

Lung Precancer Study

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

Department of Biomedical Engineering
Ulsan National Institute of Science and Technology

jwlee230@unist.ac.kr

2021-09-15

Overview

1 Introduction

2 Materials

3 Methods

4 Results

5 Discussion

6 References

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

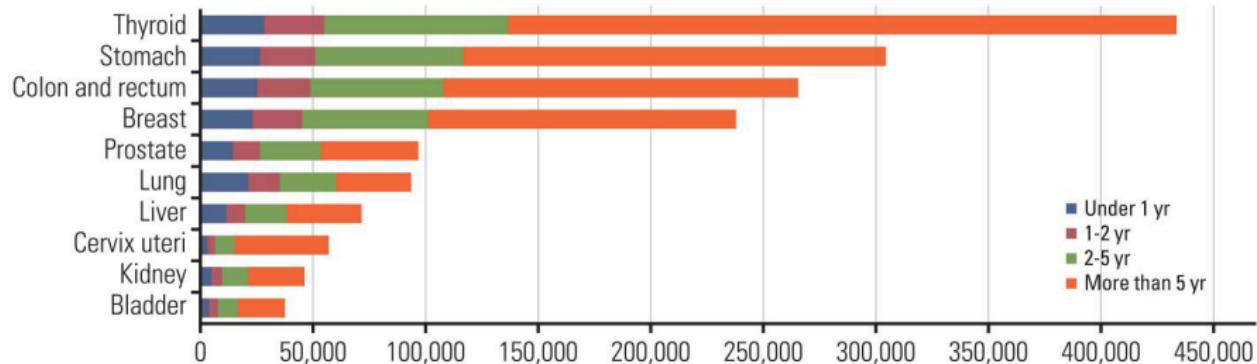


Figure: Common cancer survival rates (Hong et al., 2021)

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)

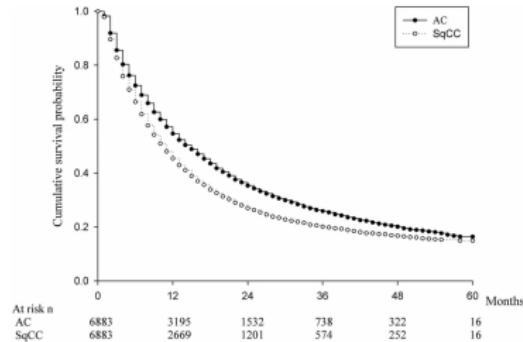
(Vincent et al., 1977; Collins, Haines, Perkel, & Enck, 2007)

ADC vs. SQC I

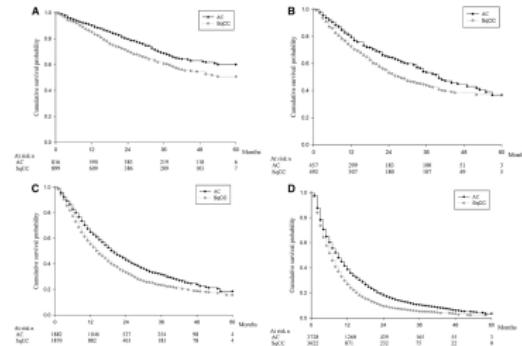


Figure: ADC and SQC histology in Lung cancer (Travis, 2002)

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

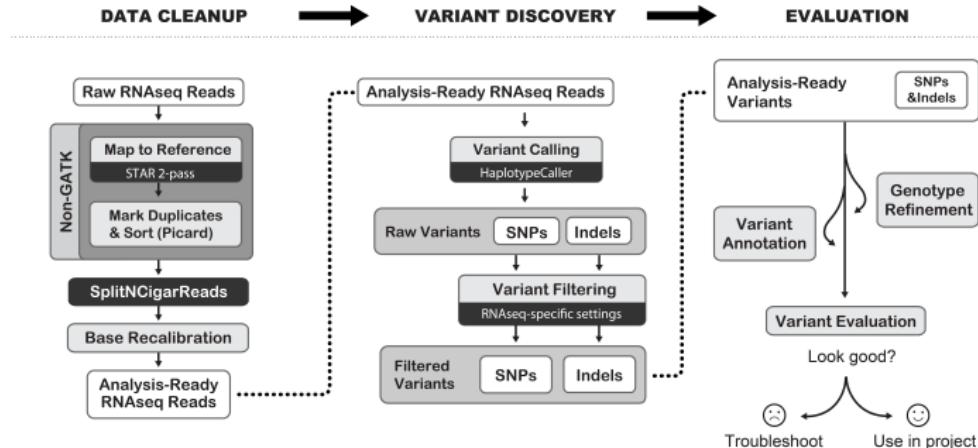


Figure: RNA-seq short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

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

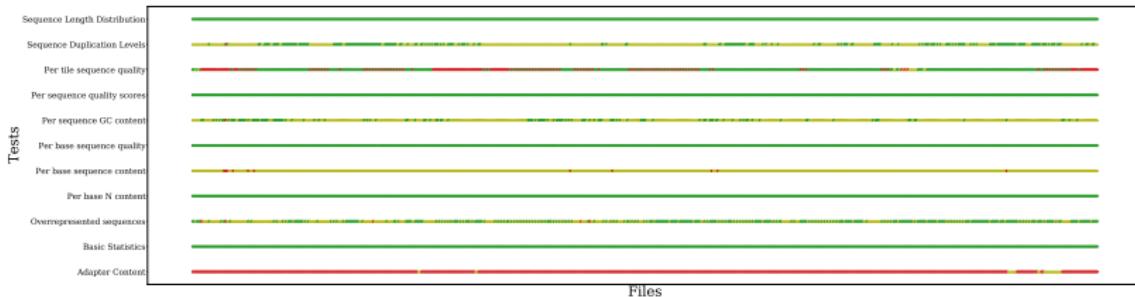


Figure: FastQC with WES data

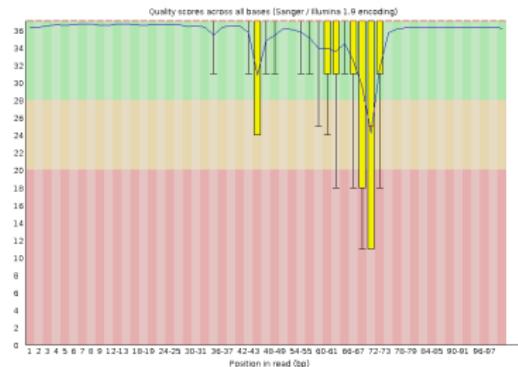
Failure on 33P1 sample

33P1 is excluded at further analysis.

Failure on 33P1 I



(a) 33N



(b) 33P1

Figure: Per Base Sequence Quality Results

Failure on 33P1 II

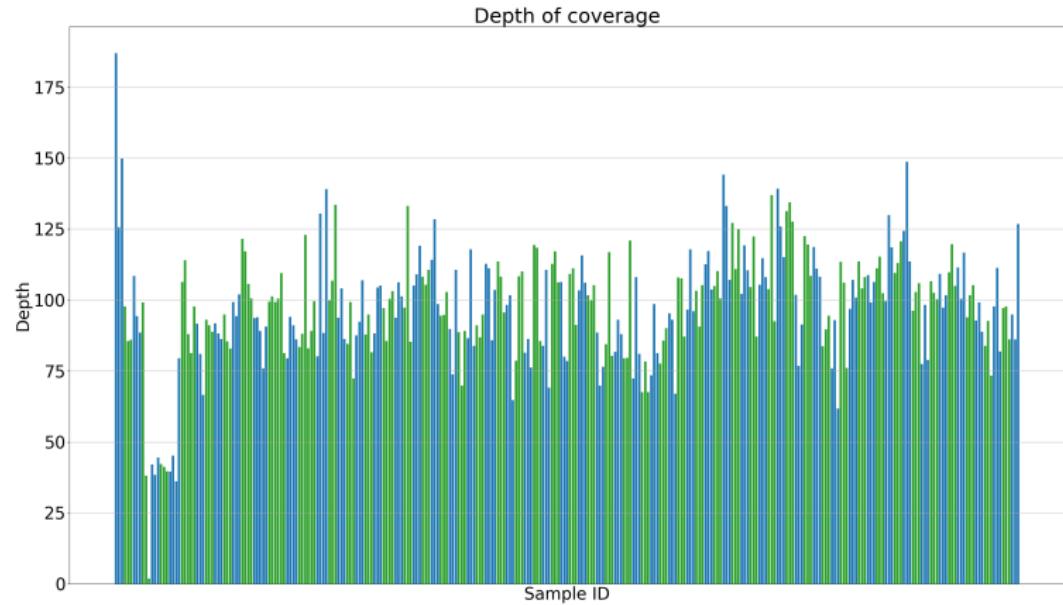


Figure: Coverage Depth Plot

FastQC on WTS

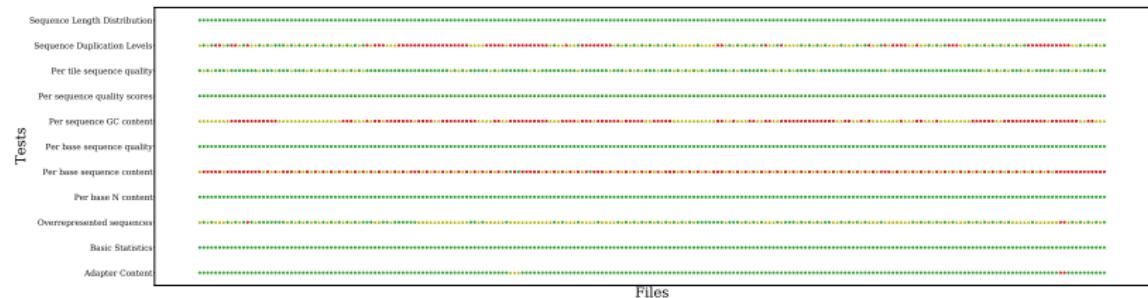


Figure: FastQC with WTS data

All sample are good to analysis

∴ No sample has more than 5 failures.

Results

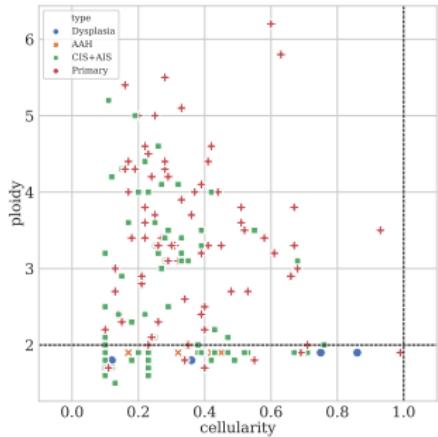
Copy Number Variations

Sequenza?

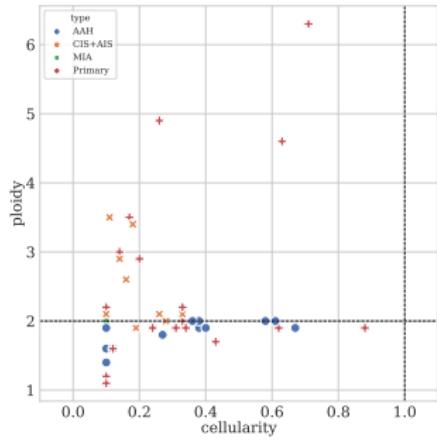


Figure: Representative Output of the Sequenza (Favero et al., 2015)

Cellularity & Ploidy on WES



(a) SQC Samples



(b) ADC Samples

Figure: Cellularity and Ploidy from Sequenza

Genome View on Patient #57

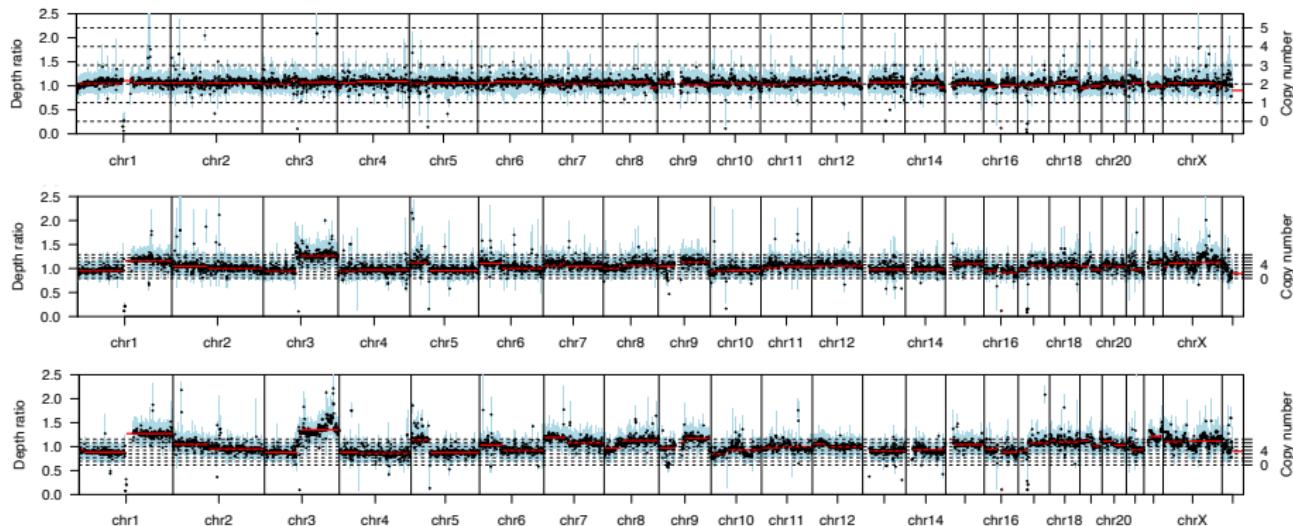


Figure: Dysplasia-CIS-Primary Tumor on Patient #57

CNVs of SQC

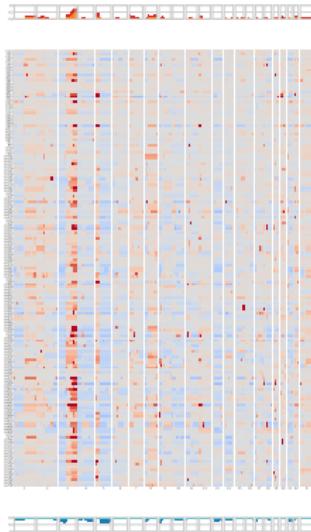


Figure: CNV Plot with SQC Patients

CNVs of ADC

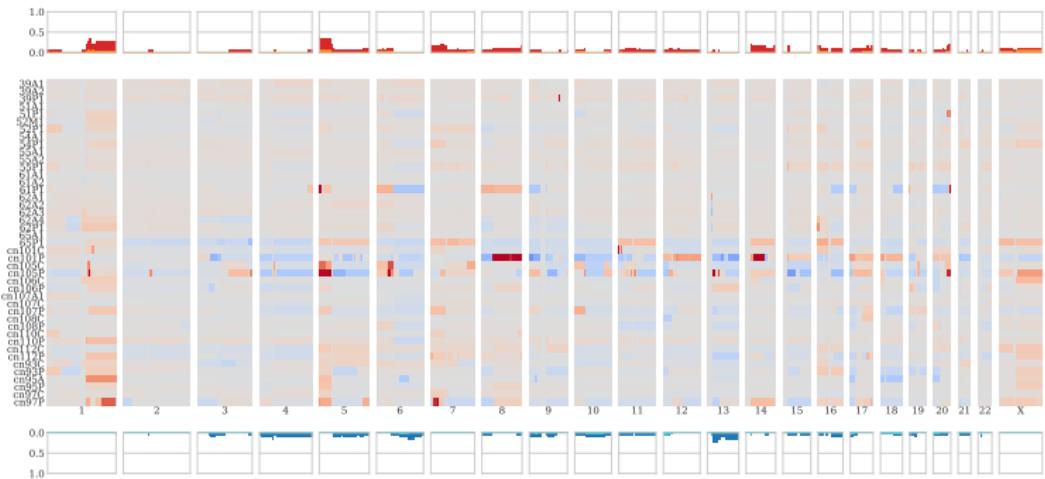


Figure: CNV Plot with ADC Patients

SQC vs. ADC in CNV Plot

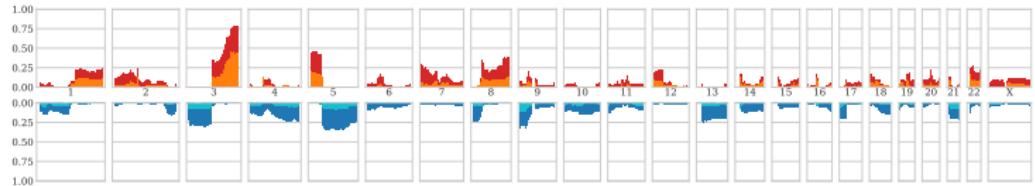


Figure: Simple CNV Plot with SQC Patients

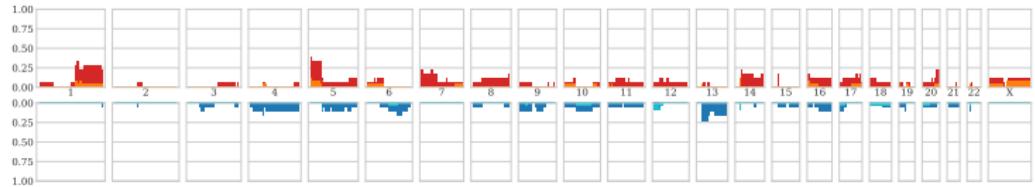


Figure: Simple CNV Plot with ADC Patients

Findings in Sequenza

Results

SNVs Analysis

Mutect2?

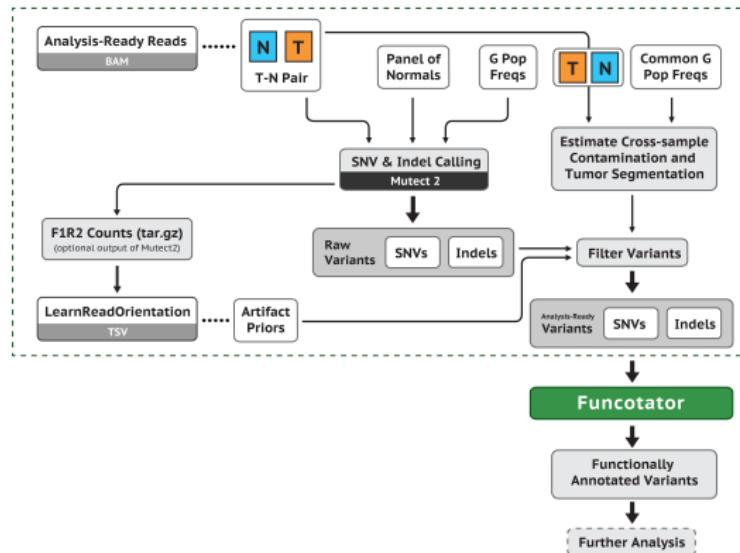
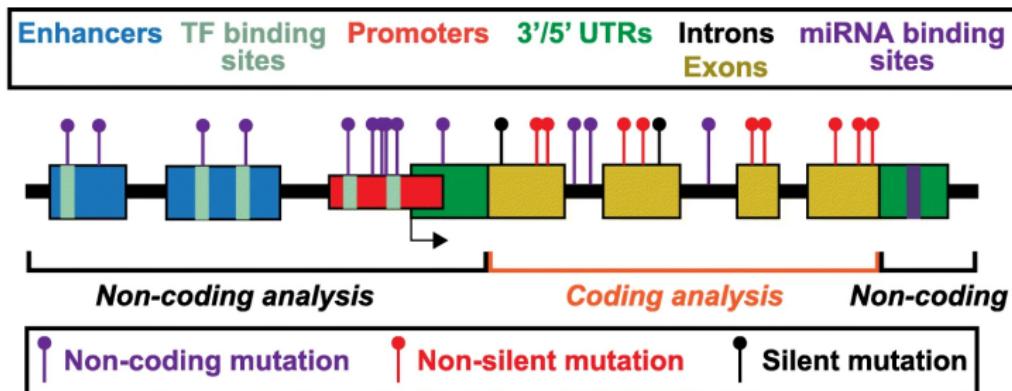


Figure: Somatic short variant discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

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)

Driver Gene Selection Strategy

Fisher FDR

Fisher FDR < 0.05

Fisher P-value

Fisher P-value < 0.05

Gene P-value

Gene P-value < 0.05

Somatic Variant in SQC

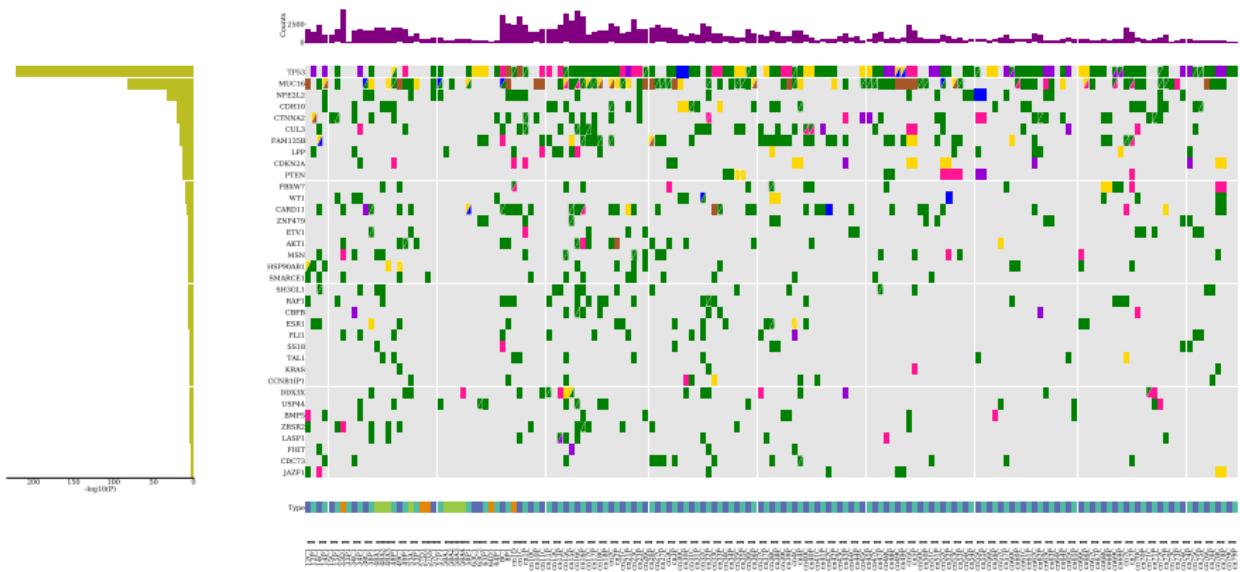


Figure: CoMut Plot with SQC Patients

Somatic Variant in ADC



Figure: CoMut Plot with ADC Patients

Findings in SNVs Analysis

Results

VAF Analysis

VAF?

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

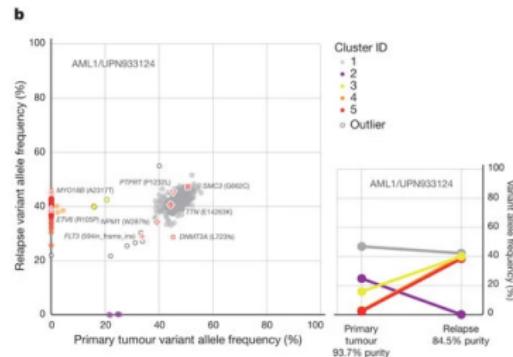


Figure: VAF distribution of validated mutations (Ding et al., 2012)

VAF Plots I

PyClone?

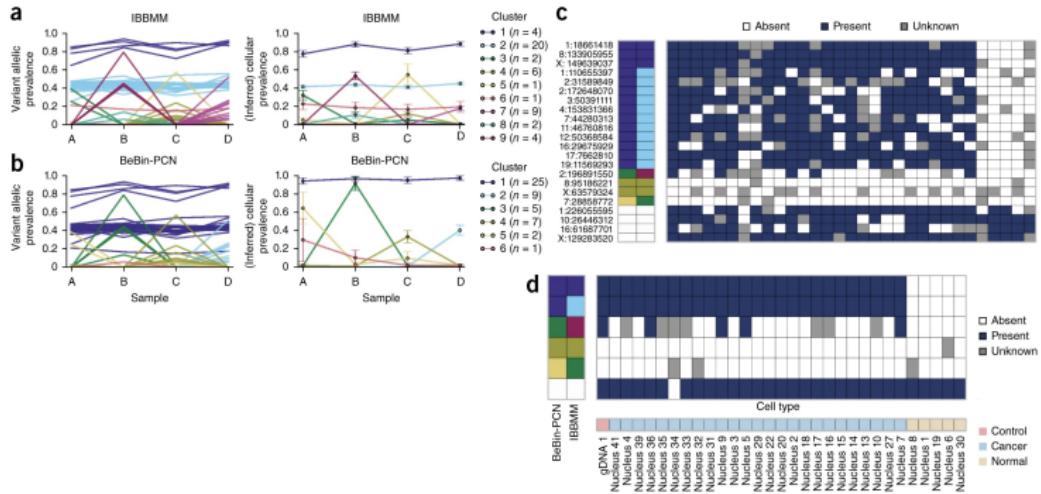


Figure: Analysis of multiple samples by PyClone (Roth et al., 2014)

PyClone Plots I

Findings in VAF Analysis

Results

Tumor Evolution Trajectories Analysis

Revolver?

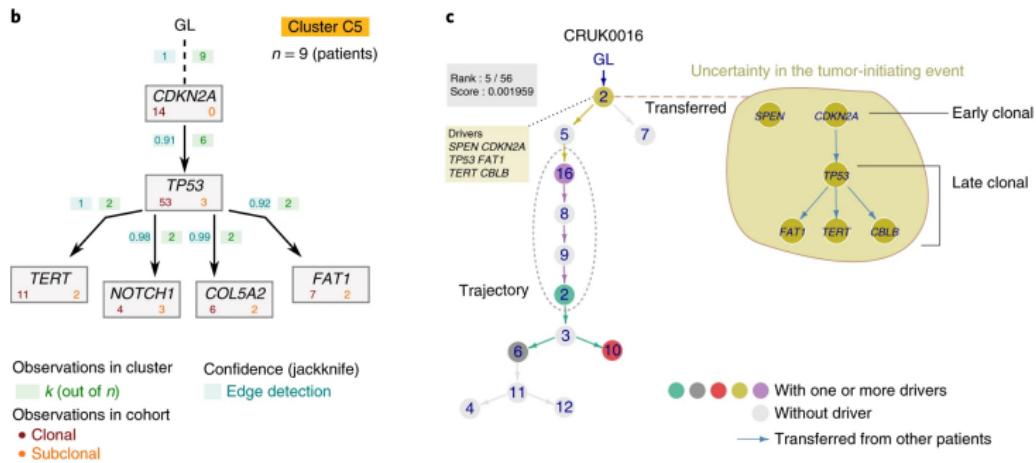


Figure: Repeated Evolutionary Trajectories (Caravagna et al., 2018)

Findings in Tumor Evolution Trajectories Analysis

Results

Differences in Gene Expression Levels

RSEM?

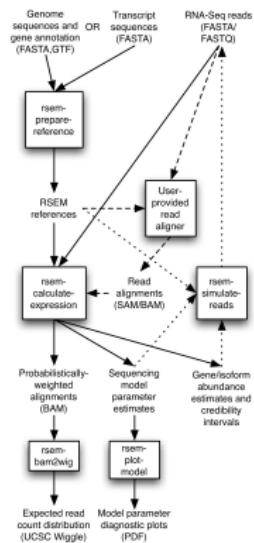


Figure: RSEM workflow (Li & Dewey, 2011)

DESeq2?

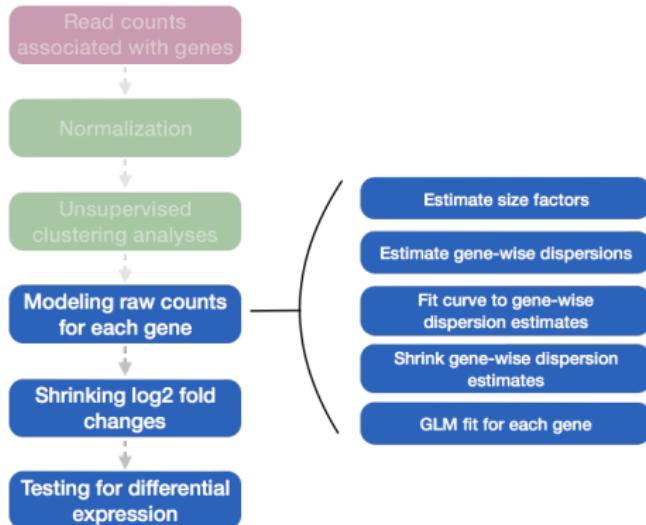


Figure: DESeq2 workflow (Love, Huber, & Anders, 2014)

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

$$P_{adj} < 0.05$$

Enrichr?

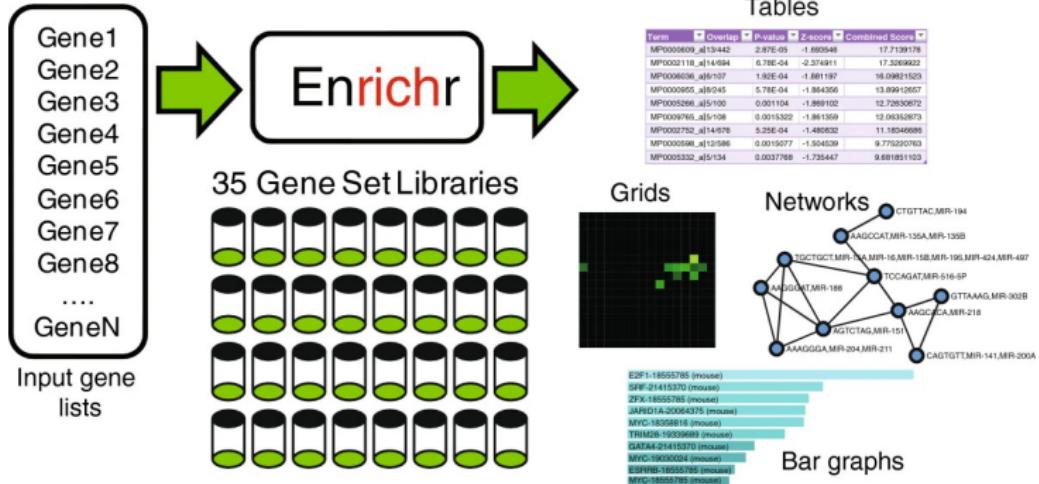


Figure: Enrichr workflow (Chen et al., 2013; Kuleshov et al., 2016)

Gene-set Library

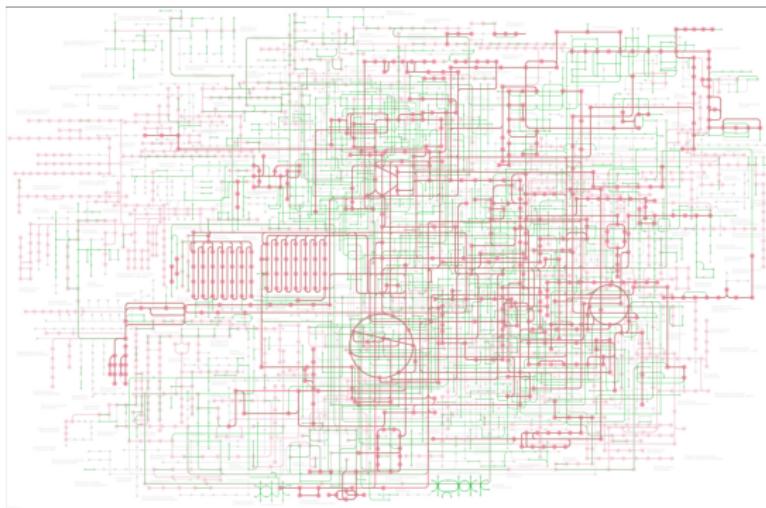


Figure: The global map of metabolic pathways by KEGG (Kanehisa et al., 2021)

KEGG

KEGG 2021 Human

WTS Data Composition I

Table: Number of WTS samples

Cancer Subtype	Stage	Number of Samples
SQC (n=89)	Normal	17
	Dysplasia	2
	CIS	33
	Primary	35
ADC (n=30)	Normal	12
	AAH	1
	AIS	9
	MIA	0
	Primary	8

WTS Data Composition II

Table: Number of WTS SQC samples

Recurrence?	Stage	Number of Samples
Recurrence (n=13)	Normal	1
	Dysplasia	1
	CIS	5
	Primary	6
Non-recurrence (n=74)	Normal	16
	Dysplasia	1
	CIS	28
	Primary	29

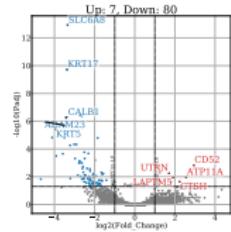
WTS Data Composition III

Table: Number of WTS ADC samples

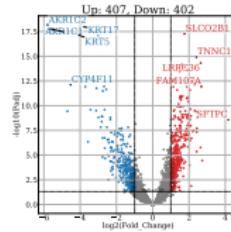
Recurrence?	Stage	Number of samples
Recurrence (n=4)	Normal	1
	AAH	0
	AIS	2
	MIA	0
	Primary	1
Non-recurrence (n=26)	Normal	11
	AAH	1
	AIS	7
	MIA	0
	Primary	7

DEG Volcano Plots in SQC

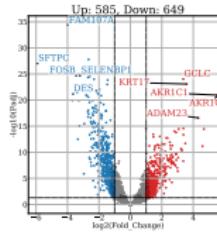
Normal → Dysplasia → CIS → Primary (SQC)



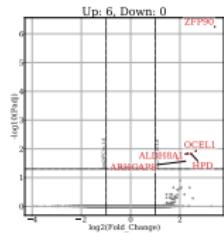
(a) Normal-Dysplasia



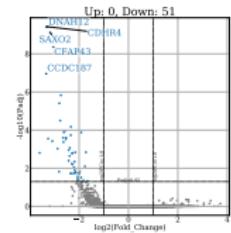
(b) Normal-CIS



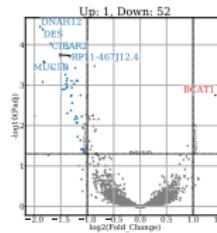
(c) Normal-Primary



(d) Dysplasia-CIS



(e) Dysplasia-Primary

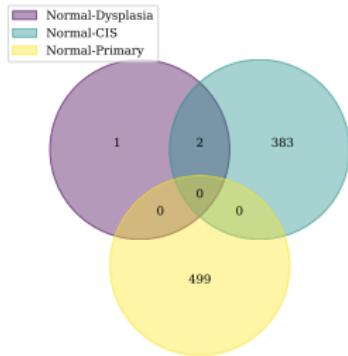


(f) CIS-Primary

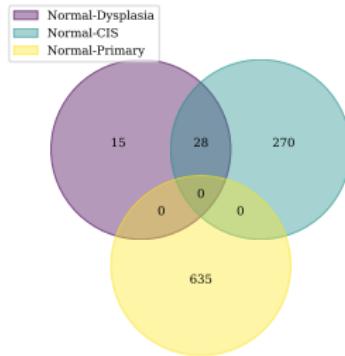
Figure: DEG Volcano Plots in SQC

DEG Venn Diagram with STAR in SQC

Normal → Dysplasia → CIS → Primary (SQC)



(a) Up-regulated



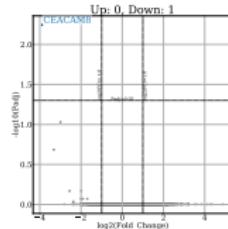
(b) Down-regulated

Figure: DEG Venn Diagram in SQC

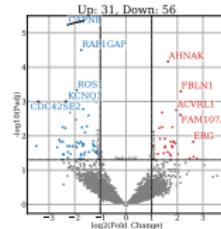
Enrichment test with KEGG in SQC

DEG Volcano Plots with STAR in ADC

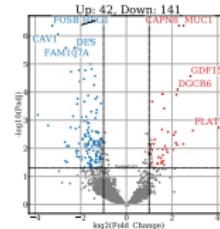
Normal → AAH → AIS → MIA (★ No WTS) → Primary (ADC)



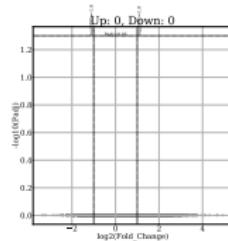
(a) Normal-AAH



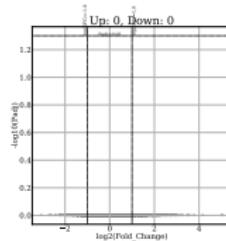
(b) Normal-AIS



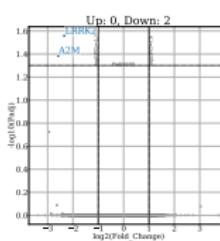
(c) Normal-Primary



(d) AAH-AIS



(e) AAH-Primary

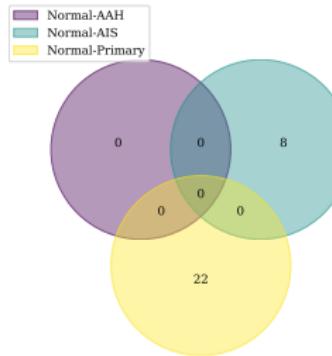


(f) AIS-Primary

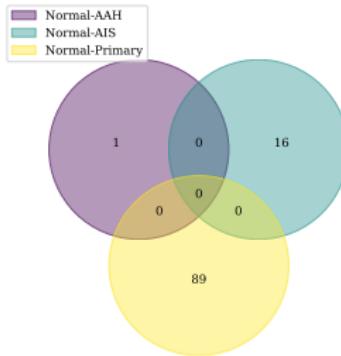
Figure: DEG Volcano Plots in ADC

DEG Venn Diagram with Bowtie2 in ADC

Normal → AAH → AIS → MIA (★ No WTS) → Primary (ADC)



(a) Up-regulated



(b) Down-regulated

Figure: DEG Venn Diagram in ADC

Enrichment test with KEGG in ADC

Findings in DEG Analysis

Results

Bulk Cell Deconvolution

Single-cell data as Reference

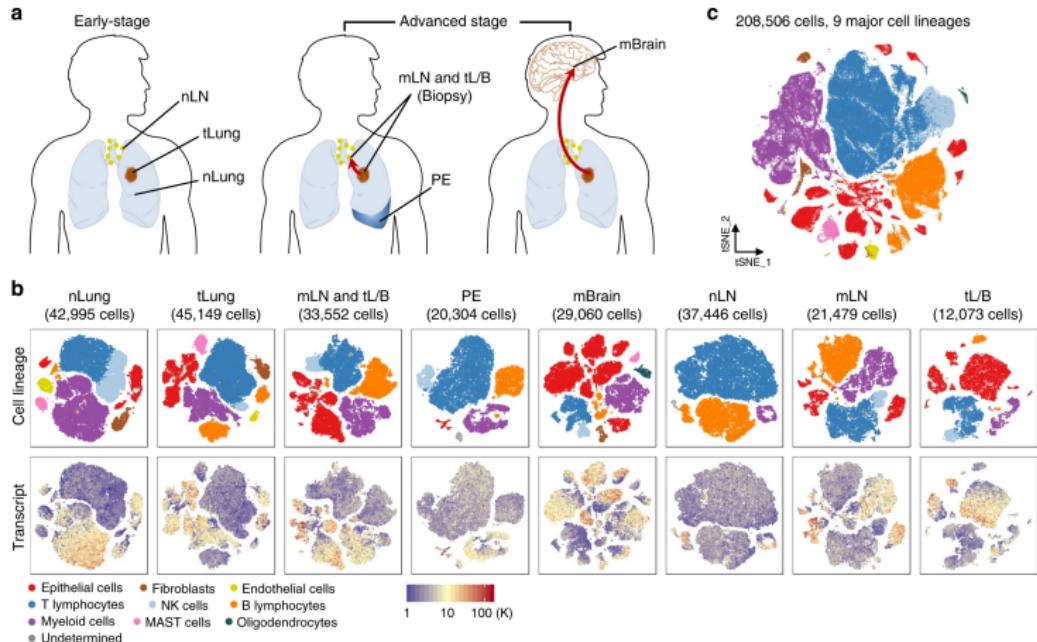


Figure: Comprehensive dissection and clustering of 208,506 single cells from LUAD patients (Kim et al., 2020)

MuSiC?

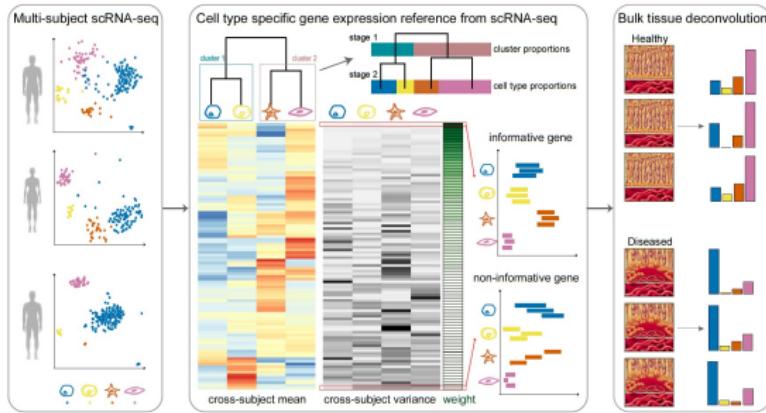


Figure: Workflow for MuSiC (X. Wang, Park, Susztak, Zhang, & Li, 2019)

Cluster Plot with STAR in SQC

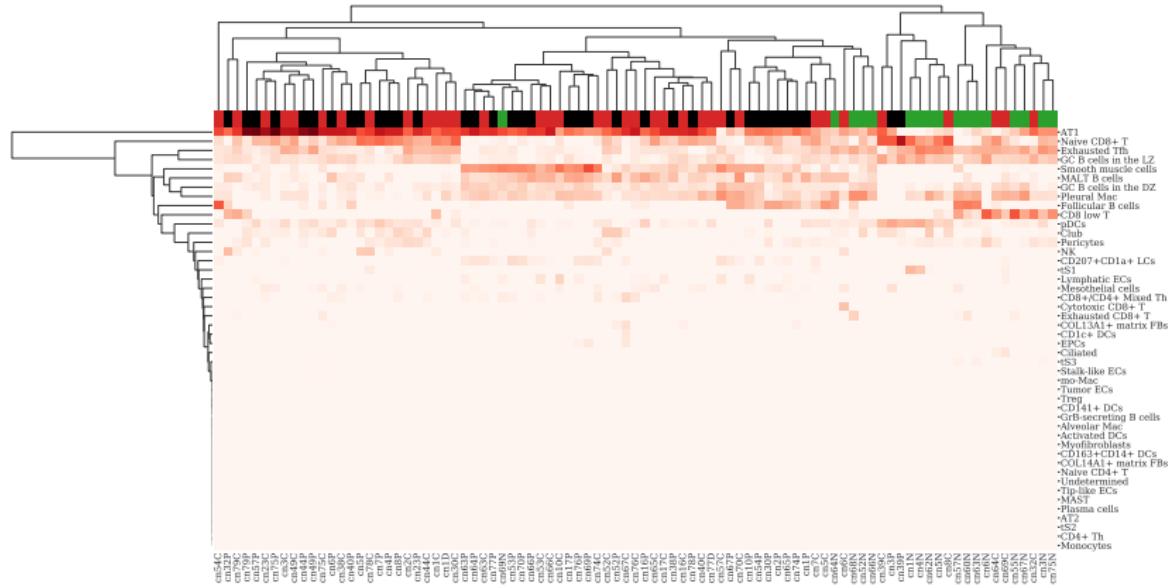


Figure: Cluster Plot in SQC

Cluster Plot with STAR in ADC

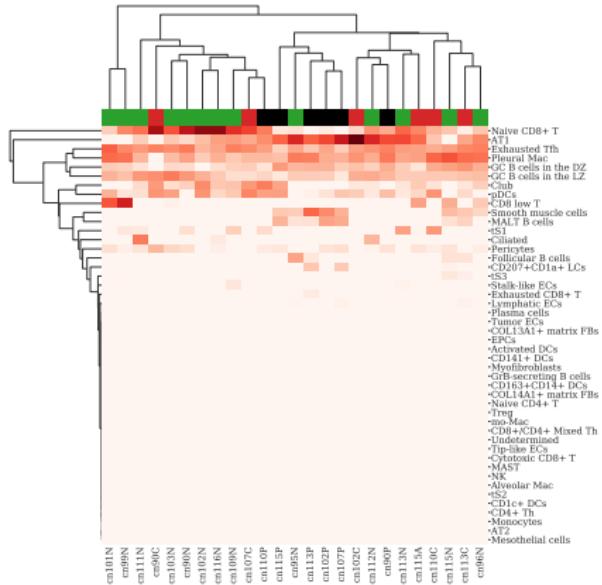


Figure: Cluster Plot in ADC

Findings in Bulk Cell Deconvolution

Results

Discovery of Gene Fusion

Arriba?

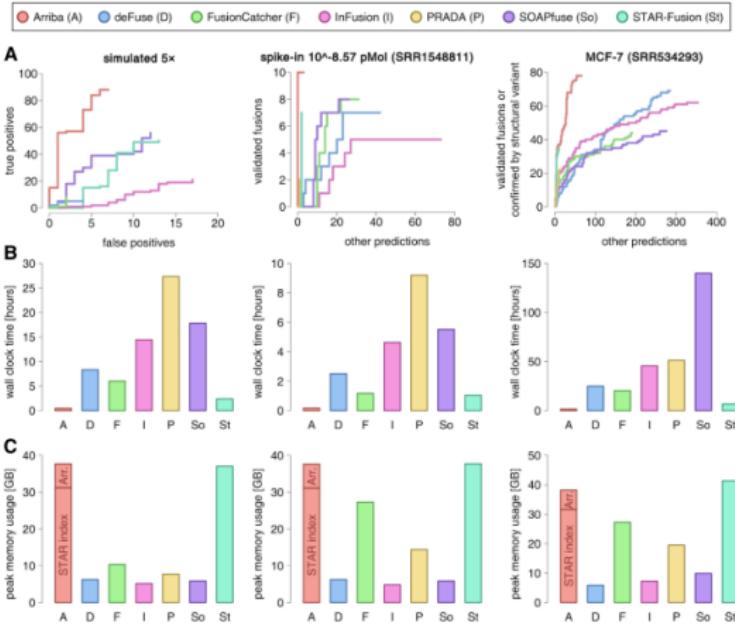


Figure: Benchmark of Arriba versus alternative methods (Uhrig et al., 2021)

Findings in Gene Fusion Discovery

Discussion

References

References I

- Andrews, S., Krueger, F., Segonds-Pichon, A., Biggins, L., Krueger, C., & Wingett, S. (2012, January). *FastQC*. Babraham Institute. Babraham, UK.
- Caravagna, G., Giarratano, Y., Ramazzotti, D., Tomlinson, I., Graham, T. A., Sanguinetti, G., & Sottoriva, A. (2018). Detecting repeated cancer evolution from multi-region tumor sequencing data. *Nature methods*, 15(9), 707–714.
- Chen, E. Y., Tan, C. M., Kou, Y., Duan, Q., Wang, Z., Meirelles, G. V., ... Ma'ayan, A. (2013). Enrichr: interactive and collaborative html5 gene list enrichment analysis tool. *BMC bioinformatics*, 14(1), 1–14.
- Collins, L. G., Haines, C., Perkel, R., & Enck, R. E. (2007). Lung cancer: diagnosis and management. *American family physician*, 75(1), 56–63.

References II

- 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.
- Ding, L., Ley, T. J., Larson, D. E., Miller, C. A., Koboldt, D. C., Welch, J. S., ... others (2012). Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing. *Nature*, 481(7382), 506–510.
- 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.

References III

- Hong, S., Won, Y.-J., Lee, J. J., Jung, K.-W., Kong, H.-J., Im, J.-S., ... others (2021). Cancer statistics in korea: Incidence, mortality, survival, and prevalence in 2018. *Cancer Research and Treatment: Official Journal of Korean Cancer Association*, 53(2), 301.
- Kanehisa, M., Furumichi, M., Sato, Y., Ishiguro-Watanabe, M., & Tanabe, M. (2021). Kegg: integrating viruses and cellular organisms. *Nucleic acids research*, 49(D1), D545–D551.
- Kim, N., Kim, H. K., Lee, K., Hong, Y., Cho, J. H., Choi, J. W., ... others (2020). Single-cell rna sequencing demonstrates the molecular and cellular reprogramming of metastatic lung adenocarcinoma. *Nature communications*, 11(1), 1–15.
- Kuleshov, M. V., Jones, M. R., Rouillard, A. D., Fernandez, N. F., Duan, Q., Wang, Z., ... others (2016). Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic acids research*, 44(W1), W90–W97.

References IV

- 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.
- 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.
- Minna, J. D., Roth, J. A., & Gazdar, A. F. (2002). Focus on lung cancer. *Cancer cell*, 1(1), 49–52.
- Roth, A., Khattra, J., Yap, D., Wan, A., Laks, E., Biele, J., ... Shah, S. P. (2014). Pyclone: statistical inference of clonal population structure in cancer. *Nature methods*, 11(4), 396–398.
- Soltis, A. R., Dalgard, C. L., Pollard, H. B., & Wilkerson, M. D. (2020). Mutenricher: a flexible toolset for somatic mutation enrichment analysis of tumor whole genomes. *BMC bioinformatics*, 21(1), 1–8.

References V

- Travis, W. D. (2002). Pathology of lung cancer. *Clinics in chest medicine*, 23(1), 65–81.
- Uhrig, S., Ellermann, J., Walther, T., Burkhardt, P., Fröhlich, M., Hutter, B., ... others (2021). Accurate and efficient detection of gene fusions from rna sequencing data. *Genome research*, 31(3), 448–460.
- 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.
- Vincent, R. G., Pickren, J. W., Lane, W. W., Bross, I., Takita, H., Houten, L., ... Rzepka, T. (1977). The changing histopathology of lung cancer. a review of 1682 cases. *Cancer*, 39(4), 1647–1655.

References VI

- Wang, B.-Y., Huang, J.-Y., Chen, H.-C., Lin, C.-H., Lin, S.-H., Hung, W.-H., & Cheng, Y.-F. (2020). The comparison between adenocarcinoma and squamous cell carcinoma in lung cancer patients. *Journal of cancer research and clinical oncology*, 146(1), 43–52.
- Wang, X., Park, J., Susztak, K., Zhang, N. R., & Li, M. (2019). Bulk tissue cell type deconvolution with multi-subject single-cell expression reference. *Nature communications*, 10(1), 1–9.