

INCREASED SENSITIVITY IN MIXTURES AND LOW LEVEL SAMPLES USING OSIRIS

George Riley, Robert Goor, Douglas Hoffman

National Center for Biotechnology Information, National Library of Medicine, NIH

Sensitive detection of minor peaks is critical in mixture and low-level sample analysis. The detection of a minor peak can make the difference between including a contributor at a reasonable probability or reporting either a low probability, or worse, “inconclusive”. Laboratories are using analytical thresholds at or below 30 RFU where justified by the signal to noise ratio in their system. Even a few RFU higher or lower can make the difference between a peak being above the analytical threshold or below it. Increasing sensitivity results in detection of low level peaks that would otherwise fall below the analytical threshold.

OSIRIS has an increased sensitivity in comparison to GeneMapper IDX, as noted by users that employ both systems or have done comparisons. OSIRIS analysis produces taller peaks than analysis with GeneMapper IDX. Peaks analyzed with OSIRIS average about 8% higher RFU than with GeneMapper IDX analysis. The difference varies with peak height. Large peaks average about 5% taller, with small peaks averaging about 8-14% taller in OSIRIS analysis than GeneMapper IDX. These analysis differences are largely due to three major differences between OSIRIS and GeneMapper IDX: smoothing, baselining, and to a lesser extent, calculation of peak height. The GeneMapper IDX default is to use smoothing to reduce the impact of noise on the peaks, which reduces peak heights¹. OSIRIS employs a different strategy, fitting a mathematical curve to the raw data, eliminating the impact of noise on the analyzed data without the need for smoothing. GeneMapper IDX baselining strategy can bias the normalization of the baseline where the peaks are closely spaced and the signal does not return to the actual baseline, thus over-subtracting, which reduces peak heights¹. OSIRIS uses a sophisticated algorithm that avoids peaks when sampling the baseline, resulting in a highly accurate baseline normalization, and avoiding unnecessarily reducing peak heights. Finally, with OSIRIS, the center of the mathematical curve that is fitted to the raw data peak usually falls between the raw data points collected by the Genetic Analyzer CE. The analyzed curve peak center may therefore be slightly higher than the collected raw data points on either side. These three factors mean that OSIRIS gives a more accurate reflection of the DNA signal's true height. While the increase in peak height is relatively small, the difference is critical when the peak height is close to the analytical threshold.

¹ GeneMapper®ID-X Software Version 1.5 Reference Guide