Introduction

Microsatellites, also known as short tandem repeats (STRs), are polymorphic DNA loci that contain a repeat sequence of 2–7 nucleotides. The number of repeats for a given locus may vary, resulting in alleles of differing lengths. Data for allelic variation, the number of repeats, and allelic frequencies are available for thousands of markers across numerous organisms. The ability to choose from such a large selection of highly informative markers has made microsatellite analysis a widely accepted tool for linkage mapping studies, association studies, and identification of organisms.

When choosing a capillary-based genetic analysis system for this application, several factors must be considered. Reaction optimization, proper run conditions, and efficient assay design are critical to ensure high-quality data and to minimize the need for costly reruns. In addition, managing large amounts of microsatellite data requires an efficient and highly automated workflow for every step, from data production and analysis to allele-scoring.

This Fact Sheet discusses the factors that must be considered for successful microsatellite analysis, and the many reasons why the Applied Biosystems 3130 and 3130xl Genetic Analyzers, in combination with optimized chemistries and the new GeneMapper® Software v3.7 provide a complete solution for microsatellite analysis.

Optimization of Microsatellite Reactions

One of the most common problems encountered during microsatellite analysis is poor or non-specific amplification. Microsatellite analysis projects often necessitate the interrogation of hundreds of loci per sample. Managing large numbers of reactions requires efficient primer design and robust universal reaction conditions, which minimize the need for costly reruns caused by failed PCR reactions.

When performing custom microsatellite applications, these variables must be addressed, and optimization may be necessary to ensure the best possible results. Applied Biosystems manufactures a suite of microsatellite-based kits that provide the most robust performance possible.

Microsatellite Reaction Artifacts

Most PCR-based systems produce artifacts that complicate data interpretation. Two well-characterized artifacts of PCR amplification observed in microsatellite analysis are stutter and non-templated 3’ A nucleotide additions, sometimes called “Plus A” additions.

Applied Biosystems uses a combination of specialized chemistries and advanced software features to differentiate these artifacts from true allele peaks.
**Stutter Artifacts**

Stutter artifacts are observed as multiple peaks preceding the true allele peak. The number of peaks and their intensities are proportional to the length of the repeat and the number of repeats in the PCR product. Although stutter is well-characterized and reproducible, it can complicate data analysis. Applied Biosystems GeneMapper® software is designed to identify and filter out stutter artifacts for accurate scoring of true alleles (Figure 1).

**Plus A Additions**

Plus A additions, caused by incomplete A nucleotide addition, also increase the complexity of the peak pattern, which makes recognition of true allele peaks more difficult (Figure 2). Reaction conditions greatly impact these locus-dependent artifacts, but to minimize the occurrence of Plus A additional peaks, most Applied Biosystems microsatellite kits promote Plus A additions, thus generating a more consistent allele peak pattern. The combination of optimized reactions that promote Plus A additions, and the ability of GeneMapper® software to filter out stutter and Plus A peaks, results in accurate identification of true alleles.

**Multiplexing**

The costs associated with microsatellite analysis projects and the time they require can be minimized by co-electrophoresis of multiple markers within each capillary. The ability to multiplex depends on the optimization of run conditions, differences in fragment sizes, and the number of dye labels compatible with a capillary electrophoresis (CE) system. Fluorescent labeling permits the analysis of multiple loci in the same capillary injection. The technology uses color and size to distinguish between fragments. The number of dyes available for fragment labeling and the selection of spectrally resolved dyes are essential for preventing analysis complications caused by spectral overlap. While most CE systems can analyze four-dye chemistries, the Applied Biosystems five-dye chemistry system increases multiplexing capacity (Figure 3), improves spectral resolution, and reduces project costs by increasing throughput 33%.
Data Collection

Data production is another important component of the microsatellite project workflow. The ability of a system to produce reliable, high-quality, automated results impacts the time required to complete a project, as well as its cost. Applied Biosystems 3130 Series Systems allow easy scheduling and run prioritization for maximal flexibility. Data Collection software promotes quick set-up with minimal hands-on intervention, enabling faster turnaround times for data production.

Ensuring Superior Precision and Resolution

Precision and high resolution in microsatellite analysis depend upon the following factors:

- Fragment size
- Dye chemistry
- Enhancements to the sizing algorithm
- Optimization of electrophoretic separation conditions

When optimizing separation conditions, it is important to consider four factors:

1. Temperature regulation
2. Polymer type
3. Capillary array length
4. Consumables quality and their ease-of-use

DNA Fragment Sizing

A common misconception about DNA fragment-sizing is that the calculated size of a DNA fragment is equivalent to the length (bp) of the fragment. Because the electrophoretic mobility of DNA is sequence-dependent, DNA fragments of the same length can have different mobilities and, therefore, can vary in calculated size. Size is calculated by the mobility of the fragment and not by its base-pair length, therefore reproducibility and precision are crucial aspects in microsatellite analysis. The 3130 and 3130xl systems provide the most reproducible data possible for all microsatellite applications (Figure 4). These systems also contain the following new enhancements that facilitate more efficient polymer handling and significantly reduce set-up time:

- Enabling 3130 POP-7™ Polymer
- A detection cell heater to improve migration uniformity through better thermal control
- An Automated Polymer Delivery System

Data Analysis and Management

Microsatellite analysis projects can involve data from hundreds or thousands of samples, depending on whether a few individuals are screened with many loci, or many individuals are screened with a few. Because an efficient data management workflow is crucial, fragment analysis software should offer the following advanced data analysis and management features:

- Flexibility
- Precise fragment sizing and allele scoring
- Automation

Figure 4. Overlay of 16 GeneScan™-500 LIZ™ size-standard electropherograms shows sizing precision on the 3130 Series Systems.

Figure 5. Advanced data management and analysis software produces intuitive data tables for fast allele scoring and data review.
GeneMapper® software provides all these features and easily manages a wide variety of projects, regardless of size.

Advanced algorithms in GeneMapper® software recognize and filter amplification chemistry artifacts, including Plus A and stutter peaks, by differentiating among microsatellite repeats of varying lengths. Additionally, the software uses genotyping quality (GQ) scores to flag lower quality sample files for manual review, and produces tables that can be sorted in a format that is easy to interpret. These software benefits allow researchers to review large amounts of microsatellite data accurately and rapidly (Figure 5).

**Conclusion**

The sensitivity, improved data quality, and precision of the 3130 Series Systems, in combination with their five-color chemistry and pre-optimized run conditions, make these systems ideal for microsatellite analysis. In addition, analysis software with flexible and intuitive data management options should enable both small- and large-scale studies to be performed. The integration of these instruments with GeneMapper® software allows one-button operation for data collection, fragment size calling, and allele scoring in both graphical and tabular form. These features help generate large amounts of high-quality data with minimal hands-on time or manual data review, dramatically reducing the time and cost required for a microsatellite project.

### Ordering Information

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<thead>
<tr>
<th>Description</th>
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<tbody>
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*contact your sales representative.