Doctoral Thesis

<Lung Pre-cancer Analysis>

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Abstract

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

1.2 Precancer

1.3 Study Objectives

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 Structure & Count

III Methods

3.1 Workflows

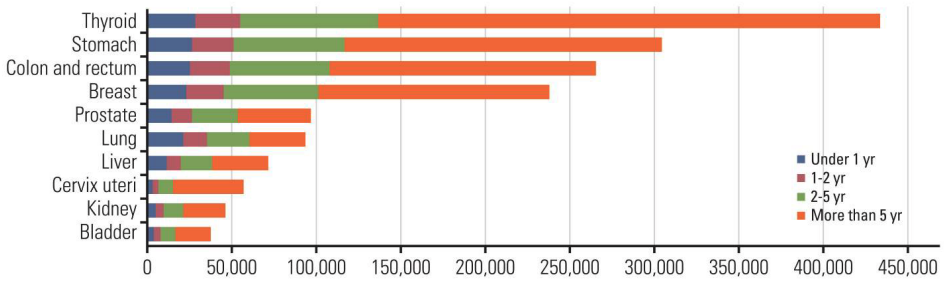


Figure 1: Common cancer survival rates (Hong et al., 2021)

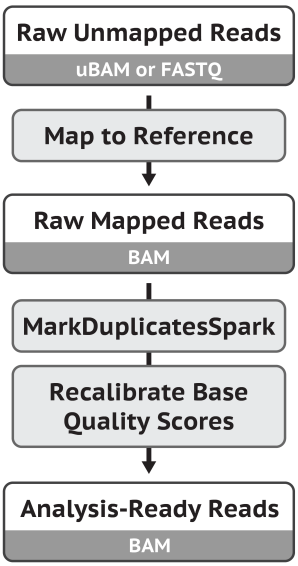


Figure 2: Workflow for data pre-processing for variant discovery (Van der Auwera et al., 2013; DePristo et al., 2011)

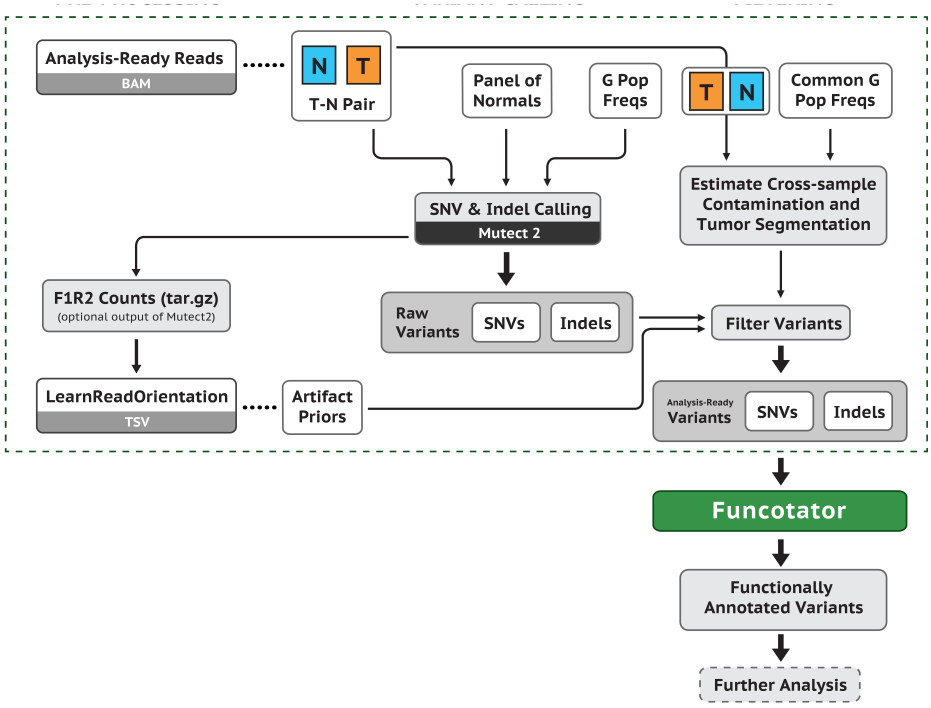
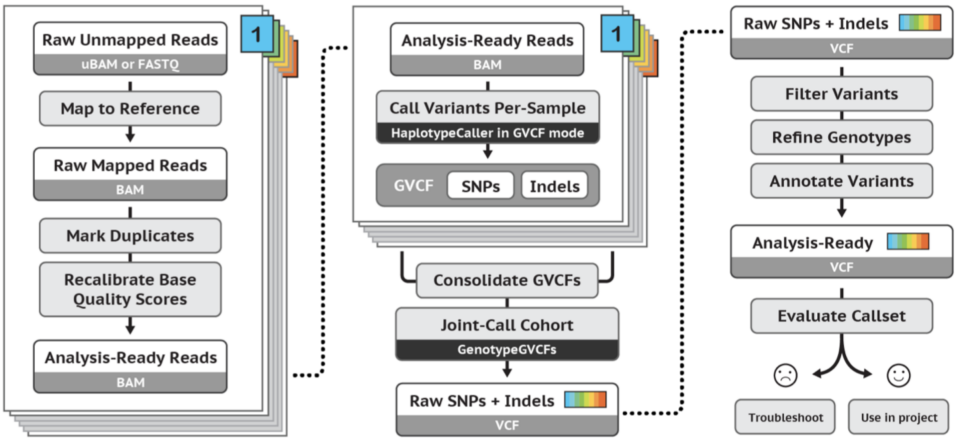


Figure 3: Somatic short variant discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)



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4.1 Quality Check

4.2 Quality Check with FastQC

Findings in Quality Check

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Copy Number Variation Analysis with Sequenza

Cellularities and Ploidies

Copy Number Variations

Findings in Copy Number Variation Analysis

4.4 Somatic Short Variation

Somatic Short Variation Analysis with Mutect2

Findings in Somatic Short Variation Analysis

4.5 Variant Allele Frequencies

4.6 Differences in Gene Expression levels

4.7 Bulk Cell Deconvolution

Single-cell Reference Data

CIBERSORTx

BisqueRNA

MuSiC

SCDC

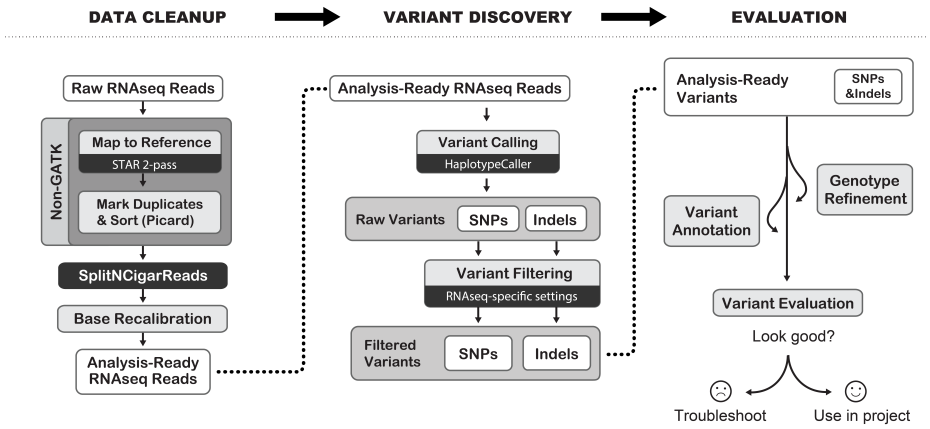


Figure 5: RNA-seq short variant discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

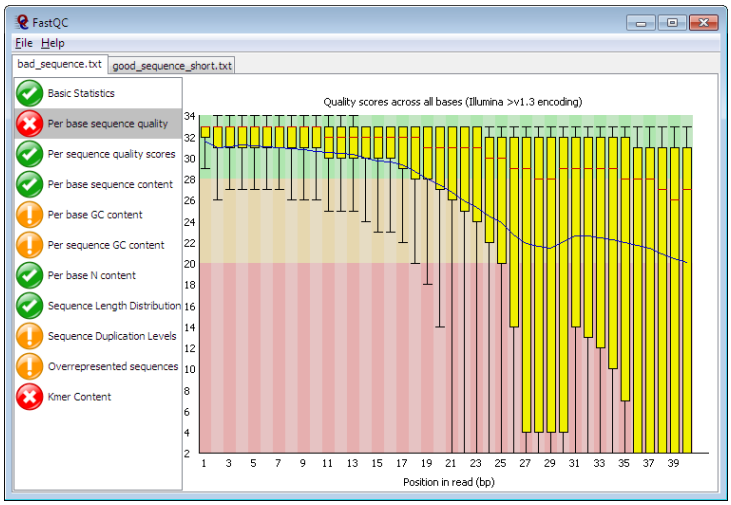


Figure 6: Example of FastQC Result (Andrews et al., 2012)

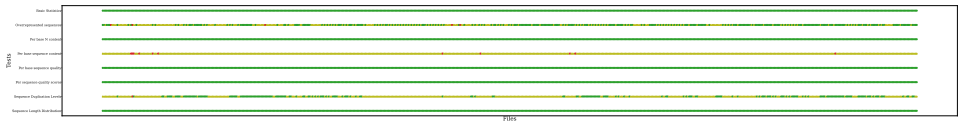


Figure 7: FastQC results with WES data

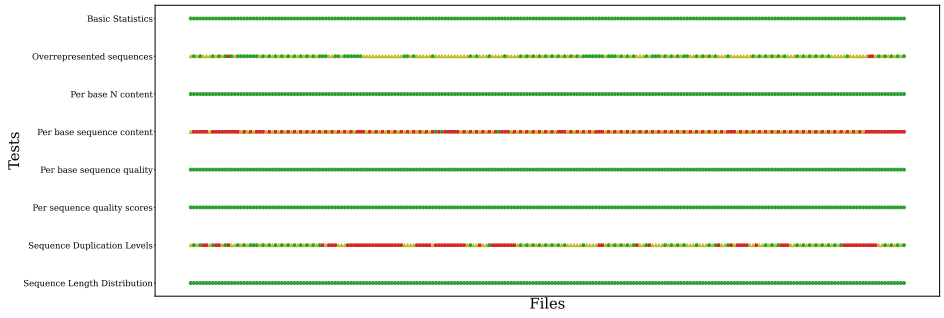
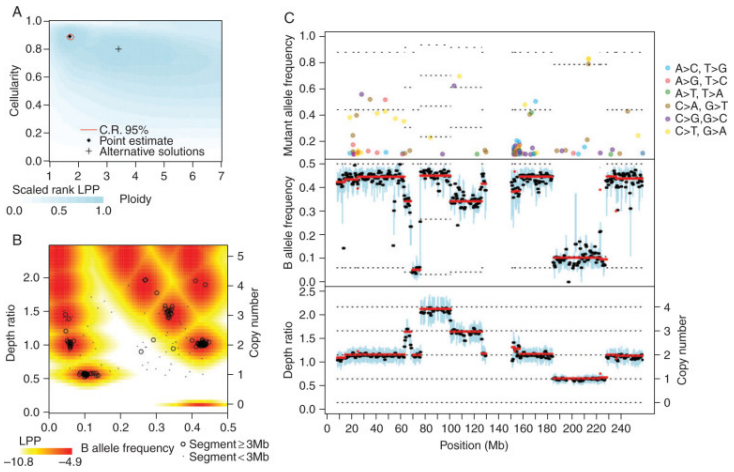


Figure 8: FastQC results with WTS data



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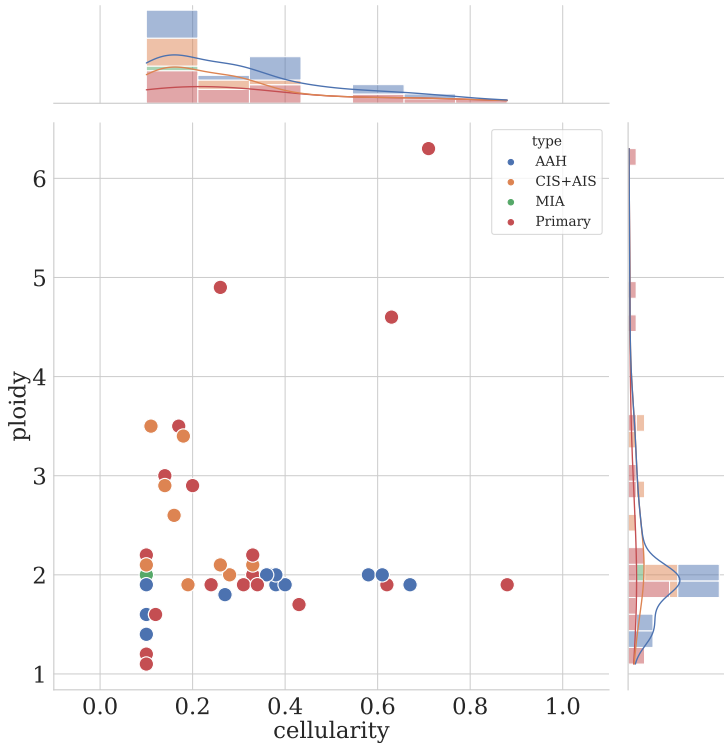


Figure 10: Cellularities and Ploidies by BWA in ADC

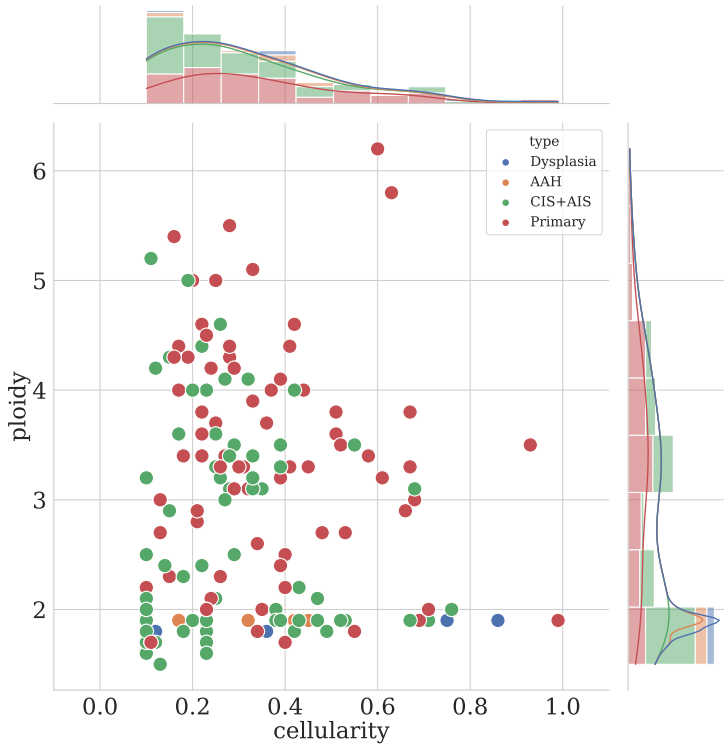


Figure 11: Cellularities and Ploidies by BWA in SQC

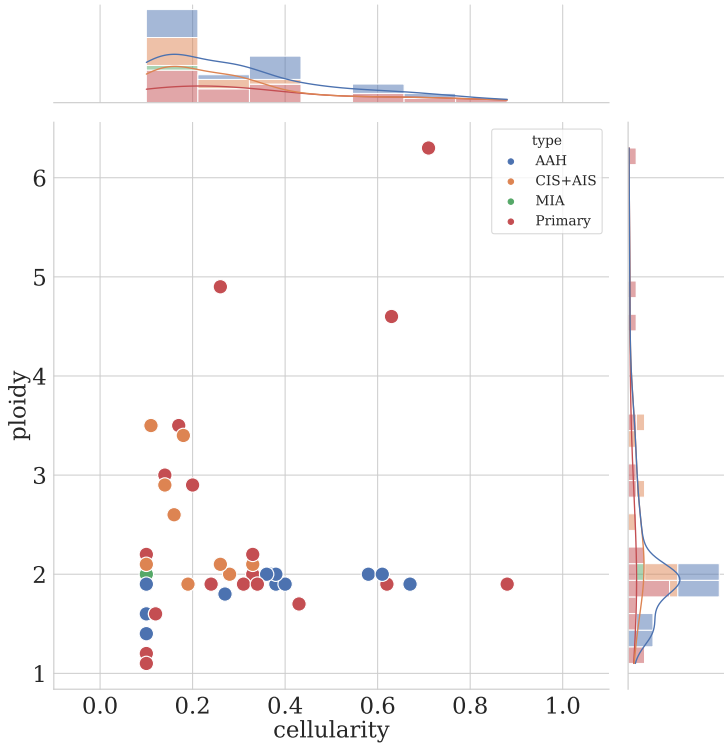


Figure 12: Cellularities and Ploidies by Bowtie2 in ADC

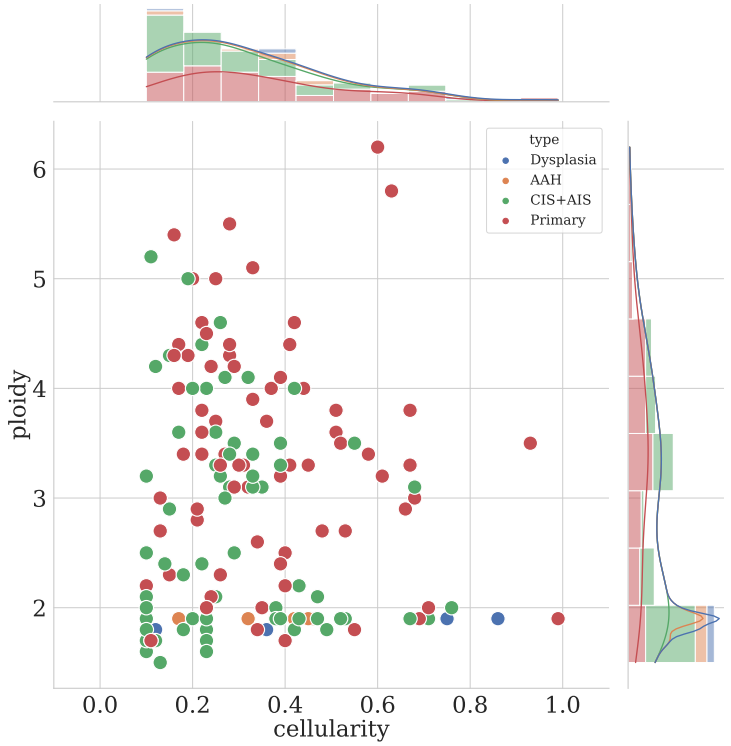


Figure 13: Cellularities and Ploidies by Bowtie2 in SQC

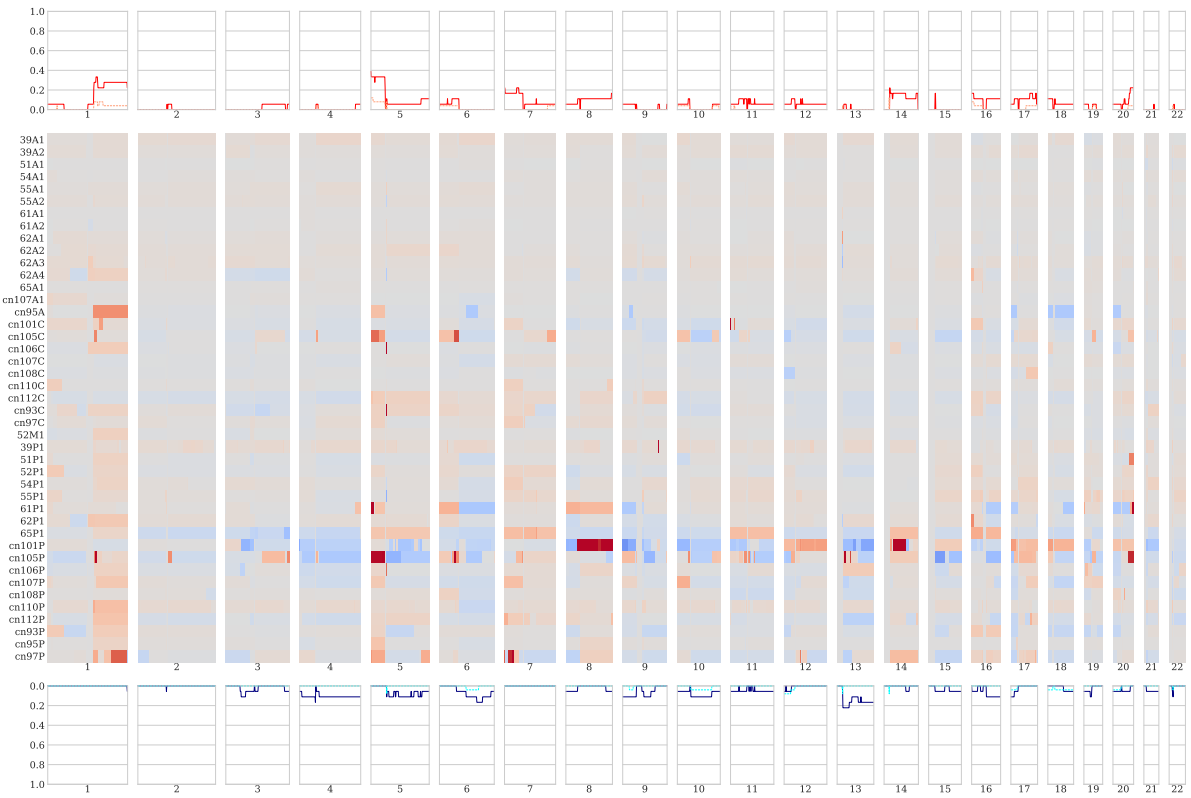
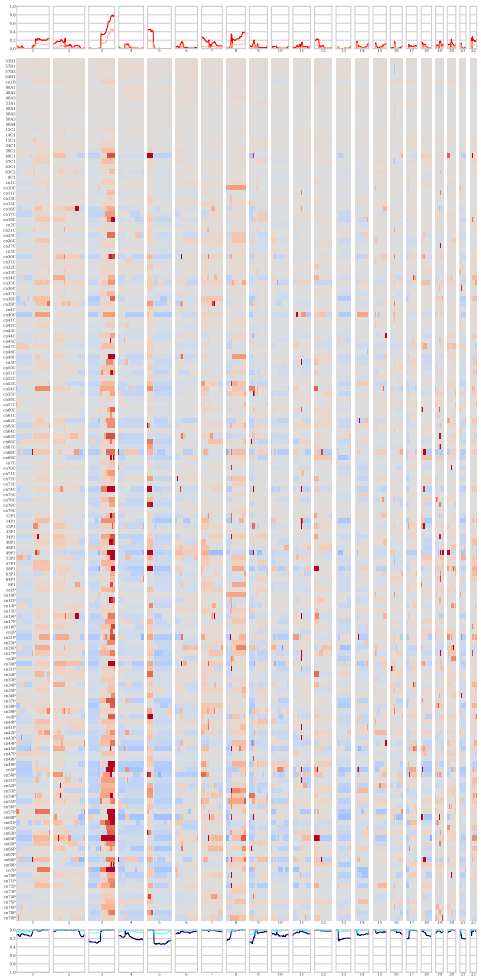


Figure 14: CNV plot by BWA in ADC



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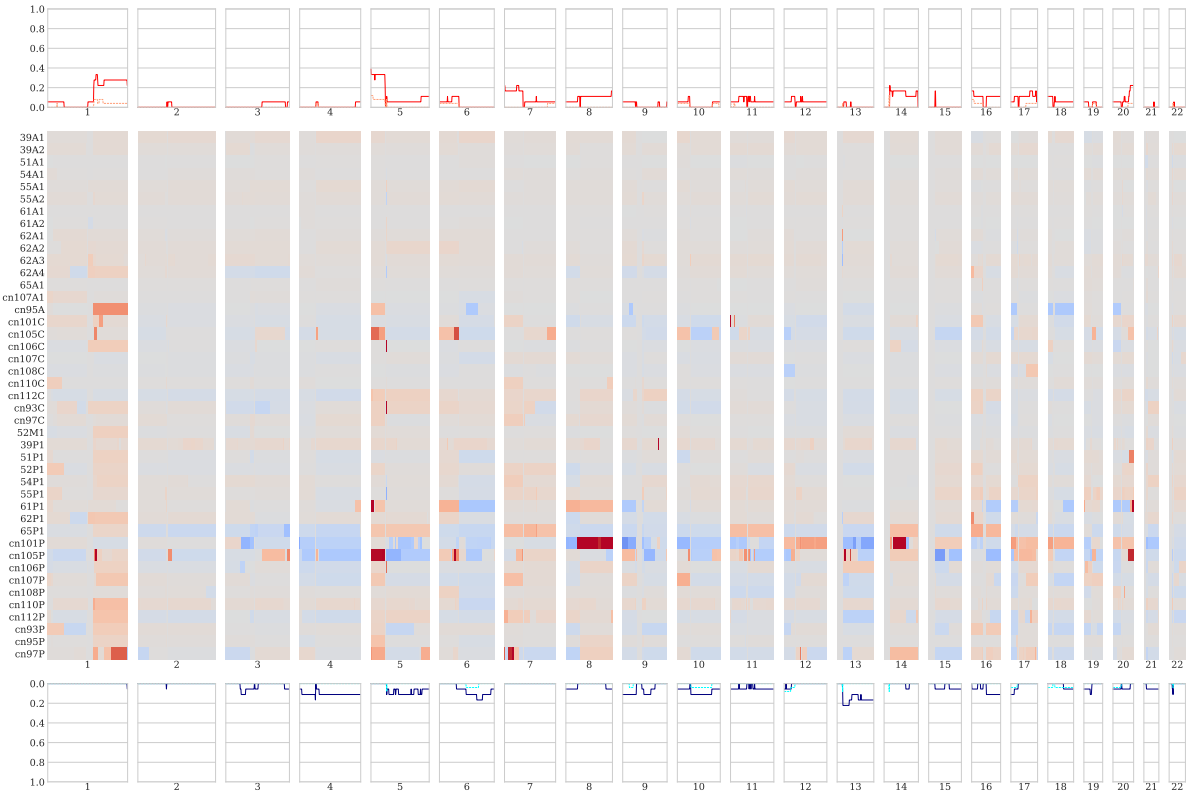
V Discussion



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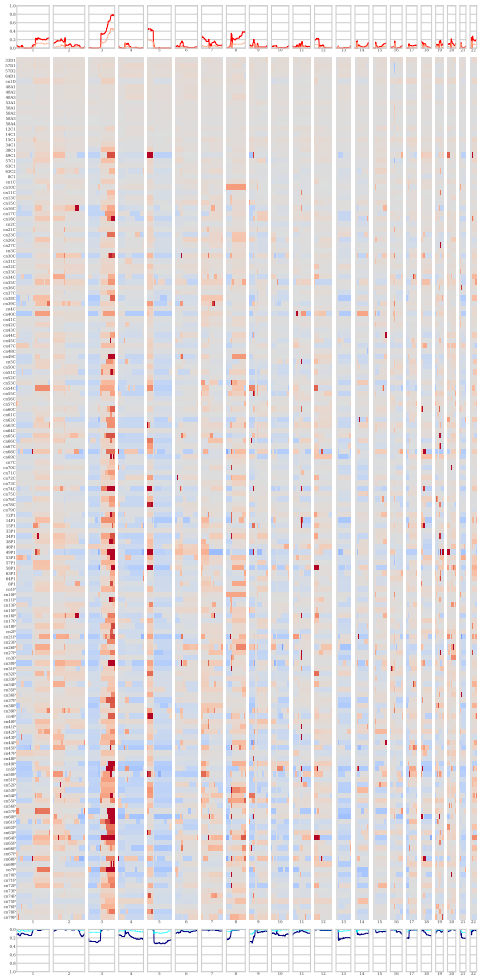


Figure 17: CNV plot by Bowtie2 in SQC

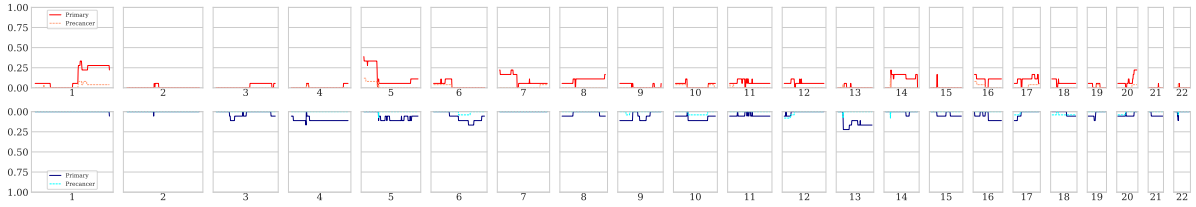


Figure 18: Simple CNV plot by BWA in ADC

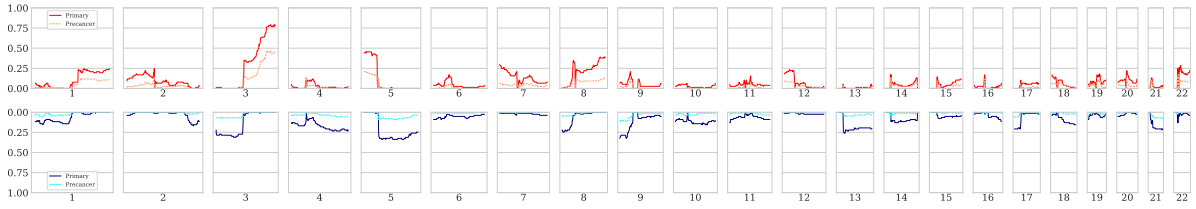


Figure 19: Simple CNV plot by BWA in SQC

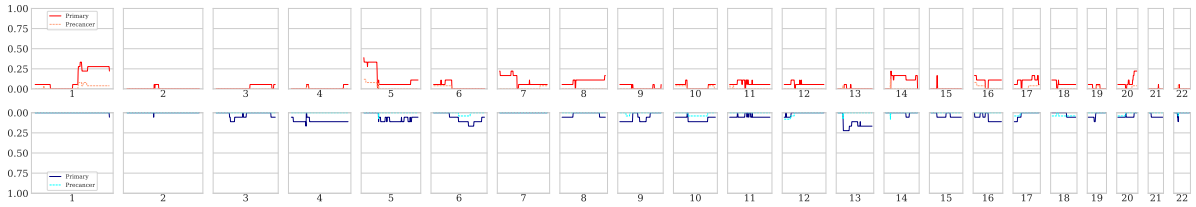


Figure 20: Simple CNV plot by Bowtie2 in ADC

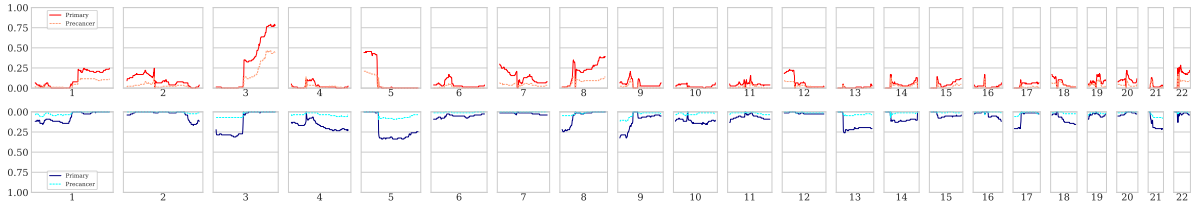


Figure 21: Simple CNV plot by Bowtie2 in SQC

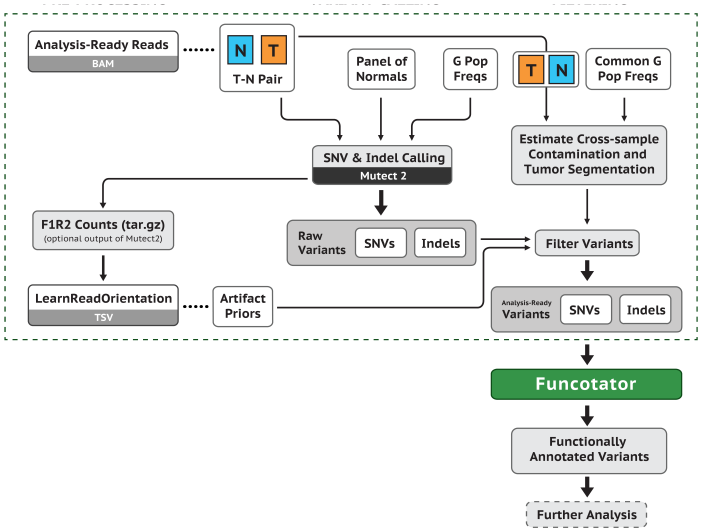


Figure 22: Somatic Short Variant Discovery Workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

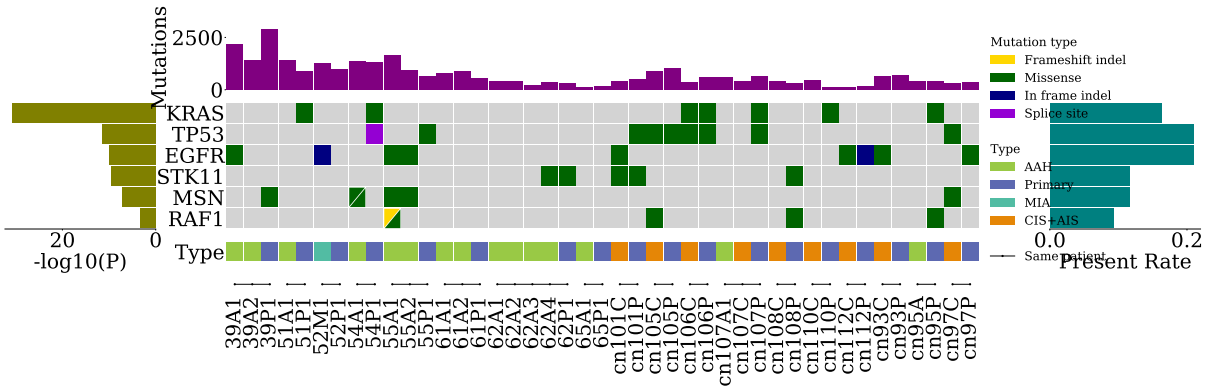


Figure 23: CoMut plot by BWA in ADC

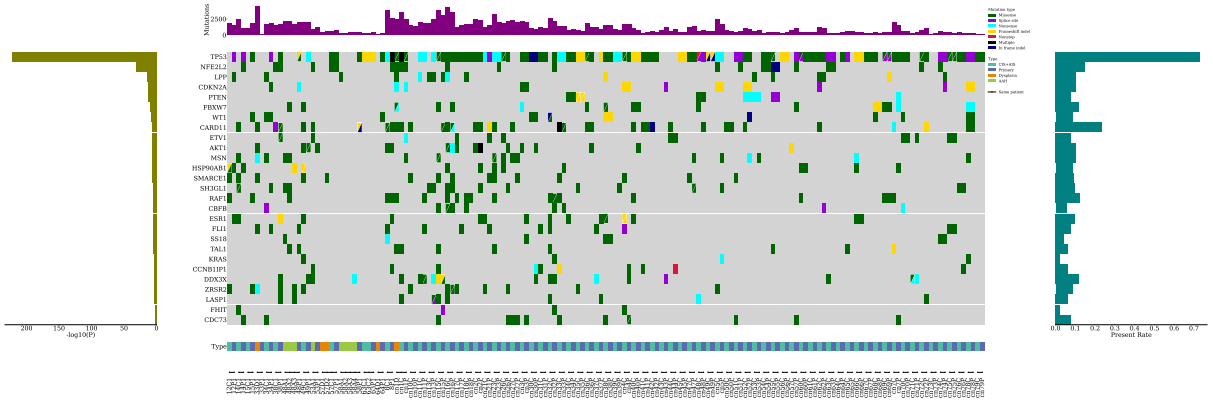


Figure 24: CoMut plot by BWA in SQC

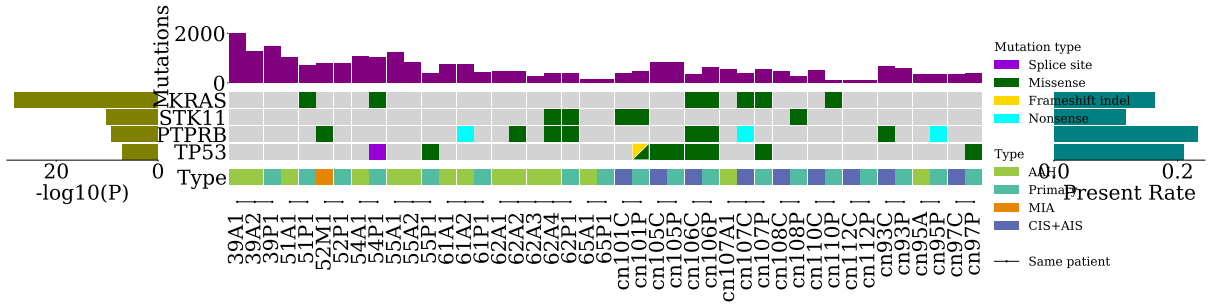


Figure 25: CoMut plot by Bowtie2 in ADC

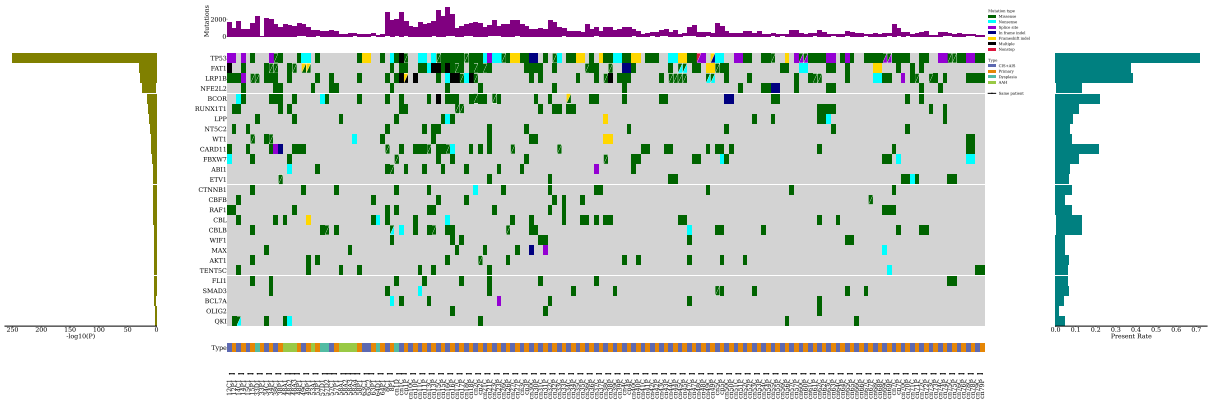
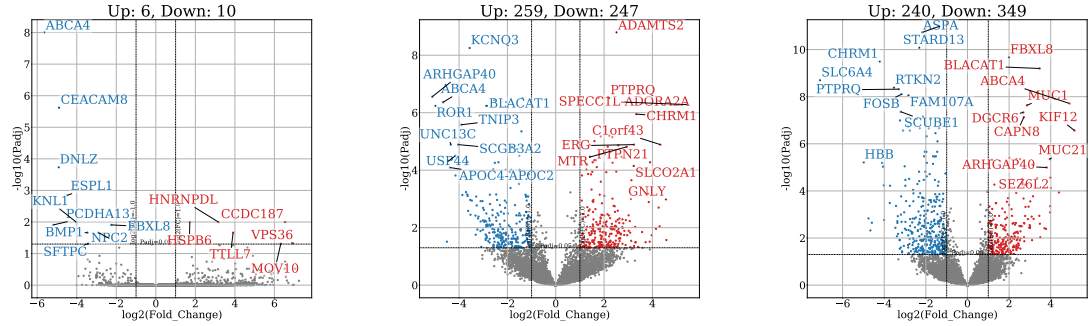


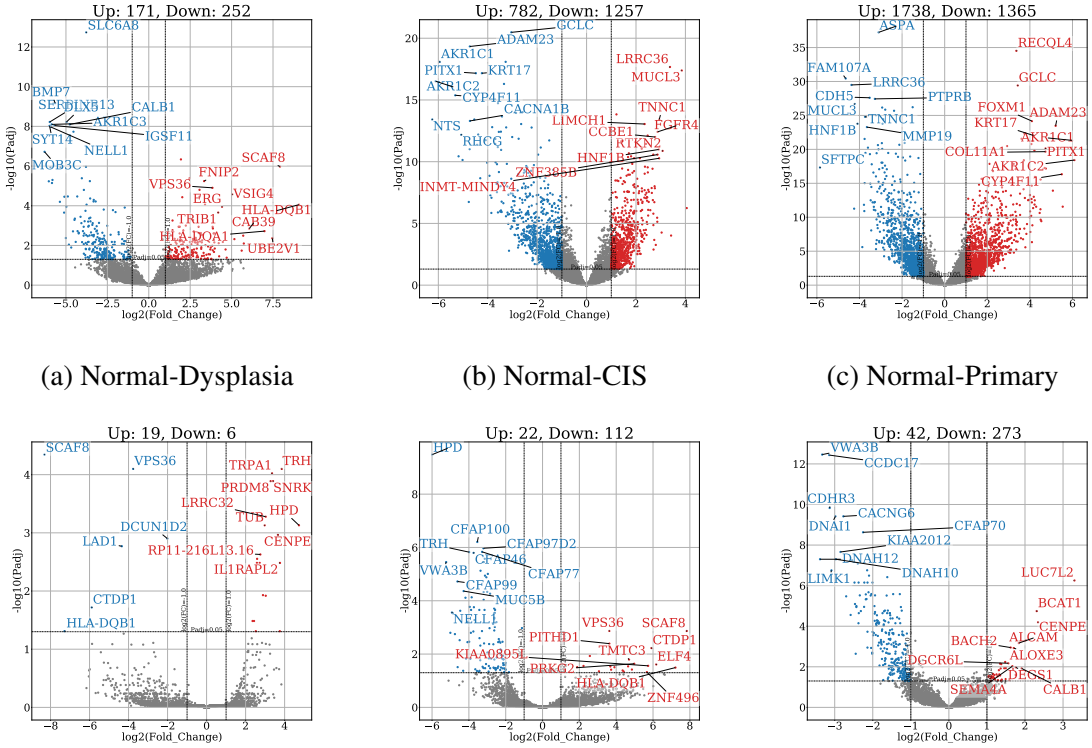
Figure 26: CoMut plot by Bowtie2‘ in SQC



(a) Normal-AAH (b) Normal-AIS (c) Normal-Primary

(d) AAH-AIS (e) AAH-Primary (f) AIS-Primary

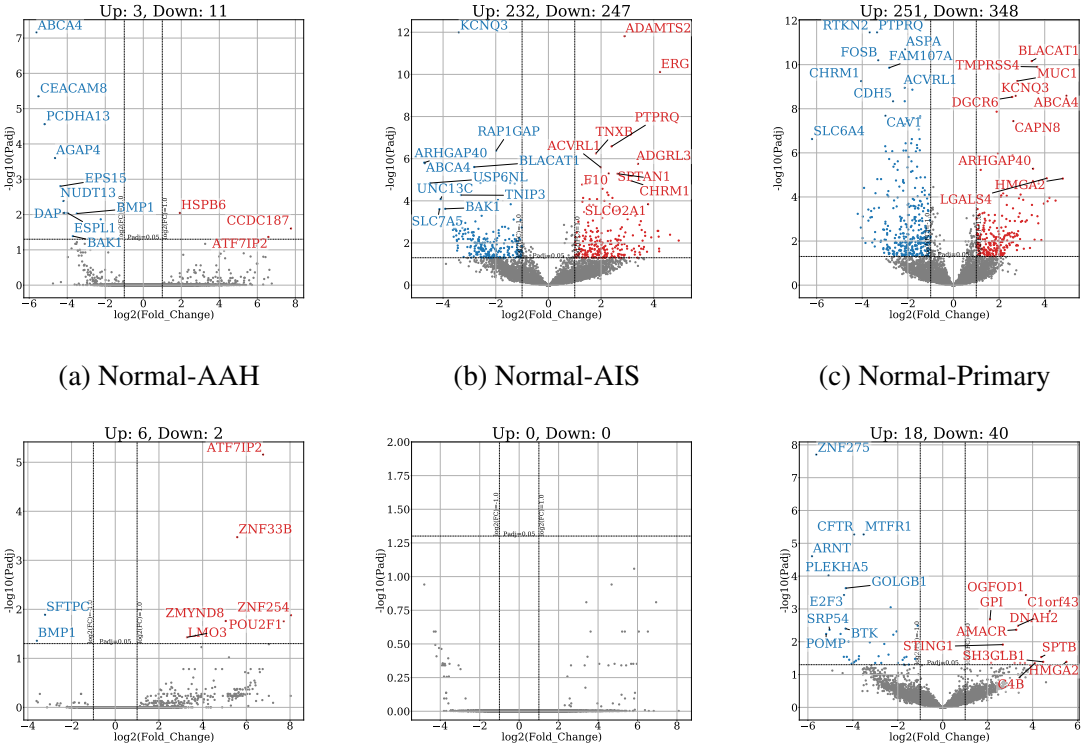
Figure 27: DEG volcano plots by Bowtie2 in ADC



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

(d) Dysplasia-CIS (e) Dysplasia-Primary (f) CIS-Primary

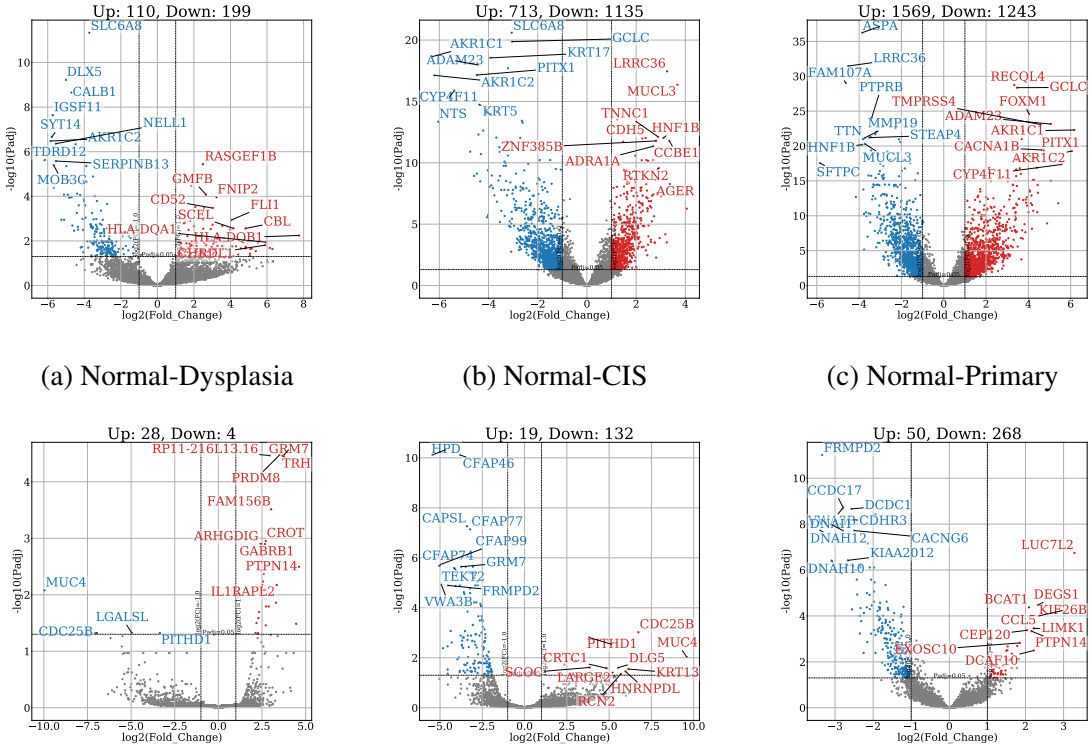
Figure 28: DEG volcano plots by Bowtie2 in SQC



(a) Normal-AAH (b) Normal-AIS (c) Normal-Primary

(d) AAH-AIS (e) AAH-Primary (f) AIS-Primary

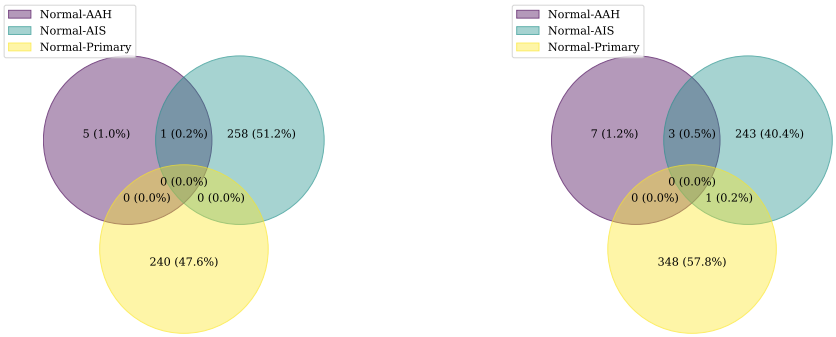
Figure 29: DEG volcano plots by STAR in ADC



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

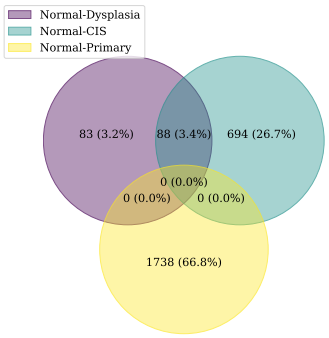
(d) Dysplasia-CIS (e) Dysplasia-Primary (f) CIS-Primary

Figure 30: DEG volcano plots by Bowtie2 in SQC

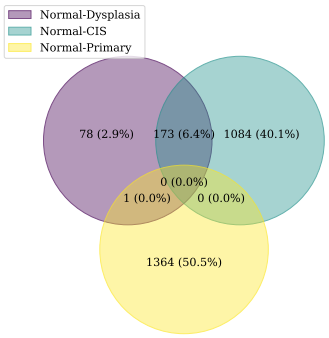


(a) Up-regulated (b) Down-regulated

Figure 31: DEG Venn Diagram by Bowtie2 in ADC

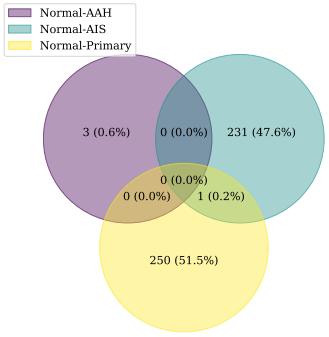


(a) Up-regulated

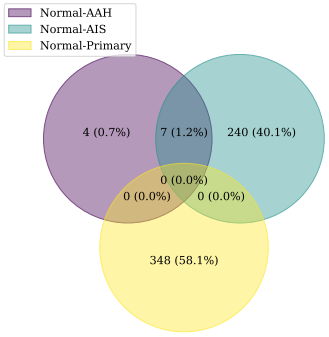


(b) Down-regulated

Figure 32: DEG Venn Diagram by Bowtie2 in SQC

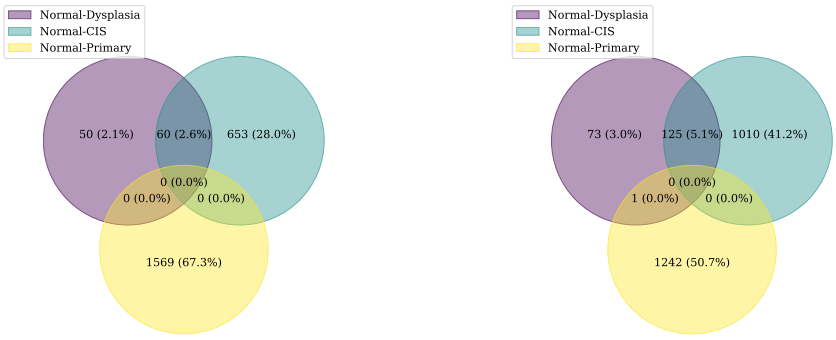


(a) Up-regulated



(b) Down-regulated

Figure 33: DEG Venn Diagram by STAR in ADC



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

Figure 34: DEG Venn Diagram by STAR in SQC

figures/CIBERSORTx/reference.jpg

Figure 35: Comprehensive dissection and clustering of 208,506 single cells from LUAD patients (Kim et al., 2020)

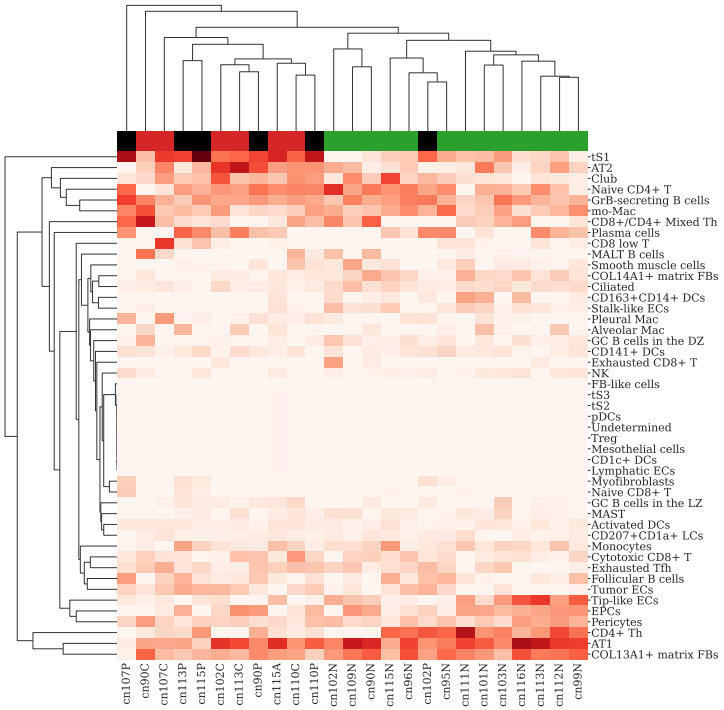


Figure 36: Cell deconvolution clustermap by Bowtie2 and CIBERSORTx in ADC

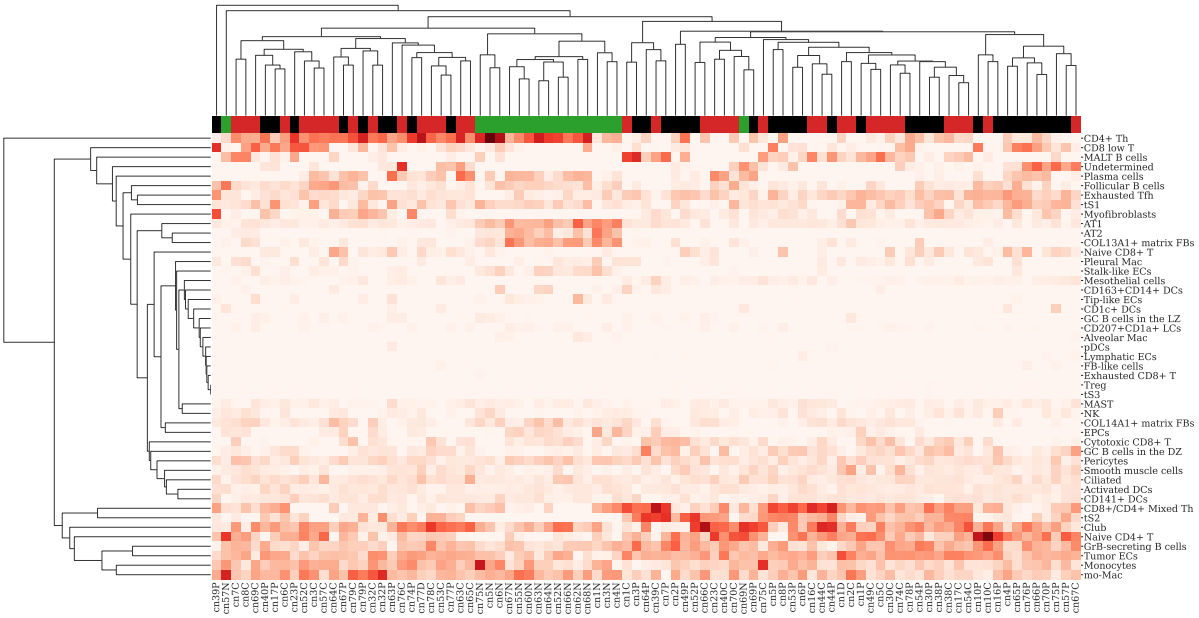


Figure 37: Cell deconvolution clustermap by Bowtie2 and CIBERSORTx in SQC

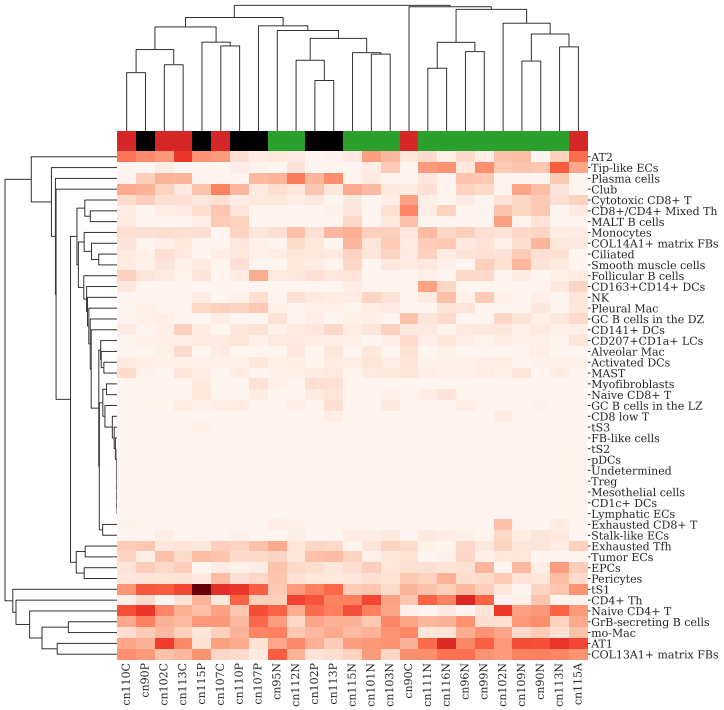


Figure 38: Cell deconvolution clustermap by STAR and CIBERSORTx in ADC

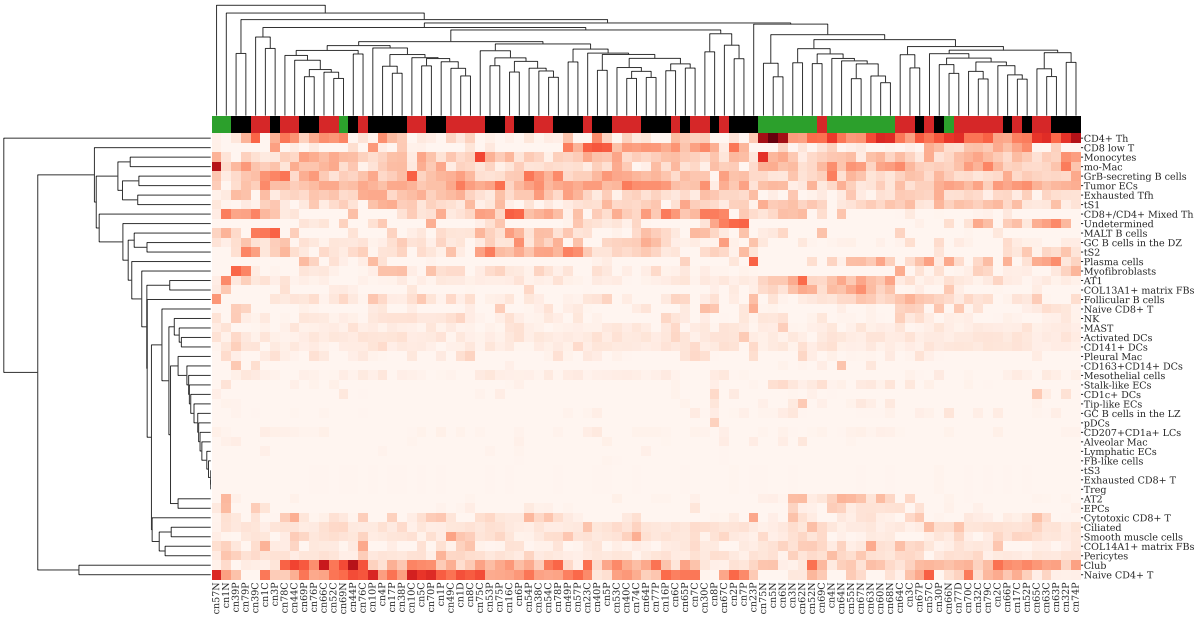


Figure 39: Cell deconvolution clustermap by STAR and CIBERSORTx in SQC

Figure 40: Cell deconvolution clustermap by Bowtie2 and BisqueRNA in ADC

Figure 41: Cell deconvolution clustermap by Bowtie2 and BisqueRNA in SQC

Figure 42: Cell deconvolution clustermap by STAR and BisqueRNA in ADC

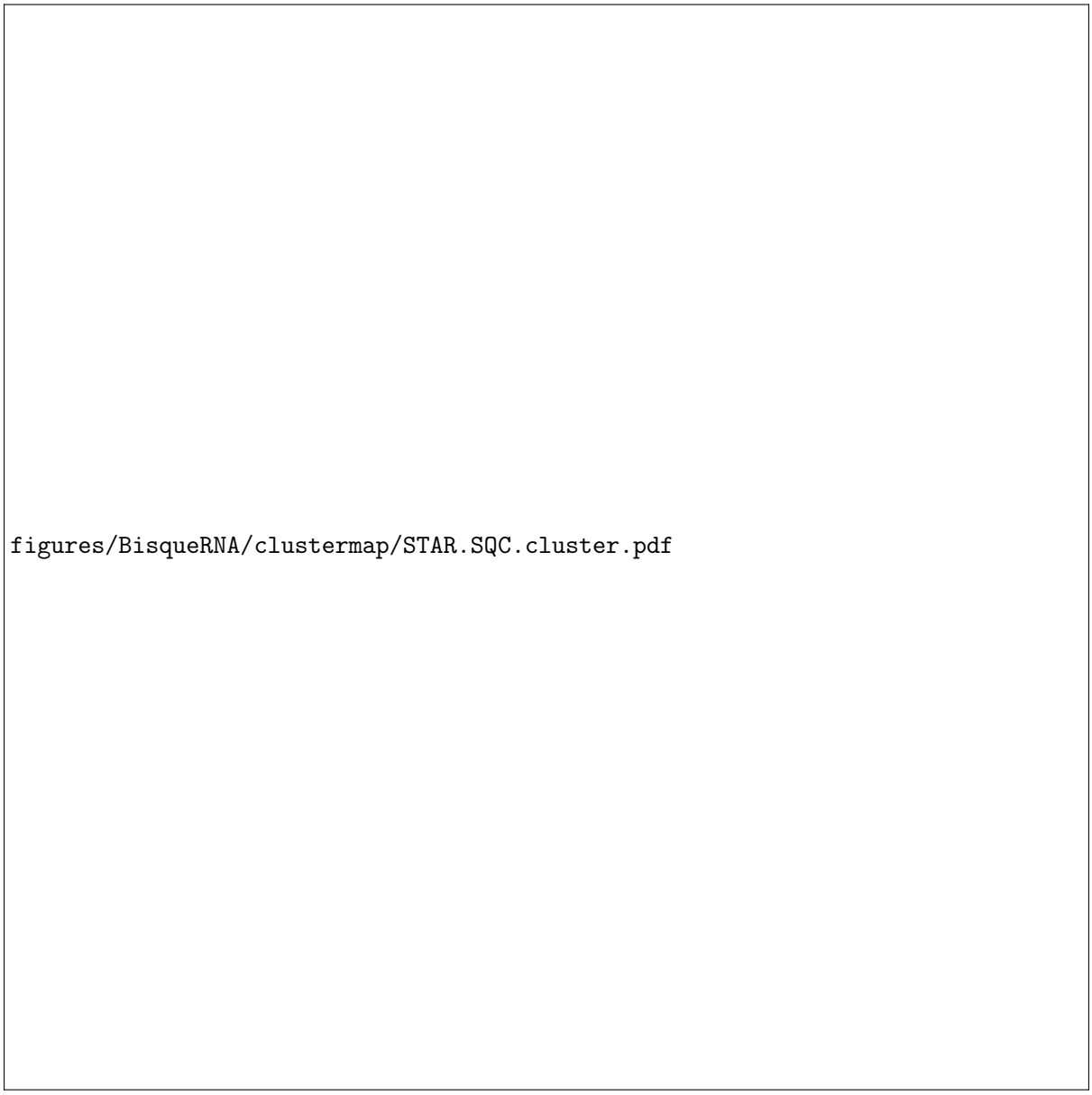


Figure 43: Cell deconvolution clustermap by STAR and BisqueRNA in SQC

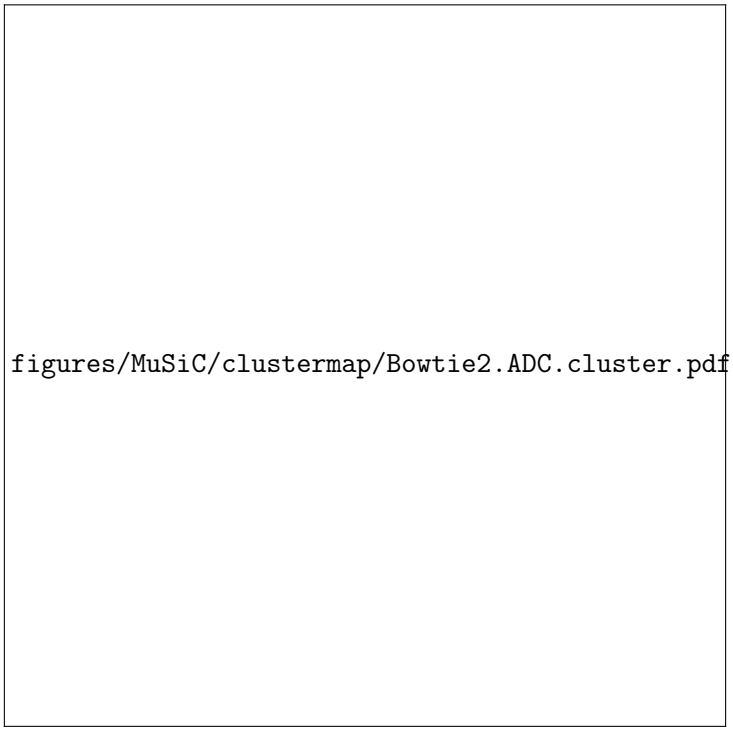


Figure 44: Cell deconvolution clustermap by Bowtie2 and MuSiC in ADC

Figure 45: Cell deconvolution clustermap by Bowtie2 and MuSiC in SQC

Figure 46: Cell deconvolution clustermap by STAR and MuSiC in ADC

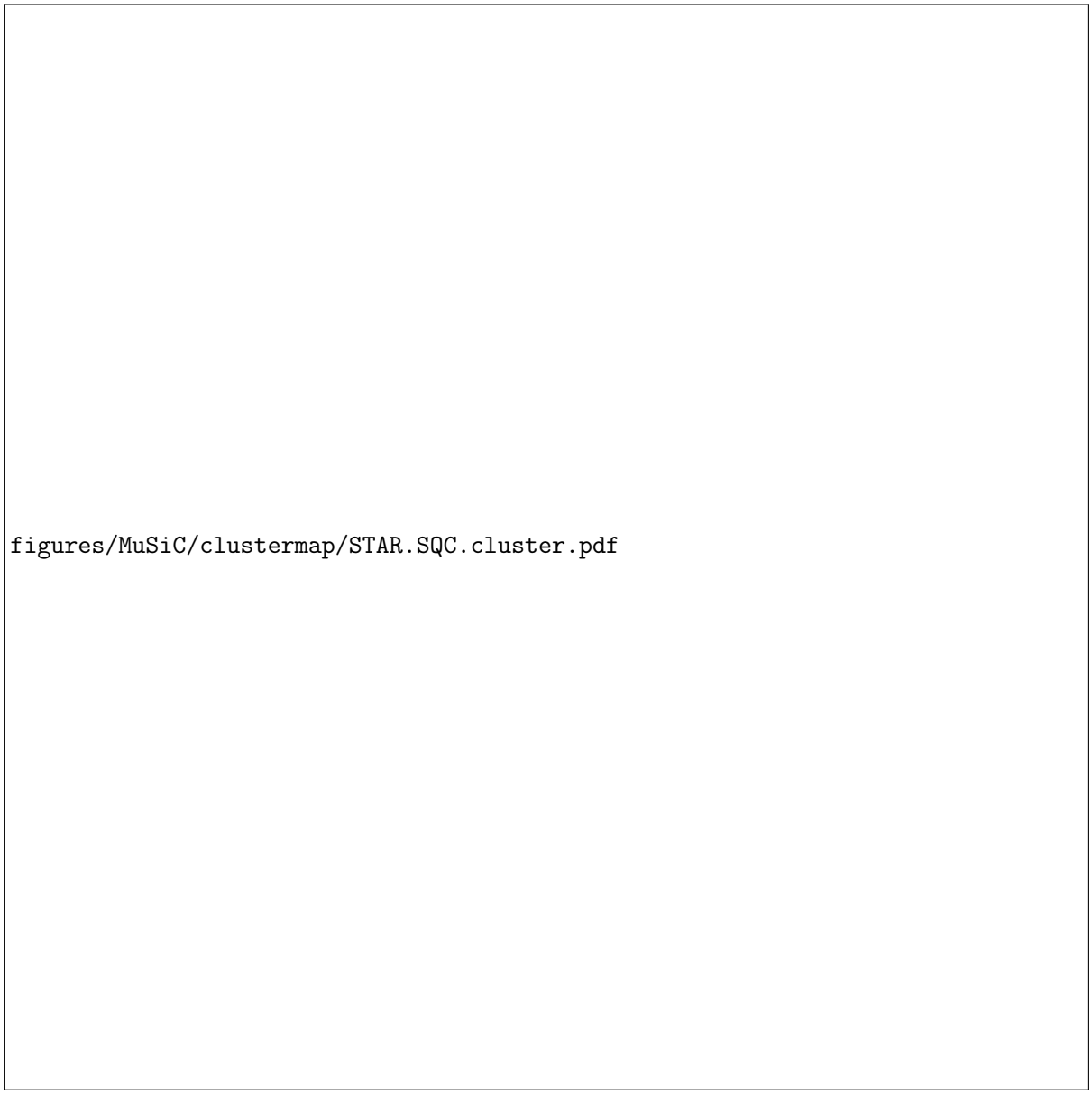


Figure 47: Cell deconvolution clustermap by STAR and MuSiC in SQC

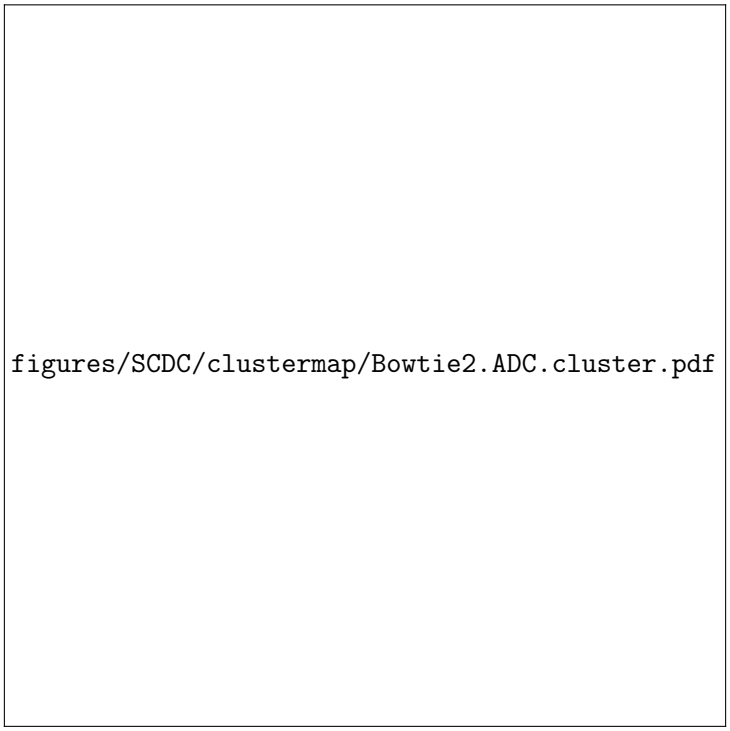


Figure 48: Cell deconvolution clustermap by Bowtie2 and SCDC in ADC

Figure 49: Cell deconvolution clustermap by Bowtie2 and SCDC in SQC

Figure 50: Cell deconvolution clustermap by STAR and SCDC in ADC

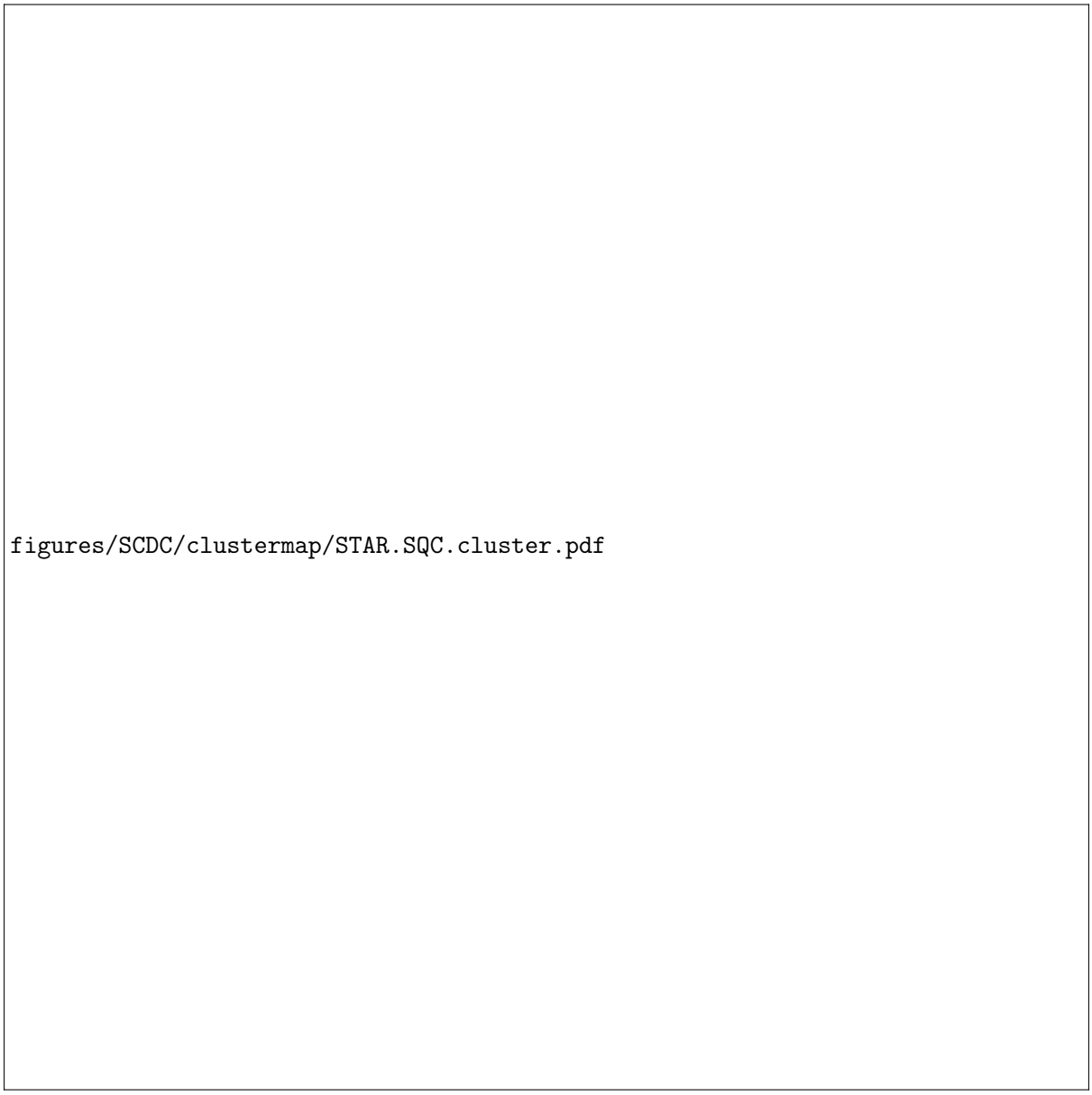


Figure 51: Cell deconvolution clustermap by STAR and SCDC in SQC

References

Andrews, S., Krueger, F., Segonds-Pichon, A., Biggins, L., Krueger, C., & Wingett, S. (2012, January). *FastQC.* Babraham Institute. Babraham, UK.

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.

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.

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.

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.

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.

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