

Data integration in proteomics

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Two use-cases, and caveats:

- ▶ Integration of mass spectrometry based proteomics and RNA-Seq transcriptomics: **mapping peptides to genome coordinates**
- ▶ Combining experimental spatial proteomics and third-party data using **transfer learning**

Different approaches to data integration (1)

Conversion to common feature identifiers and measuring co relation (or lack thereof).

Transcript and protein measurement have previously been combined and compared by linking the respective features by a **common (gene) identifier** (PMID:21179022, PMID:22068331).

Such approaches are often difficult to track and are susceptible to inconsistencies in the relation between different data sources when, for example, multiple transcripts are compared to ambiguous protein groups.

Different approaches to data integration (2)

Reference-based approaches, that map different sources of data against a common reference.

These approaches are a natural choice for data stemming from genomics, transcriptomics, epigenomics, etc that directly rely on **mapping** their data features along a **genome reference**.

Different approaches to data integration (3)

Model- or network-based approaches that identify common patterns in different data sources.

Very versatile and rely on experiment-wide clustering/modelling and crucially depend on reliably linking features (explicitly via common identifiers or through functional contextualisation).

Integrating MS-based proteomics and RNA-Seq data

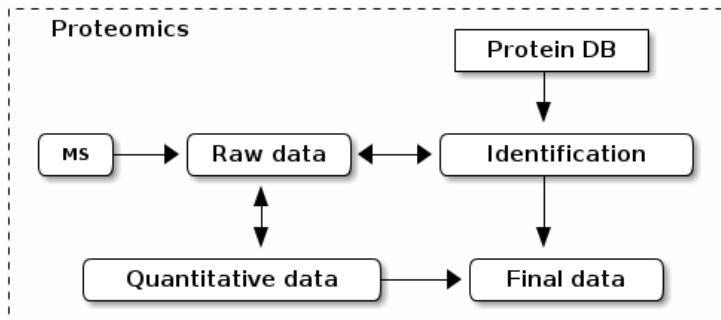
March 7, 2016

Use case 1: mapping peptides

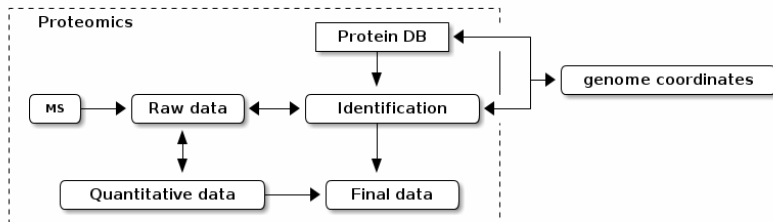
Mapping of *peptides along protein sequences* (although not explicitly considered a mapping exercise) and *short reads along genome coordinates*.

Mapping protein and gene identifiers

The protein database and the genome are *independent*, i.e. the proteins do not make explicitly reference to the genome they originate from.



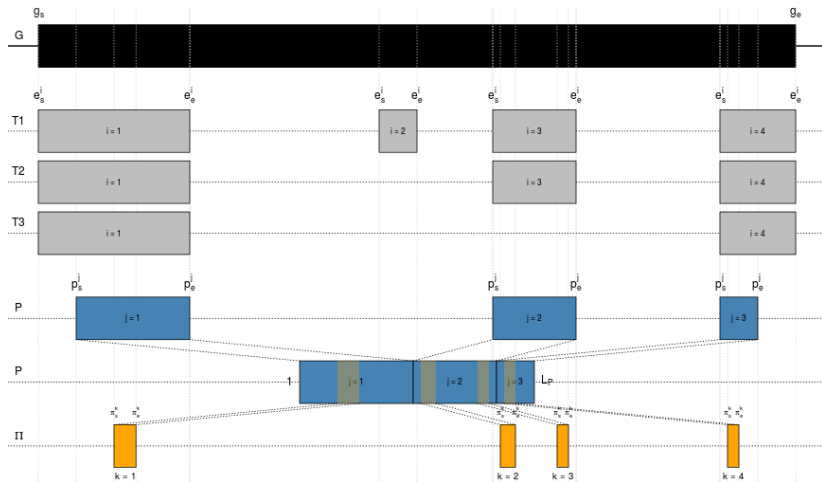
Mapping protein and gene identifiers



If we want to map UniProt accession numbers to genomic identifiers (Ensembl transcript identifiers)

Mapping peptides to genomic coordinates

The **goal** is to map peptides from protein coordinates (1 to L_p) to genomic coordinates.



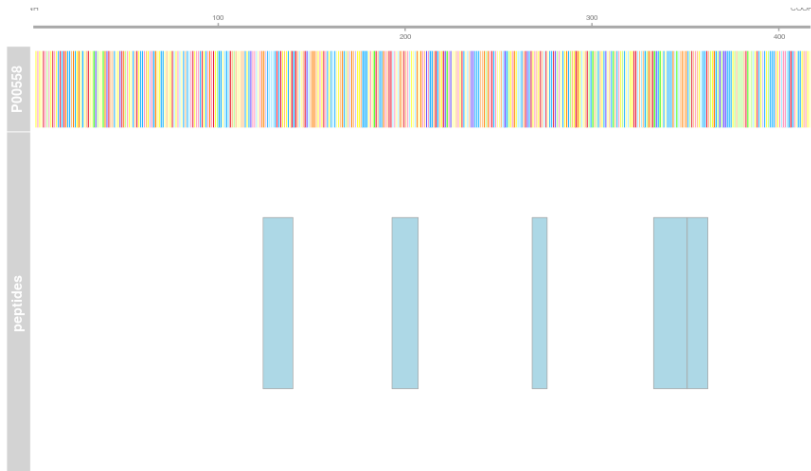
Data

Illustration with the Pbase Bioconductor package.

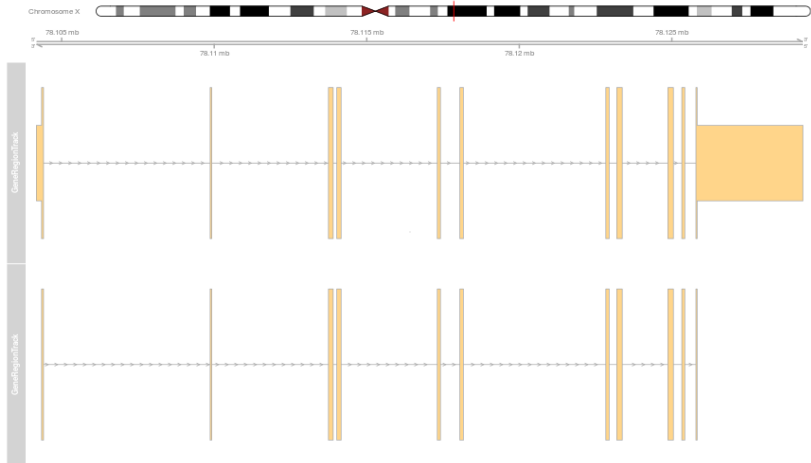
We have an example data composed of 9 proteins, with UniProt accession numbers and Ensembl transcript identifiers and each protein has a set experimentally observed peptides (see table below). This object was generated from the protein database (fasta file) and the MS identification results (mzIdentML file) against this very same protein database.

Acc	ENST	npep
A4UGR9	ENST00000409195	36
A6H8Y1	ENST00000358731	23
O43707	ENST00000252699	6
O75369	ENST00000295956	13
P00558	ENST00000373316	5
P02545	ENST00000368300	12
P04075	ENST00000338110	21
P04075-2	ENST00000395248	20
P60709	ENST00000331789	1

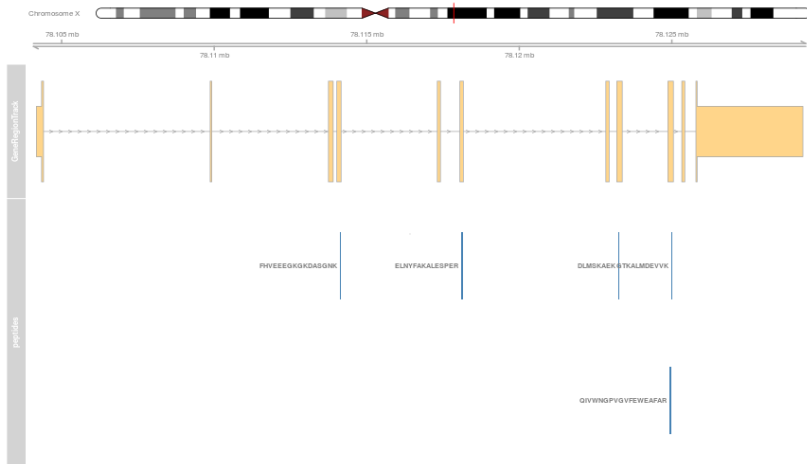
For example, P00558:



Genomic coordinates of the transcripts/exons

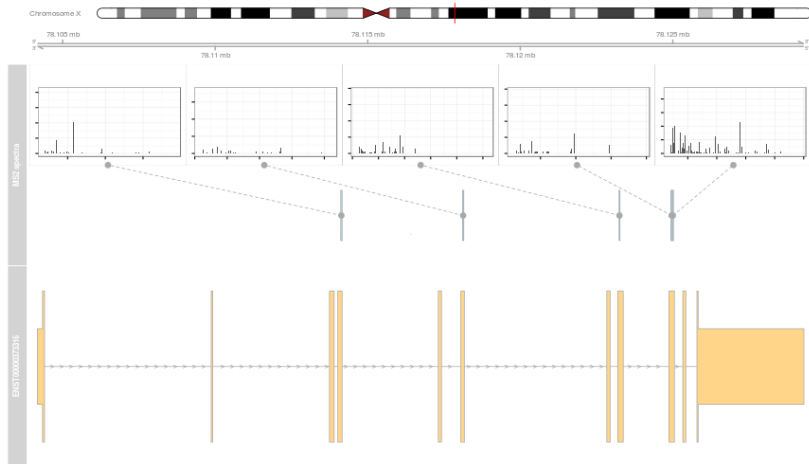


Mapping peptides to the genome



Detailed annotation tracks

Maintaining access to the raw MS data (used as input with the fasta file to generate the identification results).

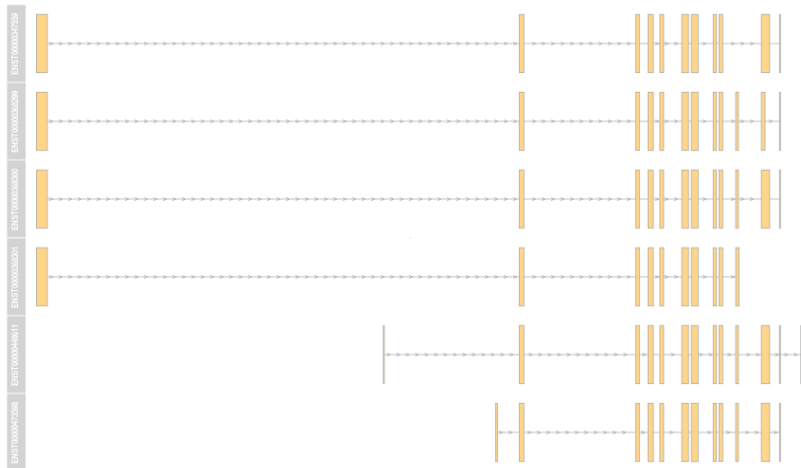


Multiple transcripts per protein

If we hadn't the curated UniProt accession/Ensembl transcript identifier maps, we would, for example, query an online repository such as the Ensembl Biomart instance. For example

UNIPROTKB	ENSEMBL_TRANSCRIPT
P02545	ENST00000347559
P02545	ENST00000368299
P02545	ENST00000368300
P02545	ENST00000368301
P02545	ENST00000448611
P02545	ENST00000473598

Genomic coordinates



Discriminating transcripts

We extract the transcript sequences, translate them into protein sequences and align each to our original protein sequence.

```
## ENST00000347559 ENST00000368299 ENST00000368300 ENST00000368301
##      0.9548193      0.9246988      1.0000000      0.8614458
## ENST00000448611 ENST00000473598
##      0.8298193      0.8358434
```

```
## Global PairwiseAlignmentsSingleSubject (1 of 1)
## pattern: [1] METPSQRRATRSGAQASSTPLSPTRITRLQEK...GGGSFGDNLVTRSYLLGNSSPRTQSPQNCSIM
## subject: [1] METPSQRRATRSGAQASSTPLSPTRITRLQEK...GGGSFGDNLVTRSYLLGNSSPRTQSPQNCSIM
## score: 2843.652
```

ENST00000368300

ENST00000368300



Mapping MS peptides and RNA-Seq short reads

The last step of the mapping process is to combine the newly mapped peptides and reads from RNA-Seq experiments. The figures below illustrate this with data from Sheynkman et al. (PMID: 23629695, 25149441) from the Jurkat cell line (TIB-152). The mass spectrometry (PASS00215) and RNA-Seq (SRR791580) were processed with standard pipelines.

Chromosome 17



chr17:301200000-301200000





For all details/code see the Pbase package mapping vignette
<http://bioconductor.org/packages/Pbase>

Use case 1: mapping peptides

Mapping of *peptides along protein sequences* (although not explicitly considered a mapping exercise) and *short reads along genome coordinates*.

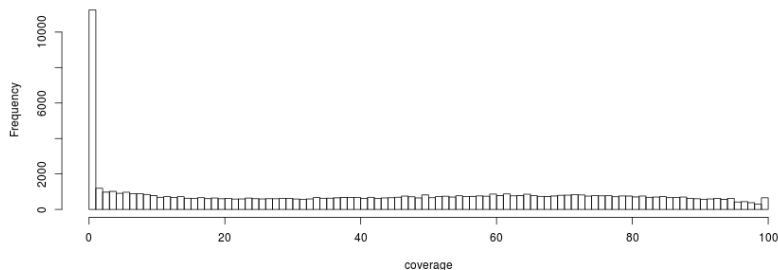
But...

- ▶ coverage
- ▶ protein inference
- ▶ identifier mapping
- ▶ missing values

Coverage

- ▶ Coverage in proteomics in %
- ▶ Coverage in RNA-Seq in fold X

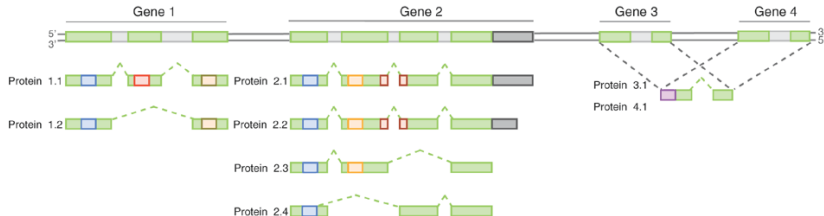
The following values are higher bounds, *without* peptide filtering for about 80000 *gene groups*



And

- ▶ the majority of peptides map to a minority of proteins different
- ▶ peptides within one protein can be differently detectable in MS acquisitions

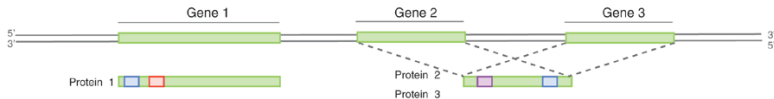
Protein inference



Eukaryotes

Prokaryotes

Class	Protein sequence(s)	Protein isoform(s)	Gene(s)
1a	Unambiguous	Unambiguous	Unambiguous
1b	Unambiguous	Ambiguous	Unambiguous
2a	Ambiguous	Ambiguous	Unambiguous
2b	Ambiguous	Ambiguous	Unambiguous
3a	Unambiguous	Ambiguous	Ambiguous
3b	Ambiguous	Ambiguous	Ambiguous



From Qeli and Ahrens (2010). See also Nesvizhskii and Aebersold (2005).

Protein groups

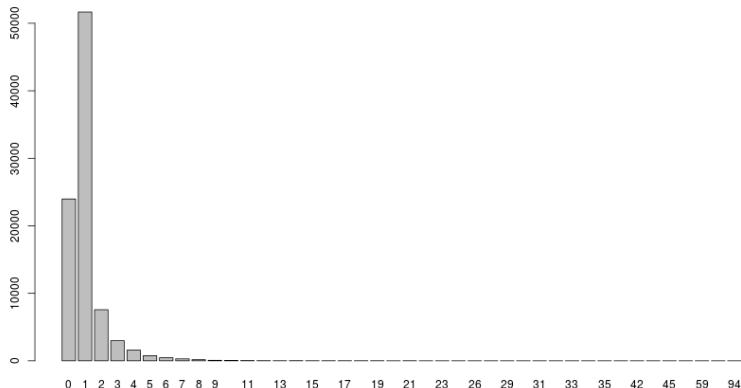
Often, in proteomics experiments, the features represent single proteins and **groups** of indistinguishable or non-differentiable proteins identified by shared (non-unique) peptides.

Caveat: Mapping between protein groups and unique transcripts?

Mapping identifiers

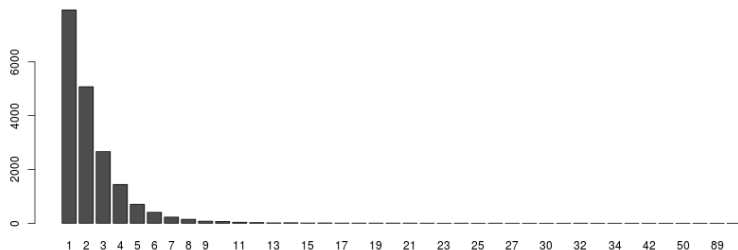
The UniProt human proteome (release 2015_02) has 89796 entries.
Using UniProt.ws:

- ▶ 23972 have no transcript identifier
- ▶ 51673 have a unique transcript identifier
- ▶ 14151 have more than one transcript identifier



Using biomaRt:

Mapping 18911 identifiers, of which



Caveat: Mapping between single protein and unique transcripts?

Missing values

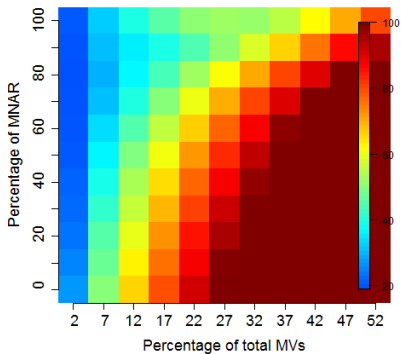
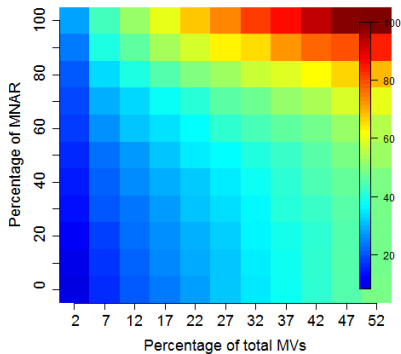
Options are:

- ▶ Filtering: Remove missing values, or at least features or samples with excessive number of missing values:
- ▶ Data imputation: inferring plausible values for missing data.

Data imputation

There are two types of mechanisms resulting in missing values in LC/MSMS experiments.

- ▶ Missing values resulting from absence of detection of a feature, despite ions being present at detectable concentrations. For example in the case of ion suppression or as a result from the stochastic, data-dependent nature of the MS acquisition method. These missing value are expected to be randomly distributed in the data and are defined as **missing at random** (MAR) or **missing completely at random** (MCAR).
- ▶ Biologically relevant missing values, resulting from the *absence* of the low abundance of ions (below the limit of detection of the instrument). These missing values are not expected to be randomly distributed in the data and are defined as **missing not at random** (MNAR).



MNAR features should ideally be imputed with a **left-censor** (minimum value (right), but not zero, ...) method. Conversely, it is recommended to use **hot deck** methods (nearest neighbour (left), maximum likelihood, ...) when data are missing at random.

Summary

- ▶ mapping peptides along genomic coordinates
- ▶ protein/transcript mapping
- ▶ protein groups
- ▶ missing values and coverage

References

Laurent Gatto and Sebastian Gibb (2016). Pbase: Manipulating and exploring protein and proteomics data. R package version 0.11.3. <https://github.com/ComputationalProteomicsUnit/Pbase>

Lazar C, Gatto L, Ferro M, Bruley C, and Burger T. Accounting for the Multiple Natures of Missing Values in Label-Free Quantitative Proteomics Data Sets to Compare Imputation Strategies. Publication Date: February 23, 2016 DOI: 10.1021/acs.jproteome.5b00981

Pang et al. Tools to covisualize and coanalyze proteomic data with genomes and transcriptomes: validation of genes and alternative mRNA splicing. J Proteome Res. 2014 Jan 3;13(1):84-98. doi: 10.1021/pr400820p. Epub 2013 Nov 12. PubMed PMID: 24152167.

Sheynkman GM, Johnson JE, Jagtap PD, Shortreed MR, Onsongo G, Frey BL, Griffin TJ, Smith LM. Using Galaxy-P to leverage RNA-Seq for the discovery of novel protein variations. BMC Genomics. 2014 Aug 22;15:703. doi: 10.1186/1471-2164-15-703. PubMed PMID: 25149441.

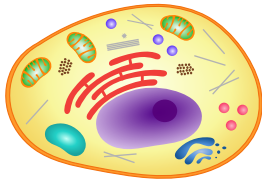
Qeli E, Ahrens CH. PeptideClassifier for protein inference and targeted quantitative proteomics. Nat Biotechnol. 2010 Jul;28(7):647-50. doi: 10.1038/nbt0710-647. PubMed PMID: 20622826.

Boekel J, Chilton JM, Cooke IR, Horvatovich PL, Jagtap PD, Käll L, Lehtiö J, Lukasse P, Moerland PD, Griffin TJ. Multi-omic data analysis using Galaxy. Nat Biotechnol. 2015 Feb 6;33(2):137-9. doi: 10.1038/nbt.3134. PubMed PMID: 25658277.

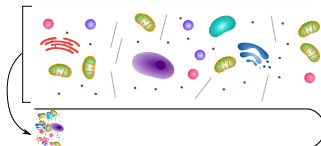
Evans VC, Barker G, Heesom KJ, Fan J, Bessant C, Matthews DA. De novo derivation of proteomes from transcriptomes for transcript and protein identification. Nat Methods. 2012 Dec;9(12):1207-11. doi: 10.1038/nmeth.2227. Epub 2012 Nov 11. PubMed PMID:23142869; PubMed Central PMCID:PMC3581816.

Learning from heterogeneous data sources: an application in spatial proteomics

March 6, 2016

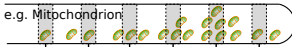


Cell lysis



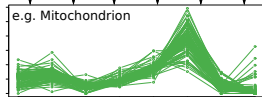
Fractionation/centrifugation

e.g. Mitochondrion



Quantitation/identification
by mass spectrometry

e.g. Mitochondrion



Quantitation data and organelle markers

	Fraction ₁	Fraction ₂	...	Fraction _m	markers
p ₁	q _{1,1}	q _{1,2}	...	q _{1, m}	unknown
p ₂	q _{2,1}	q _{2,2}	...	q _{2, m}	<i>loc₁</i>
p ₃	q _{3,1}	q _{3,2}	...	q _{3, m}	unknown
p ₄	q _{4,1}	q _{4,2}	...	q _{4, m}	<i>loc_i</i>
⋮	⋮	⋮	⋮	⋮	⋮
p _j	q _{j,1}	q _{j,2}	...	q _{j, m}	unknown

Visualisation and classification

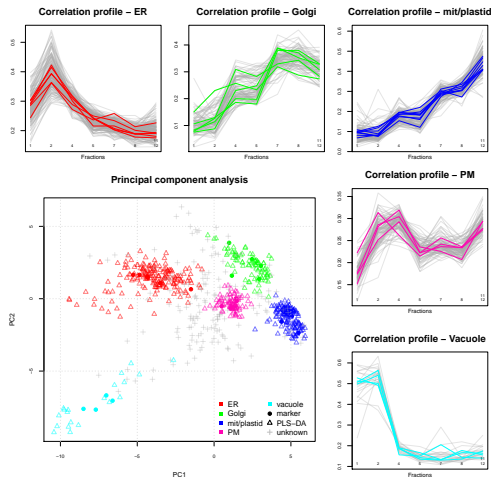


Figure : From Gatto et al. (2010), *Arabidopsis thaliana* data from Dunkley et al. (2006)

What about annotation data from repositories such as GO, sequence features, signal peptide, transmembrane domains, images, protein-protein interactions,

- ▶ From a user perspective: "**free/cheap**" vs. expensive
- ▶ Abundant (all proteins, 100s of features) vs. (experimentally) limited/**targeted** (1000s of proteins, 6 – 20 of features)
- ▶ For localisation in system at hand: *low* vs. high **quality**
- ▶ **Static** vs. **dynamic**

number GO features \gg experimental fractions
 \Rightarrow dilution of experimental data

Goal

Support/complement the primary target domain (experimental data) with auxiliary data (annotation) features without compromising the integrity of our primary data.

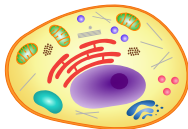
Updated experimental design for

- ▶ primary/experimental data

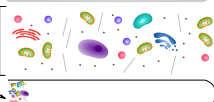
and

- ▶ auxiliary/annotation data

PRIMARY EXPERIMENTAL DATA



Cell lysis

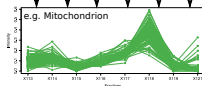


Fractionation/centrifugation

e.g. Mitochondrion

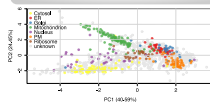


Quantitation/identification
by mass spectrometry



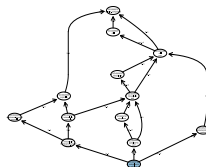
	X110	X114	X115	X116	X117	X118	X119	X121
CDMT7	0.180	0.130	0.082	0.187	0.277	0.1478	0.0380	0.0039
PR1488	0.1914	0.205	0.0848	0.185	0.237	0.0986	0.0180	0.0777
CERT3A3	0.1287	0.201	0.0548	0.188	0.290	0.1883	0.0206	0.0060
GRU5C1	0.1908	0.197	0.0519	0.184	0.158	0.0000	0.0000	0.0000

Visualisation



Database query

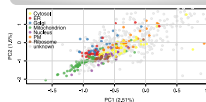
Extract GO CC terms



Convert terms to binary

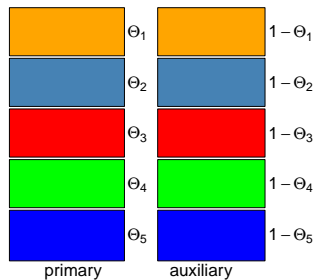
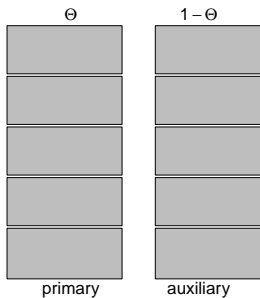
	GO:0005822	GO:0005789	GO:0005783	GO:
CDMT7	1	1	1	...
PR1488	1	1	1	...
CERT3A3	1	1	1	...
GRU5C1	1	1	1	...

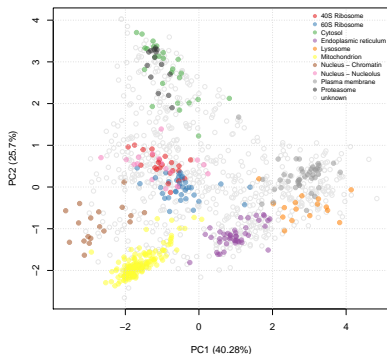
Visualisation



AUXILIARY DRY DATA

Weighting





Data from mouse stem cells (E14TG2a)

We use a **class-weighted** kNN transfer learning algorithm to combine primary and auxiliary data, based on Wu and Dietterich (2004):

$$V(c_i)_j = \theta^* n_{ij}^P + (1 - \theta^*) n_{ij}^A$$

Classes and weights

$$\mathbb{C} = \{c_{i=1}, \dots, c_{i=l}\}; \Theta = \{0, 0.5, 1\}$$

Primary data

$$L_P = \begin{bmatrix} q_{1,1} & q_{1,2} & \dots & q_{1,m} \\ q_{2,1} & q_{2,2} & \dots & q_{2,m} \\ \vdots & \vdots & \ddots & \vdots \\ q_{j,1} & q_{j,2} & \dots & q_{j,m} \end{bmatrix}; \begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_j \end{bmatrix}; k_P$$

Auxiliary data

$$L_A = \begin{bmatrix} b_{1,1} & b_{1,2} & \dots & \dots & b_{1,n} \\ b_{2,1} & b_{2,2} & \dots & \dots & b_{2,n} \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ b_{j,1} & b_{j,2} & \dots & \dots & b_{j,n} \end{bmatrix}; \begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_j \end{bmatrix}; k_A$$

Neighbour matrices

$$N_P = \begin{bmatrix} c_{i=1} & \dots & c_{i=l} \\ n_{1,1}^P & \dots & n_{1,l}^P \\ n_{2,1}^P & \dots & n_{2,l}^P \\ \vdots & \vdots & \vdots \\ \vdots & \vdots & \vdots \end{bmatrix}; N_A = \begin{bmatrix} c_{i=1} & \dots & c_{i=l} \\ n_{1,1}^A & \dots & n_{1,l}^A \\ n_{2,1}^A & \dots & n_{2,l}^A \\ \vdots & \vdots & \vdots \\ \vdots & \vdots & \vdots \end{bmatrix}$$

Classes and weights

$$\mathbb{C} = \{c_{i=1}, \dots, c_{i=l}\}; \Theta = \{0, 0.5, 1\}$$

Primary data

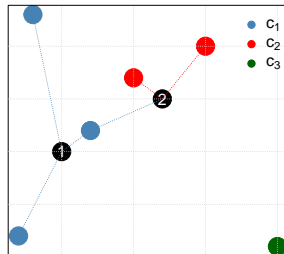
$$L_P = \begin{bmatrix} q_{1,1} & q_{1,2} & \dots & q_{1,m} \\ q_{2,1} & q_{2,2} & \dots & q_{2,m} \\ \vdots & \vdots & \ddots & \vdots \\ q_{j,1} & q_{j,2} & \dots & q_{j,m} \end{bmatrix}; \begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_j \end{bmatrix}; k_P$$

Auxiliary data

$$L_A = \begin{bmatrix} b_{1,1} & b_{1,2} & \dots & \dots & b_{1,n} \\ b_{2,1} & b_{2,2} & \dots & \dots & b_{2,n} \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ b_{j,1} & b_{j,2} & \dots & \dots & b_{j,n} \end{bmatrix}; \begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_j \end{bmatrix}; k_A$$

Neighbour matrices

$$N_P = \begin{bmatrix} c_{i=1} & \dots & c_{i=l} \\ n_{1,1}^P & \dots & n_{1,l}^P \\ n_{2,1}^P & \dots & n_{2,l}^P \\ \vdots & \vdots & \vdots \end{bmatrix}; N_A = \begin{bmatrix} c_{i=1} & \dots & c_{i=l} \\ n_{1,1}^A & \dots & n_{1,l}^A \\ n_{2,1}^A & \dots & n_{2,l}^A \\ \vdots & \vdots & \vdots \end{bmatrix}$$



$$N_P = \begin{matrix} & c_1 & c_2 & c_3 \\ \begin{matrix} p_1 \\ p_2 \end{matrix} & \begin{bmatrix} \frac{3}{3} & 0 & 0 \\ \frac{1}{3} & \frac{2}{3} & 0 \\ \vdots & \vdots & \vdots \end{bmatrix} \end{matrix}$$

Classes and weights

$$\mathbb{C} = \{c_{i=1}, \dots, c_{i=I}\}; \Theta = \{0, 0.5, 1\}$$

Primary data

$$L_P = \begin{bmatrix} q_{1,1} & q_{1,2} & \dots & q_{1,m} \\ q_{2,1} & q_{2,2} & \dots & q_{2,m} \\ \vdots & \vdots & \ddots & \vdots \\ q_{j,1} & q_{j,2} & \dots & q_{j,m} \end{bmatrix}; \begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_j \end{bmatrix}; k_P$$

Auxiliary data

$$L_A = \begin{bmatrix} b_{1,1} & b_{1,2} & \dots & \dots & b_{1,n} \\ b_{2,1} & b_{2,2} & \dots & \dots & b_{2,n} \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ b_{j,1} & b_{j,2} & \dots & \dots & b_{j,n} \end{bmatrix}; \begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_j \end{bmatrix}; k_A$$

Neighbour matrices

$$N_P = \begin{bmatrix} c_{i=1} & \dots & c_{i=I} \\ n_{1,1}^P & \dots & n_{1,I}^P \\ n_{2,1}^P & \dots & n_{2,I}^P \\ \vdots & \vdots & \vdots \\ \vdots & \vdots & \vdots \end{bmatrix}; N_A = \begin{bmatrix} c_{i=1} & \dots & c_{i=I} \\ n_{1,1}^A & \dots & n_{1,I}^A \\ n_{2,1}^A & \dots & n_{2,I}^A \\ \vdots & \vdots & \vdots \\ \vdots & \vdots & \vdots \end{bmatrix}$$

Weights matrix (labelled)

$$\begin{matrix} & c_1 & c_2 & c_3 \\ \theta_1 & \begin{bmatrix} 0 & 0 & 0 \end{bmatrix} \\ \theta_2 & \begin{bmatrix} 0 & 0 & 1 \end{bmatrix} \\ \theta_i & \begin{bmatrix} \vdots & & \vdots \end{bmatrix} \\ \vdots & \begin{bmatrix} 1 & 1 & 0 \end{bmatrix} \\ \theta_{\Theta^I} & \begin{bmatrix} 1 & 1 & 1 \end{bmatrix} \end{matrix} \begin{bmatrix} F_{1_1} \\ F_{1_2} \\ F_{1_i} \\ \vdots \\ F_{1_{\Theta^I}} \end{bmatrix}$$

$$\theta^* = \{1, 0, 1\}$$

(♥ BiocParallel)

Classes and weights

$$\mathbb{C} = \{c_{i=1}, \dots, c_{i=l}\}; \Theta = \{0, 0.5, 1\}$$

Primary data

$$L_P = \begin{bmatrix} q_{1,1} & q_{1,2} & \dots & q_{1,m} \\ q_{2,1} & q_{2,2} & \dots & q_{2,m} \\ \vdots & \vdots & \ddots & \vdots \\ q_{j,1} & q_{j,2} & \dots & q_{j,m} \end{bmatrix}; \begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_j \end{bmatrix}; k_P$$

Auxiliary data

$$L_A = \begin{bmatrix} b_{1,1} & b_{1,2} & \dots & \dots & b_{1,n} \\ b_{2,1} & b_{2,2} & \dots & \dots & b_{2,n} \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ b_{j,1} & b_{j,2} & \dots & \dots & b_{j,n} \end{bmatrix}; \begin{bmatrix} y_1 \\ y_2 \\ \vdots \\ y_j \end{bmatrix}; k_A$$

Neighbour matrices

$$N_P = \begin{bmatrix} c_{i=1} & \dots & c_{i=l} \\ n_{1,1}^P & \dots & n_{1,l}^P \\ n_{2,1}^P & \dots & n_{2,l}^P \\ \vdots & \vdots & \vdots \\ \vdots & \vdots & \vdots \end{bmatrix}; N_A = \begin{bmatrix} c_{i=1} & \dots & c_{i=l} \\ n_{1,1}^A & \dots & n_{1,l}^A \\ n_{2,1}^A & \dots & n_{2,l}^A \\ \vdots & \vdots & \vdots \\ \vdots & \vdots & \vdots \end{bmatrix}$$

Class-weighted classifier (unlabelled)

$$V(c_i)_j = \theta^* n_{ij}^P + (1 - \theta^*) n_{ij}^A$$

$$\begin{matrix} c_{i=1} & \dots & c_{i=l} \end{matrix} \begin{bmatrix} 1 \\ 2 \\ 3 \\ \vdots \\ j \end{bmatrix} \begin{matrix} V(c_i)_j \end{matrix}$$

$$y_j = \operatorname{argmax}(V(c_i)_j)$$

Class-weighted classifier (unlabelled)

$$\theta^* = \{1, 0, 1\} \quad N_P = \begin{matrix} & c_1 & c_2 & c_3 \\ p_1 & \frac{3}{3} & 0 & 0 \\ p_2 & \frac{1}{3} & \frac{2}{3} & 0 \\ & \vdots & \vdots & \vdots \end{matrix}$$

$$V(c_1)_1 = 1 \times \frac{3}{3} + (1 - 1) \times n_{1,1}^A$$

$$V(c_2)_1 = 0 \times 0 + (1 - 0) \times n_{1,2}^A$$

$$V(c_3)_1 = 1 \times 0 + (1 - 1) \times n_{1,3}^A$$

$$V(c_1)_2 = 1 \times \frac{1}{3} + (1 - 1) \times n_{1,1}^A$$

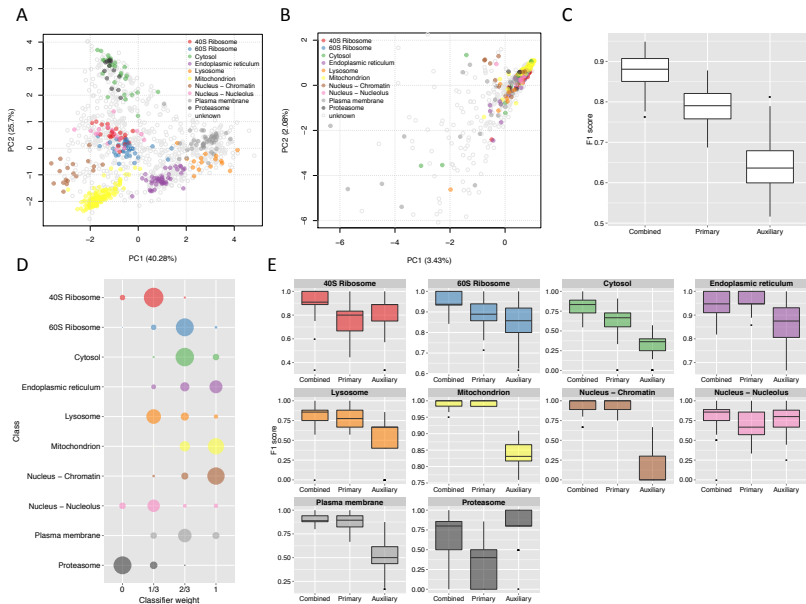
$$V(c_2)_2 = 0 \times \frac{2}{3} + (1 - 0) \times n_{1,2}^A$$

$$V(c_3)_2 = 1 \times 0 + (1 - 1) \times n_{1,3}^A$$

$$V(c_i)_j = \theta^* n_{ij}^P + (1 - \theta^*) n_{ij}^A$$

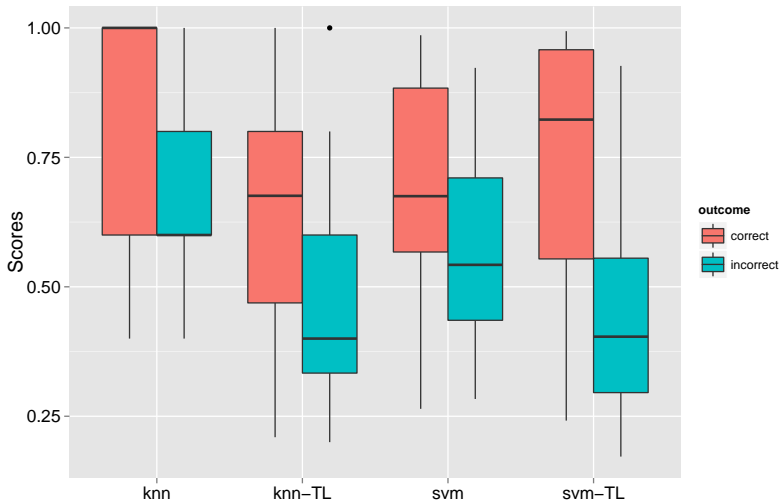
$$\begin{matrix} & c_1 & c_2 & c_3 \\ 1 & V(c_1)_1 & V(c_2)_1 & V(c_3)_1 \\ 2 & V(c_1)_2 & V(c_2)_2 & V(c_3)_2 \\ \vdots & & \vdots & \\ j & & & \end{matrix}$$

$$y_j = \operatorname{argmax}(V(c_i)_j)$$

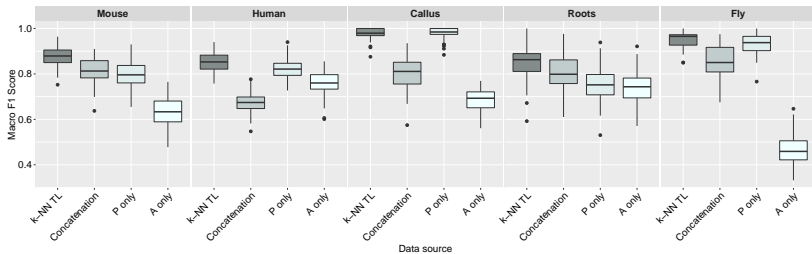


Data from mouse stem cells (E14TG2a).

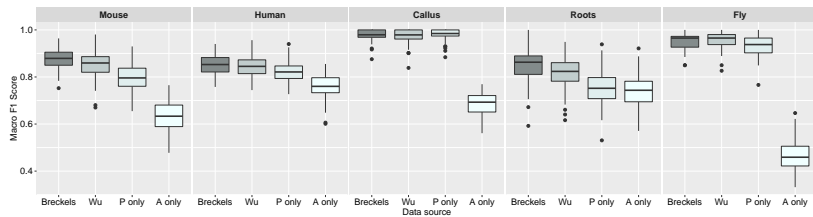
Discrimination power



Negative transfer



Class-level weights



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