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Mass spectrometric analysis of human microsatellite markers

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1. Introduction

Genotyping based on the analysis of multiple short tandem repeat (STR) loci has been validated for routine use in human identification, parentage testing as well as in diagnosis of hereditary diseases. Currently, the analysis of the polymorphic variations in allele length of these genetic markers depends on electrophoretic separation of fluorescently labeled PCR products. Another technique accepted for sizing and typing of microsatellite markers is capillary electrophoresis which, compared to slab gel electrophoresis, is capable of a more automated procedure with increased resolution of the separation and reduced time for an electrophoretic run. Despite the improvement toward automation and throughput, difficulties remain associated with electrophoretic methods. Mainly the size determination which is correlated to the mobility of an internal standard in the gel matrix is error prone due to sequence-specific effects and unresolved secondary structures.

With the introduction of gentle ionization techniques for large biomolecules such as electrospray (ESI) and matrix-assisted laser desorption/ionization (MALDI), mass spectrometry (MS) has become an alternative method for the size determination of DNA fragments. The generation of intact gas phase ions for separation and detection offers the advantage of short analysis time and the ability to obtain molecular weight information without the need for labeling and size standards. However, mass analysis of PCR products is complicated by the presence of various salts as well as of surplus nucleotide triphosphates and primers used in the amplification reaction of relevant regions in the genome. Thus, purification of the PCR reaction is a severe demand for the use of both MALDI and ESI MS.

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2. Results

For the analysis of human microsatellite markers using ESI ion trap MS, a new purification method based on reversible binding of PCR products onto magnetic particles is applied. This approach has the capability for fully automated handling and is directly compatible with ESI ion trap MS analysis.

The purified PCR products derived from several polymorphic STR loci dissociate during ionization resulting in detection of the sense and the anti-sense strand with a mass accuracy of less than 0.5%, sufficient to determine a single base substitution in a mass range of 30 kDa. The sensitivity of this approach is below 500 fmol/μl.