

SomaticCancerAlterations

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1 Motivation

Over the last years, large efforts have been taken to characterize the somatic landscape of cancers. Many of the conducted studies make their results publicly available, providing a valuable resource for investigating beyond the level of individual cohorts. The *SomaticCancerAlterations* package collects mutational data of several tumor types, currently focusing on the TCGA calls sets, and aims for a tight integration with *R* and *Bioconductor* workflows. In the following, we will illustrate how to access this data and give examples for use cases.

2 Data Sets

The Cancer Genome Atlas (TCGA)¹ is a consortium effort to analyze a variety of tumor types, including gene expression, methylation, copy number changes, and somatic mutations². With the *SomaticCancerAlterations* package, we provide the callsets of somatic mutations for all publically available TCGA studies. Over time, more studies will be added, as they become available and unrestricted in their usage.

To get started, we get a list of all available data sets and access the metadata associated with each study.

```
all_datasets = scaListDatasets()
print(all_datasets)
```

¹<http://cancergenome.nih.gov>

²<https://wiki.nci.nih.gov/display/TCGA/TCGA+Home>

```
## [1] "gbm_tcga" "hnesc_tcga" "kirc_tcga" "luad_tcga" "lusc_tcga" "ov_tcga" "skcm_tcga"
## [8] "thca_tcga"

meta_data = scaMetadata()
print(meta_data)

##           Cancer_Type      Center NCBI_Build Sequence_Source Sequencing_Phase
## gbm_tcga      GBM broad.mit.edu      37          WXS          Phase_I
## hnesc_tcga    HNSC broad.mit.edu      37          Capture        Phase_I
## kirc_tcga     KIRC broad.mit.edu      37          Capture        Phase_I
## luad_tcga     LUAD broad.mit.edu      37          WXS          Phase_I
## lusc_tcga     LUSC broad.mit.edu      37          WXS          Phase_I
## ov_tcga       OV broad.mit.edu      37          WXS          Phase_I
## skcm_tcga     SKCM broad.mit.edu      37          Capture        Phase_I
## thca_tcga     THCA broad.mit.edu      37          WXS          Phase_I
##
##           Sequencer Number_Samples Number_Patients
## gbm_tcga  Illumina GAIIX           291           291
## hnesc_tcga Illumina GAIIX           319           319
## kirc_tcga  Illumina GAIIX           297           293
## luad_tcga  Illumina GAIIX           538           519
## lusc_tcga  Illumina GAIIX           178           178
## ov_tcga    Illumina GAIIX           142           142
## skcm_tcga  Illumina GAIIX           266           264
## thca_tcga  Illumina GAIIX           406           403
##
##           Cancer_Name
## gbm_tcga      Glioblastoma multiforme
## hnesc_tcga    Head and Neck squamous cell carcinoma
## kirc_tcga     Kidney Chromophobe
## luad_tcga     Lung adenocarcinoma
## lusc_tcga     Lung squamous cell carcinoma
## ov_tcga       Ovarian serous cystadenocarcinoma
## skcm_tcga     Skin Cutaneous Melanoma
## thca_tcga     Thyroid carcinoma
```

Next, we load a single dataset with the `scaLoadDataset` function.

```
ov = scaLoadDatasets("ov_tcga", merge = TRUE)
```

3 Exploring Mutational Data

The somatic variants of each study are represented as a object, ordered by genomic positions. Additional columns describe properties of the variant and relate it to the affected gene, sample, and patient.

```
head(ov, 3)

## GRanges with 3 ranges and 14 metadata columns:
##           seqnames           ranges strand | Hugo_Symbol Entrez_Gene_Id
##           <Rle>             <IRanges> <Rle> |   <factor>      <integer>
##   ov_tcga      1 [1334552, 1334552]   * |     CCNL2         81669
##   ov_tcga      1 [1961652, 1961652]   * |     GABRD          2563
##   ov_tcga      1 [2420688, 2420688]   * |     PLCH2          9651
##           Variant_Classification Variant_Type Reference_Allele Tumor_Seq_Allele1
```

```
##           <factor>      <factor>      <factor>      <factor>
## ov_tcga      Silent      SNP          C          C
## ov_tcga      Silent      SNP          C          C
## ov_tcga      Missense_Mutation  SNP          C          C
##           Tumor_Seq_Allele2 Verification_Status Validation_Status Mutation_Status
##           <factor>      <factor>      <factor>      <factor>
## ov_tcga      T          Unknown      Valid      Somatic
## ov_tcga      T          Unknown      Valid      Somatic
## ov_tcga      G          Unknown      Valid      Somatic
##           Patient_ID      Sample_ID      index      Dataset
##           <factor>      <factor> <integer> <factor>
## ov_tcga TCGA-24-2262 TCGA-24-2262-01A-01W-0799-08      3901 ov_tcga
## ov_tcga TCGA-24-1552 TCGA-24-1552-01A-01W-0551-08      3414 ov_tcga
## ov_tcga TCGA-13-1484 TCGA-13-1484-01A-01W-0545-08      1567 ov_tcga
## ---
## seqlengths:
##           1           2           3           4 ... GL000192.1 NC_007605      hs37d5
##           249250621 243199373 198022430 191154276 ...      547496      171823      35477943
```

```
with(mcols(ov), table(Variant_Classification, Variant_Type))
```

```
##           Variant_Type
## Variant_Classification DEL INS SNP
## 3'UTR                  0  0  3
## 5'Flank                 0  0  1
## 5'UTR                   0  0  1
## Frame_Shift_Del        79  0  0
## Frame_Shift_Ins         0 16  0
## IGR                     0  0  5
## In_Frame_Del           26  0  0
## In_Frame_Ins            0  1  0
## Intron                  0  0 34
## Missense_Mutation       0  0 4299
## Nonsense_Mutation       0  0  285
## Nonstop_Mutation        0  0   6
## RNA                     0  0   1
## Silent                   0  0 1417
## Splice_Site              9  2  121
## Translation_Start_Site  1  0   1
```

With such data at hand, we can identify the samples and genes harboring the most mutations.

```
head(sort(table(ov$Sample_ID), decreasing = TRUE))
##
## TCGA-09-2049-01D-01W-0799-08 TCGA-13-0923-01A-01W-0420-08 TCGA-09-2050-01A-01W-0799-08
##           119           118           111
## TCGA-25-1326-01A-01W-0492-08 TCGA-25-1313-01A-01W-0492-08 TCGA-23-1110-01A-01D-0428-08
##           110           104           102

head(sort(table(ov$Hugo_Symbol), decreasing = TRUE), 10)
##
## TP53      TTN PCDHAC2 MUC16 MUC17 PCDHGC5 USH2A CSMD3 CD163L1 DYNC1H1
## 118      30 14 12 9 9 9 8 7 7
```

4 Exploring Multiple Studies

Instead of focusing on an individual study, we can also import several at once. The results are stored as a *GRangesList* in which each element corresponds to a single study. This can be merged into a single *GRanges* object with `merge = TRUE`.

```
three_studies = scaLoadDatasets(all_datasets[1:3])
print(elementLengths(three_studies))

## gbm_tcga hnscc_tcga kirc_tcga
##      22166      73766      26265

class(three_studies)

## [1] "SimpleGenomicRangesList"
## attr(,"package")
## [1] "GenomicRanges"
```

```
merged_studies = scaLoadDatasets(all_datasets[1:3], merge = TRUE)
class(merged_studies)

## [1] "GRanges"
## attr(,"package")
## [1] "GenomicRanges"
```

We then compute the number of mutations per gene and study:

```
gene_study_count = with(mcols(merged_studies), table(Hugo_Symbol, Dataset))
gene_study_count = gene_study_count[order(apply(gene_study_count, 1, sum), decreasing = TRUE),
]
gene_study_count = addmargins(gene_study_count)
head(gene_study_count)
```

| | Dataset | | | | |
|----------------|----------|------------|-----------|------|--|
| ## Hugo_Symbol | gbm_tcga | hnscc_tcga | kirc_tcga | Sum | |
| ## Unknown | 29 | 899 | 630 | 1558 | |
| ## TTN | 121 | 401 | 125 | 647 | |
| ## TP53 | 101 | 323 | 8 | 432 | |
| ## MUC16 | 68 | 155 | 46 | 269 | |
| ## ADAM6 | 0 | 173 | 63 | 236 | |
| ## MUC4 | 17 | 32 | 130 | 179 | |

Further, we can subset the data by regions of interests, and compute descriptive statistics only on the subset.

```
tp53_region = GRanges("17", IRanges(7571720, 7590863))
tp53_studies = subsetByOverlaps(merged_studies, tp53_region)
```

For example, we can investigate which type of somatic variants can be found in TP53 throughout the studies.

```
addmargins(table(tp53_studies$Variant_Classification, tp53_studies$Dataset))

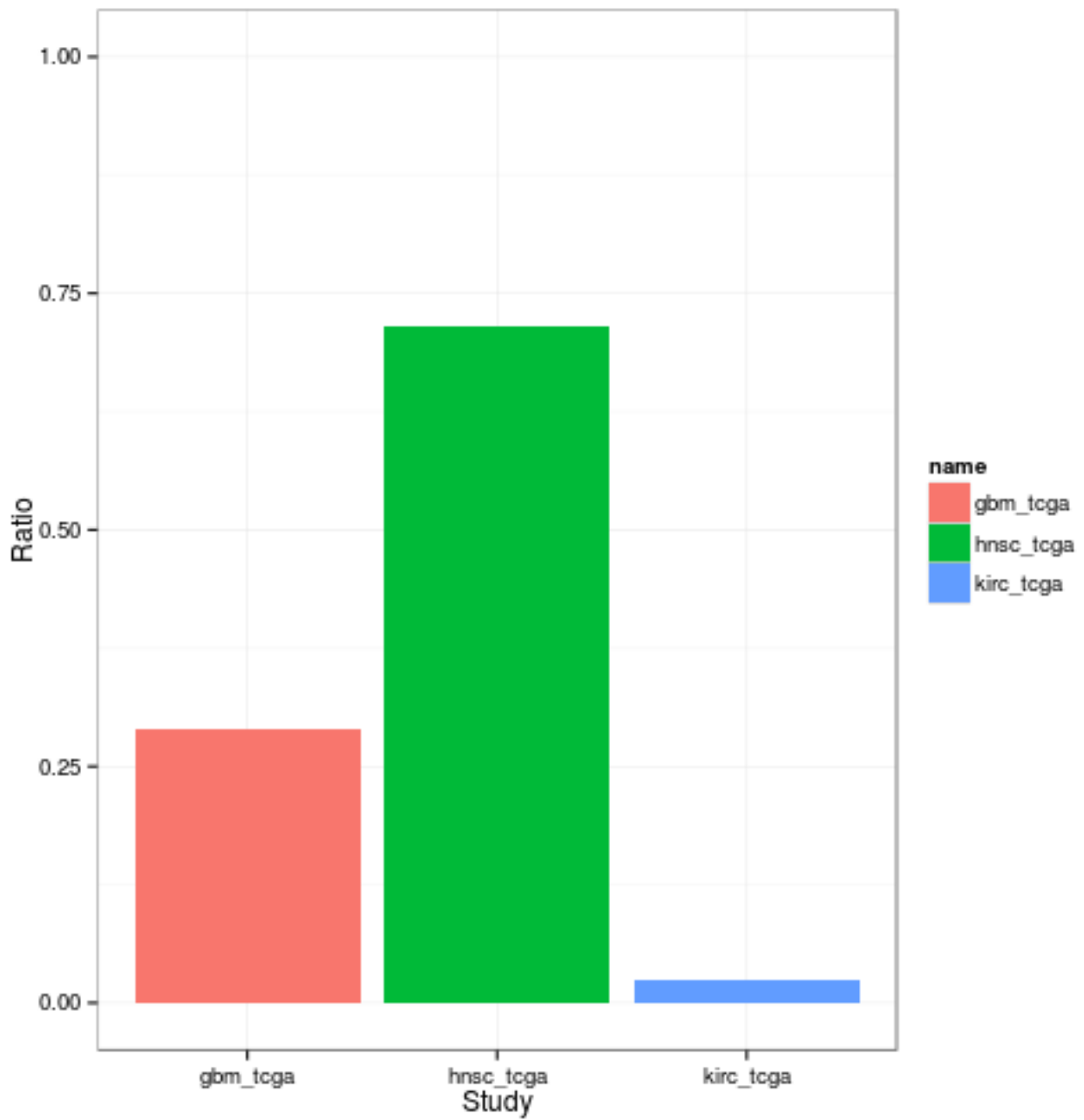
##
##           gbm_tcga hnscc_tcga kirc_tcga Sum
## Frame_Shift_Del           6           41           0  47
```

| | | | | | |
|----|------------------------|-----|-----|---|-----|
| ## | Frame_Shift_Ins | 1 | 11 | 0 | 12 |
| ## | In_Frame_Del | 2 | 7 | 0 | 9 |
| ## | In_Frame_Ins | 0 | 2 | 0 | 2 |
| ## | Missense_Mutation | 81 | 183 | 6 | 270 |
| ## | Nonsense_Mutation | 4 | 54 | 0 | 58 |
| ## | Nonstop_Mutation | 0 | 0 | 0 | 0 |
| ## | Silent | 1 | 6 | 1 | 8 |
| ## | Splice_Site | 6 | 19 | 1 | 26 |
| ## | Translation_Start_Site | 0 | 0 | 0 | 0 |
| ## | RNA | 0 | 0 | 0 | 0 |
| ## | Sum | 101 | 323 | 8 | 432 |

To go further, how many patients have mutations in TP53 for each cancer type?

```
fraction_mutated_region = function(y, region) {
  s = subsetByOverlaps(y, region)
  m = length(unique(s$Patient_ID))/metadata(s)$Number_Patients
  return(m)
}
mutated_fraction = sapply(three_studies, fraction_mutated_region, tp53_region)
mutated_fraction = data.frame(name = names(three_studies), fraction = mutated_fraction)
```

```
library(ggplot2)
p = ggplot(mutated_fraction) + ggplot2::geom_bar(aes(x = name, y = fraction, fill = name),
  stat = "identity") + ylim(0, 1) + xlab("Study") + ylab("Ratio") + theme_bw()
print(p)
```



5 Data Provenance

5.1 TCGA Data

When importing the mutation data from the TCGA servers, we checked the data for consistency and fix common ambiguities in the annotation.

5.1.1 Processing

1. Selection of the most recent somatic variant calls for each study. These were stored as *.maf files in the TCGA data directory³. If both manually curated and automatically generated variant calls were available, the curated version was chosen.
2. Importing of the *.maf files into R and checking for consistency with the TCGA MAF specifications⁴. Please note that these guidelines are currently only suggestions and most TCGA files violate some of these.
3. Transformation of the imported variants into a GRanges object, with one row for each reported variant. Only columns related to the genomic origin of the somatic variant were stored, additional columns describing higher-level effects, such as mutational consequences and alterations at the protein level, were dropped. The seqlevels information defining the chromosomal ranges were taken from the 1000genomes phase 2 reference assembly⁵.
4. The patient barcode was extracted from the sample barcode.
5. Metadata describing the design and analysis of the study was extracted.
6. The processed variants were written to disk, with one file for each study. The metadata for all studies were stored as a single, separate object.

5.1.2 Selection Criteria of Data Sets

We included data sets in the package that were

- conducted by the Broad Institute.
- cleared for unrestricted access and usage⁶.
- sequenced with Illumina platforms.

5.1.3 Consistency Check

According to the TCGA specifications for the MAF files, we screened and corrected for common artifacts in the data regarding annotation. This included:

- Transferring of all genomic coordinates to the NCBI 37 reference notation (with the chromosome always depicted as 'MT')
- Checking of the entries against all allowed values for this field (currently for the columns Hugo_Symbol, Chromosome, Strand, Variant_Classification, Variant_Type, Reference_Allele, Tumor_Seq_Allele1, Tumor_Seq_Allele2, Verification_Status, Validation_Status, Sequencer).

6 Alternatives

The TCGA data sets can be accessed in different ways. First, the TCGA itself offers access to certain types of its collected data⁷. Another approach has been taken by the cBioPortal for Cancer Genomics⁸ which has performed high-level analyses of several TCGA data sources, such as gene expression and copy number changes. This summarized data can be queried through an R interface⁹.

7 Session Info

```
## R version 3.0.2 (2013-09-25)
## Platform: x86_64-unknown-linux-gnu (64-bit)
##
## locale:
```

³https://tcga-data.nci.nih.gov/tcgafiles/ftp_auth/distro_ftpusers/anonymous/tumor/

⁴[https://wiki.nci.nih.gov/display/TCGA/Mutation+Annotation+Format+\(MAF\)+Specification](https://wiki.nci.nih.gov/display/TCGA/Mutation+Annotation+Format+(MAF)+Specification)

⁵ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/technical/reference/phase2_reference_assembly_sequence/

⁶<http://cancergenome.nih.gov/abouttcga/policies/publicationguidelines>

⁷<https://tcga-data.nci.nih.gov/tcga/tcgaDownload.jsp>

⁸<http://www.cbioportal.org/public-portal>

⁹http://www.cbioportal.org/public-portal/cgds_r.jsp

```
## [1] LC_CTYPE=en_US.UTF-8      LC_NUMERIC=C          LC_TIME=en_US.UTF-8
## [4] LC_COLLATE=C                LC_MONETARY=en_US.UTF-8 LC_MESSAGES=en_US.UTF-8
## [7] LC_PAPER=en_US.UTF-8       LC_NAME=C            LC_ADDRESS=C
## [10] LC_TELEPHONE=C            LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] parallel stats graphics grDevices utils datasets methods base
##
## other attached packages:
## [1] ggbio_1.10.0                ggplot2_0.9.3.1
## [3] GenomicRanges_1.14.1      XVector_0.2.0
## [5] IRanges_1.20.0            BiocGenerics_0.8.0
## [7] SomaticCancerAlterations_0.99.20
##
## loaded via a namespace (and not attached):
## [1] AnnotationDbi_1.24.0      BSgenome_1.30.0         Biobase_2.22.0
## [4] BiocStyle_1.0.0          Biostrings_2.30.0       DBI_0.2-7
## [7] GenomicFeatures_1.14.0   Hmisc_3.12-2           MASS_7.3-29
## [10] RColorBrewer_1.0-5      RCurl_1.95-4.1         RSQLite_0.11.4
## [13] Rsamtools_1.14.1        VariantAnnotation_1.8.2 XML_3.98-1.1
## [16] biomaRt_2.18.0          biovizBase_1.10.0      bitops_1.0-6
## [19] cluster_1.14.4          colorspace_1.2-4       dichromat_2.0-0
## [22] digest_0.6.3            evaluate_0.5.1         exomeCopy_1.8.0
## [25] formatR_0.9             grid_3.0.2             gridExtra_0.9.1
## [28] gtable_0.1.2            highr_0.2.1            knitr_1.5
## [31] labeling_0.2            lattice_0.20-24        munsell_0.4.2
## [34] plyr_1.8                proto_0.3-10           reshape2_1.2.2
## [37] rpart_4.1-3             rtracklayer_1.22.0     scales_0.2.3
## [40] stats4_3.0.2           stringr_0.6.2          tools_3.0.2
## [43] zlibbioc_1.8.0
```