

large fragment **70** has not yet reached the temperature of beginning denaturation. According to the invention, the high temperature T_2 is adjusted such that separation of the most stable double-helix section or the G:C clamp does not yet occur. During electrophoresis the temperature T_1 , however, is adjusted from low temperature to steadily increasing temperature, at maximum until $T_1=T_2$ is reached. In this way, each molecule passes the temperature gradient, regardless of its migration velocity or size.

b) Renaturation experiments as schematically described in FIGS. **16b** and **16c** provide sharp band signals only in cases where not merely a spatial temperature gradient alone is passed. Indeed, in this case an undesired effect of band broadening occurs in foldback which is all the more disturbing, the steeper the course of the denaturation curve. In this case, the front of the band will be strongly accelerated with respect to the end, since lower temperatures are present behind. As a result, the band is blurred. On the contrary, this effect is very desirable where, vice versa, the band front in migration direction migrates relatively at higher temperatures. According to the invention, this may be achieved also with renaturation experiments by combining space and time gradients. Instead of renaturation in a linear T gradient as conducted, e.g., using a device according to FIG. **1**, the sample may be subjected to electrophoresis and analyzed in a relatively increasing gradient between T_1 and T_2 (FIG. **10**) within the time interval t_0 to t_3 (FIGS. **16b** and **c**), both temperatures, however, being jointly decreased (FIG. **16b**), or only T_1 is decreased (FIG. **16c**) to an extent that the sample having shorter running time experiences lower temperature, the band front, however, always has higher temperature than its rear side. In this way, bands grow sharper according to the invention.

The process according to the invention and the device of the invention may be used for analytical detection and quantitative detection as well as for preparation of components from material mixtures. Analysis and preparation may be performed simultaneously with many samples; detection and evaluation may be effected automatically. In particular, process and device are suited for preparation and analysis of viroids, viral nucleic acids, satellite RNA, for analysis of mutations in nucleic acids, for analysis of mutations in proteins, and for analysis of protein nucleic acid complexes.

Temperature gradient gel electrophoresis proves particularly advantageous for the preparation of variants, since direct sequencing of variants is possible without prior cloning. This is permitted by elution of exceedingly small amounts of a specific variant subjected to enzymatic amplification and subsequent sequencing. Such modes of operation will gain importance in future, since working with vectors otherwise used for amplification, and working with recombinant organisms is hampered by safety injunctions.

We claim:

1. A process for the quantitative and qualitative detection of mutants or specific gene sequences comprising the steps of:

- a) adding, to a mixture of different nucleotide sequences, in which one of the different nucleotide sequences has a known concentration, a marker-carrying nucleic acid sequence, the sequence of which is identical to one of the nucleotide sequences, in an amount less than said

known concentration, wherein the marker can emit a detectable signal;

- b) subjecting the mixture to at least one denaturation/renaturation cycle to obtain hybrids of the different nucleotide sequences with the marker-carrying nucleic acid sequence;

- c) analyzing the mixture to detect a mutant or a specific gene sequence by separating nucleic acids in the mixture, using (i) time-controlled temperature gradient gel electrophoresis, wherein the temperature gradient is built up by varying the temperature with time, or (ii) a combination of the time-controlled temperature gradient gel electrophoresis and spatial-controlled temperature gradient gel electrophoresis and examining the separated nucleic acids for the marker signal and measuring relative intensities of detected signals.

2. The process according to claim **1**, wherein the marker-carrying nucleic acid sequence hybridizes to the nucleotide sequence having a known concentration.

3. The process according to claim **1**, wherein the mixture of different nucleotide sequences has been obtained by enzymatic amplification.

4. The process according to claim **1** using the combination of time-controlled and spatial-controlled temperature gradient gel electrophoresis, wherein the temperature gradient is oriented perpendicularly to the direction of the electrical field.

5. The process according to claim **1** using the combination of time-controlled and spatial-controlled temperature gradient gel electrophoresis, wherein the temperature gradient is oriented parallel to the direction of the electrical field.

6. The process according to claim **1**, wherein one of the different nucleotide sequences is a mutation-carrying nucleotide sequence, which forms a heteroduplex with the marker-carrying nucleic acid sequence such that the mutation is located in a thermodynamically unstable region of the heteroduplex.

7. The process according to claim **5**, wherein the time-controlled temperature gradient sets off, at the cathode side of the electrical field, from a temperature level higher than the melting point of the thermodynamically unstable region of the heteroduplex and is time-controlled in the direction of decreasing temperature toward the anode side of the electrical field.

8. The process according to claim **1**, wherein the spatial-controlled temperature gradient is oriented perpendicularly to the direction of the electrical field.

9. The process according to claim **1**, wherein the spatial-controlled temperature gradient is oriented parallel to the direction of the electrical field.

10. A process according to claim **1**, wherein the anode side temperature level of the spatial-controlled temperature gradient is higher than the temperature level at the cathode side.

11. A process according to claim **1**, wherein the cathode side temperature level of the spatial-controlled temperature gradient is higher than the temperature level at the anode side.

12. A process according to claim **1**, wherein the sequence having a known concentration is the mutation-bearing sequence.

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