

SomaticCancerAlterations

Julian Gehring, EMBL Heidelberg

October 18, 2014

Contents

1	Motivation	1
2	Data Sets	1
3	Exploring Mutational Data	2
4	Exploring Multiple Studies	4
5	Data Provenance	6
5.1	TCGA Data	6
5.1.1	Processing	7
5.1.2	Selection Criteria of Data Sets	7
5.1.3	Consistency Check	7
6	Alternatives	7
7	Session Info	7

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.

¹<http://cancergenome.nih.gov>

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

```

all_datasets = scaListDatasets()
print(all_datasets)

## [1] "gbm_tcga" "hnsk_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
## hnsk_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
## hnsk_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
## hnsk_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 object 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
##   -----
##   seqinfo: 86 sequences from an unspecified genome
```

```
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)
}

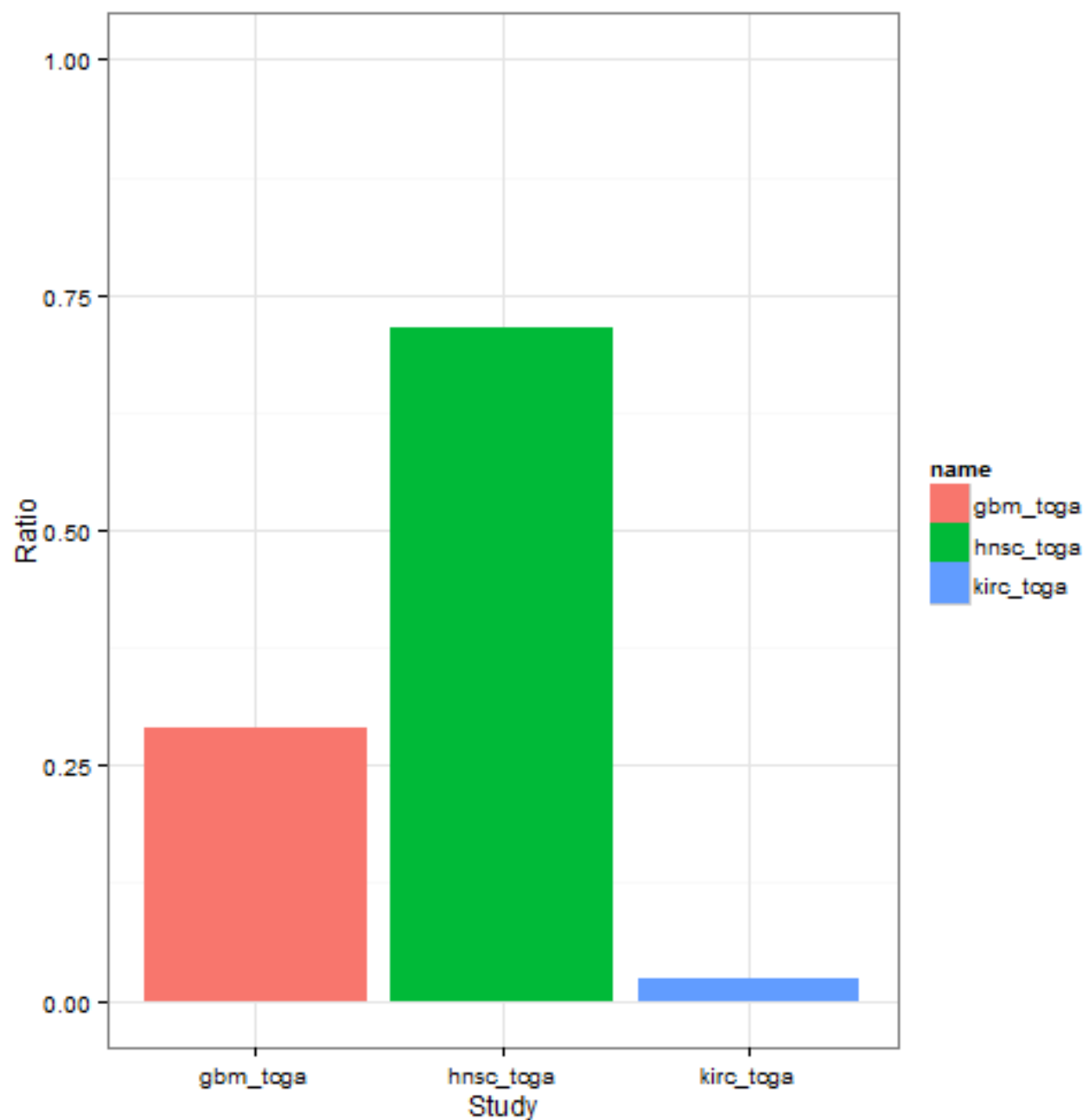
mutated_fraction = sapply(three_studies, fraction_mutated_region, tp53_region)

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

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

```
## R version 3.1.1 Patched (2014-09-24 r66678)
## Platform: i386-w64-mingw32/i386 (32-bit)
##
## locale:
## [1] LC_COLLATE=C                      LC_CTYPE=English_United States.1252
## [3] LC_MONETARY=English_United States.1252 LC_NUMERIC=C
## [5] LC_TIME=English_United States.1252
##
## attached base packages:
## [1] stats4      parallel  stats      graphics  grDevices  utils      datasets  methods
## [9] base
##
## other attached packages:
## [1] ggbio_1.14.0                      ggplot2_1.0.0
## [3] GenomicRanges_1.18.1              GenomeInfoDb_1.2.0
## [5] IRanges_2.0.0                     S4Vectors_0.4.0
## [7] BiocGenerics_0.12.0               SomaticCancerAlterations_1.1.1
##
## loaded via a namespace (and not attached):
## [1] AnnotationDbi_1.28.0              BBmisc_1.7                      BSgenome_1.34.0
## [4] BatchJobs_1.4                    Biobase_2.26.0                  BiocParallel_1.0.0
## [7] BiocStyle_1.4.1                  Biostrings_2.34.0               DBI_0.3.1
## [10] Formula_1.1-2                    GGally_0.4.8                    GenomicAlignments_1.2.0
## [13] GenomicFeatures_1.18.0           Hmisc_3.14-5                    MASS_7.3-35
## [16] OrganismDbi_1.8.0                RBGL_1.42.0                     RColorBrewer_1.0-5
## [19] RCurl_1.95-4.3                  RSQLite_0.11.4                  Rcpp_0.11.3
## [22] Rsamtools_1.18.0                 VariantAnnotation_1.12.0         XML_3.98-1.1
## [25] XVector_0.6.0                    acepack_1.3-3.3                 base64enc_0.1-2
## [28] biomaRt_2.22.0                   biovizBase_1.14.0               bitops_1.0-6
## [31] brew_1.0-6                       checkmate_1.4                   cluster_1.15.3
## [34] codetools_0.2-9                  colorspace_1.2-4                 dichromat_2.0-0
## [37] digest_0.6.4                     evaluate_0.5.5                   exomeCopy_1.12.0
## [40] fail_1.2                         foreach_1.4.2                   foreign_0.8-61
## [43] formatR_1.0                      graph_1.44.0                     grid_3.1.1
## [46] gridExtra_0.9.1                  gtable_0.1.2                     highr_0.3
## [49] iterators_1.0.7                  knitr_1.7                        labeling_0.3
## [52] lattice_0.20-29                  latticeExtra_0.6-26              munsell_0.4.2
## [55] nnet_7.3-8                       plyr_1.8.1                       proto_0.3-10
## [58] reshape_0.8.5                    reshape2_1.4                     rpart_4.1-8
## [61] rtracklayer_1.26.1               scales_0.2.4                     sendmailR_1.2-1
## [64] splines_3.1.1                    stringr_0.6.2                   survival_2.37-7
## [67] tools_3.1.1                      zlibbioc_1.12.0
```