AFFYMETRIX®

Technical Note

Analysis of Temperature Performance in the GeneChip® Hybridization Oven 645

This Technical Note describes the temperature performance of the GeneChip® Hybridization Oven 645 and provides data demonstrating the stability of the oven when confronted with several sources of potential variance. Testing performed by Affymetrix has demonstrated that a number of potential error sources, individually or combined, pose a minimal challenge to the performance of the new oven.

Introduction

Appropriate control of temperature variance is important for reducing variance in many biological processes. The Affymetrix® GeneChip® Hybridization Oven 645 promotes improved uniformity in GeneChip® experiments by limiting all contributors to temperature variance.

The most common sources of variance in laboratory ovens include:

- Calibration accuracy
- Accuracy of response to changes in set point
- Internal non-uniformity
- Susceptibility to changes in laboratory ambient temperature
- Short- and long-term thermal drift

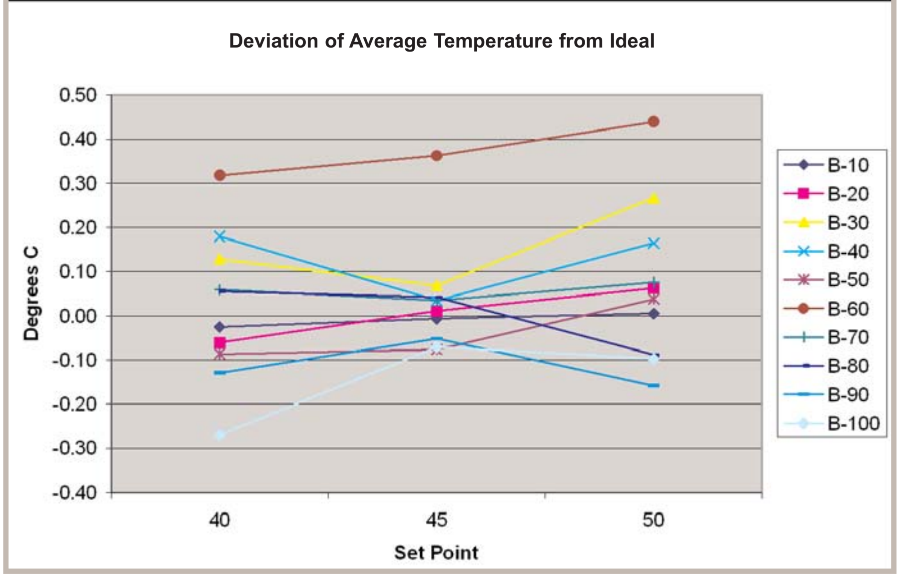
In adopting the 645 design, Affymetrix investigated each of these potential contributors to ensure that individually or combined, none would pose a significant threat to appropriate temperature performance within the oven.

This Technical Note addresses each of the potential variances in turn, presenting data collected during product evaluation testing.

Methodology

All temperature data was obtained with NIST traceable ValProbe® temperature sensors accurate to 0.1°C. Two sensors were located in the cartridge carrier, one each on the left and right sides of the rotating chip rotisserie. Average temperature was calcu-

Figure 1: Average temperatures measured inside 10 GeneChip® Hybridization 645 Ovens operating under normal laboratory conditions at +/-5°C around 45°C calibration set point. A 5 °C change in either direction introduced less than 0.2°C additional error.



lated from the mean of the left and right readings. Ten ovens were employed in the study; the ovens were serialized B-10, B-20, etc., through B-100.

Testing and Results

SET POINT CALIBRATION ACCURACY AND RESPONSE TO CHANGES IN SET POINT

Inaccuracies in calibration contribute directly to the overall inaccuracy of an oven. However, the use of NIST traceable sources and careful technique can largely eliminate this source. The average temperatures of all ovens were calibrated at 45.0°C to ±0.1°C. The set points were then changed to 40.0°C and 50.0°C and the deviation of average temperature from these set points was recorded and charted.

Figure 1 illustrates that the 5°C degree change in either direction introduced less than 0.2°C additional error. The mean error and increase in standard deviation of all ovens in each direction were both well under 0.1°C. Assuming ±0.25°C as a worst-case scenario, additional error across any 5°C change produces an error coefficient of ±0.05°C per degree of change in set point away from 45.0°C. Note that all ovens except B-60 (which was not recalibrated) deviate less than 0.1°C from ideal at the 45°C calibration point. This attribute was greatly improved during Beta testing by the introduction of new oven firmware that reduced the systemic deviation to near zero.

Although the test sample demonstrates that it is possible to calibrate all ovens to within 0.1°C of 45.0°C, prior production line testing experience demonstrates that ±0.3°C is a more practical limit across manufacturing lots. Combining these two variances produces a worst-case variance of ±0.3°C at the 45°C set point and ±0.55°C at 40°C and 50°C set points.

INTERNAL NON-UNIFORMITY

Non-uniformity is the maximum difference in temperature from the warmest to the coolest array locations in the rotisserie. Non-uniformity is typically worst when the difference between internal and external temperature is greatest. Figure 2 illustrates temperatures taken at four separate locations within three ovens, with a relatively high set point of 50.0°C and an external ambient temperature of 15°C, representing the coolest likely temperatures that will be encountered in a controlled laboratory environment.

Even in this relatively extreme case, no location experiences a temperature deviating greater than ±0.2°C from the average temperature, or a maximum left-right delta

Figure 2: The measured and average temperature at four locations for three randomly selected Hybridization 645 Ovens (B-10, B-30 and B-50). Testing performed at 50°C set point and 15°C ambient. All locations are less than ±0.2°C from the average temperature. Maximum left-right difference was less than 0.4°C.

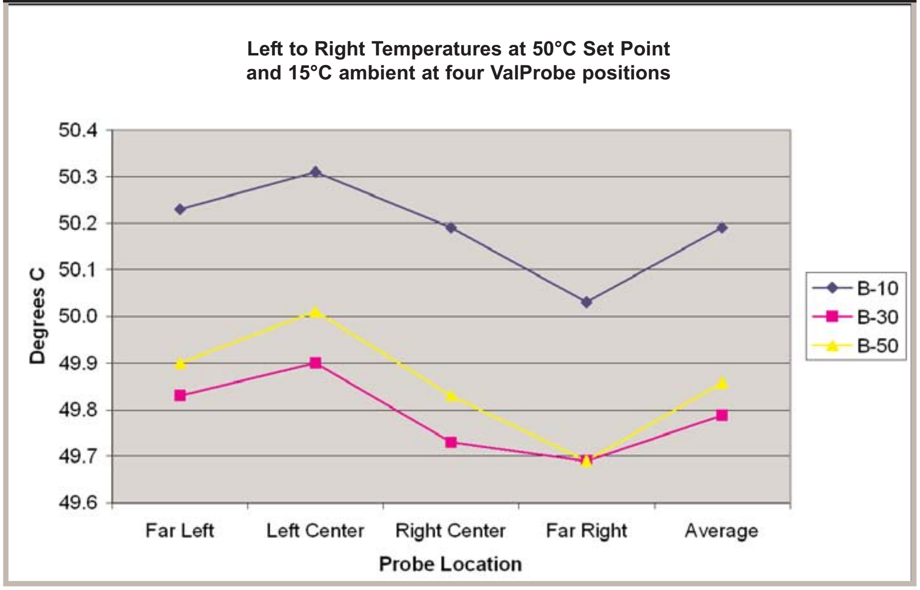


Figure 3: The susceptibility of set point accuracy to changes in ambient temperature. Three set point levels were tested; lower set points were affected slightly more than higher ones. Typical susceptibilities observed were between -0.01°C and -0.03°C of change per degree of ambient change, depending upon oven and set point.

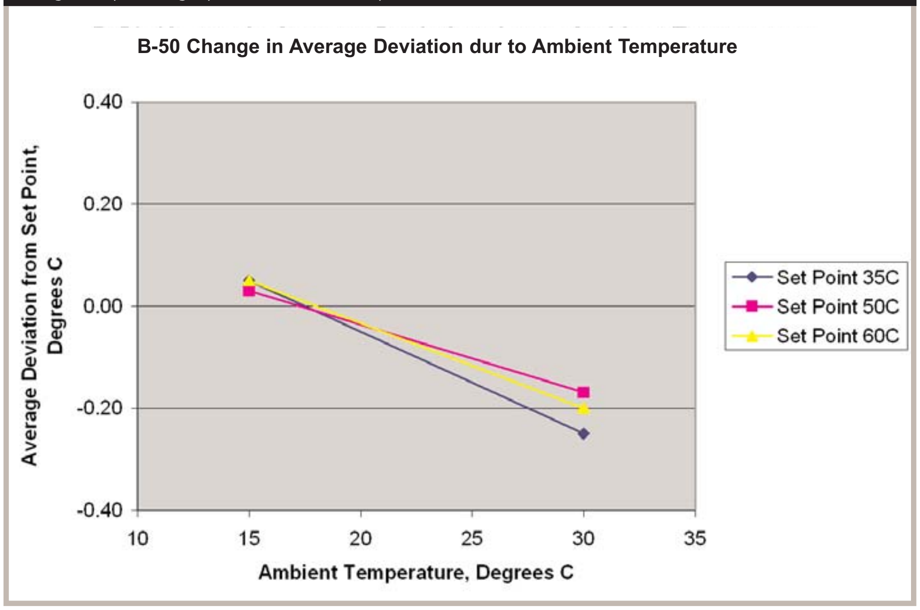
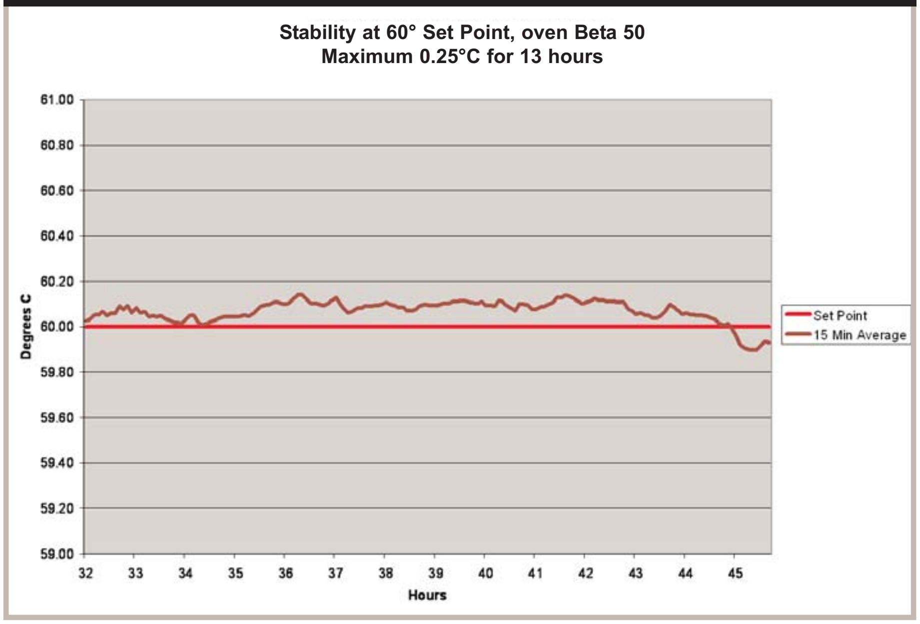


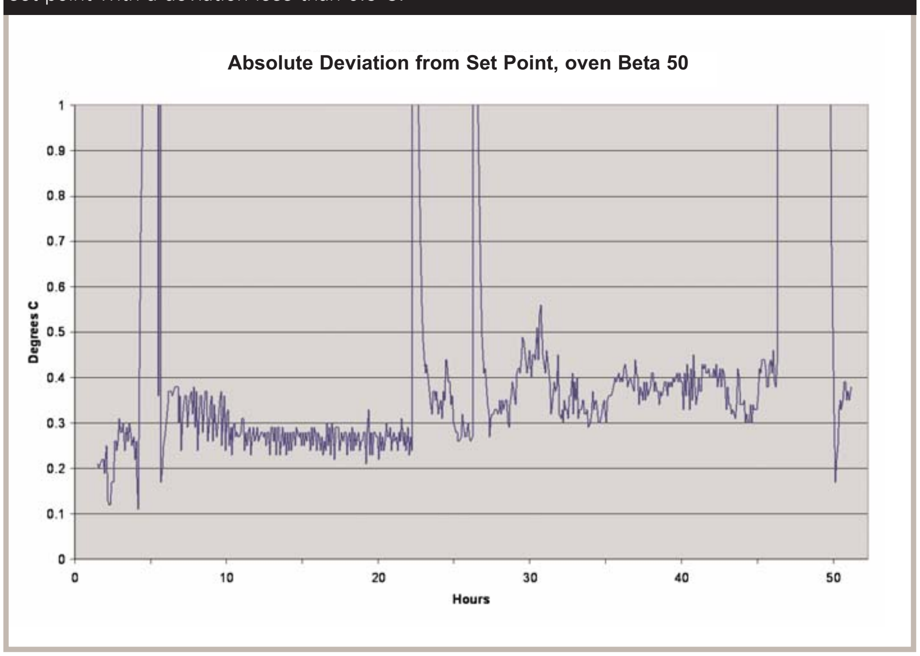
Figure 4: The typical temperature variability over an extended hybridization period (45 hours). Short-term variation was typical of the oven population at less than 0.25°C variation.



of 0.4°C well centered about the average. The data also suggests strong repeatability between ovens, in both the magnitude and pattern of non-uniformity.

A check of left-right delta on all 10 test ovens under normal laboratory ambient conditions at set points of 40.0°C, 45.0°C and 50°C supports this conclusion, with no oven exceeding a 0.4°C delta. It is therefore reasonable to assign a 0.5°C delta or ±0.25°C deviation from average to deviation due to internal non-uniformity.

Figure 5: Absolute deviation from set point across 50-hour test period on B-50. Large deviations represent periods when oven was shut off and restarted. Note that oven always returned to the set point with a deviation less than 0.5°C.



IMMUNITY TO AMBIENT CHANGES

The behavior of the ovens and other heating devices in response to ambient change is typically very consistent. The effect of higher ambient temperature is always to slightly reduce the true oven temperature. The susceptibility of the Model 645 ovens, however, was found to be low—between -0.01 and -0.03 °C of change per degree of ambient change, depending upon oven and set point. Figure 3 illustrates ambient susceptibility on one typical oven. Assuming a 15°C worst-case change in laboratory ambient temperature and a worst-case susceptibility of -0.04°C, the worst-case change can be bounded to under 0.6°C. Given that oven calibrations are performed under more typical midpoint ambient conditions of 20°C to 25°C, it is reasonable to express the variance due to ambient susceptibility as ±0.3°C from set point.

SHORT-TERM VARIATION DURING A TYPICAL HYBRIDIZATION CYCLE

Ovens B-10, 30 and 50 were run at 35°C with ambient at maximum, and at 60°C with ambient at minimum while operating in the environmental chamber. Worst-case drift in average temperature occurred at 60°C.

- Oven B-10 displayed 0.11°C of variation
- Oven B-30 displayed 0.16°C of variation
- Oven B-50 displayed 0.25°C of variation

A temperature plot from oven B-50 illustrates this behavior (Figure 4). It is reasonable to expand this value to assume a worst-case deviation of ±0.2°C during any hybridization session.

LONG-TERM DRIFT

A calculation of all drift factors within the oven control system predicts a worst-case variance of ±0.143°C over a one-year recommended recalibration cycle. This value is supported by experimentation on similar control systems and the absence of drift observed during the several-month experimentation period (data not shown).

LOADING AND ROTATION SPEED

Additional testing found no significant influence on average temperature and slight influence on non-uniformity (on the order of 0.1°C) due to the number of arrays in the oven, their loading pattern within the rotisserie or the rotisserie rotation speed (data not shown).

DISCUSSION

Testing and evaluation of the new Hybridization Oven 645 focused on quantifying the sources that contribute to temperature accuracy. Maximum error levels are summarized below:

- Set Point: ±0.55°C
- Non-uniformity: ±0.25°C
- Ambient Immunity: ±0.30°C
- Short-term Variation: ±0.20°C
- Long-term Drift: ±0.143°C
- Chip Load, Rotisserie Speed: ±0.10°C

Even if one assumes the extreme and very unlikely case that each of these errors are both worst-case and additive in the same direction, the deviation from set point is 1.55°C, still within the ±2.0°C published specification of the oven.

However, in typical situations errors are non-additive, so temperature performance well within ±1.0°C of set point is expected. This is supported by maximum temperature variations within the ovens tested. In Figure 5, the worst-case deviations from set point in oven B-50 are plotted under a wide variety of ambient conditions and set points during a 50-hour test. The large spikes represent deliberate change in state or periods when the oven was shut off. In all settled, normal operation, the oven maintained all temperatures within 0.5°C of the programmed set point.

Conclusion

The Affymetrix® GeneChip® Hybridization Oven 645 promotes improved uniformity in GeneChip® experiments by limiting all contributors to temperature variance. Testing performed by Affymetrix has demonstrated that a number of potential error sources, individually or combined, pose a minimal challenge to the stated performance of the new oven. The superior temperature performance of the Hybridization Oven 645 is expected to deliver superior conditions for hybridization of GeneChip® microarrays.

Ordering Information

GeneChip® Hybridization Oven 645

GeneChip® Hybridization Oven 645

00-0331 GeneChip® Hybridization Oven 645 (110 or 220V; includes eight probe array carriers)

90-0356 GeneChip® Probe Array Carriers Red (quantity two)

90-0357 GeneChip® Probe Array Carriers Green (quantity two)

90-0358 GeneChip® Probe Array Carriers Purple (quantity two)

90-0359 GeneChip® Probe Array Carriers White (quantity two)

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General Purpose Laboratory Instrument.

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