CytoSure

A Sysmex Group Company

What binds us, makes us.

What's wrong with my arrays?

Category	Problem	Identifier	Solution
DNA Sample QC	Low A260/280 values	Protein Contamination	Re-purify samples using proteinase steps
DNA Sample QC	High A260/280 values	RNA Contamination	Ensure that your DNA extraction protocol includes RNase
DNA Sample QC	Low A260/230 values	Contamination of salts or solvents (e.g. Phenol)	Re-purified by ethanol precipitation Resuspending the DNA in TE buffer
DNA Sample QC	Inaccurate Sample Concentration	High DNA concentration (>350ng/µl)	Dilute DNA 1:2 in water or suitable buffer
Labelling reaction	Low Dye Incorporation– (poor pmol/µl or DNA concentration values)	The wrong temperatures or times are being used	Check temperatures with a calibrated thermometer Check incubation times against protocol
		Incorrect volumes used in mastermix preparation	Check correct volumes are being added Check pipettes are calibrated correctly
		Insufficient mixing of samples, reagents and mastermixes	Gently vortex all reagent tubes (except Klenow) Flick mix Klenow tube Briefly spin to drive contents off tube walls
		Too much exposure to light or air	Use a closed thermal cycler with heated lid
		Loss of solution from evaporation	Use PCR machine with a heated lid If using tubes, make sure lids are tightly closed If using plates, use caps not a plate sealer
Hybridisation and Wash	Black holes on array	Low volume of Hybridisation solution	Ensure the correct volume of hybridisation solution has been used Check no leakage of hybridisation solution has occurred
Hybridisation and Wash	Non-uniform signal intensities	Split, deformity or crack in backing slide	Check the backing slide seal is intact and has not cracked Report any gasket slide failures to support@ogt.com
Hybridisation and Wash	Bubble Scarring/Scotching	Jig assembly untouched for too long after hybridisation Oven rotation malfunction	Check oven rotators are working Remove jigs from oven one at a time Disassemble under wash buffer rapidly
Hybridisation and Wash	Fluorescent smears across the slide	Wash-step contamination with fluorescent material Dried-out arrays during the hybridisation or wash steps	Ensure dishes are regularly cleaned with appropriate solvent Ensure clean gloves, forceps and dishes Carry out additional acetonitrile wash for 1min at room temp
Hybridisation and Wash	High Background Signal	Wash-step contamination with fluorescent material Wash conditions not stringent enough	Ensure dishes are regularly cleaned with appropriate solvent Ensure clean gloves and forceps Check stirrer is producing a vortex prior to adding slides in wash buffer Check temperature of oven and washes
Hybridisation and Wash	Poor Signal intensity	Overly stringent wash or hybridisation conditions Cy5-labelled DNA was exposed to light	Check protocol for correct wash instructions Cover tubes with foil or use amber tubes Check temperature of oven and washes
Ozone	Low Cy5 signal towards the edges of a feature	Wet ozone: outer edges of features dry quicker than inside, exposing edges to ozone	Ensure slides are scanned immediately after washing Enclose scanners in a box with ozone scrubbers
Ozone	Low Cy5 signal gradient with more signal loss at one end of the slide	Dry ozone: Degradation during scanning, with exposed end degrading quicker	

Important QC metrics

DLRS values

This is perhaps the most important QC metric and calculates the probe-to-probe log ratio noise of an array. A poor Derivative Log Ratio Spread (DLRS) will mean that it is more difficult to accurately call amplifications or deletions. The DLRS value should be <0.3. Higher values can indicate poor quality DNA.

To detect very small aberrations, a DLRS value of <0.2 may be required. An excellent array would have a DLRS value of around 0.15; although for some sample types (e.g., formalin fixed paraffin embedded), this may be difficult to achieve. Check the quality of the DNA on a high percentage agarose gel for degradation. If the DNA is degraded, shown by a smear on the gel, re-extract the sample.

Signal to Noise

This value is calculated by dividing the signal intensity by the background noise and indicates how clearly the spots can be detected above the background level. This metric is dependent on how well the sample labelling and washing steps worked.

It is often easier to look at this metric first and then, if it does not pass, identify where the problem occurred by looking at the background noise and the signal intensity.

An excellent value for signal to noise would be above 100, between 100 and 30 is good but below 30 is poor. It is difficult to reliably detect aberrations on arrays where the Signal-to-Noise is <30.

Background Noise

This metric is calculated as the standard deviation of negative control probes on the array. The values are recorded for both the green and red channel and can be classified into Excellent, Good and Poor.

The values will depend on the array format being used. A poor background does not necessarily indicate that the array has failed. This is a secondary metric as it is incorporated into the Signal-to-Noise metric.

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