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Restriction Enzymes

Restriction enzymes are identified by three-letter abbreviations for the parent organism (for example, Hin for Haemophilus influenzae or Bam for Bacillus amyloliquefaciens).

From: Insect Molecular Genetics (Third Edition), 2013

Related terms:

Agarose, Plasmid, Restriction Enzyme, Nested Gene, Methylation, Cloning, Mutation, Digestion

Restriction Endonucleases and Modification Methylases

Hyone-Myong Eun, in Enzymology Primer for Recombinant DNA Technology, 1996

iv. DNA Conformation.

Restriction enzymes are not only sequence specific but also structure sensitive and may exhibit either enhanced or inhibited cleavage activity. The restriction sites located in nuclease SI-sensitive, conformationally flexible junctions, for example, between contiguous B-DNA and Z-DNA or in non-B-DNA conformation, are resistant to cleavage by many restriction enzymes such as *Bam*HI, *Eco*RI, *Hind*III, and *Pst*I (6, 7). The 5′-GCGC-3′ site of *Hha*I is not cleaved when the sequence is within the stretch of alternating GC sequences that adopt a Z-DNA conformation (8). In contrast, *Mbo*I (GATC) has an enhanced cleavage reactivity at the B-Z junction (9). Modulation of DNA conformation by DNA intercalating drugs, oligonucleotides, or oligonucleotide analogs thus provides an efficient way for achieving controlled cleavages by restriction enzymes (see below).

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Neurobiology of Steroids

Michael Karl, ... George P. Chrousos, in Methods in Neurosciences, 1994

Restriction Enzyme Analysis

Restriction enzymes are enzymes that bind to specific recognition sequences to cleave double-stranded DNA (38). Mutations creating or abolishing such recognition sites can, therefore, be investigated by employment of restriction enzymes. Once a change in a recognition site has been identified in the DNA sequence of a patient, specific PCR-amplified fragments of the DNA obtained from relatives or controls, potentially harboring the base change, are incubated with the specific restriction enzyme. The cut or uncut segments can be visualized by gel electrophoresis to determine whether an individual is a carrier of such a mutation or bears the wild-type sequence. Unfortunately, only a small number of mutations can be identified by this technique.

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Techniques for Nucleic Acid Engineering

Şükrü Tüzmen, ... Candan Hızel, in Omics Technologies and Bio-Engineering, 2018

14.2.2 Where Are They Found?

Restriction enzymes (endonucleases) are “Molecular scissors” that are found in and harvested from bacteria and archaea, which cut DNA strands at predetermined locations on DNA. The importance of these enzymes resides in the fact that they do not cut a strand of DNA arbitrarily. Preferably, they recognize a unique pattern of bases and then excise only at that specific site. Bacteria and archaea use restriction enzymes as part of their defense mechanisms to cut up the invading DNA of bacteriophages (Arber and Linn, 1969; Krüger and Bickle, 1983). Bacteriophages can be described as viruses that infect bacteria and use bacterial DNA to reproduce their own. As part of bacterial defense system, restriction enzymes cut (digest) any foreign DNA they encounter, by binding at their unique recognition sites. DNA that has been cut with these enzymes becomes inoperative (Ausubel et al., 1998; New England Biolabs Inc., 2014). Meanwhile, the host DNA is safeguarded by a modification enzyme (a methylase), which alter the bacterial DNA, and prevent it

from being cleaved. Considering that the nucleotide sequences recognized by the restriction enzymes are very short, usually varying between 4 and 8 nucleotides, the bacterium itself undoubtedly has plenty of these nucleotide sequences existing in its own DNA. Consequently, to stop destruction of its own DNA by its own restriction enzymes, the bacterium label its own DNA by attaching methyl groups (CH_3) to its cytosine or adenine nucleotides (Capuano et al., 2014). This alteration should not obstruct the DNA base-pairing, for that reason, generally only a few specific nucleotides are modified on each DNA strand (Kobayashi, 2001). There exists over 3000 restriction enzymes that are studied in detail thus far, and more than 600 of these are commercially available (Roberts et al., 2007). Each enzyme is named after the bacterium from which the enzyme is isolated. The number that comes after the letters represents the order in which the enzyme is isolated. For example, EcoR1 restriction endonuclease was the first restriction enzyme to be isolated from the bacteria *Escherichia coli* (Table 14.4). Similarly, HindIII is the third enzyme isolated from *Haemophilus influenza* bacteria (Smith and Nathans, 1973; Ausubel et al., 1998).

Table 14.4. Derivation of EcoRI Name (Smith and Nathans, 1973)

Abbreviation	Meaning	Description
E	*Escherichia*	Genus
co	*coli*	Specific epithet
R	RY13	Strain
I	First identified	Order of identification
		In the bacterium

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General Considerations

Gregory A. Denomme PhD, ... Marion E. Reid PhD, in Molecular Protocols in Transfusion Medicine, 2000

2.2.10 Restriction Enzymes

Restriction enzymes are endonucleases, that is, enzymes that digest nucleic acids. Restriction enzymes recognize specific sequences of nucleotides in a DNA strand. Their use allows the detection of point mutations in DNA and eliminates the need for subcloning and sequencing. For instance, the *Alu* I restriction enzyme recognizes and cleaves the DNA sequence AGCT. If a DNA segment containing the AGCT sequence is subjected to *Alu* I treatment, it will be cleaved into two fragments. These fragments can be visualized after electrophoresis in agarose or acrylamide gel. If any one of the four nucleotides is replaced (e.g., GGCT or any other combination) the enzyme will no longer recognize the site and will not digest the DNA. There are enzymes originating from different sources (therefore different enzymes) that cleave within the same target sequence. These enzymes are known as iso-schizomers.

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Molecular Anatomic Pathology

Marina N. Nikiforova, Yuri E. Nikiforov, in Diagnostic Immunohistochemistry (Third Edition), 2011

Restriction Fragment Length Polymorphism Analysis

Restriction enzymes (restriction endonucleases) are enzymes that cut DNA at specific nucleotide sequences known as *restriction sites*. The restriction sites are usually 4 to 8 nt long and are palindromic (i.e., the DNA sequences are the same in both directions). Restriction fragment length polymorphism (RFLP) analysis exploits the ability of restriction enzymes to cut DNA at these specific sites. If a DNA sequence variation such as a point mutation alters (creates or destroys) the restriction site for a specific enzyme, it will change the size of the PCR product. This can be detected by gel electrophoresis.

RFLP is frequently used to detect known point mutations or single nucleotide polymorphisms (SNP).[14] It can also be used to separate two amplified sequences that are highly similar in nucleotide composition. Figure 2.6 illustrates the usage of PCR-RFLP to differentiate between *SYT/SSX1* and *SYT/SSX2* rearrangements, which are common in synovial sarcomas.

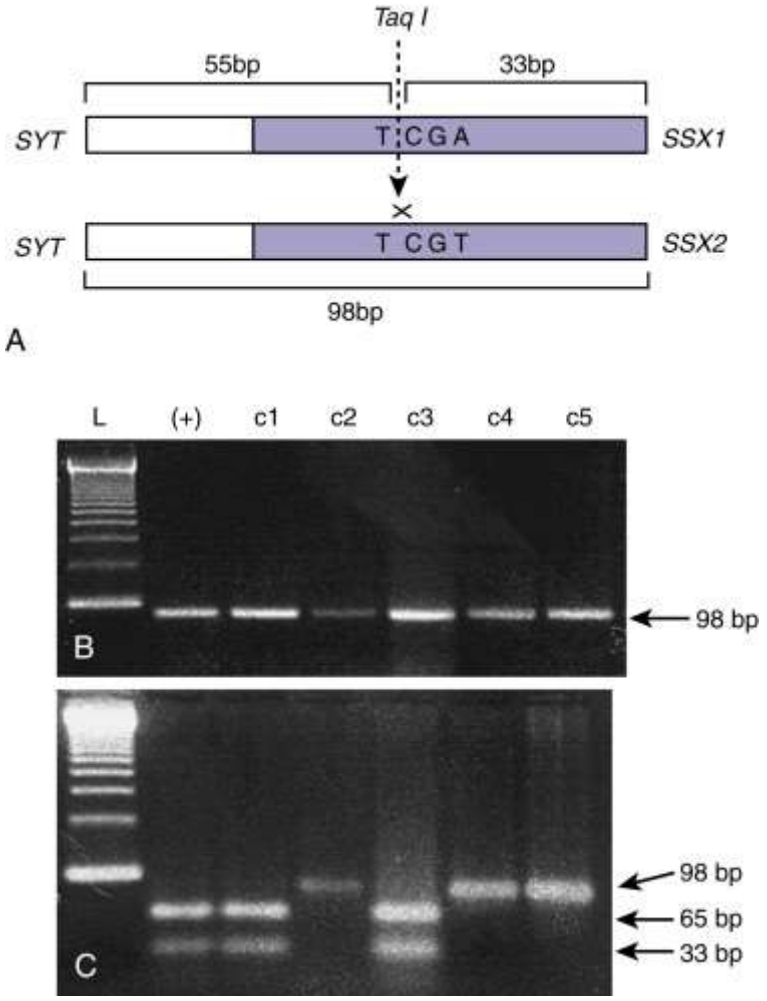

FIGURE 2.6. PCR-RFLP detection of *SYT/SSX1* and *SYT/SSX2* rearrangements in synovial sarcoma. **(A)** *Taq I* restriction enzyme cuts the *SYT/SSX1* fusion DNA into two fragments, 55 bp and 33 bp long. The *SYT/SSX2* fragment remains uncut, 98 bp in size. **(B)** Five synovial sarcoma DNA samples (c1 to c5) are PCR amplified with primers complementary to both *SYT/SSX1* and *SYT/SSX2* rearrangement types and reveal a 98-bp amplification band in the agarose gel. **(C)** After digestion with *Taq I,* the PCR products from tumors c1 and c3, as well as from the *SYT/SSX1* positive control (+), are cut into two fragments, indicating the presence of *SYT/SSX1* rearrangement, whereas the rest of the tumor samples remain uncut, consistent with *SYT/SSX2*.

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Characterization of Nucleic Acids and Proteins

Chang-Hui Shen, in Diagnostic Molecular Biology, 2019

Different Sources of Type II Restriction Endonucleases

Restriction enzymes isolated from different bacteria may recognize and cut DNA at the same site. This type of enzymes is known as isoschizomers. Typical examples of isoschizomers are *Bsp*EI from a *Bacillus* species and *Acc*III from *Acinetobacter calcoaceticus*. They both bind the same DNA sequence and cut at the same sites. On the other hand, some restriction enzymes recognize and bind to the same sequence of DNA but cleave at different positions, producing different single-stranded extensions. These are neoschizomers, such as *Nar*I from *Nocardia* argentinensis and *Sfo*I from *Serratia* fonticola. They bind the same DNA sequence but cut at different sites. Isocaudomers are restriction endonucleases that produce the same nucleotide extensions but have different recognition sites. The examples are *Nco*I from *Nocardia corallina* and *Pag*I from *Pseudomonas alcaligenes*. They bind different DNA sequences but produce the same sticky ends. In some cases, a restriction endonuclease will cleave a sequence only if one of the nucleotides in the recognition site is methylated.

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Approaches to Detecting DNA Base Modification in the Brain

X. Li, W. Wei, in DNA Modifications in the Brain, 2017

Restriction Enzymes for DNA Modifications

Restriction enzymes are one of the easiest approaches to detect modified DNA at specific genomic sites. Cleavage of DNA by a restriction enzyme may be blocked or impaired when a particular base in the recognition site is modified. For example, MspI and HpaII recognize the same sequence (CCGG); however, they are sensitive to different modification status: when the external C in the sequence CCGG is methylated, MspI and HpaII cannot cleave. Unlike HpaII, MspI can cleave the sequence when the internal C residue is methylated (Bird & Southern, 1978). Another enzyme, Pvurts1I, only cleave the sequence $^{hm}CN_{11–12}/N_{9–10}G$, which contains 5hmC (Asgar Abbas, Monika, Honorata, & Matthias, 2014; Evelina &

Giedrius, 2014; Sun et al., 2015). The combination of DpnI and DpnII is use to detect m6dA; both recognize the consensus sequence GATC, but only DpnI will cleave at this site if the adenine is methylated (Fu et al., 2015; Greer et al., 2015; Heyn & Esteller, 2015; Ratel, Ravanat, Berger, & Wion, 2006). Thus, using different restriction enzymes, we could detect DNA modification beyond familiar cytosine, including 5mC and 5hmC. In addition, this approach is cost-effective and fast; however, it is still limited by the number and distribution of restriction sites in the genome. Dai et al. (2002) found that a maximum of 4100 sites can be accessed by the restriction enzymes known to be DNA modification sensitive, and specific sites of interest that are not located at restriction sites cannot be investigated using this method.

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FGF Mutants

Xiaokun Li, ... Zhiyong Sheng, in Fibroblast Growth Factors, 2018

2.1 Reagent

Restriction enzymes *Nde*I, *BamH*I, and *Bgl*II were purchased from NEB Company (USA); *Pyrobest* DNA polymerase was from Dalian Takara Company (China); PCR puriWcation kit, gel extraction kit, and plasmid miniprep kit were obtained from Shanghai Biocolors Company (China); CM–Sepharose and heparin–Sepharose were from Pharmacia (USA); The plasmid pUC-haFGF, expression vector pET3c, and *Escherichia coli* strain BL21(DE3) were kept by Biopharmaceutical Research and Development Center of Jinan University; Primers were synthesized by Shanghai Sangon Company (China).

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URL: https://www.sciencedirect.com/science/article/pii/B9780128161425000035

Molecular and Cytogenetic Analysis

Letizia Foroni, ... Sandra Hing, in Dacie and Lewis Practical Haematology (Twelfth Edition), 2017

Principle

Restriction enzymes (REs) cleave DNA at short specific sequences. Because many REs are available, it is not uncommon for a single point mutation to coincidentally

create or destroy an RE recognition sequence. If this is the case, digestion of the appropriate PCR product prior to agarose gel electrophoresis enables the mutation to be identified. A difference in the size of the restriction fragments seen in normal and mutant samples can be predicted from a restriction map of the amplified fragment and the site of the mutation that changes a restriction site. The observed fragments should be consistent with either the mutant or the normal pattern. An example of this technique is shown in Figure 8-2.

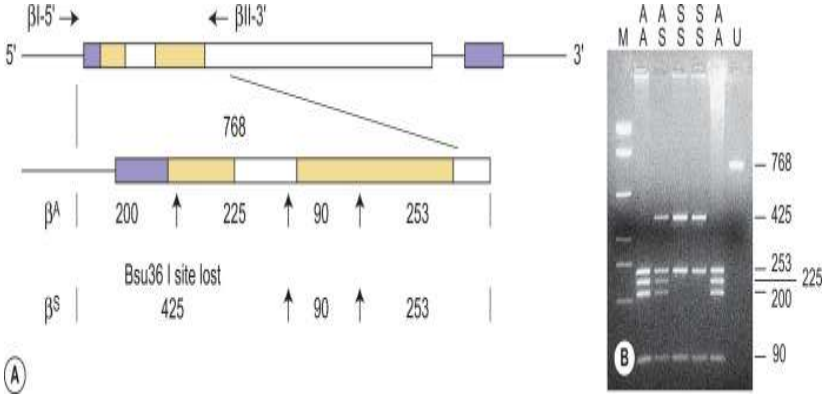

Figure 8-2. Detection of the sickle cell mutation using restriction enzyme analysis.

A, A sketch of the β globin gene shows the position of the primers used to amplify a 768 bp fragment in a polymerase chain reaction *(PCR)*. The sequence of β I–5′ is

5′ TAAGCCAGTGCCAGAAAGAGCC 3′ and that of β II-3′ is

5′ CATTCGTCTGTTTCCCATTCTA 3′. Maps of the Bsu36 I restriction sites and the fragment sizes from βA and βS genes are shown below. **B,** An ethidium bromide–stained minigel illustrates the fragment sizes generated by Bsu36 I digestion of the PCR product from normal *(A/A)*, sickle cell trait *(A/S)* and sickle cell anaemia *(S/S)* individuals, along with the undigested amplified fragment *(U)* and the molecular size marker *(M)*.

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Oxygen Sensing

Paul J. Kemp, ... Chris Peers, in Methods in Enzymology, 2004

Ligation of Cloned Channels into pcDNA3.1-TOPO

Restriction enzymes available for cutting the vector at the multiple cloning site are detailed in the manufacturer's instructions. The enzyme(s) of choice will be determined by the restriction sites available on both the vector and the DNA insert; a combination of *Not*I and *Hind*III is often suitable, but a restriction map of the particular insert must be generated before such experiments can be performed usefully. To prevent recircularization of digested plasmid DNA in the ligation reaction, the 5′ end phosphate groups are removed using calf intestinal alkaline phosphatase (New England Biolabs Inc., Hitchin, Herts., UK). Ligation is carried out using the using T4 DNA ligase (Promega, Southampton, Hants., UK) with a 1:3 vector:insert ratio.

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