Determination of PCR Products by Capillary Electrophoresis with Contactless Conductivity Detection

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Keywords: Capillary electrophoresis · Contactless conductivity detection · PCR

The detection of polymerase chain reaction (PCR) products is widely used in DNA analysis for such diverse tasks as the determination of paternity, genetic fingerprinting in forensics, identification of pathogens, diagnosis of genetic diseases, recognition of banned genetically modified food items or the identity of meat samples. The standard method for this analysis is planar gel electrophoresis in which the DNA fragments are separated by their size. This method is well established, but it is hard to automate, time consuming, and requires staining for visualization on the plate. Data processing requires awkward optical scanning of the plate.

Capillary electrophoresis (CE) is an alternative which allows faster separations and the direct electronic acquisition of electropherograms with characteristic peaks whose areas are directly related to the amount of the species in question. The method is relatively simple as only the application of a high voltage is required and high pressure pumps as in column chromatography are not needed. Contactless conductivity detection is a fully electronic universal technique which is much easier to implement than optical detection on the narrow capillaries needed in electrophoresis which have inner diameters of typically 50 µm. The electrodes are placed on the outside of the capillary and thus cannot deteriorate by contact with solutions. The conductivity measurement of the solution inside the tubing is enabled by capacitive coupling of an AC-voltage into the capillary and the similar coupling of the resulting current out of the capillary on a pair of tubular electrodes. The term C^4D, for capacitively coupled contactless conductivity detection, has become widely accepted for this technique. Due to the low power requirements it also possible to construct complete portable CE-C^4D instruments which can be run from batteries.

As an example of the application, the identification of genetically modified soybean (Roundup Ready), which is banned from importation into Switzerland, was carried out. PCR primers were designed to yield a fragment of 400 base-pairs for the GM-soybeans.

The method allows the relatively fast analysis of PCR products yielding the results in a standard electropherogram on a simple and inexpensive instrument.

References


Electropherograms obtained by CE-C^4D for the PCR-product of the Roundup Ready soybean (a) and a mixture of mass standards (ladder) (b), shown together with a picture of the stained plate for the separations carried out by conventional planar gel electrophoresis.

Received: April 16, 2013

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