

**PRECISION AND AMBIENT TEMPERATURE EFFECTS
ON THE ABI PRISM[®] 3100 GENETIC ANALYZER**

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It has been well documented that precision in the measurement of STR allele sizes can be affected by room temperature fluctuations over the course of a run on the capillary electrophoresis-based ABI PRISM[®] 310 Genetic Analyzer. Temperature variation seems to have the same effect on the sizing precision of the ABI PRISM[®] 3100 Genetic Analyzer. Standard practice when analyzing with the Local Southern sizing method is to exclude the use of the 250 bp peak of the GS500 size standard due to its anomalous migration relative to the other size standard peaks and the sample peaks when temperature changes. Data has shown that also excluding the 340 bp peak can improve sizing precision and, in addition, switching to the Global Southern sizing method further improves precision.

Ninety-six injections of AmpF/STR[®] Identifier[®] Allelic Ladder were made and analyzed with GeneScan[®] 3.7 NT software. Data from these injections were analyzed using various sizing methods. Data was first analyzed by the Local Southern sizing method excluding only the 250 bp peak. The mean size and standard deviation was calculated for all 19,680 alleles. When calculations were made for each individual capillary across an entire plate of 96 wells, the standard deviation values ranged from 0.01 – 0.23 bp. Because the Genotyper macro commonly used creates an allele calling window, which is +/- 0.5bp, standard deviations should ideally be ≤ 0.16 bp. In this study, higher standard deviations occurred when alleles were greater than 300 bp in size. When the same data was analyzed by capillary using the Local Southern size calling method excluding both the 250 and 340 bp peaks, the range of standard deviation values decreased to 0.01 – 0.15 bp. Re-analysis using the Global Southern size calling method and excluding both the 250 and 340 bp peaks improved the precision slightly more than the Local Southern method under the same conditions. With this method, the standard deviations ranged from 0.01 – 0.13 bp.

Data was also analyzed separately for each 16 capillary injection set by Local Southern excluding only the 250 bp peak. If ambient temperature variation was the cause of the sizing variation observed, then the “within run” (16 simultaneous injections) precision should be much better. This was the case as data calculated in this manner resulted in a maximum standard deviation of only 0.09 bp, suggesting strongly that the 3100 is susceptible to mobility fluctuations as a result of ambient temperature variation. Applied Biosystems recommends including an allelic ladder with each injection set. Our data suggests that an alternative approach to avoiding temperature related genotyping problems when using the 3100 is to choose a different method of size calling (Global Southern) and/or excluding the 340 bp peak, along with the 250bp peak, of the GS500 size standard.