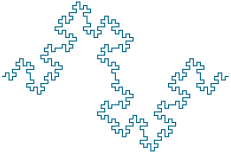
High-Throughput Sequencing Course

Introduction

Biostatistics and Bioinformatics



Summer 2018

Duke University School of Medicine

DUKE BIOSTATISTICS & BIOINFORMATICS

From Raw Unaligned Reads

```
owzar001@cox: ~/CURRENT/hts-course-stat/CURRENT/Slides
owzar001@cox: ~/CURRENT/hts-course-stat/CURRENT/Slides 85x24
@SRR546799.1 HWI-1KL120:92:C0F56ACXX:1:1101:1203:2232 length=50
CATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAAC
+SRR546799.1 HWI-1KL120:92:C0F56ACXX:1:1101:1203:2232 length=50
B@CFFFEFHHHHHFGHIGHHIJIHIIIJJIHBFHG=FFCEIIEAACECDE
@SRR546799.2 HWI-1KL120:92:C0F56ACXX:1:1101:1152:2242 length=50
CTCGTGAACTCATCTCCGGGGGTAGAGCACTGTTTCGGCAAGGGGGTCAT
+SRR546799.2 HWI-1KL120:92:C0F56ACXX:1:1101:1152:2242 length=50
@=?DBDBDFHHDDGHGHGIGGG77BFHIFIHIIIIGHGHHFF=?ADDB<A
@SRR546799.3 HWI-1KL120:92:C0F56ACXX:1:1101:1429:2119 length=50
ACCACGTGTCCCGCCCTACTCATCGAGCTCACAGCATGTGCATTTTTGTG
+SRR546799.3 HWI-1KL120:92:C0F56ACXX:1:1101:1429:2119 length=50
@@@FFFDFHHHH:EGIHGIEHEGHHHEHFHCFGCGGFHGHHIIHIIIIII
@SRR546799.4 HWI-1KL120:92:C0F56ACXX:1:1101:1376:2136 length=50
GTTAATCGGGGCAGGGTGAGTCGACCCCTAAGGCGAGGCCGAAAGGCGTA
+SRR546799.4 HWI-1KL120:92:C0F56ACXX:1:1101:1376:2136 length=50
@?@FFFFFHGHHHHJJ9CBGGHIIIJJJFGIGIIIBGHHFFDDDDDDD;?
@SRR546799.5 HWI-1KL120:92:C0F56ACXX:1:1101:1417:2140 length=50
CTGGGTTGTTTCCCTCTTCACGACGGACGTTAGCACCCGCCGTGTGTCTC
+SRR546799.5 HWI-1KL120:92:C0F56ACXX:1:1101:1417:2140 length=50
B?BFFFFDFHHHGJFHHGIGJFGHJGBGHIIJIGFHGIGGIHHEDDECEE
@SRR546799.6 HWI-1KL120:92:C0F56ACXX:1:1101:1320:2224 length=50
CCCAGAGCCTGAATCAGTGTGTGTGTTAGTGGAAGCGTCTGGAAAGGCGC
+SRR546799.6 HWI-1KL120:92:C0F56ACXX:1:1101:1320:2224 length=50
:
```

To Aligned Reads

```
owzar001@cox: ~/CURRENT/hts-course-stat/CURRENT/Slides
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SRR546799.9380746       0       AE005174-1      130     1       50M     *       0
       0       ACTTTAACCAATATAGGCATAGCGCACAGACAGATAAAAATTACAGAGTA      CCCFFFFFHHGHHJ
JJJJJJJJJJJJJJJJIJJJJJJJJJJJJJJJIJJJFG      AS:i:0  XS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:
i:0  NM:i:0  MD:Z:50 YT:Z:UU
SRR546799.8210755       0       AE005174-1      164     1       50M     *       0
       0       TAAAAATTAGAGAGTACACAACATCCATGAAACGCATTAGCACCACCATT      BBCFFFFFH?DHHJ
EGIJJJJJJJJJJJIIJIIGIJJJJJJIJJJJJJIJI      AS:i:-5 XS:i:-5 XN:i:0  XM:i:1  XO:i:0  XG:
i:0  NM:i:1  MD:Z:9C40       YT:Z:UU
SRR546799.6023888       0       AE005174-1      165     1       50M     *       0
       0       AAAAATTACAGAGTACACAACATCCATGAAACGCATTAGCACCACCATTA      CCCFFFFFHHHHHG
IJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ      AS:i:0  XS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:
i:0  NM:i:0  MD:Z:50 YT:Z:UU
SRR546799.6299012       0       AE005174-1      165     1       50M     *       0
       0       AAAAATTACAGAGTACACAACATCCATGAAACGCATTAGCACCACCATTA      CCCFFFFFHHHHHG
HIJJJJJJJJJJJJJJJJJJJJJJJJJJJIIGIJJJJJG      AS:i:0  XS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:
i:0  NM:i:0  MD:Z:50 YT:Z:UU
SRR546799.4423177       0       AE005174-1      179     1       50M     *       0
       0       ACACAACATCCATGAAACGCATTAGCACCACCATTACCACCACCATCACC      BC@DDFFFHHHHHJ
JJJ@GHIJJFIIJJJIJIJJJJJGEIJJJJJJJJIF      AS:i:0  XS:i:0  XN:i:0  XM:i:0  XO:i:0  XG:
i:0  NM:i:0  MD:Z:50 YT:Z:UU
SRR546799.151531        0       AE005174-1      182     0       50M     *       0
       0       CAACATCCATGAAACGCATTAGCACCACCATTACCACCACCATCACCATT      CCCFFFFFHHHHHI
JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJI      AS:i:-12        XS:i:-12        XN:i:0  XM:
:
```

To Counts

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

```
> 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

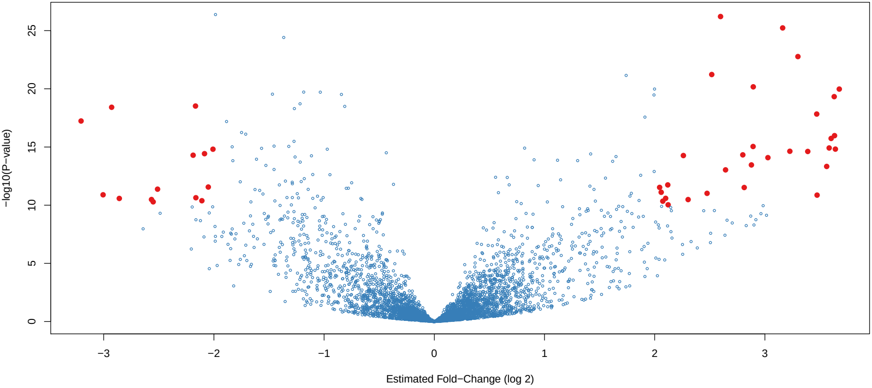
owzar001@cox: ~/CURRENT/hts-course-stat/CURRENT/Slides

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

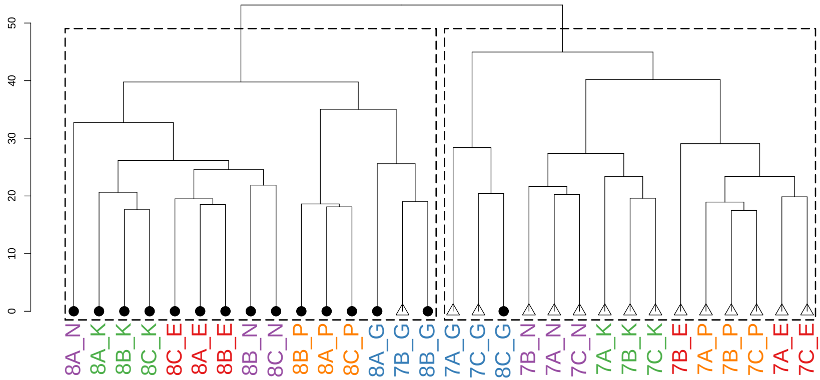
owzar001@cox: ~/CURRENT/hts-course-stat/CURRENT/Slides 85x23

```
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



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

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)
  - Supervised learning (classification and regression)
  - Unsupervised learning (class discovery)
  - Multiple testing
  - Pathway/Gene-Set Analysis
  - Meta-Analysis
  - 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))