THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

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UAB Metabolomics Workshop
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Designing a metabolomics experiment

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Metabolomics and other -Omics

- False discovery occurs in all the –omics, metabolomics is no different
- Many of the non-biologic effects come from the design of the experiment
- Very important to carefully consider the sources of variation and if they cannot be changed, at least ensure that their effects are evenly distributed across experimental groups
- Discuss the experiment with a statistician before starting out

Types of Studies

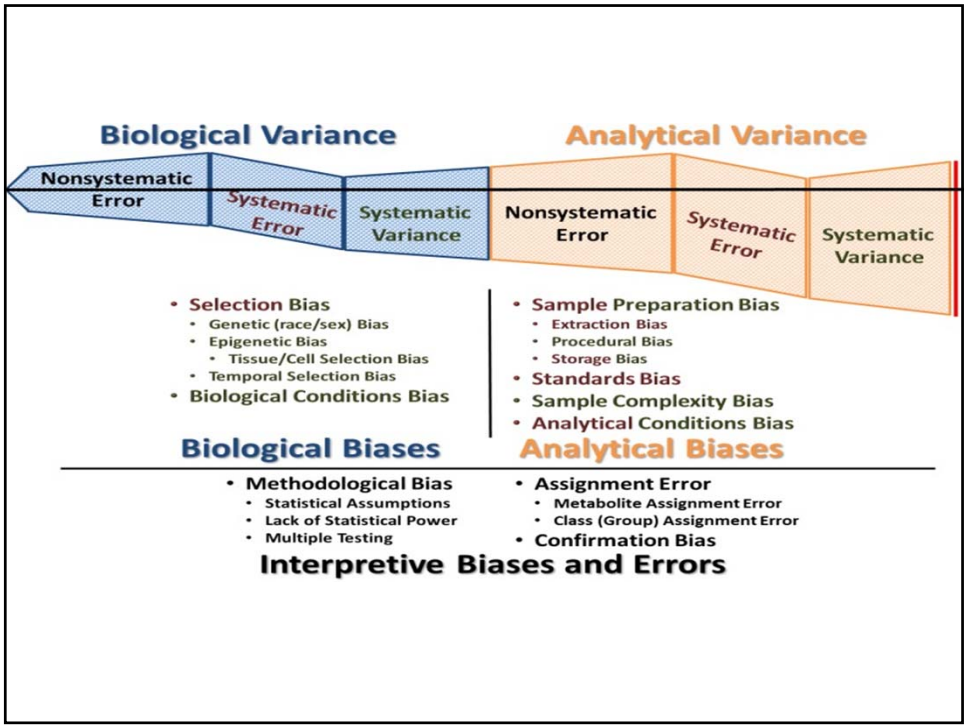
1. To classify samples into subgroups based on profiles of metabolites
   - E.g., cancer subtypes
2. Looking for metabolite signatures that are associated with a disease/condition
   - E.g., diseased vs controls; treated mice with control mice
3. Study mechanisms of certain disease/condition at metabolite level
   - E.g., drug effect mechanism

Design Considerations for Different Types of Metabolomics Studies

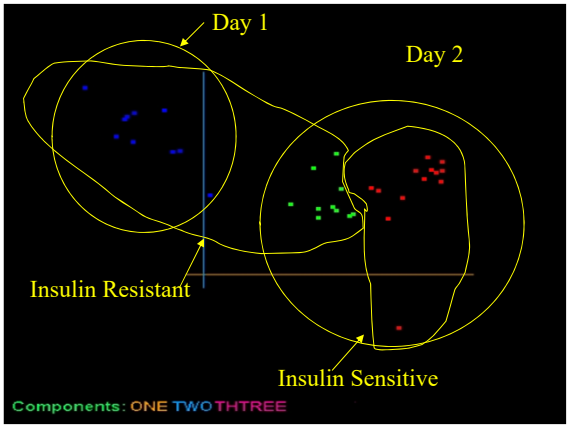
- Classification studies
  - I. relatively larger sample size
  - II. No replicates
  - III. Training set vs testing set
- Comparing disease/treatment vs control for different metabolite profiles
  - I. Comparison groups
  - II. Sample size in each group
  - III. Can we pool samples?

Design Considerations for All Metabolomics Studies

- Biological and Technical variability
- Balancing (Blocking)
- Randomization



UMSA Analysis



How to Solve This Problem?

- **Process all samples in the same day.**
- **Process half sensitive samples and half resistant samples in each day (balance sample groups against days– "treat each day as a block" in statistical terms).**

Known sources of non-biological biases (not exhaustive) that must be addressed

- Technician / post-doc
- Reagent lot
- Temperature
- Protocol
- Date
- Location
- Cage/ Field positions

Too Many factors to balance? -- Randomize

- Number the objects to be randomized and then randomly draw the numbers.

Example: Assign two treatments, Special Diet and control, to 6 mice

1 2 3 4 5 6

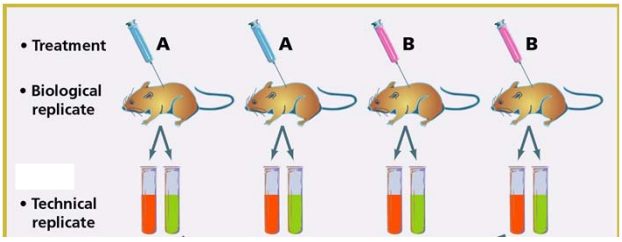
Special Diet : 1, 3, 4
Control : 2, 5, 6

Randomization in Metabolomics Experiments

- Randomize samples in respect to treatments
- Randomize the order of handling samples.
- Randomize batches/runs/days in respect to samples
- Randomize over any other variable procedures.

Replication and Sample Size for Identifying Differential Metabolites

- Replications should not be confused with repeated measurements.



How Many to Replicate? --- Sample Size

- Depends on $$
- Variability – Variability increases, N increases.
- Difference to be detected – Difference increases, N decreases.
- Desired Power – Power increases, N increases.
- Significance level – Significance level increases, N increases.

Can I pool my treatment samples?

- It is rarely recommended unless it is necessary, e.g., working with fruit flies.
- It has potential benefits (reduce biological variability) and drawbacks (lack of measure of variability across individuals).
- Definitely not pooling all your treatment samples into one big pool and your control samples into one big pool.

Thank you – Questions?