SNADRIF pipeline

- 1) Go to https://gdc.cancer.gov/about-data/publications/PanCan-CellOfOrigin
- 2) Download the file Analyte level annotations merged sample quality annotations.tsv
- 3) Using information in the column aliquot_barcode, delete all aliquots named TCGA-XX-XXXX-YYX-XXXX-XXX, where YY any number, except 01, 03 and 09 (see https://docs.gdc.cancer.gov/Encyclopedia/pages/TCGA Barcode/ and table https://gdc.cancer.gov/resources-tcga-users/tcga-code-tables/sample-type-codes), and also, using information in the column Do_not_use, delete all aliquots with False value, and save the resulting file as merged_sample_quality_annotations_do_not_use.tsv
- 4) Download the file Public mutation annotation file mc3.v0.2.8.PUBLIC.maf.gz, extract to mc3.v0.2.8.PUBLIC.maf and rename to mc3.v0.2.8.PUBLIC.tsv
- 5) Using information in the column **Tumor_Sample_Barcode**, delete from the file **mc3.v0.2.8.PUBLIC.tsv** all aliquots named TCGA-XX-XXXX-YYX-XXXX-XXXX-XXX, where YY any number, except 01, 03 и 09; all aliquots that do not have the PASS value in the column **FILTER**; and all aliquots present in the file **merged_sample_quality_annotations_do_not_use.tsv**, and save the resulting file as **mc3.v0.2.8.PUBLIC_primary_whitelisted.tsv**
- 6) In the file mc3.v0.2.8.PUBLIC_primary_whitelisted.tsv, replace zeros in the column Entrez_Gene_Id with actual Entrez gene IDs, determined from the corresponding ENSEMBL gene IDs in the column Gene using external database (ftp://ftp.ncbi.nih.gov/gene/DATA/GENE_INFO/Mammalia/Homo_sapiens.gene_info.gz), and save the file as mc3.v0.2.8.PUBLIC primary whitelisted Entrez.tsv
- 7) Using the file mc3.v0.2.8.PUBLIC_primary_whitelisted_Entrez.tsv classify all SNAs according to the column Variant_Classification and the following table:

Variant_Classification	Possible effect
De_novo_Start_InFrame	hyperactivating
De_novo_Start_OutOfFrame	passenger
Frame_Shift_Del	inactivating
Frame_Shift_Ins	inactivating
IGR	unclear
In_Frame_Del	hyperactivating
In_Frame_Ins	hyperactivating
Intron	unclear
Missense_Mutation	hyperactivating
Nonsense_Mutation	inactivating
Nonstop_Mutation	inactivating
RNA	unclear
Silent	passenger
Splice_Site	unclear
Targeted_Region	unclear
Translation_Start_Site	inactivating
3'Flank	unclear
3'UTR	unclear
5'Flank	unclear
5'UTR	unclear

- Save the classification results as the file SNA_classification_patients.tsv, with columns Tumor_Sample_Barcode, Hugo_Symbol, Entrez_Gene_Id, Gene, Number of hyperactivating SNAs, Number of inactivating SNAs, Number of SNAs with unclear role, Number of passenger SNAs.
- 8) Using the file SNA_classification_patients.tsv, for each gene calculate the sum of all alterations in all patients. Remove genes that contain only SNAs with unclear role (noncoding genes) and save the results as SNA_classification_genes.tsv with columns Hugo_Symbol, Entrez_Gene_Id, Gene, Number of hyperactivating SNAs, Number of inactivating SNAs, Number of SNAs with unclear role, Number of passenger SNAs. Also remove noncoding genes from SNA_classification_patients.tsv
- 9) Calculate the "nonsynonymous SNA enrichment index" as

$$NSEI = \frac{Number\ of\ hyperactivating\ SNAs +\ Number\ of\ inactivating\ SNAs + 1}{Number\ of\ passenger\ SNAs + 1}$$

and the "hyperactivating to inactivating SNA ratio" as

$$HISR = \frac{\text{Number of hyperactivating SNAs} + 1}{\text{Number of inactivating SNAs} + 1}$$

using the file **SNA_classification_genes.tsv** and add it as additional columns to that file. Remove genes for which the sum of hyperactivating, inactivating and passenger SNAs is less than 10 (to ensure sufficient precision of NSEI and HISR calculation) and save it as **SNA_classification_genes_NSEI_HISR.tsv.**

- 10) Using the file SNA_classification_patients.tsv, construct the gene-patient matrix SNA_matrix.tsv with columns Hugo_Symbol, Entrez_Gene_Id, Gene and individual Tumor Sample Barcodes, encoding the Number of hyperactivating SNAs, Number of inactivating SNAs, Number of SNAs with unclear role and Number of passenger SNAs as one number separated by dots (e.g. 2.0.1.1). If data for a given gene is absent in a given patient, encode as 0.0.0.0
- 11) By drawing statuses randomly with replacement (bootstrapping) 10000 times from *any* cell of **SNA_matrix.tsv**, fill the table **SNA_matrix_bootstrapped.tsv** with columns **Iteration** and individual Tumor Sample Barcodes
- 12) Calculate the sums of statuses in SNA_matrix_bootstrapped.tsv for each iteration separately, calculate the corresponding NSEI and HISR indices (see step 9). Calculate null hypothesis P-value for each iteration as the number of NSEI values higher than a given iteration's NSEI value and divided by 10000. Save the results as SNA_bootstrapped_NSEI_HISR.tsv with columns Iteration, Number of hyperactivating SNAs, Number of inactivating SNAs, Number of SNAs with unclear role, Number of passenger SNAs, NSEI, HISR, P value. Plot a histogram with the distribution of P values (x axis P values with 0.05 precision, y axis the number of occurrences of a given value) under the null hypothesis.
- 13) Calculate P-value for each gene as the number of NSEI values in SNA_bootstrapped_NSEI_HISR.tsv higher than its NSEI value in SNA_classification_genes_NSEI_HISR.tsv and divided by 10000. Add it as an additional column to SNA_classification_genes_NSEI_HISR.tsv and save the file as SNA_classification_genes_NSEI_HISR_Pvalues.tsv

- 14) Apply Benjamini—Hochberg procedure with FDR(Q)=5% to P-values in SNA_classification_genes_NSEI_HISR_Pvalues.tsv, remove the genes that do not pass and save the rest as SNA_driver_gene_list_FDR5.tsv
- 15) Using the file SNA_classification_genes_NSEI_HISR_Pvalues.tsv, plot histograms with the distribution of P values (x axis P values with 0.05 precision, y axis the number of occurrences of a given value), NSEI values (x axis NSEI values with 0.5 precision, y axis the number of occurrences of a given value) and HISR values (x axis HISR values with 0.5 precision, y axis the number of occurrences of a given value).
- 16) Using the file **SNA_driver_gene_list_FDR5.tsv**, classify driver genes into oncogenes (OG; HISR>5) and tumor suppressor genes (TSG; HISR<5), add the results as an additional column and save the file as **SNA_driver_gene_list_FDR5_OG_TSG.tsv**