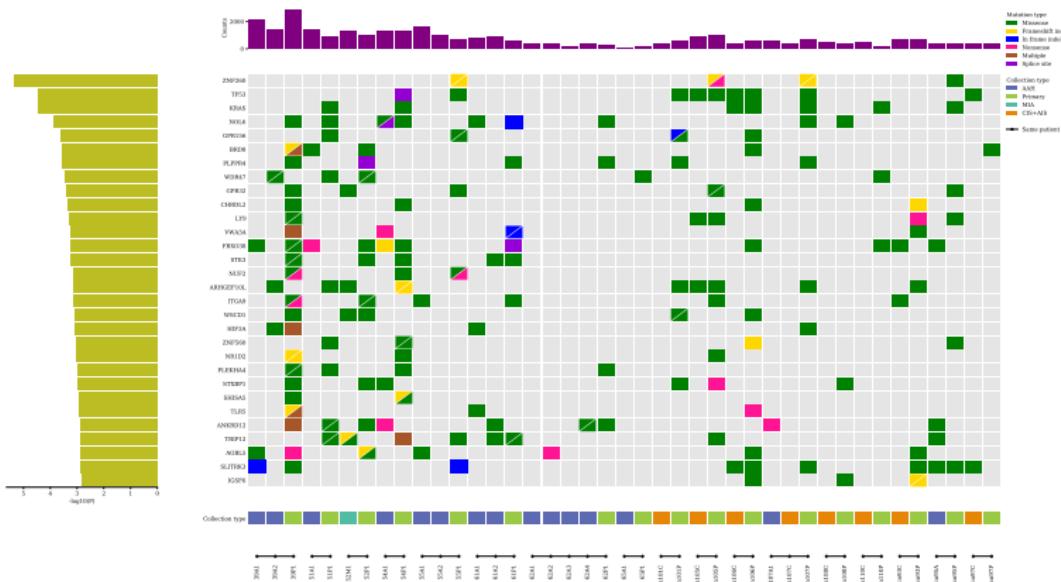


Figure: CoMut Plot with SQC Patients

## Somatic Variant in ADC



## Figure: CoMut Plot with ADC Patients

# Results

## RSEM

# RSEM?

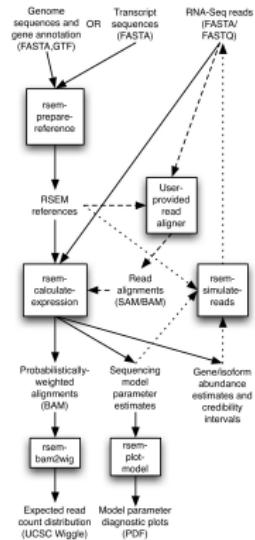


Figure: The RSEM workflow (B. Li & Dewey, 2011)

# Volcano Plot in SQC

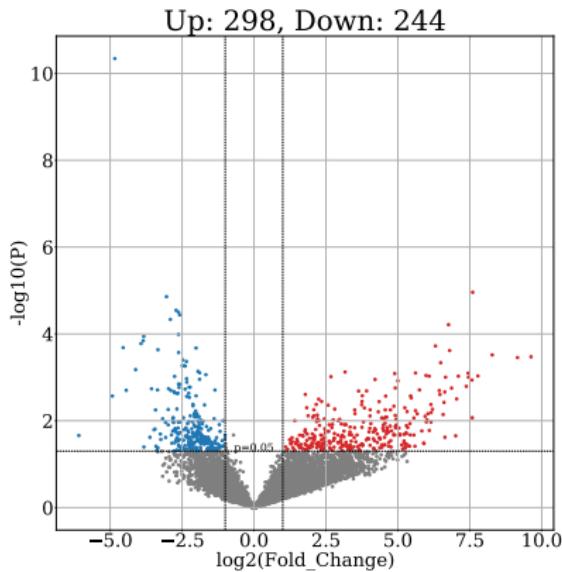


Figure: Volcano Plot in SQC

# Volcano Plot in ADC

# Results

## Tumor Evolution with MesKit

# MesKit?

# Results

## Estimated Cell Types with CIBERSORTx

# CIBERSORTx?

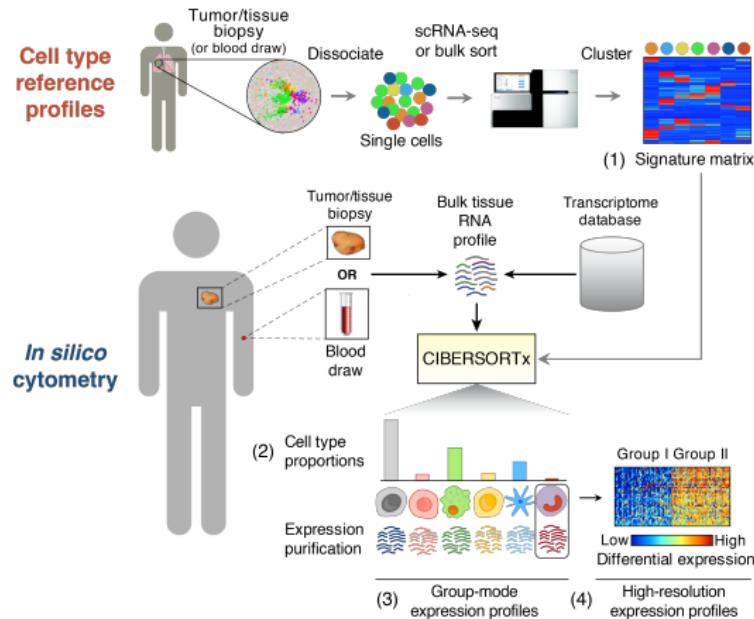
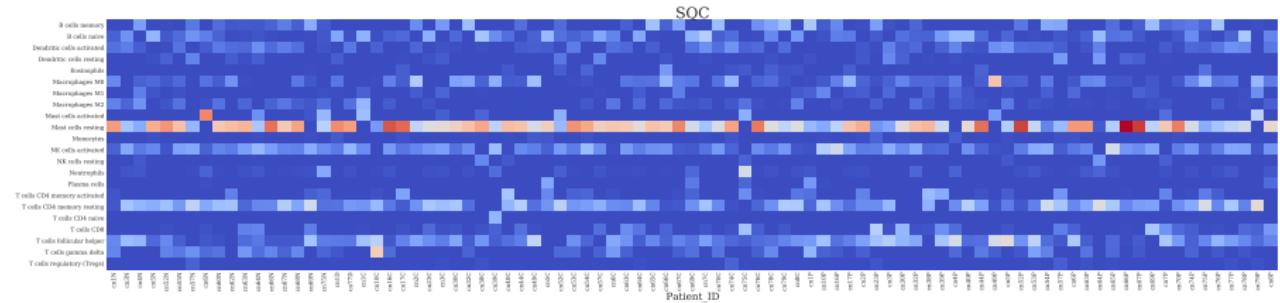


Figure: CIBERSORTx workflow (Steen et al., 2020)

CIBERSORTx with SQC



## Figure: Estimated Cell Types with SQC Samples

# CIBERSORTx with ADC

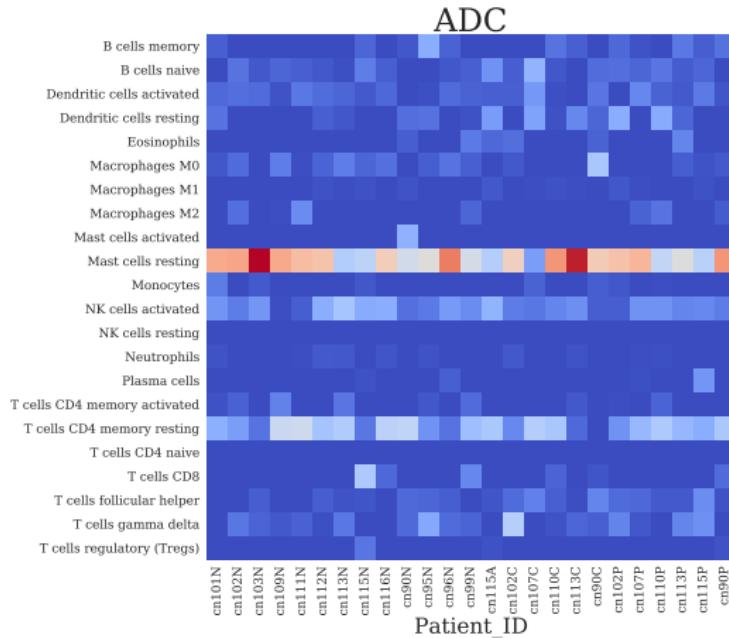


Figure: Estimated Cell Types with ADC Samples

# Discussion

# References I

- Andrews, S., Krueger, F., Segonds-Pichon, A., Biggins, L., Krueger, C., & Wingett, S. (2012, January). *FastQC*. Babraham Institute. Babraham, UK.
- Crowdis, J., He, M. X., Reardon, B., & Van Allen, E. M. (2020). Comut: visualizing integrated molecular information with comutation plots. *Bioinformatics*, 36(15), 4348–4349.
- Danecek, P., Bonfield, J. K., Liddle, J., Marshall, J., Ohan, V., Pollard, M. O., ... Li, H. (2021, 02). Twelve years of SAMtools and BCFtools. *GigaScience*, 10(2). Retrieved from <https://doi.org/10.1093/gigascience/giab008> (giab008) doi: 10.1093/gigascience/giab008
- DePristo, M. A., Banks, E., Poplin, R., Garimella, K. V., Maguire, J. R., Hartl, C., ... others (2011). A framework for variation discovery and genotyping using next-generation dna sequencing data. *Nature genetics*, 43(5), 491.

## References II

- Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., ... Gingeras, T. R. (2013). Star: ultrafast universal rna-seq aligner. *Bioinformatics*, 29(1), 15–21.
- Favero, F., Joshi, T., Marquard, A. M., Birkbak, N. J., Krzystanek, M., Li, Q., ... Eklund, A. C. (2015). Sequenza: allele-specific copy number and mutation profiles from tumor sequencing data. *Annals of Oncology*, 26(1), 64–70.
- Hunter, J. D. (2007). Matplotlib: A 2d graphics environment. *IEEE Annals of the History of Computing*, 9(03), 90–95.
- Jiang, L., Zheng, J., Kwan, J. S., Dai, S., Li, C., Li, M. J., ... others (2019). Witer: a powerful method for estimation of cancer-driver genes using a weighted iterative regression modelling background mutation counts. *Nucleic acids research*, 47(16), e96–e96.

## References III

- Kandoth, C., Gao, J., qwangmsk, Mattioni, M., Struck, A., Boursin, Y., ... Chavan, S. (2018, February). *mskcc/vcf2maf: vcf2maf v1.6.16*. Zenodo. Retrieved from  
<https://doi.org/10.5281/zenodo.1185418> doi:  
10.5281/zenodo.1185418
- Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with bowtie 2. *Nature methods*, 9(4), 357.
- Li, B., & Dewey, C. N. (2011). Rsem: accurate transcript quantification from rna-seq data with or without a reference genome. *BMC bioinformatics*, 12(1), 1–16.
- Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with bwa-mem. *arXiv preprint arXiv:1303.3997*.
- Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with burrows–wheeler transform. *bioinformatics*, 25(14), 1754–1760.

## References IV

- Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., ... Durbin, R. (2009). The sequence alignment/map format and samtools. *Bioinformatics*, 25(16), 2078–2079.
- Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for rna-seq data with deseq2. *Genome biology*, 15(12), 1–21.
- McLaren, W., Gil, L., Hunt, S. E., Riat, H. S., Ritchie, G. R., Thormann, A., ... Cunningham, F. (2016). The ensembl variant effect predictor. *Genome biology*, 17(1), 1–14.
- Nilsen, G., Liestol, K., & Lingjaerde, O. (2013). Copynumber: Segmentation of single-and multi-track copy number data by penalized least squares regression. *R package version*, 1(0).

## References V

- Nilsen, G., Liestøl, K., Van Loo, P., Vollan, H. K. M., Eide, M. B., Rueda, O. M., ... others (2012). Copynumber: efficient algorithms for single-and multi-track copy number segmentation. *BMC genomics*, 13(1), 1–16.
- pandas development team, T. (2020, February). *pandas-dev/pandas: Pandas*. Zenodo. Retrieved from <https://doi.org/10.5281/zenodo.3509134> doi: 10.5281/zenodo.3509134
- Picard toolkit. (2019). <http://broadinstitute.github.io/picard/>. Broad Institute.
- Steen, C. B., Liu, C. L., Alizadeh, A. A., & Newman, A. M. (2020). Profiling cell type abundance and expression in bulk tissues with cibersortx. In *Stem cell transcriptional networks* (pp. 135–157). Springer.

## References VI

- Van der Auwera, G. A., Carneiro, M. O., Hartl, C., Poplin, R., Del Angel, G., Levy-Moonshine, A., ... others (2013). From fastq data to high-confidence variant calls: the genome analysis toolkit best practices pipeline. *Current protocols in bioinformatics*, 43(1), 11–10.
- Waskom, M., & the seaborn development team. (2020, September). *mwaskom/seaborn*. Zenodo. Retrieved from <https://doi.org/10.5281/zenodo.592845> doi: 10.5281/zenodo.592845
- Wes McKinney. (2010). Data Structures for Statistical Computing in Python. In Stéfan van der Walt & Jarrod Millman (Eds.), *Proceedings of the 9th Python in Science Conference* (p. 56 - 61). doi: 10.25080/Majora-92bf1922-00a