

artMS

Analytical R Tools
for Mass Spectrometry

<http://artms.org>



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Stanford
MEDICINE

Cardiovascular Medicine
Ashley Lab


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Analytical R Tools
for Mass Spectrometry

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Goals

- 1 **Facilitate** the analysis of **Mass-Spectrometry** based **Proteomics** data using the  **programming language**
- 2 **Provide** different levels of analysis to ensure **data quality, quantification,** and frame results in a **biological context.**

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Quantification Methods

Label Free (+/- Fractionation)
SILAC

Analysis of

+Global Proteome

+PostTranslational Modifications

Phosphorylation

Ubiquitination

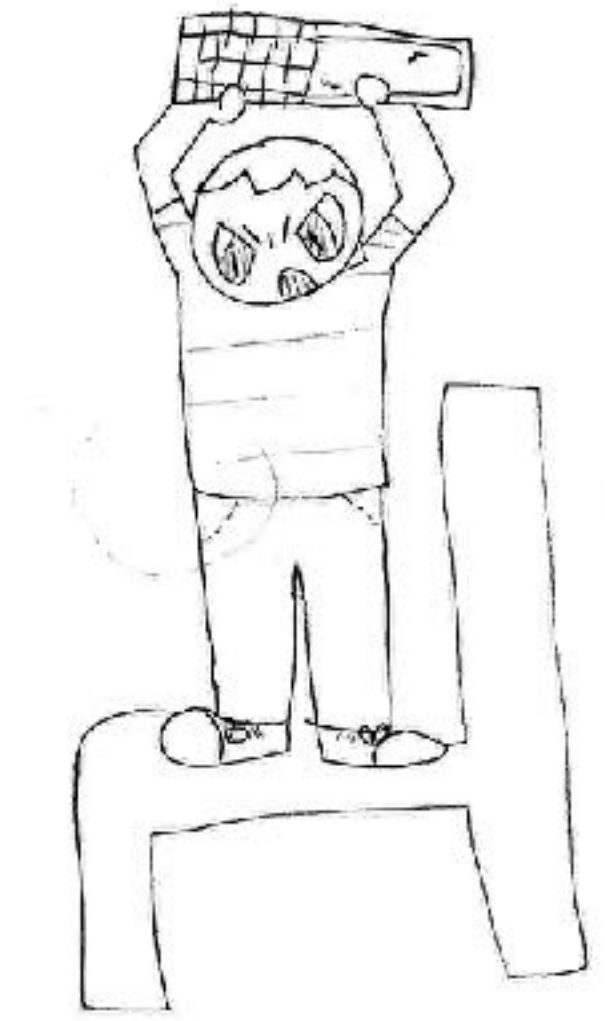
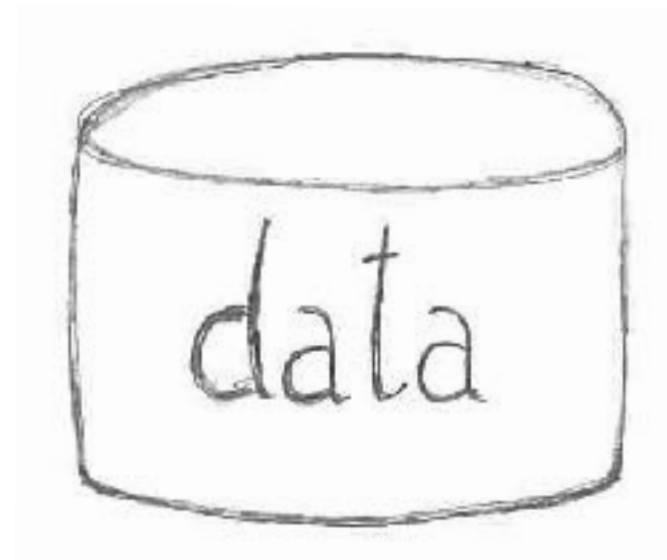
Acetylation ← (coming soon)



MS-Proteomics experts

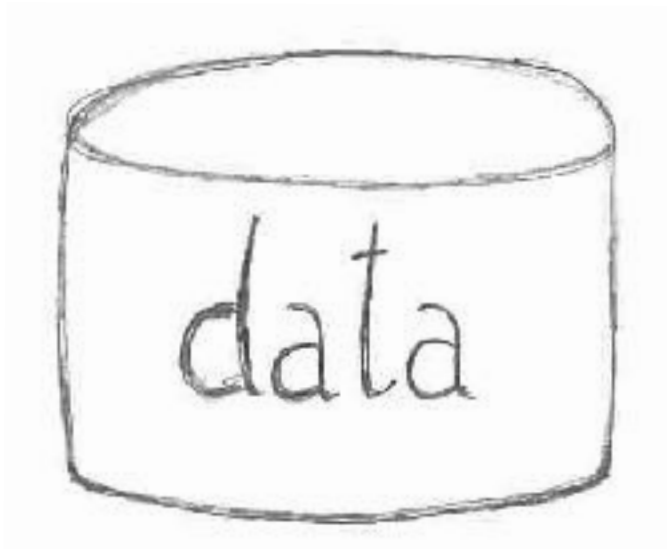


MS



System biologists
Data scientists

MS



Knowledge



MS-Proteomics experts

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Analytical R Tools
for Mass Spectrometry

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for Mass Spectrometry



System biologists
Data scientists

Cancer

samples

WT

Proteomics pipeline
PH, UB, AB, APMS...

Experimental



Mass Spectrometry

Computational

.raw files

output files



MaxQuant

Peptide Identification

- Tool widely used
- Algorithms yield high mass accuracy and precision
- It covers a wide variety of proteomics experiments
- Simplified user interface
- It can now run on **Linux** (improved performance)

Cancer

samples

WT

Proteomics pipeline
PH, UB, AB, APMS...



Mass Spectrometry

Experimental

Computational

.raw files

output files



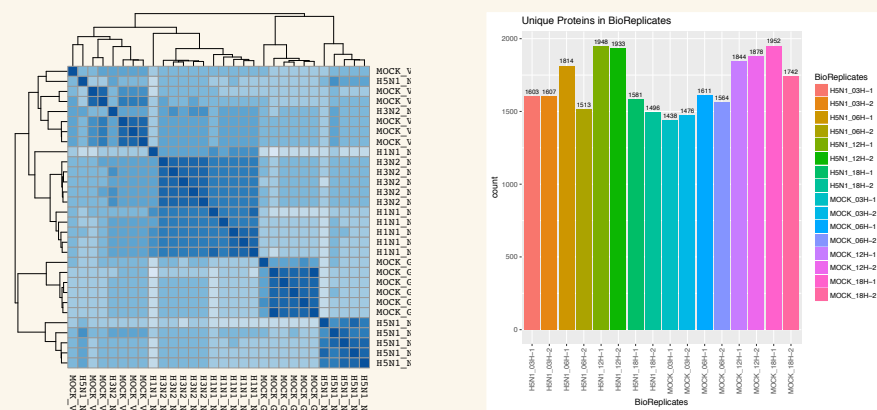
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Quality control



Relative
quantification

evidence + keys + contrast

log2FC / p-value / adj.pvalues

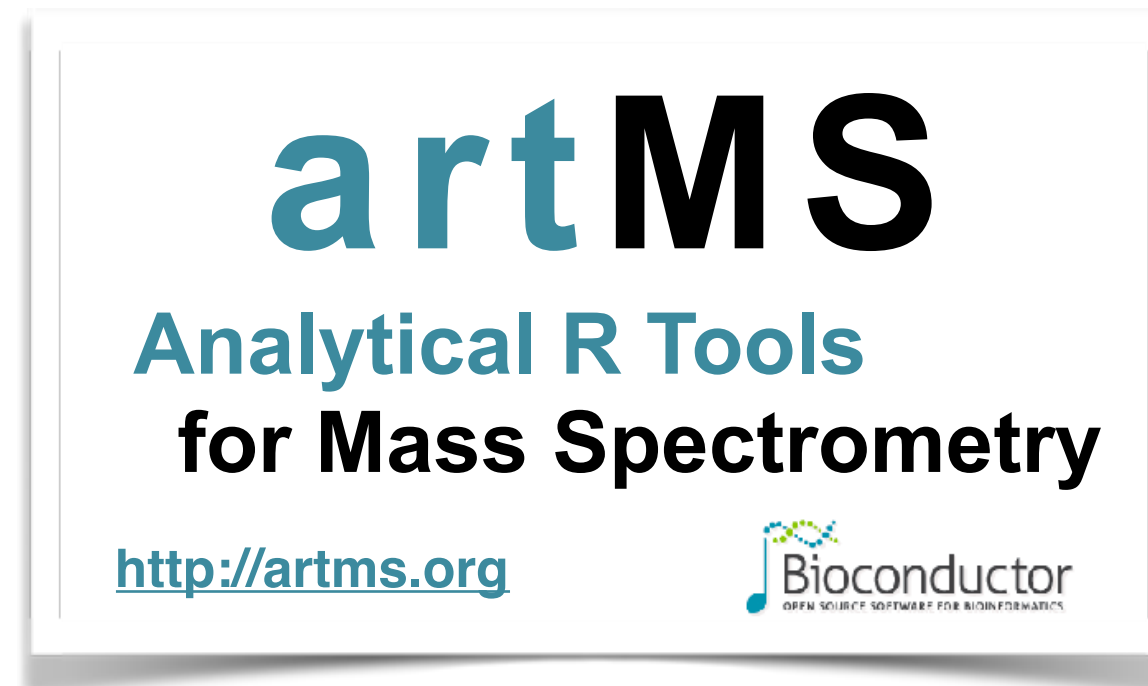
Functional analysis

Data integration
Imputation
Enrichment analysis
Pathway analysis
Network generation

Miscellaneous

SAINTq
SAINTexpress
PHOTON
Phosfate

How does it work?



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evidence.txt

+

keys.txt

+

contrast.txt

Configuration

config.yaml

Quality control

artmsQualityControlEvidenceBasic()
artmsQualityControlEvidenceExtended()
artmsQualityControlSummaryExtended()



Relative
quantification

artmsQuantification()

Functional analysis

artmsAnalysisQuantifications()

Miscellaneous

artmsEvidenceToSAINTq()
artmsEvidenceToSaintExpress()
artmsPhosphateOutput()
artmsPhotonOutput()
etc

Basic Functions

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Input Files

 evidence.txt

+

keys.txt

+

contrast.txt

Configuration

config.yaml

Experimental design

	A	B	C	D	E
1	RawFile	IsotopeLabelType	Condition	BioReplicate	Run
2	qx006145	L	Cal33	Cal33-1	1
3	qx006146	L	Cal33	Cal33-2	2
4	qx006147	L	Cal33	Cal33-3	3
5	qx006148	L	Cal33	Cal33-4	4
6	qx006154	L	HSC6	HSC6-1	5
7	qx006151	L	HSC6	HSC6-2	6
8	qx006152	L	HSC6	HSC6-3	7
9	qx006153	L	HSC6	HSC6-4	8
10					

- Two cell cancer cell lines: Cal33, HSC6
- 4 biological replicates each


Quality control



Relative
quantification

Functional analysis

Miscellaneous

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 evidence.txt

+


keys.txt

+

contrast.txt

Configuration
config.yaml

Quality control

 Relative quantification


Functional analysis

Miscellaneous

Relative quantification
between conditions

Cal33-HSC6

- Two cell cancer cell lines: **Cal33, HSC6**
- 4 biological replicates each

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 evidence.txt

+


keys.txt

+

contrast.txt

Configuration
config.yaml

Quality control

 Relative quantification

Functional analysis

Miscellaneous

Relative quantification
between conditions

- A-B
- A-C
- A-D
- D-E
- D-F
- D-G
- etc

- Two cell cancer cell lines: **A, B, C, D, E, F, G**
- 4 biological replicates each

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Quality control



Relative
quantification

Functional analysis

Miscellaneous

```
config.yaml
1 files :
2   evidence : '~/ph_ms/evidence.txt'
3   keys : '~/ph_ms/keys.txt'
4   contrasts : '~/ph_ms/contrast.txt'
5   output : '~/ph_ms/phglobal/phglobal-results.txt'
6 qc:
7   basic: 1
8   extended: 1
9 data:
10  enabled: 1
11  fractions:
12    enabled: 0
13  silac:
14    enabled: 0
15  filters:
16    enabled: 1
17    contaminants: 1
18    protein_groups: remove
19    modifications: PH
20  sample_plots: 1
21 msstats:
22  enabled: 1
23  msstats_input:
24  profilePlots: none
25  normalization_method: equalizeMedians
26  normalization_reference:
27  summaryMethod: TMP
28  censoredInt: NA
29  cutoffCensored: minFeature
30  MBimpute: 1
31  feature_subset: all
32 output_extras:
33  enabled: 1
34  annotate:
35    enabled: 1
36    specie: HUMAN
37  plots:
38    volcano: 1
39    heatmap: 1
40    LFC: -1.5 1.5
41    FDR: 0.05
42    heatmap_cluster_cols: 0
43    heatmap_display: log2FC
```

Files

Configuration

config.yaml

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Quality control



Relative
quantification

Functional analysis

Miscellaneous

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config.yaml
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```

Configuration

config.yaml

QC


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Quality control

 Relative
quantification

Functional analysis

Miscellaneous

Configuration

config.yaml

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config.yaml
1 files :
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6 qc:
7   basic: 1
8   extended: 1
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10  enabled: 1
11  fractions:
12    enabled: 0
13  silac:
14    enabled: 0
15  filters:
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18    protein_groups: remove
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Proteomics
details

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Quality control



Relative
quantification

Functional analysis

Miscellaneous

Configuration

config.yaml

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12    enabled: 0
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39     heatmap: 1
40     LFC: -1.5 1.5
41     FDR: 0.05
42     heatmap_cluster_cols: 0
43     heatmap_display: log2FC
```

Relative Quantification

(MSstats)

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Quality control



Relative
quantification

Functional analysis

Miscellaneous

Configuration


config.yaml

```
config.yaml
1 files :
2   evidence : '~/ph_ms/evidence.txt'
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4   contrasts : '~/ph_ms/contrast.txt'
5   output : '~/ph_ms/phglobal/phglobal-results.txt'
6 qc:
7   basic: 1
8   extended: 1
9 data:
10  enabled: 1
11  fractions:
12    enabled: 0
13  silac:
14    enabled: 0
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24  profilePlots: none
25  normalization_method: equalizeMedians
26  normalization_reference:
27  summaryMethod: TMP
28  censoredInt: NA
29  cutoffCensored: minFeature
30  MBimpute: 1
31  feature_subset: all
32  output_extras:
33    enabled: 1
34    annotate:
35      enabled: 1
36      specie: HUMAN
37    plots:
38      volcano: 1
39      heatmap: 1
40      LFC: -1.5 1.5
41      FDR: 0.05
42      heatmap_cluster_cols: 0
43      heatmap_display: log2FC
```

Outputs / extras

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Input Files

 evidence.txt

keys.txt

Quality control

artmsQualityControlEvidenceBasic()
artmsQualityControlEvidenceExtended()
artmsQualityControlSummaryExtended()

 Relative
quantification

Functional analysis

Miscellaneous

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for Mass Spectrometry
<http://artms.org>
Bioconductor

Input Files
evidence.txt
keys.txt

Quality control

artmsQualityControlEvidenceBasic()
artmsQualityControlEvidenceExtended()
artmsQualityControlSummaryExtended()

MS stats
Relative quantification

artmsQuantification()

Functional analysis

artmsAnalysisQuantifications()

Miscellaneous

artmsEvidenceToSAINTq()
artmsEvidenceToSaintExpress()
artmsPhosphateOutput()
artmsPhotonOutput()
etc

```

Console Terminal x
~/
> library(artMS)
> artmsQualityControlEvidenceBasic(evidence_file = "evidence.txt",
+                                   keys_file = "keys.txt")

QUALITY CONTROL -----
>> LOADING FILES
>> MERGING FILES
    It might take a long time
    (depending on the size of the evidence file)
>> GENERATING THE INTENSITY DISTRIBUTION PLOTS
>> CONTAMINANTS CON__IREV__ REMOVED
>> GENERATING THE REPRODUCIBILITY PLOTS
    (Warning: it might take some time)
|=====| 100%
>> GENERATING CORRELATION MATRICES
--- NO Technical Replicates detected
--- By Biological replicates
--- By Conditions
>> GENERATING INTENSITY STATS PLOTS
--- AB PROCESSED
>> BASIC QUALITY CONTROL ANALYSIS COMPLETED!
  
```

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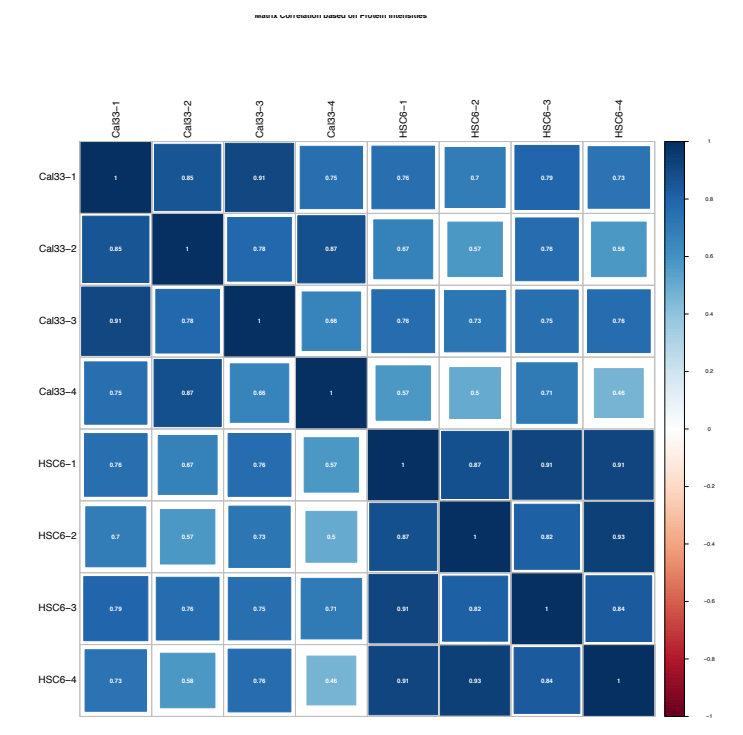
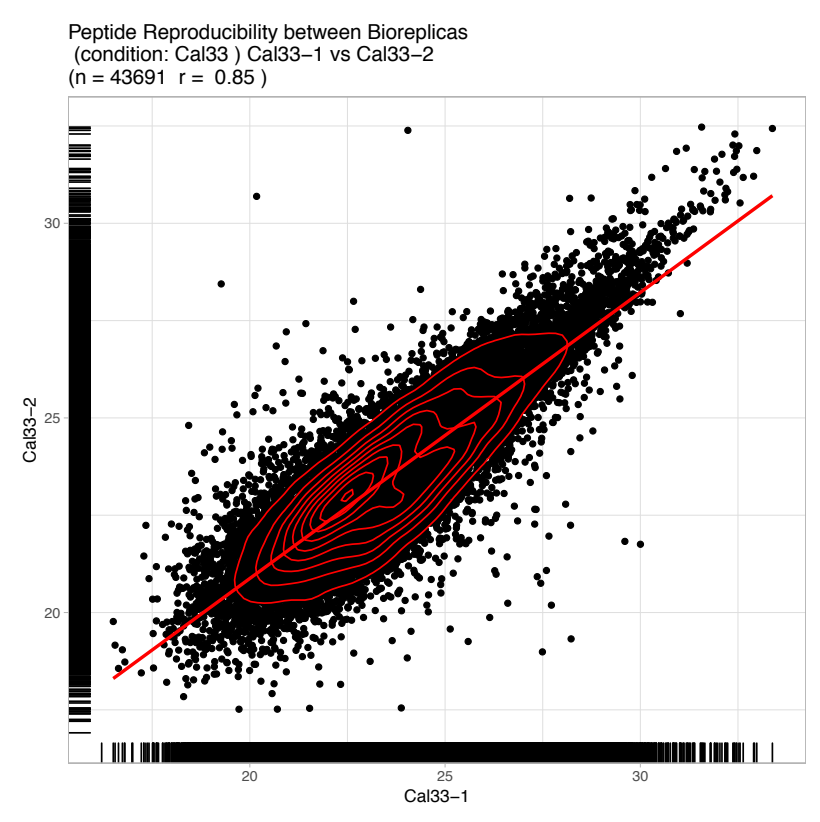
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for Mass Spectrometry

<http://artms.org>

Input Files

evidence.txt

keys.txt

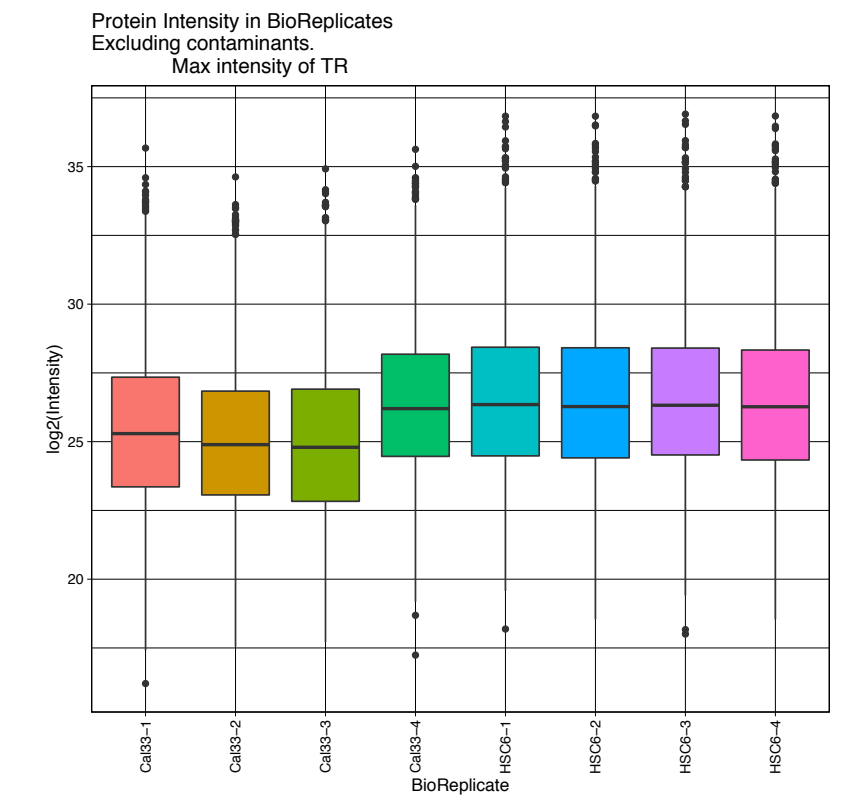
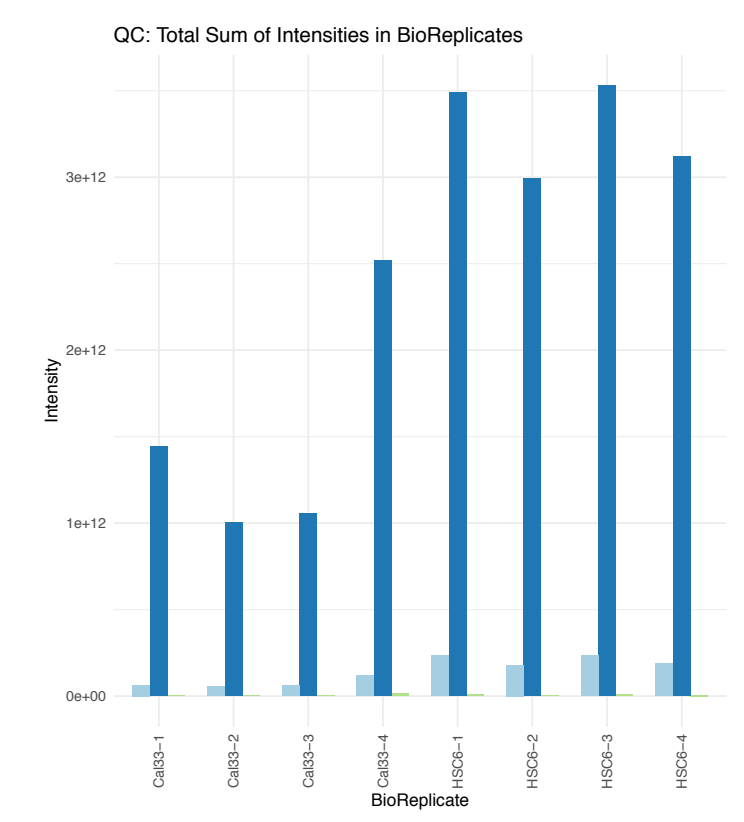
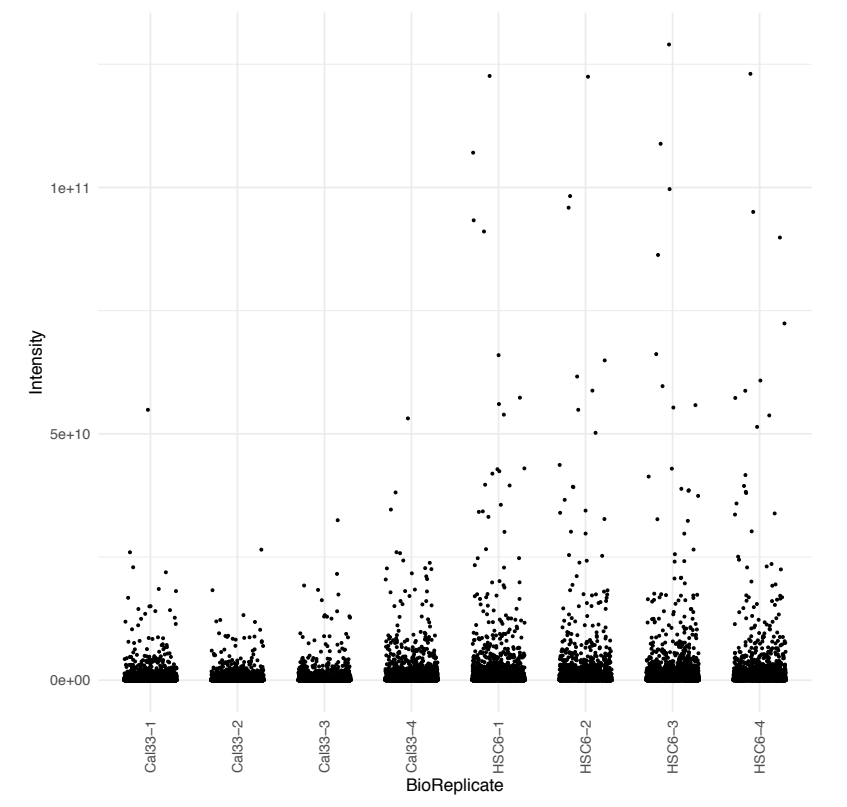


Quality control

`artmsQualityControlEvidenceBasic()`

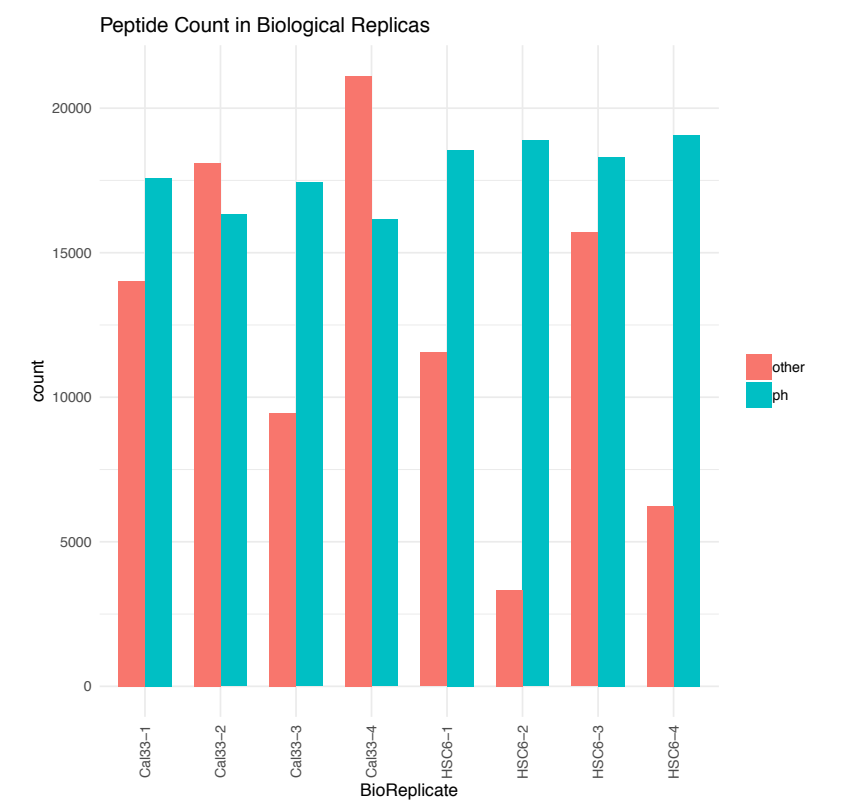
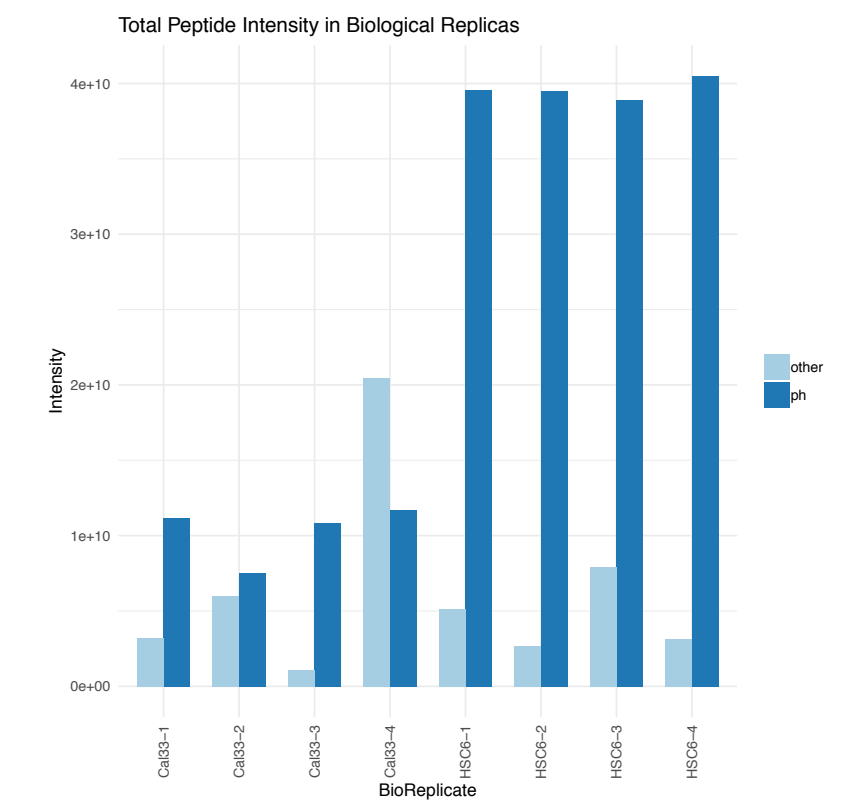
- Reproducibility
- MS Intensity
- Spectral Counts
- Contaminants
- PTMs

Relative quantification



Functional analysis

Miscellaneous



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for Mass Spectrometry
<http://artms.org>
Bioconductor

Input Files

Mo evidence.txt

keys.txt

Quality control

artmsQualityControlEvidenceBasic()
artmsQualityControlEvidenceExtended()

MS stats
Relative quantification

artmsQuantification()

Functional analysis

artmsAnalysisQuantifications()

Miscellaneous

artmsEvidenceToSAINTq()
artmsEvidenceToSaintExpress()
artmsPhosphateOutput()
artmsPhotonOutput()
etc

```


Console Terminal x
~/
> artmsQualityControlEvidenceExtended(evidence_file = "evidence.txt",
+                                     keys_file = "keys.txt")

>>EXTENDED QUALITY CONTROL ANALYSIS
(evidence.txt based)-----
>> MERGING FILES
    It might take a long time
    (depending on the size of the evidence file)
>> GENERATING QC PLOTS
--- Plot PSM done
--- Plot IONS done
--- Plot TYPE done
--- Plot PEPTIDES done
--- Plot PROTEINS done
--- Plot Plot Ion Oversampling done
--- Plot Charge State done
--- Plot Mass Error done
--- Plot Mass-over-Charge distribution done
--- Plot Peptide Intensity CV done
--- Plot Peptide Detection (using modified.sequence) done
--- Plot Protein Intensity CV done
--- Plot Protein Detection done
--- Plot ID overlap done
--- Plot Inter-Correlation done
--- Plot Sample Preparation done
  
```

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Input Files

`evidence.txt`

`keys.txt`

Quality control

Relative quantification

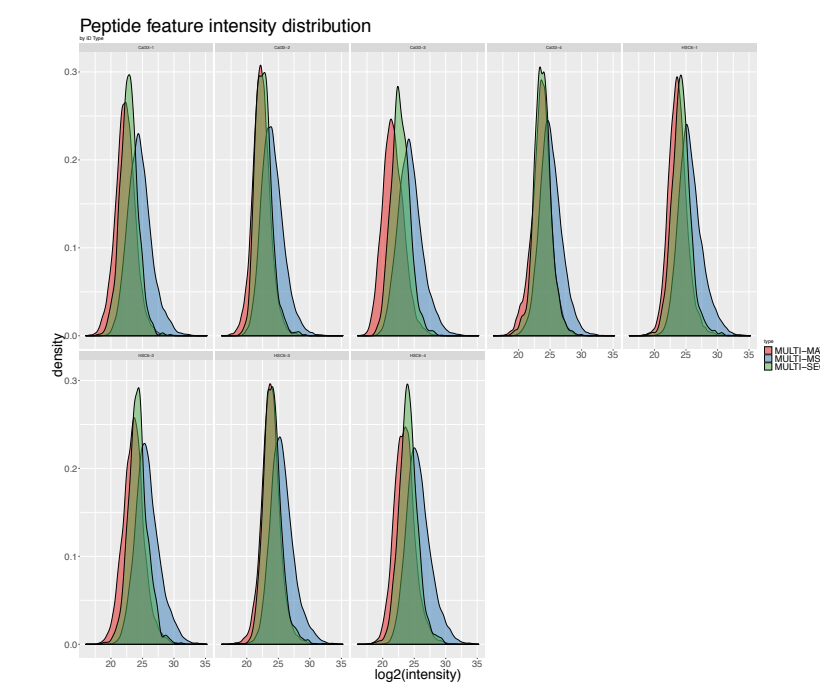
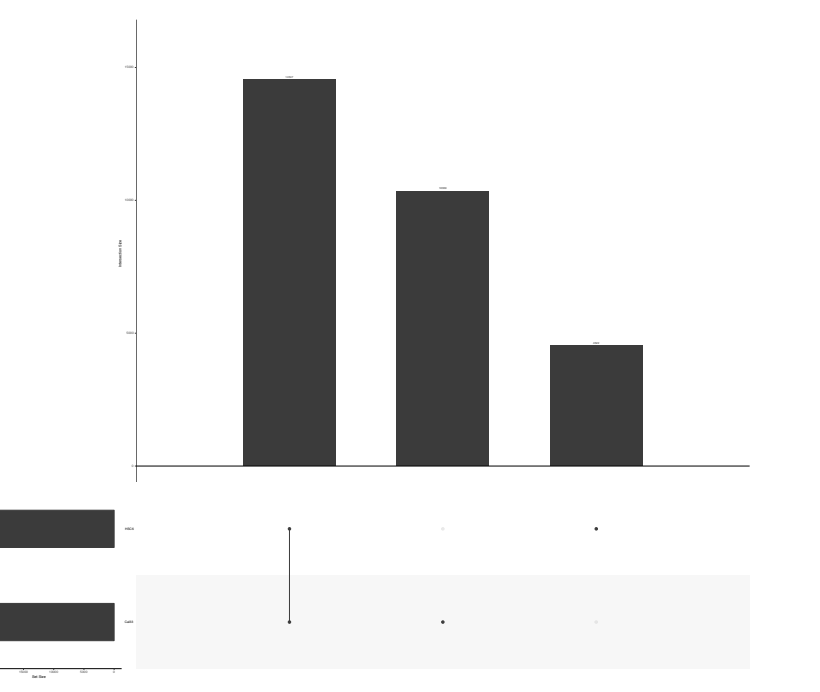
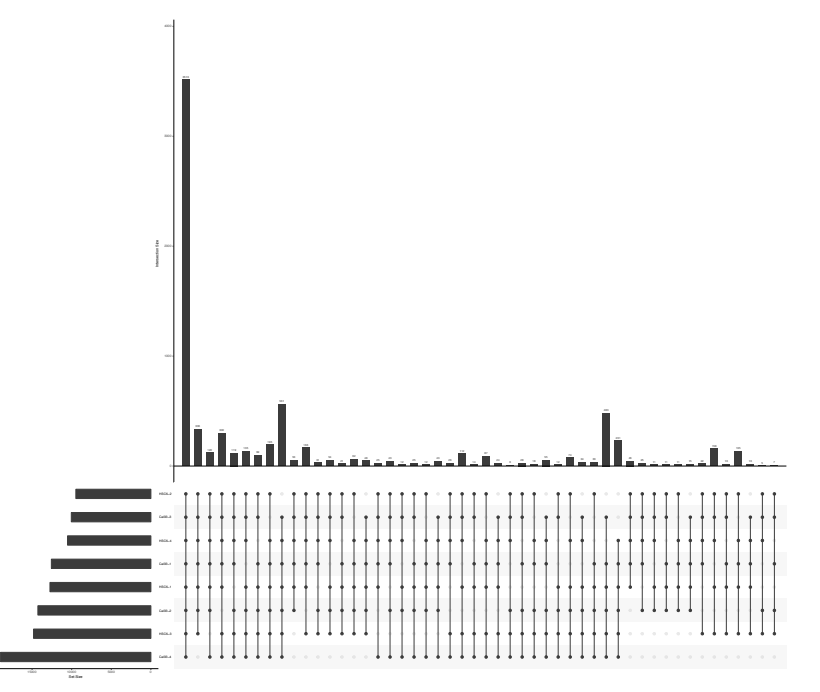
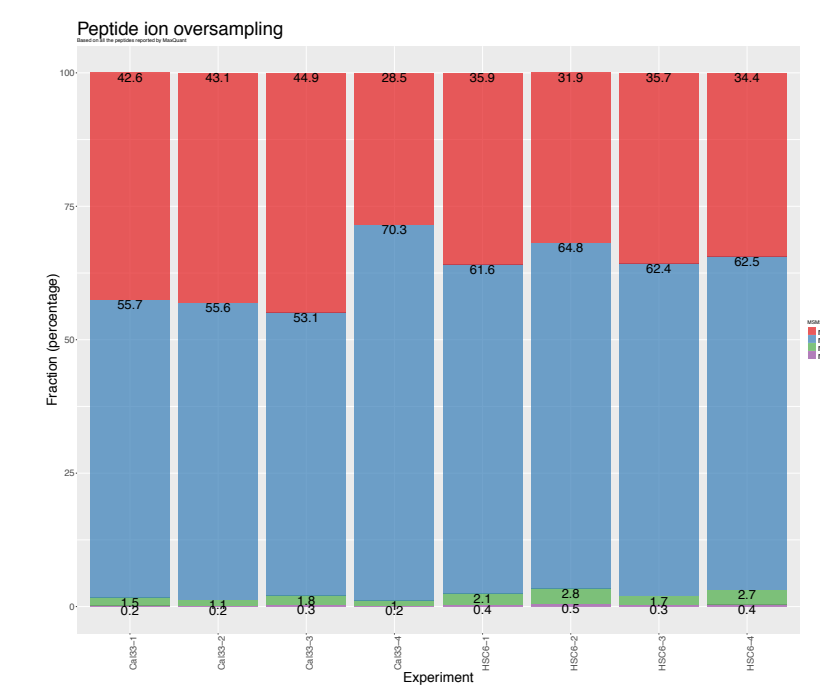
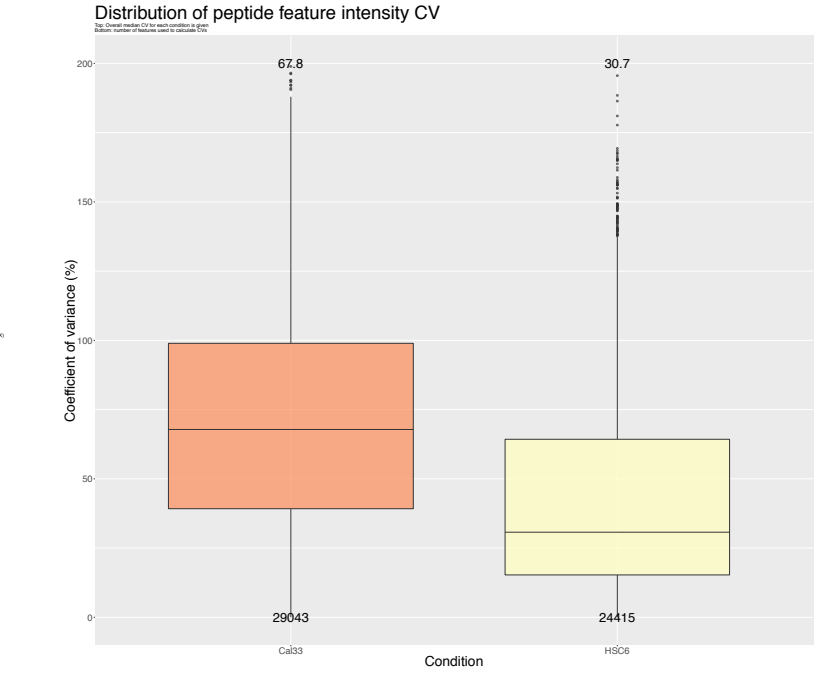
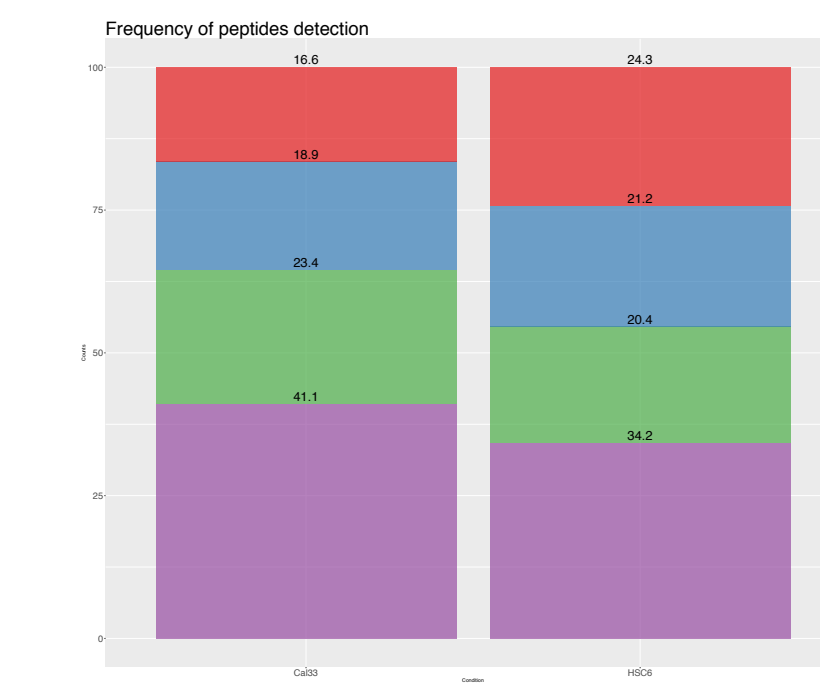
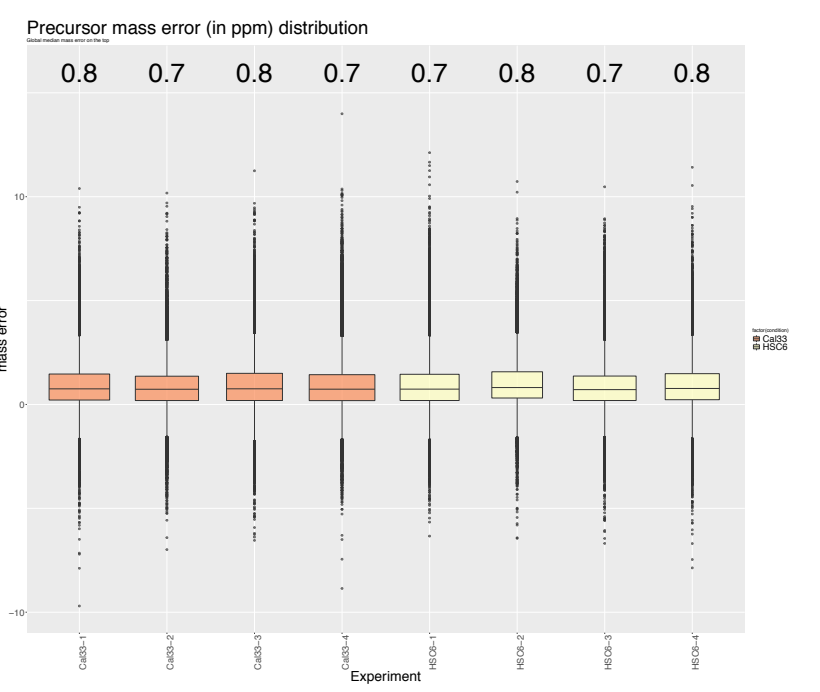
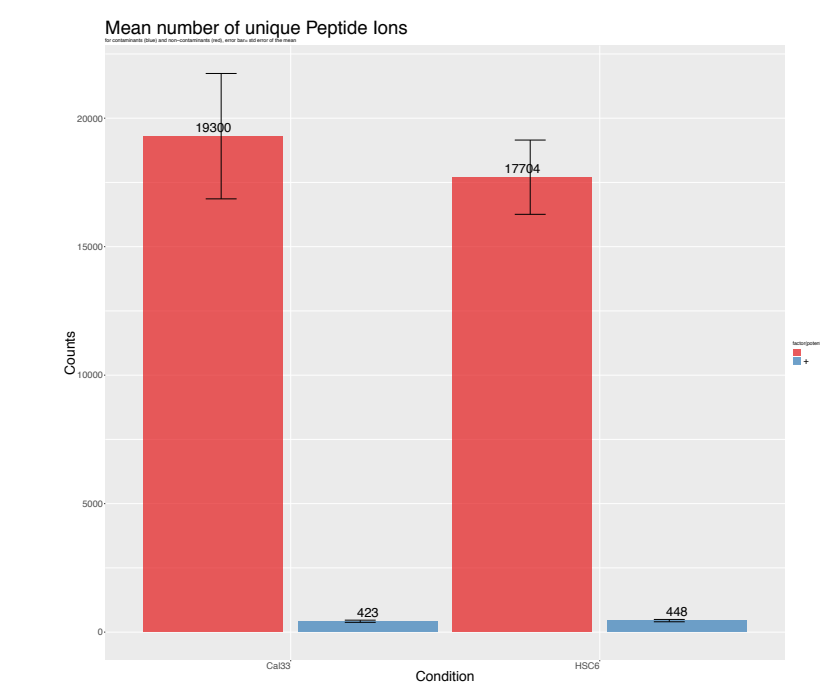
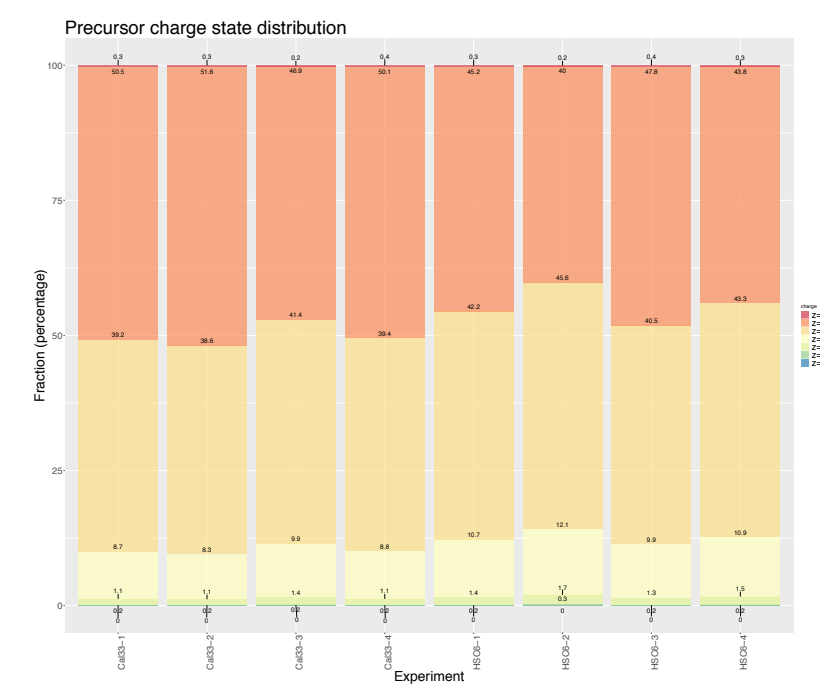
Functional analysis


Miscellaneous


```
artmsQualityControlEvidenceBasic()
artmsQualityControlEvidenceExtended()
```

- Charge State Distribution
- ID overlap
- Peptide Ion Statistics
- Precursor Mass Error
- Precursor m/z error
- Frequency Peptide Detection
- Peptide Intensity CV
- Peptide Statistics
- Peptide Ion oversampling
- Protein detection frequency
- Protein Statistics / CV
- Peptide-spectrum matches
- MaxQuant Type statistics

```
artmsAnalysisQuantifications()
artmsEvidenceToSintExpress()
artmsHostateOutput()
artmsPhotonOutput()
etc
```




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Input Files
 evidence.txt
keys.txt

Quality control

artmsQualityControlEvidenceBasic()
artmsQualityControlEvidenceExtended()
artmsQualityControlSummaryExtended()

 Relative quantification


artmsQuantification()


Functional analysis

artmsAnalysisQuantifications()

Miscellaneous


artmsEvidenceToSAINTq()
artmsEvidenceToSaintExpress()
artmsPhosphateOutput()
artmsPhotonOutput()
etc

artMS
Analytical R Tools
for Mass Spectrometry
<http://artms.org> 

Input Files
 evidence.txt
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Quality control

artmsQualityControlEvidenceBasic()
artmsQualityControlEvidenceExtended()
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 Relative quantification

artmsQuantification()

Functional analysis

artmsAnalysisQuantifications()


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artmsEvidenceToSAINTq()
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etc


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Input Files

 evidence.txt

keys.txt

Quality control



Relative quantification

`artmsQuantification()`

Functional analysis

Miscellaneous



<http://msstats.org/>

MSstats is an open-source R package for **statistical relative quantification of proteins** and peptides in global, targeted, and data-independent proteomics.

- It uses a family of **linear mixed models** that attempts to:
- minimize bias and inefficiencies in spectrometry-based proteomics,
 - distinguish the systematic variation from random artifacts,
 - maximize the reproducibility of the results

Developed by Meena Choi and **Olga Vitek**

Used in  Skyline

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Bioconductor

Input Files

- evidence.txt
- keys.txt
- contrast.txt
- config.yaml

Quality control

Relative quantification

Functional analysis

Miscellaneous

artmsQuantification("config.yaml") →

TXT FILES

- quantification-results.txt
- quantification-results-annotated.txt
- Normalized abundance
 - * results_ModelQC.txt
 - * results_RunlevelData.txt
 - * results-mss-groupQuant.txt
 - * results-mss-normalized.txt
 - * results-mss-sampleQuant.txt
- results_sampleSize.txt
- results_experimentPower.txt

PDF FILES

- results-heatmap.pdf
- results-peptidecounts-perBait.pdf
- results-peptidecounts.pdf
- results-sign.pdf
- results-volcano.pdf

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Input Files

 evidence.txt

keys.txt

contrast.txt

config.yaml

Quality control

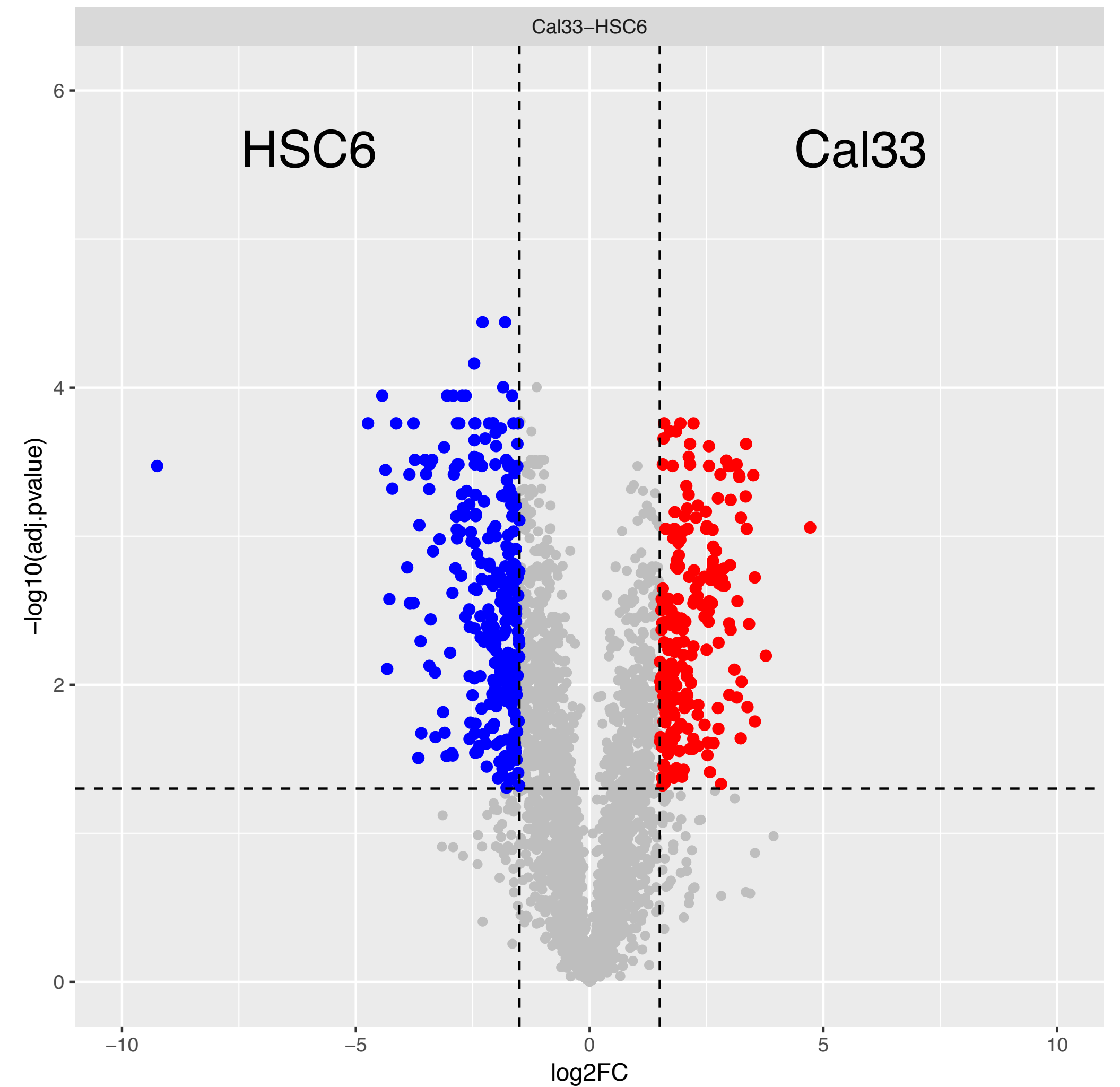


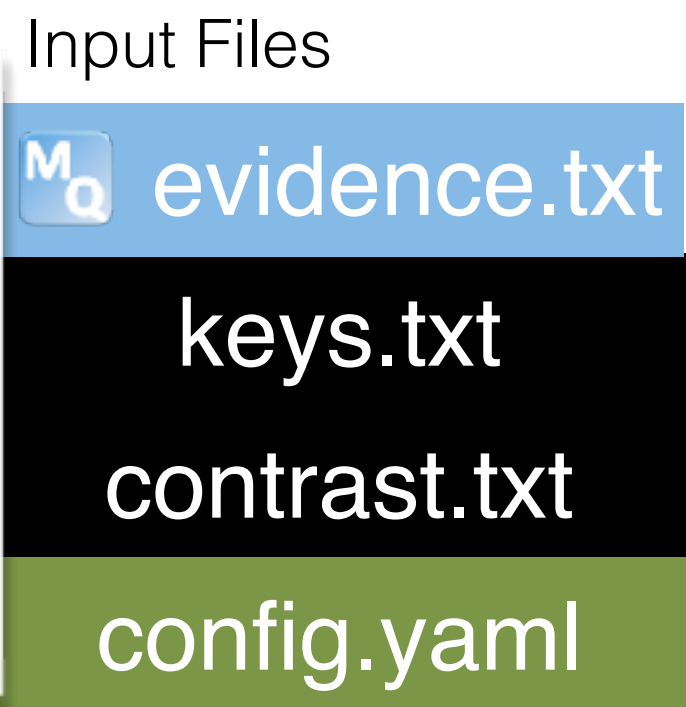
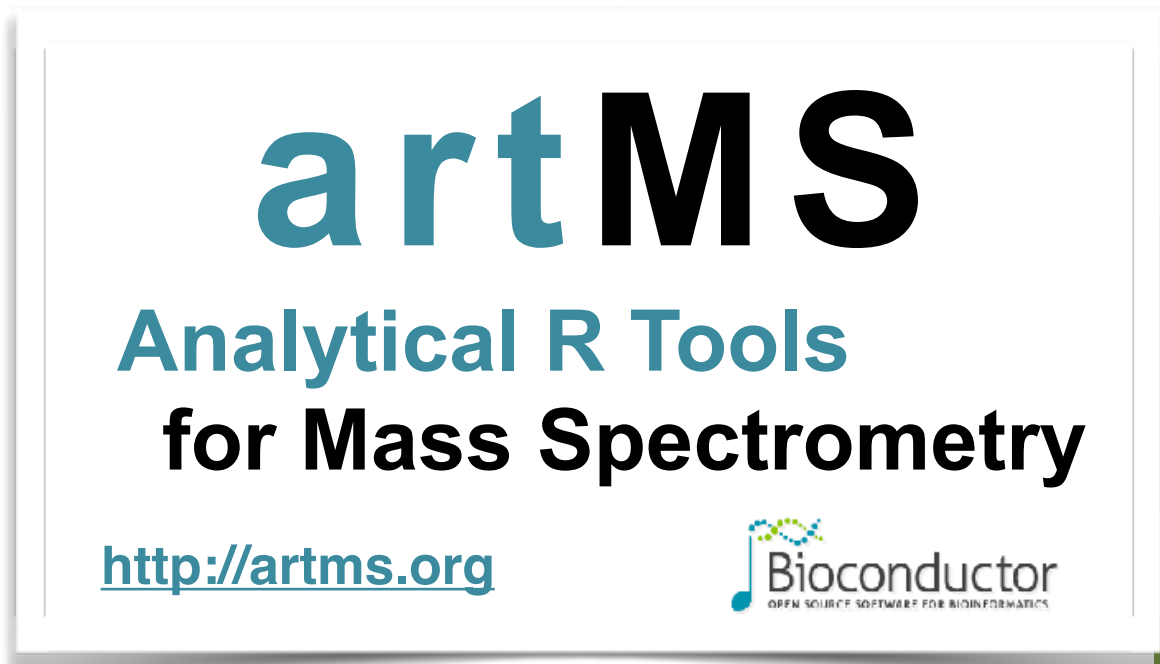
Relative
quantification

`artmsQuantification("config.yaml")`

Functional analysis

Miscellaneous





- Annotations
- Summary files in different format (xls, txt) and shapes
- Numerous summary plots
- Enrichment analysis using Gprofiler
- PCA of protein abundance
- PCA of quantifications
- Clustering analysis

Functional analysis

artmsAnalysisQuantifications() →

Miscellaneous

artmsEvidenceToSAINTq()
 artmsEvidenceToSaintExpress()
 artmsPhosphateOutput()
 artmsPhotonOutput()
 etc

EXCEL

-results-summary.xlsx

TXT FILES

- results-abundance-long.txt
- results-abundance-wide.txt
- results-enrich-MAC-allsignificants-corum.txt
- results-enrich-MAC-allsignificants.txt
- results-enrich-MAC-negatives-corum.txt
- results-enrich-MAC-negatives.txt
- results-enrich-MAC-positives-corum.txt
- results-enrich-MAC-positives.txt
- results-log2fc-long.txt
- results-log2fc-wide.txt
- results.log2fc-clusterheatmap-enriched.txt
- results.log2fc-clusterheatmap.txt

PDF FILES

- results-enrich-MAC-allsignificants-corum.pdf
- results-enrich-MAC-negatives-corum.pdf
- results-enrich-MAC-positives-corum.pdf
- results-pca-correlations.pdf
- results-pca-pca01.pdf
- results-pca-pca02.pdf
- results-pca-pca03.pdf
- results.clustering.abundance.all-overview.pdf
- results.clustering.abundance.all-zoom.pdf
- results.clustering.log2fc.all-overview.pdf
- results.clustering.log2fc.all-zoom.pdf
- results.clustering.log2fcSign.all-overview.pdf
- results.clustering.log2fcSign.all-zoom.pdf
- results.distributions.pdf
- results.distributionsFil.pdf
- results.imputation.pdf
- results.log2fc-clusterheatmap.pdf
- results.log2fc-clusters.pdf
- results.log2fc-corr.pdf
- results.log2fc-dendro.pdf
- results.log2fc-individuals-pca.pdf
- results.TotalQuantifications.pdf
- results.correlationConditions.pdf
- results.correlationQuantifications.pdf
- results.relativeABUNDANCE.pdf
- results.reproducibilityAbundance.pdf

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Input Files

`evidence.txt`

`keys.txt`

`contrast.txt`

`config.yaml`

- Annotations
- Summary files in different format (xls, txt) and shapes
- Numerous summary plots
- Enrichment analysis using Gprofiler
- PCA of protein abundance
- PCA of quantifications
- Clustering analysis

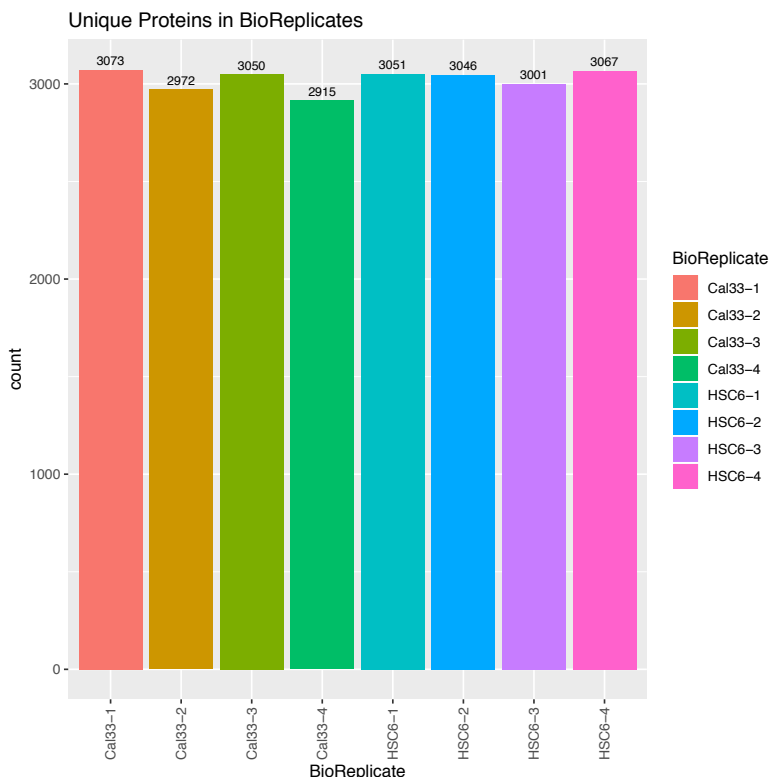
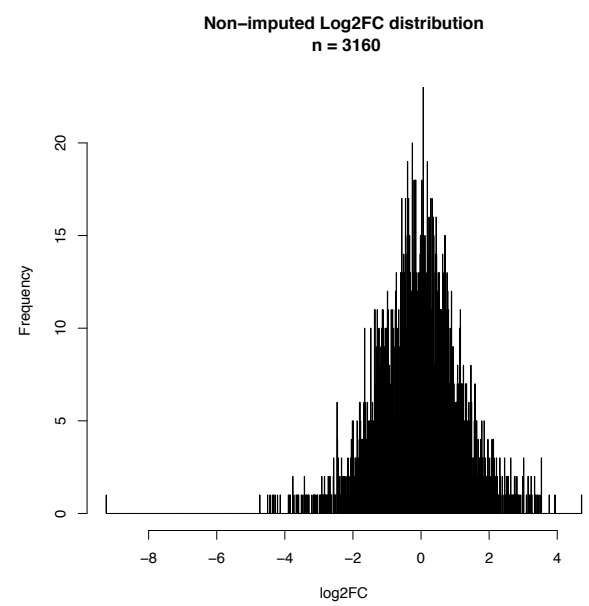
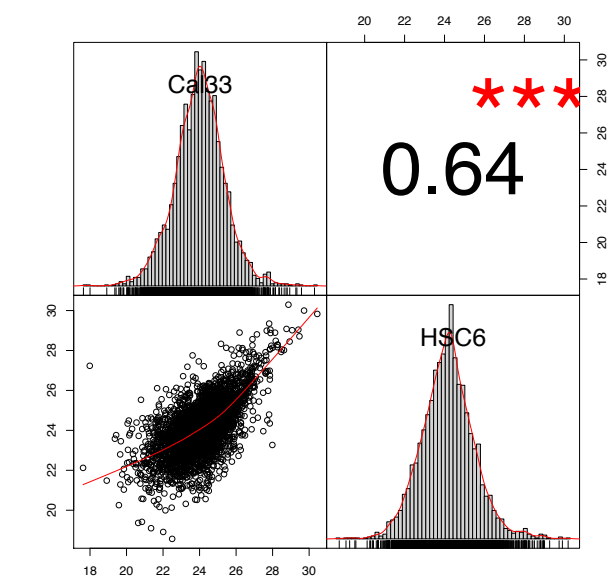
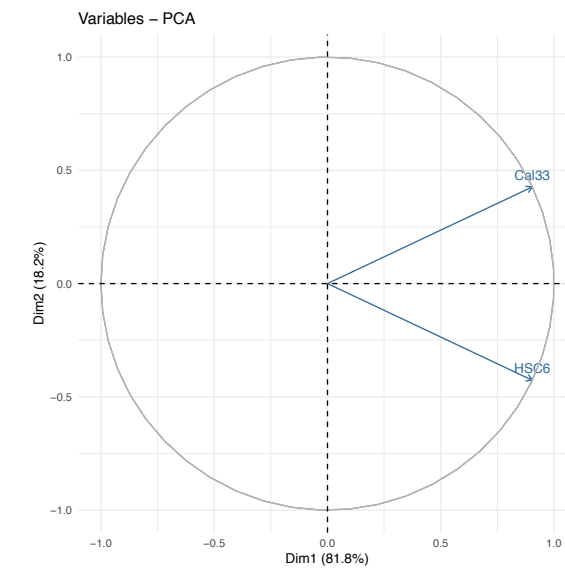
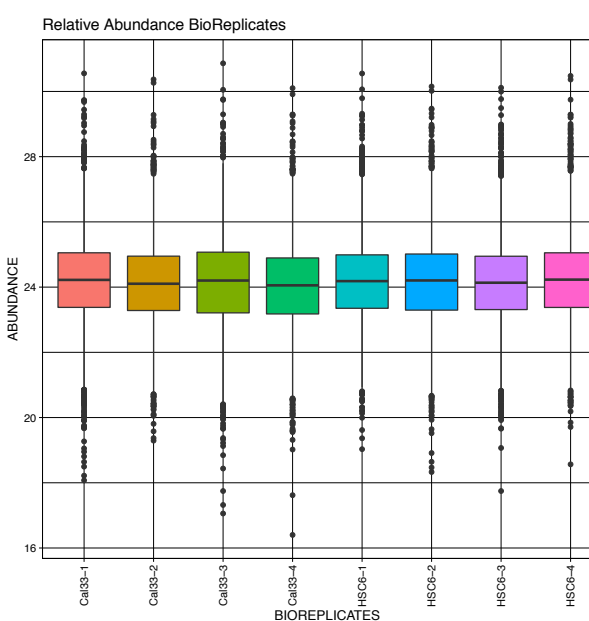
Functional analysis

`artmsAnalysisQuantifications()`

ANT Protein Complex Enrichment

- MYC-MAX complex
- TNF-alpha/NF-kappa B signaling complex 9
- YY1-MDM2-p53 complex
- TAM1-EBF1-EPHA2 complex
- Sturpantin-CRM1-RanGTP complex
- SLC2A1-DMTN-ADD2 complex
- SAPCD2-Galpha-LGN complex
- SIP1-c-Met-ITGB4 complex, HGF- or SIP-induced
- p300-MDM2-p53 protein complex
- NUMB-TP53-MDM2 complex
- MYC-MAX-BLCL131 complex
- MYC-DNAH3A-ZBTB17 complex
- HSP90-CDC37-LRRK2 complex
- Er-alpha-p53-hdm2 complex
- ER-alpha-GRIPI-c-Jun complex
- c-Fos-c-Jun-SAF-1 complex
- CDKN1A-TP53-CDK1-PCNA complex
- SNARE complex (STX4, VAMP8, VAMP3, SNAP23)
- SDCBP-CTNMB1-CTNNA1-CDH1 complex
- POC-1-SRP40-SRP55-SRP75 complex
- ITGA6-ITGB4-SHC1-GRB2 complex
- CDC37-HSP90A1-HSP90AB1-MAP3K11 complex
- Elongator holo complex
- TNF-alpha/NF-kappa B signaling complex 8
- Emerin architectural complex
- Mis12 centromere complex
- BLM complex III
- BLM complex II
- WWTR1 homodimer
- TLE1 homodimer complex
- TERF1 homodimer complex
- p53 homotetramer complex
- N-WASP homomer
- HDAC4-ERK2 complex
- BANF1 homodimer protein
- DZIP1-GLIS complex

Cal33-HSC6



Protein	Gene	ProteinName	EntrezID	Uniprot_PTM	Comparison	log2FC	pvalue	adj pvalue	imputed	iLog2FC	iPvalue	Cal33	HSC6	CMA	UniprotIDRef	Species
A0AVK6	E2F8	E2F transcription factor 8	79733	A0AVK6_S358	Cal33-HSC6	0.714364541	0.068971677	0.144027182	no	0.71484541	0.144027182	3	4	Cal33	A0AVK6	human
A0AVK6	F2F8	F2F transcription factor 8	79733	A0AVK6_S417	Cal33-HSC6	-1.643170638	0.020905156	0.063710769	no	-1.643170638	0.063710769	3	4	HSC6	A0AVK6	human
A0AVK6	E2F8	E2F transcription factor 8	79733	A0AVK6_S71	Cal33-HSC6	-0.692523032	0.740104187	0.809434278	no	-0.692523032	0.809434278	2	2	HSC6	A0AVK6	human
A0FGRR	FSYT2	extended synaptotagmin 2	57488	A0FGRR_S676	Cal33-HSC6	-2.4675906	0.352698385	0.468371968	no	-2.4675906	0.468371968	1	7	HSC6	A0FGRR	human
A0FGRR	ESYT2	extended synaptotagmin 2	57488	A0FGRR_S651	Cal33-HSC6	-0.198128315	0.405278123	0.518470215	no	-0.198128315	0.518470215	3	3	HSC6	A0FGRR	human
A0FGRR	FSYT2	extended synaptotagmin 2	57488	A0FGRR_S651;A0FGRR_S693	Cal33-HSC6	#NUM!			yes	3.66610745	0.0198395	7	0	Cal33	A0FGRR	human
A0FGRR	ESYT2	extended synaptotagmin 2	57488	A0FGRR_S653	Cal33-HSC6	0.950304175	0.15494643	0.254530287	no	0.950304175	0.254530287	4	4	Cal33	A0FGRR	human
A0FGRR	FSYT2	extended synaptotagmin 2	57488	A0FGRR_S736	Cal33-HSC6	-0.019383937	0.932856234	0.952326477	no	-0.019383937	0.952326477	4	3	HSC6	A0FGRR	human
A0FGRR	ESYT2	extended synaptotagmin 2	57488	A0FGRR_S743	Cal33-HSC6	#NUM!			yes	-7.793852072	0.0244517	0	4	HSC6	A0FGRR	human
A0FGRR	FSYT2	extended synaptotagmin 2	57488	A0FGRR_S758;A0FGRR_S761	Cal33-HSC6	-0.388729606	0.906408456	0.934755075	no	-0.388729606	0.934755075	1	3	HSC6	A0FGRR	human
A0FGRR	ESYT2	extended synaptotagmin 2	57488	A0FGRR_S761	Cal33-HSC6	-0.710786851	0.002631059	0.016113125	no	-0.710786851	0.016113125	2	4	HSC6	A0FGRR	human
A0FGRR	FSYT2	extended synaptotagmin 2	57488	A0FGRR_T701;A0FGRR_T705	Cal33-HSC6	-1.705399144	0.01777448	0.05442631	no	-1.705399144	0.05442631	4	4	HSC6	A0FGRR	human
A0JLT2	MED19	mediator complex subunit 1	219541	A0JLT2_S226	Cal33-HSC6	-0.628616166	0.213593755	0.32335184	no	-0.628616166	0.32335184	4	4	HSC6	A0JLT2	human

Miscellaneous

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<https://bioconductor.org/packages/release/bioc/html/artMS.html>



GitHub

<https://github.com/biodavidjm/artMS>

available at:



Documentation:

and everything else...

<http://artms.org>



artMS: Analytical R Tools for Mass Spectrometry-based Proteomics

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In preparation

1. Stanford
2. SBP
3. UCSF

a c k n o w l e d g e m e n t s



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