

Measuring (nucleic acid concentration and) dye incorporation rates

Dye incorporation rates vary according to the dye being used (*e.g.*, Cy3 vs. Cy5) and the protocol employed (*e.g.*, direct vs. indirect). We can measure dye incorporation rates with the Nanodrop ND-1000 spectrophotometer (<http://www.nanodrop.com>). We do not routinely make such measurements because this would leave insufficient material for a hybridisation.

Protocol

1. Open the Nanodrop icon and select 'Microarray Measurement Mode'.
2. Add 2 μ l of solvent the labelled-sample has been dissolved in ("the solvent"); the instrument will then initialise.
3. After each and all subsequent measurements, clean the pedestal by wiping with a dry lint-free tissue.
4. Add 2 μ l of solvent and press 'Blank'
5. Repeat the blanking until there is a stable baseline, close to zero
6. Confirm that the baseline is correct by measuring 2 μ l of solvent, as if it were your first sample by pressing 'Measure'
7. Add 2 μ l of the first sample making sure to add the sample ID (or name) to the 'Sample ID' field and then press 'Measure'
8. Repeat step 3 and then 7 for all samples
9. Confirm that the baseline is correct after taking all measurements by measuring 2 μ l of solvent, as if it were your last sample by pressing 'Measure'
10. Each of the measurements is automatically saved by the instrument

Detection limits

Lower and upper detection limits for dye incorporation measurements of labelled-hybridisation extracts. The upper limit assumes the labelled-hybridisation extract has now been diluted.

Sample type (dye)	Lower limit (pmol/ μ l)	Upper limit (pmol/ μ l)
Cy3, Cy3.5, Alexa_555, Alexa_660	0.20	100
Cy5, Cy5.5, Alexa_647	0.12	60
Alexa_488, Alexa_594	0.40	215
Alexa_546	0.30	145

R. Auburn (20-02-2006).