

# Pseudo-Alignment with kallisto

## Efficient Probabilistic Quantification of RNA-Seq Reads

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### 1 Pseudo-Alignment of RNA-Seq Reads

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The present project concerns the re-analysis of transcriptomic data from the study described in the paper “Developmental regulation of human cortex transcription and its clinical relevance at base resolution”, Jaffe *et al.*, *Nature Neuroscience*. In order to quantify RNA-Seq reads, alignment against a reference transcriptome must be performed, a procedure which results in tables of read counts for use in downstream statistical analysis. Here, we take advantage of **pseudo-alignment**, a novel development in sequencing algorithms, to probabilistically align reads. Below, we describe pseudo-alignment and the results of its application to the Jaffe *et al.* data.

#### 1.1 The Pseudo-Alignment Process

Pseudo-alignment is a novel process for quantifying a set of samples of RNA-Seq reads by performing partial matching against a reference transcriptome. The novel pseudo-alignment process, implemented in the command line tool **kallisto**, takes into account all of the information contained in a set of reads while reducing the computational burden imposed by more traditional alignment techniques. The **kallisto** tool provides results similar to that produced by other alignment software (*e.g.*, **bowtie**), while taking only a fraction of the time. For a complete description of pseudo-alignment, consult the paper “Near-optimal probabilistic RNA-seq quantification”, Bray *et al.*, *Nature Biotechnology*.

#### 1.2 The Results of Pseudo-Alignment

The pseudo-alignment procedure was implemented on the Jaffe *et al.* data through the use of the **kallisto** command line tool. Using a publicly available transcriptome assembled from the GRCh38 (hg19) *Homo sapiens* genome, sets of paired-end RNA-Seq reads for each of the 12 subjects involved in the study were pseudo-aligned, resulting in count tables mapping each set of reads to **173,259** transcriptomic objects. *Please note that while similar quantification tools (e.g., Cufflinks) produce estimates of mappings of isoforms, kallisto provides estimates of transcript abundance.* Though this is the first time that I have used **kallisto** for alignment and RNA-Seq quantification, elementary searches for the “run.info.json” output file (from other unrelated projects) seem to suggest that the produced transcript abundances are normal for **kallisto**. Tables of counts are produced

for each set of paired-end RNA-Seq reads for each subject in a tab-separated file format; these files are suitable for concatenation into a single count table containing quantification results for all subjects, which can be used as input to a set of statistical analysis scripts after appropriate data cleaning.