Batch effect

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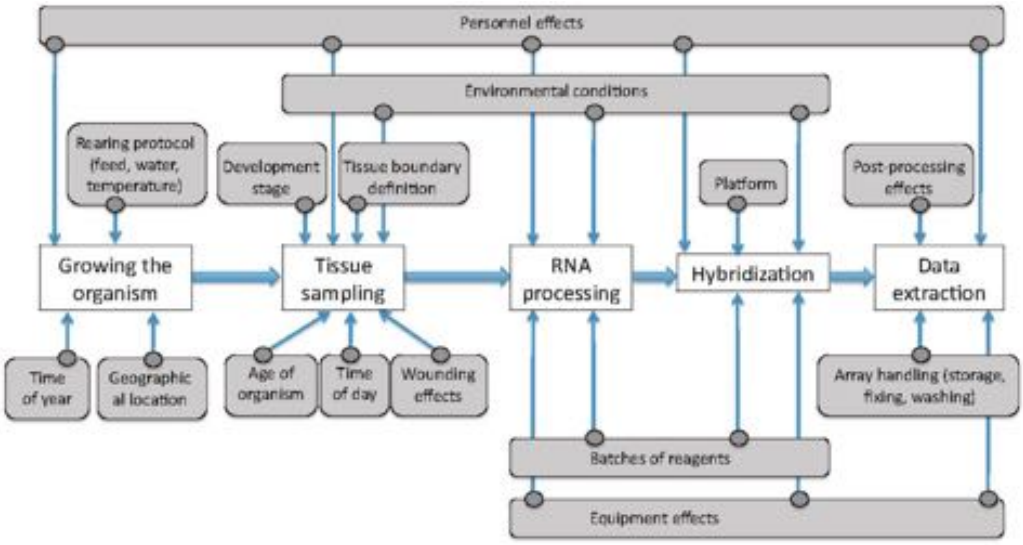
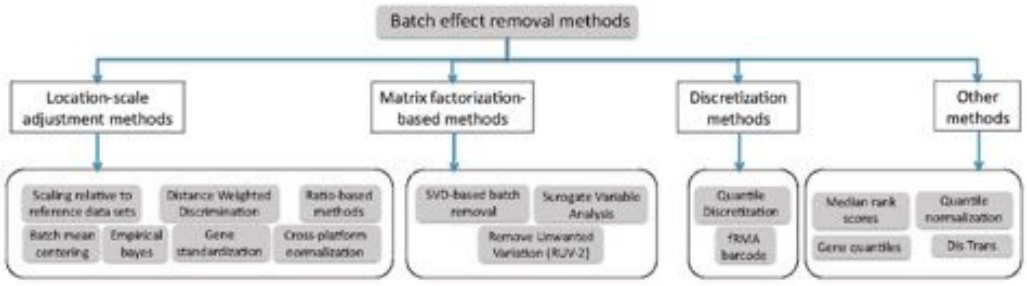
Batch effects

- Batch effects are widespread in high-throughput biology. They are artifacts not related to the biological variation of scientific interests.
- For instance, two microarray experiments on the same technical replicates processed on two different days might present different results due to factors such as room temperature or the two technicians who did the two experiments.
- Batch effects can substantially confound the downstream analysis, especially meta-analysis across studies.

Batch sources



Batch removal methods



Lazar et.al., "**Batch effect removal methods for microarray gene expression data integration: a survey**" Brief Bioinform 2013
http://bib.oxfordjournals.org/content/14/4/469.long

The effect of batch removal

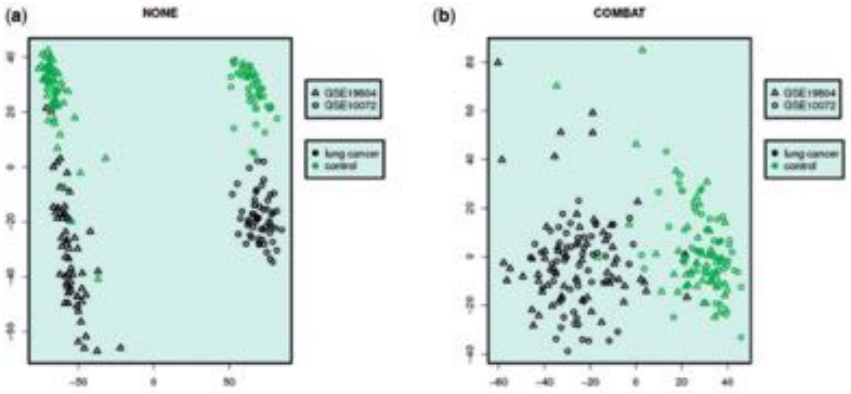


Figure 7: Illustration of PCA plots as validation tools for batch effect removal. Plot of first two principal components: (a) before batch effect removal and (b) after batch effect removal (using EB method).

Lazar et.al., "**Batch effect removal methods for microarray gene expression data integration: a survey**" Brief Bioinform 2013
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Accounting for batch effects

- In statistical modeling, batch effects can be included as covariates (additional predictors) in the model (preferred method).
- For exploratory analysis, we often attempt to “eliminate” or “adjust for” such unwanted variation in advance, by subtracting the estimated effect from each variable (ComBat, SVA).

Batch removal methods

Two main approaches:

- **Location-scale (LS)**
- **Matrix-factorization (MF)**

Batch removal methods

Location-scale

- LS method assumes a model for the location (mean) and/or scale (variance) of the data within the batches.
- Adjusts the batches in order to agree with these models

Batch removal methods

Matrix-factorization

- MF techniques assume that the variation in the data corresponding to batch effects is independent on the variation corresponding to the biological variable of interest
- Capture non-biological variability in a small set of factors
- Factors can be estimated through some matrix factorization methods

ComBat

- “Eliminate” the impact of the (known) batch variable on the observed values
- Can provide information about variables of interest, whose effect should be retained in the data
- Works best if batch and variable of interest are not confounded

ComBat

ComBat - Location-scale method

The core idea of ComBat was that the observed measurement Y_{ijg} for the expression value of gene g for sample j from batch i can be expressed as

$$Y_{ijg} = \alpha_g + X\beta_g + \gamma_{ig} + \delta_{ig}\epsilon_{ijg}$$

where X consists of covariates of scientific interests, while γ_{ig} and δ_{ig} characterize the additive and multiplicative batch effects of batch i for gene g.

https://www.bu.edu/jlab/wp-assets/ComBat/Abstract.html

ComBat

After obtaining the estimators from the above linear regression, the raw data Y_{ijg} can be adjusted to Y^*_{ijg}:

$$Y^*_{ijg} = \frac{Y_{ijg} - \hat{\alpha_g} - X\hat{\beta_g} - \hat{\gamma_{ig}}}{\hat{\delta_{ig}}} + \hat{\alpha_g} + X\hat{\beta_g}$$

For real application, an empirical Bayes method was applied for parameter estimation.

https://www.bu.edu/jlab/wp-assets/ComBat/Abstract.html

What if the batch variable is unknown?

- Manifests as systematic “unwanted variation” in data
- Can be identified using e.g. control genes (“housekeeping” genes, spike-ins)
- Represent themselves as residuals after eliminating known signal

Methods to account for

- Include estimated unwanted variation as covariate(s) in the statistical model
- `RUV`, `sva` packages commonly used in genomics

SVA

When batches were unknown, the surrogate variable analysis (SVA) was developed.

The main idea was to separate the effects caused by covariates of our primary interests from the artifacts not modeled.

Now the raw expression value Y_{jg} of gene g in sample j can be formulated as:

$$Y_{jg} = \alpha_g + X\beta_g + \sum_{k=1}^{K} \lambda_{kg}\eta_{kj} + \epsilon_{jg}$$

where η_{kj}s represent the unmodeled factors and are called as “surrogate variables”.

SVA

Once again, the basic idea was to estimate η_{kj}s and adjust them accordingly.

An iterative algorithm based on singular value decomposition (SVD) was derived to iterate between estimating the main effects $\hat{\alpha_g} + X\hat{\beta_g}$ given the estimation of surrogate variables and estimating surrogate variables from the residuals $r_{jg} = Y_{jg} - \hat{\alpha_g} - X\hat{\beta_g}$

`sva` package in Bioconductor

- Contains `ComBat` function for removing effects of known batches.
- Assume we have:
  - `edata`: a matrix for raw expression values
  - `batch`: a vector named for batch numbers.

```
modcombat = model.matrix(~1, data=as.factor(batch))

combat_edata = ComBat(dat=edata, batch=batch, mod=modcombat, par.prior=TRUE, prior.plot=FALSE)
```

https://bioconductor.org/packages/release/bioc/html/sva.html

SVASEQ

For sequencing data, `svaseq`, the generalized version of SVA, suggested applying a moderated log transformation to the count data or fragments per kilobase of exon per million fragments mapped (FPKM) first to account for the nature of discrete distributions

Instead of a direct transformation on the raw counts or FPKM, remove unwanted variation (RUV) adopted a generalized linear model for Y_{jg}

BatchQC - Batch Effects Quality Control

A Bioconductor package with a GUI (shiny app).

https://github.com/mani2012/BatchQC

What to use

“ComBat, an Empirical Bayes method, outperformed the other five programs by most metrics”

Chen C et.al., **"Removing Batch Effects in Analysis of Expression Microarray Data: An Evaluation of Six Batch Adjustment Methods"** PLoS ONE 2011 http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0017238

References

- Johnson, W. Evan, Cheng Li, and Ariel Rabinovic. “**Adjusting Batch Effects in Microarray Expression Data Using Empirical Bayes Methods.**” Biostatistics 2007 http://biostatistics.oxfordjournals.org/content/8/1/118.long
- Lazar, Cosmin, et.al. “**Batch Effect Removal Methods for Microarray Gene Expression Data Integration: A Survey.**” Briefings in Bioinformatics 2013 http://bib.oxfordjournals.org/content/14/4/469.long
- Batch effects and the importance of EDA, https://kbroman.wordpress.com/2012/04/25/microarrays-suck/