## **PALDRIC GENE** pipeline

To obtain the necessary files, run SNADRIF, GECNAV and ANDRIF pipelines before executing PALDRIC GENE pipeline.

- 1) Go to <a href="https://gdc.cancer.gov/node/905/">https://gdc.cancer.gov/node/905/</a>
- 2) Download Clinical with Follow-up clinical PANCAN patient with followup.tsv
- 3) Remove from clinical\_PANCAN\_patient\_with\_followup.tsv all patients with icd\_o\_3\_histology different from XXXX/3 (primary malignant neoplasm) and all patients not present (at the level TCGA-XX-XXXX) simultaneously in mc3.v0.2.8.PUBLIC\_primary\_whitelisted\_Entrez.tsv, ISAR\_GISTIC.all\_thresholded.by\_genes\_primary\_whitelisted.tsv and Primary\_whitelisted\_arms.tsv and save the resulting file as clinical\_PANCAN\_patient\_with\_followup\_primary\_whitelisted.tsv
- 4) A) Manually convert the outputs of third-party driver *mutation* prediction algorithms to tsv files with columns **HUGO symbol, Ensembl Transcript ID**, **mutation**, **cohort**, removing all results with q-value >0.05
  - B) Manually convert the outputs of third-party driver *gene* prediction algorithms to tsv files with columns **HUGO symbol**, **cohort**, removing all results with q-value >0.05
- 5) Find Entrez Gene IDs using HUGO symbols and external database <a href="ftp://ftp.ncbi.nih.gov/gene/DATA/GENE\_INFO/Mammalia/Homo\_sapiens.gene\_info.gz">ftp://ftp.ncbi.nih.gov/gene/DATA/GENE\_INFO/Mammalia/Homo\_sapiens.gene\_info.gz</a> and update the file
- 6) A) For lists of driver mutations, remove all entries from mc3.v0.2.8.PUBLIC\_primary\_whitelisted\_Entrez.tsv except those that satisfy the following conditions simultaneously: Transcript\_ID matches Ensembl Transcript ID in the driver list; nucleotide/amino acid substitution matches the one in the driver list; cancer type (identified by matching Tumor\_Sample\_Barcode with bcr\_patient\_barcode and acronym in clinical\_PANCAN\_patient\_with\_followup.tsv) matches cohort in the driver list or the driver list is for pancancer analysis; Variant\_Classification column contains one of the following values: De\_novo\_Start\_InFrame, Frame\_Shift\_Del, Frame\_Shift\_Ins, In\_Frame\_Del, In\_Frame\_Ins, Missense\_Mutation, Nonsense\_Mutation, Nonstop\_Mutation, Translation\_Start\_Site.

Save the results as **AlgorithmName\_output\_SNA.tsv** with columns **TCGA Barcode**, **HUGO Symbol**, **Entrez Gene ID** 

B) For lists of driver *genes*, remove all entries from

mc3.v0.2.8.PUBLIC\_primary\_whitelisted\_Entrez.tsv except those that satisfy the following conditions simultaneously: Entrez\_Gene\_ID matches Entrez Gene ID in the driver list; cancer type (identified by matching Tumor\_Sample\_Barcode with bcr\_patient\_barcode and acronym in clinical\_PANCAN\_patient\_with\_followup.tsv) matches cohort in the driver list or the driver list is for pancancer analysis; Variant\_Classification column contains one of the following values: De\_novo\_Start\_InFrame, Frame\_Shift\_Del, Frame\_Shift\_Ins, In\_Frame\_Del, In\_Frame\_Ins, Missense\_Mutation, Nonsense\_Mutation, Nonstop\_Mutation, Translation Start Site.

Save the results as **AlgorithmName\_output\_SNA.tsv** with columns **TCGA Barcode**, **HUGO Symbol**, **Entrez Gene ID** 

- 7) Remove all entries from
  - ISAR\_GISTIC.all\_thresholded.by\_genes\_primary\_whitelisted.tsv except those that satisfy the following conditions simultaneously: Locus ID matches Entrez Gene ID in the driver list; cancer type (identified by matching Tumor Sample Barcode with bcr\_patient\_barcode and acronym in clinical\_PANCAN\_patient\_with\_followup.tsv) matches cohort in the driver list or the driver list is for pancancer analysis; CNA values are 2,1, -1 or -2. Convert these data from the matrix to a list format (with columns TCGA Barcode, HUGO Symbol, Entrez Gene ID) and save as AlgorithmName\_output\_CNA.tsv.
- 8) Combine AlgorithmName\_output\_SNA.tsv and AlgorithmName\_output\_CNA.tsv, remove duplicate TCGA Barcode-Entrez Gene ID pairs, and save as AlgorithmName\_output.tsv
- 9) Choose desired AlgorithmName\_output.tsv files and fill the columns TCGA Barcode, HUGO Symbol and Entrez Gene ID of AnalysisName\_genes\_level0.tsv, removing duplicate TCGA Barcode-Entrez Gene ID pairs and patients not present in clinical\_PANCAN\_patient\_with\_followup\_primary\_whitelisted.tsv. If overlap was chosen by the user, remove all TCGA Barcode-Entrez Gene ID pairs present in fewer chosen AlgorithmName\_output.tsv files than the user-chosen overlap number.
- 10) Use data from SNA\_classification\_patients.tsv to fill the columns Number of hyperactivating SNAs and Number of inactivating SNAs in AnalysisName\_genes\_level0.tsv; if a given TCGA Barcode-Entrez Gene ID pair is absent in SNA\_classification\_patients.tsv, write zeros. Use data from SNA\_classification\_genes\_NSEI\_HISR.tsv to fill the HISR column; if a given Entrez Gene ID is absent in SNA\_classification\_genes\_NSEI\_HISR.tsv, leave the cell empty. Use data from ISAR\_GISTIC.all\_thresholded.by\_genes\_primary\_whitelisted\_RNAfiltered.tsv to fill the CNA status column; if a given TCGA Barcode-Entrez Gene ID pair is absent in ISAR\_GISTIC.all\_thresholded.by\_genes\_primary\_whitelisted\_RNAfiltered.tsv write zero. Save the results as AnalysisName\_genes\_level1.tsv
- 11) Use data from **AnalysisName\_genes\_level1.tsv** to classify driver alterations according to the following table:

Driver type	Number of hyperactivating SNAs + inactivating SNAs	Number of inactivating SNAs	HISR	CNA status	Count as driver event(s)
SNA-based oncogene	≥1	0	>5	0	1
CNA-based oncogene	0	0	>5	1 or 2	1
Mixed oncogene	≥1	0	>5	1 or 2	1
SNA-based tumor suppressor	≥1	≥0	≤5	0	1
CNA-based tumor suppressor	0	0	≤5	-1 or -2	1
Mixed tumor suppressor	≥1	≥0	≤5	-1 or -2	1
Passenger	0	0		0	0
Low-probability driver	All the rest				0

and fill the columns CNA status, Driver type and Count as ... driver event(s) of AnalysisName\_genes\_level1.tsv, saving it as AnalysisName\_genes\_level2.tsv

12) Use data from AnalysisName\_genes\_level2.tsv, Chromosome\_drivers\_FDR5.tsv µ
Arm\_drivers\_FDR5.tsv, to count for each patient the number of driver events of
various classes (Number of SNA-based oncogenic events, Number of CNA-based
oncogenic events, Number of Mixed oncogenic events, Number of SNA-based tumor
suppressor events, Number of CNA-based tumor suppressor events, Number of
Mixed tumor suppressor events. Number of Driver chromosome losses, Number of

Driver chromosome gains, Number of Driver arm losses, Number of Driver arm gains, Total number of driver events), counting each tumor suppressor as 2 events (see table above). Add patients without any identified drivers (i.e. whose TCGA barcodes are absent in AnalysisName\_genes\_level2.tsv, Chromosome\_drivers\_FDR5.tsv and Arm\_drivers\_FDR5.tsv, but present in

**clinical\_PANCAN\_patient\_with\_followup\_primary\_whitelisted.tsv**) writing zero values for them, saving the results as **AnalysisName\_patients.tsv**.

Write individual gene or chromosome names for each patient and driver class into **AnalysisName\_patients\_genes.tsv.** 

Use data from clinical\_PANCAN\_patient\_with\_followup\_primary\_whitelisted.tsv to fill the columns Cancer type (acronym), Gender (gender), Age (age\_at\_initial\_pathologic\_diagnosis), and Tumor stage (pathologic\_stage, if data absent then clinical\_stage, if data absent then pathologic\_T, if data absent then clinical\_T, convert to Arabic number) in both files.

- 13) Use data from AnalysisName\_patients.tsv to count the number of patients with each integer total number of driver events (0,1,...,99, 100) for each cancer type, also for males and females separately, and save as AnalysisName\_distribution\_events.tsv, AnalysisName\_distribution\_events\_males.tsv and AnalysisName\_distribution\_events\_females.tsv. For each file, plot a multicolor cumulative histogram "Cancer type distribution by total number of driver events per patient".
- 14) Use data from AnalysisName\_patients.tsv to count the average number of various types of driver events in each cancer type (ACC,..., UVM, PANCAN), also for males and females separately, and save as AnalysisName\_distribution\_cohorts.tsv, AnalysisName\_distribution\_cohorts\_males.tsv and AnalysisName\_distribution\_cohorts\_females.tsv. For each file, plot a multicolor cumulative histogram "Driver event distribution by cancer type"

Use data from **AnalysisName\_patients\_genes.tsv** to count the number of various types of driver events in individual genes or chromosomes in each cancer type (ACC,..., UVM, PANCAN), also for males and females separately, and save as

AnalysisName\_distribution\_cohorts\_genes.tsv,

AnalysisName\_distribution\_cohorts\_males\_genes.tsv and

**AnalysisName\_distribution\_cohorts\_females\_genes.tsv**. For each cancer type and gender, plot a histogram of top 10 driver events in each class and overall.

15) Use data from **AnalysisName\_patients.tsv** to count the average number of various types of driver events in patients with each total number of driver events (1,2,...,99, 100), also for males and females separately, and save as

AnalysisName\_distribution\_events\_detailed.tsv,

AnalysisName\_distribution\_events\_detailed\_males.tsv and

AnalysisName\_distribution\_events\_detailed\_females.tsv. For each file, plot a multicolor cumulative histogram "Driver event distribution by total number of driver events per patient".

Use data from **AnalysisName\_patients\_genes.tsv** to count the number of various types of driver events in individual genes or chromosomes in patients with each total number of driver events (1,2,..., 99, 100), also for males and females separately, and save as

AnalysisName\_distribution\_events\_detailed\_genes.tsv,
AnalysisName\_distribution\_events\_detailed\_males\_genes.tsv and
AnalysisName\_distribution\_events\_detailed\_females\_genes.tsv. For each total number of driver events and gender, plot a histogram of top 10 driver events in each class and overall.

Calculate Driver Strength Index (DSI)

$$DSI_A = \sum_{i=1}^{100} \frac{p_{A i}}{i p_i}$$

Where  $p_{A\ i}$  = number of patients with a driver event in the gene/chromosome A amongst patients with i driver events in total;  $p_i$  = number of patients with i driver events in total.

Take  $p_{A\ i}$  data from AnalysisName\_distribution\_events\_detailed\_genes.tsv, AnalysisName\_distribution\_events\_detailed\_males\_genes.tsv and AnalysisName\_distribution\_events\_detailed\_females\_genes.tsv.

Take  $p_i$  data from the PANCAN row of **AnalysisName\_distribution\_events.tsv**, **AnalysisName distribution events males.tsv** and

AnalysisName\_distribution\_events\_females.tsv.

Save results as AnalysisName\_distribution\_events\_detailed\_genes\_DSI.tsv, AnalysisName\_distribution\_events\_detailed\_males\_genes\_DSI.tsv and AnalysisName\_distribution\_events\_detailed\_females\_genes\_DSI.tsv.

Use these data to combine lists of drivers from various classes, removing drivers with lower DSI in case of duplicates and removing all drivers with DSI<0.05, fetch Entrez Gene IDs from

<u>ftp://ftp.ncbi.nih.gov/gene/DATA/GENE\_INFO/Mammalia/Homo\_sapiens.gene\_info.gz</u> and save the results as

AnalysisName\_distribution\_events\_detailed\_genes\_DSI\_top.tsv,
AnalysisName\_distribution\_events\_detailed\_males\_genes\_DSI\_top.tsv and
AnalysisName\_distribution\_events\_detailed\_females\_genes\_DSI\_top.tsv.

Calculate Normalized Driver Strength Index (NDSI)

AnalysisName\_distribution\_cohorts\_females\_genes.tsv.

$$NDSI_{A} = \frac{\sum_{i=1}^{100} \frac{p_{A i}}{i p_{i}}}{\sum_{i=1}^{100} \frac{p_{A i}}{p_{i}}}$$

Where  $p_{A\ i}$  = number of patients with a driver event in the gene/chromosome A amongst patients with i driver events in total;  $p_i$  = number of patients with i driver events in total.

Take  $p_A$  i data from AnalysisName\_distribution\_events\_detailed\_genes.tsv, AnalysisName\_distribution\_events\_detailed\_males\_genes.tsv and AnalysisName\_distribution\_events\_detailed\_females\_genes.tsv. Remove genes/chromosomes if present in less than 10 patients in each driver event type, as counted in the PANCAN row of AnalysisName\_distribution\_cohorts\_genes.tsv, AnalysisName\_distribution\_cohorts males\_genes.tsv and

Take  $p_i$  data from the PANCAN row of **AnalysisName distribution events.tsv**,

AnalysisName\_distribution\_events\_males.tsv and

AnalysisName\_distribution\_events\_females.tsv.

Save results as AnalysisName\_distribution\_events\_detailed\_genes\_NDSI.tsv,

AnalysisName\_distribution\_events\_detailed\_males\_genes\_NDSI.tsv and

AnalysisName\_distribution\_events\_detailed\_females\_genes\_NDSI.tsv.

Use these data to combine lists of drivers from various classes, removing drivers with lower NDSI in case of duplicates and removing all drivers with NDSI<0.05, fetch Entrez Gene IDs from

ftp://ftp.ncbi.nih.gov/gene/DATA/GENE\_INFO/Mammalia/Homo\_sapiens.gene\_info.gz and save the results as

AnalysisName\_distribution\_events\_detailed\_genes\_NDSI\_top.tsv,
AnalysisName\_distribution\_events\_detailed\_males\_genes\_NDSI\_top.tsv and
AnalysisName\_distribution\_events\_detailed\_females\_genes\_NDSI\_top.tsv.

16) Use data from **AnalysisName\_patients.tsv** to count the average number of various types of driver events for males and females separately, and save as **AnalysisName\_distribution\_gender.tsv.** Plot a multicolor cumulative histogram "Driver event distribution by gender".

Use data from **AnalysisName\_patients\_genes.tsv** to count the number of various types of driver events in individual genes or chromosomes for males and females separately, and save as **AnalysisName\_distribution\_gender\_genes.tsv**. For each gender, plot a histogram of top 10 driver events in each class and overall.

17) Use data from **AnalysisName\_patients.tsv** to count the average number of various types of driver events for each tumor stage (1,2,3,4), also for males and females separately, and save as **AnalysisName\_distribution\_stage.tsv**,

AnalysisName distribution stage males.tsv and

**AnalysisName\_distribution\_stage\_females.tsv**. For each file, plot a multicolor cumulative histogram "Driver event distribution by cancer stage".

Use data from **AnalysisName\_patients\_genes.tsv** to count the number of various types of driver events in individual genes or chromosomes for each tumor stage (1,2,3,4), also for males and females separately, and save as

AnalysisName\_distribution\_stage\_genes.tsv,

AnalysisName\_distribution\_stage\_males\_genes.tsv and

**AnalysisName\_distribution\_stage\_females\_genes.tsv**. For each stage and gender, plot a histogram of top 10 driver events in each class and overall.

18) Use data from AnalysisName\_patients.tsv to count the average number of various types of driver events for each age group (<25, 25-29,...,≥85), also for males and females separately, and save as AnalysisName\_distribution\_age.tsv,

AnalysisName distribution age males.tsv and

**AnalysisName\_distribution\_age\_females.tsv**. For each file, plot a multicolor cumulative histogram "Driver event distribution by age".

Use data from **AnalysisName\_patients\_genes.tsv** to count the number of various types of driver events in individual genes or chromosomes for each age group (<25, 25-29,...,≥85), also for males and females separately, and save as

AnalysisName distribution age genes.tsv,

AnalysisName\_distribution\_age\_males\_genes.tsv and

**AnalysisName\_distribution\_age\_females\_genes.tsv**. For each age group and gender, plot a histogram of top 10 driver events in each class and overall.