Feature selection in metaproteomics: how to deal with missing values? Toward a more qualitative analysis

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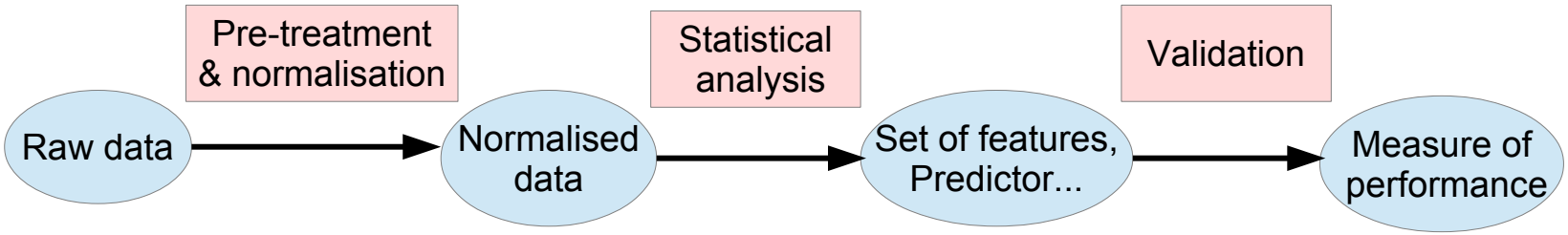

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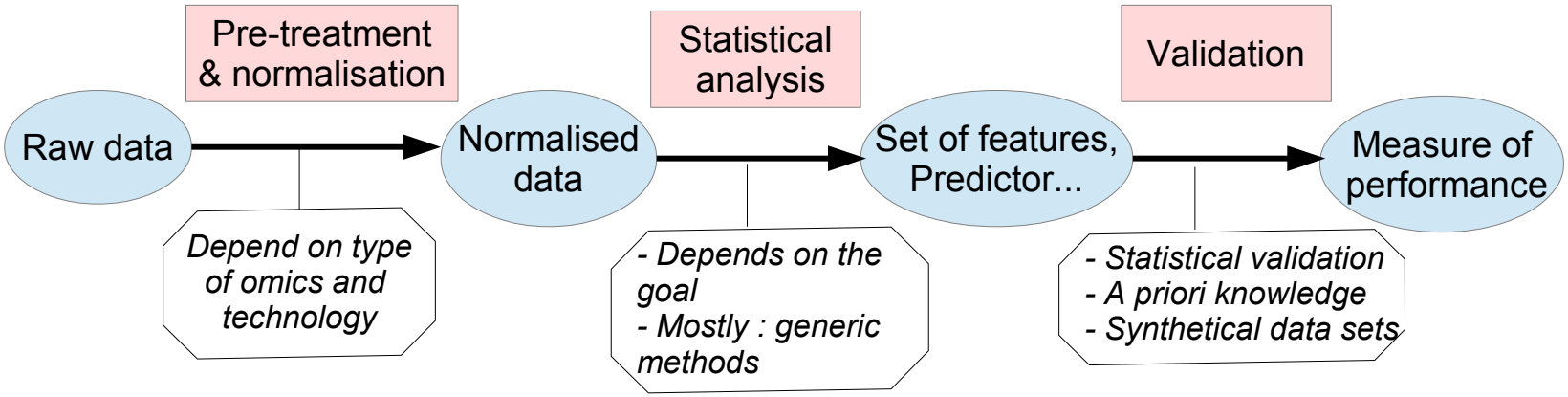

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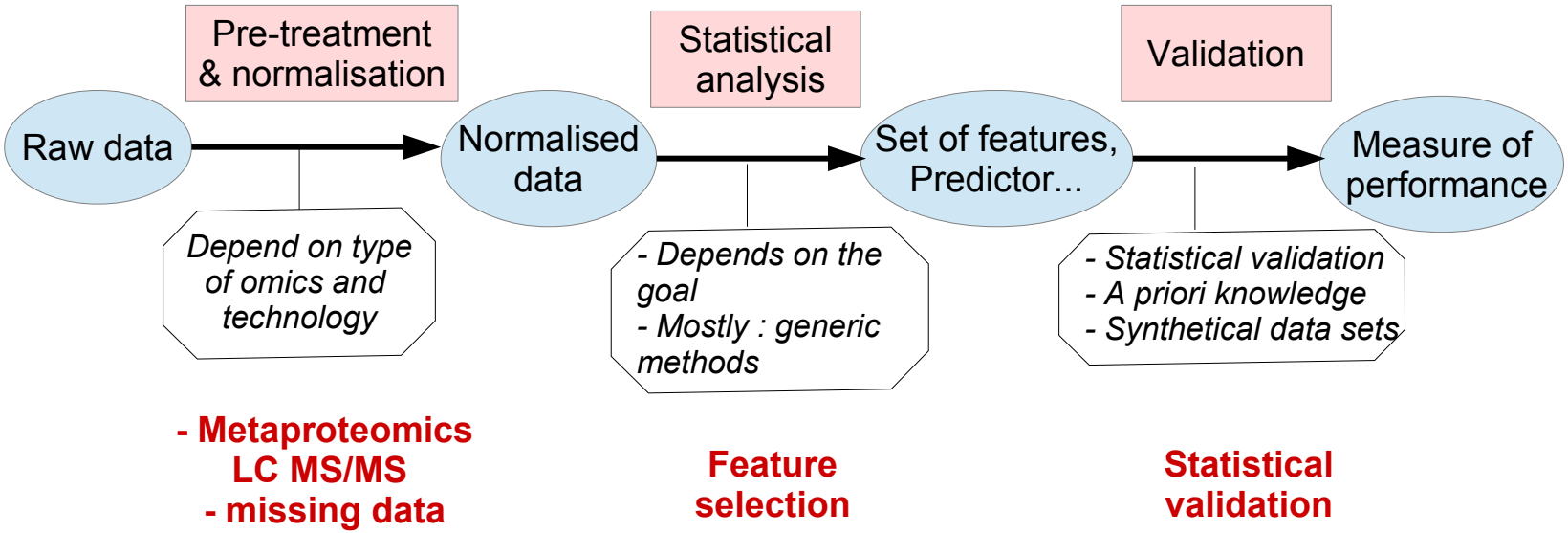
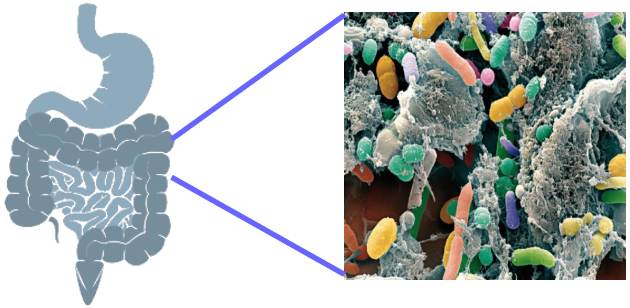


Metaproteomics

- Gut microbiote = complex bacterial ecosytem
  - Highly specific of the individual



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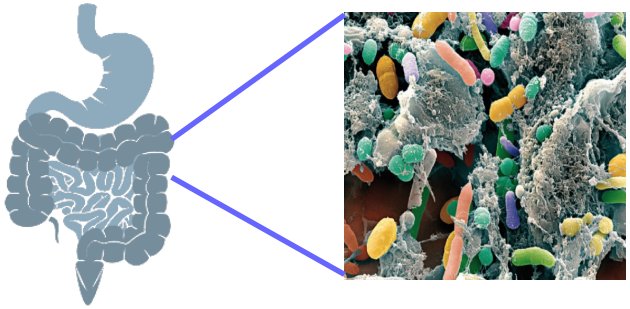

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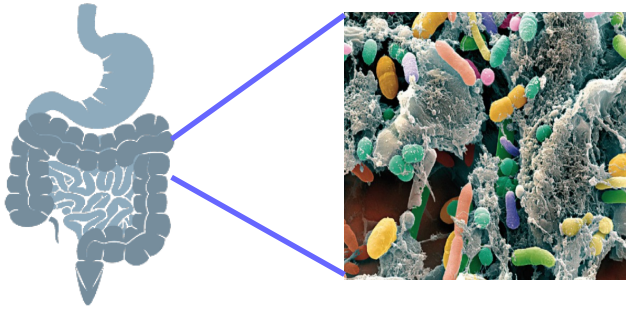
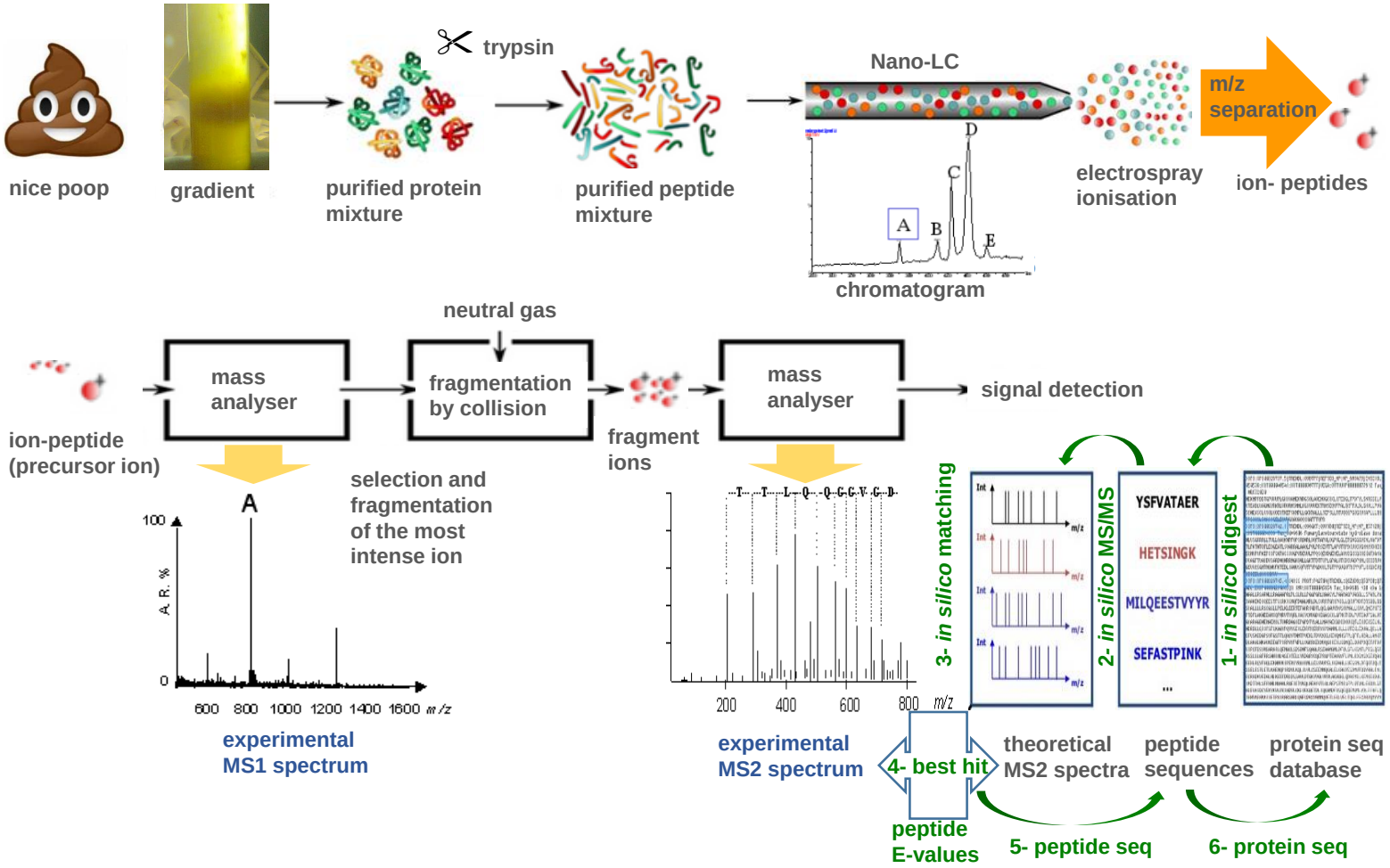
- Metaproteomics : measurements of all proteins in a sample
  - proteins actually expressed = functions realised in the gut



PROTEIN IDENTIFICATION USING MS/MS DATA



DATA ACQUISITION

DATA INTERPRETATION

A. Bassignani (2019)

Complex sources of technical variability

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These mechanisms lead to missing values

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In practise : impossible to distinguish between these types of missingness

Handling missing data in (meta)-proteomics in literature

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- Statistical analyses : imputed and observed intensities treated equally
  - Problem with a large proportion of missing value

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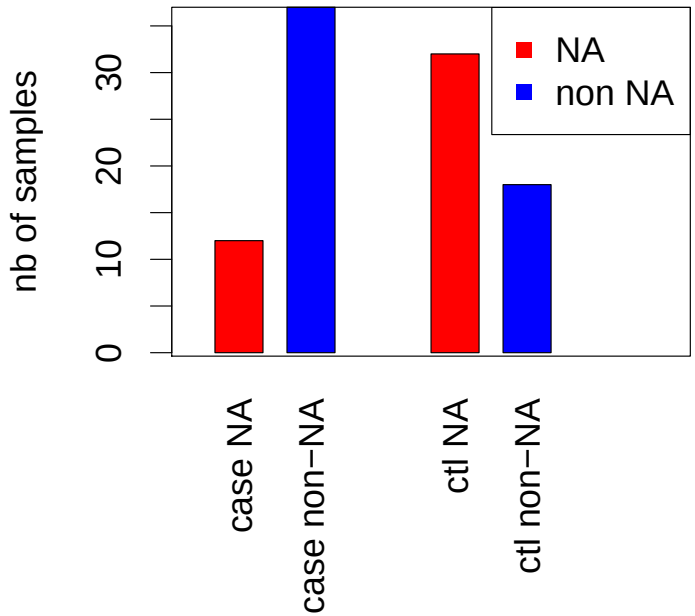

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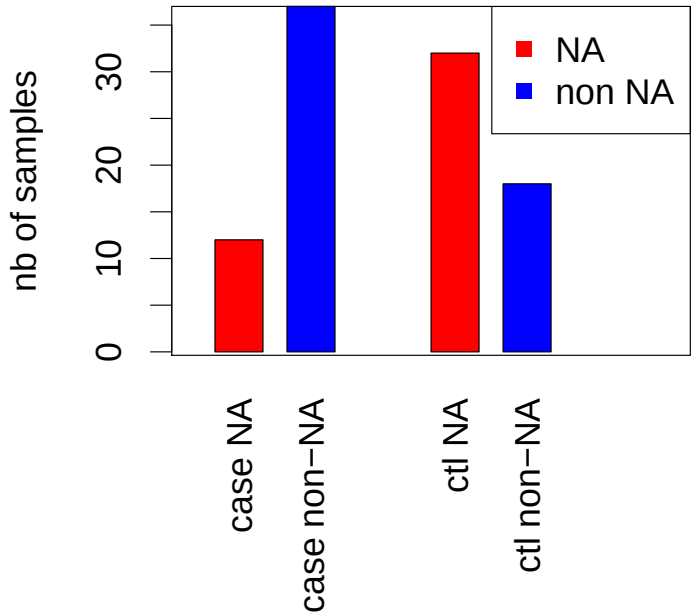
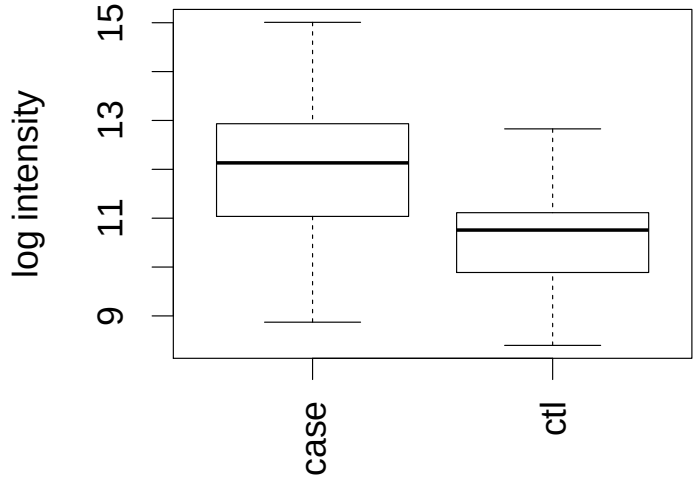
  (b) Difference in intensities among non-missing value [MAR]





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    - ★ Technical detail : distribution under H_0 is assumed identical for all proteins with same proportion of NA

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- Data sets from the ProteoCardis project
  - Metaproteome from 99 individuals in two groups (50 cases / 49 controls)
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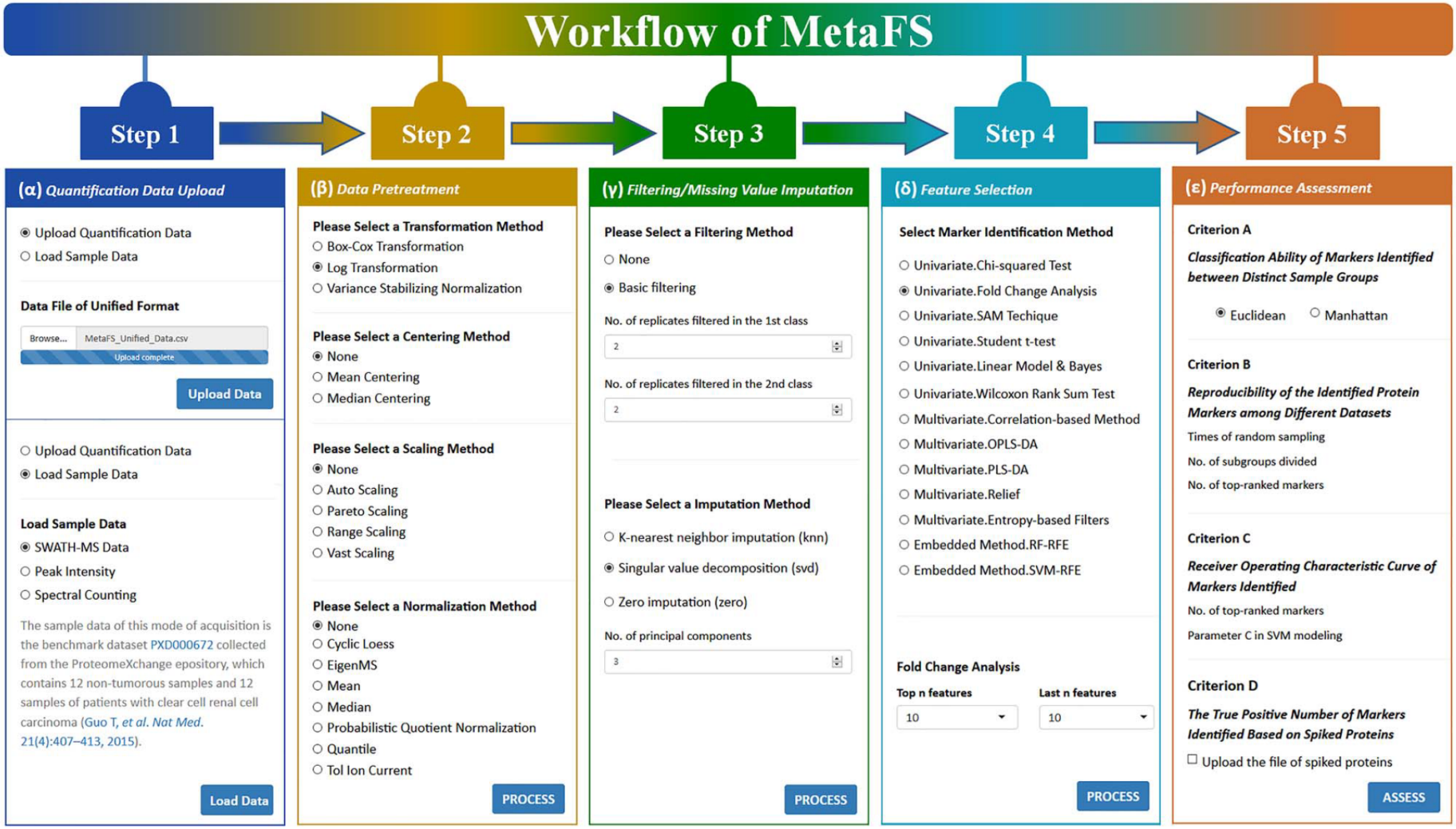
  (1) log-transformation + filter 20 non-NA + KNN + t-test [MAR]
  (2) log-transformation + filter 20 non-NA+ Single value imputation + t-test [MNAR]
  (3) log-transformation + filter 20 non-NA+ combined test [MAR + MNAR]

Comparison of feature selection methods in literature



Tang *et al* (2020), Briefings in Bioinformatics

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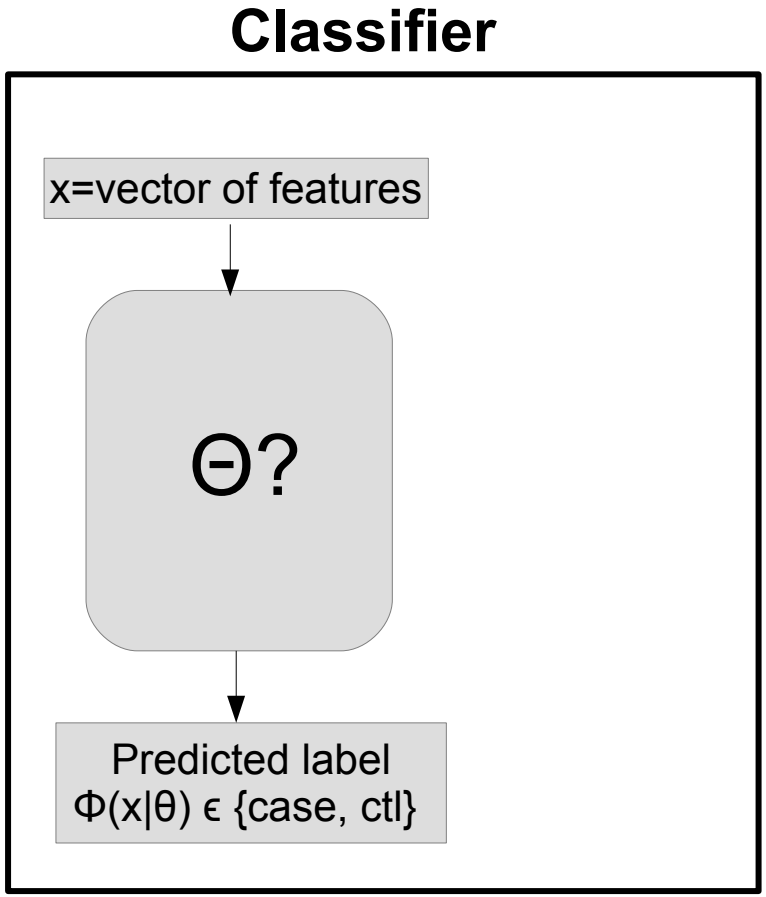

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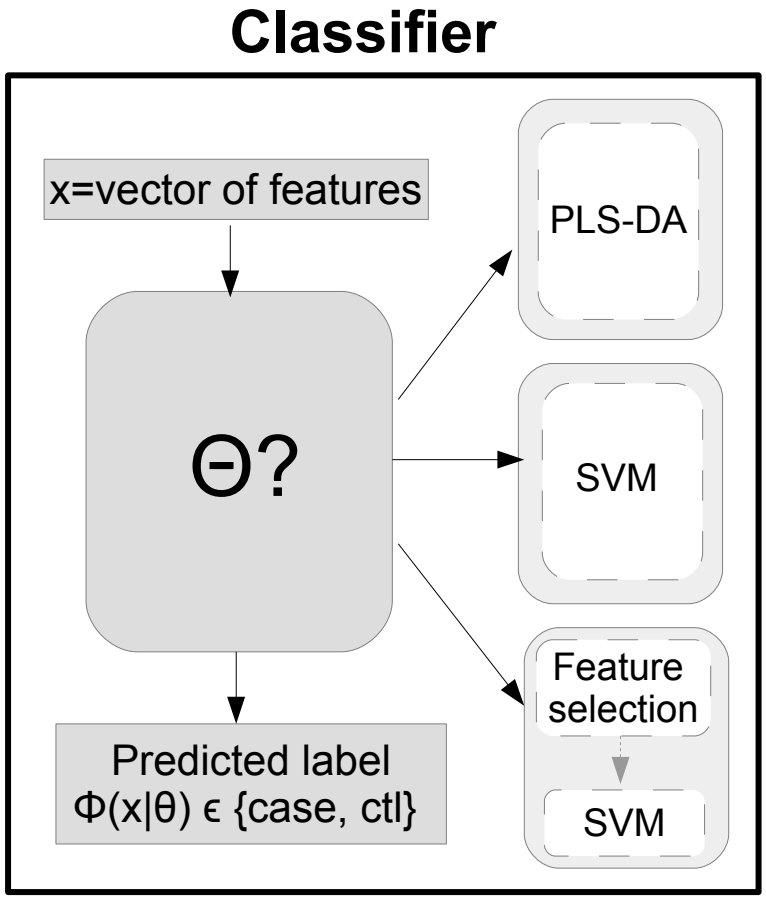

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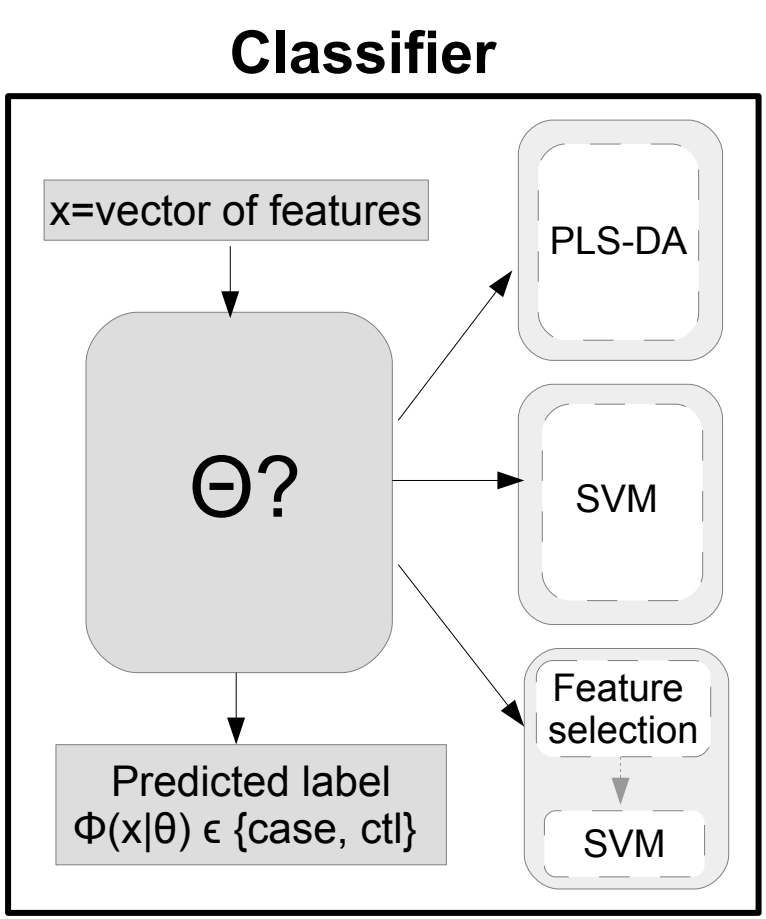

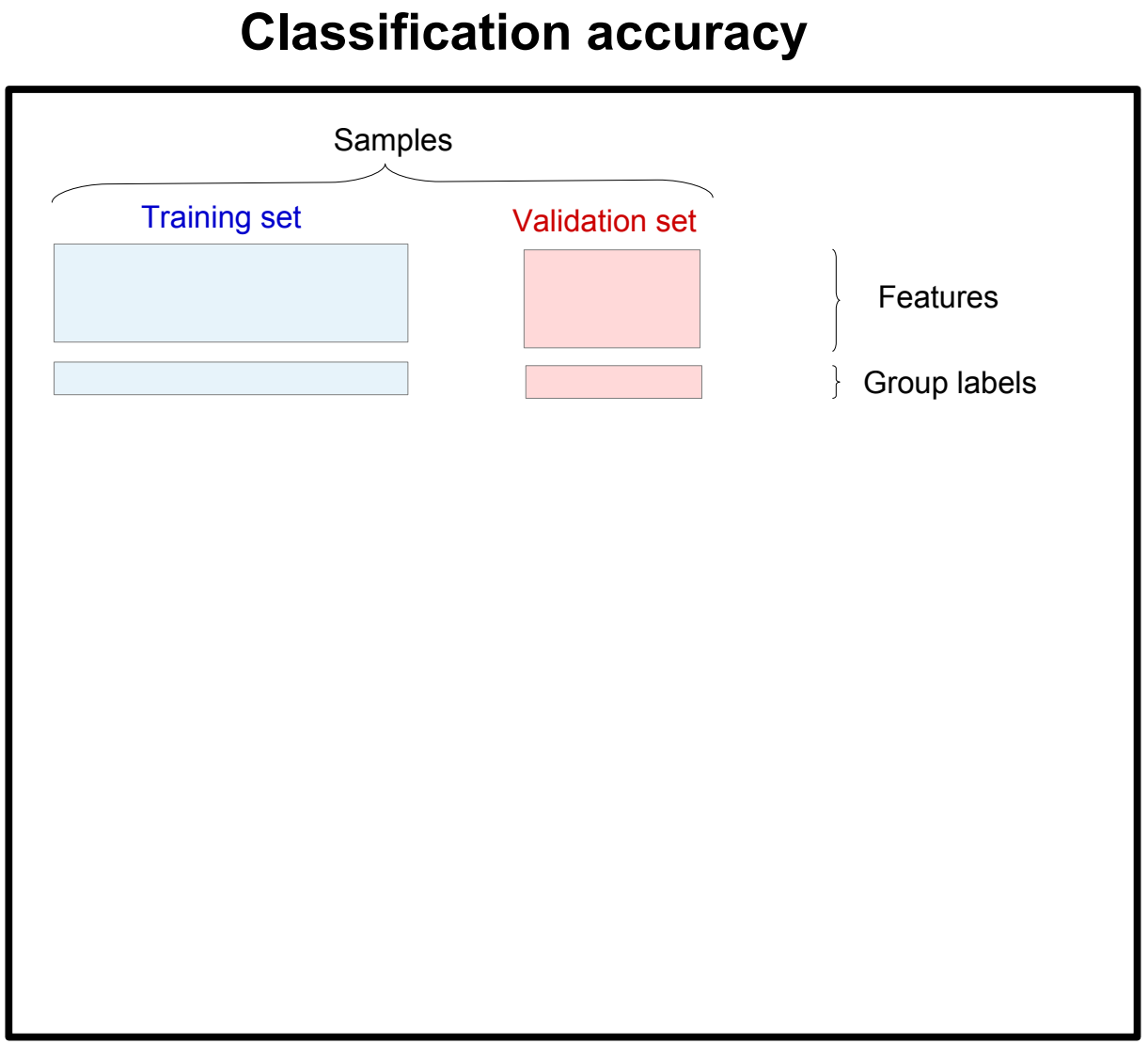

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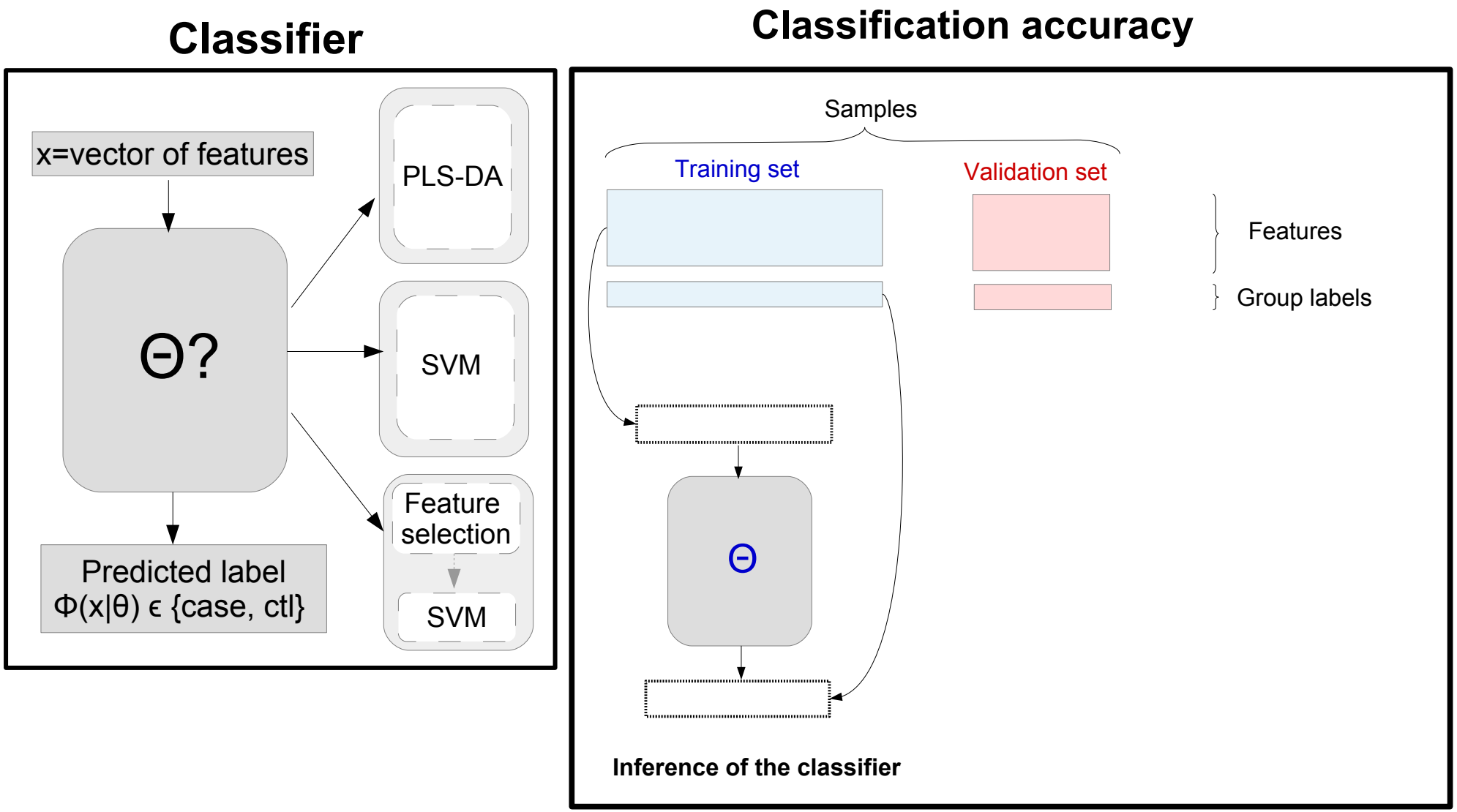

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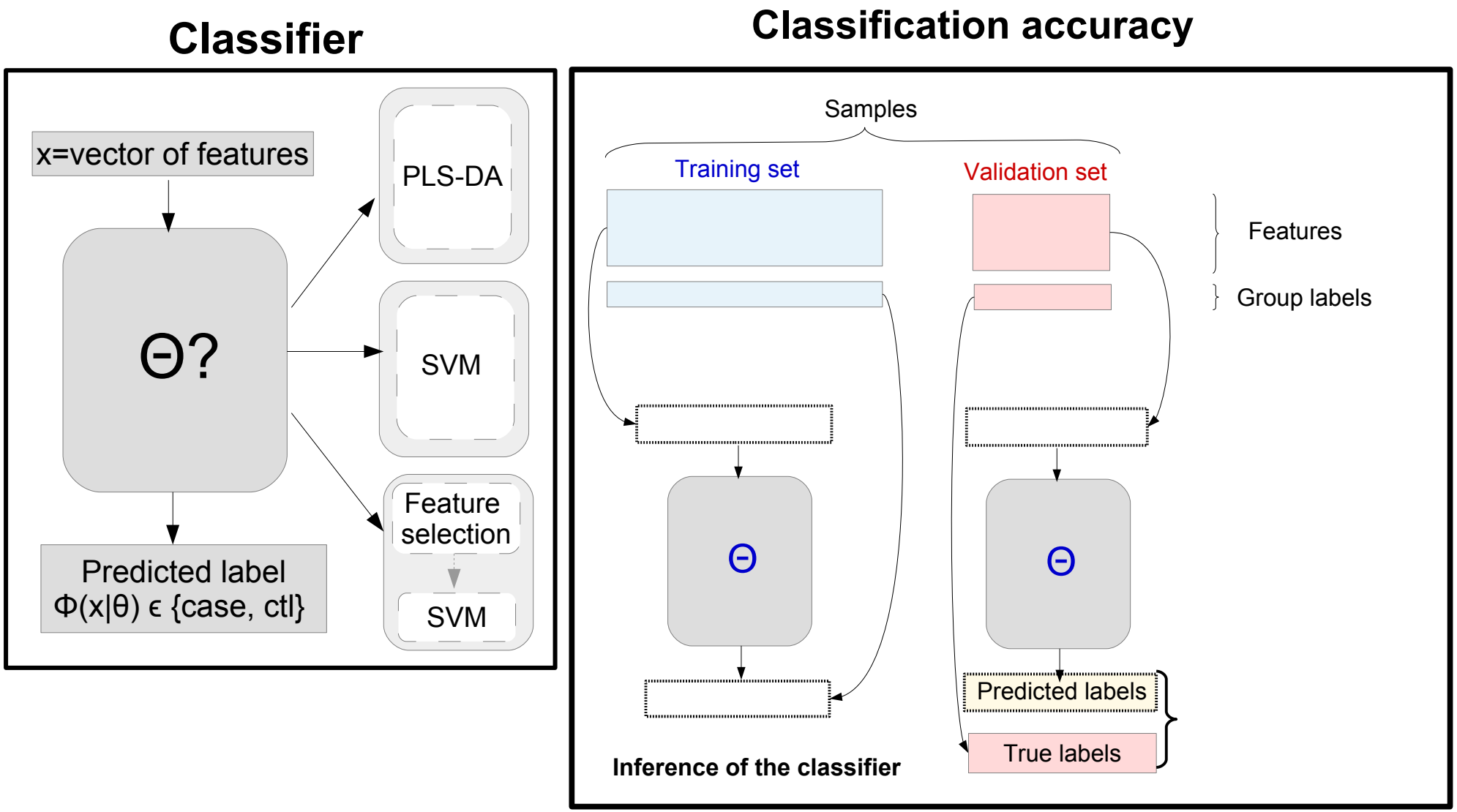

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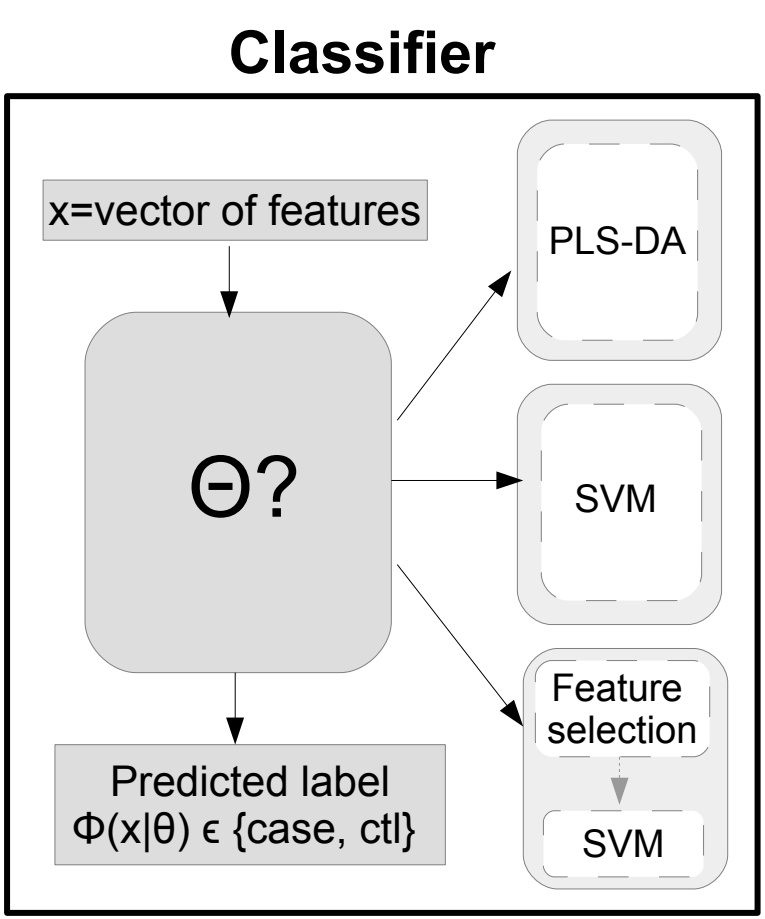

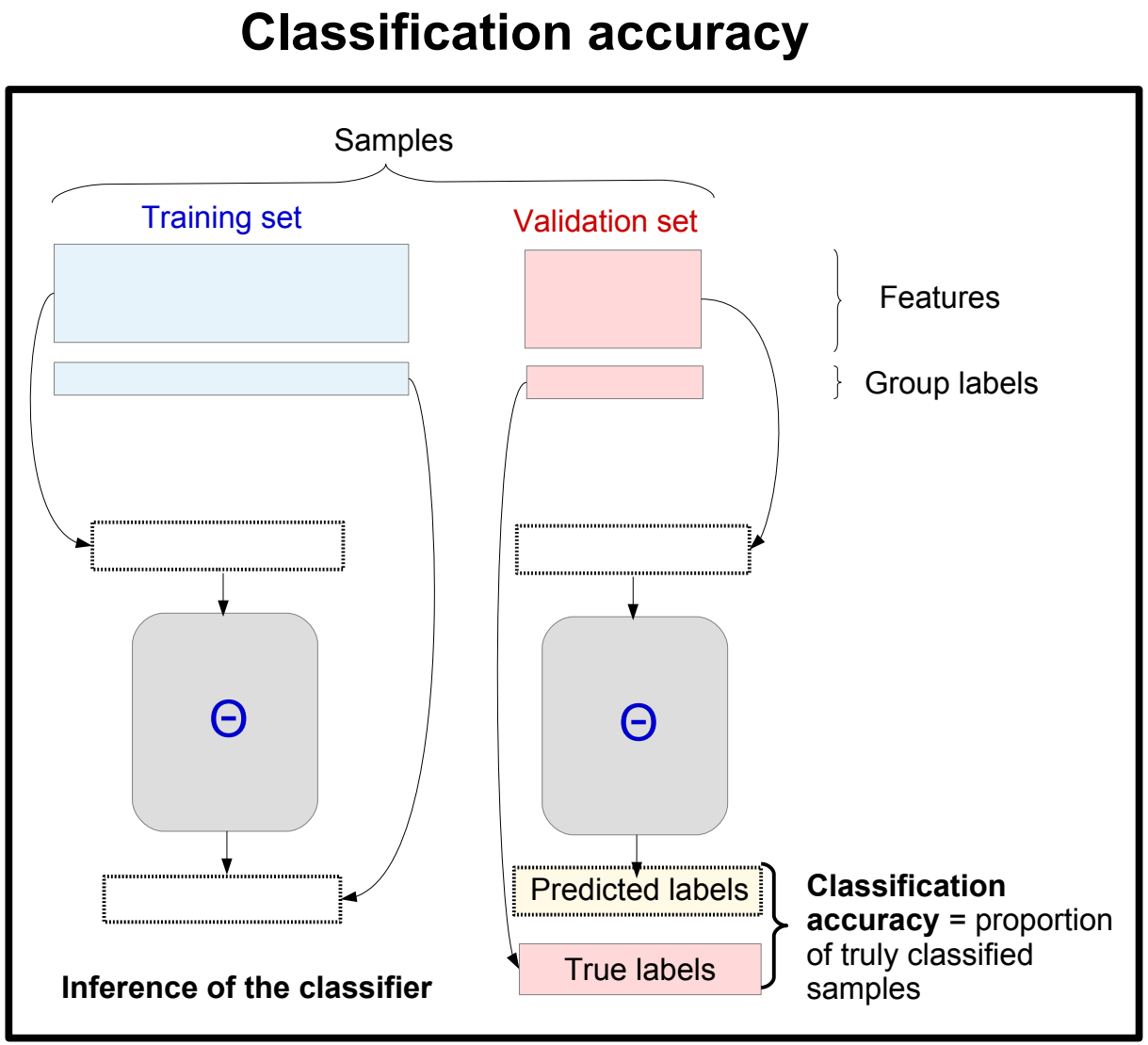

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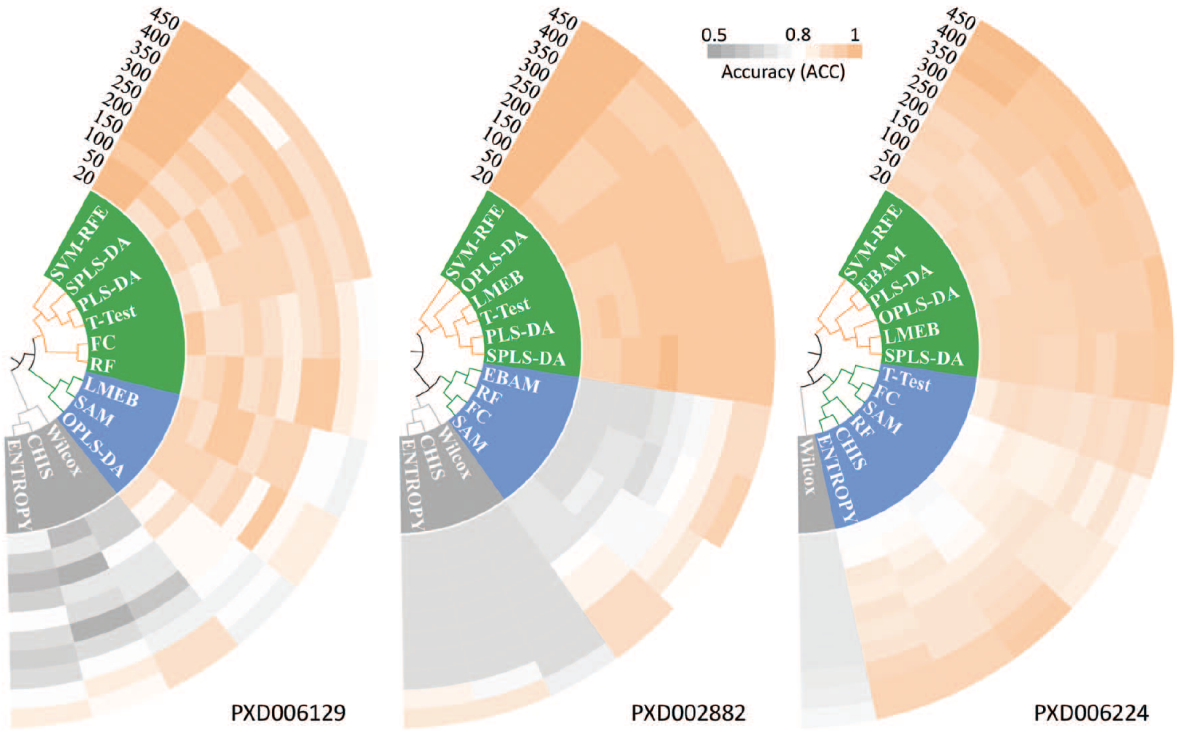
- (Arbitrary) choice of classifier to compute prediction accuracy : SVM

Classification accuracy on 3 data sets



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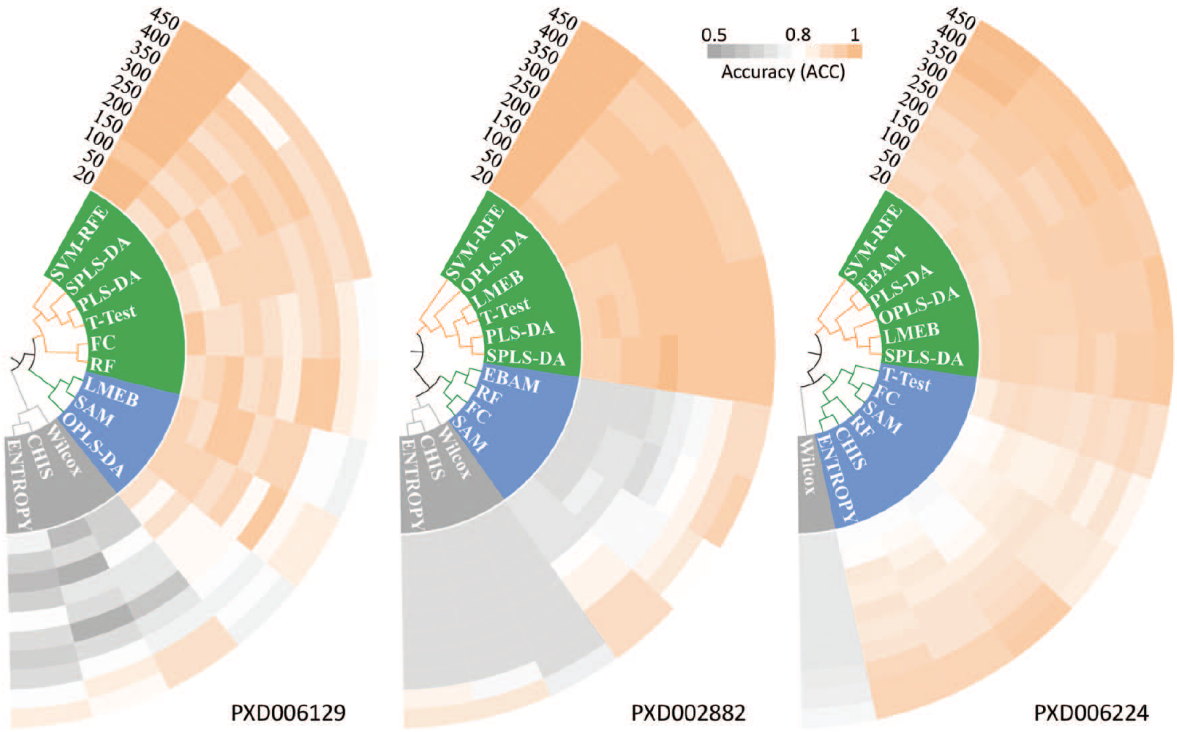

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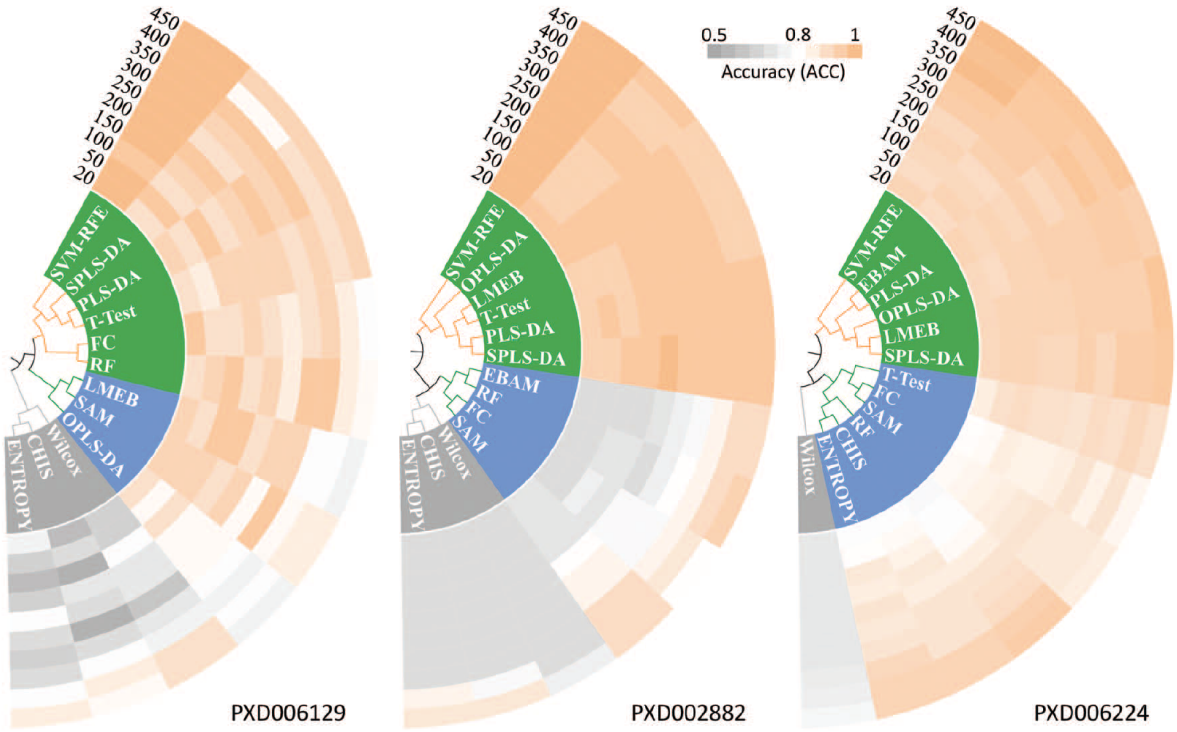

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- Coincidence? I think not...

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	Proteins		Specific peptides	
	NA = 0 for RF	KNN imput for RF	NA = 0 for RF	KNN imput for RF
FSM1	0.71	0.63	0.72	**0.72**
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- Method ranking depends on data set and classifier
- Similar performances

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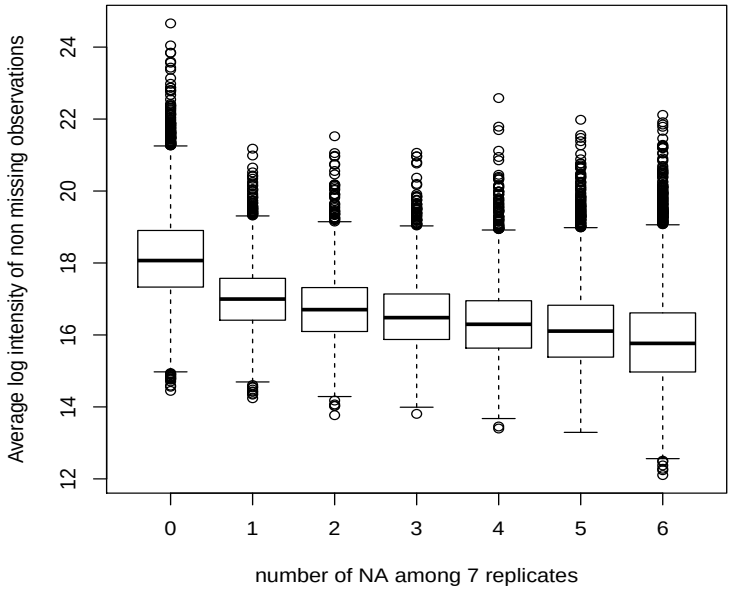

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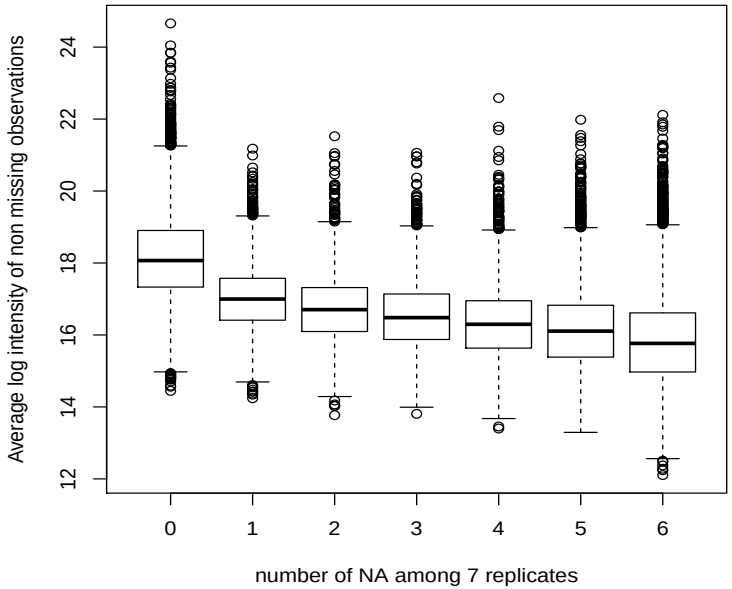

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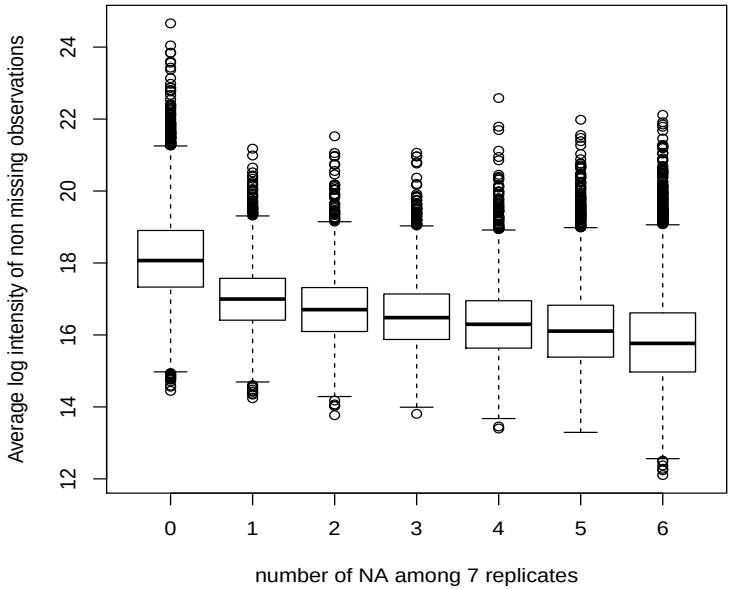

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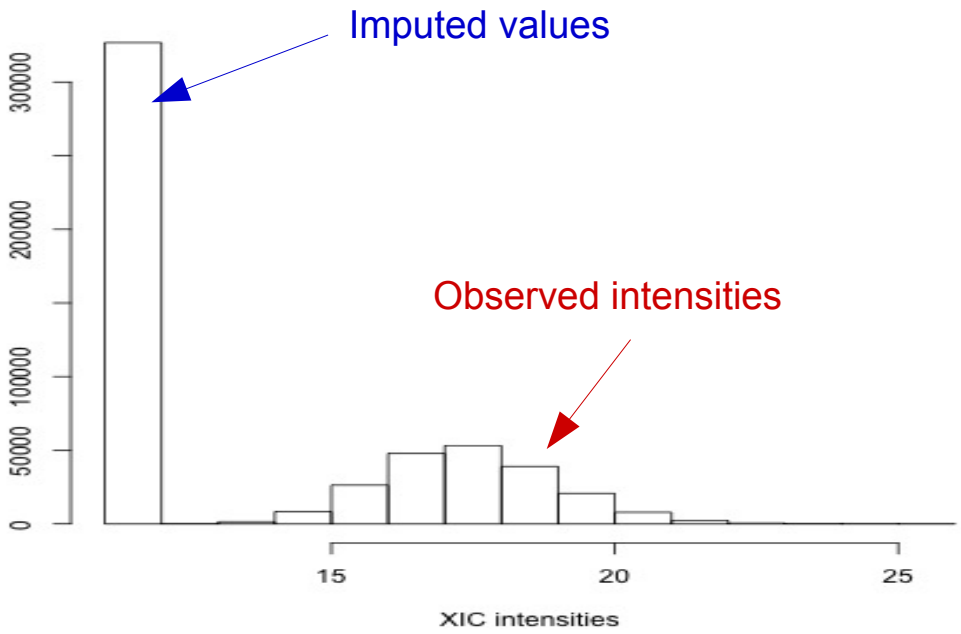
Both MAR and MNAR

Single value imputation

- Imputed values = smallest observed intensity

  Histogram of intensities after single value imputation



Single value imputation

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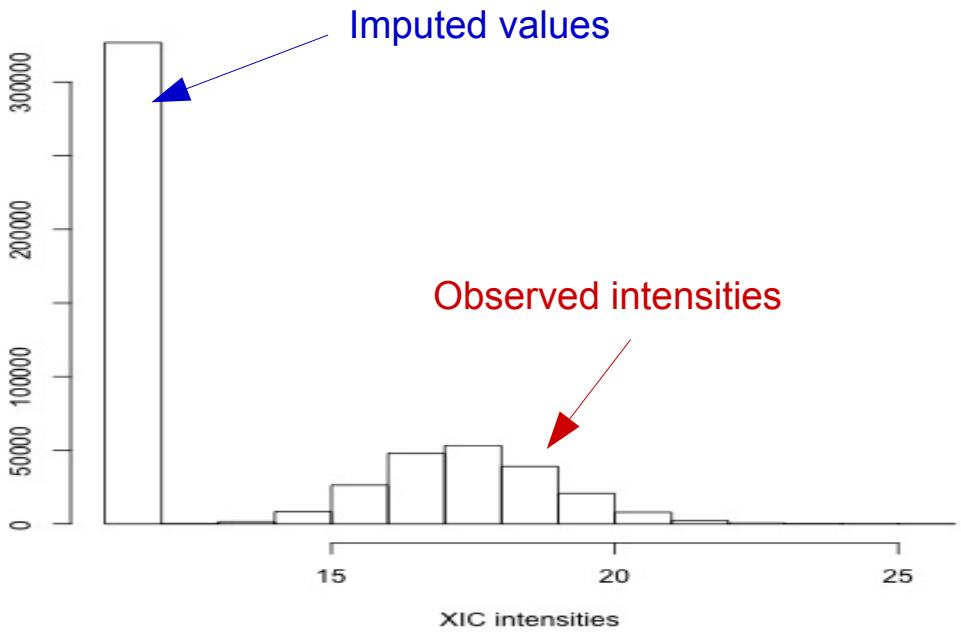
  Histogram of intensities after single value imputation

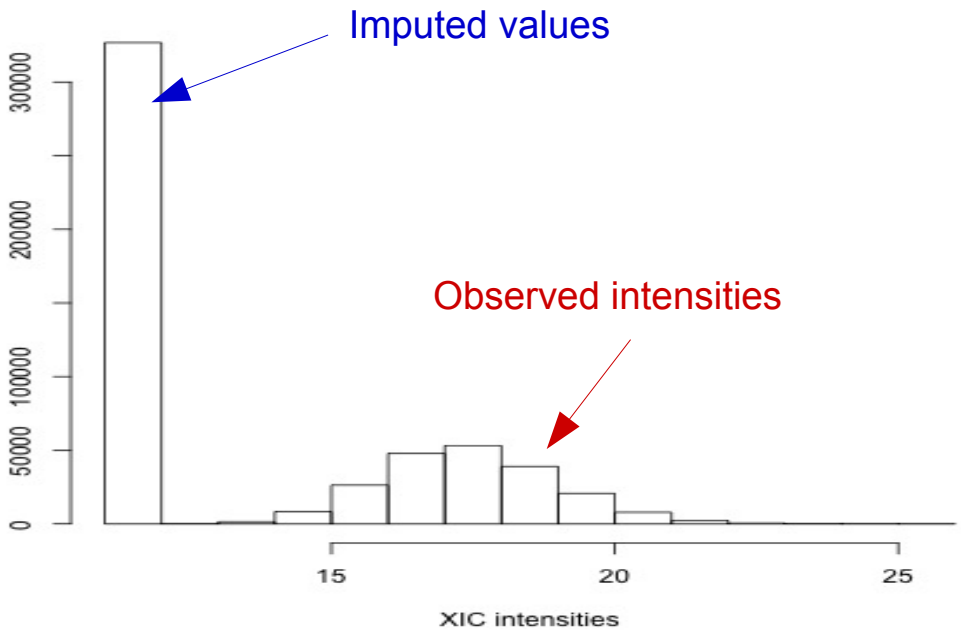


- Imputed value = too small

Single value imputation

- Imputed values = smallest observed intensity

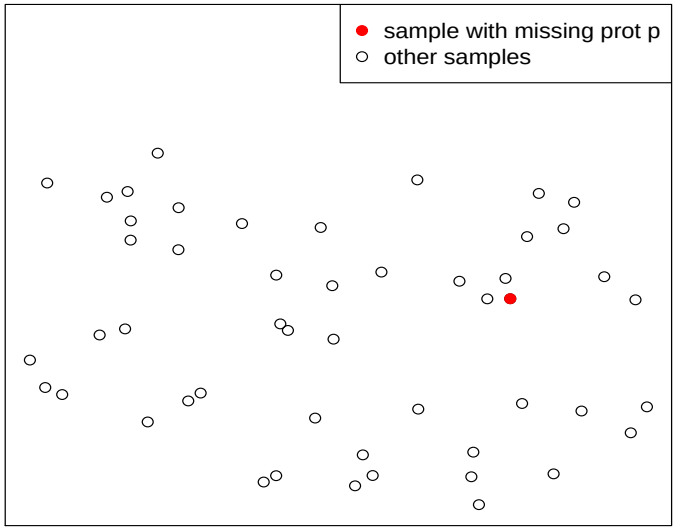
Histogram of intensities after single value imputation



- Imputed value = too small
- Rather a decreasing probability of detection than a threshold

K Nearest Neighbors

- KNN imputation depends on
  - number of neighbors k
  - distance



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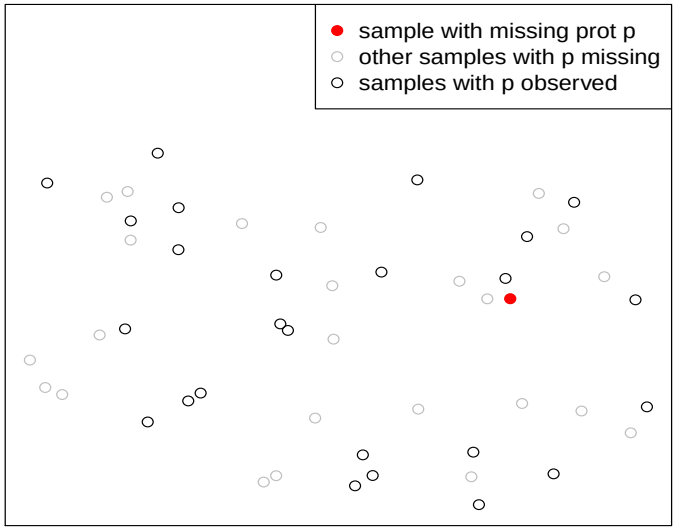

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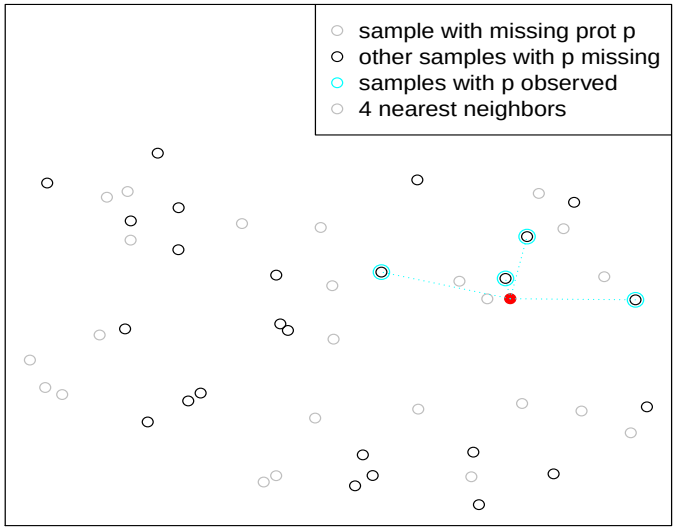

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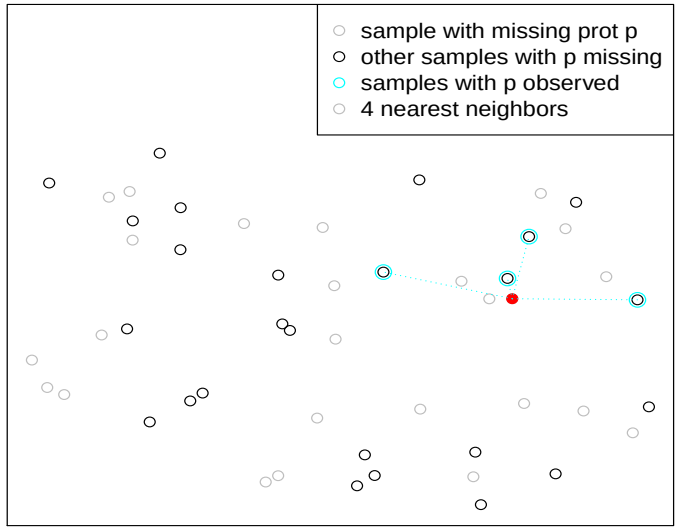
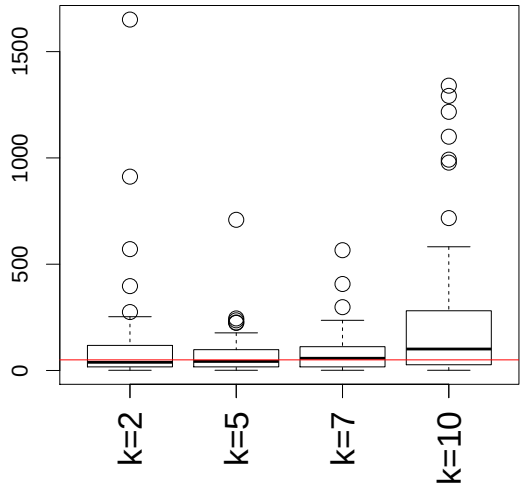
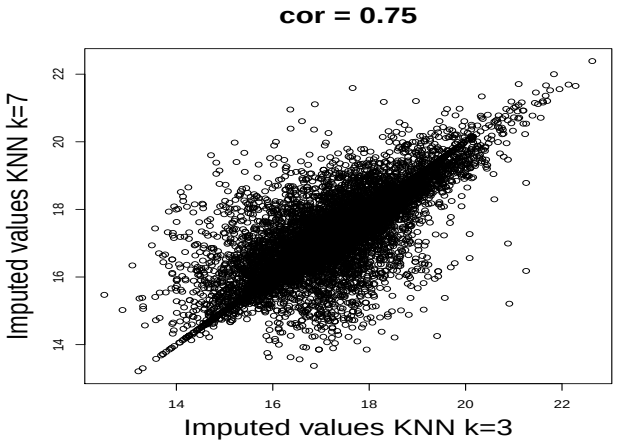


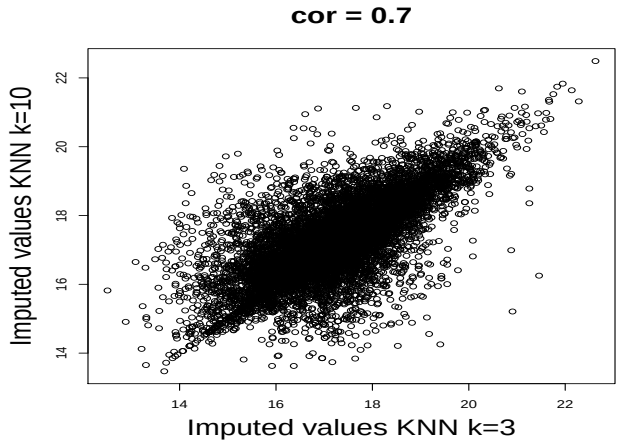
- Impact of k on feature selection

- KNN + t-test with $k_0 = 3$: rank variables
- KNN + t-test with $k \neq k_0$: extract top 50
- Look at order of the top 50 in the first ranking (with k_0)

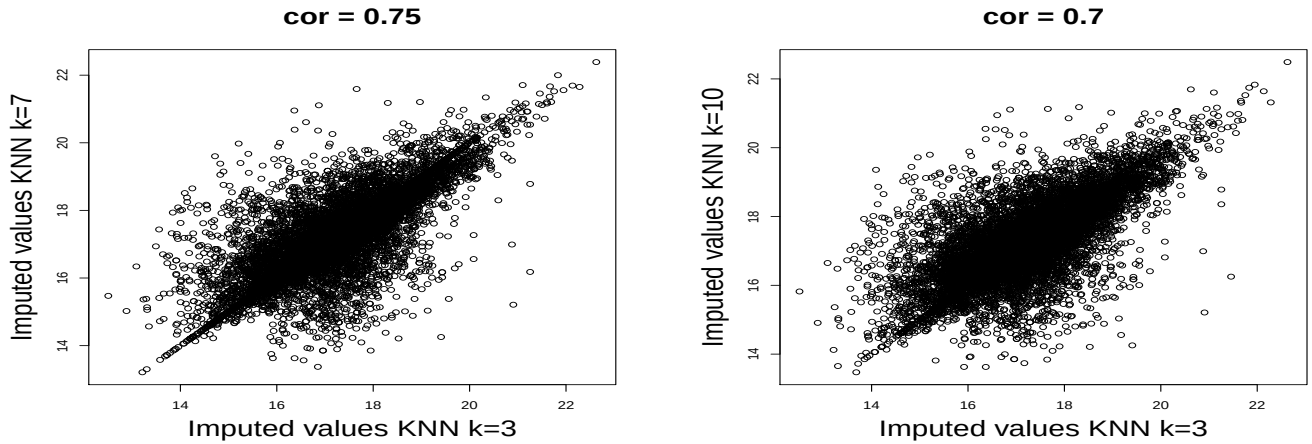


- Impact of k on imputed values





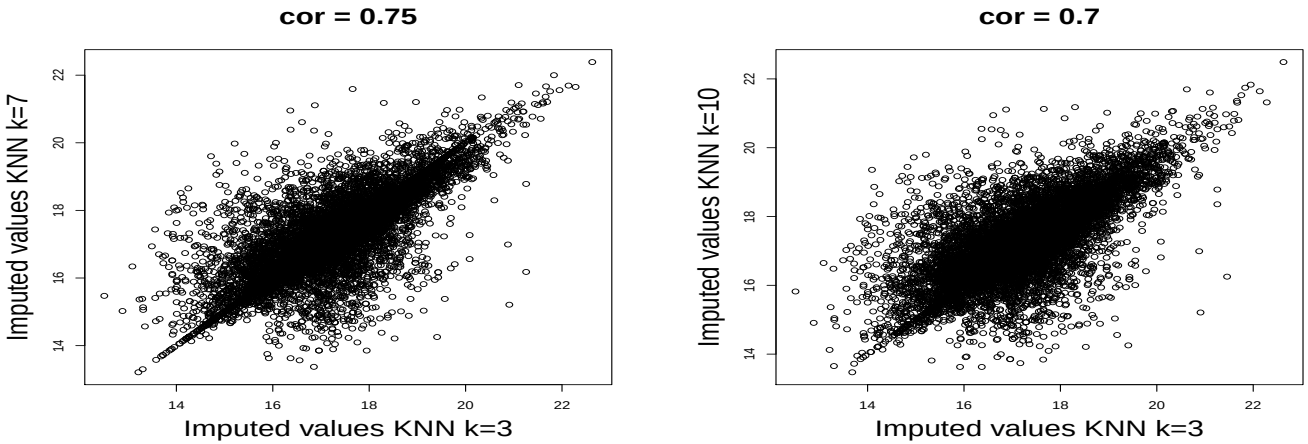
- Impact of k on imputed values



- Comparison with technical noise.
  - For each protein p and each replicated sample s,

$$\text{mean of } |\log X_{srp} - \log X_{sr'p}| \begin{cases} \text{on pairs with both observed: } d_{sp}^{\text{obs}} \\ \text{on pairs with at least one imputed: } d_{sp}^{\text{imput}} \end{cases}$$

- Impact of k on imputed values



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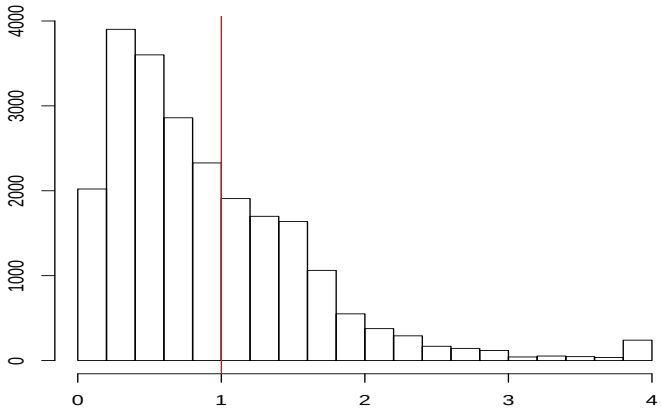
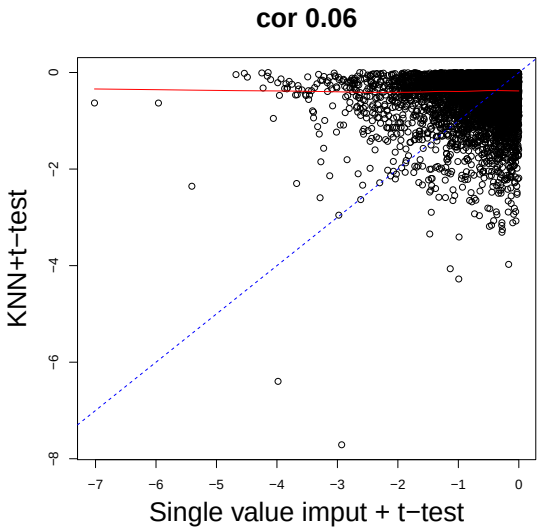
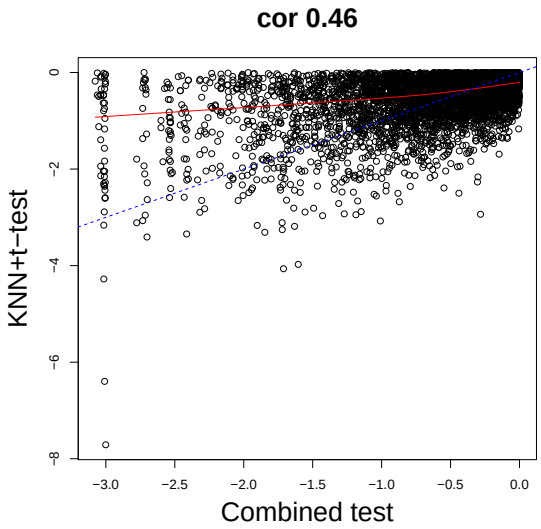
$$\text{mean of } |\log X_{srp} - \log X_{sr'p}| \begin{cases} \text{on pairs with both observed: } d_{sp}^{\text{obs}} \\ \text{on pairs with at least one imputed: } d_{sp}^{\text{imput}} \end{cases}$$

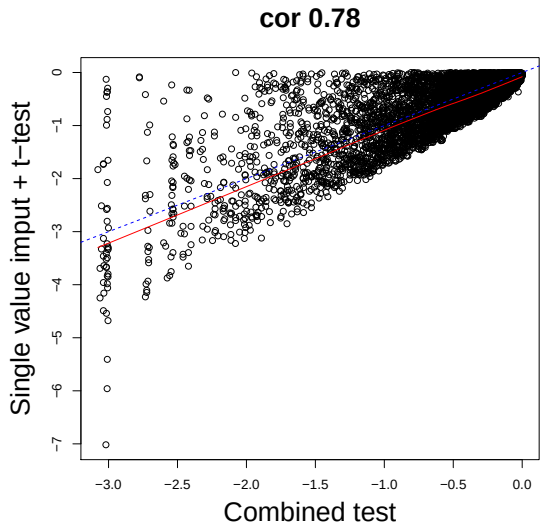


- Histogram of $d_{sp}^{\text{obs}} / d_{sp}^{\text{imput}}$
- Imputation increases moderately variability

log10-p-values for the 3 procedures

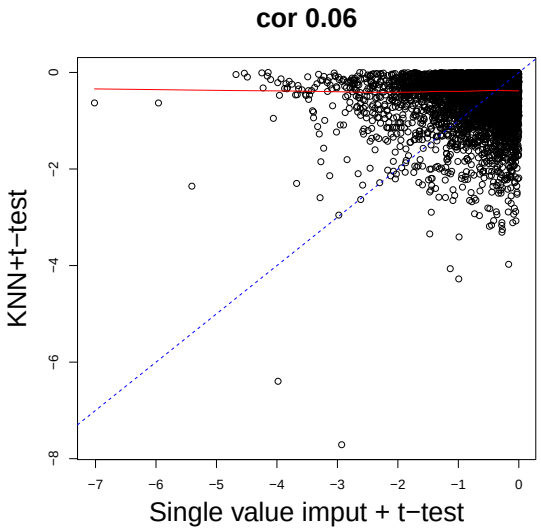


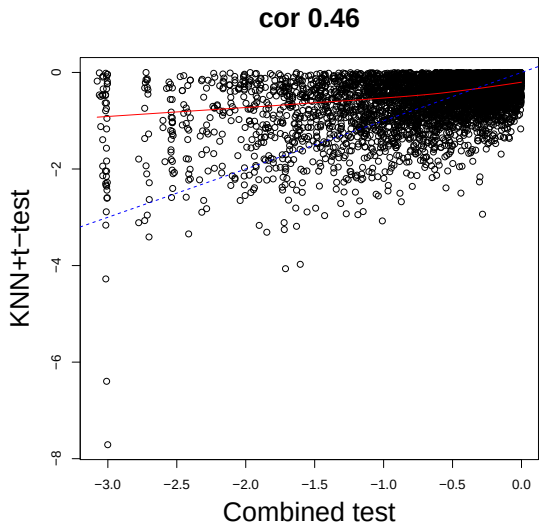


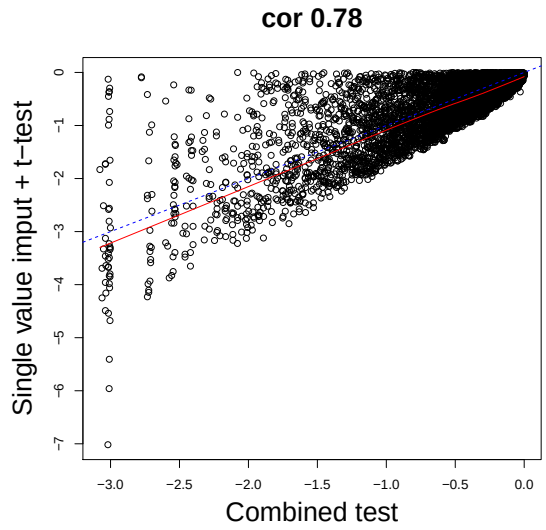


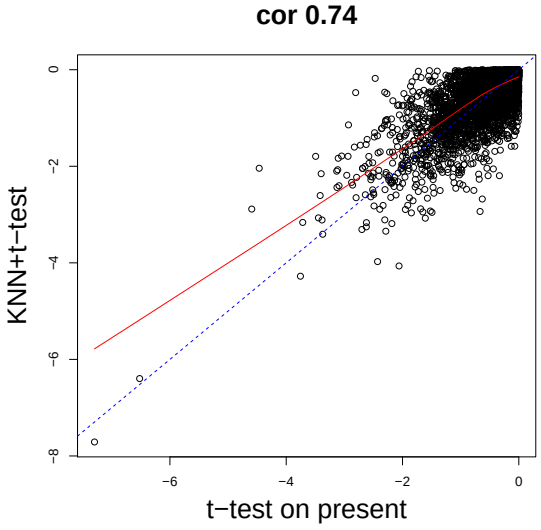
- KNN and single value: very different pv

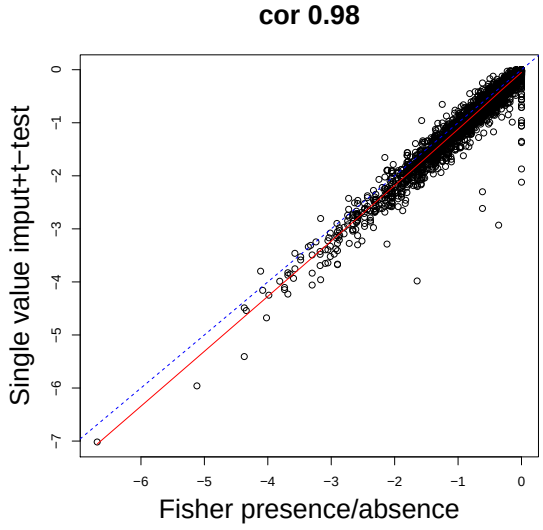
log10-p-values for the 3 procedures





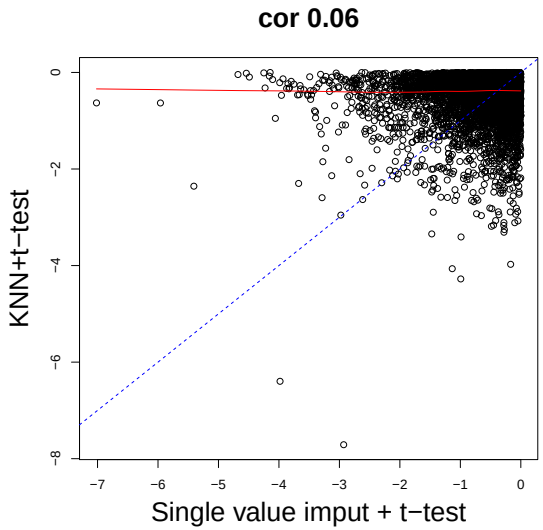


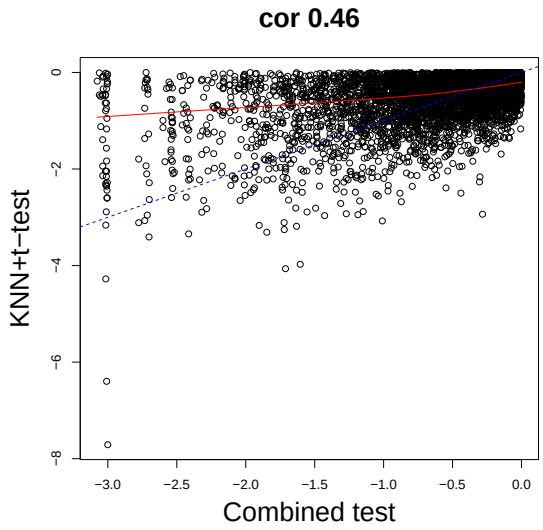


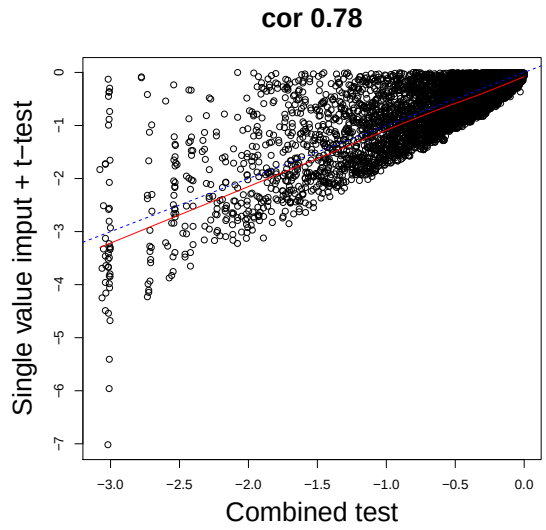


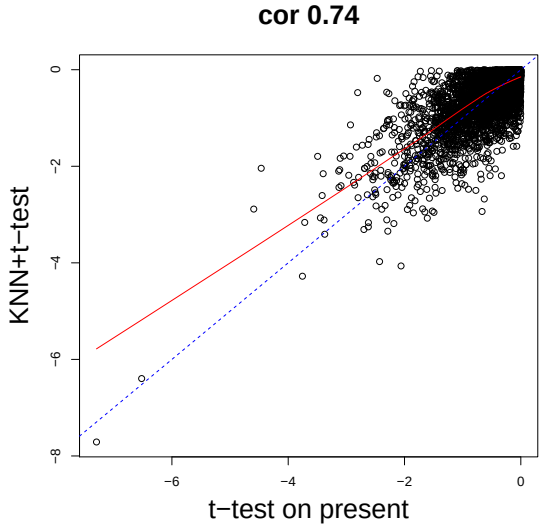
- KNN and single value: very different pv

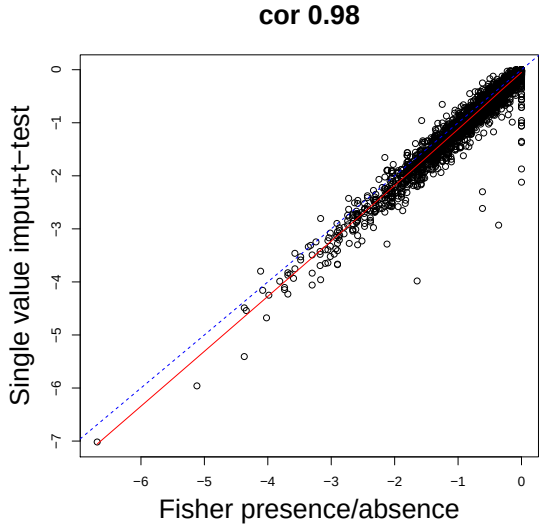
log10-p-values for the 3 procedures











- KNN and single value: very different pv
- Combined test ≈ recover results with KNN and single value

Summary of qualitative analysis

NA imputation

- ⊕ Flexible: enables any statistical analysis
- ⊖ The information of missingness is "lost"
- ⊖ Results highly dependent on methods and parameters
- ⊖ Only MAR or MNAR
- ⊕/⊖ KNN: makes use of correlation structure between variables

Combined test

- ⊖ Less flexible: only univariate statistical analysis
- ⊕ Preserve information of missingness
- ⊕ Both MAR and MNAR
  - Recover variables from KNN and single value imput.
- ⊕/⊖ Do not use correlation structure between variables
- ⊖ **Require sufficient sample size**

(Subjective) conclusion on comparison of statistical methods

- Quantitative performances: of interest but should be considered cautiously
  - Ranking varies with data set
  - Criteria may depend on (arbitrary) parametrisation
  - Cross-validation may be erroneous
- Complementary qualitative analysis
  - Examine underlying assumption/modelling
    - ⋆ Combine skills of biologist/biochemist and statistician/mathematician
  - If possible: produce technical replicates
- Implement various statistical strategies
  - More robust biological findings