Introduction to Transcriptomics Analysis

Class 10 - Practice about Gene Expression Quantification.

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Outline of Topics

- Exercise 1: Running Stringtie on the mapped reads.
- Exercise 2: Running HTSeq to calculate expression

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A- RNASeq Analysis pipeline with Hisat2-StringTie-Ballgown

PROTOCOL

Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown

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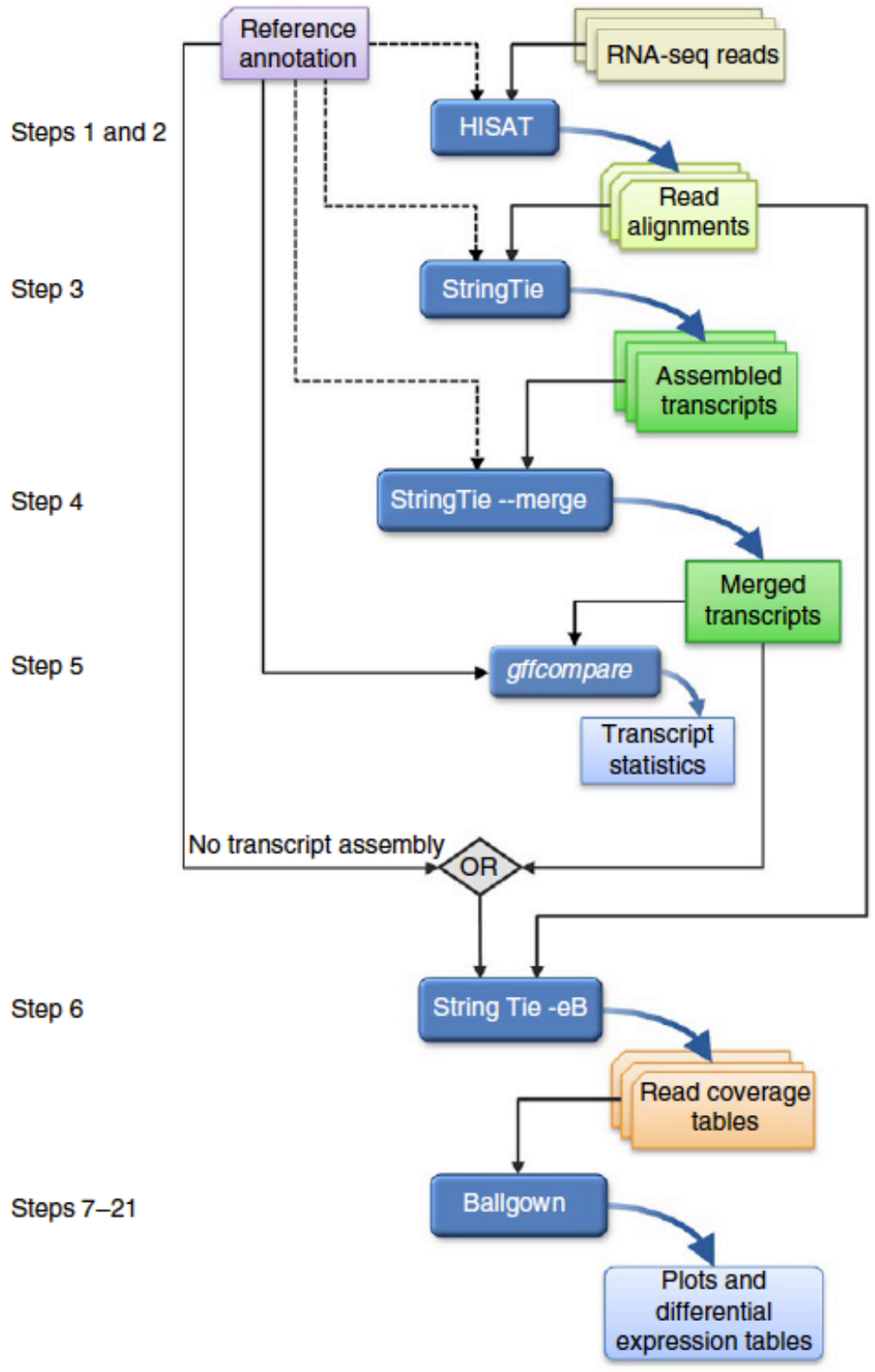
Published online 11 August 2016; doi:10.1038/nprot.2016.095

Pertea, Mihaela, et al. "Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown." *Nature protocols* 11.9 (2016): 1650.

A- RNASeq Analysis pipeline with Hisat2-StringTie-Ballgown



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- Exercise 1: Running Stringtie on the mapped reads.

Goal: Generate the expression tables for Stringtie. Stringtie is divided in three steps: 1- Transcript discovery; 2- Comparison and 3- Quantification

Input:

- Reads mapped in BAM format (Artha_*.bam files)
- GFF file with the annotations (Araport11_GFF3_genes_transposons.201606.gtf)

Recommended commands:

```
stringtie -p 48 -G ref.gtf -o my_sample.gtf -l my_sample_label my_sample.bam

ls | grep gtf > merged_list.txt

stringtie --merge -p 8 -G ref.gtf -o stringtie_merged.gtf merged_list.txt

gffcompare Stringtie_merged.gtf -r ref.gtf

stringtie -e -B -p 48 -G stringtie_merged.gtf -o my_sample/count.gtf
my_sample.bam
```

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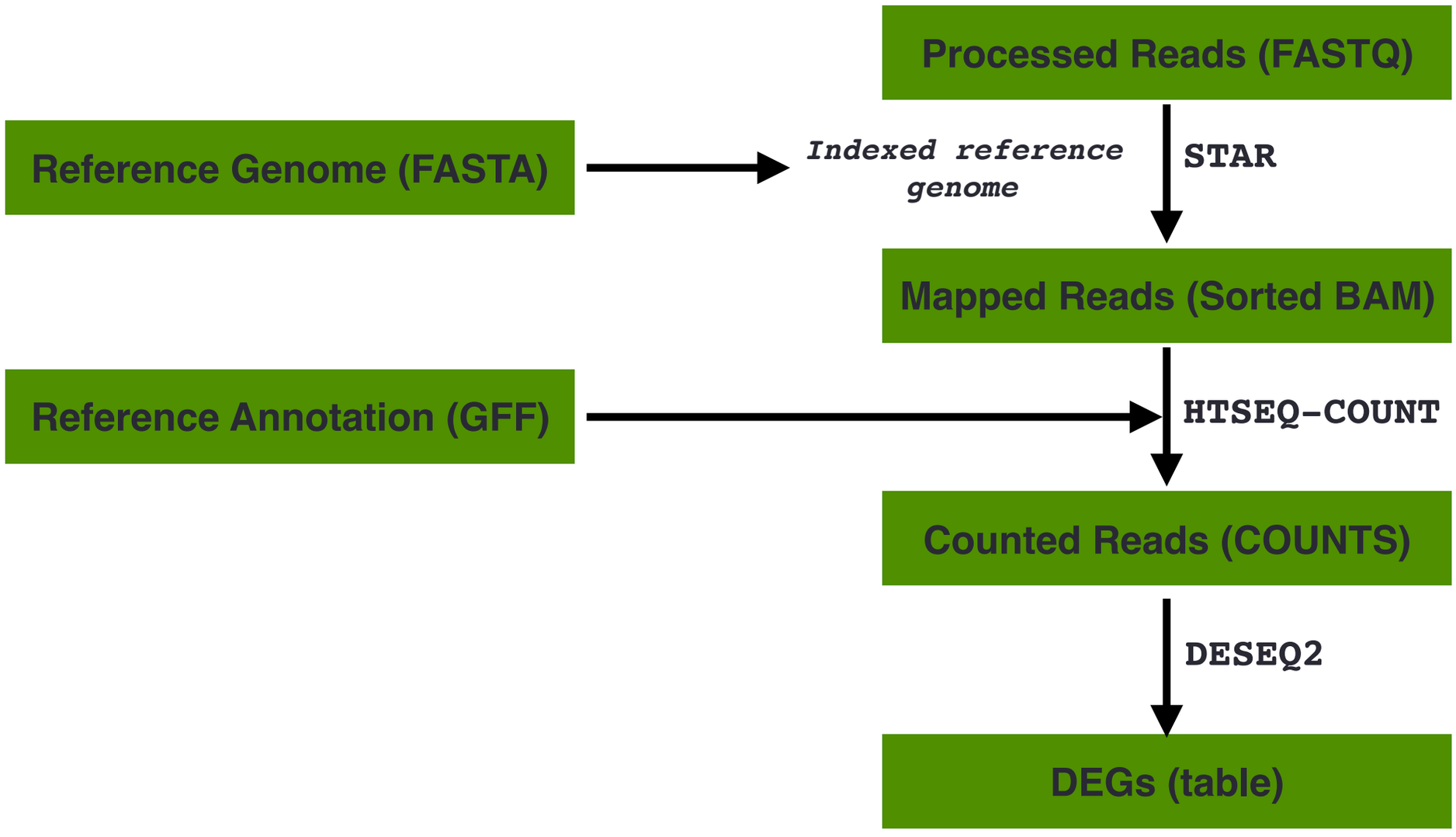
B- RNASeq Analysis pipeline with STAR-HTSeqCount—DESeq2



- Exercise 2: Running HTSeq to calculate expression

Goal: Generate the expression tables with HTSeq. It will be a single step.

Input:

- Reads mapped in BAM format (Artha_*.bam files)
- GFF file with the annotations (Araport11_GFF3_genes_transposons.201606.gff)

Recommended command:

```
htseq-count -f bam -m union -r pos -i ID -a 10 --type=gene --stranded=no
my_star_align.bam my_ref.gff > my_htseq.counts
```