APPLICATION NOTE

Assessing the Effects of Humidity on FISH Using the Nick Translation DNA Labeling System 2.0

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NICK TRANSLATION DNA LABELING SYSTEM 2.0 (ENZ-GEN111)
GREEN 496 dUTP (ENZ-42831)

INTRODUCTION

Labeling DNA with fluorescent dye dUTPs is well recognized as superior to labeling DNA with indirect two-step labeling methods. Coupled with the Nick Translation DNA Labeling System 2.0, fluorescent dUTPs provide a simple and efficient approach to fluorescent labeling for FISH applications.

Accommodating a wide range of fluorophore-labeled, biotin-labeled, and digoxigenin-labeled nucleotides, the Nick Translation DNA Labeling System 2.0 is the recommended method for labeling double-stranded DNA larger than 1 kb for applications including fluorescent *in situ* hybridization (FISH). FISH is a well-documented technique used to label DNA sequences in chromosomes for research and clinical applications.

Relatively humid environmental conditions are necessary for proper fluorescent *in situ* hybridization; however, the optimal humidity for FISH is not yet known. The main objective of this study was to demonstrate the capabilities of the Nick Translation System 2.0 for FISH under varying levels of humidity with the Boekel Scientific RapidFISH Slide Hybridization Oven.

MATERIALS

- Nick Translation DNA Labeling System 2.0 (Enzo Life Sciences, ENZ-GEN111)
- Green 496 dUTP (Enzo Life Sciences, ENZ-42831)
- Bacmid DNA for c-myc
- Nanodrop ND-1000 (Thermo Fisher)
- QIAquick® PCR Purification Kit (Qiagen, 28104, 28106)
- Cot-1 DNA (Thermo Fisher, 15279011)
- VECTASHIELD® Antifade Mounting Medium with DAPI (Vector Laboratories, H-1200)
- RapidFISH Slide Hybridization Oven (Boekel Scientific, 240200)
- CGH Metaphase Target Slides (Abbot Molecular, 06J96-001)
- Fluorescence Microscope (Olympus, BX51)
- Temperature and Humidity Data Logger (Madgetech, RHTemp101A)

METHODS

Fluorescent Labeling via Nick Translation

DNA was labeled with Green 496 dUTP according to the following procedure, summarized in Tables 1 and 2. To eliminate any effects of pipetting error, a master mix containing 3 µg of bacmid DNA, reagents from the Nick Translation Labeling System 2.0 kit, and 5.2 µL Green 496 dUTP (at a concentration of 0.3 mM) was prepared.

Step	Add Reagent	Amount
1.	DNA (3 µg)	78.3 µL
2.	Reaction Buffer	15.5 µL
3.	dNTP Mix (dATP, dGTP, dCTP)	15.5 µL
4.	dTTP	10.3 µL
5.	Fluorophore-dUTP	5.2 µL
6.	Nick Translation Enzyme Mix	15.5 µL
7.	Add H_20	to 155 µL

Table 1. Reagent Mix for Nick Translation Reaction

Step	Condition
1.	Add Nick Translation Reagents in Order of Table 1.
2.	Carefully mix and briefly centrifuge
3.	Incubate at 15 °C for 1 hour
4.	Place reactants on ice
5.	Stop reaction with 5 µL Stop Buffer and heat at 65 °C for 5 min.
6.	Place on ice for 5 minutes

Table 2. Nick Translation Reaction Summary

Purification

The fluorescent-dye labeled bacmid DNA must be purified prior to hybridization. The DNA probe was purified using the QIAquick® PCR Purification Kit. Samples were gently mixed with 275 µL of provided PB buffer (Qiagen), applied to in the column and centrifuged at 16,100 x g for 1 minute at room temperature. Flow-through was discarded and 750 µL of provided PE buffer (Qiagen) was added for second centrifugation. DNA was eluted by using 10 µL of EB buffer (Qiagen), allowing the mixture to stand for one minute, followed by a centrifugation for one minute at 16,100 x g.

Determining DNA Fluorescent Dye Incorporation

The Thermo Fisher Nanodrop® ND-1000 UV-VIS Spectrometer was used in the Microarray Measurement Mode to determine the incorporation of Green 496 dUTP in each prepared batch.

Hybridization & Scanning

Human male metaphase slides from Abbott (Prod. No. 06J96-001) were warmed to room temperature and prepared for hybridization (2 µL from Batch 1 or 0.5 µL from Batch 2). Purified Green 496 probe and 3 µg (1 µg/µL) Cot-1 DNA were added to each sample. Varying amounts of nuclease-free water were added to the oven tray for each hybridization. All samples were immediately placed into the Boekel RapidFISH Slide Hybridization Oven for overnight incubation at 37 °C.

Hybridization quality was tested at 0 mL, 5 mL, 10 mL, 15 mL, 20 mL, 25 mL, and 30 mL. Each volume was tested in duplicate. A temperature and humidity data logger (Madgetech) was used to measure operating temperature and relative humidity at a sampling rate of 30 s. After incubation, each sample was washed and carefully stained using 8 µL of VECTASHIELD® H-1200 mounting medium containing DAPI (1.5 µg/mL) with antifade.

The hybridized samples were scanned immediately after hybridization and washing using an Olympus BX51 Fluorescence Microscope with a 60X objective lens. All images were collected using 1 s exposure with both DAPI and FITC fluorochrome filters to best assess the effects of humidity on hybridization for FISH while minimizing photobleaching.

Results

Green 496 fluorescent dUTP was sufficiently incorporated into the bacmid DNA using the Nick Translation DNA Labeling System 2.0. Hybridization for FISH using the Boekel Scientific RapidFISH Slide Hybridization Oven yielded bright results with as little as 5 mL added moisture. Results were similar for moisture levels greater than 20 mL.

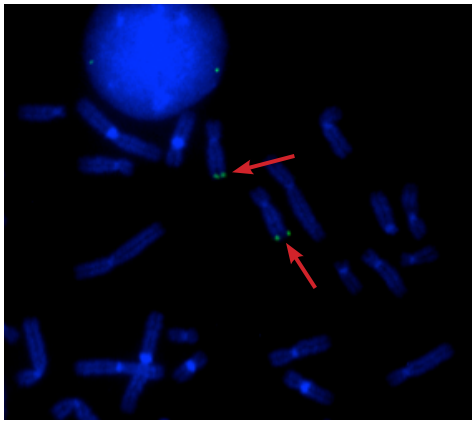


Figure 1. Green 496 dUTP probe (44.245 ng/µL) labels human chromosome 8 after 37 °C FISH hybridization with 25 mL nuclease-free water. Blue DAPI counterstain was applied prior to visualization. Note the clarity and intensity of the green fluorescence.

Fluorescent *in situ* Hybridization (FISH) Conditions: 25 mL of moisture, 44.25 ng/µL of probe

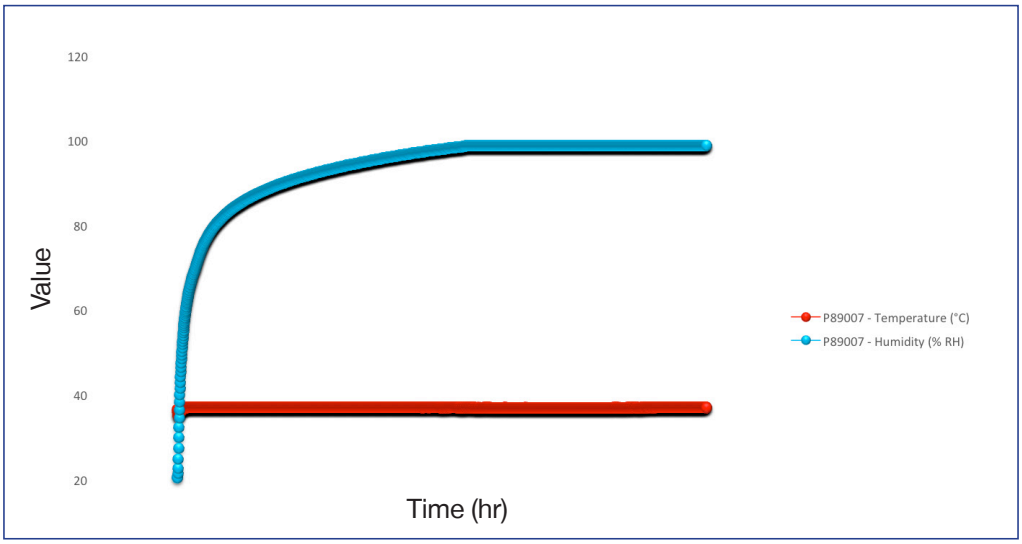


Figure 2. Temperature and relative humidity conditions were collected continuously at 30 s sampling rate using a Madgetech RHTemp101A data logger throughout hybridization. The Boekel RapidFISH Hybridization Oven boasts quick warm-up and stable conditions across the duration of hybridization.

Hybridization Incubation Humidity Conditions

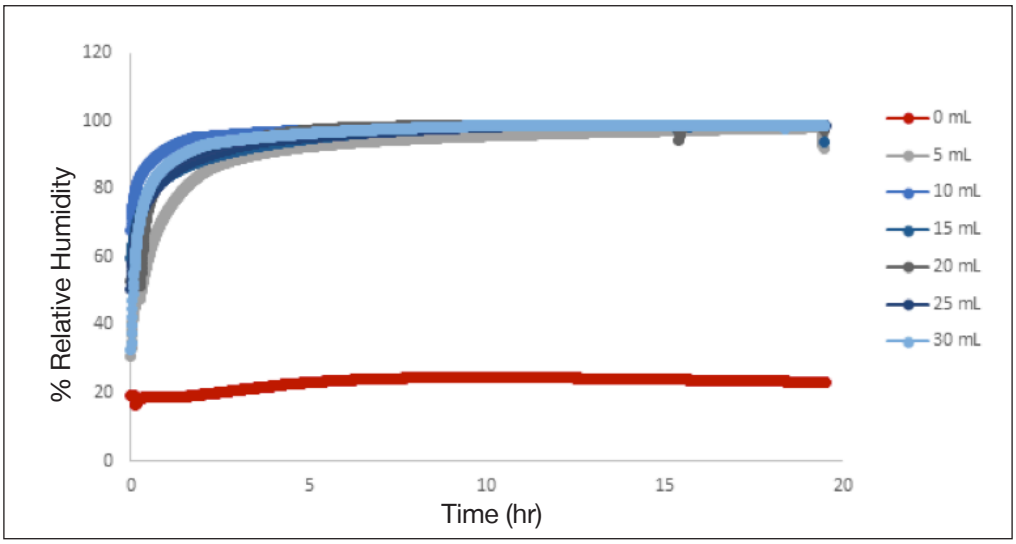


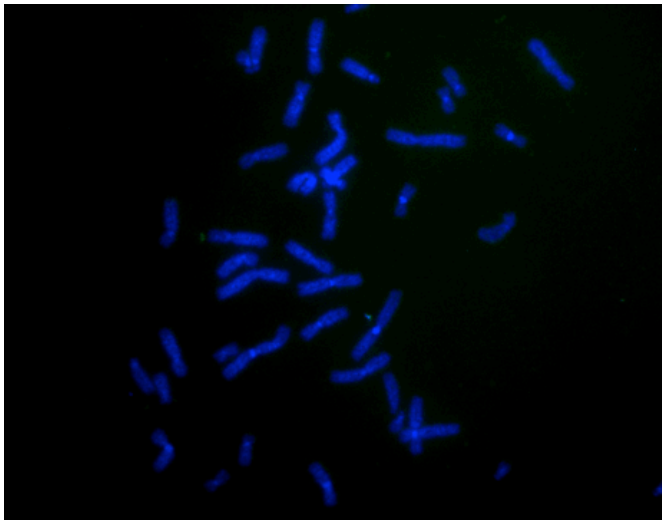
Figure 3. Relative humidity for 0-30 mL during incubation was collected at a sampling rate of 30 s. Data from two runs were averaged for each increment of added moisture (5 mL – 30 mL). Low relative humidity is observed with no added moisture, indicative of poor hybridization conditions.

CONCLUSION

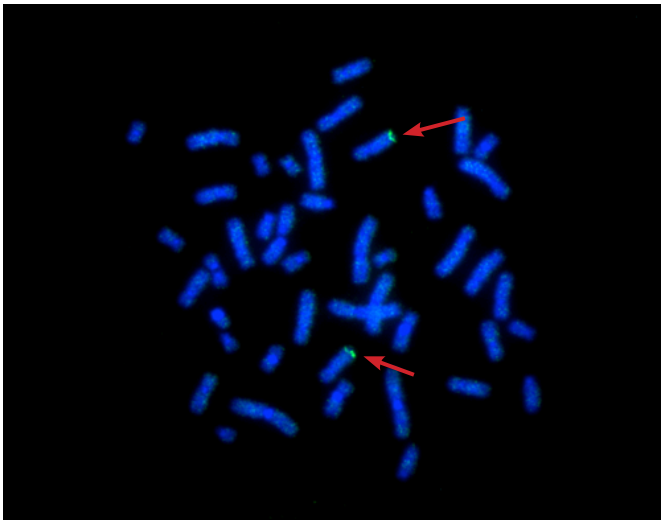
We conducted hybridization for FISH using two separate batches of Green 496 bacmid DNA probe under varying levels of humidity to identify optimal conditions to prepare samples for FISH applications. The Boekel RapidFISH Slide Hybridization Oven in conjunction with our Nick Translation DNA Labeling System 2.0 proved to be a robust platform for producing fast, predictable, and repeatable results. By virtue of its proprietary self-locking tray mechanism, the Boekel RapidFISH Slide Hybridization Oven maintained temperature and humidity conditions throughout all experiments where moisture was added to the slide hybridization tray. Only when no moisture was added to the hybridization tray was the relative humidity low and fluctuating during incubation.

When using the Boekel RapidFISH Slide Hybridization Oven, we recommend adding no less than 20 mL of nuclease-free water to ensure bright signal.

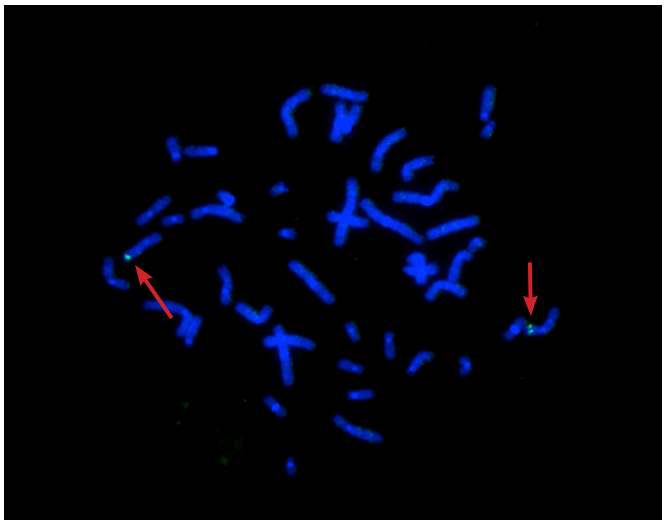
APPENDIX A: Additional FISH Pictures



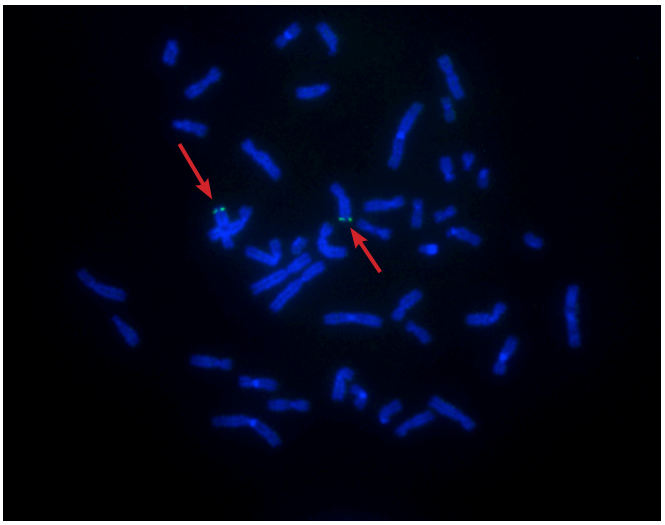
No water added



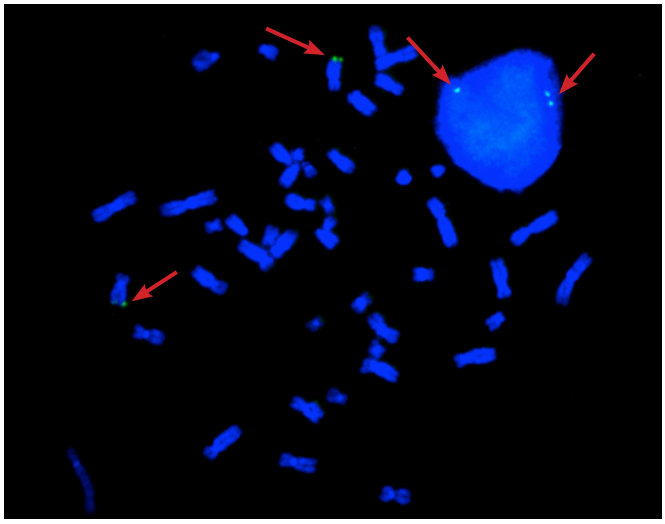
5 mL



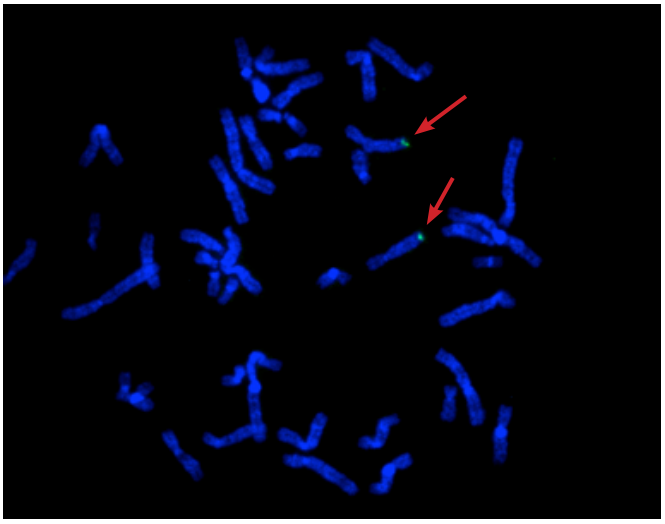
5 mL



10 mL



25 mL



30 mL

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