

Additional file 3: The parameters for each tool

1 polyester

1.1 simulate_experiment_countmat():

```
paired = TRUE,  
readlen = 100,  
error_rate = 0.005,  
bias = "none",  
strand_specific = TRUE,  
seed = 12345
```

2 TopHat2-Cufflinks pipeline

2.1 TopHat2

```
-num-threads 4
```

2.2 Cuffdiff2

```
-emit-count-tables  
-max-bundle-frags 100000000  
-num-threads 6
```

3 HISAT2-StringTie-Ballgown pipeline

3.1 HISAT2

```
-seed 12345  
-new-summary true  
-no-mixed true  
-threads 2
```

3.2 StringTie

```
-e  
-B
```

4 STAR-RSEM-EBSeq pipeline

4.1 STAR

```
-readFilesCommand zcat
```

```
--readNameSeparator "'|'"
--limitBAMsortRAM 48000000000
--outReadsUnmapped Fastx
--outSAMtype "BAM SortedByCoordinate"
--quantMode TranscriptomeSAM
--outSAMattributes All
--outSAMstrandField intronMotif
--outSAMheaderHD "'@HD VN1.4 SOcoordinate'"
--runThreadN 2
--runRNGseed 777
```

1 4.2 RSEM

```
--bam
--estimate-rspd
--calc-ci
--seed 12345
--no-bam-output
--num-threads 4
--ci-memory 61440
```

2 5 Kallisto-Seleuth

3 5.1 Kallisto

```
--seed 12345
-b 100
--pseudobam
--genomebam
```

4 5.2 Sleuth

5 5.2.1 *sleuth_results()*:

```
test_type = "wt",
show_all = FALSE,
pval_aggregate = FALSE
```

6 6 Salmon-DESeq2 pipeline

7 6.1 Salmon

8 Execute with default parameters.

9 6.2 DESeq2

10 6.2.1 *nbinomWaldTest()*:

11 Execute with default parameters.