Research into the future of FISH with minimal volumes of Cytocell® FISH probes

Introduction

As the demand for FISH analysis continues to grow, pressures to reduce cost and increase output are forcing labs to find more efficient ways to generate a greater number of assays. In an effort to keep current with the needs of our customers we wanted to research the outcomes associated with reduced probe volumes in contrast to standard protocol recommendations. This research would confirm the robust nature of our product despite changing probe volumes.

Smart technologies, such as the BioDot CellWriter, allow automation to decrease processing time and implement small probe volumes to reduce costs. This research describes the analytical study carried out to evaluate how the automation technology performs, when used with Oxford Gene Technology's Cytocell FISH probes in research applications to produce high quality results with strong signals and minimal background.

Materials

Low volume of OGT's Cytocell probes with high quality signals, minimal background

OGT designs, manufactures and distributes Cytocell FISH probes for haematology, haematopathology, pathology and constitutional sample applications. Our high-quality FISH probes come conveniently packaged in hybridisation solution to minimize pipetting errors, streamline reagent setup by removing the need to mix multiple solutions and reduce wastage of additional vials. Our FISH probes can be utilised effectively with traditional or manual methods of FISH processing, or with high-throughput, automated FISH processing platforms, and in this research, we seek to confirm successful results with limited probe volumes by FISHArray on the BioDot CellWriter.

Cytocell RUO probes* and reagents utilised in the study:

Probe Name	Catalogue Number
IGH Breakapart Probe	RU-LPH 014 (100µl)
D13S319 *Plus* Deletion Probe	RU-LPH 068 (100µl)
12cen in Blue Spectrum, Satellite Enumeration Probe (with Hyb Sol B)	RU-LPE 012B (100µl)
PML/RARα Translocation, Dual Fusion Probe	RU-LPH 023 (100µl)
MYB Deletion Probe	RU-LPH 016 (100µl)
P53/ATM Probe Combination	RU-LPH 052 (100µl)
BCR/ABL *Plus* Translocation, Dual Fusion Probe	RU-LPH 038 (100µl)

BioDot CellWriter materials required:

- BioDot CellWriter S2 Platform (Catalogue # CellWriter S2)
- BioDot Phaselink Software (Catalogue # Phaselink)
- BioDot Probe Inventory Management Software (Catalogue # PIMS)

Additional equipment required:

- ThermoBrite®
- Olympus BX61 with ASI GenASIs software
- 8-Well FISHArray™ Microscope Slide (BioDot Catalogue # 0004-0047)
- Thermo Scientific™ Ultra Frost Gold Seal™ Microscope Slides (ThermoSci Catalogue # 3063-002)
- Normal Human Bone Marrow Specimens (in Carnoy's solution (3:1 methanol/acetic acid) fixative)

Slide Pretreatment:

Bath 1: 2xSSC at 37°C for 2 minutes

Bath 2: Protease Solution (Pepsin) at 37°C solution for 10 minutes

Bath 3: 1x PBS at Room Temperature for 5 minutes

Bath 4: 1% Formaldehyde Solution at Room Temperature for 5 minutes

Bath 5: 1x PBS at Room Temperature for 5 minutes

Bath 6: 70% EtOH at Room Temperature for 1 minute

Bath 7: 85% EtOH at Room Temperature for 1 minute

Bath 8: 100% EtOH at Room Temperature for 1 minute

Methods

Sample and Slide Preparation:

1. FISH assays were processed using 3 month old normal human bone marrow research specimens fixed in 3:1 methanol:acetic acid. The pellets were spun down, the supernatant was removed, and they were supplied with fresh fixative prior to use.
2. Manual FISH slides were prepared by dropping 20µl of sample onto the slides (Thermo Scientific) and allowing them to air dry on the benchtop. A total of 10µl Cytocell FISH probe was used for each manual FISH assay.
3. FISHArray slides were prepared on the BioDot CellWriter S2 platform with integrated pellet normalisation; a total of 2µl specimen was deposited onto each target well. On the CellWriter platform, the total amount of specimen used per slide may vary depending on the concentration of the sample. The CellWriter has an integrated reader that allows the system to dynamically determine the total drop volume per well; the baseline is 1µl total per target well. All seven FISH probes were processed on a single FISHArray slide; a total of 0.45µl probe was applied to each target well.

   *Prior to probe-spotting the slides underwent the BioDot FISH Pre-treatment protocol; this process helps ensure consistently clean slides with low amounts of debris.

Methods continued

FISH Assay Conditions

1. Both manual slides and FISHArray slides were denatured and hybridised using the BioDot protocol on Thermobrites (note that the latest version of the CellWriter S2 platform has a heated nest allowing on-board denature/hybridisation).
2. Two sets of slides were prepared: overnight hyb (all seven probes listed above) and 2hr hyb (BCR/ABL *Plus* and PML/RARα only).
3. Overnight Hyb: the slides and probes were codenatured at 78°C for 3 minutes and hybridised at 37°C for 12 hrs.
4. 2hr Hyb: the slides and probes were codenatured at 78°C for 3 minutes and hybridised at 37°C for 2hrs.

BioDot Recommended Post Hybridisation Wash Protocol

1. 0.4x SSC with 0.3% IGEPAL, pH 7 at 73°C for 2 minutes
2. di$H_2$0 at Room Temperature for 5 seconds
3. 70% EtOH at Room Temperature for 5 seconds
4. 85% EtOH at Room Temperature for 5 seconds
5. 100% EtOH at Room Temperature for 5 seconds

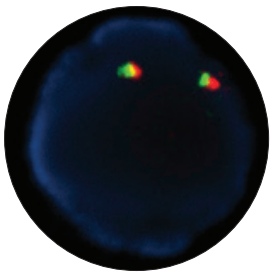
Results

Good results were observed on nanoFISH FISHArray multi-welled slides (0.45µl of Cytocell FISH probe); signal brightness was assessed and results were comparable to those obtained on interphase cell comparisons of manual FISH (10µl of Cytocell FISH probe). Strong signals were observed for all seven probes listed below after an overnight hybridisation. BCR/ABL *Plus* Translocation, Dual Fusion and PML/RARα Translocation, Dual Fusion probes showed strong signals as well after a 2hr hybridisation.

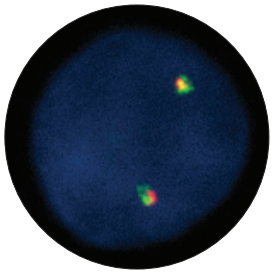
Overnight Hybridisations

IGH Breakapart Probe

IGHC, 14q32.33, Red
IGHV, 14q32.33, Green



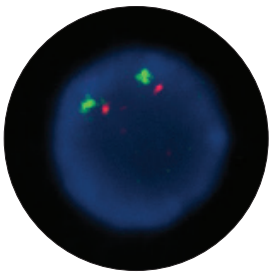
10µl control



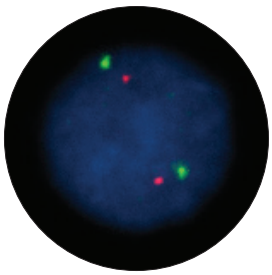
0.45µl FISHArray

D13S319 *Plus* Deletion Probe

D13S319, 13q14.2-14.3, Red
13qter, 13q34, Green



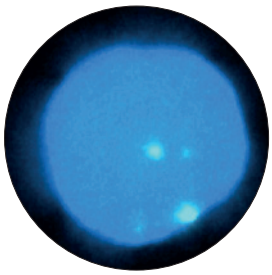
10µl control



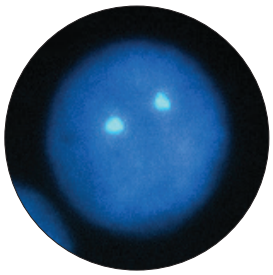
0.45µl FISHArray

12Cen in Blue Spectrum

D12Z3, 12p11.1-q11.1, Blue



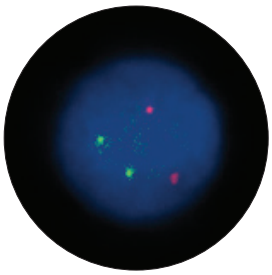
10µl control



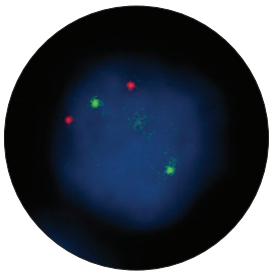
0.45µl FISHArray

P53/ATM Probe Combination

P53, 17p13.1, Red
ATM, 11q22.3, Green



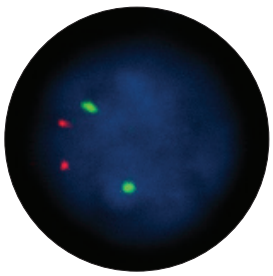
10µl control



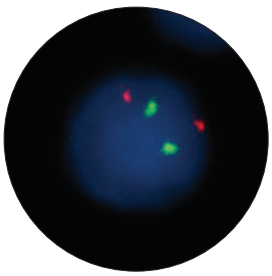
0.45µl FISHArray

MYB Deletion Probe

MYB, 6q23.3, Red
D6Z1, 6p11.1-q11.1, Green



10µl control

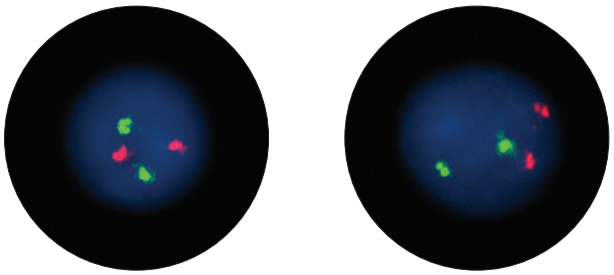


0.45µl FISHArray

Overnight Hybridisation vs 2 Hour Hybridisation

PML/RARα Translocation, Dual Fusion - overnight hybridisation

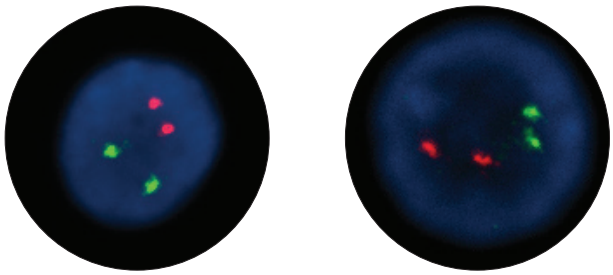
PML, 15q24.1, Red
RARα, 17q21.1-q21.2, Green



10µl control | 0.45µl FISHArray

PML/RARα Translocation, Dual Fusion - 2hr hybridisation

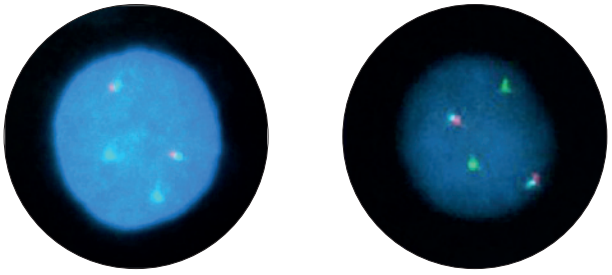
PML, 15q24.1, Red
RARα, 17q21.1-q21.2, Green



10µl control | 0.45µl FISHArray

BCR/ABL *Plus* Translocation, Dual Fusion - overnight hybridisation

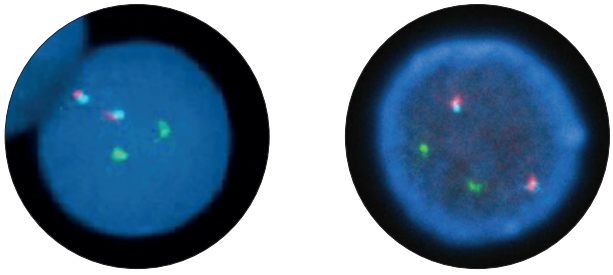
ABL1, 9q34.11-q34.12, Red
BCR, 22q11.22-q11.23, Green
ASS1, 9q34.11-q34.12, Blue



10µl control | 0.45µl FISHArray

BCR/ABL *Plus* Translocation, Dual Fusion - 2hr hybridisation

ABL1, 9q34.11-q34.12, Red
BCR, 22q11.22-q11.23, Green
ASS1, 9q34.11-q34.12, Blue



10µl control | 0.45µl FISHArray

Conclusion

In conclusion, our research has demonstrated that the Cytocell FISH probes highlighted in this study, when utilised on the BioDot CellWriter system using FISHArray slides, can still deliver highly concordant results despite reduced probe quantities, potentially allowing research laboratories to reduce costs and increase sample throughput while still maintaining consistency of results.

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