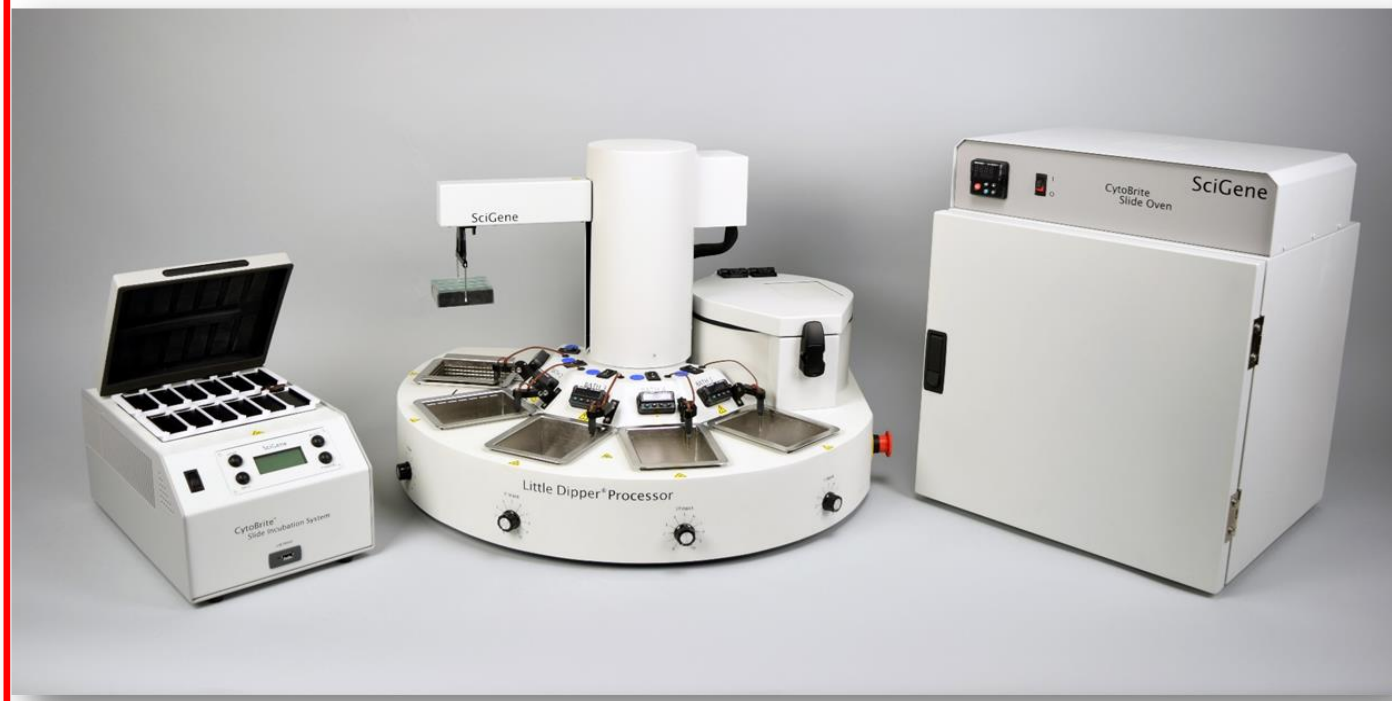
Simplifying FISH

The SciGene MicroFISH System for Cytogenomics

AlphaMetrix Biotech

The MicroFISH Assay System

Fluorescence in situ hybridization (FISH) detects the presence, copy numbers and/or location of a DNA sequence within a set of metaphase chromosomes or an interphase nucleus.
It is an important molecular cytogenetic technique widely used in diagnosing various genetic disorders and/or to further confirm chromosomal abnormalities found by other assays such as karyotyping and array-based comparative genomic hybridization (aCGH).
FISH, also known as whole chromosome painting, is valuable in defining rearrangements among different chromosomes and the origin of ring and marker chromosomes that are not clearly deciphered by karyotype analysis.



The MicroFISH Assay System combines a new generation of microvolume, multiwell slides designed for cytogenetic applications with a simple workflow using SciGene's slide hybridization and processing equipment to form a simple and economical system for performing routine cellular FISH. The throughput can vary from very few tests being manually processed to high troughput being highly automated processed.

The MicroFISH Assay System is a clinically validated method combining microvolume, multiwell slides with SciGene slide processing instruments to economically perform routine cellular FISH in cytogenetic laboratories

SciGene
SIMPLIFYING FISH

MicroFISH Clinical Validation

Clinically Validated and in Routine Clinical Use at Genetics Associates, Nashville, TN

Clinical Validation Study (Poster+Talk @ ACMG 2016 -Tampa)

We have previously shown (Crawford et al., ACMG 2015 poster #182) that multi-well slides with a hydrophobic coating (MicroFISH; SciGene) used in a simplified workflow allowed for more rapid processing of FISH samples using 1 µl probe volumes. We report here the completion of the clinical validation of the platform and automation of the slide preparation steps in the workflow.

For the clinical validation study, blood cell samples from 100 patients were analyzed in parallel utilizing the MicroFISH slides and workflow vs. the laboratory's standard method. Each slide set was scored by two readers who graded overall cell quality on a 1 (failure) to 5 (very good) scale.

The average quality grade for MicroFISH slides was 3.77 compared to 3.73 for our standard method. Both methods had identical probe spot failure rates of 1.4% and 100% concordance on successfully analyzed pairs of spots interpreted as abnormal. Significant variation was noted in one run which was attributed to oversaturation of the humidity chambers during hybridization. In serial hybridizations, 100 ml total volume of water provided optimal signal quality in a MicroFISH humidity chamber.

	MicroFISH	GAI Method
# Probes	361	361
Avg. Quality Score (1-5)	3.7	3.7
# Probe Failures (%)	5 (1.3%)	7 (1.9%)
# Abnormals	21	20

GAI Standard Method							
Probe	Normal	1r2g	1r1g	2r1g	3r3g	1r1g12f	1r1g1f
5q	95%	2%	1%				
7q	95%	1%	2%	1%			
8	95%				1%		
20q	93%	1%	2%	1%			
BCR;ABL1	92%					1%	3%
MicroFISH Method							
Probe	Normal	1r2g	1r1g	2r1g	3r3g	1r1g12f	1r1g1f
5q	94%	2%	1%				
7q	95%	1%	2%	1%			
8	94%				1%		
20q	95%	1%	3%	1%			
BCR;ABL1	93%					1%	3%

MicroFISH Assay System - Workflow

1

Add fixed cells

Pipette 1 µl fixed cells / well

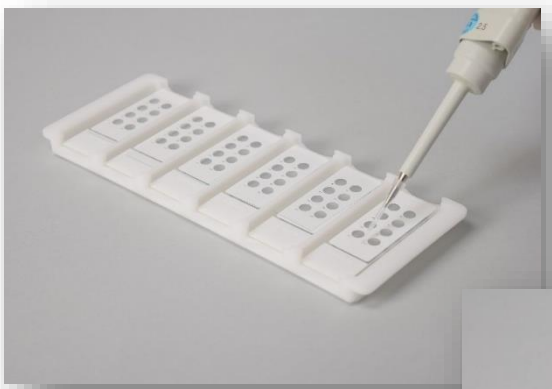
2

Age samples

Heat at 90°C / 10 minutes

3

Add probe(s)



Pipette 1 µl probe

Place coverslip
No sealant required

MicroFISH Assay System - Workflow

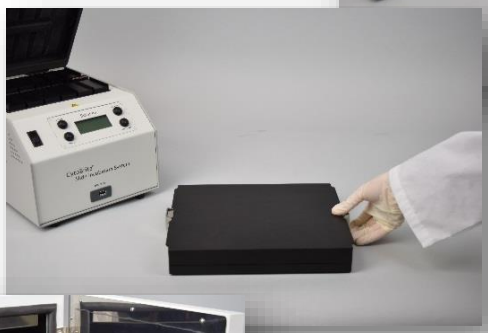
Denature

4

Heat at 76°C / 2 minutes

Hybridize

5



Transfer trays to humidified chamber.
Place in CytoBrite Oven overnight.

Transfer slides and wash

6

Coverslips shake off
and slides washed

Coverslips retrieved from basket

The MicroFISH Assay Method

Materials and Methods

Probes and Reagents:
Probes (Cytocell, Cambridge UK) were
prepared following the manufacturer's recommendations. Slides were washed in 0.4X SSC; 0.3% NP40 (VWR) at the prescribed temperature and counterstained with DAPI (Vector).

Equipment:
A CytoBrite Slide Incubation System and CytoBrite Slide Oven (SciGene) were used for performing denaturation and incubation steps respectively followed by a Little Dipper Processor (SciGene) for automated coverslip removal and post-hybridization washing and drying. Slides were imaged using standard methods (Cytovision) after manual examination.

Manual Slide Preparation:
1 µl of fixed blood or bone marrow cells in fresh Carnoy's was dropped in each well to be used on MicroFISH slides held in the CytoBrite slide tray. Slide trays were then transferred to a 90oC oven for 10 minutes. 1 µl of probe was then placed in the center of each well containing cells and a single 22 x 50 mm coverslip placed over all eight wells. No coverslip sealant was used.

Hybridization:
CytoBrite slide trays with coverslipped MicroFISH slides were transferred to a CytoBrite Slide Incubation System and heated at 76°C for 2 minutes. Trays were then transferred to a sealed chamber with absorbent pad moistened with 100 ml of water and placed in a CytoBrite Slide Oven set at 37°C overnight.

Post-Hybridization Processing:
After incubation, the sealed humidity chamber was taken from the oven and CytoBrite slide trays removed. MicroFISH slides were immediately placed in a slide rack for the Little Dipper Processor (SciGene) to process: MicroFISH slides were removed from the rack, a bead of DAPI applied down the center of each slide and a 22x50 mm coverslip placed over the wells.

Imaging:
MicroFISH slides were imaged under 100X oil objective and scored following standard methods.

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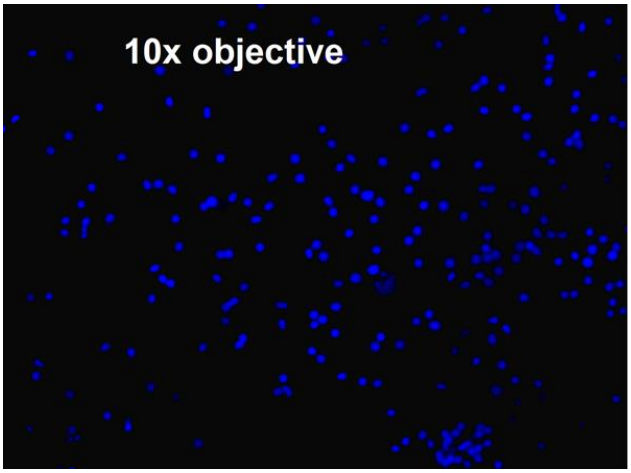
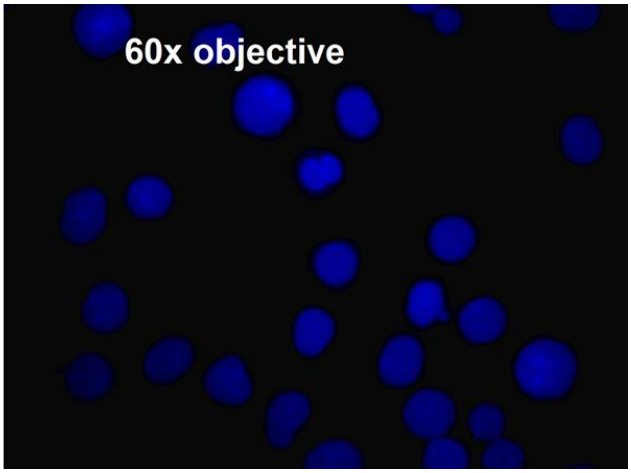
1 Genetics Associates, Nashville, TN, 2 Art Robbins Instruments, Sunnyvale, CA and 3 SciGene, Sunnyvale, CA

MicroFISH Results

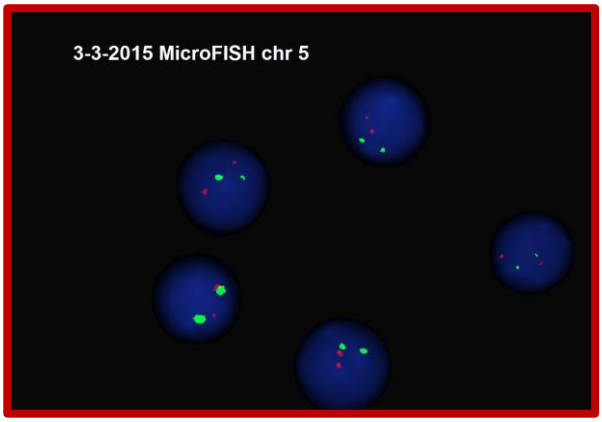
Benchmark Studies : 1000-2000 cells / well
Sufficient number of cells to study in 5mm wells

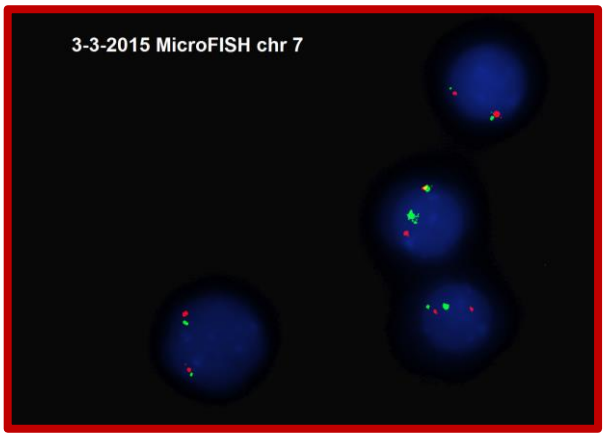




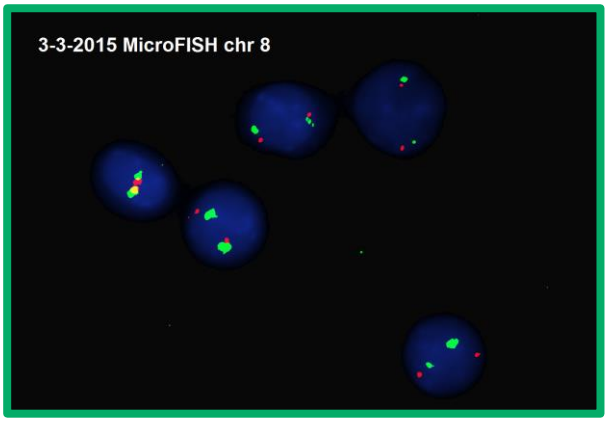
Coverslip Seal without Rubber Cement

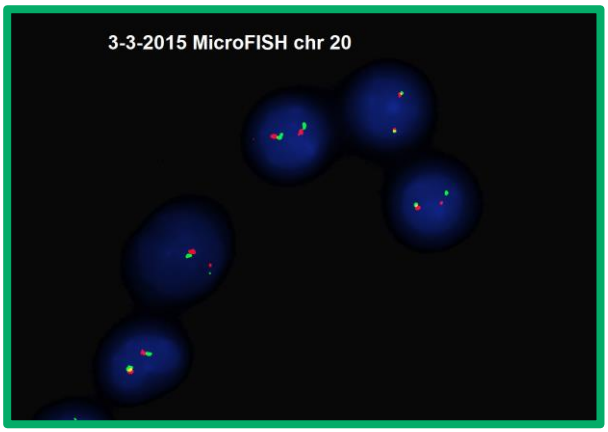
With Rubber Cement





Without Rubber Cement





MicroFISH Ecomomics

Probe Economics of the MicroFISH System

Probe Supplier A – 8 € per µl Probe

	Annual Probe Cost		
# Cases per Year	**Standard Method ***	**MicroFISH**	**Annual Savings**
1.000	192.000 €	48.000 €	144.000 €
5.000	960.000 €	240.000 €	720.000 €
10.000	1.920.000 €	480.000 €	1.440.000 €

*** 4 µl Probe per Sample / 6 Probe Panel**

SciGene
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MicroFISH Required Equipment

Required Equipment

CytoBrite Slide Incubation System with 2x slide trays
SciGene cat. #2019-00-1

CytoBrite Slide Oven — preheated to Hybridisation Temperature
SciGene cat. #2019-70-2

Little Dipper Processor for MicroFISH
SciGene cat. #1080-70-X

Little Dipper Coverslip Catcher Bath Insert
SciGene cat. #1080-10-9

MicroFISH Hybridization Chamber
SciGene cat. #2040-38-0

MicroFISH Hybridization Chamber Absorbent Pads
SciGene cat. #2040-32-1

Single Channel Pipettors (2 and 100 µl)

Coverslips, 22 x 50 mm

Required Materials and Reagents

12x MicroFISH Assay Slides, 8 well
SciGene cat. #2040-01-1

FISH Wash Buffer 1 , 4L (0.4x SSC; 0.3% IGEPAL, pH 5.0)
SciGene cat. #2010-00-1

Carnoy's Solution (3:1 methanol:acetic acid; prepared fresh)

Fixed blood/bone marrow cells (per your lab method)

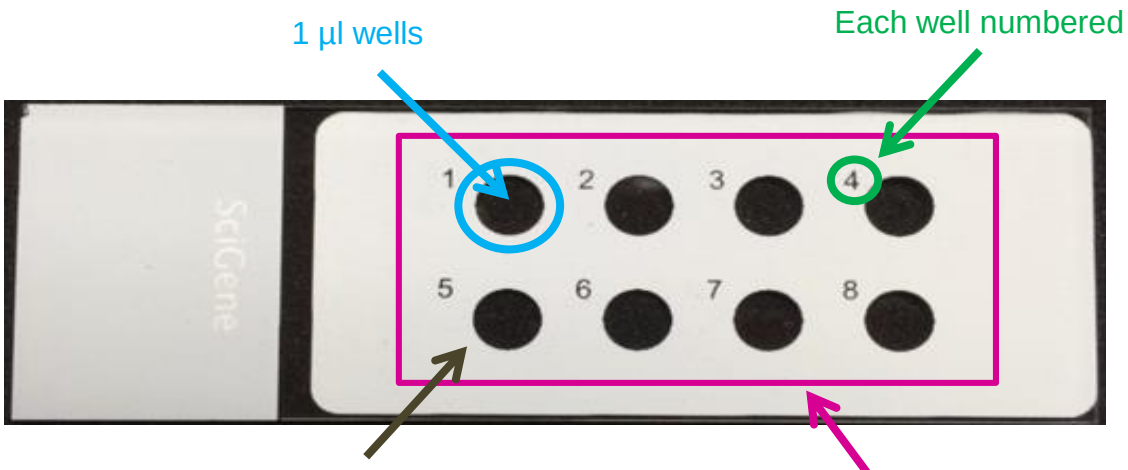
FISH probes (prepared per manufacturer instructions)

DAPI

MicroFISH Slides and Sealed Chamber

MicroFISH Assay Slides, 8 well

The MicroFISH slides are 8-well (each well 5mm Ø) slides with a special hydrophobic coating that eliminates coverslip sealant and only requires 1 µl cell samples (dry and age cells) and 1 µl of probe solution during the hybridization process. The slides are MicroFISH Assay slides, developed and produced by SciGene, Sunnyvale, CA..



SciGene cat. #2040-01-1

CytoBrite Incubation System

CytoBrite Incubation System

Programmable system with superior temperature control for performing FISH/ISH hybridization protocols on 1 to 12 slides. Uses rapid Peltier heating and cooling to process FFPE, bone marrow, cell lines and other samples with FISH probes from Abbott, Agilent, Cymogen and Cytocell. Accurately detects slide temperatures using SlideSense technology for more reproducible results. Records a time/temperature datalog to USB. Removable slide trays reduce handling from assay setup through hybridization. MicroFISH Slide trays can be transferred to a CytoBrite Slide Oven for extended or overnight probe denaturation.

- Achieves accurate and reliable temperatures run-to-run
- Built-in temperature calibration eliminates CLIA certification cost
- Removable 6-slide trays streamline set up and handling
- Slides incubated dry – No humidity strips needed
- Run data captured on USB drive

SciGene cat. #2019-00-1

CytoBrite Slide Oven

CytoBrite Slide Oven

High capacity convection oven with superior temperature regulation and accuracy for overnight incubation of up to 60 FISH slides. Slides are uniformly heated to ± 0.5°C from set point eliminating slide-to-slide variability seen on older generation hybridizers. After denaturation, slides are transferred to the oven's five pull-out drawers using up to 4 MicroFISH casettes with 3 trays each. Built-in temperature calibration system eliminates the cost of third party calibration required for CLIA compliance.

- Slides denatured with CytoBrite System then transferred to oven
- Slides uniformly heated; eliminates slide-to-slide variation
- Removable 6-slide trays speed transfers between instruments
- Slides incubated dry – No humidity chambers required
- Built-in temperature calibration eliminates CLIA certification cost

SciGene cat. #2019-70-2 and SciGene cat. #2040-38-0

Little Dipper for FISH

Little Dipper Processors for MicroFISH and G-Banding

A SciGene Little Dipper Microarray Processor is a programmable, robotic instrument that automates the various hybridization processing steps used with FISH and G-Banding samples. The Little Dipper instrument improves data quality by eliminating sample handling and by controlling wash buffer/reagent temperatures and times, agitation rates and centrifuge/drying conditions. Each system arrives configured for your platform and programmed with recommended protocols.

Accurate Control Improves Assay Reproducibility

Precise control of timing and washing movement is critical for obtaining consistent low backgrounds and good contrast staining. The instrument is designed for use by CLIA-certified labs with a built-in system for verifying and calibrating accurate conditions.

Simple Training and Operation

After filling baths, and activating stir bars, slides are loaded into either a 12 or 24-position rack and mounted onto the robotic arm. Staining and washing action is achieved through the movement of the robotic arm and stir bar vortex. The instrument performs programmed wash and dry steps and holds the slides in the dark ready for imaging. The simplicity of design makes training a snap and ensures consistent day-to-day results by different operators.

SciGene cat. #1080-70-X

MicroFISH Reagents

CytoZyme®
Stabilized Pepsin

CytoZyme Stabilized Pepsin is a liquid formulation of purified pepsin with enhanced shelf life used for preparing tissue samples for FISH assays.

Provided as a concentrate ready for dilution in CytoZyme Reaction Buffer, the activity of each lot is assayed to ensure consistent lot-to-lot activity eliminating the need for laboratories to evaluate new lots. Priced over 80% less than Abbott Kits, it significantly reduces FISH cost per test.

CytoZyme is available in high (HC) and standard activity formulations.

CytoZyme HC is a high potency solution used for digesting any tissue by diluting the working solution to match the tissue type; eliminating the inconvenience and added cost of purchasing different reagent kits. Standard activity CytoZyme is also available with an activity equivalent to Abbott Pretreatment Kit I.

Convenient, purified liquid pepsin

- CytoZyme HC – a single formulation for all Tissue types
- Priced over 80% less than Abbott Kits
- Lot tested to ensure consistent activity eliminates checking lots
- Retains full activity for >12 months at 4˚C

MicroFISH Reagents

Sodium Thiocyanate

A convenient 1M liquid formulation of NaSCN. Use for pre-treating tissue samples prior to application of nucleic acid probes for cytogenetic assays, including FISH. Priced 20% less than the comparable product from Abbott saving the typical laboratory hundreds of dollars annually. Available in two sizes: one liter bottle for use with Coplin jars or staining dishes and four liter container with flow control spout for filling baths on the Little Dipper Processor and Abbott VP 2000 instruments.

- Convenient ready-to-use liquid formulation
- Priced 20% less than Abbott
- Available in 1L bottle or 4L container with flow control spout
- No dilution required
- Store at room temperature

FISH Wash Buffers

Ready-to-use FISH Wash Buffer 1 (0.4xSSC/0.3% IGEPAL, pH 7) and FISH Wash Buffer 2 (2xSSC/0.1% IGEPAL, pH 7) for post-hybridiza□on slide washing. Provided in convenient 4L containers with flow control spouts.

Bringing Cells into Focus

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