GS01 0163
Analysis of Microarray Data

Keith Baggerly and Brad Broom
Department of Bioinformatics and Computational Biology
UT M. D. Anderson Cancer Center
kabagg@mdanderson.org
bmbroom@mdanderson.org

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Lecture 23: Batch Effects

- What are batch effects? How do we plan for them?
- How do we detect them?
- How do we correct for them?
- DWD – Benito et al, Bioinf 20:105-14, 2004
- COMBAT – Johnson et al, Biostat 8:118-27, 2007
- TCGA batches and genes

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Batch Effect Sources and Planning

This is a free-for-all slide, in that we've talked about these several times now.

Some causes?

Some ways of planning?

An ovarian cancer case study:
Dressman et al, JCO 2007
Baggerly et al, JCO 2008.

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Signatures and Pathways for Response in Ovarian Cancer

An Integrated Genomic-Based Approach to Individualized Treatment of Patients With Advanced-Stage Ovarian Cancer

Holly K. Dressman, Andrew Berchuck, Gina Chan, Jun Zhai, Andrea Bild, Robyn Sayer, Janiel Cragun, Jennifer Clarke, Regina S. Whitaker, LiHua Li, Jonathan Gray, Jeffrey Marks, Geoffrey S. Ginsburg, Anil Potti, Mike West, Joseph R. Nevins, and Johnathan M. Lancaster

JCO, Feb 10, 2007.

Using profiles of 119 ovarian tumors, they looked for signatures of response to cisplatin-based chemo. The also looked at the deregulation levels of 5 pathways (Src, β-catenin, Myc, E2F3, and Ras), trying to relate them to survival.

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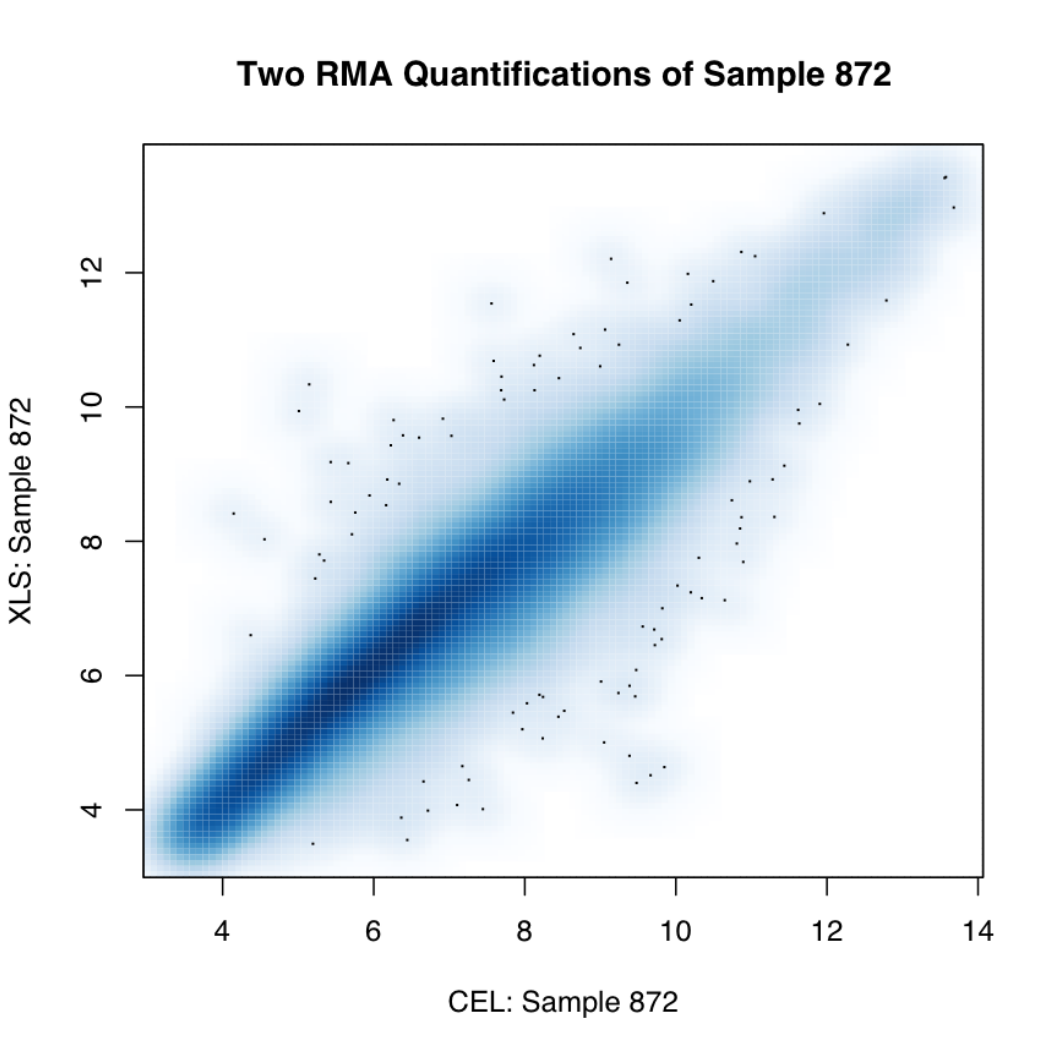
Checking Agreement



Ours vs theirs. We expected better (fewer outliers).

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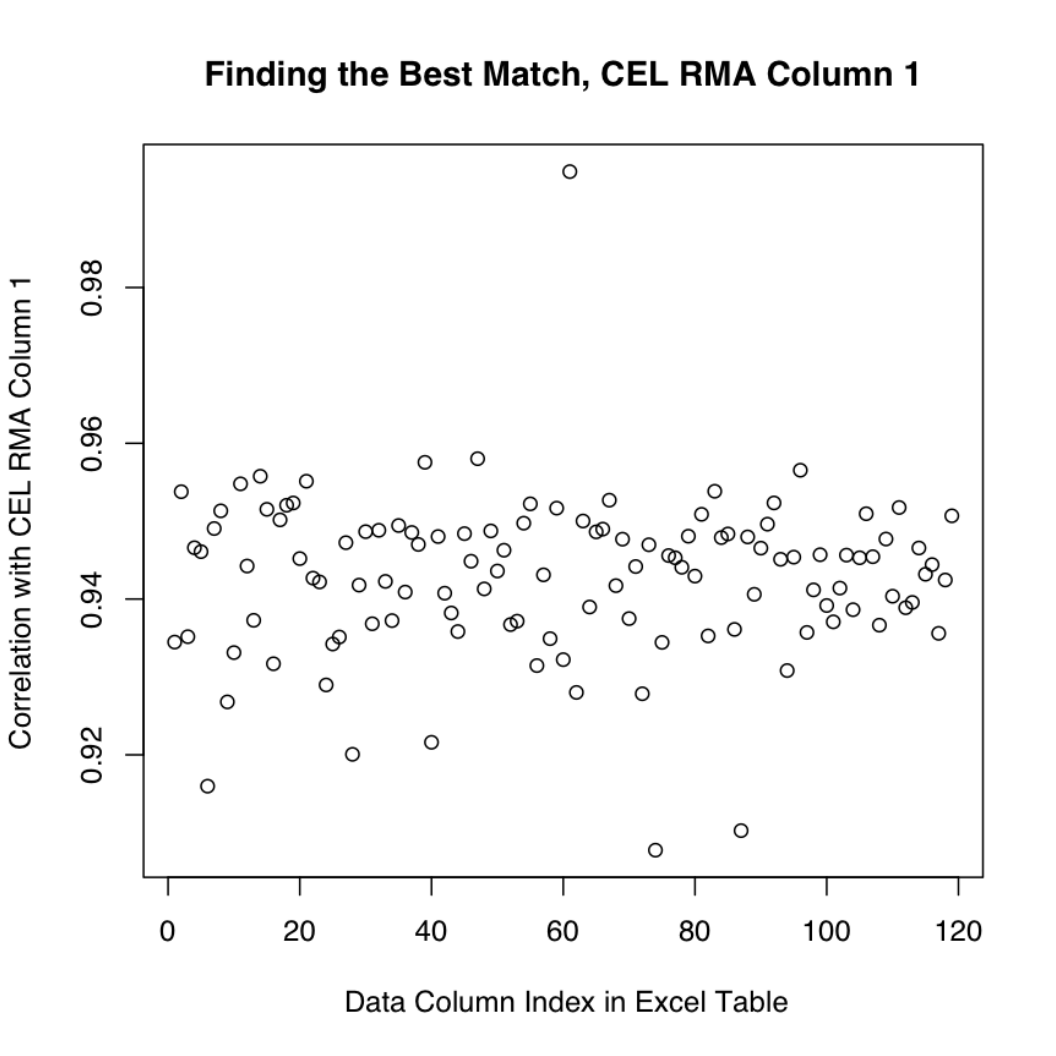
Looking at Their Other Quants



Which one would you pick?

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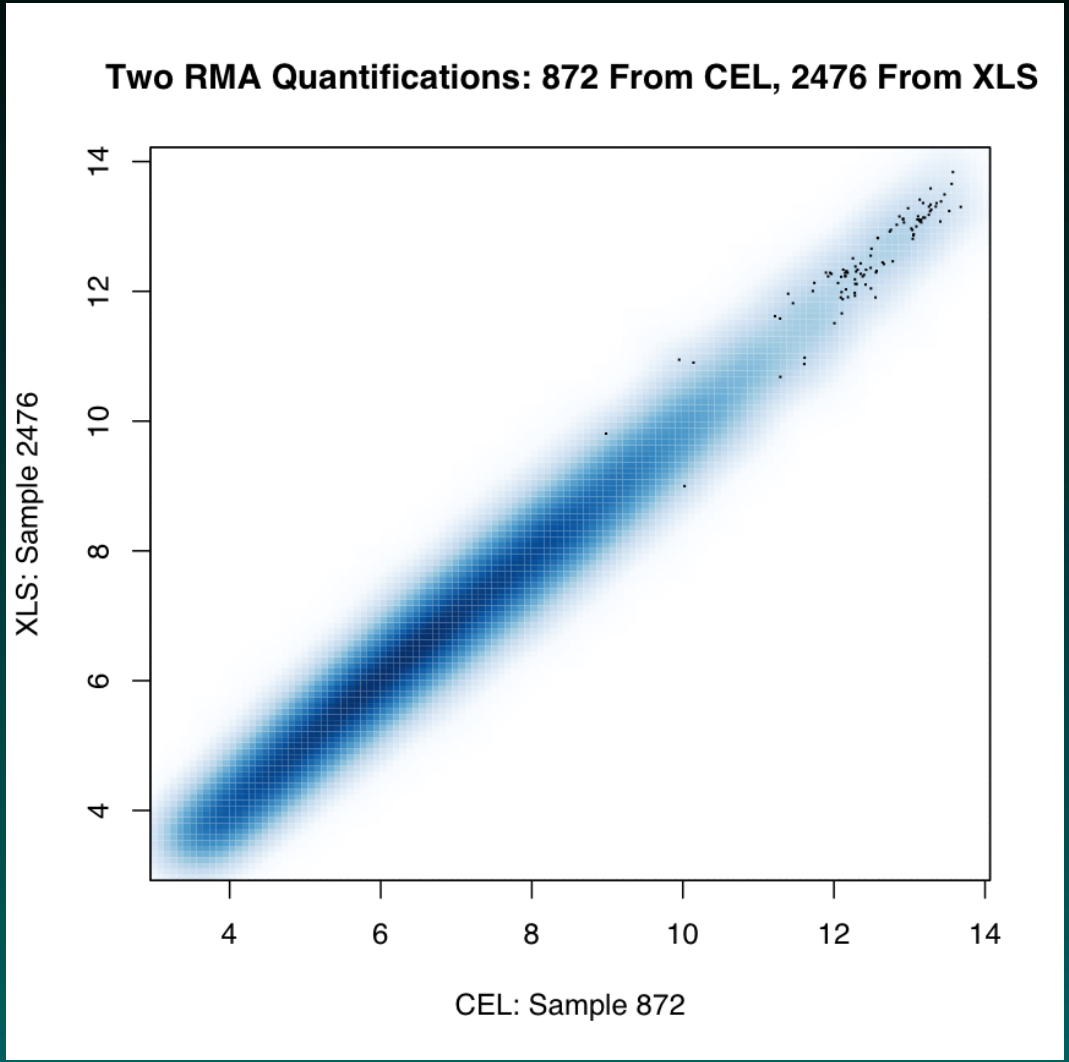
Looking at The "Best" Fit



Same array. *Different* names (2476 from XLS, 872 from CEL).

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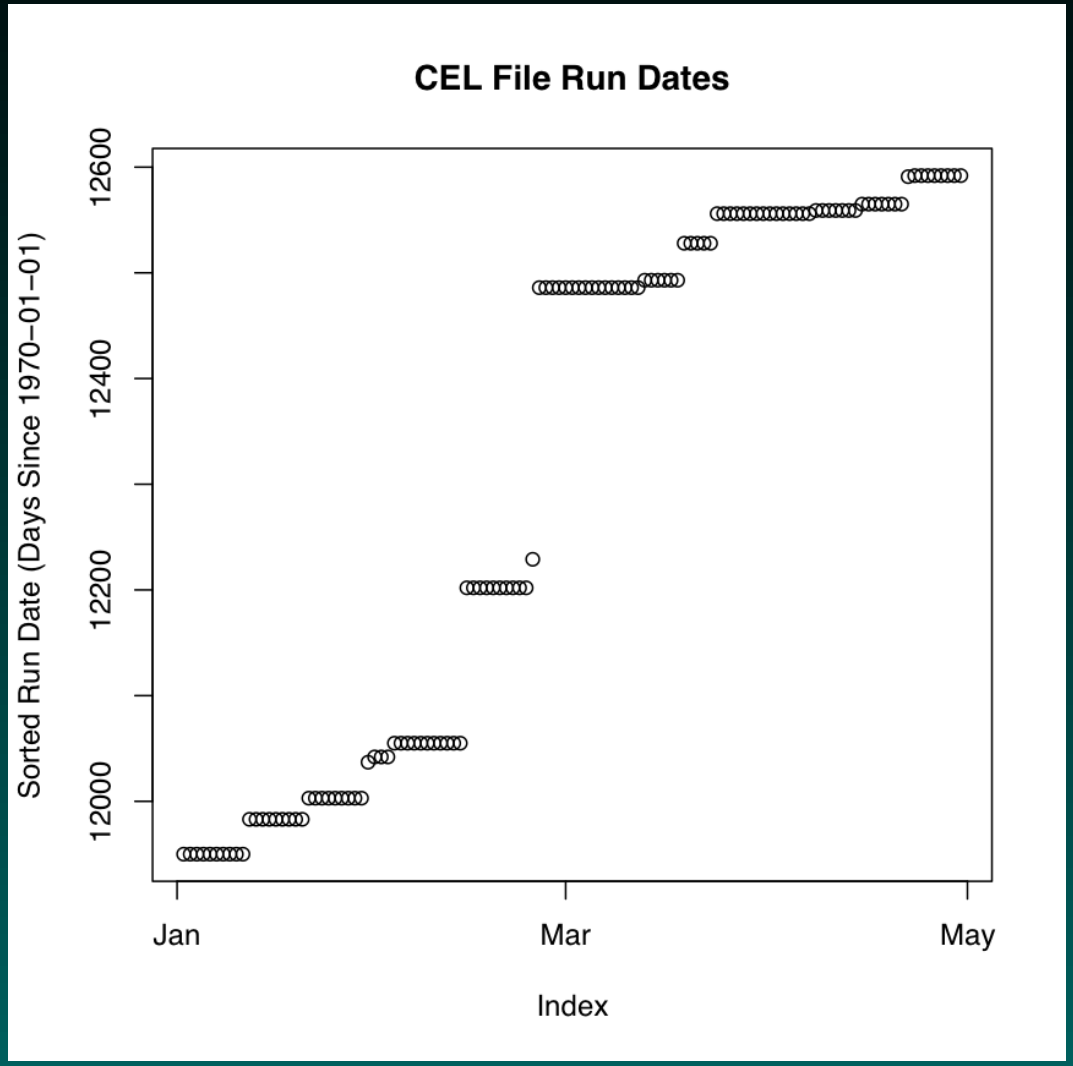
Grab One More Thing: Run Date



Should we be worried?

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Some Berchuck et al Clinical Info

2392	Early Stage
2393	Early Stage
1772	Long
1773	Long
1774	Long
1775	Long
1776	Long
1777	Long
1778	Long
1779	Long
1780	Long
1781	Long
1900	Long

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The Berchuck et al Dat Headers

```
0074_1772_h133a_872.cel:DatHeader=[0..37764] .. 09/20/02 11
0074_1773_h133a_922.cel:DatHeader=[0..33251] .. 09/20/02 11
0074_1774_h133a_1451.cel:DatHeader=[0..43335] .. 09/20/02 1
0074_1775_h133a_1526.cel:DatHeader=[0..45012] .. 09/20/02 1
0074_1776_h133a_1784.cel:DatHeader=[0..46104] .. 09/20/02 1
0074_1777_h133a_1834.cel:DatHeader=[0..42469] .. 09/20/02 1
0074_1778_h133a_1846.cel:DatHeader=[0..36713] .. 09/20/02 1
0074_1779_h133a_2075.cel:DatHeader=[0..37459] .. 09/20/02 1
0074_1780_h133a_2204.cel:DatHeader=[0..43583] .. 09/20/02 1
0074_1781_h133a_2419.cel:DatHeader=[0..46101] .. 09/20/02 1
0074_1827_h133a_.08.cel:DatHeader=[0..46104] .. 10/23/02 12
0074_1828_h133a_860.cel:DatHeader=[0..46102] .. 10/23/02 12
```

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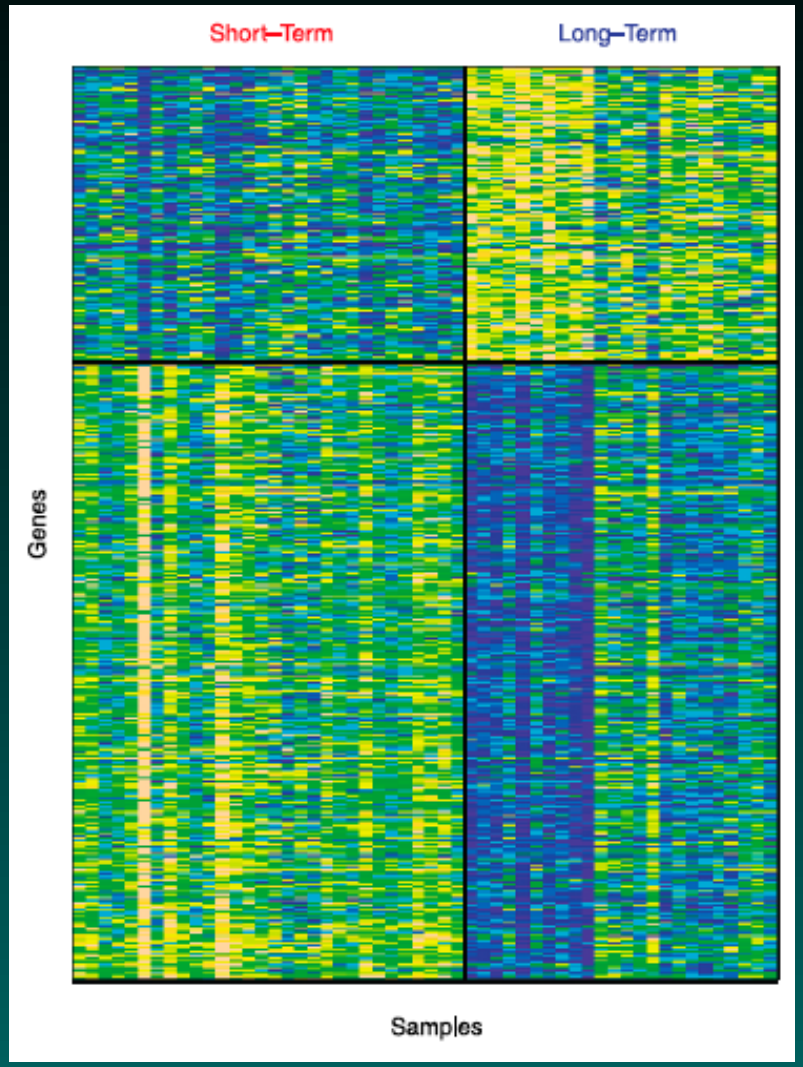
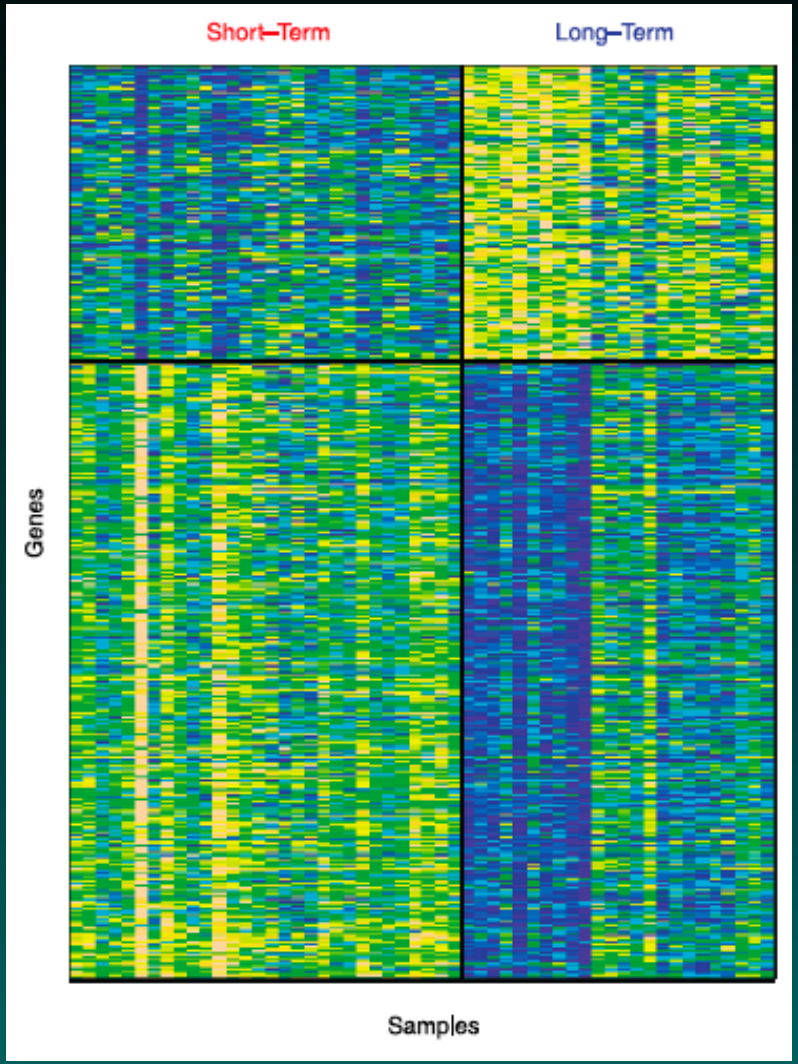
The Berchuck et al Heatmap



Can you spot clusters?

The Berchuck et al Heatmap



Can you spot clusters? Expression tracks with batch.

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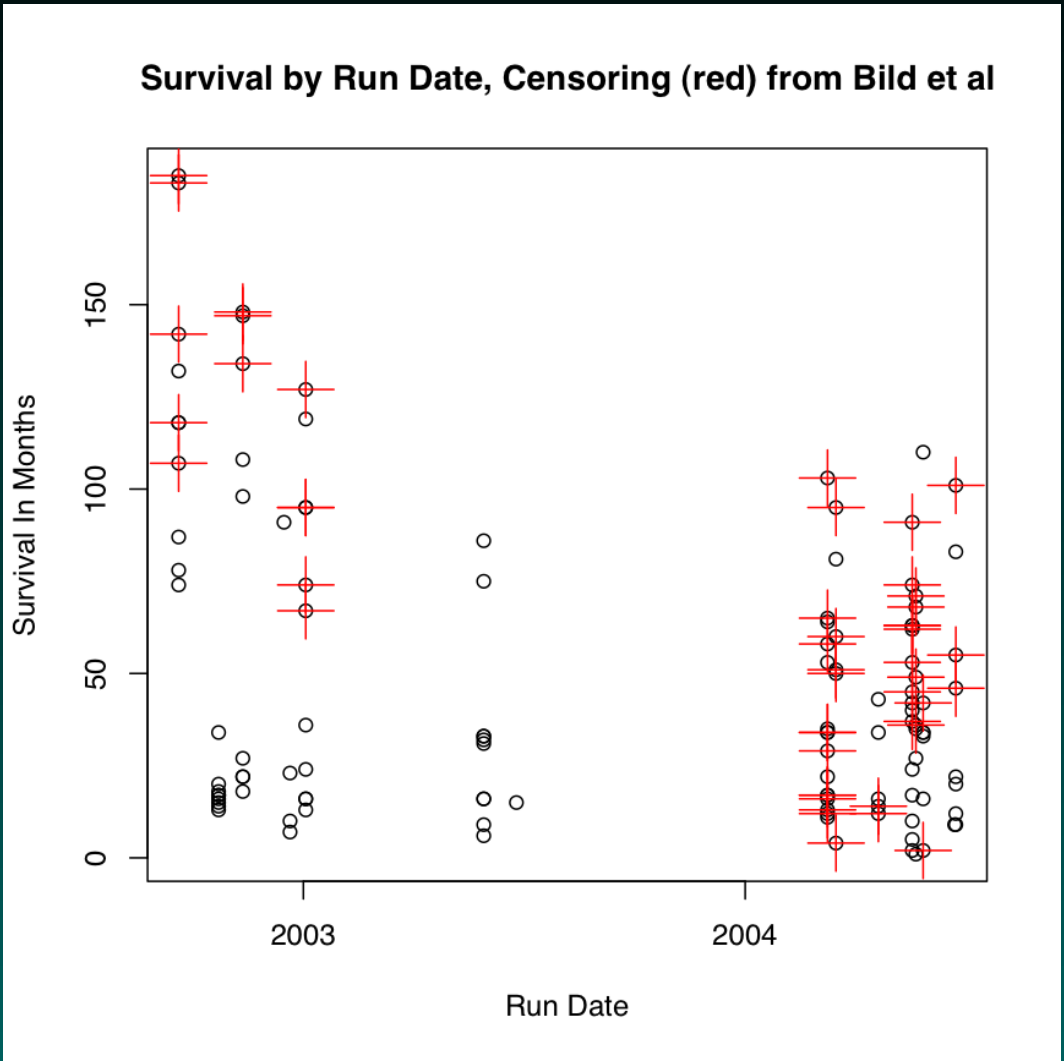
Survival By Date (Bild Censoring)



Survival is confounded with date.

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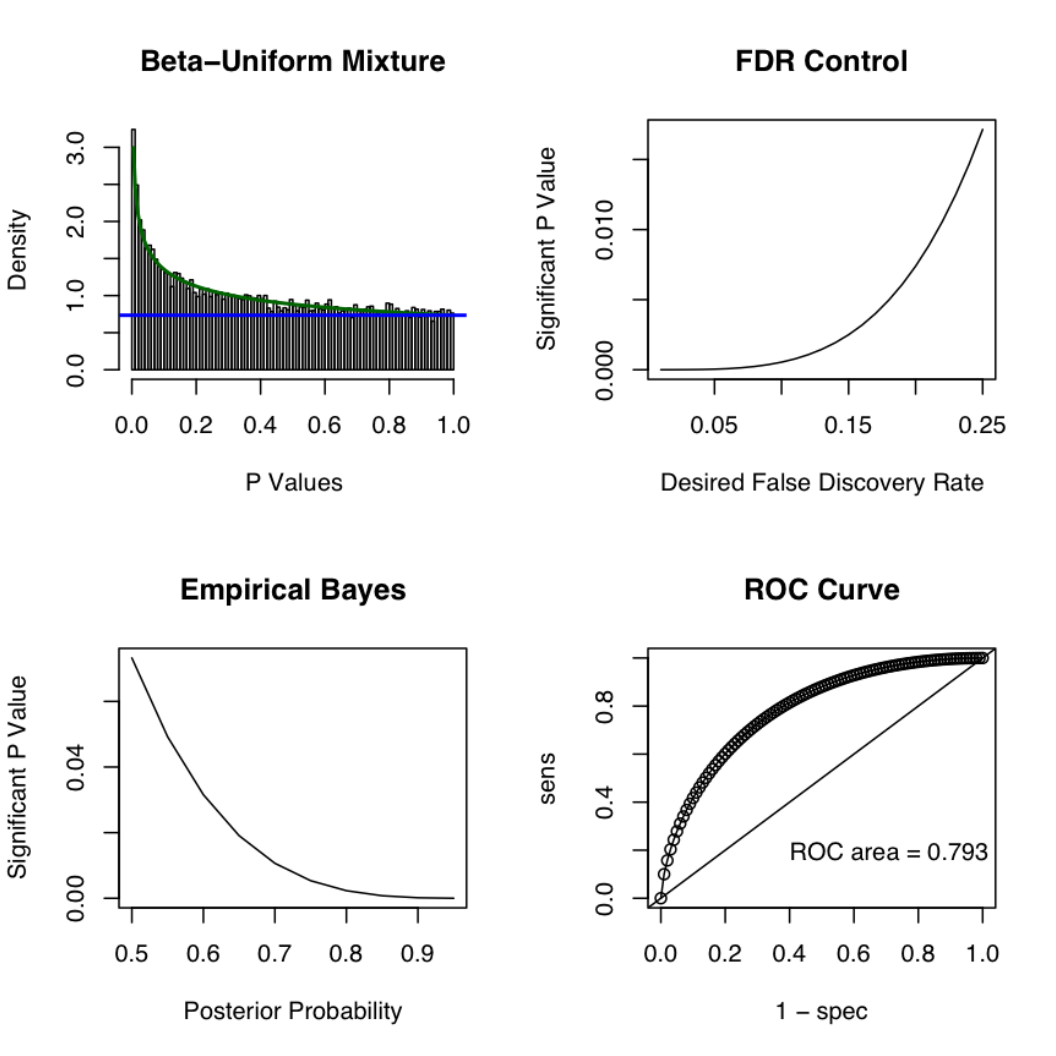
Is There Division by CR/NR?



Maybe something.

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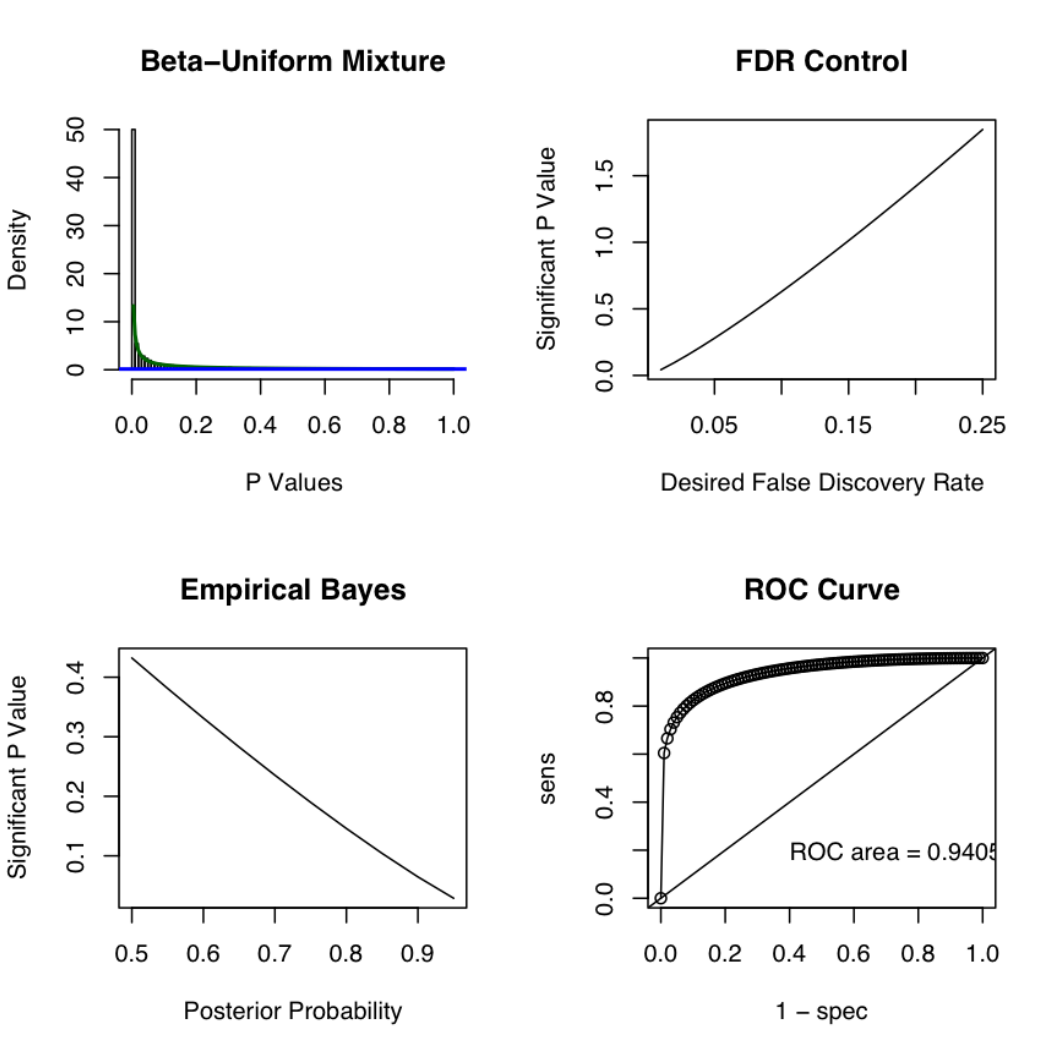
Is There Division by Date?



Erm, yep.

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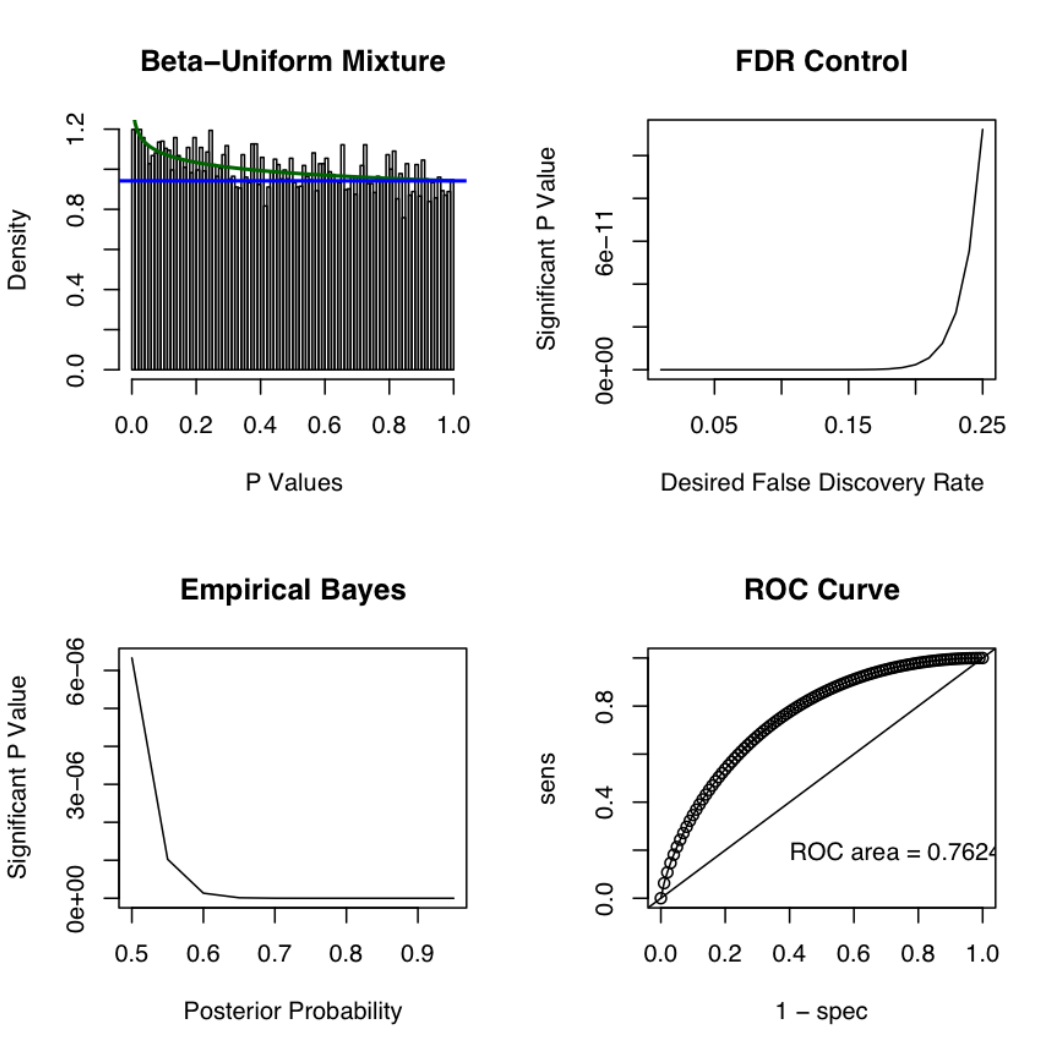
Division by CR/NR After Date?



Not really.

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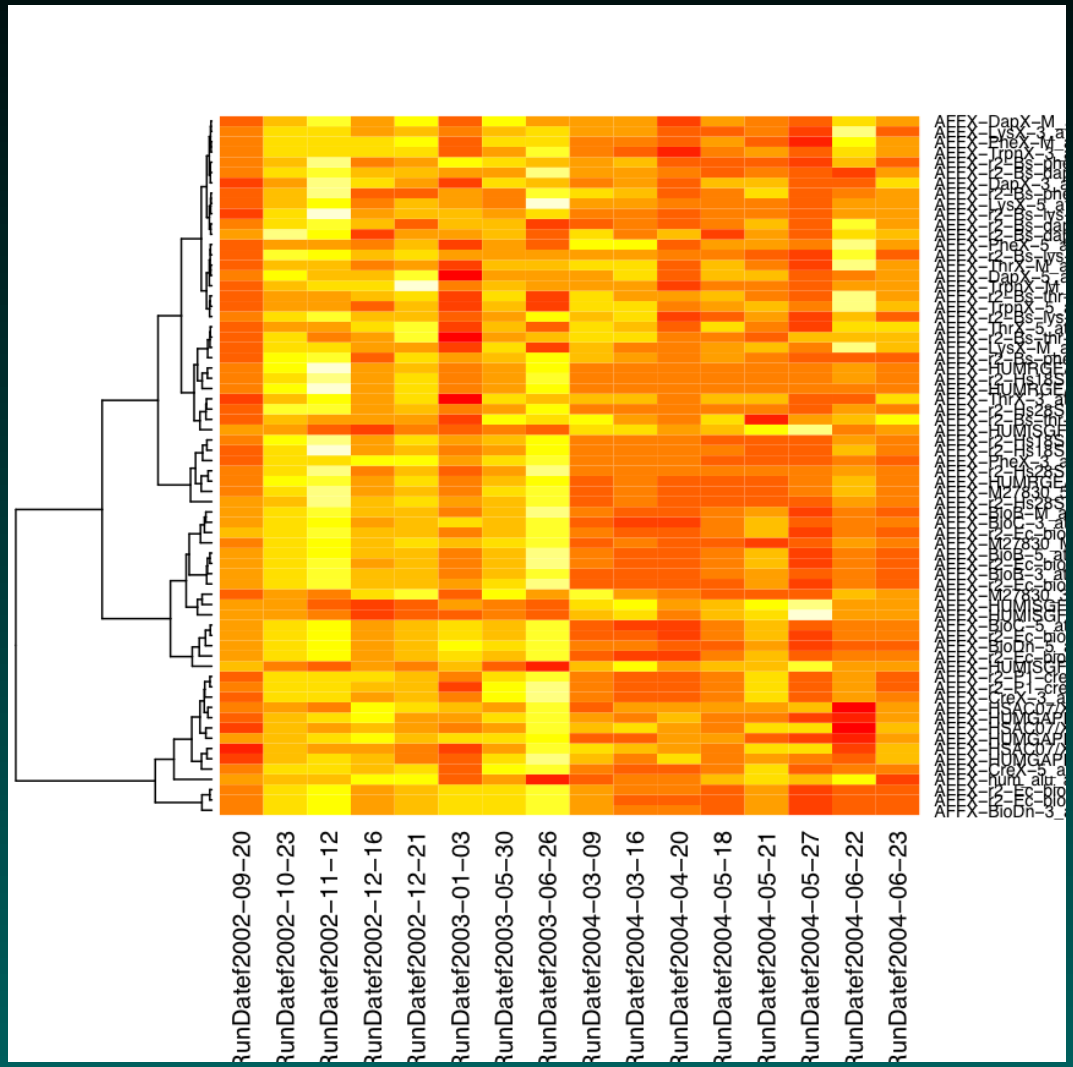
How Many Batches? (Controls)



Using Affy controls, we see 7 blocks.

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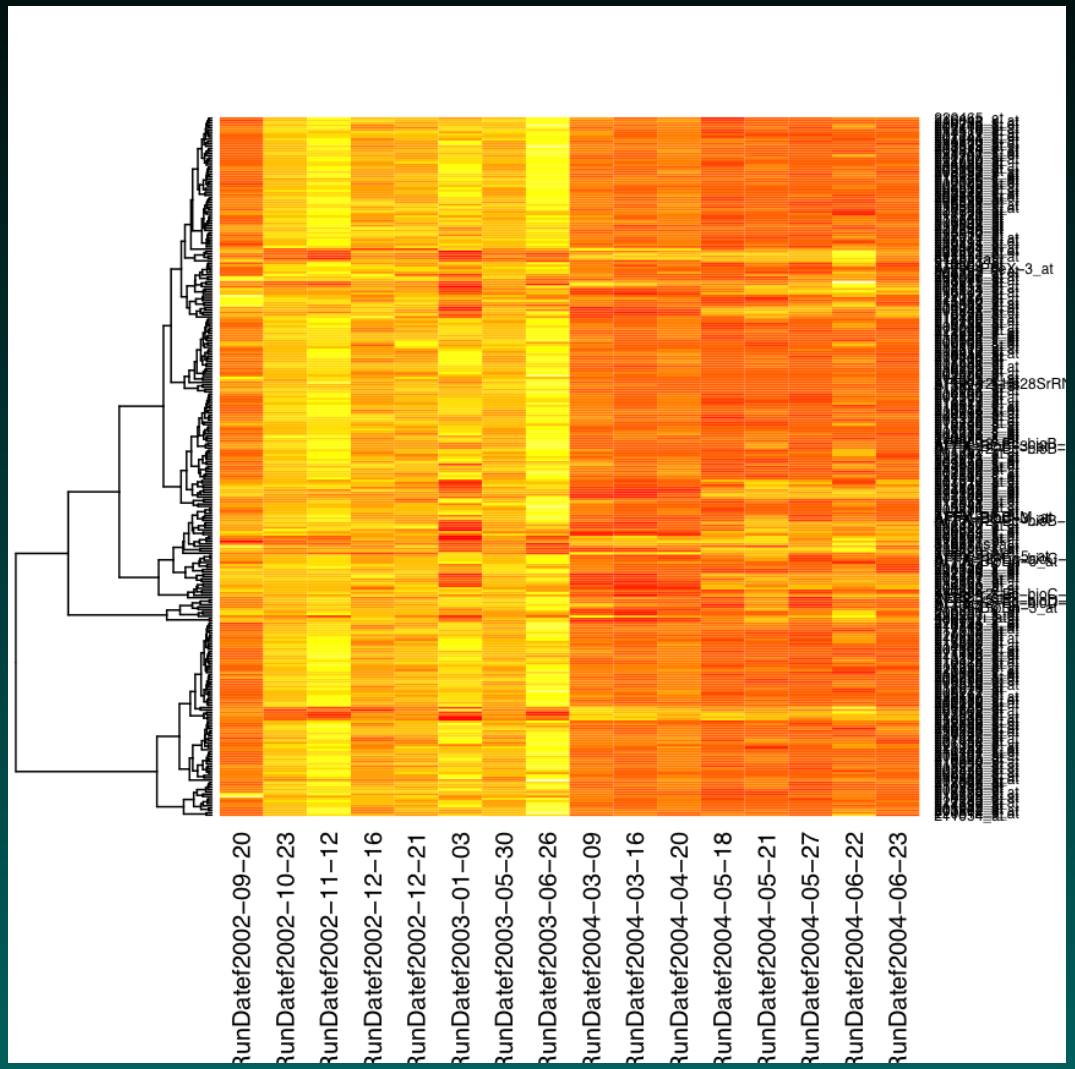
How Many Batches? (Top ANOVAs)



The smallest ANOVA p-values show the same.

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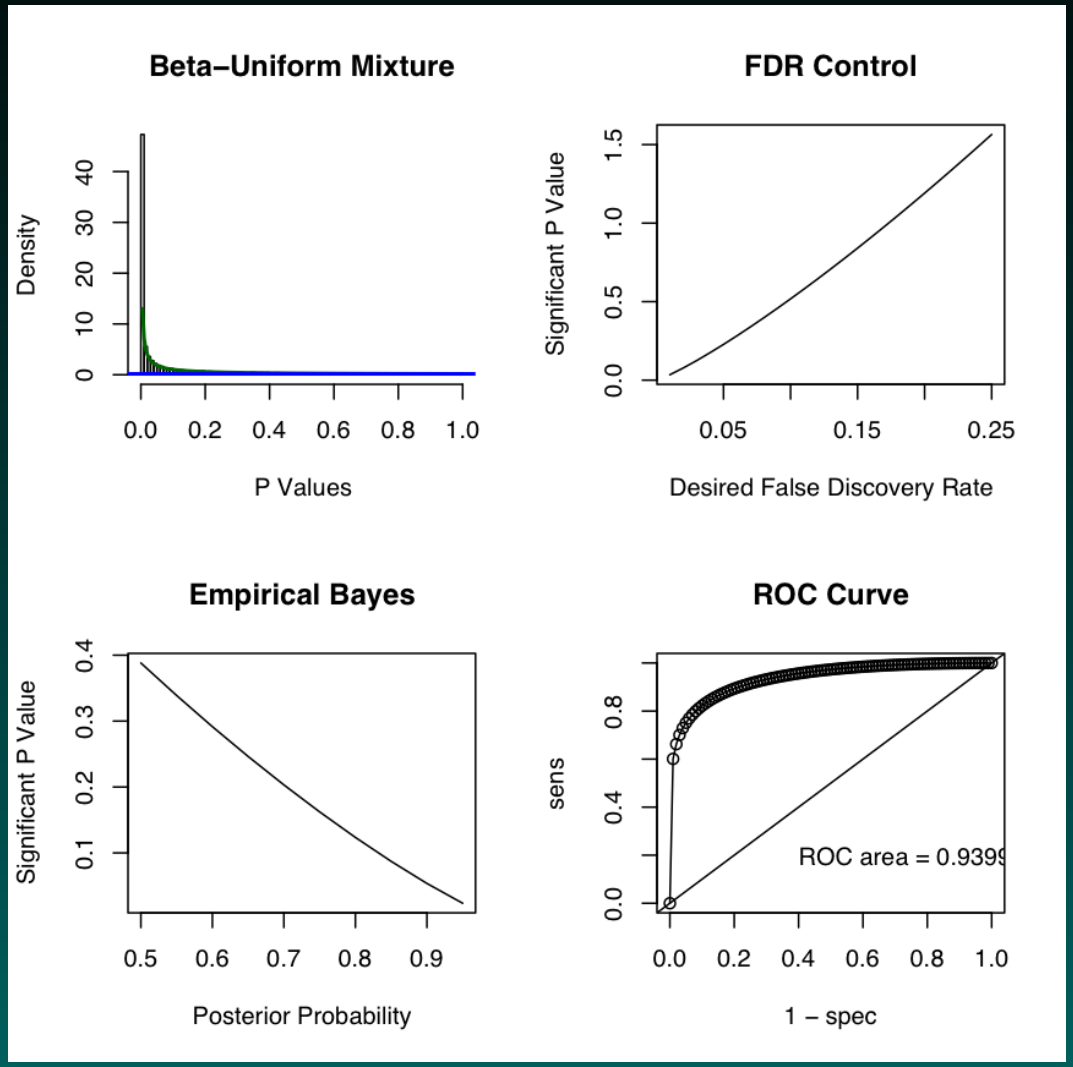
Is There Division by Batch?



We think we caught most of it.

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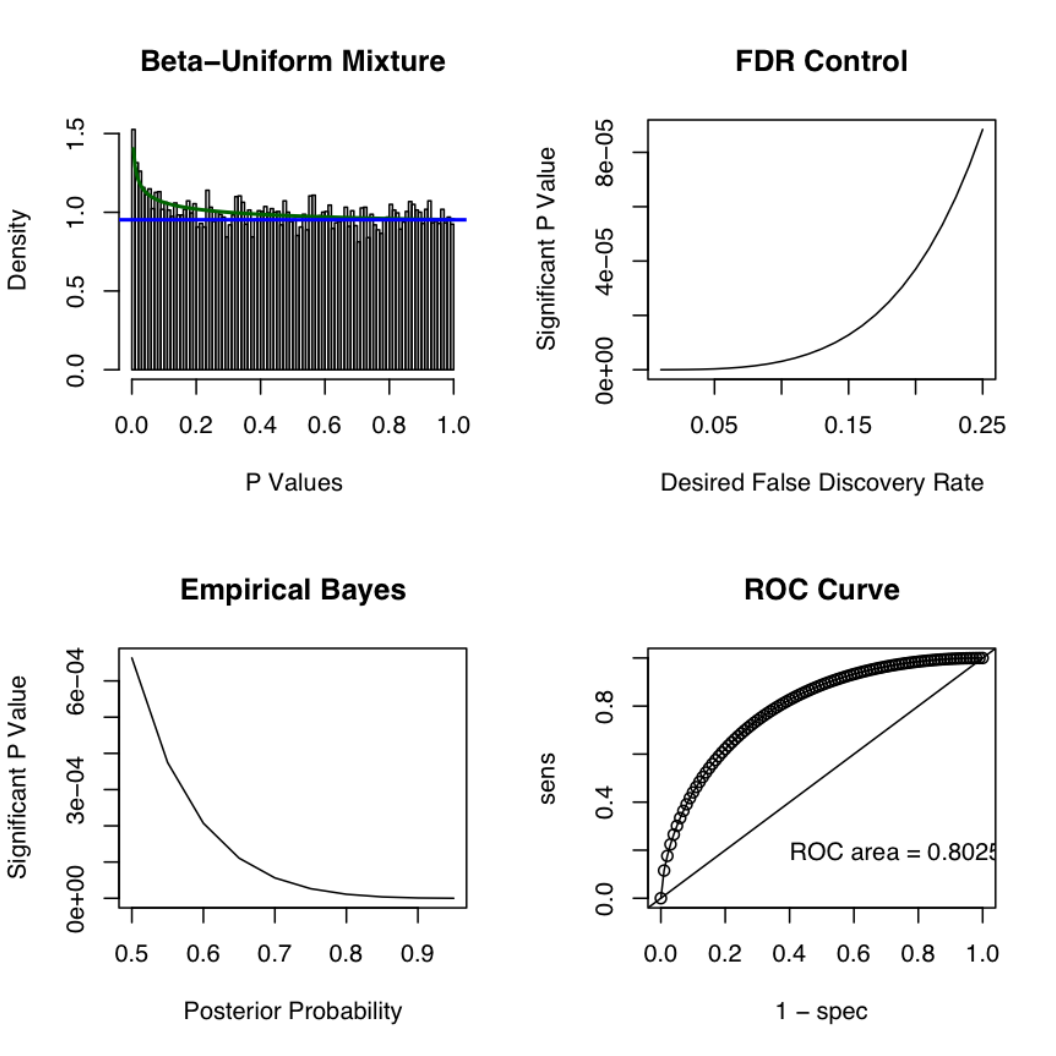
Division by CR/NR After Batch?



Maybe some, but the FDR isn't small.

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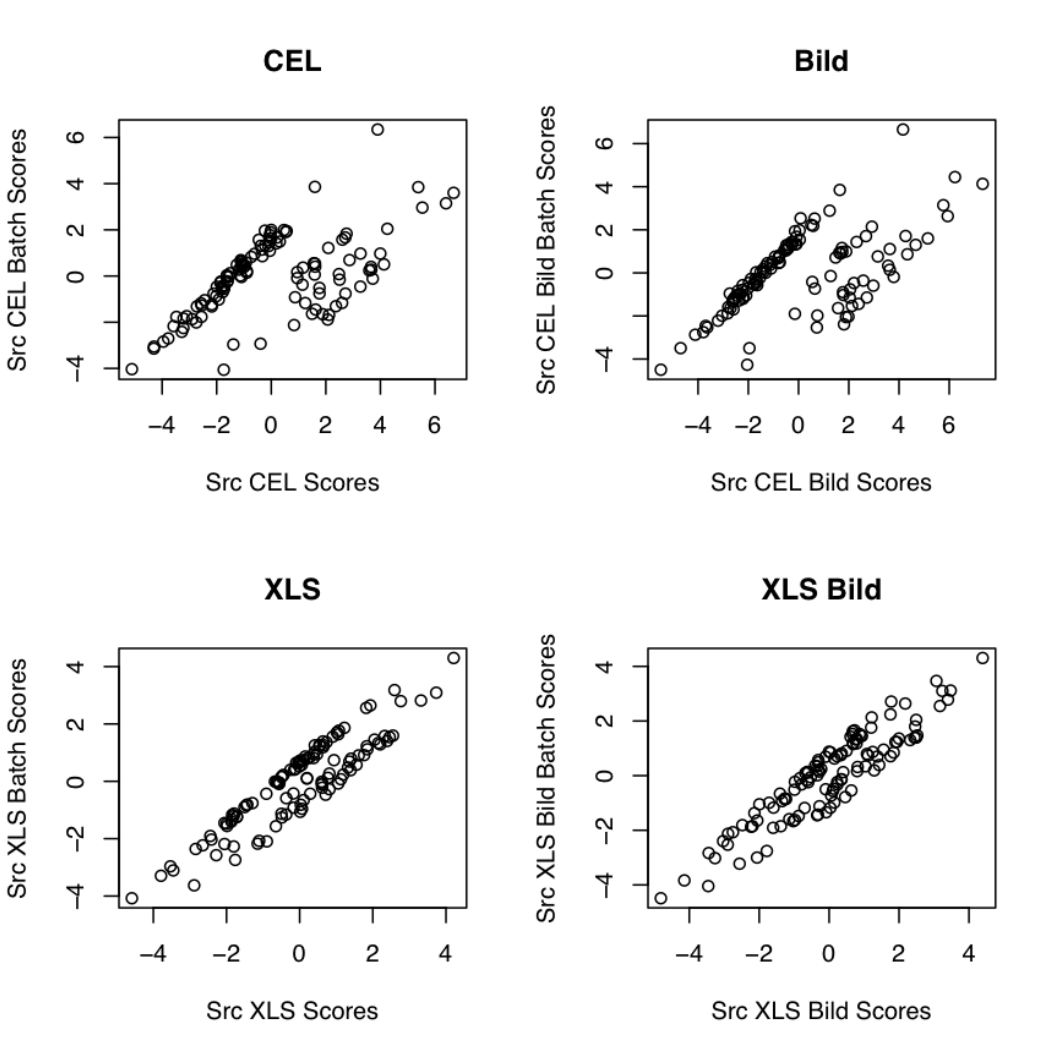
Batches Affect Scores



Offsets are quite visible

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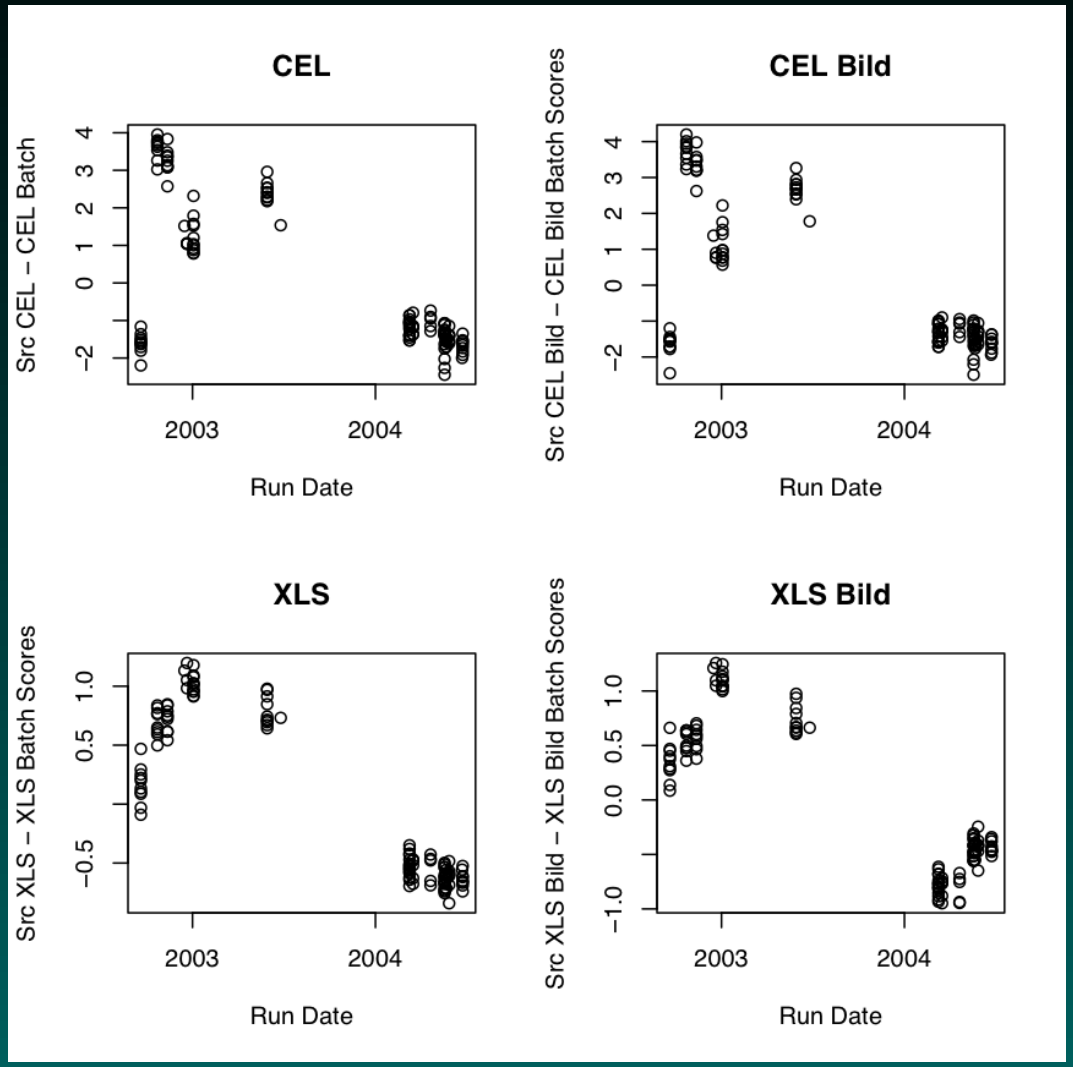
Early Batch Effects Are Larger



Remember, batches are confounded with Survival!

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So, how did we get Run Date?

```
> celDatHeaders <- celFiles
> for (i1 in 1:length(celDatHeaders)) {
      temp <- read.celfile.header(file.path("DukeWe
          celFiles[i1]), info = "full")
      celDatHeaders[i1] <- temp$DatHeader
  }
```

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Eyeballing DatHeader Lines

```
> celDatHeaders[1]
872
 "[0..37764]  0074_1772_H133A_872:CLS=4733
  RWS=4733 XIN=3  YIN=3  VE=17        2.0
  09/20/02 11:43:50    \024  \024 HG-U133A.1sq
  \024  \024  \024  \024  \024  \024  \024  \024
  \024 6"
celDatHeaders[119]
M810
 "[0..29187]  Robyn 810 CR-2:CLS=5391 RWS=5391
  XIN=2  YIN=2  VE=30        2.0 04/20/04
  11:29:32 50101330  M10    \024  \024 HG-U133A.1s
  \024  \024  \024  \024  \024  \024  \024  \024
  \024 6"
```

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Extracting Dates

```
> tempDate1 <- strsplit(celDatHeaders, "2\\.0 ")
> tempDate2 <- unlist(lapply(tempDate1, function(x
    x[2]
 }))
> tempDate3 <- unlist(lapply(tempDate2, function(x
    substr(x, 1, 17)
 }))
> tempDate3[1]
                 872
"09/20/02 11:43:50"
```

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Formatting Dates

```
> celRunDate <- as.Date(tempDate3,
    format = "%m/%d/%y %H:%M:%S")
> names(celRunDate) <- names(tempDate3)
> celRunDate <- celRunDate[rownames(clinicalInfo)]
> celRunDate[1:3]
      0.08        860        872
"2002-10-23" "2002-10-23" "2002-09-20"
```

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Tabulating Dates

```
> table(celRunDate)
celRunDate
2002-09-20 2002-10-23 2002-11-12 2002-12-16 2002-1
        10          9          9          1
2003-06-26 2004-03-09 2004-03-16 2004-04-20 2004-0
         1         16          6          5
2004-06-22 2004-06-23
         1          8
```

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Using Dates

```
> names(celRunDate[celRunDate < "2004-03-09"])
 [1] "0.08" "860"  "872"  "922" ...
[51] "3102" "3107" "3142" "3249"
> names(celRunDate[celRunDate >= "2004-03-09"])
 [1] "D1805" "D1837" "D1859" "D2098" ...
[64] "M6199" "M810"
> sum(celRunDate < "2004-03-09")
[1] 54
> table(celRunDate, clinicalInfo$Response)
celRunDate   CR NR
  2002-09-20 10  0
  2002-10-23  2  7
  2002-11-12  8  1
  2002-12-16  1  0
```

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So, how did we Correct for Batch?

```
> runBatch <- rep(6, 119)
> runBatch[celRunDate == "2002-09-20"] <- 1
> runBatch[celRunDate == "2002-10-23"] <- 2
> runBatch[celRunDate == "2002-11-12"] <- 2
> runBatch[celRunDate == "2002-12-16"] <- 3
> runBatch[celRunDate == "2002-12-21"] <- 3
> runBatch[celRunDate == "2003-01-03"] <- 3
> runBatch[celRunDate == "2003-05-30"] <- 4
> runBatch[celRunDate == "2003-06-26"] <- 4
> runBatch[celRunDate == "2004-03-09"] <- 5
> runBatch[celRunDate == "2004-03-16"] <- 5
> runBatch[celRunDate == "2004-04-20"] <- 5
> runBatch[celRunDate == "2004-05-18"] <- 6
> runBatch[celRunDate == "2004-05-21"] <- 6
```

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```
> runBatch[celRunDate == "2004-05-27"] <- 6
> runBatch[celRunDate == "2004-06-22"] <- 6
> runBatch[celRunDate == "2004-06-23"] <- 6
> runBatch <- as.factor(runBatch)
> names(runBatch) <- names(celRunDate)
```

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Modeling Residuals

Fit gene expression values as a function of runBatch, then fit residuals after correcting for batch.

```
> batchModelForm <- Y ~ runBatch
> batchModelLMAll <-
    MultiLinearModel(batchModelForm,
    data.frame(runBatch = runBatch),ovcaRMAFromCEL
> ovcaRMAFromCELResids <- ovcaRMAFromCEL -
    t(batchModelLMAll@predictions)
> responseModelForm <- Y ~ Response
> responseModelLMAll <-
    MultiLinearModel(responseModelForm,
    data.frame(Response = clinicalInfo$Response),
       ovcaRMAFromCELResids)
```

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Other Approaches

Principal Components/Singular Value Decompostion

Excellent for getting a high-level view of the structure in your data, and seeing if there are outliers or outlying clusters.

Hence, useful for revealing batches, but not so useful for removing them – it doesn't use labels at all.

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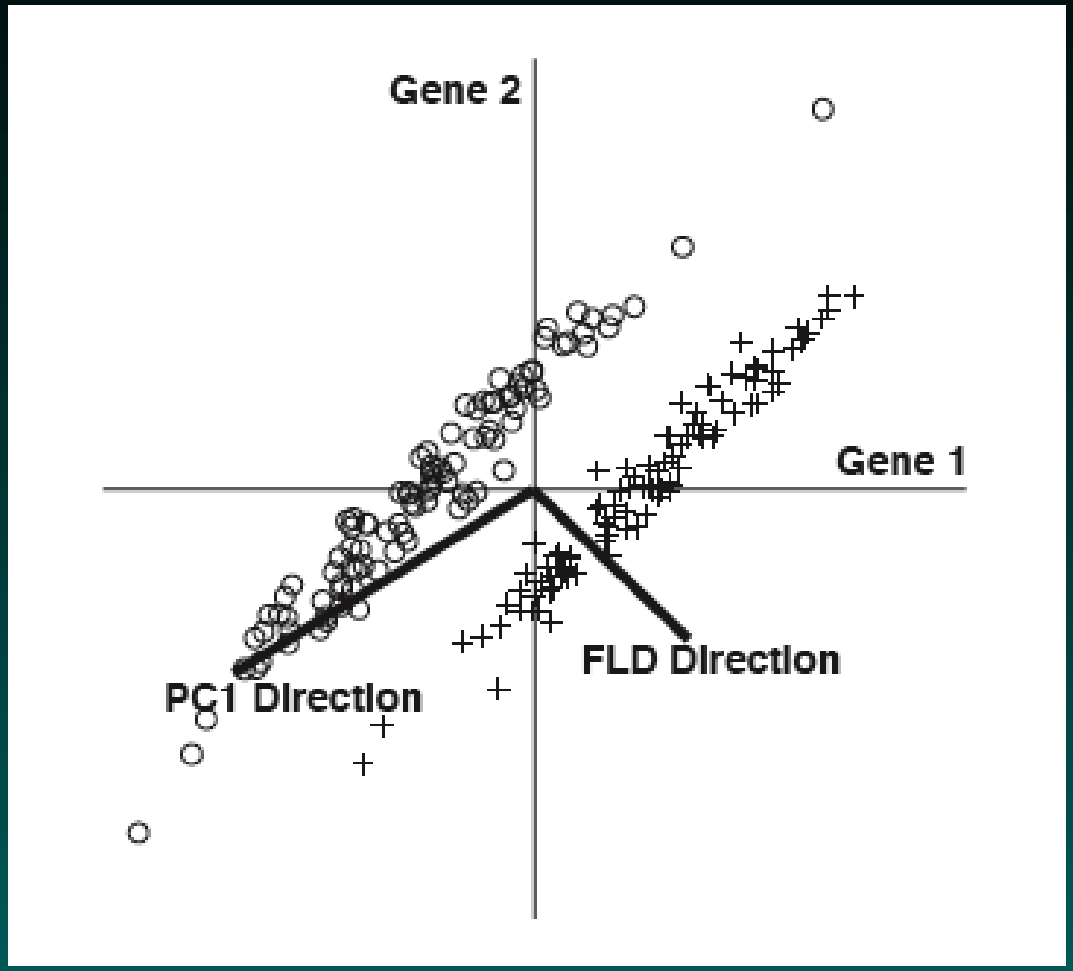
Where SVD Breaks



Benito et al, Bioinf 2004, fig 1. The batch effect isn't clearly dominant. LDA is ok here.

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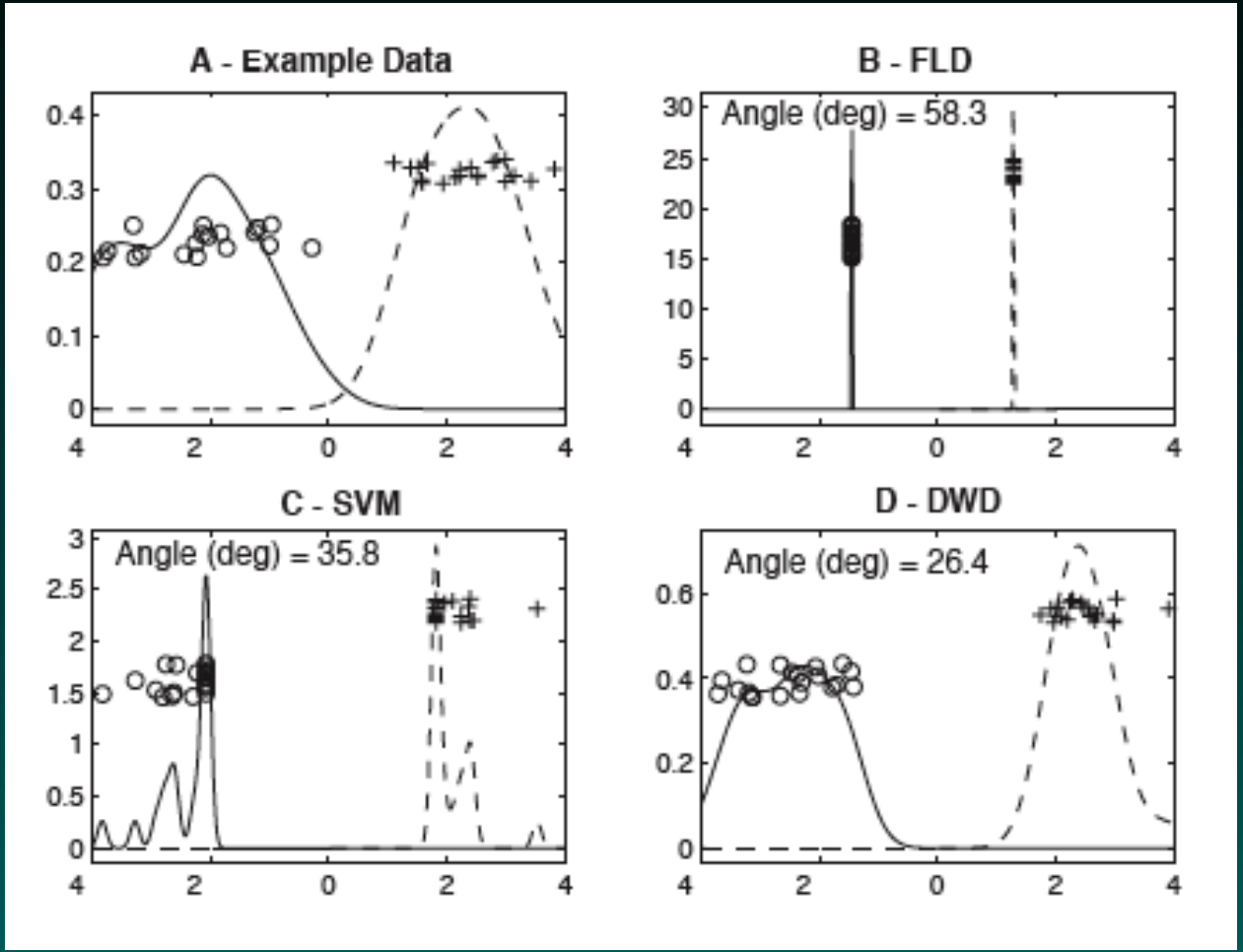
Where LDA Breaks



Benito et al, Bioinf 2004, fig 2. Low-d structure in high-d data. LDA sidetracked.

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Distance Weighted Discrimination (DWD)

Similar to a Support Vector Machine (SVM) approach, but not solely driven by extrema (maximizing the minimum distance). Instead, maximize the sum of the inverse distances — lets all points contribute, but still stays robust to outliers.

Matlab implementation code exists, involves some sophisticated optimization algorithms.

Focuses on two groups, works better if groups are moderately large.

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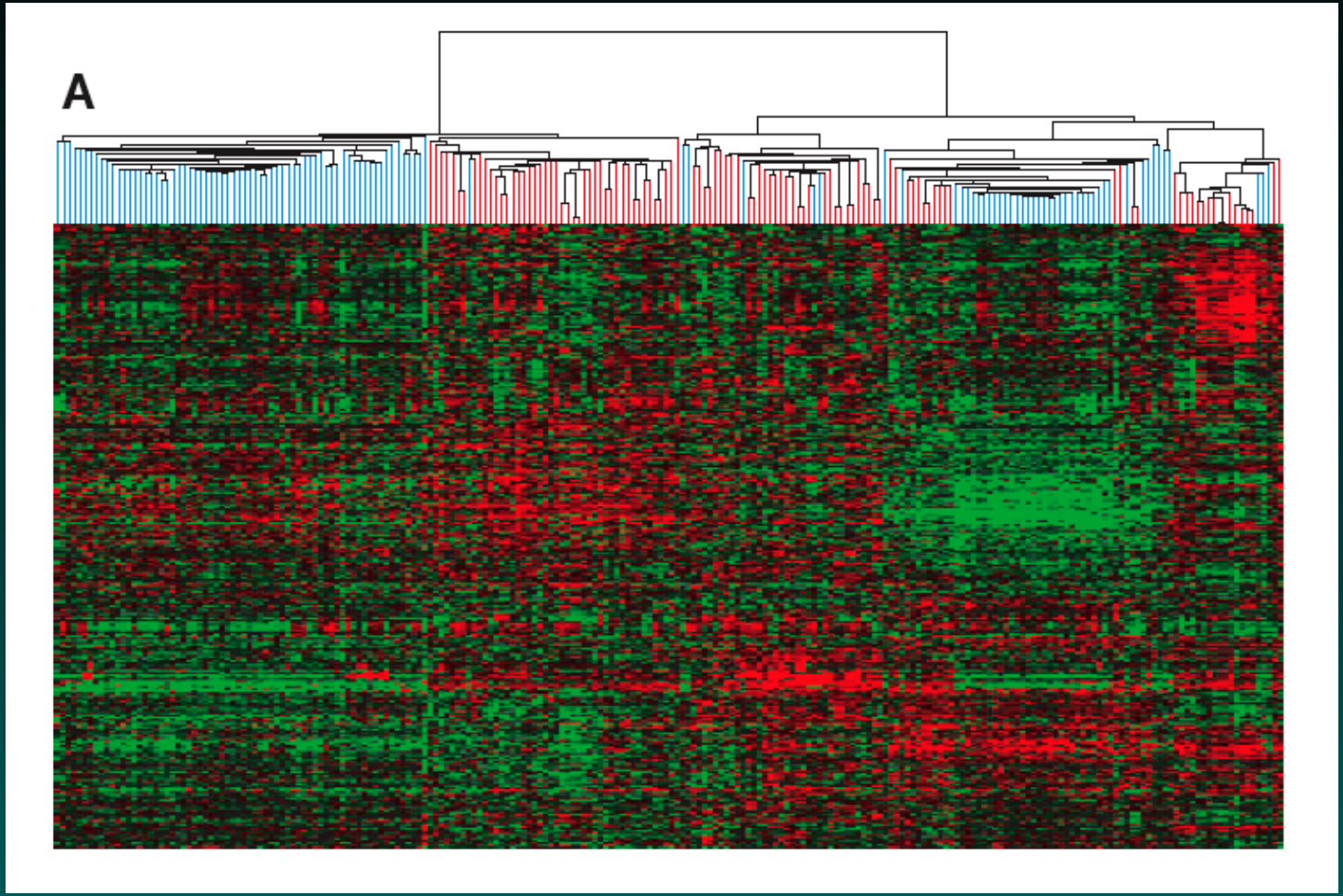
Real Data pre DWD



Benito et al, Bioinf 2004, fig 6a. Datasets from two platforms (logratio), each median centered.

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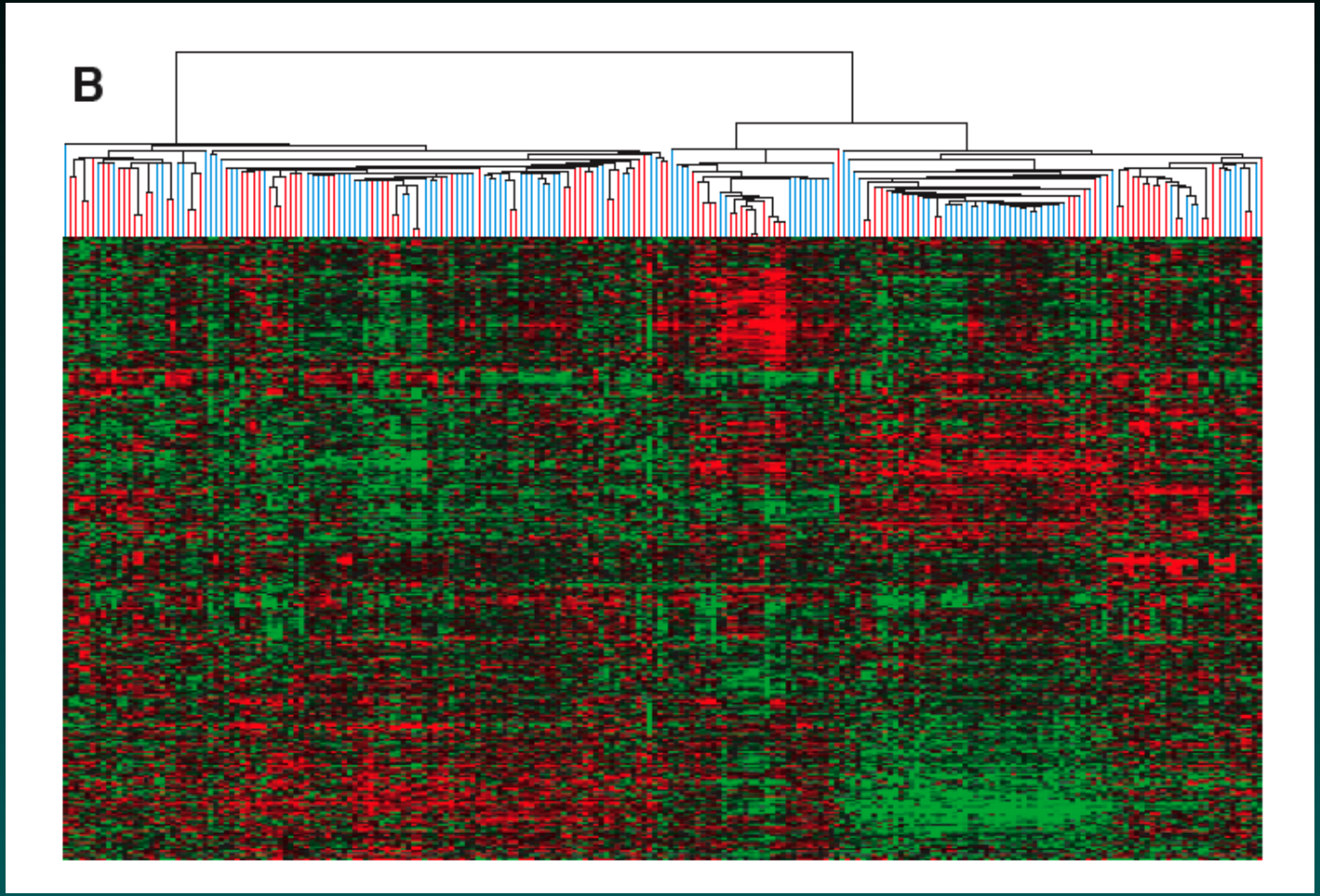
Real Data post DWD



Benito et al, Bioinf 2004, fig 6b. Datasets after DWD adjustment. More coherent.

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An Alternative – COMBAT

Starts with an adjustment similar to what we tried in the first place with the linear models, with two shifts.

First, it allows for shifts in scale as well as center.

Second, it borrows strength across genes and shrinks towards the central location and scale adjustments.

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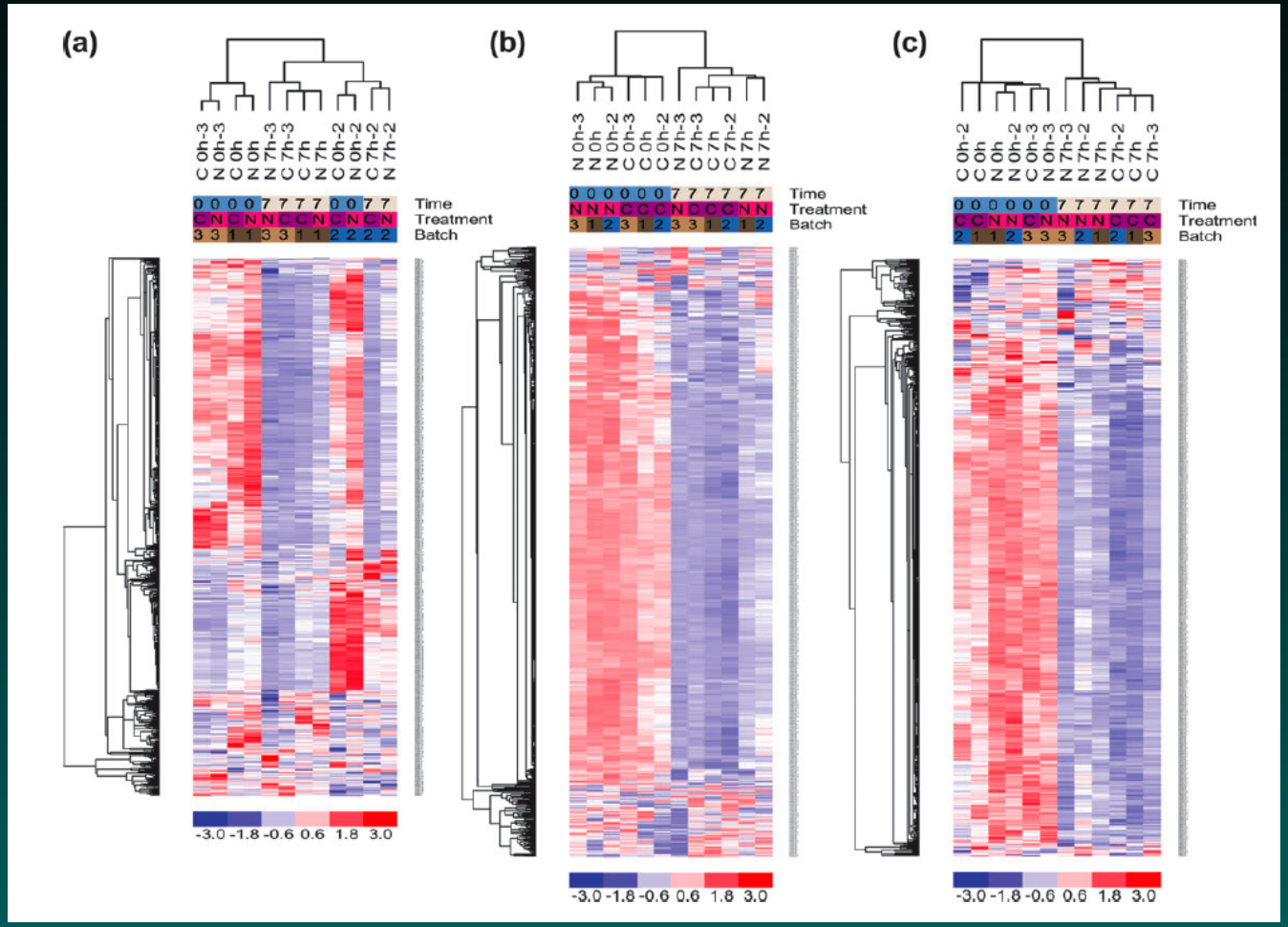
A COMBATive Triptych



Bad, Good, Good

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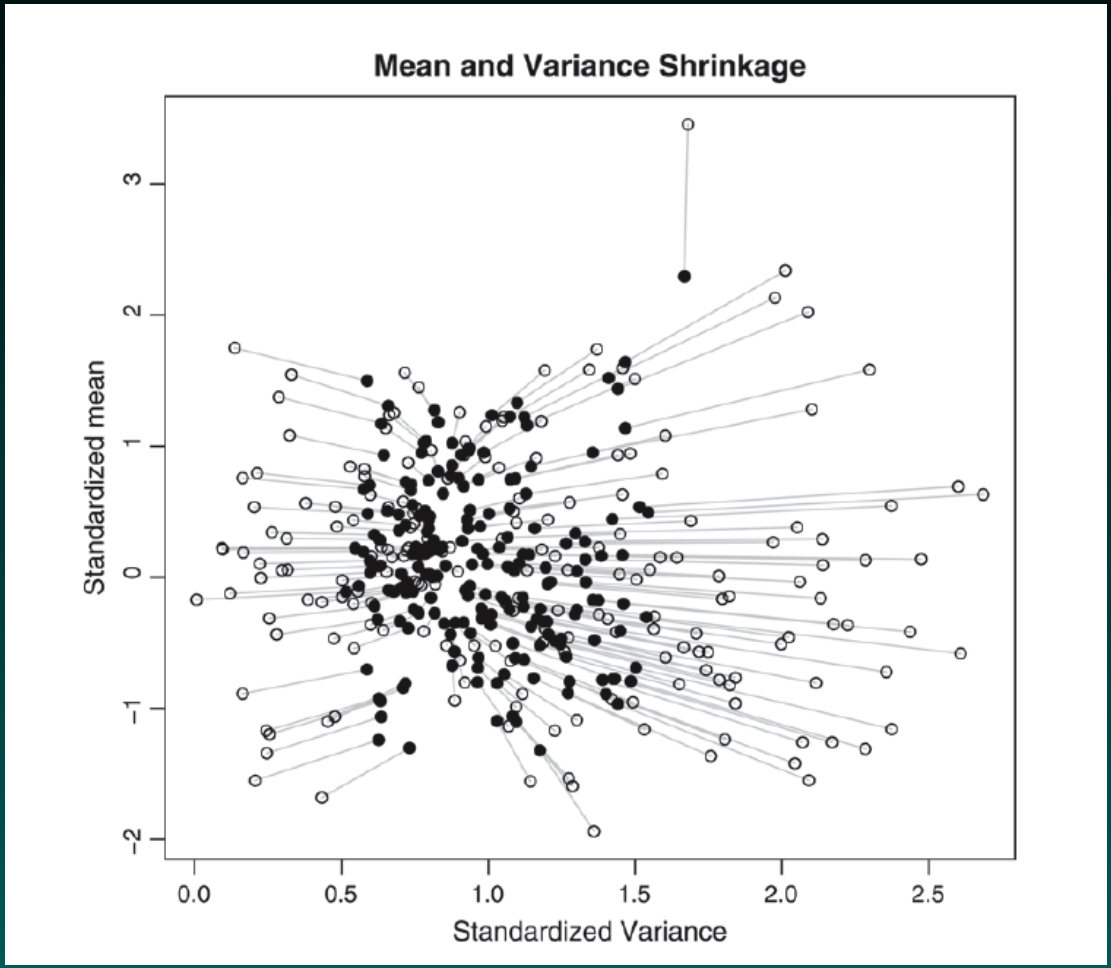
A Shrinking Black Hole



Which values are “better”? Why?

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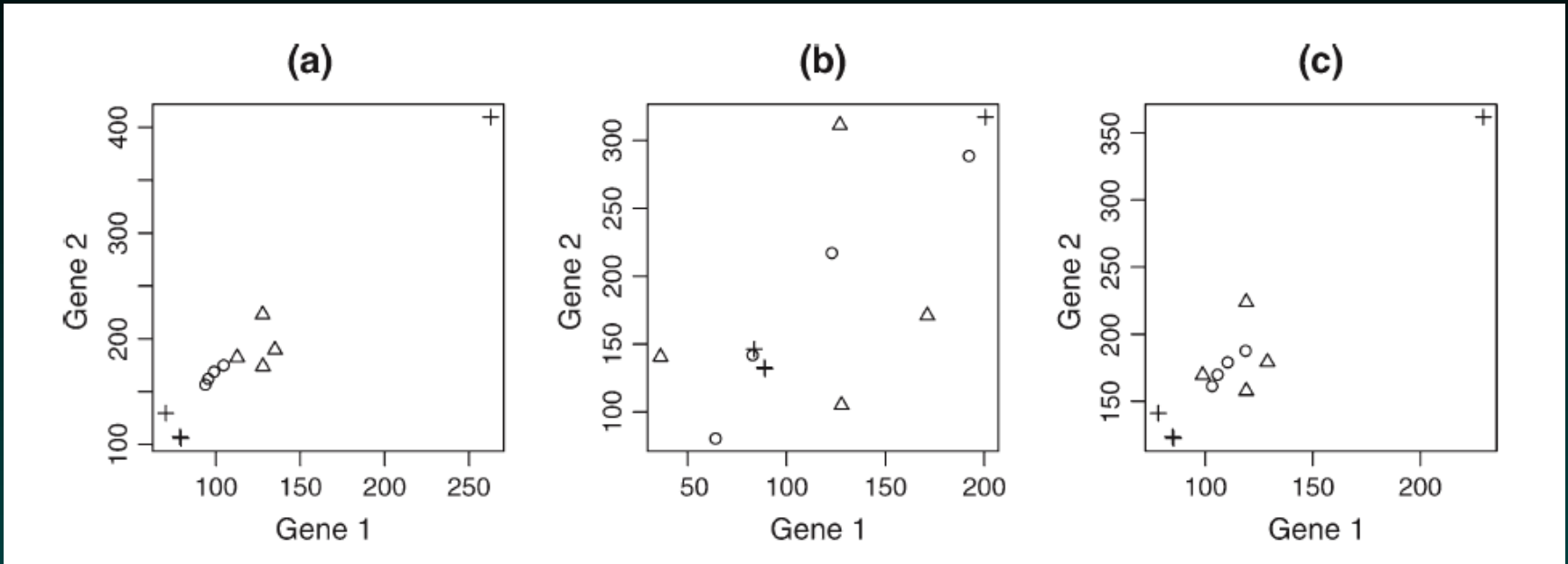
The Value of Shrinkage



Borrowing can let you recognize (and downweight) outliers.

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Implementing COMBAT

This is actually pretty easy, since a set of R scripts is available from Evan Johnson's web site at BYU:

http://statistics.byu.edu/johnson/ComBat

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Which do I Prefer?

I like them all, but at present I lean towards either linear model approaches or ComBat.

This is for reasons of relative simplicity and ease of implementation.

In general, I think the choice of a particular method of batch adjustment is an order of magnitude less important than recognizing that batches may be present in the first place, and coming up with reasonable ideas as to what they are.

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TCGA Batches

The TCGA samples are processed in batches.

This is largely by necessity, because not all of the samples are coming into the processing center at once, and they don't want to wait for all 500 before starting.

Batches typically involve about 30 samples.

The batches have mostly (but not always!) been assembled with balance in mind.

For some batches, mild correction will likely be necessary.

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TCGA Genes

"GBM Phase I + II.xls" (from the TCGA web site)

Phase	Name	Chr	Start	Stop
Phase1	ABI1	chr10	27077005	27190298
Phase1	ABL1	chr9	132579028	132751391
Phase1	ADAM15	chr1	153289987	153302102

locusLinkId	omimId	proteinAcc
10006	603050	NP_005461
25	189980	NP_009297
8751	605548	NP_003806

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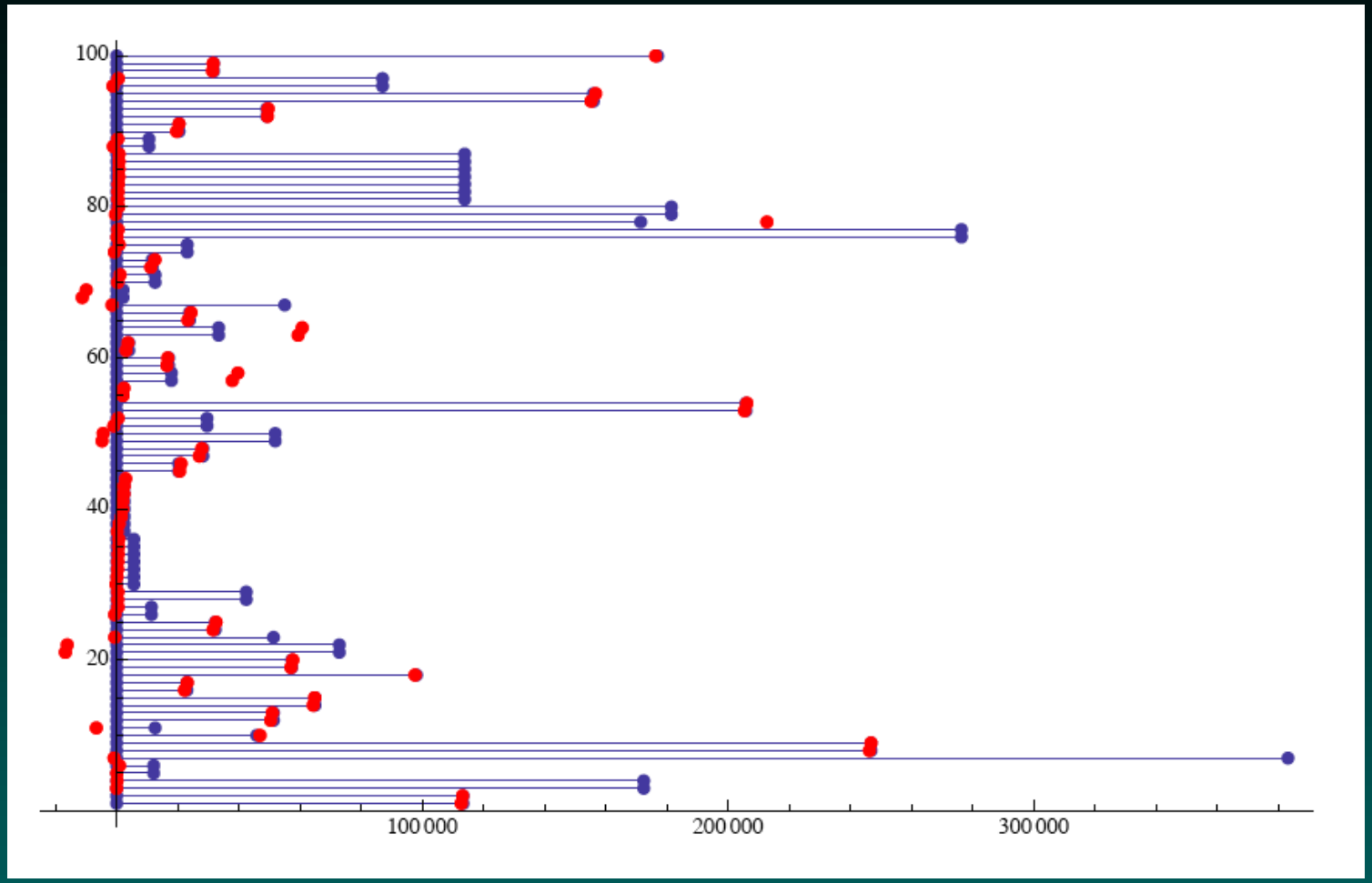
TCGA Positions?



Shouldn't they line up?

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