

Abstract

DNA ladders are considered as one of the most essential tools in molecular biology laboratories. They are used to estimate the size of the DNA by comparing the amount of electrophoretic movement of the unknown fragment and the marker after electrophoresis. Typically, a DNA sample and DNA ladder are loaded in the agarose gel in adjacent wells. The DNA is separated in the gel electrophoresis and the size of sample fragments is determined by comparing the amount of movement with the known bands of DNA Ladder. The purpose of this study was comparison of polymerase chain reaction method and enzymatic digestion for the construction of DNA ladder. In PCR method, 10 primer pairs were designed for amplifying DNA fragments in the range between 100 to 1000 bp. The bacterial plasmid pCAMBIA 1305.2 was used as a template DNA in PCR. First, PCR products were purified using PCR purification kit, and then their absorption were analyzed in 260 nm wavelength. The PCR products were combined to obtain the DNA ladder and purified by PCR product purification kit and finally compared with a commercial marker. In the enzyme digestion method, 1000 bp fragment again was amplified using the same primer in PCR. The primers contained the cleavage site of *XbaI* and *HindIII* enzymes. The 1000 base pair fragment containing the *XbaI* and *HindIII* enzymes restriction sites was digested and then ligated to the digested pTZ57R/T plasmid. Finally, the recombinant plasmid was digested with *HindIII* and *XbaI* enzymes and compared with a commercial DNA marker. Therefore, the enzyme digestion method could be introduced as a fast and inexpensive method compared to the polymerase chain reaction method in order to construct a DNA ladder in molecular biology laboratories.

key words :DNA Ladder, Enzyme digestion, Cloning, Polymerase Chain Reaction



University of zabol

Graduate school

Faculty of science

Department of Biology

**The Thesis submitted for the Degree of M.Sc
(in the field of Genetic)**

Title:

Comparison of PCR and enzyme digestion methods in order to construct
DNA Ladder

Supervisor:

Dr. H. kamaladini

Dr. F. Haddadi

Advisor:

M. Seyedi

By:

Hosnieh Dadipour

May 2018