

MSnbase development

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August 27, 2012

Abstract

This vignette describes the classes implemented in **MSnbase** package. It is intended as a starting point for developers or users who would like to learn more or further develop/extend **pSet**.

Keywords: Mass Spectrometry (MS), proteomics, infrastructure.

Contents

1	Introduction	2
2	MSnbase classes	2
2.1	pSet : a virtual class for raw mass spectrometry data and meta data	2
2.2	MSnExp : a class for MS experiments	3
2.3	MSnSet : a class for quantitative proteomics data	4
2.4	MSnProcess : a class for logging processing meta data	4
2.5	MIAPe : Minimum Information About a Proteomics Experiment	5
2.6	Spectrum <i>et al.</i> : classes for MS spectra	7
2.7	ReporterIons : a class for isobaric tags	8
2.8	NAnnotatedDataFrame : multiplexed AnnotatedDataFrames	9
3	Miscellaneous	9
4	Session information	10

Foreword

MSnbase is under active development; current functionality is evolving and new features will be added. This software is free and open-source software. If you use it, please support the project by citing it in publications:

Laurent Gatto and Kathryn S. Lilley. *MSnbase - an R/Bioconductor package for isobaric tagged mass spectrometry data visualization, processing and quantitation*. *Bioinformatics* 28, 288-289 (2011).

You are welcome to contact me for questions, bugs, typos or suggestions about MSnbase. If you wish to reach a broader audience for general questions about proteomics analysis using R, you may want to use the Bioconductor mailing list¹.

1 Introduction

This document is not a replacement for the individual manual pages, that document the slots of the MSnbase classes. It is a centralised high-level description of the package design.

MSnbase aims at being compatible with the Biobase infrastructure [Gentleman et al. \(2004\)](#). Many meta data structures that are used in eSet and associated classes are also used here. As such, knowledge of the *Biobase development and the new eSet vignette*² would be beneficial.

The initial goal is to use the MSnbase infrastructure for labelled quantitation using reporter ions (iTRAQ ([Ross et al., 2004](#)) and TMT ([Thompson et al., 2003](#))). Spectral counting should be trivial to apply with current features, as long as identification data is at hand. Currently, no effort is invested to streamline label-free quantitative proteomics, although some effort has been done to keep the infrastructure flexible enough to accommodate more designs.

2 MSnbase classes

All classes have a `__classVersion__` slot, of class `Versioned` from the Biobase package. This slot documents the class version for any instance to be used for debugging and object update purposes. Any change in a class implementation should trigger a version change.

2.1 pSet: a virtual class for raw mass spectrometry data and meta data

This virtual class is the main container for mass spectrometry data, i.e spectra, and meta data. It is based on the eSet implementation for genomic data.

¹<https://stat.ethz.ch/mailman/listinfo/bioconductor>

²The vignette can directly be accessed with `vignette("BiobaseDevelopment", package="Biobase")` once Biobase is loaded.

The main difference with `eSet` is that the `assayData` slot is an environment containing any number of `Spectrum` instances (see section 2.6).

One new slot is introduced, namely `processingData`, that contains one `MSnProcess` instance (see section 2.4). and the `experimentData` slot is now expected to contain MIAPE data (see section 2.5). The `annotation` slot has not been implemented, as no prior feature annotation is known in shotgun proteomics.

```
> getClass("pSet")
```

```
Virtual Class "pSet" [package "MSnbase"]
```

```
Slots:
```

Name:	assayData	phenoData	featureData
Class:	environment	NAnnotatedDataFrame	AnnotatedDataFrame
Name:	experimentData	protocolData	processingData
Class:	MIAxE	AnnotatedDataFrame	MSnProcess
Name:	.cache	.__classVersion__	
Class:	environment	Versions	

```
Extends: "Versioned"
```

```
Known Subclasses: "MSnExp"
```

Future work Currently, few setters have been implemented.

2.2 MSnExp: a class for MS experiments

`MSnExp` extends `pSet` to store MS experiments. It does not add any new slots to `pSet`. Accessors and setters are all inherited from `pSet` and new ones should be implemented for `pSet`. Methods that manipulate actual data in experiments are implemented for `MSnExp` objects.

```
> getClass("MSnExp")
```

```
Class "MSnExp" [package "MSnbase"]
```

```
Slots:
```

Name:	assayData	phenoData	featureData
Class:	environment	NAnnotatedDataFrame	AnnotatedDataFrame
Name:	experimentData	protocolData	processingData
Class:	MIAxE	AnnotatedDataFrame	MSnProcess

```

Name:          .cache    .__classVersion__
Class:         environment    Versions

Extends:
Class "pSet", directly
Class "Versioned", by class "pSet", distance 2

```

2.3 MSnSet: a class for quantitative proteomics data

This class stores quantitation data and meta data after running `quantify` on an `MSnExp` object. The quantitative data is in form of a $n \times m$ matrix, where m is the number of features/spectra originally in the `MSnExp` used as parameter in `quantify` and m is the number of reporter ions (see section 2.7).

This prompted to keep a similar implementation as the `ExpressionSet` class, while adding the proteomics-specific annotation slot introduced in the `pSet` class, namely `processingData` for objects of class `MSnProcess` (see section 2.4).

The `MSnSet` class extends the virtual `eSet` class to provide compatibility for `ExpressionSet`-like behaviour. The experiment meta-data in `experimentData` is also of class `MIAPE` (see section 2.5). The `annotation` slot, inherited from `eSet` is not used.

```

> getClass("MSnSet")

Class "MSnSet" [package "MSnbase"]

Slots:

Name:      experimentData    processingData    qual
Class:     MIAPE             MSnProcess         data.frame

Name:      assayData         phenoData         featureData
Class:     AssayData AnnotatedDataFrame AnnotatedDataFrame

Name:      annotation        protocolData    .__classVersion__
Class:     character AnnotatedDataFrame    Versions

Extends:
Class "eSet", directly
Class "VersionedBiobase", by class "eSet", distance 2
Class "Versioned", by class "eSet", distance 3

```

2.4 MSnProcess: a class for logging processing meta data

This class aims at recording specific manipulations applied to `MSnExp` or `MSnSet` instances. The `processing` slot is a `character` vector that describes major

processing. Most other slots are of class `logical` that indicate whether the data has been centroided, smoothed, ...although many of the functionality is not implemented yet. Any new processing that is implemented should be documented and logged here.

It also documents the raw data file from which the data originates (`files` slot) and the `MSnbase` version that was in use when the `MSnProcess` instance, and hence the `MSnExp/MSnSet` objects, were originally created.

```
> getClass("MSnProcess")
```

```
Class "MSnProcess" [package "MSnbase"]
```

```
Slots:
```

Name:	files	processing	merged	cleaned
Class:	character	character	logical	logical
Name:	removedPeaks	smoothed	trimmed	normalised
Class:	character	logical	numeric	logical
Name:	MSnbaseVersion	.__classVersion__		
Class:	character	Versions		

```
Extends: "Versioned"
```

2.5 MIAPE: Minimum Information About a Proteomics Experiment

The Minimum Information About a Proteomics Experiment ([Taylor et al., 2007, 2008](#)) MIAPE class describes the experiment, including contact details, information about the mass spectrometer and control and analysis software.

Raw data is currently imported from `mzXML` files ([Pedrioli et al., 2004](#)) < using the `xcms::rampRawData` and `xcms::rampRawDataMSn` functions from the `xcms` package ([Smith et al., 2006](#)). These functions do not give access to the meta data. New importer functions are under development (see for instance `mzR`³) that will hopefully give programmatic access to meta data stored in the data file to populate the MIAPE object.

```
> getClass("MIAPE")
```

```
Class "MIAPE" [package "MSnbase"]
```

```
Slots:
```

Name:	title	url
-------	-------	-----

³<https://github.com/sneumann/mzR/blob/master/DESCRIPTION>

Class:	character	character
Name:	abstract	pubMedIds
Class:	character	character
Name:	samples	preprocessing
Class:	list	list
Name:	other	dateStamp
Class:	list	character
Name:	name	lab
Class:	character	character
Name:	contact	instrumentModel
Class:	character	character
Name:	instrumentManufacturer	instrumentCustomisations
Class:	character	character
Name:	softwareName	softwareVersion
Class:	character	character
Name:	switchingCriteria	isolationWidth
Class:	character	numeric
Name:	parameterFile	ionSource
Class:	character	character
Name:	ionSourceDetails	analyser
Class:	character	character
Name:	analyserDetails	collisionGas
Class:	character	character
Name:	collisionPressure	collisionEnergy
Class:	numeric	character
Name:	detectorType	detectorSensitivity
Class:	character	character
Name:	.__classVersion__	
Class:	Versions	

Extends:
Class "MIAxE", directly

Class "Versioned", by class "MIAxE", distance 2

2.6 Spectrum *et al.*: classes for MS spectra

`Spectrum` is a virtual class that defines common attributes to all types of spectra. MS1 and MS2 specific attributes are defined in the `Spectrum1` and `Spectrum2` classes, that directly extend `Spectrum`.

The choices of attributes has been dictated by the `xcms::rampRawData` and `xcms::rampRawDataMSn` functions and what data from the `mzXML` file they gave access to. It is expected that some hopefully minor changes might come up here when migrating to other data import packages, that allow random access to `mzXML` data and support `mzML` (Martens et al., 2010).

```
> getClass("Spectrum")
```

Virtual Class "Spectrum" [package "MSnbase"]

Slots:

Name:	msLevel	peaksCount	rt	acquisitionNum
Class:	integer	integer	numeric	integer
Name:	scanIndex	mz	intensity	fromFile
Class:	integer	numeric	numeric	integer
Name:	centroided	__classVersion__		
Class:	logical	Versions		

Extends: "Versioned"

Known Subclasses: "Spectrum2", "Spectrum1"

```
> getClass("Spectrum1")
```

Class "Spectrum1" [package "MSnbase"]

Slots:

Name:	polarity	msLevel	peaksCount	rt
Class:	integer	integer	integer	numeric
Name:	acquisitionNum	scanIndex	mz	intensity
Class:	integer	integer	numeric	numeric
Name:	fromFile	centroided	__classVersion__	
Class:	integer	logical	Versions	

```

Extends:
Class "Spectrum", directly
Class "Versioned", by class "Spectrum", distance 2

> getClass("Spectrum2")

Class "Spectrum2" [package "MSnbase"]

Slots:

Name:          merged          precScanNum          precursorMz
Class:         numeric         integer           numeric

Name: precursorIntensity  precursorCharge  collisionEnergy
Class:         numeric         integer           numeric

Name:          msLevel          peaksCount          rt
Class:         integer         integer           numeric

Name:          acquisitionNum    scanIndex          mz
Class:         integer         integer           numeric

Name:          intensity          fromFile          centroided
Class:         numeric         integer           logical

Name:    __classVersion__
Class:    Versions

Extends:
Class "Spectrum", directly
Class "Versioned", by class "Spectrum", distance 2

```

2.7 ReporterIons: a class for isobaric tags

The iTRAQ and TMT (or any other peak of interest) are implemented `ReporterIons` instances, that essentially defines an expected MZ position for the peak and a width around this value as well a names for the reporters.

```

> getClass("ReporterIons")

Class "ReporterIons" [package "MSnbase"]

Slots:

Name:          name          reporterNames          description          mz
Class:         character         character           character           numeric

```


Name:	col	width	__classVersion__
Class:	character	numeric	Versions

Extends: "Versioned"

2.8 NAnnotatedDataFrame: multiplexed AnnotatedDataFrames

The simple expansion of the `AnnotatedDataFrame` classes adds the `multiplex` and `multiLabel` slots to document the number and names of multiplexed samples.

```
> getClass("NAnnotatedDataFrame")
```

```
Class "NAnnotatedDataFrame" [package "MSnbase"]
```

Slots:

Name:	multiplex	multiLabels	varMetadata	data
Class:	numeric	character	data.frame	data.frame

Name:	dimLabels	__classVersion__
Class:	character	Versions

Extends:

Class "AnnotatedDataFrame", directly

Class "Versioned", by class "AnnotatedDataFrame", distance 2

3 Miscellaneous

Unit tests MSnbase implements unit tests with the `testthat` package.

Processing methods Methods that process raw data, i.e. spectra should be implemented for `Spectrum` objects first and then `eapply`'ed (or similar) to the `assayData` slot of an `MSnExp` instance in the specific method.

Speed and memory requirements Raw mass spectrometry file are generally several hundreds of MB large and most of this is used for binary raw spectrum data. As such, data containers can easily grow very large and thus require large amounts of RAM. This requirement is being tackled by avoiding to load the raw data into memory and using on-disk random access to the content of `mzXML`/`mzML` data files on demand. When focusing on reporter ion quantitation, a direct solution for this is to trim the spectra using the `trimMz` method to select the area of interest and thus substantially reduce the size of the `Spectrum` objects. This is illustrated in section ?? on page ?? of the `MSnbase-demo` vignette.

The independent handling of spectra is ideally suited for parallel processing. The `quantify` method now performs reporter peaks quantitation in parallel. More functions are being updated.

4 Session information

- R version 2.15.1 (2012-06-22), i386-pc-mingw32
- Locale: LC_COLLATE=C, LC_CTYPE=English_United States.1252, LC_MONETARY=English_United States.1252, LC_NUMERIC=C, LC_TIME=English_United States.1252
- Base packages: base, datasets, grDevices, graphics, methods, stats, tools, utils
- Other packages: Biobase 2.16.0, BiocGenerics 0.2.0, MSnbase 1.4.1, Rcpp 0.9.13, cacheSweave 0.6-1, codetools 0.2-8, filehash 2.2-1, formatR 0.6, ggplot2 0.9.1, highlight 0.3.2, mzR 1.2.2, parser 0.0-16, pgfSweave 1.3.0, stashR 0.3-5
- Loaded via a namespace (and not attached): BiocInstaller 1.4.7, IRanges 1.14.4, MASS 7.3-20, RColorBrewer 1.0-5, affy 1.34.0, affyio 1.24.0, colorspace 1.1-1, dichromat 1.2-4, digest 0.5.2, grid 2.15.1, labeling 0.1, lattice 0.20-10, limma 3.12.1, memoise 0.1, munsell 0.3, plyr 1.7.1, preprocessCore 1.18.0, proto 0.3-9.2, reshape 0.8.4, reshape2 1.2.1, scales 0.2.1, stats4 2.15.1, stringr 0.6.1, tikzDevice 0.6.2, vsn 3.24.0, zlibbioc 1.2.0

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