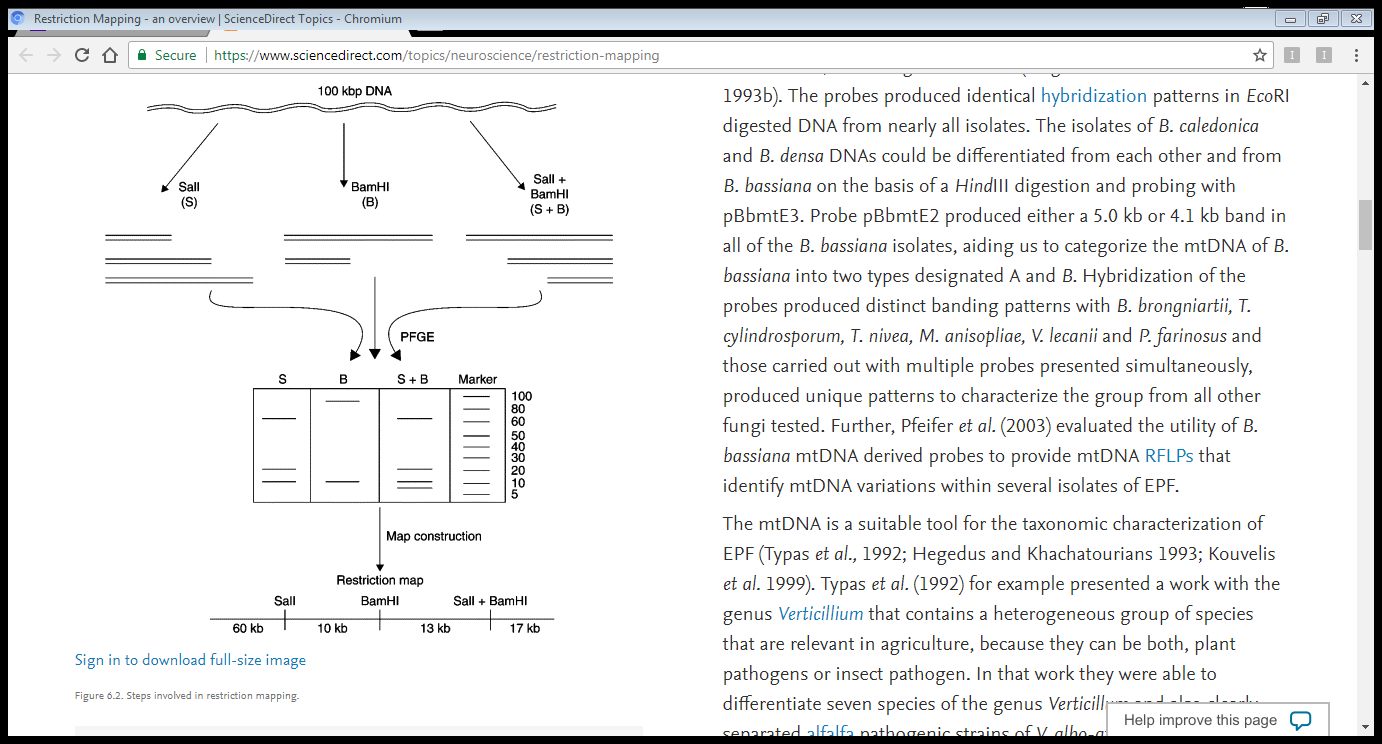
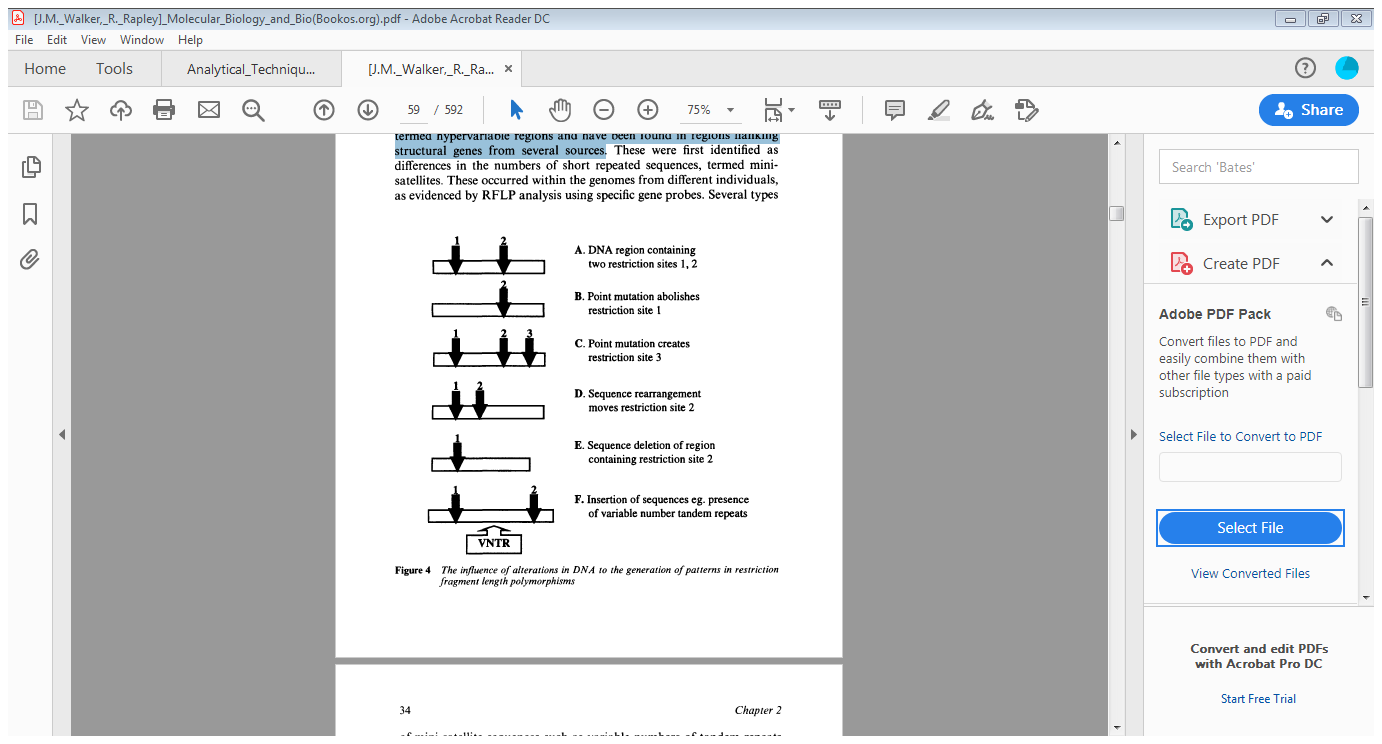
RESTRICTION MAPPING

Restriction mapping is a physical mapping technique which is used to determine the relative location of restriction sites on a DNA fragment to give a restriction map. Restriction enzymes are endonucleases that recognize specific sequences on DNA and make specific cuts. Restriction mapping involves the positioning of relative locations of restriction sites on a DNA fragment. Rare cutting enzymes are useful for genome mapping as they generate relatively less DNA fragments.

After digestion, DNA fragments are separated in agarose gel using a special technique called Pulse Field Gel Electrophoresis (PFGE). Using this technique, DNA fragments of up to 10 Mbp can be separated as against less than 40 kbp which can be separated using conventional agarose gel electrophoresis.

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Restriction mapping of the genome is useful only for smaller genomes such as viruses and bacteria. Comparison of the lengths of fragments obtained allows their relative positions within the DNA fragment to be deduced. Any mutation which creates, destroys or moves the recognition sequence for a restriction enzyme leads to a *restriction fragment polymorphism* (RFLP). An RFLP can be detected by examining the profile of restriction fragments generated during digestion.

Routine RFLP analysis of genomic DNA samples generally also involves hybridization with labelled gene probes to detect a specific gene fragment. The first useful RFLP was described for the detection of sickle cell anaemia. In this caseHhaI could be identified between DNA samples from normal individuals and patients with the disease. This polymorphism was later shown to be the result of a single base substitution in the gene for Q-globin which changed a codon, GAG, specific for the amino acid glutamine, to GTG, which encodes valine.

RFLPs may arise by a number of different means which alter the relative position of restriction endonuclease recognition sequences **.** In general most polymorphisms are randomly distributed throughout a genome, however there are certain regions where a particularly high concentration of polymophisms exist. These are termed hypervariable regions and have been found in regions flanking structural genes from several sources.

