Restriction fragment length polymorphism

One of the oldest and still most widely used techniques in DNA diagnostics is undoubtedly **restriction analysis** (restriction fragment length polymorphism, **RFLP**). It utilizes bacterial endonucleases (restricases), which can cleave DNA if it contains a certain precisely defined sequence of nucleotides. Bacteria use these enzymes to defend themselves against virus infection: viral DNA can be easily cleaved, unlike its own nucleic acid, which is protected from degradation by methylation.

The stretch of DNA that will be recognized and cleaved by the endonuclease is usually only a few base pairs long and is often a palindromic sequence. This allows it to create a loop that is easier for the enzyme to find and cut. If one allele of a particular gene contains a recognition sequence while the other does not, the DNA of the first allele will be cleaved, while the DNA of the second allele will remain intact. During electrophoresis of the fragments, we find two shorter sections for the cleaved sequence, whereas the uncleaved DNA forms only one strip in the gel corresponding to the longer sequence of the original length.

An example can be the endonuclease KpnI. Its source is the bacterium *Klebsiella pneumoniae OK8*. The sequence that this endonuclease can recognize is GGTACC (note that this is a palindrome), breaking both strands of the double helix between cytosines (ie the DNA double helix will have a "bumpy end" when cleaved):

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5´- G G T A C|C -3´
3´- C|C A T G G -5´
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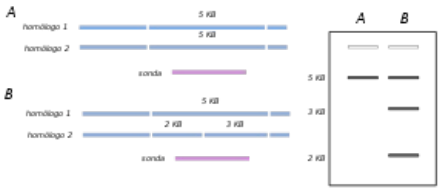
In restriction enzyme tables, it will usually be characterized by the notation GGTAC^C.



The endonuclease did not cleave a single allele in A, while one allele was cleaved in B. On electrophoresis, the restriction segments appear as two new lines.

Individual endonucleases differ in the conditions under which they work optimally. Many of them work at higher temperatures, in solutions with a higher salt content, etc. E.g. TaiI is an endonuclease isolated from the thermophilic bacterium *Thermus aquaticus Cc1-331*. Its recognition sequence is ACGT^ (again a palindrome). It requires a temperature of around 65°C and a buffer with a higher ionic strength to work. Information about individual restriction endonucleases, microorganisms from which they were isolated, target sequences, etc. is collected by REBASE (http://rebase.neb.com/rebase/rebase.html) (The Restriction Enzyme Database).

External links

- RFLP at NCBI (https://www.ncbi.nlm.nih.gov/probe/docs/techrflp/)
- RFLP on the Genetics - Biology website (http://www.genetika-biologie.cz/rflp)