

MICROARRAY RESOURCE FACILITY

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Analysis of Labeling Reaction for Spotted Array Sample Preparation

Note: For duo-color method (spotted array). Suitable for any dye that gives similar fluorescence wavelength to Cy3 (550 nm) or Cy5 (650 nm).

1. Use a 50 μL quartz MicroCuvette to analyze the entire undiluted sample in a spectrophotometer.
2. wash the cuvette with water and dry completely.
3. pipette sample into cuvette and place cuvette in spectrophotometer.
4. For each sample measure absorbance at 260 nm and either 550 nm for Cy3 or 650 nm for Cy5, as appropriate.
5. Pipette sample from cuvette back into the original sample tube.
6. For each sample calculate the total picomoles of cDNA synthesized using:

$$\text{Pmol nucleotides} = \frac{[\text{OD}_{260} * \text{volume } (\mu\text{L}) * 37 \text{ ng}/\mu\text{L} * 1000 \text{ pg}/\text{ng}]}{324.5 \text{ pg}/\text{pmol}}$$

Note: 1 OD 260 = 37 ng/ μL for cDNA; 324.5 pg/pmol average molecular weight of a dNTP.

7. For each sample calculate the total picomoles of dye incorporation (Cy3 or Cy5 accordingly) using:

$$\text{Pmol Cy3} = \frac{\text{OD}_{550} * \text{volume } (\mu\text{L})}{0.15}$$

$$\text{pmol Cy5} = \frac{\text{OD}_{650} * \text{volume } (\mu\text{L})}{0.25}$$

$$\text{nucleotides / dye ratio} = \frac{\text{pmol cDNA}}{\text{pmol Cy dye}}$$

Note: >100 pmol of dye incorporation per sample and a ratio of 25-50 nucleotides / dye molecules is optimal for hybridizations. However, our hybridization instrument can detect signal as low as 30 pmol incorporation.

Note: low dye incorporation is often due to mis-handled or aged cy dyes. You need to obtain fresh dyes and repeat the sample preparation again.

8. After analysis mix together the two differentially labeled probes (Cy3 vs. Cy5) which will be hybridized to the same microarray slide for study of relative gene expression.
9. Reduce the volume to 50 μL using Microcon column.
10. Sample is ready for submission . Please bring the OD data with the sample(s).