Project

This is using yeast data to understand how to analyze non-human RNA-seq samples

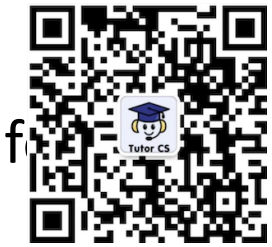
Background

Status	Public on Oct 2
Title	Sequencing of mutants with or without phosphate depletion
Organism	Saccharomyces cerevisiae
Experiment type	Expression profiling by high throughput sequencing
Summary	Targeting of yeast with genetic or nutrient perturbation to assess mitochondrial membrane potential and its regulation.
Overall design	RNA-seq (3 replicates of WT, prototrophic WT, pho85, rpo41, or rpo41/pho85 deletion in yeast, and bar1 deletion in W303 yeast) in samples with or without depletion of phosphate, nitrogen, and/or uracil.

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE212790

Data

- **Experiments**:

  grow with **no phosphate** f 

grow with **phosphate** for 4hr

- **Data**

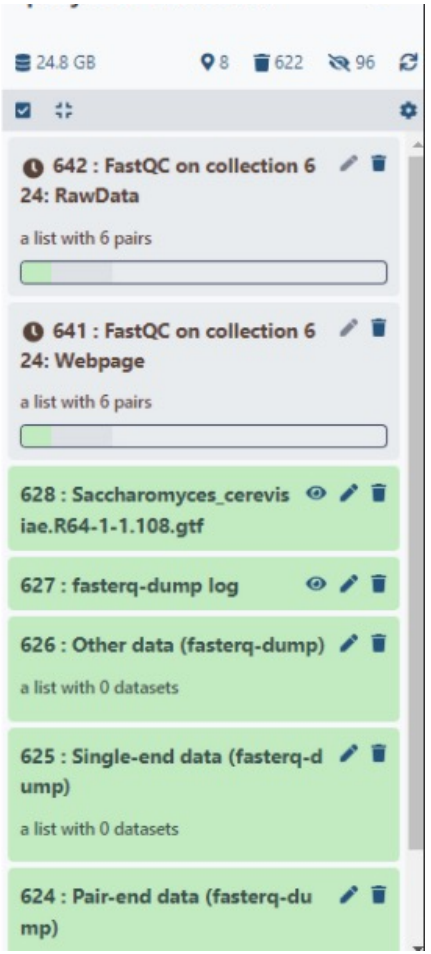
https://ncbi.nlm.nih.gov/Traces/study/?acc=SRP395898&o=acc_s%3Aa&s=SRR21445048,SRR21445049,SRR21445050,SRR21445042,SRR21445043,SRR21445044

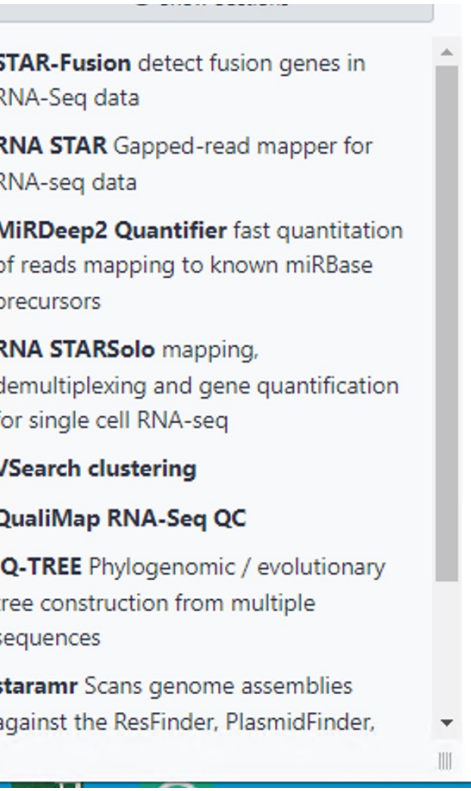
☑ 19	SRR21445042	SAMN30693178	6.57 G	1.94 Gb	SRX17448893	wild-type	GSM6552448	GSM6552448	Saccharomyces cerevisiae (BY)	grow with no phosphate for 4hr
☑ 20	SRR21445043	SAMN30693179	7.81 G	2.31 Gb	SRX17448892	wild-type	GSM6552447	GSM6552447	Saccharomyces cerevisiae (BY)	grow with no phosphate for 4hr
☑ 21	SRR21445044	SAMN30693180	6.69 G	1.96 Gb	SRX17448891	wild-type	GSM6552446	GSM6552446	Saccharomyces cerevisiae (BY)	grow with no phosphate for 4hr
☐ 22	SRR21445045	SAMN30693181	6.49 G	1.92 Gb	SRX17448890	wild-type	GSM6552445	GSM6552445	Saccharomyces cerevisiae (BY)	no treatment (ctrl for no ura)
☐ 23	SRR21445046	SAMN30693182	7.89 G	2.31 Gb	SRX17448889	wild-type	GSM6552444	GSM6552444	Saccharomyces cerevisiae (BY)	no treatment (ctrl for no ura)
☐ 24	SRR21445047	SAMN30693183	6.08 G	1.81 Gb	SRX17448888	wild-type	GSM6552443	GSM6552443	Saccharomyces cerevisiae (BY)	no treatment (ctrl for no ura)
☑ 25	SRR21445048	SAMN30693184	6.70 G	2.01 Gb	SRX17448887	wild-type	GSM6552442	GSM6552442	Saccharomyces cerevisiae (BY)	grow with phosphate for 4hr
☑ 26	SRR21445049	SAMN30693185	7.06 G	2.06 Gb	SRX17448886	wild-type	GSM6552441	GSM6552441	Saccharomyces cerevisiae (BY)	grow with phosphate for 4hr
☑ 27	SRR21445050	SAMN30693186	10.38 G	3.03 Gb	SRX17448885	wild-type	GSM6552440	GSM6552440	Saccharomyces cerevisiae (BY)	grow with phosphate for 4hr

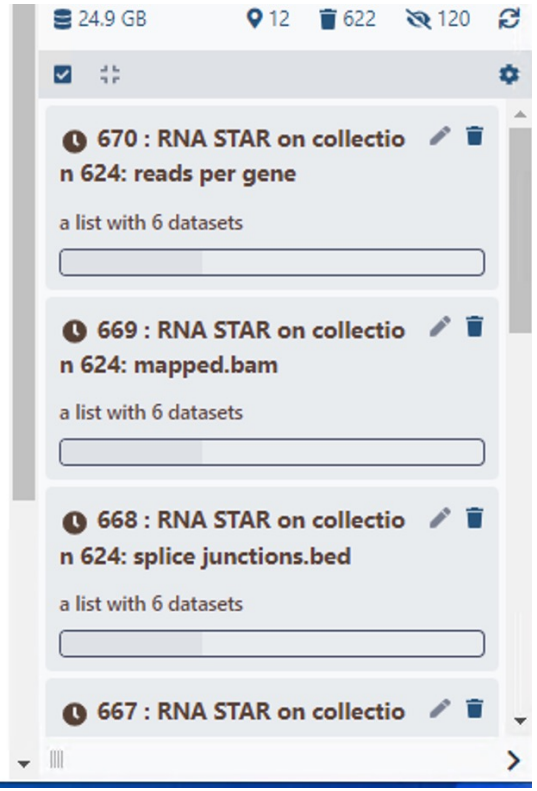
Objectives

1. Quality control (Galaxy 'FastQC') of the RNA-seq raw reads (https://usegalaxy.org/).
2. Mapping RNA-seq reads to **sac**[illegible]y 'STAR', use **Galaxy Version 2.7.8a** for this analysis, use '**sacCer3.ensGene.gtf**' as the G[illegible], be aware that the RNA-seq data is **paired-end**).
3. Differential gene expression analysis (DE-analysis) between two conditions (with and without phosphate) (Use 'DESeq2' in R, **prefiltering out lowly expressed genes** before the DE-analysis)
4. Functional analysis (both GSEA and GSOA) using KEGG pathways, GO_BP (biological process), and GO_MF (molecular function) gene sets in Gene Ontology ('ClusterProfiler' in R)
5. **Questions to answer in your report:**
   a) What do you think about the quality of the sequencing reads based on FastQC reports?
   b) Compared to samples without, how many up-regulated genes and down-regulated genes did you find, respectively (cutoff: BH-adjusted p-value < 0.05 and |log2 fold change| > 1)?
   c) Based on the literature review and the functional analysis result, discuss the altered biological functions in response to the depletion of phosphate.

example







Submission

1. FastQC reports in '.html' fo[illegible]ective 1**). Take a screenshot of your analyses on galaxy as well, save into jp[illegible]
2. The STAR mapping results [illegible] Galaxy in '.tabular' format (**Objective 2**).
3. A table summarizing the results of differential gene expression analysis phosphate depleted culture and control samples in 'csv' format (**Objective 3**).
4. Tables summarizing GSOA and GSEA results, and save into '.csv' format (Objective 4).
5. Enrichment maps for GSOA and GSEA and save into in '.pdf' format (**Objective 4**).
6. Programming code in R script or R Markdown format and/or Galaxy snapshots (for every step on galaxy).

Report

1. A Word file summarizing your answers to the questions in **Objective 5,** with proper figures and proper citation.
2. This report has to be at least one page excluding the reference page .