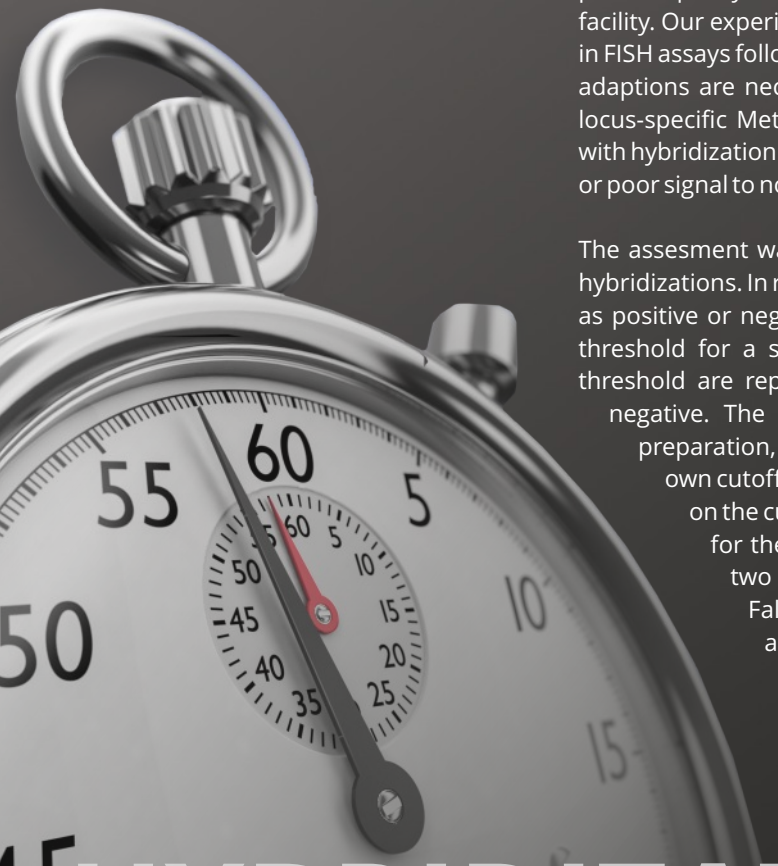
RapidHyb!

Flexible Hybridization Times by MetaSystems!

Demand more than excellent performance...

Fluorescence in situ hybridization has become an essential detection assay in today´s routine diagnostics. However, long hybridization times of many hours to overnight are still a restrictive factor. Several attempts have been made to overcome this limiting factor, special FISH probes have been designed for short-time hybridization, protocols have been adapted and new buffers have been invented.
At MetaSystems Probes, the production process was refined to reduce background and artefacts and to improve the signal to noise ratio, particularly in short-time hybridization. Since mid-2015, one hour hybridization on lymphocytes is an integral part of quality control for all XCyting locus-specific probes at our manufacturing facility. Our experience shows that short-time hybridization can be successfully used in FISH assays following the recommended sample preparation and FISH protocol. No adaptions are necessary other than shortening the hybridization time. Usually all locus-specific MetaSystems probes hybridized to lymphocytes are easily analyzed with hybridization times of one hour under optimal conditions. In case of weak signals or poor signal to noise ratio, hybridization time might be increased to 2-4h.

The assesment was done by analyzing cutoff values from overnight and one hour hybridizations. In routine diagnostics, cutoff values are used to designate a FISH result as positive or negative. The cutoff value for any particular FISH probe defines the threshold for a specific signal pattern in normal individuals. Results above the threshold are reported as positive, results below the threshold are reported as negative. The cutoff value depends on several parameters such as sample preparation, equipment and other factors. Laboratories usually determine their own cutoff values under local conditions. Also the probe design has an impact on the cutoff. Dual fusion assays normally have a very low false positive rate for the typical aberrant signal constellation one orange, one green and two colocalization/fusion signals due to the complexity of the pattern. False positive single fusions as a result of coincidental signal overlap are much more frequent. Break apart probes are more prone to false positivity than dual fusion assays. The patterns are less complex and the probability of loss of one complete fusion signal is comparatively high. Also, deletion probes with two independent locus-specific signals usually have a higher false positive rate than dual fusion assays for the typical aberrant signal pattern.

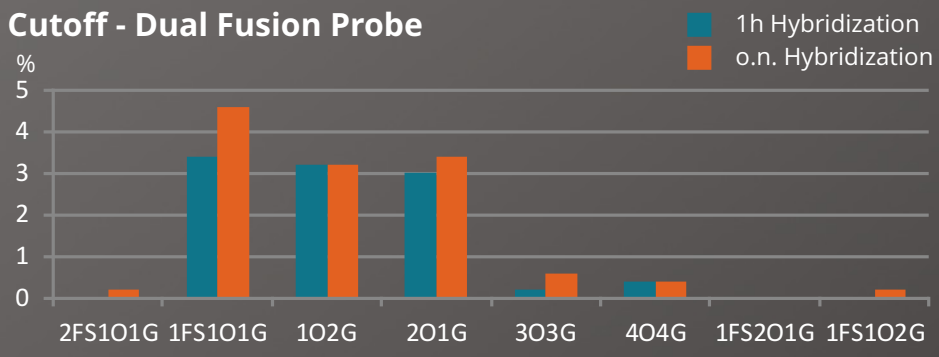


HYBRIDIZATION

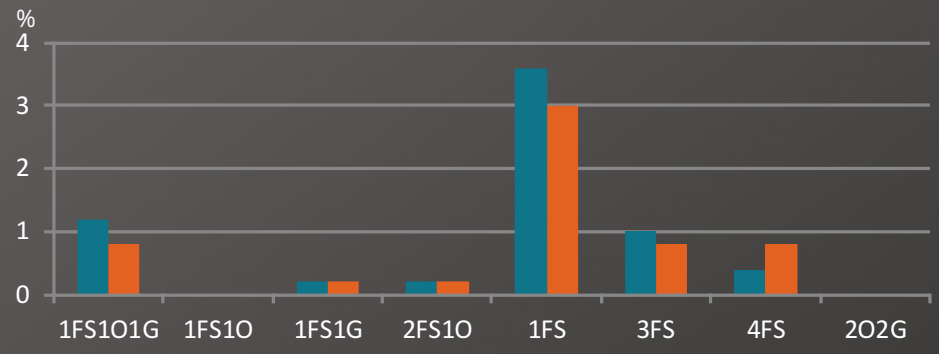
MetaSystems Probes

The cutoff values (500 cells, binominal treatment) for representative probes from our portfolio have been compared. One probe from the categories amplification/deletion, break apart and dual fusion were tested in overnight and one hour hybridizations on lymphocytes. The results are comparable, indicating that MetaSystems XCyting locus-specific probes are suitable for short time hybridization on blood slides. This study did not include clinical samples and the results obtained with lymphocytes are not applicable to FFPE tissue sections.

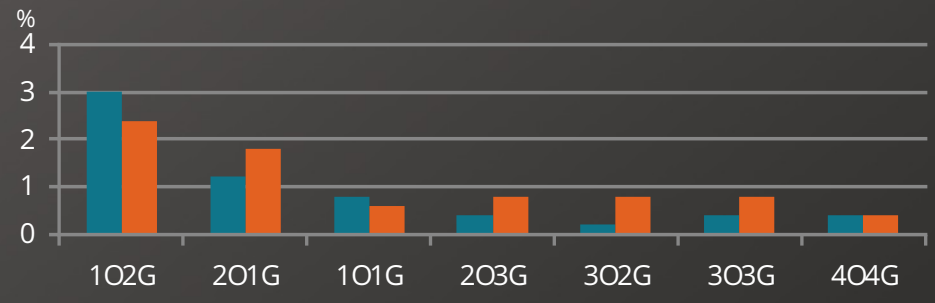
Cutoff - Dual Fusion Probe



Cutoff - Break Apart Probe



Cutoff - Amplification/Deletion Probe



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HYBRIDIZATION