Detection of ecDNA in DLP+ Single Cell DNA Sequencing of Esophageal Adenocarcinoma Organoids

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Background

Esophageal Adenocarcinoma (EAC) incidence is increasing, while 5-year survival rates stubbornly remain below 15%. A large contribution to this low survival rate is the relatively late diagnosis, with most EAC presenting at an advanced stage with numerous large scale genomic copy number alterations (CNA). Furthermore >50% of cases feature extrachromosomal circular DNAs (ecDNA), a stand-alone circular DNA fragment that is replicated with the genome but inherited stochastically. This random inheritance yields a varying expression of the genes contained within, which is clinically relevant given ecDNA frequently contains treatment resistance genes.

Direct Library Preparation (DLP+) is a whole genome amplification (WGA)-free, single cell DNA sequencing method that provides even sequencing coverage across thousands of cells. Since DLP+ randomly generates genetic fragments across the genome and barcodes them prior to PCR amplification, DLP+ allows for an unbiased representation of the global CNA states throughout the genome. Here, we employed DLP+ to track CNA states in EAC organoids.

Findings

In our work we demonstrate that DLP+ has enough sensitivity to:

- Monitor clonal selection across the long-term maintainence of the organoids
- Independently identify and accurately reconstruct genomic leisons
- Quantify absolute number of ecDNA fragments on a per cell level.

Materials & Methods

All data was generated from a patient derived EAC orgnaoid, named CAM277. The DLP+ data was generated at passage 4 and 15, while the whole genome sequencing data and the Oxford Nanopore long read sequencing data were generated at passage 15. EcDNA assembly for both the ONT and DLP+ data was performed by Amplicon Architect.

Conclusion

Despite the shallow per-cell coverage of the DLP+ data, in aggregate, we were able to independently detect and reconstruct the ecDNA fragments assembled by the ONT data. EACs experience high rates of CNA, increasing the chance of producing ecDNA. Given ecDNA's outsized impact on gene expression, these findings demonstrate that we will be able to successfully reconstruct ecDNA found in clinical samples.

Detecting Clonal Selection

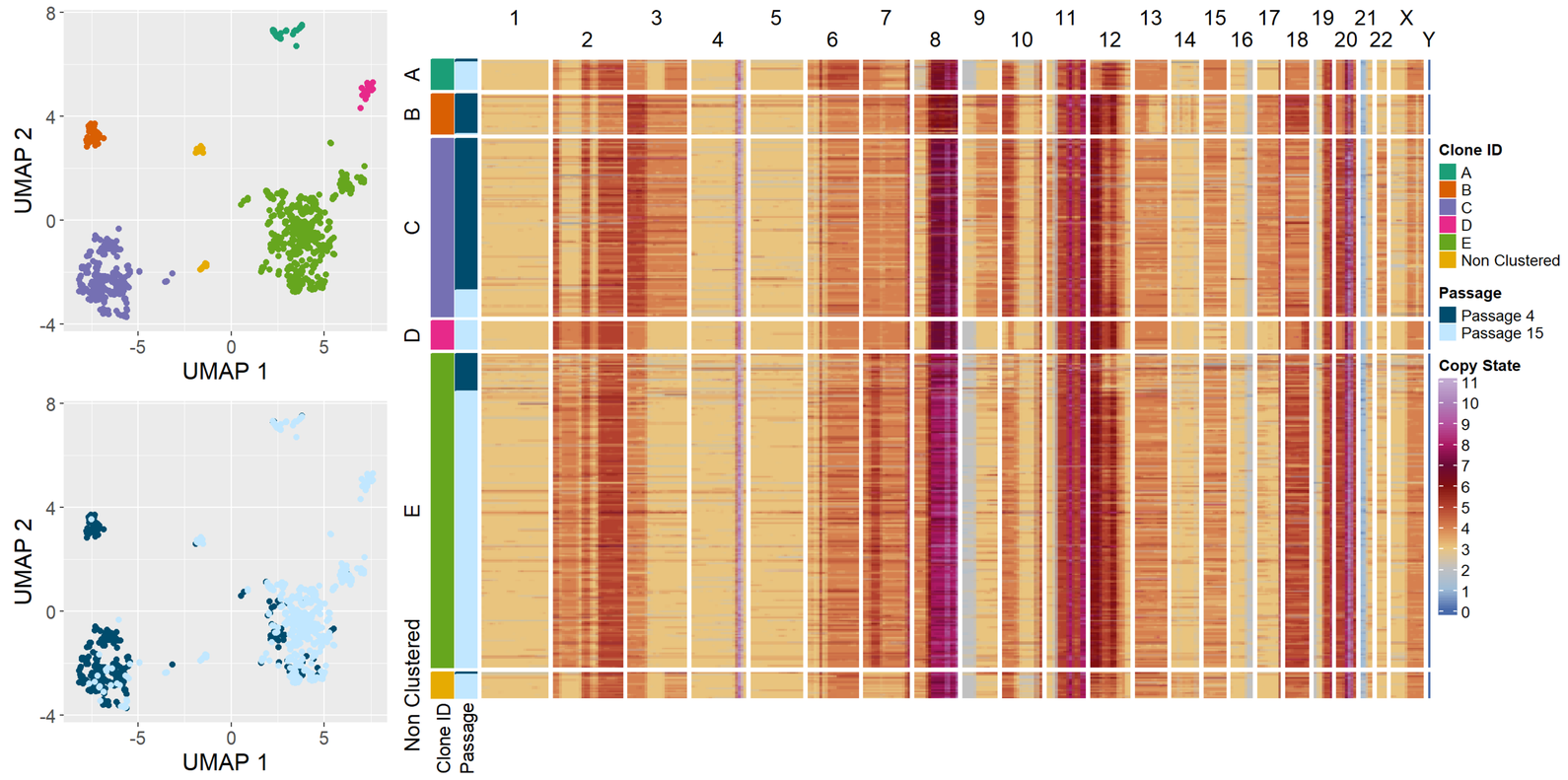

Figure 1: Clonal composition of CAM277 over two timepoints

ecDNA Reconstruction

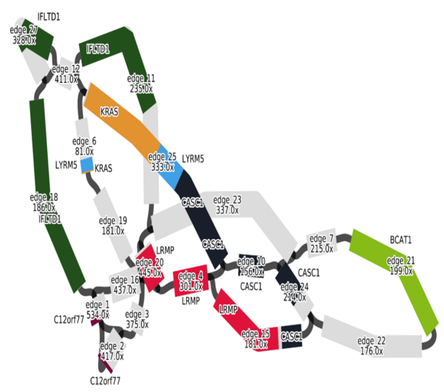

Figure 2: Oxford Nanopore Constructed ecDNA. The multi-cyclic structure is due to unresolved junction ambiguities

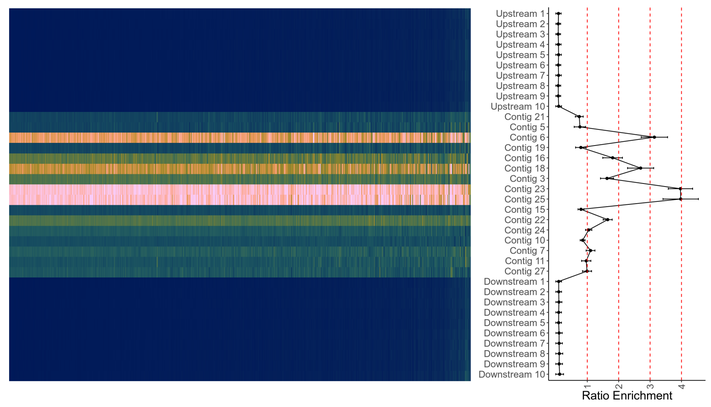

Figure 3: Relative frequencies of ONT detected ecDNA fragments and the genomic region flanking the ecDNA. Constant ratios between ecDNA fragments indicate one unified ecDNA.

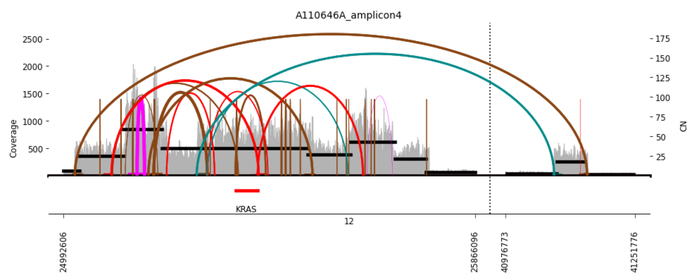

Figure 4: DLP+ assembled ecDNA in passage 4

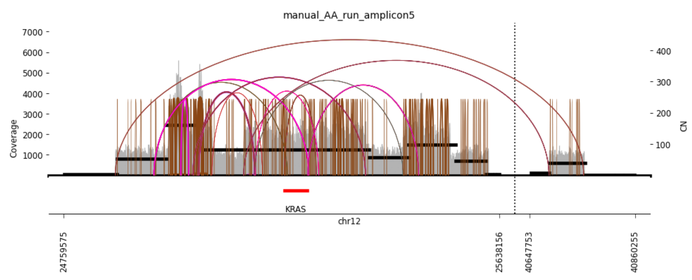

Figure 5: DLP+ assembled ecDNA in passage 15

Per Cell ecDNA Counts

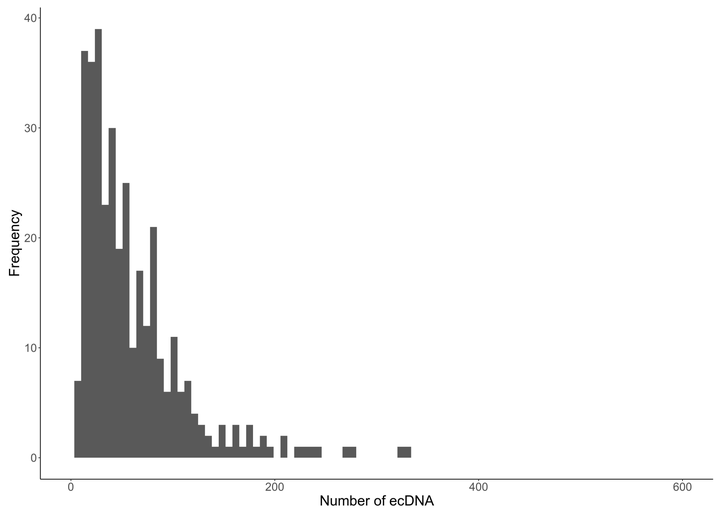

Figure 6: Absolute ecDNA counts in passage 4

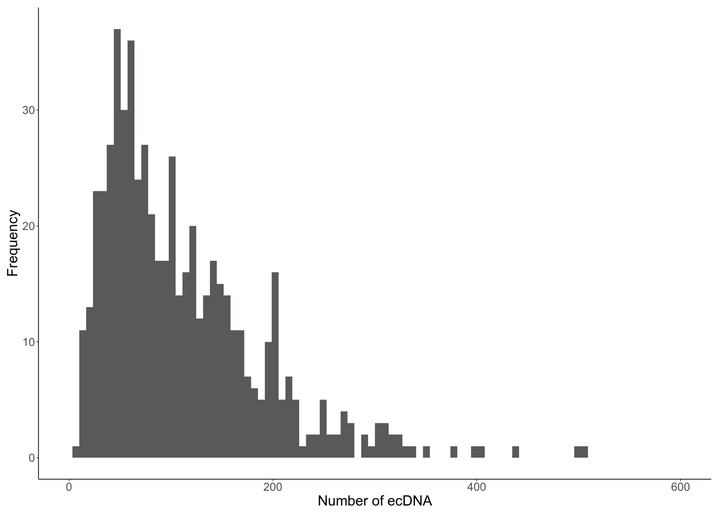

Figure 7: Absolute ecDNA counts in passage 15

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