

LABORATORY EXERCISE #3. Detection of genetically modified food products by gel electrophoresis

Introduction

PCR products of differing lengths can be separated from each other based on size by an analytical method called gel electrophoresis. In this method charged macromolecules (such as DNA, RNA or proteins) migrate through a porous gel typically made from agarose (polysaccharide polymer derived from sea weed) or polyacrylamide when an electrical current is applied. The DNA sample is loaded into a well within the gel and placed near the anode (negative charge). As DNA has a very high negative charge due to the presence of the ionized phosphate groups on its backbone, it will migrate away from the negative anode toward the positively charged cathode as electrophoresis takes place. During the migration, smaller DNA fragments will pass through the gel pores more easily and thus migrate through the gel faster. Larger DNA molecules take longer to get through the pores and will migrate slower. The separated DNA "bands" or fragments can be visualized within the gel by staining with **ethidium bromide**. This dye intercalates (inserts) itself into the DNA and will fluoresces under ultraviolet light. In other words, DNA fragments will appear as fluorescent bands in the gel when exposed to UV light (Fig 1).

Concept check: If you compared two DNA fragments, one of 200-base pairs (bp) and the other of 1000 bp, which fragment will appear lower on the gel/have migrated faster?

Q: During gel electrophoresis, DNA is separated based on what two properties?

A: Size and charge

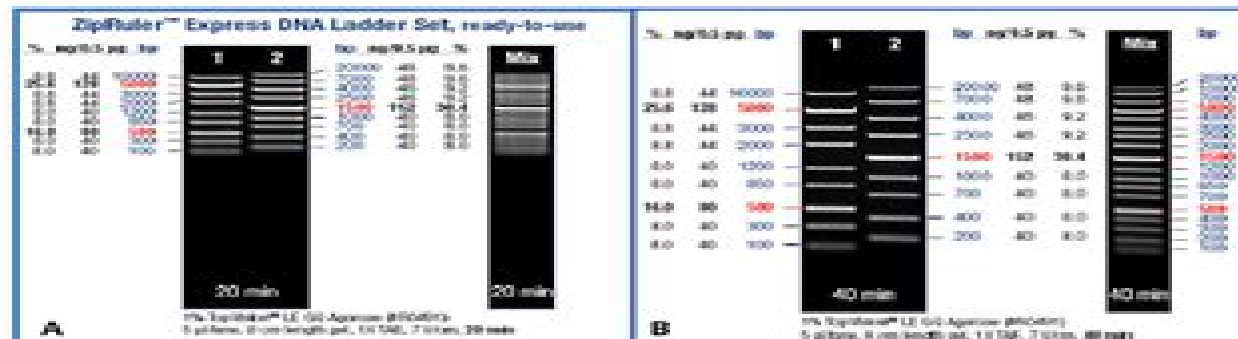


Figure 1. DNA fragments of known size are separated on an agarose gel for 20 min (A) or 40 min (B). By comparing the results, you can see how at 20 min, the DNA fragments are not well separated but by 40 min, the individually sized fragments are well separated and the size is easily determined. Figure adapted from Biogen.

Electrophoresis Laboratory Manual

RC Schank



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