FISH SLIDE HYBRIDIZER TEMPERATURE VARIABILITY

Paul Scott and James Stanchfield, Ph.D.

SciGene • 1287 Reamwood Avenue, Sunnyvale, CA 94089 USA • (408) 733-7337 • techserv@scigene.com

Introduction

Molecular analysis methods that employ FISH (fluorescence in situ hybridization) of DNA probes to chromosomes are used in every cytogenetic laboratory for diagnosing a wide variety of cancers and other genetic abnormalities.

A key step in performing a FISH-based assay is the successful co-denaturation of the fluorescent probe with the cell or tissue sample so that hybridization of the probe to the target DNA sequences can occur. The co-denaturation step calls for heating the probe solution, in contact with the slide sample, for a specific temperature and time as instructed by the probe manufacturer. Failure to achieve the specified co-denaturation temperature for the prescribed time can lead to low fluorescence signals or generate unclear signal patterns that confound interpretation.

During the development of the CytoBrite® Slide Incubation System, we were challenged by the current tools available for accurately measuring metal surface temperature at each slide position. Using individual thermocouples, we ended up with a tangle of wires that were heated to varying degrees under the instrument cover that affected temperature readings. Despite taking great care to set up these tests, we found the precision of the measurements using this approach to be no better than ± 1°C.

Searching for another method, we discovered a miniature (size of a dime) NIST-traceable, wireless sensor with integrated datalogger that measures and stores slide position temperature, and also provides better precision and accuracy, than a traditional thermocouple.

In this report, we describe the validation of this measurement system and its application for determining the temperature accuracy and uniformity of slide hybridization instruments. Using this superior method, we collected the temperature profiles from over 50 Thermobrite®/HYBrite® instruments in 18 cytogenetics laboratories throughout the United States. We report here the findings of this extensive study on the temperature accuracy and uniformity of these instruments.

Materials & Methods

The wireless iButton® Thermochron sensors and 1-Wire® adapter used in this study were purchased from www.ibuttonlink.com (Figure 1).

- Thermodata Viewer Package ($106.00)
- iButton DS1922T-F5# Thermochrons ($89.99 each)

Figure 1. iButton sensors and adapter

Figure 2. iButtons placed in ThermoBrite

Method for Collecting Data on Slide Hybridizers

Using the Thermodata Viewer Software and adapter, iButtons were configured to log at 10 second intervals at a high resolution of 0.1°C. The sensors were then placed at slide positions 1 and 2 of the Hybridizer and a programmed protocol run on the instrument: 75°C for 2 minutes; 37°C for 40 hours.

(Note: A few institutions ran denaturation temperatures of 73°C, 80°C and 82°C).

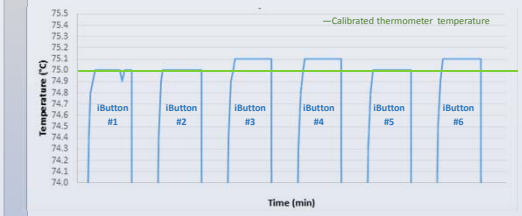
After the protocol had returned to the incubation temperature of 37°C, the program was stopped, sensors were moved to positions 5 and 6 and the same program was run again. Sensors were then moved a final time to positions 11 and 12 and the program repeated again. Data was downloaded through the Thermodata Viewer Software using the 1-Wire adapter. Time and temperature information was then compiled into a spreadsheet and analyzed.

Results

Accuracy and Precision of the Wireless Temperature Logger

The manufacturer of the wireless temperature logger specifies the system has an accuracy of ± 0.5°C and offers a certificate of compliance for each iButton. We independently verified both the accuracy and precision of each iButton used in this study by placing them on a well regulated heatblock set at 75°C (Hybex® Microsample Incubator, SciGene) connected to a NIST-traceable digital thermometer calibrated to a temperature standard. For each iButton, data was collected for 20 minutes and then an individual temperature profile was downloaded and analyzed. The steady state temperature achieved by all six sensors (Figure 3) was within ± 0.1°C of the calibrated digital thermometer in good agreement with the manufacture's specifications. The precision of the method, defined as the range in temperatures reported by the six using iButton sensors, was also ± 0.1°C . These results demonstrate that the iButton logger provides excellent accuracy and precision in this application; performance far superior to wired thermocouple methods we used previously.

Figure 3. iButton Temperature Accuracy and Precision

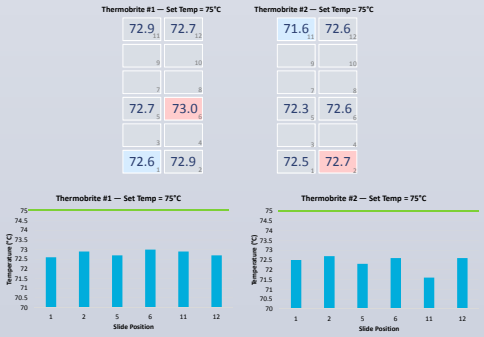


Temperature Analysis of Thermobrite/HYBrite Slide Hybridizers

After incorporating the use of iButtons into the quality system for manufacturing our CytoBrite family of instruments, we took the system to 18 cytogenetics laboratories across the United States and collected temperature profiles from each lab's Thermobrite and HYBrite instruments. Slide position temperatures were logged using iButtons while running the laboratory's preferred FISH program. After downloading the data, the denaturation temperature that was attained for each slide position was extracted from the temperature profile. Data from this extensive study of 43 Thermobrite and 14 HYBrite instruments is shown in Table 1. Data from some individual instruments is shown in Figures 4 and 5.

The highest and lowest slide position temperatures and the maximum variation from the programmed temperature across the instruments is reported for each laboratory. None of the instruments reached the programmed denaturation temperature, with deviations across instruments in a single laboratory ranging from -2.0°C (Lab "M") to - 16.4°C (Lab "F"). Although both instrument models showed significantly lower temperatures from the programmed temperature, the largest deviations were observed in the older HYBrite instruments.

Figure 4. Examples of Thermobrite Temperature Variation



Results (continued)

Figure 5. Examples of HYBrite Temperature Variation

Table 1. Temperature Variation across 57 Instruments at 18 Laboratories

Lab	Set Temp (°C)	# Themobrites	# Hybrites	Highest Temp Slide Position	Lowest Temp Slide Position	Max Obs. Variation from Set Temp
A	75	1	1	74.8	58.6	-16.4
B	75	1	1	72.3	58.9	-16.1
C	73	4	2	73.0	57.4	-15.6
D	75	6	2	74.3	60.6	-14.4
E	75	1	1	74.8	61.0	-14.0
F	75	2	3	72.6	61.0	-14.0
G	75	1	1	72.7	61.2	-13.8
H	75	3	0	74.3	62.1	-12.9
I	75	1	1	74.5	62.5	-12.5
J	82	1	1	81.6	70.3	-11.7
K	75	2	1	74.5	65.7	-9.3
L	82	2	0	80.7	76.8	-5.2
M	73	6	0	71.7	68.8	-4.2
N	75	5	0	74.3	71.5	-3.5
O	75	3	0	74.1	71.6	-3.4
P	75	1	0	73.8	72.2	-2.8
Q	75	1	0	73.9	72.3	-2.7
R	75	2	0	75.1	73.0	-2.0
	Totals:	43	14			

Conclusions

We describe here an accurate and precise system to measure the temperature of slide positions in FISH slide hybridization instruments using a miniature wireless temperature sensor with datalogger (iButton). The system is simple to use, reliable, available commercially and affordable.

We have used the iButton system to measure the slide position temperatures on 57 Thermobrite/HYBrite systems in 18 cytogenetics laboratories across the United States. Slide position temperatures were found to be consistently lower than the programmed temperature with deviations within instruments in a single laboratory ranging from 2.0 to 16.4 degrees below the target temperature. So in practice, this means the operator thinks the instrument is getting to the temperature on the display but it's actually significantly cooler with the older HYBrite units frequently 10 degrees below the programmed temperature. The iButton system has solved the mystery behind the "good" and "bad" instruments commonly referred to in cytogenetics laboratories.

The various suppliers of FISH probes specify that the "melt" temperature when performing co-denaturation of probe with sample that should not vary more than 1°C. The variability in slide position temperatures among the many slide hybridization instruments analyzed in this study clearly show these instruments do not meet probe supplier requirements and are a significant (and unnecessary) source of FISH assay variability.

The CytoBrite Slide Incubation System from SciGene meets FISH probe supplier requirements for slide temperature accuracy for performing a FISH assay.

SciGene and CytoBrite are registered trademarks of SciGene Corporation. All other marks are the property of their respective owners. © 2015