Design and analysis of microarray experiments

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Microarray Analysis Group

- University of Adelaide
  - Patty Solomon
  - Gary Glonek
  - Jonas Lloyd (Ph.D.)
  - Michael Calvert (Grad. Dip.)
  - Adam Kister (Grad. Dip./Ph.D.)
- Hanson Institute, IMVS
  - Anna Tsykin

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  - Anna Tsykin
- http://www.maths.adelaide.edu.au/MAG
  - ★ Preprints, talks, workshop materials, links, ...

Major collaborative projects

- Aim to understand the genetic basis of disease, and it is hoped, to find treatments or cures:
  - ◇ Osteoarthritis
    - ⋆ Nick Fazzalari (IMVS), David Findlay (RAH)
  - ◇ Leukaemia, cancer, asthma . . .
    - ⋆ Richard D'Andrea, Brenton Reynolds (WCH), Tom Gonda (Bionomics)
    - ⋆ Mark Guthridge (IMVS)
  - ◇ Angiogenesis
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  - Animal and plant breeding
    - Waite, Roseworthy
  - . . .

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- *Design microarray experiments.*
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- *Image processing:*
  - ⋆ quantifying expression.
- *Normalisation:*
  - ⋆ Removing dye and other systematic biases.
  - ⋆ Robust normalisation, Calvert et al.
- *Statistical analysis:*
  - ⋆ Detecting differential or co-expression in complex experiments
  - ⋆ Reynolds et al., Lloyd et al., Cox & Solomon 2002.
- *No single method of analysis can be appropriate for all experiments.*

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  - Should samples from individual animals or people be compared *directly* or via a common *reference* mRNA sample?*
- Should tissue samples from animals be *pooled* then compared, or should different animals be hybridised to different slides?*
- Which sample should be labelled with one dye and which with the other?
  - Should dye-swapped replicates be made on different extractions?

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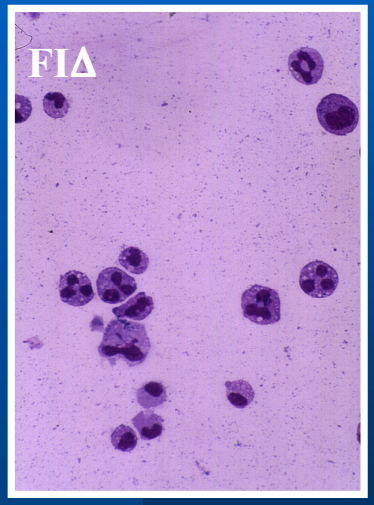
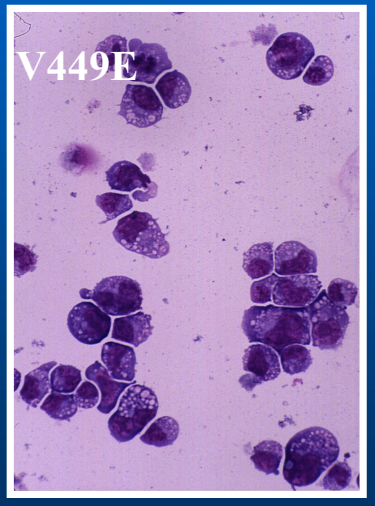
- *Our premise is that the most appropriate way to find differentially expressed genes is to prescribe a design subject to*
  - the key contrasts and parameters of interest
  - and the practical constraints of the problem.

Design case study

Biological function of the activated mutants in FDB cells





⇒ Different signals generated by the two mutants

- *Aim: to identify genes that play an important role in receptor signalling and leukaemogenesis in mutant mice.*

A 2x2 factorial design

- *Hypothesis:* that there is a set of genes induced specifically in response to expression of V449E that results in its leukaemic effects.
  - ◇ Compare two cell populations: FIΔ and V449E at two times 0 hours and 24 hours

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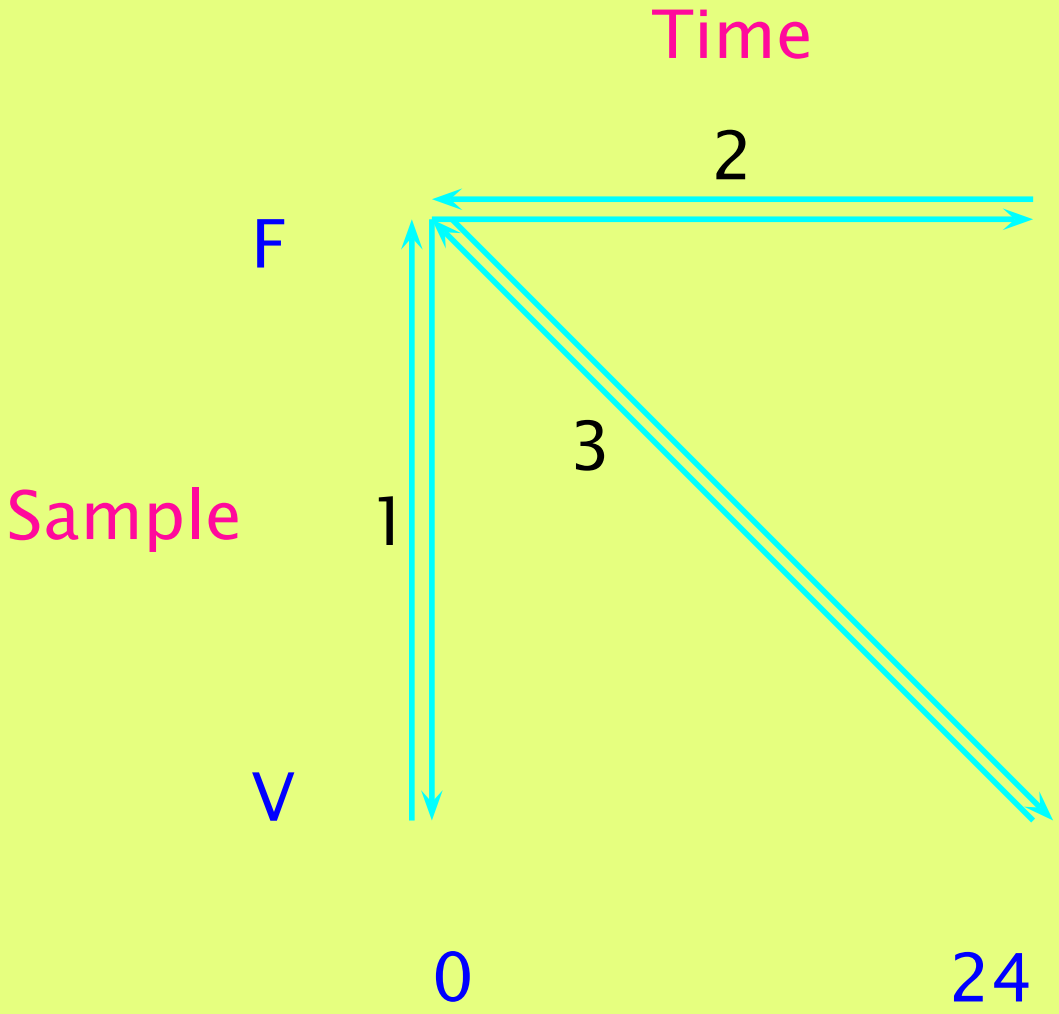
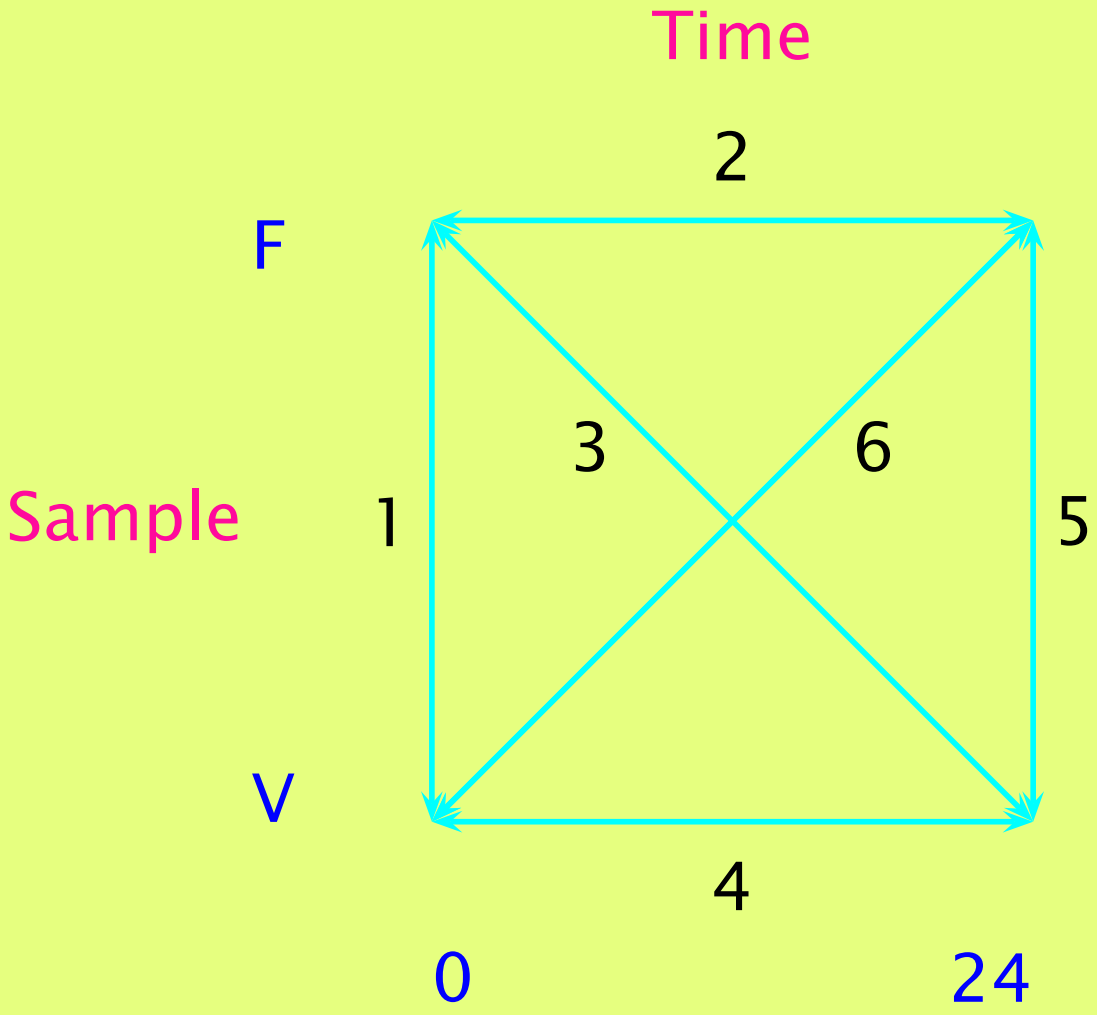
- *Interaction of primary interest:*
  e.g. genes that are differentially expressed in the two samples at time 24 hours, but not at time 0 hours.

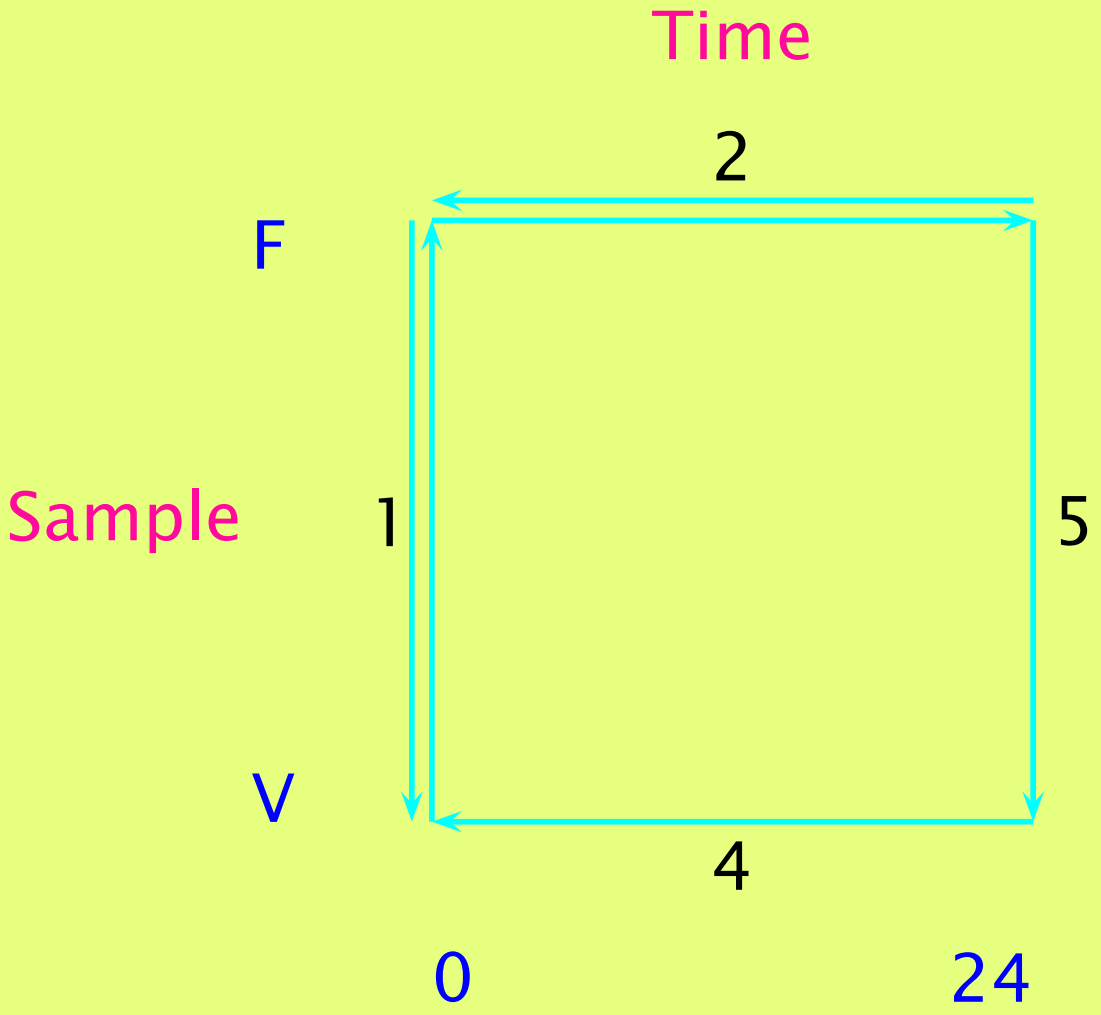
The reference design



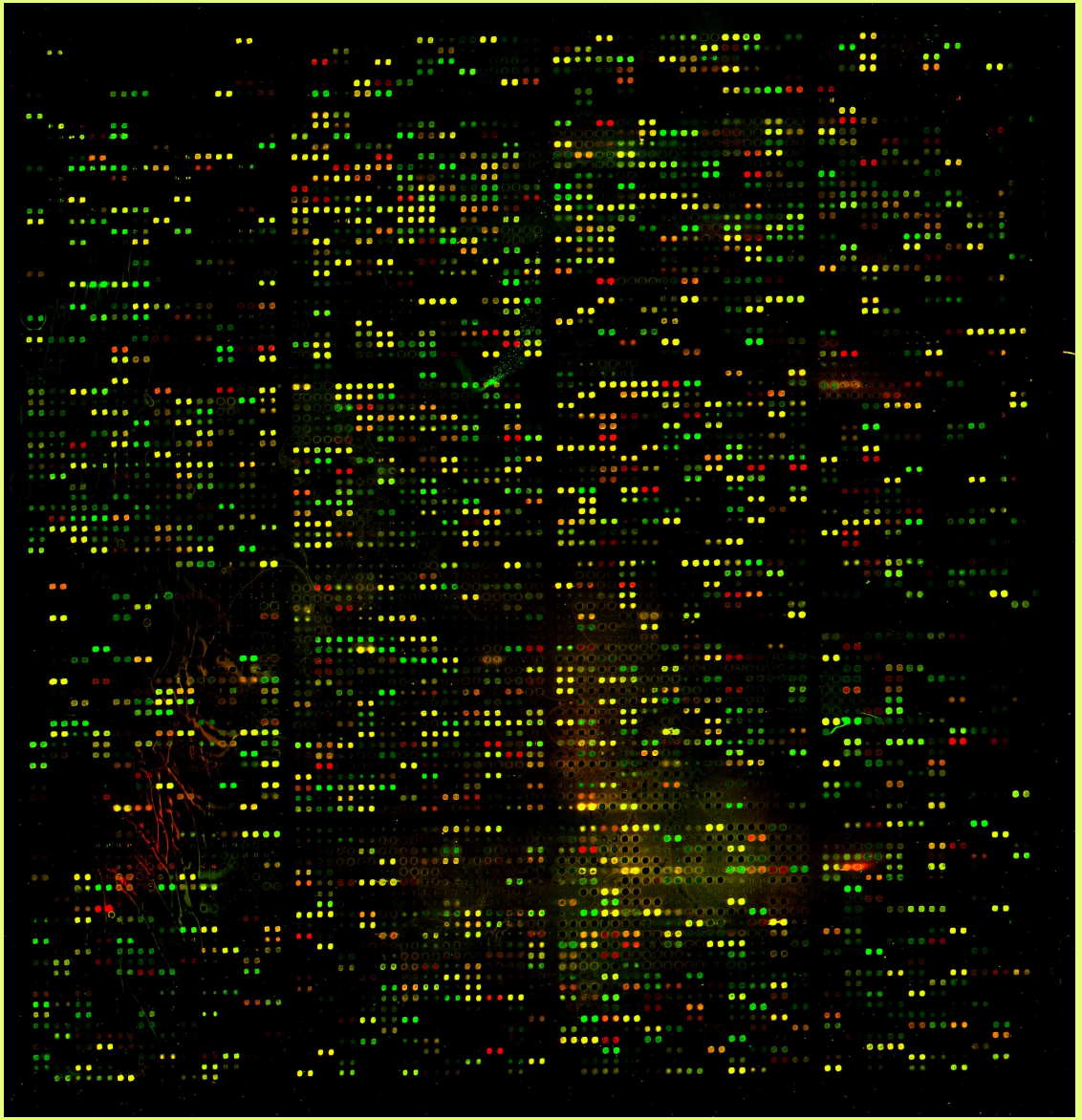
All comparisons design: 6 slides



Optimal design: 6 slides



Human clones with MCF-7 and Jurkatt probes



Quantifying expression

- For each spot on the array, calculate the background-adjusted intensities:

red intensity $R \rightarrow R - Rb$

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- and combine them in the log base 2 ratio:

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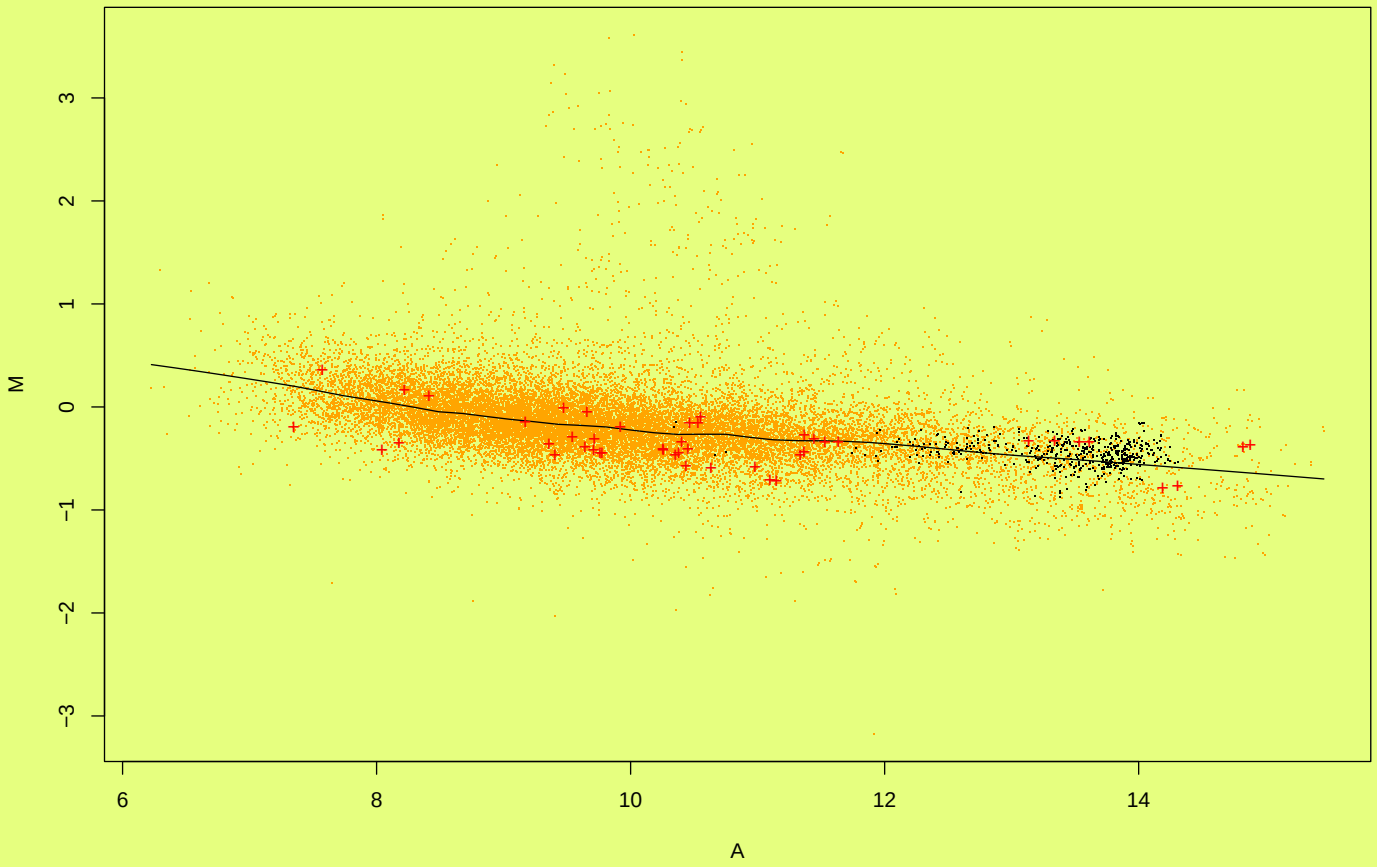
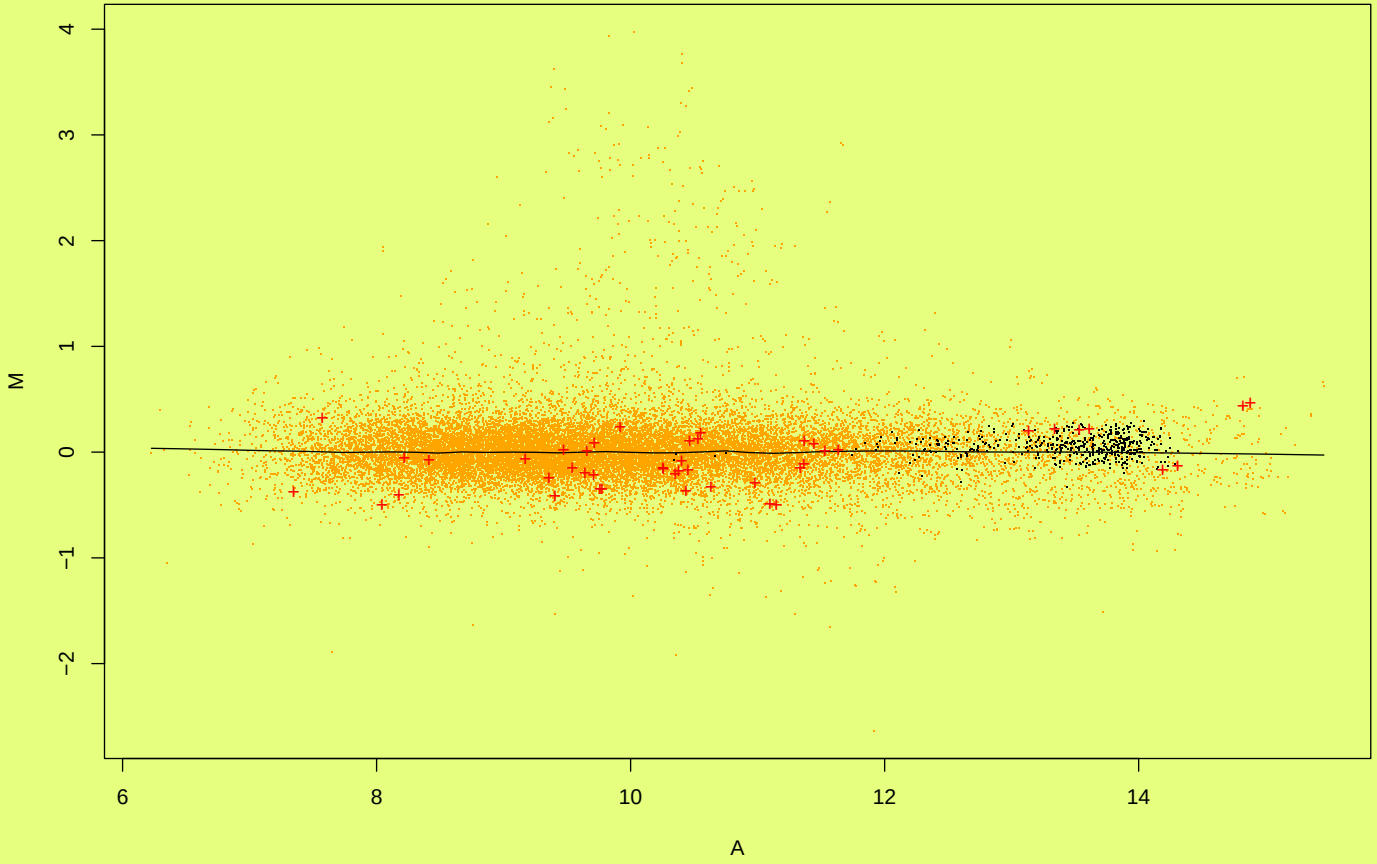
- *Background is a big issue.*

Pre-processing angiogenesis: time 0 versus .5 hour



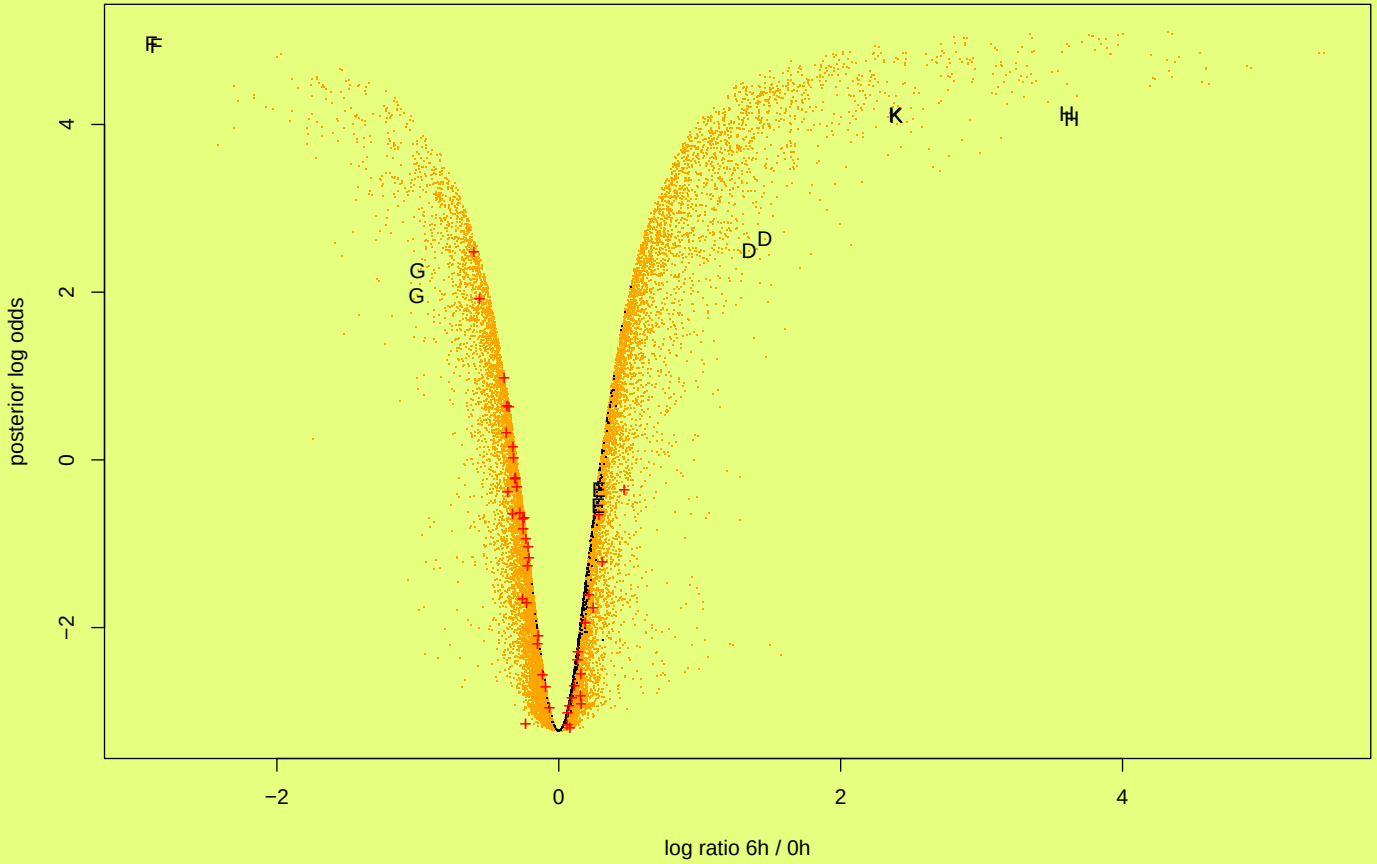
M is intensity and spatially dependent

Angiogenesis: after printtip normalisation



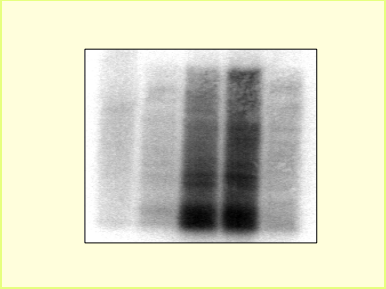
(Robust normalisation: Calvert et al.)

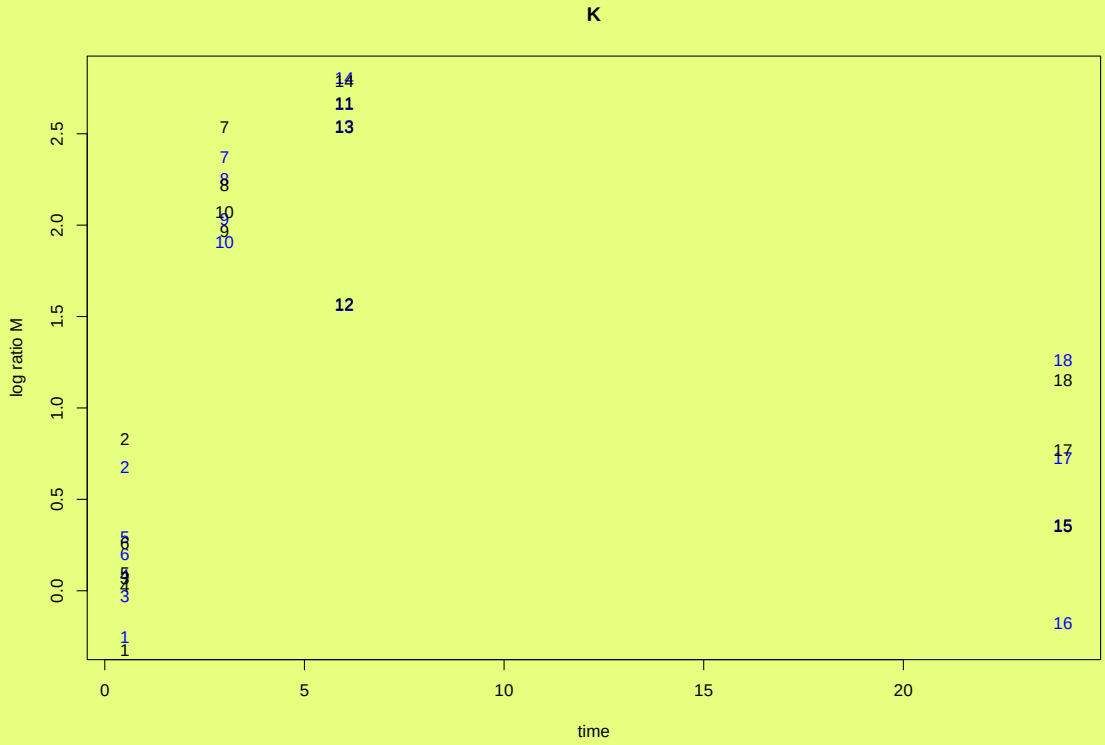
Bayesian analysis of differential expression



Angiogenesis: 0 vs 6 hours, 4 dye-swapped replicates

Angiogenesis: control K time course





Some ‘truths’

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- *The interface between statistics, biology, computer science and medicine has gone from information-poor to information-mega-rich*.
  - ◇ Statistics has a central role to play in processing that information and making it intelligible.

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- '*When we entered the era of high technology, we entered the era of mathematical technology*' [1].
- *The interface between statistics, biology, computer science and medicine has gone from information-poor to information-mega-rich*.
  - ◇ Statistics has a central role to play in processing that information and making it intelligible.
- *Biology looks set to dominate statistics at the beginning of this century, just as it did at the beginning of the last one.*

---

[1] Ad hoc Committee on Resources for the Mathematical Sciences, US National Research Council, 1981.

Acknowledgements

- Adelaide Women's and Children's Hospital
  - Brenton Reynolds
  - Richard D'Andrea
- Hanson Institute, IMVS
  - Greg Goodall
  - Chris Hahn
  - Jenifer Gamble
  - Nick Fazzalari
  - Mark Guthridge
- Bionomics
  - Tom Gonda

Further reading and web sites

- Bioconductor Project: Open source bioinformatics using R. http://www.bioconductor.org
- MJ Buckley. The Spot user's guide. CSIRO Mathematical and Information Sciences, August 2000. http://www.cmis.csiro.au/IAP/Spot.htm
- G Glonek & PJ Solomon (2002). Factorial designs for microarray experiments. Submitted. http://maths.adelaide.edu.au/MAG
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- sma v. 0.5.6 (November 2001), B Bolstad, S Dudoit, YH Yang. *http://www.stat.berkeley.edu/users/terry/zarray/Software/smacode.html*.
- G Smyth, et al. (2002) Statistical issues in cDNA microarray data analysis. Research Report, WEHI.
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- TP Speed. Preprints, information and software (sma) www.stat.berkeley.edu/users/terry/zarray
- TP Speed & YH Yang (2002). Direct versus indirect designs for cDNA microarray experiments. Preprint.

THE END