BIOPUCES SEMINAR 12/06/2025

ACCOUNTING FOR CONFOUNDING VARIATION IN MULTI-OMICS DATA INTEGRATION

APPLICATION TO THE STUDY OF LOW-DOSE RADIATION EFFECTS IN CHORNOBYL TREE FROGS

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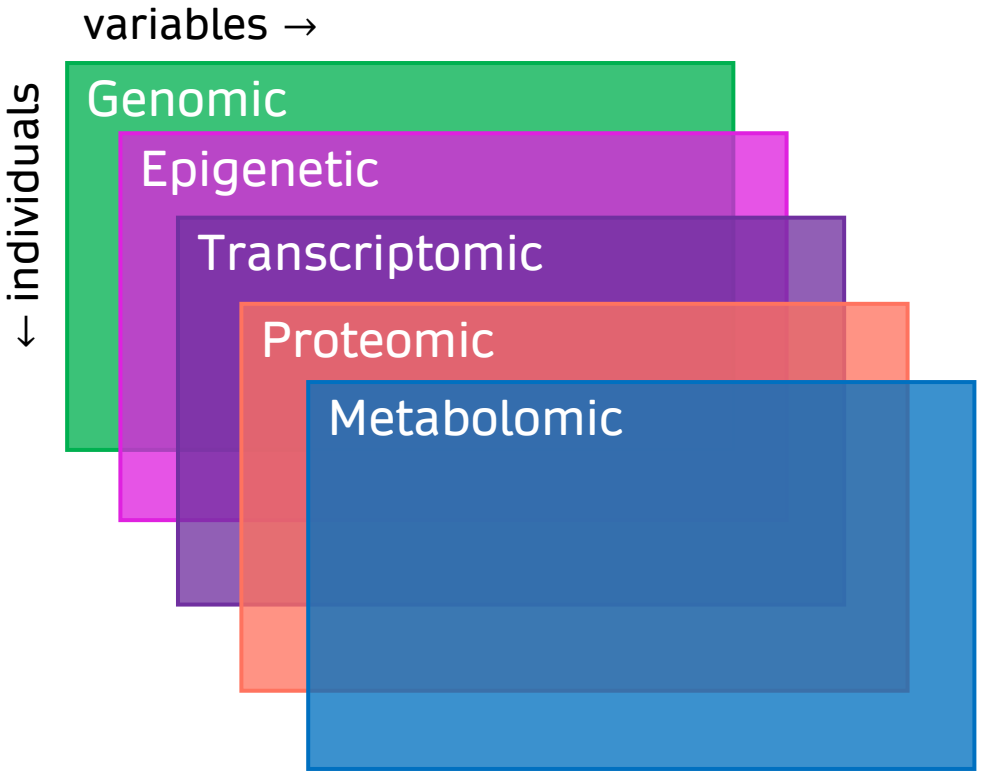
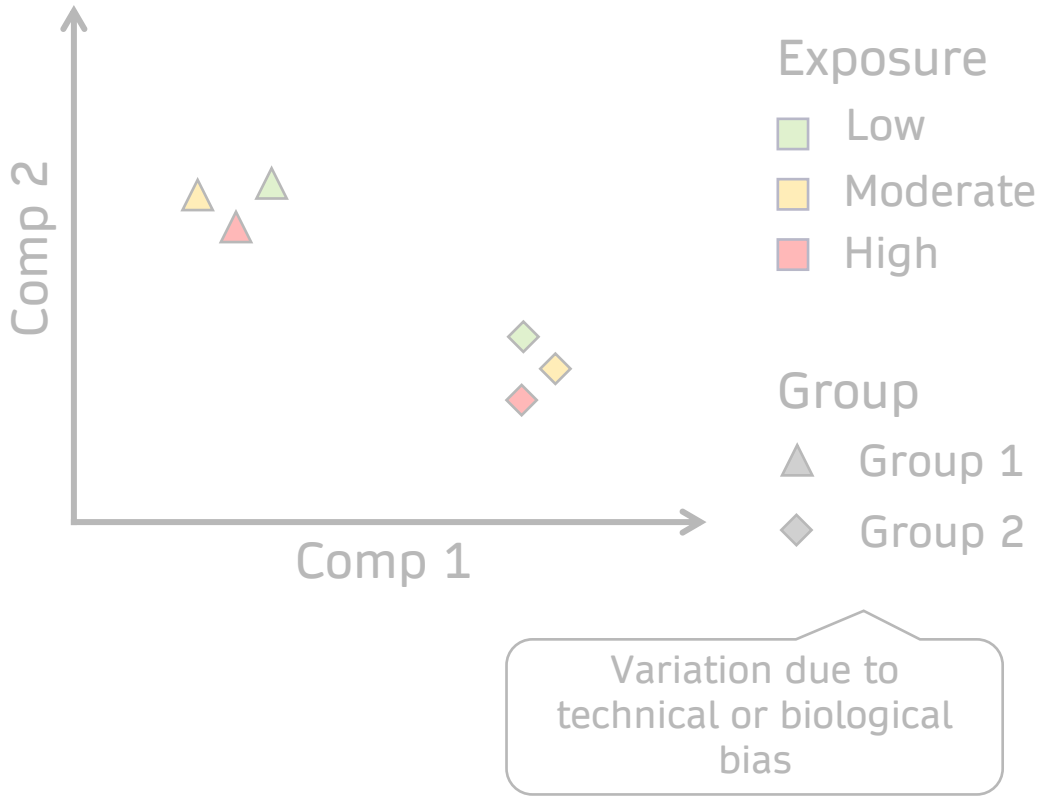
2. CentraleSupélec, CNRS, Université Paris-Saclay, Laboratoire des Signaux et Systèmes (L2S)
3. ASNR-IRSN, Laboratoire de recherche sur les effets des radionucléides sur les écosystèmes (LECO)

METHODOLOGICAL CHALLENGES

How to integrate several omics datasets in a joint analysis?



How to manage the effect of undesirable or confounding variables?



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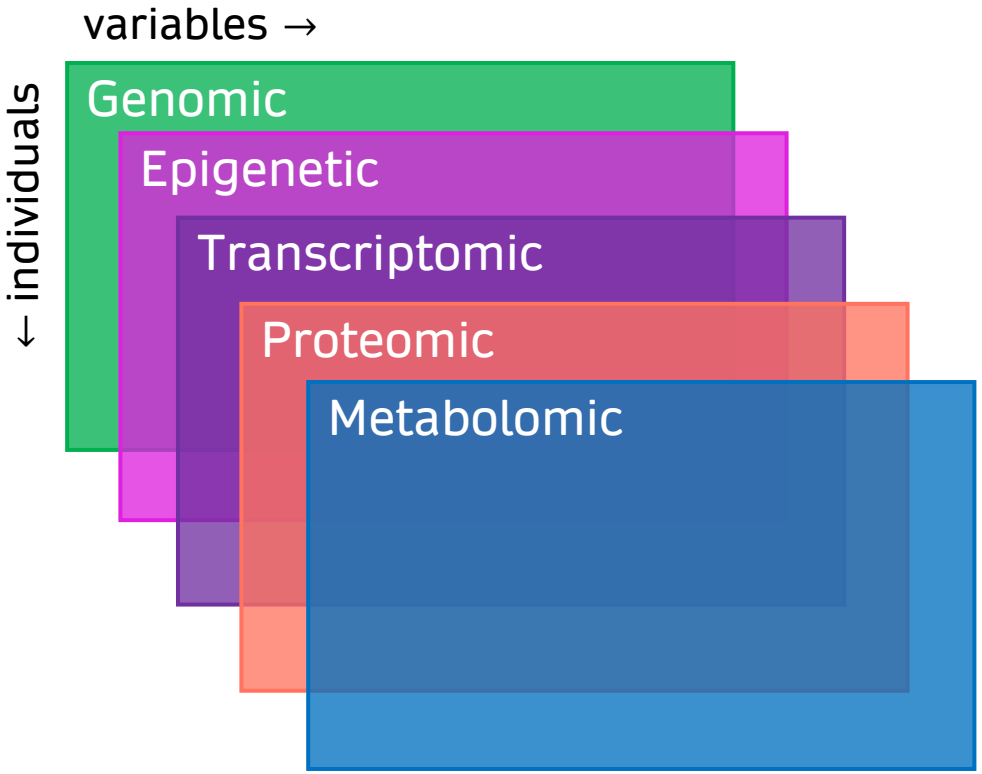

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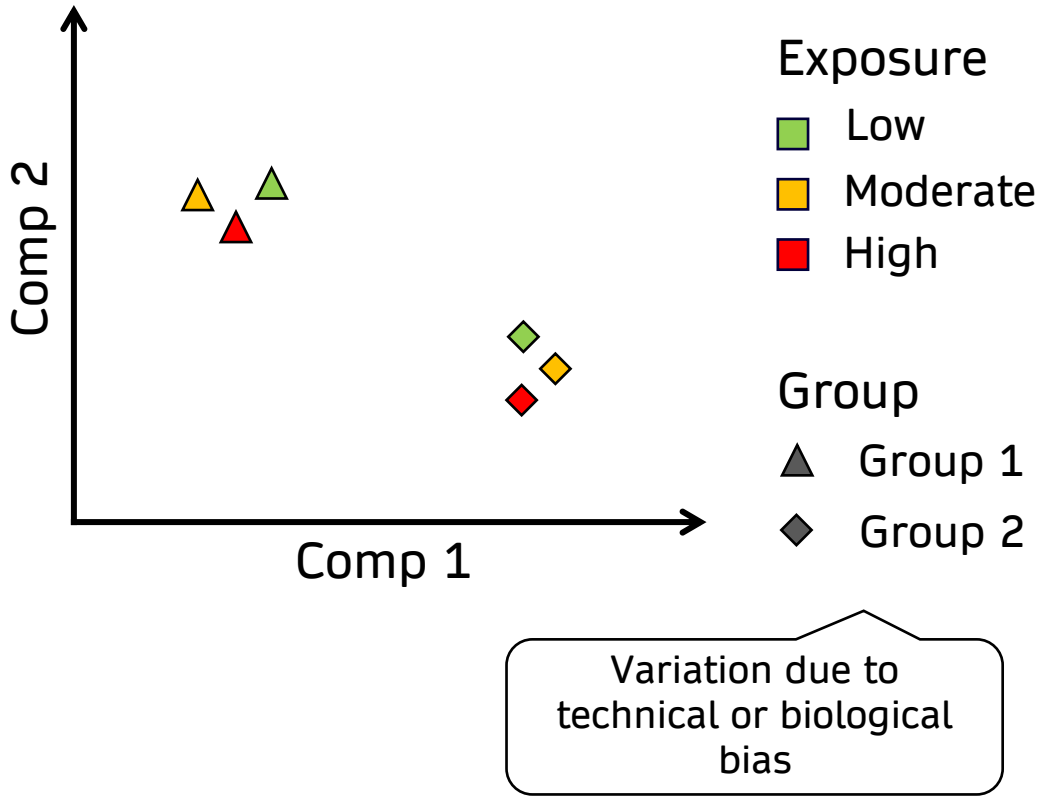


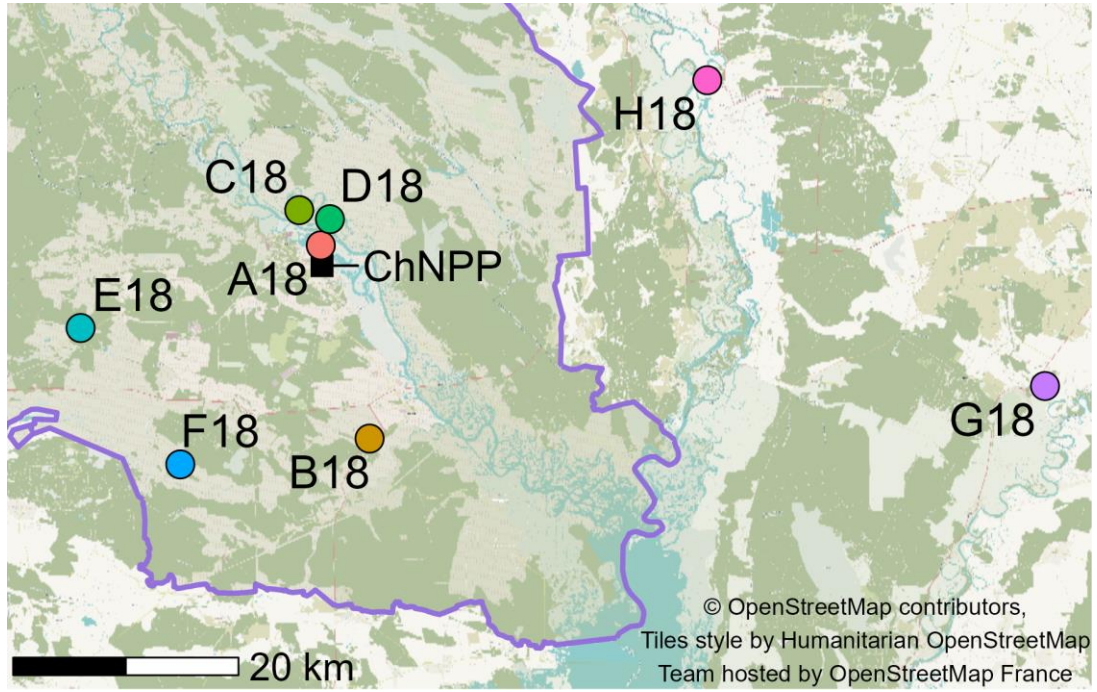
CONTEXT OF THE RADIOPROTECTION STUDY
CHORNOBYL TREE FROGS

Can we identify molecular signatures of chronic exposure to low doses?

2018: sampling of *Hyla orientalis* tree frog populations [Burraco 2021, Car 2022, Car 2023]

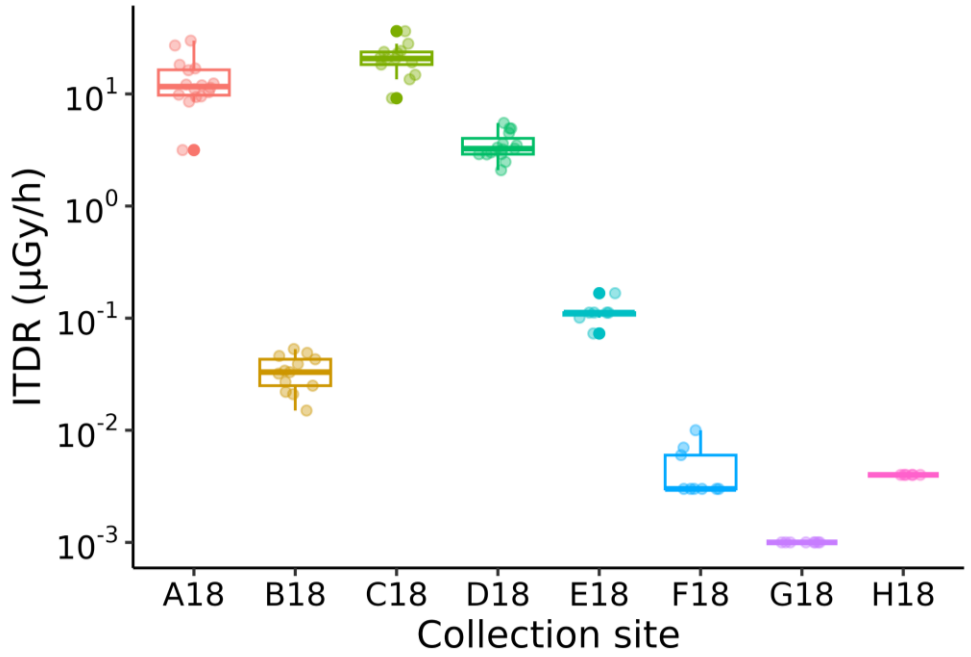
Types of data collected:

- Dosimetry (ITDR = Individual Total Dose Rate, including internal and external contributions from ^{137}Cs and ^{90}Sr)
- Age, phenotype (mass, dimensions)
- Genomics
- Transcriptomics
- Proteomics

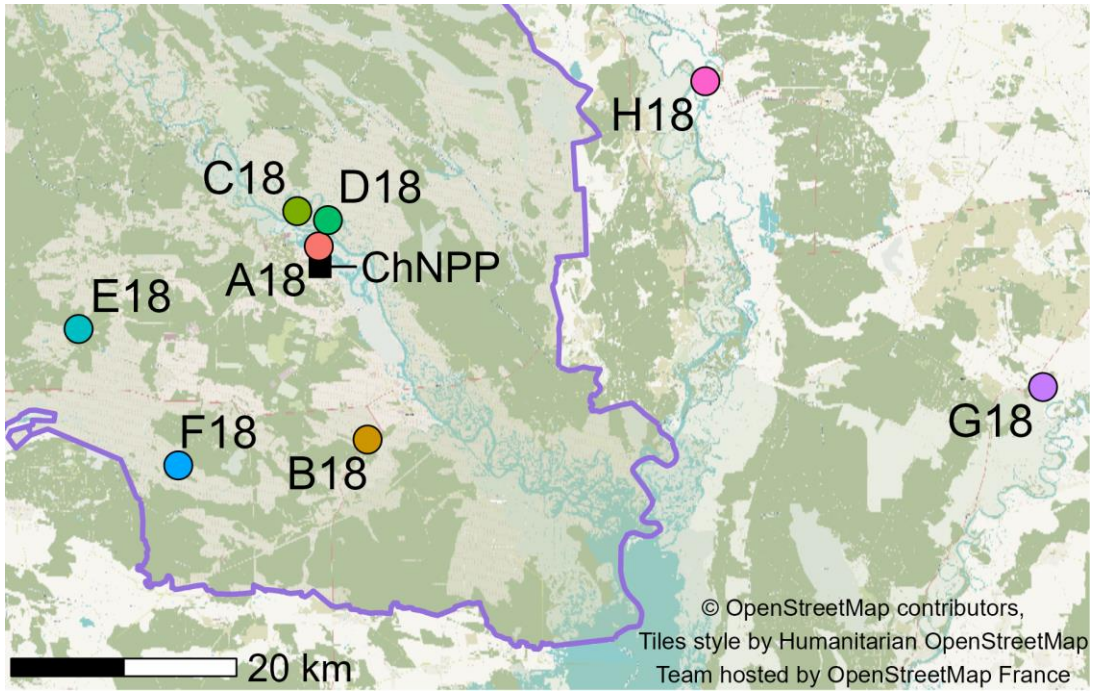


Collection sites inside and outside the Chornobyl Exclusion Zone (CEZ: —, ChNPP = Chornobyl nuclear power plant)

CONTEXT
FROGS' COLLECTION SITES AND RADIATION DOSE RATE

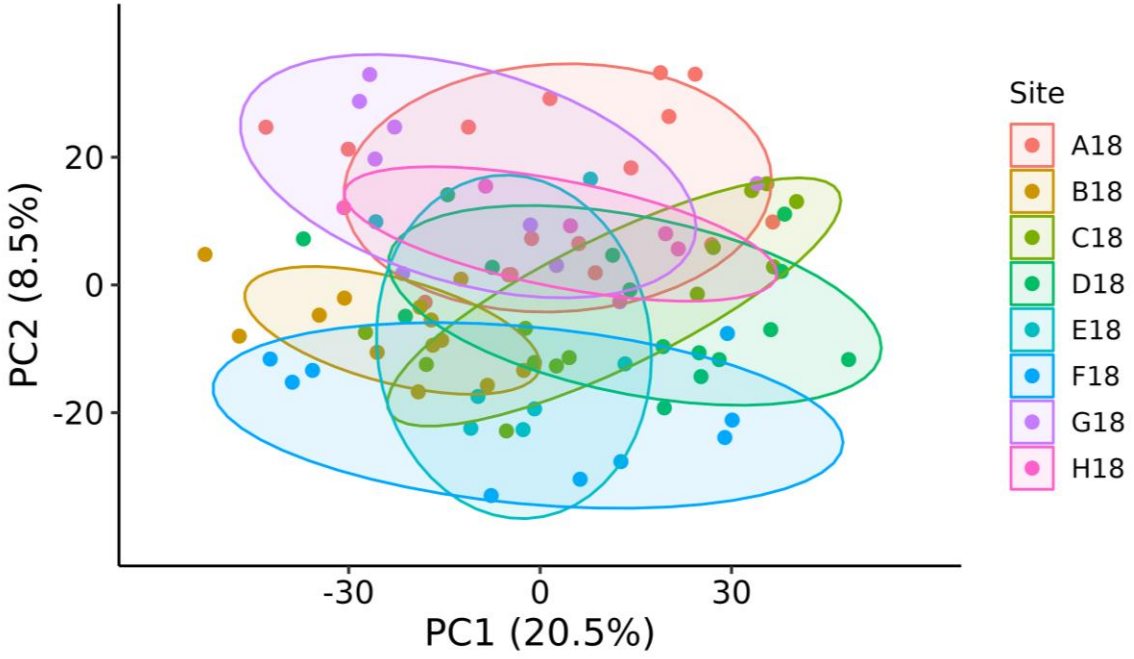


Individual total dose rate (ITDR) distribution among sites

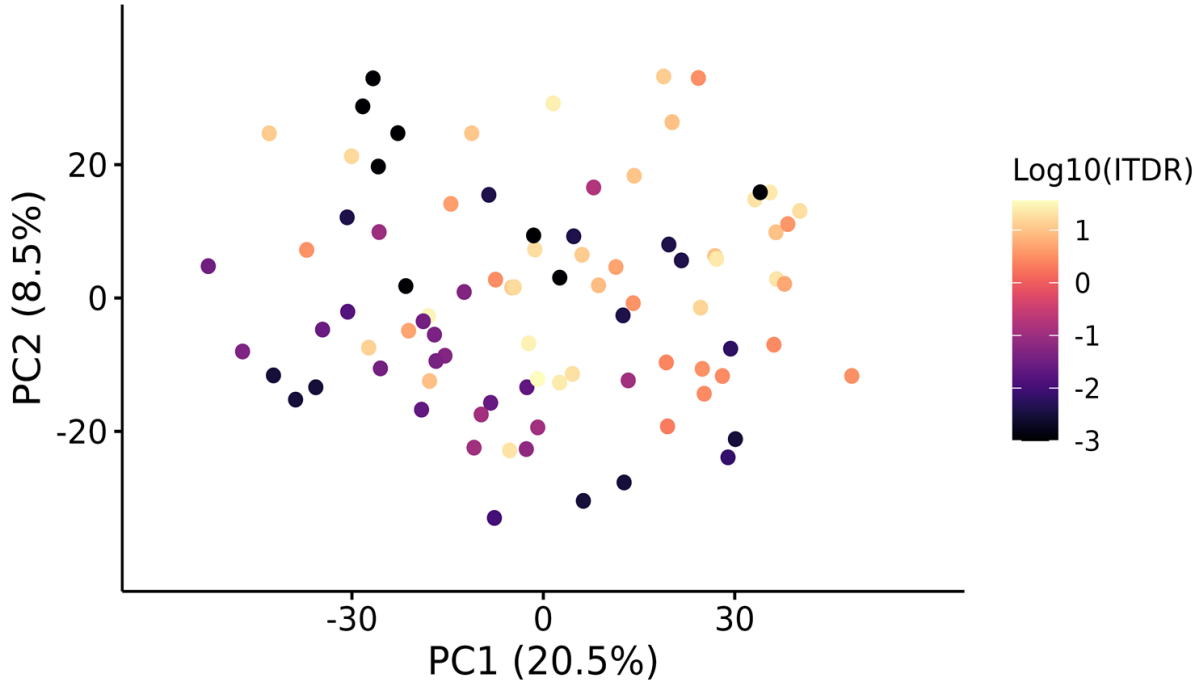


Collection sites inside and outside the Chornobyl Exclusion Zone (CEZ — , ChNPP = Chornobyl nuclear power plant)

CONTEXT
CONFOUNDING EFFECT OF THE SITE ON RNA-SEQ DATA



Individuals in the first factorial plane, colored by site



Individuals in the first factorial plane, colored by dose rate

OBJECTIVES

Advance our understanding of low-dose radiation effects

→ Design an integrative approach for the joint analysis of multi-omics data

→ Handle the confounding effect of the collection site

OUTLINE

HANDLING THE SITE EFFECT IN THE FROGS RNA-SEQ DATA

TOWARDS ACCOUNTING FOR COVARIATES IN MULTI-OMICS ANALYSIS

OUTLINE

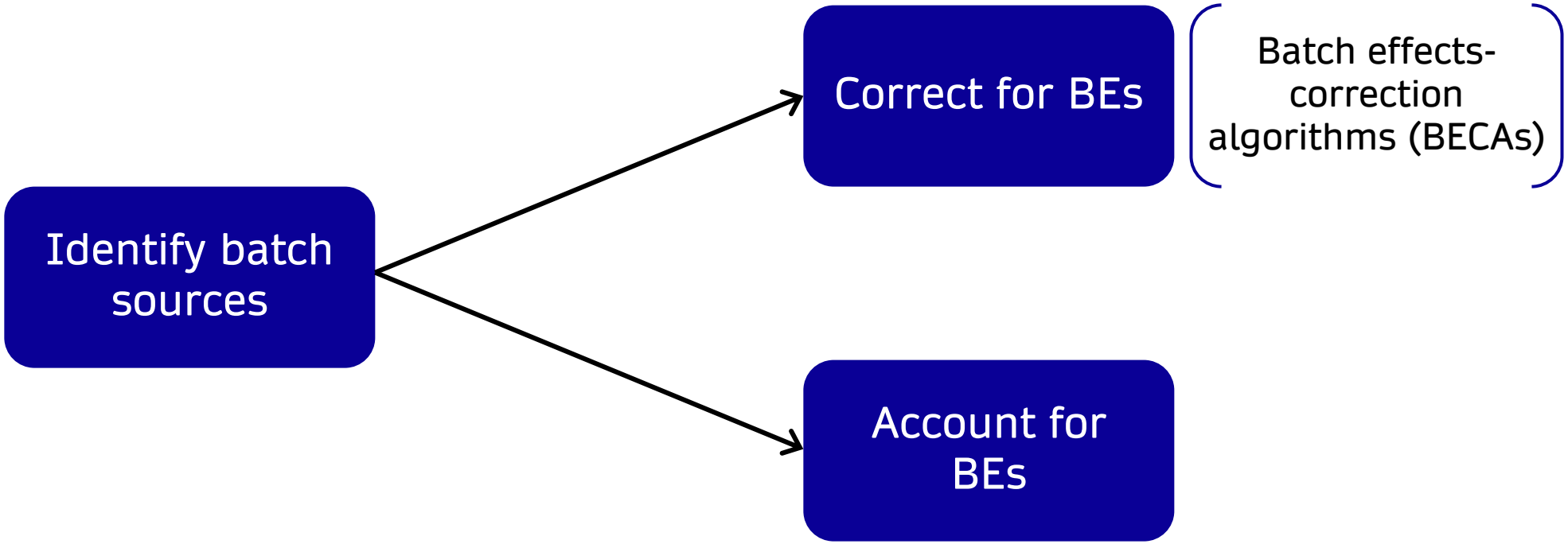
HANDLING THE SITE EFFECT IN THE FROGS RNA-SEQ DATA

TOWARDS ACCOUNTING FOR COVARIATES IN MULTI-OMICS ANALYSIS

HANDLING BATCH EFFECTS (BEs)

Batch effects = biases arising from technical variation [Leek 2010, Goh 2022]

⟶ Can interfere with the analysis of omics data



BATCH EFFECT-CORRECTION ALGORITHMS

Residualization

[Sims 2008, García 2020]
R package `stats` or `limma`

Removes linear relationship between variables and batch

For gene g:

gene expression

batch

biological condition

$$X_g = \hat{\beta}_g^\top z + \hat{\alpha}_g\, y + \varepsilon_g$$

residuals

$$X_g^* = \hat{\alpha}_g y + \varepsilon_g$$

batch-corrected gene expression

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batch-corrected gene expression

ComBat_seq

[Zhang 2020]
R package `sva`

Corrects data distribution to fit a batch-free distribution

For gene g, sample j, batch i:

$$X_{gij} \sim NB(\mu_{gij}, \phi_{gi})$$

$$\log \mu_{gij} = \alpha_g + \beta_g y_i + \gamma_{gi} + \log N_j$$

average level of expression

biological condition

batch term

library size

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gene expression · batch · biological condition · residuals

$$X_g^* = \hat{\alpha}_g y + \varepsilon_g$$

batch-corrected gene expression

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average level of expression · biological condition · batch term · library size

Surrogate Variable Analysis (SVA)
[Leek 2007]
R package `sva`

Identifies unobserved sources of expression variation

For gene g:

$$X_g = \hat{\alpha}_g y + \varepsilon_g$$

biological condition · residuals

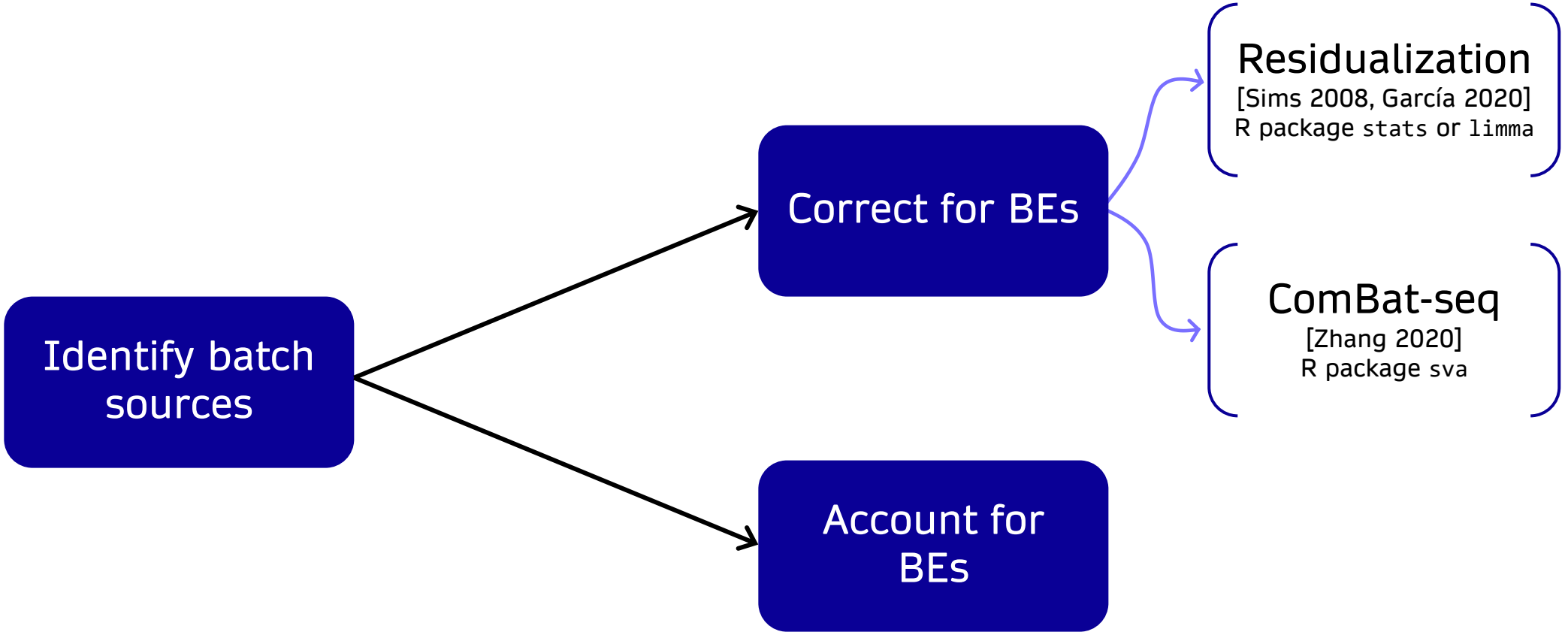
$$X_g^* = \varepsilon_g$$

singular value decomposition

$$X^* = U\Sigma V^\top$$

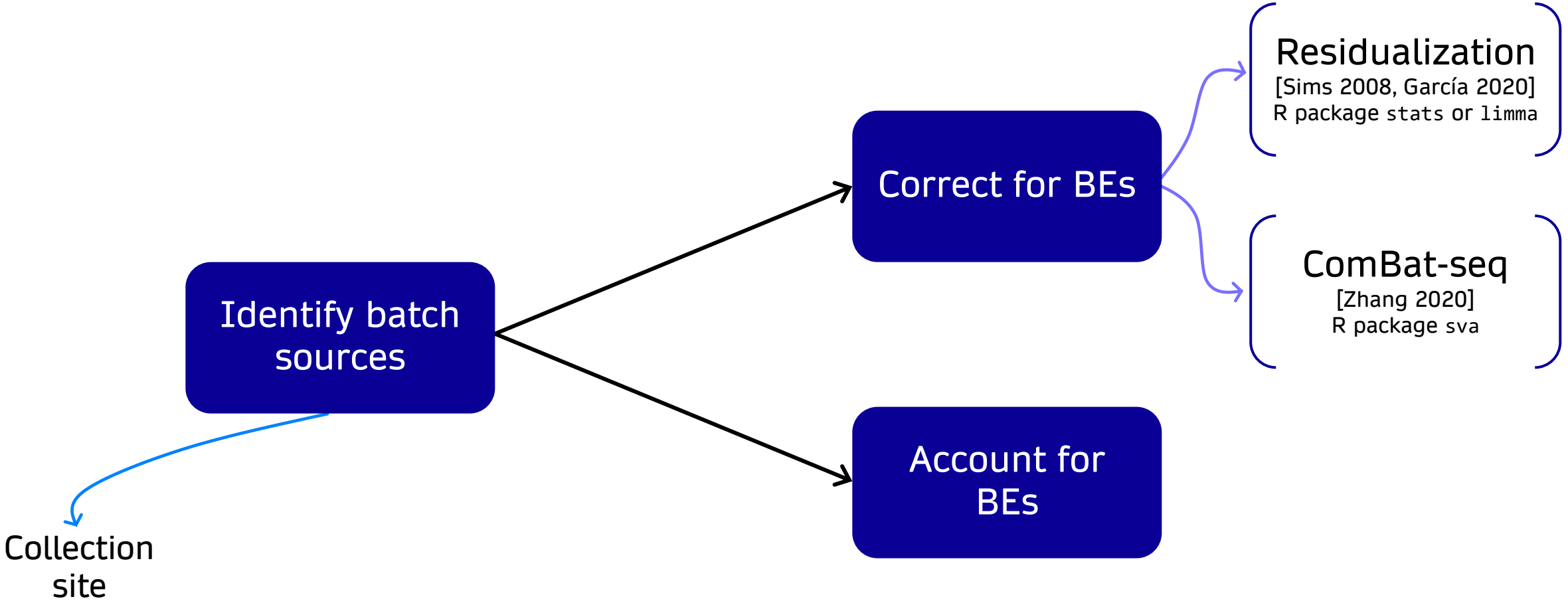
extract surrogate variables

CORRECTION STRATEGIES
RNA-SEQ DATA



Further information in [Goujon 2024, Car 2023]

CORRECTION STRATEGIES
RNA-SEQ DATA



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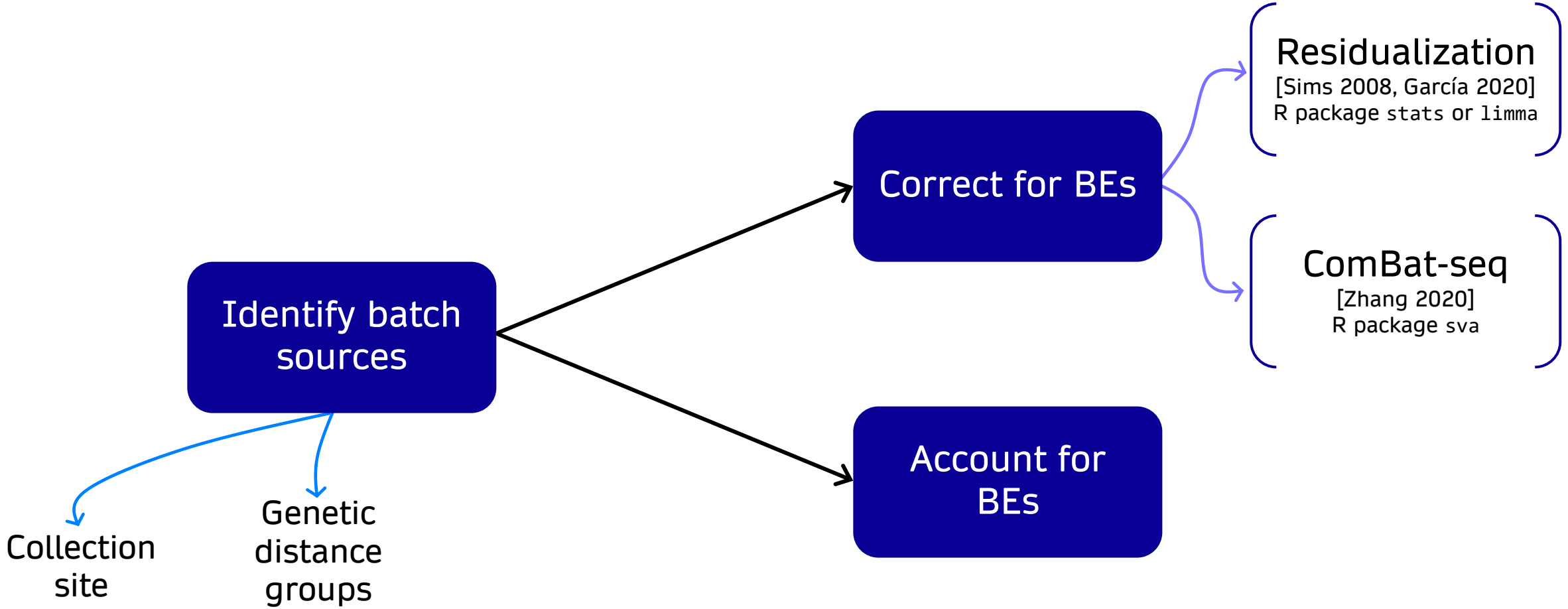
RNA-SEQ DATA



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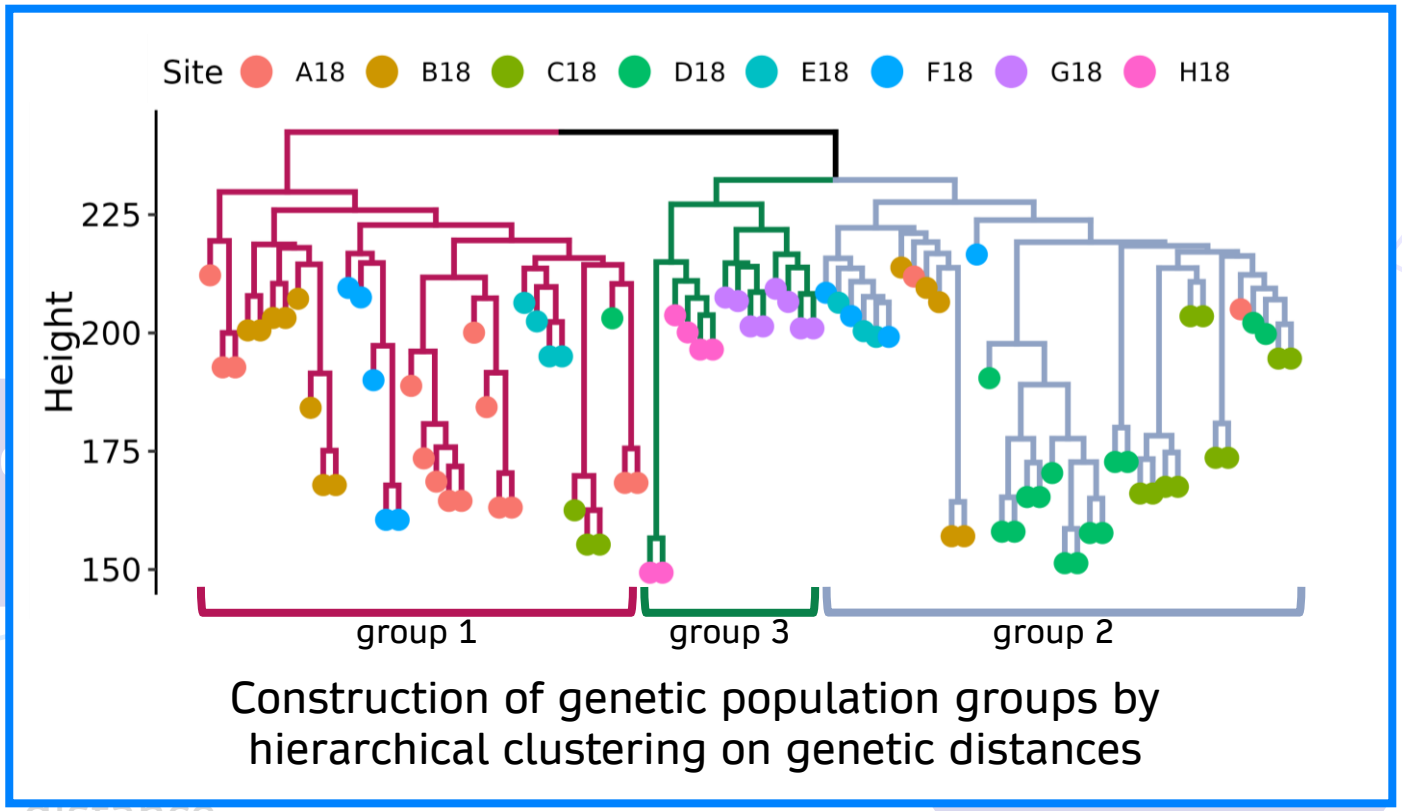


Construction of genetic population groups by hierarchical clustering on genetic distances

Residualization
[Sims 2008, García 2020]
R package stats or limma

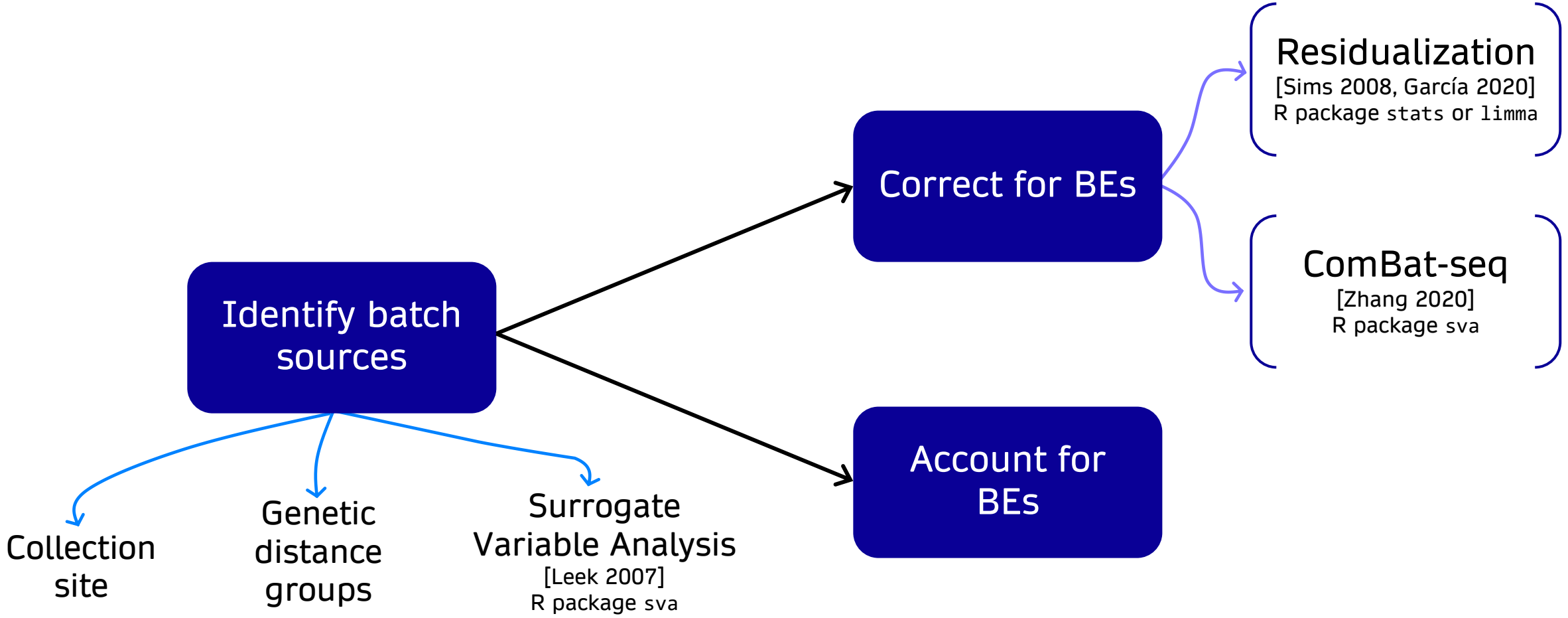
ComBat-seq
[Zhang 2020]
R package sva

Collection site

distance groups

Further information in [Goujon 2024, Car 2023]

CORRECTION STRATEGIES
RNA-SEQ DATA



Further information in [Goujon 2024, Car 2023]

METHODS

Batch effect assessment:

- principal components analysis (PCA) → visualization
  → quantification

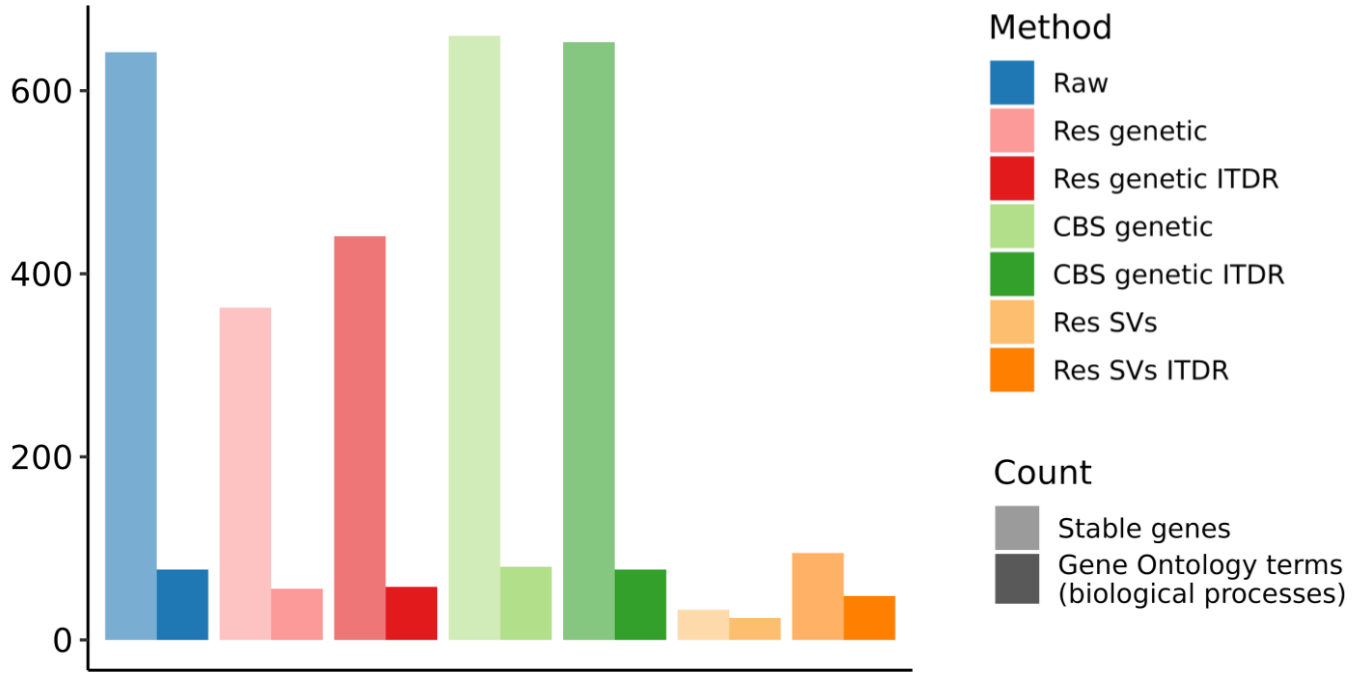
Batch effect correction:

- BECAs: residualization, ComBat-seq, SVA
  - ➢ both with and without preservation of radiocontamination effects

Performance evaluation:

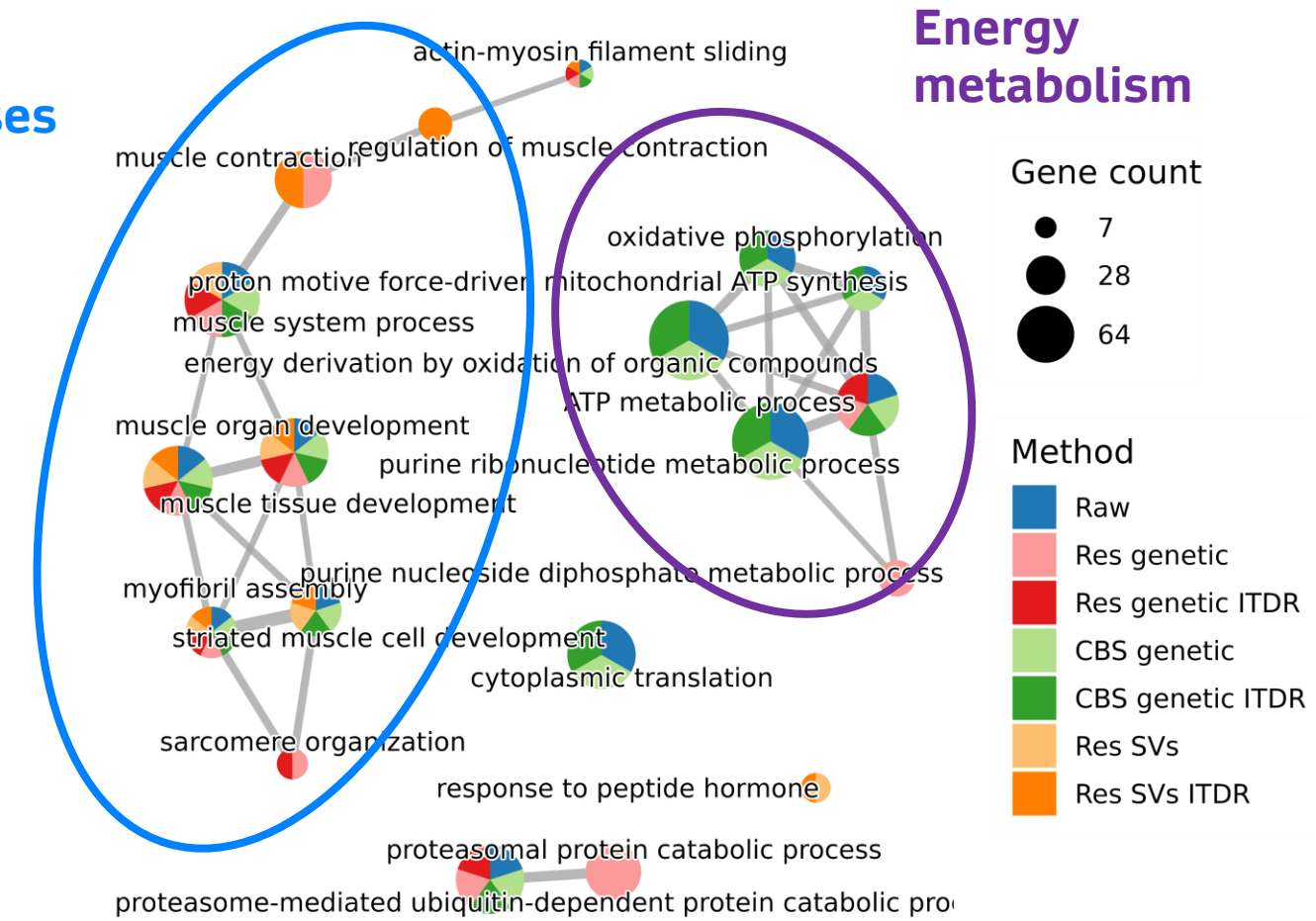
- visualization and summary statistics
- biological interpretation
  - ➢ sparse PCA followed by functional enrichment analysis

RESULTS
BIOLOGICAL INFORMATION IN CORRECTED DATASETS



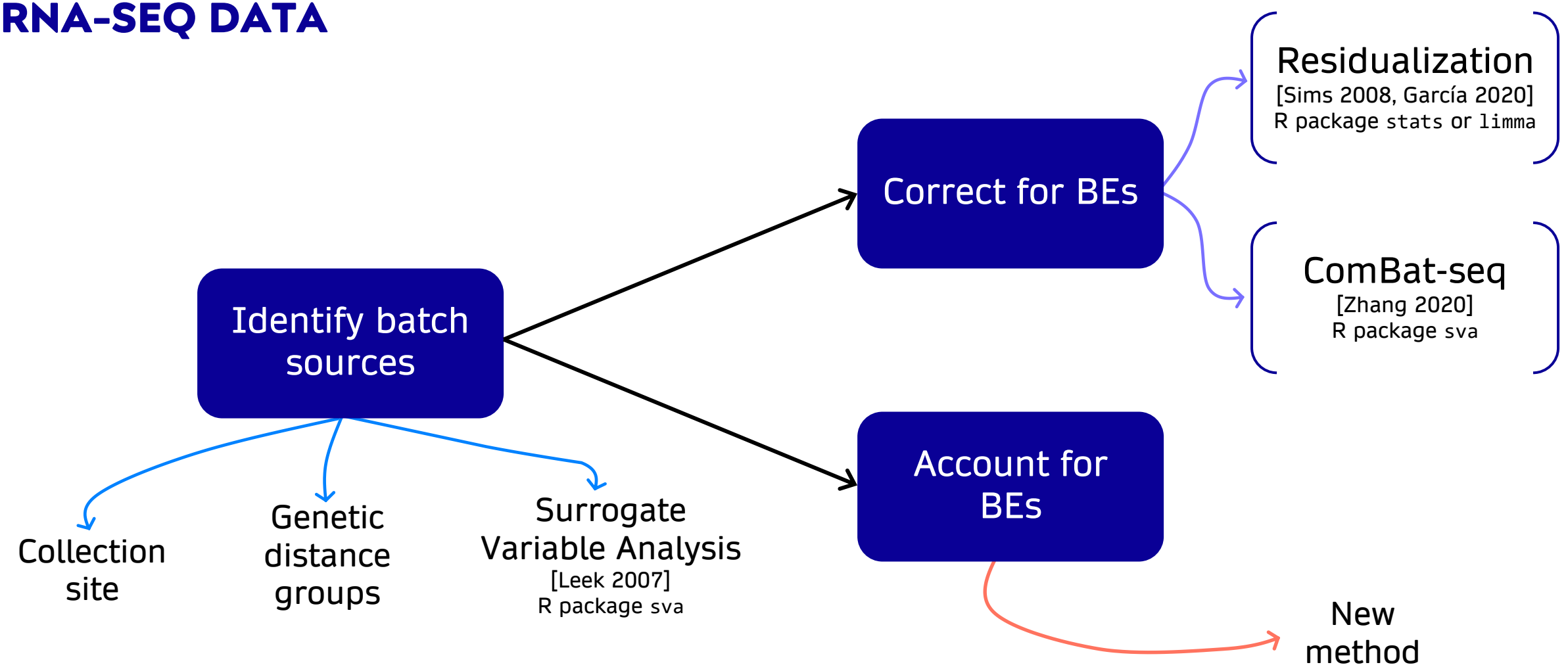
Number of stable genes selected in PCs 1 and 2, and enriched GO terms

RESULTS
ENRICHED GENE ONTOLOGY TERMS



Network of enriched GO terms after the different correction strategies

CORRECTION STRATEGIES
RNA-SEQ DATA



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OUTLINE

HANDLING THE SITE EFFECT IN THE FROGS RNA-SEQ DATA

TOWARDS ACCOUNTING FOR COVARIATES IN MULTI-OMICS ANALYSIS

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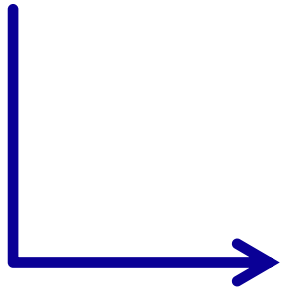
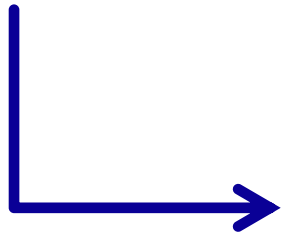
partial correlation coefficient

$r(x_1, x_2|z)$



partial CCA [Rao 1969]

$\mathbf{X}_1, \mathbf{X}_2 \mid \mathbf{Z}$



generalization to multi-block component analysis

AC-PCA

SIMULTANEOUS DIMENSION REDUCTION AND ADJUSTMENT FOR CONFOUNDING [LIN 2016]

⟶ Penalty scheme used to take confounding factors into account

- dataset $\mathbf{X} \in \mathcal{M}_{n,p}$
- confounders matrix $\mathbf{Y} \in \mathcal{M}_{n,l}$
- kernel matrix $\mathbf{K} = \mathbf{Y}\mathbf{Y}^\top$
- parameter $\lambda > 0$

$$\max_{\mathbf{v} \in \mathbb{R}^p} \mathbf{v}^\top \mathbf{X}^\top \mathbf{X} \mathbf{v} - \lambda \mathbf{v}^\top \mathbf{X}^\top \mathbf{Y}\mathbf{Y}^\top \mathbf{X} \mathbf{v}$$
$$\text{subject to } \|\mathbf{v}\|_2^2 \leq 1$$

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$\mathbf{Y}^\top \mathbf{X}\mathbf{v} = \text{cov}(\mathbf{Y}, \mathbf{X}\mathbf{v})$

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- parameter $\lambda > 0$

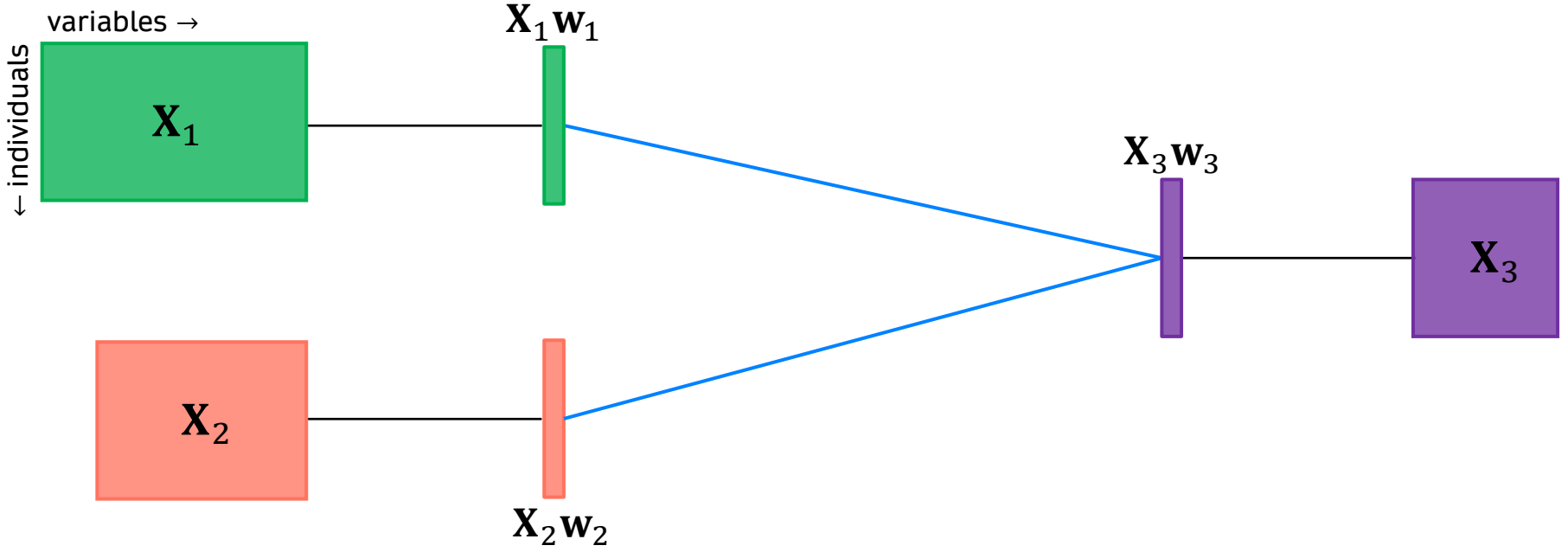
or another positive definite matrix

$$\max_{\mathbf{v} \in \mathbb{R}^p} \mathbf{v}^\top \mathbf{X}^\top \mathbf{X}\mathbf{v} - \lambda \mathbf{v}^\top \mathbf{X}^\top \mathbf{K} \mathbf{X}\mathbf{v}$$
$$\text{subject to } \|\mathbf{v}\|_2^2 \leq 1$$

MULTI-BLOCK RGCCA FRAMEWORK

REGULARIZED GENERALIZED CANONICAL CORRELATION ANALYSIS [TENENHAUS 2014, 2017]

⟶ Statistical framework for multi-omics integration



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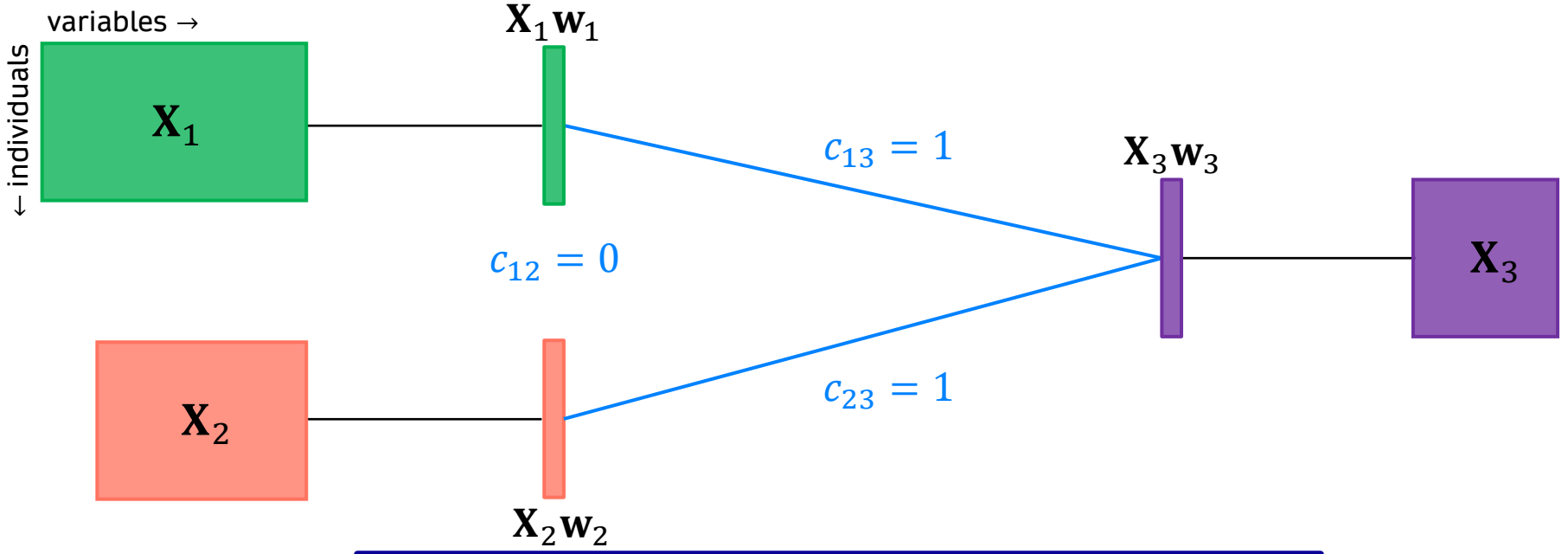

$$\max_{\mathbf{w}_1,\ldots,\mathbf{w}_J} \sum_{j,k=1}^{J} c_{jk}\, g\left(\mathrm{cov}\left(\mathbf{X}_j\mathbf{w}_j, \mathbf{X}_k\mathbf{w}_k\right)\right)$$

under constraints on $\mathbf{w}_j, j = 1, \ldots, J$

- $c_{jk} = 1$ if $\mathbf{X}_j \leftrightarrow \mathbf{X}_k$, else 0
- g = convex continuous function

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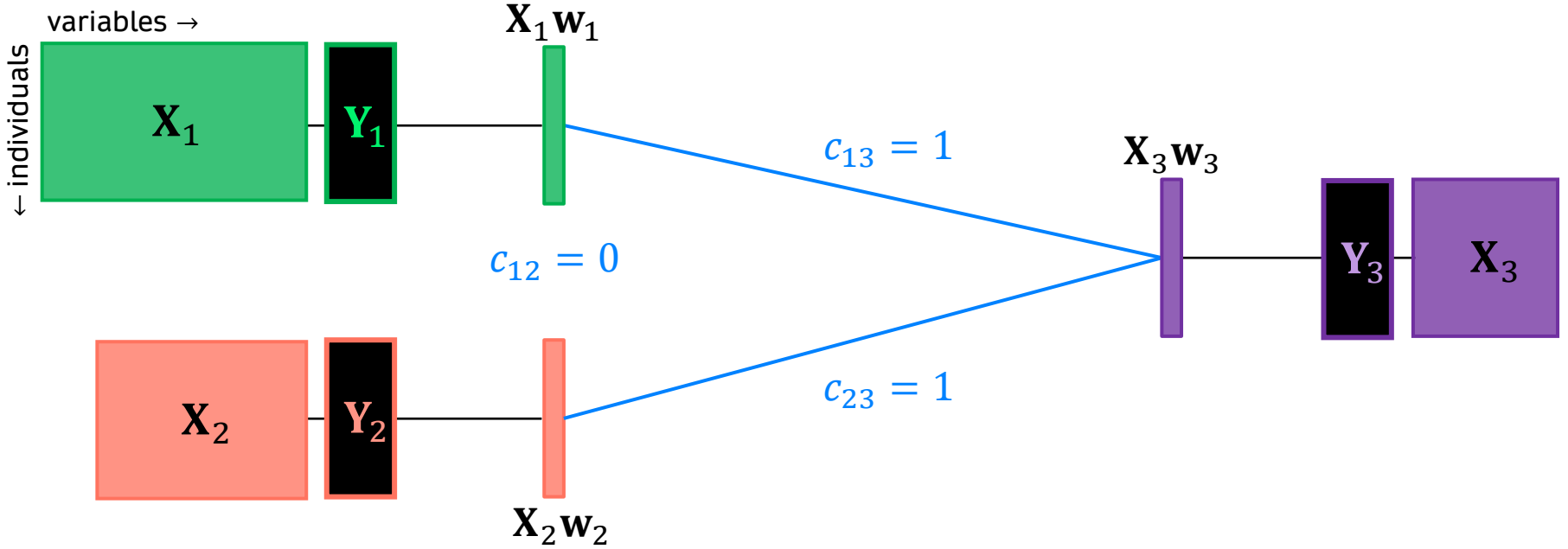

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- g = convex continuous function

EXTENSION TO AC-RGCCA

⟶ Include the constraint in the multi-block RGCCA framework

penalty parameter

kernel matrix built from the confounders

$$\max_{w_1,\dots,w_J} \sum_{j,k=1}^{J} c_{jk}\, g\left(\mathrm{cov}\left(\mathbf{X}_j\mathbf{w}_j, \mathbf{X}_k\mathbf{w}_k\right)\right) - \sum_{l=1}^{J} \frac{\lambda_l}{n} \mathbf{w}_l^\top \mathbf{X}_l^\top \mathbf{K}_l \mathbf{X}_l \mathbf{w}_l$$

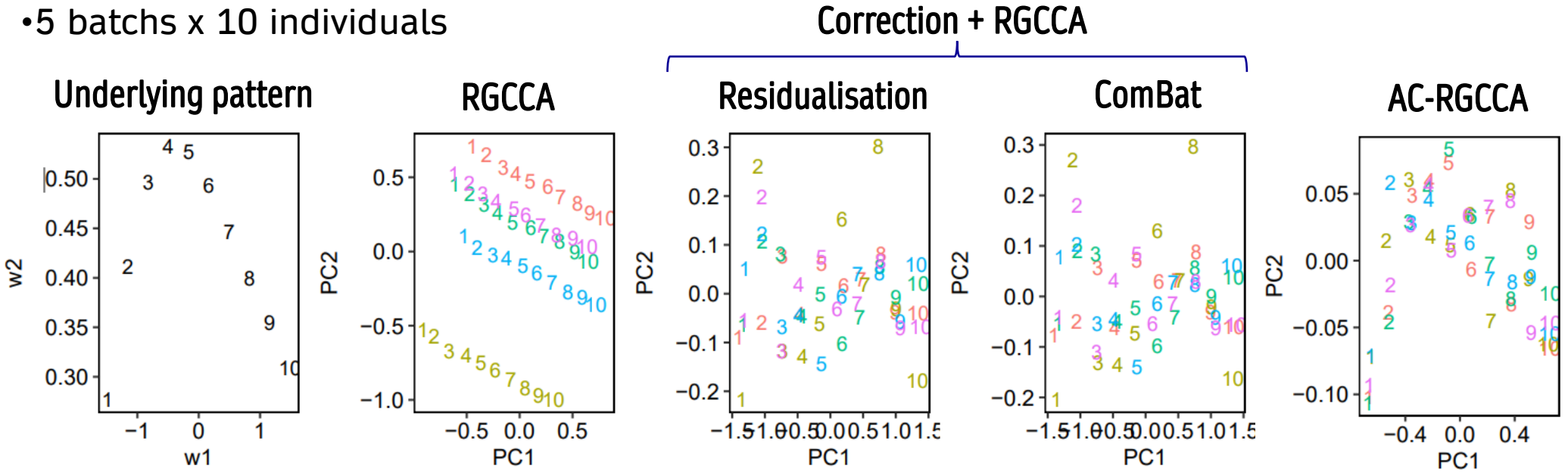
$$\text{under constraints on } \mathbf{w}_j = 1\,, j = 1, \dots, J$$

Iterative algorithm based on block relaxation and Lagragian method
Implementation in R on the *GitHub* of package RGCCA

RESULTS ON SIMULATED DATA

- Method validation
  - Reproduction of AC-PCA's results

- Evaluation of results in multi-block simulations
  - Simulation scenario:
    - 3 blocks
    - 5 batchs x 10 individuals



CONCLUSION & PERSPECTIVES

AXE 1: HANDLING THE SITE EFFECT IN THE FROGS RNA-SEQ DATA

AXE 2: INTEGRATED APPROACHES TO ADJUST FOR CONFOUNDERS

- Multi-group RGCCA [Tenenhaus 2014] (https://github.com/rgcca-factory/RGCCA/tree/multigroup)
- AC-RGCCA

Current work:

- Application to the Chornobyl tree frogs study
- AC-RGCCA: finish writing the article
- Thesis manuscript

VALORIZATIONS

PUBLICATION

BMC BIOLOGY, 2023

“Population transcriptogenomics highlights impaired metabolism and small population sizes in tree frogs living in the Chernobyl Exclusion Zone”
Clément Car, André Gilles, **Elen Goujon**, *et al*.

CONFERENCE PROCEEDINGS PAPER AND ORAL COMMUNICATION

CMSB 2024 (PRÉSENTATION ORALE)

CMSB 2024

“Batch Effect Correction in a Confounded Scenario: a Case Study on Gene Expression of Chornobyl Tree Frogs”
Elen Goujon, Olivier Armant, Clément, Car, Jean-Marc Bonzom, Arthur Tenenhaus, and Imène Garali

POSTERS

ICRER 2024

“Leveraging multi-omics data integration in the study of Chornobyl tree frogs”
Elen Goujon, Olivier Armant, Mélodie Noyau, Sandrine Frelon, Luc Camoin, Jean-Marc Bonzom, Arthur Tenenhaus, and Imène Garali

6th International Conference on Radioecology & Environmental Radioactivity
MARSEILLE, FRANCE
24TH–29TH NOVEMBER 2024

JOBIM 2023 (POSTER ET FLASH-TALK)

“Handling confounding factors in analyzing the transcriptomic data from Chornobyl tree frogs”
Elen Goujon, Olivier Armant, Jean-Marc Bonzom, Arthur Tenenhaus, and Imène Garali

JOBIM 2023
27>30 JUIN
Édition multisite

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