

Doctoral Thesis

<Lung Precancer Analysis>

<Jaewoong Lee>

<Department of Biomedical Engineering>

Ulsan National Institute of Science and Technology

<2022>

<Lung Precancer Analysis>

<Jaewoong Lee>

<Department of Biomedical Engineering>

Ulsan National Institute of Science and Technology

## **Abstract**



## Contents

I	Introduction . . . . .	1
1.1	Lung Cancer . . . . .	1
1.2	Non-small Cell lung cancer . . . . .	1
1.3	Lung Precancer . . . . .	1
1.4	Study Objectives . . . . .	1
II	Materials . . . . .	3
2.1	List of IPNs . . . . .	3
2.2	Data Composition . . . . .	3
III	Methods . . . . .	5
3.1	Workflows . . . . .	5
IV	Results . . . . .	8
4.1	Quality Checks . . . . .	8
4.2	Copy Number Variation Analyses . . . . .	8
4.3	Single Nucleotide Variation Analyses . . . . .	8
4.4	Variant Allele Frequency Analyses . . . . .	8
4.5	Bulk Cell Deconvolution Analyses . . . . .	8
4.6	Mutational Signature Analyses . . . . .	8

4.7	Point Mutation Analyses with Clinical Data . . . . .	8
4.8	Differentially Expressed Genes Analyses . . . . .	8
4.9	Gene Fusion Analyses . . . . .	8
V	Discussion . . . . .	25
5.1	General Conclusions . . . . .	25
5.2	Plan for Future . . . . .	25
5.3	Future Perspective . . . . .	25
	References . . . . .	26
	Acknowledgements . . . . .	27

## List of Figures

1	Common cancer survival rates (Hong et al., 2021) . . . . .	2
2	Lung cancer classification (Gridelli et al., 2015) . . . . .	2
3	Workflow for data pre-processing for variant discovery (Van der Auwera et al., 2013; DePristo et al., 2011) . . . . .	6
4	Somatic short variant discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011) . . . . .	6
5	Germline short variant discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011) . . . . .	7
6	RNA-seq short variant discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011) . . . . .	7
7	FastQC results with WES data . . . . .	9
8	FastQC results with WTS data . . . . .	9
9	Depths plot with WES data . . . . .	9
10	Quality Distribution by Samples . . . . .	10
11	Sequenza Cellularity and Ploidy Plots . . . . .	11
12	PureCN Purity and Ploidy Plots . . . . .	12
13	Sequenza LUSC Genome View Plot . . . . .	13
14	PureCN LUSC Genome View Plot . . . . .	14
15	CNVkit LUSC Genome View Plot . . . . .	15

16	Sequenza LUAD Genome View Plot . . . . .	16
17	PureCN LUAD Genome View Plot . . . . .	16
18	CNVkit LUAD Genome View Plot . . . . .	17
19	Sequenza LUSC Violin Plots . . . . .	17
20	PureCN LUSC Violin Plots . . . . .	18
21	Sequenza LUAD Violin Plots . . . . .	18
22	PureCN LUAD Violin Plots . . . . .	19
23	Comut Plot by LUSC . . . . .	19
24	Comut Plot by LUAD . . . . .	20
25	BisqueRNA clustermap plot with LUSC samples upon GSE131907 . . . . .	21
26	MuSiC clustermap plot with LUSC samples upon GSE131907 . . . . .	22
27	SCDC clustermap plot with LUSC samples upon GSE131907 . . . . .	23
28	BisqueRNA clustermap plot with LUAD samples upon GSE131907 . . . . .	24
29	MuSiC clustermap plot with LUAD samples upon GSE131907 . . . . .	24
30	SCDCSiC clustermap plot with LUAD samples upon GSE131907 . . . . .	24

## **List of Tables**

1	WES Data Composition . . . . .	4
2	WTS Data Composition . . . . .	4

# **I Introduction**

## **1.1 Lung Cancer**

Lung cancer is the most common form of cancer as 12.3 % of all cancers (Minna, Roth, & Gazdar, 2002).

## **1.2 Non-small Cell lung cancer**

**Lung Adenocarcinoma (LUAD)**

**Lung Squamous Cell Carcinoma (LUSC)**

**LUSC vs. LUAD**

## **1.3 Lung Precancer**

## **1.4 Study Objectives**

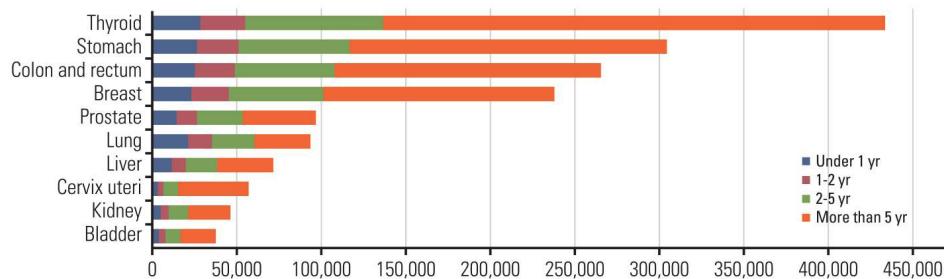


Figure 1: Common cancer survival rates (Hong et al., 2021)

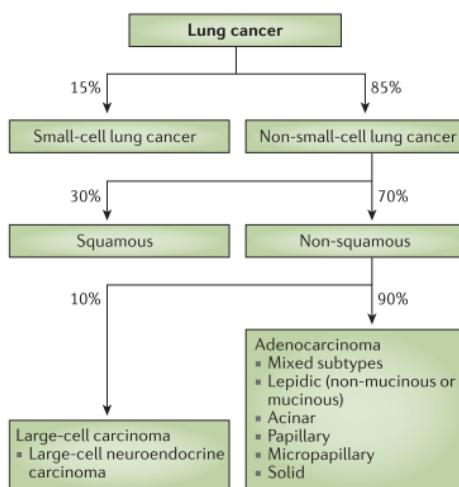


Figure 2: Lung cancer classification (Gridelli et al., 2015)

## **II Materials**

### **2.1 List of IPNs**

#### **Carcinoma *in situ***

Carcinoma *in situ* (CIS)

#### **Adenocarcinoma *in situ***

Adenocarcinoma *in situ* (AIS)

#### **Atypical Adenomatous Hyperplasia**

Atypical adenomatous hyperplasia (AAH)

#### **Dysplasia**

#### **Minimally Invasive Adenocarcinoma**

Minimally invasive adenocarcinoma (MIA)

### **2.2 Data Composition**

Table 1: WES Data Composition

Cancer Subtype	Stage	Number of Samples	
LUSC	Normal	77	
	Dysplasia	5	
	AAH	8	
	CIS+AIS	73	
	Primary	77	
	Total	240	
LUAD	Normal	18	
	AAH	15	
	CIS+AIS	9	
	MIA	1	
	Primary	18	
	Total	61	

Table 2: WTS Data Composition

Cancer Subtype	Stage	Number of Samples	
LUSC	Normal	17	
	Dysplasia	2	
	CIS+AIS	34	
	Primary	36	
	Total	89	
LUAD	Normal	13	
	AAH	1	
	CIS+AIS	5	
	Primary	6	
	Total	25	

### **III Methods**

#### **3.1 Workflows**

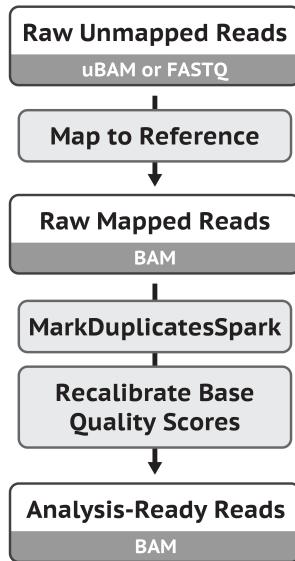


Figure 3: Workflow for data pre-processing for variant discovery (Van der Auwera et al., 2013; DePristo et al., 2011)

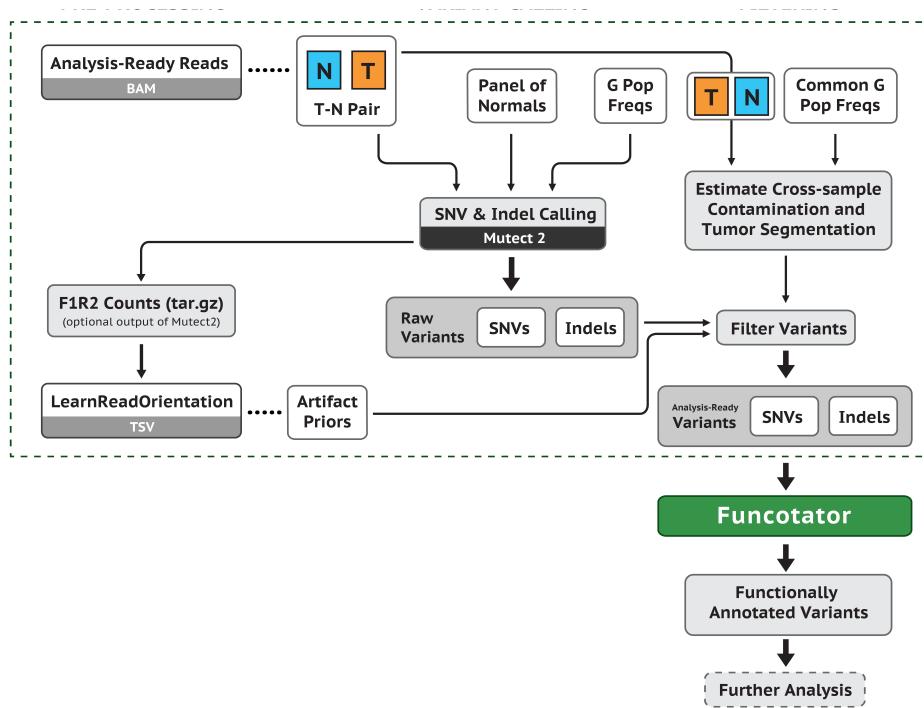


Figure 4: Somatic short variant discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

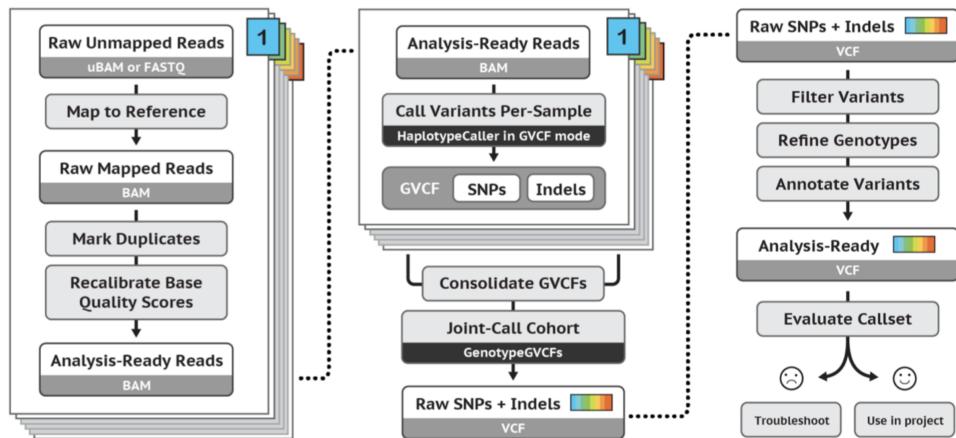


Figure 5: Germline short variant discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

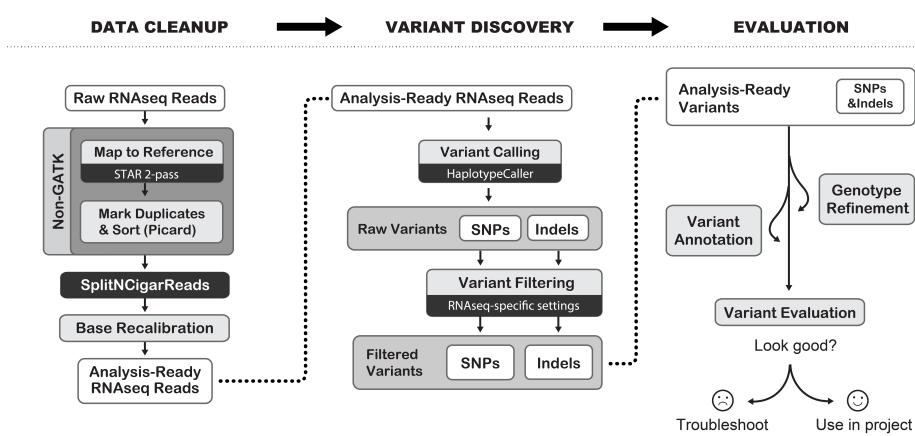


Figure 6: RNA-seq short variant discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

## **IV Results**

### **4.1 Quality Checks**

**Quality Checks with FastQC**

**Quality Checks with Depths**

**Quality Checks with Picard**

**Findings in Quality Checks**

### **4.2 Copy Number Variation Analyses**

**Purity and Ploidy**

**Copy Number Variation Analyses**

**Copy Number Variation Analyses with Recurrence**

**Copy Number Variation Analyses with Smoking History**

**Gistic Analyses**

**Gistic Analyses with Recurrence**

**Gistic Analyses with Smoking History**

**Findings in Copy Number Variation Analyses**

### **4.3 Single Nucleotide Variation Analyses**

**Somatic Short Variation Analyses with Mutect2**

**Somatic Short Variant with Clinical Data**

**Findings in Somatic Short Variation Analyses**

### **4.4 Variant Allele Frequency Analyses**

**Findings in Variant Allele Frequency Analyses**

### **4.5 Bulk Cell Deconvolution Analyses**

**Single-cell Reference Data**

**GSE131907 as Reference**

**GSE162498 as Reference**

**GSE179994 as Reference**

**Findings in Bulk Cell Deconvolution Analyses**

### **4.6 Mutational Signature Analyses**

**Single Base Substitutions**

**Double Base Substitutions**

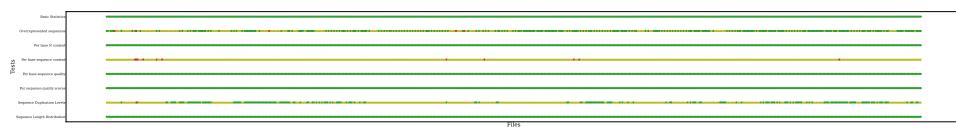


Figure 7: FastQC results with WES data

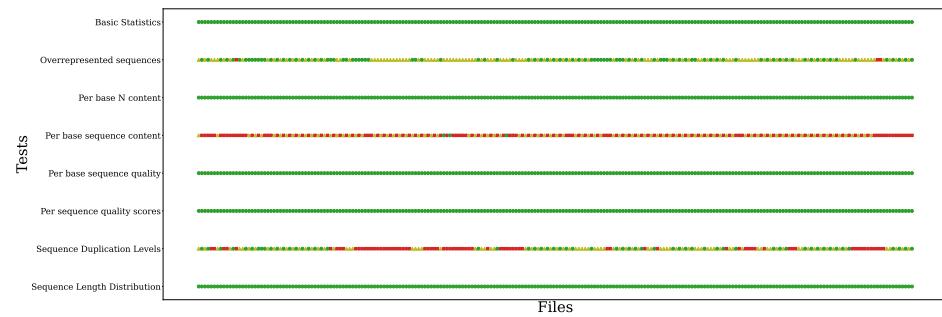


Figure 8: FastQC results with WTS data

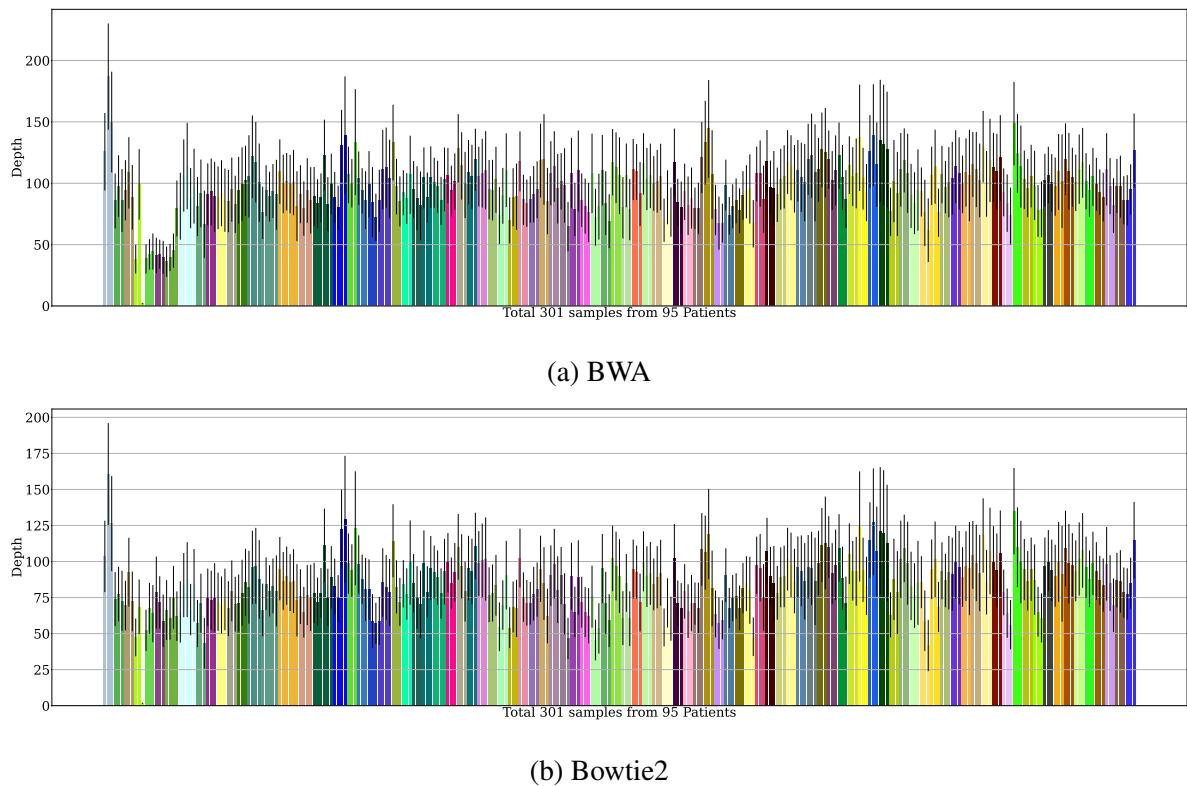
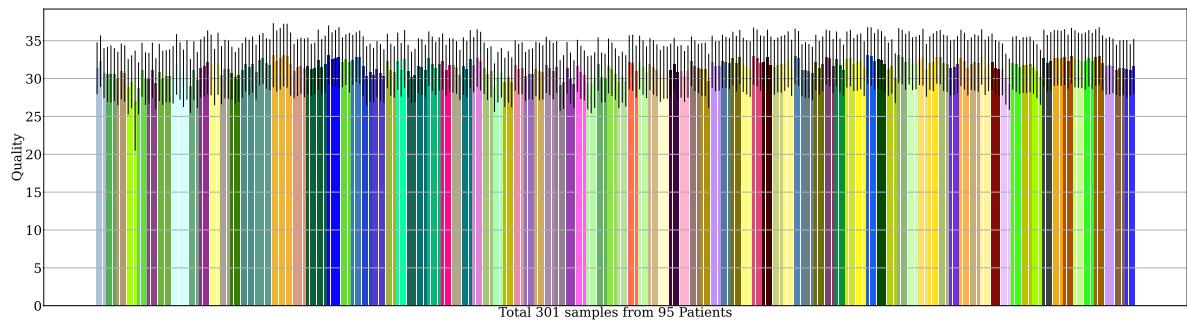
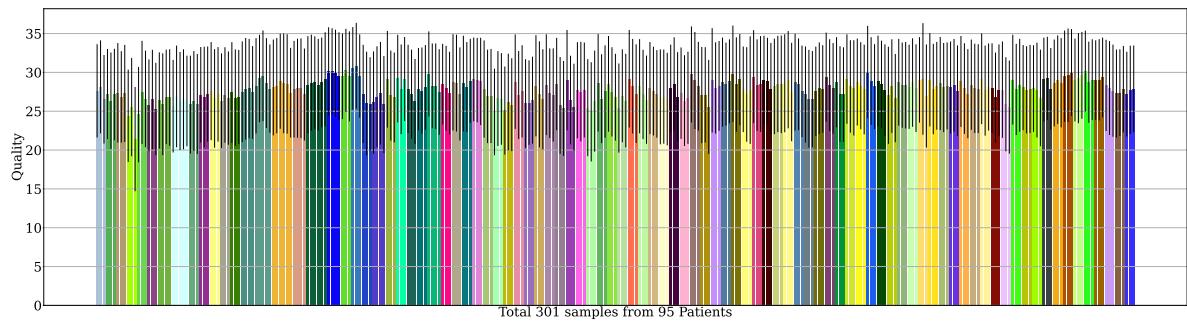


Figure 9: Depths plot with WES data



(a) BWA



(b) Bowtie2

Figure 10: Quality Distribution by Samples

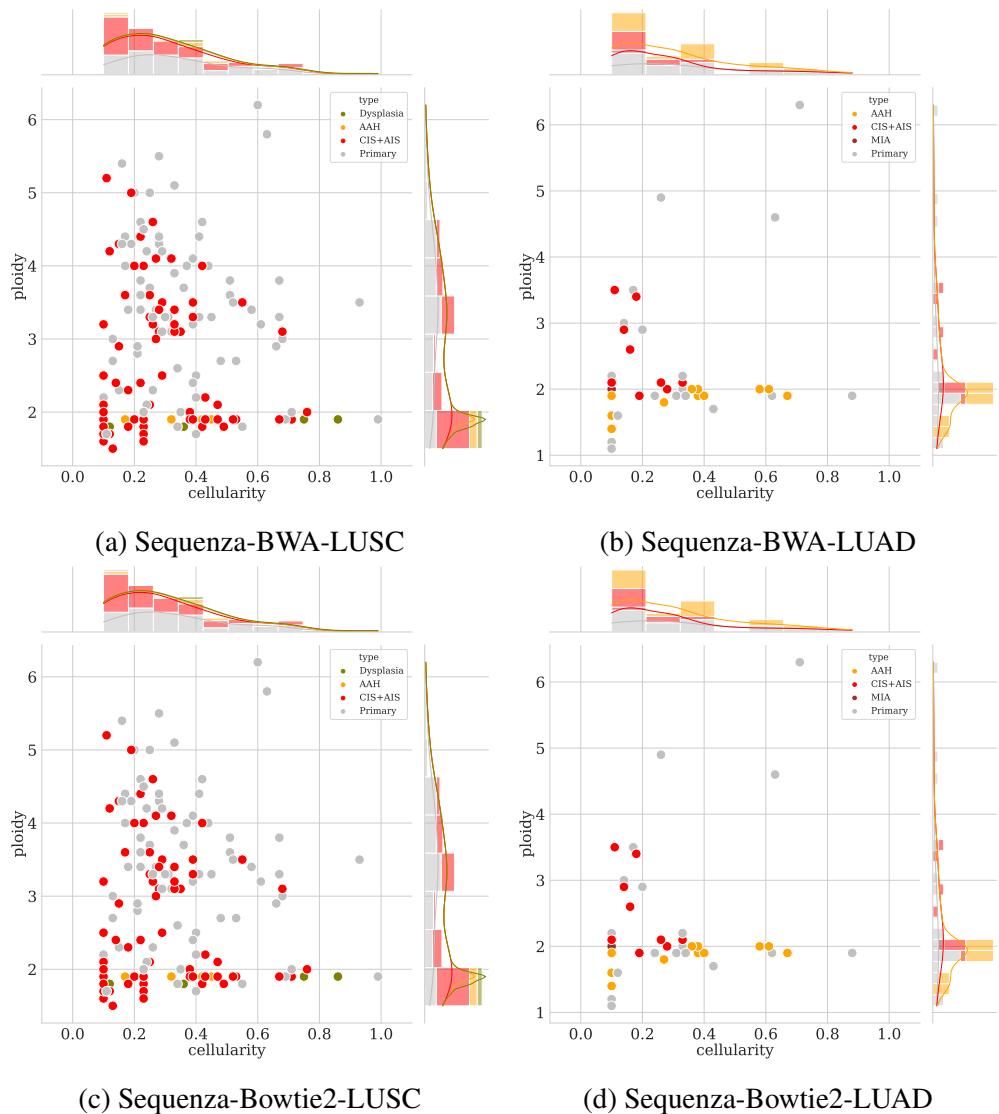


Figure 11: Sequenza Cellularity and Ploidy Plots

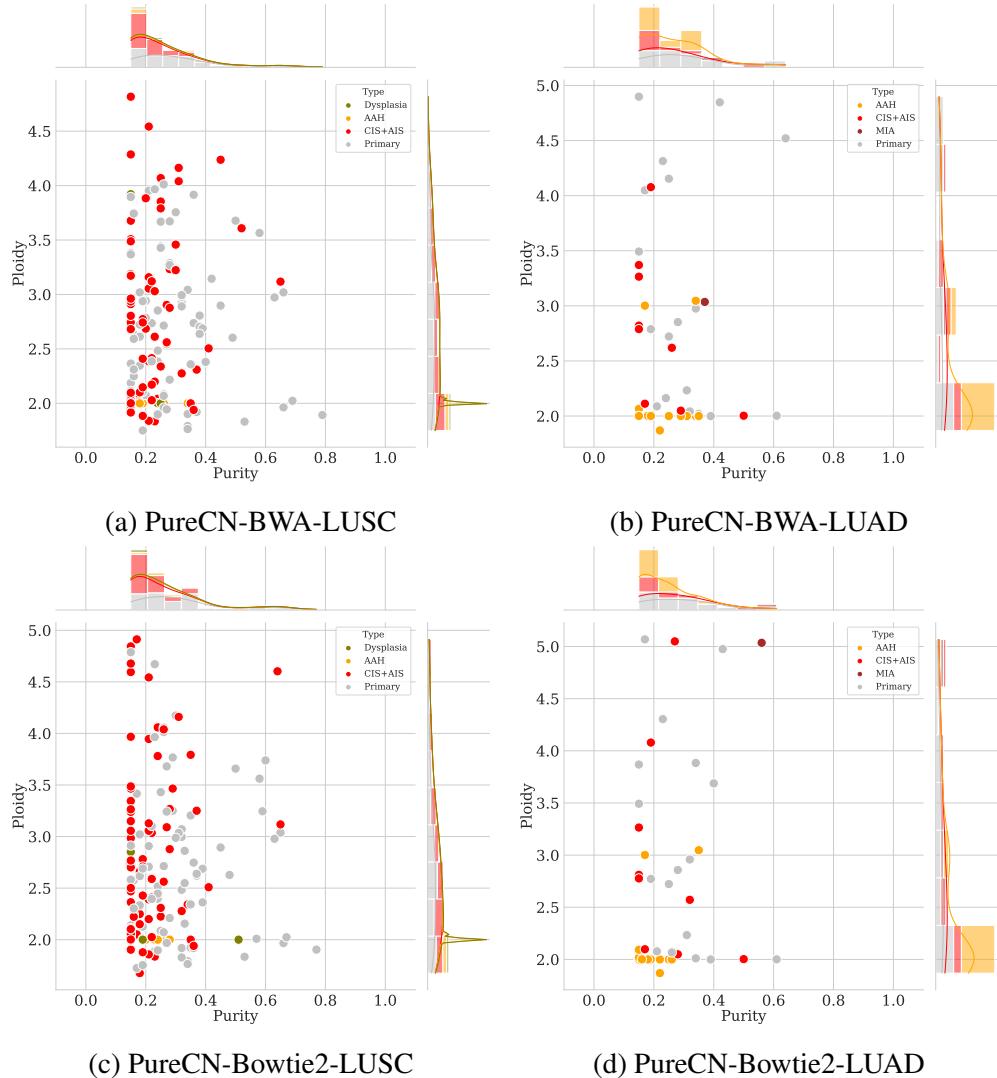


Figure 12: PureCN Purity and Ploidy Plots

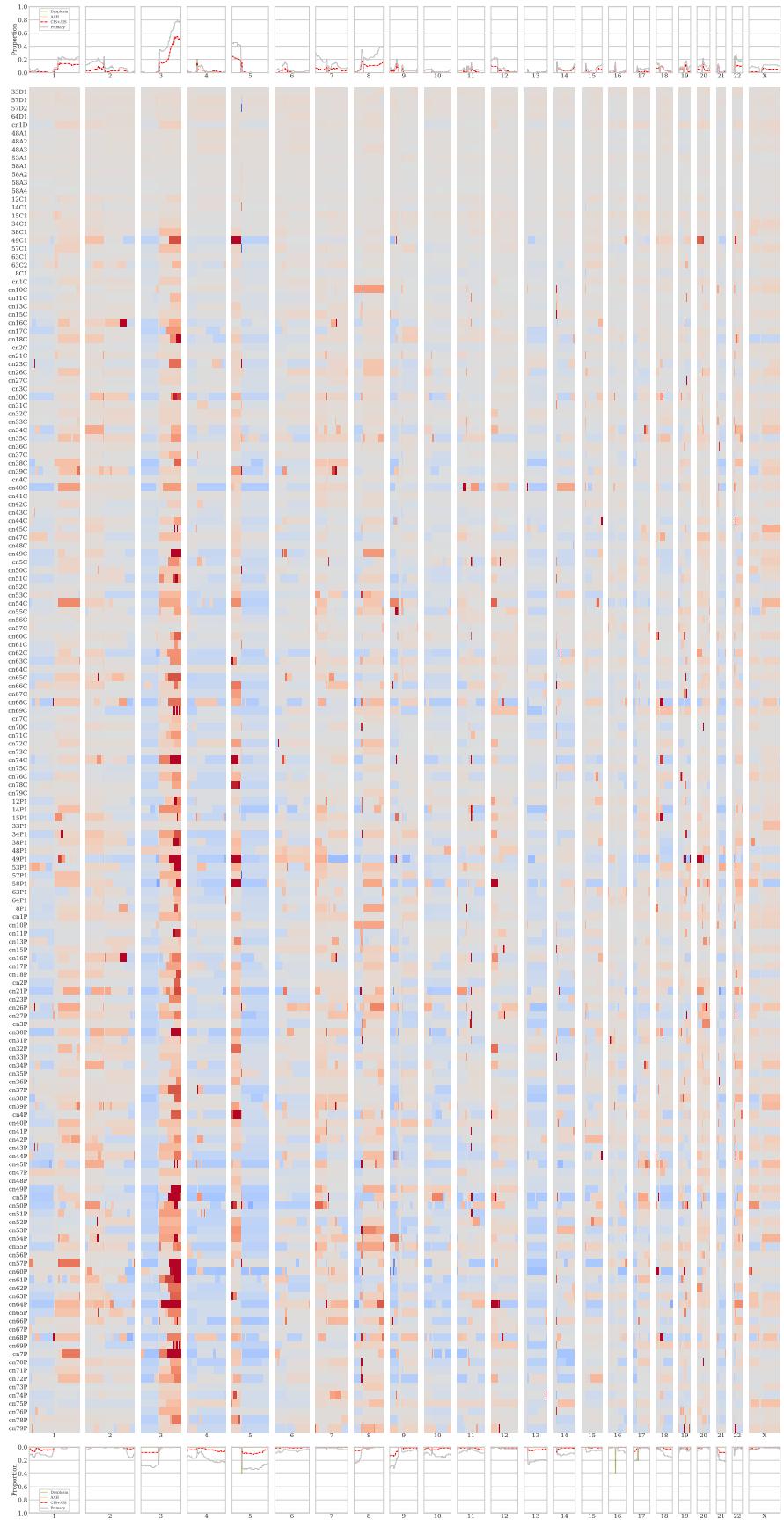


Figure 13: Sequenza LUSC Genome View Plot

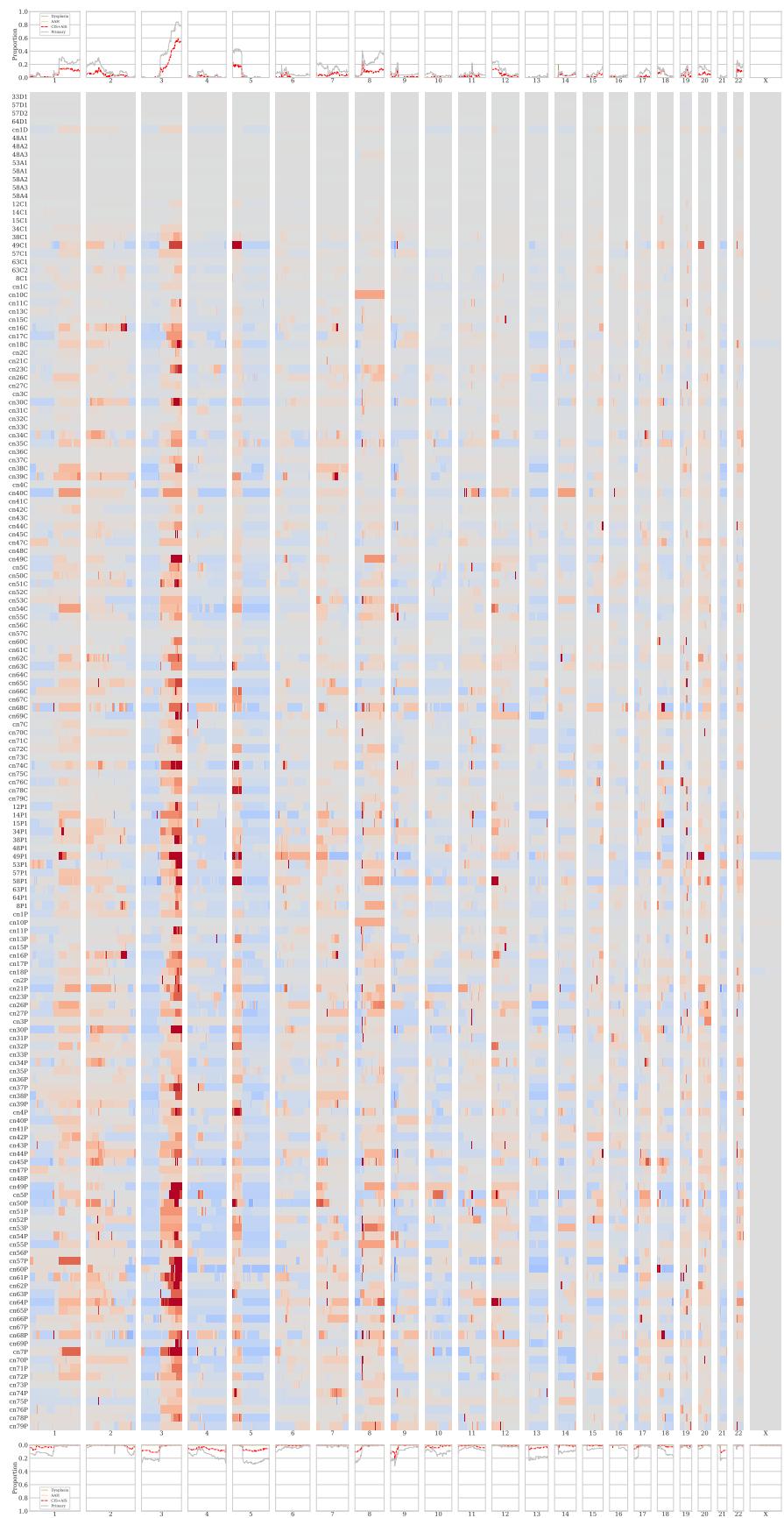


Figure 14: PureCN LUSC Genome View Plot

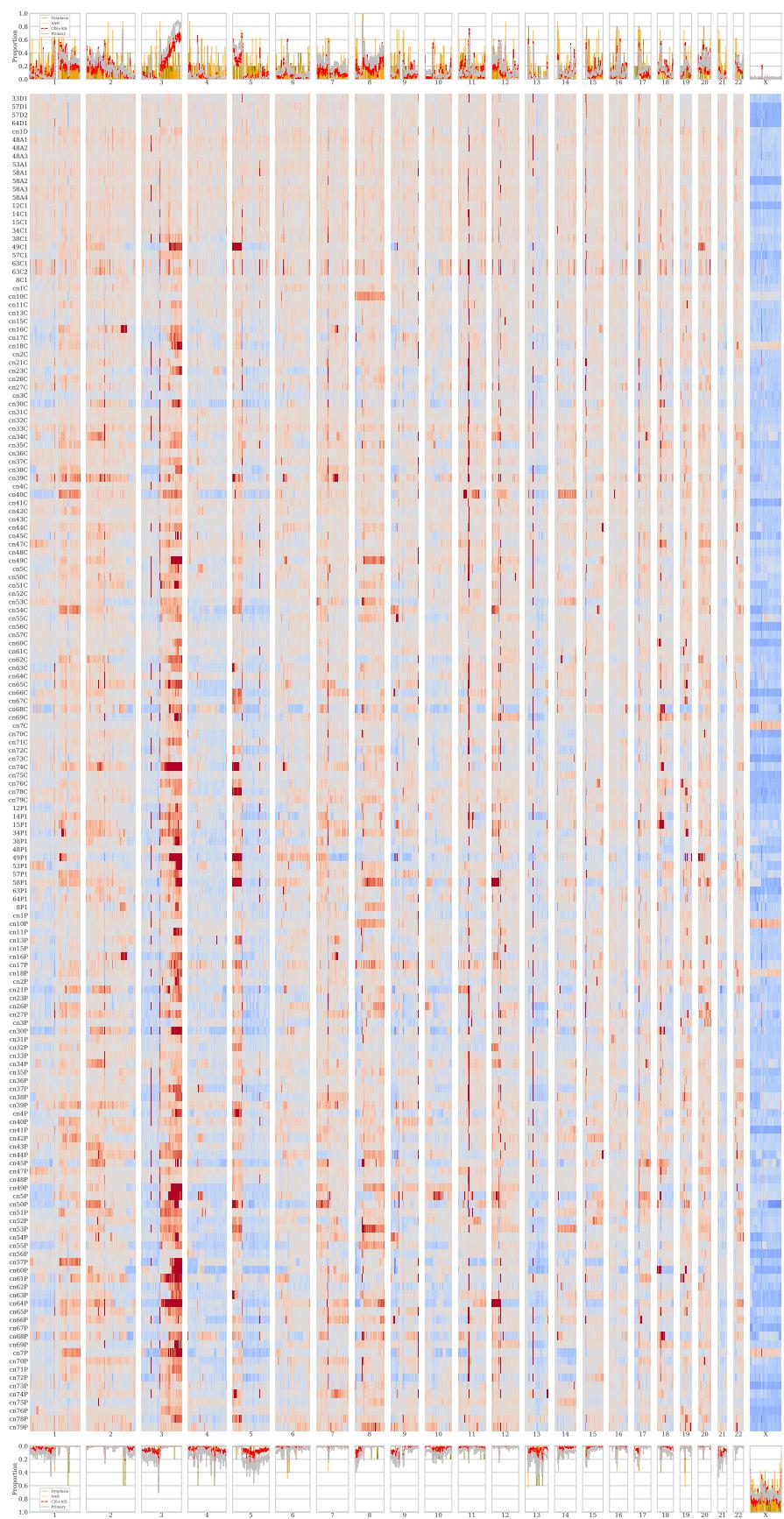


Figure 15: CNVkit LUSC Genome View Plot

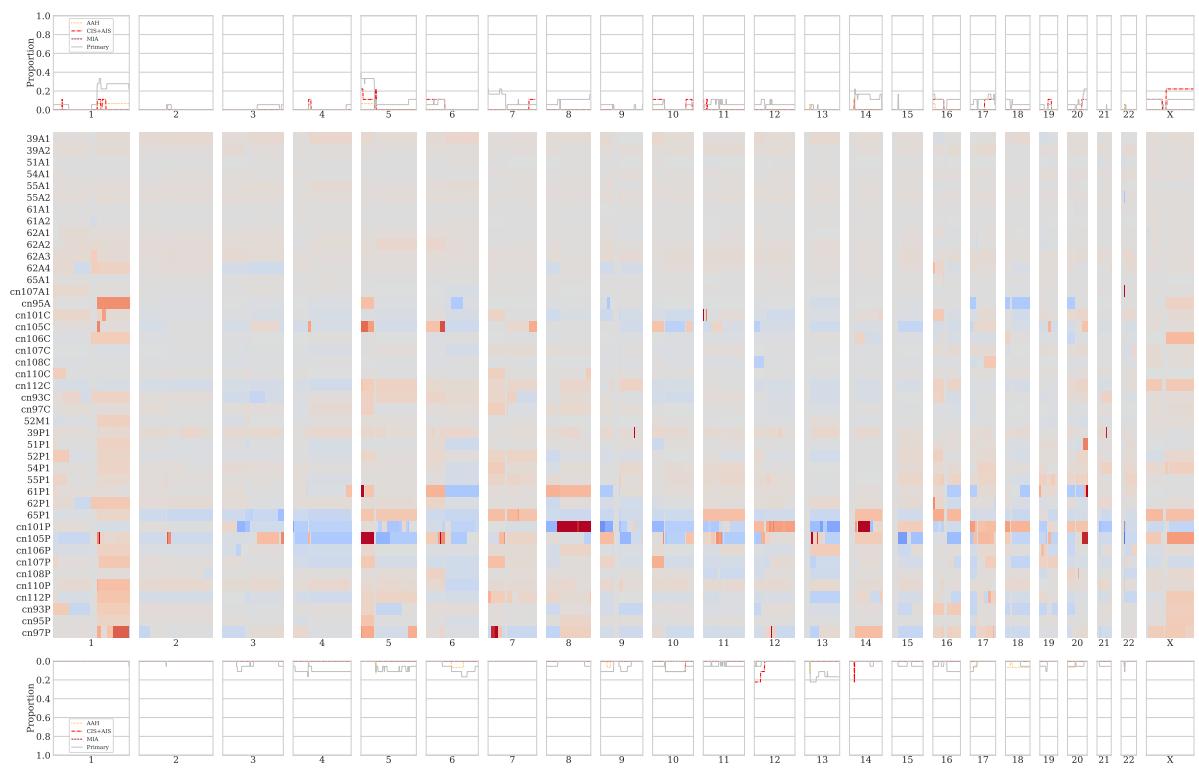


Figure 16: Sequenza LUAD Genome View Plot

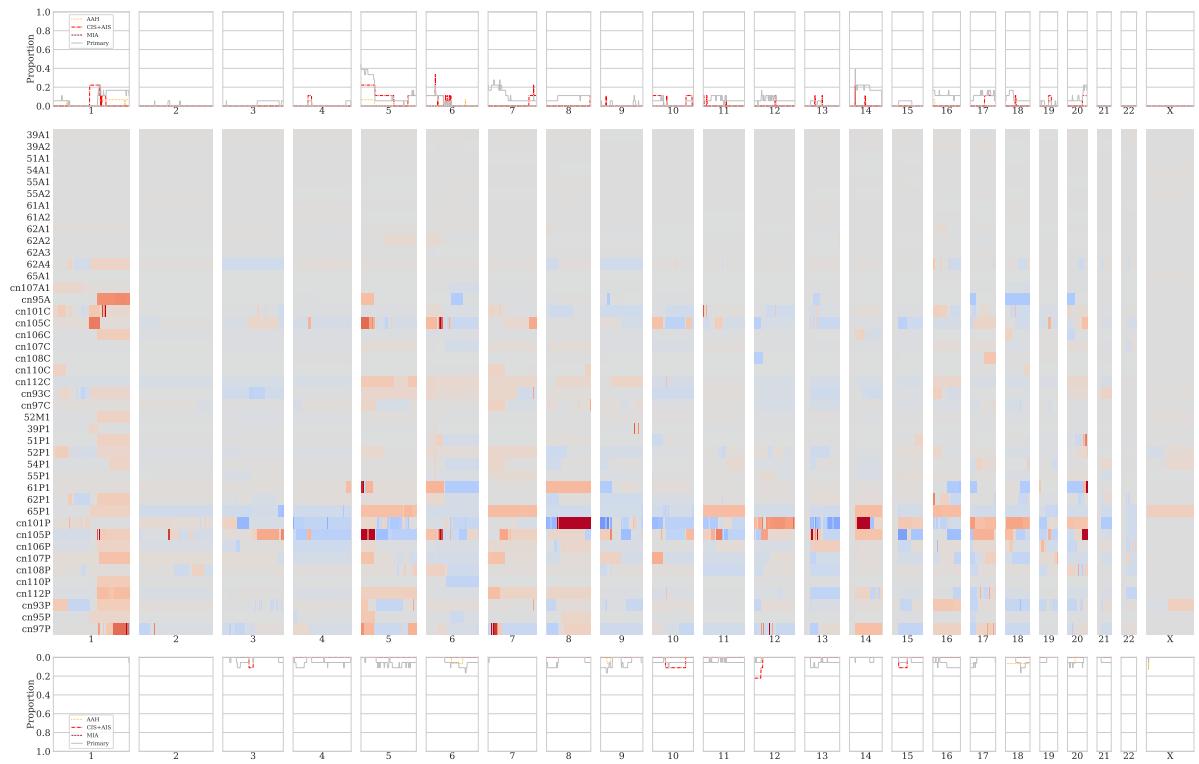


Figure 17: PureCN LUAD Genome View Plot

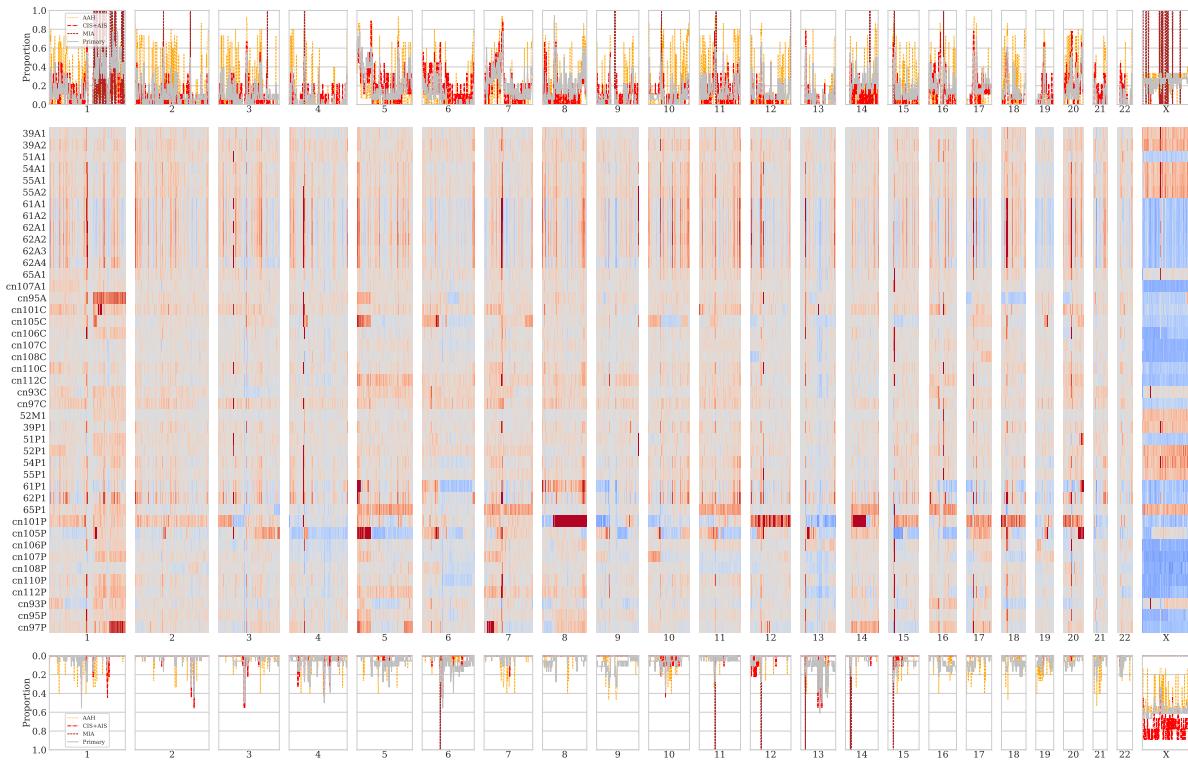


Figure 18: CNVkit LUAD Genome View Plot

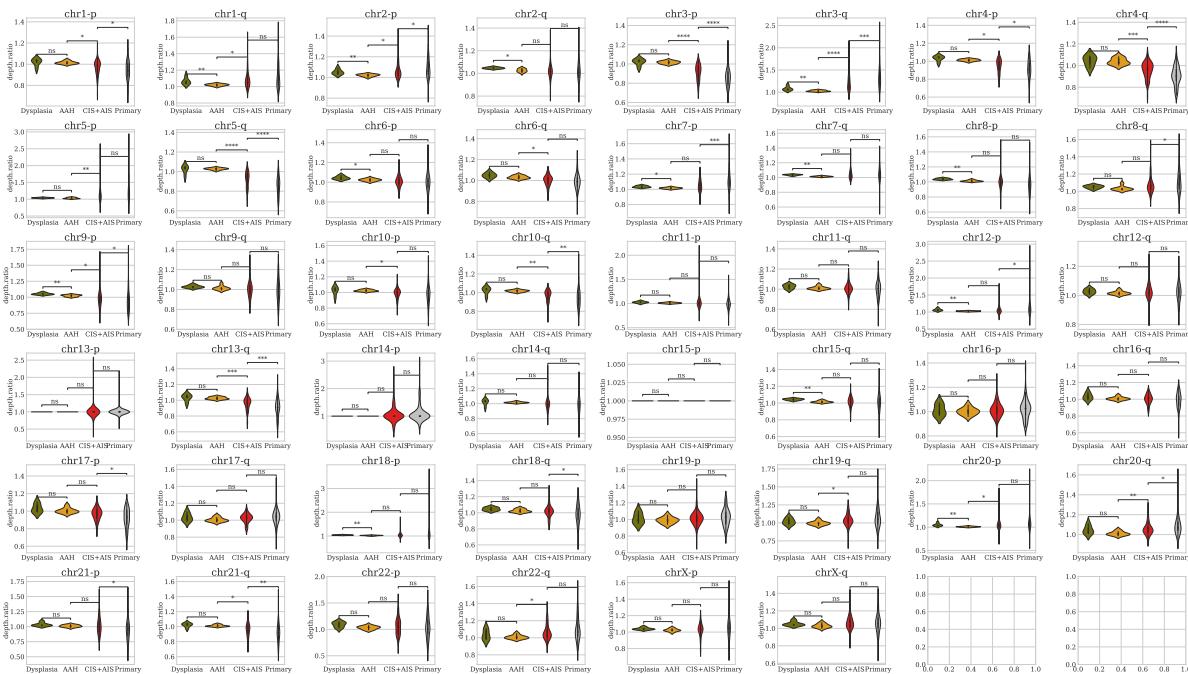


Figure 19: Sequenza LUSC Violin Plots

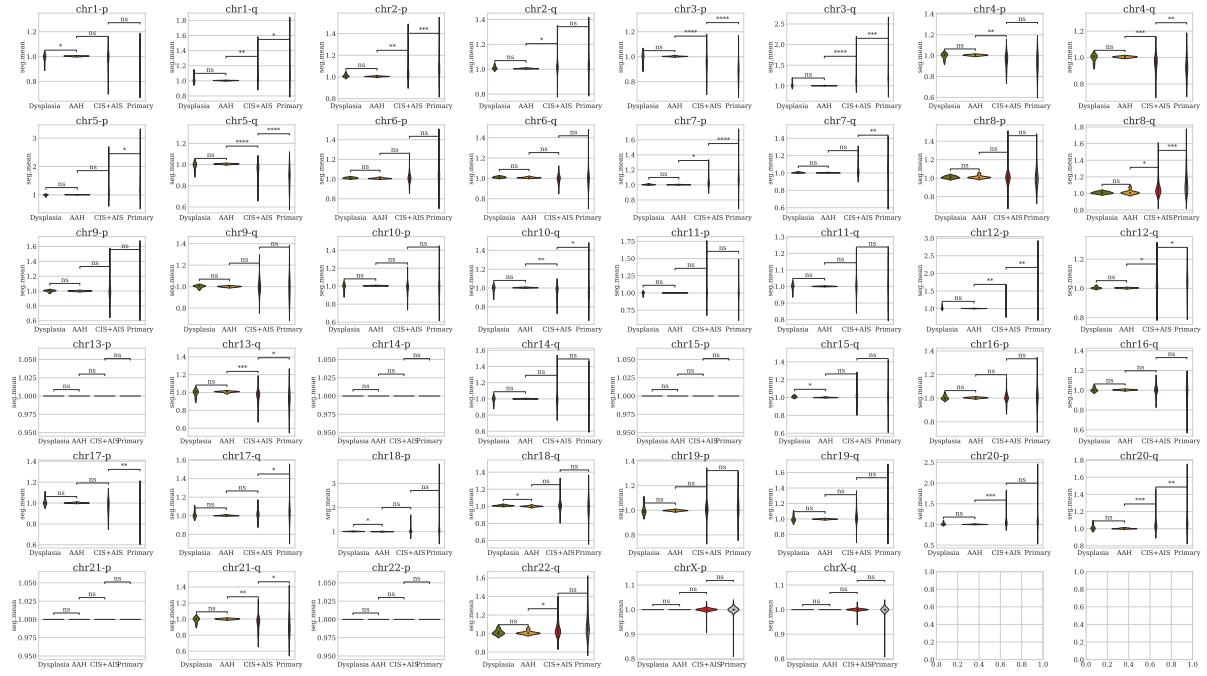


Figure 20: PureCN LUSC Violin Plots

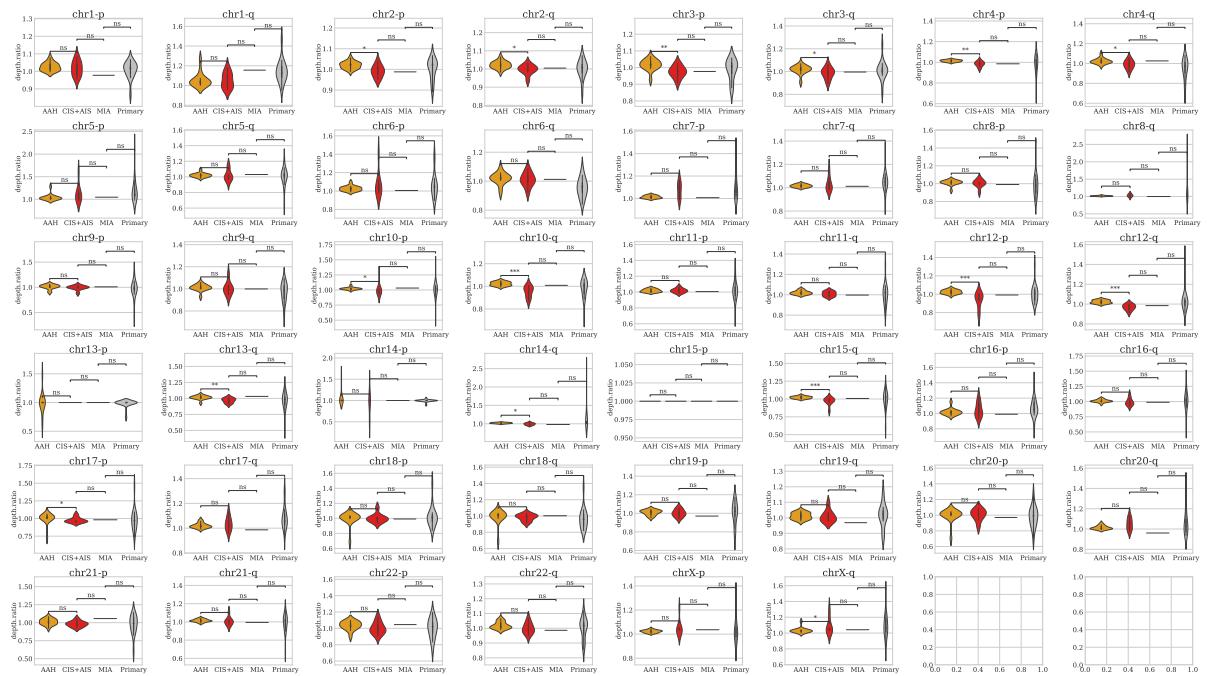


Figure 21: Sequenza LUAD Violin Plots

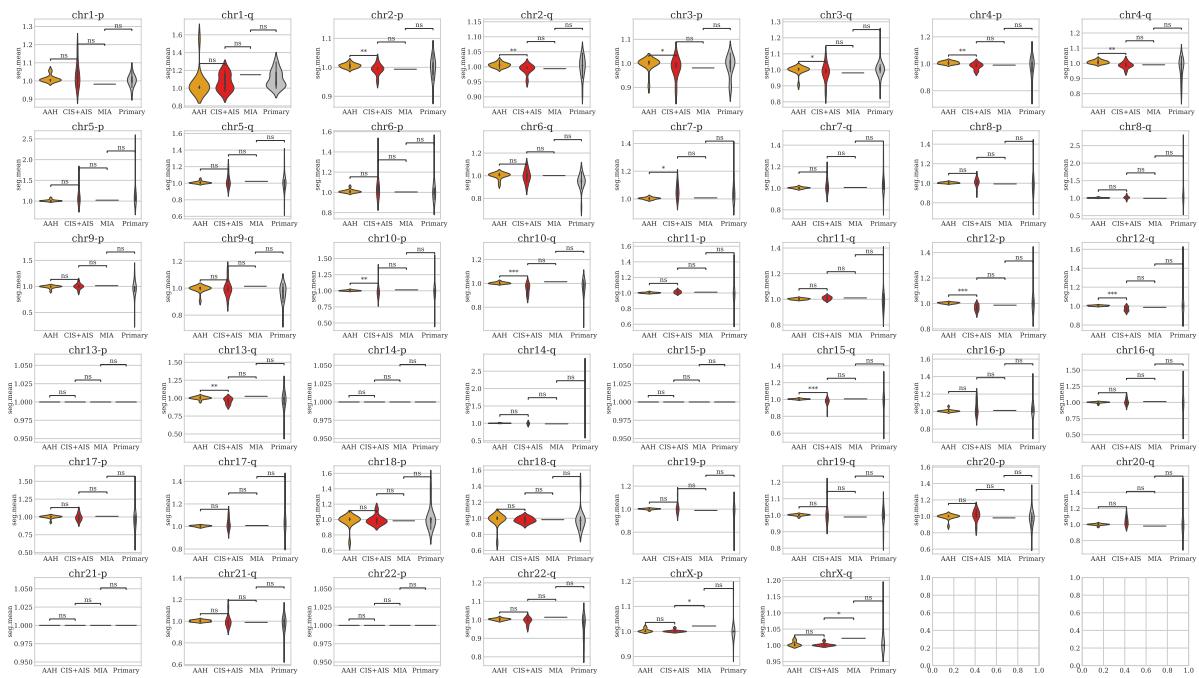


Figure 22: PureCN LUAD Violin Plots

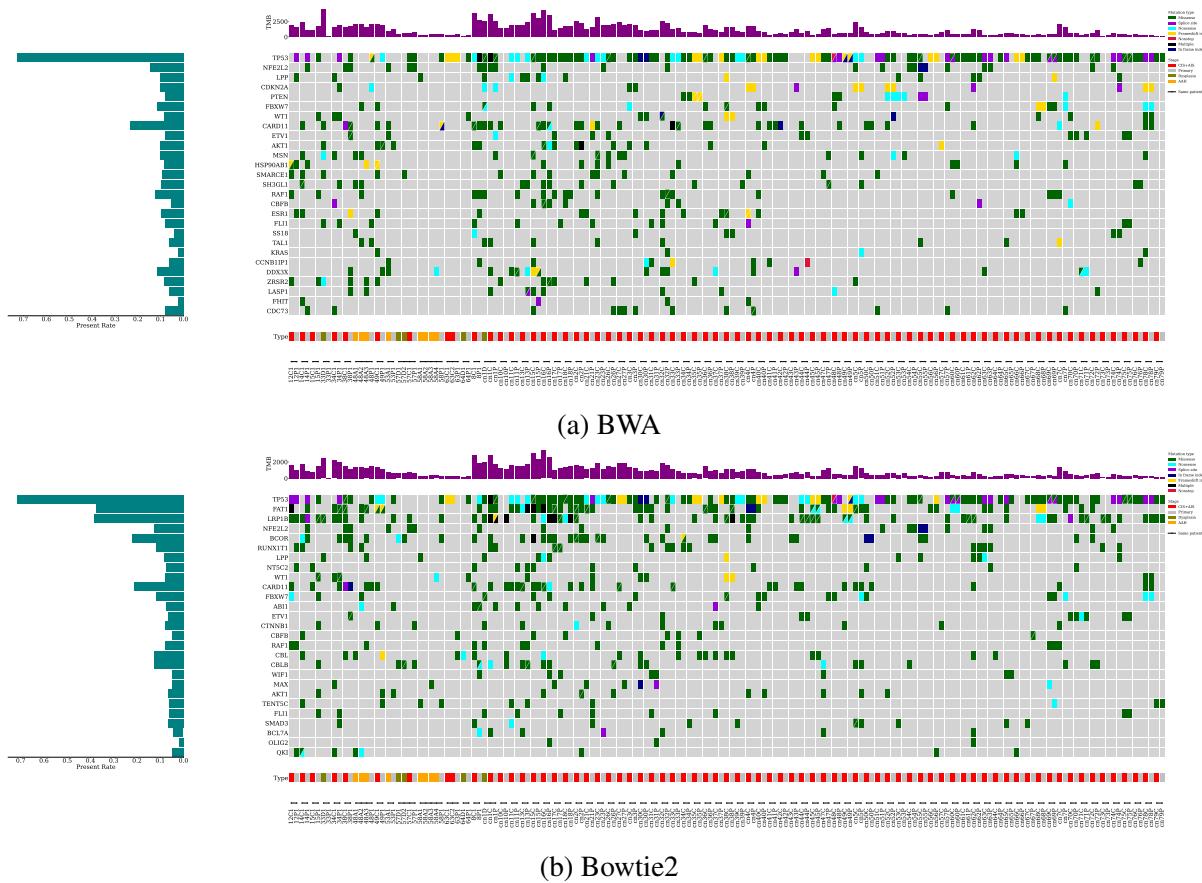


Figure 23: Comut Plot by LUSC

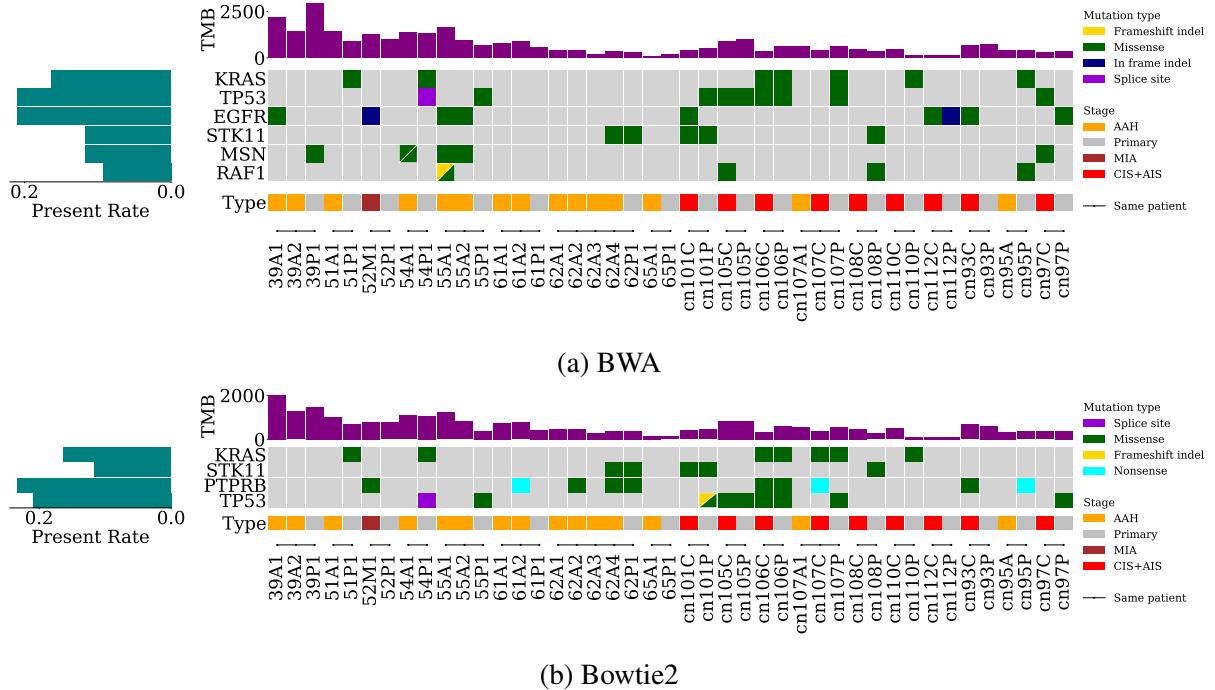


Figure 24: Comut Plot by LUAD

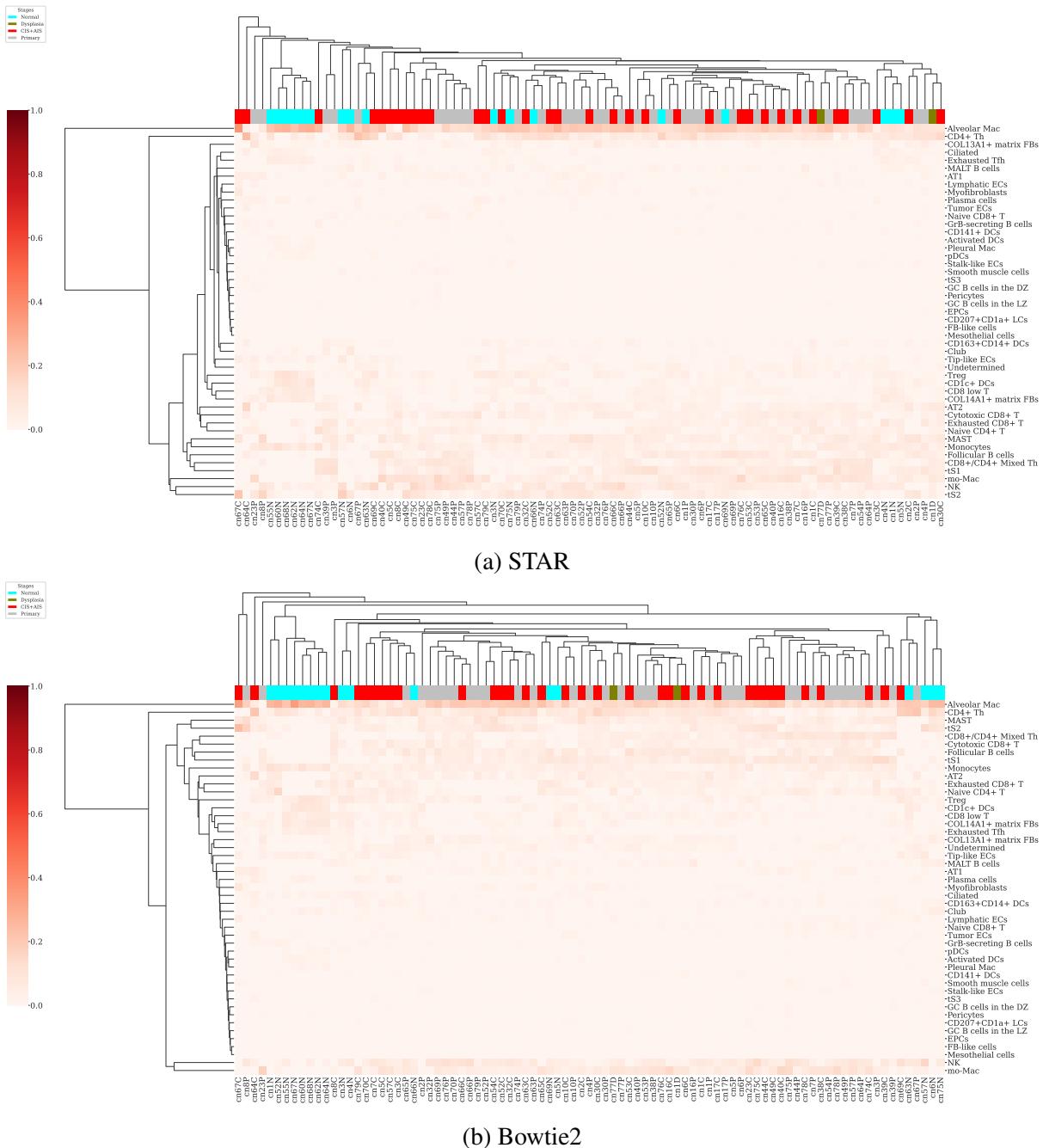


Figure 25: BisqueRNA clustermap plot with LUSC samples upon GSE131907

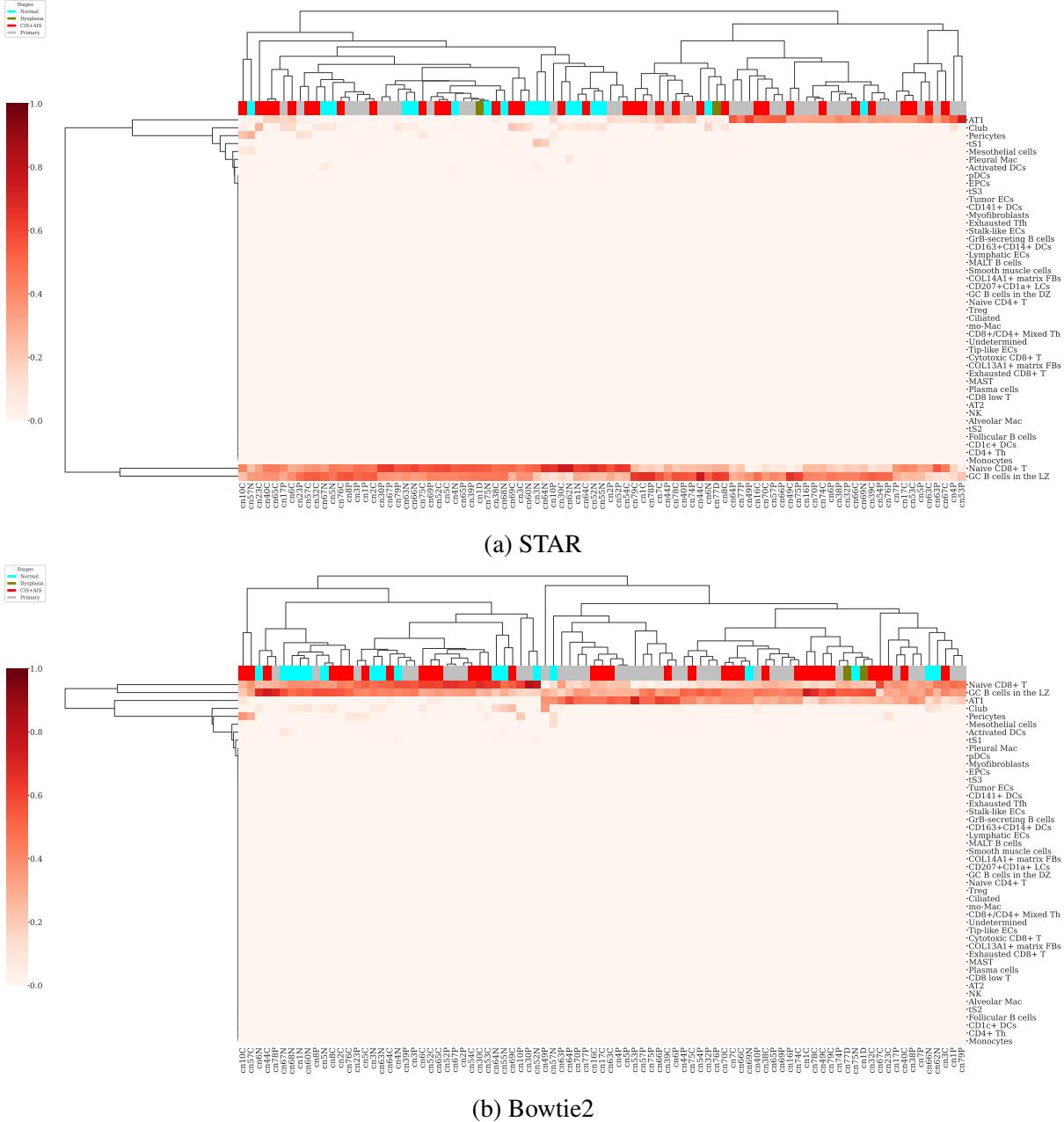


Figure 26: MuSiC clustermap plot with LUSC samples upon GSE131907

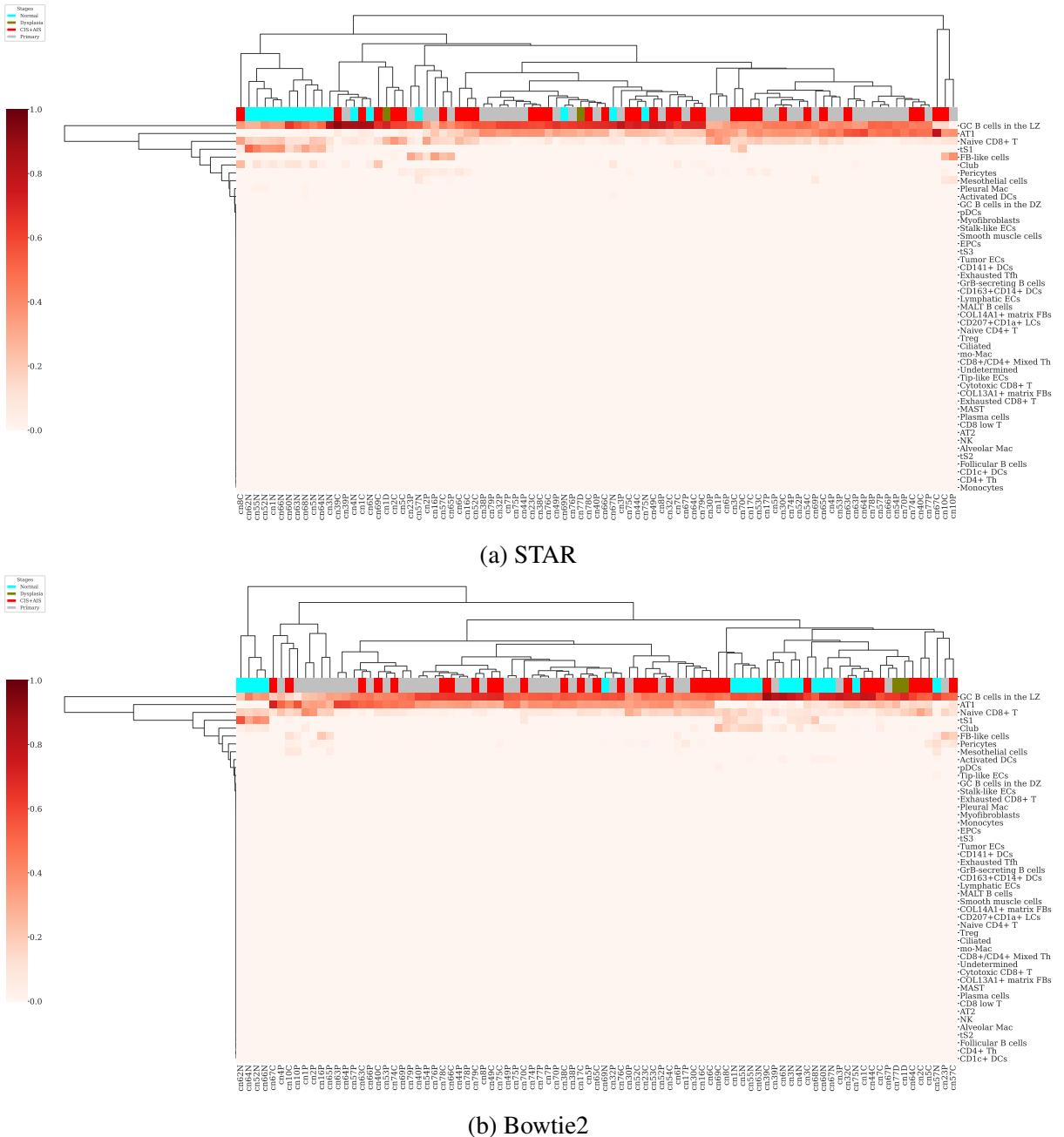


Figure 27: SCDC clustermap plot with LUSC samples upon GSE131907

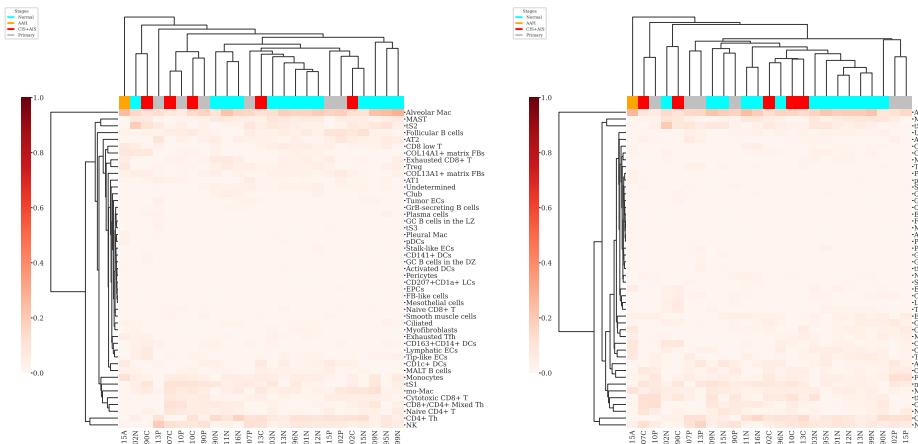


Figure 28: BisqueRNA clustermap plot with LUAD samples upon GSE131907

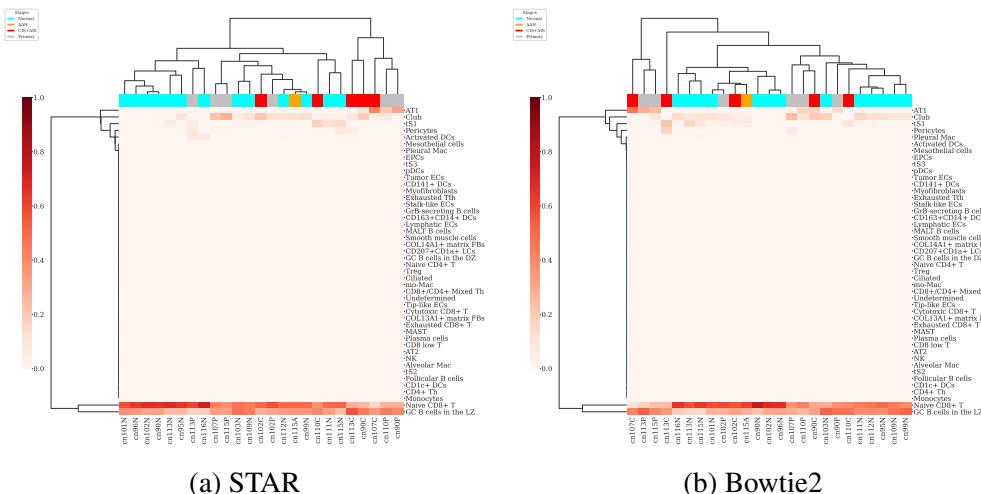


Figure 29: MuSiC clustermap plot with LUAD samples upon GSE131907

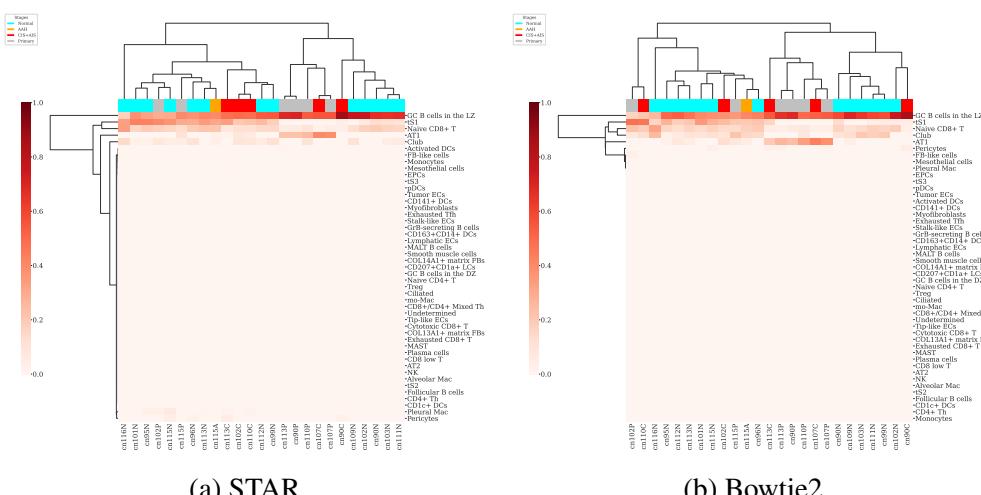


Figure 30: SCDCSiC clustermap plot with LUAD samples upon GSE131907

## **V Discussion**

**5.1 General Conclusions**

**5.2 Plan for Future**

**5.3 Future Perspective**

# References

- 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.
- Gridelli, C., Rossi, A., Carbone, D. P., Guarize, J., Karachaliou, N., Mok, T., ... Rosell, R. (2015). Non-small-cell lung cancer. *Nature reviews Disease primers*, 1(1), 1–16.
- 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.
- Minna, J. D., Roth, J. A., & Gazdar, A. F. (2002). Focus on lung cancer. *Cancer cell*, 1(1), 49–52.
- 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.

## **Acknowledgements**

Thank you very much.

