SciGene

APPLICATION NOTE

Automated Processing of Agilent SureFISH™ Slides

Overview

Fluorescence in situ hybridization (FISH) is the preferred method for validating genetic aberrations detected by chromosome microarray analysis (CMA) through direct visualization and mapping of these genetic aberrations within the genome. With the introduction of Agilent SureFISH™ Probes,[1] cytogeneticists can now perform high resolution genomic mapping superior to that achieved using BAC probes. Genetic alterations in regions of the genome under 50kb and near repetitive sequences can now be reliably detected using these oligonucleotide probes.[2]

Advantages of Automated FISH Slide Processing

Strict adherence to the slide washing conditions specified for FISH-based assays is required to achieve the high probe signals and low backgrounds necessary for clear interpretation of test results. Precise control of time, agitation and temperature of wash buffers during the process is critical. Of particular importance is controlling the high temperature wash step (normally 72°C) to within 1°C. The manual process of moving slides between Coplin jars of wash buffers in and out of laboratory waterbaths that fluctuate in temperature contribute to the day to day variability of FISH based assays.

The Little Dipper® Processor is a widely used instrument that automates the processing of slides used in CMA and FISH-based tests. Following pre-installed or user derived protocols, the instrument automates the movement and agitation of 1 to 24 slides between baths containing solutions and into an integrated centrifuge for drying. Buffer temperatures are regulated to within 0.5°C of set point providing precise control over probe signal levels and enhancing the day to day reliability of results.

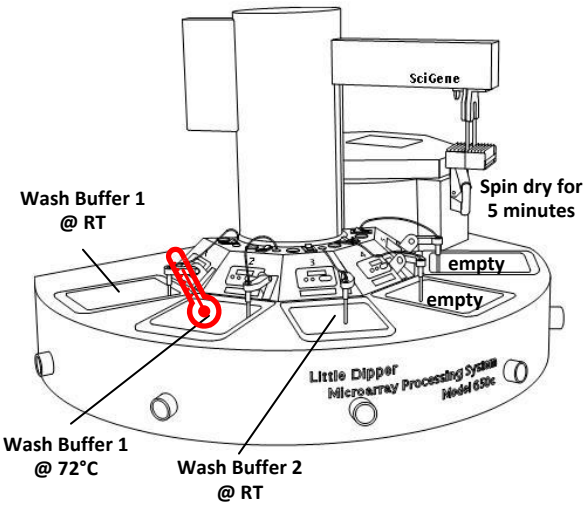

Figure 1. Little Dipper® Processor setup for 1-24 SureFISH™ slides.

Automated Processing Method for SureFISH™ Slides

The Little Dipper Processor (SciGene cat. #1080-70-X) was set up as shown in Figure 1. FISH wash buffer 1 (0.4X SSC/0.3% Igepal) was added to baths 1 and 2 and FISH wash buffer 2 (2X SSC/0.1% Igepal) was added to bath 3. The temperature of bath 2 was allowed to stabilize at 72°C (~ 10 min) before slide processing. Following hybridization and removal of coverslips, slides were loaded onto the instrument and the SureFISH™ processing protocol initiated. Following this method, slides were submerged in bath 1 for two minutes with agitation, moved to bath 2 for two minutes without agitation, to bath 3 for one minute and then finally centrifuged dry for 5 minutes. Slides were then counter-stained with DAPI and visualized.

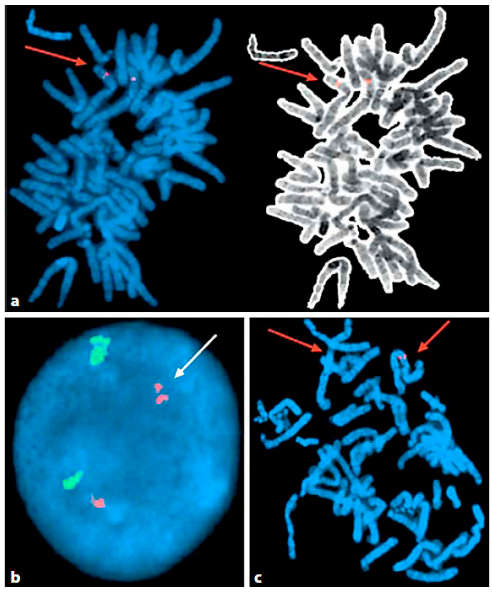

*Figure 2. From Yamada et al [2]: **a**) 6.7-kb region at 6p22.2 is detected using oligonucleotide-based FISH with DAPI counterstain (left), and inverted DAPI stain confirming the chromosomal location (right); **b**) Confirmation of a 79.5-kb 9q34 duplication in chromosome 9; **c**) Deletion of NPHP1 region in 2q13. Arrows indicate chromosomes 2, with the chromosome to the left showing the deletion (no red FISH signal).*

Results

The images in Figure 2 are patient samples subjected to high-resolution FISH analysis using SureFISH™ probes processed on the Little Dipper Processor (Yamada, N.A. et al).[2] High intensity signals can be observed from single gene targets as small as 6.7kb that would be difficult to visualize with clone-based FISH probes.

The ability of the Little Dipper Processor to automate and finely control FISH post-hybridization slide processing conditions ensures laboratories will receive the benefits of a new generation of high resolution FISH assays powered by Agilent SureFISH™ probes.

References:

(**1**) Agilent website (*www.agilent.com*)
(**2**) ***Visualization of Fine-Scale Genomic Structure by Oligonucleotide-Based High -Resolution FISH***; Yamada, N.A. et al; Cytogenetic and Genome Research, 2011 132(4):248-54

SciGene · 1287 Reamwood Ave, Sunnyvale, CA 94089 USA · Tel 408-733-7337 · Fax 408-733-7336 · sales@scigene.com