Integration example 6: Partial Least Squares

Sonia Tarazona

starazona@cipf.es

Genomics of Gene Expression Group

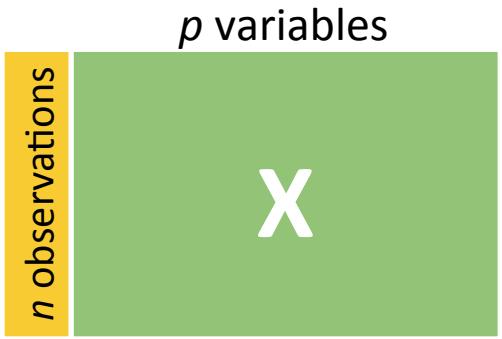
PRINCIPE FELIPE
CENTRO DE INVESTIGACION

PLS-like methods

- PLS regression
- Sparse PLS
- N-PLS
- O2-PLS
- PLS-DA

Partial Least Squares (PLS)

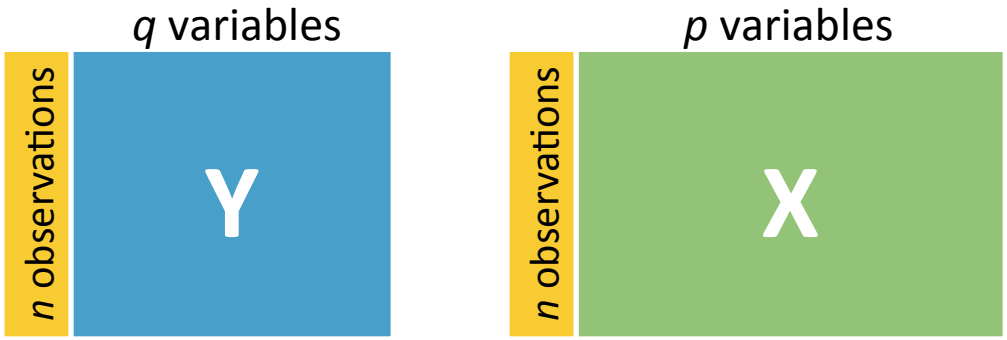
Principal Component Analysis (PCA)



- Goal: Reduce the number of variables describing the variability in X

- PCA is a multivariate dimension-reduction method that transforms data from potentially correlated variables into uncorrelated principal components (PCs)
- $X = TP^t + E$
- Loadings (P) = Weights of original variables in PCs
- Scores (T) = Weights of observations in PCs
- Residuals (E)
- Iterative process → Different algorithms

Principal Component Regression (PCR)

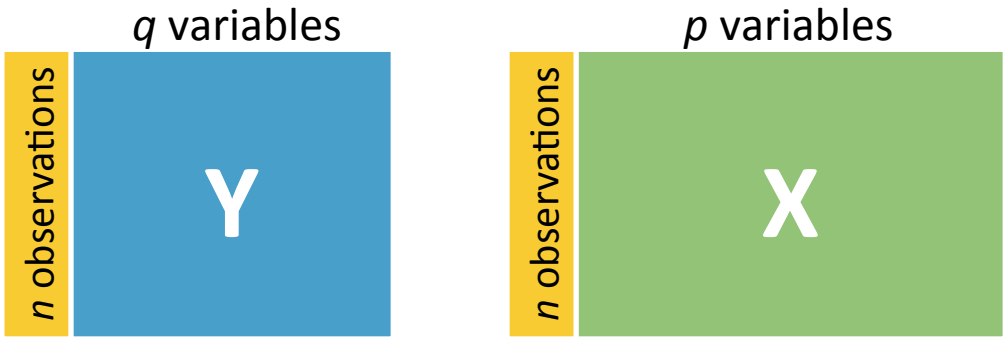


- Goal: Predicting Y from X
- Classical regression models: $p < n$
- PCR can handle $p >> n$!!

- PCR is a multivariate dimension-reduction method that first computes PCA on X matrix and then uses the resulting PCs (scores T) as regressors on Y → $Y = TB + E$
- BUT the PCs are chosen to explain X rather than Y so it could happen that they are not really relevant for Y.

https://www.youtube.com/watch?v=AxmqUKYeD-U

Partial Least Squares (PLS)



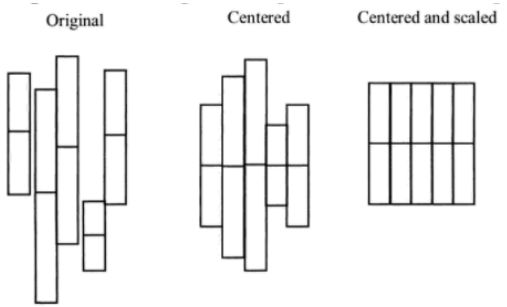
- Goal: Predicting Y from X and describing their common structure → $Y = XB + E$
- Classical regression models: $p < n$
- PLS can handle $p >> n$!!

- PLS is a multivariate dimension-reduction method that maximizes the covariance between Y and X by finding linear combinations of the variables (latent variables) from both
- $X = TP^t + E$; $Y = UQ^t + F$
- Loadings (P and Q) = Weights of original variables in latent variables
- Scores (T and U) = Weights of observations in latent variables
- Residuals (E and F)
- Iterative process → Different algorithms

https://www.youtube.com/watch?v=AxmqUKYeD-U

Scaling data for multivariate methods

- Centering
  - Subtracting the variable mean from the variable values
- Scaling (or weighting)
  - Dividing the variable by its standard deviation
  - Variables become comparable when they are measured in different units and therefore a variable with different range will not dominate the others
- Autoscaling: mean centering + scaling



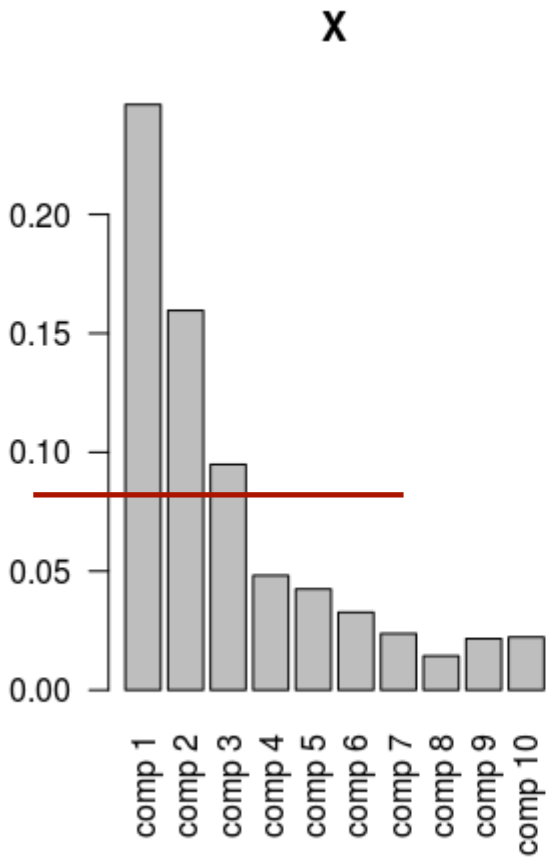
- Warning! Most of the multivariate R functions apply autoscaling by default.
- Some times, e.g. genes, all variables are measured in the same units and it is interesting to allow them for having different variability
- A preliminary exploration of data (e.g. with PCA) can help to choose the best option.
- Use scale() R function to prepare the data prior to multivariate analysis instead of letting the multivariate method to perform the scaling.

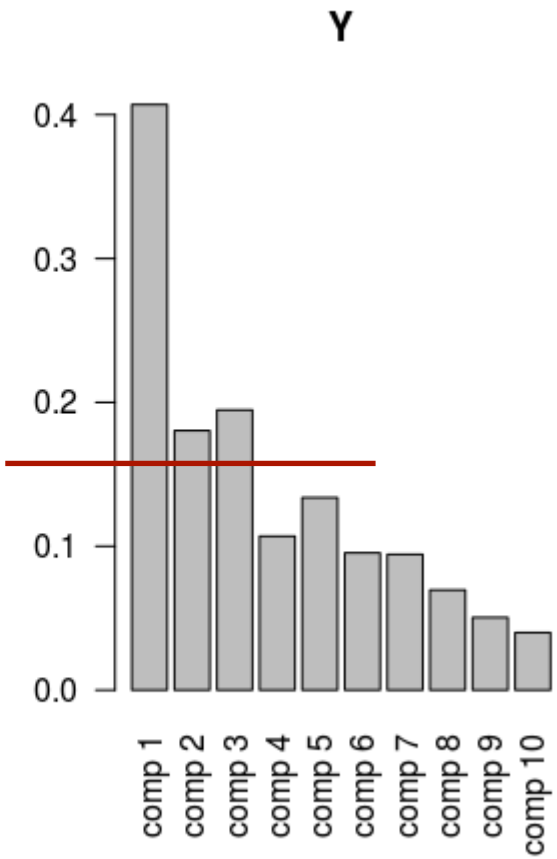
PLS example: Liver toxicity

- HandsOn/IE6_PLS/IE6_Examples.R
- Study from Bushel et al., 2007 (taken from mixOmics R library)
- Organism: rat
  - 64 male rats
  - Samples from liver after necropsy
- Types of data:
  - Expression of 3,116 genes
  - Measurements for 10 clinical variables (markers for liver injury)
- Experimental design:
  - Doses of acetaminophen: 50 mg/kg (non-toxic), 150 mg/kg (non-toxic), 1500 mg/kg (moderately toxic), 2000 mg/kg (severely toxic)
  - 6, 18, 24 and 48 hours after exposure

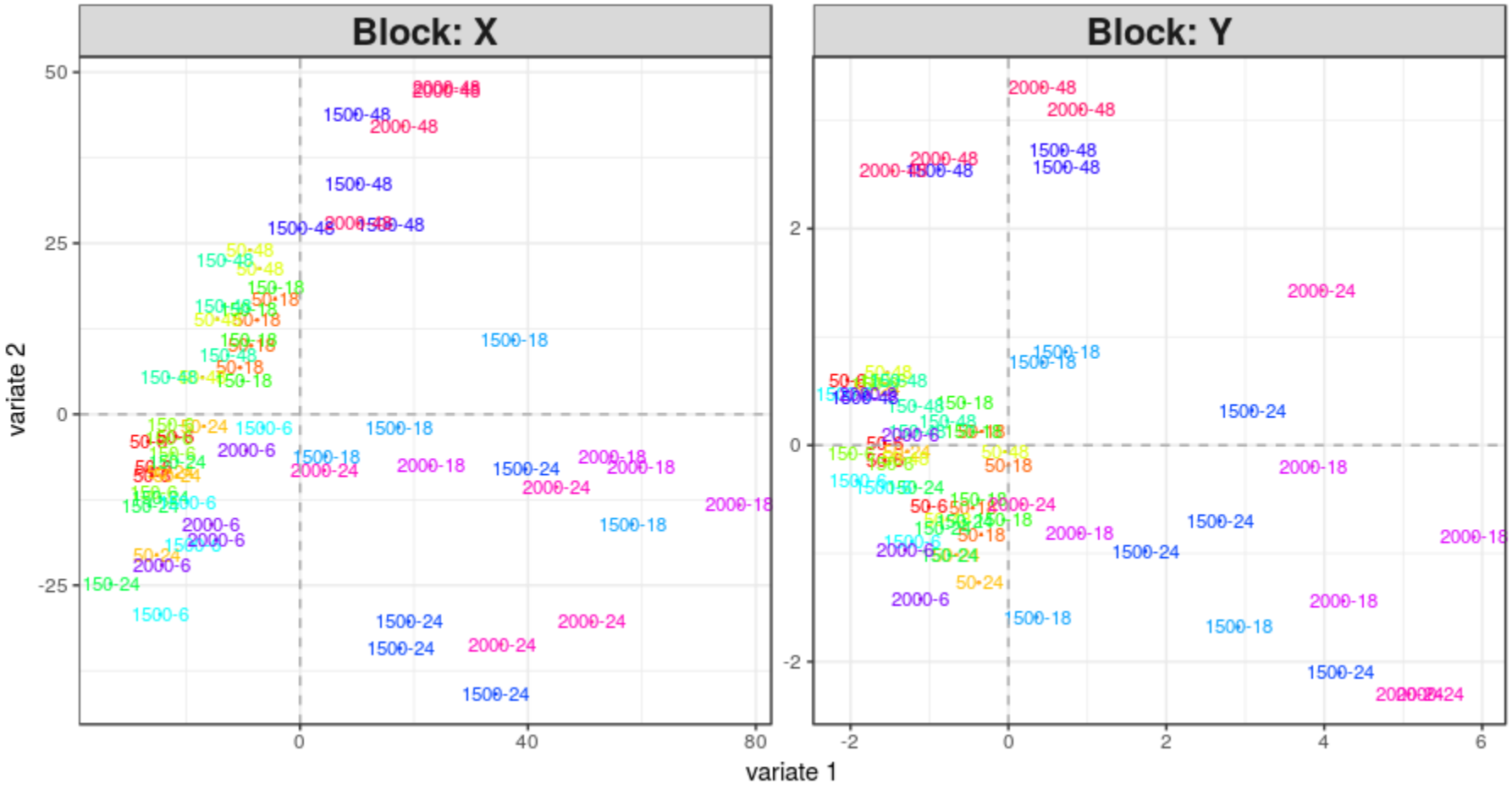
PLS example: Liver toxicity

Choosing the number of components → **ncomp = 3**

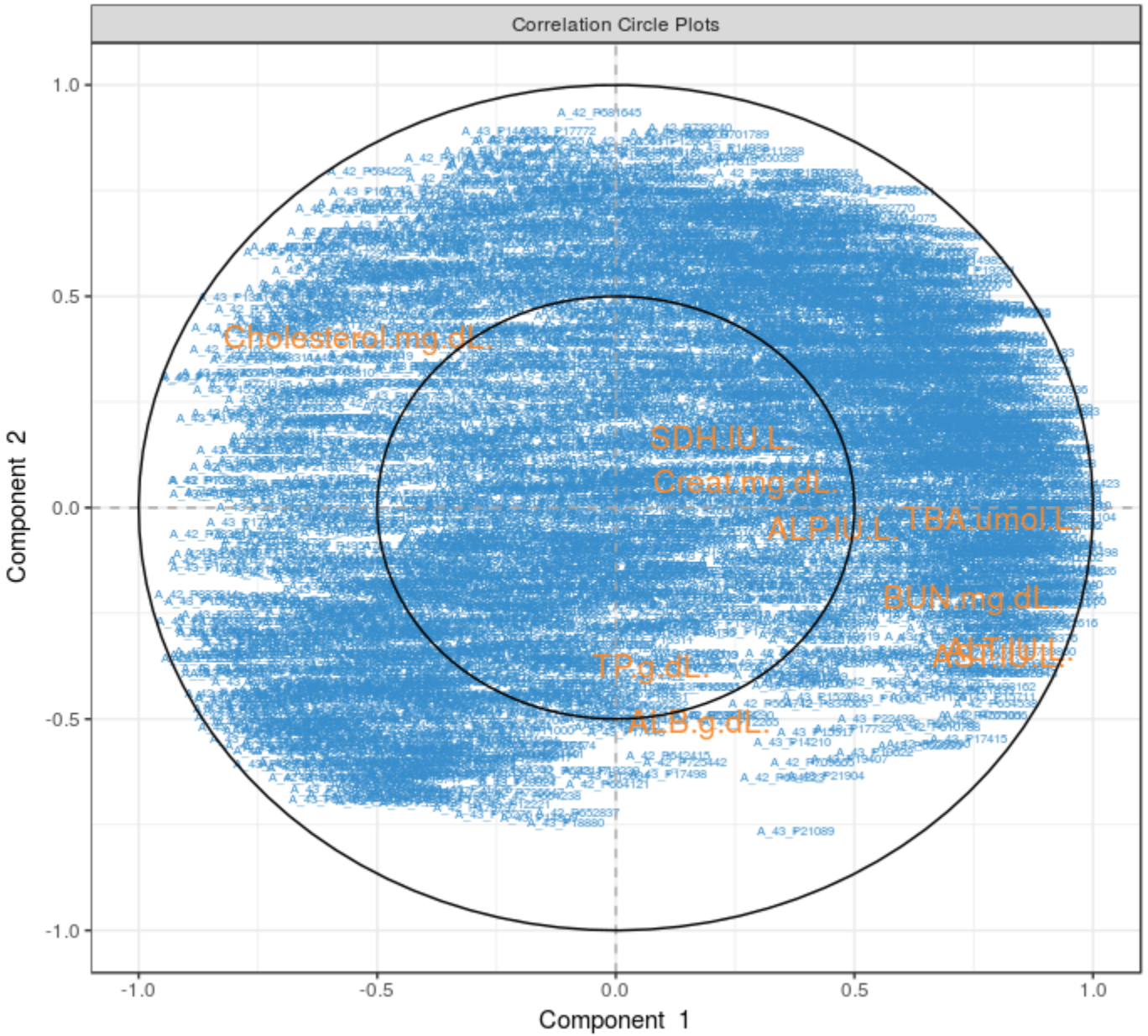




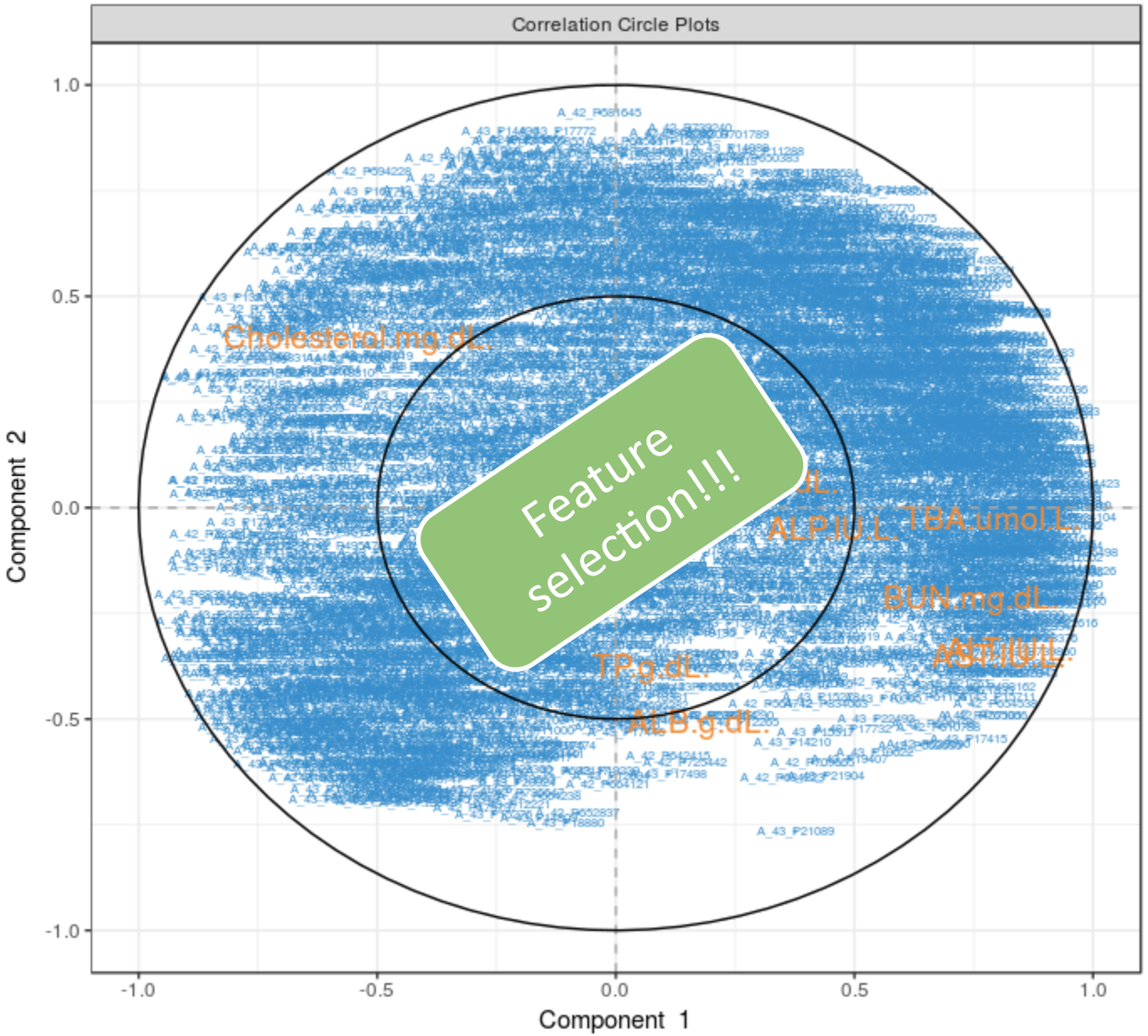
PLS example: Liver toxicity



PLS example: Liver toxicity



PLS example: Liver toxicity



PLS performance

How to measure the prediction error:

- R^2: Variance of Y explained by the PLS model
- Residual Sum of Squares (RSS): $RSS_h^k = \sum_{i=1}^{n}(y_i^k - \hat{y}_{hi}^k)^2$
- PRediction Error Sum of Squares (PRESS) or Mean Square Error of Prediction (MSEP): $PRESS_h^k = \sum_{i=1}^{n}(y_i^k - \hat{y}_{h(-i)}^k)^2$
- Root Mean Square Error of Prediction (RMSEP)
- Q_h^2: Marginal contribution of latent variable *h* (or score vector) to the predictive power of the model

$$Q_h^2 = 1 - \frac{\sum_{k=1}^{q} PRESS_{kh}}{\sum_{k=1}^{q} RSS_{k(h-1)}}$$

  - Rule: Latent variable h is selected if $Q_h^2 \geq (1 - 0.95^2) = 0.0975$

Cross-validation

- Model validation allows for assessing whether the generated model will be suitable for prediction given a new independent data set.
- Validation = Divide the data into two subsets: *training* set and *testing* set → Requires a large number of observations
- Small number of observations → **Cross-validation (CV)**: Multiple rounds of validation → Prediction error is averaged for all the rounds
  - **Leave-one-out CV** (LOOCV): Training set = n-1 observations, Test set = 1 observation → Recommended for small sample sizes
  - **K-fold CV**: The data are divided in k equal sized subsets. Training set = k-1 subsets, Test set = 1 subset

Feature selection in PLS

Feature selection in PLS

- Based on loadings, that measure the relative importance of the variables in the model.
- VIP (Variable Importance in Projection) measures the importance of X-variables in the model for each component (computed from the loadings for each component).
  - "Greater than one" rule is generally used
- Permutation strategies.
- Elastic Net regularization (Zou and Hastie, 2005).
- Sparse PLS (Lê Cao et al., 2008)
- ...

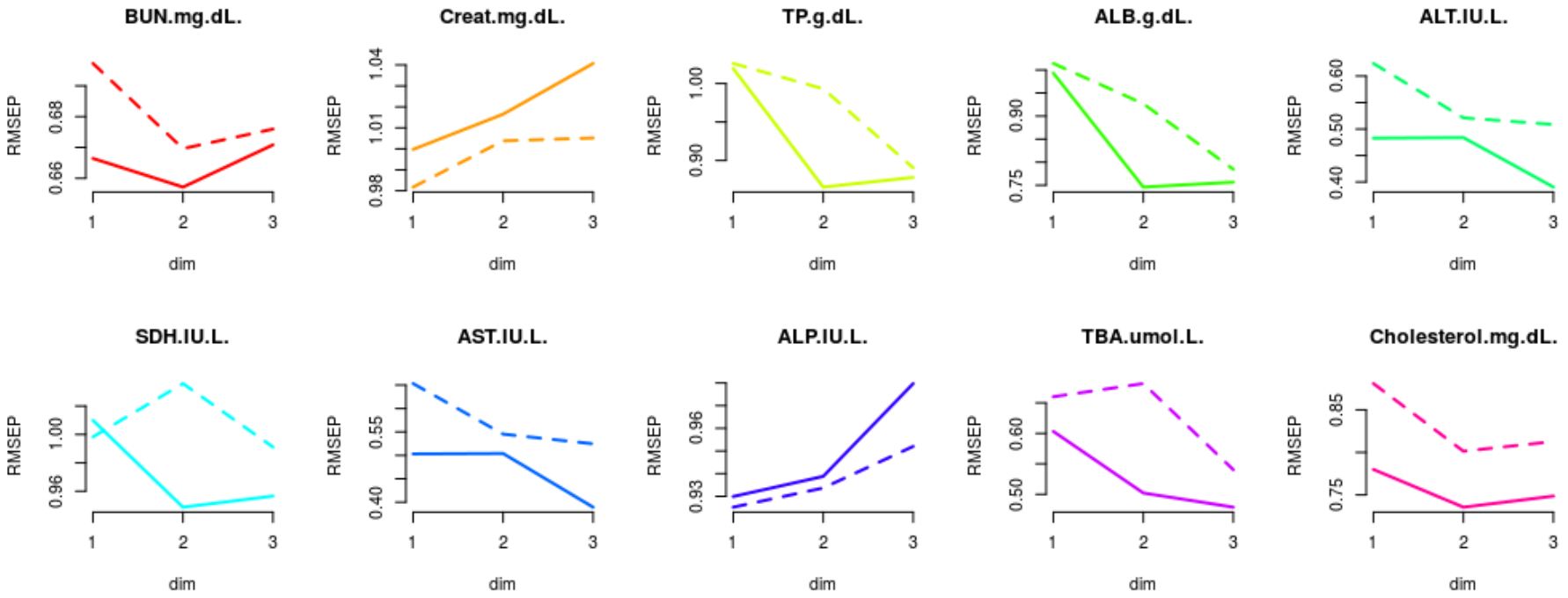
Tarazona et al. *Variable selection for multifactorial genomic data*. Chemometrics and Intelligent Laboratory Systems, 2012

Sparse PLS (*Lê Cao et al., 2008*)

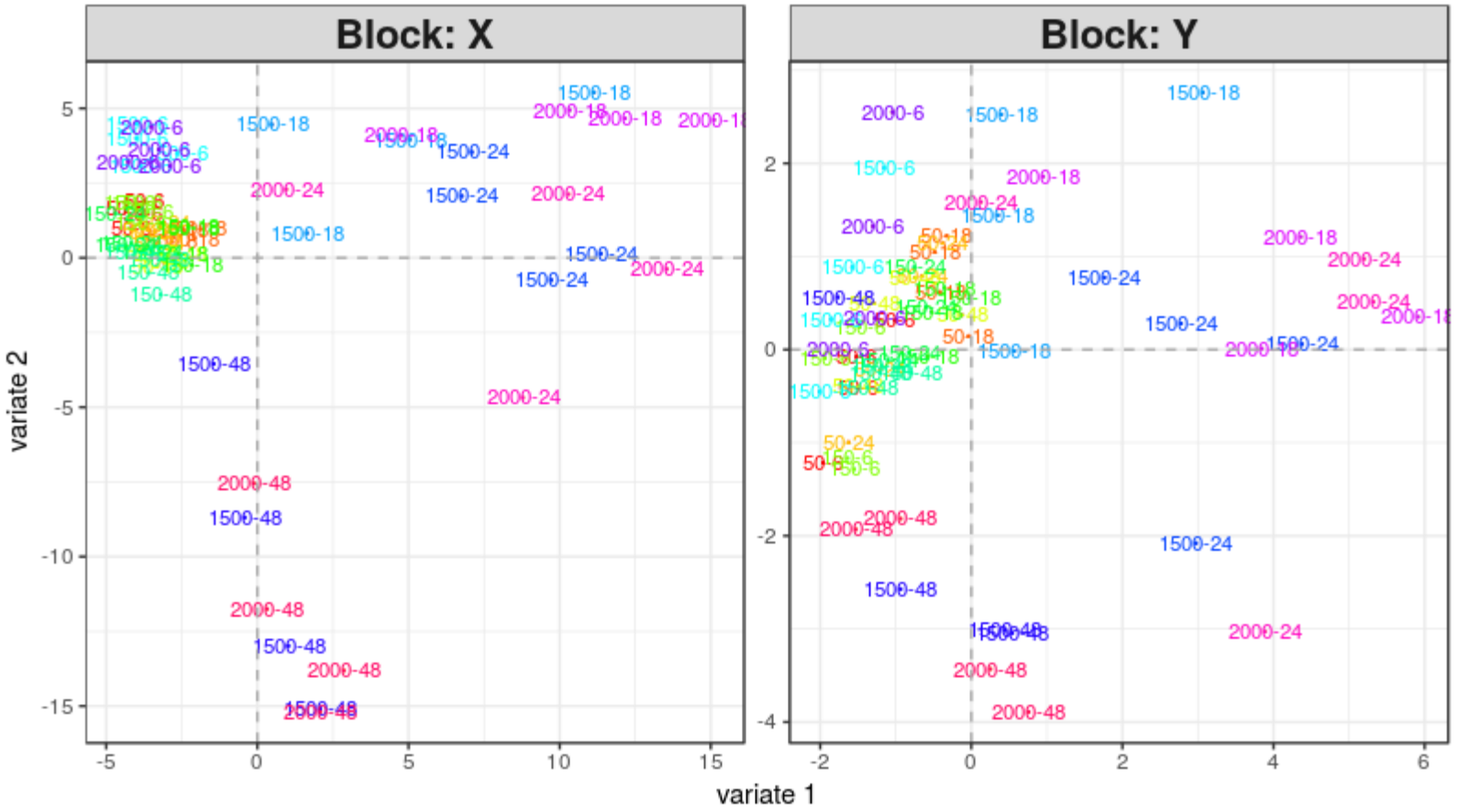
- Idea: Select the X and Y variables that are contributing most to the PLS model
- How: Penalizing loadings → Lasso penalization
- The user chooses the number of variables to be retained = number of variables with non-zero loading for each component and for X and Y
- Implemented in mixOmics package

Example on sPLS

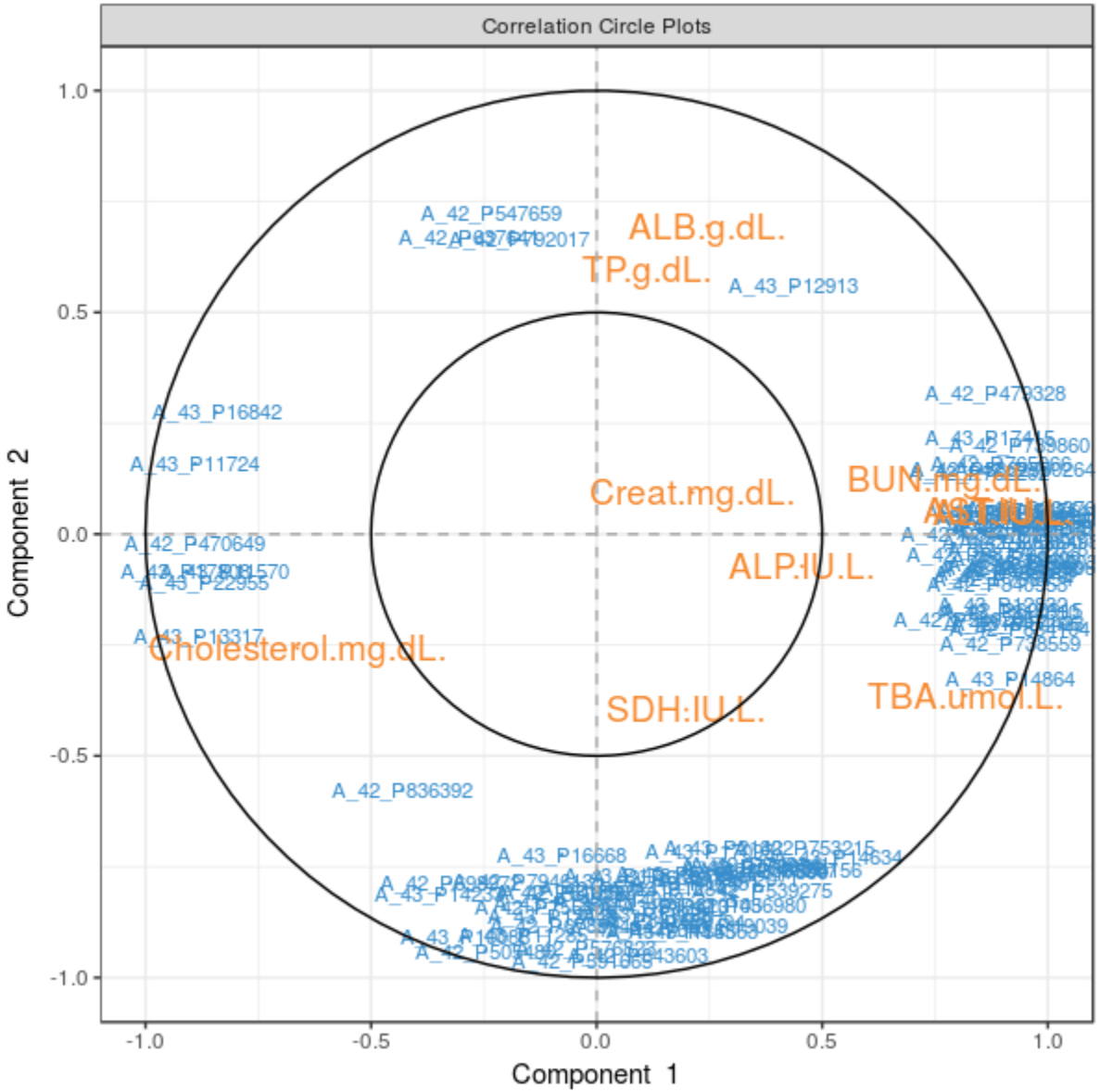
- HandsOn/IE6_PLS/IE6_Examples.R
- Prediction error tends to be lower for sPLS (full line) than for PLS (dashed line)
- This plot can be used to either choose the number of components or the number of features to retain



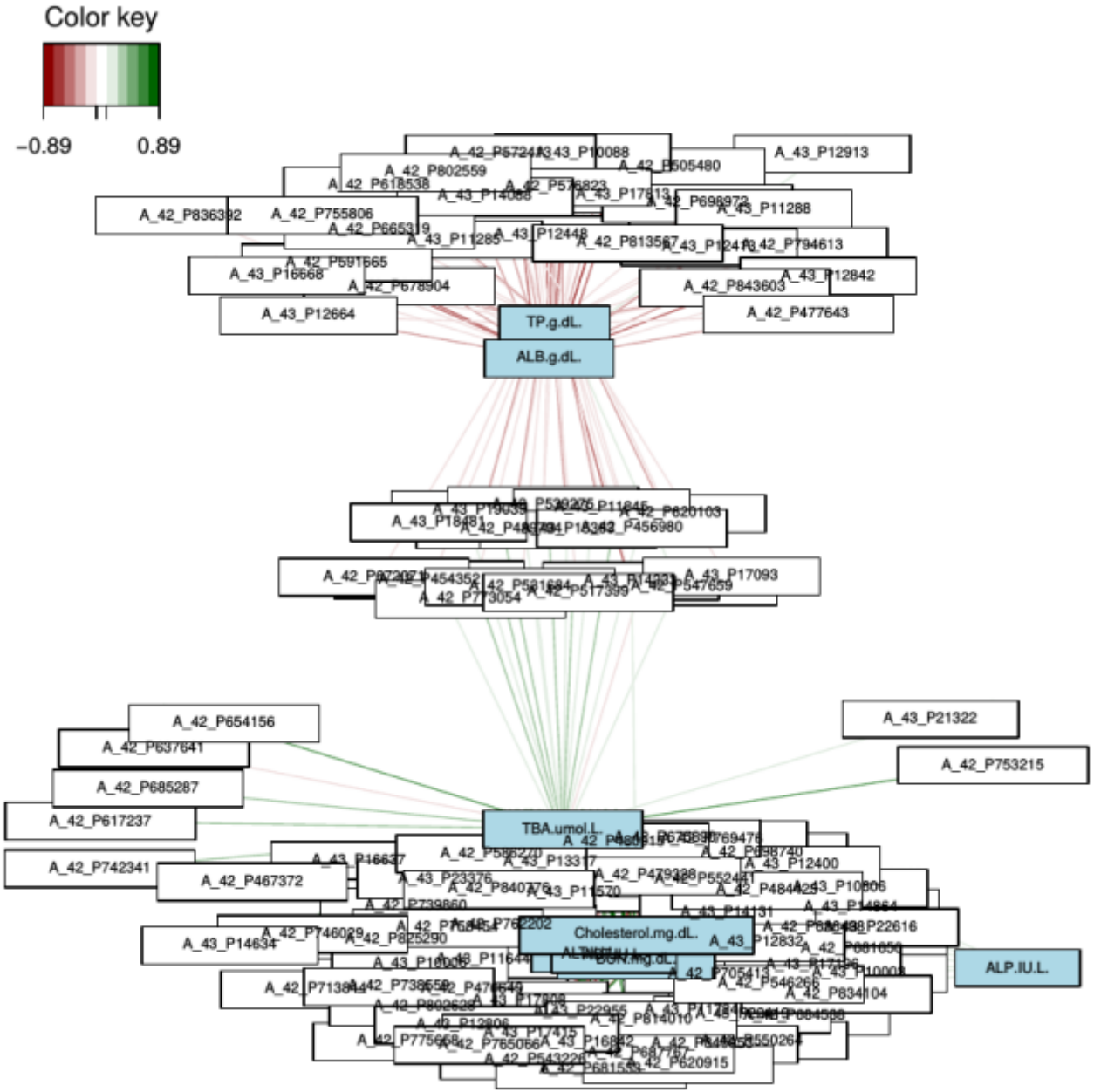
sPLS example: Samples plot



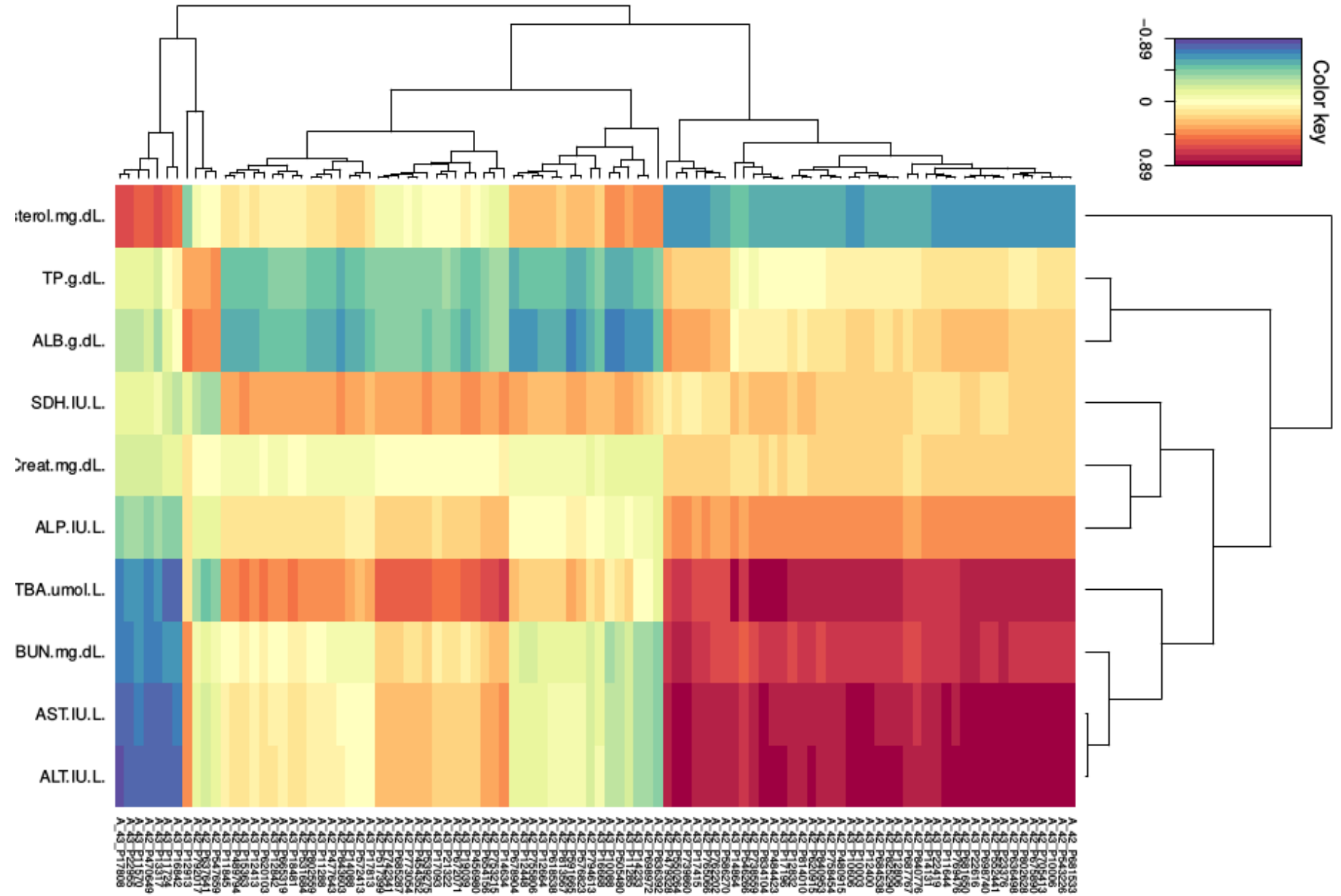
sPLS: Loadings plot



sPLS: Other graphical representations



sPLS: Other graphical representations



PLS

E. coli data

- Dataset for Escherichia coli K-12 strains (Ishii et al., 2007).
- mRNA, protein, and metabolite concentrations
- Knockout strains in which a single enzyme gene is knocked out in each strain + Wild type
- Datasets are normalized, and the effects of differences due to the sampling dates and arrays were eliminated. However, there are still missing values in some datasets.

HandsOn/IE_PLS/EcoliData

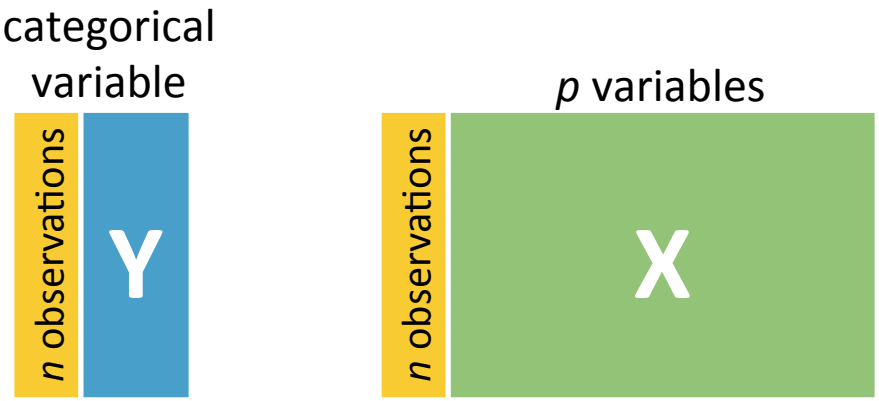
E. coli data

1. Missing value treatment
   - The following tasks can be performed with missing values (accepted by mixOmics functions)
   - However, make sure you do not have entire rows or columns with missing values
2. Explore **expression** and **protein** data, and decide the best scaling option
   - Boxplots, PCA, etc.
3. Generate an appropriate PLS model
   1. Number of components?
   2. Sparse or not?
4. Conclusions from PLS model

What if Y-variable is categorical?

PLS-DA

PLS Discriminant Analysis (PLS-DA)



- Goal: Predicting Y from X, where Y is a categorical variable

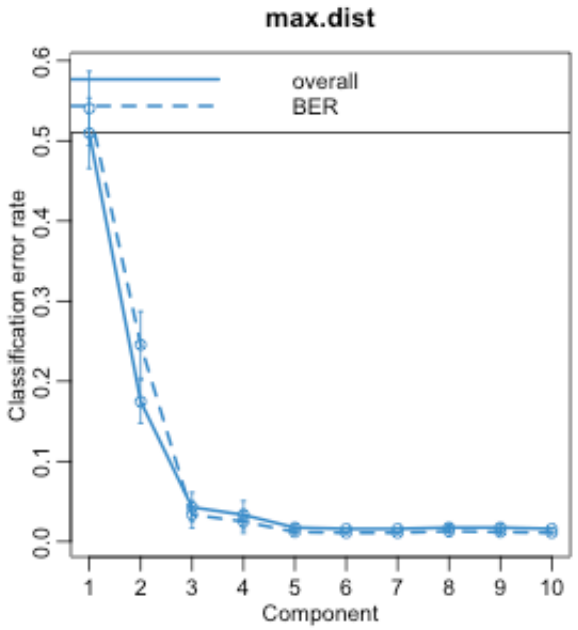
- Y is recoded as a dummy block matrix
- The number of components is usually set to k-1, where k is the number of classes in the categorical variable Y
- Sparse PLS-DA (Lê Cao, BMC Bioinformatics 2011) allows for variable selection and it is implemented in mixOmics R package

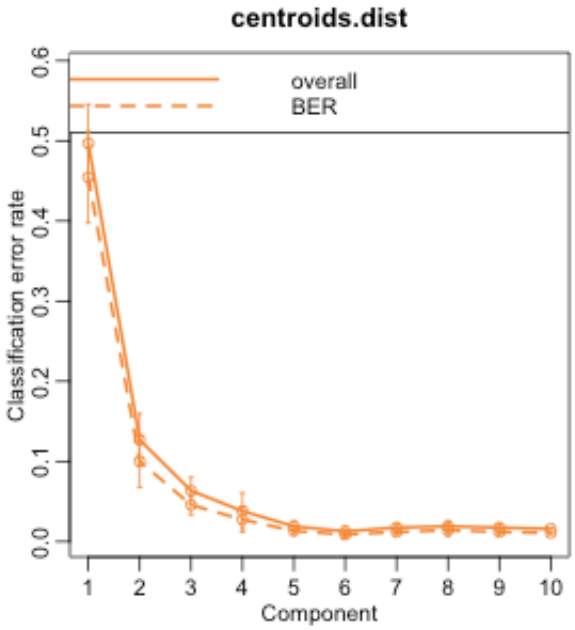
PLS-DA example: Small Round Blue Cell Tumors

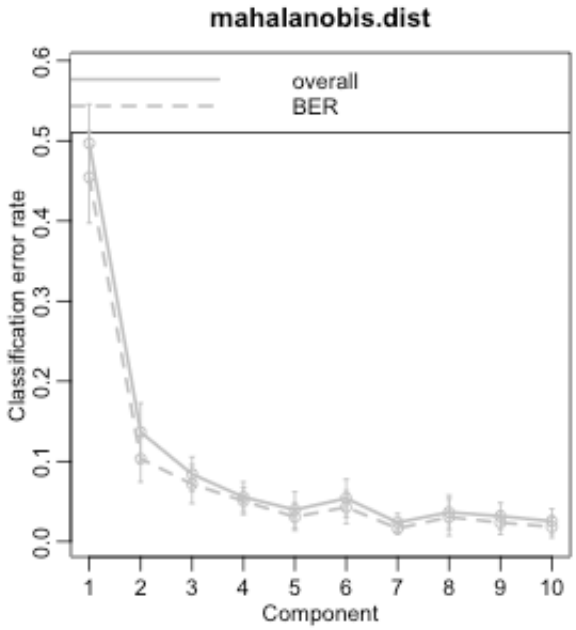
- HandsOn/IE6_PLS/IE6_Examples.R
- Study from Kahn et al., 2001
- Expression levels for 2,308 genes
- 63 samples distributed in 4 classes
  - 8 Burkitt Lymphoma (BL)
  - 23 Ewing Sarcoma (EWS)
  - 12 neuroblastoma (NB)
  - 20 rhabdomyosarcoma (RMS)

Choosing the number of components

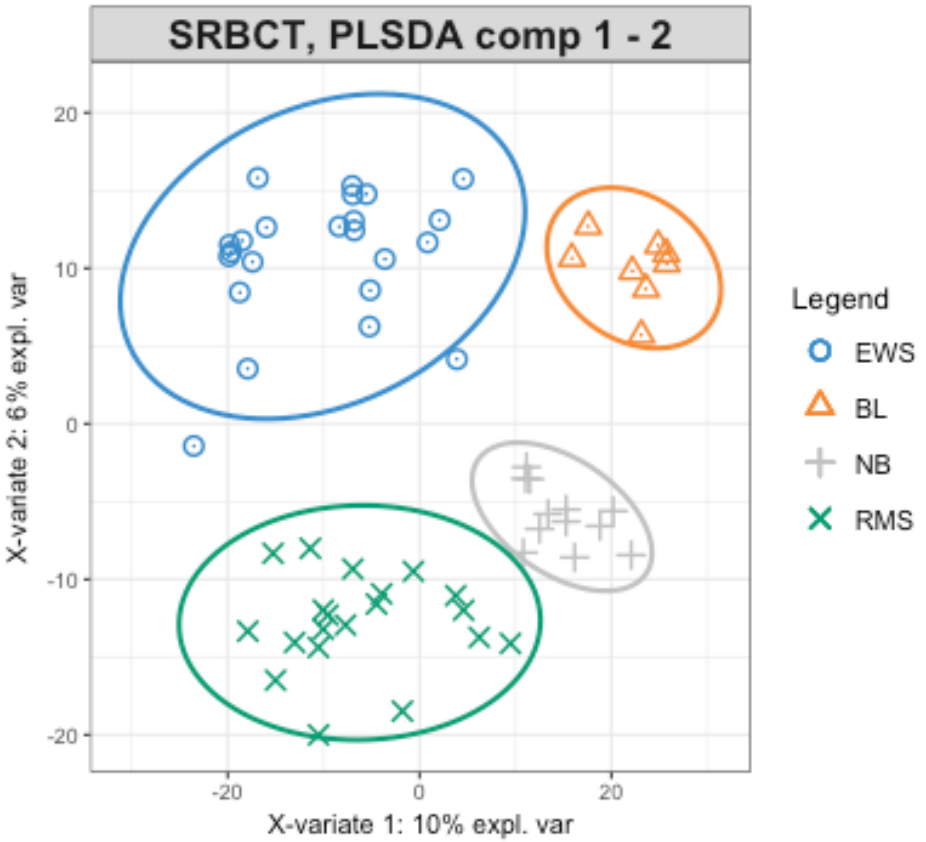
1. Compute the model with a high number of components, e.g. *ncomp = 10*.
2. Perform cross-validation.
3. Look at the error rate results to choose the number of components.
   - BER = Balanced Error Rate → Used when the number of samples per group is not balanced

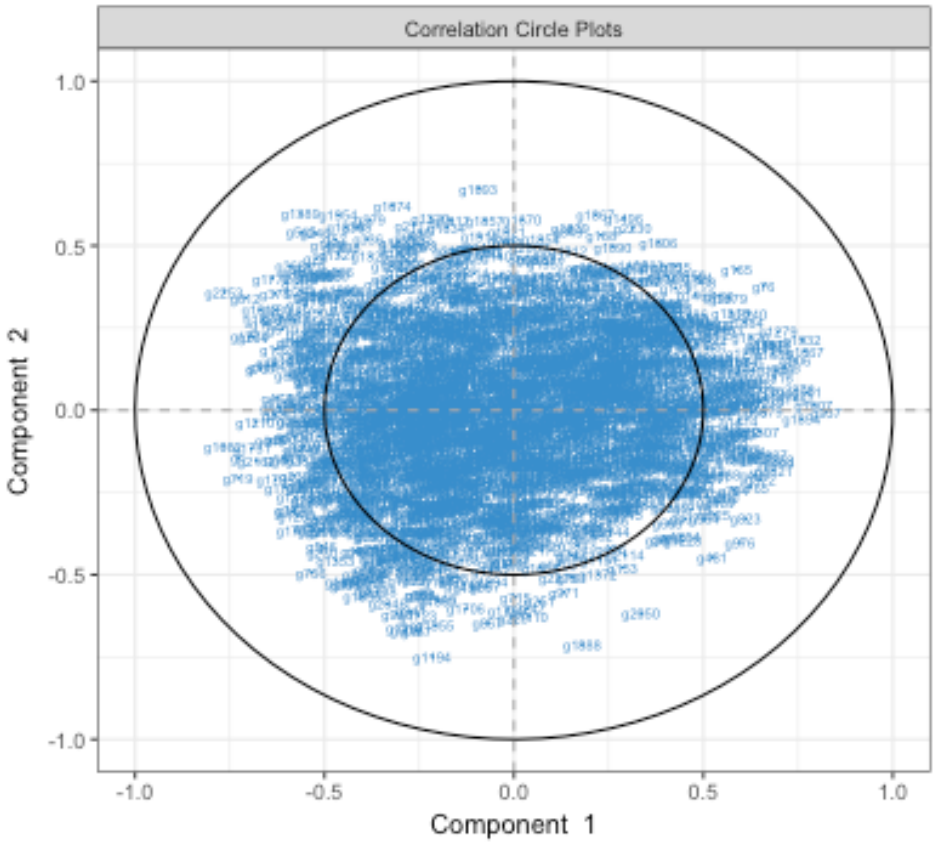




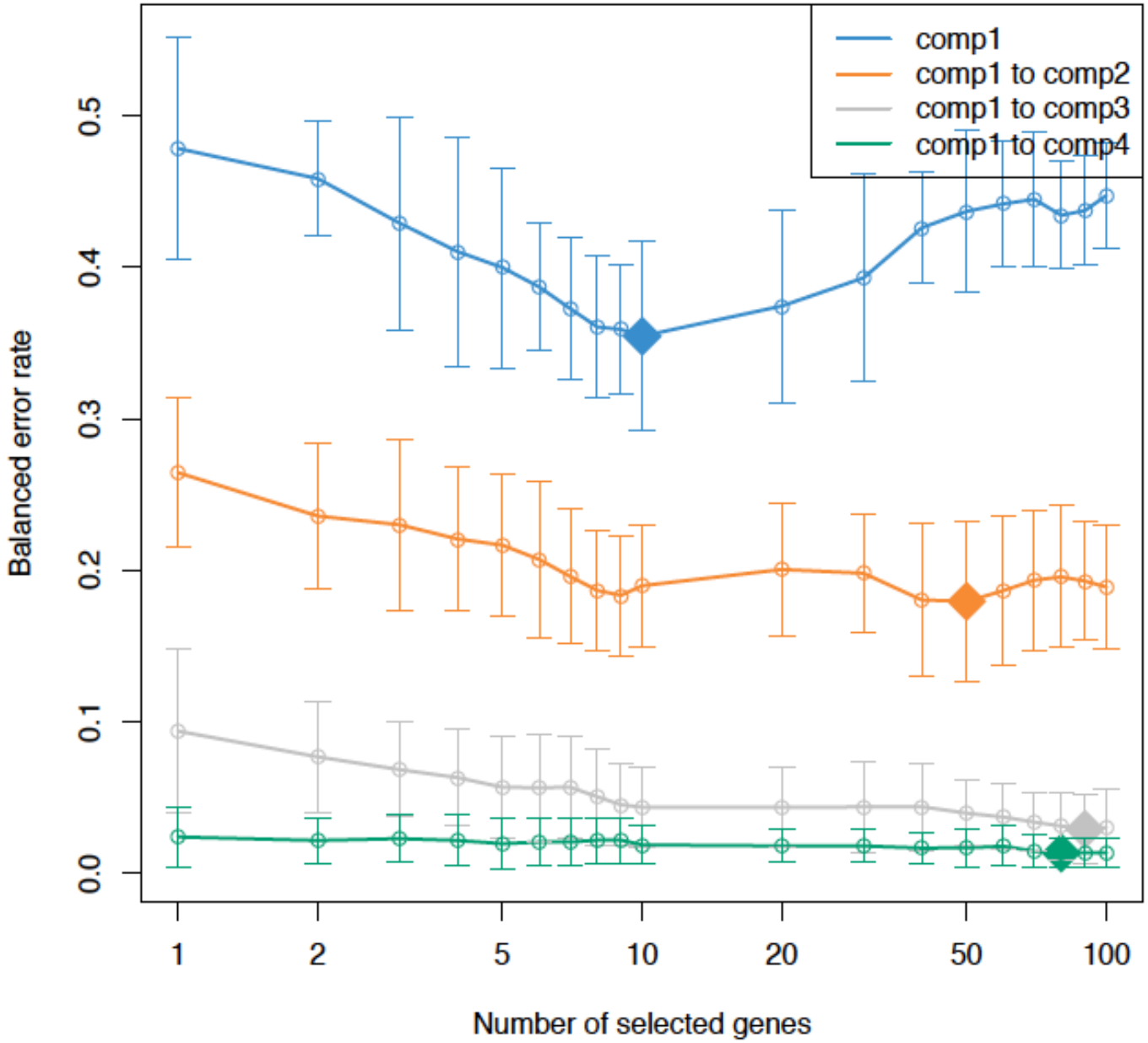


PLS-DA example: Model results

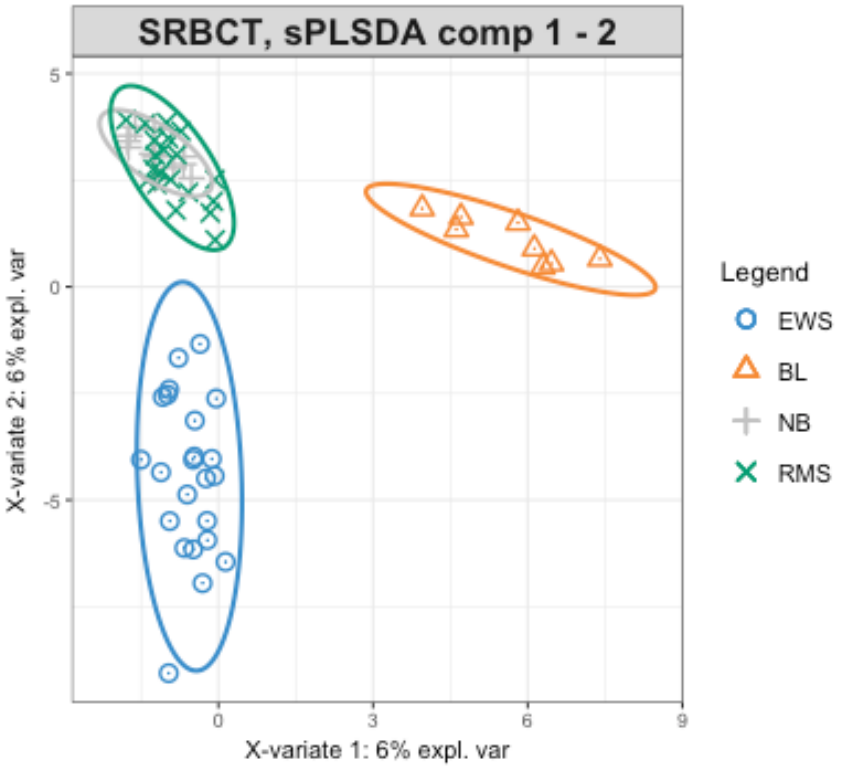


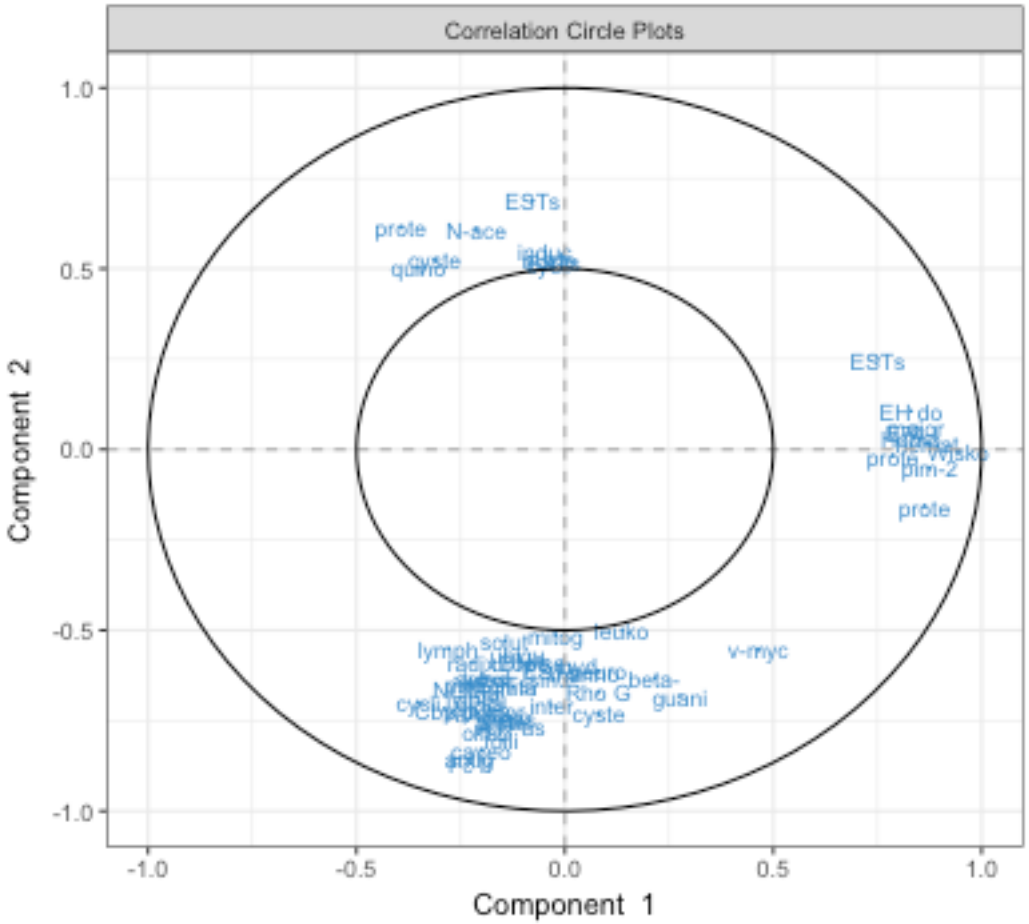


Sparse PLS-DA example: Tuning the model



Sparse PLS-DA example: Final model



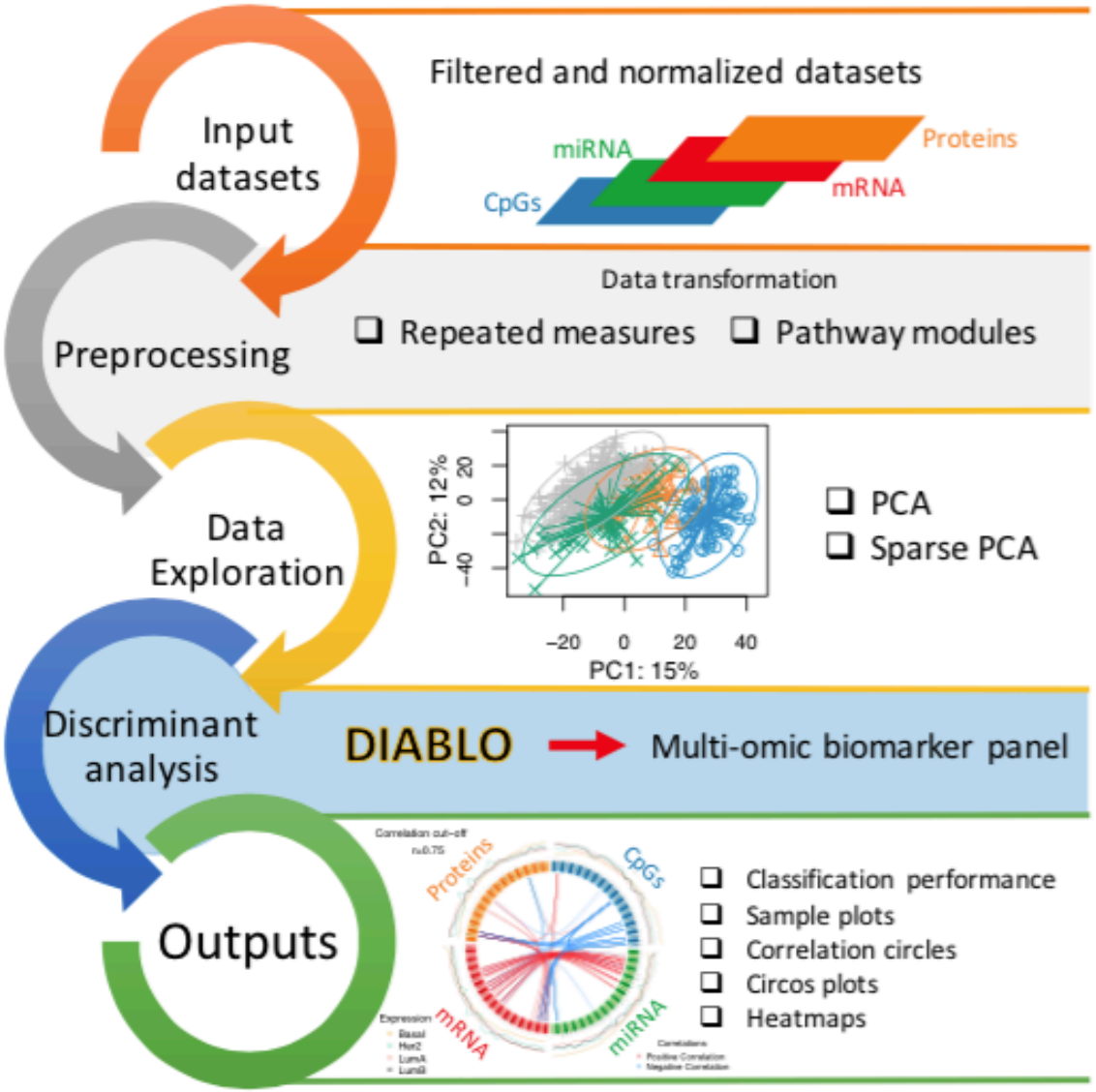


PLS-DA for multi-omics integration

Different omics measured on the same samples → Multi-omic signature to predict outcome

- Concatenate omics in X + PLS-DA
  - Different number of features for each omic → Unbalanced signature
- Model-based integration (e.g. ensemble classifiers)
- DIABLO (mixOmics)
  - http://www.biorxiv.org/content/biorxiv/early/2016/08/03/067611.full.pdf
  - Categorical Y + different omic data X_1, X_2, etc.
  - Still requires a minimum sample size in order to have train and test data
  - Recommended to pre-filter omic data: variability filter, features with significant changes, PCA, sparse PCA,...

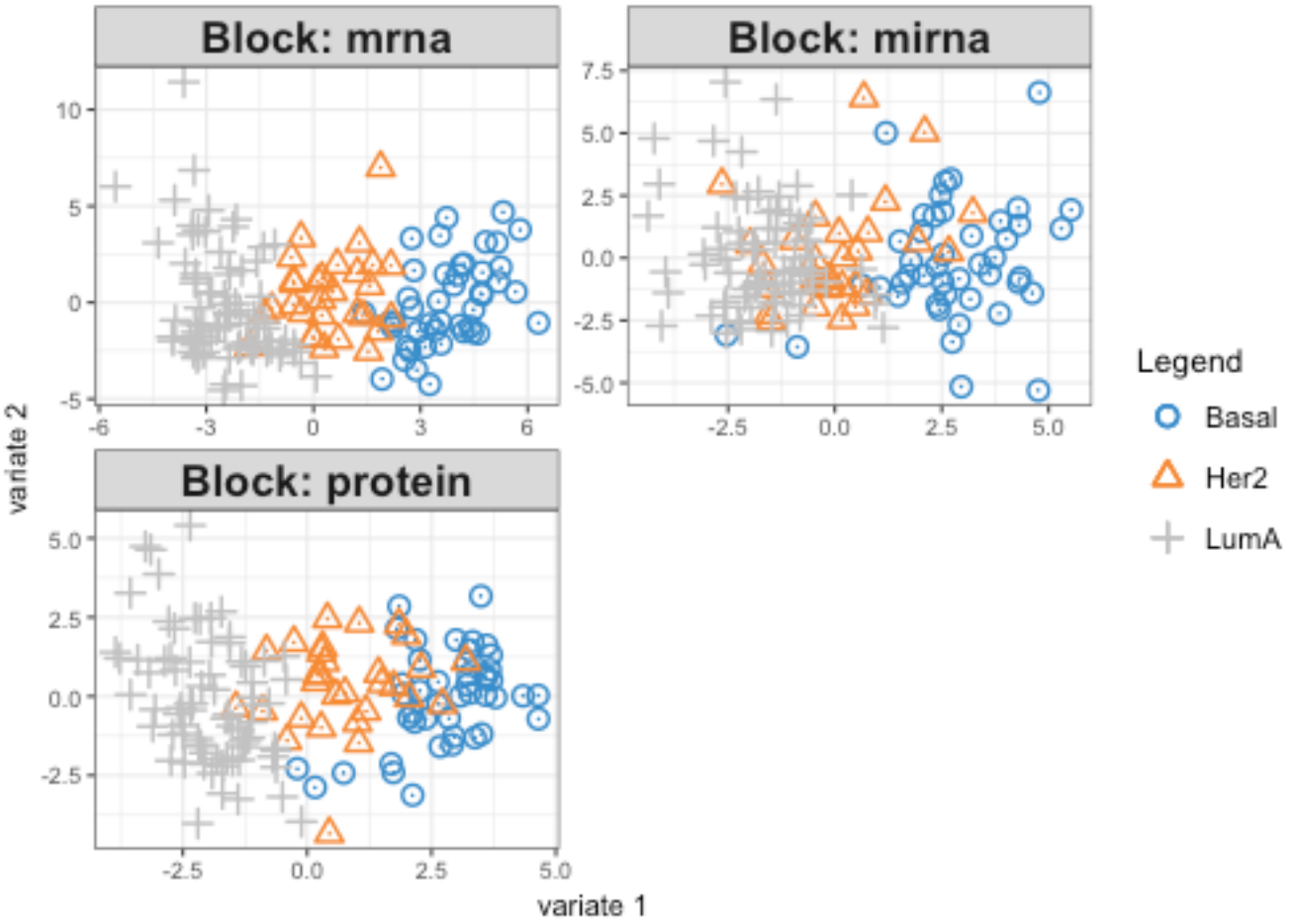
DIABLO (mixOmics R package)



DIABLO example: Breast TCGA

- HandsOn/IE6_PLS/IE6_Examples.R
- Data from The Cancer Genome Atlas (https://cancergenome.nih.gov/)
- Omic data types:
  - mRNAs
  - miRNAs
  - proteins
- 220 samples from 3 cancer subtypes
  - Basal
  - Her2
  - LumA

DIABLO example: Results



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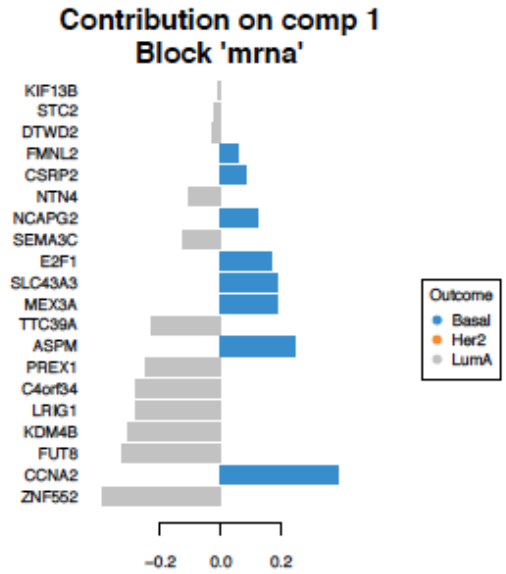
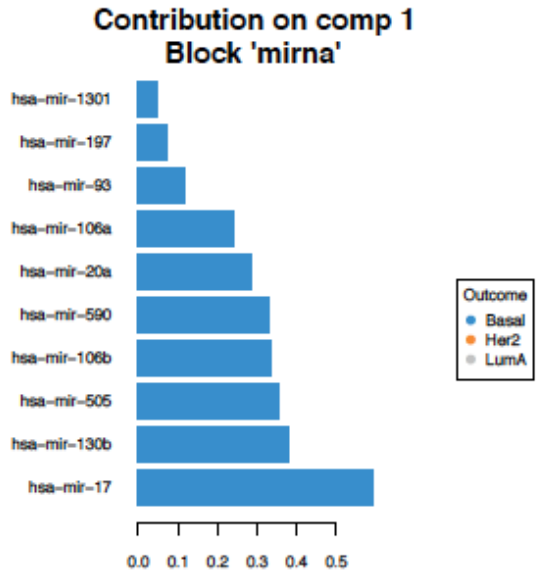
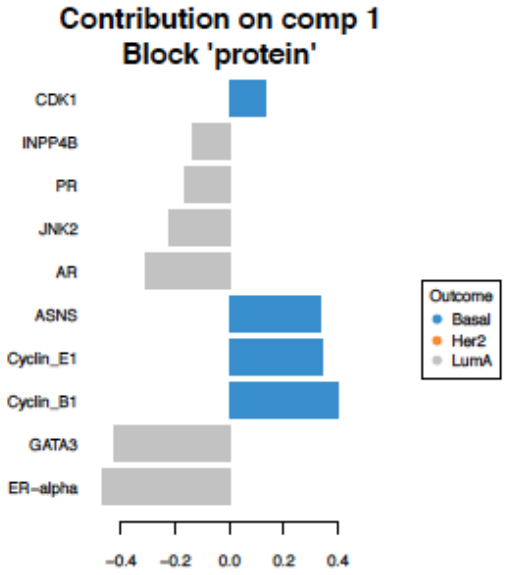
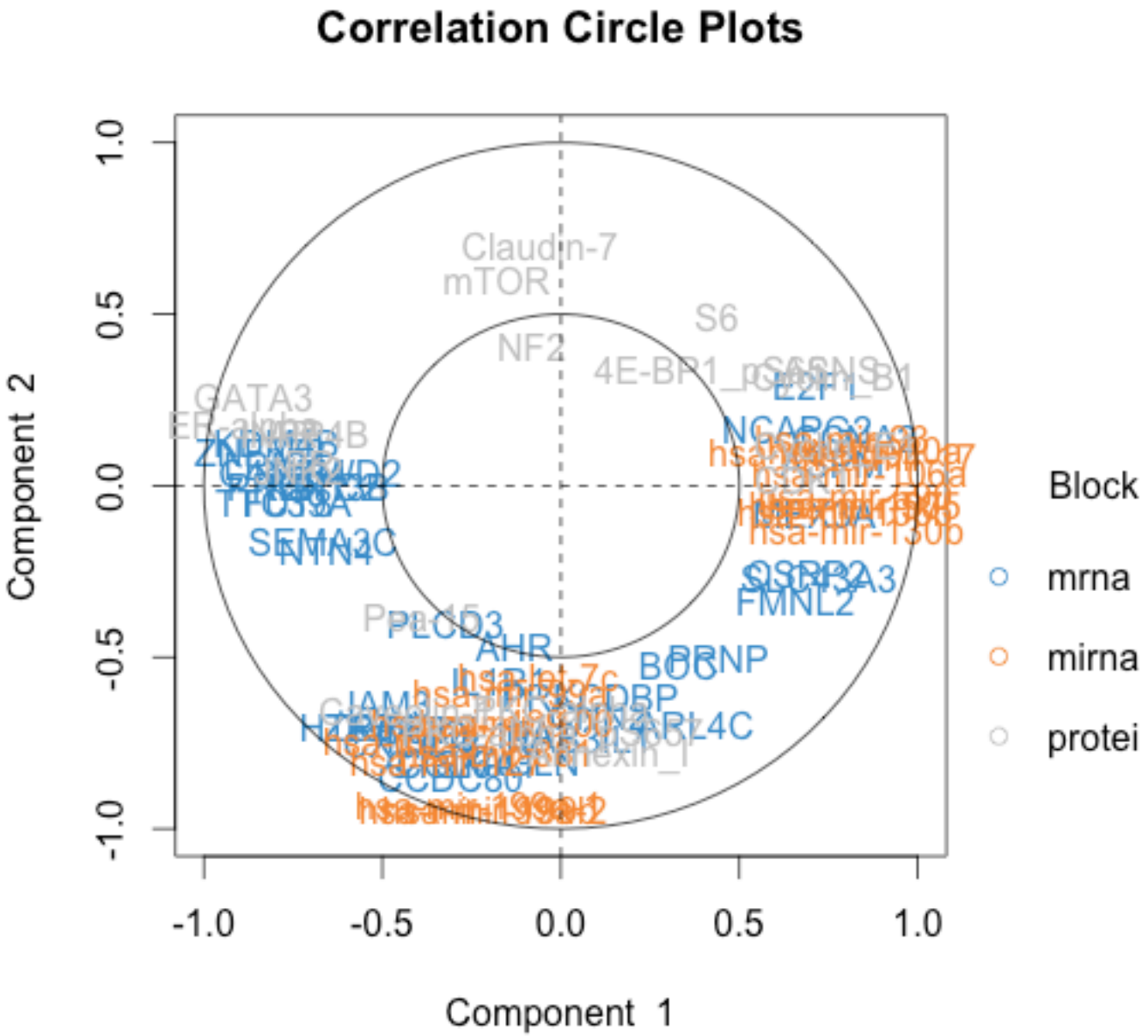






DIABLO example: Results



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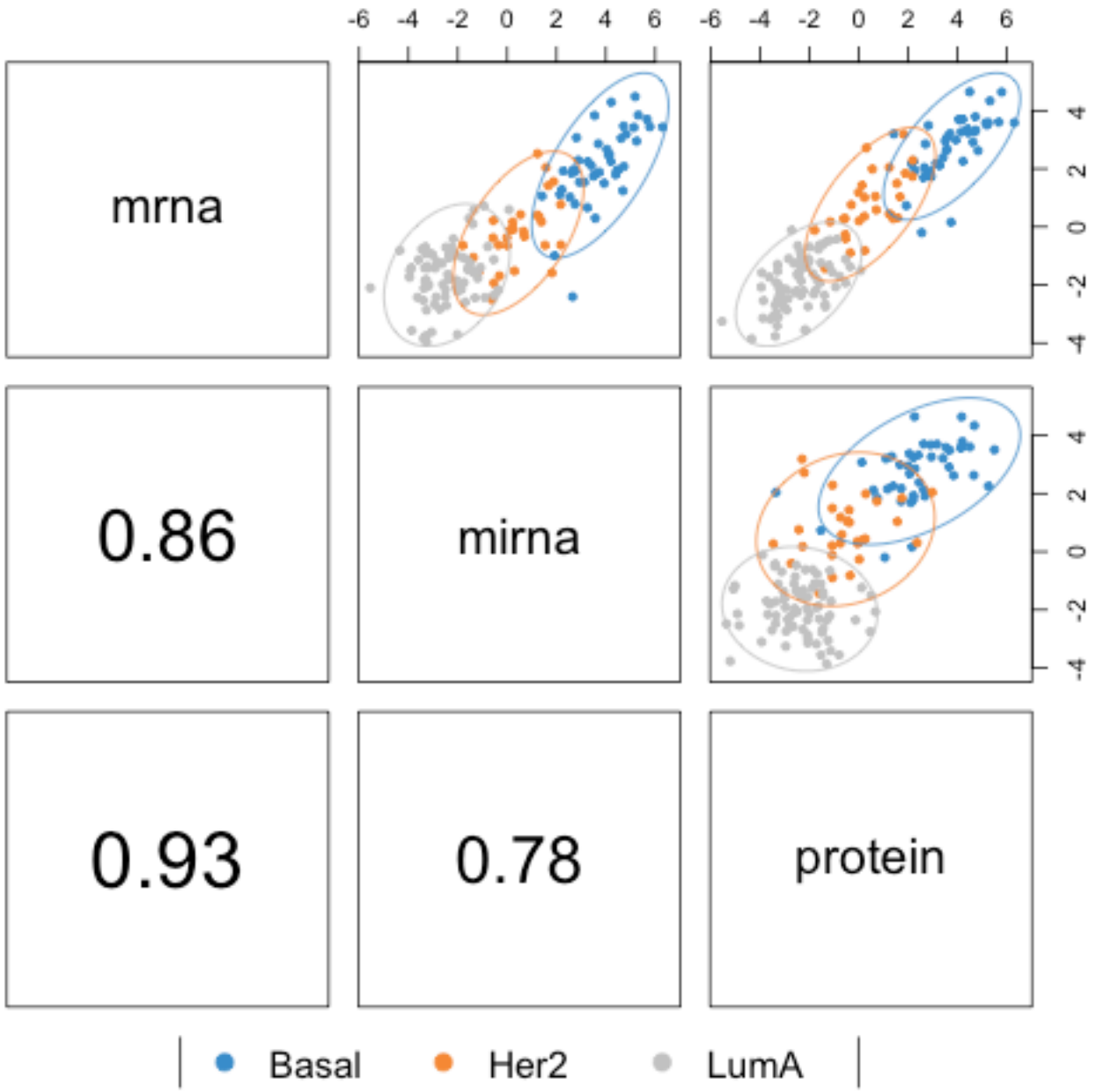

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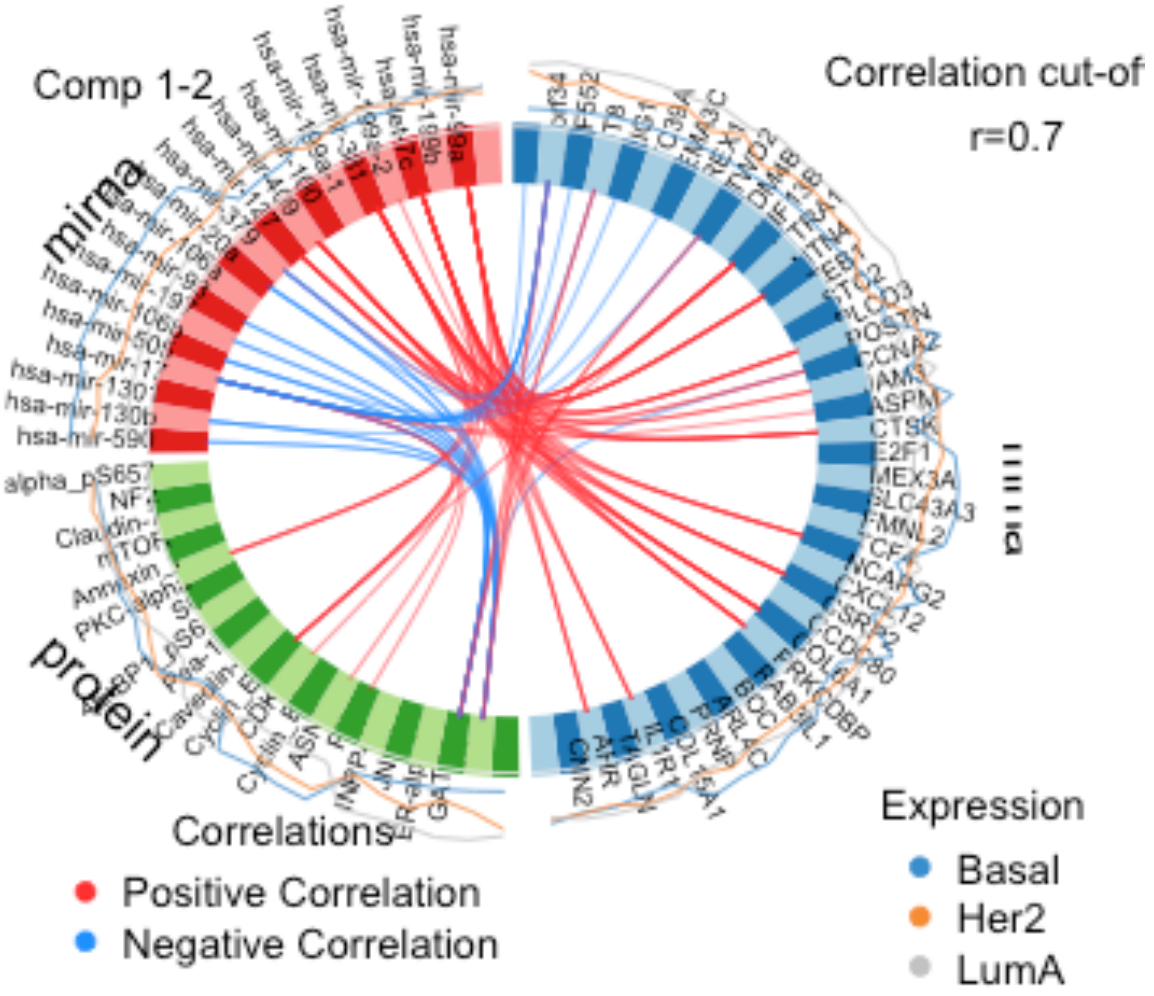
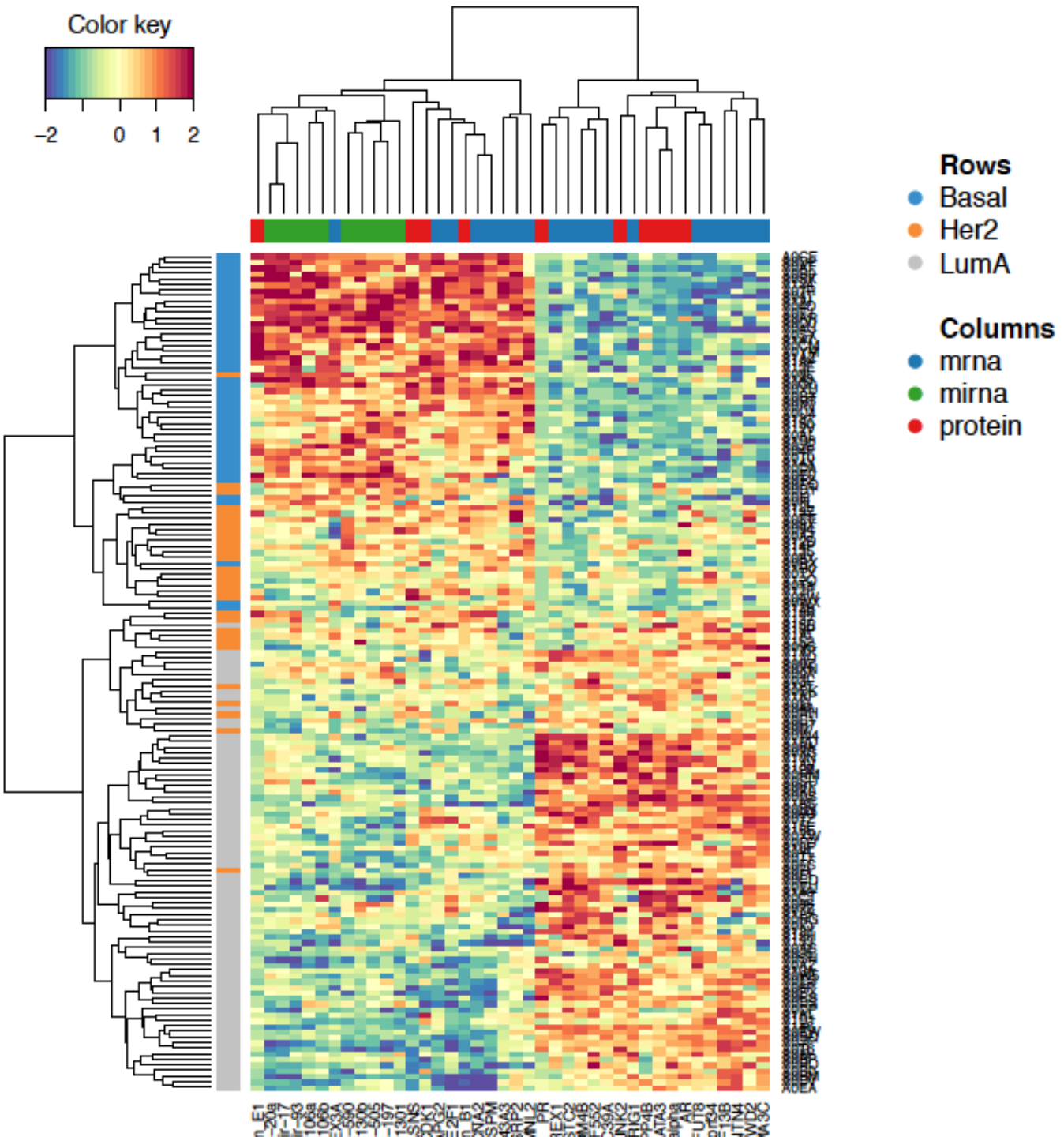


DIABLO example:

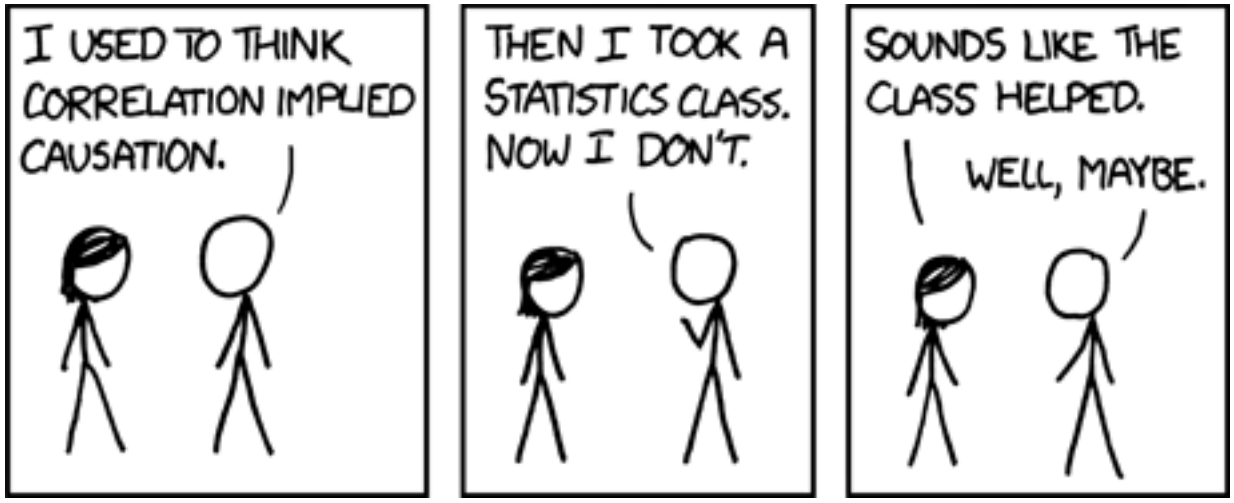


Glioblastoma data from TCGA

- Data from STAegRa R package: *data("STATegRa_S3")*
- Gene expression, miRNA expression
- Processed data from TCGA (Van Deun et al., 2012)
  - Glioblastoma multiforme (GBM) → Tumor subtypes:
    - Classical
    - Mesenchymal
    - Neural
    - Proneural
  - 600 genes with significant changes
  - 300 miRNAs with significant changes.

Glioblastoma data from TCGA

1. Select the train data and apply DIABLO to obtain a multiomic signature to predict the different cancer subtypes.
   - Which is the optimal number of components?
   - Which is the optimal number of variables to keep from each data set?
2. Generate the appropriate plots for the final model and interpret the results.
3. Apply the obtained signature to the test data and discuss how well the fitted model predicts the cancer subtype.


I USED TO THINK CORRELATION IMPLIED CAUSATION.
THEN I TOOK A STATISTICS CLASS. NOW I DON'T.
SOUNDS LIKE THE CLASS HELPED.
WELL, MAYBE.