



Alignment and splice junction identification

RNA-Seq Hands-on Practical

Gayle Philip, VLSCI

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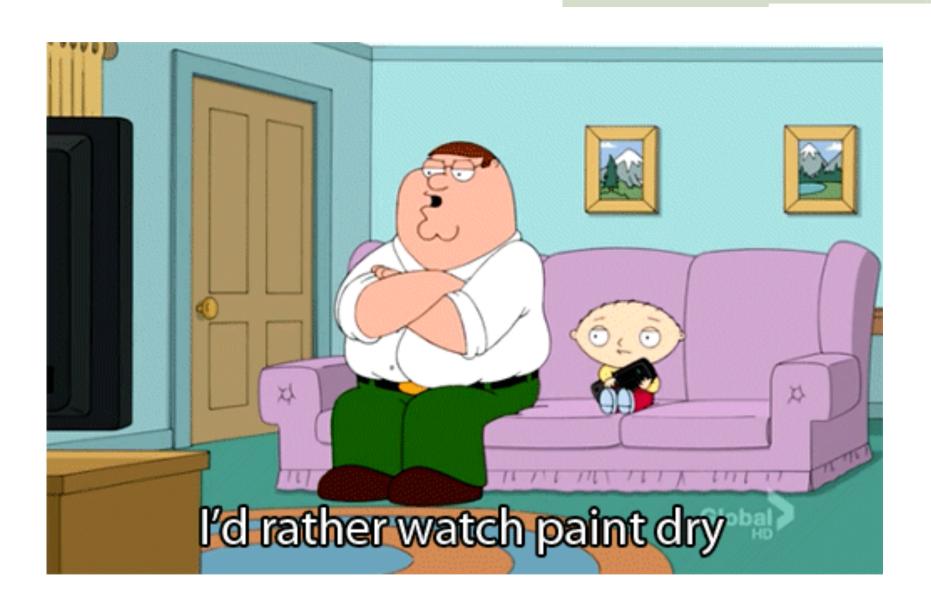


Introduction

- The goal of this hands-on session is to perform some basic tasks in the analysis of RNA-seq data. We will start by aligning RNA-seq data to the zebrafish genome using Tophat2.
 - Prepare the environment
 - Tophat alignment
 - Viewing the alignment using IGV

Preparing the alignment

- Sample
 - Zebrafish (Danio rerio)
 - 2 conditions : 2 cells and 6h
- Type of sequencing
 - 76bp pair-end from polyA selected RNA
 - Illumina
- File name and formats
 - Fastq file
 - 2cells 1.fastq and 2cells 2.fastq (RNA-seq data of a 2-cell zebrafish embryo)
 - 6h 1.fastq and 6h 2.fastq (RNA-seq data of zebrafish embryos
 6h post fertilization)



DO NOT Run!



The command to create an index is as follows. You DO NOT need to run this command yourself - we have done this for you.

1 bowtie2-build genome/Danio_rerio.Zv9.66.dna.fa genome/ZV9

- Some commands take a **LONG** time to run. For example, indexing a genome can take a few hours to finish. **The genome index has been pre-computed for you.**
- Sometimes, the **same** command has to be **repeated** multiple times on different input files. E.g. Data from case and control conditions have to be aligned independently in the same manner. Pre-computed files have been provided for some of the samples.

Alignment

- Reference genome
 - Danio_rerio.Zv9.66
 - Index has already been created
- Alignment program and parameters
 - The 2cells data is pre-aligned
 - Alignment needs to be done for 6h dataset

Alignment Visualisation in IGV

- The Integrative Genomics Viewer (IGV) is able to provide a visualisation of read alignments given a reference sequence and a BAM file.
 - Visualise the alignment
 - Look at the splice junctions
- Interpreting the alignment in IGV
 - http://www.broadinstitute.org/igv/AlignmentData