Application of Mass Spectrometry in DNA Analysis

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Mass spectrometers have been used for DNA analysis since 1988 (Hillenkamp and Karas, Tanaka). The process is described as matrix-assisted laser desorption/ ionization time-of-flight (MALDI-TOF).

The substance to be measured (DNA) is mixed with a matrix and irradiated with a laser beam. The matrix absorbs part of the laser energy which is transferred to the DNA fragments. The fragments are thus converted to the gaseous phase and ionized. The ions are accelerated in an electronic field and subsequently move in a field-free drift space, the flight tube, where the masses are separated: smaller fragments fly faster than larger ones. When the fragments reach the detector they generate a signal which is digitalized and transmitted to a computer for evaluation.

The time difference between the laser discharge and the collision of the DNA fragments with the detector is determined, and the mass is derived from the time difference. The actual measuring process is very rapid, one DNA sample is measured in a matter of 5 – 10 sec, and the mass is determined with an accuracy rate of 0.1 % (for a 30mer fragment with roughly 300 Dalton per base this corresponds to about ± 9 Dalton).

The DNA sample and matrix are placed on a silicon chip by piezo-electric pump. The chip is then measured automatically in the mass spectrometer. The data are stored in a data bank.

Conventional analytical methods such as gel electrophoresis may be encumbered by various problems: for example, different primer labelling or DNA composition might result in different gel electrophoretic mobility. Mass spectrometry determines intrinsically absolute values, namely molecular mass, which are not subject to relative fluctuation.

SCHEMA OF THE ANALYTICAL PROCESS:

Specific fragments of isolated genomic DNA are amplified by PCR. The double stranded PCR product is then bound to a solid phase (paramagnetic beads) and subsequently denaturated. A specific primer is annealed to the single strand and extended in a so-called "Primer Oligo Base Extension (PROBE)" reaction, whereby the DNA polymerase is admixed with a dideoxynucleotide and three deoxynucleotides. The extension of the PROBE primer will terminate after the addition of the dideoxynucleotide. Since the sequence of the fragment to be analyzed is known, the PROBE primer and the combination of nucleotides are compared with the expected results. This will result in a specific product, depending upon the genotype and/or allele. The masses of all potential products are calculated (so-called "look-up tables") and compared with the values measured. Each Genotype correlates with one of the calculated masses and can therefore be specifically allocated.

Sequenom has applied this process successfully in a number of different analyses:

- Analysis of single nucleotide polymorphisms (SNPs); for example: SNPs in the SOX9 and GPDa genes
- Analysis of different mutations;
  an example is the human hemoglobin, where one PROBE Primer in one reaction permits the differentiation between the wild type: HbA, HbS, a two-base deletion, and Thal Cod5 DCT.
- Analysis of microsatellites; different microsatellites were analyzed: dinucleotide repeats, trinucleotide repeats and tetranucleotide repeats. The number of repeats is determined by comparing the measured masses with calculated values. Also incomplete repeats such as 9.3 of TH01 are identified.
- Mixtures of male/female DNA can be identified by analysis of the Amelogenin gene, even with a ratio of 1:25.

Sequenom has developed an Automated Process Line (APL) for the analysis of large numbers of samples. The APL consists of three functional modules:

In the first module, PCRs are set up by distributing genomic DNAs plus a master mix (buffer, primer, and enzyme) with a pipetting robot in the wells of a microtiter plate. This pre-PCR unit is physically separated from the two other modules of the line, i.e. the PCR module and the post-PCR module. Air filters prevent PCR products from entering this area. A robot running on a rail transports the microtiter plates to the APL instruments. Following the combination of the PCR components, the microtiter plate is sealed with a lid and set into the cycler. Following amplification, the robot

moves the plate to the lid park station where the lid is removed. Beads are placed into each well of the microtiter plate by another pipetting robot, and the plate is incubated in a shaker to bind the PCR products. The bound products are then washed at a 96-channel pipetting robot equipped with automatic magnets. This pipetting robot also washes out residual salt in the PROBE products. All other solutions required for the PROBE reactions are added by another 96-channel pipetting robot. The PROBE is then incubated in a cycler. It is possible to analyze simultaneously 96 different DNAs in one microtiter plate, or 96 different analyses with one and the same DNA can be conducted, or any other combination. The specific products are removed from the template and transferred to the wells in a chip by a nanoliter pipetting robot. The chip is then inserted in the mass spectrometer for automatic analysis.