

University of Surrey Microarray Experiment Guidelines

Version 1 (Feb 2008)

Copyright: © 2008 E. Laing, G. Bucca, C. P. Smith. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original authors and source are credited.

Funding: This material is based on work supported by the European Commission FP6 ActinoGEN programme grant no. 005224 and the BBSRC. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Address: Faculty of Health and Medical Sciences, University of Surrey, Guildford, GU2 7XH, UK.

Version 2 of this document will include a section on ChIP-on-chip analysis.

Table of Contents

Background	2
Why Microarrays?	2
What are microarrays?	2
Microarray experiment workflow	5
Decision to do a microarray experiment	6
Designing a microarray experiment	7
Important checks during the experiment	8
Normalisation and differential expression analysis	9
Normalisation	9
Differential expression analysis	14
Storage of Microarray data: MIAME compliance	15
Why do I need to document my experiment?	15
What is MIAME?	15
How can I document my experiment?	16
How to fill in the Excel Workbook template	16
Source Worksheet	17
Samples Worksheet	17
Extracts Worksheet	18
Hybridisation Worksheet	19
Protocols Worksheet	20

Background

Why Microarrays?

Capable of simultaneously measuring the expression levels for thousands of genes, microarrays provide a large quantity of information about an organism/cell/tissue – whether it be mutational studies (monitoring the effects of gene expression by knocking out/in a particular gene), conditional (monitoring the effects on gene expression when presenting the organism/cell to a particular environment/stress) and/or comparative (compare the presence/absence of genes in different organisms/strains). The advantage of studying many genes and many transcripts at the same time is that it provides a systematic view of how organisms/cells/tissues react in response to certain stimuli, or a global view of genome organisation (presence/absence of genes).

What are microarrays?

As a large-scale, parallel version of Northern blots (or Southern blots for comparative genomics), the process of hybridisation where two nucleic acid strands hybridise if they are complementary to each other is fundamental to monitoring the expression of genes in microarray techniques; the use of Northern (for detecting RNA) and Southern blotting (for detecting DNA), ‘one-gene-at-a-time’ techniques, has been somewhat overshadowed since the emergence of the larger scale hybridisation techniques of microarrays but are not redundant.

Briefly, a microarray is a solid surface (slide) to which DNA (either PCR products, oligonucleotides or *in situ* synthesised) is immobilised upon; each fragment of DNA fixed on to the microarray slide is called a probe and is designed to be uniquely complementary to the RNA/DNA target present in a given sample of interest. The RNA/DNA target is labelled with fluorophores and is then hybridised to the array, as depicted in Figure 1.

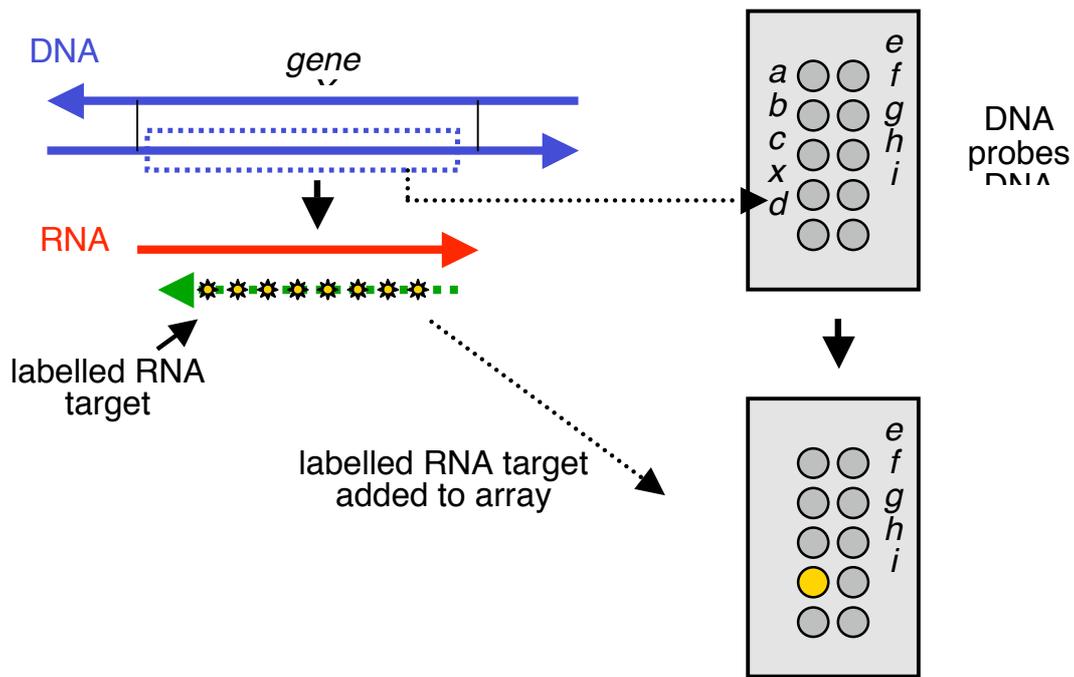


Figure 1 A microarray slide

Spotted (either PCR products or oligonucleotides spotted onto the microarray slide) or Ink-jet *in situ* synthesised, IJISS (DNA synthesised directly on to the microarray slide) microarrays are advantageous as they enable the semi-quantification of gene expression for thousands of genes in a single experiment. Unlike Affymetrix GeneChip® arrays, spotted/IJISS arrays allow two conditions to be tested simultaneously through the use of two fluorophores. Spotted Oligo, spotted PCR and IJISS microarrays are all available from the University of Surrey, design details of which can be found at www.surrey.ac.uk/SBMS/Fgenomics/Microarrays/.

By using two fluorophores, typically Cy3 (fluoresces as green) and Cy5 (fluoresces as red), to label different samples it is possible to co-hybridise samples such that they can be directly compared on a single array, as depicted in Figure 2. Thus, when the microarray is scanned by a laser scanner and the fluorophores are excited by the laser (at the specific wavelengths) it is possible to determine which genes are more expressed in sample A than in sample B, less expressed in sample A than in sample B or equally expressed in sample A and B by observing the spot colours.

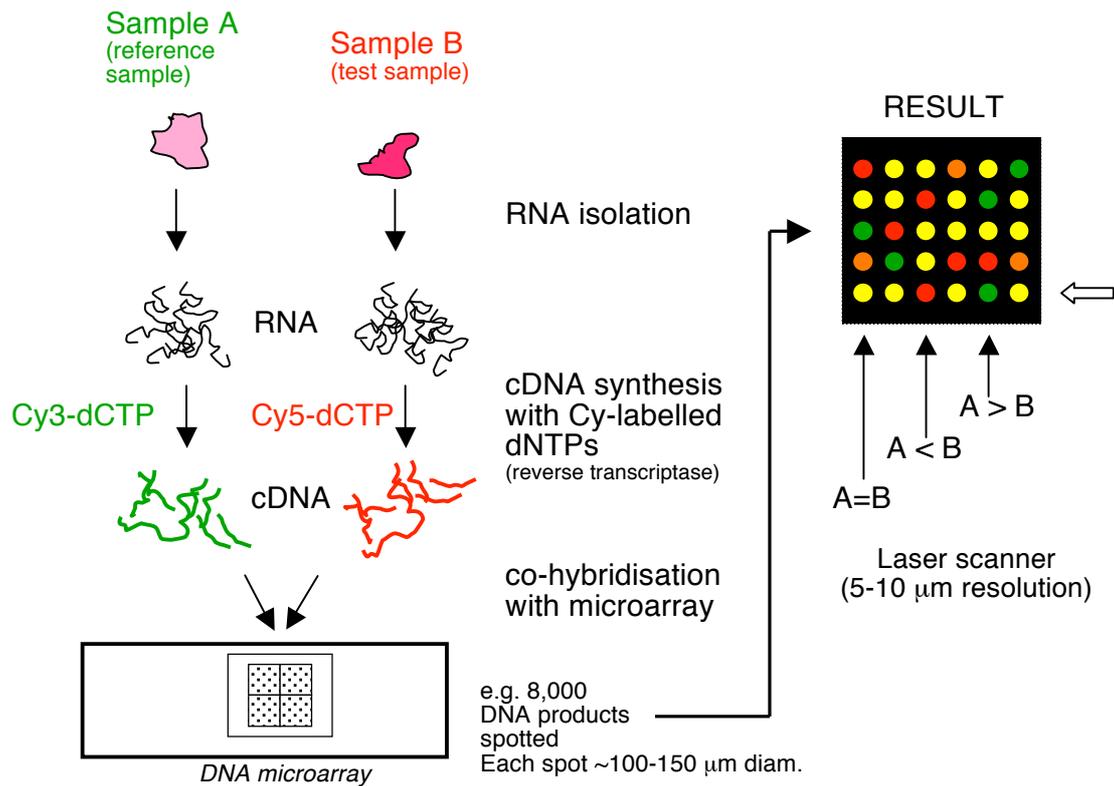


Figure 2 Two colour microarray experiment

Observing spot colours is not accurate; instead the image output of the scanner is further processed using feature extraction software (e.g. Bluefuse or Agilent feature extraction) to quantify the signal intensities at each spot on the microarray. The ratio of the signal intensities of each channel (red and green) for a spot/gene is therefore the change in expression between samples A and B. This can then be repeated across biological replicates/conditions to produce an expression matrix that represents expression profiles for rows of genes at each replicate/condition (given in the columns), as seen in Figure 3. It is this expression matrix that can then be used to identify genes that are differentially expressed between two conditions (or present/absent between strains) and/or co-regulated.

Biological Replicate 1			
	Sample A	Sample B	Ratio A/B
Gene 1	2500	1250	2.0
Gene 2	250	250	1.0
Gene 3	100	500	0.2
.....

Biological Replicate 2			
	Sample A	Sample B	Ratio A/B
Gene 1	1250	1250	1.0
Gene 2	625	250	2.5
Gene 3	1500	500	3.0
.....

Gene expression matrix			
	Biological replicate 1	Biological replicate 2
Gene 1	2.0	1.0
Gene 2	1.0	2.5
Gene 3	0.2	3.0
.....

Figure 3 Gene expression matrix.

Microarray experiment workflow

Figure 4 depicts the experimental workflow identified by the group at University of Surrey. Each of the points in Figure 4 represent checkpoints that need to be gone through for a successful microarray experiment; this not only ensures that microarray users obtain the right help but also aids the standardisation of data across laboratories and users within the consortium. Several of these checkpoints are discussed in the following sections.

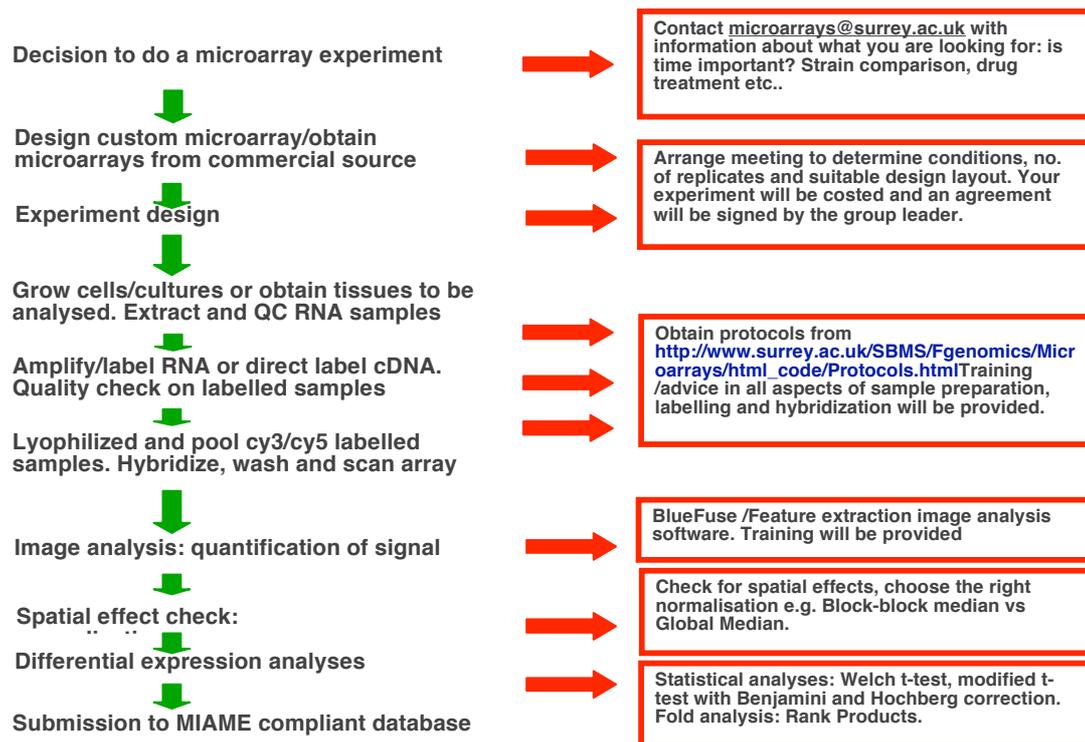


Figure 4 Workflow of a Microarray experiment

Decision to do a microarray experiment

To make the decision on whether to conduct a microarray experiment or not it is important to understand what it is you're looking for. **Do you know which strains/cells/tissues you would like to compare?** I.e. are you comparing different conditions e.g. drug treatment: treated vs. untreated? Effects of manipulation of a single gene (over-expression/ knockout)? Or monitoring gene expression changes during the life cycle? **Be selective!!** Know the specific biological questions you would like answered, this simplifies the design, the actual experiment and further downstream analysis.

Other factors that are important in making the decision and have an effect on the design of the experiment and in some cases the results are:

- Do you know at which stage/time-point strain differences become apparent? (no need to waste money/time by doing unnecessary time-points on arrays)
- Would you like to take time into consideration? (reduces/increases the number of arrays needed in the experiment)
- Have you considered what should be used as reference samples? (will a common reference be used? Or a WT? which WT?)

- Have you considered how much source material you have? (this will affect the number of arrays that can be hybridised and the design of the experiment)
- Are your reference and samples grown under the same conditions? (are the samples directly comparable? Will the downstream analysis make sense?)
- Have you considered how many biological replicates you need for your experiment? (at least 3 biological replicates are normally required for a microarray experiment – more replicates allow the detection of more subtle gene expression changes)

Once these questions are answered the next step is to contact microarrays@surrey.ac.uk to discuss the design of your experiment.

Designing a microarray experiment

Different biological questions, number of replicates and/or conditions will affect the design of your microarray experiment (as discussed above). Most importantly it is the labelling bias (Cy3 incorporates more efficiently than Cy5) and biological variance that needs to be minimised during the experimental design; there are two microarray experimental designs that are typically exploited at UoS that achieve this: Common reference design and Balanced-block design; each of these are discussed in turn.

Common reference design

Comparison: e.g. Wild-type vs. Mutant

Conditions: Know which time-points are the most important and get good biological replication.

Biological question: Want to know which genes are up or down across time in both samples.

Design: Reference design incorporating 3 biological replicates for each time point of interest using RNA vs. common reference (e.g. genomic DNA (gDNA)). Example shown in Figure 5, three time-points in each strain meaning six arrays for each biological replicate, 18 arrays in total for complete experiment.

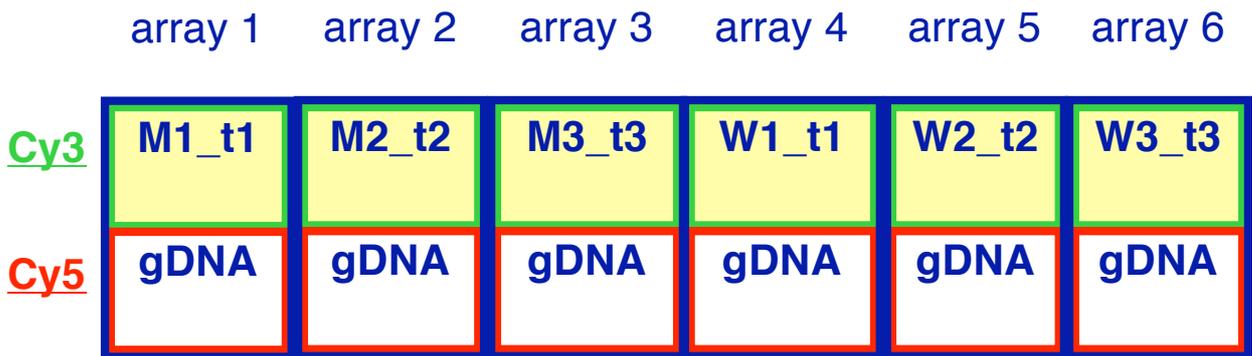


Figure 5 Common reference design. Nomenclature of Strain_timepoint.

Balanced-block design

Comparison: e.g. Wild-type vs. Mutant

Conditions: Know which time-points are the most important and get good biological replication.

Biological question: Want to know which genes are up or down between Mutant and Wild-type (across time not important and neither absolute expression level).

Design: Balanced block design incorporating 4 biological replicates for time points of interest using RNA vs. RNA; the design is shown in Figure 6, four arrays in total for complete experiment (one time point).



Figure 5 Common reference design. Nomenclature of Strain_timepoint.

The best design for your experiment can be discussed by contacting microarrays@surrey.ac.uk.

Important checks during the experiment

Below are several checks identified in the microarray experiment workflow that will aid a successful experiment:

- For *Streptomyces* experiments you will require 10µg RNA for each labelling reaction, this usually provides sufficient labelled target for several arrays.
- For eukaryotic systems aim to start from a **minimum of 5 × 10⁶ cells** or 30 mg of tissue in order **to get enough total RNA** for your experiment (this depends on the specific tissue/cell line).
- The ratio between the absorbance (using the Nanodrop spectrophotometer) of each RNA sample at 260/ 280nm and 260/230nm gives an indication of the purity of your sample. **RNA with A260/280 ratio between 1.8 and 2.1 and A260/230 between 1 and 2 are routinely used in microarray experiments.**
- Microfluidic Lab-on-a-Chip technology is used to check the integrity of RNA (RIN) where a number from 0-10 is assigned to each sample, **only RNA with a RIN number >7 is taken forward for microarray experiments.**
- **A minimum of 5 micrograms of total RNA or 1 microgram of mRNA is typically used per labelling reaction in a microarray experiment for eukaryotic systems, for *Streptomyces* 10-15µg of total RNA is needed;** go to www.surrey.ac.uk/SBMS/Fgenomics/Microarrays for exact protocol.
- **Calculate the frequency of incorporation of Cy dyes into your sample** using the Nanodrop spectrophotometer.
- Make sure that ranges or print runs of arrays do not only appear in a single condition (i.e. only WT or only Mutant): **Be random when selecting arrays to hybridise your samples!**

Normalisation and differential expression analysis

Normalisation

In addition to non-specific binding errors, noise in a microarray experiment can arise from technical errors such as dust on the surface, irregular spot printing (for spotted arrays), and non-uniform light intensity detection (where parts of an array seem to have more fluorophore than the others, not due to biological variation), to name a few. Thus, it is important to subtract background or noise from the signal intensity measurements obtained after scanning and feature extraction of the microarray slide(s); it is only when the data has been normalised (reduction of noise) can any real interpretation/analysis of the data be conducted.

Firstly, the main goal of “within-array” (applied to each individual slide based on values of that slide) normalisation is to take all of the raw ratios (ratios of Sample/reference values obtained directly after feature extraction) seen in a slide and push their mean value to 1 (0 in \log_2) as it is assumed that very few genes are differentially represented in each of the two channels in the array. Box plots of array data before and after normalisation can be seen in Figure 6. As with any normalisation the dynamic range or spread of data is compressed (see Figure 6).

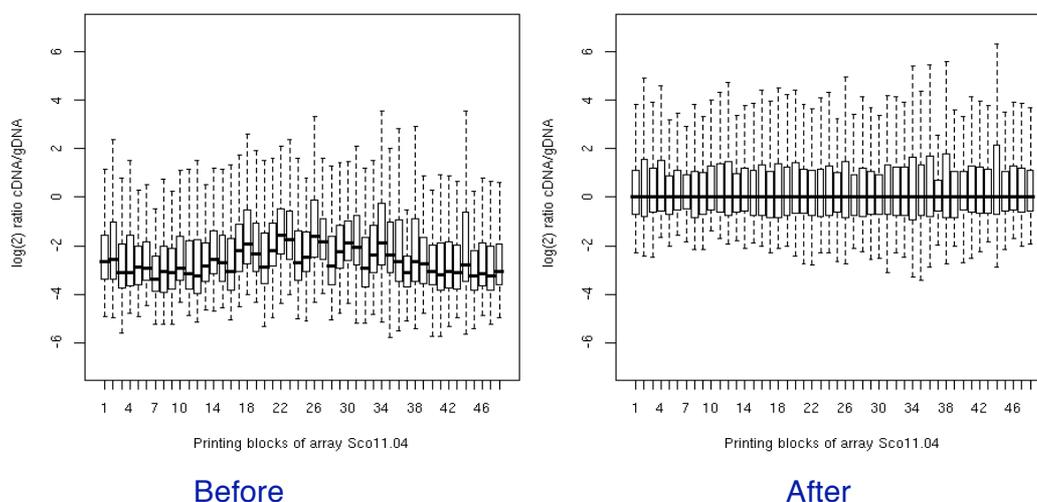


Figure 6 Box plots depicting the effects of normalisation on the \log_2 ratios of each printing block on a spotted oligonucleotide array.

There are different within-array normalisation strategies that are applied to microarrays, each making subtly different assumptions of the data; the choice of normalisation strategy for your data is dependent on the hybridisations performed (RNA vs. RNA or RNA vs. a common reference) and if any spatial effects (such as that seen in Figure 7) are observed.

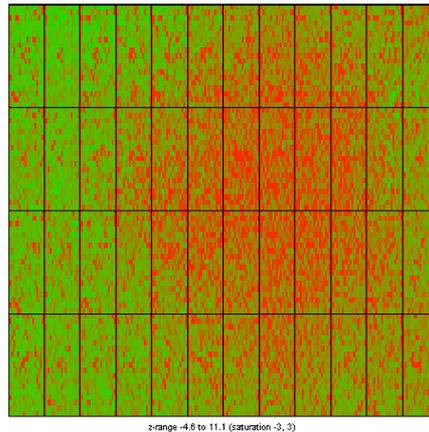


Figure 7 Image reconstruction of an array with a spatial effect. A patch in the centre of the array where non-uniform hybridisation has occurred.

The common strategies applied for within-array normalisation are:

- **Global Median** is typically applied to arrays that use a common reference design or where there should be no similarity in expression between the samples hybridised and no spatial effect is observed. This normalisation does not assume that the two channels (Cy3 and Cy5) have the same distribution, it does not force them to be similar, it assumes only that the signal across the array should be uniform (upheld as the probes on the array are spotted/printed randomly).
- **Block-Median** is typically applied to arrays that use a common reference design or where there should be no similarity in expression between the samples hybridised and a spatial effect is observed (such as that in Figure 7). Again, this normalisation does not assume that the two channels have the same distribution, it assumes only that the signal within each block (a sub-grid of spots on the array) should be uniform.
- **Lowess (sometimes written as Loess)** is typically applied to arrays where there is similarity in expression between the two samples hybridised on the array (e.g. RNA vs. RNA or Comparative Genomic Hybridisations) and no spatial effect is observed. This normalisation assumes that the two channels across the array have the same distribution.
- **Print-tip Lowess (sometimes written as Loess)** is typically applied to arrays where there is similarity in expression between the two samples hybridised on the array (e.g. RNA vs. RNA or Comparative Genomic Hybridisations) and a spatial effect is observed. This normalisation

assumes that the two channels within each block of the array have the same distribution.

The effect on the distribution of values in each of the channels of an array that the different normalisation strategies have been applied to can be seen in Figure 8.

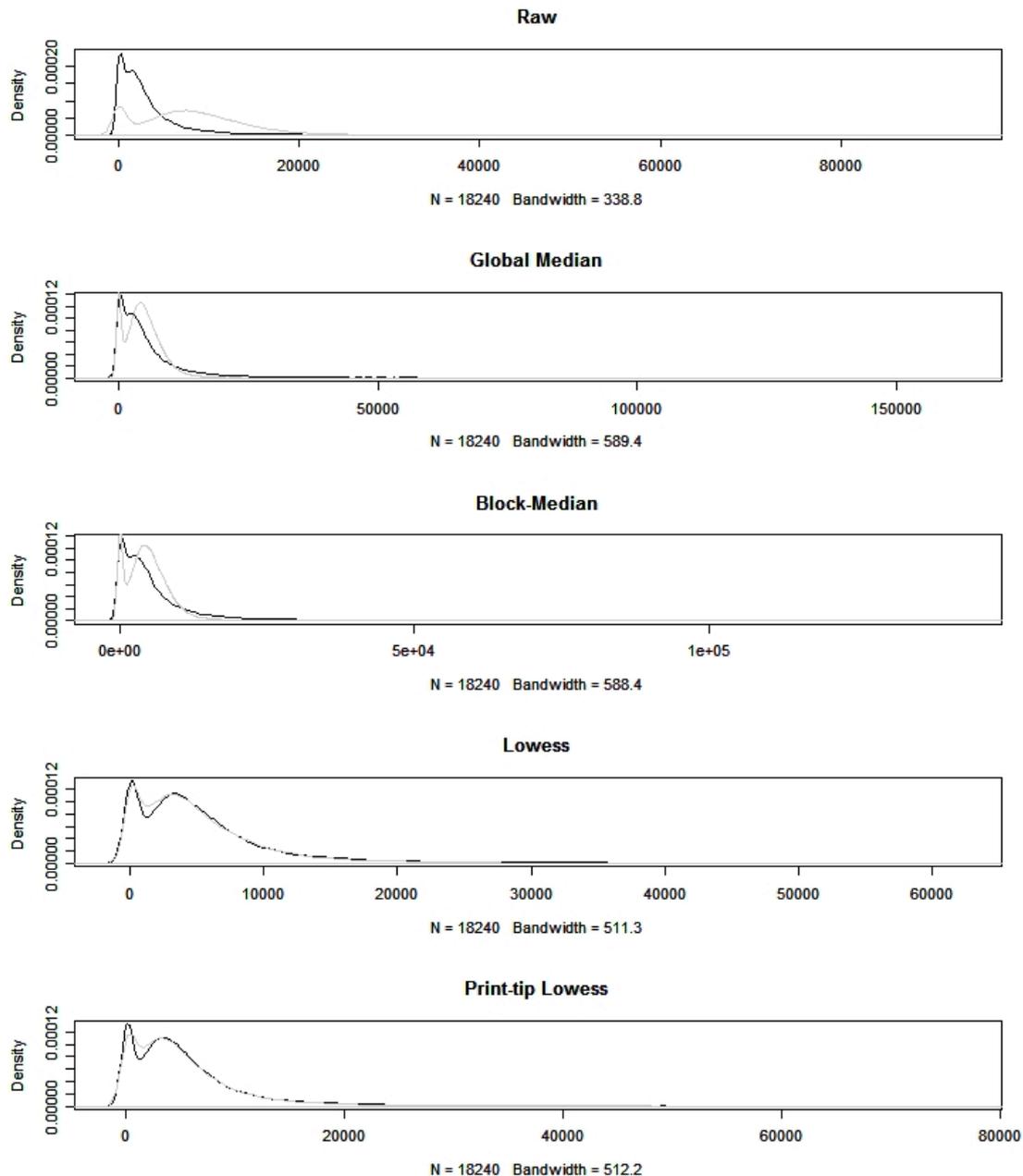


Figure 8 Distributions of Channel intensities on the array. Raw intensities (without normalisation) and intensities after different normalisations (Global Median, Block-Median, Lowess and Print-tip Lowess) are shown. Black distribution represents the Cy5 channel on the array, whilst grey represents Cy3.

Finally, after within-array normalisation it is important to decide whether to apply across-array (a value applied to each array based on values of all arrays) normalisation. It is important because a further normalisation step will further compress your data. However, if the normalisation is not applied and the arrays in your experiment have wildly different distributions then you may get spurious results when looking for differentially expressed genes (many more genes would be classed as significant when the across-array normalisation isn't applied). Thus, the distributions of your data must be first looked at before making your decision, then, if different distributions are observed (such as that seen in Figure 9 "Before") and the null hypothesis is that there is no difference in expression between these samples (arrays) then it is correct to apply across-array normalisation. The across-array normalisation will scale your data such that the arrays will have similar distributions (seen in Figure 9 "After").

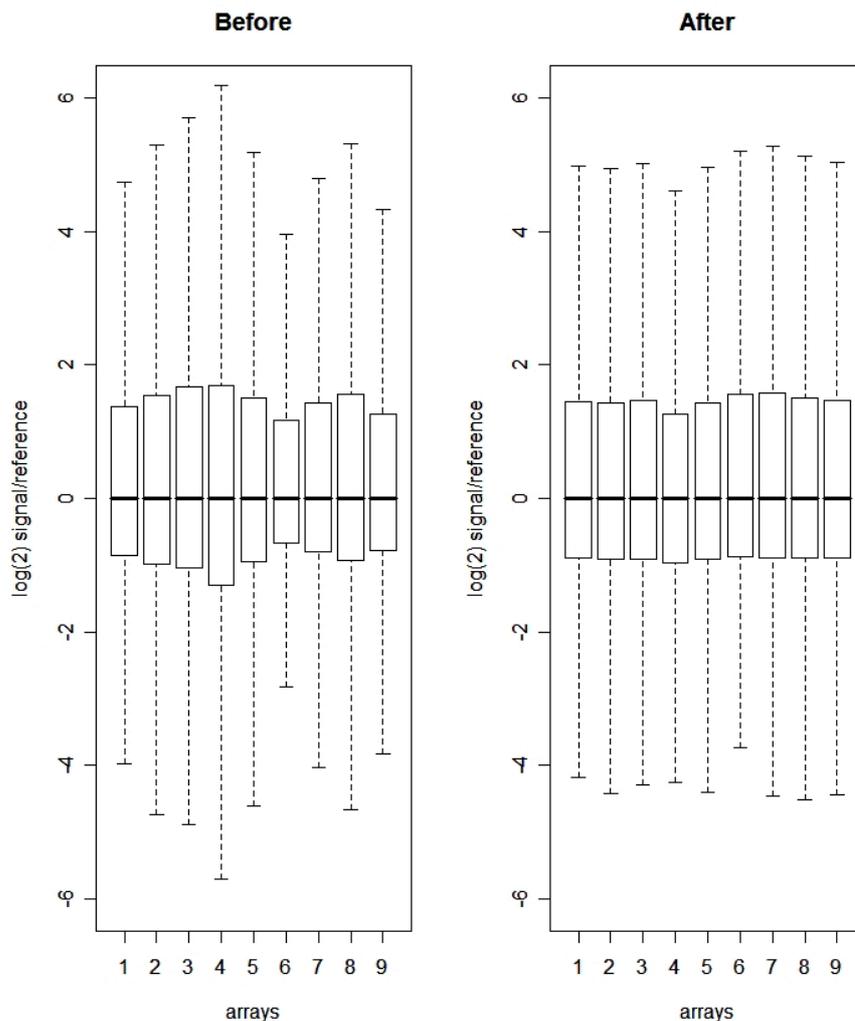


Figure 9 Box-plots of before and after across-array normalisation.

Once normalised, the data can then be filtered such that all bad spots (flagged spots dependent on the threshold of outliers you apply) are removed. Replicate probe spots on the array for each individual gene are then averaged as well as technical replicate arrays. After filtering and averaging (leaving data for genes that have good values across each biological replicate for each condition (if any)) the data is then ready to be analysed for differential expression / interpretation / presence or absence of genes.

Differential expression analysis

Historically the differential expression (or absence in the case of comparative genomic hybridisations) of a gene has been identified by 2 Fold analysis, where genes with a 2 fold (arbitrary threshold) increase/decrease in expression between conditions/samples are selected for further interrogation. Nowadays, unless there is much validation (many RT-PCRs and/or knockout (of identified genes) studies) a significant, as determined by application of statistics, differential expression needs to be identified. Typically the statistical significance of a gene being differentially expressed is calculated using two sample statistics e.g. a gene in WT vs. the same gene in Mutant or a gene at time-point x vs. the same gene at time-point y.

Within the group at Surrey two tests are applied to identify statistically significant differentially expressed genes between two conditions:

- **T-test**, commonly the Welch T-test, an adapted Student's t-test that does not assume that the samples (e.g. expression ratios in WT and Mutant) have equal variance; an associated p-value from the T statistic is obtained. As this test is run many times over a gene list (e.g. list of genes that passed filtering, discussed previously) the generated p-values need to be corrected to control the false discovery rate (i.e. the more times the T-test is run the more likely that the genes you first identify as being significant are not), this is achieved through the use of the Benjamini and Hochberg algorithm, the more lenient version of p-value correctors. After correction a gene is significantly differentially expressed if its p-value is less than 0.05. **Note: The more variance between biological replicates the less significant genes will be obtained, the t-test is not that robust when it comes to large variances: a modified T-test (e.g. eBayes) is more robust and may be more suitable for your data.**

- **Rank Product analysis**, a test found to be more robust (than T-test) when biological replicates have large variance. This test does not use the actual ratios themselves to compute significance, only (as the name suggests) ranks; it checks whether the most up-regulated gene is the most up-regulated gene across biological replicates and similarly the most down-regulated (or absent in the case of comparative genomic hybridisation) gene is the same across replicates. With this test the pfp (percentage of false positives) value is used to determine significant differential expression between conditions; if a gene has a pfp value less than 0.1 (equivalent to you being wrong 10% of the time if class this gene as being differentially expressed) then it is significant. A pfp value is generated for both up- and down-regulation. A web tool for conducting Rank Product analysis is available at <http://strep-microarray.sbs.surrey.ac.uk/RankProducts>.

At University of Surrey the final significantly-differentially-expressed-gene-list is obtained by combining the two lists generated by the above tests. This list can then be further interrogated e.g. clustering of genes to identify common expression profiles, over-representation of annotation, identification of common regulatory motifs etc.

Storage of Microarray data: MIAME compliance

Why do I need to document my experiment?

There are many reasons why you should document your microarray experiment; the most important three being: (1) if you should leave, someone in your lab can see how/when/why your experiment was done, (2) It allows you to share your data with people – collaborators or readers of a related paper and (3) It is now required that upon submission of a microarray related paper to journals that your data be documented in an appropriate manner and deposited in a database – both of which are under MIAME guidelines.

What is MIAME?

MIAME is the **Minimum Information About a Microarray Experiment** as set out by the MGED group (www.mged.org). Briefly, the MGED group is a group of people that got together to form a standard for describing microarray experiments. This standard describes what the minimum information about a microarray experiment is such that other people can understand and repeat

the experiment if necessary and provides a fixed vocabulary (ontology) with which to do this.

How can I document my experiment?

There are many ways in which you can document your experiment and the choice is primarily dependent upon where you will store the information. There are 2 MIAME compliant databases that are recognised by journals, ArrayExpress (www.ebi.ac.uk/arrayexpress/) and GEO (www.ncbi.nlm.nih.gov/geo/); these databases are the most targeted. Typically, UK submissions go to ArrayExpress and American submissions to GEO. Within this consortium we focus on ArrayExpress, where we already have experience of successful submissions.

Submission of data to ArrayExpress can be via one of three ways:

1. MIAMExpress, a web tool that has a user-interface for describing your experiment.
2. Tab2MAGE, fill a spreadsheet and then have to convert to MAGE-ML (a mark-up language for describing microarray experiments).
3. Submission of MAGE-ML from a different database.

Here we recommend the submission of MAGE-ML from a different (non ArrayExpress) database (option 3) as it requires less knowledge of the MGED ontologies. The database specifically for the *Streptomyces* community is StreptoBASE (<http://streptobase.org/>), this allows private (only for the experimenter/consortium) and public (everyone) data viewing.

To get data into StreptoBASE you now have 2 choices:

1. Direct use of the maxdLoad software to fill a database created for you (needs contact (microarrays@surrey.ac.uk) prior to wanting to do this). This requires knowledge of the software and some knowledge of MGED ontologies.
2. Fill out an Excel workbook template and send it along with your measurement data to microarrays@surrey.ac.uk.

How to fill in the Excel Workbook template

Text highlighted in yellow indicates choices of ontology typically chosen to describe a *S. coelicolor* experiment.

Source Worksheet

Source_Name: The name of the source (strain) you used

Description: Free text, a description of the source (strain) you used

Source_Type: Choice of either: agar_stab, freeze_dried_sample, fresh_sample, **frozen_sample**, paraffin_sample or urine

Material_Type: Choice of either: DNA, cell, cell_lystate, cytoplasmic_RNA, genomic_DNA, molecular_mixture, nuclear_RNA, organellar_DNA, organellar_RNA, organism_part, polyA_RNA, protein, synthetic_DNA, synthetic_RNA, total_RNA, virus or **whole_organism**

Organisation_of_Provider: Where did you get the source from?

Person_who_provided: The name of the person who gave you the source

Type DB: NCBI_taxonomy

Type Ref: NCBI taxonomy accession number for your organism e.g. 100226 for *Streptomyces coelicolor* (different for other organisms)

Type URL:

Samples Worksheet

Sample Type (Untreated or Treated): Is the sample Treated or Untreated?

Sample_Name: The name of the sample

Name of where comes from (untreated sample name or treated sample name or source): The name of where your sample comes from, this can be the source (for an untreated sample), an untreated sample (for a treated sample) or a treated sample (for a treated, treated sample).

Protocol: The name of the protocol you used to create the sample (must be included in the Protocols worksheet)

Type_of_Action: Choice of either: PCR_amplification, acclimatization, behavioral_stimulus, compound_based_treatment, decontaminate, dissect, fractionate, genetic_modification, grow, harvest, histological_slide_preparation, incubate, infect, inoculate, irradiate, linear_amplification, pool, preservation, purify, reverse_transcription, sacrifice, specified_biomaterial_action, split, starvation, store, transfect or unknown_protocol_type

Performed_by: The name of the person who took the sample

Comments: Free text commenting on the sample (not compulsory)

Description: Free text describing the sample (not compulsory)

Material_Type: Choice of either: DNA, cell, cell_lystate, cytoplasmic_RNA, genomic_DNA, molecular_mixture, nuclear_RNA, organellar_DNA, organellar_RNA, organism_part, polyA_RNA, protein, synthetic_DNA, synthetic_RNA, total_RNA, virus or **whole_organism**

Sample_Type: Choice of either: extract or not_extract

Extracts Worksheet

Name of extract: The name of the extract

Name of treated sample: The name of the treated sample from which the extract comes from (must be included in the Samples worksheet)

Extraction_protocol: The name of the protocol used for extraction (must be included in the Extracts worksheet)

Performed by: The name of the person who made the extract

Extraction comments: Free text commenting on the extract (not compulsory)

Action of extraction: Choice of either: PCR_amplification, acclimatization, array_manufacturing, behavioral_stimulus, biological_fluid_collection, compound_based_treatment, decontaminate, dissect, element_design, feature_extraction, fractionate, genetic_modification, grow, harvest, histological_slide_preparation, hybridization, image_acquisition, incubate, infect, inoculate, irradiate, labelling, linear_amplification, **nucleic_acid_extraction**, pool, preservation, purify, reverse_transcription, sacrifice, specified_biomaterial_action, split, starvation, store, transfect, unknown_protocol_type or wash

Extracted_Material_Type: Choice of either: DNA, cell, cell_lystate, cytoplasmic_RNA, genomic_DNA, molecular_mixture, nuclear_RNA, organellar_DNA, organellar_RNA, organism_part, polyA_RNA, protein, synthetic_DNA, synthetic_RNA, **total_RNA**, virus or whole_organism

Description of extraction: Free text describing the extract (not compulsory)

Name of labelled extract: The name of the labelled extract

labelling protocol: The name of the protocol used for labelling (must be included in the Protocols worksheet)

Labelling comments: Free text commenting on the labelling reactions (not compulsory)

Action of labelling: labeling

Labelled_Material_Type: Choice of either: DNA, cell, cell_lystate, cytoplasmic_RNA, genomic_DNA, molecular_mixture, nuclear_RNA, organellar_DNA, organellar_RNA, organism_part, polyA_RNA, protein, synthetic_DNA, synthetic_RNA, **total_RNA**, virus or whole_organism

Labelling Compound: Choice of either: **Cy3, Cy5**, _32P, _33P, alexa_350, alexa_430, alexa_488, alexa_532, alexa_546, alexa_568, alexa_594, alexa_633, alexa_660, alexa_680 or biotin

Quantity of Labelled material: The amount of material in numbers

Quantity of labelled material_unit: Choice of either: kg, g, mg, **µg**, ng, pg, fg or other

Hybridisation Worksheet

Name of hybridisation: The name of the hybridisation

Arrays used in hybridisation: The actual arrays (print run number_slide number) used for the hybridisation

ArrayType (gal file used): The array type/format used (e.g. SCo3, SCp26)

Labelled extracts used in hybridisation: The name of the labelled extracts that were hybridised to the array (must be included in the Extracts Worksheet)

Hybridisation_protocol: The name of the hybridisation protocol used (must be included in the Protocols Worksheet)

Performed by: The name of the person who did the hybridisation

Comments on hybridisation: Free text commenting on the hybridisation (not compulsory)

Description of hybridisation: Free text describing the hybridisation (not compulsory)

Image_Name: The name of the image

Scanning_protocol: The name of the scanning protocol used (must be included in the Protocols Worksheet)

Comments on image acquisition: Free text describing what was done (not compulsory)

Description of image: Free text describing the image (not compulsory)

Image URL: URL of where the image is stored

Supplementary_image: URL of where a second image (i.e. the other channel) is stored

Image Format: Choice of either: Affymetrix_DAT, GIF, JPEG, PNG or **TIFF**

Measurement_Name: The name of the measurement e.g. WTBioRep1_vs_MutantBioRep1_meas

ImageAnalysis_protocol: The name of the image analysis protocol used (must be included in the Protocols Worksheet)

File_to_get_measurements: The name of the file where to get the relevant measurements from e.g. WT_vs_Mut_output.xls

URL of measurement file: The URL of the measurement file.

Comment on measurement: Free text commenting on the measurement (not compulsory)

Description of measurements: Free text describing the measurement (not compulsory)

Protocols Worksheet

Protocol_For: Choice of either: Sampling, Treatment, Extraction, Labelling, Scanning, ImageAnalysis or Hybridisation.

Given_Protocol_Name: The name of your protocol e.g. Growth on ONA

Protocol_Type: Choice of either: PCR_amplification, acclimatization, array_manufacturing, behavioral_stimulus, biological_fluid_collection, compound_based_treatment, decontaminate, dissect, element_design, feature_extraction, fractionate, genetic_modification, grow, harvest, histological_slide_preparation, hybridization, image_acquisition, incubate, infect, inoculate, irradiate, labelling, linear_amplification, nucleic_acid_extraction, pool, preservation, purify, reverse_transcription, sacrifice, specified_biomaterial_action, split, starvation, store, transfect, unknown_protocol_type or wash

Description: Free text describing what was done.