DGGE

A very sensitive technique for searching for mutations is electrophoresis in a gradient denaturing gel (**denaturing gradient gel electrophoresis**, **DGGE**). It uses the fact that the rate of DNA denaturation depends on the number of hydrogen bonds. DNA strands will separate more easily at sites rich in AT pairs, while sections rich in CGs will be more stable. A polyacrylamide gel with a gradually increasing concentration of denaturing substances (formamid and urea) are used for electroforesis. The examined DNA travels in the electric field at a speed corresponding to its molecular weight, until the moment when the two strands begin to separate from each other. The denatured strands travel much slower during electrophoresis, so the easier a certain section is denatured, the closer to the start the DNA sample stops. Since completely separated ssDNA would create fuzzy bands, primers with a so-called CG-clamp are used for DNA amplification. PCR product then contains double helices that have only CG pairs at one end; at this point the chains are not easily denatured. The sensitivity of DGGE is close to 100%.

TGGE

A similar technique is **TGGE** (**temperature gradient gel electrophoresis**). Instead of a gel with a gradually increasing concentration of denaturing substances, gradually increasing gel temperatures are used.

Kategorie:Molekulární biologie Kategorie:Biochemie Kategorie:Genetika Kategorie:Základy DNA diagnostiky