Group assignment Bioinformatics

Group members:

Alexandra Ristani r0603823

Nikola Sklenovská r0474833

Shana Verschuere r0307152

Transcriptome Profiling of Activated T Cells

T cells play a central role in the cell-mediated immune system and their **activation**, which is based on a strictly controlled **network of genes and proteins**, is an essential response of the immune response. The molecular and cellular events underlying this complex process are not fully understood though.

In an attempt to determine the trascriptional profile of activated T-cells the differential expression of *in vitro* activated T cells by means of **RNA-sequencing** (RNAseq) was studied (for a detailed description, see **section 1**). In order to check if the *in vitro* transcriptional profile accurately represents what happens *in vivo*, a differential expression analysis in a **microarray** dataset of patients with an inflammatory disease, in this case asthma, was performed (for a detailed description, see **section 2**).

1 RNAseq

Transcriptome Profiling of *in vitro* Activated T Cells

Referring to a study which can be found under PMCID: PMC3894192.

RNAseq was used for the purpose of transcriptome profiling of RNA samples from a human T cell activation experiment. Here, CD4+ memory T cells were stimulated with both anti-CD3 and anti-CD28, after which RNA was isolated at a total of 6 time points, with two biological replicates per time point.The dataset can be found in te SRA database found on ncbi under the number **SRP026389**.

Below, we described the first part of the RNAseq analysis, involving the processing of the RNAseq data (see **section 1**). The second part constituting **differential expression analysis** can be found in the **Addendum**.

1.1 Processing RNAseq data

1.1.1 Retrieving SRA data

First we made a cache directory for the SRA toolkit.

In [4]:

```
mkdir /home/luna.kuleuven.be/r0307152/assignment/~/data/ncbi2
vdb-config -s /repository/user/main/public/root=~/data/ncbi2
```

Now, we ensured that we are in a work directory.

In [5]:

```
mkdir /home/luna.kuleuven.be/r0307152/data/assignment && cd /home/luna.kuleuven.be/r0307152
```

We downloaded the SRA data, which can be found on the SRA database found on ncbi under the number SRP026389.

In [6]:

```
fastq-dump --split-files SRR924538
```

```
Read 45645666 spots for SRR924538
Written 45645666 spots for SRR924538
```

In [7]:

```
fastq-dump --split-files SRR924537
```

```
Read 48629616 spots for SRR924537
Written 48629616 spots for SRR924537
```

In [8]:

```
fastq-dump --split-files SRR924536
```

```
Read 47388222 spots for SRR924536
Written 47388222 spots for SRR924536
```

In [9]:

```
fastq-dump --split-files SRR924535
```

```
Read 47397849 spots for SRR924535
Written 47397849 spots for SRR924535
```

In [10]:

```
fastq-dump --split-files SRR924534
```

```
Read 52336874 spots for SRR924534
Written 52336874 spots for SRR924534
```

In [11]:

```
fastq-dump --split-files SRR924533
```

```
Read 46253538 spots for SRR924533
Written 46253538 spots for SRR924533
```

In [12]:

```
fastq-dump --split-files SRR924532
```

```
Read 49222642 spots for SRR924532
Written 49222642 spots for SRR924532
```

In [13]:

```
fastq-dump --split-files SRR924531
```

```
Read 50046772 spots for SRR924531
Written 50046772 spots for SRR924531
```

In [14]:

```
fastq-dump --split-files SRR924530
```

```
Read 45812679 spots for SRR924530
Written 45812679 spots for SRR924530
```

In [15]:

```
fastq-dump --split-files SRR924529
```

```
Read 51972705 spots for SRR924529
Written 51972705 spots for SRR924529
```

In [16]:

```
fastq-dump --split-files SRR924528
```

```
Read 47255516 spots for SRR924528
Written 47255516 spots for SRR924528
```

In [17]:

```
fastq-dump --split-files SRR924146
```

```
Read 54255978 spots for SRR924146
Written 54255978 spots for SRR924146
```

In [18]:

```
cd /home/luna.kuleuven.be/r0307152/data/assignment
```

We symlinked the fastq files by creating a symbolic link.

In [19]:

```
ln -sf /mnt/nfs/data/work/SRP026389/*.fastq /home/luna.kuleuven.be/r0307152/data/assignment
ls -l
```

```
total 347605080
drwxr-xr-x 3 r0307152 domain users        4096 nov 23 22:04 ~
lrwxrwxrwx 1 r0307152 domain users          36 nov 25 10:43 *.fastq -> /mnt/
nfs/data/work/SRP026389/*.fastq
-rw-r--r-- 1 r0307152 domain users 16474020950 nov 25 03:52 SRR924146_1.fast
q
-rw-r--r-- 1 r0307152 domain users 16474020950 nov 25 03:52 SRR924146_2.fast
q
-rw-r--r-- 1 r0307152 domain users 14346141286 nov 25 02:08 SRR924528_1.fast
q
-rw-r--r-- 1 r0307152 domain users 14346141286 nov 25 02:08 SRR924528_2.fast
q
-rw-r--r-- 1 r0307152 domain users 15780044866 nov 25 00:39 SRR924529_1.fast
q
-rw-r--r-- 1 r0307152 domain users 15780044866 nov 25 00:39 SRR924529_2.fast
q
-rw-r--r-- 1 r0307152 domain users 13907034480 nov 24 23:00 SRR924530_1.fast
q
-rw-r--r-- 1 r0307152 domain users 13907034480 nov 24 23:00 SRR924530_2.fast
q
-rw-r--r-- 1 r0307152 domain users 15194725688 nov 24 21:22 SRR924531_1.fast
q
-rw-r--r-- 1 r0307152 domain users 15194725688 nov 24 21:22 SRR924531_2.fast
q
-rw-r--r-- 1 r0307152 domain users 14943138186 nov 24 19:45 SRR924532_1.fast
q
-rw-r--r-- 1 r0307152 domain users 14943138186 nov 24 19:45 SRR924532_2.fast
q
-rw-r--r-- 1 r0307152 domain users 14041392542 nov 24 18:09 SRR924533_1.fast
q
-rw-r--r-- 1 r0307152 domain users 14041392542 nov 24 18:09 SRR924533_2.fast
q
-rw-r--r-- 1 r0307152 domain users 15893046732 nov 24 12:27 SRR924534_1.fast
q
-rw-r--r-- 1 r0307152 domain users 15893046732 nov 24 12:27 SRR924534_2.fast
q
-rw-r--r-- 1 r0307152 domain users 14388382530 nov 24 10:44 SRR924535_1.fast
q
-rw-r--r-- 1 r0307152 domain users 14388382530 nov 24 10:44 SRR924535_2.fast
q
-rw-r--r-- 1 r0307152 domain users 14385931344 nov 24 09:15 SRR924536_1.fast
q
-rw-r--r-- 1 r0307152 domain users 14385931344 nov 24 09:15 SRR924536_2.fast
q
-rw-r--r-- 1 r0307152 domain users 14763432342 nov 24 01:02 SRR924537_1.fast
q
-rw-r--r-- 1 r0307152 domain users 14763432342 nov 24 01:02 SRR924537_2.fast
q
-rw-r--r-- 1 r0307152 domain users 13856431400 nov 23 23:28 SRR924538_1.fast
q
-rw-r--r-- 1 r0307152 domain users 13856431400 nov 23 23:28 SRR924538_2.fast
q
```

1.1.2 Quality control using FASTQC

We ran the FASTQC program on every .fastq file.

In [20]:

```
/usr/local/bin/FastQC/fastqc -o . SRR924538_1.fastq SRR924538_2.fastq
```

```
Started analysis of SRR924538_1.fastq
Approx 5% complete for SRR924538_1.fastq
Approx 10% complete for SRR924538_1.fastq
Approx 15% complete for SRR924538_1.fastq
Approx 20% complete for SRR924538_1.fastq
Approx 25% complete for SRR924538_1.fastq
Approx 30% complete for SRR924538_1.fastq
Approx 35% complete for SRR924538_1.fastq
Approx 40% complete for SRR924538_1.fastq
Approx 45% complete for SRR924538_1.fastq
Approx 50% complete for SRR924538_1.fastq
Approx 55% complete for SRR924538_1.fastq
Approx 60% complete for SRR924538_1.fastq
Approx 65% complete for SRR924538_1.fastq
Approx 70% complete for SRR924538_1.fastq
Approx 75% complete for SRR924538_1.fastq
Approx 80% complete for SRR924538_1.fastq
Approx 85% complete for SRR924538_1.fastq
Approx 90% complete for SRR924538_1.fastq
Approx 95% complete for SRR924538_1.fastq
Analysis complete for SRR924538_1.fastq
Started analysis of SRR924538_2.fastq
Approx 5% complete for SRR924538_2.fastq
Approx 10% complete for SRR924538_2.fastq
Approx 15% complete for SRR924538_2.fastq
Approx 20% complete for SRR924538_2.fastq
Approx 25% complete for SRR924538_2.fastq
Approx 30% complete for SRR924538_2.fastq
Approx 35% complete for SRR924538_2.fastq
Approx 40% complete for SRR924538_2.fastq
Approx 45% complete for SRR924538_2.fastq
Approx 50% complete for SRR924538_2.fastq
Approx 55% complete for SRR924538_2.fastq
Approx 60% complete for SRR924538_2.fastq
Approx 65% complete for SRR924538_2.fastq
Approx 70% complete for SRR924538_2.fastq
Approx 75% complete for SRR924538_2.fastq
Approx 80% complete for SRR924538_2.fastq
Approx 85% complete for SRR924538_2.fastq
Approx 90% complete for SRR924538_2.fastq
Approx 95% complete for SRR924538_2.fastq
Analysis complete for SRR924538_2.fastq
```

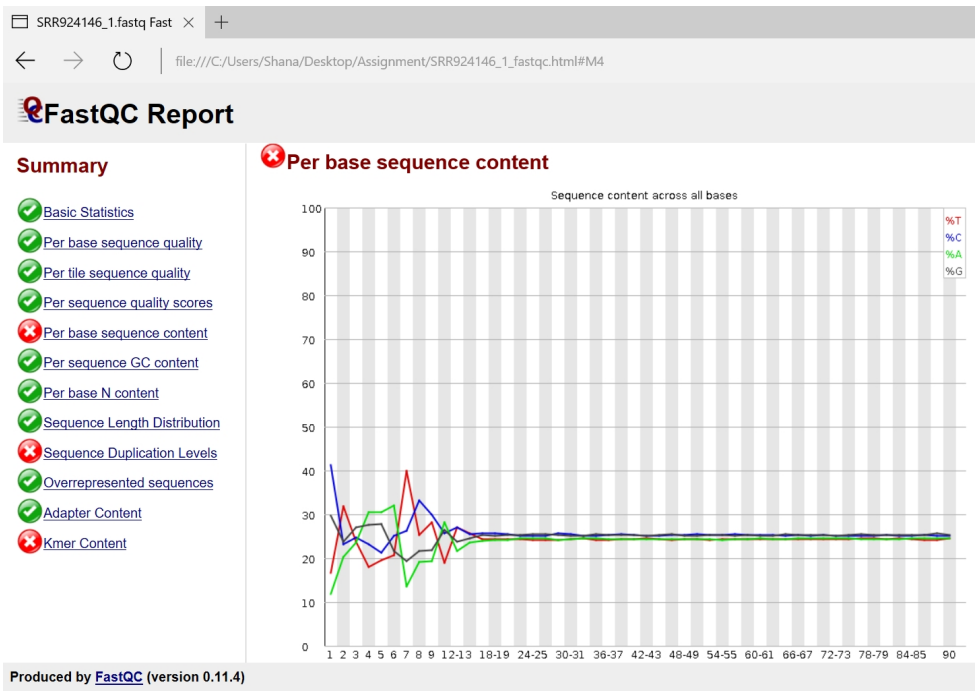
In [21]:

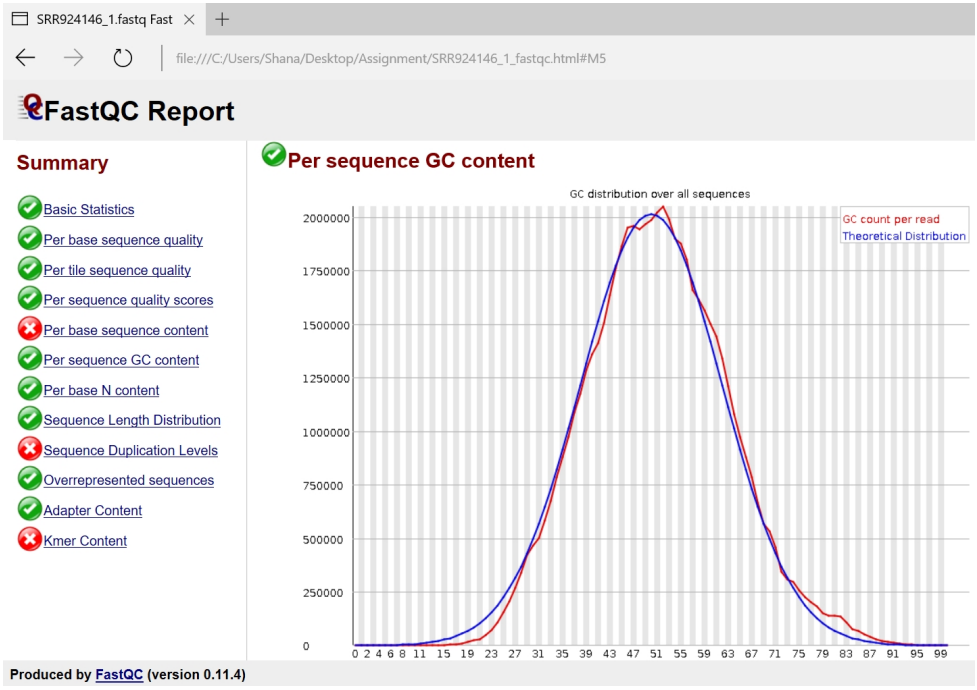
```
/usr/local/bin/FastQC/fastqc -o . SRR924537_1.fastq SRR924537_2.fastq SRR924536_1.fastq SRR
```

The FastQC reports showed that most samples have a problem with: (1) **Per base sequence content** : there is a bias at the beginning of the reads, which is common for RNA-Seq data. This occurs during RNA-seq library preparation, when “random” primers are annealed to the start of sequences. (2) **Sequence duplication level** : while analysing transcriptome sequencing data, we should not remove these duplicates because we don’t know whether they represent PCR duplicates or high gene expression of our samples.

All samples have good **Per base sequence quality scores** and **Per sequence quality scores** . All samples have good **Per sequence GC content distribution** .

Below there is an example of what the FastQC report partly looks like for sample SRR924146_1.





1.1.3 Mapping to genome

We loaded the genome into the memory.

In [22]:

```
STAR --genomeLoad LoadAndExit --genomeDir /mnt/nfs/mfiers/STAR/hg19_star_db
```

```
Nov 25 14:08:07 ..... started STAR run
Nov 25 14:08:07 ..... loading genome
```

Mapping the reads from all the samples against the reference genome at the same time didn't work.

In [23]:

```
STAR --genomeDir /mnt/nfs/mfiers/STAR/hg19_star_db \
     --genomeLoad LoadAndKeep \
     --runThreadN 2 \
     --readFilesIn SRR924538_1.fastq SRR924538_2.fastq SRR924537_1.fastq SRR924537_2.fastq
     --outFileNamePrefix allsamples2.
```

```
Segmentation fault (core dumped)
```

So instead, we mapped the reads from one sample at a time against the reference genome.

In [25]:

```
STAR --genomeDir /mnt/nfs/mfiers/STAR/hg19_star_db \
     --genomeLoad LoadAndKeep \
     --runThreadN 2 \
     --readFilesIn SRR924538_1.fastq SRR924538_2.fastq \
     --outFileNamePrefix SRR924538.
```

```
Nov 25 14:24:10 ..... started STAR run
Nov 25 14:24:10 ..... loading genome
Nov 25 14:24:10 ..... started mapping
Nov 25 14:58:44 ..... finished successfully
```

In [26]:

```
STAR --genomeDir /mnt/nfs/mfiers/STAR/hg19_star_db \
     --genomeLoad LoadAndKeep \
     --runThreadN 2 \
     --readFilesIn SRR924537_1.fastq SRR924537_2.fastq \
     --outFileNamePrefix SRR924537.
```

```
Nov 25 14:58:46 ..... started STAR run
Nov 25 14:58:46 ..... loading genome
Nov 25 14:58:46 ..... started mapping
Nov 25 15:31:52 ..... finished successfully
```

In [27]:

```
STAR --genomeDir /mnt/nfs/mfiers/STAR/hg19_star_db \
     --genomeLoad LoadAndKeep \
     --runThreadN 2 \
     --readFilesIn SRR924536_1.fastq SRR924536_2.fastq \
     --outFileNamePrefix SRR924536.
```

```
Nov 25 21:43:29 ..... started STAR run
Nov 25 21:43:29 ..... loading genome
Nov 25 21:43:29 ..... started mapping
Nov 25 22:15:53 ..... finished successfully
```

In [28]:

```
STAR --genomeDir /mnt/nfs/mfiers/STAR/hg19_star_db \
     --genomeLoad LoadAndKeep \
     --runThreadN 2 \
     --readFilesIn SRR924535_1.fastq SRR924535_2.fastq \
     --outFileNamePrefix SRR924535.
```

```
Nov 25 22:15:55 ..... started STAR run
Nov 25 22:15:55 ..... loading genome
Nov 25 22:15:55 ..... started mapping
Nov 25 22:49:56 ..... finished successfully
```

In [29]:

```
STAR --genomeDir /mnt/nfs/mfiers/STAR/hg19_star_db \
     --genomeLoad LoadAndKeep \
     --runThreadN 2 \
     --readFilesIn SRR924534_1.fastq SRR924534_2.fastq \
     --outFileNamePrefix SRR924534.
```

```
Nov 25 22:49:57 ..... started STAR run
Nov 25 22:49:57 ..... loading genome
Nov 25 22:49:58 ..... started mapping
Nov 25 23:24:27 ..... finished successfully
```

In [30]:

```
STAR --genomeDir /mnt/nfs/mfiers/STAR/hg19_star_db \
     --genomeLoad LoadAndKeep \
     --runThreadN 2 \
     --readFilesIn SRR924533_1.fastq SRR924533_2.fastq \
     --outFileNamePrefix SRR924533.
```

```
Nov 26 00:26:12 ..... started STAR run
Nov 26 00:26:12 ..... loading genome
Nov 26 00:26:12 ..... started mapping
Nov 26 00:58:22 ..... finished successfully
```

In [31]:

```
STAR --genomeDir /mnt/nfs/mfiers/STAR/hg19_star_db \
     --genomeLoad LoadAndKeep \
     --runThreadN 2 \
     --readFilesIn SRR924532_1.fastq SRR924532_2.fastq \
     --outFileNamePrefix SRR924532.
```

```
Nov 26 00:58:23 ..... started STAR run
Nov 26 00:58:23 ..... loading genome
Nov 26 00:58:23 ..... started mapping
Nov 26 01:34:23 ..... finished successfully
```

In [32]:

```
STAR --genomeDir /mnt/nfs/mfiers/STAR/hg19_star_db \
     --genomeLoad LoadAndKeep \
     --runThreadN 2 \
     --readFilesIn  SRR924531_1.fastq SRR924531_2.fastq \
     --outFileNamePrefix SRR924531.
```

```
Nov 26 01:34:25 ..... started STAR run
Nov 26 01:34:25 ..... loading genome
Nov 26 01:34:25 ..... started mapping
Nov 26 02:10:08 ..... finished successfully
```

In [33]:

```
STAR --genomeDir /mnt/nfs/mfiers/STAR/hg19_star_db \
     --genomeLoad LoadAndKeep \
     --runThreadN 2 \
     --readFilesIn  SRR924530_1.fastq SRR924530_2.fastq \
     --outFileNamePrefix SRR924530.
```

```
Nov 26 02:10:10 ..... started STAR run
Nov 26 02:10:10 ..... loading genome
Nov 26 02:10:10 ..... started mapping
Nov 26 02:45:13 ..... finished successfully
```

In [34]:

```
STAR --genomeDir /mnt/nfs/mfiers/STAR/hg19_star_db \
     --genomeLoad LoadAndKeep \
     --runThreadN 2 \
     --readFilesIn  SRR924529_1.fastq SRR924529_2.fastq \
     --outFileNamePrefix SRR924529.
```

```
Nov 26 02:45:15 ..... started STAR run
Nov 26 02:45:15 ..... loading genome
Nov 26 02:45:15 ..... started mapping
Nov 26 03:21:41 ..... finished successfully
```

In [35]:

```
STAR --genomeDir /mnt/nfs/mfiers/STAR/hg19_star_db \
     --genomeLoad LoadAndKeep \
     --runThreadN 2 \
     --readFilesIn  SRR924528_1.fastq SRR924528_2.fastq \
     --outFileNamePrefix SRR924528.
```

```
Nov 26 08:15:36 ..... started STAR run
Nov 26 08:15:36 ..... loading genome
Nov 26 08:15:36 ..... started mapping
Nov 26 08:49:58 ..... finished successfully
```

In [36]:

```
STAR --genomeDir /mnt/nfs/mfiers/STAR/hg19_star_db \
     --genomeLoad LoadAndKeep \
     --runThreadN 2 \
     --readFilesIn  SRR924146_1.fastq SRR924146_2.fastq \
     --outFileNamePrefix SRR924146.
```

```
Nov 26 08:50:00 ..... started STAR run
Nov 26 08:50:00 ..... loading genome
Nov 26 08:50:00 ..... started mapping
Nov 26 09:28:11 ..... finished successfully
```

1.1.4 SAM file structure

We wanted to see what one of these files looks like.

In [37]:

```
head -40 SRR924146.Aligned.out.sam | grep '^@'
```

```
@HD     VN:1.4
@SQ     SN:chrM LN:16571
@SQ     SN:chr1 LN:249250621
@SQ     SN:chr2 LN:243199373
@SQ     SN:chr3 LN:198022430
@SQ     SN:chr4 LN:191154276
@SQ     SN:chr5 LN:180915260
@SQ     SN:chr6 LN:171115067
@SQ     SN:chr7 LN:159138663
@SQ     SN:chr8 LN:146364022
@SQ     SN:chr9 LN:141213431
@SQ     SN:chr10        LN:135534747
@SQ     SN:chr11        LN:135006516
@SQ     SN:chr12        LN:133851895
@SQ     SN:chr13        LN:115169878
@SQ     SN:chr14        LN:107349540
@SQ     SN:chr15        LN:102531392
@SQ     SN:chr16        LN:90354753
@SQ     SN:chr17        LN:81195210
@SQ     SN:chr18        LN:78077248
@SQ     SN:chr19        LN:59128983
@SQ     SN:chr20        LN:63025520
@SQ     SN:chr21        LN:48129895
@SQ     SN:chr22        LN:51304566
@SQ     SN:chrX LN:155270560
@SQ     SN:chrY LN:59373566
@PG     ID:STAR PN:STAR VN:STAR_2.5.2b  CL:STAR   --runThreadN 2   --genomeD
ir /mnt/nfs/mfiers/STAR/hg19_star_db   --genomeLoad LoadAndKeep   --readFile
sIn SRR924146_1.fastq   SRR924146_2.fastq      --outFileNamePrefix SRR92414
6.
@CO     user command line: STAR --genomeDir /mnt/nfs/mfiers/STAR/hg19_star_d
b --genomeLoad LoadAndKeep --runThreadN 2 --readFilesIn SRR924146_1.fastq SR
R924146_2.fastq --outFileNamePrefix SRR924146.
```

In [38]:

```
head -34 SRR924146.Aligned.out.sam | grep -v '^@'
```

```
SRR924146.1     99      chr9    130704859       255     90M     =       1307
04894   125     GGGGNCTGAGGTCATGGCTCCCAGGAAGAGGCCGCCAGCAGGGGCCCCAGGACACAAGGA
AGGGACAGCTGAAGCACTAAGGAGTCAGAC  ???D%2==<D?:DEBBBEEEI12C?38?;;D1C?@6)07BD=
((55:AAD))6@AA?;=>;2),558?=3::A:)+<:AA%%%%%%%%%        NH:i:1  HI:i:1  AS:
i:167   nM:i:5
SRR924146.1     147     chr9    130704894       255     90M     =       1307
04859   -125    CAGCAGGTCCCCCAGGACACAAGGAAGGGACAGCTGAAGCACTAAGCAGTCAGACAGTCA
CAGGTGGCTGGATTCCGGAGGCGGTCTGGC  %%%%%%%%%%%%%%%@A>;3CB;>CE?7.?=*=4CD@88.8*@
=*=AHCF4EEHDF9IHDAEEHD::+D@BGGE1HHFF6FDDEDD@7@  NH:i:1  HI:i:1  AS:i:167
        nM:i:5
SRR924146.2     99      chrX    47511285        255     10M185N64M5427N16M
        =       47511531        5905    CTCTAAGCCCCTCTAGCAACATGTGGATATGGGCTT
TGATATTCATGGAGGCCTTGGTGAGGCTGTTGCTGAGCAGCACTGTGTCAACGA  ?@@DDA4+:+CDFFCBHIEH
IICBHHC9EF4CC8DDBBBDDGI:++?+99*?;FGGCG=CFFHIIHI<EHHCAE;*6);AC;>>@>;A;?  NH:
i:1     HI:i:1  AS:i:175        nM:i:3
SRR924146.2     147     chrX    47511531        255     13M5427N68M142N9M
        =       47511285        -5905   GCTGTTGCTGAGCACCACTGTGTCAACGAAGAAGTT
ACAGCCCAAATCCACCTGCATATATAACCCCGAGTGCTTAGCTTCCTGGAGTCG  <B?<5:ACCA@@;;CAAA;;
7*?>FCFHHIIHAGE@FF=.(>>EGFBEBBDGB9BIGD9B:*C?*C8C3<+A<4F>@?;FHFDDDAD??=  NH:
i:1     HI:i:1  AS:i:175        nM:i:3
SRR924146.3     99      chr9    135895238       1       90M     =       1358
95246   98      ATTTGAAGCTGGTATCTCCAAGAATGGGCAGACCCGAGAGCATGCCCTTCTGGCTTACAC
ACTGGGTGTGAAACAACTAATTGTCGGTGT  @@@FADDFHHGHHGGIGHIIIIBEEHIIIIIIIEF<?6?@AH<?
8DFFGIIIIIAACGGE3;C:;A@DD=CC6;A=AACC@ACACBB;<@  NH:i:3  HI:i:1  AS:i:178
        nM:i:0
SRR924146.3     147     chr9    135895246       1       90M     =       1358
95238   -98     CTGGTATCTCCAAGAATGGGCAGACCCGAGAGCATGCCCTTCTGGCTTACACACTGGGTG
TGAAACAACTAATTGTCGGTGTTAACAAAA  C:@>C>3?:CCDC>>-3DCC>CADFFHFFHC=.=-=ACEF>HDD
<HGGFB+HGH?IIIGGF>IGGIGHEEGHGEG@GHHFHHDBDDD@@@  NH:i:3  HI:i:1  AS:i:178
        nM:i:0
```

1.1.5 BAM file

We sorted the SAM files and converted them to BAM files.

In [39]:

```
samtools sort -o SRR924538.bam SRR924538.Aligned.out.sam
```

```
[bam_sort_core] merging from 38 files...
```

In [40]:

```
samtools sort -o SRR924537.bam SRR924537.Aligned.out.sam
```

```
[bam_sort_core] merging from 40 files...
```

In [41]:

```
samtools sort -o SRR924536.bam SRR924536.Aligned.out.sam
```

```
[bam_sort_core] merging from 39 files...
```

In [42]:

```
samtools sort -o SRR924535.bam SRR924535.Aligned.out.sam
```

```
[bam_sort_core] merging from 39 files...
```

In [43]:

```
samtools sort -o SRR924534.bam SRR924534.Aligned.out.sam
```

```
[bam_sort_core] merging from 43 files...
```

In [44]:

```
samtools sort -o SRR924533.bam SRR924533.Aligned.out.sam
```

```
[bam_sort_core] merging from 38 files...
```

In [45]:

```
samtools sort -o SRR924532.bam SRR924532.Aligned.out.sam
```

```
[bam_sort_core] merging from 40 files...
```

In [46]:

```
samtools sort -o SRR924531.bam SRR924531.Aligned.out.sam
```

```
[bam_sort_core] merging from 41 files...
```

In [47]:

```
samtools sort -o SRR924530.bam SRR924530.Aligned.out.sam
```

```
[bam_sort_core] merging from 37 files...
```

In [48]:

```
samtools sort -o SRR924529.bam SRR924529.Aligned.out.sam
```

```
[bam_sort_core] merging from 43 files...
```

In [57]:

```
samtools sort -o SRR924528.bam SRR924528.Aligned.out.sam
```

```
[bam_sort_core] merging from 38 files...
```

In [58]:

```
samtools sort -o SRR924146.bam SRR924146.Aligned.out.sam
```

```
[bam_sort_core] merging from 44 files...
```

We generated an index using samtools.

In [59]:

```
samtools index SRR924538.bam
```

In [60]:

```
samtools index SRR924537.bam
```

In [61]:

```
samtools index SRR924536.bam
```

In [62]:

```
samtools index SRR924535.bam
```

In [63]:

```
samtools index SRR924534.bam
```

In [64]:

```
samtools index SRR924533.bam
```

In [65]:

```
samtools index SRR924532.bam
```

In [66]:

```
samtools index SRR924531.bam
```

In [67]:

```
samtools index SRR924530.bam
```

In [68]:

```
samtools index SRR924529.bam
```

In [69]:

```
samtools index SRR924528.bam
```

In [70]:

```
samtools index SRR924146.bam
```

We checked if all the files were there.

In [87]:

```
ls -l SRR924538*[bs]a[mi]
```

```
-rw-r--r-- 1 r0307152 domain users 25014402019 nov 25 14:58 SRR924538.Aligne
d.out.sam
-rw-r--r-- 1 r0307152 domain users  5688324784 nov 26 11:52 SRR924538.bam
-rw-r--r-- 1 r0307152 domain users     2948792 nov 27 04:46 SRR924538.bam.ba
i
```

In [86]:

```
ls -l SRR924537*[bs]a[mi]
```

```
-rw-r--r-- 1 r0307152 domain users 26779648697 nov 25 15:31 SRR924537.Aligne
d.out.sam
-rw-r--r-- 1 r0307152 domain users  6067543265 nov 26 12:16 SRR924537.bam
-rw-r--r-- 1 r0307152 domain users     2962728 nov 27 04:48 SRR924537.bam.ba
i
```

In [72]:

```
ls -l SRR924536*[bs]a[mi]
```

```
-rw-r--r-- 1 r0307152 domain users 26225142814 nov 25 22:15 SRR924536.Aligne
d.out.sam
-rw-r--r-- 1 r0307152 domain users  5956618590 nov 26 12:40 SRR924536.bam
-rw-r--r-- 1 r0307152 domain users     2973360 nov 27 04:49 SRR924536.bam.ba
i
```

In [73]:

```
ls -l SRR924535*[bs]a[mi]
```

```
-rw-r--r-- 1 r0307152 domain users 26056204290 nov 25 22:49 SRR924535.Aligne
d.out.sam
-rw-r--r-- 1 r0307152 domain users  6051889205 nov 26 13:03 SRR924535.bam
-rw-r--r-- 1 r0307152 domain users     2978680 nov 27 04:51 SRR924535.bam.ba
i
```

In [74]:

```
ls -l SRR924534*[bs]a[mi]
```

```
-rw-r--r-- 1 r0307152 domain users 28675472065 nov 25 23:24 SRR924534.Aligne
d.out.sam
-rw-r--r-- 1 r0307152 domain users  6554343488 nov 26 13:29 SRR924534.bam
-rw-r--r-- 1 r0307152 domain users     2913248 nov 27 04:53 SRR924534.bam.ba
i
```

In [75]:

```
ls -l SRR924533*[bs]a[mi]
```

```
-rw-r--r-- 1 r0307152 domain users 25208506126 nov 26 00:58 SRR924533.Aligne
d.out.sam
-rw-r--r-- 1 r0307152 domain users  5906977480 nov 26 13:51 SRR924533.bam
-rw-r--r-- 1 r0307152 domain users     2920360 nov 27 04:54 SRR924533.bam.ba
i
```

In [76]:

```
ls -l SRR924532*[bs]a[mi]
```

```
-rw-r--r-- 1 r0307152 domain users 26556587204 nov 26 01:34 SRR924532.Aligne
d.out.sam
-rw-r--r-- 1 r0307152 domain users  6152650521 nov 26 14:35 SRR924532.bam
-rw-r--r-- 1 r0307152 domain users     2992568 nov 27 04:57 SRR924532.bam.ba
i
```

In [77]:

```
ls -l SRR924531*[bs]a[mi]
```

```
-rw-r--r-- 1 r0307152 domain users 27039066331 nov 26 02:10 SRR924531.Aligne
d.out.sam
-rw-r--r-- 1 r0307152 domain users  6237915278 nov 26 15:05 SRR924531.bam
-rw-r--r-- 1 r0307152 domain users     2949792 nov 27 05:01 SRR924531.bam.ba
i
```

In [78]:

```
ls -l SRR924530*[bs]a[mi]
```

```
-rw-r--r-- 1 r0307152 domain users 24711202058 nov 26 02:45 SRR924530.Aligne
d.out.sam
-rw-r--r-- 1 r0307152 domain users  5851673669 nov 26 15:30 SRR924530.bam
-rw-r--r-- 1 r0307152 domain users     3010696 nov 27 05:03 SRR924530.bam.ba
i
```

In [79]:

```
ls -l SRR924529*[bs]a[mi]
```

```
-rw-r--r-- 1 r0307152 domain users 28412576508 nov 26 03:21 SRR924529.Aligne
d.out.sam
-rw-r--r-- 1 r0307152 domain users  6464535414 nov 26 15:59 SRR924529.bam
-rw-r--r-- 1 r0307152 domain users     3045008 nov 27 05:06 SRR924529.bam.ba
i
```

In [80]:

```
ls -l SRR924528*[bs]a[mi]
```

```
-rw-r--r-- 1 r0307152 domain users 25360136625 nov 26 08:49 SRR924528.Aligne
d.out.sam
-rw-r--r-- 1 r0307152 domain users  5654358539 nov 27 04:18 SRR924528.bam
-rw-r--r-- 1 r0307152 domain users     2922440 nov 27 05:10 SRR924528.bam.ba
i
```

In [81]:

```
ls -l SRR924146*[bs]a[mi]
```

```
-rw-r--r-- 1 r0307152 domain users 29287253565 nov 26 09:28 SRR924146.Aligne
d.out.sam
-rw-r--r-- 1 r0307152 domain users  6516590248 nov 27 04:45 SRR924146.bam
-rw-r--r-- 1 r0307152 domain users     3101216 nov 27 05:11 SRR924146.bam.ba
i
```

We viewed the reads that map to each chromosome using samtools idxstats.

In [82]:

```
samtools idxstats SRR924538.bam
```

```
chrM    16571   2649102 0
chr1    249250621       9000870 0
chr2    243199373       5658956 0
chr3    198022430       4016180 0
chr4    191154276       2225934 0
chr5    180915260       3881160 0
chr6    171115067       6264808 0
chr7    159138663       4260954 0
chr8    146364022       2634808 0
chr9    141213431       3423528 0
chr10   135534747       3023888 0
chr11   135006516       5146672 0
chr12   133851895       6753572 0
chr13   115169878       1280402 0
chr14   107349540       2441992 0
chr15   102531392       2961050 0
chr16   90354753        4075158 0
chr17   81195210        5728326 0
chr18   78077248        854748  0
chr19   59128983        6112174 0
chr20   63025520        1750228 0
chr21   48129895        842930  0
chr22   51304566        2366180 0
chrX    155270560       3379020 0
chrY    59373566        182594  0
*       0       0       0
```

We viewed the distribution of the mapping flags with samtools flagstat.

In [83]:

```
samtools flagstat SRR924538.bam
```

```
90915234 + 0 in total (QC-passed reads + QC-failed reads)
4647904 + 0 secondary
0 + 0 supplementary
0 + 0 duplicates
90915234 + 0 mapped (100.00% : N/A)
86267330 + 0 paired in sequencing
43133665 + 0 read1
43133665 + 0 read2
86267330 + 0 properly paired (100.00% : N/A)
86267330 + 0 with itself and mate mapped
0 + 0 singletons (0.00% : N/A)
0 + 0 with mate mapped to a different chr
0 + 0 with mate mapped to a different chr (mapQ>=5)
```

We then visualized the alignment with IGV. By inspecting the SRP02638.gene.counts.tsv with Excel, we chose a gene that has a high gene count number, for example *TPRG1L* and inspected it in sample SRR924530.



1.1.6 Reads to gene count

We made a symbolic link to the full human annotation.

In [84]:

```
ln -sf /mnt/nfs/data/RNA-seq/gencode.v19.nopseudo.plus.sort.gtf
ls -l *gtf
```

```
lrwxrwxrwx 1 r0307152 domain users 56 nov 27 10:58 gencode.v19.nopseudo.plu
s.sort.gtf -> /mnt/nfs/data/RNA-seq/gencode.v19.nopseudo.plus.sort.gtf
```

In [85]:

```
head -10 gencode.v19.nopseudo.plus.sort.gtf
```

```
##description: evidence-based annotation of the human genome (GRCh37), versi
on 19 (Ensembl 74)
##provider: GENCODE
##contact: gencode@sanger.ac.uk
##format: gtf
##date: 2013-12-05
chr1    HAVANA  exon    29554   30039   .       +       .       gene_id "ENS
G00000243485.2"; transcript_id "ENST00000473358.1"; gene_type "lincRNA"; gen
e_status "NOVEL"; gene_name "MIR1302-11"; transcript_type "lincRNA"; transcr
ipt_status "KNOWN"; transcript_name "MIR1302-11-001"; exon_number 1;  exon_i
d "ENSE00001947070.1";  level 2; tag "not_best_in_genome_evidence"; havana_g
ene "OTTHUMG00000000959.2"; havana_transcript "OTTHUMT00000002840.1";
chr1    HAVANA  gene    29554   31109   .       +       .       gene_id "ENS
G00000243485.2"; transcript_id "ENSG00000243485.2"; gene_type "lincRNA"; gen
e_status "NOVEL"; gene_name "MIR1302-11"; transcript_type "lincRNA"; transcr
ipt_status "NOVEL"; transcript_name "MIR1302-11"; level 2; havana_gene "OTTH
UMG00000000959.2";
chr1    HAVANA  transcript      29554   31097   .       +       .       gene
_id "ENSG00000243485.2"; transcript_id "ENST00000473358.1"; gene_type "lincR
NA"; gene_status "NOVEL"; gene_name "MIR1302-11"; transcript_type "lincRNA";
 transcript_status "KNOWN"; transcript_name "MIR1302-11-001"; level 2; tag
 "not_best_in_genome_evidence"; havana_gene "OTTHUMG00000000959.2"; havana_t
ranscript "OTTHUMT00000002840.1";
chr1    HAVANA  exon    30267   30667   .       +       .       gene_id "ENS
G00000243485.2"; transcript_id "ENST00000469289.1"; gene_type "lincRNA"; gen
e_status "NOVEL"; gene_name "MIR1302-11"; transcript_type "lincRNA"; transcr
ipt_status "KNOWN"; transcript_name "MIR1302-11-002"; exon_number 1;  exon_i
d "ENSE00001841699.1";  level 2; tag "not_best_in_genome_evidence"; havana_g
ene "OTTHUMG00000000959.2"; havana_transcript "OTTHUMT00000002841.2";
chr1    HAVANA  transcript      30267   31109   .       +       .       gene
_id "ENSG00000243485.2"; transcript_id "ENST00000469289.1"; gene_type "lincR
NA"; gene_status "NOVEL"; gene_name "MIR1302-11"; transcript_type "lincRNA";
 transcript_status "KNOWN"; transcript_name "MIR1302-11-002"; level 2; tag
 "not_best_in_genome_evidence"; havana_gene "OTTHUMG00000000959.2"; havana_t
ranscript "OTTHUMT00000002841.2";
```

In [88]:

```
featureCounts \
     -Q 10 \
     -g gene_name \
     -a /mnt/nfs/data/RNA-seq/gencode.v19.nopseudo.plus.sort.gtf \
     -o SRP02638.counts\
     SRR924146.bam SRR924528.bam SRR924529.bam SRR924530.bam SRR924531.bam SRR924532.bam
```

In [89]:

```
ls -l *.counts*
```

```
-rw-r--r-- 1 r0307152 domain users 9673003 nov 27 11:31 SRP02638.counts
-rw-r--r-- 1 r0307152 domain users     973 nov 27 11:31 SRP02638.counts.summ
ary
```

We inspected both files.

In [92]:

```
head -20 SRP02638.counts.summary | column -t
```

```
Status                      SRR924146.bam  SRR924528.bam  SRR924529.bam  SRR9
24530.bam  SRR924531.bam  SRR924532.bam  SRR924533.bam  SRR924534.bam  SRR92
4535.bam  SRR924536.bam  SRR924537.bam  SRR924538.bam
Assigned                    78493768       68424120       78648744       6813
8126       74331258       73621724       71350631       81239709       75553
012       76142841       76494037       71320341
Unassigned_Ambiguity       5621847        5008735        5758077        5035
676        5562630        5340883        5249822        5976220        56082
06        5728570        6021270        5733123
Unassigned_MultiMapping    0              0              0              0
           0              0              0              0              0
          0              0              0
Unassigned_NoFeatures      11118025       9103459        9096969        8092
548        9227912        8760661        7140419        7757383        50814
74        5152607        6156879        5808154
Unassigned_Unmapped        0              0              0              0
           0              0              0              0              0
          0              0              0
Unassigned_MappingQuality  11327664       9722060        9894530        8659
794        9237972        8902502        7919090        9293926        84349
38        8276152        8620356        8053616
Unassigned_FragmentLength  0              0              0              0
           0              0              0              0              0
          0              0              0
Unassigned_Chimera         0              0              0              0
           0              0              0              0              0
          0              0              0
Unassigned_Secondary       0              0              0              0
           0              0              0              0              0
          0              0              0
Unassigned_Nonjunction     0              0              0              0
           0              0              0              0              0
          0              0              0
Unassigned_Duplicate       0              0              0              0
           0              0              0              0              0
          0              0              0
```

In [94]:

```
head -20 SRP02638.counts.summary
```

```
Status  SRR924146.bam   SRR924528.bam   SRR924529.bam   SRR924530.bam   SRR9
24531.bam       SRR924532.bam   SRR924533.bam   SRR924534.bam   SRR924535.ba
m       SRR924536.bam   SRR924537.bam   SRR924538.bam
Assigned        78493768        68424120        78648744        68138126
        74331258        73621724        71350631        81239709        7555
3012    76142841        76494037        71320341
Unassigned_Ambiguity    5621847 5008735 5758077 5035676 5562630 5340883 5249
822     5976220 5608206 5728570 6021270 5733123
Unassigned_MultiMapping 0       0       0       0       0       0       0
        0       0       0       0       0
Unassigned_NoFeatures   11118025        9103459 9096969 8092548 9227912 8760
661     7140419 7757383 5081474 5152607 6156879 5808154
Unassigned_Unmapped     0       0       0       0       0       0       0
        0       0       0       0       0
Unassigned_MappingQuality       11327664        9722060 9894530 8659794 9237
972     8902502 7919090 9293926 8434938 8276152 8620356 8053616
Unassigned_FragmentLength       0       0       0       0       0       0
        0       0       0       0       0       0
Unassigned_Chimera      0       0       0       0       0       0       0
        0       0       0       0       0
Unassigned_Secondary    0       0       0       0       0       0       0
        0       0       0       0       0
Unassigned_Nonjunction  0       0       0       0       0       0       0
        0       0       0       0       0
Unassigned_Duplicate    0       0       0       0       0       0       0
        0       0       0       0       0
```

We seperated the columns on the gene structure and the columns with the actual counts.

In [95]:

```
cut -f-6 SRP02638.counts > SRP02638.genedata.tsv
```

In [96]:

```
head SRP02638.genedata.tsv
```

```
# Program:featureCounts v1.5.0-p1; Command:"featureCounts" "-Q" "10" "-g" "g
ene_name" "-a" "/mnt/nfs/data/RNA-seq/gencode.v19.nopseudo.plus.sort.gtf" "-
o" "SRP02638.counts" "SRR924146.bam" "SRR924528.bam" "SRR924529.bam" "SRR924
530.bam" "SRR924531.bam" "SRR924532.bam" "SRR924533.bam" "SRR924534.bam" "SR
R924535.bam" "SRR924536.bam" "SRR924537.bam" "SRR924538.bam"
Geneid  Chr     Start   End     Strand  Length
MIR1302-11      chr1;chr1;chr1  29554;30267;30976       30039;30667;31109
        +;+;+   1021
FAM138A chr1;chr1;chr1  34554;35245;35721       35174;35481;36081       -;-;
-       1219
OR4F5   chr1    69091   70008   +       918
RP11-34P13.7    chr1;chr1;chr1;chr1;chr1;chr1;chr1      89295;92091;110953;1
12700;120721;129055;133374      91629;92240;111357;112804;120932;129223;1335
66      -;-;-;-;-;-;-   3569
RP11-34P13.8    chr1;chr1       89551;90287     90050;91105     -;-     1319
AL627309.1      chr1;chr1       134901;137621   135802;139379   -;-     2661
RP11-34P13.14   chr1;chr1       139790;140075   139847;140339   -;-     323
RP11-34P13.13   chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1;chr1       1414
74;146386;155767;164263;165884;168100;168610;169049;172557;173753       1430
11;149707;155831;164791;165942;168165;168767;169264;172688;173862       -;-;
-;-;-;-;-;-;-;- 6195
```

In [97]:

```
cut -f1,7- SRP02638.counts | grep -v '^#' > SRP02638.gene.counts
```

In [98]:

```
head SRP02638.gene.counts
```

```
Geneid  SRR924146.bam   SRR924528.bam   SRR924529.bam   SRR924530.bam   SRR9
24531.bam       SRR924532.bam   SRR924533.bam   SRR924534.bam   SRR924535.ba
m       SRR924536.bam   SRR924537.bam   SRR924538.bam
MIR1302-11      0       0       0       0       0       0       0       0
        0       0       0       0
FAM138A 0       0       0       0       0       0       0       0       0
        0       0       0
OR4F5   0       0       0       0       0       0       0       0       0
        0       0       0
RP11-34P13.7    6       3       4       16      2       4       4       2
        2       0       0       0
RP11-34P13.8    0       0       0       0       0       0       0       0
        0       0       0       0
AL627309.1      165     158     194     155     100     154     95      68
        26      40      83      95
RP11-34P13.14   0       2       2       2       0       0       0       1
        2       0       1       1
RP11-34P13.13   11      8       9       14      5       10      2       2
        1       3       8       6
RNU6-1100P      0       0       0       0       0       0       0       0
        0       0       0       0
```

We downloaded the SRP02638.gene.counts to our computer and changed it to SRP02638.gene.counts.tsv for inspection with Excel.

1.2 Differential expression analysis ¶

For differential expression analysis using DESeq2: see Addendum section A

1.3 Gene Regulatory Network

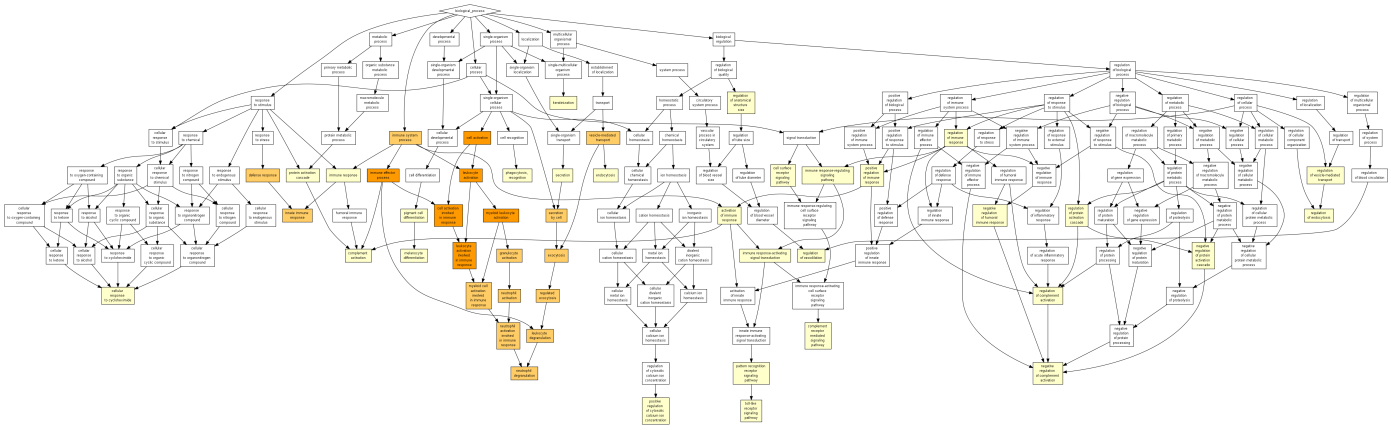
1.3.1 Gene Ontology (GO) enrichment analysis on gene signatures

For the analysis the DEGs of the 24h versus 0h was used.

The tool GOrilla was used to identify and visualize enriched GO terms in a ranked list of 3313 genes that were found to be significantly differentially expressed.

GORilla's output: Species used: Homo sapiens The system has recognized 2743 genes out of 3313 gene terms entered by the user. 2743 genes were recognized by gene symbol and 0 genes by other gene IDs . Only 2462 of these genes are associated with a GO term.

GORilla produces an overview diagram, showing the processes that the DEGs of our dataset are implicated in:



DEGs appear to be implicated in a lot of processes and mainly in immune system processes and cell activation pathways including leukocyte, granulocyte and neutrophil activation (main cells activated during immune response). This comes as no surprise as the T cells are a key component of the cell-mediated immune response.

1.3.2 Functional analysis of significant modules in the interaction network

We use the iRegulon software to predict regulators and targets within our dataset.

The top scored regulon corresponds to the transcription factor with the code name: V$NFKAPPAB_01, and has a score of 3.622 with 105 direct targets for differential expression of genes in inflammation.This matches annotation for **NF-κB**. NF-κB is a transcriptional factor involved in cellular responses to stimuli such as stress, cytokines etc. It has a key role in the regulation of the immune response to infection (bacterial, viral), and incorrect regulation of NF-κB has been linked to inflammatory and autoimmune disease, viral infection and improper immune development. [7]

2 Microarray analysis

Transcriptome Profiling of *in vivo* Activated T Cells in patients with Severe Asthma

Referring to a study which can be found under PMID: 21917308.

Asthma is a chronic lung disease characterised by inflamation and narrowing of the airways. Previously, it has been shown that the immune system is a key element of asthma progression and lots of inflammatory cells participate in its progression.

Microarray analysis was performed in order to detect differentially expressed genes (DEGs) in CD8+ T cells of patients with Severe Asthma (SA) as compared to healthy controls (HC). For that reason, the gene expression profile **GSE31773** was downloaded from Gene Expression Omnibus(GEO) database. This dataset includes 40 samples: 20 of circulating CD4+ cells and 20 CD8+ T cell specimens, isolated from 8 HC, 4 patients with non-severe asthma and 8 patients with SA. For our analysis only **16 samples of CD8+ T cell specimens, isolated from 8 HC and 8 SA patients** , are used. Therefore this study investigates gene expression changes in CD8+ T cells in humans suffering from SA. Differentially expressed genes (DEGs) were screened out using limma package in R.

For the execution of the microarray analysis: see **Addendum section B**.

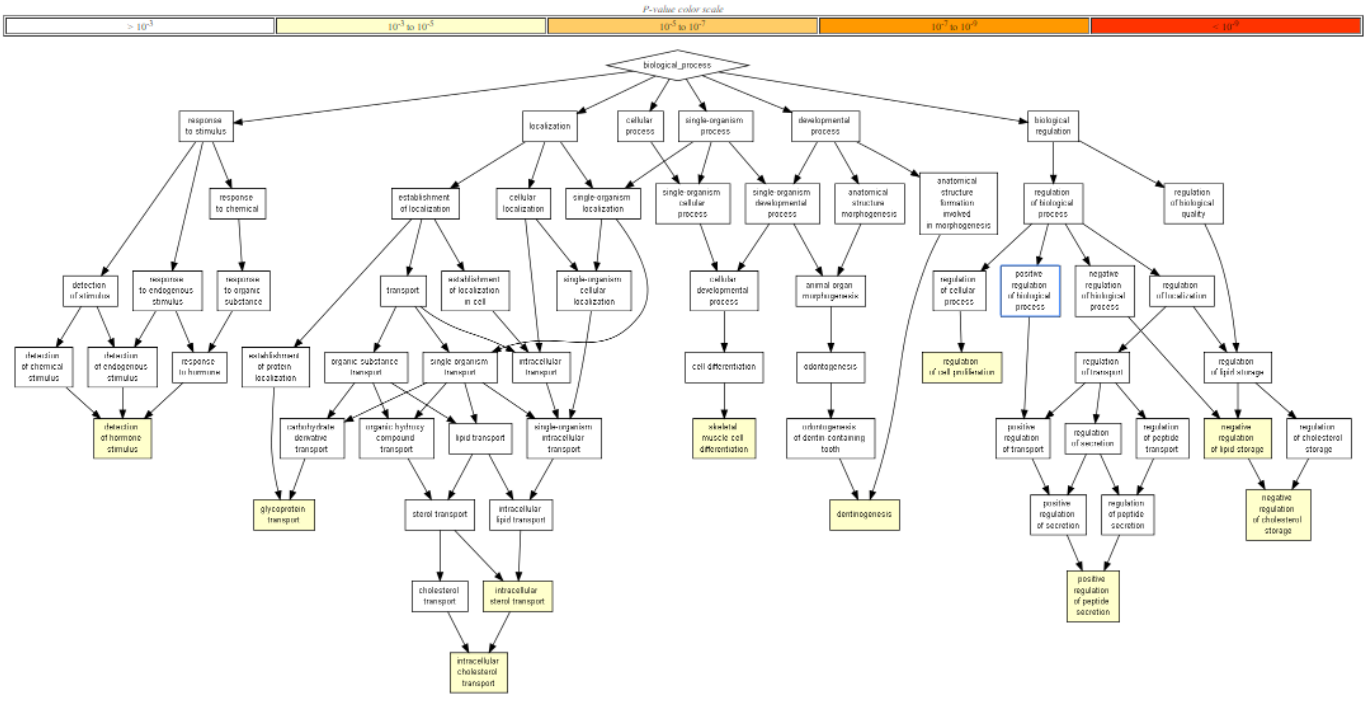
Gene Regulatory Network

Gene Ontology (GO) enrichment analysis on gene signatures

The tool GOrilla was used to identify and visualize enriched GO terms in a ranked list of 3167 genes that were found to be significantly differentially expressed on the microarray dataset.

GORilla's output: Species used: Homo sapiens The system has recognized 3059 genes out of 3167 gene terms entered by the user. 3059 genes were recognized by gene symbol and 0 genes by other gene IDs. 1 duplicate genes were removed (keeping the highest ranking instance of each gene) leaving a total of 3058 genes. Only 2623 of these genes are associated with a GO term.

GORilla produces an overview diagram, showing the processes that the DEGs of our dataset are implicated in:



DEGS in T cells are implicated in pathways such as detection of hormone stimulus, glycoprotein transport, intracellular sterol and cholesterol transport, regulation of cell proliferation, negative regulation of lipid and cholesterol storage and positive regulation of peptide secretion. Asthma is a chronic inflammatory disease characterized by complex cellular interactions. Therefore, a variety of genes associated with diverse cellular pathways can be implicated in the disease development. Pathways involving the regulation, storage and transport of lipids can be implicated since important inflammatory modulators such as eicosanoids, leukotrienes (LTs) and prostaglandins (PGs) are lipid derived. Moreover, lately lipid mediators acting as anti-inflammatory and pro-resolution mediators of allergic airway inflammation has been characterized, revealing the important connection between lipids and immunity. [2] Factors regulating the proliferation of immune cells are also implicated in states of inflammation and asthma. [3]

Functional analysis of significant modules in the interaction network

We use the iRegulon software to predict regulators and targets within our dataset.

The top scored regulon corresponds to the **EIF5A2** enriched with a score of 4.214 with 795 direct targets for differential expression of genes in asthma. EIF5A is a transcription factor and is not a surprise that a transcription factor of all the DEGs is the one that is interacting the most with the rest of the DEGs. EIF5A2 or Eukaryotic translation initiation factor 5A-2 is a mRNA-binding protein essential for translation elongation, that has recently been described as an oncogene in many types of human cancer. [3] EIF5A has been though to contribute to the production of proinflammatory cytokines, promoting inflammation. [4] Inflammatory response is a key component of asthma pathogenesis, suggesting that eIF5A might indeed play a role in the disease progression. [2]

3 Conclusion

Based on the gene regulatory network analysis done in both the RNAseq and microarray data it seems that different pathways are implicated in the *in vivo* and the *in vitro* situation. But is that the case? Probably not. The different results obtained are probably due to the fact that in the RNAseq analysis the genomic profiling of CD4+ T cells was studied, whereas in the microarray analysis that of the CD8+ T cells.

There is established cooperation between CD8+ and CD4+ T cells during the immune response. [6]

CD4+ cells are helper cells are the ones helping in the activation of other immune cells, possibly also CD8+ cells, therefore CD4+ cells are mainly implicated in activation pathways, whereas CD8+ cells as cytotoxic T cells are implicated in cascades such as peptide secretion, lipid transfer and cellular response to hormone stimulus.

4 References

1. Tsitsiou E, Williams A, Moschos S, Patel K, Rossios C, Jiang X et al. Transcriptome analysis shows activation of circulating CD8+ T cells in patients with severe asthma. Journal of Allergy and Clinical Immunology. 2012;129(1):95-103.
2. Murdoch JR, Lloyd CM. Chronic inflammation and asthma. Mutation Research. 2010;690(1-2):24-39. doi:10.1016/j.mrfmmm.2009.09.005.
3. Waal Malefyt R., Haanen J., Spits H., Roncarolo M.G., te Velde A., Figdor C., Johnson K., Kastelein R., Yssel H., de Vries J.E. Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. J. Exp. Med. 1991;174:915–924.

4. Wang F, Guan X, Xie D. Roles of Eukaryotic Initiation Factor 5A2 in Human Cancer. International Journal of Biological Sciences. 2013;9(10):1013-1020.
5. Maier B, Ogihara T, Trace A, Tersey S, Robbins R, Chakrabarti S et al. The unique hypusine modification of eIF5A promotes islet β cell inflammation and dysfunction in mice. Journal of Clinical Investigation. 2010;120(6):2156-2170.
6. Castellino, Flora and Ronald N. Germain. "COOPERATION BETWEEN CD4+ AND CD8+ T CELLS: When, Where, And How". Annual Review of Immunology 24.1 (2006): 519-540.
7. Castellino, Flora and Ronald N. Germain. "COOPERATION BETWEEN CD4+ AND CD8+ T CELLS: When, Where, And How". Annual Review of Immunology 24.1 (2006): 519-540.