Experiment 23	**Restriction Enzyme Analysis of plasmid-DNA**
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Reading	Chapters in BBOM 10th: 10.12 BBOM: Madigan M.T., J.M. Martinko and J. Parker: "Brock - Biology of Microorganisms", 10th Edition (2003), Prentice Hall.
Objectives	• Digesting plasmid-DNA. • Calculating fragment sizes of digested plasmid-DNA • Identifying different bacteria on the basis of their plasmid-DNA • Seperation of different DNA-fragments by gel electrophoresis
Background	The function of the restriction enzymes is to recognize certain sequences of DNA and cut the DNA. These enzymes have proven enormously valuable in many of the techniques of in vitro DNA manipulation. Indeed, the discovery of these enzymes was the beginning of what came to be called genetic engineering. A DNA molecule will be cut at specific sequences by a given restriction enzyme. Because the base sequences recognized by many restriction enzymes are four to six nucleotides long, there will generally be only a limited number of such sequences in piece of DNA. After cleaving the DNA, the fragments generated can be separated from each other by gel electrophoresis. Electrophoresis is the procedure by which charged molecules migrate in an electrical field, the rate of migration being determined by the charge on the molecule and by its size. Small or compact DNA molecules migrate more rapidly than larger DNA molecules. After a defined period of migration time the gel can be stained with a compound that binds to DNA. Because a given restriction enzyme always cuts at the same site, the banding pattern of a given DNA is reproducible and, using a standard, the size of the fragments can be determined.
Literature	The chapters of BBOM 10th mentioned above
www Links	• http://www.people.virginia.edu/~rjh9u/restdna1.html • http://www.erin.utoronto.ca/~w3bio/bio215/lecture03_2002/
Practical work	We will digest isolated plasmid-DNA (from experiment 22) We will seperate digested DNA by gel electrophoresis
Material and Methods	1. pipette in a microcentrifuge tube: 12µl H_2O 5µl DNA 2µl NEB buffer 1µl ECO RI

	2. centrifuge briefly 3. incubate the solution containing the digest at 37 °C for 30 min 4. add loading dye to digested DNA 5. load the electrophoresis gel with the digested DNA 6. Add an appropriate DNA size marker mixture to one or two wells of the gel 7. run separation 8. stain the gel in ethidium bromide bath
Experinces gained	• learning how to calculate fragment sizes of digested DNA • loading a electrophoresis gel • detect different bacteria groups with Restriction Enzyme Analysis
Lboratory Rules and Precautions	Use good laboratory practice! Do not contaminate yourself, others or the laboratory environment. All waste must be sterilized before disposal. It is necessary to work cautiously and, where necessary aseptically. Ethidium bromide is extremely toxic. Wear gloves and handle the solution and the gels only in the hood which is assigned for this procedure. Wash your hands before you leave the room and desinfect bench surfaces with 70% ethanol.
Timing	90 minutes
Reporting	Note the different fragment sizes of digested DNA and the number of different DNA types.
Questions to be answered	How many DNA fragments do you expect for a DNA-sample? How long are these fragments ? Discuss virtual digestion patterns.