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How Good Is My Microarray Experiment?

A quick guide to checking the quality of microarray data.

Introduction

Data quality is one of the most important aspects of any microarray experiment. This technical note outlines the ways that users can check the quality of their microarray data using BlueFuse[®] Multi software, to assess laboratory procedures, before proceeding with downstream analyses. Checking the data quality enables users to assess whether the genomic changes seen in a sample are real or artefacts.

This document should be used as a guide only. It remains the responsibility of the laboratory personnel to make the final decision on the usability of array data.

Visualizing the Array and Checking the Grid Placement

The original scan can be visualized by clicking the **Subarray QC** shortcut. The zoom function can be accessed by right-clicking the image. Similarly, the user can double-check that the grid has been placed correctly by right-clicking the image and selecting **View Grids** from the pull-down menu (Figure 1).



Table 1: Oligo Array QC Metrics

The scan should show a good signal-to-background ratio and clearly defined features, with the red and green images well registered and the colors evenly balanced (not too green or red).

Quality Control Metrics (Oligo Microarrays)

The Quality Control measures are displayed in the Report (click the **Report** shortcut). Examples are shown in Table 1 and Figure 2. The main measures to check are:

- Standard Deviation (SD Robust), which should ideally be below 0.17. The SD autosome is calculated on every clone on the array; the robust SD is used for calling after outliers have been removed.
- Derivative Log Ratio (DLR Raw/Fused), which should be below 0.2.
- The signal-to-background ratio (SBR), which should be greater than 3.
- The background-subtracted signal (Amplitude), which should be roughly balanced between the two channels, and in the region of 300-700 (for an Agilent scanner). This is only an indication and will vary among scanners and array types.

Tips

- "Speckling" can be caused by incomplete resuspension in hybridization buffer or precipitation if the array is cold.
- The powder in latex gloves can exhibit significant fluorescence.
- If the hybridization chamber volume was too low (either too little solution was added or the chamber dried out because the gasket was breached), the center of the array will display a dark area.
- Ensure that the hybridization oven or water bath is at the correct temperature.
- Ensure that the room temperature wash is around 20°C. This can be an issue in winter.
- Ensure good agitation in the first wash.
- Ensure that the final wash is at the correct temperature, as this determines the stringency of washing.

Parameter	Good Oligo Array Result	Marginal Oligo Array Result	Failed Oligo Array Result
SD Autosome/Robust	0.12/0.09 (good)	0.19/0.15 (good)	0.27/0.19 (poor)
DLR Raw/Fused	0.15/0.14 (good)	0.21/0.21 (borderline)	0.28/0.28 (too high)
SBR ch1/ch2	8.02/9.62 (good)	63.63/22.52 (too high)	8.55/7.70 (good)
Amplitude ch1/ch2	326/430 (acceptable)	6250/7279 (too high)	712/1152 (good)
Notes	Amplitude lower than a "good" array, but still acceptable.	Massively over-scanned and therefore suppressing some ratios. Need to reduce PMT gain.	Very poor SD, very high DLR; probably DNA-related as the SBR is good.



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