ing the gene of interest or the pathology of that gene. Instead diagnosis is based upon a neighboring DNA marker that can be used to follow the inheritance of a putative gene (GnRH) as it segregates through a family. A gene that is closely linked to the gene for GnRH on chromosome 8 is the gene for plasminogen activator, tissue (PLAT or tPA).[12] Non-coding sequences in (introns) and around (flanking) the gene for PLAT are known to be polymorphic. Differences in the nucleotide sequence for a given locus on homologous chromosomes create dimorphisms that are unique for an individual or within members of the same family. These DNA polymorphisms or markers are sometimes called alleles. To date, these alleles do not demonstrate a recognizable function like those alleles or genes coding for a protein, but they are transmitted in Mendelian fashion. In the family under study, the DNA of both parents are cut with a restriction enzyme and probed with the linked PLAT gene. On a Southern blot, each parent has two bands, namely, two polymorphisms or alleles. The parents are informative because their DNA contain two different bands, one at 10 kb and a lower-molecular-weight 5 kb band. They are heterozygotes for the tPA polymorphism. Since the tPA probe hybridized with this gene on chromosome 8, we can infer that one band is linked to one chromosome 8 and the other to its homologue. This benign variation in an intron or non-coding flanking sequence at this locus now enables us to follow the segregation of these two chromosome-8 homologues. Since each parent has two alleles, or is dimorphic for this sequence, they are said to be "informative." In order to be homozygous for GnRH deficiency, the affected child must have received one copy of the mutant gene from each parent. The only information we are lacking is whether the mutant gene is linked to and segregating with the 10 kb-RFLP or the 5 kb-RFLP. This linkage or "phase" can be determined by analyzing the DNA of an affected individual in the family. Southern analysis of the DNA of the affected adult female using the tPA probe reveals only the intense 10 kb band. This indicates that the mutant phenotype is segregating with the 10 kb band. Each parent has contributed one 10 kb allele to make the child homozygous for the deficiency of GnRH. This can be inferred without any knowledge of the precise mutation affecting the GnRH gene or even regulatory regions of the gene. The diagnosis and phase is further corroborated by similar analysis of the DNA of the other two children. One child is homozygous for the 5 kb whereas the other has received a 10 kb allele from one parent and 5 kb allele from the other parent. The former child is phenotypically and genotypically normal, whereas the latter is phenotypically normal but a carrier like her parents. It is apparent now that the use of RFLPs for diagnosis requires the DNA of at least one affected individual in the pedigree in order to establish phase. The latter is a strong argument for extracting and freezing DNA on everyone for future comparative studies and diagnosis. Prior to specific gene isolation RFLPs have been used for the diagnosis of Huntington's disease, factor VIII and IX deficiencies, some cases of β-thalassemia, cystic fibrosis, and phenylketonuria. There is a RFLP linked to the C4A complement gene on chromosome 6, which is closely linked to the gene for 21-OH. Potentially, this fragment or polymorphism could also be used to diagnose and follow the inheritance of the mutant gene for 21-OH. The versatility of DNA technology is enormous, since a disease like congenital adrenal hyperplesia (CAH) might potentially be diagnosed by any one of the three described techniques, digestion with specific restriction enzyme, ASO probing, or RFLP analysis.

It is good to point out that the same restriction fragment polymorphisms can be used to determine the parental origin of the extra chromosome in aneuploidic situations such as Trisomy 21. As more and more of these polymorphisms are recognized and RFLPs are identified to cover the entire human genome, every monogenic disorder is potentially diagnosable by this linkage technique.

The overall advantage of the technique of RFLPs is that a gene can be linked with one of its flanking DNA polymorphisms. In humans, with about $3 \times 10^9$ bp in the genome, calculations indicate there would be several million detectable differences in the DNA between any two individuals. This would appear to be more than sufficient DNA variation to be used to establish linkage of any defective gene to an RFLP. By following an RFLP that co-segregates with a mutant phenotype, it should be possible to recognize recessive carriers, the non-penetrant dominant carrier, and delayed-onset mutant phenotypes like Kallman's syndrome, which generally do not express themselves until adolescence. The ultimate goal is to map the human genome with a series of overlapping RFLPs or at the least no greater distance apart than 1 cM*. It has been estimated that 2500 markers would be needed to construct a 1 cM RFLP map. At that point every gene of interest in the genome would be linked to one of these polymorphic loci. The recent identification of simple sequence repeat polymorphisms distributed randomly throughout the genome has rapidly expanded the number of DNA markers available to track disease.

* 1 cM – 1 centiMorgan – a unit for expressing the relative distance between genes on a chromosome. One centiMorgan (1 cM) indicates that recombination frequency between that gene and another marker gene is 1% in meiosis.