

**PROCESS AND DEVICE FOR THE
SEPARATION AND DETECTION OF
COMPONENTS OF A MIXTURE OF
MATERIALS BY TEMPERATURE
GRADIENT GEL ELECTROPHORESIS**

This application is a continuation of U.S. application Ser. No. 08/200,551, filed Feb. 22, 1994, abandoned, which is a continuation of U.S. application Ser. No. 07/847,761, filed Feb. 19, 1992, abandoned, which is a continuation-in-part of PCT Application No. PCT/EP90/01366, filed Aug. 18, 1990, abandoned.

FIELD OF THE INVENTION

Subject matter of the present invention is a process for the separation and detection of components of a mixture of materials by temperature gradient gel electrophoresis, in particular, a process for the detection of mutations of nucleic acid fragments by analysis of the heteroduplex obtained by hybridization of the nucleic acid fragment having the mutation (mutant) and the nucleic acid fragment not having the mutation (wild type), a process for sample preparation using an oligonucleotide as well as this oligonucleotide itself, and a device for performing the temperature gradient gel electrophoresis.

BACKGROUND OF THE INVENTION

Detection of mutations in genetic material or detection of phenotypical consequences of genetic mutation is an important analytical object in many fields of biological research, applied medicine, biotechnical production and criminology. On a genetic level, mutation means exchange of at least one nucleotide or base pair on the DNA or RNA level. The potential of the so-called "genetic engineering" using hybridization or sequencing techniques allows for the detection of a mutation in clonal DNA or RNA. However, these techniques are restricted to research-related use. For routine employment, a technical standard capable of standing comparison with immunological methods (ELISA, etc.), could not be achieved.

Temperature gradient gel electrophoresis, as described in German Application DE-OS 36 22 591, is a method for detecting slight structural differences or peculiarities of biological macro-molecules such as nucleic acids or proteins. However, this technique is not suitable for automatable analysis, as is necessary, for example, in the determination of many individual samples in the clinical field for the analysis of genetic diseases or in forensic analytics. Using temperature gradient gel electrophoresis technology, it was possible to make mutations visible without tedious differential hybridization (Riesner et al. (1989), *Electrophoresis* 10, p. 377-389); this technique, however, is restricted to the operation of flat-bed gel electrophoresis for research analytics. Similar results are obtained with the combination of flat-bed gel electrophoresis and a denaturing chemical gradient which, however, can hardly be formed in reproducible fashion and, thus, is out of question for automatization.

The necessary precondition for the sensitive detection of even single mutations in temperature gradient gel electrophoresis is a homogeneous temperature level within the gel perpendicular to the electrophoresis flow direction, i.e., at points of equal electrical potential. For instance, this cannot be realized sufficiently by using double-side thermostatted vertical electrophoresis according to D. R. Thatcher and B. Hodson (1981), *Biochemistry* 197, p. 105-109, since the thermostat plates in opposition to each other which are

insufficiently thermally connected do not have identical temperatures at points of equal electrical potential.

The appearance of living nature is programmed on a genetic level in the form of nucleic acids consisting either of RNA or DNA chain molecules. Changes of genetic information are referred to as mutations and are the basis for evolutionary developments, for genetically caused diseases, and other genetically caused biological properties of a virus or an organism. Most of the mutations having occurred do not have notable consequences for the system. Such mutations are referred to as neutral. Once gene-technological techniques had been introduced, it has become possible to discover a mutation, to determine when it was created, and to measure its influence on a biological function.

Using the technique of comparative sequence analysis (sequencing) of homologous sequences, a mutation can be recognized. The term mutation is understood to be a single nucleotide exchange, a deletion, or an insertion of single up to many nucleotides, or a rearrangement of chain segments. In spite of great progress in recent years, sequence analysis still is a costly technique, supported by expensive equipment, and not suited for routine analysis. Merely the search for per se known mutations described for certain genetic diseases, for example, α -1-antitrypsin deficiency (Kidd, U. J., Wallace, R. B., Hakura, K., and Woo, S. L. C. (1983), *Nature* 304, 230-234), has been technically simplified by the use of synthetic oligonucleotide probes. A number of questions, however, are nearly completely reluctant to experimental access, such as, for example, the search for unknown mutations in long gene segments not being associated with restriction fragment length polymorphisms (RFLP), or routine examinations important to medical genetics, population analysis, evolutionary relationship analysis, analysis of virus variants, etc.

Nucleic acid chains (RNA and DNA) are capable of forming double-helical structures with so-called complementary sequences, resulting in DNA/DNA, RNA/RNA, and DNA/RNA double-stranded structures. A characteristic feature of these structures is temperature-dependent denaturation (melting) of the double-strands. Melting occurs within a very narrow temperature interval, i.e., large sections of the double-stranded structures are denatured in a single-step process. Thus, it is a physical reaction taking its course in a highly cooperative manner. Loss of continuous double-stranded structure becomes manifest in mobility change (in most cases loss of mobility) of the nucleic acid concerned. Such mobility loss may be used in an electrophoretic separation process, to separate nucleic acids having different melting temperatures. Thus, thermodynamically less stable nucleic acids migrate more slowly and, therefore, less far than those having stable structures. Here, the medium used for separation must have a denaturation gradient, for instance by increasing concentration of a denaturing agent. Stability of the internal regions of base pairs depends on G/C content and on the sequence. These effects have been studied in detail (Meinkoth, S. and Wahl, G. (1984), *Analytical Biochem.* 138, 267-284).

Now, if mutation leads to sufficient changes in the according region, the mutated nucleic acid will exhibit different melting behavior than the non-mutated one. Frequently, a mutation is characterized by the exchange of only one base pair for another (transversion or transition). Therefore, the mutated nucleic acid strand is quite stable in itself and, in general, melts at similar temperatures as does the non-mutated form, so that discrimination is not possible. Such mutations, however, become visible by mixing the mutated nucleic acid and a nucleic acid not having this mutation