Module 5F: A Parametric Test

Z-scores & RNAseq analysis

Agenda:

1. Z-scores
2. RNASeq

RNAseq:

https://pluto.bio/resources/learning-series/overview-of-z-scores-in-rna-seq-experiments

In RNA-seq analysis, Z-scores are used to compare expression levels between samples

- A Z-score of 0 indicates:
  - the gene's expression level is **the same as** the mean expression across ALL samples
- A Z-score >1 indicates:
  - The gene's expression level is **higher** than the mean expression across ALL samples
- A Z-score <1 indicates:
  - The gene's expression level is **lower** than the mean expression across ALL samples

Once Z-scores are calculated, they can be used to **identify differentially** expressed genes

- For example, a Z-score > threshold (such as 2 or 3) can be considered differentially expressed
- Z-scores can be used to create a heatmap or a volcano plot to easily visualize expression patterns

Simple example of using z-scores for RNA-seq data:

Gene	Control 1	Control 2	Treat 1	Treat 2
G1	5	6	9	10
G2	200	210	220	230
G3	1.2	1.1	1.3	1.4

This data set has two controls, two treatments for three genes. Each number is log-scaled or normalized expression value.

For each gene, ask:

"How far above or below that gene's own average is each sample?"

$$z = \frac{x - \bar{x}}{s}$$

x = value for one sample

$\bar{x}$ = *mean for that gene*

s = *Standard deviation for that gene*

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$$z = \frac{x - \bar{x}}{s}$$

For Gene 1:

Sample	Value	Mean ($\bar{x}$)	Std Dev (s)	z-score
Control 1	5	7.5	≈ 2.38	(5 – 7.5)/2.38 = -1.05
Control 2	6	7.5	≈ 2.38	-0.63
Treat 1	9	7.5	≈ 2.38	0.63
Treat 2	10	7.5	≈ 2.38	1.05

-0.84

+0.84

Controls have negative z's (below average); treatments have positive z's (above average) → up-regulated with treatment. Typically, you would have many examples of treatment and control at a gene and then you would take their mean (we only have two from each category)