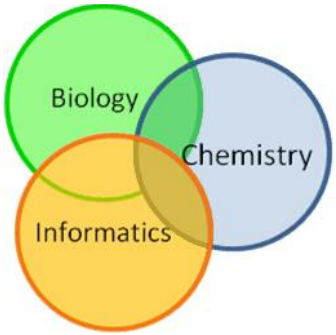


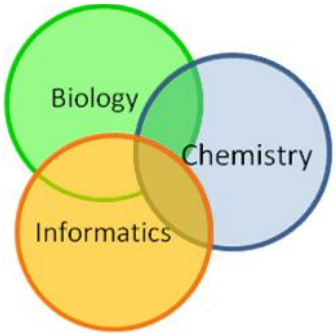
Statistical analysis of the effects of sample drying on metabolite concentrations in pumpkin leaves

Statistics

Goals: Carry out statistical tests with false discovery correction to identify metabolites, which are significantly altered between the two workup methods

Topics:

1. Statistical analysis (2 classes)
2. False discovery rate
3. Power analysis



Identify the effects of sample drying?

Statistics

Use DATA: Pumpkin data 2.csv

```
          Treatment
fresh frozen:6
lyophilized :6
```

Steps:

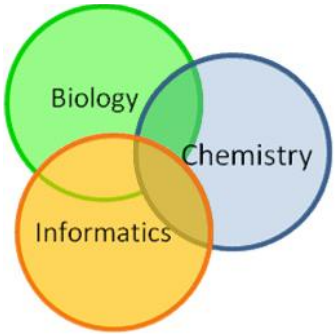
1. Use t-Test to compare metabolite means for each treatment
2. Correct for the false discovery rate (FDR) adjusted p-value
3. Estimate FDR (q-value)

Visualize:

1. Relationship between p-value and FDR adjusted p-value
2. Relationship between FDR adjusted p-value and q-value
3. Box plots for highest and lowest p-value metabolites

Questions:

1. When should you use a one-sample, two-sample or paired t-test, ANOVA?



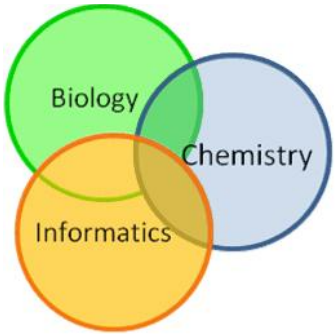
Hypothesis Testing Strategies

ID	Name	t.test_p.value	t.test_adjusted.p.valu	t.test_q.value
1	zymosterol	0.291722421	0.502033933	0.298398
2	xylose	0.030977402	0.162459872	0.095294
3	xylonic.acid	0.069986189	0.235408091	0.137851
4	valine	0.999373245	0.999373245	0.593001
5	urea	0.004614152	0.072966512	0.037578
6	tyrosine	0.803351252	0.927489763	0.539431
7	tyramine	3.84E-05	0.002843104	0.001686
8	tryptophan	0.544657821	0.767708167	0.442608
9	trehalose	0.542894016	0.767708167	0.441808

Statistics

- **One sample t-Test** is used to compare single value to a population mean
- **Two sample t-Test** is used to compare 2 independent populations
- **Paired t-Test** is used to compare the same population (intervention, repeated measures)
- **One-way ANOVA** (analysis of variance) is used to compare n populations for one factor
- **Two-way ANOVA** is used to compare n populations for 2 factors
- **Repeated Measures ANOVA** (e.g time course, intervention, cross-over)
- **ANCOVA** (analysis of covariance) is used to adjust n populations for covariate (typically continuous) prior to testing for n factors
- **Mixed effects models** are versatile analogue to linear model or ANOVA/ANCOVA and typically used to adjust for covariates or variance due to repeated measures

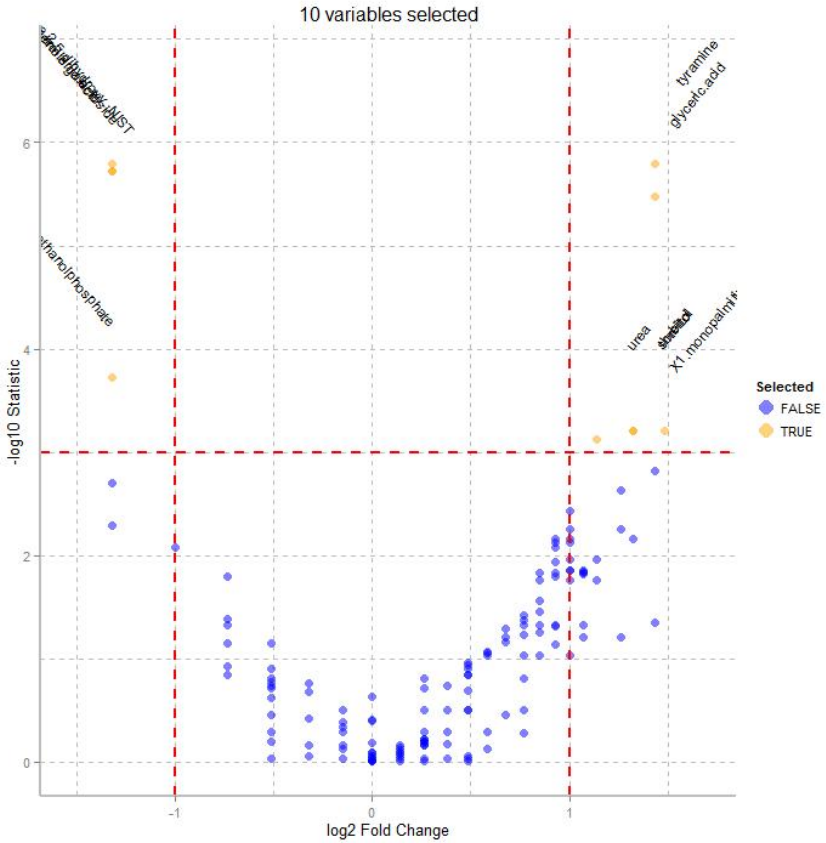
*All of the above are parametric tests, and some of which have non-parametric analogues

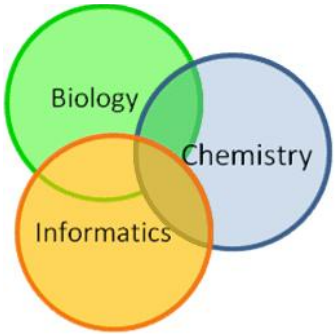


Question:

Statistics

Identify all significantly altered metabolites between the two treatments at p<0.05 and FDRp<0.05

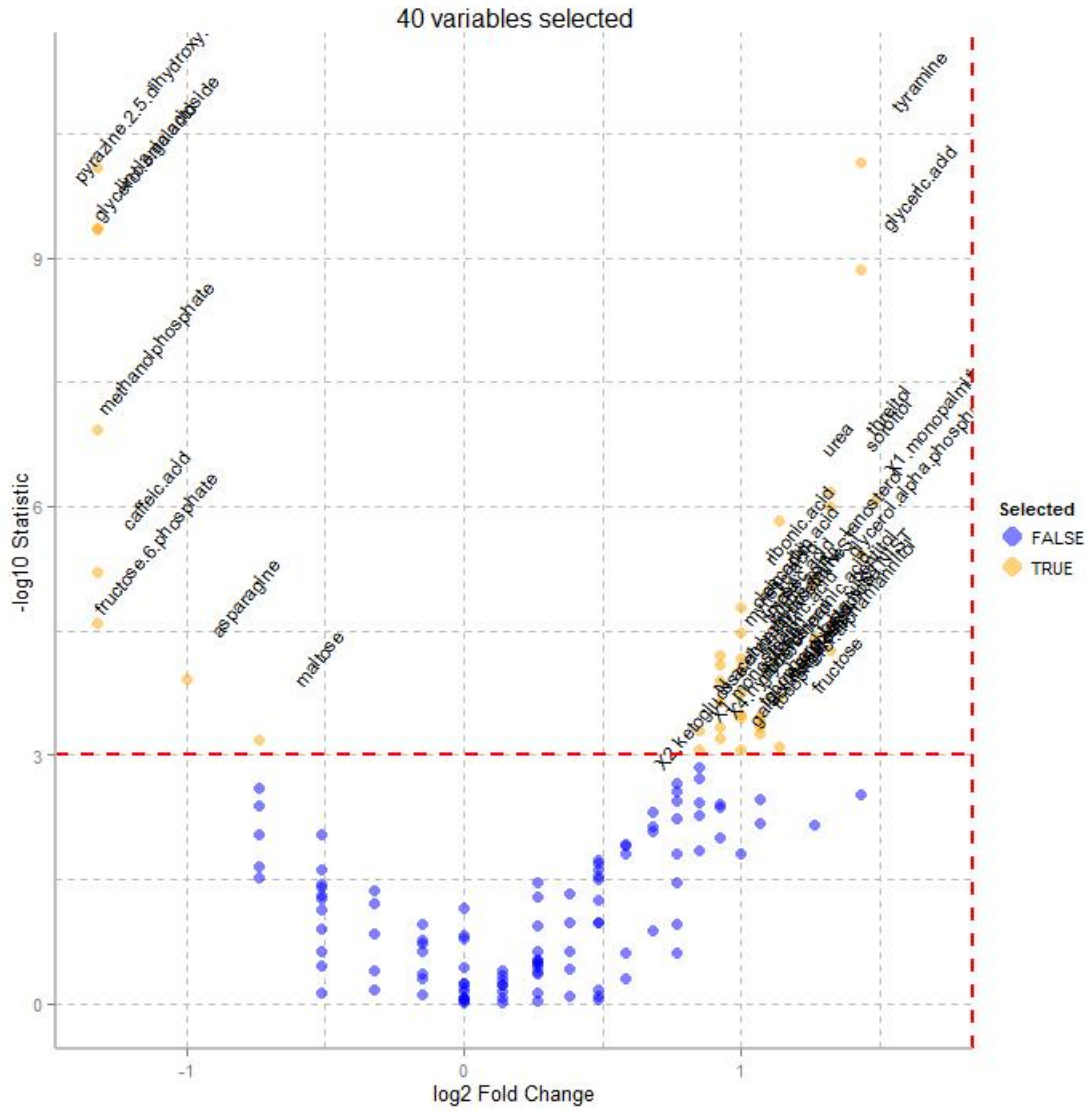


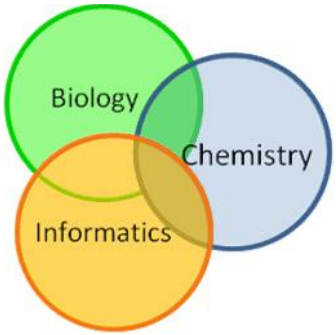


Answer:

Identify all significantly altered metabolites between the two treatments at p<0.05

Statistics

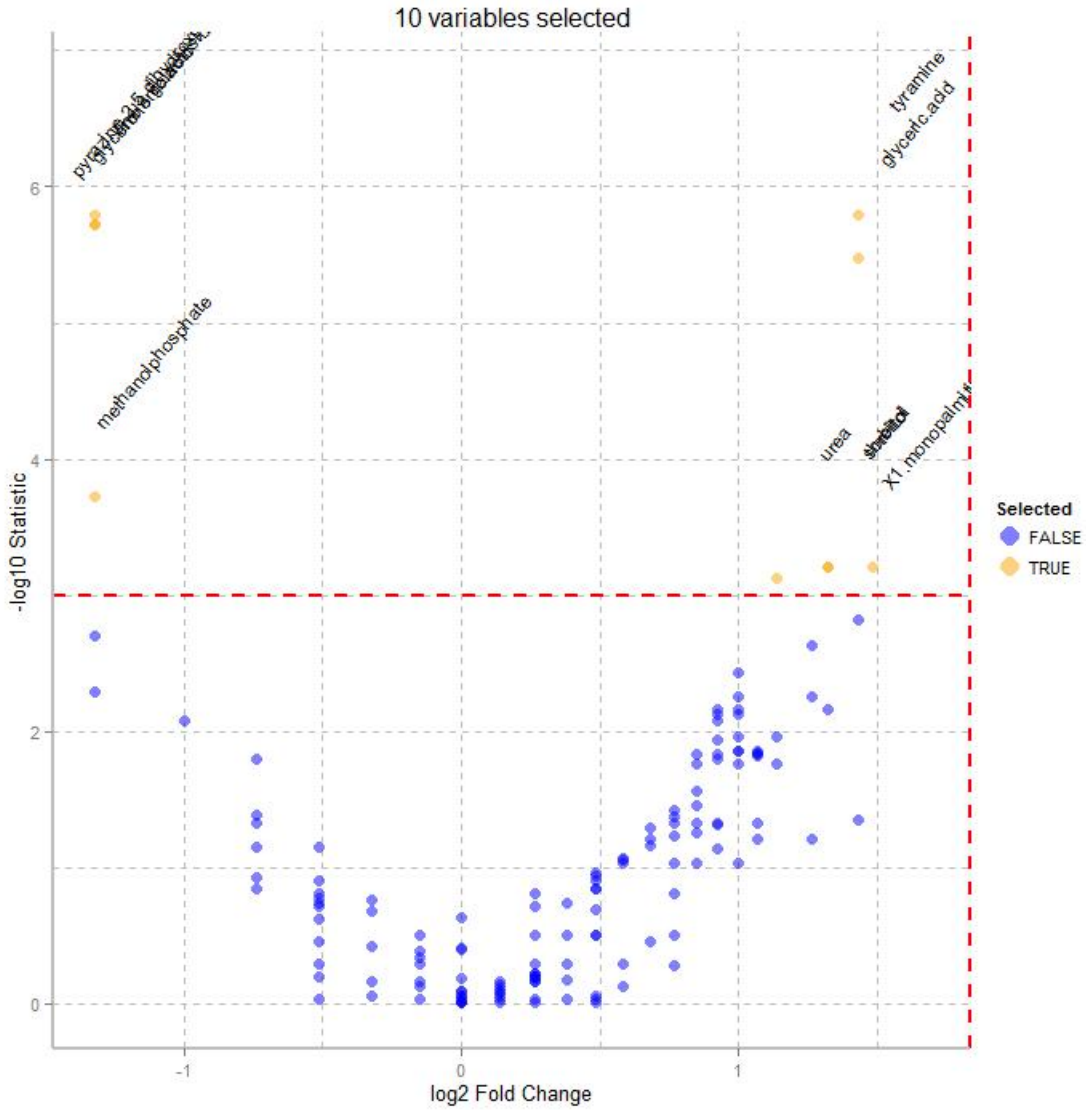


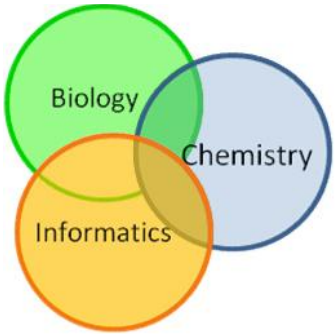


Answer:

Identify all significantly altered metabolites between the two treatments at FDRp<0.05

Statistics

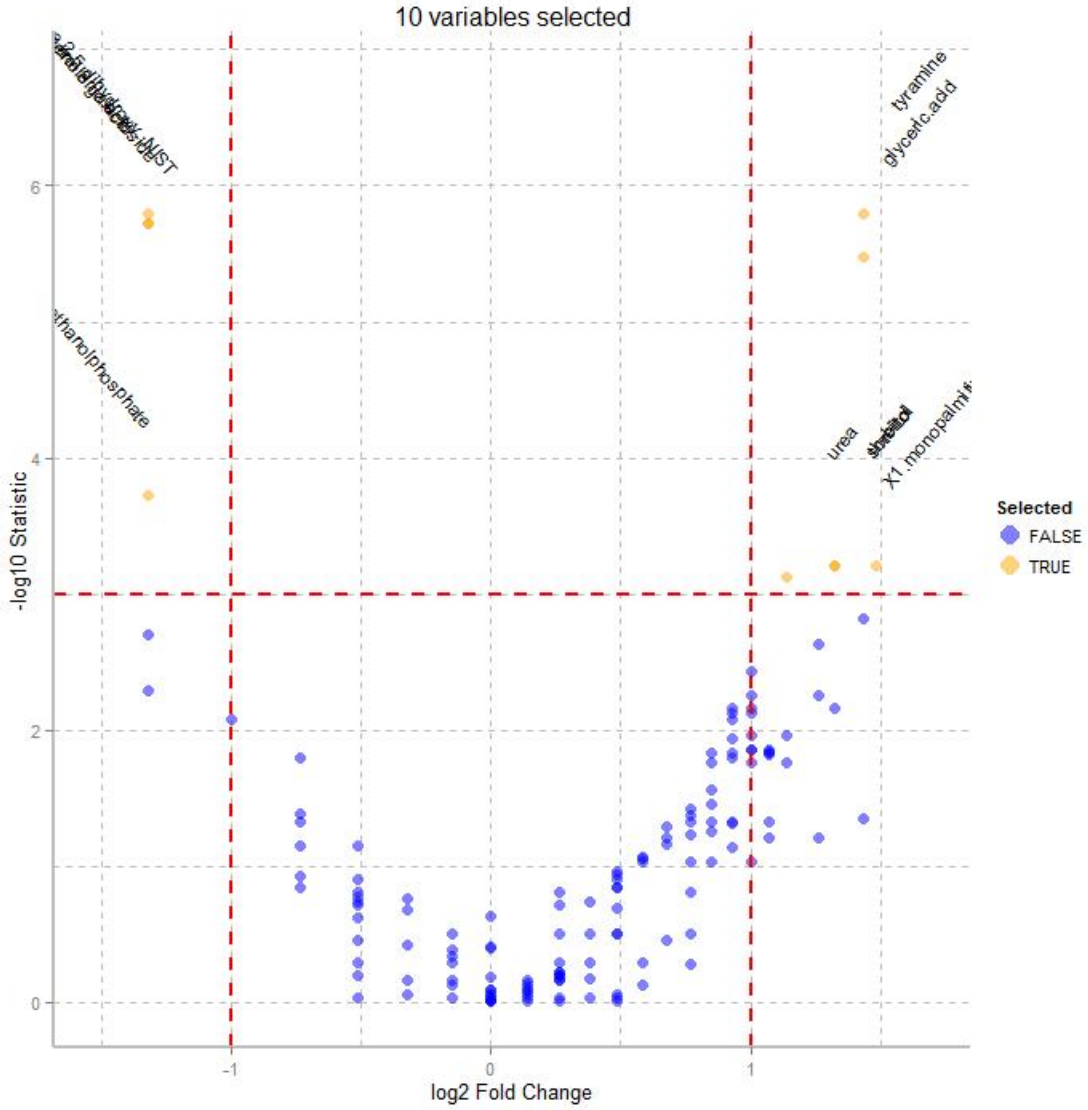


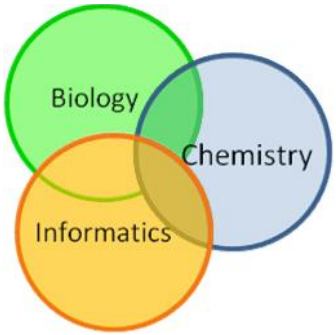


Volcano Plot

Importance based on magnitude of change and significance

Statistics

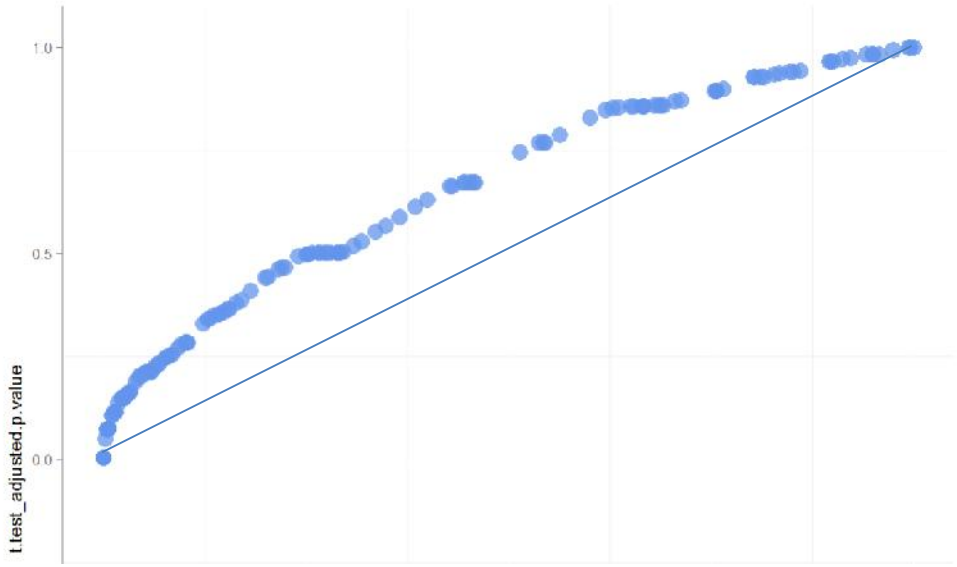


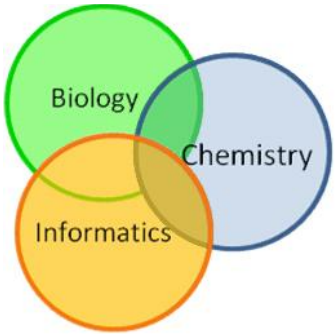


Question:

Statistics

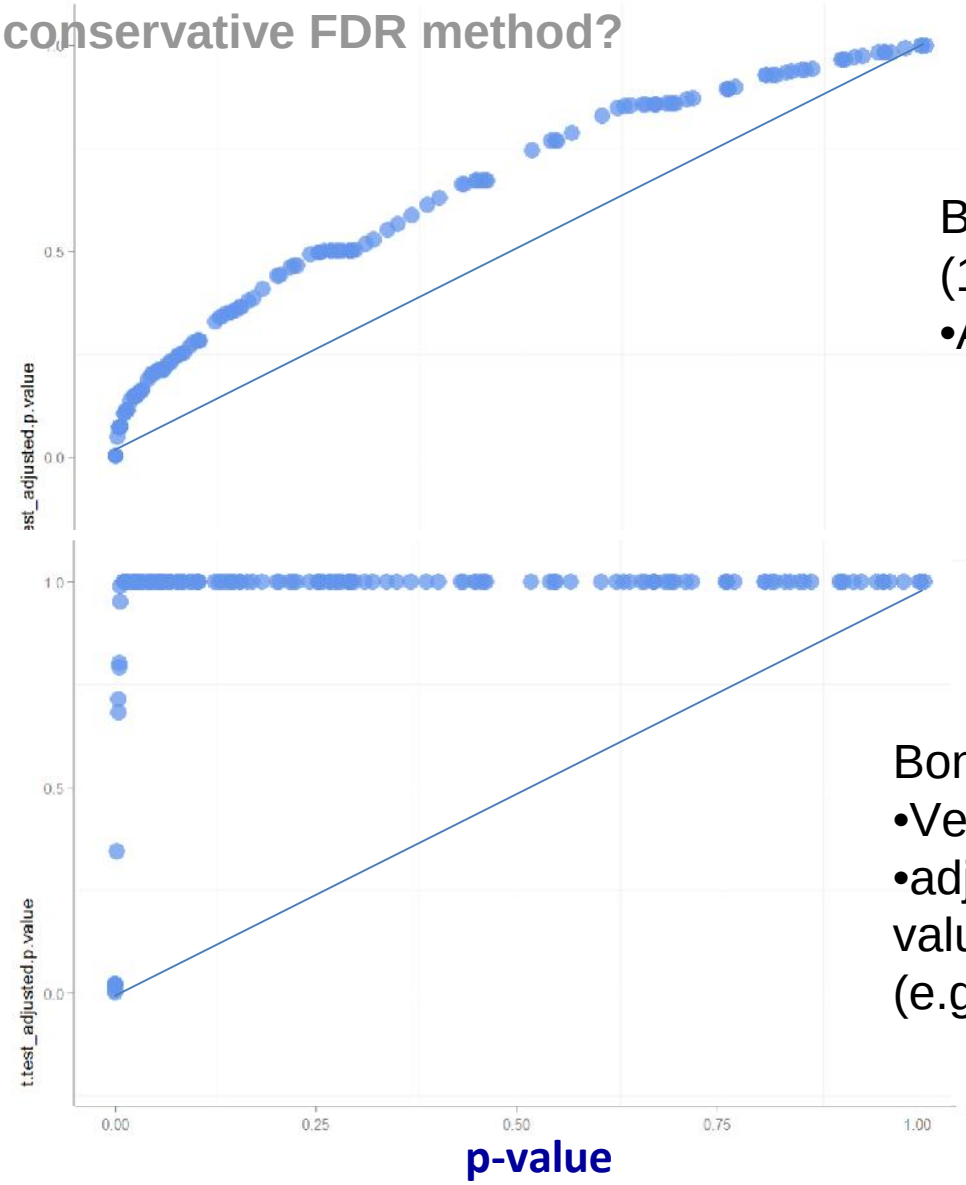
What is the relationship between p-values and FDR adjusted p-values, what is the most and least conservative FDR method?





Answer:

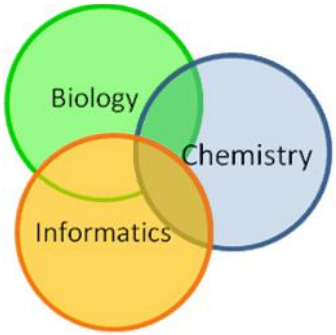
What is the relationship between p-values and FDR adjusted p-values, what is the most and least conservative FDR method?



Benjamini & Hochberg (1995) (“BH”)
•Accepted standard

Bonferroni
•Very conservative
•adjusted p-value = p-value*# of tests
(e.g. 0.005 * 148 = 0.74)

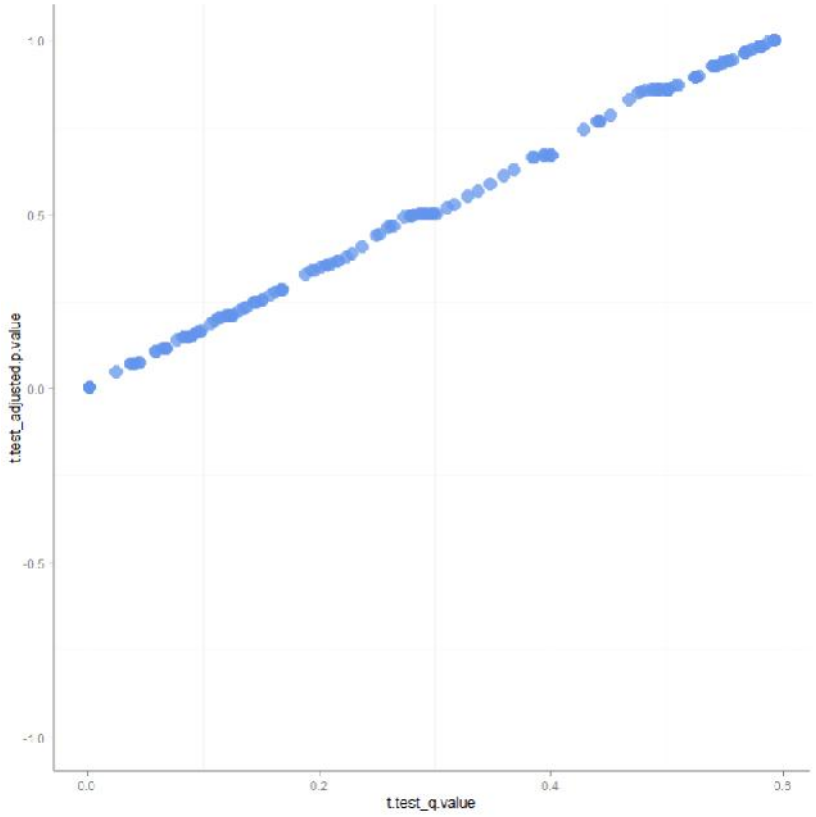
Statistics



p-value vs. q-value

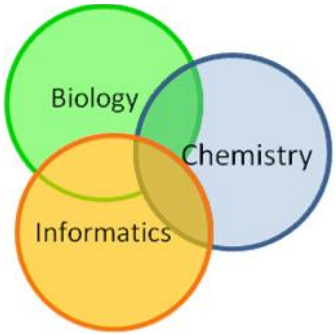
Statistics

FDR adjusted p-value



q-value

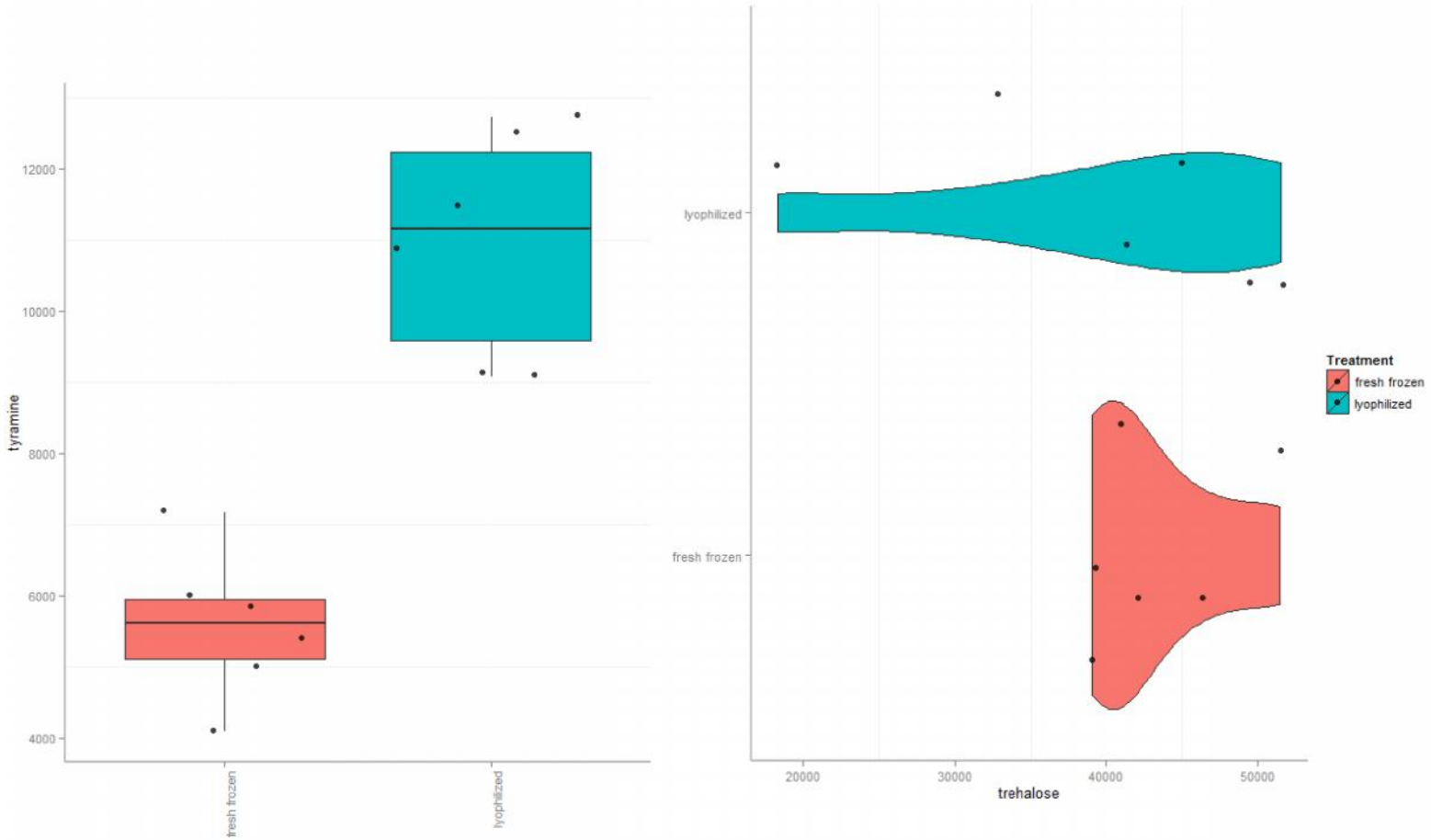
- q-value can be used to select appropriate p-value cut off for an acceptable FDR for multiple hypotheses tested
- q=0.05 nicely matches assumptions of p=0.05 for multiple hypotheses tested
- q-value≤0.2 can be acceptable

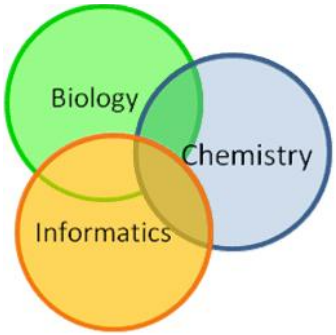


Question:

Characterize the effect size between dry and fresh treatments for any two metabolites of interest

Statistics



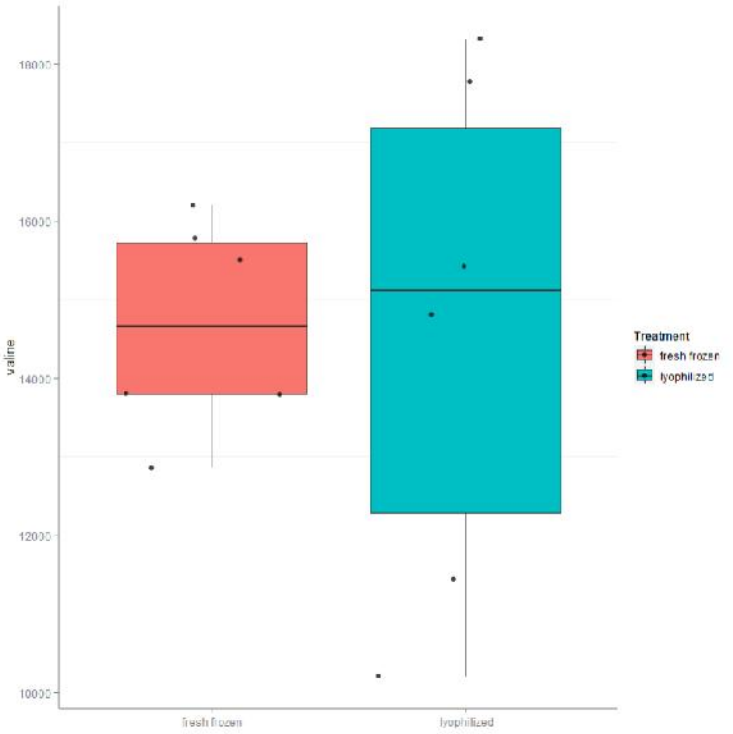


Answer:

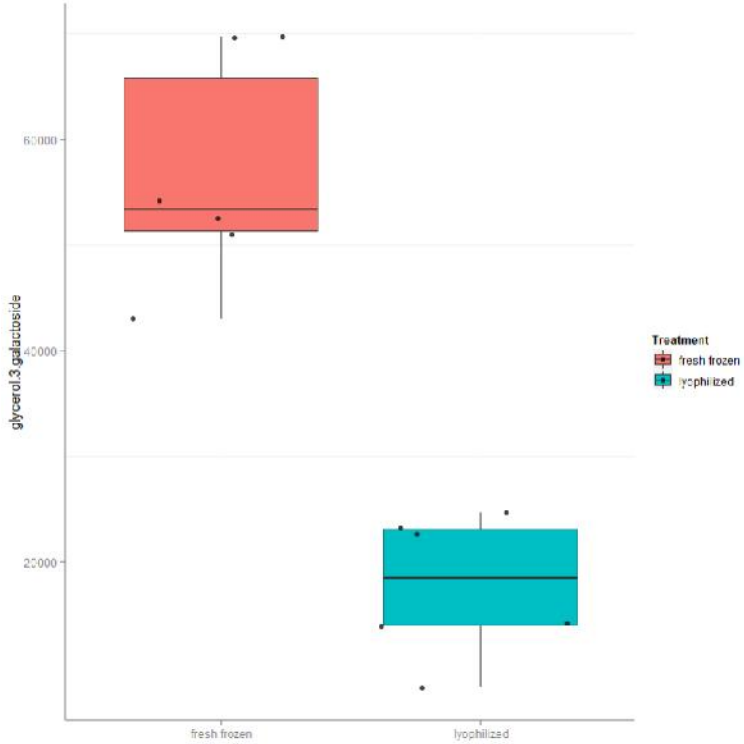
Characterize the effect size between dry and fresh treatments for any two metabolites of interest

Effect size: small large

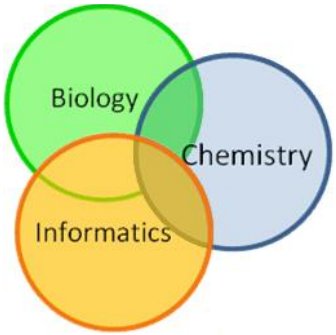
Statistics



valine



glycerol 3 glactoside

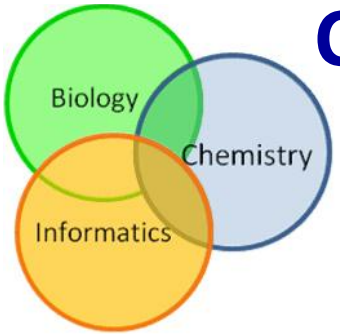


Effect of drying is minimal

However the following metabolites are significantly altered between the two work up methods

Statistics

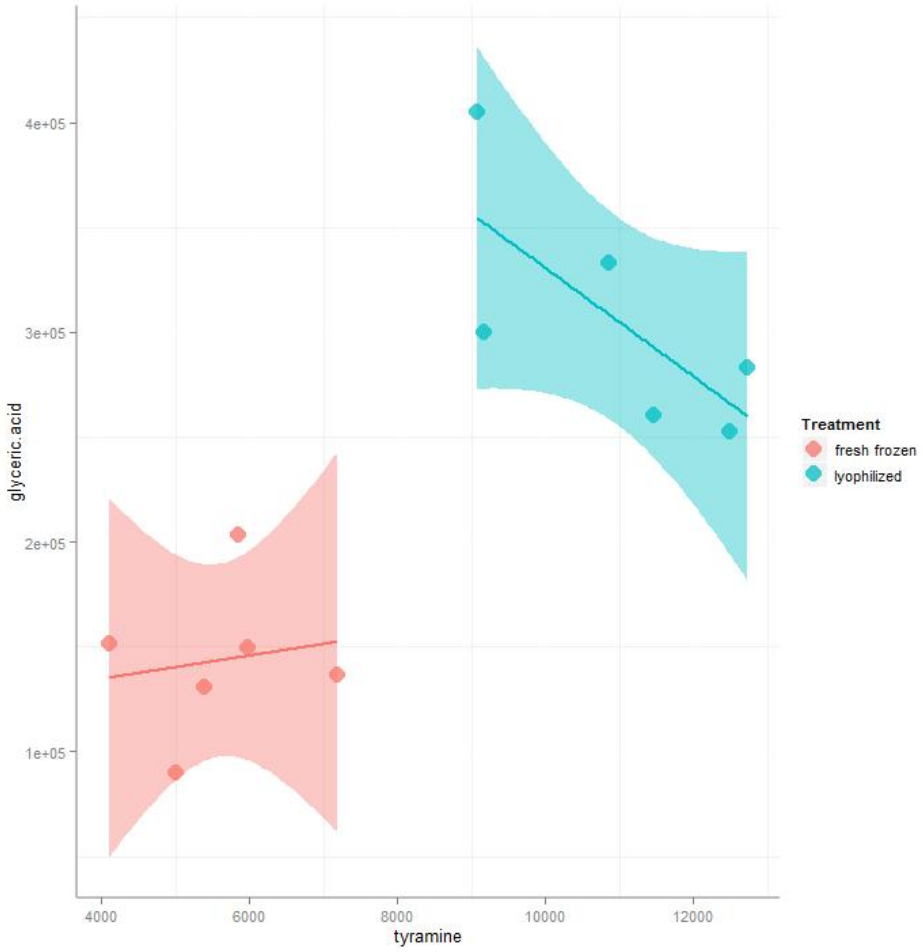
	t.test_p.value	t.test_adjusted.p.value	t.test_q.value	FC_.lyophilized_.fresh.frozen
tyramine	3.88e-05	0.00305	0.00181	2.7
pyrazine.2.5.dihydroxy..NIST	4.12e-05	0.00305	0.00181	0.4
linolenic.acid	8.65e-05	0.00326	0.00193	0.4
glycerol.3.galactoside	8.82e-05	0.00326	0.00194	0.4
glyceric.acid	1.41e-04	0.00418	0.00248	2.7
methanolphosphate	9.76e-04	0.02407	0.01323	0.4
threitol	2.08e-03	0.04060	0.02169	2.5
X1.monopalmitin	2.30e-03	0.04060	0.02291	2.8
sorbitol	2.47e-03	0.04060	0.02380	2.5
urea	2.97e-03	0.04401	0.02611	2.2
glycerol.alpha.phosphate	4.44e-03	0.05974	0.03444	2.7



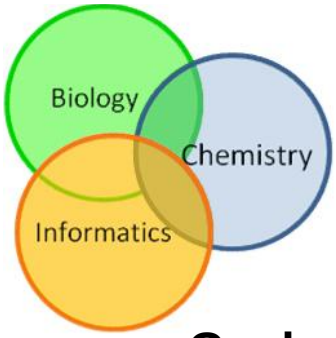
Consequence of workup methods

Different methods can affect the apparent relationships between metabolites

Statistics



- **Analysis of method performance based on metabolite physical properties can be used to spot method bias.**



Power analysis

Goals: Use power analysis to plan a follow up experiment to detect differences in metabolites due to drying treatment

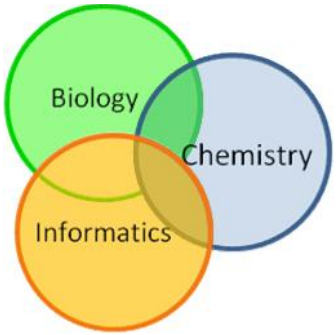
Steps:

1. Calculate effect size and power for three metabolites
2. Given the observed effect size calculate the number of samples needed to reach 80% power

Questions:

1. How would you take FDR in to account?

Statistics

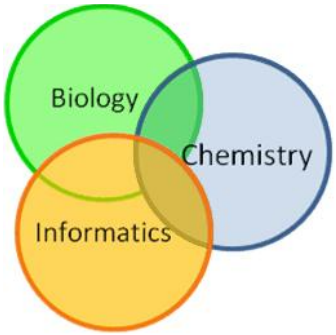


Question:

Calculate the effect size and power for any metabolites of interest

Statistics

variable	comparison	effect.size	alpha	power
zymosterol	lyophilized- fresh frozen	0.643	0.05	0.17
xylose	lyophilized- fresh frozen	1.45	0.05	0.62
xylonic.acid	lyophilized- fresh frozen	1.17	0.05	0.45
valine	lyophilized- fresh frozen	0.000465	0.05	0.05
urea	lyophilized- fresh frozen	2.1	0.05	0.9
tyrosine	lyophilized- fresh frozen	0.148	0.05	0.056
tyramine	lyophilized- fresh frozen	4.03	0.05	1
tryptophan	lyophilized- fresh frozen	0.362	0.05	0.088



Answer:

Calculate the effect size and power for any metabolites of interest

Scaled difference in means between treatments

Ability to detect a difference when it exists (control false negative rate)

variable	comparison	effect.size	alpha	power
zymosterol	lyophilized- fresh frozen	0.643	0.05	0.17
xylose	lyophilized- fresh frozen	1.45	0.05	0.62
xylonic.acid	lyophilized- fresh frozen	1.17	0.05	0.45
valine	lyophilized- fresh frozen	0.000465	0.05	0.05
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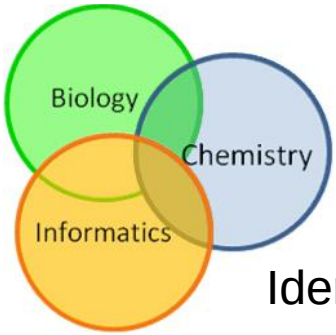
tyramine	lyophilized- fresh frozen	4.03	0.05	1
tryptophan	lyophilized- fresh frozen	0.362	0.05	0.088

Probability of being wrong when spotting a difference (control false positive rate)

Statistics



Utility of power analysis

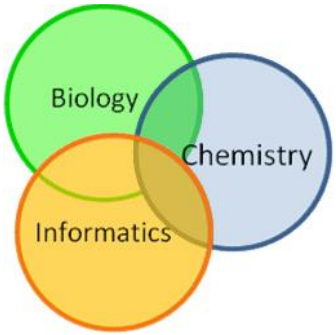
Identify optimal number of samples to detect differences at some p-value and power in follow-up experiments, given effect sizes calculated from the current experiment

Statistics

	variable	comparison	effect.size	alpha	power
1	zymosterol	lyophilized- fresh frozen	0.643	0.05	0.17
2	urea	lyophilized- fresh frozen	2.1	0.05	0.9
3	xylose	lyophilized- fresh frozen	1.45	0.05	0.62
4	xylonic.acid	lyophilized- fresh frozen	1.17	0.05	0.45
5	valine	lyophilized- fresh frozen	0.000465	0.05	0.05

	samples.per.group	effect.size	alpha	power
1	39	0.643	0.05	0.8

	samples.per.group	effect.size	alpha	power
1	9	1.45	0.05	0.8



Utility of power analysis

samples.per.group	effect.size	alpha	power
156978	0.01	0.05	0.8
1571	0.1	0.05	0.8
17	1	0.05	0.8
5	2	0.05	0.8

Statistics

The minimum fold change (FC) in means observable by the study can be calculated using RSD and estimated effect size to reach 0.8 (80%) power given the population size

RSD = 0.21 and effect size (EF) =1.2

$$EF = \frac{m1 - m2}{RSD} \qquad m1 = EF * RSD + m2 = 1.8 * 0.21 + 1 = 1.38 \qquad m2 = 1 \qquad FC = \frac{m1}{m2} = \frac{1.38}{1} = 1.38$$

We can observe a minimum of a 38% change in means at 0.8 power (p= 0.05).