## ➢ Restriction Endonucleases

Our genome is so large that it cannot go through the agarose gel pores, so it has to be simplified by fragmenting it into shorter, smaller fragments able to pass through the gel and migrate. DNA needs to be fragmented in a certain specific way; we do not want random fragmentation. This can be achieved by enzymes known as **restriction endonucleases**.

A **nuclease** is an enzyme that degrades (cleaves) nucleic acids, specifically in this case DNA. (*Endo* means inside or internal), so these endonucleases cut DNA within the DNA fragment. **Exonucleases**, on the other hand, are enzymes that cleave DNA from either end (cleaving the first nucleotide from either end).

They are called restriction endonucleases because they are restricted by where they make their cuts, meaning that they recognize specific sequences within the DNA and they cleave the DNA within these sequences by cutting the phosphodiester bond between nucleotides at these specific sequences.

The sequences which restriction endonucleases recognize are usually about 4 to 8 base-pairs long and are termed **restriction sites**. The fragments generated by restriction endonucleases are known as **restriction fragments**.

**Basically, restriction endonucleases recognize restriction sites cleaving DNA at these sites and generating restriction fragments.**

The enzyme EcoRI is a restriction endonuclease that was isolated from the bacteria *E. coli* which is famous for infecting rotten meat and can be fatal. This particular endonuclease recognizes the sequence GAATTC (and makes the cut between G and A) which means that wherever this sequence is located in DNA, EcoRI cleaves DNA at that spot. Restriction endonucleases can cut the same DNA strand at several locations generating multiple restriction fragments of different lengths.

## ➢ DNA Polymorphisms

Looking at the figure in **SLIDE 24** which demonstrates an example of adding restriction endonucleases to different alleles of a gene. Each allele generates a different number of restriction fragments when restriction endonucleases are added, this happens because of a difference in sequence between the two alleles in the middle of the strands, this difference removes a restriction site that is present in the middle of allele 1 from allele 2 thus resulting in the generation of one longer fragment in allele 2 rather than the two shorter ones as is the case for allele 1. This means that the difference between the alleles is only in the middle of the strand and the two outer fragments of the alleles are identical (the first fragment for allele 1 is identical in size to the first fragment of allele 2, and the

last fragment for allele 1 is identical in size to the last fragment of allele 2 meaning that they migrate at identical speeds in the gel and are present at the same band).

Depending on the alleles present and their sequences the following can be observed:

- If a person is **homozygous for allele 1**, adding restriction endonucleases results in 3 cuts and the generation of 4 fragments for each allele totaling four different restriction fragments.
- If a person is **homozygous for allele 2**, adding restriction endonucleases results in 2 cuts and the generation of 3 fragments for each allele totaling three different restriction fragments.
- If a person is **heterozygous**, adding restriction endonucleases results in 3 cuts on allele 1 and the generation of 4 fragments while resulting in 2 cuts on allele 2 and the generation of 3 fragments. For this person, as mentioned above, the difference between the alleles is only in the middle where allele 1 generates 2 short fragments while allele 2 generates one longer fragment. This makes for a total of five different restriction fragments.

Take a quick look at **SLIDE 27** for conformation of these observations. **Note that**: allele 1 here is allele B in SLIDE 27 and allele 2 here is allele A in SLIDE 27.

Individual variations in DNA sequence (genetic variants) may create or remove restriction-enzyme recognition sites (as demonstrated above), generating different restriction fragments. In the example above, the restriction site that was present in allele 1 but absent in allele 2 is called a **polymorphic restriction site**, which means that it is responsible for variations and differences in DNA sequences.

## ➢ Restriction Fragment Length Polymorphism (RFLP)

**RFLP** is a technique that relies on the presence of restriction endonuclease-generated DNA fragments of different lengths. Since individuals differ in DNA sequence, restriction endonucleases generate different restriction fragments of variable lengths among individuals. This is known as **Molecular Fingerprinting**. This can be detected by gel electrophoresis by itself or along with Southern blotting.

Look at **SLIDE 28**, notice how Southern blotting is used to detect these differences. Allele A generates a long fragment and a short fragment when cleaved by restriction endonucleases while allele B generates only one much longer fragment. The probe hybridizes to the specific area indicated. Notice that the hybridized fragment of allele B is longer the hybridized fragment of allele A which means that it migrates at a slower speed thus locates nearer to the top. Based on this, the following is observed:

A. In the DNA sample for a person **homozygous for allele A**, the probe hybridizes with the long fragment of allele A and **only one band is detected (low position)**

B. In the DNA fragment for a person **homozygous for allele B**, the probe hybridizes with the fragment of allele B and **only one band is detected (high position)**
C. In the DNA fragment of a **heterozygous** person, the probe hybridizes with *BOTH* fragments: the long fragment of allele A and the much longer fragment of allele B, thus **two bands are detected. (the low band of A and the high band of B)**

**RFLP** was used as diagnostic tools clinically. It is not used anymore since there are better techniques available specifically PCR. However, it was used to detect whether a person has a mutation or not, also to determine from which parent a specific allele was inherited. These are some examples:

### *I. Disease detection (Sickle cell anemia)*

*Sickle cell anemia* is caused by a mutation in one nucleotide in the globin gene that encodes for hemoglobin thus making it responsible for the mutation of hemoglobin. The location of this nucleotide happens to be within a restriction site, which enables the use of RFLP in its detection. Individuals can be:

- Homozygous for the normal alleles designated as **A**
- Heterozygous, carriers of one normal allele and one mutated allele designated as **AS**
- Homozygous for the mutated allele designated as **S**

Looking at **SLIDE 31**, in the normal allele, the probe hybridizes a fragment of length 1.15 Kb (Kilobase-pairs) but in the mutated allele, it hybridizes a fragment of length 1.35 Kb. The observations of SLIDE 28 (points A, B and C) also apply here.

### *II. Paternity Testing*

Done by taking a DNA sample from the father, a sample from the mother and samples from the children. Since DNA is inherited from the mother and the father, some of this DNA will be the same as the mother while some of it the same as the father which means that we should have a match. All DNA fragments in our bodies MUST come from our parents.

### *III. Forensics*

If we have a sample taken from a crime scene, we can use this sample to determine the culprit among the suspects because it will perfectly match only the sample from the culprit. However, the sample taken from the crime scene can be contaminated with bacterial DNA and other elements.