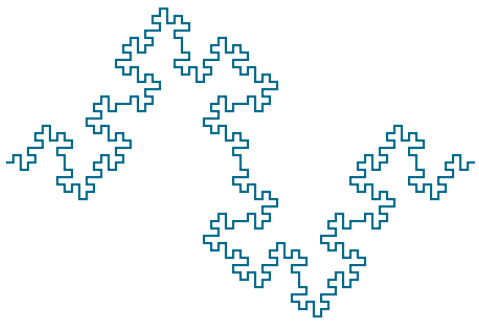
High-Throughput Sequencing Course

Introduction

Biostatistics and Bioinformatics

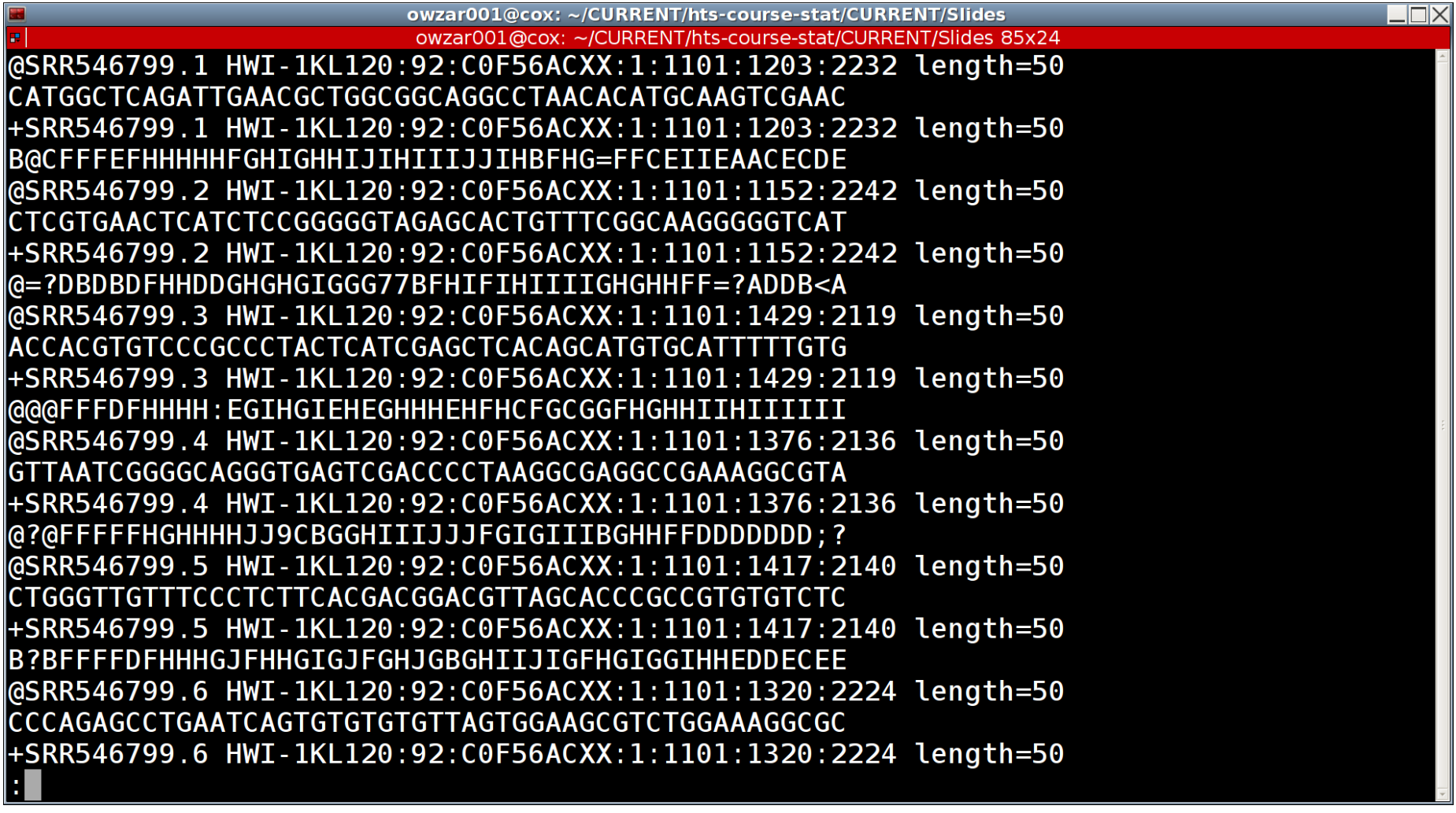


Summer 2019

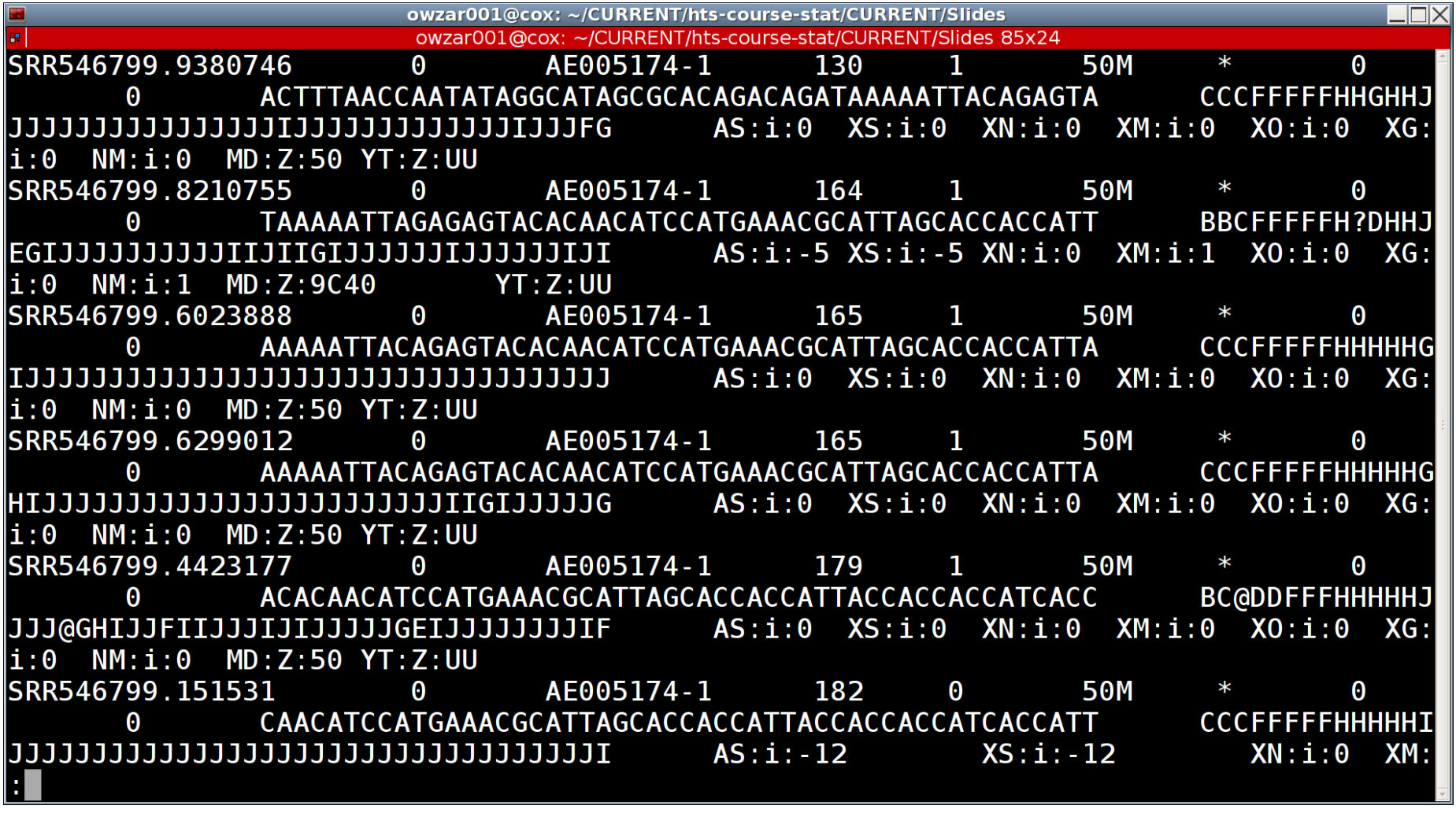
Duke University
School of Medicine

DUKE BIOSTATISTICS & BIOINFORMATICS

From Raw Unaligned Reads



To Aligned Reads



To Counts

owzar001@cox: ~/CURRENT/hts-course-stat/CURRENT/Slides

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```
> head(counts(htseq),20)[,1:15]
         7A_E 7A_G 7A_K 7A_N 7A_P 7B_E 7B_G 7B_K 7B_N 7B_P 7C_E 7C_G 7C_K 7C_N 7C_P
gene0       9   17   11   17   11   12   22   20    6    9   19   20   17    5   20
gene1     108  170   97   88  173  119  241  103   51  162  155  149  124   88  128
gene10      3    0    7    3    3    2    1    1    2    2    2    2    2    7    5
gene100    24   27   15   16   23   11   24   28    5   30   24   20   22   15   25
gene1000   11    5    8    2   13   10    8    7    2   13    8    2    5   13    9
gene1001    1    3    2    5    2    3    1    1    3    5    3    4    4    1    2
gene1002   32   11   19   12   23   31   29   19   11   34   22   20   19   12   27
gene1003   80   60  109   58   68  100   57   74   36   74   76   75   85   55   58
gene1004    1    2    1    1    3    0    5    0    0    1    1    3    1    2    0
gene1005  873  499  713  356  662 1259  575  585  236  820  937  521  486  317  809
gene1006   24   14   33   17   28   25   20   20   10   21   21   15   17   27   12
gene1007   64   29   86   46   49   79   52   57   28   65   67   22   75   38   54
gene1008   16    6   23   14   11   21   21   26   10   15   25   12   23   14   20
gene1009    9    8   17    5   14   17   13    9    2   12   18    6    5    9    7
gene101    29   39   29   42   47   46   68   40   16   41   48   80   46   28   41
gene1010    0    1    2    0    1    4    0    0    0    2    0    0    1    0    1
gene1011    0    1    0    0    0    0    0    1    0    0    2    0    0    0    1
gene1012    2    0    1    0    1    2    1    0    1    0    0    1    0    1    0
gene1013    0    0    2    0    2    0    0    0    1    1    0    0    0    0    1
gene1014    2    0    1    0    1    2    0    0    0    0    1    1    0    0    0
>
```

Differential Expression

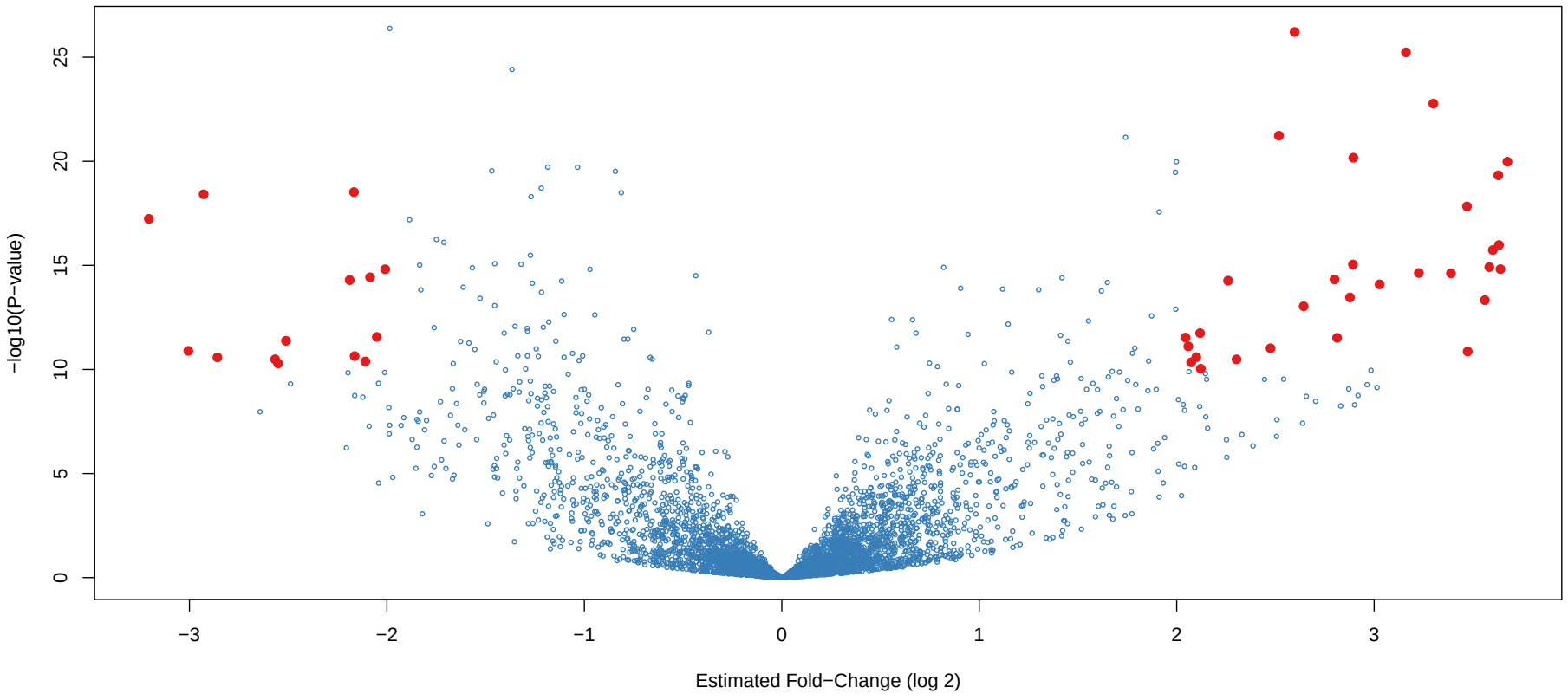
owzar001@cox: ~/CURRENT/hts-course-stat/CURRENT/Slides

owzar001@cox: ~/CURRENT/hts-course-stat/CURRENT/Slides | owzar001@cox: ~/CURRENT/hts-course-stat/CURRENT/Slides

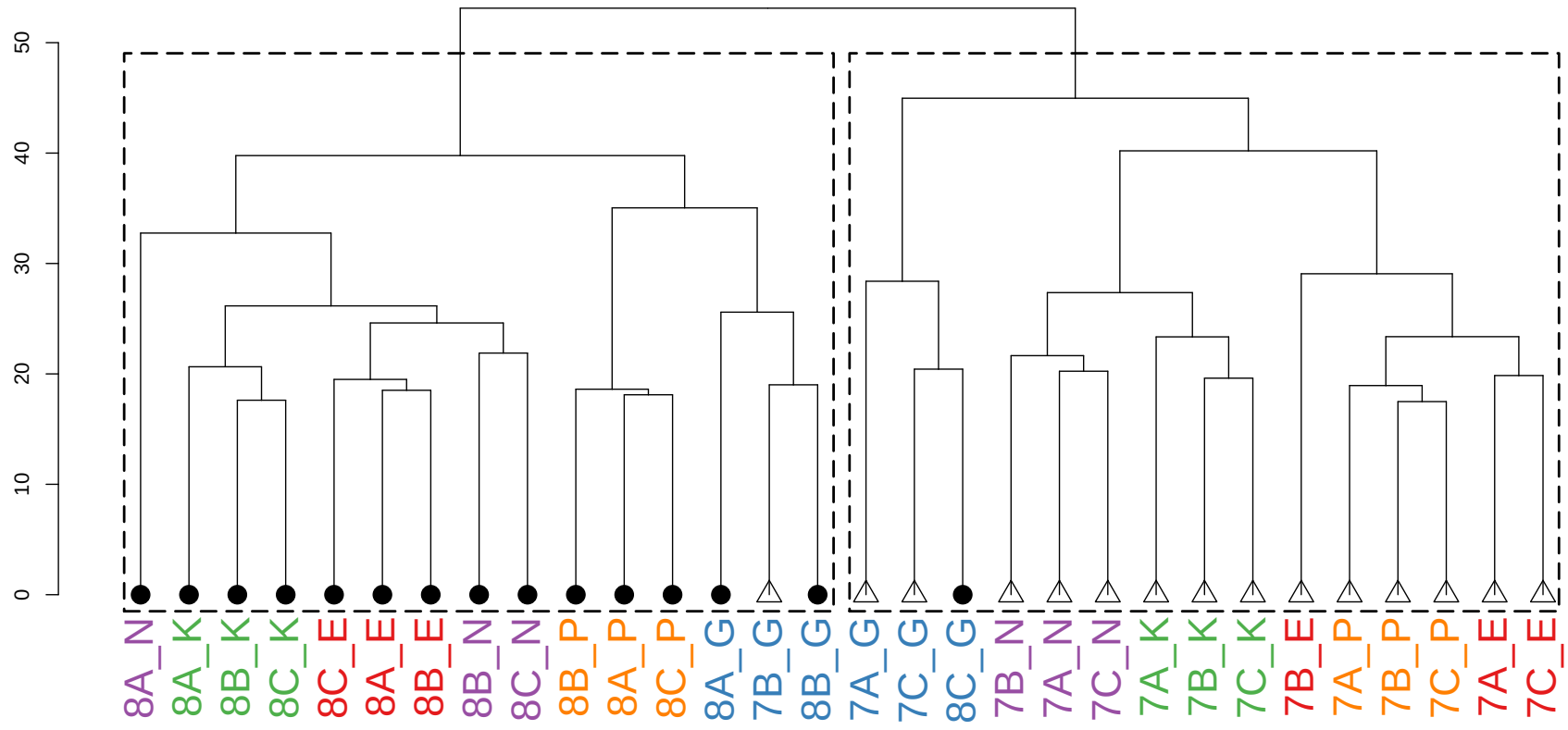
owzar001@cox: ~/CURRENT/hts-course-stat/CURRENT/Slides 85x23

```
fitting model and testing
-- replacing outliers and refitting for 46 genes
-- DESeq argument 'minReplicatesForReplace' = 7
-- original counts are preserved in counts(dds)
estimating dispersions
fitting model and testing
log2 fold change (MAP): trt 8 vs 7
Wald test p-value: trt 8 vs 7
DataFrame with 4444 rows and 6 columns
           baseMean log2FoldChange     lfcSE       stat       pvalue        padj
          <numeric>      <numeric> <numeric>  <numeric>    <numeric>   <numeric>
gene0     15.274431     0.28920009 0.2167382  1.3343292 0.1820959756 0.334270077
gene1    145.603062     0.43095114 0.1292386  3.3345378 0.0008544128 0.004147663
gene10     2.605083    -0.28595073 0.3674671 -0.7781668 0.4364706803 0.614286779
gene100   20.323396     0.08658647 0.1486582  0.5824532 0.5602614320 0.723906417
gene1000   6.582580    -0.43057986 0.2612653 -1.6480558 0.0993412243 0.214598998
...             ...            ...       ...        ...          ...         ...
gene995   1.6041044      0.6238433 0.4006699  1.5570009    0.1194703   0.2450365
gene996  10.3271263     -0.2176632 0.1992665 -1.0923221    0.2746915   0.4504187
gene997   6.8183976     -0.2618863 0.2651733 -0.9876041    0.3233466   0.5039471
gene998  29.3582205     -0.2004418 0.1752968 -1.1434424    0.2528549   0.4264820
gene999   0.6089341     -0.1343551 0.5377144 -0.2498632    0.8026931   0.8962573
> 
```

Differential Expression



Class Discovery



Recipe for Disaster: The Formula That Killed Wall Street

- This article appeared in Wired Magazine 17.03
- Written by Felix Salmon
- Claims that the naive use of the "normal copula" led to financial disaster

Copula

- Consider two outcomes say X (*e.g.*, price of a bond 1) and Y (*e.g.*, price of bond 2)
- Marginal effect of X: Statistical properties of X (*e.g.*, what is the mean value of X)
- Marginal effect of Y: Statistical properties of Y (*e.g.*, what is the median value of Y)
- Joint effect: How X and Y are related
- Copulas are functions that can be used to model the joint effect (dependency) between two outcomes
- Example: Is a change in X dependent on a change in Y
- The normal copula referenced in the article is a specific type of copula

Beautiful, simple, and, most commonly, tractable

"When you talk to market participants, they use words like *beautiful, simple, and, most commonly, tractable*. It could be applied anywhere, for anything, and was quickly adopted not only by banks packaging new bonds but also by traders and hedge funds dreaming up complex trades between those bonds."

Warnings ignored

"His method was adopted by everybody from bond investors and Wall Street banks to ratings agencies and regulators. And it became so deeply entrenched—and was making people so much money—that warnings about its limitations were largely ignored."

Devastating punch

"How could one formula pack such a devastating punch? The answer lies in the bond market, the multitrillion-dollar system that allows pension funds, insurance companies, and hedge funds to lend trillions of dollars to companies, countries, and home buyers."

The "inventor" can't be blamed

Gilkes of CreditSights: "After all, he just invented the model. Instead, we should blame the bankers who misinterpreted it. And even then, the real danger was created not because any given trader adopted it but because every trader did. In financial markets, everybody doing the same thing is the classic recipe for a bubble and inevitable bust."

Notes on article

- The "inventor" mentioned in the article did *not* invent the normal copula
- He may have introduced the concept to the finance/insurance industry
- If the premise of the article is true, then we will continue to pay for this naive use of a statistical approach
- What copulas have in common with many statistical approaches in genomics is that once somebody provides a software, anyone can conduct the analysis
- A basic understanding of the normal copula (*e.g.*, lack of tail-depency) would have immediately disqualifed its use in finance

Emphasis, Focus, Approach and Topics

- Concepts rather than on mechanics (*e.g.*, which software or method to use to fit a regression model)
- How statistical concepts are misunderstood or misinterpreted
- How and why things could go wrong
- Use simulation as a tool to illustrate these issues
- Topics:
  - Statistical Inference (testing and estimation)
  - Unsupervised learning (class discovery)
  - Multiple testing
  - Pathway/Gene-Set Analysis
  - Gene Networks
  - Distributions and regression models for counts

I hope that we can convince you that

- Bad: $P < 0.05$
- Worse: $P > 0.05$
- Statistical significance does imply biological significance
- Understand the concept of a confidence interval
- There is price to be paid every time you conduct a test
- You must distinguish between a decision and the truth
- There is a trade-off between false-positivity and false-negativity
- There is a trade-off between precision and accuracy
- You must identify the sources of variability
- You must be aware of algorithms that are self-fulfilling prophecies (most are)
- Think of sequencing data as count data

Limitations and Caveats

- This is not a complete course in Statistics
- While the presentation is *not* mathematically rigorous, some notation is used
- Exclusively focused on "frequentist" inference
- The "Bayesian" approach (plays a key role in genomics) is not covered due to time limitations

PCR/Microarray versus RNA-seq: Common objectives and challenges

- Hypothesis testing: Is the mRNA abundance related to a phenotype, or changed in response to treatment or over time
- Effect size estimation: How to quantify the effect size and then how to estimate it from data
- Classification: Predict an outcome on the basis of baseline RNA levels from multiple genes
- Class Discovery: Discover subsets on the basis of baseline levels or changes in the levels of multiple genes
- Multiplicity: how to deal with testing not a single marker but thousands if not millions of markers ($P < 0.05$ makes no sense here or anywhere)

RNA-Seq: A tool for measuring abundance of RNA from cells

- The data observed are not gene expressions (quantified on a continuum)
- We observe the number of reads mapped to each gene
- These are counts
- Microarrays: consider distributions and regression models for quantitative traits (often assume that these are normally distributed)
- RNA-Seq: consider distributions and regression models for counts

mRNA abundance, gene expressions and read counts

- Suppose that Y is the true abundance for a gene of interest
- $\hat{Y}$: the "expression" measured by microarray transcript (e.g., oligo nucleotide)
- K: The number of RNA-Seq reads mapped to the gene
- Questions:
  - Is $\hat{Y}$ close to Y (the truth)?
  - Is K close to Y (the truth)?
  - Should K even be compared with Y?

RNA-Seq: Two Approaches

- Two-stage method:
  - Convert counts to "Expression" (e.g., RPKM, FPKM, TPM)
  - plug these into a standard tests or regressions models
  - In essence: Force things into a microarray problem
- One-stage method:
  - Relate the counts directly to the phenotype
  - Use distributions and regression models for counts

Decision versus Truth

- Any statistical method will yield a decision
- Whether that conclusion of the decision is close to the truth or even reasonable will remain unknown
- We have to accept that the decision may be wrong
- Goal: Bound the probability of a wrong decision through the use of proper statistical design and methods
- and *proper* and *measured* interpretation of the results

The Simulation Method

- Simulate data from the "truth" *in silico* using computers
- Apply your proposed statistical method to the simulated (synthetic) data
- Repeatedly compare the decision at which you arrive (by virtue of the chosen statistical method) to the truth (under your control)

The Simulation Method: Noise Discovery

- Simulate data from noise
- Example: simulate treated and untreated samples from the same distribution
- Assess the proportion of times you arrive at the wrong conclusion
- Wrong conclusion: Conclude that there a treatment effect
- Important tool for identifying self-fulfilling prophecies.

On Statistics, Conclusions and Solutions

"No isolated experiment, however significant in itself, can suffice for the experimental demonstration of any natural phenomenon; for the 'one chance in a million' will undoubtedly occur, with no less and no more than its appropriate frequency, however surprised we may be that it should occur to us."

Ronald Aylmer Fisher (The Design of Experiments (1935), 16)

"Doing statistics is like doing crosswords except that one cannot know for sure whether one has found the solution."

John Wilder Tukey (Annals of Statistics, 2002:30(6))