

Strand-seq analysis Day5 scNOVA

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```
## Warning: package 'BiocGenerics' was built under R version 4.0.5
## Warning: package 'GenomeInfoDb' was built under R version 4.0.5
```

1 Introduction

In this practical session, we will analyze 77 cells of T-ALL P1 which has subclonal chromothripsis event.

```
## Download the scNOVA pipeline from the github and setup R environment
git lfs install
git clone https://github.com/jeongdo801/scNOVA.git

## We need to specify Renviroment for R_conda, let's create one folder in our individual folder
mkdir /g/korbel2/StrandSeq/Test_HJ/R_conda
vi ~/.Renvirom
R_LIBS=/g/korbel2/StrandSeq/Test_HJ/R_conda
```

2 Preparation of input files for scNOVA

After copying the pipeline, we need to put input files to the input bam and input user folder. Inside of input bam, you need to copy good quality bam files from selected folder of mosaicatcher. In the input user folder, 1) Add key result files from mosaicatcher output in the input user folder 2) Add the subclonality information 3) Add the genes within copy number changed region to mask in the infer differential expression result, if it's not provided, genes will not be masked.

```
## Change the project name in the Snakefile
cd scNOVA
vi Snakefile

## Create input_bam folder, copy bam and index files
mkdir input_bam
cp /scratch/jeong/pipeline_20190625/bam/TALL03-DEA5/selected/*.bam input_bam
cp /scratch/jeong/pipeline_20190625/bam/TALL03-DEA5/selected/*.bai input_bam

## Create input_user folder, prepare input
mkdir input_user
cp /scratch/jeong/pipeline_20190625/strand_states/TALL03-DEA5/100000_fixed_norm.selected_j0.1_s0.5_scedist20,
cp /scratch/jeong/pipeline_20190625/sv_calls/TALL03-DEA5/100000_fixed_norm.selected_j0.1_s0.5_scedist20/simp
cp input_user_example/input_subclonality_TALL03-DEA5_77cells.txt input_user/input_subclonality.txt
cp input_user_example/input_SV_affected_genes_DEA5.txt input_user/input_SV_affected_genes.txt
```

3 Install dependencies

scNOVA implements multiple R packages in the pipeline, we will install dependencies using conda environment.

```
## Let's go inside the pipeline folder
vi ~/.condarc
pkgs_dirs:
  - /g/korbel2/jeong/envs/pkgs/
envs_dirs:
  - /g/korbel2/jeong/envs/

snakemake -j 4 --use-conda --conda-create-envs-only
```

4 Running scNOVA

Now we are ready to execute scNOVA pipeline

```
## Let's go inside of the folder with output files from plotting pipeline
sbatch -t 90:00:00 -N 1 -n 1 --mem=50000 --mail-type=FAIL,BEGIN,END --mail-user=hyobin.jeong@embl.de -o outp
```

5 Explore the output folders and plots

After the function has finished, you will find several output files including **result plot** which are the most frequently used outputs for the downstream analysis.

```
##Let's explore 'result_plot' folder
Heatmap
```

- **result plot** These output plots show the sv call result with different visualizations.

```
##Single-cell SV detection result for each chromosome separately
sv_calls/RPE/100000_fixed_norm.selected_j0.1_s0.5_scedist20/plots/sv_calls

##Variant allele frequency and genomic positions of all the potential SVs
sv_calls/RPE/100000_fixed_norm.selected_j0.1_s0.5_scedist20/plots/sv_consistency

##Heatmap of SVs clustered by log likelihood score of SVs
sv_calls/RPE/100000_fixed_norm.selected_j0.1_s0.5_scedist20/plots/sv_clustering
```

6 Session Info

```
toLatex(sessionInfo())
```

- R version 4.0.3 (2020-10-10), x86_64-apple-darwin17.0
- Locale: en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
- Running under: macOS High Sierra 10.13.3
- Random number generation:
- RNG: Mersenne-Twister
- Normal: Inversion

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- Sample: Rounding
- Matrix products: default
- BLAS:
/System/Library/Frameworks/Accelerate.framework/Versions/A/Frameworks/vecLib.framework/Versions/A/libBLAS.dylib
- LAPACK:
/Library/Frameworks/R.framework/Versions/4.0/Resources/lib/libRlapack.dylib
- Base packages: base, datasets, graphics, grDevices, methods, parallel, stats, stats4, utils
- Other packages: BiocGenerics 0.36.1, breakpointR 1.8.0, breakpointRdata 1.8.0, cowplot 1.1.1, GenomInfoDb 1.26.7, GenomicRanges 1.42.0, IRanges 2.24.1, knitr 1.33, S4Vectors 0.28.1
- Loaded via a namespace (and not attached): assertthat 0.2.1, beachmat 2.6.4, Biobase 2.50.0, BiocManager 1.30.16, BiocNeighbors 1.8.2, BiocParallel 1.24.1, BiocSingular 1.6.0, BiocStyle 2.18.1, Biostrings 2.58.0, bitops 1.0-7, bluster 1.0.0, codetools 0.2-18, colorspace 2.0-2, compiler 4.0.3, crayon 1.4.1, DBI 1.1.1, DelayedArray 0.16.3, DelayedMatrixStats 1.12.3, digest 0.6.27, doParallel 1.0.16, dplyr 1.0.7, dqrng 0.3.0, edgeR 3.32.1, ellipsis 0.3.2, evaluate 0.14, fansi 0.5.0, foreach 1.5.1, generics 0.1.0, GenomInfoDbData 1.2.4, GenomicAlignments 1.26.0, ggplot2 3.3.5, glue 1.4.2, grid 4.0.3, gtable 0.3.0, gtools 3.9.2, highr 0.9, htmltools 0.5.1.1, igraph 1.2.6, irlba 2.3.3, iterators 1.0.13, lattice 0.20-44, lifecycle 1.0.0, limma 3.46.0, locfit 1.5-9.4, magrittr 2.0.1, Matrix 1.3-4, MatrixGenerics 1.2.1, matrixStats 0.59.0, munsell 0.5.0, pillar 1.6.1, pkgconfig 2.0.3, purrr 0.3.4, R6 2.5.0, Rcpp 1.0.6, RCurl 1.98-1.3, rlang 0.4.11, rmarkdown 2.9, Rsamtools 2.6.0, rsvd 1.0.5, scales 1.1.1, scan 1.18.7, scuttle 1.0.4, SingleCellExperiment 1.12.0, sparseMatrixStats 1.2.1, statmod 1.4.36, stringi 1.6.2, stringr 1.4.0, SummarizedExperiment 1.20.0, tibble 3.1.2, tidyselect 1.1.1, tools 4.0.3, utf8 1.2.1, vctrs 0.3.8, xfun 0.24, XVector 0.30.0, yaml 2.2.1, zlibbioc 1.36.0