curatedOvarianData: Clinically Annotated Data for the Ovarian Cancer Transcriptome

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1 curatedOvarianData: Clinically Annotated Data for the Ovarian Cancer Transcriptome

This package represents a manually curated data collection for gene expression meta-analysis of patients with ovarian cancer. This resource provides uniformly prepared microarray data with curated and documented clinical metadata. It allows a computational user to efficiently identify studies and patient subgroups of interest for analysis and to run such analyses immediately without the challenges posed by harmonizing heterogeneous microarray technologies, study designs, expression data processing methods, and clinical data formats.

The curatedOvarianData package is published in the journal DATABASE [1]. Note the existence also of curatedCR-CData and curatedBladderData.

Please see http://bcb.dfci.harvard.edu/ovariancancer for alternative versions of this package, differing in how redundant probe sets are dealt with.

In this vignette, we give a short tour of the package and will show how to use it efficiently.
2 Load TCGA data

Loading a single dataset is very easy. First we load the package:

```r
library(curatedOvarianData)
```

To get a listing of all the datasets, use the `data` function:

```r
data(package="curatedOvarianData")
```

Now to load the TCGA data, we use the `data` function again:

```r
data(TCGA_eset)
TCGA_eset
```

ExpressionSet (storageMode: lockedEnvironment)
- assayData: 13104 features, 578 samples
  - element names: exprs
- protocolData: none
- phenoData
  - sampleNames: TCGA.20.0987 TCGA.23.1031 ...
  - TCGA.13.1819 (578 total)
  - varLabels: alt_sample_name unique_patient_ID ...
  - uncurated_author_metadata (31 total)
- varMetadata: labelDescription
- featureData
  - featureNames: A1CF A2M ... ZZZ3 (13104 total)
  - fvarLabels: probeset gene
  - fvarMetadata: labelDescription
- experimentData: use 'experimentData(object)'
  - pubMedIds: 21720365
- Annotation: hthgu133a

The datasets are provided as Bioconductor ExpressionSet objects and we refer to the Bioconductor documentation for users unfamiliar with this data structure.

3 Load datasets based on rules

For a meta-analysis, we typically want to filter datasets and patients to get a population of patients we are interested in. We provide a short but powerful R script that does the filtering and provides the data as a list of ExpressionSet objects. One can use this script within R by first sourcing a config file which specifies the filters, like the minimum numbers of patients in each dataset. It is also possible to filter samples by annotation, for example to remove early stage and normal samples.

```r
source(system.file("extdata", + "patientselection.config",package="curatedOvarianData"))
ls()
```

[1] "TCGA_eset"
[2] "add.surv.y"
[3] "duplicates"
[4] "impute.missing"
[5] "keep.common.only"
[6] "meta.required"
[7] "min.number.of.events"
[8] "min.number.of.genes"
[9] "min.sample.size"
[10] "probes.not.mapped.uniquely"
[12] "remove.retracted"
See what the values of these variables we have loaded are. The variable names are fairly descriptive, but note that “rule.1” is a character vector of length 2, where the first entry is the name of a clinical data variable, and the second entry is a Regular Expression providing a requirement for that variable. Any number of rules can be added, with increasing identifiers, e.g. “rule.2”, “rule.3”, etc.

Here strict.checking is FALSE, meaning that samples not annotated for the variables in these rules are allowed to pass the filter. If strict.checking == TRUE, samples missing this annotation will be removed.

3.1 Cleaning of duplicate samples

The patientselection.config file loaded above contains several objects indicating which samples were removed for QC and duplicate cleaning by Waldron et al. [2]:

- tcga.lowcor.outliers: two profiles identified in the TCGA dataset with anomalously low correlation to other ovc profiles
- duplicates: samples blacklisted because they contain duplicates. In the case of duplicates, generally better-annotated samples, and samples from more recent studies, were kept.
- remove.samples: the above to vectors of samples concatenated

```r
grep("remove.samples", ls())
```

```r
> apply(ls(), function(x) if(!x %in% c("remove.samples", "duplicates")) print(get(x)))
```

ExpressionSet (storageMode: lockedEnvironment)
assayData: 13104 features, 578 samples
element names: exprs
protocolData: none
phenoData
  sampleNames: TCGA.20.0987 TCGA.23.1031 ...
  varLabels: alt_sample_name unique_patient_ID ...
  uncurated_author_metadata (31 total)
  varMetadata: labelDescription
featureData
  featureNames: A1CF A2M ... ZZZ3 (13104 total)
  fvarLabels: probeset gene
  fvarMetadata: labelDescription
experimentData: use 'experimentData(object)'
pubMedIds: 21720365
Annotation: hthgu133a
function (X)
Surv(X$days_to_death, X$vital_status == "deceased")
```

```r
[1] FALSE
[1] FALSE
[1] "days_to_death" "vital_status"
[1] 15
[1] 1000
[1] 40
[1] "drop"
[1] 0
[1] FALSE
[1] TRUE
[1] TRUE
[1] "sample_type" "tumor"
[1] FALSE
$TCGA_eset
ExpressionSet (storageMode: lockedEnvironment)
assayData: 13104 features, 578 samples
   element names: exprs
protocolData: none
phenoData
   sampleNames: TCGA.20.0987 TCGA.23.1031 ...
      TCGA.13.1819 (578 total)
   varLabels: alt_sample_name unique_patient_ID ...
      uncurated_author_metadata (31 total)
   varMetadata: labelDescription
featureData
   featureNames: A1CF A2M ... ZZZ3 (13104 total)
   fvarLabels: probeset gene
   fvarMetadata: labelDescription
experimentData: use 'experimentData(object)'
   pubMedIds: 21720365
Annotation: hthgu133a

$add.surv.y
function (X)
Surv(X$days_to_death, X$vital_status == "deceased")

$duplicates
NULL

$impute.missing
[1] FALSE

$keep.common.only
[1] FALSE

$meta.required
[1] "days_to_death" "vital_status"

$min.number.of.events
[1] 15

$min.number.of.genes
[1] 1000

$min.sample.size
[1] 40

$probes.not.mapped.uniquely
[1] "drop"

$quantile.cutoff
[1] 0

$remove.retracted
[1] FALSE

$remove.samples
NULL
Now that we have defined the sample filter, we create a list of `ExpressionSet` objects by sourcing the `createEsetList.R` file:

```r
> source(system.file("extdata", "createEsetList.R", package = "curatedOvarianData"))
```

It is also possible to run the script from the command line and then load the R data file within R:

```
R --vanilla "--args patientselection.config ovarian.eset.rda tmp.log" < createEsetList.R
```
Now we have 16 datasets with samples that passed our filter in a list of ExpressionSet objects called esets:

```r
> names(esets)
[1] "E.MTAB.386_eset"  "GSE13876_eset"
[3] "GSE14764_eset"  "GSE17260_eset"
[5] "GSE18520_eset"  "GSE19829.GPL8300_eset"
[7] "GSE26193_eset"  "GSE26712_eset"
[9] "GSE30161_eset"  "GSE32062.GPL6480_eset"
[11] "GSE49997_eset"  "GSE51088_eset"
[13] "GSE9891_eset"  "PMID17290060_eset"
[15] "TCGA.RNASeqV2_eset"  "TCGA_eset"
```

### 4 Association of CXCL12 expression with overall survival

Next we use the list of 16 datasets from the previous example and test if the expression of the CXCL12 gene is associated with overall survival. CXCL12/CXCR4 is a chemokine/chemokine receptor axis that has previously been shown to be directly involved in cancer pathogenesis.

We first define a function that will generate a forest plot for a given gene. It needs the overall survival information as Surv objects, which the `createEsetList.R` function already added in the phenoData slots of the ExpressionSet objects, accessible at the y label. The resulting forest plot is shown for the CXCL12 gene in Figure 1.

```r
> esets[[1]]$y
[1] 840.9+ 399.9+ 524.1+ 1476.0 144.0 516.9
[7] 405.0 87.0 45.9+ 483.9+ 917.1 1013.1+
[13] 69.9 486.0 369.9 2585.1+ 738.9 362.1
[19] 2031.9+ 477.9 1091.1+ 1062.0+ 720.9 1200.9+
[25] 977.1 537.9 638.1 587.1 1509.0 1619.1+
[31] 1043.1 198.9 1520.1 696.9 1140.9 1862.1+
[37] 1751.1+ 1845.0+ 1197.0 1401.0 399.0 992.1
[43] 927.9+ 1509.0 1914.0+ 591.9 426.0 1374.9+
[49] 546.9 809.1+ 480.9+ 486.0+ 642.9+ 540.9+
[55] 962.1 2025.0 473.1 1140.0 512.1 1002.9+
[61] 1731.9+ 690.0 930.0 1026.9 1193.1+ 720.9
[67] 369.0 1326.9+ 501.9+ 1677.0+ 1773.9+ 251.1
[73] 1338.9+ 35.1 1467.9+ 165.9 981.9 1200.1
[79] 1800.0+ 399.9 422.1 861.9 2010.0+ 660.0
[85] 2138.1+ 516.0+ 1001.1+ 693.9 825.0+ 815.1+
[91] 657.0+ 1013.1+ 426.0 656.1 1356.0 1610.1+
[97] 1068.9+ 1221.9+ 2388.0+ 447.9+ 602.1+ 1875.0+
[103] 920.1+ 959.1 708.0 546.0 1254.9+ 611.1+
[109] 1317.9 1899.0 1886.1 642.0 1763.1 1857.0+
[115] 540.0 852.9 498.0+ 3.9+ 836.1 1452.0
[121] 2721.0 450.9 1398.9 1481.1 2724.0+ 2061.9
[127] 651.9 2349.0+
```

```r
> forestplot <- function(esets, y="y", probeset, formula=y~probeset, + mlab="Overall", rma.method="FE", at=NULL,xlab="Hazard Ratio",...) {
+ require(metafor)
+ esets <- esets[sapply(esets, function(x) probeset %in% featureNames(x))]
+ coefs <- sapply(1:length(esets), function(i) {
+ tmp <- as(phenoData(esets[[i]]), "data.frame")
+ summary(coxph(formula=data=tmp))$coefficients[1,c(1,3)]
+ })
+ }
```
Figure 1: The database confirms CXCL12 as prognostic of overall survival in patients with ovarian cancer. Forest plot of the expression of the chemokine CXCL12 as a univariate predictor of overall survival, using all 16 datasets with applicable expression and survival information. A hazard ratio significantly larger than 1 indicates that patients with high CXCL12 levels had poor outcome. The p-value for the overall HR, found in res$pval, is 9e-10. This plot is Figure 3 of the curatedOvarianData manuscript.

```
+ res.rma <- metafor::rma(yi = coefs[1,], sei = coefs[2,],
+    method=rma.method)
+ if (is.null(at)) at <- log(c(0.25,1,4,20))
+ forest.rma(res.rma, xlab=xlab, slab=gsub("_eset$","",names(esets)),
+    atransf=exp, at=at, mlab=mlab,...)
+ return(res.rma)
+ }
```

We now test whether CXCL12 is an independent predictor of survival in a multivariate model together with success of debulking surgery, defined as residual tumor smaller than 1 cm, and Federation of Gynecology and Obstetrics (FIGO) stage. We first filter the datasets without debulking and stage information:

```
> idx.tumorstage <- sapply(esets, function(X)
+     sum(!is.na(X$tumorstage)) > 0 & length(unique(X$tumorstage)) > 1)
> idx.debulking <- sapply(esets, function(X)
+     sum(X$debulking=="suboptimal",na.rm=TRUE)) > 0
```
Figure 2: Validation of CXCL12 as an independent predictor of survival. This figure shows a forest plot as in Figure 1, but the CXCL12 expression levels were adjusted for debulking status (optimal versus suboptimal) and tumor stage. The p-value for the overall HR, found in res$pval, is 1.8e-05.

In Figure 2, we see that CXCL12 stays significant after adjusting for debulking status and FIGO stage. We repeated this analysis for the CXCR4 receptor and found no significant association with overall survival (Figure 3).
> res <- forestplot(esets=esets,probeset="CXCR4",at=log(c(0.5,1,2,4)))

---

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Hazard Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.MTAB.386</td>
<td>0.70 [0.55, 0.89]</td>
</tr>
<tr>
<td>GSE13876</td>
<td>1.06 [0.88, 1.29]</td>
</tr>
<tr>
<td>GSE14764</td>
<td>1.15 [0.72, 1.82]</td>
</tr>
<tr>
<td>GSE17260</td>
<td>0.85 [0.62, 1.16]</td>
</tr>
<tr>
<td>GSE18520</td>
<td>1.15 [0.84, 1.56]</td>
</tr>
<tr>
<td>GSE19829.GPL8300</td>
<td>0.94 [0.61, 1.45]</td>
</tr>
<tr>
<td>GSE26193</td>
<td>0.98 [0.78, 1.23]</td>
</tr>
<tr>
<td>GSE26712</td>
<td>0.92 [0.78, 1.09]</td>
</tr>
<tr>
<td>GSE30161</td>
<td>1.76 [1.17, 2.65]</td>
</tr>
<tr>
<td>GSE32062.GPL6480</td>
<td>0.90 [0.71, 1.13]</td>
</tr>
<tr>
<td>GSE49997</td>
<td>1.16 [0.89, 1.51]</td>
</tr>
<tr>
<td>GSE51088</td>
<td>1.11 [0.83, 1.48]</td>
</tr>
<tr>
<td>GSE9891</td>
<td>0.96 [0.79, 1.16]</td>
</tr>
<tr>
<td>PMID17290060</td>
<td>1.05 [0.82, 1.36]</td>
</tr>
<tr>
<td>TCGA.RNASeqV2</td>
<td>0.94 [0.81, 1.10]</td>
</tr>
<tr>
<td>TCGA</td>
<td>0.89 [0.79, 1.00]</td>
</tr>
<tr>
<td>Overall</td>
<td>0.96 [0.91, 1.01]</td>
</tr>
</tbody>
</table>

---

Figure 3: Up-regulation of CXCR4 is not associated with overall survival. This figure shows again a forest plot as in Figure 1, but here the association of mRNA expression levels of the CXCR4 receptor and overall survival is shown. The p-value for the overall HR, found in res$pval, is 0.12.
5 Batch correction with ComBat

If datasets are merged, it is typically recommended to remove a very likely batch effect. We will use the ComBat [3] method, implemented for example in the SVA Bioconductor package [4]. To combine two ExpressionSet objects, we can use the `combine()` function. This function will fail when the two ExpressionSets have conflicting annotation slots, for example annotation when the platforms differ. We write a simple `combine2` function which only considers the `exprs` and `phenoData` slots:

```r
> combine2 <- function(X1, X2) {
+   fids <- intersect(featureNames(X1), featureNames(X2))
+   X1 <- X1[fids,]
+   X2 <- X2[fids,]
+   ExpressionSet(cbind(exprs(X1),exprs(X2)),
+       AnnotatedDataFrame(rbind(as(phenoData(X1),"data.frame"),
+                               as(phenoData(X2),"data.frame")))
+  )
+}
```

In Figure 4, we combined two datasets from different platforms, resulting in a huge batch effect. Now we apply ComBat and adjust for the batch and show the boxplot after batch correction in Figure 5:

```r
> mod <- model.matrix(~as.factor(tumorstage), data=X)
> batch <- as.factor(grepl("DFCI", sampleNames(X)))
> combat_edata <- ComBat(dat=exprs(X), batch=batch, mod=mod)

Found 2 batches
Adjusting for 2 covariate(s) or covariate level(s)
Standardizing Data across genes
Fitting L/S model and finding priors
Finding parametric adjustments
Adjusting the Data
> data(E.MTAB.386_eset)
> data(GSE30161_eset)
> X <- combine2(E.MTAB.386_eset, GSE30161_eset)
> boxplot(exprs(X))

Figure 4: Boxplot showing the expression range for all samples of two merged datasets arrayed on different platforms. This illustrates a huge batch effect.
> boxplot(combat_data)

![Boxplot showing the expression range for all samples of two merged datasets arrayed on different platforms after batch correction with ComBat.](image)

Figure 5: Boxplot showing the expression range for all samples of two merged datasets arrayed on different platforms after batch correction with ComBat.
6 Non-specific probe sets

In the standard version of curatedOvarianData (the version available on Bioconductor), we collapse manufacturer probe sets to official HGNC symbols using the Biomart database. Some probe sets are mapped to multiple HGNC symbols in this database. For these probe sets, we provide all the symbols. For example 220159_at maps to ABCA11P and ZNF721 and we provide ABCA11P///ZNF721 as probe set name. If you have an array of gene symbols for which you want to access the expression data, “ABCA11P” would not be found in curatedOvarianData in this example.

The script createEsetList.R provides three methods to deal with non-specific probe sets by setting the variable probes.not.mapped.uniquely to:

- "keep": leave as-is, these have "///" in gene names,
- "drop": drop any non-uniquely mapped features, or
- "split": split non-uniquely mapped features to one per row. If this creates duplicate rows for a gene, those rows are averaged.

This feature uses the following function to create a new ExpressionSet, in which both ZNF721 and ABCA11P are features with identical expression data:

```r
> expandProbesets <- function (eset, sep = "///")
+ {
+   x <- lapply(featureNames(eset), function(x) strsplit(x, sep)[[1]])
+   eset <- eset[order(sapply(x, length)), ]
+   x <- lapply(featureNames(eset), function(x) strsplit(x, sep)[[1]])
+   idx <- unlist(sapply(1:length(x), function(i) rep(i, length(x[[i]])))))
+   xx <- !duplicated(unlist(x))
+   idx <- idx[xx]
+   x <- unlist(x)[xx]
+   eset <- eset[idx, ]
+   featureNames(eset) <- x
+   eset
+ }

> X <- TCGA_eset[head(grep("///", featureNames(TCGA_eset))),]
> exprs(X)[,1:3]

<table>
<thead>
<tr>
<th></th>
<th>TCGA.20.0987</th>
<th>TCGA.23.1031</th>
<th>TCGA.24.0979</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCB4///ABCB1</td>
<td>2.993923</td>
<td>3.600534</td>
<td>3.539278</td>
</tr>
<tr>
<td>ABCB6///ATG9A</td>
<td>4.257024</td>
<td>4.793526</td>
<td>4.909549</td>
</tr>
<tr>
<td>ABCC6P2///ABCC6P1///ABCC6</td>
<td>3.110547</td>
<td>6.699198</td>
<td>3.085545</td>
</tr>
<tr>
<td>ABHD17AP3///ABHD17AP2///ABHD17AP1 ///ABHD17AP6///ABHD17A</td>
<td>6.886997</td>
<td>6.529303</td>
<td>3.539278</td>
</tr>
<tr>
<td>ACOT1///ACOT2</td>
<td>4.702057</td>
<td>3.534889</td>
<td>5.476369</td>
</tr>
<tr>
<td>ACSM2A///ACSM2B</td>
<td>2.980667</td>
<td>2.890781</td>
<td>4.993433</td>
</tr>
</tbody>
</table>

> exprs(expandProbesets(X))[1:3]

<table>
<thead>
<tr>
<th></th>
<th>TCGA.20.0987</th>
<th>TCGA.23.1031</th>
<th>TCGA.24.0979</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCB4</td>
<td>2.993923</td>
<td>3.600534</td>
<td>3.539278</td>
</tr>
<tr>
<td>ABCB6</td>
<td>4.257024</td>
<td>4.793526</td>
<td>4.909549</td>
</tr>
<tr>
<td>ABCC6P2</td>
<td>3.110547</td>
<td>6.699198</td>
<td>3.085545</td>
</tr>
<tr>
<td>ABHD17AP3</td>
<td>6.886997</td>
<td>6.529303</td>
<td>3.539278</td>
</tr>
<tr>
<td>ACOT1</td>
<td>4.702057</td>
<td>3.534889</td>
<td>5.476369</td>
</tr>
<tr>
<td>ACSM2A</td>
<td>2.980667</td>
<td>2.890781</td>
<td>4.993433</td>
</tr>
</tbody>
</table>
```
In curatedOvarianData, probesets mapping to the same gene symbol are merged by selecting the probeset with the maximum mean across all studies of a given platform. You can see which representative probeset was chosen by looking at the featureData of the ExpressionSet, e.g.:

```r
> head(pData(featureData(GSE18520_eset)))
```

<table>
<thead>
<tr>
<th>probeset</th>
<th>gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1BG</td>
<td>229819_at</td>
</tr>
<tr>
<td>A1BG-AS1</td>
<td>232462_s_at</td>
</tr>
<tr>
<td>A1CF</td>
<td>220951_s_at</td>
</tr>
<tr>
<td>A2M</td>
<td>217757_at</td>
</tr>
<tr>
<td>A2M-AS1</td>
<td>1564139_at</td>
</tr>
<tr>
<td>A2ML1</td>
<td>1553505_at</td>
</tr>
</tbody>
</table>

The full, unmerged ExpressionSets are available through the FULLVcuratedOvarianData package at http://bcb.dfc.harvard.edu/ovariancancer/. Probeset to gene maps are again provided in the featureData of those ExpressionSets. Where official Bioconductor annotation packages are available for the array, these are stored in the ExpressionSet annotation slots, e.g.:

```r
> annotation(GSE18520_eset)
```

[1] "hgu133plus2"

so that standard filtering methods such as nsFilter will work by default.

### 8 Available Clinical Characteristics

### 9 Summarizing the List of ExpressionSets

This example provides a table summarizing the datasets being used, and is useful when publishing analyses based on curatedOvarianData. First, define some useful functions for this purpose:

```r
> source(system.file("extdata", "summarizeEsets.R", package = "curatedOvarianData"))
```

Now optionally create the table, used for Table 1 of the curatedOvarianData manuscript:

```r
> (myfile <- tempfile())
```
Figure 6: Available clinical annotation. This heatmap visualizes for each curated clinical characteristic (rows) the availability in each dataset (columns). Red indicates that the corresponding characteristic is available for at least one sample in the dataset. This plot is Figure 2 of the curatedOvarianData manuscript.

[1] "/tmp/Rtmpcs6JXJ/file6c186426d33e"
> write.table(summary.table, file=myfile, row.names=FALSE, quote=TRUE, sep=";")

10 For non-R users

If you are not doing your analysis in R, and just want to get some data you have identified from the curatedOvarianData manual, here is a simple way to do it. For one dataset:

> library(curatedOvarianData)
> library(affy)
> data(GSE30161_eset)
> write.csv(exprs(GSE30161_eset), file="GSE30161_eset_exprs.csv")
> write.csv(pData(GSE30161_eset), file="GSE30161_eset_clindata.csv")

Or for several datasets:

> data.to.fetch <- c("GSE30161_eset", "E.MTAB.386_eset")
> for (onedata in data.to.fetch){
+ print(paste("Fetching", onedata))
+ data(list=onedata)
```r
+ write.csv(exprs(get(onedata)), file=paste(onedata, "_exprs.csv", sep=""))
+ write.csv(pData(get(onedata)), file=paste(onedata, "_clindata.csv", sep=""))
+ }

11 Session Info

- R version 3.2.2 (2015-08-14), x86_64-pc-linux-gnu
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  LC_MONETARY=en_US.UTF-8, LC_MESSAGES=en_US.UTF-8, LC_PAPER=en_US.UTF-8, LC_NAME=C,
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Table 1: Datasets provided by curatedOvarianData. This is an abbreviated version of Table 1 of the manuscript; the full version is written by the write.table command above. Stage column is early/late/unknown, histology column is ser/clearcell/endo/mucinous/other/unknown.
References


