Cellular effects of splenectomy on liver regeneration after 70% resection

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Transcriptome analysis

The Illumina HiSeq 2500 sequencing used total RNA from peripheral blood and splenic monocytes obtained by magnetic sorting. The libraries were prepared from 1 μg total RNA using TruSeq RNA Sample Prep Kit v2 with 10 cycle enrichment step in accordance with the manufacturer's recommendations. The final libraries were pooled in equimolar ratios before sequencing in a 50 bp paired-end mode using Illumina HiSeq 2500 sequencing system. Raw reads were processed using RTA 1.17.21.3 and Casava 1.8.2 (Illumina). Processing of the initial data was carried out using Nextflow nf-core/rnaseq bioinformatics analysis to compare the control and one of the experimental groups. Commands for running the analysis of Blood/Blood, Spleen/Spleen groups:

./nextflow run nf-core/rnaseq --input ./mouse_rnaseq/blood_spleen.csv --genome Rnor_6.0 -profile docker

A csv file was passed as an input file (the "input" option) containing the paths to the fastq files of the samples of the two compared groups without chain specification ("strandedness" = unstranded). The reference genome M. musculus version Rnor_6.0 was retrieved from iGenomes [https://support.illumina.com/sequencing/sequencing_software/igenome.html] (the "genome" option) and docker [https://www.docker.com/] was used as a configuration profile (the "profile" option). Other parameters were set by default. The main steps of the pipeline are described in the documentation [https://nf-co.re/rnaseq/3.8.1]. Quantitative outputs for individual genes obtained by applying Salmon [Patro, 2017] to multiple alignments performed using the STAR tool [Dobin, 2013] were used in the Hobotnica pipeline [Stupnikov, 2021] to optimize the choice of tool for differential gene expression analysis. The tool selection algorithm [Stupnikov, 2021] promoted DeSeq2 as the fittest option; the results were filtered by a threshold of 0.1 for adjusted p-values.

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Gating strategy