Package 'Neoantimon'

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Title Neoantimon: A multifunctional R package for identification oftumor-specific neoantigens

Type Package

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|--|
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| Description This Package has been developed to calculate candidates neoantigens from Mutation Data (.vcf,.txt,string). |
| License MIT + file LICENSE |
| VignetteBuilder knitr |
| Encoding UTF-8 |
| Depends R (>= 3.3.0) |
| biocViews |
| Imports ensemblVEP, devtools, graphics, grDevices, stats, utils Suggests data.table, knitr, rmarkdown LazyData FALSE RoxygenNote 7.0.2 R topics documented: |
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```

Export_Summary_Entire_Fragments

Export Summary Count from Indel/SV Results

Description

Export Summary Count from Indel/SV Results

Usage

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```
Export_Summary_Entire_Fragments(
   Input,
   Mut_IC50_th = NA,
   Mut_Rank_th = NA,
   Total_RNA_th = NA,
   Tumor_RNA_th = NA,
   MutRatio_th = NA,
   WriteLongIndel = NA,
   DupCount = FALSE
)
```

Arguments

| Input | Input file generated from MainSNVClass1,2. |
|----------------|---|
| Mut_IC50_th | The threshold for mutant peptide to be neoantigen by IC50. |
| Mut_Rank_th | The threshold for mutant peptide to be neoantigen by Rank. |
| Total_RNA_th | The total RNA expression threshold. |
| Tumor_RNA_th | The tumor specific RNA expression threshold. |
| MutRatio_th | The mutation ratio threshold. |
| WriteLongIndel | If setting a file name, Write Long Indels of which the p-value is less than 0.05. |
| DupCount | Count for each different HLA type |

Value

Num_Alteration The number of evaluated alterations.

Num_Alteration_Generating_NeoAg The number of evaluated alterations that can generate neoantigen.

Num_Peptide The number of evaluated peptifdes.

Num_Peptide_Generating_NeoAg The number of evaluated peptides that can be neoantigen.

```
Export_Summary_Fragments
```

Export Summary Count from Indel/SV Results

Description

Export Summary Count from Indel/SV Results

Usage

```
Export_Summary_Fragments(
   Input,
   Mut_IC50_th = NA,
   Mut_Rank_th = NA,
   Total_RNA_th = NA,
   Tumor_RNA_th = NA,
   MutRatio_th = NA,
   WriteLongIndel = NA,
   DupCount = FALSE
)
```

Arguments

| Input | Input file generated from MainSNVClass1,2. |
|----------------|--|
| Mut_IC50_th | The threshold for mutant peptide to be neoantigen by IC50. |
| Mut_Rank_th | The threshold for mutant peptide to be neoantigen by Rank. |
| Total_RNA_th | The total RNA expression threshold. |
| Tumor_RNA_th | The tumor specific RNA expression threshold. |
| MutRatio_th | The mutation ratio threshold. |
| WriteLongIndel | If setting a file name, Write Long Indels of which the p-value is less than 0.05 . |
| DupCount | Count for each different HLA type |

Value

Num_Alteration The number of evaluated alterations.

Num_Alteration_Generating_NeoAg The number of evaluated alterations that can generate neoantigen.

Num_Peptide The number of evaluated peptifdes.

```
Export_Summary_IndelSV
```

Export Summary Count from Indel/SV Results

Description

Export Summary Count from Indel/SV Results

Usage

```
Export_Summary_IndelSV(
   Input,
   Mut_IC50_th = NA,
   Mut_Rank_th = NA,
   Total_RNA_th = NA,
   Tumor_RNA_th = NA,
   MutRatio_th = NA,
   Weight = NA,
   WriteLongIndel = NA,
   IgnoreLongIndel = 0,
   DupCount = FALSE
)
```

Arguments

| | Input | Input file generated from MainSNVClass1,2. |
|-----------------|----------------|---|
| | Mut_IC50_th | The threshold for mutant peptide to be neoantigen by IC50. |
| | Mut_Rank_th | The threshold for mutant peptide to be neoantigen by Rank. |
| | Total_RNA_th | The total RNA expression threshold. |
| | Tumor_RNA_th | The tumor specific RNA expression threshold. |
| | MutRatio_th | The mutation ratio threshold. |
| | Weight | The weight for alterations. |
| | WriteLongIndel | If setting a file name, Write Long Indels of which the p-value is less than 0.05. |
| IgnoreLongIndel | | |
| | | Ignore Indels of which p-value is less than the indicated value for counting. |
| | DupCount | Count for each different HLA type |

Value

Num_Alteration The number of evaluated alterations.

Num_Alteration_Generating_NeoAg The number of evaluated alterations that can generate neoantigen.

Num_Peptide The number of evaluated peptifdes.

```
Export_Summary_IndelSV_perFragments
```

Export Summary Count from Indel/SV Results

Description

Export Summary Count from Indel/SV Results

Usage

```
Export_Summary_IndelSV_perFragments(
   Input,
   Mut_IC50_th = NA,
   Mut_Rank_th = NA,
   Total_RNA_th = NA,
   Tumor_RNA_th = NA,
   MutRatio_th = NA,
   Weight = NA,
   WriteLongIndel = NA,
   IgnoreLongIndel = 0,
   DupCount = FALSE
)
```

Arguments

| Input | Input file generated from MainSNVClass1,2. | |
|-----------------|---|--|
| Mut_IC50_th | The threshold for mutant peptide to be neoantigen by IC50. | |
| Mut_Rank_th | The threshold for mutant peptide to be neoantigen by Rank. | |
| Total_RNA_th | The total RNA expression threshold. | |
| Tumor_RNA_th | The tumor specific RNA expression threshold. | |
| MutRatio_th | The mutation ratio threshold. | |
| Weight | The weight for alterations. | |
| WriteLongIndel | If setting a file name, Write Long Indels of which the p-value is less than 0.05. | |
| IgnoreLongIndel | | |
| | Ignore Indels of which p-value is less than the indicated value for counting. | |
| DupCount | Count for each different HLA type | |

Value

Num_Alteration The number of evaluated alterations.

Num_Alteration_Generating_NeoAg The number of evaluated alterations that can generate neoantigen.

Num_Peptide The number of evaluated peptifdes.

Export_Summary_SNV

Export Summary Count from SNV Results

Description

Export Summary Count from SNV Results

Usage

```
Export_Summary_SNV(
   Input,
   Mut_IC50_th = NA,
   Mut_Rank_th = NA,
   Wt_IC50_th = NA,
   Wt_Rank_th = NA,
   Total_RNA_th = NA,
   Tumor_RNA_th = NA,
   MutRatio_th = NA,
   DupCount = FALSE
)
```

Arguments

| Input | Input file generated from MainSNVClass1,2. |
|--------------|--|
| Mut_IC50_th | The threshold for mutant peptide to be neoantigen. |
| Mut_Rank_th | The threshold for mutant peptide to be neoantigen. |
| Wt_IC50_th | The threshold for wt peptide to be neoantigen. |
| Wt_Rank_th | The threshold for wt peptide to be neoantigen. |
| Total_RNA_th | The total RNA expression threshold. |
| Tumor_RNA_th | The tumor specific RNA expression threshold. |
| MutRatio_th | The mutation ratio threshold. |
| DupCount | Count for each different HLA type |

Value

Num_Alteration The number of evaluated alterations.

Num_Alteration_Generating_NeoAg The number of evaluated alterations that can generate neoantigen.

Num_Peptide The number of evaluated peptifdes.

MainEntireRegionClass1

Calculate A Set All Neoantigen Candidates from A Given Gene Symbol and nm_id for MHC Class1 (Not yet stably available)

Description

Calculate A Set All Neoantigen Candidates from A Given Gene Symbol and nm_id for MHC Class1 (Not yet stably available)

Usage

```
MainEntireRegionClass1(
  input_nm_id,
  group_ids = seq(1:length(input_nm_id)),
  hla_file = "here_is_a_table",
  hla_{types} = NA,
  file_name_in_hla_table = NA,
  refflat_file = paste(hmdir, "lib/refFlat.txt", sep = "/"),
  refmrna_file = paste(hmdir, "lib/refMrna.fa", sep = "/"),
  hmdir = getwd(),
  job_id = "ID",
  export_dir = paste("result", job_id, "EntireRegion1", sep = "."),
  netMHCpan_dir = paste(hmdir, "lib/netMHCpan-4.0/netMHCpan", sep = "/"),
  peptide_length = c(8, 9, 10, 11, 12, 13),
  reading_frame = 1,
  CalculateIC50 = FALSE,
  IgnoreShortPeptides = TRUE
)
```

Arguments

| | input_nm_id | (Required) An input amino acid sequence indicated as NM_ID |
|------------------------|--------------|---|
| | group_ids | flag to cluster the same group |
| | hla_file | A tab separated file indicating HLA types. The 1st column is input_file name, and the following columns indicate HLA types. |
| | | See by data(sample_hla_table_c1); sample_hla_table_c1; |
| | hla_types | Set a list of HLA types |
| file_name_in_hla_table | | a_table |
| | | If the name (1st column) in HLA table is not the same as input_file, indicate the corresponding name (Default=input_file). |
| | refflat_file | refFlat file to be used in constructing peptide. (Default=paste(hmdir, "lib/refFlat.txt", sep=""). |
| | | See "https://github.com/hase62/Neoantimon" |
| | refmrna_file | refMrna file to be used in constructing peptide (Default=paste(hmdir, "lib/refMrna.fa", sep=""). |
| | | See "https://github.com/hase62/Neoantimon" |
| | hmdir | Home directory for the analysis (Default = getwd()). |
| | | |

job_id Job-id to be attached in output files (Default = "NO_job_id").

export_dir The directory will be stored results (Default = "paste("result", file_name_in_hla_table,

job_id, sep=".")")

netMHCpan_dir The file directory to netMHCpan (Default="lib/netMHCpan-4.0/netMHCpan").

peptide_length Peptide Length to be generated (Default = 8,9,10,11,12,13).

reading_frame The starting frame of the input sequence (Default = 1)

CalculateIC50 Whether Calculate IC50 by NetMHCpan or not.

IgnoreShortPeptides

Ignore to output results of Short Peptide Less Than min(peptide_length)

MHCflurry Also output results using MHCflurry (Default=FALSE).

Value

void (Calculated Neoantigen Files will be generated as .tsv files.):

HLA: HLA type used to calculate neoantigen.

Pos: The position of a fraction of peptide used to be evaluated from the full-length peptide.

Gene: Gene symbol used to be evaluated in NetMHCpan.

Evaluated_Mutant_Peptide: The mutant peptide to be evaluated.

Evaluated_Mutant_Peptide_Core: The core peptide of the mutant peptide to be evaluated in NetMHC-pan.

Mut_IC50: IC50 value for evaluated mutant peptide.

Mut Rank: Rank value for evaluated mutanat peptide.

Chr: Chromosome Number of the mutation.

NM_ID: NM_ID used to construct peptides from the mutation.

Change: The annotation to be described in .vcf file.

Ref: reference type nucleic acid base.

Alt: alternative type nucleic acid base.

Prob: A probability of reference nucleic acid base described in .vcf file.

Mutation_Prob: A probability of alternative nucleic acid base described in .vcf file.

Exon Start: The exon start position of the corrsponding NM ID.

Exon_End: The exon end position of the corrsponding NM_ID.

Mutation_Position: The mutation position of the corrsponding NM_ID.

Total_Depth: The sum depth of the reference and alternative nucleic acid base.

Tumor_Depth: The depth of the alternative nucleic acid base.

Wt_Peptide: The full-length of the wild-type peptide.

Mutant_Peptide: The full-length of the mutant peptide.

Total_RNA: The expression amount of the corresponding RNA.

Tumor_RNA_Ratio: The variant allele frequency of the corresponding RNA.

Tumor_RNA: The modified amount of the corresponding RNA level based on RNA Reads.

Tumor_RNA_based_on_DNA: The modified amount of the corresponding RNA level based on DNA Reads.

MutRatio: The mean value of the cancer cell fraction probability.

MutRatio_Min: The 1% percentile of the cancer cell fraction probability.

MutRatio_Max: The 99% percentile of the cancer cell fraction probability.

MainEntireRegionClass2

Calculate A Set All Neoantigen Candidates from A Given Gene Symbol and nm_id for MHC Class2 (Not yet stably available)

Description

Calculate A Set All Neoantigen Candidates from A Given Gene Symbol and nm_id for MHC Class2 (Not yet stably available)

Usage

```
MainEntireRegionClass2(
  input_nm_id,
 group_ids = seq(1:length(input_nm_id)),
 hla_file = "here_is_a_table",
 hla_{types} = NA,
 file_name_in_hla_table = NA,
 refflat_file = paste(hmdir, "lib/refFlat.txt", sep = "/"),
  refmrna_file = paste(hmdir, "lib/refMrna.fa", sep = "/"),
 hmdir = getwd(),
  job_id = "ID",
 export_dir = paste("result", job_id, "EntireRegion2", sep = "."),
 netMHCIIpan_dir = paste(hmdir, "lib/netMHCIIpan-3.1/netMHCIIpan", sep = "/"),
 peptide_length = c(15),
 reading_frame = 1,
 CalculateIC50 = FALSE,
 IgnoreShortPeptides = TRUE
)
```

Arguments

| input_nm_id | (Required) An input amino acid sequence indicated as NM_ID | |
|------------------------|---|--|
| group_ids | flag to cluster the same group | |
| hla_file | A tab separated file indicating HLA types. The 1st column is input_file name, and the following columns indicate HLA types. | |
| | See by data(sample_hla_table_c1); sample_hla_table_c1; | |
| hla_types | Set a list of HLA types | |
| file_name_in_hla_table | | |
| | If the name (1st column) in HLA table is not the same as input_file, indicate the corresponding name (Default=input_file). | |
| refflat_file | refFlat file to be used in constructing peptide. (Default=paste(hmdir, "lib/refFlat.txt", sep=""). | |
| | See "https://github.com/hase62/Neoantimon" | |
| refmrna_file | refMrna file to be used in constructing peptide (Default=paste(hmdir, "lib/refMrna.fa", sep=""). | |
| | See "https://github.com/hase62/Neoantimon" | |
| hmdir | Home directory for the analysis (Default = getwd()). | |

job_id Job-id to be attached in output files (Default = "NO_job_id").

export_dir The directory will be stored results (Default = "paste("result", file_name_in_hla_table,

job_id, sep=".")")

netMHCIIpan_dir

The file directory to netMHCpan (Default="lib/netMHCIIpan-3.2/netMHCIIpan").

peptide_length Peptide Length to be generated (Default = 8,9,10,11,12,13). reading_frame The starting frame of the input sequence (Default = 1) CalculateIC50 Whether Calculate IC50 by NetMHCpan or not.

IgnoreShortPeptides

Ignore to output results of Short Peptide Less Than min(peptide_length)

Value

void (Calculated Neoantigen Files will be generated as .tsv files.):

HLA: HLA type used to calculate neoantigen.

Pos: The position of a fraction of peptide used to be evaluated from the full-length peptide.

Gene: Gene symbol used to be evaluated in NetMHCpan.

Evaluated_Mutant_Peptide: The mutant peptide to be evaluated.

Evaluated_Mutant_Peptide_Core: The core peptide of the mutant peptide to be evaluated in NetMHC-

pan.

Mut_IC50: IC50 value for evaluated mutant peptide.

Mut_Rank: Rank value for evaluated mutanat peptide.

Chr: Chromosome Number of the mutation.

NM ID: NM ID used to construct peptides from the mutation.

Change: The annotation to be described in .vcf file.

Ref: reference type nucleic acid base.

Alt: alternative type nucleic acid base.

Prob: A probability of reference nucleic acid base described in .vcf file.

Mutation_Prob: A probability of alternative nucleic acid base described in .vcf file.

Exon_Start: The exon start position of the corrsponding NM_ID.

Exon_End: The exon end position of the corrsponding NM_ID.

Mutation_Position: The mutation position of the corrsponding NM_ID.

Total_Depth: The sum depth of the reference and alternative nucleic acid base.

Tumor Depth: The depth of the alternative nucleic acid base.

Wt_Peptide: The full-length of the wild-type peptide.

Mutant_Peptide: The full-length of the mutant peptide.

Total_RNA: The expression amount of the corresponding RNA.

Tumor_RNA_Ratio: The variant allele frequency of the corresponding RNA.

Tumor RNA: The modified amount of the corresponding RNA level based on RNA Reads.

Tumor_RNA_based_on_DNA: The modified amount of the corresponding RNA level based on DNA Reads.

MutRatio: The mean value of the cancer cell fraction probability.

MutRatio_Min: The 1% percentile of the cancer cell fraction probability.

MutRatio Max: The 99% percentile of the cancer cell fraction probability.

MainINDELClass1

Calculate Neoantigen Candidates on INDELs for MHC Class1

Description

Calculate Neoantigen Candidates on INDELs for MHC Class1

Usage

```
MainINDELClass1(
  input_file,
  hla_file = "here_is_a_table",
  hla_types = NA,
  file_name_in_hla_table = input_file,
  refflat_file = paste(hmdir, "lib/refFlat.txt", sep = "/"),
  refmrna_file = paste(hmdir, "lib/refMrna.fa", sep = "/"),
  hmdir = getwd(),
  job_id = "ID",
  export_dir = paste("result", job_id, "INDEL1", sep = "."),
  rnaexp_file = NA,
  rnabam_file = NA,
  cnv_file = NA,
  purity = 1,
  netMHCpan_dir = paste(hmdir, "lib/netMHCpan-4.0/netMHCpan", sep = "/"),
  MHCflurry = NA,
  refdna_file = NA,
  samtools_dir = "samtools",
  bcftools_dir = NA,
  chr_column = NA,
  mutation_start_column = NA,
  mutation_end_column = NA,
  mutation_ref_column = NA,
  mutation_alt_column = NA,
  nm_id_column = NA,
  depth_normal_column = NA,
  depth_tumor_column = NA,
  ambiguous_between_exon = 0,
  ambiguous\_codon = 0,
  peptide_length = c(8, 9, 10, 11, 12, 13),
  IgnoreShortPeptides = TRUE,
  SNPs = NA,
  multiple_variants = FALSE,
  apply_annotation = FALSE
)
```

Arguments

```
input_file (Required) An input vcf file annotated by,
```

e.g., ANNOVAR (http://annovar.openbioinformatics.org/en/latest/) or other softwares.

See by data(sample_vcf); sample_vcf;

hla_file A tab separated file indicating HLA types. The 1st column is input_file name, and the following columns indicate HLA types.

See by data(sample_hla_table_c1); sample_hla_table_c1;

hla_types Set a list of HLA types

file_name_in_hla_table

If the name (1st column) in HLA table is not the same as input_file, indicate the corresponding name (Default=input_file).

refflat_file refFlat file to be used in constructing peptide. (Default=paste(hmdir, "lib/refFlat.txt", sep="").

See "https://github.com/hase62/Neoantimon"

 $refmrna_file \qquad refMrna\ file\ to\ be\ used\ in\ constructing\ peptide\ (Default=paste(hmdir,\ "lib/refMrna.fa",\ normalization of the paste of the$

sep="").

See "https://github.com/hase62/Neoantimon"

hmdir Home directory for the analysis (Default = getwd()).

job_id Job-id to be attached in output files (Default = "NO_job_id").

 ${\tt export_dir} \qquad \qquad {\tt The \ directory \ will \ be \ stored \ results} \ ({\tt Default = "paste("result", file_name_in_hla_table, result", file_name_in_hla_table, results})$

job_id, sep=".")")

rnaexp_file A file including RNA expressions (Default=NA). The 1st, 2nd and 3rd columns

are "GeneSymbol Chr:Exonstart-Exonend (locus) ExpressionAmount", respec-

tively. The 1st row should be any header.

See by data(sample_rna_exp); sample_rna_exp;

rnabam_file RNA bam file to calculate variant allele frequency of RNA at each mutation

(Default=NA).

cnv_file A file including copy number variation to calculate cancer cell fraction prob-

ability (CCFP) (Default=NA). The format is according to ASCAT output files. The columns are "SNPName Chromosome Position LogR segmentedLogR BAF segmentedBAF CopyNumber MinorAllele RawCopyNumber" The 1st row should

be the above header.

See data(sample_copynum); sample_copynum;

purity Tumor purity or tumor contents ratio required to calculate CCFP (Default=1).

netMHCpan_dir The file directory to netMHCpan (Default="lib/netMHCpan-4.0/netMHCpan").

MHCflurry Also output results using MHCflurry (Default=FALSE).

refdna_file refdna_file information to be used to calculate RNA VAF (Default=NA).

See "https://github.com/hase62/Neoantimon"

samtools_dir The file directory to samtools_0_x_x (Default="samtools"). It shouled be indi-

cated when you indicate RNA-bam and try to calculate RNA VAF.

bcftools_dir The file directory to netMHCpan (Default="bcftools"). It shouled be indicated

when you indicate RNA-bam and try to calculate RNA VAF. samtools 0_x_x

includes beftools in the directory.

chr_column The column number describing chromosome number in input_file (Default=NA,

but will automatically search "Chr" in header).

mutation_start_column

The column number describing mutation start Position in input_file (Default=NA, but will automatically search "Start" in header) .

mutation_end_column

The column number describing mutation end Position in input_file (Default=NA, but will automatically search "End" in header).

mutation_ref_column

The column number describing mutation Ref in input_file (Default=NA, but will automatically search "Ref" in header).

mutation_alt_column

The column number describing mutation Alt in input_file (Default=NA, but will automatically search "Alt" in header).

nm_id_column The column number describing NM IDs in input_file such as

"SLCO1C1:NM 001145944:exon7:c.692 693insG:p.L231fs" (Default=NA).

depth_normal_column

The column number describing the read count from normal cells (Default = NA).

depth_tumor_column

The column number describing the read count from tumor cells (Default = NA).

ambiguous_between_exon

The maximum number to permit the differences between Exon-Lengths from refFlat and refMrna (Default=0).

ambiguous_codon

The maximum number to permit the differences between inputfile- and refMrnaoriented translation start/end position (Default=0).

peptide_length Peptide Length to be generated (Default = 8,9,10,11,12,13).

IgnoreShortPeptides

Ignore to output results of Short Peptide Less Than min(peptide_length)

SNPs Apply indivisual SNPs on peptides by indicate a vcf file.

multiple_variants

Reflect multiple variants on a peptide, e.g., SNVs on frameshift region.

apply_annotation

Anontate by Ensembl Variant Effect Predictor (VEP).

Value

void (Calculated Neoantigen Files will be generated as .tsv files.):

HLA: HLA type used to calculate neoantigen.

Pos: The position of a fraction of peptide used to be evaluated from the full-length peptide.

Gene: Gene symbol used to be evaluated in NetMHCpan.

Evaluated_Mutant_Peptide: The mutant peptide to be evaluated.

Evaluated_Mutant_Peptide_Core: The core peptide of the mutant peptide to be evaluated in NetMHC-pan.

Mut_IC50: IC50 value for evaluated mutant peptide.

Mut_Rank: Rank value for evaluated mutanat peptide.

Chr: Chromosome Number of the mutation.

NM_ID: NM_ID used to construct peptides from the mutation.

Change: The annotation to be described in .vcf file.

Ref: reference type nucleic acid base.

Alt: alternative type nucleic acid base.

Prob: A probability of reference nucleic acid base described in .vcf file.

Mutation_Prob: A probability of alternative nucleic acid base described in .vcf file.

Exon_Start: The exon start position of the corrsponding NM_ID.

Exon_End: The exon end position of the corrsponding NM_ID.

Mutation_Position: The mutation position of the corrsponding NM_ID.

Total_Depth: The sum depth of the reference and alternative nucleic acid base.

Tumor_Depth: The depth of the alternative nucleic acid base.

Wt_Peptide: The full-length of the wild-type peptide.

Mutant_Peptide: The full-length of the mutant peptide.

Total_RNA: The expression amount of the corresponding RNA.

Tumor_RNA_Ratio: The variant allele frequency of the corresponding RNA.

Tumor_RNA: The modified amount of the corresponding RNA level based on RNA Reads.

Tumor_RNA_based_on_DNA: The modified amount of the corresponding RNA level based on DNA Reads.

MutRatio: The mean value of the cancer cell fraction probability.

MutRatio_Min: The 1% percentile of the cancer cell fraction probability.

MutRatio_Max: The 99% percentile of the cancer cell fraction probability.

MainINDELClass2

Calculate Neoantigen Candidates on INDELs for MHC Class2

Description

Calculate Neoantigen Candidates on INDELs for MHC Class2

Usage

```
MainINDELClass2(
  input_file,
  hla_file = "here_is_a_table",
  hla_{types} = NA,
  file_name_in_hla_table = input_file,
  refflat_file = paste(hmdir, "lib/refFlat.txt", sep = "/"),
  refmrna_file = paste(hmdir, "lib/refMrna.fa", sep = "/"),
  hmdir = getwd(),
  job_id = "ID",
  export_dir = paste("result", job_id, "INDEL2", sep = "."),
  rnaexp_file = NA,
  rnabam_file = NA,
  cnv_file = NA,
  purity = 1,
  netMHCIIpan_dir = paste(hmdir, "lib/netMHCIIpan-3.1/netMHCIIpan", sep = "/"),
  refdna_file = NA,
  samtools_dir = "samtools",
  bcftools_dir = NA,
  chr_column = NA,
  mutation_start_column = NA,
  mutation_end_column = NA,
  mutation_ref_column = NA,
```

mutation_alt_column = NA,

```
nm id column = NA.
      depth_normal_column = NA,
      depth_tumor_column = NA,
      ambiguous_between_exon = 0,
      ambiguous_codon = 0,
      peptide_length = c(15),
      IgnoreShortPeptides = TRUE,
      SNPs = NA,
      multiple_variants = FALSE,
      apply_annotation = FALSE
    )
Arguments
    input_file
                      (Required) An input vcf file annotated by,
                      e.g., ANNOVAR (http://annovar.openbioinformatics.org/en/latest/) or other soft-
                      wares.
                      See by data(sample_vcf); sample_vcf;
    hla_file
                      A tab separated file indicating HLA types. The 1st column is input_file name,
                      and the following columns indicate HLA types.
                      See by data(sample_hla_table_c2); sample_hla_table_c2;
    hla_types
                      Set a list of HLA types
    file_name_in_hla_table
                      If the name (1st column) in HLA table is not the same as input_file, indicate the
                      corresponding name (Default=input_file).
                      refFlat file to be used in constructing peptide. (Default=paste(hmdir, "lib/refFlat.txt",
    refflat_file
                      sep="").
                      See "https://github.com/hase62/Neoantimon"
    refmrna_file
                      refMrna file to be used in constructing peptide (Default=paste(hmdir, "lib/refMrna.fa",
                      sep="").
                      See "https://github.com/hase62/Neoantimon"
                      Home directory for the analysis (Default = getwd()).
    hmdir
                      Job-Id to be attached in output files (Default = "NO_job_id").
    job_id
                      The directory will be stored results (Default = "paste("result", file name in hla table,
    export_dir
                      job_id, sep=".")")
```

A file including RNA expressions (Default=NA). The 1st, 2nd and 3rd columns rnaexp_file

are "GeneSymbol Chr:Exonstart-Exonend (locus) ExpressionAmount", respec-

tively. The 1st row should be any header.

See by data(sample rna exp); sample rna exp;

rnabam_file RNA bam file to calculate variant allele frequency of RNA at each mutation

(Default=NA).

cnv_file A file including copy number variation to calculate cancer cell fraction prob-

> ability (CCFP) (Default=NA). The format is according to ASCAT output files. The columns are "SNPName Chromosome Position LogR segmentedLogR BAF segmentedBAF CopyNumber MinorAllele RawCopyNumber" The 1st row should

be the above header.

See data(sample_copynum); sample_copynum;

purity Tumor purity or tumor contents ratio required to calculate CCFP (Default=1). netMHCIIpan_dir

The file directory to netMHCpan (Default="lib/netMHCIIpan-3.2/netMHCpan").

refdna_file refdna_file information to be used to calculate RNA VAF (Default=NA).

See "https://github.com/hase62/Neoantimon"

samtools_dir The file directory to samtools_0_x_x (Default="samtools"). It shouled be indicated when you indicate RNA-bam and try to calculate RNA VAF.

bcftools_dir The file directory to netMHCpan (Default="bcftools"). It shouled be indicated when you indicate RNA-bam and try to calculate RNA VAF. samtools 0_x_x includes bcftools in the directory.

chr_column The column number describing chromosome number in input_file (Default=NA, but will automatically search "Chr" in header).

mutation_start_column

The column number describing mutation start Position in input_file (Default=NA, but will automatically search "Start" in header).

mutation_end_column

The column number describing mutation end Position in input_file (Default=NA, but will automatically search "End" in header).

mutation_ref_column

The column number describing mutation Ref in input_file (Default=NA, but will automatically search "Ref" in header).

mutation_alt_column

The column number describing mutation Alt in input_file (Default=NA, but will automatically search "Alt" in header).

nm_id_column The column number describing NM IDs in input_file such as "SLCO1C1:NM_001145944:exon7:c.692_693insG:p.L231fs" (Default=NA).

depth_normal_column

The column number describing the read count from normal cells (Default = NA).

depth_tumor_column

The column number describing the read count from tumor cells (Default = NA).

ambiguous_between_exon

The maximum number to permit the differences between Exon-Lengths from refFlat and refMrna (Default=0).

ambiguous_codon

The maximum number to permit the differences between inputfile- and refMrna-oriented translation start/end position (Default=0).

peptide_length Peptide Length to be generated (Default = 15 in HLA Class2).

IgnoreShortPeptides

Ignore to output results of Short Peptide Less Than min(peptide_length)

SNPs Apply indivisual SNPs on peptides by indicate a vcf file.

multiple_variants

Reflect multiple variants on a peptide, e.g., SNVs on frameshift region.

apply_annotation

Anontate by Ensembl Variant Effect Predictor (VEP).

Value

void (Calculated Neoantigen Files will be generated as .tsv files.):

HLA: HLA type used to calculate neoantigen.

Pos: The position of a fraction of peptide used to be evaluated from the full-length peptide.

Gene: Gene symbol used to be evaluated in NetMHCpan.

Evaluated_Mutant_Peptide: The mutant peptide to be evaluated.

Evaluated_Mutant_Peptide_Core: The core peptide of the mutant peptide to be evaluated in NetMHC-

pan.

Mut_IC50: IC50 value for evaluated mutant peptide.

Mut_Rank: Rank value for evaluated mutanat peptide.

Chr: Chromosome Number of the mutation.

NM_ID: NM_ID used to construct peptides from the mutation.

Change: The annotation to be described in .vcf file.

Ref: reference type nucleic acid base.

Alt: alternative type nucleic acid base.

Prob: A probability of reference nucleic acid base described in .vcf file.

Mutation_Prob: A probability of alternative nucleic acid base described in .vcf file.

Exon_Start: The exon start position of the corrsponding NM_ID.

Exon_End: The exon end position of the corrsponding NM_ID.

Mutation_Position: The mutation position of the corrsponding NM_ID.

Total_Depth: The sum depth of the reference and alternative nucleic acid base.

Tumor_Depth: The depth of the alternative nucleic acid base.

Wt_Peptide: The full-length of the wild-type peptide.

Mutant_Peptide: The full-length of the mutant peptide.

Total_RNA: The expression amount of the corresponding RNA.

Tumor_RNA_Ratio: The variant allele frequency of the corresponding RNA.

Tumor_RNA: The modified amount of the corresponding RNA level based on RNA Reads.

Tumor_RNA_based_on_DNA: The modified amount of the corresponding RNA level based on DNA Reads.

MutRatio: The mean value of the cancer cell fraction probability.

MutRatio_Min: The 1% percentile of the cancer cell fraction probability.

MutRatio_Max: The 99% percentile of the cancer cell fraction probability.

MainSeqFragmentClass1 Calculate Neoantigen Candidates from A Given Sequence for MHC Class1

Description

Calculate Neoantigen Candidates from A Given Sequence for MHC Class1

Usage

```
MainSeqFragmentClass1(
  input_sequence = NA,
  group_ids = seq(1:length(reference_nm_id)),
  hla_file = "here_is_a_table",
  hla_{types} = NA,
  file_name_in_hla_table = NA,
  refflat_file = paste(hmdir, "lib/refFlat.txt", sep = "/"),
  refmrna_file = paste(hmdir, "lib/refMrna.fa", sep = "/"),
  hmdir = getwd(),
  job_id = "ID",
  export_dir = paste("result", job_id, "SeqFragment1", sep = "."),
  netMHCpan_dir = paste(hmdir, "lib/netMHCpan-4.0/netMHCpan", sep = "/"),
  peptide_length = c(8, 9, 10, 11, 12, 13),
  reference_nm_id = NA,
  reference_gene_symbol = NA,
  IgnoreShortPeptides = TRUE
)
```

Arguments

```
input_sequence (Required) An input amino acid sequence
                  flag to cluster the same group
group_ids
hla_file
                  A tab separated file indicating HLA types. The 1st column is input_file name,
                  and the following columns indicate HLA types.
                  See by data(sample_hla_table_c1); sample_hla_table_c1;
hla_types
                  Set a list of HLA types
file_name_in_hla_table
                  If the name (1st column) in HLA table is not the same as input_file, indicate the
                  corresponding name (Default=input_file).
refflat_file
                  refFlat file to be used in constructing peptide. (Default=paste(hmdir, "lib/refFlat.txt",
                  sep="").
                   See "https://github.com/hase62/Neoantimon"
refmrna_file
                   refMrna file to be used in constructing peptide (Default=paste(hmdir, "lib/refMrna.fa",
                  sep="").
                  See "https://github.com/hase62/Neoantimon"
                  Home directory for the analysis (Default = getwd()).
hmdir
job_id
                  Job-Id to be attached in output files (Default = "NO_job_id").
```

export_dir The directory will be stored results (Default = "paste("result", file_name_in_hla_table,

job_id, sep=".")")

netMHCpan_dir The file directory to netMHCpan (Default="lib/netMHCpan-4.0/netMHCpan").

peptide_length Peptide Length to be generated (Default = 8,9,10,11,12,13).

reference_nm_id

Corresponding original sequences that the input sequence is generated. If franctions of peptides generated from the input are included in the indicated protein, such peptides are removed. It can be indicated when gene_symbol is not NA.

reference_gene_symbol

Corresponding original sequences that the input sequence is generated. If franctions of peptides generated from the input are included in the indicated protein, such peptides are removed. It can be indicated when nm id is not NA.

IgnoreShortPeptides

Ignore to output results of Short Peptide Less Than min(peptide_length)

Value

void (Calculated Neoantigen Files will be generated as .tsv files.):

HLA: HLA type used to calculate neoantigen.

Pos: The position of a fraction of peptide used to be evaluated from the full-length peptide.

Gene: Gene symbol used to be evaluated in NetMHCpan.

Evaluated Mutant Peptide: The mutant peptide to be evaluated.

Evaluated_Mutant_Peptide_Core: The core peptide of the mutant peptide to be evaluated in NetMHC-

pan.

Mut IC50: IC50 value for evaluated mutant peptide.

Mut_Rank: Rank value for evaluated mutanat peptide.

Chr: Chromosome Number of the mutation.

NM_ID: NM_ID used to construct peptides from the mutation.

Change: The annotation to be described in .vcf file.

Ref: reference type nucleic acid base.

Alt: alternative type nucleic acid base.

Prob: A probability of reference nucleic acid base described in .vcf file.

Mutation_Prob: A probability of alternative nucleic acid base described in .vcf file.

Exon_Start: The exon start position of the corrsponding NM_ID.

Exon_End: The exon end position of the corrsponding NM_ID.

Mutation_Position: The mutation position of the corrsponding NM_ID.

Total_Depth: The sum depth of the reference and alternative nucleic acid base.

Tumor_Depth: The depth of the alternative nucleic acid base.

Wt_Peptide: The full-length of the wild-type peptide.

Mutant_Peptide: The full-length of the mutant peptide.

Total_RNA: The expression amount of the corresponding RNA.

Tumor_RNA_Ratio: The variant allele frequency of the corresponding RNA.

Tumor_RNA: The modified amount of the corresponding RNA level based on RNA Reads.

Tumor_RNA_based_on_DNA: The modified amount of the corresponding RNA level based on DNA Reads.

MutRatio: The mean value of the cancer cell fraction probability.

MutRatio_Min: The 1% percentile of the cancer cell fraction probability.

MutRatio_Max: The 99% percentile of the cancer cell fraction probability.

MainSeqFragmentClass2 Calculate Neoantigen Candidates from A Given Sequence for MHC Class2

Description

Calculate Neoantigen Candidates from A Given Sequence for MHC Class2

Usage

```
MainSeqFragmentClass2(
  input_sequence = NA,
  group_ids = seq(1:length(reference_nm_id)),
  hla_file = "here_is_a_table",
  hla_types = NA,
  file_name_in_hla_table = NA,
  refflat_file = paste(hmdir, "lib/refFlat.txt", sep = "/"),
  refmrna_file = paste(hmdir, "lib/refMrna.fa", sep = "/"),
  hmdir = getwd(),
  job_id = "ID",
  export_dir = paste("result", job_id, "SeqFragment2", sep = "."),
  netMHCIIpan_dir = paste(hmdir, "lib/netMHCIIpan-3.1/netMHCIIpan", sep = "/"),
  peptide_length = c(15),
  reference_nm_id = NA,
  reference_gene_symbol = NA,
  IgnoreShortPeptides = TRUE
)
```

Arguments

refmrna_file refMrna file to be used in constructing peptide (Default=paste(hmdir, "lib/refMrna.fa",

sep="").

See "https://github.com/hase62/Neoantimon"

hmdir Home directory for the analysis (Default = getwd()).

job_id Job-Id to be attached in output files (Default = "NO_job_id").

export_dir The directory will be stored results (Default = "paste("result", file_name_in_hla_table,

job_id, sep=".")")

netMHCIIpan_dir

The file directory to netMHCpan (Default="lib/netMHCIIpan-3.2/netMHCIIpan").

peptide_length Peptide Length to be generated (Default = 8,9,10,11,12,13).

reference_nm_id

Corresponding original sequences that the input sequence is generated. If franctions of peptides generated from the input are included in the indicated protein, such peptides are removed. It can be indicated when gene_symbol is not NA.

reference_gene_symbol

Corresponding original sequences that the input sequence is generated. If franctions of peptides generated from the input are included in the indicated protein, such peptides are removed. It can be indicated when nm_id is not NA.

IgnoreShortPeptides

Ignore to output results of Short Peptide Less Than min(peptide_length)

Value

void (Calculated Neoantigen Files will be generated as .tsv files.):

HLA: HLA type used to calculate neoantigen.

Pos: The position of a fraction of peptide used to be evaluated from the full-length peptide.

Gene: Gene symbol used to be evaluated in NetMHCpan.

Evaluated_Mutant_Peptide: The mutant peptide to be evaluated.

Evaluated_Mutant_Peptide_Core: The core peptide of the mutant peptide to be evaluated in NetMHC-pan.

Mut_IC50: IC50 value for evaluated mutant peptide.

Mut Rank: Rank value for evaluated mutanat peptide.

Chr: Chromosome Number of the mutation.

NM_ID: NM_ID used to construct peptides from the mutation.

Change: The annotation to be described in .vcf file.

Ref: reference type nucleic acid base.

Alt: alternative type nucleic acid base.

Prob: A probability of reference nucleic acid base described in .vcf file.

Mutation_Prob: A probability of alternative nucleic acid base described in .vcf file.

Exon_Start: The exon start position of the corrsponding NM_ID.

Exon_End: The exon end position of the corrsponding NM_ID.

Mutation_Position: The mutation position of the corrsponding NM_ID.

Total_Depth: The sum depth of the reference and alternative nucleic acid base.

Tumor_Depth: The depth of the alternative nucleic acid base.

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Wt_Peptide: The full-length of the wild-type peptide.

Mutant_Peptide: The full-length of the mutant peptide.

Total_RNA: The expression amount of the corresponding RNA.

Tumor_RNA_Ratio: The variant allele frequency of the corresponding RNA.

Tumor RNA: The modified amount of the corresponding RNA level based on RNA Reads.

Tumor_RNA_based_on_DNA: The modified amount of the corresponding RNA level based on DNA Reads.

MutRatio: The mean value of the cancer cell fraction probability.

MutRatio_Min: The 1% percentile of the cancer cell fraction probability.

MutRatio Max: The 99% percentile of the cancer cell fraction probability.

MainSNVClass1

Calculate Neoantigen Candidates on SNVs for MHC Class1

Description

Calculate Neoantigen Candidates on SNVs for MHC Class1

Usage

```
MainSNVClass1(
  input_file,
  hla_file = "here_is_a_table",
  hla_types = NA,
  file_name_in_hla_table = input_file,
  refflat_file = paste(hmdir, "lib/refFlat.txt", sep = "/"),
  refmrna_file = paste(hmdir, "lib/refMrna.fa", sep = "/"),
  hmdir = getwd(),
  job_id = "ID",
  export_dir = paste("result", job_id, "SNV1", sep = "."),
  rnaexp_file = NA,
  rnabam_file = NA,
  cnv_file = NA,
  purity = 1,
  netMHCpan_dir = paste(hmdir, "lib/netMHCpan-4.0/netMHCpan", sep = "/"),
  MHCflurry = NA,
  refdna_file = NA,
  samtools_dir = "samtools",
  bcftools_dir = NA,
  chr_column = NA,
  mutation_start_column = NA,
  mutation_end_column = NA,
  mutation_ref_column = NA,
  mutation_alt_column = NA,
  nm_id_column = NA,
  depth_normal_column = NA,
  depth_tumor_column = NA,
  ambiguous_between_exon = 0,
```

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```
ambiguous_codon = 0,
peptide_length = c(8, 9, 10, 11, 12, 13),
IgnoreShortPeptides = TRUE,
SNPs = NA,
multiple_variants = FALSE,
apply_annotation = FALSE
)
```

Arguments

input_file (Required) An input vcf file annotated by,

e.g., ANNOVAR (http://annovar.openbioinformatics.org/en/latest/) or other soft-

wares, or apply annotation_option = TRUE.

See by data(sample_vcf); sample_vcf;

hla_file A tab separated file indicating HLA types. The 1st column is input_file name,

and the following columns indicate HLA types.

See by data(sample_hla_table_c1); sample_hla_table_c1;

hla_types Set a list of HLA types

file_name_in_hla_table

If the name (1st column) in HLA table is not the same as input_file, indicate the

 $corresponding \ name \ (Default=input_file).$

refflat_file refFlat file to be used in constructing peptide. (Default=paste(hmdir, "lib/refFlat.txt",

sep="").

See "https://github.com/hase62/Neoantimon"

refmrna_file refMrna file to be used in constructing peptide (Default=paste(hmdir, "lib/refMrna.fa",

sep="").

See "https://github.com/hase62/Neoantimon"

hmdir Home directory for the analysis (Default = getwd()).

job_id Job-id to be attached in output files (Default = "NO_job_id").

export_dir The directory will be stored results (Default = "paste("result", file_name_in_hla_table,

job_id, sep=".")")

rnaexp_file A file including RNA expressions (Default=NA). The 1st, 2nd and 3rd columns

are "GeneSymbol Chr:Exonstart-Exonend (locus) ExpressionAmount", respec-

tively. The 1st row should be any header.

See by data(sample_rna_exp); sample_rna_exp;

rnabam_file RNA bam file to calculate variant allele frequency of RNA at each mutation

(Default=NA).

cnv_file A file including copy number variation to calculate cancer cell fraction prob-

ability (CCFP) (Default=NA). The format is according to ASCAT output files. The columns are "SNPName Chromosome Position LogR segmentedLogR BAF segmentedBAF CopyNumber MinorAllele RawCopyNumber" The 1st row should

be the above header.

See data(sample_copynum); sample_copynum;

purity Tumor purity or tumor contents ratio required to calculate CCFP (Default=1).

netMHCpan_dir The file directory to netMHCpan (Default="lib/netMHCpan-4.0/netMHCpan").

MHCflurry Also output results using MHCflurry (Default=FALSE).

refdna_file refdna_file information to be used to calculate RNA VAF (Default=NA).

See "https://github.com/hase62/Neoantimon"

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samtools_dir The file directory to samtools_0_x_x (Default="samtools"). It shouled be indicated when you indicate RNA-bam and try to calculate RNA VAF.

bcftools_dir The file directory to netMHCpan (Default="bcftools"). It shouled be indicated when you indicate RNA-bam and try to calculate RNA VAF . samtools 0_x_x

includes beftools in the directory.

chr_column The column number describing chromosome number in input_file (Default=NA,

but will automatically search "Chr" in header).

mutation_start_column

The column number describing mutation start Position in input_file (Default=NA, but will automatically search "Start" in header).

mutation_end_column

The column number describing mutation end Position in input_file (Default=NA, but will automatically search "End" in header).

mutation_ref_column

The column number describing mutation Ref in input_file (Default=NA, but will automatically search "Ref" in header).

mutation_alt_column

The column number describing mutation Alt in input_file (Default=NA, but will automatically search "Alt" in header).

nm_id_column The column number describing NM IDs in input_file such as

"SLCO1C1:NM_001145944:exon7:c.692_693insG:p.L231fs" (Default=NA).

depth_normal_column

The column number describing the read count from normal cells (Default = NA).

depth_tumor_column

The column number describing the read count from tumor cells (Default = NA).

ambiguous_between_exon

The maximum number to permit the differences between Exon-Lengths from refFlat and refMrna (Default=0).

ambiguous_codon

The maximum number to permit the differences between inputfile- and refMrnaoriented translation start/end position (Default=0).

peptide_length Peptide Length to be generated (Default = 8,9,10,11,12,13).

IgnoreShortPeptides

Ignore to output results of Short Peptide Less Than min (peptide_length)

SNPs Apply indivisual SNPs on peptides by indicate a vcf file.

multiple_variants

Reflect multiple variants on a peptide, e.g., SNVs on frameshift region.

apply_annotation

Anontate by Ensembl Variant Effect Predictor (VEP).

Value

void (Calculated Neoantigen Files will be generated as .tsv files.)

HLA: HLA type used to calculate neoantigen.

Pos: The position of a fraction of peptide used to be evaluated from the full-length peptide.

Gene: Gene symbol used to be evaluated in NetMHCpan.

Evaluated_Mutant_Peptide: The mutant peptide to be evaluated.

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Mut_IC50: IC50 value for evaluated mutant peptide.

Mut_Rank: Rank value for evaluated mutanat peptide.

Evaluated_Wt_Peptide: The wild-type peptide to be evaluated.

Wt_IC50: IC50 value for evaluated wild-type peptide.

Wt_Rank: Rank value for evaluated wild-type peptide.

Chr: Chromosome Number of the mutation.

NM_ID: NM_ID used to construct peptides from the mutation.

Change: The annotation to be described in .vcf file.

Ref: reference type nucleic acid base.

Alt: alternative type nucleic acid base.

Prob: A probability of reference nucleic acid base described in .vcf file.

Mutation_Prob: A probability of alternative nucleic acid base described in .vcf file.

Exon_Start: The exon start position of the corrsponding NM_ID.

Exon End: The exon end position of the corrsponding NM ID.

Mutation_Position: The mutation position of the corrsponding NM_ID.

Total_Depth: The sum depth of the reference and alternative nucleic acid base.

Tumor_Depth: The depth of the alternative nucleic acid base.

Wt_Peptide: The full-length of the wild-type peptide.

Mutant_Peptide: The full-length of the mutant peptide.

Total_RNA: The expression amount of the corresponding RNA.

Tumor_RNA_Ratio: The variant allele frequency of the corresponding RNA.

Tumor_RNA: The modified amount of the corresponding RNA level based on RNA Reads.

Tumor_RNA_based_on_DNA: The modified amount of the corresponding RNA level based on DNA Reads.

MutRatio: The mean value of the cancer cell fraction probability.

MutRatio_Min: The 1% percentile of the cancer cell fraction probability.

MutRatio_Max: The 99% percentile of the cancer cell fraction probability.

MainSNVClass2

Calculate Neoantigen Candidates on SNVs for MHC Class2

Description

Calculate Neoantigen Candidates on SNVs for MHC Class2

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Usage

```
MainSNVClass2(
  input_file,
  hla_file = "here_is_a_table",
  hla_{types} = NA,
  file_name_in_hla_table = input_file,
  refflat_file = paste(hmdir, "lib/refFlat.txt", sep = "/"),
  refmrna_file = paste(hmdir, "lib/refMrna.fa", sep = "/"),
  hmdir = getwd(),
  job_id = "ID",
  export_dir = paste("result", job_id, "SNV2", sep = "."),
  rnaexp_file = NA,
  rnabam_file = NA,
  cnv_file = NA,
  purity = 1,
  netMHCIIpan_dir = paste(hmdir, "lib/netMHCIIpan-3.2/netMHCIIpan", sep = "/"),
  refdna_file = NA,
  samtools_dir = "samtools",
  bcftools_dir = NA,
  chr_column = NA,
  mutation_start_column = NA,
  mutation_end_column = NA,
  mutation_ref_column = NA,
  mutation_alt_column = NA,
  nm_id_column = NA,
  depth_normal_column = NA,
  depth_tumor_column = NA,
  ambiguous_between_exon = 0,
  ambiguous_codon = 0,
  peptide_length = c(15),
  IgnoreShortPeptides = TRUE,
  SNPs = NA,
  multiple_variants = FALSE,
  apply_annotation = FALSE
)
```

Arguments

```
input_file
                  (Required) An input vcf file annotated by,
                  e.g., ANNOVAR (http://annovar.openbioinformatics.org/en/latest/) or other soft-
                  wares.
                  See by data(sample_vcf); sample_vcf;
                  A tab separated file indicating HLA types. The 1st column is input file name,
hla_file
                  and the following columns indicate HLA types.
                  See by data(sample_hla_table_c2); sample_hla_table_c2;
hla_types
                  Set a list of HLA types
file_name_in_hla_table
                  If the name (1st column) in HLA table is not the same as input_file, indicate the
                  corresponding name (Default=input_file).
                  refFlat file to be used in constructing peptide. (Default=paste(hmdir, "lib/refFlat.txt",
refflat_file
                  sep="").
```

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See "https://github.com/hase62/Neoantimon"

refmrna_file refMrna file to be used in constructing peptide (Default=paste(hmdir, "lib/refMrna.fa",

sep="").

See "https://github.com/hase62/Neoantimon"

hmdir Home directory for the analysis (Default = getwd()).

job_id Job-Id to be attached in output files (Default = "NO_job_id").

export_dir The directory will be stored results (Default = "paste("result", file_name_in_hla_table,

job_id, sep=".")")

rnaexp_file A file including RNA expressions (Default=NA). The 1st, 2nd and 3rd columns

are "GeneSymbol Chr:Exonstart-Exonend (locus) ExpressionAmount", respec-

tively. The 1st row should be any header.

See by data(sample_rna_exp); sample_rna_exp;

rnabam_file RNA bam file to calculate variant allele frequency of RNA at each mutation

(Default=NA).

cnv_file A file including copy number variation to calculate cancer cell fraction prob-

ability (CCFP) (Default=NA). The format is according to ASCAT output files. The columns are "SNPName Chromosome Position LogR segmentedLogR BAF segmentedBAF CopyNumber MinorAllele RawCopyNumber" The 1st row should

be the above header.

See data(sample_copynum); sample_copynum;

purity Tumor purity or tumor contents ratio required to calculate CCFP (Default=1).

netMHCIIpan_dir

The file directory to netMHCpan (Default="lib/netMHCIIpan-3.2/netMHCpan").

refdna_file refdna_file information to be used to calculate RNA VAF (Default=NA).

See "https://github.com/hase62/Neoantimon"

samtools_dir The file directory to samtools_0_x_x (Default="samtools"). It shouled be indi-

cated when you indicate RNA-bam and try to calculate RNA VAF.

bcftools_dir The file directory to netMHCpan (Default="bcftools"). It shouled be indicated

when you indicate RNA-bam and try to calculate RNA VAF . samtools 0_x_x

includes beftools in the directory.

chr_column The column number describing chromosome number in input_file (Default=NA,

but will automatically search "Chr" in header).

 ${\it mutation_start_column}$

The column number describing mutation start Position in input_file (Default=NA, but will automatically search "Start" in header) .

mutation_end_column

The column number describing mutation end Position in input_file (Default=NA, but will automatically search "End" in header).

mutation_ref_column

The column number describing mutation Ref in input_file (Default=NA, but will automatically search "Ref" in header).

mutation_alt_column

The column number describing mutation Alt in input_file (Default=NA, but will automatically search "Alt" in header).

nm_id_column The column number describing NM IDs in input_file such as

"SLCO1C1:NM_001145944:exon7:c.692_693insG:p.L231fs" (Default=NA).

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depth_normal_column

The column number describing the read count from normal cells (Default = NA).

depth_tumor_column

The column number describing the read count from tumor cells (Default = NA).

ambiguous_between_exon

The maximum number to permit the differences between Exon-Lengths from refFlat and refMrna (Default=0).

ambiguous_codon

The maximum number to permit the differences between inputfile- and refMrnaoriented translation start/end position (Default=0).

peptide_length Peptide Length to be generated (Default = 15 in HLA Class2).

IgnoreShortPeptides

Ignore to output results of Short Peptide Less Than min (peptide_length)

SNPs Apply indivisual SNPs on peptides by indicate a vcf file.

multiple_variants

Reflect multiple variants on a peptide, e.g., SNVs on frameshift region.

apply_annotation

Anontate by Ensembl Variant Effect Predictor (VEP).

Value

void (Calculated Neoantigen Files will be generated as .tsv files.):

HLA: HLA type used to calculate neoantigen.

Pos: The position of a fraction of peptide used to be evaluated from the full-length peptide.

Gene: Gene symbol used to be evaluated in NetMHCpan.

Evaluated_Mutant_Peptide: The mutant peptide to be evaluated.

Mut_IC50: IC50 value for evaluated mutant peptide.

Mut Rank: Rank value for evaluated mutanat peptide.

Evaluated_Wt_Peptide: The wild-type peptide to be evaluated.

Wt_IC50: IC50 value for evaluated wild-type peptide.

Wt_Rank: Rank value for evaluated wild-type peptide.

Chr: Chromosome Number of the mutation.

NM_ID: NM_ID used to construct peptides from the mutation.

Change: The annotation to be described in .vcf file.

Ref: reference type nucleic acid base.

Alt: alternative type nucleic acid base.

Prob: A probability of reference nucleic acid base described in .vcf file.

Mutation_Prob: A probability of alternative nucleic acid base described in .vcf file.

Exon_Start: The exon start position of the corrsponding NM_ID.

Exon_End: The exon end position of the corrsponding NM_ID.

Mutation_Position: The mutation position of the corrsponding NM_ID.

Total_Depth: The sum depth of the reference and alternative nucleic acid base.

Tumor_Depth: The depth of the alternative nucleic acid base.

Wt_Peptide: The full-length of the wild-type peptide.

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Mutant_Peptide: The full-length of the mutant peptide.

Total_RNA: The expression amount of the corresponding RNA.

Tumor_RNA_Ratio: The variant allele frequency of the corresponding RNA.

Tumor_RNA: The modified amount of the corresponding RNA level based on RNA Reads.

Tumor_RNA_based_on_DNA: The modified amount of the corresponding RNA level based on DNA Reads.

MutRatio: The mean value of the cancer cell fraction probability.

MutRatio_Min: The 1% percentile of the cancer cell fraction probability.

MutRatio_Max: The 99% percentile of the cancer cell fraction probability.

MainSVFUSIONClass1

Calculate Neoantigen Candidates on SV fusions for MHC Class1

Description

Calculate Neoantigen Candidates on SV fusions for MHC Class1

Usage

```
MainSVFUSIONClass1(
  input_file,
  hla_file = "here_is_a_table",
  hla_{types} = NA,
  file_name_in_hla_table = input_file,
  refflat_file = paste(hmdir, "lib/refFlat.txt", sep = "/"),
  refmrna_file = paste(hmdir, "lib/refMrna.fa", sep = "/"),
  hmdir = getwd(),
  job_id = "ID",
  export_dir = paste("result", job_id, "SV1", sep = "."),
  rnaexp_file = NA,
  rnabam_file = NA,
  cnv_file = NA,
  purity = 1,
  netMHCpan_dir = paste(hmdir, "lib/netMHCpan-4.0/netMHCpan", sep = "/"),
  refdna_file = NA,
  samtools_dir = NA,
  bcftools_dir = NA,
  chr_column = NA,
  mutation_start_column = NA,
  mutation_end_column = NA,
  mutation_ref_column = NA,
  mutation_alt_bnd_column = NA,
  depth_normal_column = NA,
  depth_tumor_column = NA,
  nm_id_column = NA,
  ambiguous_between_exon = 0,
  ambiguous_codon = 0,
  peptide_length = c(8, 9, 10, 11, 12, 13),
  gene_symbol_column = NA,
```

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```
mate_id_column = NA,
  IgnoreShortPeptides = TRUE
)
```

Arguments

input_file (Required) An input vcf file (BND format) annotated by,

e.g., ANNOVAR (http://annovar.openbioinformatics.org/en/latest/) or other soft-

wares.

See by data(sample_sv_bnd); sample_sv_bnd;

hla_file (Required) A tab separated file indicating HLA types. The 1st column is in-

put_file name, and the following columns indicate HLA types. See by data(sample_hla_table_c1); sample_hla_table_c1;

hla_types Set a list of HLA types

file_name_in_hla_table

If the name (1st column) in HLA table is not the same as input_file, indicate the

corresponding name (Default=input_file).

refflat_file refFlat file to be used in constructing peptide. (Default=paste(hmdir, "lib/refFlat.txt",sep="").

See "https://github.com/hase62/Neoantimon"

refmrna_file refMrna file to be used in constructing peptide (Default=paste(hmdir, "lib/refMrna.fa",

sep="").

See "https://github.com/hase62/Neoantimon"

hmdir Home directory for the analysis (Default = getwd()).

job_id Job-Id to be attached in output files (Default = "NO job id").

export_dir The directory will be stored results (Default = "paste("result", file_name_in_hla_table,

job_id, sep=".")")

rnaexp_file A file including RNA expressions (Default=NA). The 1st, 2nd and 3rd columns

are "GeneSymbol Chr:Exonstart-Exonend (locus) ExpressionAmount", respec-

tively. The 1st row should be any header.

See by data(sample_rna_exp); sample_rna_exp;

rnabam_file RNA bam file to calculate variant allele frequency of RNA at each mutation

(Default=NA).

cnv_file A file including copy number variation to calculate cancer cell fraction prob-

ability (CCFP) (Default=NA). The format is according to ASCAT output files. The columns are "SNPName Chromosome Position LogR segmentedLogR BAF segmentedBAF CopyNumber MinorAllele RawCopyNumber" The 1st row should

be the above header.

See data(sample_copynum); sample_copynum;

purity Tumor purity or tumor contents ratio required to calculate CCFP (Default=1).

netMHCpan_dir The file directory to netMHCpan (Default="lib/netMHCpan-4.0/netMHCpan").

refdna_file (Required) refdna_file information to be used to create SVs Region (Default=NA).

See "https://github.com/hase62/Neoantimon"

samtools_dir The file directory to samtools_0_x_x (Default="samtools"). It shouled be indi-

cated when you indicate RNA-bam and try to calculate RNA VAF.

bcftools_dir The file directory to netMHCpan (Default="bcftools"). It shouled be indicated

when you indicate RNA-bam and try to calculate RNA VAF . samtools 0_x_x

includes beftools in the directory.

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chr_column The column number describing chromosome number in input_file (Default=NA, but will automatically search "Chr" in header).

mutation_start_column

The column number describing mutation start Position in input_file (Default=NA, but will automatically search "Start" in header).

mutation_end_column

The column number describing mutation end Position in input_file (Default=NA, but will automatically search "End" in header).

mutation_ref_column

The column number describing mutation Ref in input_file (Default=NA, but will automatically search "Ref" in header).

mutation_alt_bnd_column

The column number describing mutation Alt (BND format) in input_file (Default=NA, but will automatically search "Alt" in header).

depth_normal_column

The column number describing the read count from normal cells (Default = NA).

depth_tumor_column

The column number describing the read count from tumor cells (Default = NA).

nm_id_column (Required if gene_symbol_column = NA) The column number describing NM IDs in input_file such as

"SLCO1C1:NM_001145944:exon7:c.692_693insG:p.L231fs" (Default=NA).

ambiguous_between_exon

The maximum number to permit the differences between Exon-Lengths from refFlat and refMrna (Default=0).

ambiguous_codon

The maximum number to permit the differences between inputfile- and refMrnaoriented translation start/end position (Default=0).

peptide_length Peptide Length to be generated (Default = 8,9,10,11,12,13).

gene_symbol_column

(Required if nm_id_column = NA) The column number describing gene symbol in input_file (Default=NA).

mate_id_column (Required) The column indicating mateIDs or svIDs such as "SVMERGE1_1" (Default=NA).

IgnoreShortPeptides

Ignore Short Peptide Less Than min(peptide_length)

Value

void (Calculated Neoantigen Files will be generated as .tsv files.):

HLA: HLA type used to calculate neoantigen.

Pos: The position of the fraction of peptide used to be evaluated from the full-length peptide.

Gene: Gene symbol used to be evaluated in NetMHCpan.

Evaluated_Mutant_Peptide_Core: The core peptide of the mutant peptide to be evaluated in NetMHC-pan.

Evaluated_Mutant_Peptide: The mutant peptide to be evaluated.

Mut_IC50: IC50 value for evaluated mutant peptide.

Mut_Rank: Rank value for evaluated mutanat peptide.

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Chr: Chromosome Number of the mutation.

NM_ID: NM_ID used to construct peptides from the mutation.

Change: The annotation to be described in .vcf file.

Ref: reference type nucleic acid base.

Alt: alternative type nucleic acid base.

Prob: A probability of reference nucleic acid base described in .vcf file.

Mutation_Prob: A probability of alternative nucleic acid base described in .vcf file.

Exon Start: The exon start position of the corrsponding NM ID.

Exon_End: The exon end position of the corrsponding NM_ID.

Mutation_Position: The mutation position of the corrsponding NM_ID.

Total_Depth: The sum depth of the reference and alternative nucleic acid base.

Tumor_Depth: The depth of the alternative nucleic acid base.

Wt_Peptide: The full-length of the wild-type peptide.

Mutant_Peptide: The full-length of the mutant peptide.

Total_RNA: The expression amount of the corresponding RNA.

Tumor_RNA_Ratio: The variant allele frequency of the corresponding RNA.

Tumor_RNA: The modified amount of the corresponding RNA level based on RNA Reads.

Tumor_RNA_based_on_DNA: The modified amount of the corresponding RNA level based on DNA Reads.

MutRatio: The mean value of the cancer cell fraction probability.

MutRatio_Min: The 1% percentile of the cancer cell fraction probability.

MutRatio_Max: The 99% percentile of the cancer cell fraction probability.

MainSVFUSIONClass2

Calculate Neoantigen Candidates on SV fusions for MHC Class2

Description

Calculate Neoantigen Candidates on SV fusions for MHC Class2

Usage

```
MainSVFUSIONClass2(
  input_file,
  hla_file = "here_is_a_table",
  hla_types = NA,
  file_name_in_hla_table = input_file,
  refflat_file = paste(hmdir, "lib/refFlat.txt", sep = "/"),
  refmrna_file = paste(hmdir, "lib/refMrna.fa", sep = "/"),
  hmdir = getwd(),
  job_id = "ID",
  export_dir = paste("result", job_id, "SV2", sep = "."),
  rnaexp_file = NA,
  rnabam_file = NA,
```

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```
cnv_file = NA,
  purity = 1,
  netMHCIIpan_dir = paste(hmdir, "lib/netMHCIIpan-3.1/netMHCIIpan", sep = "/"),
  refdna_file = NA,
  samtools_dir = NA,
 bcftools_dir = NA,
  chr_column = NA,
 mutation_start_column = NA,
 mutation_end_column = NA,
 mutation_ref_column = NA,
 mutation_alt_bnd_column = NA,
  depth_normal_column = NA,
  depth_tumor_column = NA,
  nm_id_column = NA,
  ambiguous_between_exon = 0,
  ambiguous_codon = 0,
  peptide_length = c(15),
 gene_symbol_column = NA,
 mate_id_column = NA,
  IgnoreShortPeptides = TRUE
)
```

(Required) An input vcf file (BND format) annotated by,

Arguments

input_file

export_dir

rnaexp_file

job_id, sep=".")")

tively. The 1st row should be any header.
See by data(sample_rna_exp); sample_rna_exp;

e.g., ANNOVAR (http://annovar.openbioinformatics.org/en/latest/) or other softwares. See by data(sample_sv_bnd); sample_sv_bnd; (Required) A tab separated file indicating HLA types. The 1st column is inhla_file put_file name, and the following columns indicate HLA types. See by data(sample_hla_table_c1); sample_hla_table_c1; hla_types Set a list of HLA types file_name_in_hla_table If the name (1st column) in HLA table is not the same as input_file, indicate the corresponding name (Default=input_file). refflat_file refFlat file to be used in constructing peptide. (Default=paste(hmdir, "lib/refFlat.txt",sep=""). See "https://github.com/hase62/Neoantimon" refMrna file to be used in constructing peptide (Default=paste(hmdir, "lib/refMrna.fa", refmrna_file sep=""). See "https://github.com/hase62/Neoantimon" hmdir Home directory for the analysis (Default = getwd()). Job-Id to be attached in output files (Default = "NO_job_id"). job_id

The directory will be stored results (Default = "paste("result", file_name_in_hla_table,

A file including RNA expressions (Default=NA). The 1st, 2nd and 3rd columns

are "GeneSymbol Chr:Exonstart-Exonend (locus) ExpressionAmount", respec-

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rnabam_file RNA bam file to calculate variant allele frequency of RNA at each mutation (Default=NA). A file including copy number variation to calculate cancer cell fraction probcnv_file ability (CCFP) (Default=NA). The format is according to ASCAT output files. The columns are "SNPName Chromosome Position LogR segmentedLogR BAF segmentedBAF CopyNumber MinorAllele RawCopyNumber" The 1st row should be the above header. See data(sample_copynum); sample_copynum; Tumor purity or tumor contents ratio required to calculate CCFP (Default=1). purity netMHCIIpan_dir The file directory to netMHCpan (Default="lib/netMHCIIpan-3.2/netMHCpan"). refdna_file (Required) refdna_file information to be used to create SVs Region (Default=NA). See "https://github.com/hase62/Neoantimon" samtools_dir The file directory to samtools_0_x_x (Default="samtools"). It shouled be indicated when you indicate RNA-bam and try to calculate RNA VAF. The file directory to netMHCpan (Default="bcftools"). It shouled be indicated bcftools_dir when you indicate RNA-bam and try to calculate RNA VAF . samtools 0_x_x includes beftools in the directory. chr_column The column number describing chromosome number in input_file (Default=NA, but will automatically search "Chr" in header). mutation_start_column The column number describing mutation start Position in input_file (Default=NA, but will automatically search "Start" in header). mutation_end_column The column number describing mutation end Position in input_file (Default=NA, but will automatically search "End" in header). mutation_ref_column The column number describing mutation Ref in input file (Default=NA, but will automatically search "Ref" in header). mutation_alt_bnd_column The column number describing mutation Alt (BND format) in input_file (Default=NA, but will automatically search "Alt" in header). depth_normal_column depth_tumor_column The column number describing the read count from tumor cells (Default = NA). nm_id_column IDs in input_file such as

The column number describing the read count from normal cells (Default = NA).

(Required if gene_symbol_column = NA) The column number describing NM

"SLCO1C1:NM_001145944:exon7:c.692_693insG:p.L231fs" (Default=NA).

ambiguous_between_exon

The maximum number to permit the differences between Exon-Lengths from refFlat and refMrna (Default=0).

ambiguous_codon

The maximum number to permit the differences between inputfile- and refMrnaoriented translation start/end position (Default=0).

peptide_length Peptide Length to be generated (Default = 15 in HLA Class2). gene_symbol_column

> (Required if nm_id_column = NA) The column number describing gene symbol in input_file (Default=NA).

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mate_id_column (Required) The column indicating mateIDs or svIDs such as "SVMERGE1_1" (Default=NA).

IgnoreShortPeptides

Ignore Short Peptide Less Than min(peptide_length)

Value

void (Calculated Neoantigen Files will be generated as .tsv files.):

HLA: HLA type used to calculate neoantigen.

Pos: The position of the fraction of peptide used to be evaluated from the full-length peptide.

Gene: Gene symbol used to be evaluated in NetMHCpan.

Evaluated_Mutant_Peptide_Core: The core peptide of the mutant peptide to be evaluated in NetMHC-pan.

Evaluated_Mutant_Peptide: The mutant peptide to be evaluated.

Mut_IC50: IC50 value for evaluated mutant peptide.

Mut_Rank: Rank value for evaluated mutanat peptide.

Chr: Chromosome Number of the mutation.

NM_ID: NM_ID used to construct peptides from the mutation.

Change: The annotation to be described in .vcf file.

Ref: reference type nucleic acid base.

Alt: alternative type nucleic acid base.

Prob: A probability of reference nucleic acid base described in .vcf file.

Mutation_Prob: A probability of alternative nucleic acid base described in .vcf file.

Exon_Start: The exon start position of the corrsponding NM_ID.

Exon_End: The exon end position of the corrsponding NM_ID.

Mutation_Position: The mutation position of the corrsponding NM_ID.

Total_Depth: The sum depth of the reference and alternative nucleic acid base.

Tumor_Depth: The depth of the alternative nucleic acid base.

Wt_Peptide: The full-length of the wild-type peptide.

Mutant_Peptide: The full-length of the mutant peptide.

Total RNA: The expression amount of the corresponding RNA.

Tumor RNA Ratio: The variant allele frequency of the corresponding RNA.

Tumor_RNA: The modified amount of the corresponding RNA level based on RNA Reads.

Tumor_RNA_based_on_DNA: The modified amount of the corresponding RNA level based on DNA Reads.

MutRatio: The mean value of the cancer cell fraction probability.

MutRatio_Min: The 1% percentile of the cancer cell fraction probability.

MutRatio_Max: The 99% percentile of the cancer cell fraction probability.

36 sample_hla_table_c2

sample_copynum

A Format / Sample file for Copy Number Information

Description

A dataset containing the copy number information obtained by, e.g., ASCAT.

Usage

```
data(sample_copynum)
```

Format

A data frame with 7 rows and 9 variables

sample_hla_table_c1

A Format / Sample file for HLA CLASS1 Table

Description

A dataset containing the HLA types of patients in each row.

Usage

```
data(sample_hla_table_c1)
```

Format

A data frame with 3 rows and at most 7 variables

sample_hla_table_c2

A Format / Sample file for HLA CLASS2 Table

Description

A dataset containing the HLA types of patients in each row.

Usage

```
data(sample_hla_table_c2)
```

Format

A data frame with at least 3 row and at most 10 variables

```
sample_result_INDEL_CLASS1_ALL
```

Analyzed Result for INDEL CLASS1

Description

Analyzed Result for INDEL CLASS1

Usage

```
data(sample_result_INDEL_CLASS1_ALL)
```

```
sample_result_INDEL_CLASS2_ALL
```

Analyzed Result for INDEL CLASS2

Description

Analyzed Result for INDEL CLASS2

Usage

```
data(sample_result_INDEL_CLASS2_ALL)
```

```
sample_result_SeqFragment_CLASS1_ALL
```

Analyzed Result for A DNA Fragment CLASS1

Description

Analyzed Result for A DNA Fragment CLASS1

Usage

```
data(sample_result_SeqFragment_CLASS1_ALL)
```

```
{\tt sample\_result\_SeqFragment\_CLASS2\_ALL}
```

Analyzed Result for A DNA Fragment CLASS2

Description

Analyzed Result for A DNA Fragment CLASS2

Usage

```
data(sample_result_SeqFragment_CLASS2_ALL)
```

```
sample_result_SNV_CLASS1_ALL

Analyzed Result for SNV CLASS1
```

Description

Analyzed Result for SNV CLASS1

Usage

```
data(sample_result_SNV_CLASS1_ALL)
```

```
sample_result_SNV_CLASS2_ALL
```

Analyzed Result for SNV CLASS2

Description

Analyzed Result for SNV CLASS2

Usage

```
data(sample_result_SNV_CLASS2_ALL)
```

```
sample_result_SVFusion_CLASS1_ALL

Analyzed Result for SV Fusion CLASS1
```

Description

Analyzed Result for SV Fusion CLASS1

Usage

```
data(sample_result_SVFusion_CLASS1_ALL)
```

```
sample\_result\_SVFusion\_CLASS2\_ALL\\ Analyzed\ Result\ for\ SVFusion\ CLASS2
```

Description

Analyzed Result for SVFusion CLASS2

Usage

```
data(sample_result_SVFusion_CLASS2_ALL)
```

sample_rna_exp 39

sample_rna_exp

A Format / Sample file for RNA Expression Information

Description

A dataset containing the RNA expression amount of patient for each gene.

Usage

```
data(sample_rna_exp)
```

Format

A data frame with 22 rows and 3 variables

sample_sv_bnd

A Format / Sample file for Annotated vcf file.

Description

A dataset containing the variant information of a patient.

Usage

```
data(sample_sv_bnd)
```

Format

A data frame with 9 rows and variables including "Chr" "Start" "End" "Ref" "Alt (BND format)" "Func.refGene (exonic, intron, intergenic, ...)" "ExonicFunc.refGene (exonic nonsynonymous, synonymous, insertion, ...)" "mateID (e.g., SVMERGE1_1)"

sample_vcf

A Format / Sample file for Annotated vcf file.

Description

A dataset containing the variant information of a patient.

Usage

```
data(sample_vcf)
```

Format

A data frame with 9 rows and variables including "Chr" "Start" "End" "Ref" "Alt" "Func.refGene (exonic, intron, intergenic, ...)" "ExonicFunc.refGene (exonic nonsynonymous, synonymous, insertion, ...)" "AAChange.refGene (e.g., SLCO1C1:NM_001145944:exon7:c.692_693insG:p.L231fs ...)"

40 TestAnalysis

 ${\it TestAnalysis}$

Execute Sample Analysis

Description

Execute Sample Analysis

Usage

TestAnalysis()

Value

void

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