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# RNA-seq alignment and expression quantification
# This SLURM script performs paired-end RNA-seq alignment and gene/transcript quantification
# using RSEM with Bowtie2.
# The analysis is carried out for Saccharomyces cerevisiae samples (BY4272 and SY14), each
# with three biological replicates, using a pre-built yeast RSEM reference.

#!/bin/bash -l
#SBATCH --job-name=rnaseq_align_quant
#SBATCH --output=/scratch/grp/msc_appbio/Group2_ABCC/RNAseq/final/logs/%x_%j.out
#SBATCH --error=/scratch/grp/msc_appbio/Group2_ABCC/RNAseq/final/logs/%x_%j.err
#SBATCH --time=08:00:00
#SBATCH --mem=32G
#SBATCH --cpus-per-task=4
#SBATCH -p msc_appbio

# -----
# Activate the environment and load the necessary modules
# -----
#first make sure you have the modules with module avail function outside of slurm.

module load anaconda3
conda activate rsem_env
module load bowtie2
module load samtools

# -----
# Run RSEM alignment + quantification
# -----

# Defining input/output paths
# rsem-calculate-expression \
#   --paired-end \           # indicates paired-end reads; expects READ1 and READ2 (1.fastq and
#   2.fastq)
#   --bowtie2 \               # use Bowtie2 for alignment
#   -p 4 \                   # number of threads (should match --cpus-per-task)
#   FASTQ read 1 (specify path to the fastq file 1)
#   FASTQ read 2 (specify path to the fastq file 2)
#   RSEM reference prefix (specify path to the reference genome directory)
#   Output prefix (sample name) = BY4272_R1(example)

# -----
# Replicate 1
# -----
rsem-calculate-expression \
  --paired-end \
  --bowtie2 \
  -p 4 \
  /scratch/grp/msc_appbio/Group2_ABCC/RNAseq/R1_NEW/raw_fastq/SRR7059709_1.fastq \
  /scratch/grp/msc_appbio/Group2_ABCC/RNAseq/R1_NEW/raw_fastq/SRR7059709_2.fastq \
  /scratch/grp/msc_appbio/Group2_ABCC/RNAseq/ref_genome/yeast_rsem \
  BY4272_R1

rsem-calculate-expression \
  --paired-end \
  --bowtie2 \
  -p 4 \
  /scratch/grp/msc_appbio/Group2_ABCC/RNAseq/R1_NEW/raw_fastq/SRR7059704_1.fastq \
  /scratch/grp/msc_appbio/Group2_ABCC/RNAseq/R1_NEW/raw_fastq/SRR7059704_2.fastq \
  /scratch/grp/msc_appbio/Group2_ABCC/RNAseq/ref_genome/yeast_rsem \
  SY14_R1

# -----
# Replicate 2

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# ----

rsem-calculate-expression \
--paired-end \
--bowtie2 \
-p 4 \
/scratch/grp/msc_appbio/Group2_ABCC/RNAseq/R_2/raw_fastq/SRR7059706_1.fastq \
/scratch/grp/msc_appbio/Group2_ABCC/RNAseq/R_2/raw_fastq/SRR7059706_2.fastq \
/scratch/grp/msc_appbio/Group2_ABCC/RNAseq/ref_genome/yeast_rsem \
BY4272_R2

rsem-calculate-expression \
--paired-end \
--bowtie2 \
-p 4 \
/scratch/grp/msc_appbio/Group2_ABCC/RNAseq/R_2/raw_fastq/SRR7059705_1.fastq \
/scratch/grp/msc_appbio/Group2_ABCC/RNAseq/R_2/raw_fastq/SRR7059705_2.fastq \
/scratch/grp/msc_appbio/Group2_ABCC/RNAseq/ref_genome/yeast_rsem \
SY14_R2

# -----
# Replicate 3
# ----

rsem-calculate-expression \
--paired-end \
--bowtie2 \
-p 4 \
/scratch/grp/msc_appbio/Group2_ABCC/RNAseq/R_3/BY4272/fastq_raw/SRR7059707_1.fastq \
/scratch/grp/msc_appbio/Group2_ABCC/RNAseq/R_3/BY4272/fastq_raw/SRR7059707_2.fastq \
/scratch/grp/msc_appbio/Group2_ABCC/RNAseq/ref_genome/yeast_rsem \
BY4272_R3

rsem-calculate-expression \
--paired-end \
--bowtie2 \
-p 4 \
/scratch/grp/msc_appbio/Group2_ABCC/RNAseq/R_3/SY14/fastq_raw/SRR7059708_1.fastq \
/scratch/grp/msc_appbio/Group2_ABCC/RNAseq/R_3/SY14/fastq_raw/SRR7059708_2.fastq \
/scratch/grp/msc_appbio/Group2_ABCC/RNAseq/ref_genome/yeast_rsem \
SY14_R3

# -----
# expected output files per sample
# ----

#OUT_PREFIX.transcript.bam
#OUT_PREFIX.genes.results
#OUT_PREFIX.isoforms.results
#OUT_PREFIX.stat
#OUT_PREFIX.log

```