Comparison With Flat Bed Gel Electrophoresis

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Abstract
Microsatellite genotyping, or the measurement of microsatellite sizes across the genome in cohorts of diseased individuals and compared with their identical measurements in their non-diseased relatives, is becoming an increasingly successful tool for the identification of genes and gene regions that are associated with complex genetic diseases. In a high throughput laboratory the technique is rapid, relatively inexpensive and accurately defines regions for the more expensive SNP genotyping. Traditionally microsatellite genotyping has been performed using flat bed gel electrophoresis. The development of higher volume capillary electrophoresis instruments promises an increase in throughput with simultaneous reduction in operator involvement leading to reduced genotyping costs overall. Large cohorts and long term studies demand consistency of microsatellite sizing. Here we compared the size of PCR products amplified using a commercial primer set when determined using the Applied Biosystems 377 flat bed automated DNA sequencer and the newer 3730 capillary electrophoresis sequencing machine. Fluorescently-labeled PCR products were generated using linkage mapping set LMS-VII and electrophoresed on both polyacrylamide gels and polymer-filled capillaries. In flat bed, products were sized by comparison with a PstI digested plasmid size standard labeled with ROX. In capillaries, the same standard was labeled with LIZ. We found, with a small number of exceptions, that there tended to be good concordance between measurements of fragments between platforms although the allele bins on the 3730 were offset from 0 to -5 base pairs. Non-concordances were due to the higher sensitivity of the 3730 allowing 1 base pair fragments to be resolved which were not detected on the flat bed machine. Although our results suggest that sizing can be transposed between these two platforms, small inconsistencies observed would have us recommend that measurements of microsatellite size is still best performed on a uniform electrophoretic platform.

Non-concordance
7 (1.75%) of the 400 markers tested displayed a non-concordance between the alleles of the same marker although data from both platforms exhibited Mendelian inheritance within pedigrees groups. This non-concordance was due to 1 bp alleles being resolved in the 3730 data due to the greater sensitivity of the capillary machine. For example, a sample which yielded a genotype that was homozygous for a particular allele when run on the 3730 was found to contain a second allele 1bp apart when run on the 377 (see Figures 1 & 2). Table 3 gives an example of one of these non-concordant markers.

Conditions
PCR products were generated for 137 samples using all 400 markers of the 10m resolution ABI PRISM® Linkage Mapping Set Version2. Aliquots of these products were loaded on both the 377 and 3730 platforms using the same internal size standard (ROX/LIZ 500 bp). Samples were analysed and the genotypes produced by both platforms were compared.

The Machines

The offsetting between the markers run on both platforms was found to vary between 0 and -5 bp (see Table 2).
However, only 7 out of the 400 markers tested demonstrated a non-concordance between alleles of the same marker (see below).
Table 1 demonstrates the typical concordance within the bins of a marker run on the two different platforms.
Table 2 uses the chromosome 13 markers to demonstrate the difference in binning offsets between markers run on the two platforms.

A Strange One
Marker D6S434 allele bins shift from a consistent offset between platforms of -4 bp in the first half of its marker range to a variable offset of -3 to -17 bp, however data from both platforms exhibited Mendelian inheritance (see Table 4 and Figs 3 & 4).
This may be explained by a possible secondary structure such as a DNA loop that exists in some alleles but is not properly denatured by the 3730.

Conclusions
Data is transposable between the 377 and 3730 although it is recommended that genotyping be performed on a uniform platform to avoid confusion with non-concordant markers. The higher sensitivity of the 3730 allowed 1bp alleles to be resolved which could not be detected with the 377.