Risa: Building R objects from local ISA-Tab files

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1 Introduction

The Risa package is part of the ISA infrastructure software suite (http://isa-tools.org). It provides funcitonality to read ISA-Tab datasets, described in the following section. The source code and latest version can be found in the GitHub repository https://github.com/ISA-tools/Risa. Please, submit all 'bugs' and feature requests through https://github.com/ISA-tools/Risa/issues.

2 ISA-Tab format

The Investigation / Study / Assay (ISA) Tab-delimited (Tab) format is a general purpose framework with which to collect and communicate complex metadata (i.e. sample characteristics, technologies used, type of measurements made) from experiments employing a combination of technologies (http://isa-tools.org). In particular, ISA-Tab has been developed for - but not limited to - experiments using genomics, transcriptomics, proteomics or metabol/nomics techniques (the 'omics').

ISA-Tab uses three types of file to capture the experimental metadata:

- *Investigation file*
- *Study file*
- *Assay file* (with associated data files).

The Investigation file contains an overall description of an experiment while all experimental steps are described in the Study and in the Assay file(s). For each Investigation file there may be one or more Study files; for each Study file there may be one or more Assay files.

2.1 Investigation file

In this file, information is reported on a per-column basis and the fields are organized and divided in sections. The Investigation file is intended to meet three needs:

- to define key entities, such as factors, protocols, parameters, which may be referenced in the other files;
- to relate Assay files to Study files; and optionally,
- to relate each Study file to an Investigation (when two or more Study files need to be grouped). The declarative sections cover general information such as contacts, protocols and equipment, and also - where applicable - the description of terminologies (controlled vocabularies or ontologies) and other annotation resources that were used.

2.2 Study file

In this file, information is structured on a per-row basis with the first row being used for column headers. The Study file contains contextualizing information for one or more assays, for example; the subjects studied; their source(s); the sampling methodology; their characteristics; and any treatments or manipulations performed to prepare the specimens.

2.3 Assay file

In this file, as for the Study file, fields are organized on a per-row basis with the first row being used for column headers. The Assay file represents a portion of the experimental graph (i.e., one part of the overall structure of the workflow); each Assay file must contain assays of the same type, defined by the type of measurement (i.e. gene expression) and the technology employed (i.e. DNA microarray). Assay-related information includes protocols, additional information relating to the execution of those protocols and references to data files (whether raw or processed).

For easy transfer, ISA-Tab files and associated data files can be packaged into an ISArchive, using a standalone Java application named ISAcreator (http://isatab.sourceforge.net). In order to facilitate identification of ISA-Tab components in an ISArchive, specific extensions have been created as follows:

- *i_iname.txt* for identifying the Investigation file
- *s_sname.txt* for identifying Study file (s)
- *a_aname.txt* for identifying Assay file (s)

where 'iname', 'sname', 'aname' are the user-given names for the investigation, study/ies, assay(s), respectively.

3 The Risa package

The Risa package is used to build R objects from an ISA archive or dataset. The output is a list of objects containing, for example, the investigation, studies and assays filenames, the contents of their files, the list of samples, among other things.

These objects can then be used by downstream Bioconductor packages for data analysis and visualization (i.e, xcms). The package currently includes the function processAssayXcmsSet that, for a specific mass spectrometry assay, builds an xcmsSet object.

3.1 Building an R object from a local ISA dataset

If you have your own ISA archive, you can use the function `readISAtab` to convert it into an R object. The arguments for the function `readISAtab` are:

- path the name of the directory containing ISAtab files. The default is the working directory.
- verbose a boolean indicating to show messages for the different steps, if TRUE, or not to show them, if FALSE

As an example, we can use the *faahKO* dataset, whose version 1.2.11 contains an ISA dataset describing the experiment. First, it is required to load the *Risa* package, and the *faahKO* package must have been installed.

```
> library(Risa)
> require(faahKO)
```

Then, we read the ISA-Tab data set from the *faahKO* package:

```
> faahkoISA <- readISAtab(find.package("faahKO"))
```

The object `faahkoISA` belongs to the *ISAtab* class, and contains the following elements:

- path - the path of the ISA-Tab dataset,
- investigation.filename - the name of the Investigation file
- investigation.file - a data frame with the contents of the Investigation file
- study.identifiers - the list of study identifiers
- study.filenames - the names of the study files
- study.files - a list of data frames wiht the contents of the study files
- assay.filenames - the names of the assay files
- assay.filenames.per.study - the names of the assay files according to the study they belong to
- assay.files - a list of data frames with the contents of the assay files
- assay.files.per.study - a list of data frames with the contents of the assay files divided per study they belong to
- assay.technology.types - a list with the technology types corresponding to each assay
- assay.measurement.types - a list with the measurement types corresponding to each assay
- data.filenames - a list with the names of the data files
- samples - a list with the names of the samples
- samples.per.assay.filename - the samples classified according to the assay filename they belong to
- assay.filenames.per.sample - the names of the assay files classified per sample name
- sample.to.rawdatafile - the association between samples and raw data files
- sample.to.assayname - the association between samples and assay names
- rawdatafile.to.sample - the association between raw data files and samples
- assayname.to.sample - the association between assay names and samples

Additionally, the ISA dataset could be compressed in a .zip file. If that is the case, the function `readISAtab` can be used, passing the `zipfile` as parameter. The only condition is that the ISA-Tab files are contained directly into the zip file, i.e. not inside additional folders.

In this case, the parameters for the function `readISAtab` will be:

- zipfile a zip archive containing ISAtab files.
- path the name of the directory in which the files from the zip archive will be extracted. The default is the working directory.
- verbose a boolean indicating to show messages for the different steps, if TRUE, or not to show them, if FALSE

Building xcmsSets for mass spectrometry assays

The function `processAssayXcmsSet` allows to build an xcmsSet (object defined in the xcms package) from the information in an assay file.

The parameters for this function are:

- isa: an ISA object, as retrieved by the function `readISAtab`
- assay.filename the name of the assay file with information about the relevant assay
- ... extra arguments that can be passed down to the xcmsSet function from the xcms package

Using the *faahKO* package as an example, we select the name of assay file, and use the `processAssayXcmsSet` to build a object of type *xcmsSet*:

```
> assay.filename <- faahkoISA["assay.filenames"][1]
> faahkoXset <- processAssayXcmsSet(faahkoISA, assay.filename)
```

Augmenting the ISA-Tab dataset after analysis

The Risa package also provides the functionality to augment the original ISA-Tab dataset with more information after analysis.

The function `updateAssayMetadata` allows to modify the metadata in a particular assay file. The arguments are:

- isa An isatab object, as retrieved by the `readISAtab` function.
- assay.filename the filename of the assay file to be augmented/modified
- col.name the name of the column of the assay file to be modified
- values the values to be added to the column of the assay file: it could be a single value, and in this case the value is repeated across the column, or it could be a list of values (whose length must match the number of rows of the assay file)

To continue with our example using the *faahKO* data package, we will assume that the results of analysis are stored in the file *faahkoDSDF.txt*. Then, we will update the ISA-Tab dataset adding the result file into the 'Derived Spectral Data File' column of the assay file.

```
> updateAssayMetadata(faahkoISA, assay.filename,"Derived Spectral Data File","faahkoDSDF.txt" )

An object of class "ISATab"
Slot "path":
[1] "/home/biocbuild/bbs-3.9-bioc/R/library/faahKO"

Slot "investigation.filename":
[1] "i_Investigation.txt"

Slot "investigation.file":
                                      V1
1                 ONTOLOGY SOURCE REFERENCE
2                          Term Source Name
3                          Term Source File
4                       Term Source Version
5                 Term Source Description
6                            INVESTIGATION
7                  Investigation Identifier
```

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8                                    Investigation Title
9                                Investigation Description
10                           Investigation Submission Date
11                        Investigation Public Release Date
12                    Comment [Created with configuration]
13                Comment [Last Opened With Configuration]
14                               INVESTIGATION PUBLICATIONS
15                                  Investigation PubMed ID
16                           Investigation Publication DOI
17                   Investigation Publication Author List
18                         Investigation Publication Title
19                        Investigation Publication Status
20 Investigation Publication Status Term Accession Number
21       Investigation Publication Status Term Source REF
22                                    INVESTIGATION CONTACTS
23                           Investigation Person Last Name
24                          Investigation Person First Name
25                        Investigation Person Mid Initials
26                                Investigation Person Email
27                                Investigation Person Phone
28                                  Investigation Person Fax
29                              Investigation Person Address
30                          Investigation Person Affiliation
31                                Investigation Person Roles
32       Investigation Person Roles Term Accession Number
33             Investigation Person Roles Term Source REF
34                                                    STUDY
35                                       Study Identifier
36                                            Study Title
37                                      Study Description
38                                   Study Submission Date
39                                Study Public Release Date
40                                        Study File Name
41                                 STUDY DESIGN DESCRIPTORS
42                                        Study Design Type
43                  Study Design Type Term Accession Number
44                        Study Design Type Term Source REF
45                                       STUDY PUBLICATIONS
46                                         Study PubMed ID
47                                    Study Publication DOI
48                            Study Publication Author List
49                                  Study Publication Title
50                                Study Publication Status
51          Study Publication Status Term Accession Number
52                Study Publication Status Term Source REF
53                                           STUDY FACTORS
54                                      Study Factor Name
55                                      Study Factor Type
56                  Study Factor Type Term Accession Number
57                        Study Factor Type Term Source REF
58                                           STUDY ASSAYS
59                               Study Assay Measurement Type
60            Study Assay Measurement Type Term Source REF
61       Study Assay Measurement Type Term Accession Number
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                          Study Assay Technology Type
          Study Assay Technology Type Term Source REF
    Study Assay Technology Type Term Accession Number
                       Study Assay Technology Platform
                                Study Assay File Name
                                      STUDY PROTOCOLS
                                  Study Protocol Name
                                  Study Protocol Type
            Study Protocol Type Term Accession Number
                  Study Protocol Type Term Source REF
                           Study Protocol Description
                                   Study Protocol URI
                                Study Protocol Version
                         Study Protocol Parameters Name
Study Protocol Parameters Name Term Accession Number
      Study Protocol Parameters Name Term Source REF
                         Study Protocol Components Name
                         Study Protocol Components Type
Study Protocol Components Type Term Accession Number
      Study Protocol Components Type Term Source REF
                                      STUDY CONTACTS
                                Study Person Last Name
                               Study Person First Name
                             Study Person Mid Initials
                                    Study Person Email
                                    Study Person Phone
                                      Study Person Fax
                                 Study Person Address
                               Study Person Affiliation
                                    Study Person Roles
             Study Person Roles Term Accession Number
                   Study Person Roles Term Source REF
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Enzymes regulate biological processes through the conversion of specific substrates to product

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A 2:1:1 CHCl3/MeOH/1% NaCl solution (8 mL per brain and 4 mL per spinal cord in 8 mL vials) wa

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LC-MS analysis was performed using an Agilent 1100 LC-MSD SL instrument. For the LC analysis,

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Slot "investigation.identifier":
[1] ""

Slot "study.identifiers":
[1] "Global metabolite profiling of faah(-/-) mice"

Slot "study.titles":
[1] "Global metabolite profiling of faah(-/-) mice"

Slot "study.descriptions":
[1] "Enzymes regulate biological processes through the conversion of specific substrates to produ

Slot "study.contacts":
[1] "  "

Slot "study.contacts.affiliations":
[1] ""

Slot "study.filenames":
Global metabolite profiling of faah(-/-) mice
     "s_Proteomic_profiling_of_yeast_TFs.txt"

Slot "study.files":
$`Global metabolite profiling of faah(-/-) mice`
       Source Name Characteristics[NEWT:Organism LC] Term Source REF
1   Saghantelian_1              Mus musculus (Mouse)             NEWT
2   Saghantelian_2              Mus musculus (Mouse)             NEWT
3   Saghantelian_3              Mus musculus (Mouse)             NEWT
4   Saghantelian_4              Mus musculus (Mouse)             NEWT
5   Saghantelian_5              Mus musculus (Mouse)             NEWT
6   Saghantelian_6              Mus musculus (Mouse)             NEWT
7   Saghantelian_7              Mus musculus (Mouse)             NEWT
8   Saghantelian_8              Mus musculus (Mouse)             NEWT
9   Saghantelian_9              Mus musculus (Mouse)             NEWT
10 Saghantelian_10              Mus musculus (Mouse)             NEWT
11 Saghantelian_11              Mus musculus (Mouse)             NEWT
12 Saghantelian_12              Mus musculus (Mouse)             NEWT
   Term Accession Number Characteristics[tissue] Term Source REF
1                  10090             spinal cord              MA
2                  10090             spinal cord              MA
3                  10090             spinal cord              MA
4                  10090             spinal cord              MA
5                  10090             spinal cord              MA
6                  10090             spinal cord              MA
7                  10090             spinal cord              MA
8                  10090             spinal cord              MA
9                  10090             spinal cord              MA
10                 10090             spinal cord              MA
11                 10090             spinal cord              MA
12                 10090             spinal cord              MA
   Term Accession Number      Protocol REF Sample Name Factor Value[Genotype]
```

```
1                    216 sample collection         KO1                      KO
2                    216 sample collection         KO2                      KO
3                    216 sample collection         KO3                      KO
4                    216 sample collection         KO4                      KO
5                    216 sample collection         KO5                      KO
6                    216 sample collection         KO6                      KO
7                    216 sample collection         WT1                      WT
8                    216 sample collection         WT2                      WT
9                    216 sample collection         WT3                      WT
10                   216 sample collection         WT4                      WT
11                   216 sample collection         WT5                      WT
12                   216 sample collection         WT6                      WT
   Term Source REF Term Accession Number
1                NA                    NA
2                NA                    NA
3                NA                    NA
4                NA                    NA
5                NA                    NA
6                NA                    NA
7                NA                    NA
8                NA                    NA
9                NA                    NA
10               NA                    NA
11               NA                    NA
12               NA                    NA


Slot "assay.filenames":
                V2
"a_metabolite.txt"

Slot "assay.filenames.per.study":
$`Global metabolite profiling of faah(-/-) mice`
$`Global metabolite profiling of faah(-/-) mice`[[1]]
[1] "a_metabolite.txt"



Slot "assay.files":
$a_metabolite.txt
   Sample Name Protocol REF Extract Name Protocol REF Labeled Extract Name
1          KO1   extraction          KO1     labeling                    NA
2          KO2   extraction          KO2     labeling                    NA
3          KO3   extraction          KO3     labeling                    NA
4          KO4   extraction          KO4     labeling                    NA
5          KO5   extraction          KO5     labeling                    NA
6          KO6   extraction          KO6     labeling                    NA
7          WT1   extraction          WT1     labeling                    NA
8          WT2   extraction          WT2     labeling                    NA
9          WT3   extraction          WT3     labeling                    NA
10         WT4   extraction          WT4     labeling                    NA
11         WT5   extraction          WT5     labeling                    NA
12         WT6   extraction          WT6     labeling                    NA
   Label Term Source REF Term Accession Number      Protocol REF
```

```
1     NA              NA                    NA mass spectrometry
2     NA              NA                    NA mass spectrometry
3     NA              NA                    NA mass spectrometry
4     NA              NA                    NA mass spectrometry
5     NA              NA                    NA mass spectrometry
6     NA              NA                    NA mass spectrometry
7     NA              NA                    NA mass spectrometry
8     NA              NA                    NA mass spectrometry
9     NA              NA                    NA mass spectrometry
10    NA              NA                    NA mass spectrometry
11    NA              NA                    NA mass spectrometry
12    NA              NA                    NA mass spectrometry
   Parameter Value[instrument] Term Source REF Term Accession Number
1       Agilent 1100 LC-MSD SL              NA                    NA
2       Agilent 1100 LC-MSD SL              NA                    NA
3       Agilent 1100 LC-MSD SL              NA                    NA
4       Agilent 1100 LC-MSD SL              NA                    NA
5       Agilent 1100 LC-MSD SL              NA                    NA
6       Agilent 1100 LC-MSD SL              NA                    NA
7       Agilent 1100 LC-MSD SL              NA                    NA
8       Agilent 1100 LC-MSD SL              NA                    NA
9       Agilent 1100 LC-MSD SL              NA                    NA
10      Agilent 1100 LC-MSD SL              NA                    NA
11      Agilent 1100 LC-MSD SL              NA                    NA
12      Agilent 1100 LC-MSD SL              NA                    NA
   Parameter Value[ion source] Term Source REF Term Accession Number
1      electrospray ionization              MS                1000073
2      electrospray ionization              MS                1000073
3      electrospray ionization              MS                1000073
4      electrospray ionization              MS                1000073
5      electrospray ionization              MS                1000073
6      electrospray ionization              MS                1000073
7      electrospray ionization              MS                1000073
8      electrospray ionization              MS                1000073
9      electrospray ionization              MS                1000073
10     electrospray ionization              MS                1000073
11     electrospray ionization              MS                1000073
12     electrospray ionization              MS                1000073
   Parameter Value[detector] Term Source REF Term Accession Number
1                         NA              NA                    NA
2                         NA              NA                    NA
3                         NA              NA                    NA
4                         NA              NA                    NA
5                         NA              NA                    NA
6                         NA              NA                    NA
7                         NA              NA                    NA
8                         NA              NA                    NA
9                         NA              NA                    NA
10                        NA              NA                    NA
11                        NA              NA                    NA
12                        NA              NA                    NA
   Parameter Value[ionization mode] Term Source REF Term Accession Number
1                     positive mode              NA                    NA
2                     positive mode              NA                    NA
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3                     positive mode              NA                     NA
4                     positive mode              NA                     NA
5                     positive mode              NA                     NA
6                     positive mode              NA                     NA
7                     positive mode              NA                     NA
8                     positive mode              NA                     NA
9                     positive mode              NA                     NA
10                    positive mode              NA                     NA
11                    positive mode              NA                     NA
12                    positive mode              NA                     NA
   MS Assay Name Raw Spectral Data File Protocol REF Normalization Name
1        lc-ms-1      ./cdf/KO/ko15.CDF           NA                   NA
2        lc-ms-2      ./cdf/KO/ko16.CDF           NA                   NA
3        lc-ms-3      ./cdf/KO/ko18.CDF           NA                   NA
4        lc-ms-4      ./cdf/KO/ko19.CDF           NA                   NA
5        lc-ms-5      ./cdf/KO/ko21.CDF           NA                   NA
6        lc-ms-6      ./cdf/KO/ko22.CDF           NA                   NA
7        lc-ms-7      ./cdf/WT/wt15.CDF           NA                   NA
8        lc-ms-8      ./cdf/WT/wt16.CDF           NA                   NA
9        lc-ms-9      ./cdf/WT/wt18.CDF           NA                   NA
10      lc-ms-10      ./cdf/WT/wt19.CDF           NA                   NA
11      lc-ms-11      ./cdf/WT/wt21.CDF           NA                   NA
12      lc-ms-12      ./cdf/WT/wt22.CDF           NA                   NA
   Data Transformation Name Derived Spectral Data File Factor Value[Genotype]
1                        NA             faahkoDSDF.txt                     KO
2                        NA             faahkoDSDF.txt                     KO
3                        NA             faahkoDSDF.txt                     KO
4                        NA             faahkoDSDF.txt                     KO
5                        NA             faahkoDSDF.txt                     KO
6                        NA             faahkoDSDF.txt                     KO
7                        NA             faahkoDSDF.txt                     WT
8                        NA             faahkoDSDF.txt                     WT
9                        NA             faahkoDSDF.txt                     WT
10                       NA             faahkoDSDF.txt                     WT
11                       NA             faahkoDSDF.txt                     WT
12                       NA             faahkoDSDF.txt                     WT
   Term Source REF Term Accession Number
1               NA                    NA
2               NA                    NA
3               NA                    NA
4               NA                    NA
5               NA                    NA
6               NA                    NA
7               NA                    NA
8               NA                    NA
9               NA                    NA
10              NA                    NA
11              NA                    NA
12              NA                    NA


Slot "assay.files.per.study":
$`Global metabolite profiling of faah(-/-) mice`
$`Global metabolite profiling of faah(-/-) mice`[[1]]
```

```
   Sample Name Protocol REF Extract Name Protocol REF Labeled Extract Name
1          KO1   extraction          KO1     labeling                   NA
2          KO2   extraction          KO2     labeling                   NA
3          KO3   extraction          KO3     labeling                   NA
4          KO4   extraction          KO4     labeling                   NA
5          KO5   extraction          KO5     labeling                   NA
6          KO6   extraction          KO6     labeling                   NA
7          WT1   extraction          WT1     labeling                   NA
8          WT2   extraction          WT2     labeling                   NA
9          WT3   extraction          WT3     labeling                   NA
10         WT4   extraction          WT4     labeling                   NA
11         WT5   extraction          WT5     labeling                   NA
12         WT6   extraction          WT6     labeling                   NA
   Label Term Source REF Term Accession Number      Protocol REF
1     NA              NA                    NA mass spectrometry
2     NA              NA                    NA mass spectrometry
3     NA              NA                    NA mass spectrometry
4     NA              NA                    NA mass spectrometry
5     NA              NA                    NA mass spectrometry
6     NA              NA                    NA mass spectrometry
7     NA              NA                    NA mass spectrometry
8     NA              NA                    NA mass spectrometry
9     NA              NA                    NA mass spectrometry
10    NA              NA                    NA mass spectrometry
11    NA              NA                    NA mass spectrometry
12    NA              NA                    NA mass spectrometry
   Parameter Value[instrument] Term Source REF Term Accession Number
1       Agilent 1100 LC-MSD SL              NA                    NA
2       Agilent 1100 LC-MSD SL              NA                    NA
3       Agilent 1100 LC-MSD SL              NA                    NA
4       Agilent 1100 LC-MSD SL              NA                    NA
5       Agilent 1100 LC-MSD SL              NA                    NA
6       Agilent 1100 LC-MSD SL              NA                    NA
7       Agilent 1100 LC-MSD SL              NA                    NA
8       Agilent 1100 LC-MSD SL              NA                    NA
9       Agilent 1100 LC-MSD SL              NA                    NA
10      Agilent 1100 LC-MSD SL              NA                    NA
11      Agilent 1100 LC-MSD SL              NA                    NA
12      Agilent 1100 LC-MSD SL              NA                    NA
   Parameter Value[ion source] Term Source REF Term Accession Number
1      electrospray ionization              MS               1000073
2      electrospray ionization              MS               1000073
3      electrospray ionization              MS               1000073
4      electrospray ionization              MS               1000073
5      electrospray ionization              MS               1000073
6      electrospray ionization              MS               1000073
7      electrospray ionization              MS               1000073
8      electrospray ionization              MS               1000073
9      electrospray ionization              MS               1000073
10     electrospray ionization              MS               1000073
11     electrospray ionization              MS               1000073
12     electrospray ionization              MS               1000073
   Parameter Value[detector] Term Source REF Term Accession Number
1                         NA              NA                    NA
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2                          NA              NA                     NA
3                          NA              NA                     NA
4                          NA              NA                     NA
5                          NA              NA                     NA
6                          NA              NA                     NA
7                          NA              NA                     NA
8                          NA              NA                     NA
9                          NA              NA                     NA
10                         NA              NA                     NA
11                         NA              NA                     NA
12                         NA              NA                     NA
   Parameter Value[ionization mode] Term Source REF Term Accession Number
1                     positive mode              NA                     NA
2                     positive mode              NA                     NA
3                     positive mode              NA                     NA
4                     positive mode              NA                     NA
5                     positive mode              NA                     NA
6                     positive mode              NA                     NA
7                     positive mode              NA                     NA
8                     positive mode              NA                     NA
9                     positive mode              NA                     NA
10                    positive mode              NA                     NA
11                    positive mode              NA                     NA
12                    positive mode              NA                     NA
   MS Assay Name Raw Spectral Data File Protocol REF Normalization Name
1        lc-ms-1      ./cdf/KO/ko15.CDF           NA                  NA
2        lc-ms-2      ./cdf/KO/ko16.CDF           NA                  NA
3        lc-ms-3      ./cdf/KO/ko18.CDF           NA                  NA
4        lc-ms-4      ./cdf/KO/ko19.CDF           NA                  NA
5        lc-ms-5      ./cdf/KO/ko21.CDF           NA                  NA
6        lc-ms-6      ./cdf/KO/ko22.CDF           NA                  NA
7        lc-ms-7      ./cdf/WT/wt15.CDF           NA                  NA
8        lc-ms-8      ./cdf/WT/wt16.CDF           NA                  NA
9        lc-ms-9      ./cdf/WT/wt18.CDF           NA                  NA
10      lc-ms-10      ./cdf/WT/wt19.CDF           NA                  NA
11      lc-ms-11      ./cdf/WT/wt21.CDF           NA                  NA
12      lc-ms-12      ./cdf/WT/wt22.CDF           NA                  NA
   Data Transformation Name Derived Spectral Data File Factor Value[Genotype]
1                        NA                         NA                     KO
2                        NA                         NA                     KO
3                        NA                         NA                     KO
4                        NA                         NA                     KO
5                        NA                         NA                     KO
6                        NA                         NA                     KO
7                        NA                         NA                     WT
8                        NA                         NA                     WT
9                        NA                         NA                     WT
10                       NA                         NA                     WT
11                       NA                         NA                     WT
12                       NA                         NA                     WT
   Term Source REF Term Accession Number
1               NA                    NA
2               NA                    NA
3               NA                    NA
```

```
4               NA                    NA
5               NA                    NA
6               NA                    NA
7               NA                    NA
8               NA                    NA
9               NA                    NA
10              NA                    NA
11              NA                    NA
12              NA                    NA


Slot "assay.names":
$a_metabolite.txt
   MS Assay Name
1         lc-ms-1
2         lc-ms-2
3         lc-ms-3
4         lc-ms-4
5         lc-ms-5
6         lc-ms-6
7         lc-ms-7
8         lc-ms-8
9         lc-ms-9
10       lc-ms-10
11       lc-ms-11
12       lc-ms-12


Slot "assay.technology.types":
[1] "mass spectrometry"

Slot "assay.measurement.types":
[1] "metabolite profiling"

Slot "data.filenames":
$a_metabolite.txt
   Raw Spectral Data File Derived Spectral Data File
1        ./cdf/KO/ko15.CDF             faahkoDSDF.txt
2        ./cdf/KO/ko16.CDF             faahkoDSDF.txt
3        ./cdf/KO/ko18.CDF             faahkoDSDF.txt
4        ./cdf/KO/ko19.CDF             faahkoDSDF.txt
5        ./cdf/KO/ko21.CDF             faahkoDSDF.txt
6        ./cdf/KO/ko22.CDF             faahkoDSDF.txt
7        ./cdf/WT/wt15.CDF             faahkoDSDF.txt
8        ./cdf/WT/wt16.CDF             faahkoDSDF.txt
9        ./cdf/WT/wt18.CDF             faahkoDSDF.txt
10       ./cdf/WT/wt19.CDF             faahkoDSDF.txt
11       ./cdf/WT/wt21.CDF             faahkoDSDF.txt
12       ./cdf/WT/wt22.CDF             faahkoDSDF.txt


Slot "samples":
 [1] "KO1" "KO2" "KO3" "KO4" "KO5" "KO6" "WT1" "WT2" "WT3" "WT4" "WT5" "WT6"
```

```
Slot "samples.per.study":
$`Global metabolite profiling of faah(-/-) mice`
 [1] "KO1" "KO2" "KO3" "KO4" "KO5" "KO6" "WT1" "WT2" "WT3" "WT4" "WT5" "WT6"


Slot "samples.per.assay.filename":
$a_metabolite.txt
 [1] "KO1" "KO2" "KO3" "KO4" "KO5" "KO6" "WT1" "WT2" "WT3" "WT4" "WT5" "WT6"


Slot "assay.filenames.per.sample":
[[1]]
[[1]][[1]]
[1] "a_metabolite.txt"


[[2]]
[[2]][[1]]
[1] "a_metabolite.txt"


[[3]]
[[3]][[1]]
[1] "a_metabolite.txt"


[[4]]
[[4]][[1]]
[1] "a_metabolite.txt"


[[5]]
[[5]][[1]]
[1] "a_metabolite.txt"


[[6]]
[[6]][[1]]
[1] "a_metabolite.txt"


[[7]]
[[7]][[1]]
[1] "a_metabolite.txt"


[[8]]
[[8]][[1]]
[1] "a_metabolite.txt"


[[9]]
[[9]][[1]]
```

```
[1] "a_metabolite.txt"


[[10]]
[[10]][[1]]
[1] "a_metabolite.txt"


[[11]]
[[11]][[1]]
[1] "a_metabolite.txt"


[[12]]
[[12]][[1]]
[1] "a_metabolite.txt"



Slot "sample.to.rawdatafile":
[[1]]
   Sample Name Raw Spectral Data File
1          KO1      ./cdf/KO/ko15.CDF
2          KO2      ./cdf/KO/ko16.CDF
3          KO3      ./cdf/KO/ko18.CDF
4          KO4      ./cdf/KO/ko19.CDF
5          KO5      ./cdf/KO/ko21.CDF
6          KO6      ./cdf/KO/ko22.CDF
7          WT1      ./cdf/WT/wt15.CDF
8          WT2      ./cdf/WT/wt16.CDF
9          WT3      ./cdf/WT/wt18.CDF
10         WT4      ./cdf/WT/wt19.CDF
11         WT5      ./cdf/WT/wt21.CDF
12         WT6      ./cdf/WT/wt22.CDF


Slot "sample.to.assayname":
[[1]]
   Sample Name MS Assay Name
1          KO1       lc-ms-1
2          KO2       lc-ms-2
3          KO3       lc-ms-3
4          KO4       lc-ms-4
5          KO5       lc-ms-5
6          KO6       lc-ms-6
7          WT1       lc-ms-7
8          WT2       lc-ms-8
9          WT3       lc-ms-9
10         WT4      lc-ms-10
11         WT5      lc-ms-11
12         WT6      lc-ms-12


Slot "rawdatafile.to.sample":
```

```
[[1]]
   Raw Spectral Data File Sample Name
1        ./cdf/KO/ko15.CDF         KO1
2        ./cdf/KO/ko16.CDF         KO2
3        ./cdf/KO/ko18.CDF         KO3
4        ./cdf/KO/ko19.CDF         KO4
5        ./cdf/KO/ko21.CDF         KO5
6        ./cdf/KO/ko22.CDF         KO6
7        ./cdf/WT/wt15.CDF         WT1
8        ./cdf/WT/wt16.CDF         WT2
9        ./cdf/WT/wt18.CDF         WT3
10       ./cdf/WT/wt19.CDF         WT4
11       ./cdf/WT/wt21.CDF         WT5
12       ./cdf/WT/wt22.CDF         WT6


Slot "assayname.to.sample":
[[1]]
   MS Assay Name Sample Name
1         lc-ms-1         KO1
2        lc-ms-10         WT4
3        lc-ms-11         WT5
4        lc-ms-12         WT6
5         lc-ms-2         KO2
6         lc-ms-3         KO3
7         lc-ms-4         KO4
8         lc-ms-5         KO5
9         lc-ms-6         KO6
10        lc-ms-7         WT1
11        lc-ms-8         WT2
12        lc-ms-9         WT3


Slot "factors":
[[1]]
[[1]]$`Factor Value[Genotype]`
 [1] KO KO KO KO KO KO WT WT WT WT WT WT
Levels: KO WT



Slot "treatments":
$`Factor Value[Genotype]`
[1] KO WT
Levels: KO WT


Slot "groups":
[[1]]
[[1]][[1]]
[1] "KO1" "KO2" "KO3" "KO4" "KO5" "KO6"

[[1]][[2]]
[1] "WT1" "WT2" "WT3" "WT4" "WT5" "WT6"
```

```
Slot "assay.tabs":
[[1]]
An object of class "MSAssayTab"
Slot "path":
[1] "/home/biocbuild/bbs-3.9-bioc/R/library/faahKO"

Slot "study.filename":
[1] "s_Proteomic_profiling_of_yeast_TFs.txt"

Slot "study.identifier":
[1] "Global metabolite profiling of faah(-/-) mice"

Slot "assay.filename":
[1] "a_metabolite.txt"

Slot "assay.file":
   Sample Name Protocol REF Extract Name Protocol REF Labeled Extract Name
1          KO1   extraction          KO1     labeling                   NA
2          KO2   extraction          KO2     labeling                   NA
3          KO3   extraction          KO3     labeling                   NA
4          KO4   extraction          KO4     labeling                   NA
5          KO5   extraction          KO5     labeling                   NA
6          KO6   extraction          KO6     labeling                   NA
7          WT1   extraction          WT1     labeling                   NA
8          WT2   extraction          WT2     labeling                   NA
9          WT3   extraction          WT3     labeling                   NA
10         WT4   extraction          WT4     labeling                   NA
11         WT5   extraction          WT5     labeling                   NA
12         WT6   extraction          WT6     labeling                   NA
   Label Term Source REF Term Accession Number      Protocol REF
1     NA              NA                    NA mass spectrometry
2     NA              NA                    NA mass spectrometry
3     NA              NA                    NA mass spectrometry
4     NA              NA                    NA mass spectrometry
5     NA              NA                    NA mass spectrometry
6     NA              NA                    NA mass spectrometry
7     NA              NA                    NA mass spectrometry
8     NA              NA                    NA mass spectrometry
9     NA              NA                    NA mass spectrometry
10    NA              NA                    NA mass spectrometry
11    NA              NA                    NA mass spectrometry
12    NA              NA                    NA mass spectrometry
   Parameter Value[instrument] Term Source REF Term Accession Number
1       Agilent 1100 LC-MSD SL              NA                    NA
2       Agilent 1100 LC-MSD SL              NA                    NA
3       Agilent 1100 LC-MSD SL              NA                    NA
4       Agilent 1100 LC-MSD SL              NA                    NA
5       Agilent 1100 LC-MSD SL              NA                    NA
6       Agilent 1100 LC-MSD SL              NA                    NA
7       Agilent 1100 LC-MSD SL              NA                    NA
8       Agilent 1100 LC-MSD SL              NA                    NA
```

```
9       Agilent 1100 LC-MSD SL              NA                     NA
10      Agilent 1100 LC-MSD SL              NA                     NA
11      Agilent 1100 LC-MSD SL              NA                     NA
12      Agilent 1100 LC-MSD SL              NA                     NA
   Parameter Value[ion source] Term Source REF Term Accession Number
1      electrospray ionization              MS                1000073
2      electrospray ionization              MS                1000073
3      electrospray ionization              MS                1000073
4      electrospray ionization              MS                1000073
5      electrospray ionization              MS                1000073
6      electrospray ionization              MS                1000073
7      electrospray ionization              MS                1000073
8      electrospray ionization              MS                1000073
9      electrospray ionization              MS                1000073
10     electrospray ionization              MS                1000073
11     electrospray ionization              MS                1000073
12     electrospray ionization              MS                1000073
   Parameter Value[detector] Term Source REF Term Accession Number
1                         NA              NA                     NA
2                         NA              NA                     NA
3                         NA              NA                     NA
4                         NA              NA                     NA
5                         NA              NA                     NA
6                         NA              NA                     NA
7                         NA              NA                     NA
8                         NA              NA                     NA
9                         NA              NA                     NA
10                        NA              NA                     NA
11                        NA              NA                     NA
12                        NA              NA                     NA
   Parameter Value[ionization mode] Term Source REF Term Accession Number
1                     positive mode              NA                     NA
2                     positive mode              NA                     NA
3                     positive mode              NA                     NA
4                     positive mode              NA                     NA
5                     positive mode              NA                     NA
6                     positive mode              NA                     NA
7                     positive mode              NA                     NA
8                     positive mode              NA                     NA
9                     positive mode              NA                     NA
10                    positive mode              NA                     NA
11                    positive mode              NA                     NA
12                    positive mode              NA                     NA
   MS Assay Name Raw Spectral Data File Protocol REF Normalization Name
1        lc-ms-1      ./cdf/KO/ko15.CDF           NA                  NA
2        lc-ms-2      ./cdf/KO/ko16.CDF           NA                  NA
3        lc-ms-3      ./cdf/KO/ko18.CDF           NA                  NA
4        lc-ms-4      ./cdf/KO/ko19.CDF           NA                  NA
5        lc-ms-5      ./cdf/KO/ko21.CDF           NA                  NA
6        lc-ms-6      ./cdf/KO/ko22.CDF           NA                  NA
7        lc-ms-7      ./cdf/WT/wt15.CDF           NA                  NA
8        lc-ms-8      ./cdf/WT/wt16.CDF           NA                  NA
9        lc-ms-9      ./cdf/WT/wt18.CDF           NA                  NA
10      lc-ms-10      ./cdf/WT/wt19.CDF           NA                  NA
```

```
11      lc-ms-11      ./cdf/WT/wt21.CDF           NA                  NA
12      lc-ms-12      ./cdf/WT/wt22.CDF           NA                  NA
   Data Transformation Name Derived Spectral Data File Factor Value[Genotype]
1                        NA                          NA                     KO
2                        NA                          NA                     KO
3                        NA                          NA                     KO
4                        NA                          NA                     KO
5                        NA                          NA                     KO
6                        NA                          NA                     KO
7                        NA                          NA                     WT
8                        NA                          NA                     WT
9                        NA                          NA                     WT
10                       NA                          NA                     WT
11                       NA                          NA                     WT
12                       NA                          NA                     WT
   Term Source REF Term Accession Number
1               NA                    NA
2               NA                    NA
3               NA                    NA
4               NA                    NA
5               NA                    NA
6               NA                    NA
7               NA                    NA
8               NA                    NA
9               NA                    NA
10              NA                    NA
11              NA                    NA
12              NA                    NA

Slot "assay.technology.type":
[1] "mass spectrometry"

Slot "assay.measurement.type":
[1] "metabolite profiling"

Slot "assay.names":
   MS Assay Name
1        lc-ms-1
2        lc-ms-2
3        lc-ms-3
4        lc-ms-4
5        lc-ms-5
6        lc-ms-6
7        lc-ms-7
8        lc-ms-8
9        lc-ms-9
10      lc-ms-10
11      lc-ms-11
12      lc-ms-12

Slot "data.filenames":
   Raw Spectral Data File Derived Spectral Data File
1       ./cdf/KO/ko15.CDF                          NA
2       ./cdf/KO/ko16.CDF                          NA
```

```
3       ./cdf/KO/ko18.CDF                       NA
4       ./cdf/KO/ko19.CDF                       NA
5       ./cdf/KO/ko21.CDF                       NA
6       ./cdf/KO/ko22.CDF                       NA
7       ./cdf/WT/wt15.CDF                       NA
8       ./cdf/WT/wt16.CDF                       NA
9       ./cdf/WT/wt18.CDF                       NA
10      ./cdf/WT/wt19.CDF                       NA
11      ./cdf/WT/wt21.CDF                       NA
12      ./cdf/WT/wt22.CDF                       NA
```

For an example for a real use case, please refer to https://github.com/sneumann/mtbls2/.

Writing ISA-Tab datasets

The Risa package offers functions to write the whole ISA-Tab dataset or part of it back to disk. These functions are `write.ISAtab`, `write.investigation.file`, `write.study.file`, `write.assay.file`.

So, after updating the assay file as indicated above, we can save it back to disk, using the following command:

```
> temp = tempdir()
> write.ISAtab(faahkoISA, temp)
> #write.assay.file(faahkoISA, assay.filename, temp)
```

Session Info

```
> toLatex(sessionInfo())
```

- R version 3.6.0 (2019-04-26), `x86_64-pc-linux-gnu`
- Locale: `LC_CTYPE=en_US.UTF-8`, `LC_NUMERIC=C`, `LC_TIME=en_US.UTF-8`, `LC_COLLATE=C`, `LC_MONETARY=en_US.UTF-8`, `LC_MESSAGES=en_US.UTF-8`, `LC_PAPER=en_US.UTF-8`, `LC_NAME=C`, `LC_ADDRESS=C`, `LC_TELEPHONE=C`, `LC_MEASUREMENT=en_US.UTF-8`, `LC_IDENTIFICATION=C`
- Running under: `Ubuntu 18.04.2 LTS`
- Matrix products: default
- BLAS: `/home/biocbuild/bbs-3.9-bioc/R/lib/libRblas.so`
- LAPACK: `/home/biocbuild/bbs-3.9-bioc/R/lib/libRlapack.so`
- Base packages: base, datasets, grDevices, graphics, methods, parallel, stats, stats4, utils
- Other packages: Biobase 2.44.0, BiocGenerics 0.30.0, BiocParallel 1.18.0, MSnbase 2.10.0, ProtGenerics 1.16.0, Rcpp 1.0.1, Risa 1.26.0, S4Vectors 0.22.0, affy 1.62.0, biocViews 1.52.0, faahKO 1.23.1, mzR 2.18.0, xcms 3.6.0
- Loaded via a namespace (and not attached): BiocManager 1.30.4, DEoptimR 1.0-8, IRanges 2.18.0, MALDIquant 1.19.2, MASS 7.3-51.4, MassSpecWavelet 1.50.0, Matrix 1.2-17, R6 2.4.0, RANN 2.6.1, RBGL 1.60.0, RColorBrewer 1.1-2, RCurl 1.95-4.12, RUnit 0.4.32, XML 3.98-1.19, affyio 1.54.0, assertthat 0.2.1, bitops 1.0-6, codetools 0.2-16, colorspace 1.4-1, compiler 3.6.0, crayon 1.3.4, digest 0.6.18, doParallel 1.0.14, dplyr 0.8.0.1, foreach 1.4.4, ggplot2 3.1.1, glue 1.3.1, graph 1.62.0, grid 3.6.0, gtable 0.3.0, impute 1.58.0, iterators 1.0.10, lattice 0.20-38, lazyeval 0.2.2, limma 3.40.0, magrittr 1.5, multtest 2.40.0,

munsell 0.5.0, mzID 1.22.0, ncdf4 1.16.1, pcaMethods 1.76.0, pillar 1.3.1, pkgconfig 2.0.2, plyr 1.8.4, preprocessCore 1.46.0, purrr 0.3.2, rlang 0.3.4, robustbase 0.93-4, scales 1.0.0, splines 3.6.0, survival 2.44-1.1, tibble 2.1.1, tidyselect 0.2.5, tools 3.6.0, vsn 3.52.0, zlibbioc 1.30.0

Further information

For further information about the ISA software infrastructure, please visit our website http://isa-tools.org.