Title of experiment	**Molecular analysis of microorganisms from snow and glacier ice**
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Textbook reading	Brock Biology of Microorganisms (10th Edition). 2002. Michael T. Madigan, John Martinko, Jack Parker (Authors). ISBN: 0-13-066271-2. Contents in chapters 2, 7, 8, 11 to 15, and 18. Pages 1-38. Part One: Basic methodology. in Ehrlich, H.A (Ed.) (1992) PCR Technology: Principles and applications for DNA amplification. Oxford University Press, Inc. Bergey's Manual of Determinative Bacteriology by Bergey, John G. Holt (Editor), Noel R. Krieg, Peter H.A. Sneath, D. Bergy. 2-nd edition. Taxonomic Outline of the Archaea and Bacteria.
Research questions and objectives	Who is out there? How many different microorganisms are living and are well-adapted to life in these cold extreme habitats? Can we find new microorganisms? To which phylogenetic groups do the microorganisms belong to? How are they genotypically related to each other? We are going to do molecular identification of the microorganisms living in alpine snow and glaciel cryoconites and study their phylogenetic positions
Background	Attempts to find unique microorganisms possessing special physiological and ecological traits leads people to explore many extreme environments e.g. habitats dominated by high-temperature, high-pressure, low-temperature, high-salinity, basic and acid environments, etc. In this project we will focus on studying microbial communities from cold, high-mountain habitats. This might lead to the discovery of new and only recently described psychrophiles or psychrotolerant organisms The applications of accurate, robust, and powerfull molecular approaches e.g. PCR (Polymerase Chain Reaction) and recombinant DNA methods allow one to assess bacterial diversity without the need for cultivation. These procedures are highly developed and experimentally very demanding. The methods allow us to characterize many unculturable prokaryotes and microscopic eukaryotes and searching for new groups in the phylogenetic tree of organismic domains. The common approach is to sequence 16S-rRNA gene fragments and study the molecular phylogeny of the microorganisms based on sequence similarity. The composition and structure of the cold-loving communities will lead to ideas what could be exploited from cold extreme habitats for biotechnological purposes.
Selected literature	Overview: A Phylogenetic Backbone and Taxonomic Framework for Prokaryotic Systematics. Wolfgang Ludwig and Hans-Peter Klenk. p: 49-66. Hugenholtz, P. and N.R. Pace. 1996. Identifying microbial diversity in the natural environment: a molecular phylogenetic approach. Trends in

	Biotechnol. 14: 190-197. Woese, C.R. 1987. Bacterial evolution. Microbiol. Rev. 51: 221-271
www.Links	Sequence Blast program: http://www.ncbi.nlm.nih.gov/BLAST Phylogenetic Analysis with ARB: http://www.arb-home.de
Practical work	Purification of genomic DNA, PCR amplification of 16S-rRNA gene fragments from total community DNA and clones. Cloning of PCR products from total community DNA. Restriction Fragment-Length Polymorphisms (PCR-RFLP) analysis of the 16S-rRNA genes from selected clones. Sequencing, sequence analyses and phylogenetic analysis of unique clones.
Materials and experimental protocols	Basic molecular analyses : preparing the solutions, pipetting, contamination-free work. DNA extraction: work in acid room, waterbath, centrifuge PCR: PCR reagents, setting up PCR machine, gel electrophoresis and gel documentation apparatus PCR-RFLP: restriction enzymes and buffer, incubator, running the acrylamide gels in a TTGE apparatus Cloning of PCR-products: cloning kit (Invitrogen), LB medium, antibiotic selection plates, IPTG X-gal indicator plates. PCR for sequencing with Big Dye terminator, purification of PCR products with microcon column and Sephadex G-50, sequencing, sequence analysis: sequencer, computers with appropriate programs.
Goals and experiences gained	Familiarity with the basic knowledges and skills of molecular techniques, handling the sequence data, processing the data and phylogenetic positioning of the sequences from environmental samples
Timing	Molecular analyses and the data processing planned can be performed during the duration of the course.
Reporting	Oral presentation at the minisymposium and written report to be turned in by December 19, the latest.
Laboratory rules and precautions	Standard working techniques and precautions are required: no eating, no drinking, smoking and applying the cosmetics in the laboratory. Avoid contaminations during your work. All the waste and contaminated material needs to be sterilized before disposal. Molecular techniques, e.g. PCR, are sensitive methods. To avoid false positive results, be aware not to contaminate your samples during preparations: clean the place before using it, wear clean gloves, use DNAse-free tubes and pipettes, prepare the negative, positive, internal and "poison" controls. Some of the chemicals that will be used are dangerous to human health: acrylamide, ethidium bromide stains (carcinogenic). Please always wear gloves when handling them and dispose of them savely. The UV-radiation of the gel viewing apparatus can cause damage to eyes and skin, use protective glasses and wear a coat with long sleeves when viewing gels.