



*Setting standards  
in analytical science*

# Bio-Reference Standards/Materials

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**International Symposium on  
Reference Materials for Genetic  
Testing**  
**IRMM, November 29/30 2005**

# Genomics Standards & Standardisation Projects



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- Array performance indicators
  - Reference materials, protocols and toolkits to improve array based measurements
- Quality metrics to improve confidence in array data
  - Focus on Toxicogenomics
- Standardisation of gene expression measurements
  - Focus on QRT-PCR measurements
  - Reference methods and materials
  - internationally accepted measurement unit for gene expression
- Genotyping/haplotyping standards
  - Generic reference material for
    - platform evaluation
    - analyst training
    - assay QC
- qPCR Standards
  - LGC/NIST collaboration
  - Performance standard
- Oligo Quant standards
  - MS based quantification
  - synthetic DNA and primers
  - high order traceability

[www.mfbprog.org.uk](http://www.mfbprog.org.uk)



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# Microarray performance Indicators



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- Aims:
  - Develop and validate protocols, toolkits and reference materials to measure and improve performance of array based assays
  - International harmonisation of approaches to standardisation
- Reference standards
  - Assist in assay optimisation, QC, validation and comparability
  - Based on panels of probes containing increasing numbers of mismatches from core reference sequences
  - Complements ERCC standards



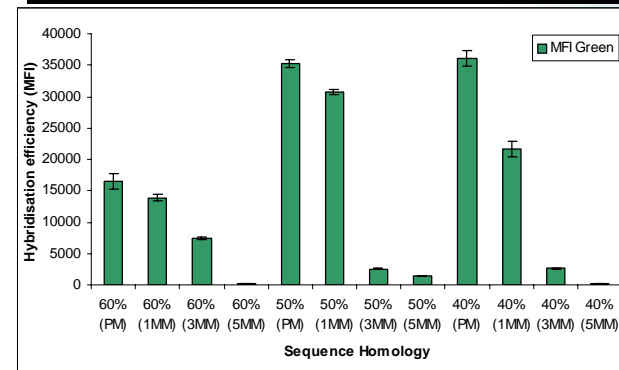
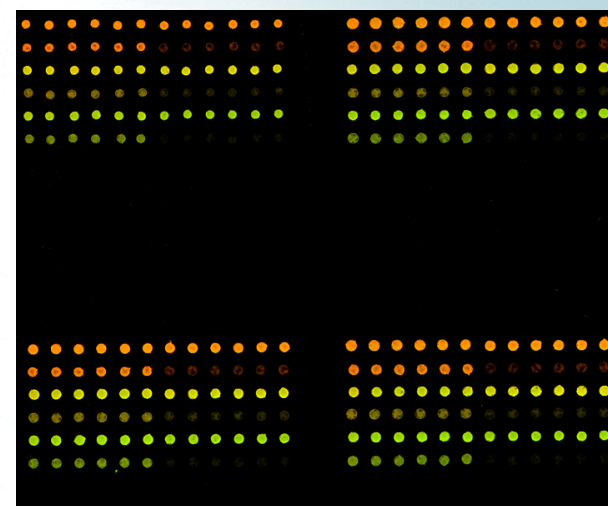
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# Specificity Standards and Performance Indicators for microarrays



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- Multiple sets of standards (high, med, low GC content)
- Four standards within each set differing by 1,3 and 5 mismatches
  - Additional panels ready to be added
- Fluorescently labelled reverse complements (Cy3 and Cy5) spiked-in
  - Dose response curve
- Discriminatory power of assay calculated
- Variations within and between arrays measured
  - Assist development, optimisation, validation and comparability
  - spatial, pin and spot distribution variability identified
  - Used to monitor hybridisation efficiency and specificity
- Useful for SNP genotyping and Expression arrays
- Complements ERCC standards
- Inter-lab trial underway with 10 labs

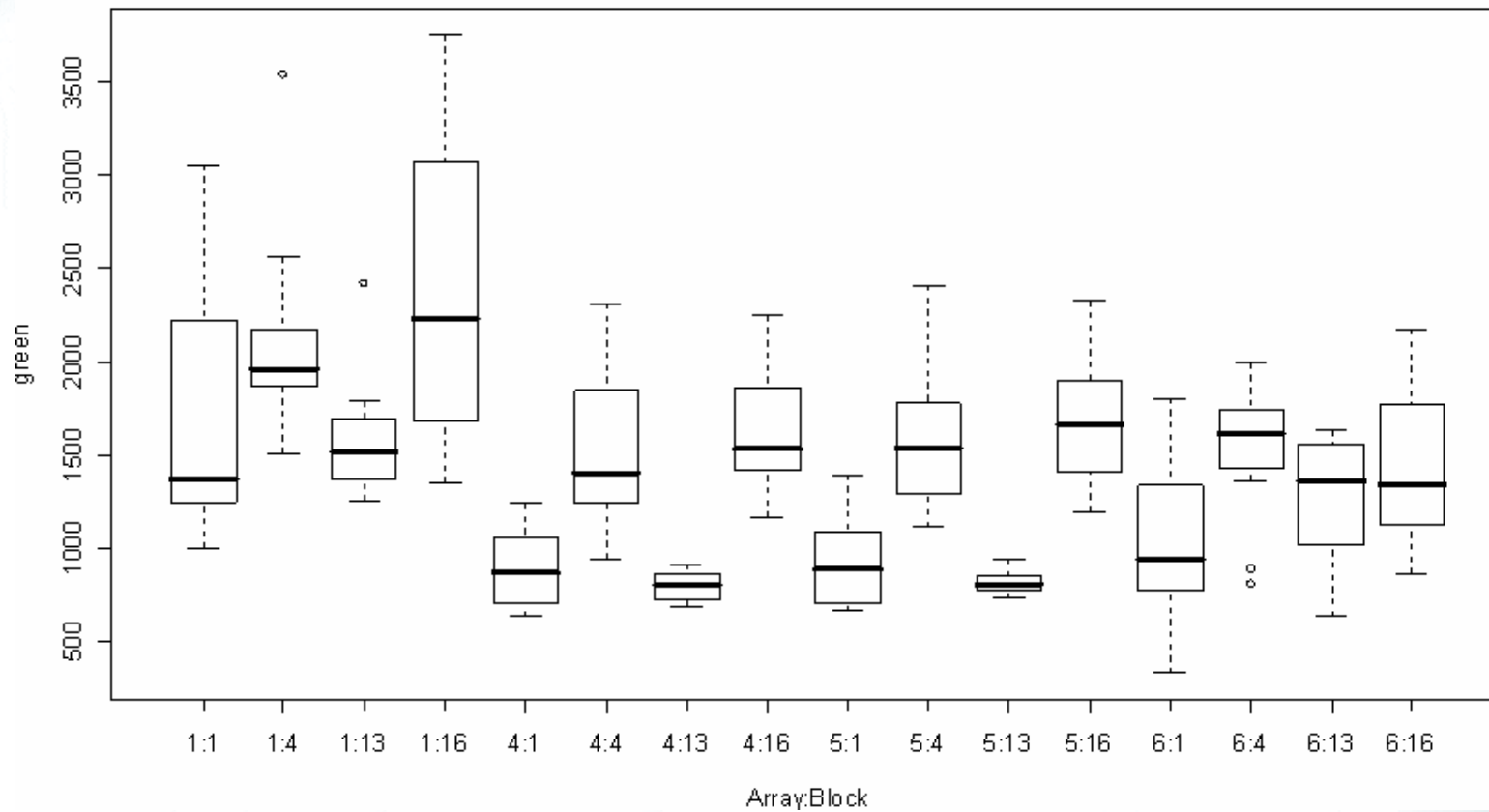


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# Array Performance



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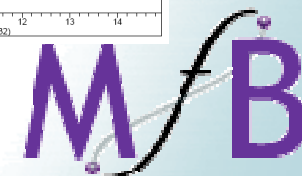
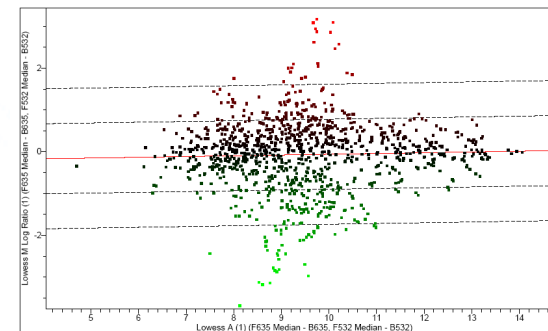
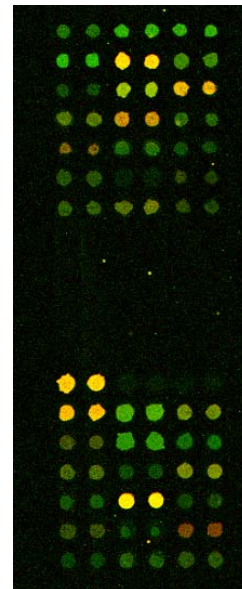
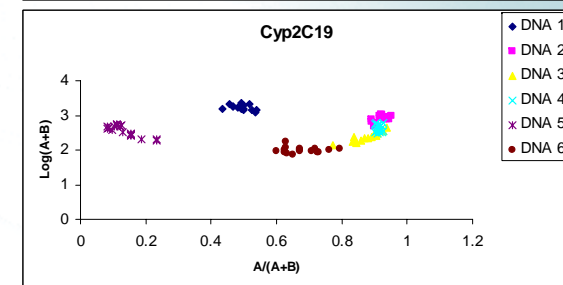
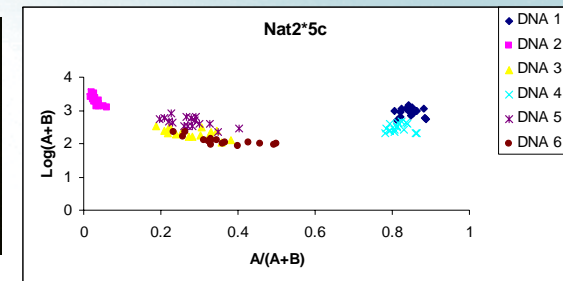
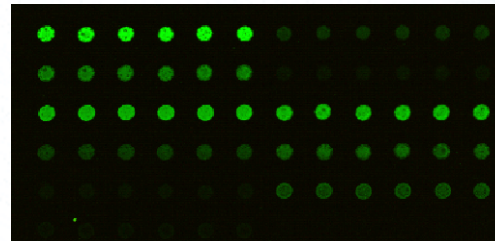
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# Current Status



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- Model SNP genotyping array
- Model Expression profiling array
- Algorithm developed for slide QC
- In-house evaluation and validation nearing completion
- Inter-laboratory trial underway



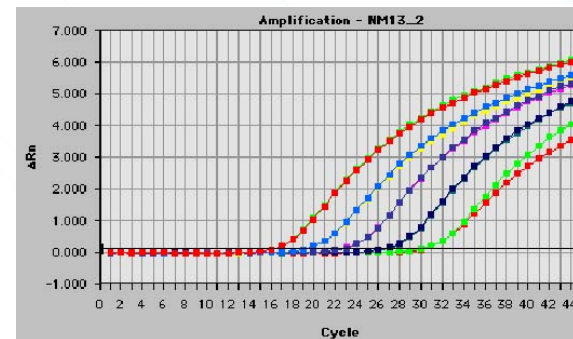
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# Standardisation of gene expression measurements



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- Working group created using MfB website discussion forum to evaluate & recommend possible approaches
- Practical assessments of potential strategies for standardising measurements underway
  - identification of “Best Practice”
- Considerations:
  - RT-PCR standardisation (priming strategy, random, oligo dT, gene specific), 3' and 5' bias
  - amplification and detection
  - standard curves
  - ‘housekeeping’/reference genes
  - calibrators
  - data analysis
- Complements ERCC initiative
- Reference transcript, primer and probe
  - Standard primer and probe sequences
  - *In vitro* transcripts from cloned cDNA sequences



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# Genotyping/haplotyping standards



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- Aims
  - To investigate requirements for generic reference panel of genotype/haplotype-validated reference materials
  - To develop a preliminary reference panel and demonstrate feasibility and utility as a measurement performance indicator /QC tool
    - platform evaluation
    - analyst training
    - assay QC
- Approach
  - Stakeholder consultation undertaken
  - Generic reference material under development
  - Non-human (Arabidopsis)
    - Two strains selected which differ by 12 SNPs in ~1500bp fragment
    - Fragments cloned and amplified
    - May provide PCR primers
  - Poly A tail and T7 promoter added to allow IVT for expression



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# Ist Pilot Study CCQM P44 - NIST/LGC

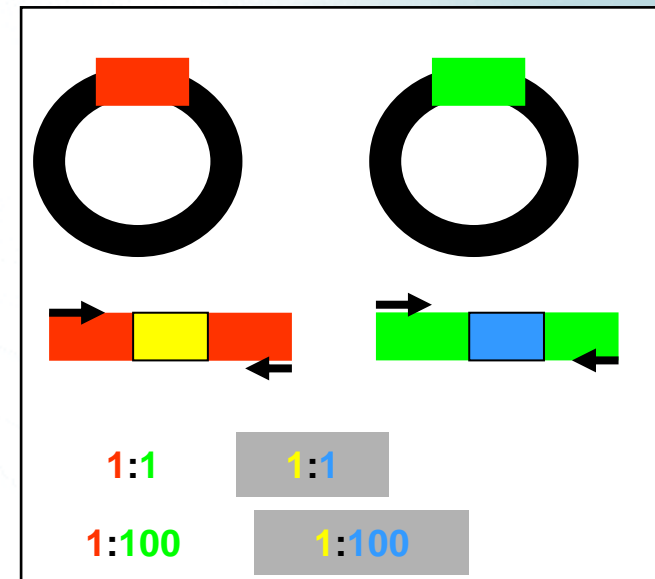
## Q PCR calibration



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### Develop measurement capability in QPCR

- Purpose: to determine major factors contributing to interlaboratory variability in quantification of a DNA sequence (eg. GM insert, viral load) using QPCR.
- How: By providing plasmid-based DNA calibration and testing materials. Designed as a independent monitor of equipment and technical staff performance
- Goal: Construct a comparability matrix of data from participating laboratories using different platforms and detection systems



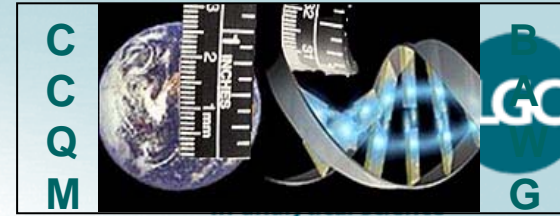
**Generic nucleic acid  
ratio assay -  
performance indicator**



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# P44.1 - round 2



## Graph of Equivalence - Sample A

