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RNA-Seq Data Analysis
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Taught module for DPhil programme in
Genomic Medicine and Statistics

Organised by Bioinformatics Core at WHG:

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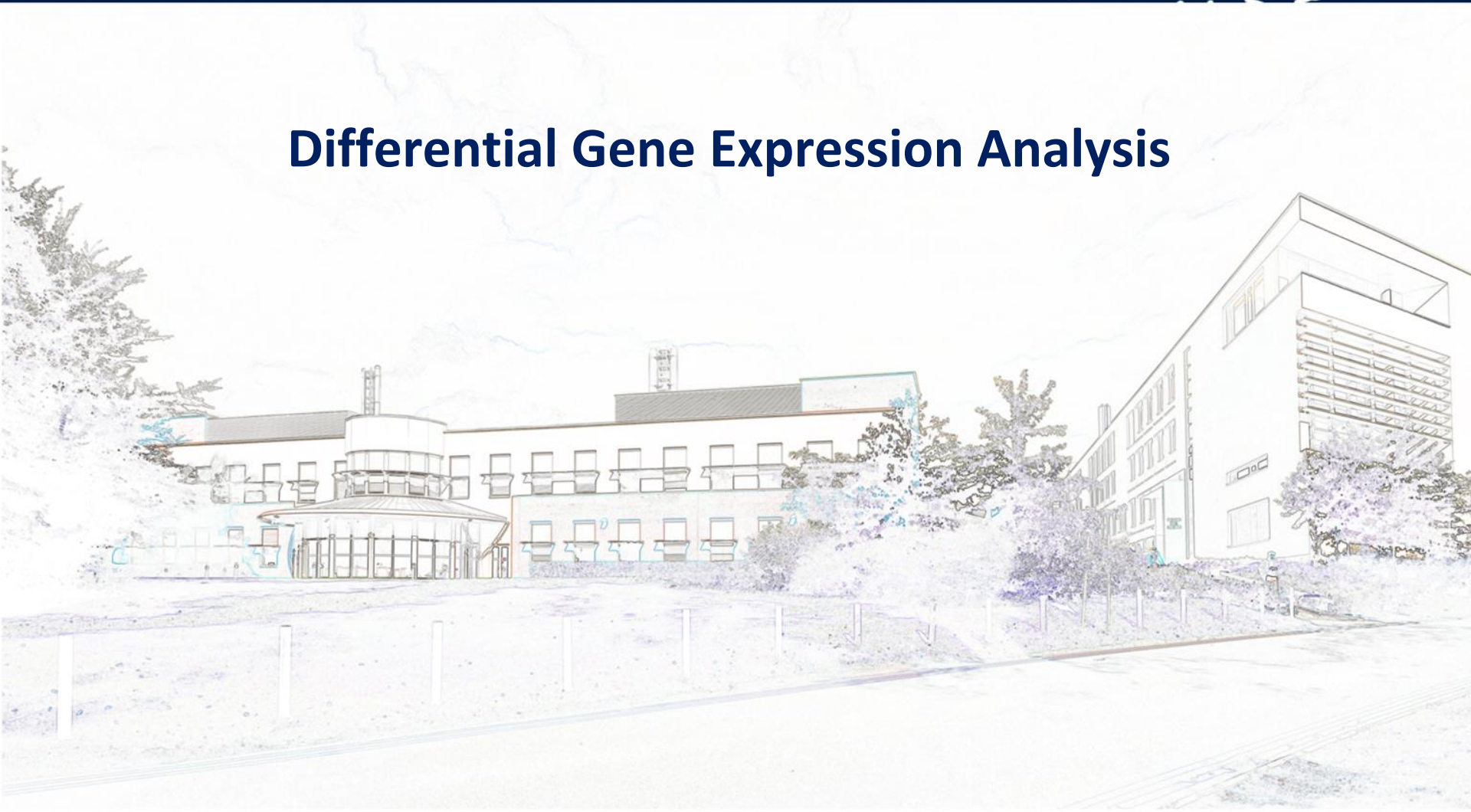
Differential Gene Expression Analysis



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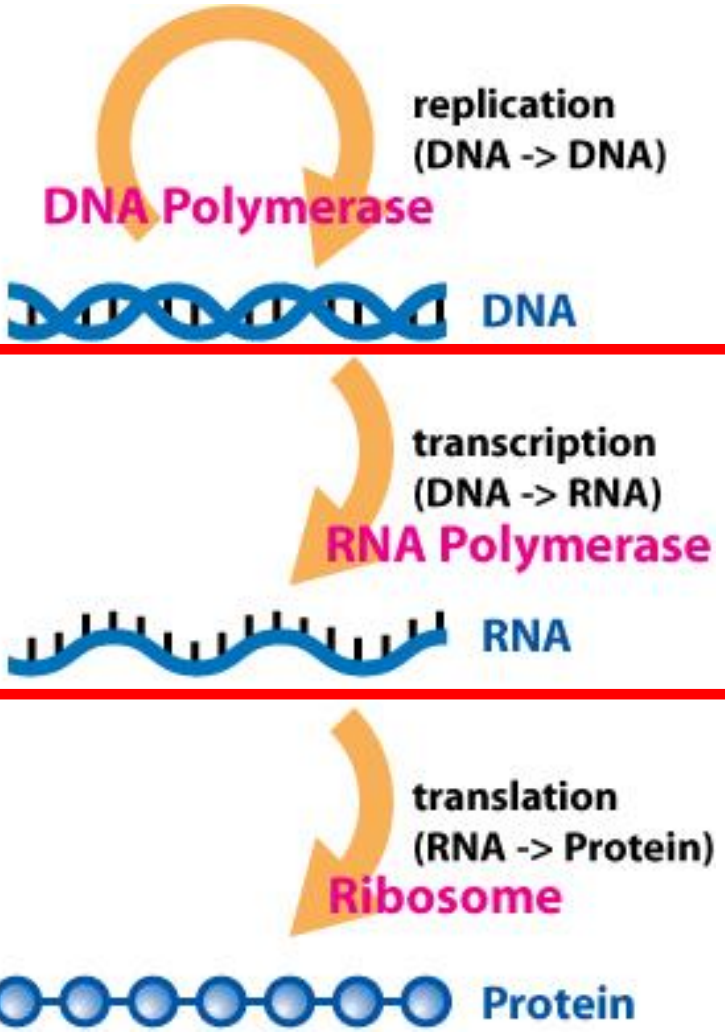
Overview

- General remarks on gene expression technology and data (RNA-Seq and microarray)
- Experimental design considerations
- Hypothesis testing
- Differential expression analysis using R/BioConductor
- Practical session working with a cancer dataset

A brief history of gene expression

Transcriptome Profiling



Transcriptome can be measured by microarrays or RNA-Seq

Widely-used techniques, provide insight into biological system, albeit a snapshot – highly dynamic and complex process (splicing, gene methylation, RNA stability/degradation, miRNA regulation etc)

Two key technologies

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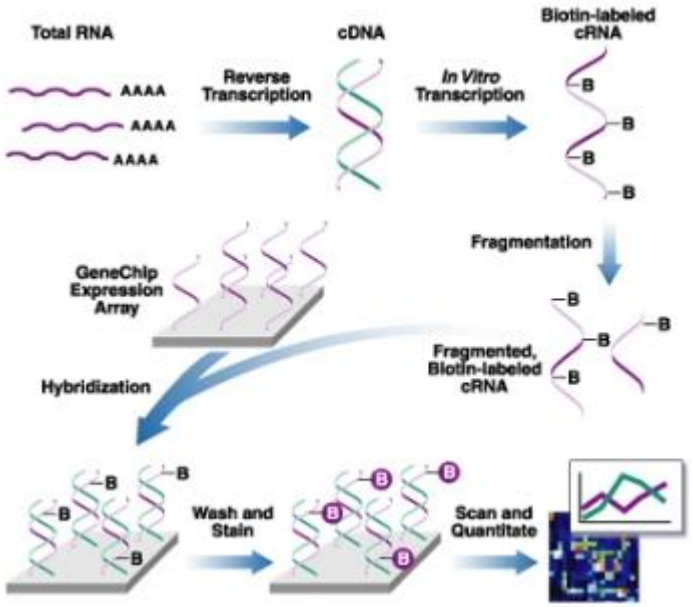
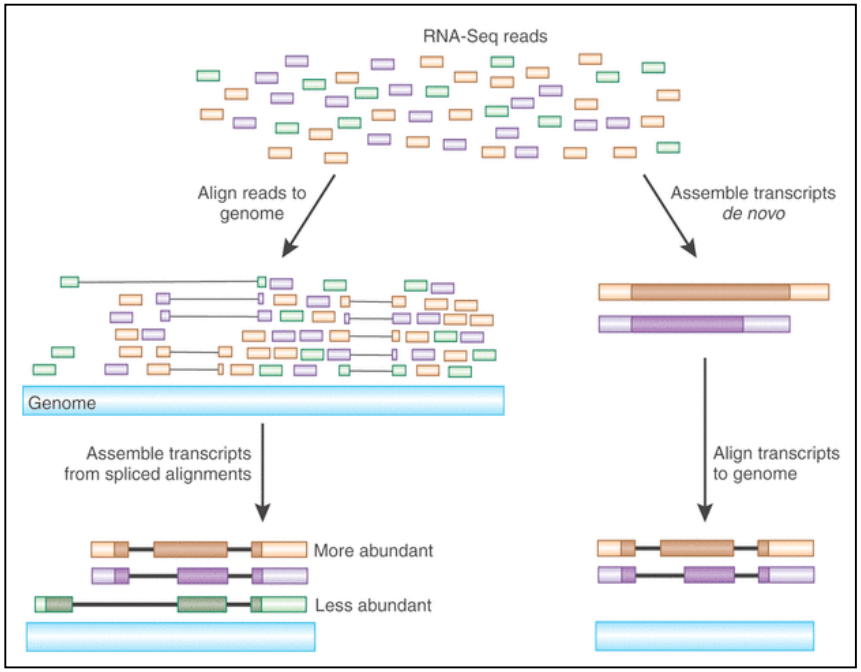
Microarrays



Complementary hybridisation
early 1990s onwards

RNA-Seq



Next-generation sequencing
2007 onwards

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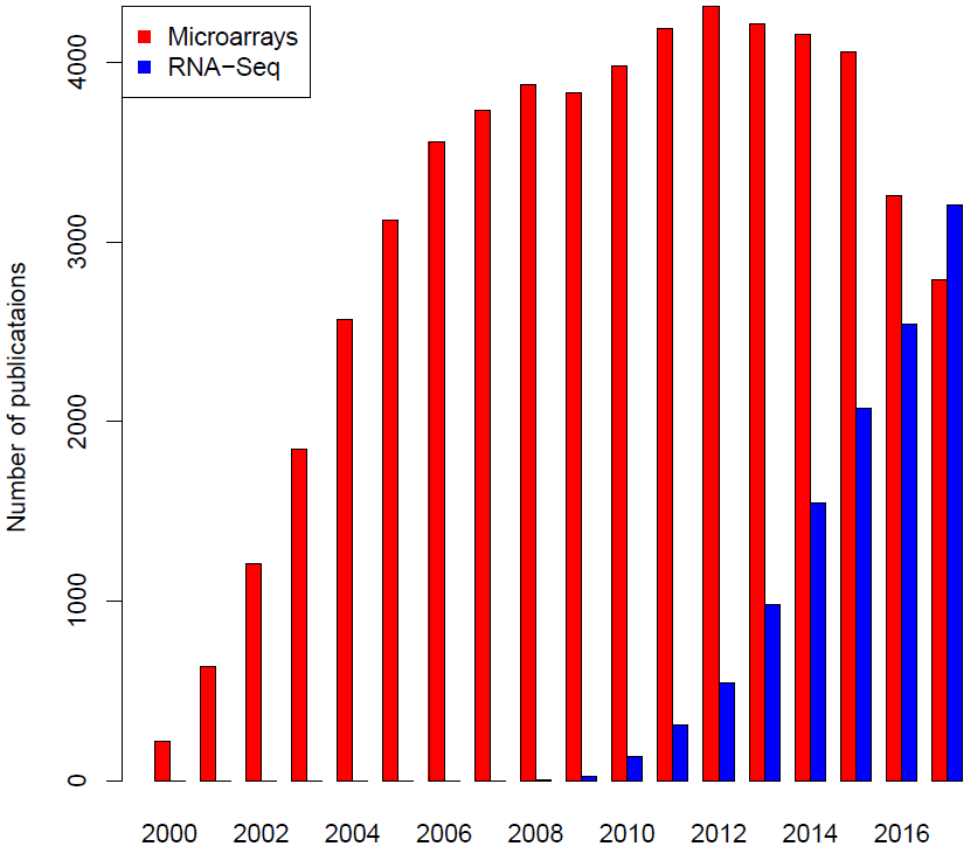
Publications by Technology



Which technology to use?

- Microarrays and RNA-Seq are complementary technologies (despite common perception that RNA-Seq superior)

- Choice usually depends how detailed a characterisation of the transcriptome is required
  - Gene level changes => microarrays sufficient, reliable and cheap
  - Isoform structure, splicing, novel transcripts => RNA-Seq
  - Note that exon arrays can also assess splicing

- Both report relative gene expression level estimates, influenced by a range of factors and biases inherent to each technology
- Fold-change concordance reasonably high between arrays and RNA-Seq

RNA-Seq Myths and Caveats

- Can detect low expressed genes better than arrays
  - Possibly but may need prohibitively expensive sequencing depth
  - In typical designs, up to half of all genes are too low expressed to be reliably detected (if at all)
  - Additional sequencing will still tend to be of highly expressed genes, so lower end hard to interrogate

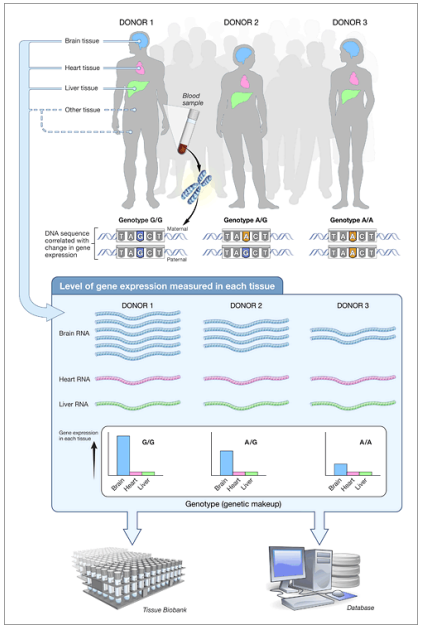
- The issue of low counts is even more problematic for splicing analysis where you may be comparing exons or junction-spanning reads

- What you sequence in an RNA-Seq library influences your data for all genes – very inter-dependent in a way that arrays are not

Large-scale gene expression projects

- ENCODE
- Allen brain atlas
- Genotype-Tissue Expression Project (GTEx)
- TGCA

- Public repositories
  - Gene Expression Omnibus (GEO)
    http://www.ncbi.nlm.nih.gov/geo/
  - Sequence Read Archive (SRA)
  - http://www.ncbi.nlm.nih.gov/sra



Typical experimental designs

- Disease vs control
- Gene knockdown/knockout vs wildtype
- Effect of treatment/stimulus/drug

- Clinical applications
  - Tumour-normal pairs
  - Good prognosis vs poor prognosis
  - Patient subgroups responding to different treatments
  - ‘Gene signature’ to predict who will respond well to a given treatment
- Time course
- Different tissues/stages of development

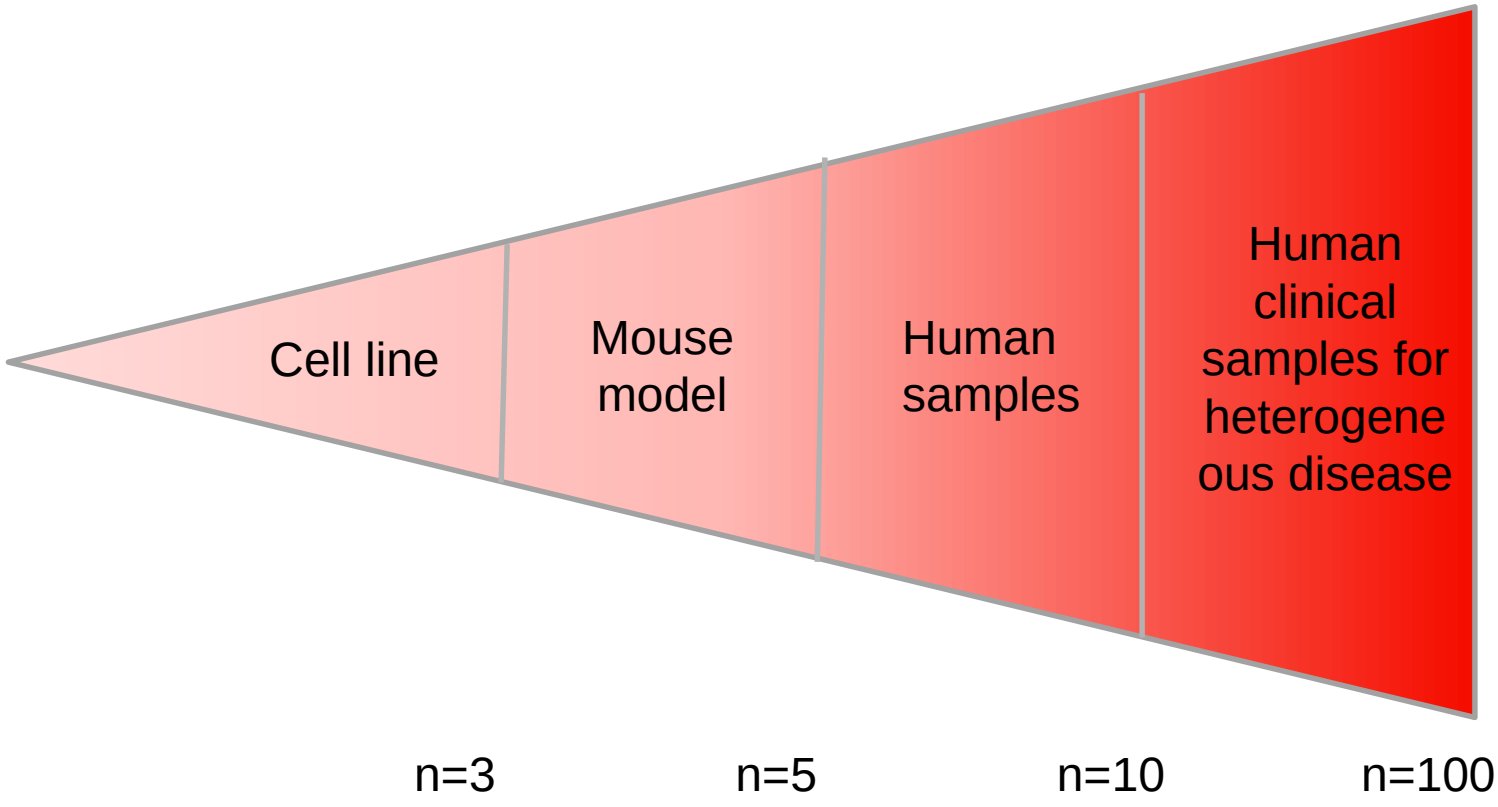
Limitations of transcriptomic profiling

- Comprehensive but inherently limited to descriptive results, no matter how well experiment performed or data analysed
- Produce large amounts of information; subjective interpretation, can be mined in different ways, always much left untouched (often publically available)
- Expensive and time-consuming so often published as a stand-alone experiment
- However best used as starting point for further work - following up hypotheses from gene expression data to uncover mechanistic/causal effects can produce elegant studies

Experimental design considerations

Replication

- Depends on context – type of sample, size of effect, heterogeneity within conditions



Sequencing Depth

- Number of reads required per sample depends on experimental question

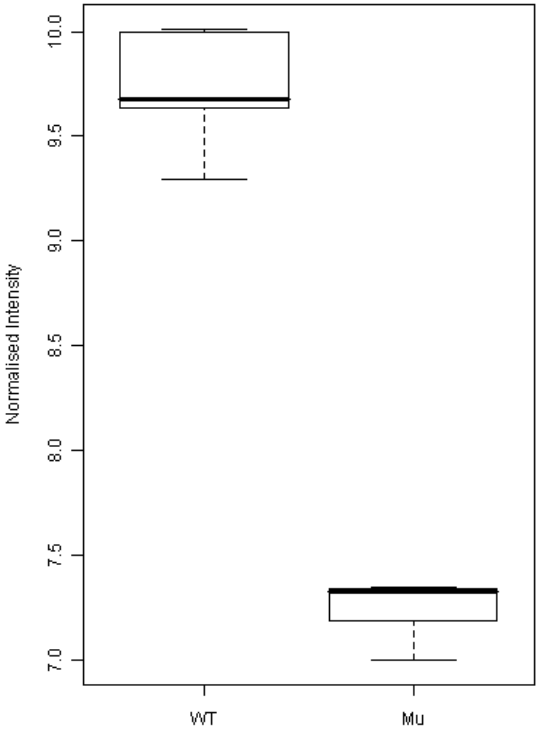
- HiSeq4000 – one lane = 250 million reads
- Multiplexing e.g. 10-plex human samples gives ~25m reads for each, plenty for quantifying gene expression (except for very low/unexpressed genes)

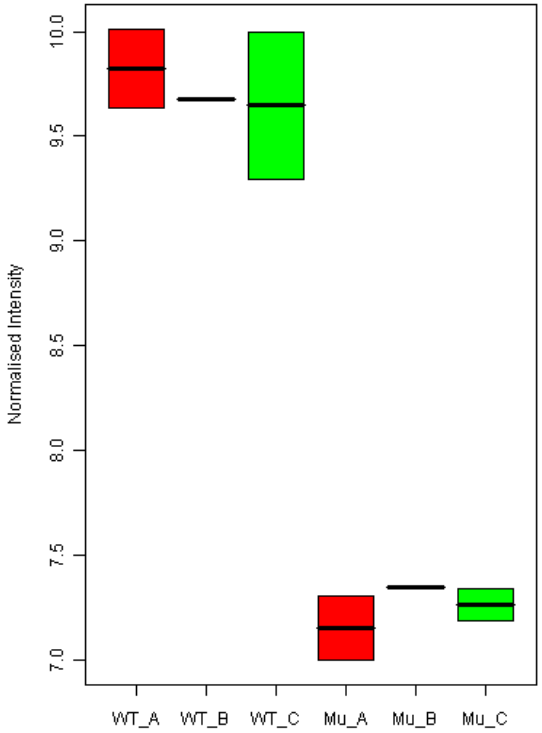
- Higher depth required in some situations e.g. for splicing analysis, certain library prep methods (ribo-depletion)

Potential confounds and covariates

- Gene expression data highly sensitive to many factors
  - Lab operator/conditions, day performed, sample collection methods, RNA extraction day and so on
  - Often influence the data to greater extent than any experimental effects
  - Any step where treated and control samples are handled differently could confound the experiment
  - If split into batches containing mix of treated/control samples, can account for potential effects in analysis
- Also be aware of potential effects from factors unrelated to the experiment on the data, which may need to be accounted for to optimise analysis

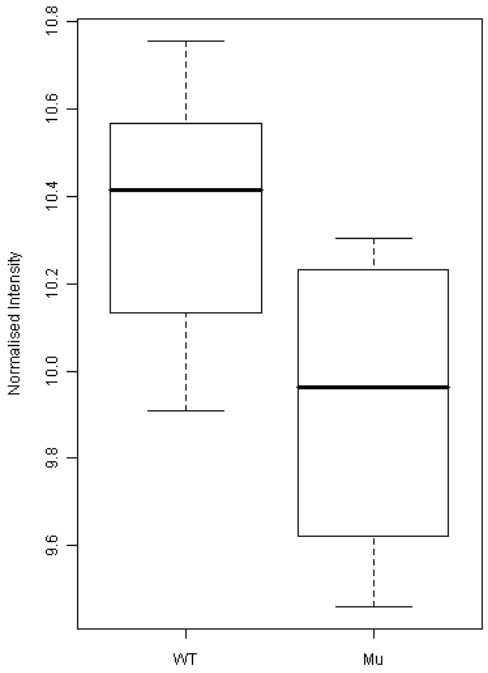
Gene not influenced by litter

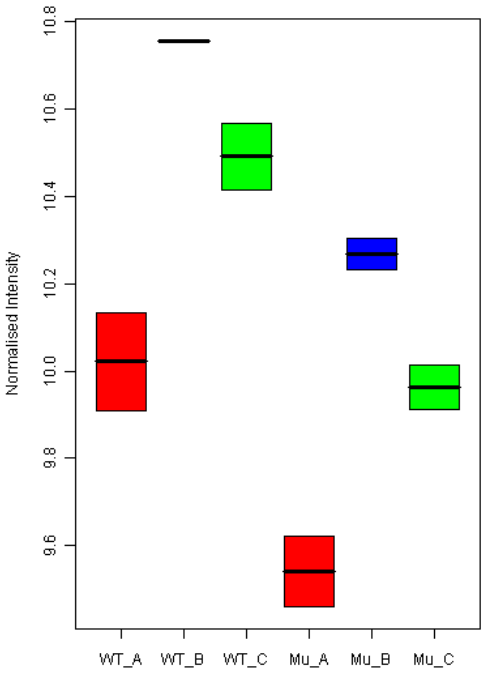




- Wt and Mut groups
- Three different litters
- Top gene ~ 5x higher expression in Wt compared to Mut
- Similarly expressed across litters in both genotypes

Gene with strong litter effect





- Within litters, consistent pattern of higher expression in WT vs Mut
- Within genotypes, B>C>A – expression depends on litter
- Accounting for this source of variability increases power to detect changes of interest