Experiment 22	**Isolation of plasmid-DNA from bacteria**
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Reading	Chapters in BBOM 10th: 10.8 BBOM : Madigan M.T., J.M. Martinko and J. Parker: "Brock - Biology of Microorganisms", 10th Edition (2003), Prentice Hall.
Objectives	• Isolation of plasmid-DNA from different bacteria clones • Handling of bacteria clones • Different markers on plasmids
Background	Almost all of the known plasmids are double-stranded DNA. Most plasmids are circular, but many linear plasmids are also known. Naturally occuring plasmids vary in size from 1 to more than 1000 kilobase pairs. The typical plasmid is a circular double-standed DNA molecule less than 1/20 the size of the chromosome. Most of the plasmid-DNA isolated from cells is in the supercoiled configuration, which is the most compact form within the cell. Isolation of plasmid-DNA can generally be readily accomplished by making use of the physical properties of supercoiled DNA molecules. Although chromosomes are also supercoiled inside the cell, isolation of chromosomal DNA almost always leads to breakage of the strands and consequent loss of supercoiling. Separation can then proceed by a variety of techniques, including ultracentrifugation and electrophoresis on agarose gels. Among the most widespread and well-studied groups of plasmids are the resistance plasmids which confer resistance to antibiotics and various other inhibitors of growth.
Literature	The chapters of BBOM 10th mentioned above
www. Links	http://www.accessexcellence.org/AB/GG/plasmid.html http://histmicro.yale.edu/mainfram.htm http://gweb1.ucsf.edu:8080/shared_databases/plasmids/
Practical work	We will isolate plasmid-DNA from different bacteria clones
Material and Methods	1. Centrifugation of bacteria at 5000rpm for 1min. 2. Resuspend pelleted bacterial cells in 250 µl buffer P1. 3. Add 250 µl buffer P2 and invert the tube gently 4-6 times to mix. 4. Add 350 µl buffer N3 and invert the tube immediately but gently 4-6 times. 5. Centrifuge for 10 min at maximum speed in a tabletop microcentrifuge.

	6. Apply the supernatant from step 5 to the column by decanting. 7. Centrifuge for 60 s. Discard the flowthrough. 8. Wash the spin column by adding 0.75ml buffer PE and centrifuging for 60 s. 9. Discard the flowthrough, and centrifuge for an additional 1 min to remove residual wash buffer. 10. Place the column in a clean 1.5ml microcentrifuge tube. To elute DNA, add 50μl buffer EB to the center of the column, let stand for 1 min, and centrifuge for 1min.
Experinces gained	• Handling antibiotic resistant bacteria • Learning how to isolate plasmids
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. Wash your hands before you leave the room and desinfect bench surfaces with 70% ethanol.
Timing	90 minutes
Reporting	Report the different functions of the different buffers used!
Questions to be answered	How is it possible to seperate chromosomal DNA from plasmid-DNA?