Lung Precancer Study

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Overview

1. Introduction

1. Introduction

1.1. Lung Cancer

Lung Cancer?

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

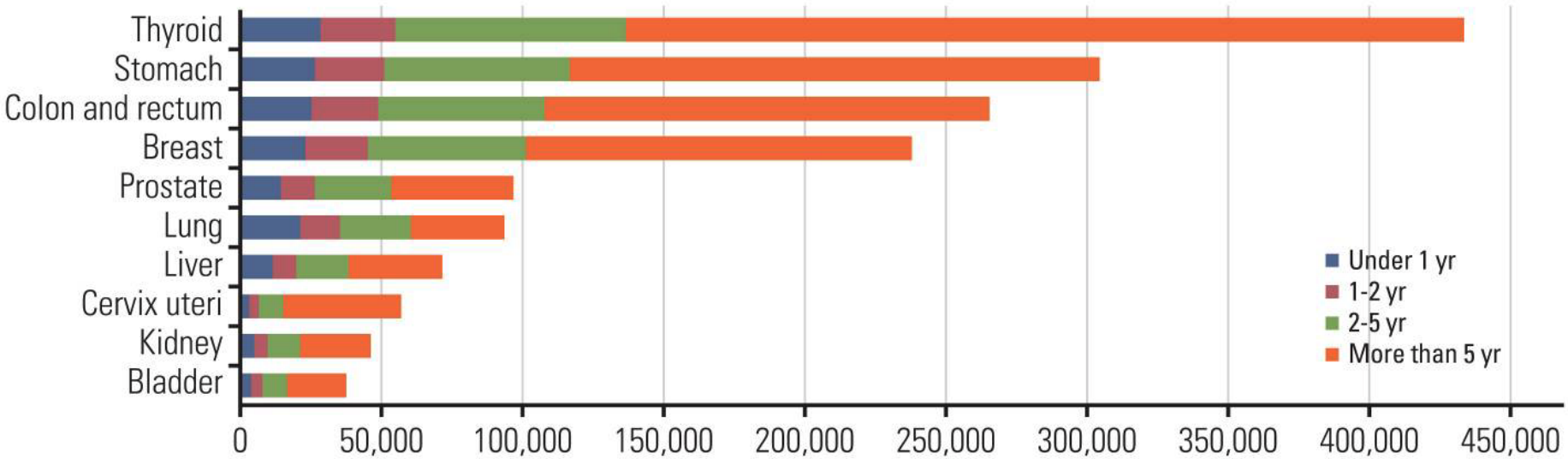


Figure: Common cancer survival rates (Hong et al., 2021)

Survival rate (More than 5 yr)

- Tyroid: 68.4 %
- Lung: 35.4 %

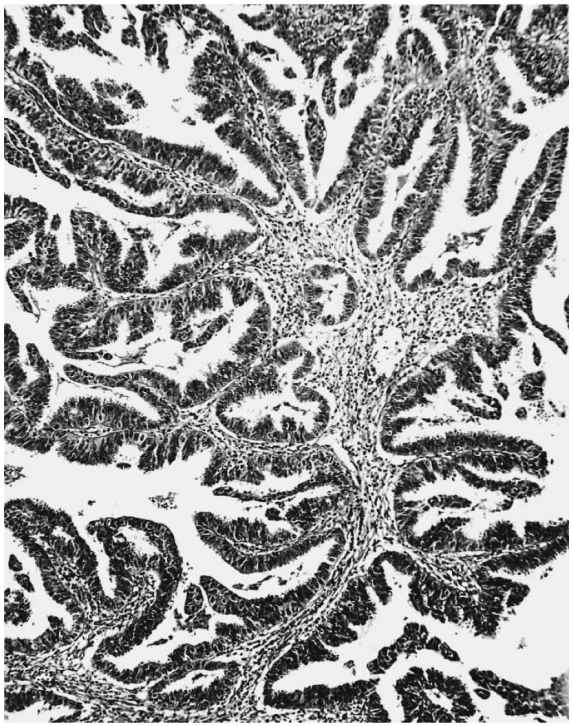
Type of Lung Cancer

Types of lung cancer:

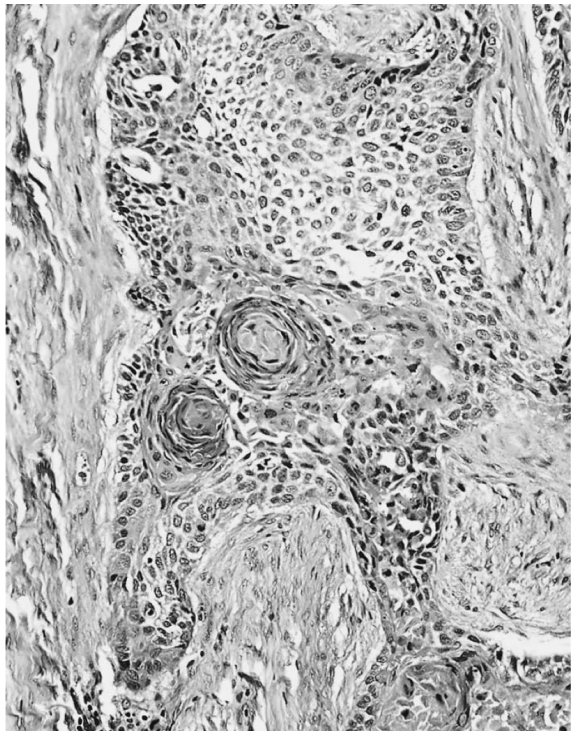
1. Adenocarcinoma (LUAD) (40 %) ★
2. Squamous cell carcinoma (LUSC) (25 %) ★
3. Small cell carcinoma (20 %)
4. Large cell carcinoma (10 %)
5. Adenosquamous carcinoma (< 5 %)
6. Carcinoid (< 5 %)
7. Bronchioalveolar (Bronchial gland carcinoma)

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

LUAD vs. LUSC I



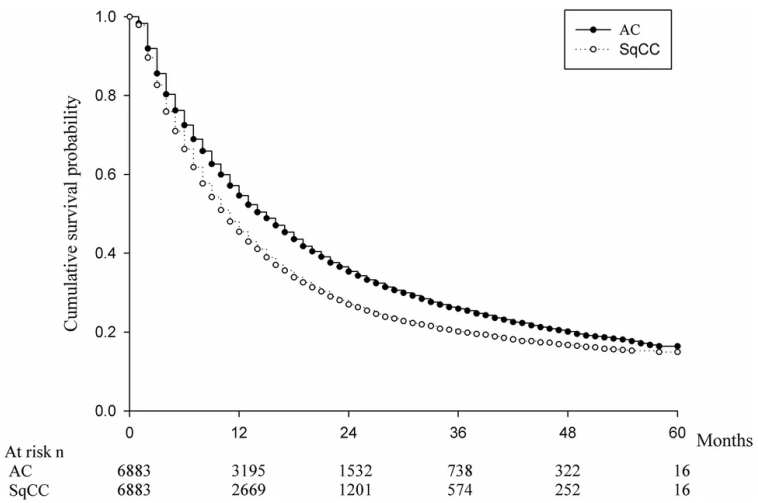
(a) LUAD



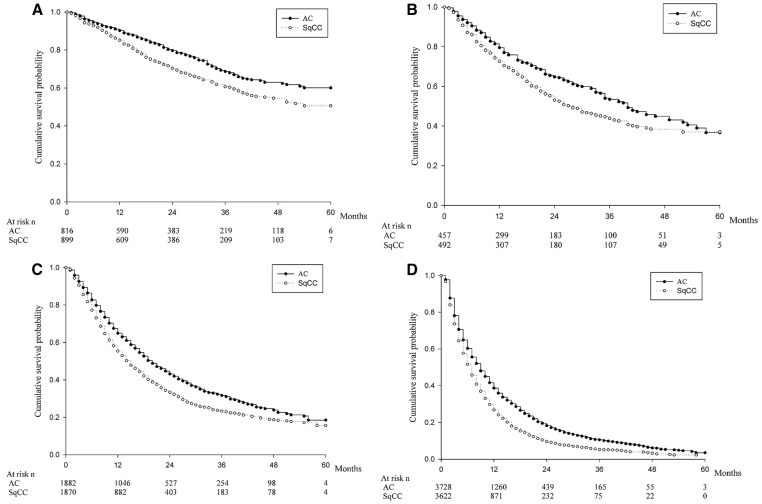
(b) LUSC

Figure: LUAD and LUSC histology in Lung cancer (Travis, 2002)

LUAD vs. LUSC II



(a) All patients



(b) By cancer stages

Figure: Kaplan-Meiere survival curves for LUAD & LUSC (Wang et al., 2020)

Findings

LUSC is more dangerous than LUAD. $\because p < 0.001$

1. Introduction

1.2. 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

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

3. Methods

3. Methods

3.1. Workflows

Data pre-processing for variant discovery

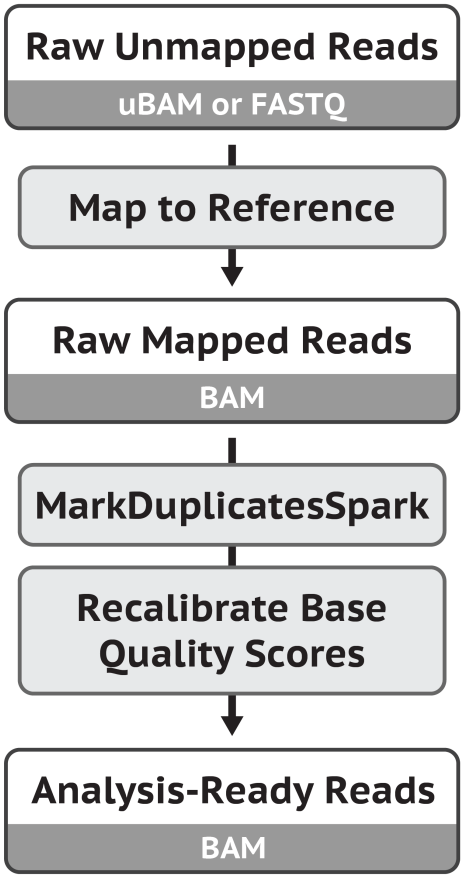


Figure: Data pre-processing for variant discovery (Van der Auwera et al., 2013; DePristo et al., 2011)

Somatic short variant discovery

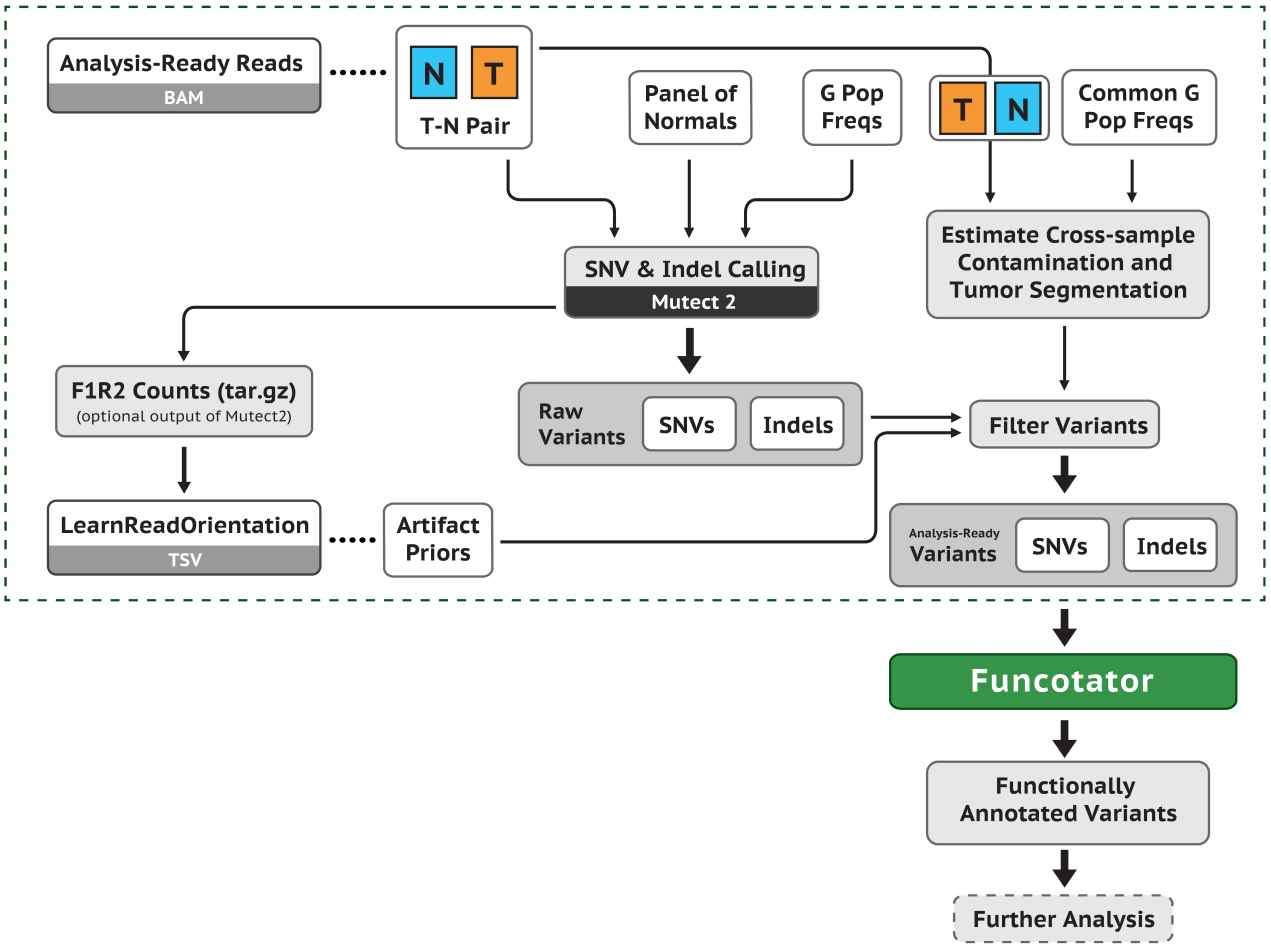


Figure: Somatic short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

Germline short variant discovery

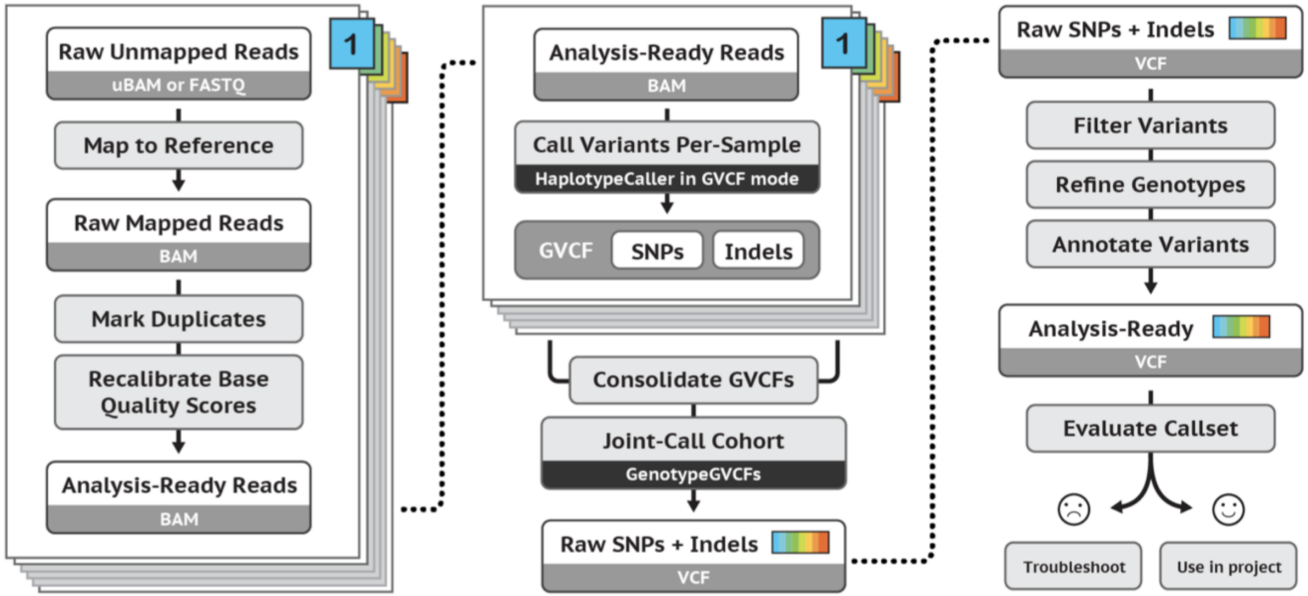


Figure: Germline short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

RNA-seq short variant discovery

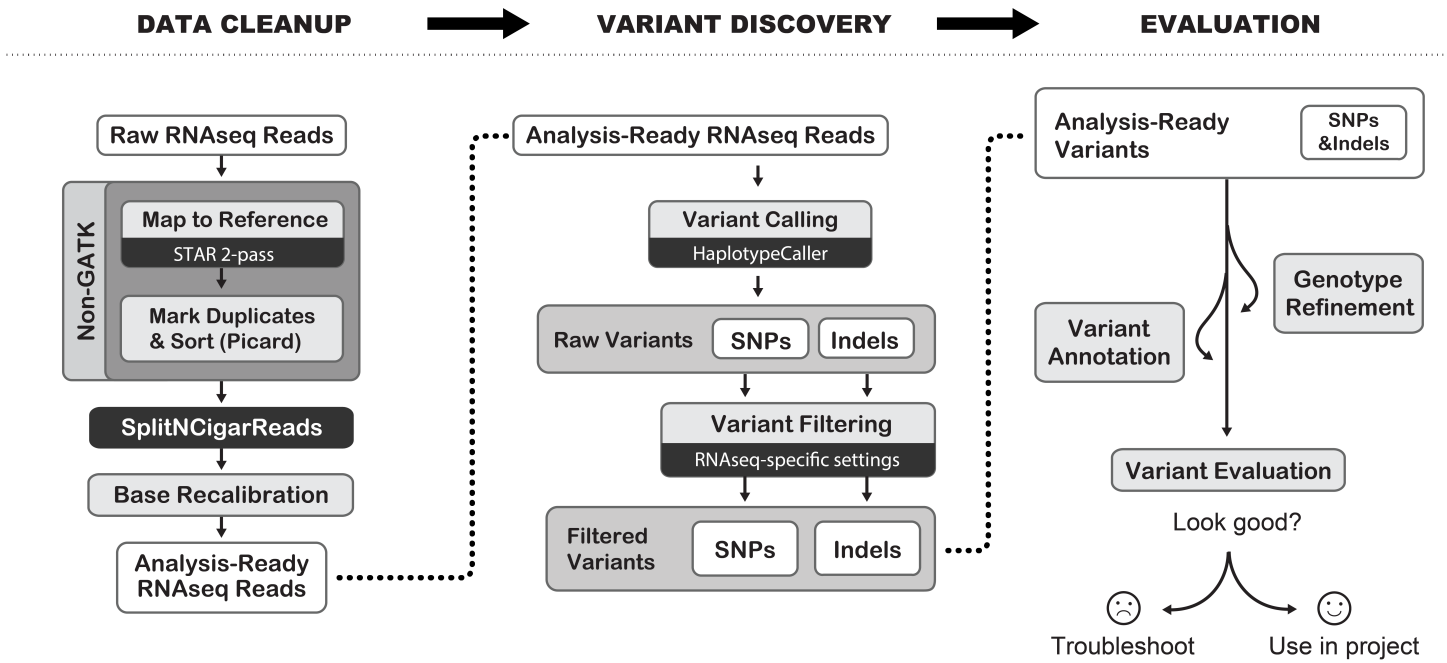


Figure: RNA-seq short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

4. Results

4. Results

4.1. Quality Checks

FastQC?

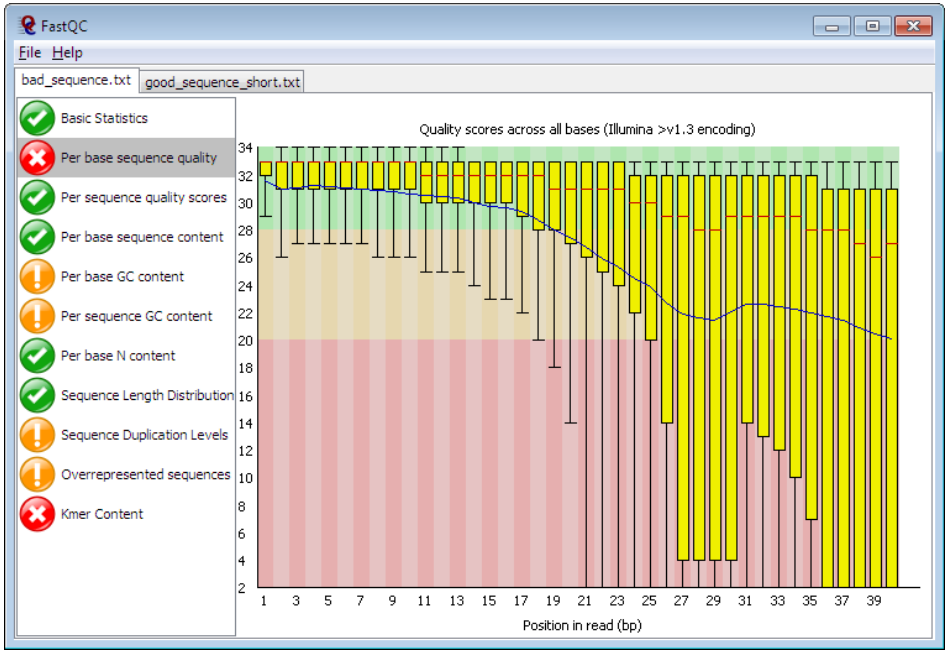


Figure: Example of FastQC Result (Andrews et al., 2012)

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

FastQC on WES

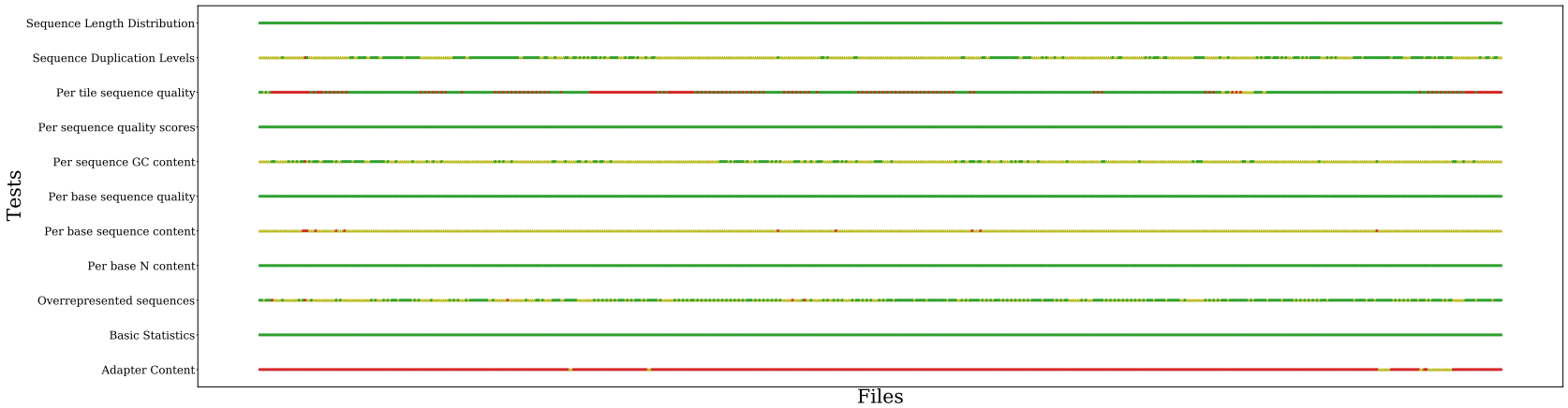
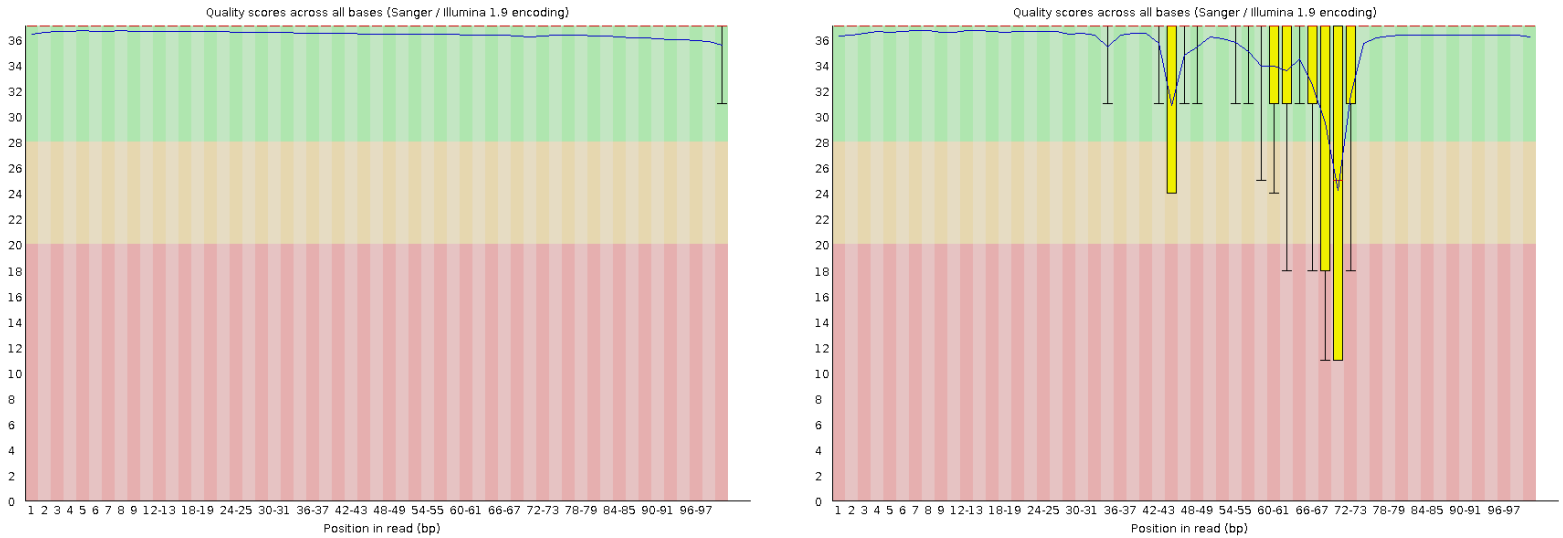


Figure: FastQC with WES data

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

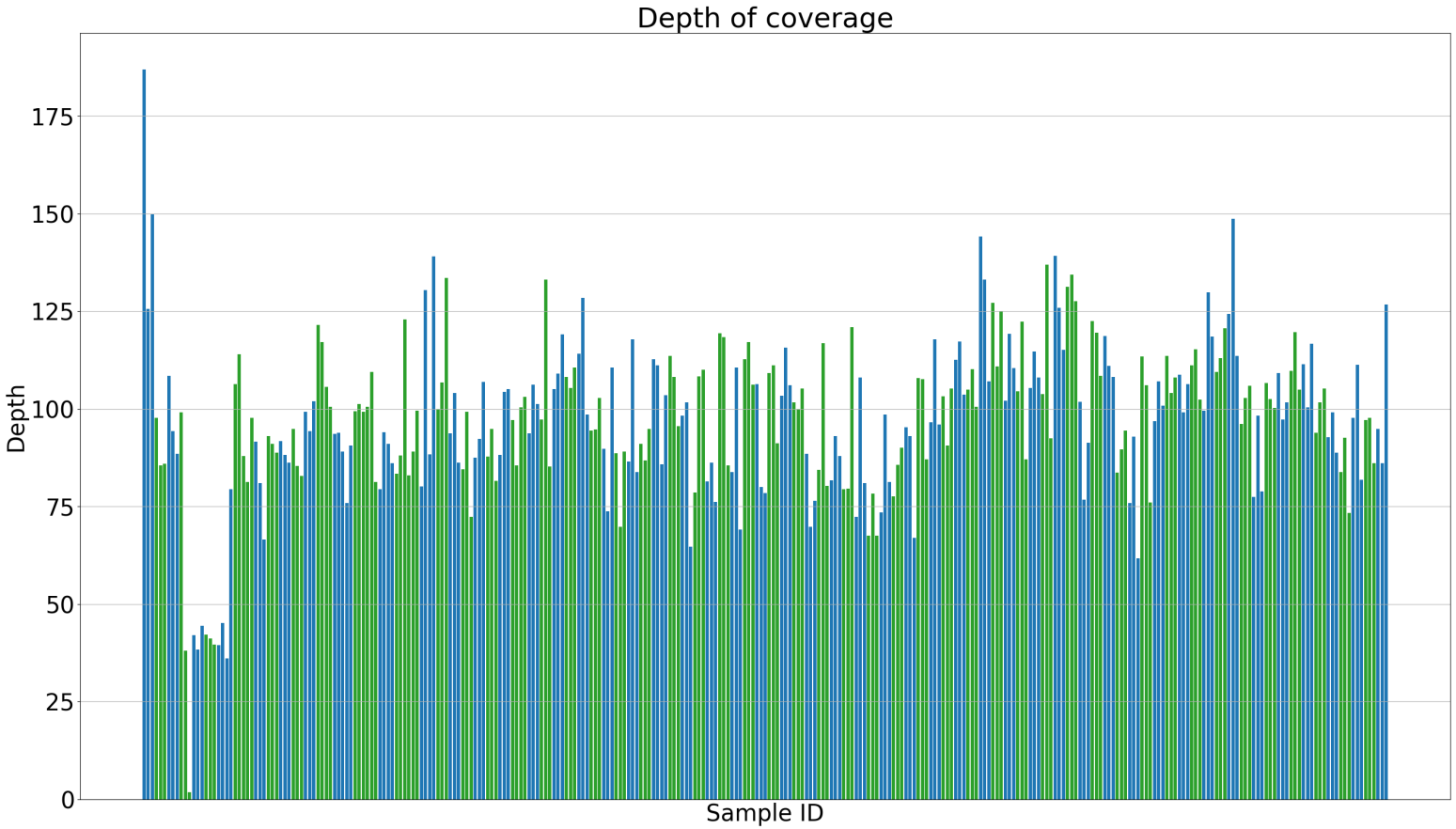


Figure: Coverage Depth Plot

FastQC on WTS

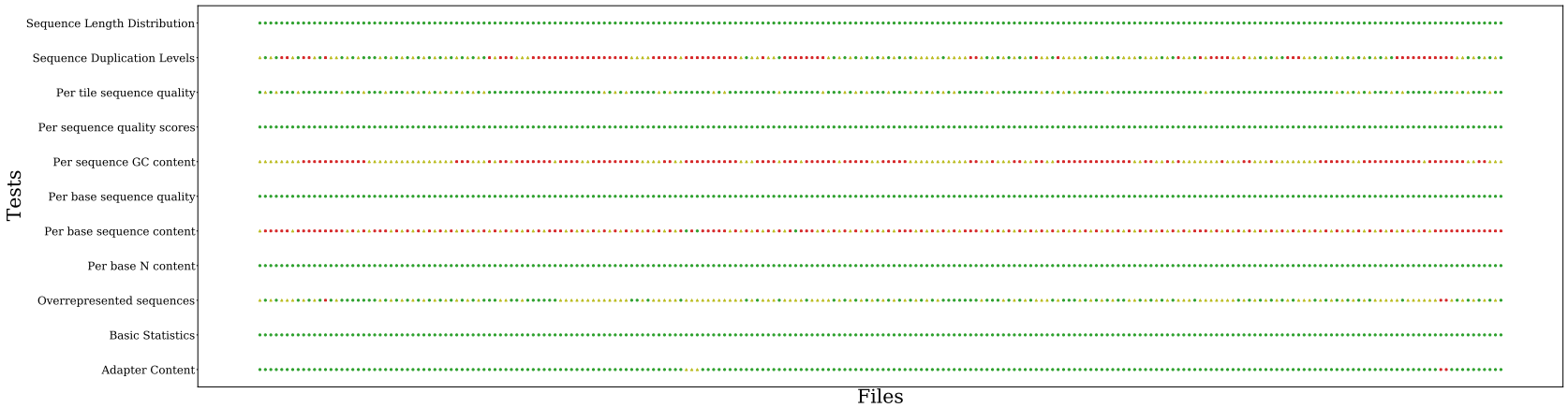


Figure: FastQC with WTS data

All sample are good to analysis

∵ No sample has more than 5 failures.

4. Results

4.2. Copy Number Variations

Sequenza?

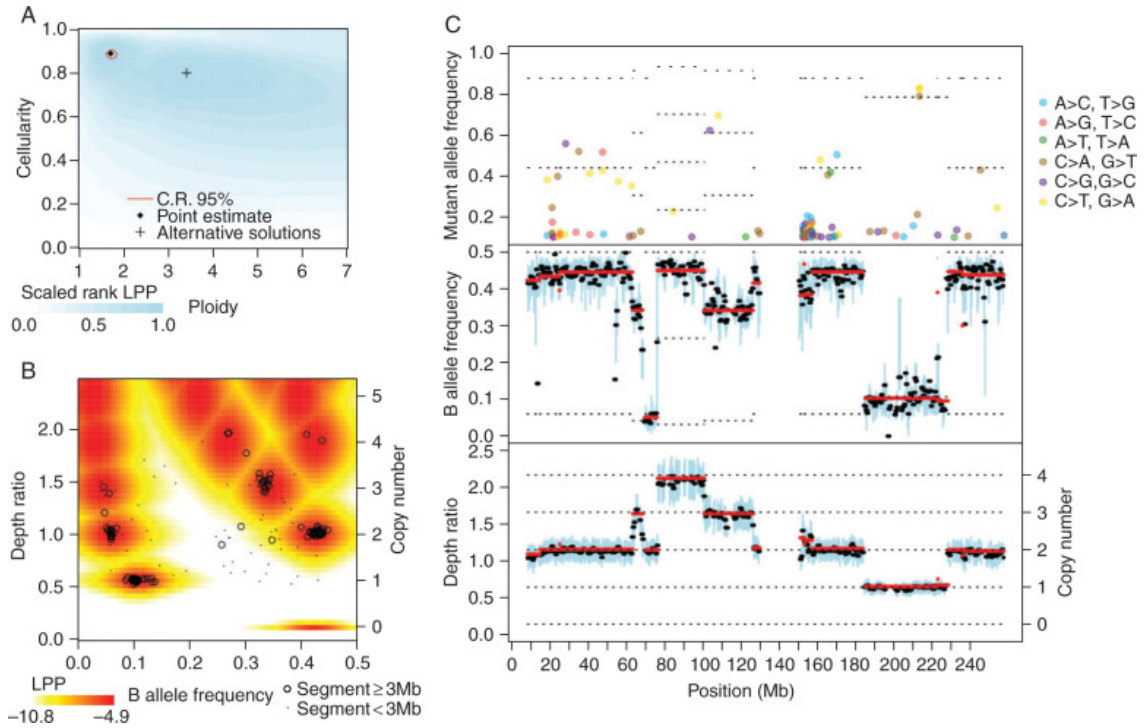
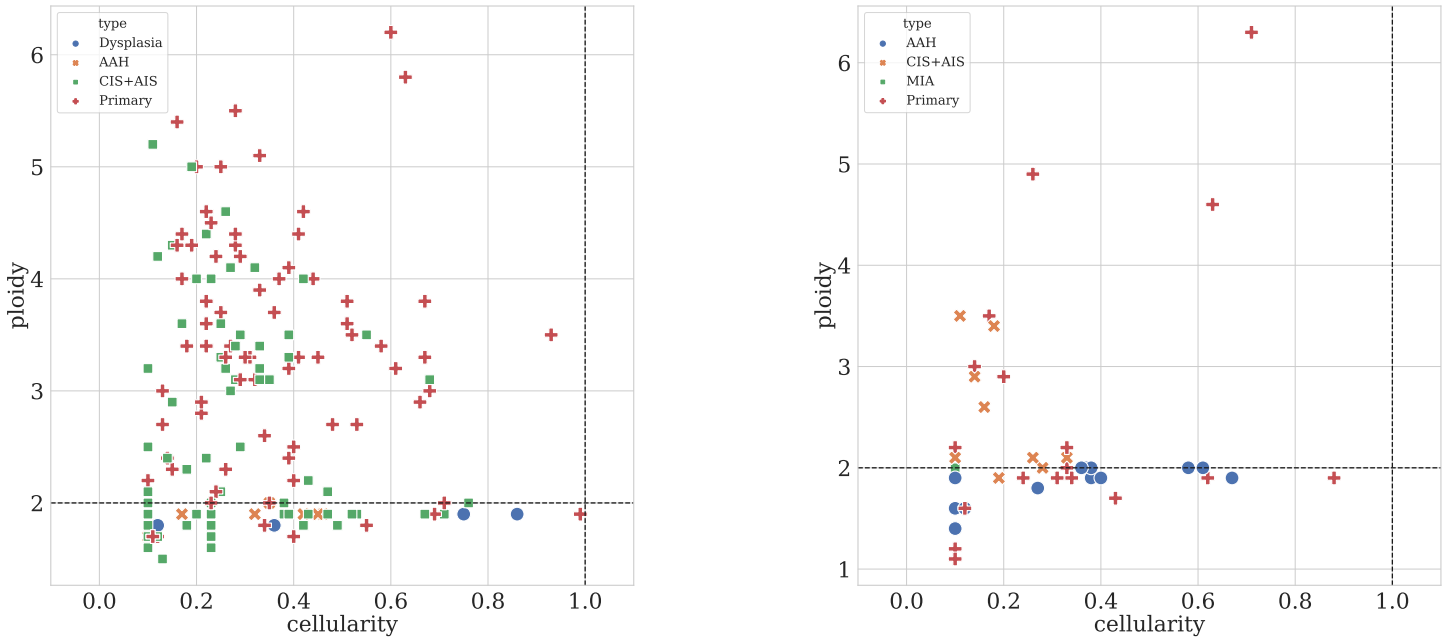


Figure: Representative Output of the Sequenza (Favero et al., 2015)

Cellularity & Ploidy on WES



(a) LUSC Samples (b) LUAD Samples

Figure: Cellularity and Ploidy from Sequenza

Genome View on Patient #57

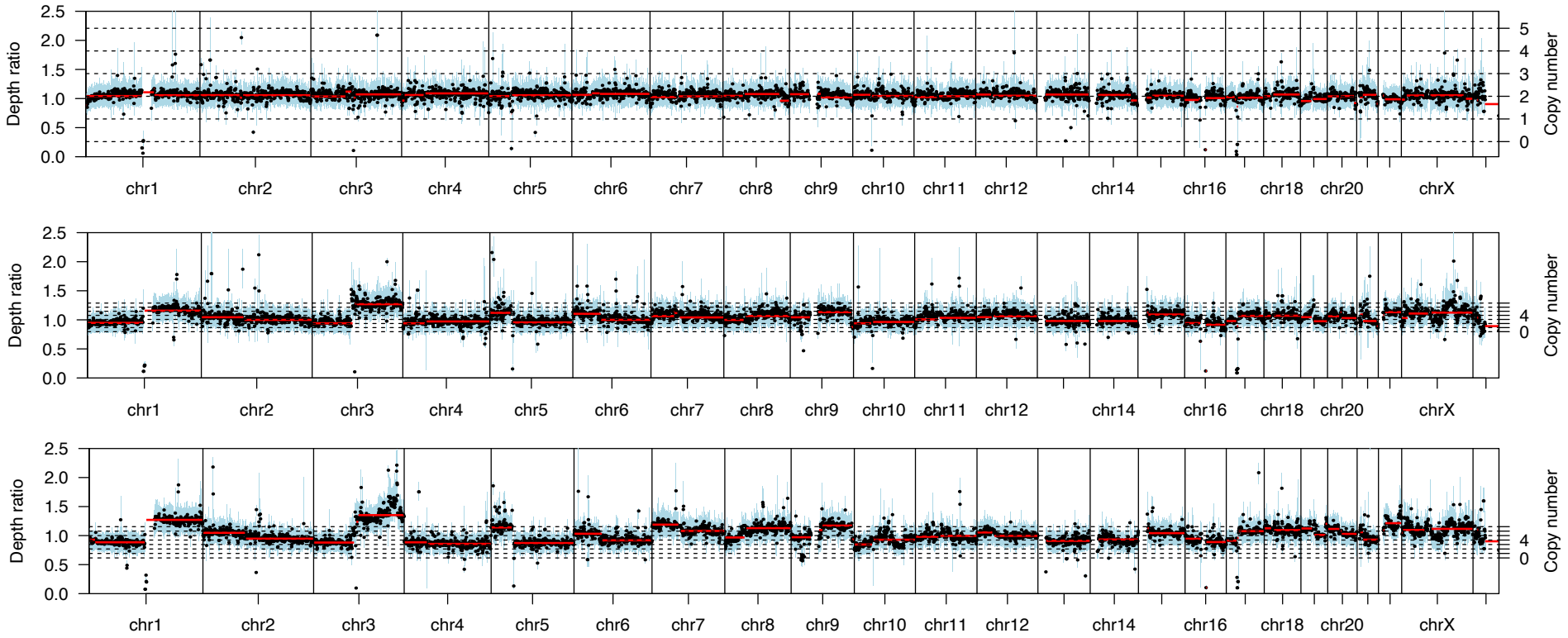


Figure: Dysplasia-CIS-Primary Tumor on Patient #57

CNVs of LUSC

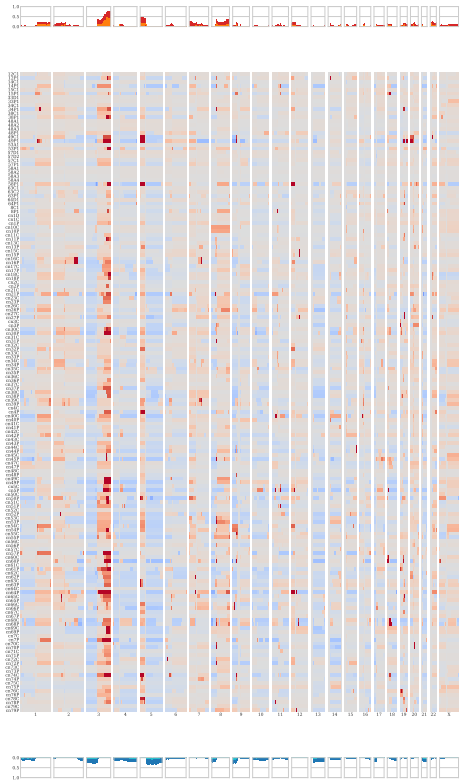


Figure: CNV Plot with LUSC Patients

CNVs of LUAD

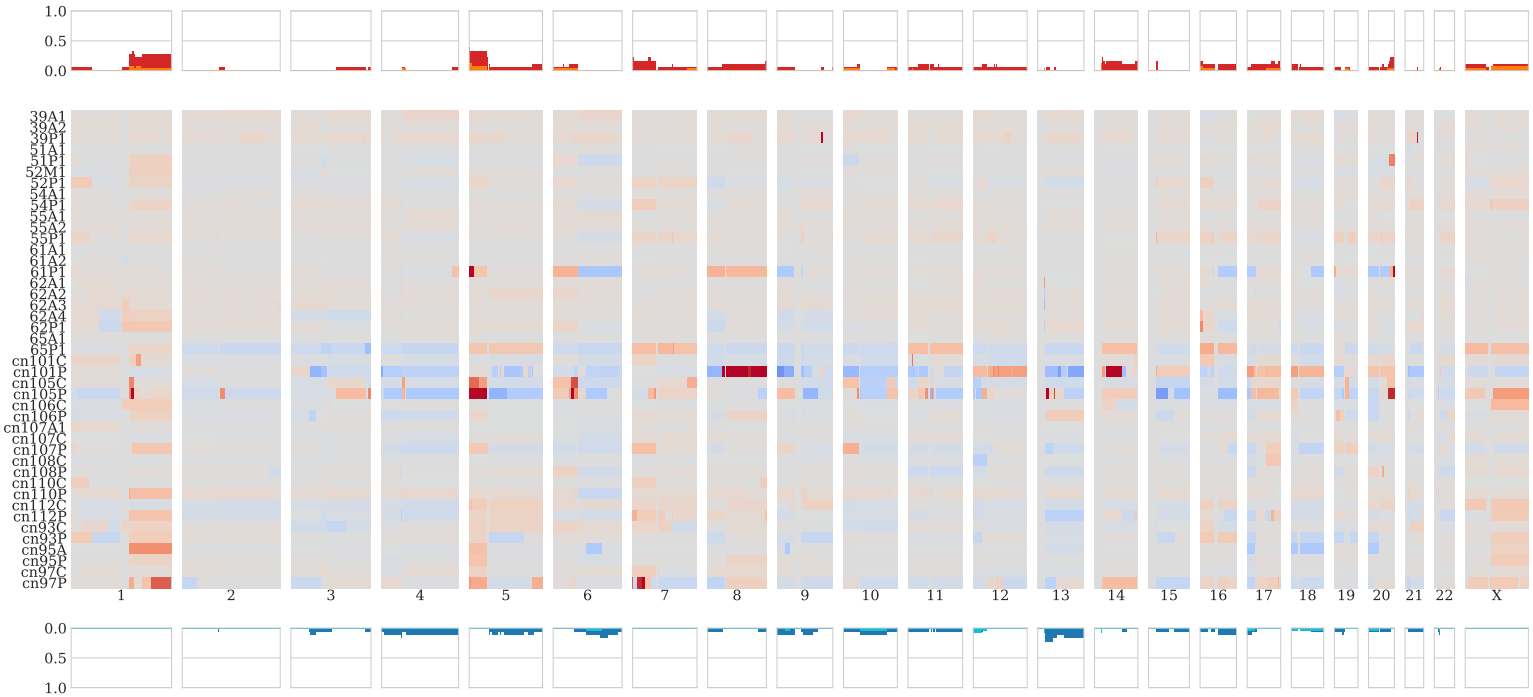


Figure: CNV Plot with LUAD Patients

LUSC vs. LUAD in CNV Plot

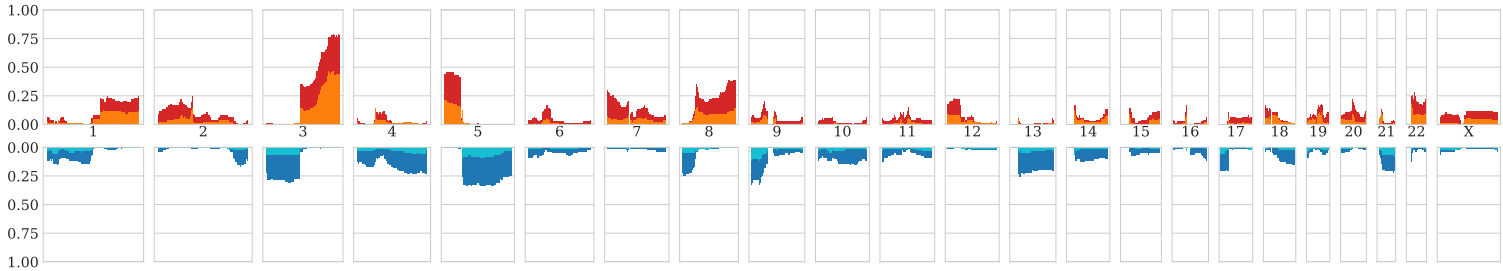


Figure: Simple CNV Plot with LUSC Patients

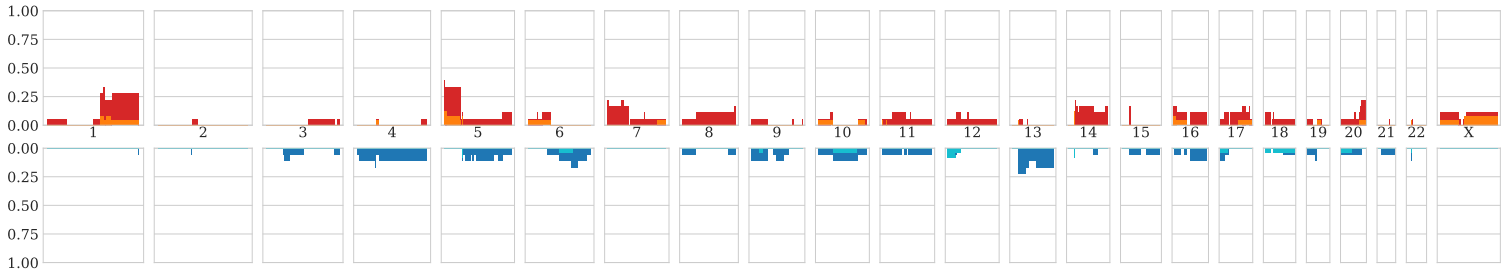


Figure: Simple CNV Plot with LUAD Patients

Findings in Sequenza

4. Results

4.3. SNVs Analysis

Mutect2?

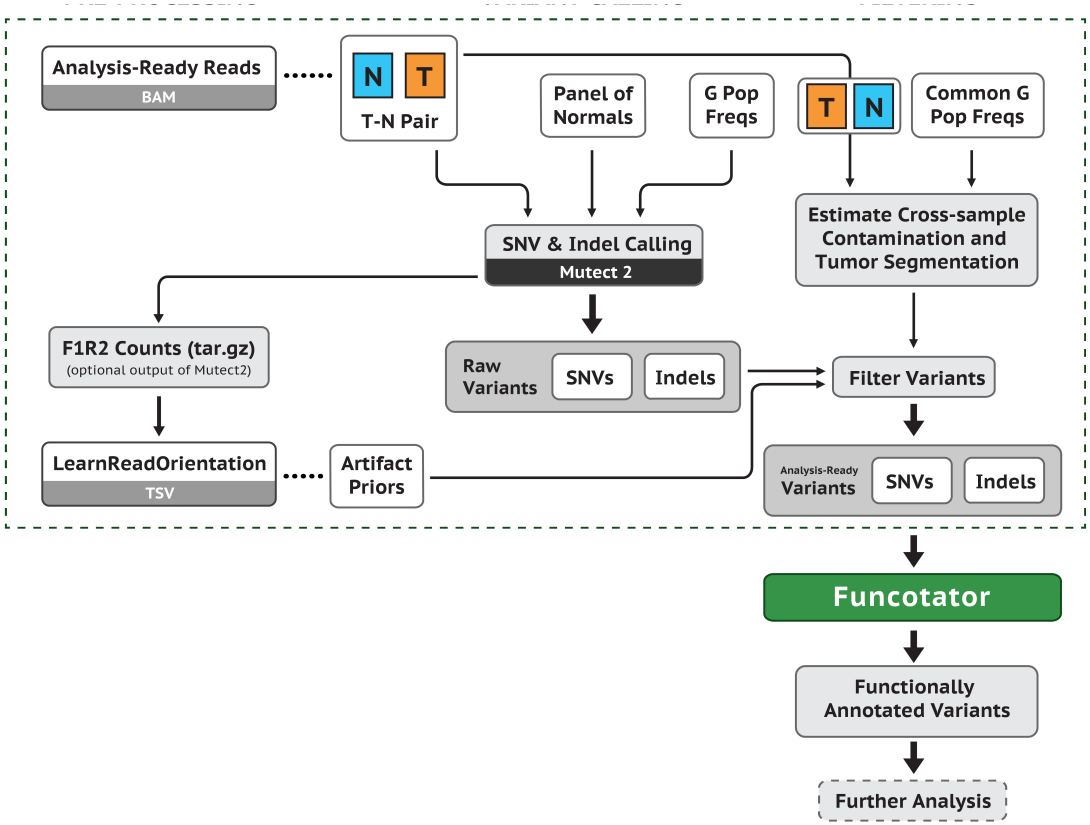


Figure: Somatic short variant discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

MutEnricher?

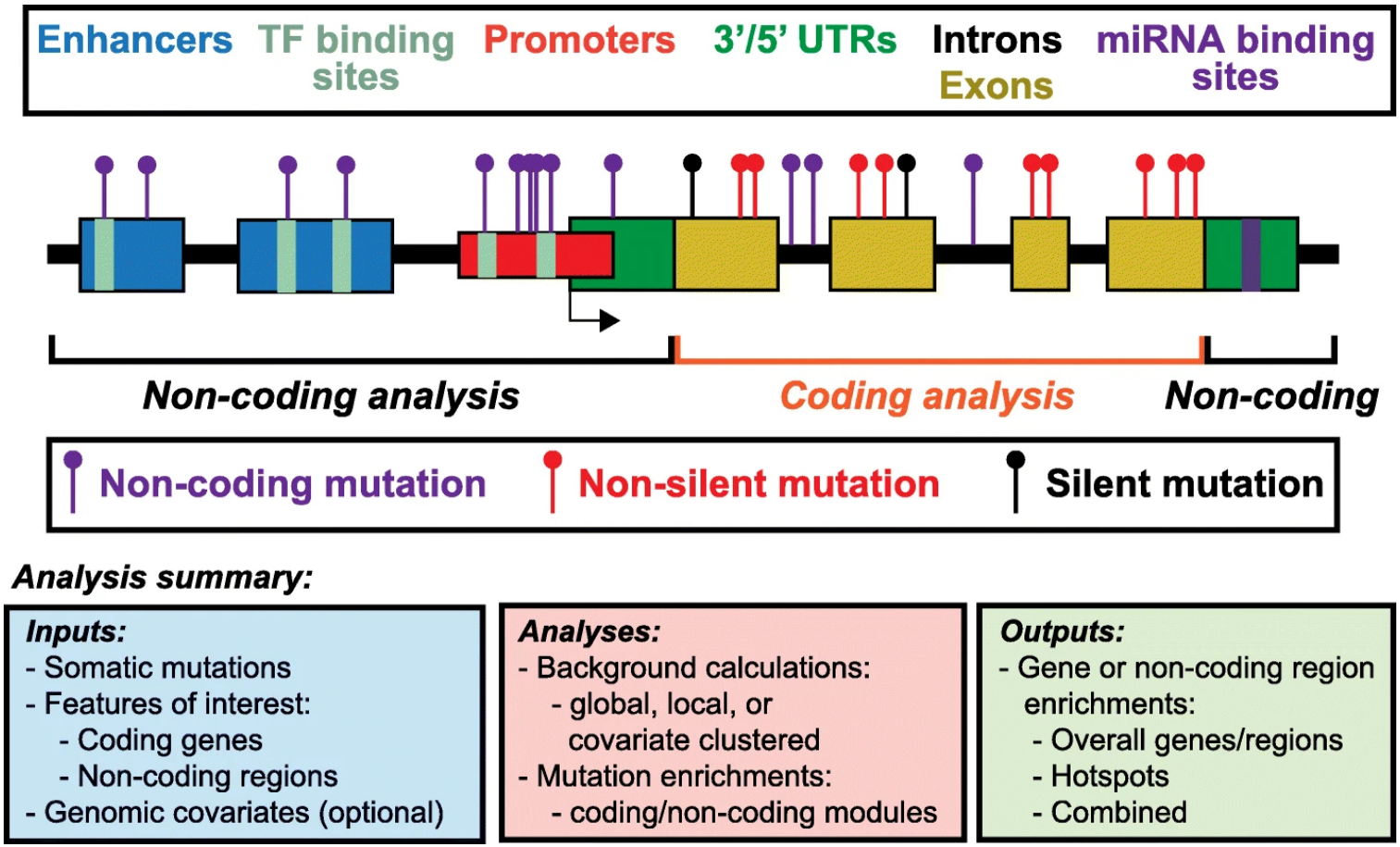


Figure: Schematic representation of MunEnricher's analysis procedures (Soltis et al., 2020)

Driver Gene Selection Strategy

COSMIC Cancer Gene Census (Tate John et al., 2018)

Gene $\in$ CGC Tier 1 set

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 LUSC

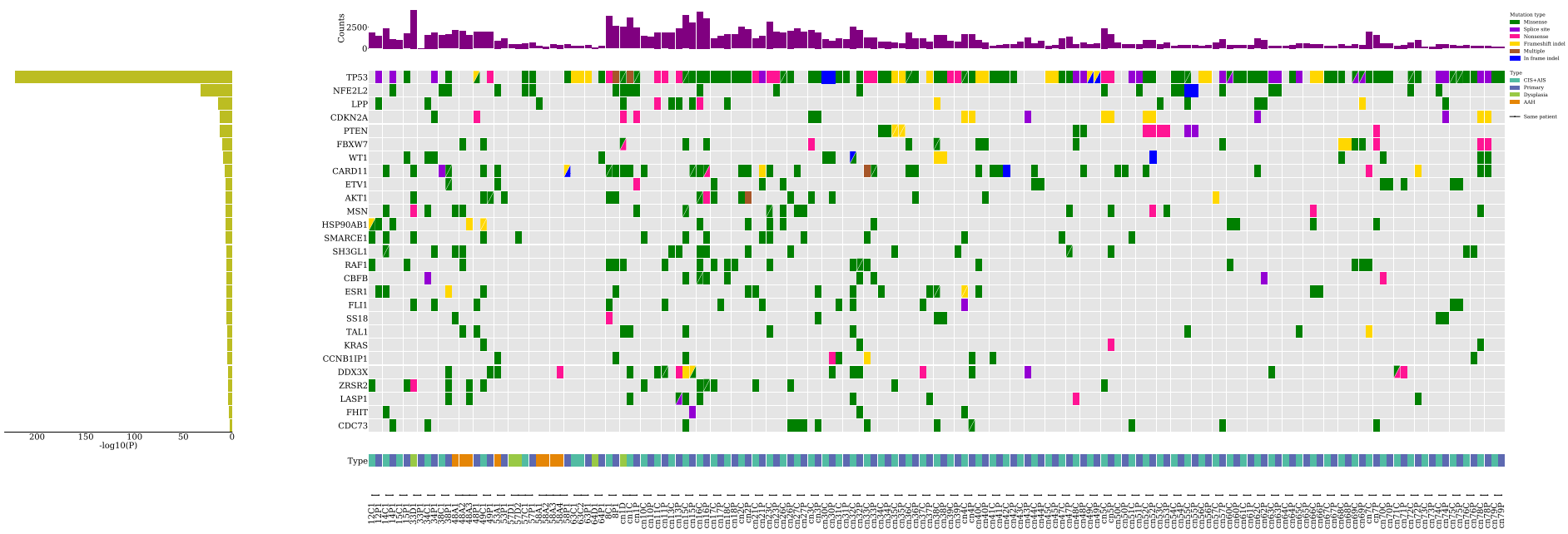


Figure: CoMut Plot with LUSC Patients

Somatic Variant in LUAD

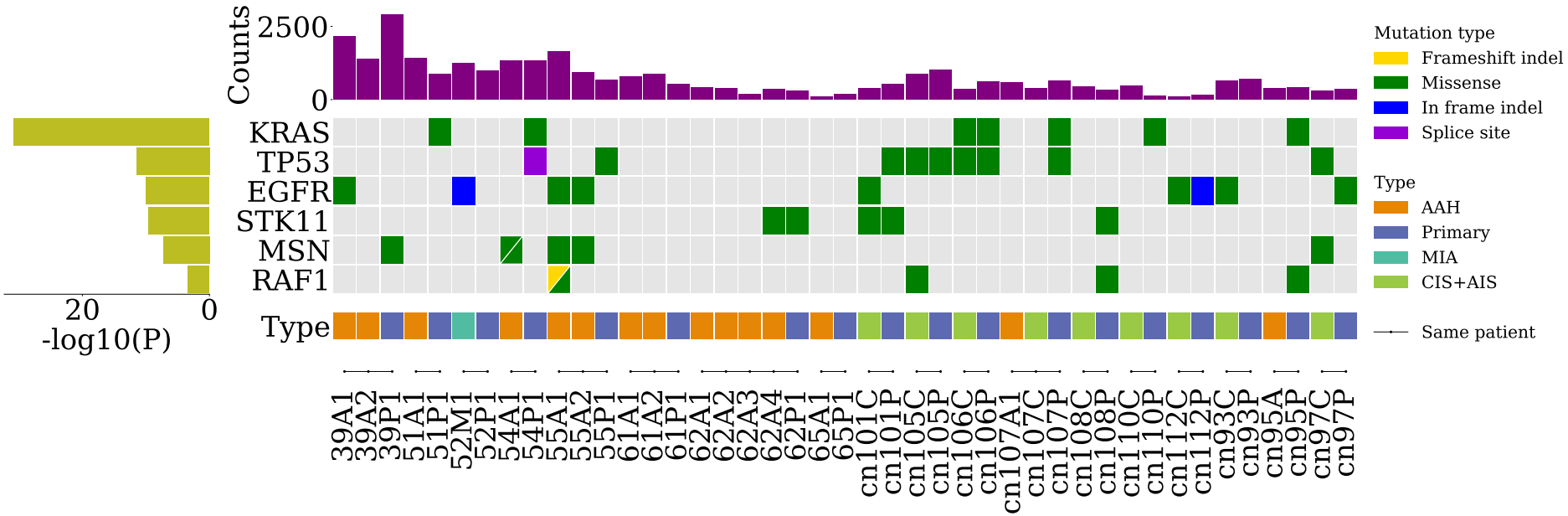


Figure: CoMut Plot with LUAD Patients

Findings in SNVs Analysis

4. Results

4.4. VAF Analysis

VAF?

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

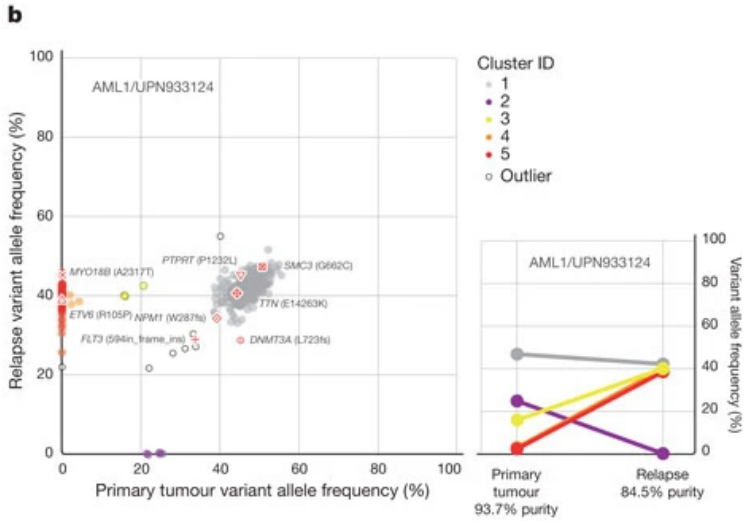


Figure: VAF distribution of validated mutations (Ding et al., 2012)

VAF Plots I

PyClone?

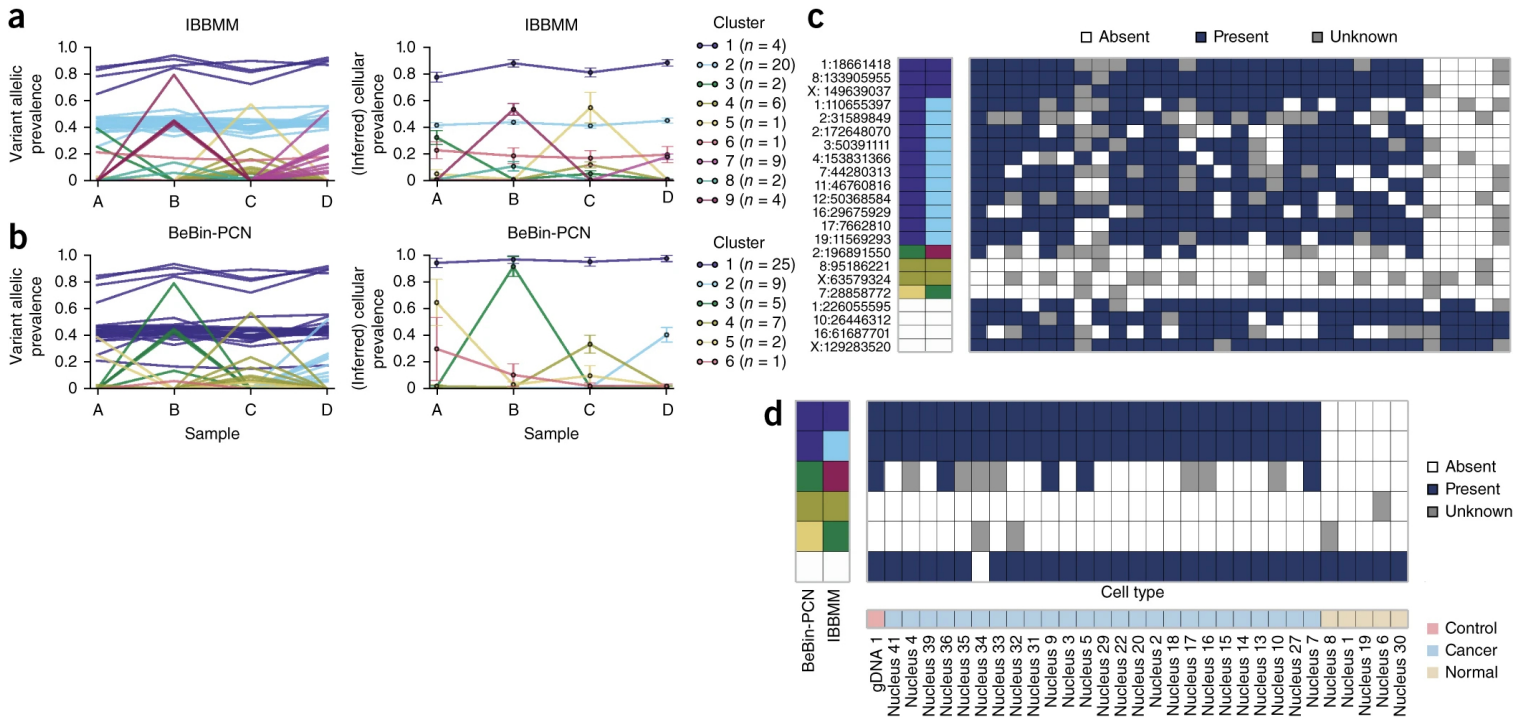


Figure: Analysis of multiple samples by PyClone (Roth et al., 2014)

PyClone Plots I

Findings in VAF Analysis

4. Results

4.5. Tumor Evolution Trajectories Analysis

Revolver?

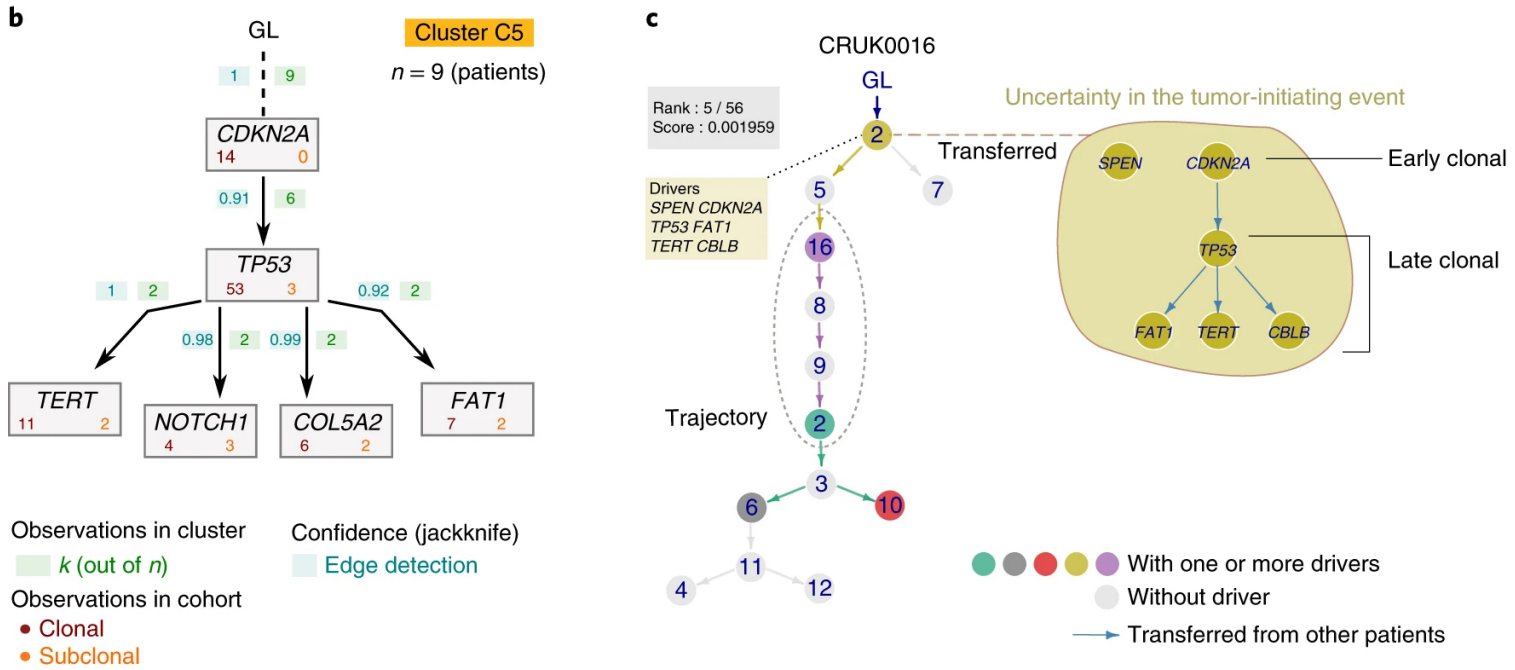


Figure: Repeated Evolutionary Trajectories (Caravagna et al., 2018)

Findings in Tumor Evolution Trajectories Analysis

4. Results

4.6. Differences in Gene Expression Levels

RSEM?

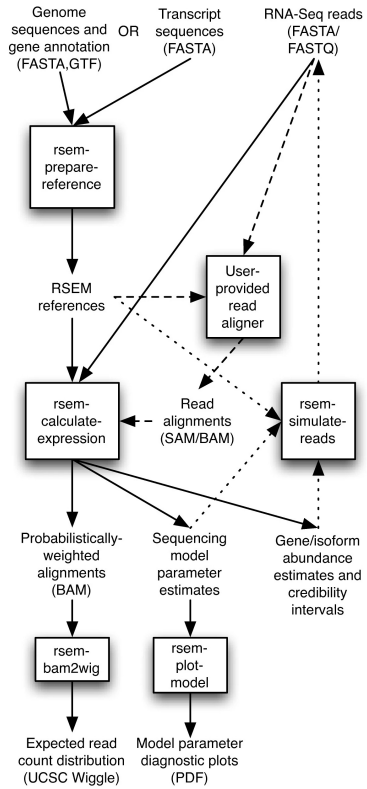


Figure: RSEM workflow (Li & Dewey, 2011)

DESeq2?

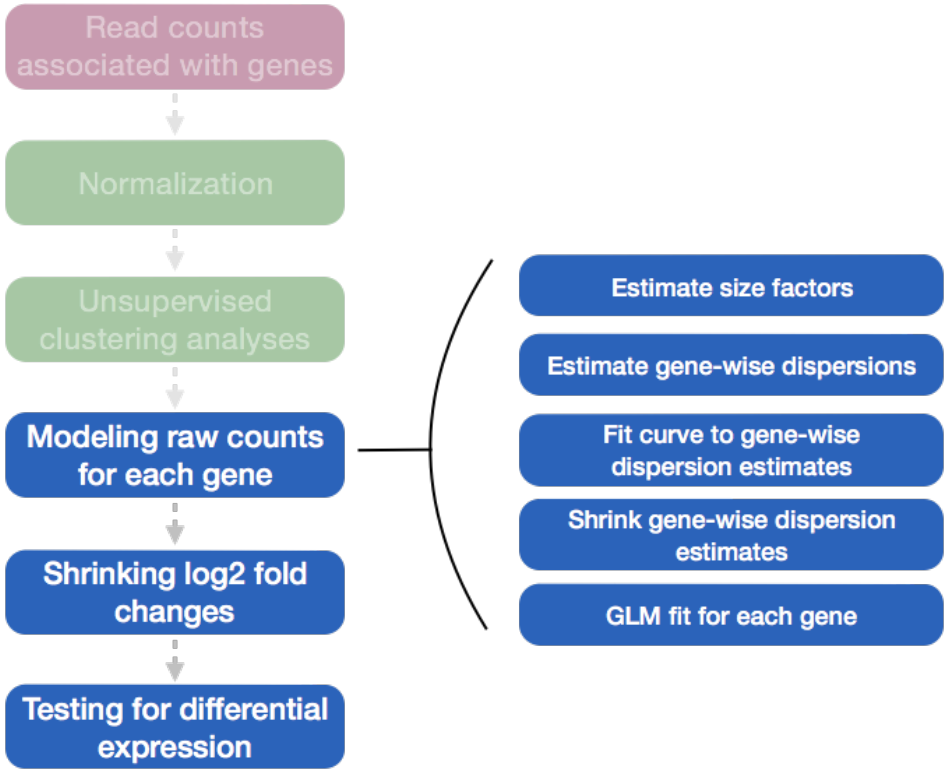


Figure: DESeq2 workflow (Love, Huber, & Anders, 2014)

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-value < 0.05

Adjusted P-value

Padj < 0.05

Enrichr?

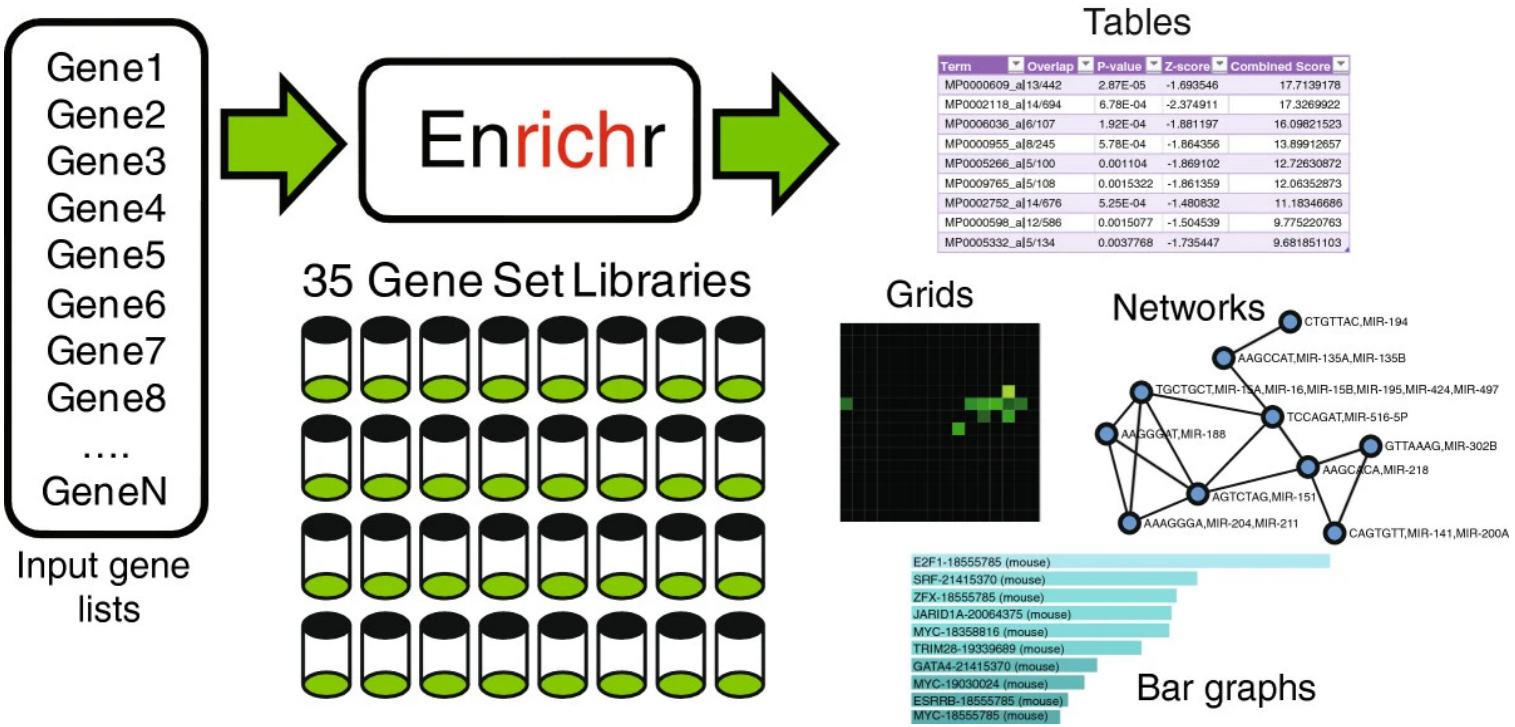


Figure: Enrichr workflow (Chen et al., 2013; Kuleshov et al., 2016)

Gene-set Library

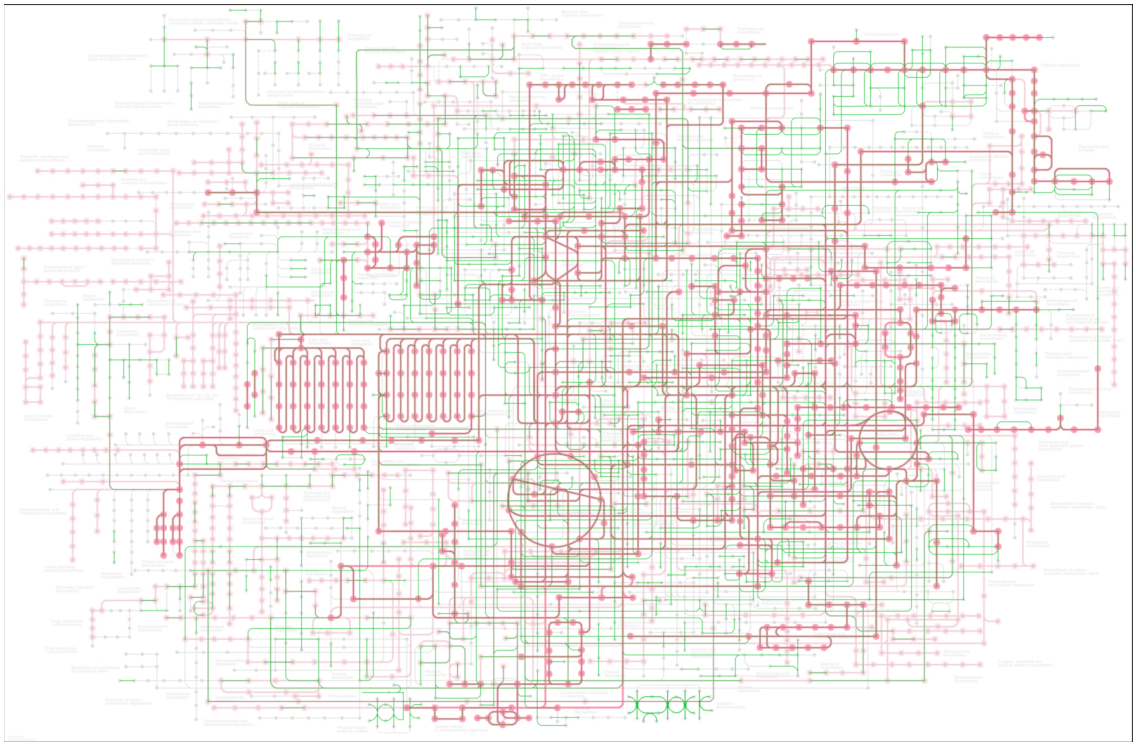


Figure: The global map of metabolic pathways by KEGG (Kanehisa et al., 2021)

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 LUSC 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 LUAD 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

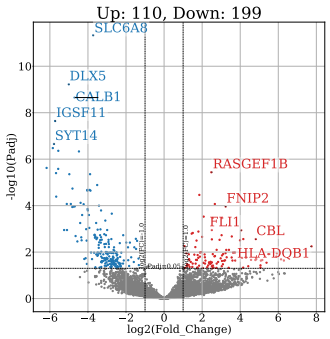
4. Results

4.6. Differences in Gene Expression Levels

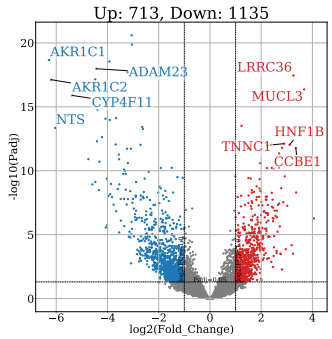
4.6.1. Comparing cancer stage in LUSC

DEG Volcano Plots in LUSC

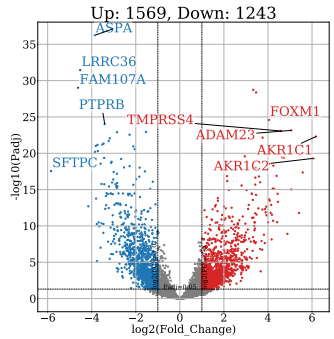
Normal → Dysplasia → CIS → Primary (LUSC)



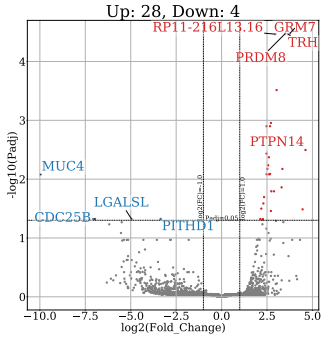
(a) Normal-Dysplasia



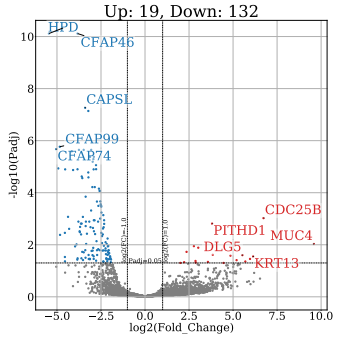
(b) Normal-CIS



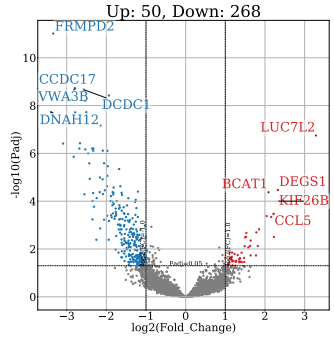
(c) Normal-Primary



(d) Dysplasia-CIS



(e) Dysplasia-Primary

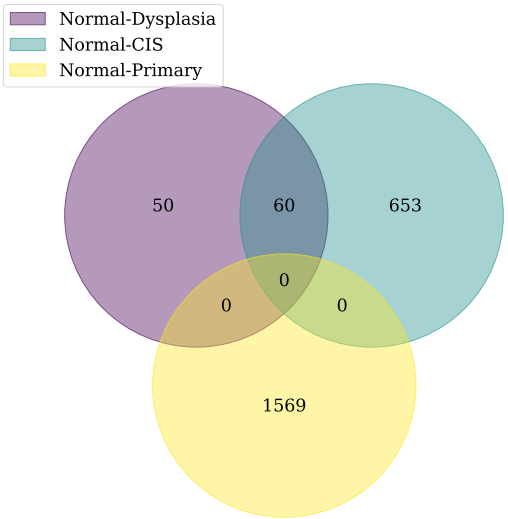


(f) CIS-Primary

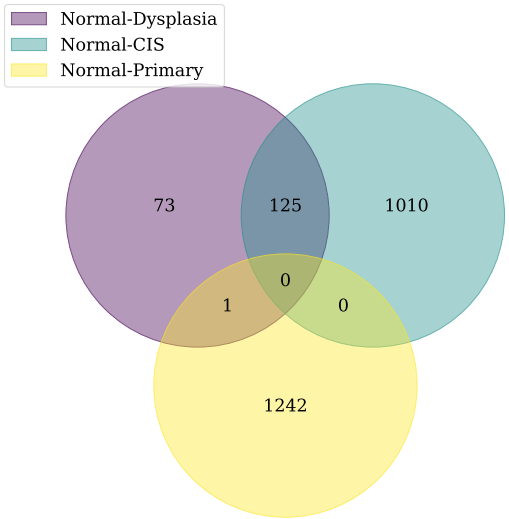
Figure: DEG Volcano Plots in LUSC

DEG Venn Diagram in LUSC

Normal → Dysplasia → CIS → Primary (LUSC)



(a) Up-regulated



(b) Down-regulated

Figure: DEG Venn Diagram in LUSC

Enrichment test with Normal vs. Dysplasia in LUSC

Table: Up-regulated Pathways on Normal vs. Dysplasia

Term name	Adjusted p-value
Leishmaniasis	6.72e-03
Lysosome	6.72e-03
Phagosome	1.15e-02

Table: Down-regulated Pathways on Normal vs. Dysplasia

Term name	Adjusted p-value
NaN	NaN

Enrichment test with Normal vs. CIS in LUSC

Table: Up-regulated Pathways on Normal vs. CIS

Term name	Adjusted p-value
Hematopoietic cell lineage	7.22e-08
Malaria	1.16e-06
Cell adhesion molecules	1.16e-06

Table: Down-regulated Pathways on Normal vs. CIS

Term name	Adjusted p-value
Metabolism of xenobiotics by cytochrome P450	9.34e-06
Drug metabolism	9.06e-05
Cell cycle	1.68e-04

Enrichment test with Normal vs. Primary in LUSC

Table: Up-regulated Pathways on Normal vs. Primary

Term name	Adjusted p-value
Cell cycle	1.53e-04
Glutathione metabolism	1.53e-04
DNA replication	1.72e-04

Table: Down-regulated Pathways on Normal vs. Primary

Term name	Adjusted p-value
Hematopoietic cell lineage	7.33e-09
Malaria	7.33e-09
Hypertrophic cardiomyopathy	1.24e-08

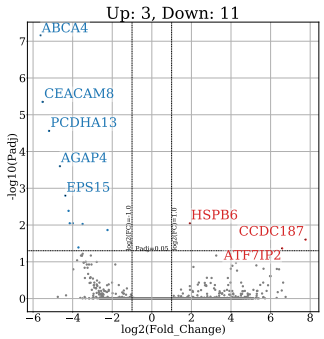
4. Results

4.6. Differences in Gene Expression Levels

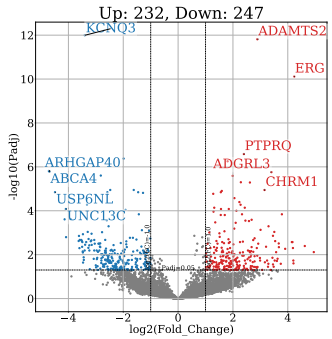
4.6.2. Comparing cancer stage in LUAD

DEG Volcano Plots in LUAD

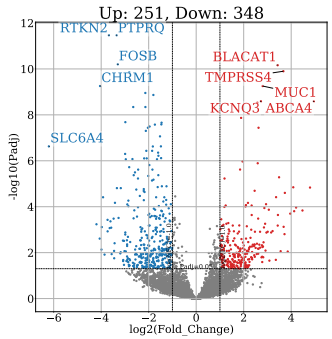
Normal → AAH → AIS → Primary (LUAD)



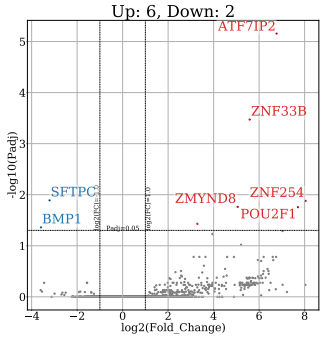
(a) Normal-AAH



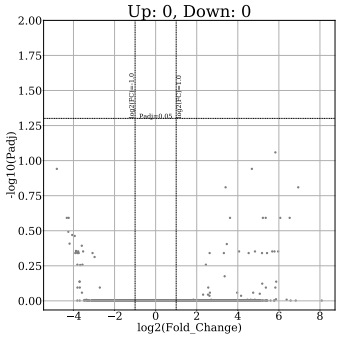
(b) Normal-AIS



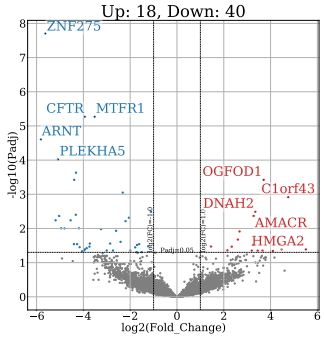
(c) Normal-Primary



(d) AAH-AIS



(e) AAH-Primary

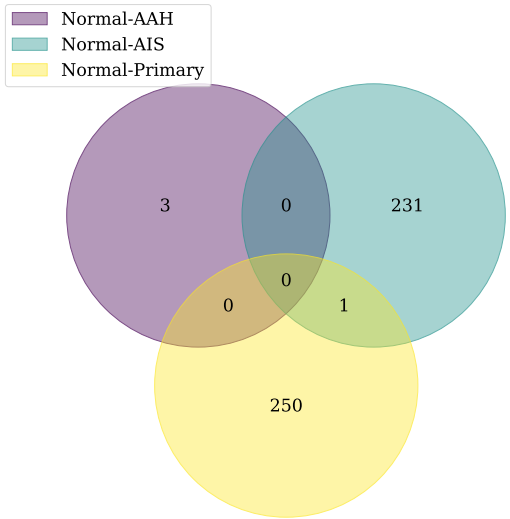


(f) AIS-Primary

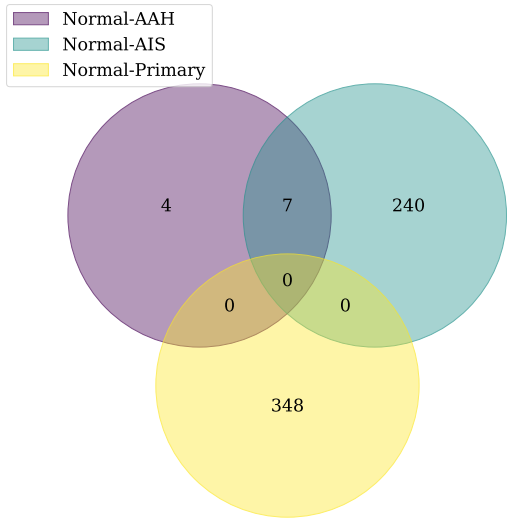
Figure: DEG Volcano Plots in LUAD

DEG Venn Diagram in LUAD

Normal $\rightarrow$ AAH $\rightarrow$ AIS $\rightarrow$ Primary (LUAD)



(a) Up-regulated



(b) Down-regulated

Figure: DEG Venn Diagram in LUAD

Enrichment test with Normal vs. AAH in LUAD

Table: Up-regulated Pathways on Normal vs. AAH

Term name	Adjusted p-value
NaN	NaN

Table: Down-regulated Pathways on Normal vs. AAH

Term name	Adjusted p-value
NaN	NaN

Enrichment test with Normal vs. AIS in LUAD

Table: Up-regulated Pathways on Normal vs. AIS

Term name	Adjusted p-value
Calcium signaling pathway	2.49e-02
Cell adhesion molecules	3.55e-02

Table: Down-regulated Pathways on Normal vs. AIS

Term name	Adjusted p-value
NaN	NaN

Enrichment test with Normal vs. Primary in LUAD

Table: Up-regulated Pathways on Normal vs. Primary

Term name	Adjusted p-value
NaN	NaN

Table: Down-regulated Pathways on Normal vs. Primary

Term name	Adjusted p-value
Vascular smooth muscle contraction	1.38e-04
ECM-receptor interaction	3.58e-04
Calcium signaling pathway	4.03e-04

4. Results

4.6. Differences in Gene Expression Levels

4.6.3. Recur vs. Non-recur in LUSC

LUSC Data Composition

Table: Number of WTS LUSC samples

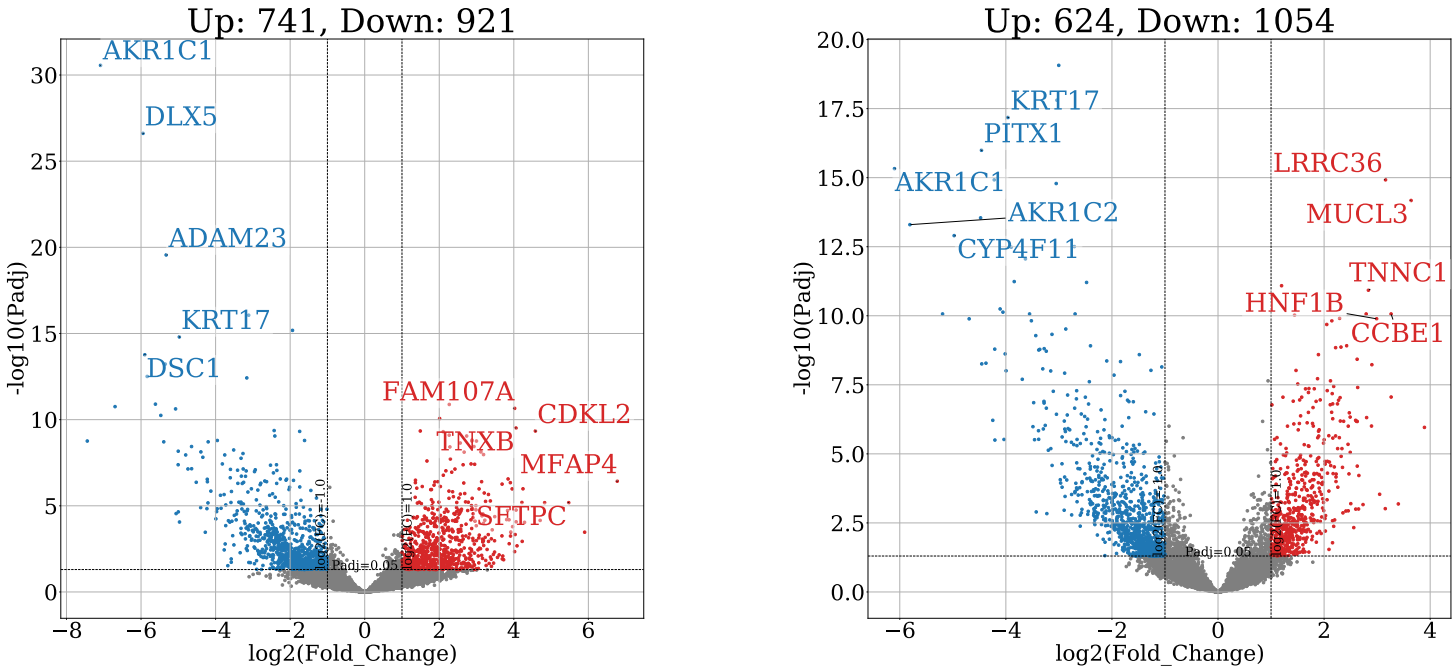
Recurrence?	Stage	Number of Samples
Recurrence (n=13)	Normal	1
	Dysplasia	1
	CIS	5
	Primary	6
Non-recurrence (n=74)	Normal	16
	Dysplasia	1
	CIS	28
	Primary	29

Normal samples

In order to compare with Normal stage, merging Normal samples.

$\because$ Insufficient number of Normal samples in Recur.

DEG Volcano Plots Recur vs. Non-recur with CIS in LUSC

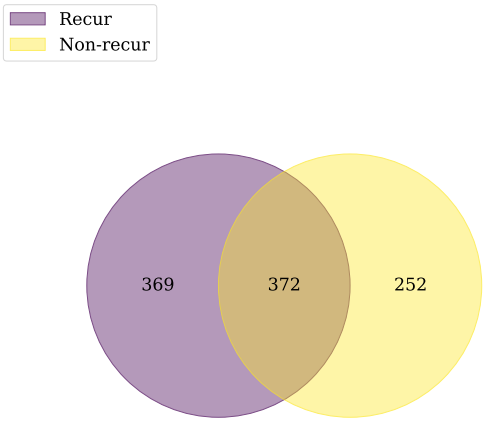


(a) Recurrence

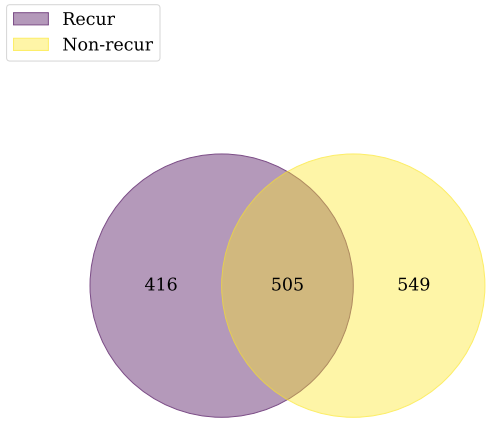
(b) Non-recurrence

Figure: DEG Volcano Plots Recur vs. Non-recur with CIS

DEG Venn Diagram Recur vs. Non-recur with CIS in LUSC



(a) Up-regulated



(b) Down-regulated

Figure: DEG Venn Diagram Recur vs. Non-recur with CIS

Enrichment test for Recur with CIS in LUSC

Table: Up-regulated Pathways on Recur with CIS in LUSC

Term name	Adjusted p-value
NaN	NaN

Table: Down-regulated Pathways on Recur with CIS in LUSC

Term name	Adjusted p-value
Huntington disease	6.36e-06
Amyotrophic lateral sclerosis	1.62e-05
Parkinson disease	1.62e-05

Enrichment test for Non-recur with CIS in LUSC

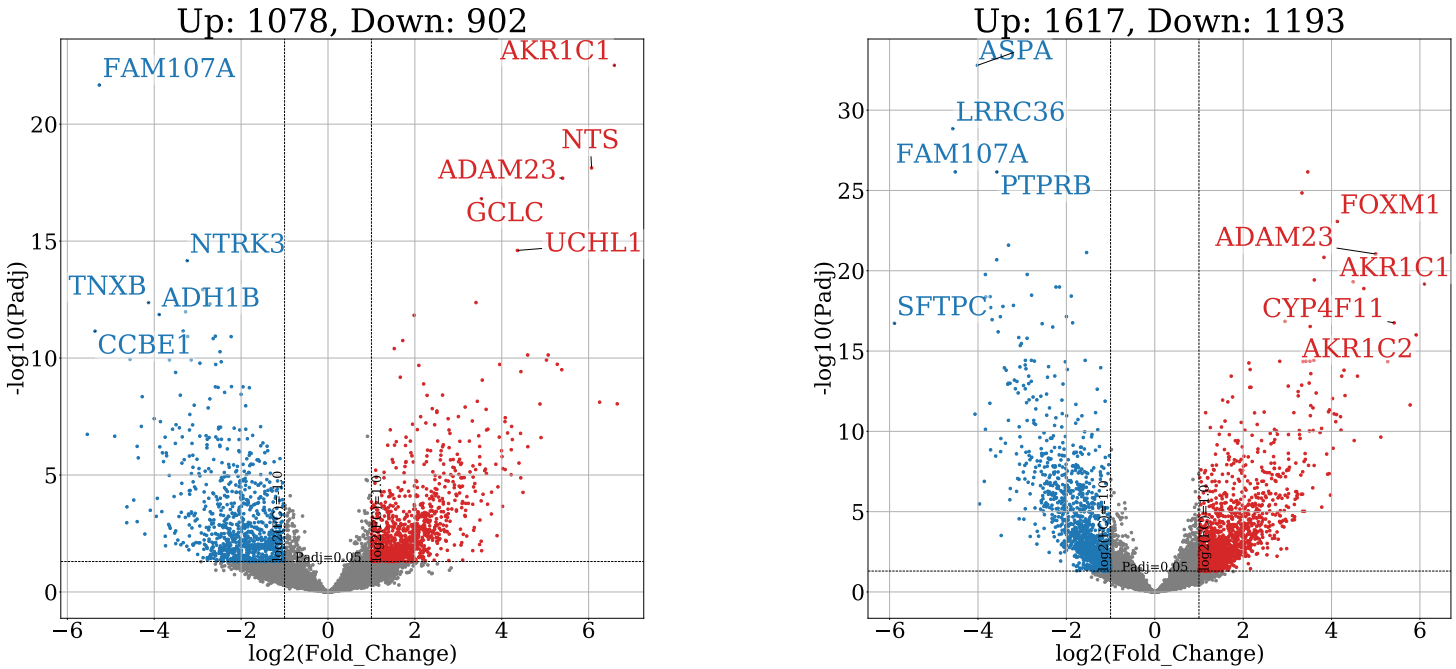
Table: Up-regulated Pathways on Non-recur with CIS in LUSC

Term name	Adjusted p-value
Malaria	7.76e-03
Th1 and Th2 cell differentiation	1.15e-02
Transcriptional misregulation in cancer	1.15e-02

Table: Down-regulated Pathways on Non-recur with CIS in LUSC

Term name	Adjusted p-value
NaN	NaN

DEG Volcano Plots R vs. NR with Primary in LUSC

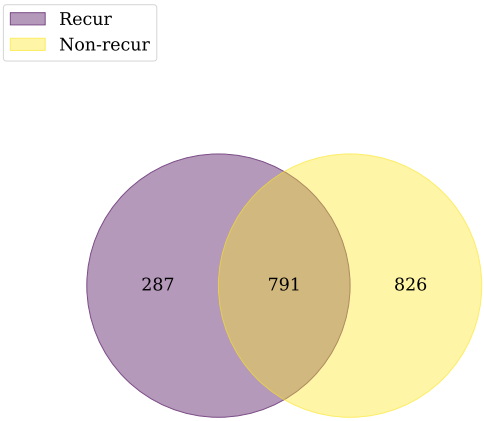


(a) Recurrence

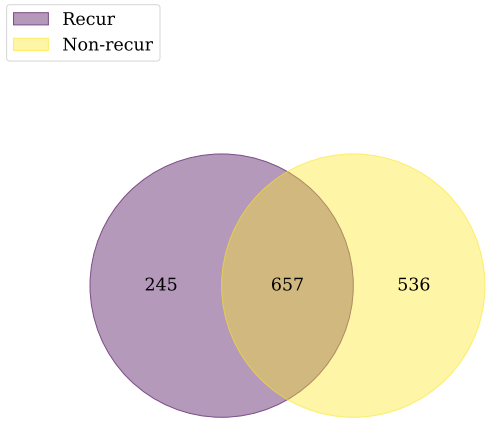
(b) Non-recurrence

Figure: DEG Volcano Plots Recur vs. Non-recur with Primary

DEG Venn Diagram R vs. NR with Primary in LUSC



(a) Up-regulated



(b) Down-regulated

Figure: DEG Venn Diagram Recur vs. Non-recur with Primary

Enrichment test for Recur with Primary in LUSC

Table: Up-regulated Pathways on Recur with Primary in LUSC

Term name	Adjusted p-value
Amyotrophic lateral sclerosis	4.85e-03
RNA transport	6.11e-03
mRNA surveillance pathway	6.11e-03

Table: Down-regulated Pathways on Recur with Primary in LUSC

Term name	Adjusted p-value
NaN	NaN

Enrichment test for Non-recur with Primary in LUSC

Table: Up-regulated Pathways on Non-recur with Primary in LUSC

Term name	Adjusted p-value
Homologous recombination	1.00e-02

Table: Down-regulated Pathways on Non-recur with Primary in LUSC

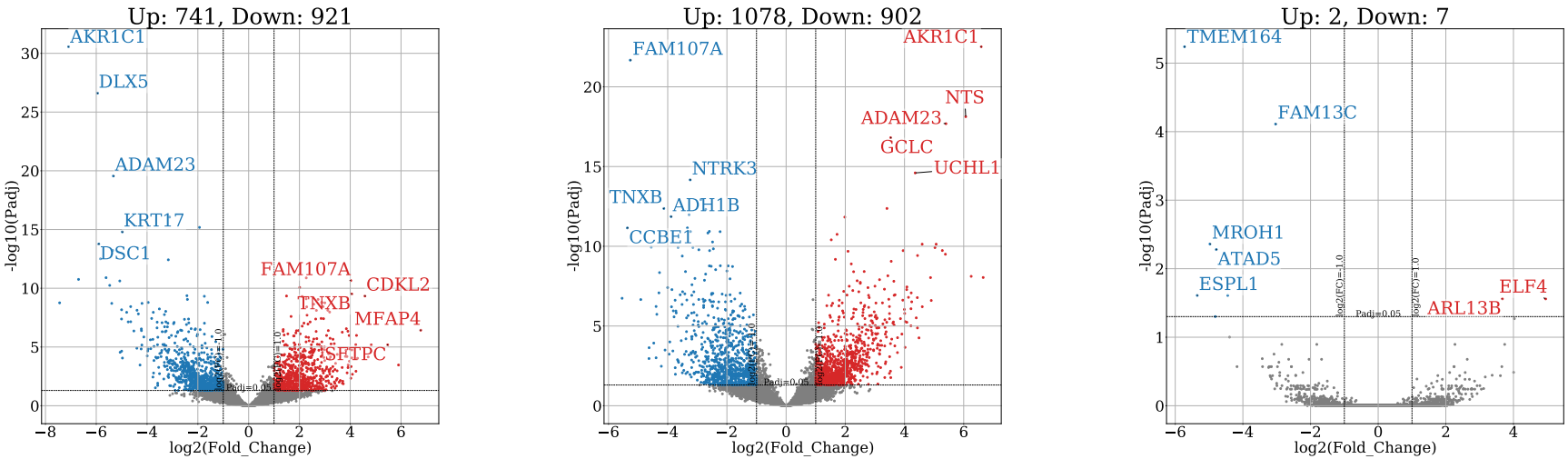
Term name	Adjusted p-value
Staphylococcus aureus infection	5.37e-05
Hematopoietic cell lineage	5.37e-05
Leishmaniasis	4.30e-04

4. Results

4.6. Differences in Gene Expression Levels

4.6.4. Within Recur in LUSC

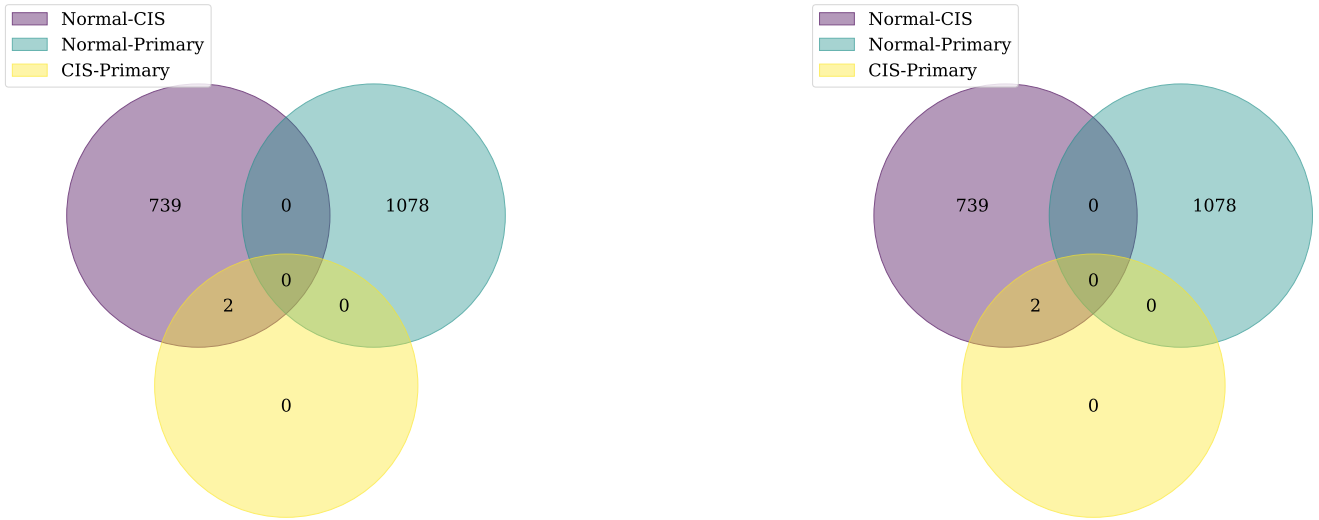
DEG Volcano Plots with Recur in LUSC



(a) Normal-CIS (b) Normal-Primary (c) CIS-Primary

Figure: DEG Volcano Plots with Recur samples in LUSC

DEG Venn Diagram with Recur in LUSC



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

Figure: DEG Venn Diagram with Recur samples in LUSC

Enrichment test with Normal vs. CIS for Recur

Table: Up-regulated Pathways on Normal vs. CIS for Recur in LUSC

Term name	Adjusted p-value
Hematopoietic cell lineage	1.87e-05
Cell adhesion molecules	1.87e-05
Hypertrophic cardiomyopathy	9.66e-05

Table: Down-regulated Pathways on Normal vs. CIS for Recur in LUSC

Term name	Adjusted p-value
Parkinson disease	2.11e-05
Alzheimer disease	2.11e-05
Huntington disease	2.11e-05

Enrichment test with Normal vs. Primary for Recur

Table: Up-regulated Pathways on Normal vs. Primary for Recur in LUSC

Term name	Adjusted p-value
Glycolysis / Gluconeogenesis	1.90e-05
RNA transport	2.66e-05
Drug metabolism	2.66e-05

Table: Down-regulated Pathways on Normal vs. Primary for Recur in LUSC

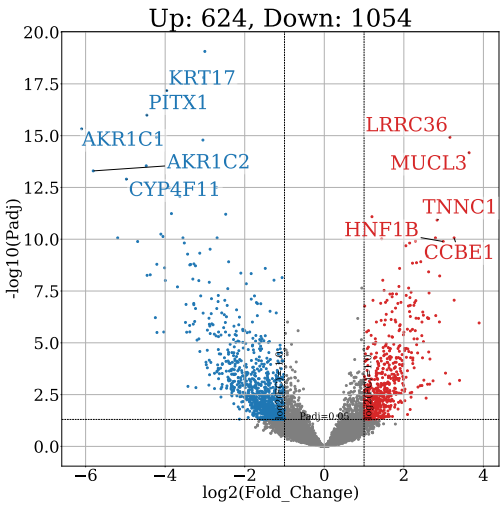
Term name	Adjusted p-value
Dilated cardiomyopathy	2.19e-06
Hypertrophic cardiomyopathy	2.19e-06
Arrhythmogenic right ventricular cardiomyopathy	4.12e-06

4. Results

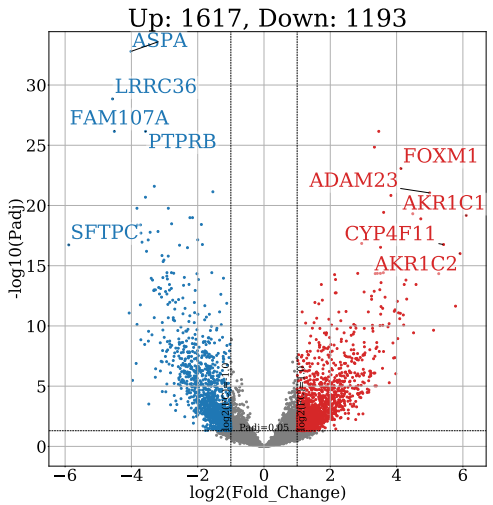
4.6. Differences in Gene Expression Levels

4.6.5. Within Non-recur in LUSC

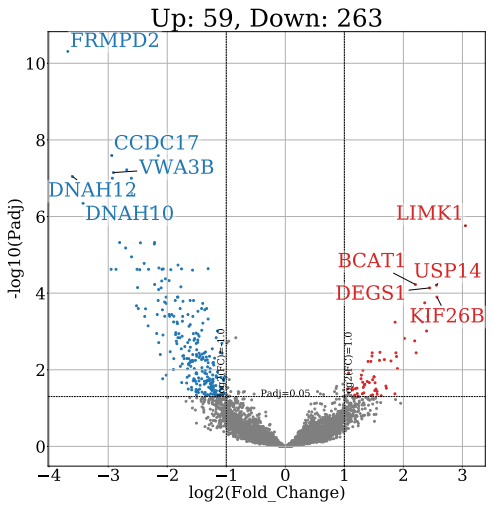
DEG Volcano Plots with Non-recur in LUSC



(a) Normal-CIS



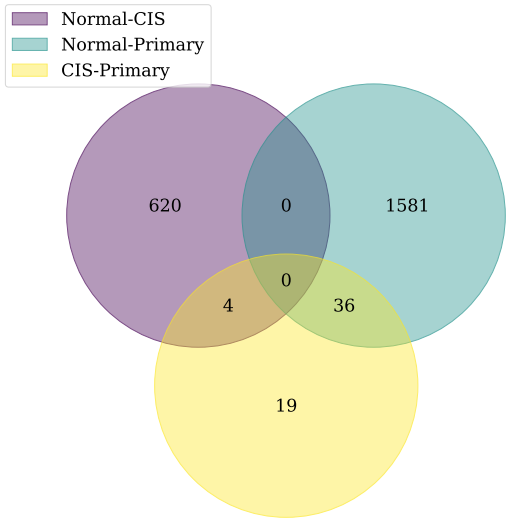
(b) Normal-Primary



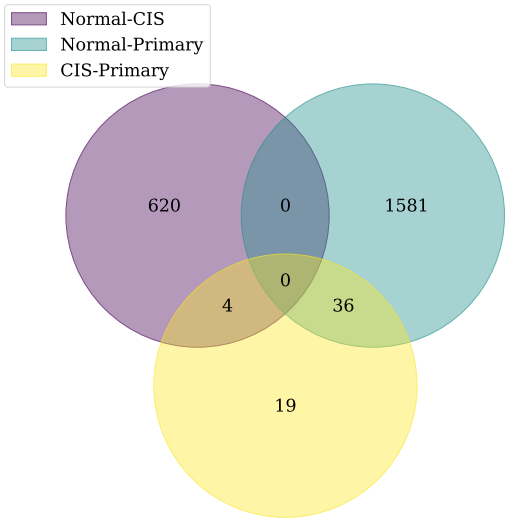
(c) CIS-Primary

Figure: DEG Volcano Plots with Non-recur samples in LUSC

DEG Venn Diagram with Non-recur in LUSC



(a) Up-regulated



(b) Down-regulated

Figure: DEG Venn Diagram with Non-recur in LUSC

Enrichment test with Normal vs. CIS for Non-recur

Table: Up-regulated Pathways on Normal vs. CIS for Non-recur in LUSC

Term name	Adjusted p-value
Malaria	6.53e-08
Hematopoietic cell lineage	2.01e-07
Hypertrophic cardiomyopathy	1.53e-06

Table: Down-regulated Pathways on Normal vs. CIS for Non-recur in LUSC

Term name	Adjusted p-value
Metabolism of xenobiotics by cytochrome P450	9.67e-05
Drug metabolism	1.18e-04
Cell cycle	1.89e-04

Enrichment test with Normal vs. Primary for Non-recur

Table: Up-regulated Pathways on Normal vs. Primary for Non-recur in LUSC

Term name	Adjusted p-value
Cell cycle	3.04e-06
DNA replication	6.47e-06
Homologous recombination	3.33e-05

Table: Down-regulated Pathways on Normal vs. Primary for Non-recur in LUSC

Term name	Adjusted p-value
Hematopoietic cell lineage	6.65e-10
Malaria	3.57e-09
Hypertrophic cardiomyopathy	5.12e-09

4. Results

4.6. Differences in Gene Expression Levels

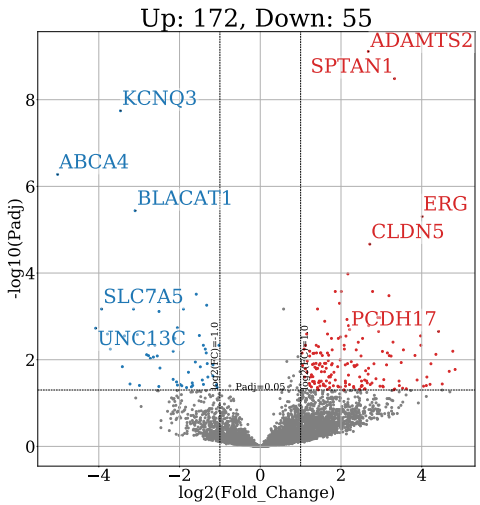
4.6.6. Within Non-recur in LUAD

LUAD Data Composition

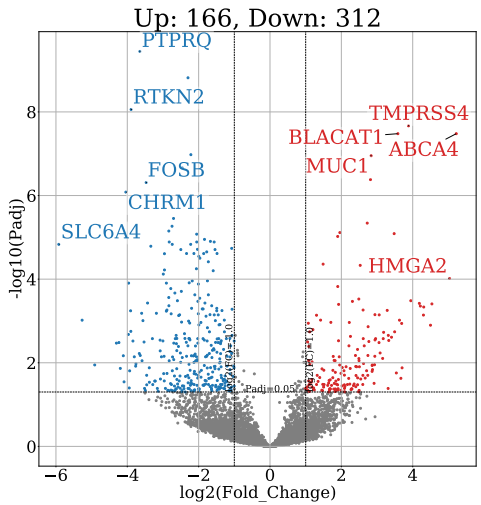
Table: Number of WTS LUAD samples

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

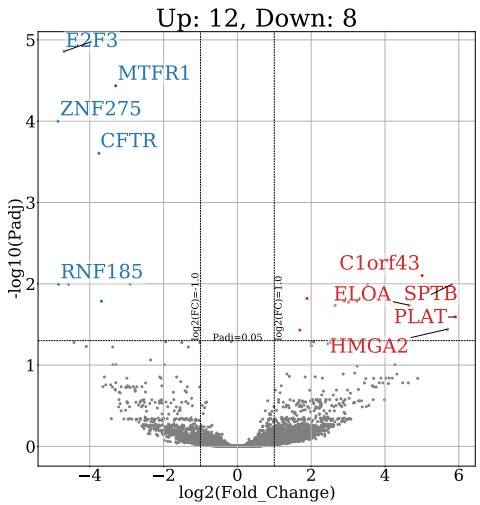
DEG Volcano Plots with Non-recur in LUAD



(a) Normal-AIS



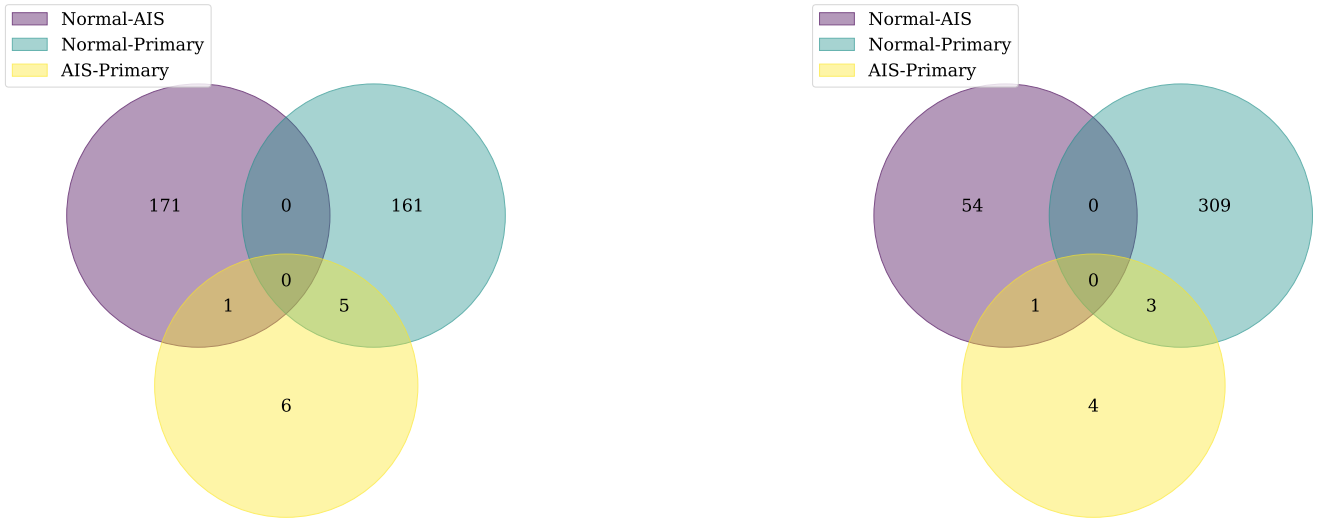
(b) Normal-Primary



(c) AIS-Primary

Figure: DEG Volcano Plots with Non-recur samples in LUAD

DEG Venn Diagram with Non-recur in LUAD



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

Figure: DEG Venn Diagram with Non-recur in LUAD

Enrichment test with Normal vs. AIS in LUAD

Table: Up-regulated Pathways on Normal vs. AIS for Non-recur in LUAD

Term name	Adjusted p-value
Calcium signaling pathway	3.90e-02

Table: Down-regulated Pathways on Normal vs. AIS for Non-recur in LUAD

Term name	Adjusted p-value
NaN	NaN

Enrichment test with Normal vs. Primary in LUAD

Table: Up-regulated Pathways on Normal vs. Primary for Non-recur in LUAD

Term name	Adjusted p-value
NaN	NaN

Table: Down-regulated Pathways on Normal vs. Primary for Non-recur in LUAD

Term name	Adjusted p-value
ECM-receptor interaction	2.05e-03
Vascular smooth muscle contraction	4.98e-03
Calcium signaling pathway	7.82e-03

Findings in DEG Analysis

4. Results

4.7. Bulk Cell Deconvolution

Single-cell data as Reference

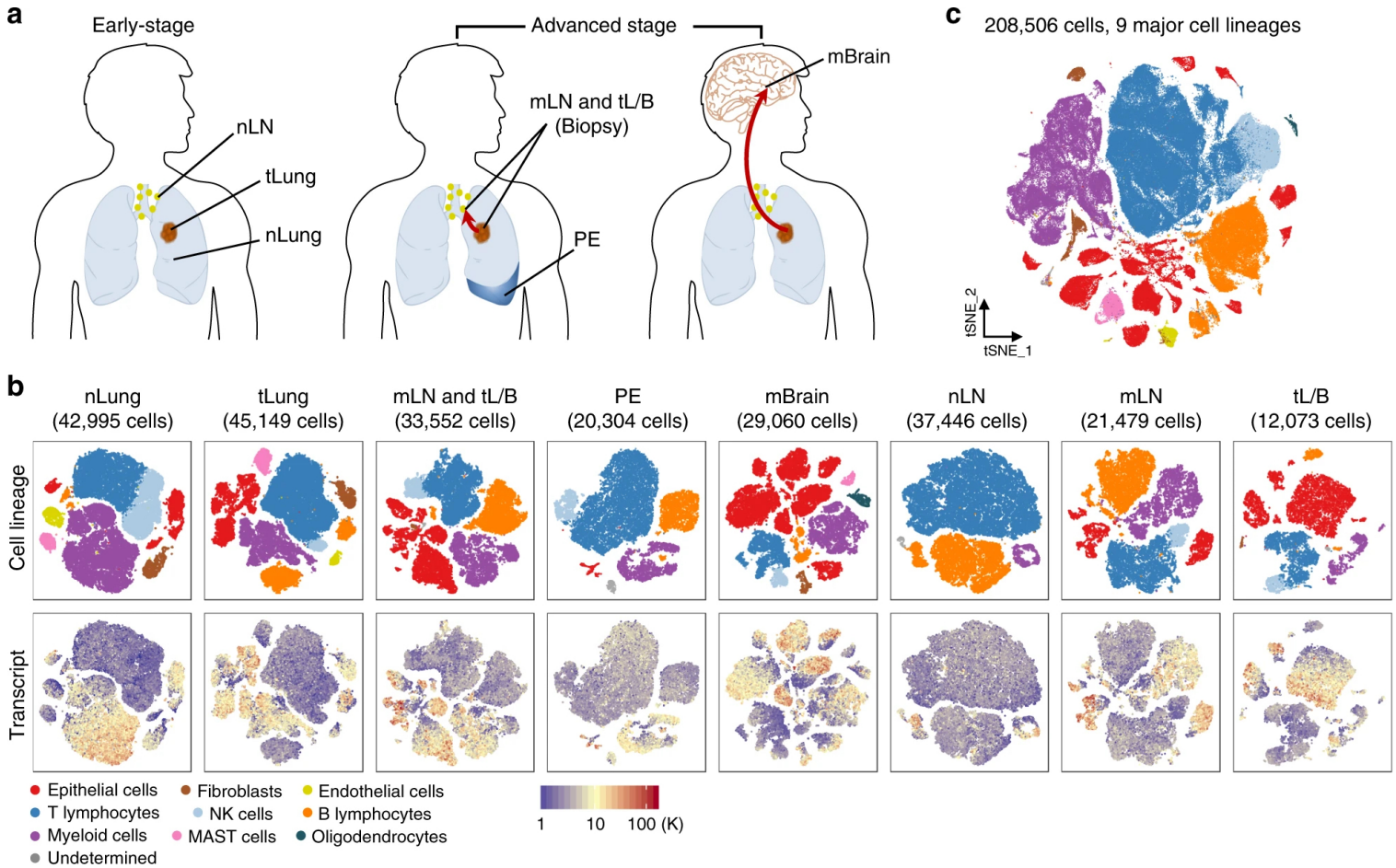


Figure: Comprehensive dissection and clustering of 208,506 single cells from LUAD patients (Kim et al., 2020)

BisqueRNA?

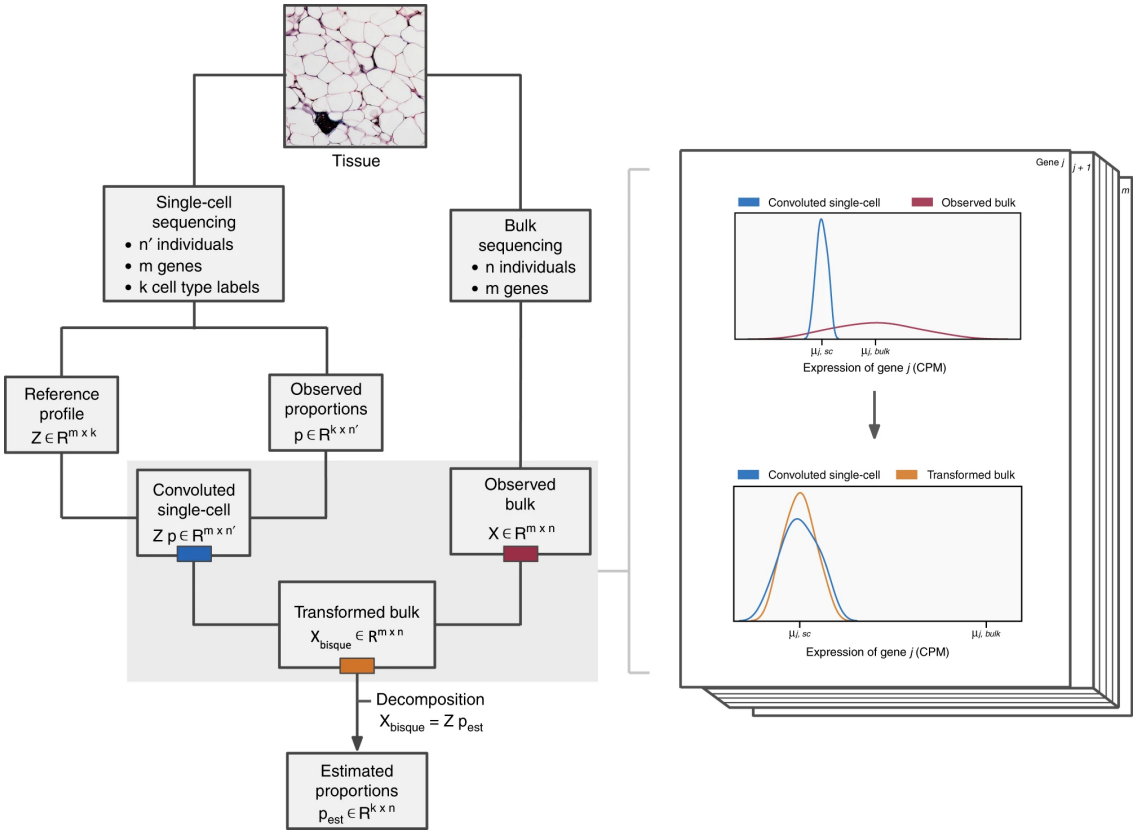


Figure: Workflow for BisqueRNA (Jew et al., 2020)

Cluster Plot in LUSC

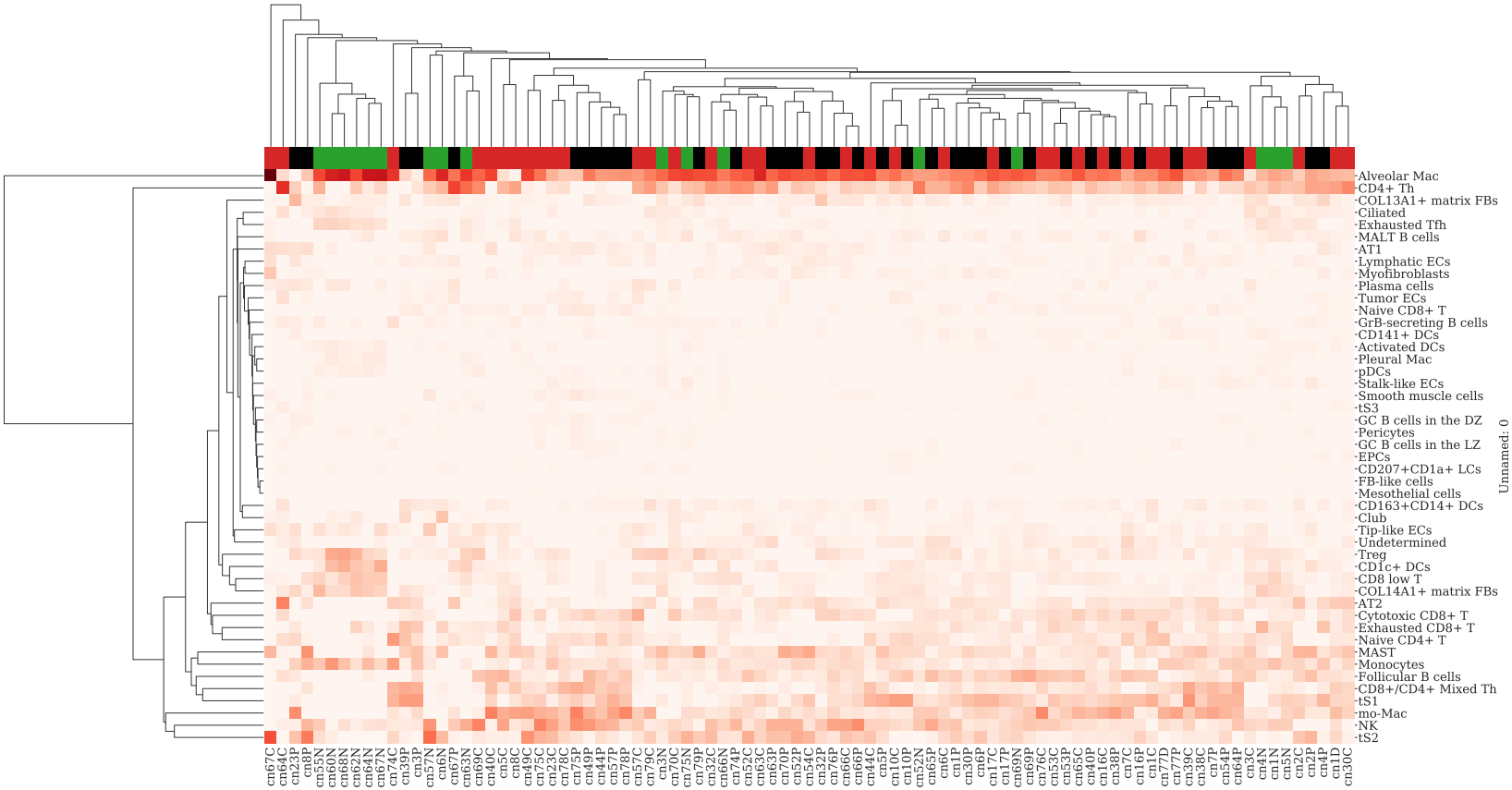


Figure: Cluster Plot in LUSC

Violin Plots in LUSC I

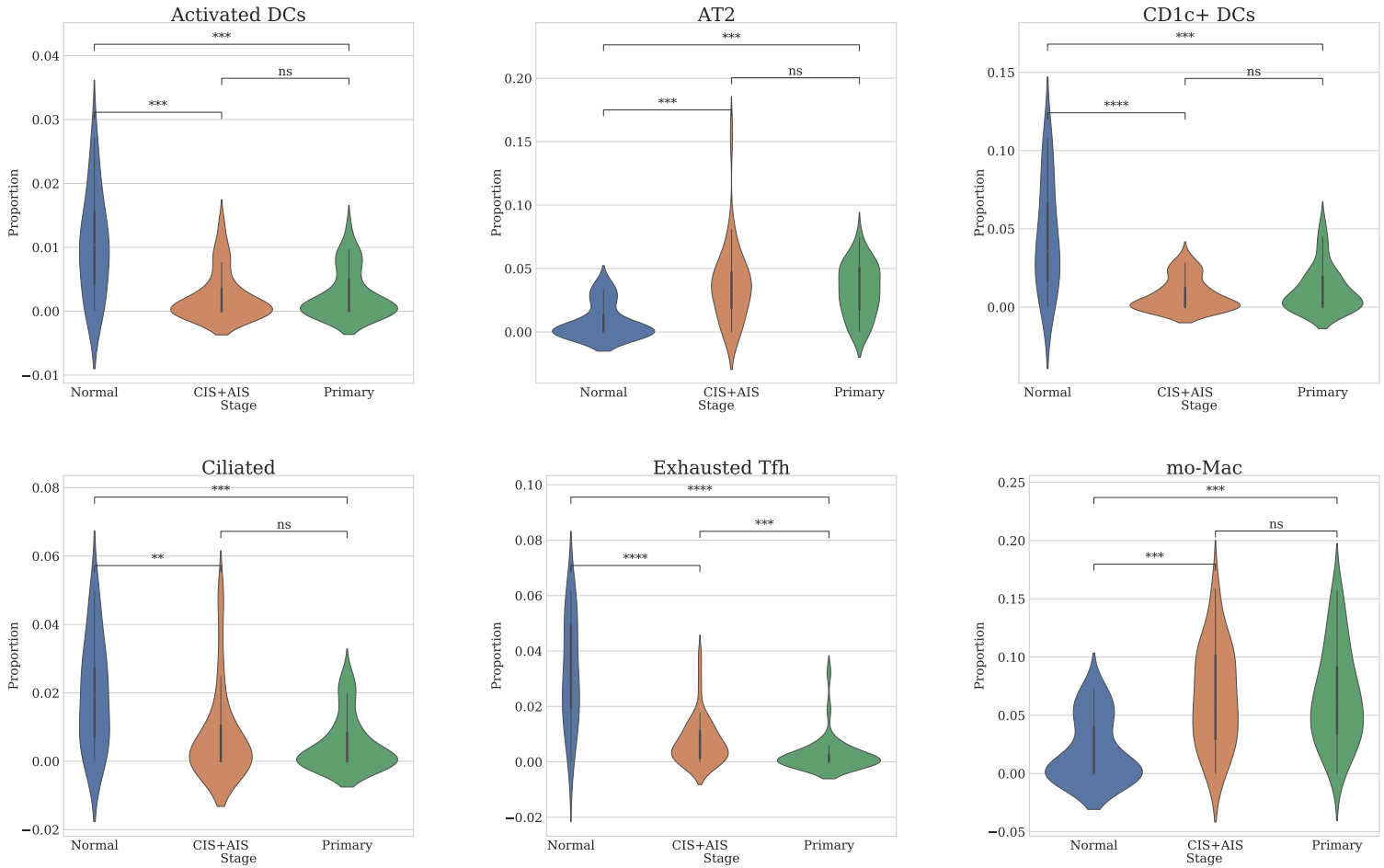


Figure: Violin Plots in LUSC

Violin Plots in LUSC II

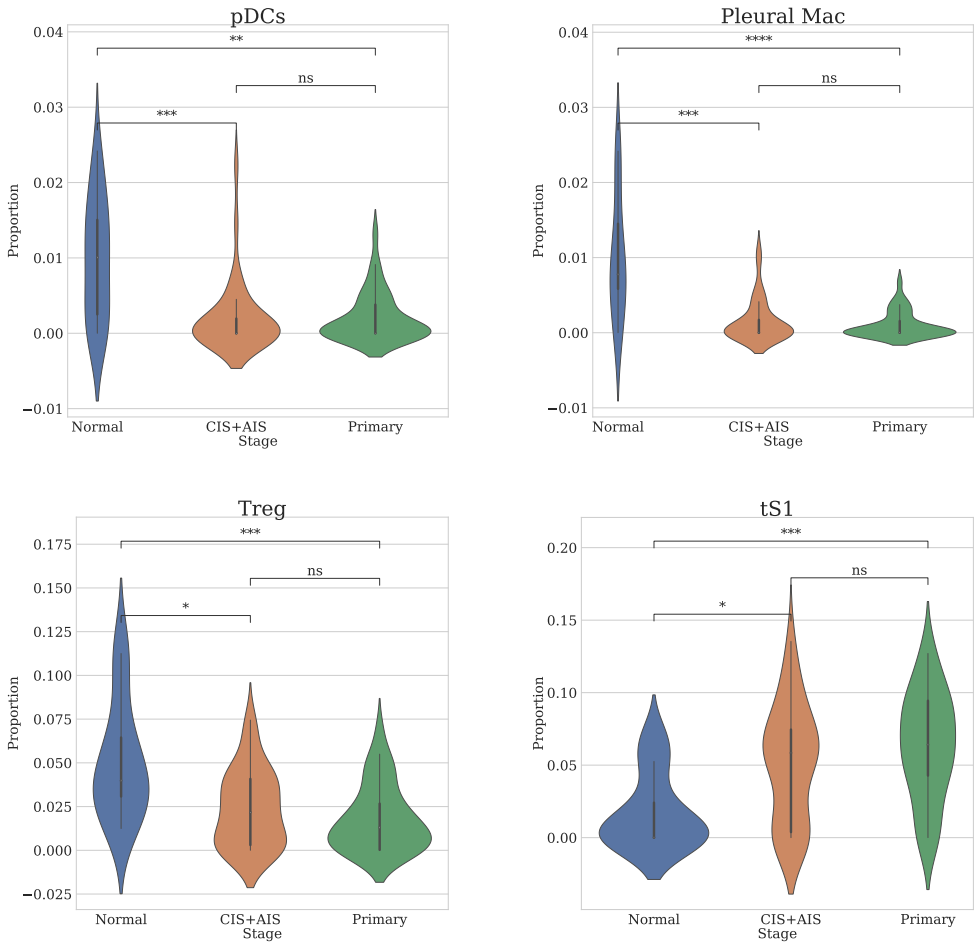


Figure: Violin Plots in LUSC

Cluster Plot in LUAD

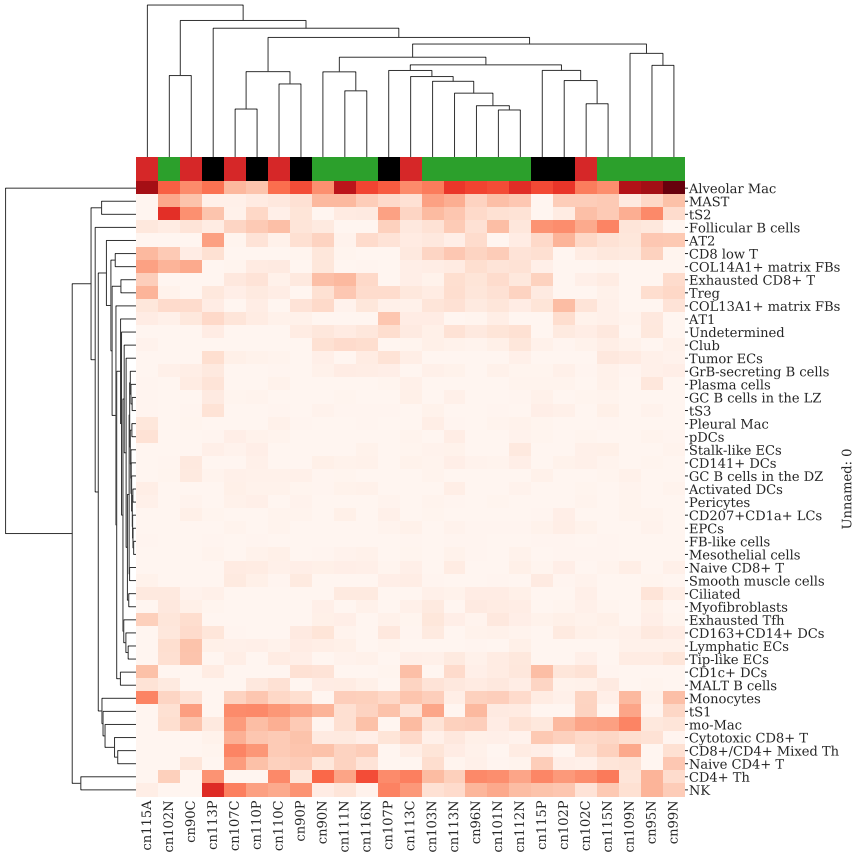


Figure: Cluster Plot in LUAD

Violin Plots in LUAD

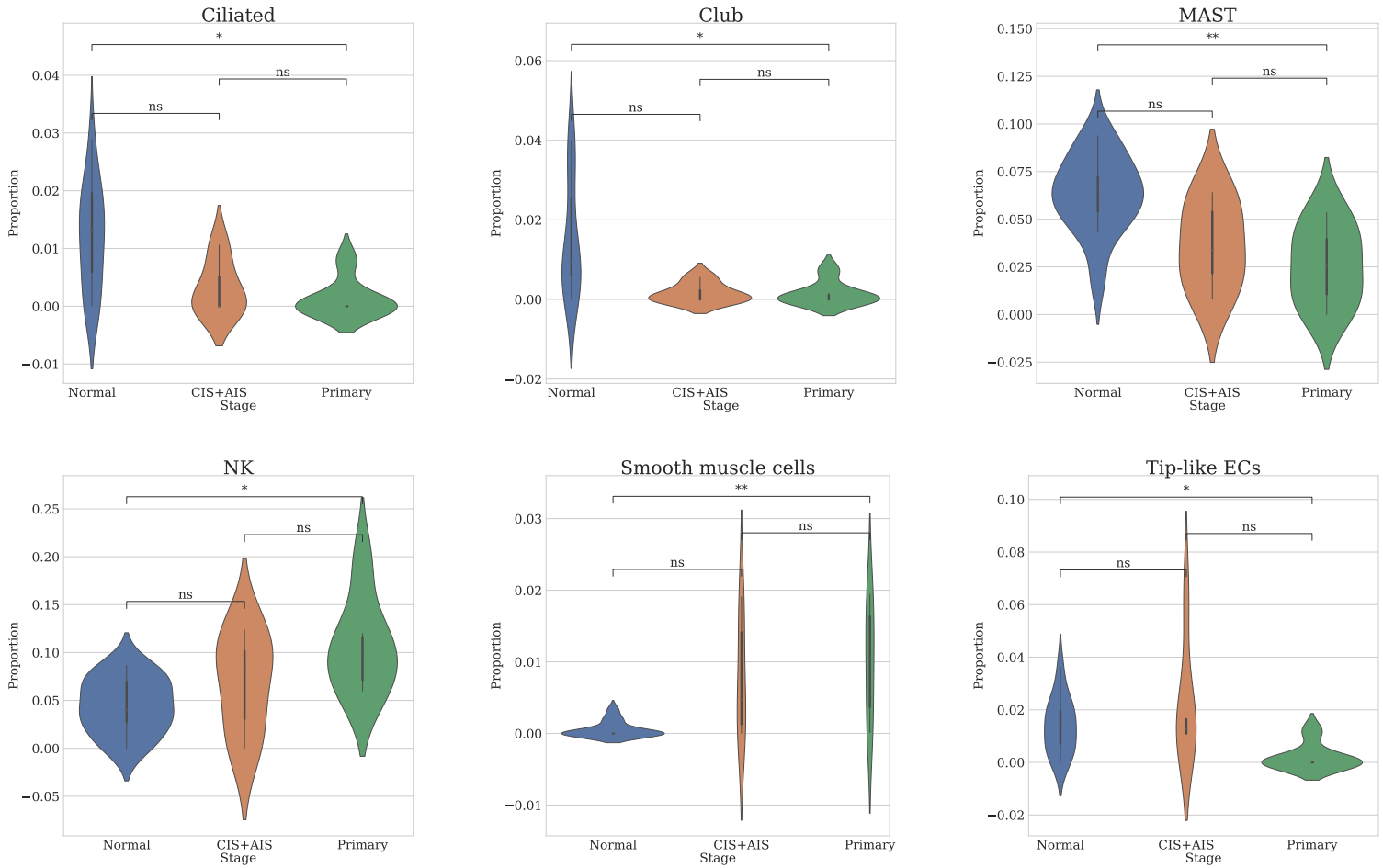


Figure: Violin Plots in LUAD

Findings in Bulk Cell Deconvolution

4. Results

4.8. Discovery of Gene Fusion

Arriba?

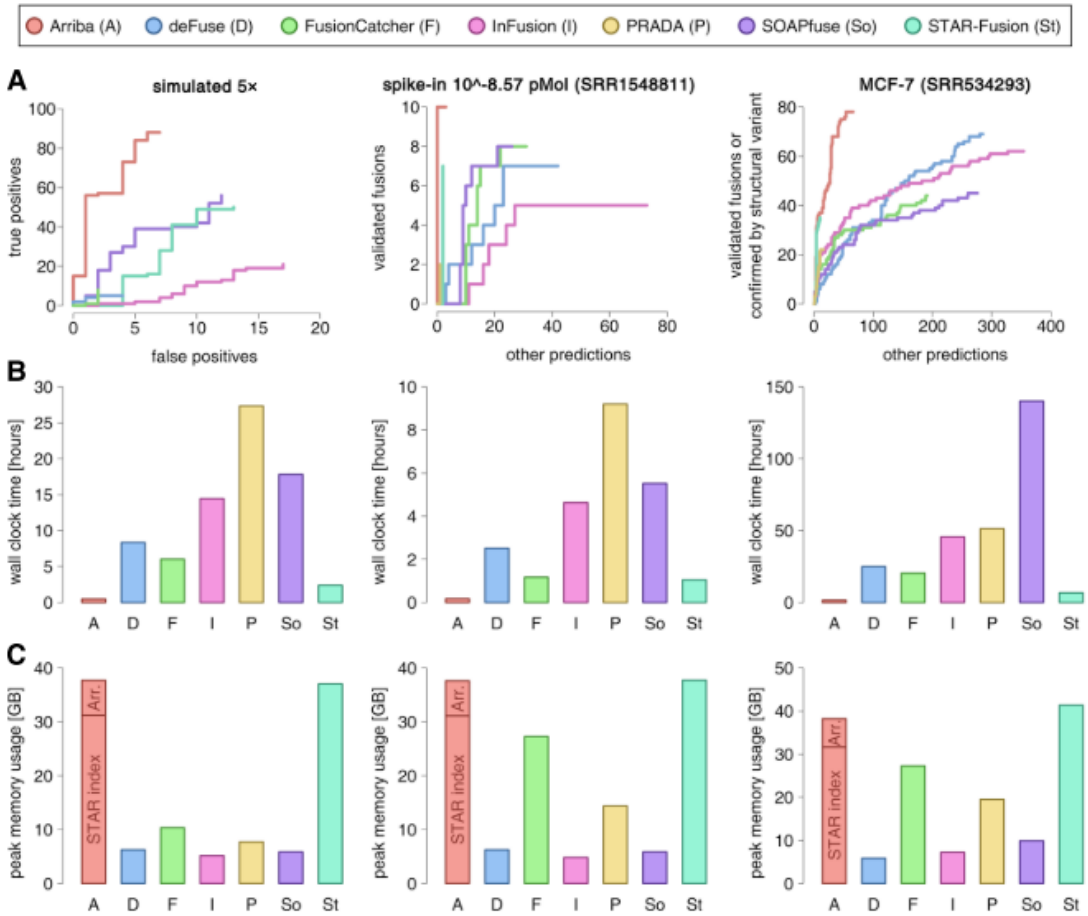


Figure: Benchmark of Arriba versus alternative methods (Uhrig et al., 2021)

Findings in Gene Fusion Discovery

5. Discussion

6. References

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