



*Setting standards
in analytical science*

Bio-Reference Standards/Materials

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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



Measurements for Biotechnology

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



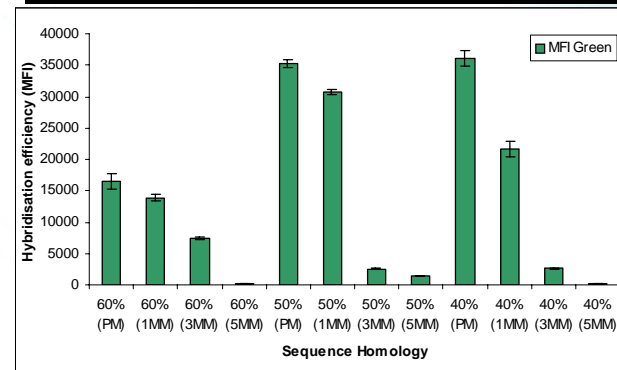
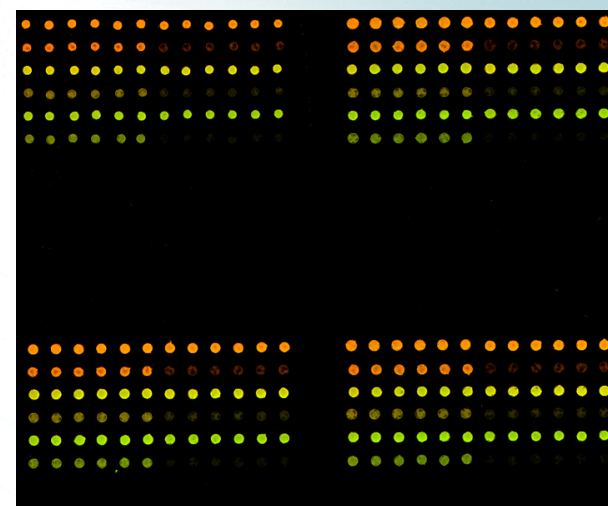
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Specificity Standards and Performance Indicators for microarrays



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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

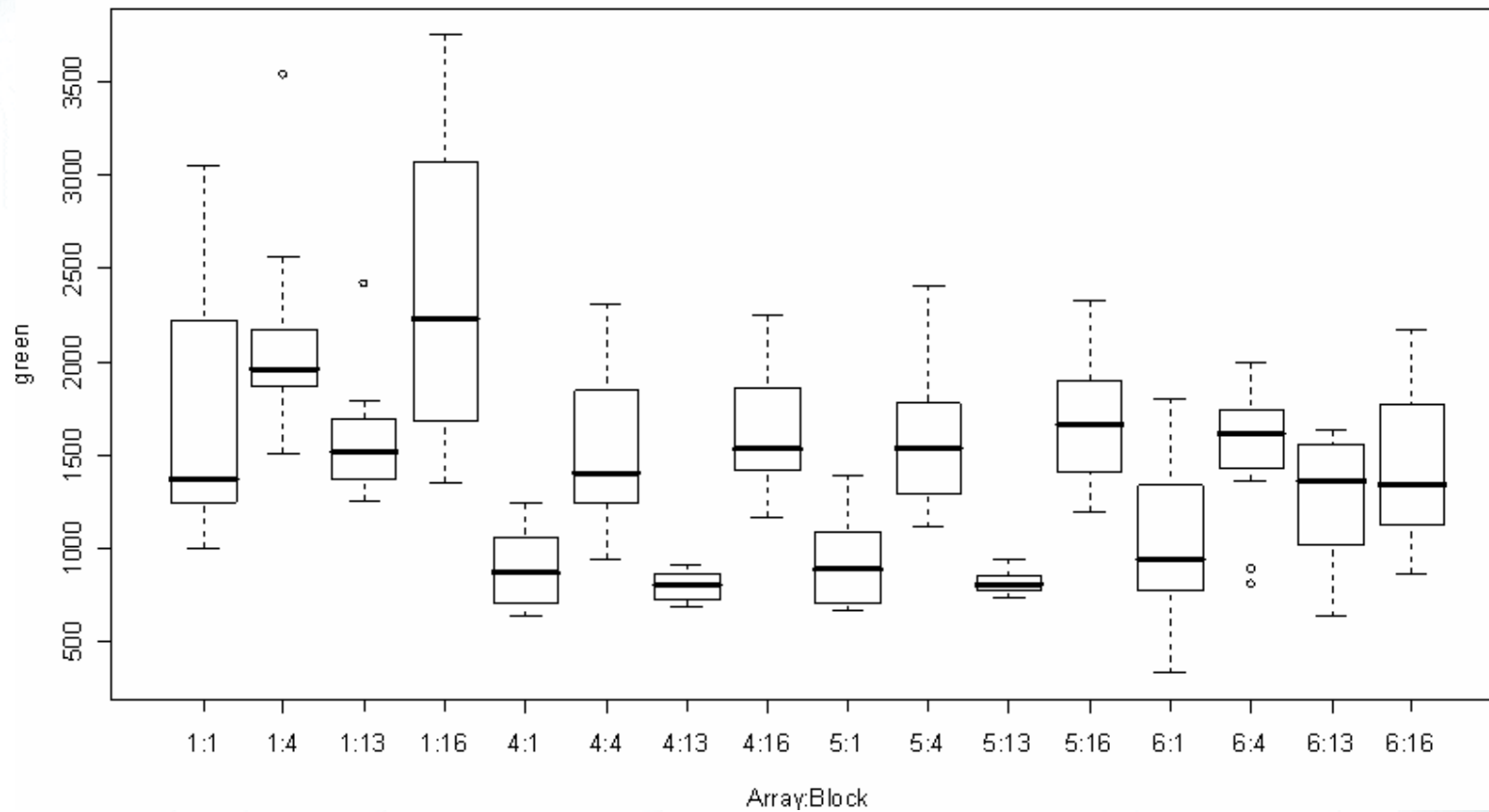


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Array Performance



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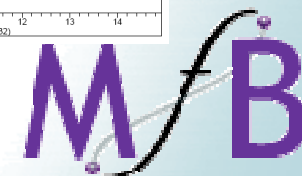
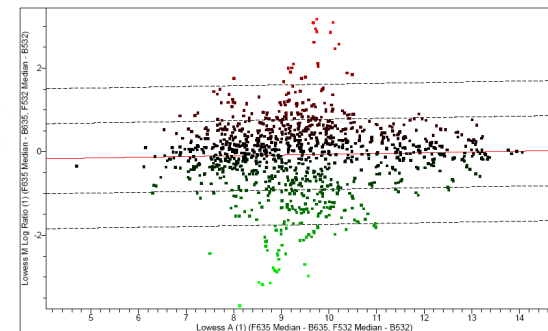
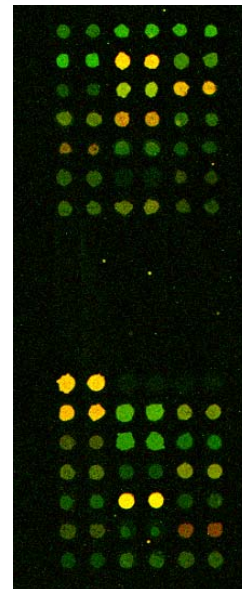
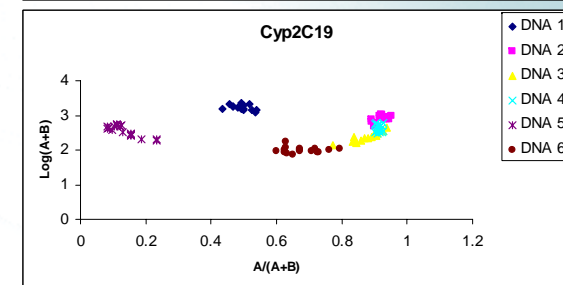
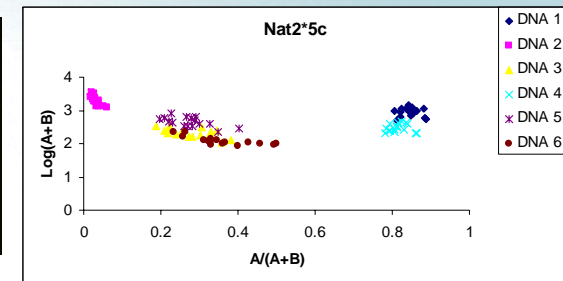
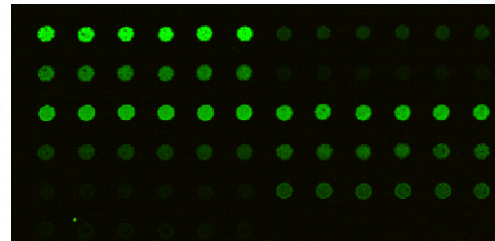


Current Status



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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



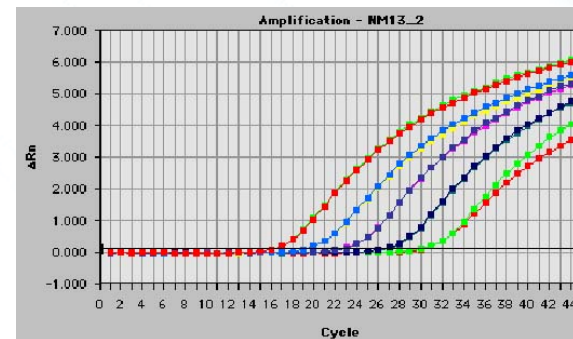
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Standardisation of gene expression measurements



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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



Measurements for Biotechnology

Ist Pilot Study CCQM P44 - NIST/LGC

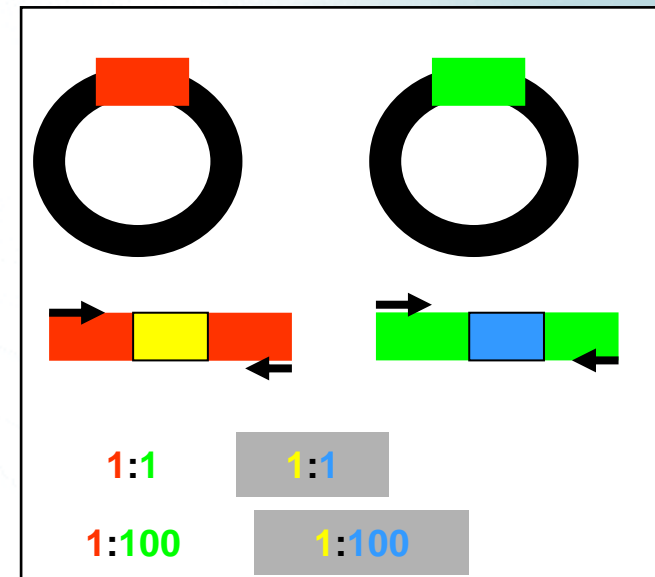
Q PCR calibration



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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

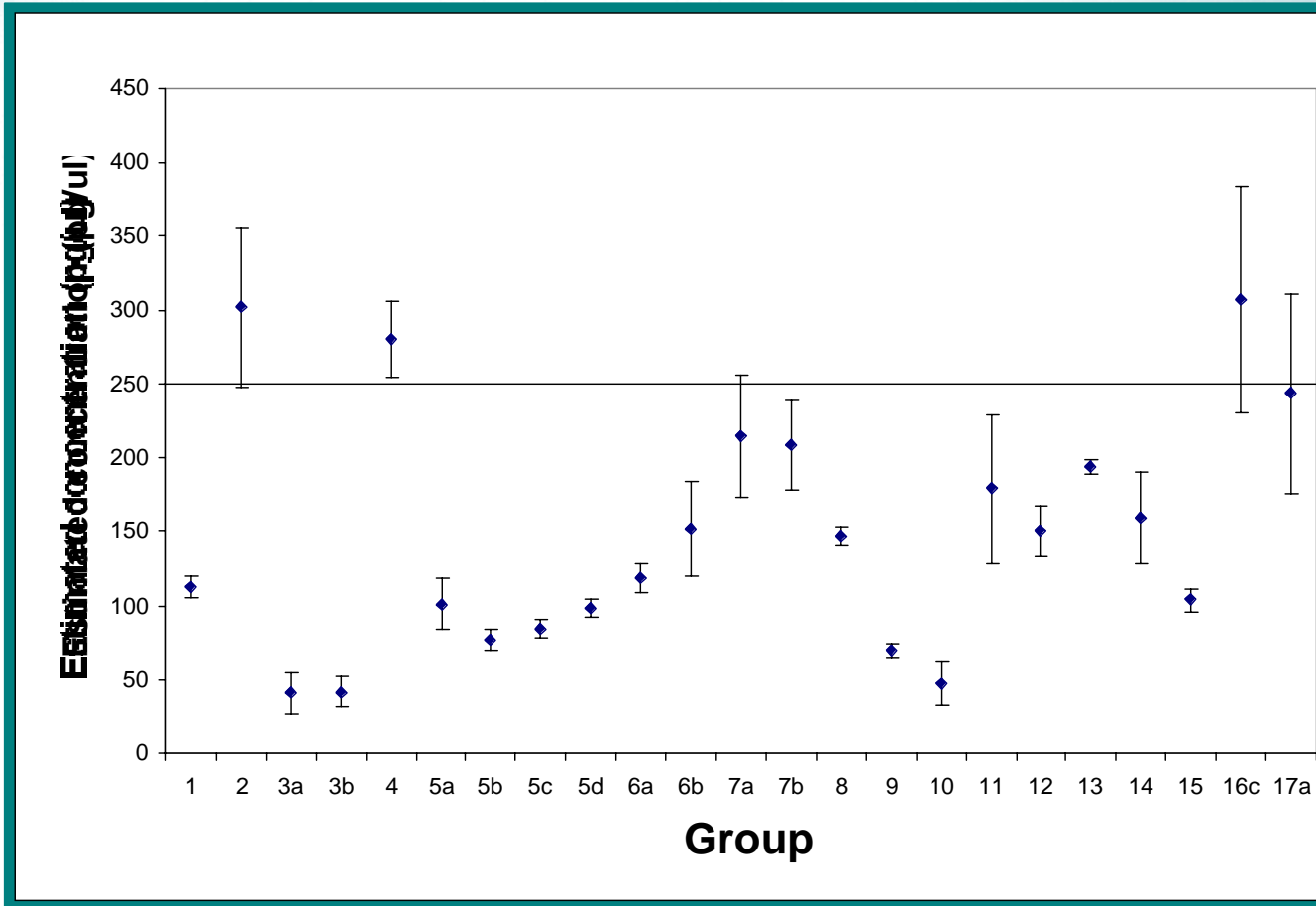
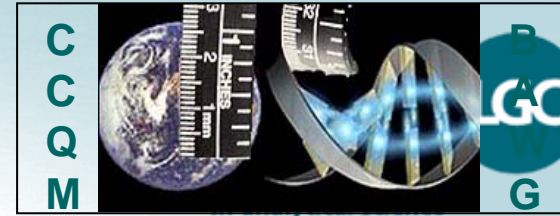


**Generic nucleic acid
ratio assay -
performance indicator**



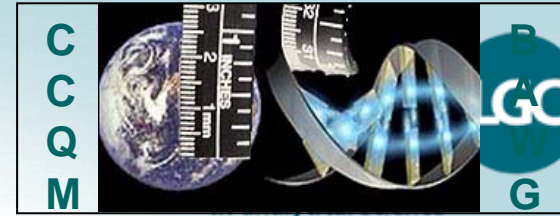
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1st round - Method performance



Group means and 95% confidence intervals are displayed on the graph

P44.1 - round 2



Graph of Equivalence - Sample A

