The Bioconductor Project for Reproducible Analysis of High Throughput Genomic Data

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Analysis and Comprehension of High Throughput Genomic Data

Hallmarks of effective computational software

1. Extensive: data, annotation
2. Statistical: volume, technology, experimental design
3. Reproducible: long-term, multi-participant science
4. Leading edge: novel, technology-driven
5. Accessible: affordable, transparent, usable

1. Extensive Data and Annotation

Data

- Expression, tiling, methylation, custom arrays.
- Sequence analysis, e.g., ChIP-, RNA-seq
- Other high-throughput assays, e.g., flow cytometry, mass spec., imaging
- Public repositories, e.g., GEO, ArrayExpress

Annotation, e.g.,

- Well-curated: NCBI, Biomart, UCSC, MsigDB, GO, KEGG
- Loosely curated: emerging, specialized, & lab-based
- Consortium: HapMap, 1000 genomes, TCGA

Bioconductor

Goal Help biologists understand their data

Focus

- Expression and other microarray; flow cytometry
- High-throughput sequencing

Themes

- Contributions from ‘core’ members and (primarily academic) user community
- Based on the *R* programming language – statistics, visualization, interoperability
- Reproducible – scripts, *vignettes*, packages
- Open source / open development

Success $>$ 400 packages; publications; 8,000 web visits / week; 75,000 unique IP downloads / year; very active mailing list; annual conferences; courses; . . .

Bioconductor: Sample Work Flow

```
> ##
> ## Pre-processing
> library(affy)
> eset <- just.rma()
> ##
> ## Quality assessment
> library(arrayQualityMetrics)
> arrayQualityMetrics(eset)
> ##
> ## Differential expression
> library(limma)
> status <-
+   c("Trt", "Trt", "Trt", "Ctrl", "Ctrl", "Ctrl")
> design <- model.matrix( ~status )
> fit <- eBayes(lmFit(eset, design))
> topTable(fit, coef=2)
```

2. Statistical

Technology

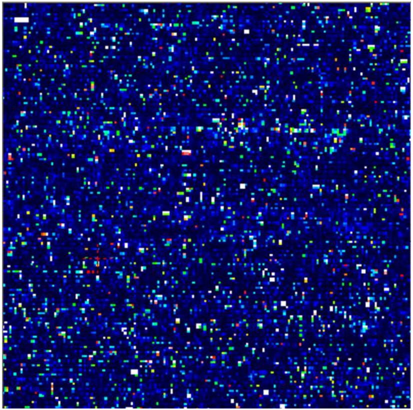
- Acknowledging artifacts and biases
- Accomodate using statistical models, e.g., RMA

Volume of data

- Data reduction essential

Experimental design

- Exploratory analysis
- Hypothesis-driven; designed experiments
- Cost-effective, but not too clever



Expression array. Pseudocolors represent hybridisation intensities of RNA to features. Source: url

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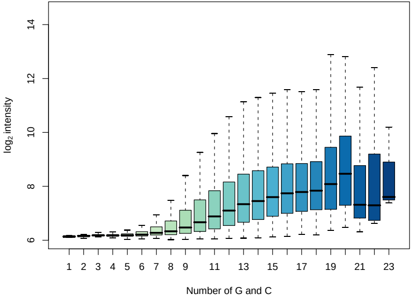

Measured intensity increases with GC content; Chronic Lymphocytic Leukemia (CLL) dataset.

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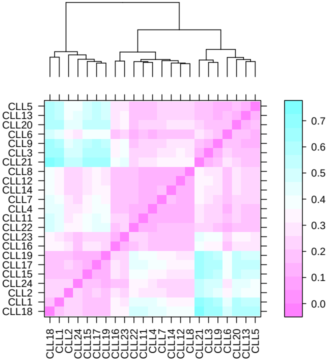

Heatmap summarizing distance between CLL arrays

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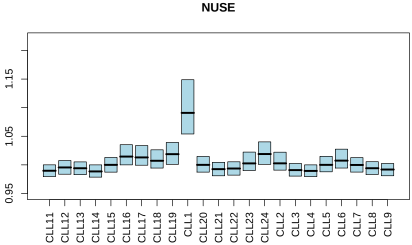

Normalized unscaled standard error (NUSE) suggests array CLL1 is an outlier.

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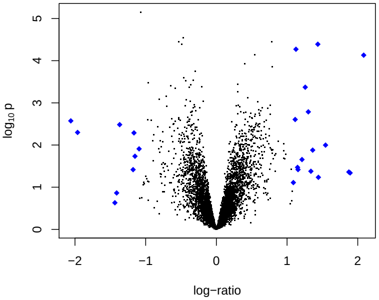

'Progressive' vs. 'stable' status. log P vs. log-fold change, CLL data set. Probe sets with extreme differentiation highlighted.

3. Reproducible Research

Long-term

- Returning to analysis after days, weeks, months of other activity

Multi-participant: communicating with...

- Other statisticians / bioinformaticians
- Biologists and others without specialized statistical knowledge

Science: reproducibility...

- Facilitates third-party verification
- Allows critical assessment
- Challenging, even in high-profile journals requiring archived raw data (Ioannidis *et al.*, 2009, Nat Genet 41: 149-155).

Reproducible Research: Case Study

Original research

- Potti *et al.*, 2006; Hsu *et al.*, 2007
- NCI60 cell line drug sensitivity signature
- Clinical trial allocation

Reproducibility

- Baggerly & Coombes, 2009
- Off-by-one cisplatin gene signature
- Four ‘interesting’ genes not supported by analysis (two not on array)

References

- Potti *et al.* 2006 Nat Med 12: 1294-1300; (retracted)
- Hsu *et al.* 2007 J Clin Oncol 25: 4350-4357. (retracted)
- Baggerly & Coombes 2009 Ann Appl Stat 3: 1309-1334

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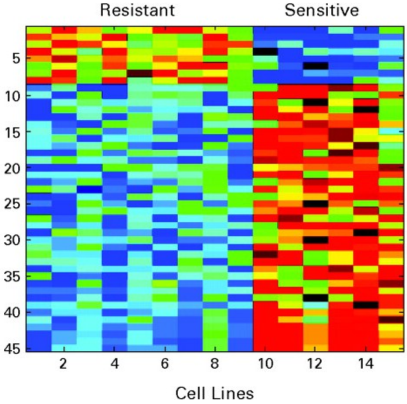

Hsu *et al.*, cisplatin, fig. 1a

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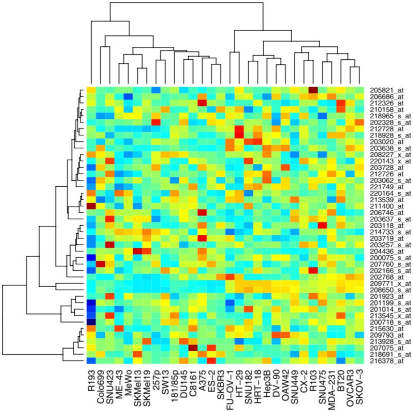

Baggerly & Coombes, fig. 2a

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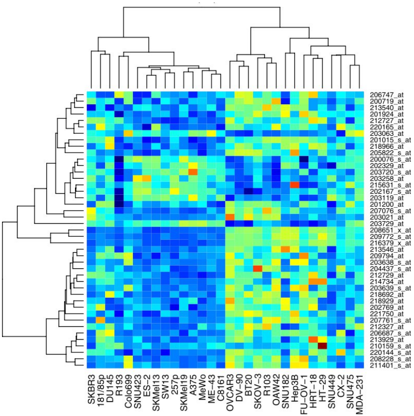

Baggerly & Coombes, fig. 2b

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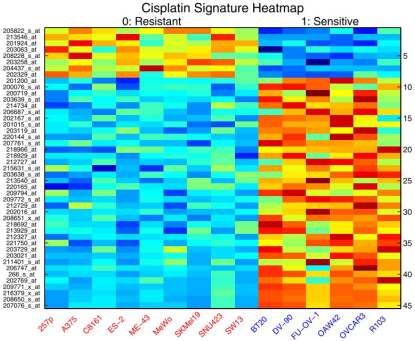

Baggerly & Coombes, fig. 2d

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. . . results incorporate several simple errors that may be putting patients at risk. One theme that emerges is that the most common errors are simple (e.g., row or column offsets); conversely, it is our experience that the most simple errors are common – Baggerly & Coombes, 2009

Reproducible Research: *Bioconductor*

Script-based	Data transformations *necessarily* documented
'Literate programming'	Text documents embed scripts, scripts *evaluated* when text document processed
Versioned software and repositories	Record which package versions used, and retrieve from *Bioconductor* archives
Integrated data containers	Sample descriptions and expression data in a single object. Subsetting expression data automatically subsets sample descriptions

The ALL dataset

```
> library(ALL); data(ALL); ALL
```

```
ExpressionSet (storageMode: lockedEnvironment)
assayData: 12625 features, 128 samples
  element names: exprs
protocolData: none
phenoData
  sampleNames: 01005 01010 ... LAL4
    (128 total)
  varLabels: cod diagnosis ... date
    last seen (21 total)
  varMetadata: labelDescription
featureData: none
experimentData: use 'experimentData(object)'
  pubMedIds: 14684422 16243790
Annotation: hgu95av2
```

4. Leading Edge

Technological innovations

- E.g., SNP, miRNA arrays
- E.g., lab sequencing platforms; novel protocols

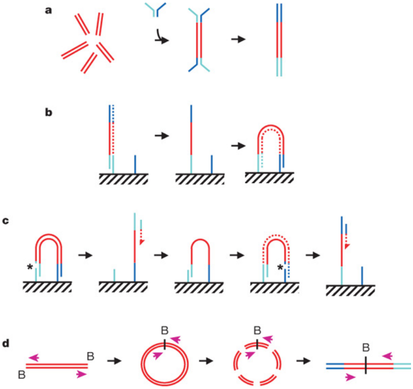
Fast-changing

- Commercial software products not yet developed, or already out-of-date
- Research questions require novel solutions

Leading Edge: Illustration

Sequencing technologies

- Historically (e.g., 2 years ago): short reads, low 'tail' quality, tail base call bias, data volume
- Current: count models, read bias, designed experiments, variant representations, annotation



Bentley et al., 2008, Nature 456: 53-9

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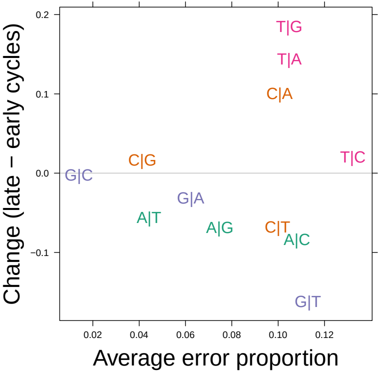

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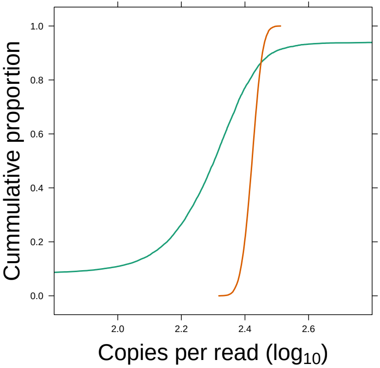

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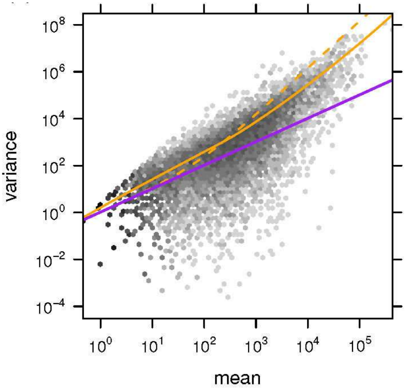

Poisson (purple) and negative binomial (orange) fit to RNA-seq data. Anders & Huber, 2010, Genome Biol, 11:R106

5. Accessible

Affordable

- Purchase / licensing; time

Transparent

- Algorithms, e.g., RMA
- Code reuse

Challenges and solutions

- Research questions requiring ‘one-off’ solutions
- Software bugs

Usable

- Documentation
- Training, such as today!

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Documentation

- Help pages
- Vignettes
- Archived course and conference material
- Mailing list

BioC2011

- Annual conference – user and scientific presentations, workshops, poster session
- Seattle July 27-29

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