

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	Deferentially Expressed Genes Analyses	8
4.9	Gene Fusion Analyses	8
V	Discussion	28
5.1	General Conclusions	28
5.2	Plan for Future	28
5.3	Future Perspective	28
	References	29
	Acknowledgements	30

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
31	BisqueRNA clustermap plot with LUSC samples upon GSE162498	25
32	MuSiC clustermap plot with LUSC samples upon GSE162498	25
33	SCDC clustermap plot with LUSC samples upon GSE162498	26
34	BisqueRNA clustermap plot with LUAD samples upon GSE162498	26
35	MuSiC clustermap plot with LUAD samples upon GSE162498	26
36	SCDC clustermap plot with LUAD samples upon GSE162498	27

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

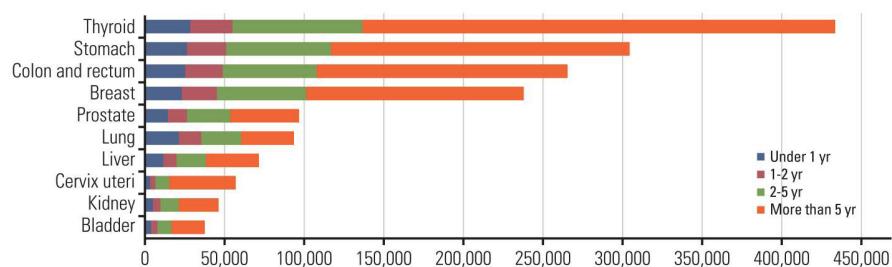


Figure 1: Common cancer survival rates (Hong et al., 2021)

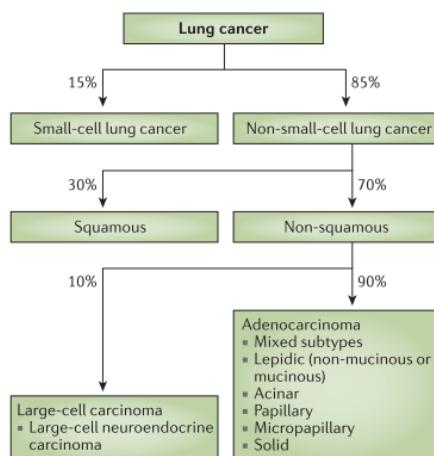


Figure 2: Lung cancer classification (Gridelli et al., 2015)

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	Number of Samples	
	Stage	
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	Number of Samples	
	Stage	
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

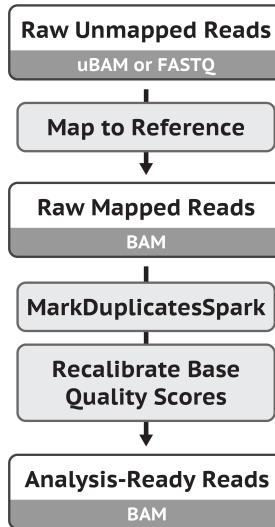


Figure 3: Workflow for data pre-processing for variant discovery (Van der Auwera et al., 2013; DePristo et al., 2011)

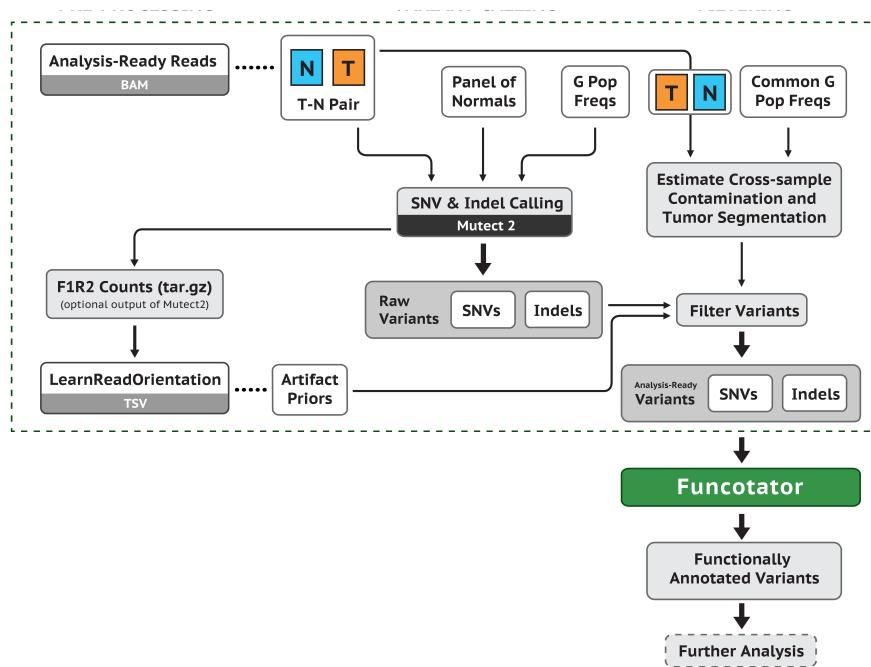


Figure 4: Somatic short variant discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

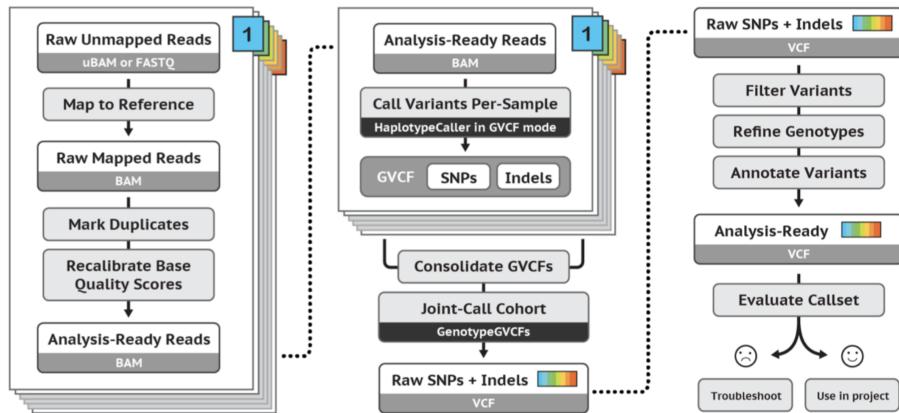


Figure 5: Germline short variant discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

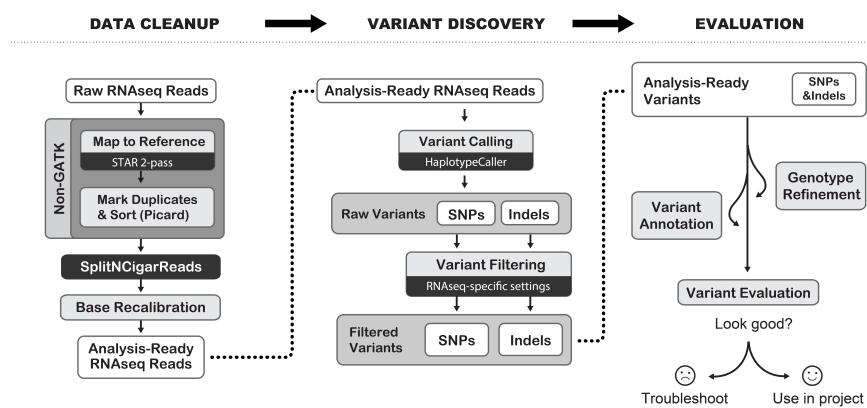


Figure 6: RNA-seq short variant discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

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

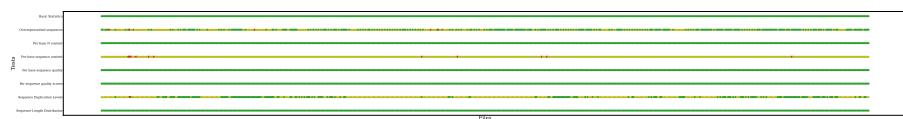


Figure 7: FastQC results with WES data

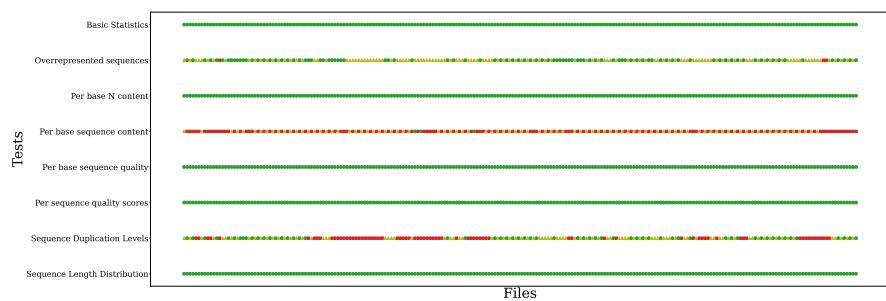
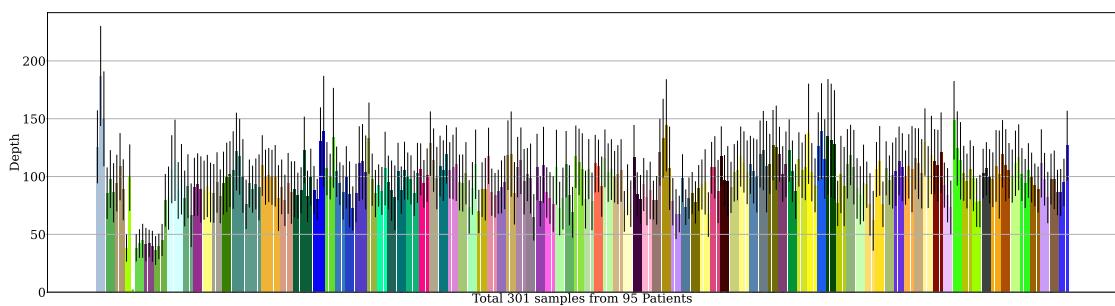
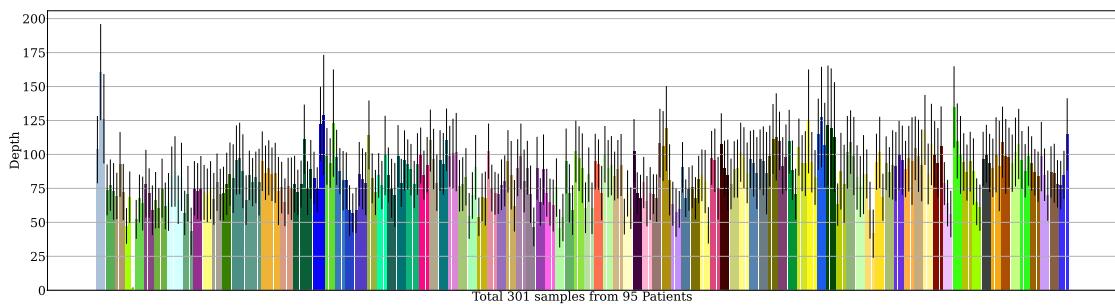


Figure 8: FastQC results with WTS data

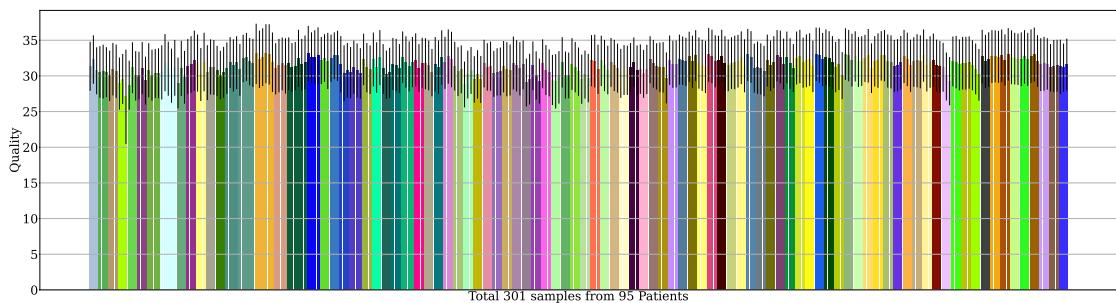


(a) BWA

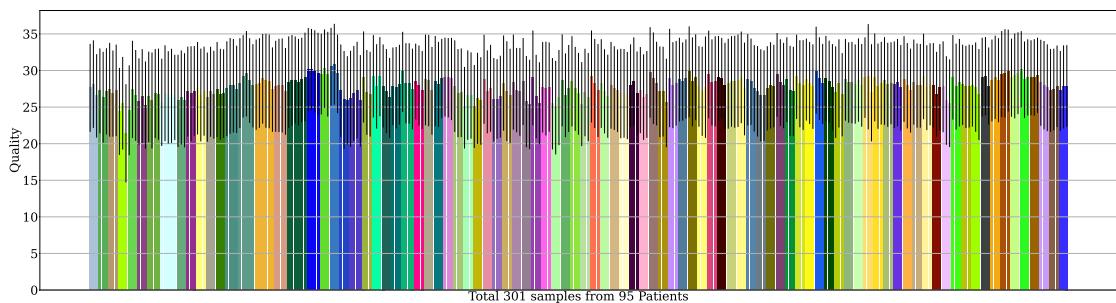


(b) Bowtie2

Figure 9: Depths plot with WES data



(a) BWA



(b) Bowtie2

Figure 10: Quality Distribution by Samples

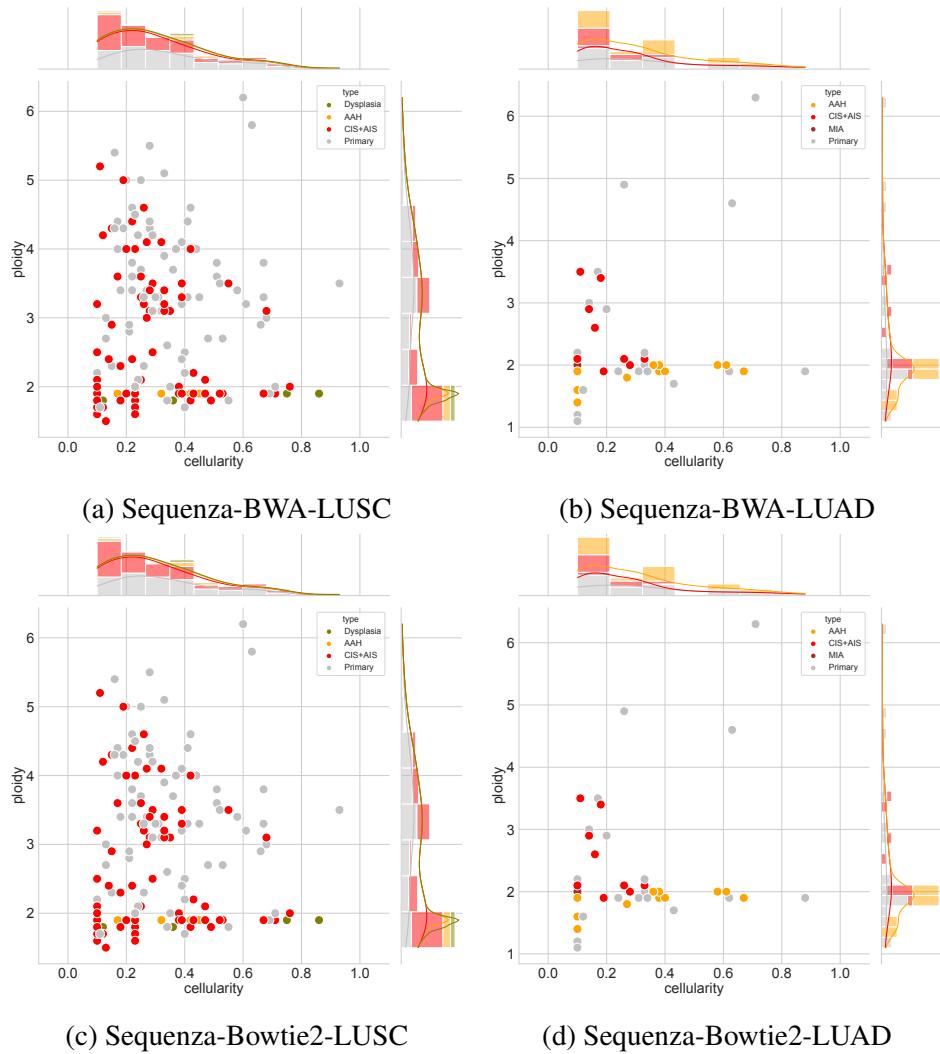


Figure 11: Sequenza Cellularity and Ploidy Plots

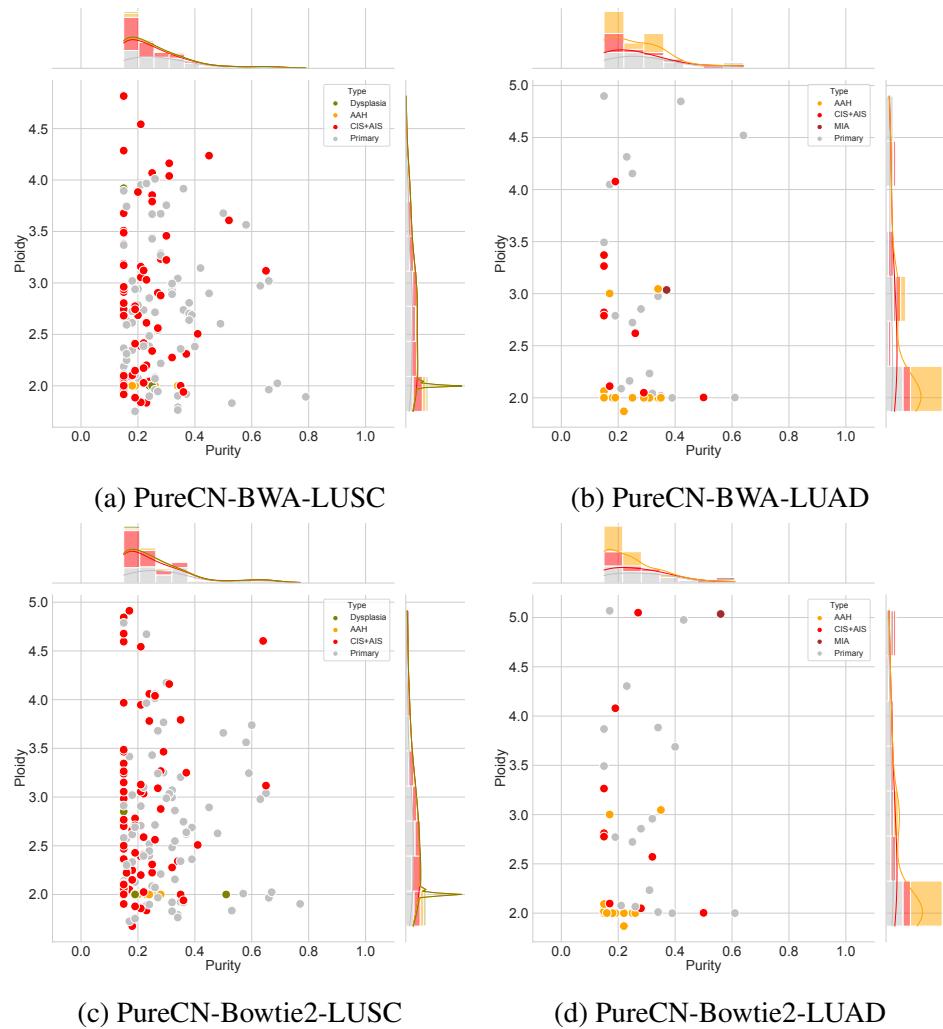


Figure 12: PureCN Purity and Ploidy Plots

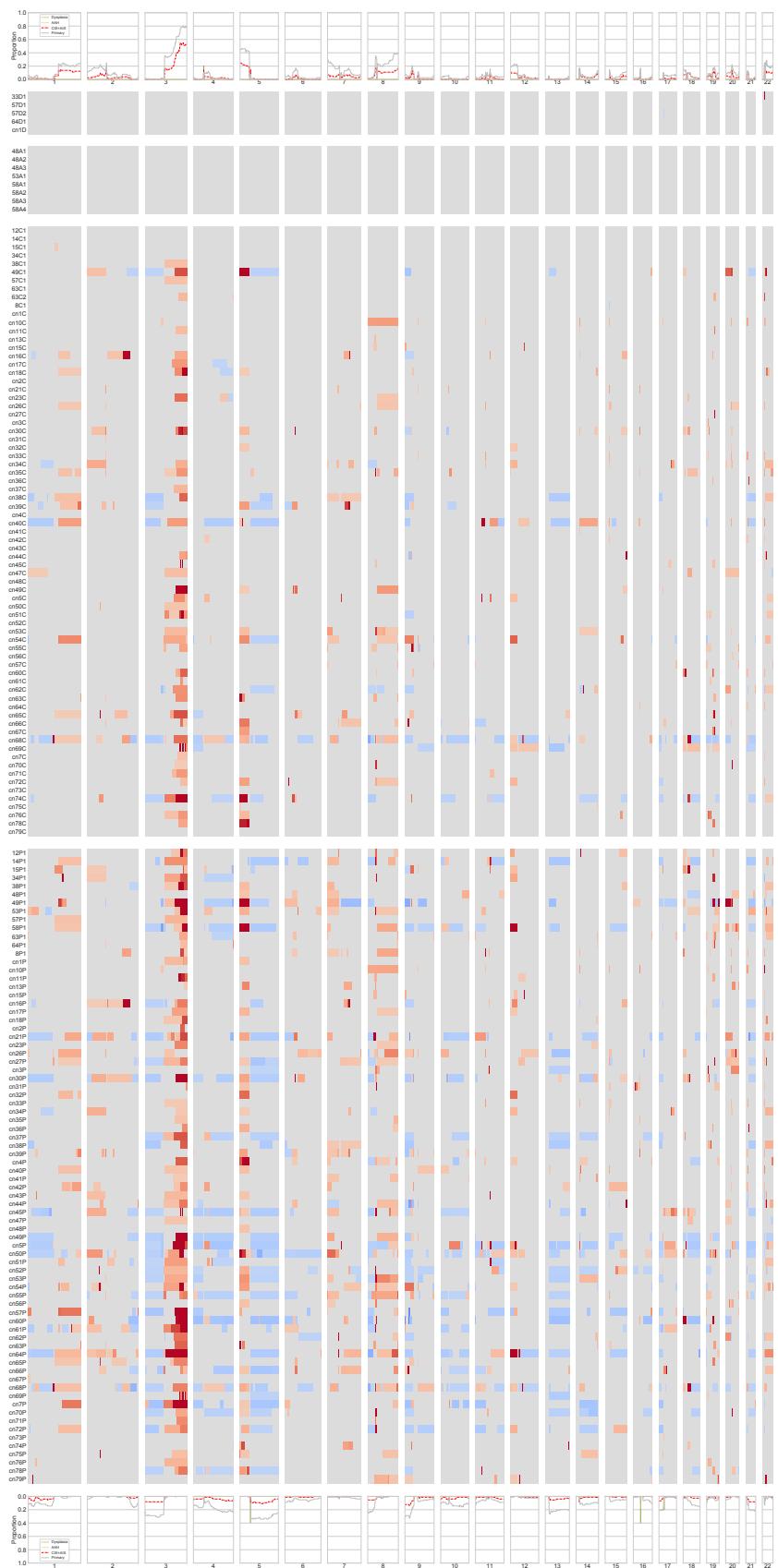


Figure 13: Sequenza LUSC Genome View Plot

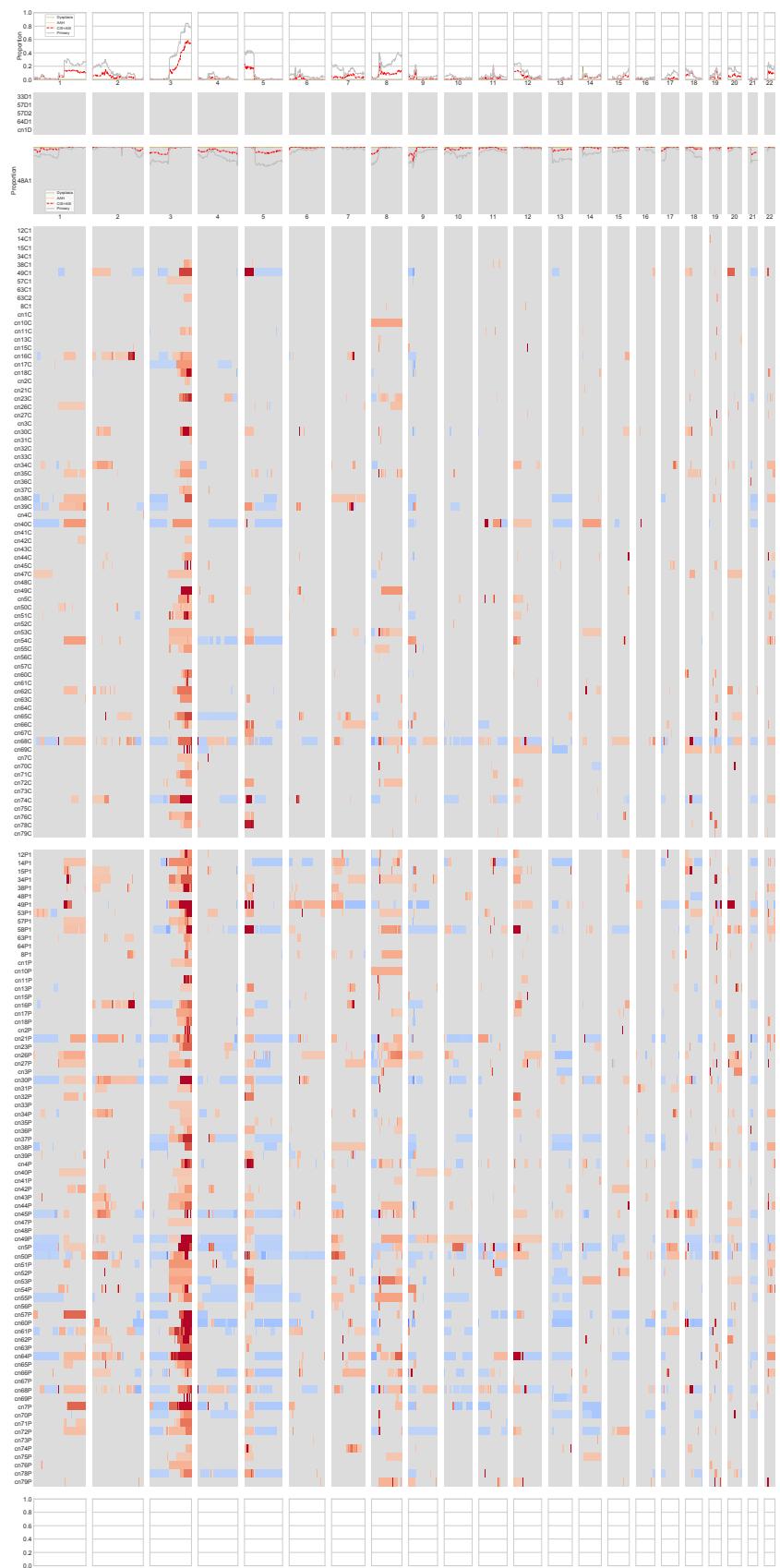


Figure 14: PureCN LUSC Genome View Plot

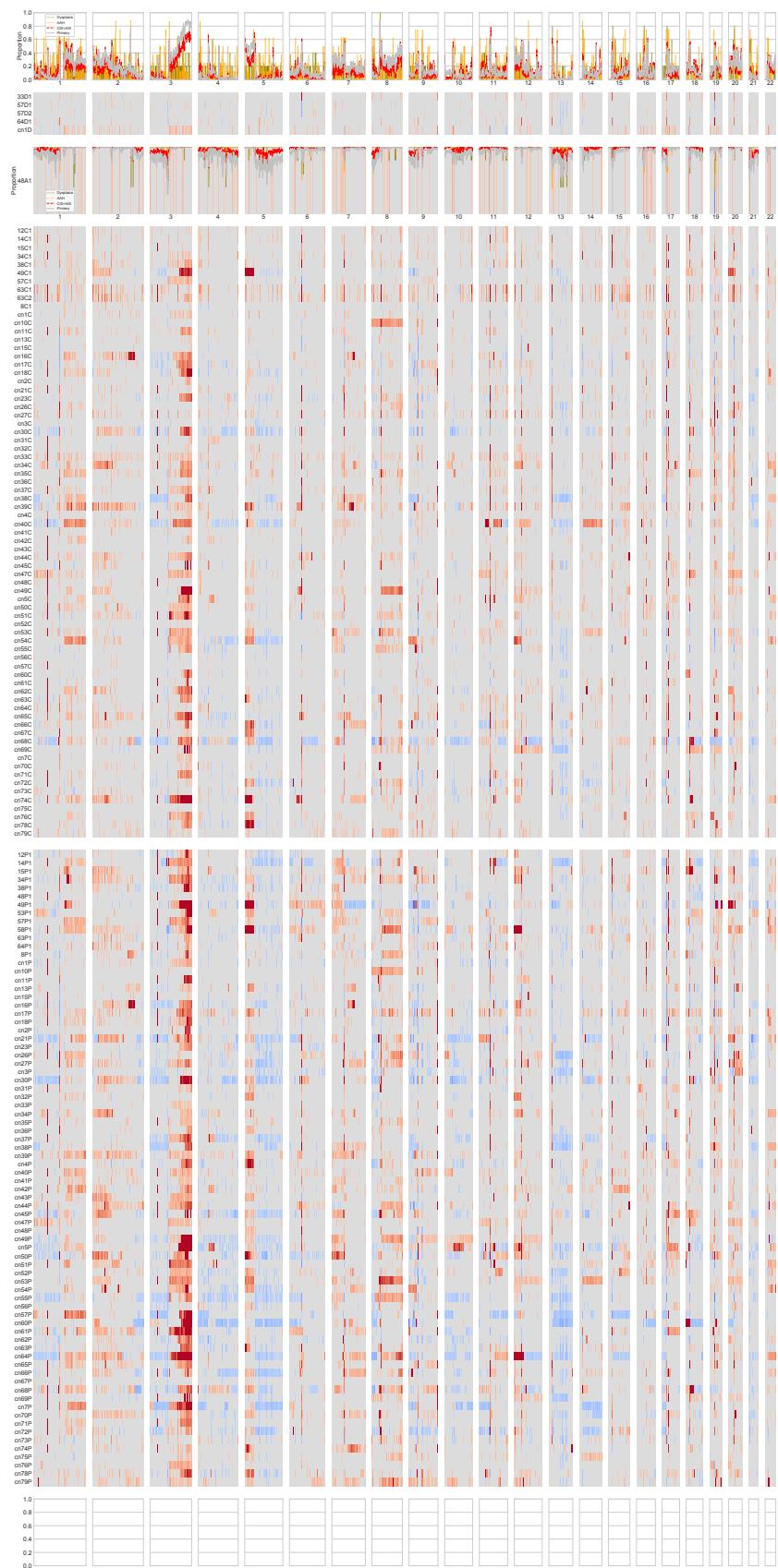


Figure 15: CNVkit LUSC Genome View Plot

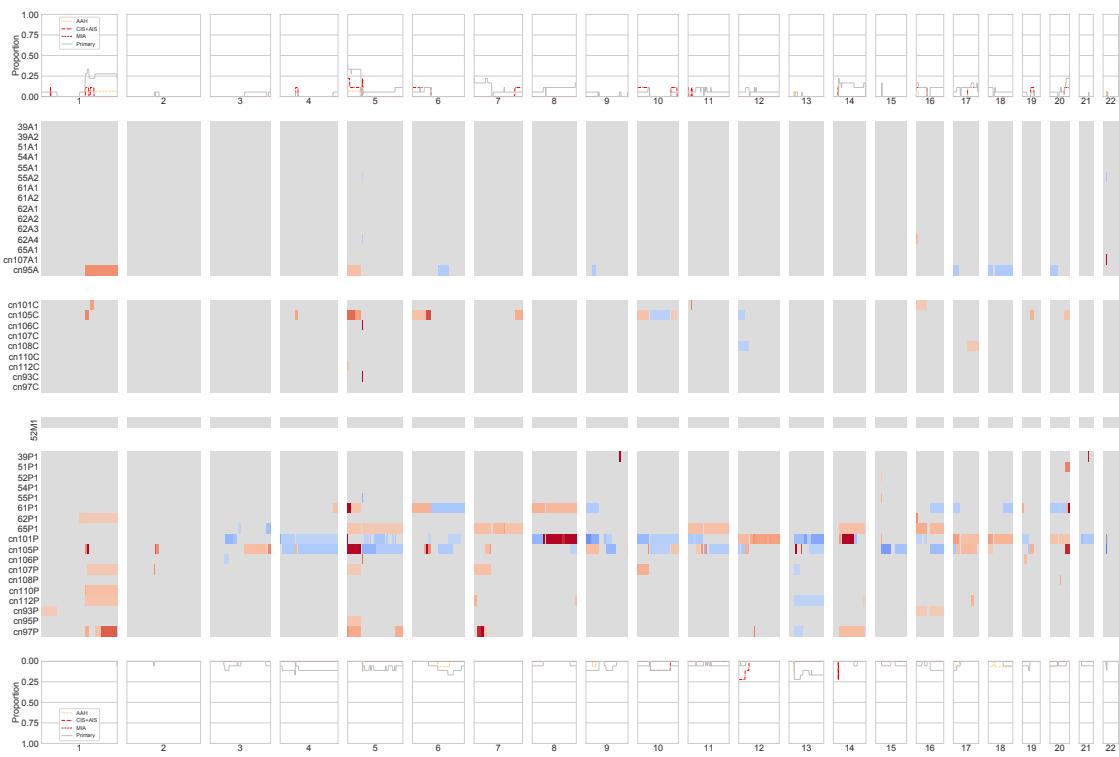


Figure 16: Sequenza LUAD Genome View Plot

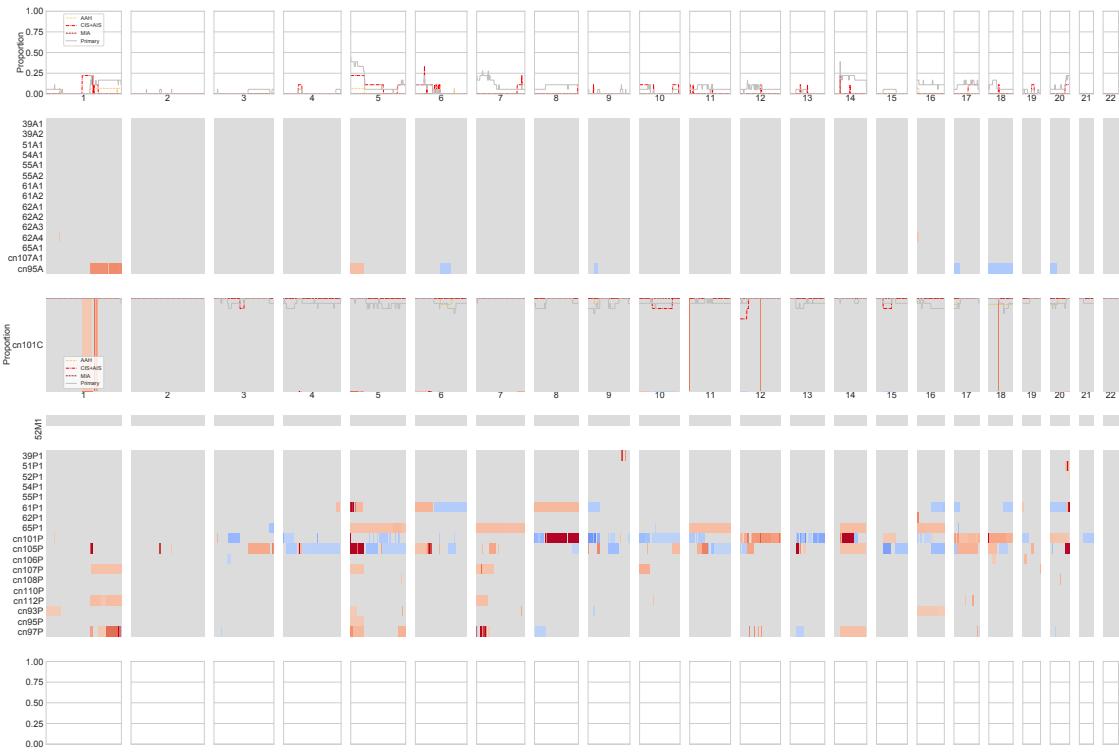


Figure 17: PureCN LUAD Genome View Plot

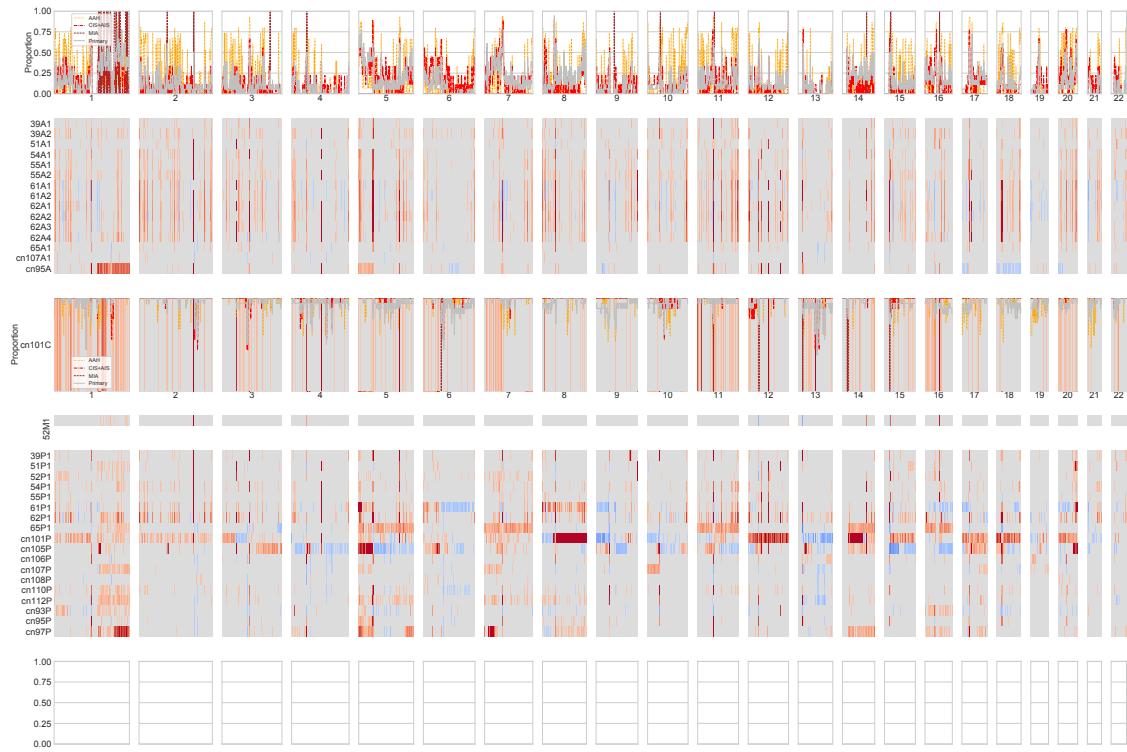


Figure 18: CNVkit LUAD Genome View Plot

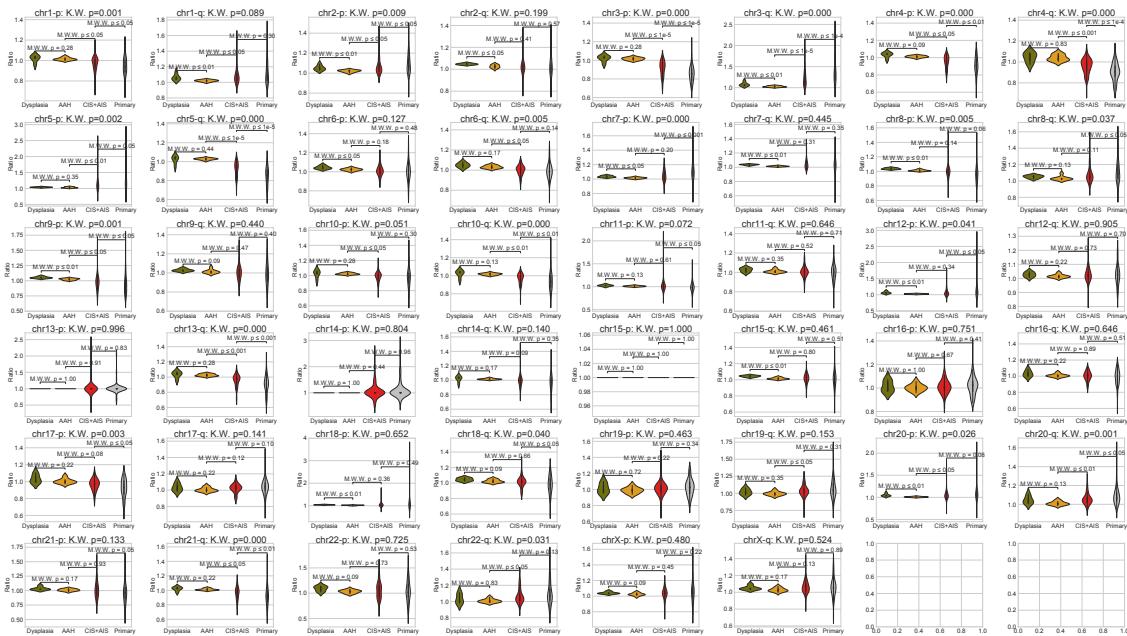


Figure 19: Sequenza LUSC Violin Plots

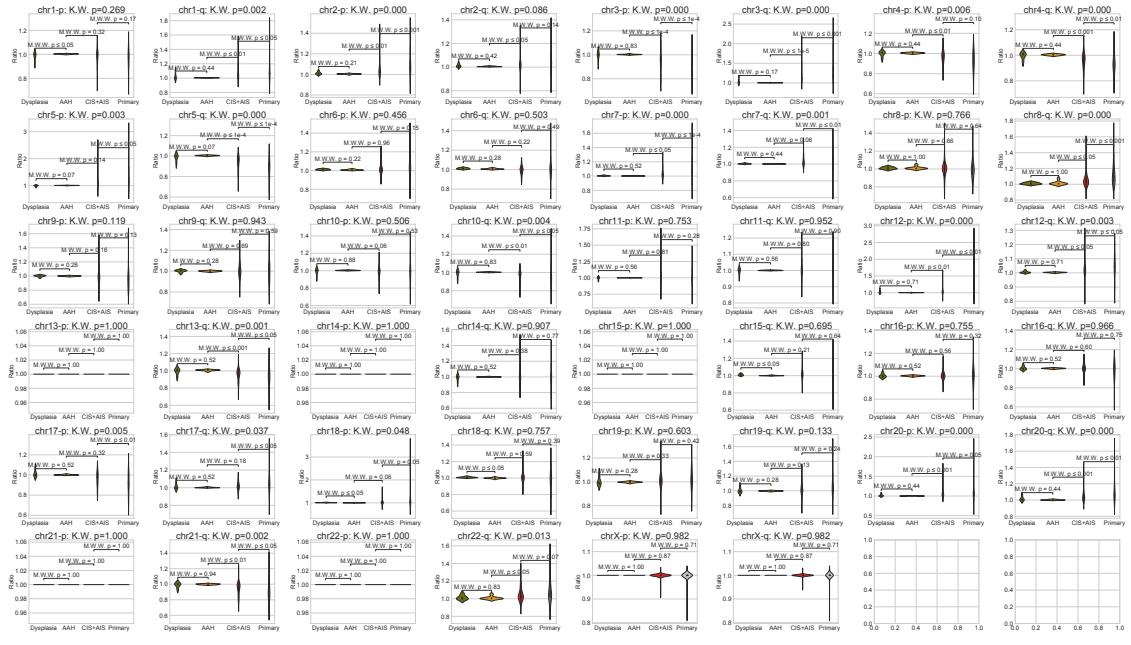


Figure 20: PureCN LUSC Violin Plots

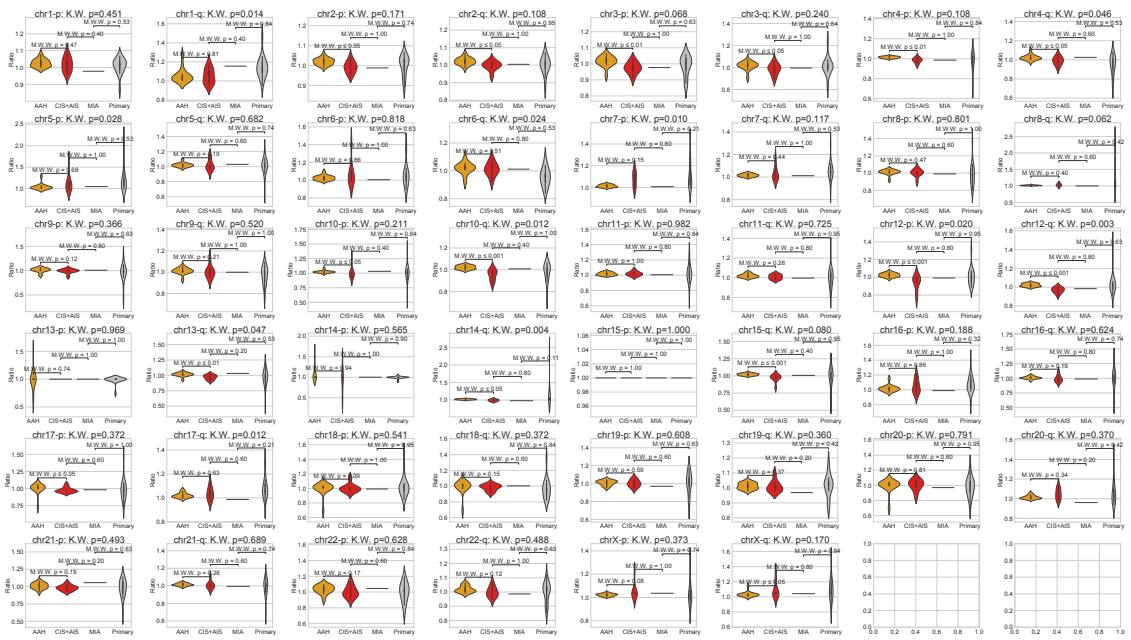


Figure 21: Sequenza LUAD Violin Plots

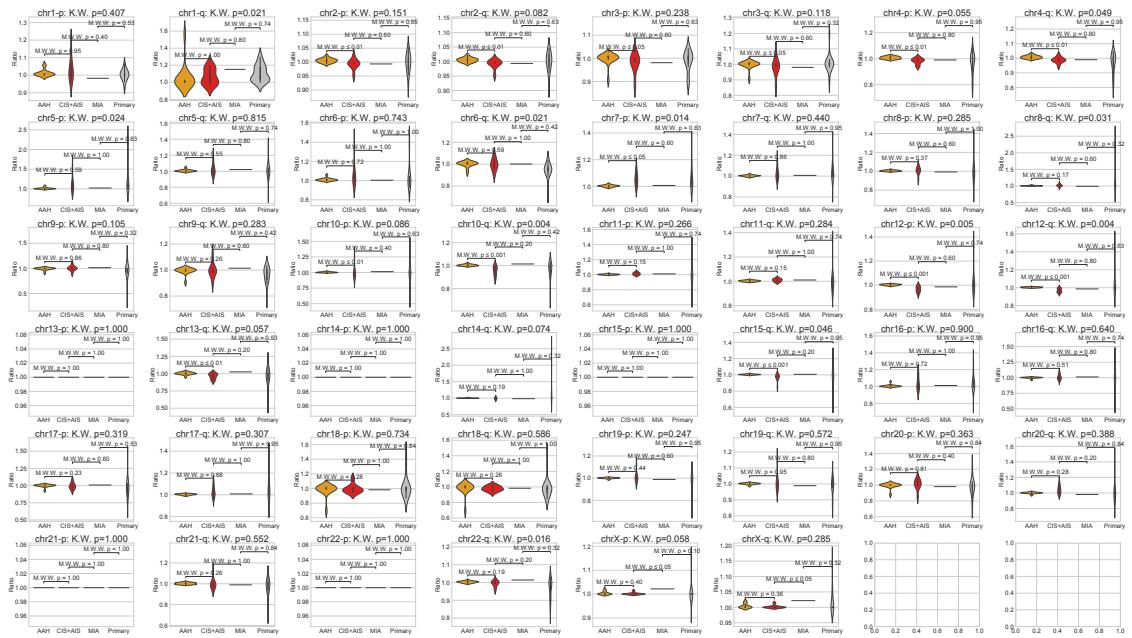


Figure 22: PureCN LUAD Violin Plots

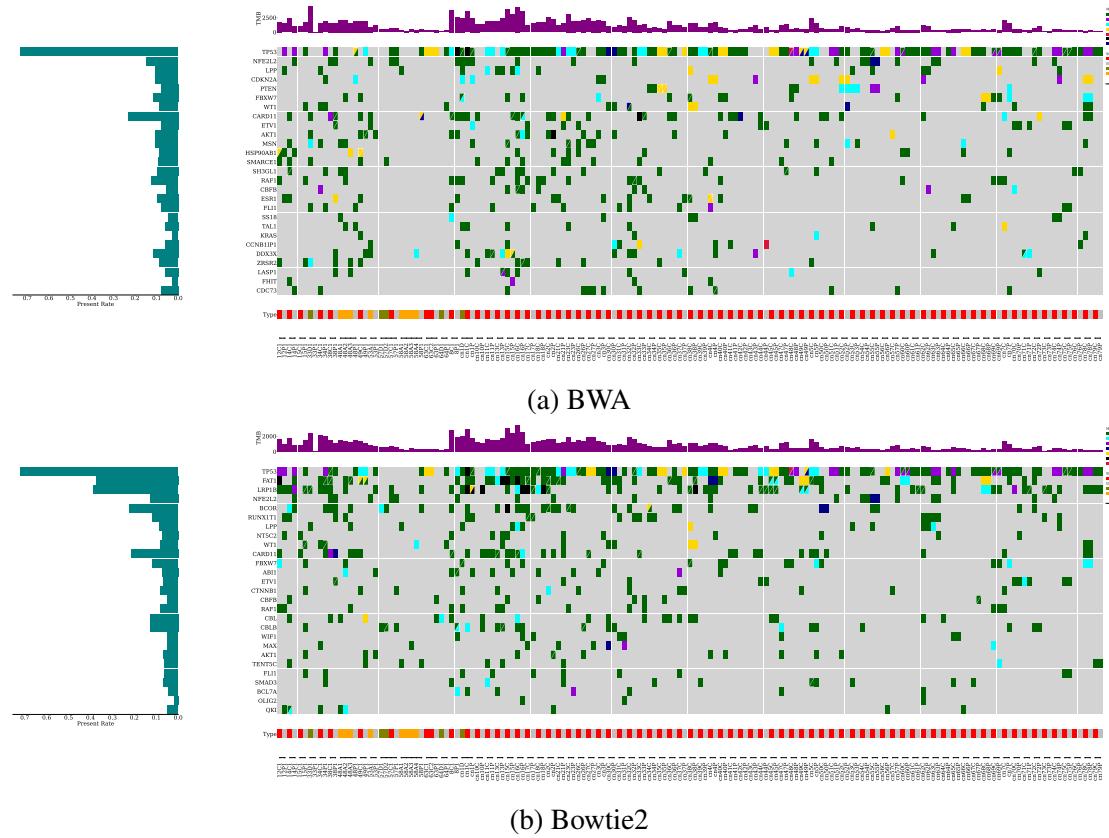


Figure 23: Comut Plot by LUSC

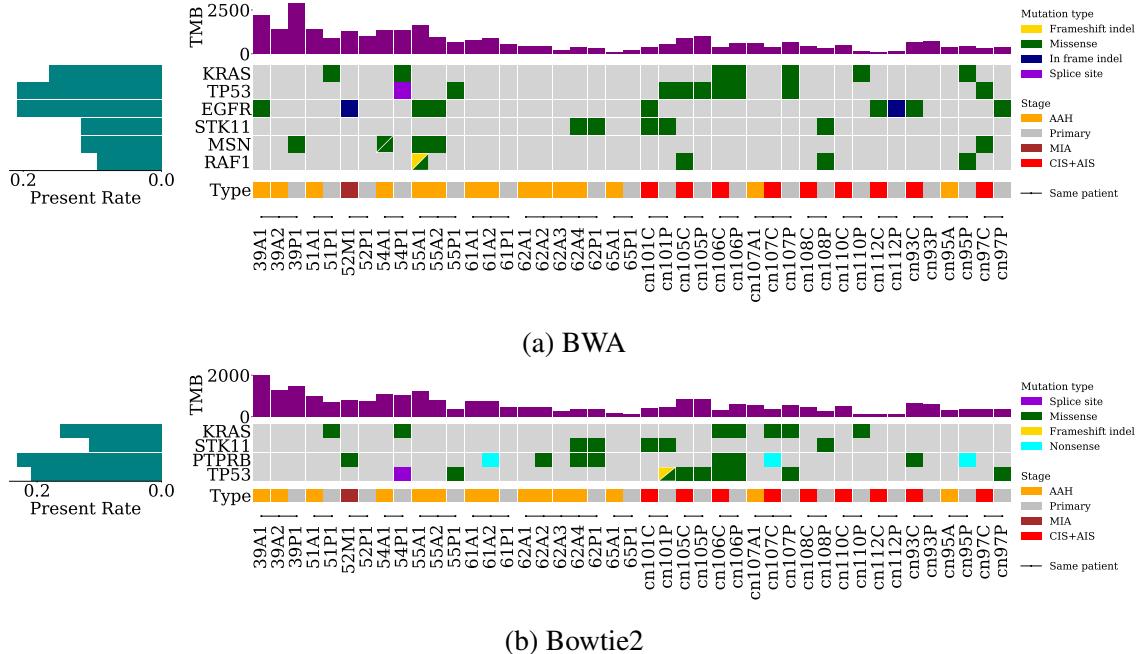


Figure 24: Comut Plot by LUAD

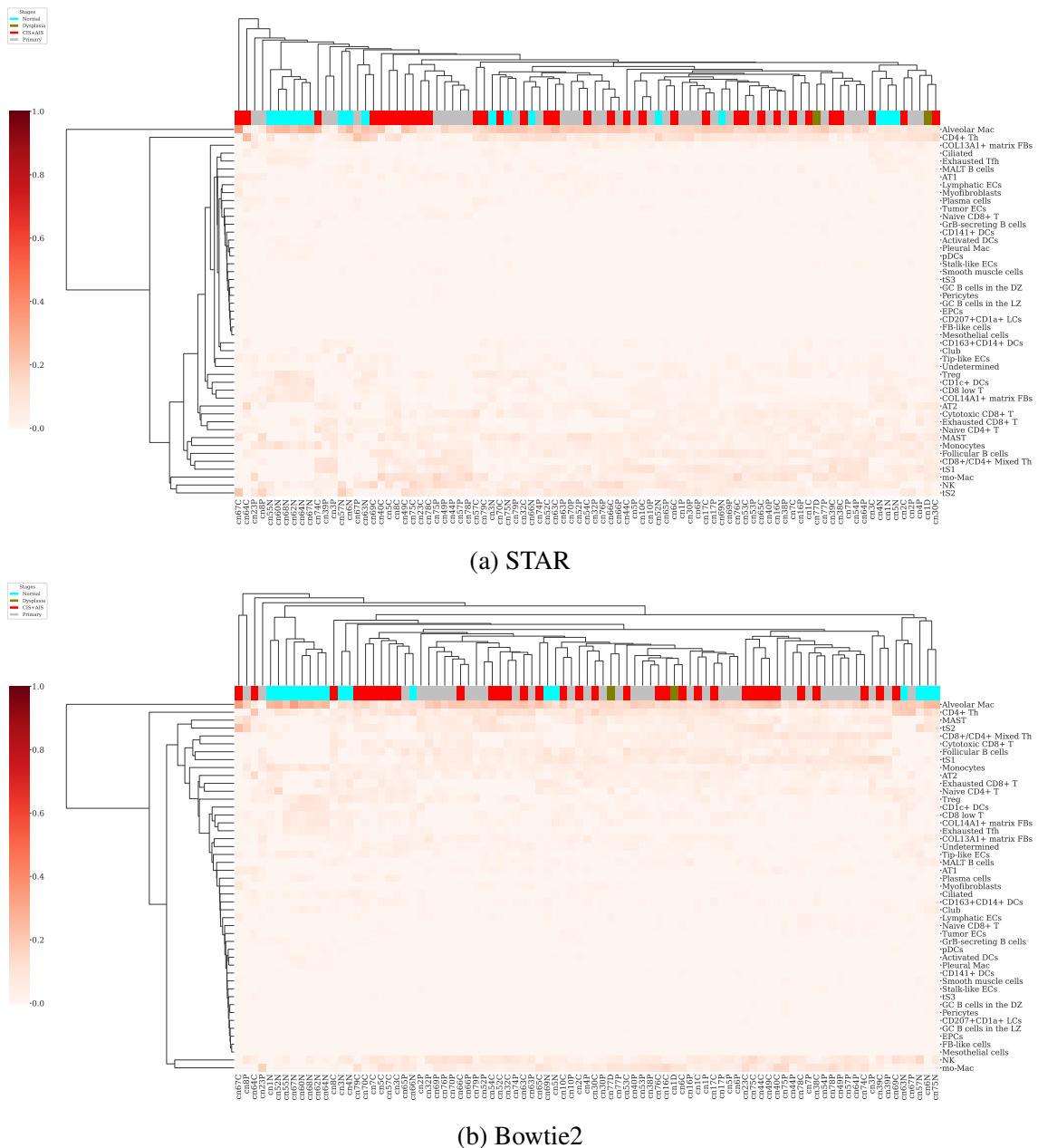


Figure 25: BisqueRNA clustermap plot with LUSC samples upon GSE131907

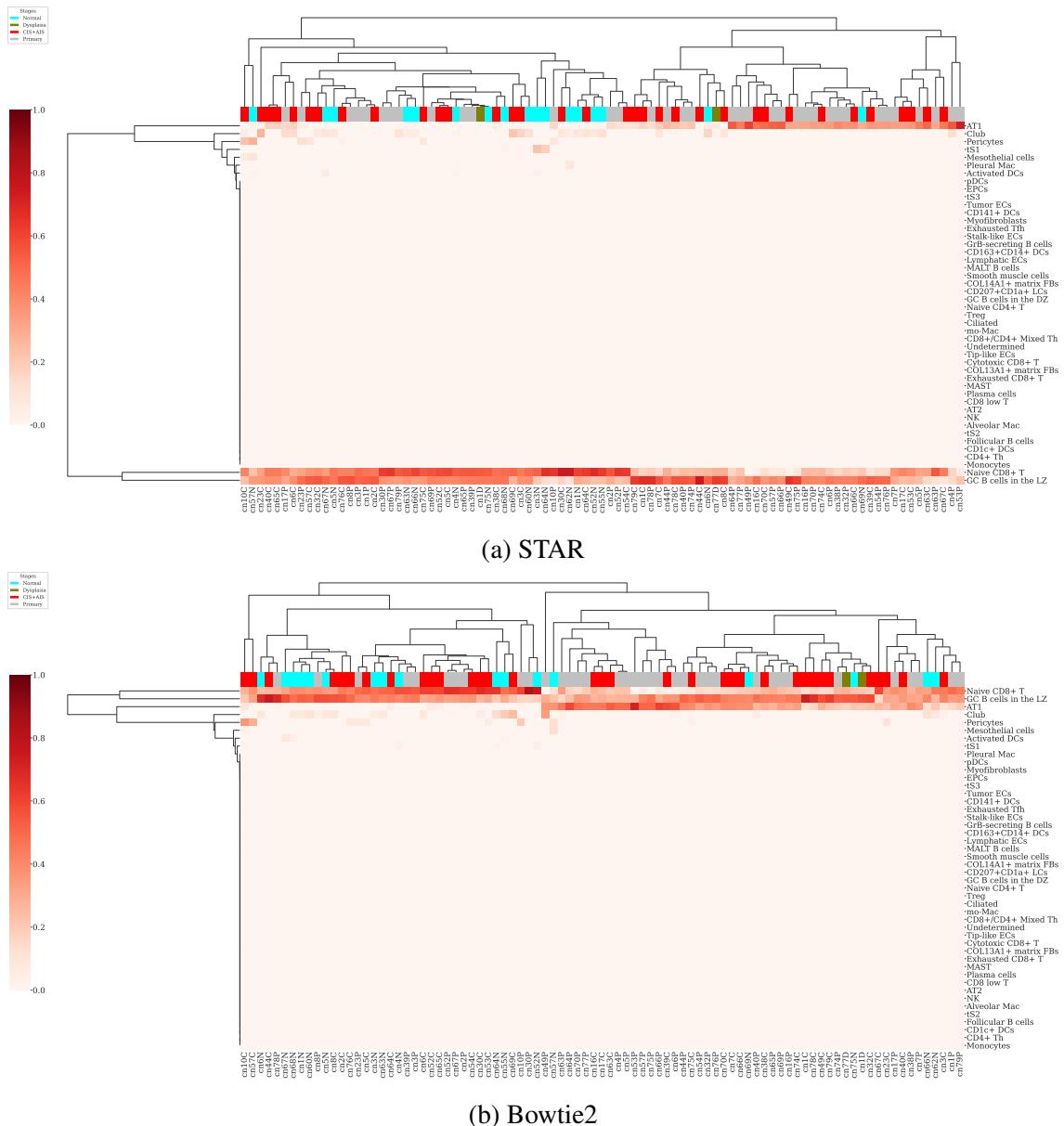


Figure 26: MuSiC clustermap plot with LUSC samples upon GSE131907

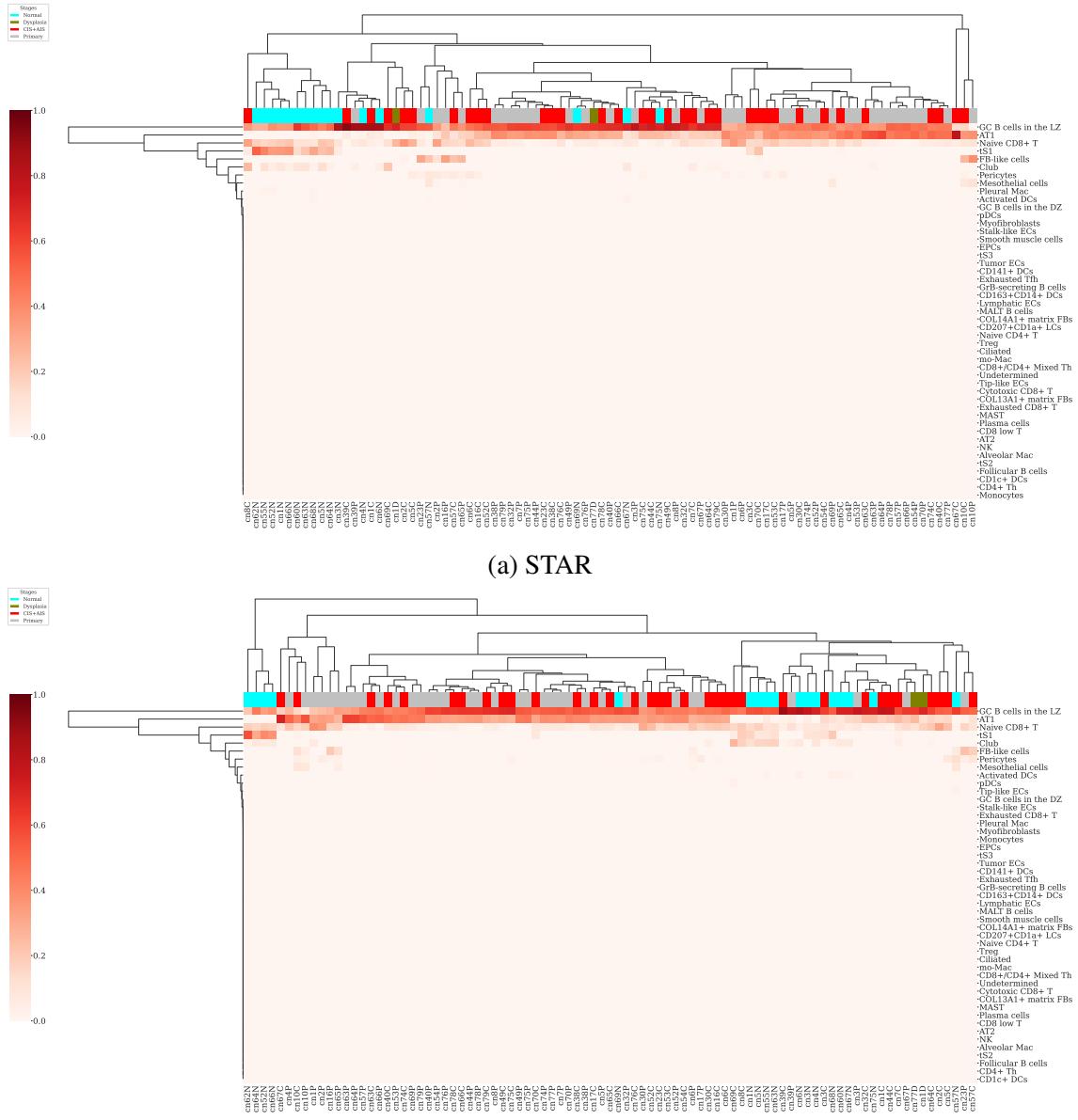


Figure 27: SCDC clustermap plot with LUSC samples upon GSE131907

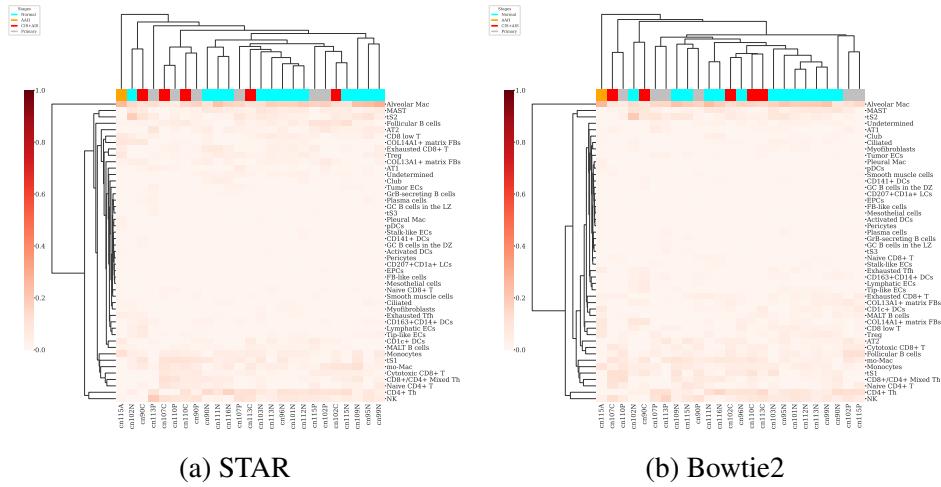


Figure 28: BisqueRNA clustermap plot with LUAD samples upon GSE131907

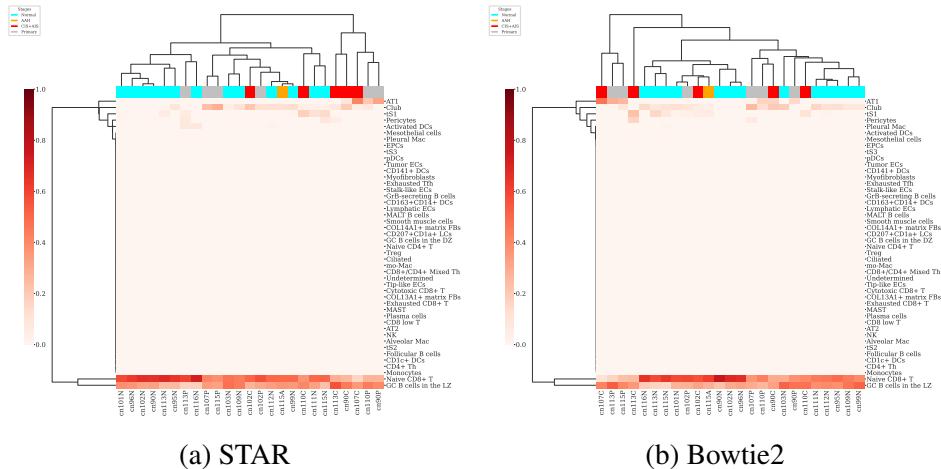


Figure 29: MuSiC clustermap plot with LUAD samples upon GSE131907

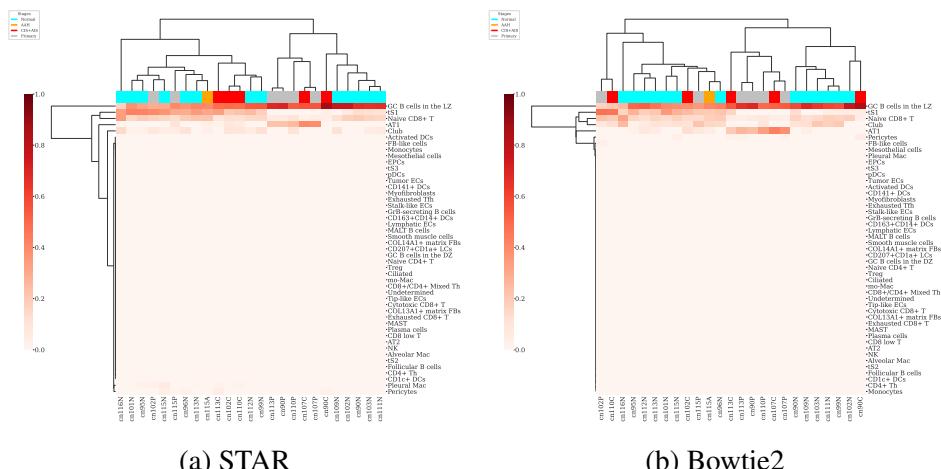


Figure 30: SCDCSiC clustermap plot with LUAD samples upon GSE131907

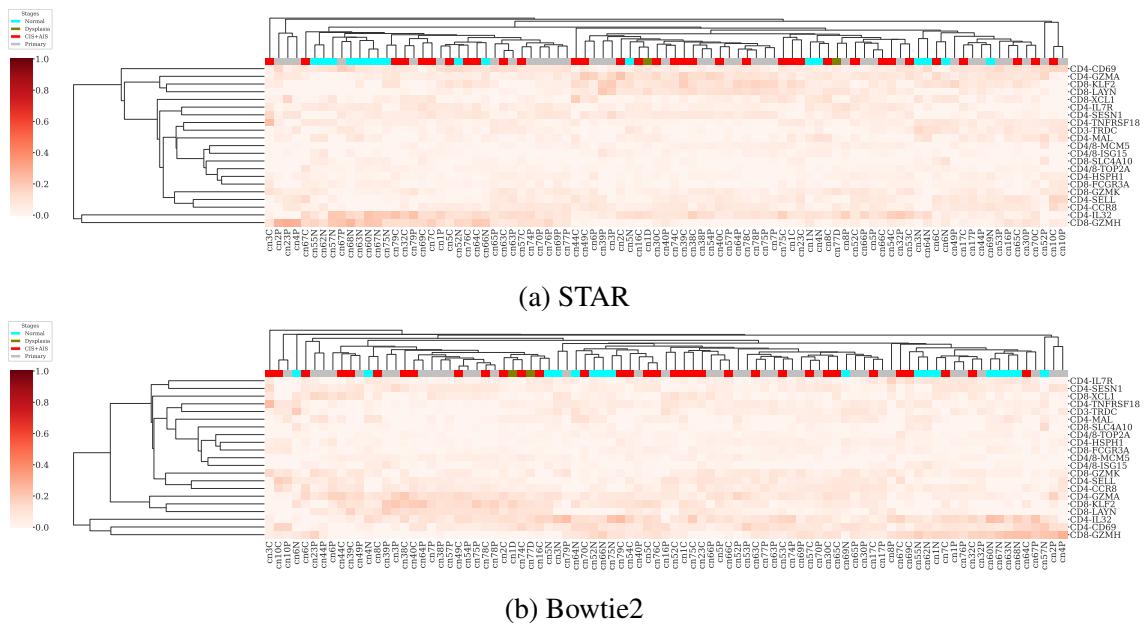


Figure 31: BisqueRNA clustermap plot with LUSC samples upon GSE162498

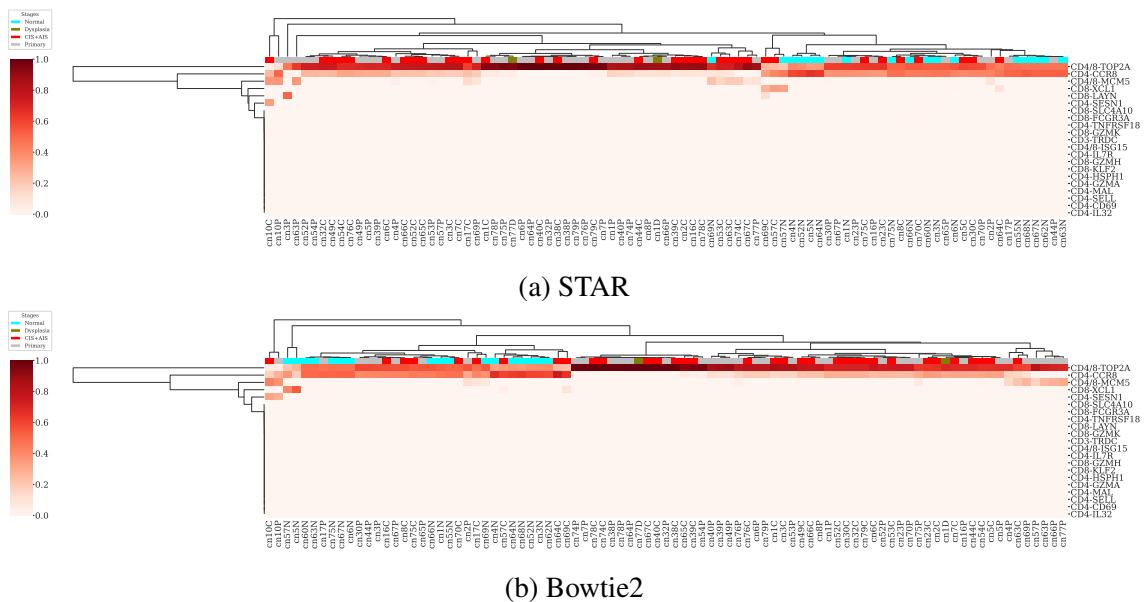


Figure 32: MuSiC clustermap plot with LUSC samples upon GSE162498

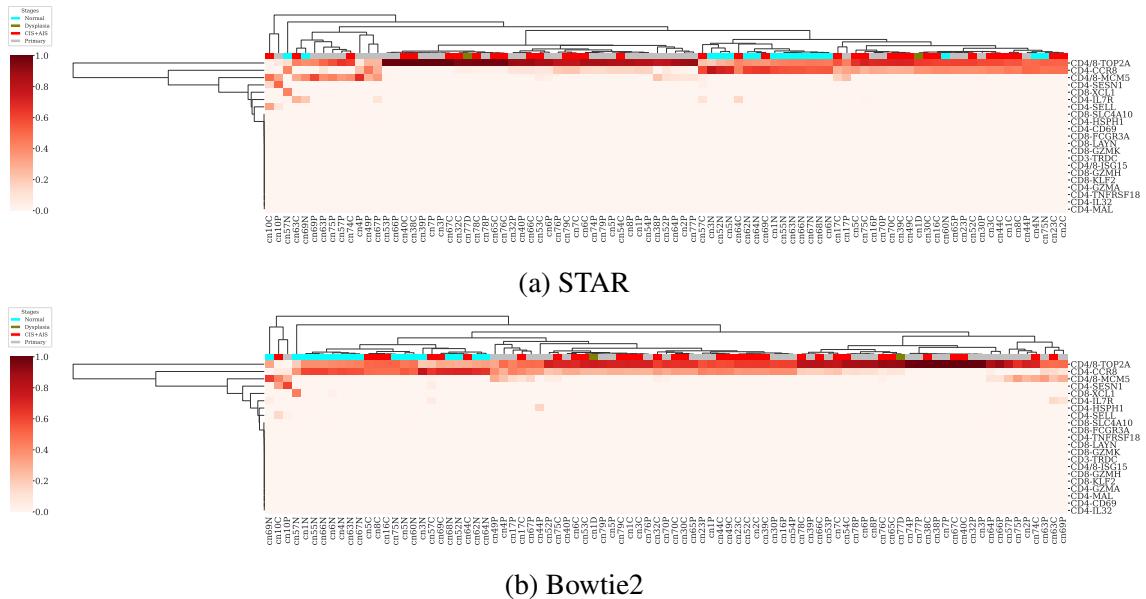


Figure 33: SCDC clustermap plot with LUSC samples upon GSE162498

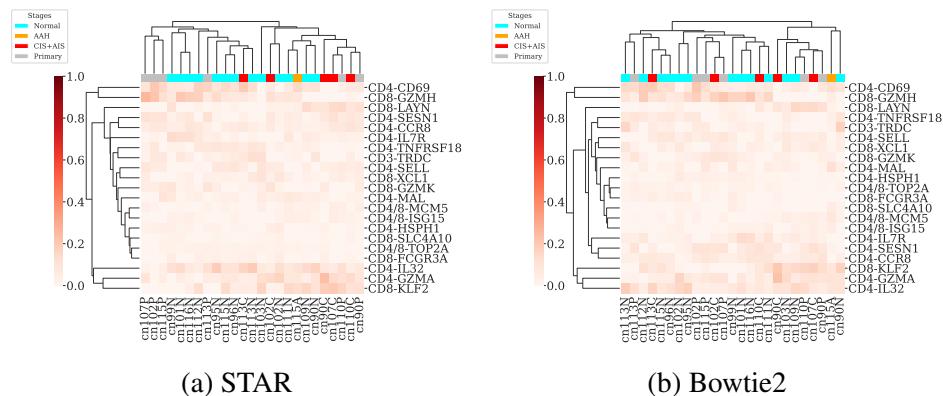


Figure 34: BisqueRNA clustermap plot with LUAD samples upon GSE162498

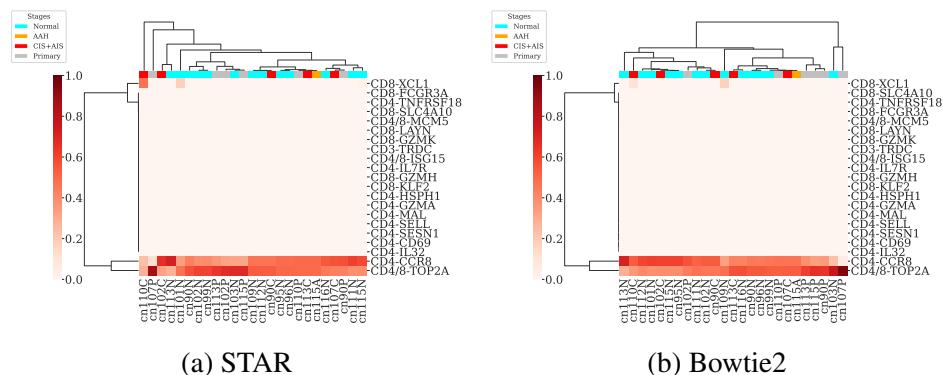


Figure 35: MuSiC clustermap plot with LUAD samples upon GSE162498

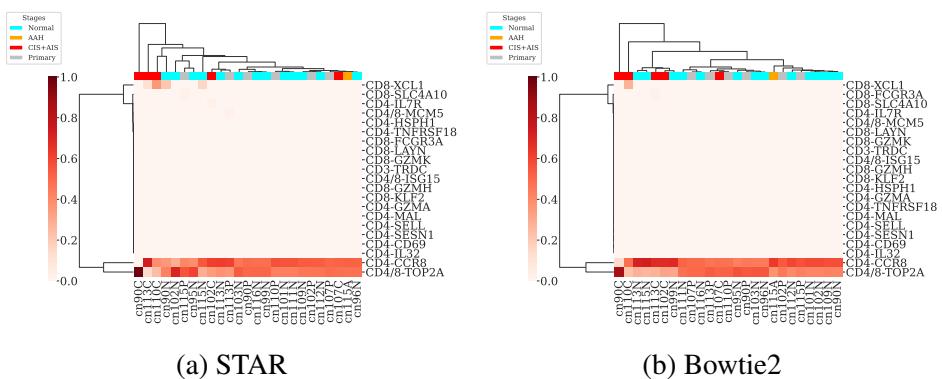


Figure 36: SCDC clustermap plot with LUAD samples upon GSE162498

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.

