Transcriptomic Effects of Retinoblastoma Somatic DNA Copy Number Alterations Deduced by Single Cell RNA and DNA Sequencing

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Introduction

Recurrent somatic DNA copy number alterations (SCNAs) contribute to the emergence and progression of human cancers but SCNA driver genes and transcriptomic effects are often poorly defined.

Tumor heterogeneity hinders understanding of SCNA effects due to variability in copy number and genomic and microenvironmental features[1].

SCNA effects may be defined by comparing transcriptomes of cells with different SCNAs within a single heterogeneous tumor.

We aim to define transcriptomic changes that accompany recurrent SCNAs in the childhood retinal tumor, retinoblastoma.

Here, we employ a recently developed method for simultaneous single cell RNA and DNA sequencing termed GTO (Genome Transcriptome One-Tube) that yields shallow coverage of genomic DNA to generate SCNA profiles and deep coverage of full-length cDNAs to generate single cell transcriptome profiles (N Anaparthy *et al., in preparation*).

We applied this method to early passage retinoblastoma cells that exhibit SCNA diversity prior to cell line establishment.

Objectives

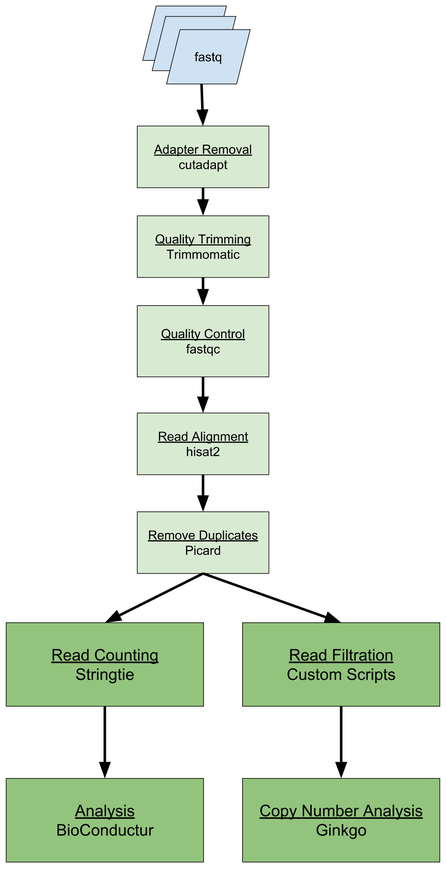
Apply Genome Transcriptome One-Tube (GTO) to early passage retinoblastoma cells in culture.

Assess fidelity and resolution of SCNA profiles for subsequent studies of transcriptomic changes.

Define clonal diversity of early passage retinoblastoma cell lines by clustering of SCNAs

Assess SCNA transcriptomic effects by comparing single cells differing in a specific SCNA.

Bioinformatic Workflow



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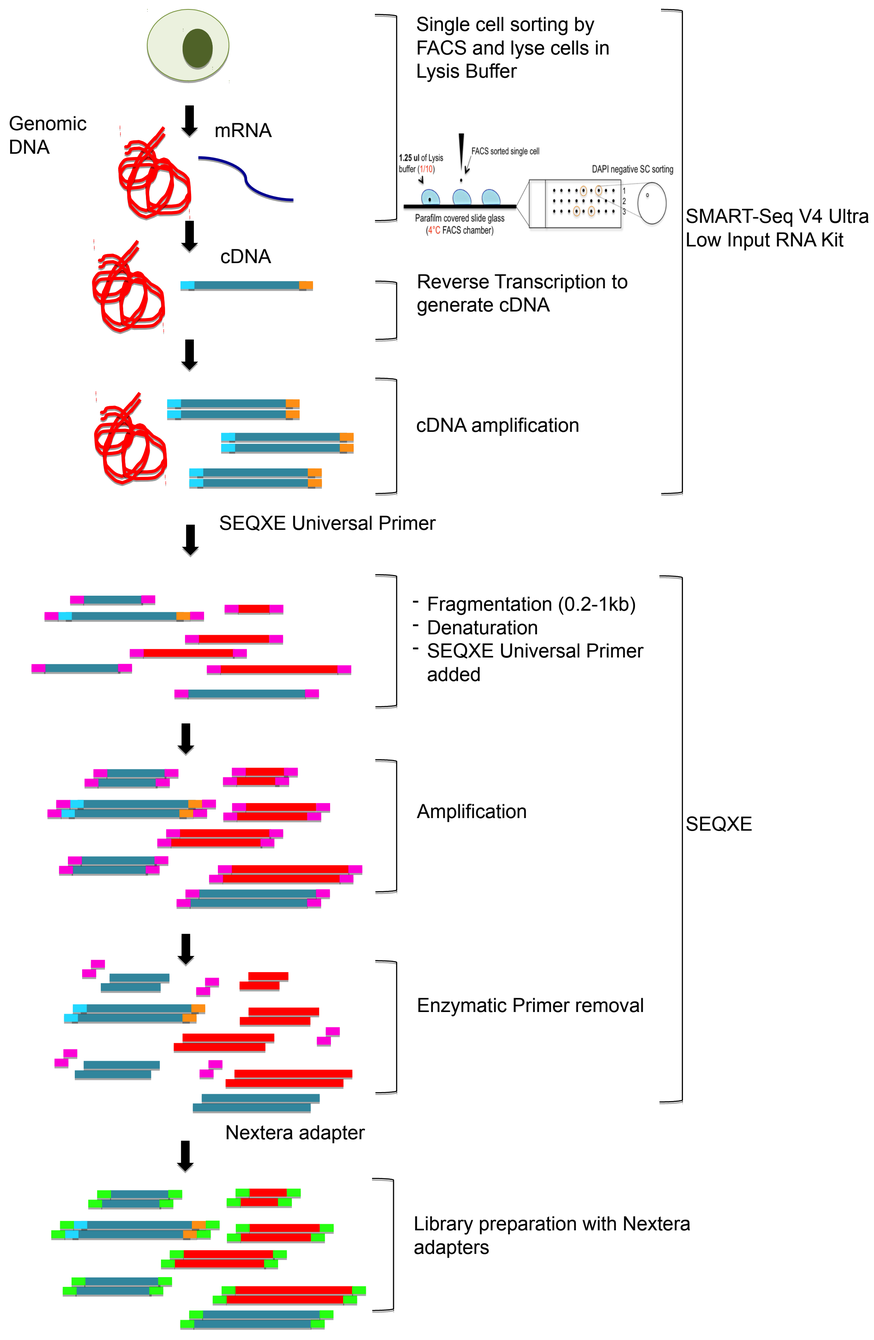
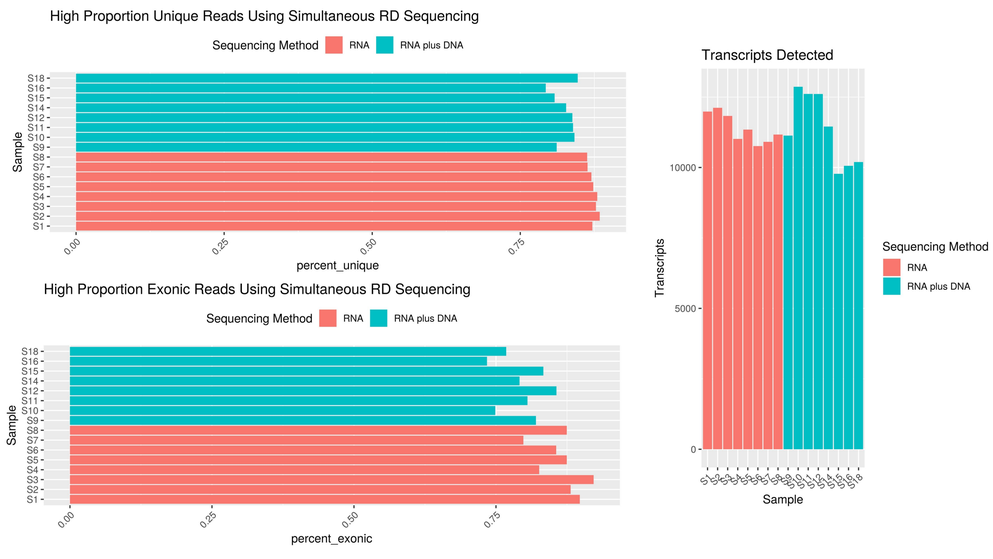
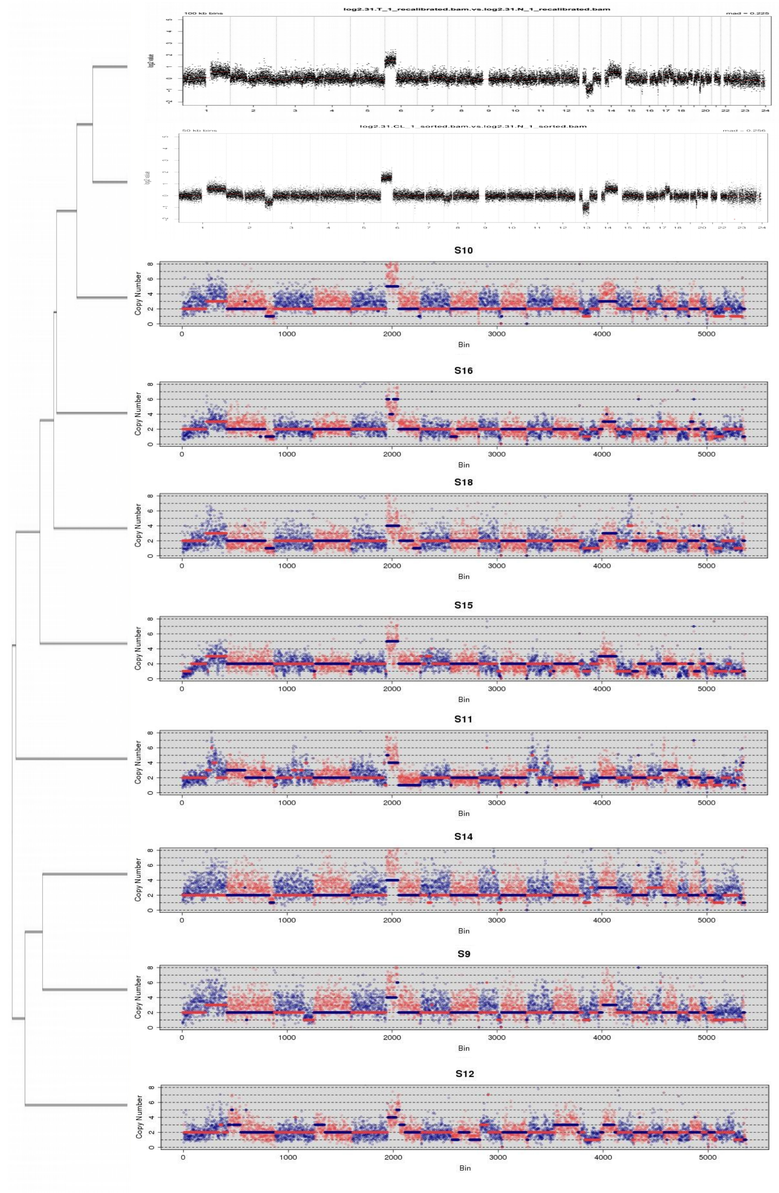
Single cell RNA &DNA sequencing Genome Transcriptome One-Tube (GTO)



Transcript Aligment



Bulk and Single Cell SCNA Profiles after GTO



Conclusions

Early passage cultured cells were comprised of several subclones that diverged from the major primary tumor cell populations.

Bulk cell line profiles were more uniform than tumor, while single cell samples from early passage cell lines showed diversity in individual SCNAs

Analyses of transcriptomic differences of the different subpopulations relative to each other and to the primary tumor are in progress.

Our findings suggest that combined single cell RNA and DNA sequencing may be used to dissect the transcriptomic effects of specific SCNAs in heterogeneous tumor cell populations.

References

1. Ho, Y.-J. et al. SAKE (Single-cell RNA-Seq Analysis and Klustering Evaluation) Identifies Markers of Resistance to Targeted BRAF Inhibitors in Melanoma Cell Populations. bioRxiv 239319 (2017). doi:10.1101/239319

2. Baslan, T. et al. Optimizing sparse sequencing of single cells for highly multiplex copy number profiling. Genome Research 125, 714–724 (2015).

3. Garvin, T. et al. Interactive analysis and assessment of single-cell copy-number variations. Nature Methods 12, 1058–1060 (2015).

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