Devya Gurung

Gen 811: Final Project

Dr. Miller

**Project: RNA seq analysis in Mouse Breast Cancer**

**Background**:

Cancer is a byproduct of many dysregulations, both within the cell and in the tumor microenvironment. It can occur due to mutations, or environmental factors. Breast cancer is one of the most common cancers found in women. In the United States alone, 255,000 cases of breast cancer are diagnosed in women and 2300 in men (CDC). Breast cancer is caused when cells in breast grow uncontrollably. In the study where I gathered raw fastq data from studied the breast cancer in genetically engineered mouse models. The mouse models carried Trp53-mutated breast cancer, along with Brca1 and Brca2 associated breast cancer and E-cadherin (Cdh1) mutated lobular breast cancer.

This study was done in mice model to further understand the mechanism in human breast cancer. Cancer compromises the functions of the immune system, therefore understanding how it regulates cells and the microenvironment is vital in developing cancer immunotherapy.

**Citations**

1. <https://www.cdc.gov/cancer/breast/basic_info/index.htm>
2. Varela I, Klijn C, Stephens PJ, et al. Somatic structural rearrangements in genetically engineered mouse mammary tumors. Genome Biology. 2010 ;11(10):R100. DOI: 10.1186/gb-2010-11-10-r100. PMID: 20942901; PMCID: PMC3218656

Various Tools and Packages were installed while performing RNA seq analysis.

**Methods:**

RNA-Seq Analysis Protocol

STAR Alignment

Raw data: Paired wise (2per/Sample)

Fastq files, Ref. Genome(Idx), Samples

Bam files (1per/Sample)

Samtools used on bam files.

1

Features (GTF)

featureCounts

CountMatrix

Column Data

Goal: RLD: normalized exp. Data

Res: Sig diff exp genes

DESeq2

#Work through the pipelines

Methods Steps:

1. 1st get the fastq raw data (RNA seq) from public databases.

2. Then run fastqc to check the quality of your reads.

3. Fastqc files were checked in Filezilla. Filezilla was installed in computer

4. Next, perform trimming.

5. Trimming was done via trim\_scriptV2 which has trimming scripts and parameters inside

6. Next, Fastqc were performed in trimming files. The trimmed fastqc files were checked in Filezilla

7. Then, Genome Index were created using STAR

8. STAR was also used to do alignments of the sequence. Ran pair-end reads

9. After STAR, output files such as .bam were generated which was used in next step.

10. FeatureCounts was performed on .bam files output

11. Samtools was used on bam files to generate bam stats files, to create bam plot files.

12. The output from featureCounts were used in R to see files.

Fastqc were run on raw fastq files.

Then the ouput files were viewed in Filezilla to see the sequence quality.

Filezilla is a tool that allows you to visualize files, but you need to connect to Ron.

Raw Trimmed

Graphical user interface, chart

Description automatically generated

Raw Trimmed

Chart, bar chart

Description automatically generated

Untrimmed file FastQc Report

Chart

Description automatically generated

**Trimmed file FastQc Report**

Chart

Description automatically generated

To generate genome index for Mouse or Human, use Gencode.

For this project, Mouse genome was used.

<https://www.gencodegenes.org/> options to filter: Use GTF file of (regions: CHR) Comprehensive gene annotation and Fasta files (Genome sequence, Regions: ALL).

Graphical user interface, application

Description automatically generated

Once the fasta files and gtf files were downloaded.

Star was performed for index and alignment.

Star: Generating genome index

Text

Description automatically generated

Alignment Output files: 1st Pair

Text

Description automatically generated with medium confidence

Alignment Output files: 2nd Pair

Text

Description automatically generated

less ERR015623Log.final.out

Text

Description automatically generated

less ERR015618Log.final.out

Text

Description automatically generated

Output of FeatureCounts

Text

Description automatically generated

Text

Description automatically generated

**Samtools output files from bam files such as png files were viewed in Filezilla.**

**Fig below:** Indels: Insertion/deletions stats

Chart, line chart

Description automatically generated Chart, line chart

Description automatically generated

**Fig below: indel cycles**

Chart, line chart

Description automatically generated Chart, line chart

Description automatically generated

**Fig below:** GC depth

Chart, line chart

Description automatically generated Chart

Description automatically generated

**Fig below:** Quality per read (Reverse and Forward)

Chart

Description automatically generated

**Analysis in R:**

**The featureCounts output file were used in R.**

Summary of ERR015623Aligned.out.bam

Text

Description automatically generated

**Summary** of ERR015618Aligned.out.bam

Graphical user interface, text, application

Description automatically generated

Table

Description automatically generated