## Strategies for Bulk RNA-seq Analysis


$\checkmark$ Genome
$\checkmark$ GTF (annotation)


## Alignment to Genome:

 HISAT2, STARmultiple BAMs (+known GTF)

Count reads
associated with genes:
htseq-count,
featureCounts
Count Matrix

DGE with R:
DESeq2, EdgeR,
limma:voom

## $\checkmark$ Genome <br> $\checkmark$ GTF (annotation)?

Sequence reads
FASTQ
Quality control
FASTQ $\quad \begin{gathered}\text { (+reference genome index) } \\ \text { (+known GTF, optional) }\end{gathered}$
Alignment to Genome:
HISAT2, STAR
Reference-based transcriptome assembly and quantitation with StringTie
multiple BAMs
(+known GTF)

Count reads
associated with genes:
htseq-count, featureCounts

Count Matrix

DGE with R:
DESeq2, EdgeR,
limma:voom

## $\checkmark$ Transcriptome (FASTA)


$\checkmark$ Genome
$\checkmark$ GTF (annotation)?
$\checkmark$ Genome?
$\checkmark$ GTF (annotation)?


Martin J.A. and Wang Z., Nat. Rev. Genet. (2011) 12:671-682


## Quantitation from assembled reads

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