Differential gene expression analysis between triple-negative breast cancer (TNBC) tumours and healthy tissues.

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Abstract

The aim is to find genes differentially expressed in triple-negative breast cancer (TNBC) when compared to healthy breast tissues and to identify gene ontology (GO) terms enriched for those genes. To do so, the reads were mapped to the reference genome and the number of reads per gene were count. The counts were used to perform a differential expression analysis and a overrepresentation analysis. A lot of the main GO terms identified involved the immune system.

1 Introduction

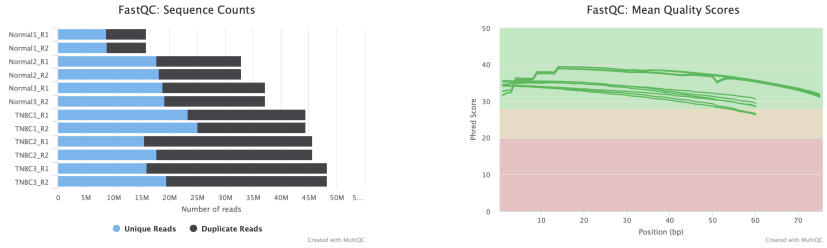
TNBC is a subtype of breast cancer were tumours lack expression of the Estrogen receptor (ER), the progesterone-receptor (PR) and the Human Epidermal Growth Factor Receptor 2 (HER2). This subtype, which can be differentiated from other breast cancer subtypes immunohistochemically, arrises more frequently to younger patients and is characterised by its increased aggressiveness with a shorter survival periods and a higher recurrence rates. [2]

2 Material and methods

2.1 Data

Data used is a subset from Eswaran et al. 2012 [2], composed of fastq files which were downloaded through the Gene Expression Omnibus (GEO), accession GSE52194. The libraries were sequenced on an Illumina HiSeq 2000 in paired-end mode.

The subset includes 3 replicates from TNBC human breast tumors and 3 healthy samples. The quality of the subset were assessed with FastQC [10]



(a) (b)

Figure 1: MultiQC summaries of FASTQC reports on the quality of samples.

(v. 0.11.9). The reference genome (assembly GRCh38) and associated annotation were download from the Ensembl ftp site. Checksums were computed and compared to the values in the CHECKSUMS file on the ftp server.

2.2 Mapping reads to the reference genome.

The reference genome was indexed using Hisat2 [4] (v. 2.2.1). Then the reads were mapped to the reference genome also using Hisat2. SAMtools [6] (v. 1.10) was then used to convert the resulting sam files to bam format. Bam files were then sorted and indexed, also using SAMtools. A table of counts containing the number of reads per gene was then produced using featureCounts [7], the bam files and the annotation file.

2.3 Analysis

The table of counts was load in R [9] (v. 4.1.0) and the package DESeq2 [8] (v. 1.32.0) was used to perform the differential expression analysis. After removing the dependence of the variance on the mean using `DESeq2::vst()`, a heatmap of the 20 more expressed genes across all the samples was produced using `pheatmap` [5] (v. 1.0.12). The differential expression results were extracted using `DESeq2::results`.

To identify GO terms that contain more differentially expressed genes, the `enrichGO` function from the clusterProfiler [11] (v. 4.0.5) package was used, along with the Bioconductor package org.Hs.eg.db [1] containing the genome wide annotation for Human. The results were visualized using the `dotplot` function from the enrichplot (v. 1.12.2) package [12].

3 Results

3.1 Data quality

MultiQC [3] (v. 1.8) was used to summarize the FastQC reports (see figure 1). The average base quality was good but with a tendency to decrease a little near

the end of the reads (see figure 1b).

3.2 Mapping reads to the reference genome.

When mapping to the reference genome, further differences were noticed between the `Normal` and the `TNBC` samples. First the alignment rates was $\sim 96.4\%$ for the `Normal` samples, and $\sim 87.5\%$ for the `TNBC` samples. For the `Normal` samples, $\sim 87\%$ of the reads aligned concordantly exactly once and $\sim 5\%$ aligned concordantly more than one time. For the `TNBC1` sample 50.38% of the reads aligned concordantly exactly one time, and 28.20% aligned concordantly more than one time. For `TNBC2` and `TNBC3`, it is only $\sim 32\%$ of the reads that aligned concordantly exactly once and $\sim 41\%$ that aligned concordantly more than one time (see `output_mapping_hisat2.md` in the Supplementary materials). So there is a strong evidence of multimapped reads for the `TNBC` samples.

When the number of reads per gene were count with featureCounts, the average proportion of reads overlapping with annotated genes for `Normal` samples is around 70%, whereas the successfully assigned alignments drop to $\sim 8\%$ for the `TNBC` samples, and almost exclusively because multimapping reads were unassigned ($\sim 80\%$ unassigned due to multimapping for `TNBC` samples against only $\sim 18\%$ for `Normal` samples, see `featureCounts_summary.md` in the Supplementary materials).

3.3 Analysis

For the differential expression analysis, one can see in figure 2 that samples from the same experimental group show similar gene expression patterns.

13170 genes were differentially expressed in the pairwise comparison between `TNBC` samples and `Normal` tissue (with a $p_{adj} < 0.05$ and a absolute value of `log2FoldChange` larger than 1). Of them, 8716 were up-regulated and 4454 were down-regulated.

The expression levels for the estrogen receptor, progesterone receptor and HER2 were investigated to confirm the triple negative status of our `TNBC` samples (see figure 3).

For the overrepresentation analysis, one can see in figure 4 the top GO terms (all subontology combined). One can see that a lot of them are involved in the immune system.

4 Discussion

Even if I got results at the end, the large amount of unassigned reads due to multimapping concerns me and could have mess up the downstream analysis. It is something that I will need to understand better in a future work.

Furthermore, the choice for comparing a subtype of breast cancer to normal breast tissue was not the most thrilling I made, especially for the aim of better understanding the TNBC subtype.

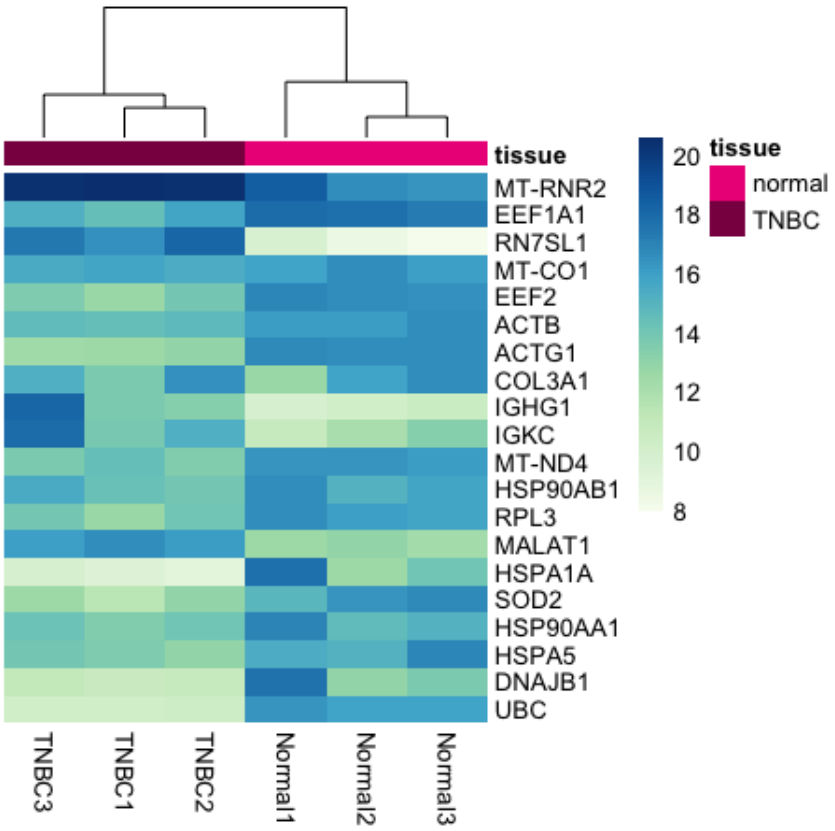


Figure 2: Heatmap of the more expressed genes across all samples.

Supplementary materials

All scripts used for this project and other supplementary materials can be found here: `https://github.com/saskia-droid/summer_breast_de`.

References

[1] Marc Carlson. *org.Hs.eg.db: Genome wide annotation for Human*. R package version 3.13.0. 2021.

[2] Jeyanthy Eswaran et al. "Transcriptomic landscape of breast cancers through mRNA sequencing". In: *Scientific Reports* 2.1 (Feb. 2012), p. 264. ISSN: 2045-2322. DOI: 10.1038/srep00264. URL: https://doi.org/10.1038/srep00264.

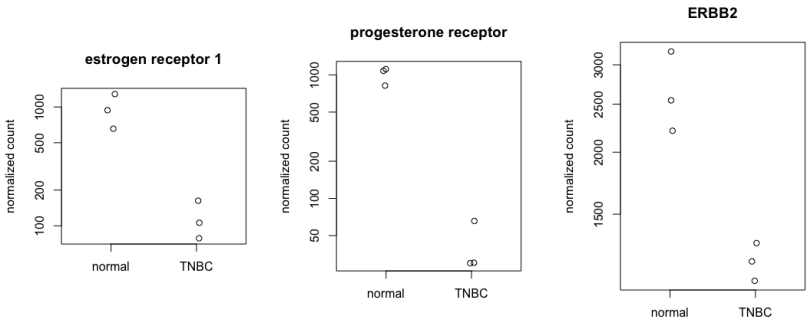


Figure 3: Expression levels of ER, PR and HER2.

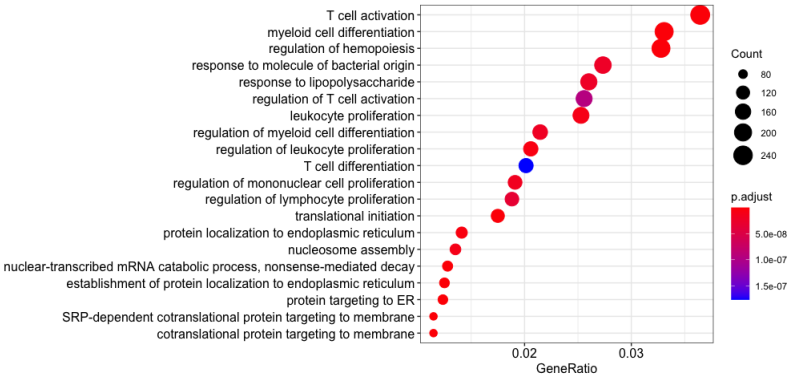


Figure 4: Top GO terms.

[3] Philip Ewels et al. "MultiQC: summarize analysis results for multiple tools and samples in a single report". In: *Bioinformatics* 32.19 (June 2016), pp. 3047–3048. DOI: 10.1093/bioinformatics/btw354. URL: https://doi.org/10.1093/bioinformatics/btw354.

[4] Daehwan Kim, Ben Langmead, and Steven L Salzberg. "HISAT: a fast spliced aligner with low memory requirements". In: *Nature methods* 12.4 (2015), pp. 357–360.

[5] Raivo Kolde. *pheatmap: Pretty Heatmaps*. R package version 1.0.12. 2019. URL: https://CRAN.R-project.org/package=pheatmap.

[6] H Li et al. "The Sequence Alignment/Map format and SAMtools". In: *Bioinformatics* 25.16 (Aug. 2009), pp. 2078–2079. DOI: 10.1093/bioinformatics/btp352. URL: https://www.ncbi.nlm.nih.gov/pubmed/19505943.

[7] Y. Liao, G. K. Smyth, and W. Shi. "featureCounts: an efficient general purpose program for assigning sequence reads to genomic features". In:

Bioinformatics 30.7 (Nov. 2013), pp. 923–930. DOI: 10.1093/bioinformatics/btt656. URL: https://doi.org/10.1093/bioinformatics/btt656.

[8] Michael I. Love, Wolfgang Huber, and Simon Anders. "Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2". In: *Genome Biology* 15 (12 2014), p. 550. DOI: 10.1186/s13059-014-0550-8.

[9] R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. Vienna, Austria, 2021. URL: https://www.R-project.org/.

[10] Andrews S. *FASTQC. A quality control tool for high throughput sequence data.* URL: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

[11] Tianzhi Wu et al. "clusterProfiler 4.0: A universal enrichment tool for interpreting omics data". In: *The Innovation* 2.3 (2021), p. 100141. DOI: 10.1016/j.xinn.2021.100141.

[12] Guangchuang Yu. *enrichplot: Visualization of Functional Enrichment Result*. R package version 1.12.2. 2021. URL: https://yulab-smu.top/biomedical-knowledge-mining-book/.