4 General Aspects of Sample Preparation

Susanne Hummel and Bernd Herrmann

This introductory chapter on sample preparation and analysis will give a brief overview of the different types and origins of ancient DNA (aDNA) and the different states of preservation. Independent of the handling of the source material, work on aDNA follows some basic principles of sample treatment, DNA preparation, and DNA analysis which are presented on the assumption that the polymerase chain reaction (PCR) technique will be applied. This is especially true for the discussion of sources and effects of inhibitory and contaminating substances. However, one should be aware that some of the basic difficulties associated with ancient materials may also occur when applying other analytical techniques such as hybridization.

1. Types and Origins of Ancient Nucleic Acids

In living organisms almost all cells contain nucleic acids. Under certain circumstances, the different types of tissue and thus the cells and their contents may remain more or less unaltered by diagenetic effects (cf. Chapter 2 and Chapter 17).

The cells of eukaryotic organisms such as animals, plants, and fungi possess different types of nucleic acids. The nucleus of a eukaryotic cell contains linear double-stranded DNA which is condensed by histones within the chromosomes, with genes as subunits. The chromosomes and therefore the chromosomal DNA is inherited from both parents. The nucleus also contains RNA, which is single-stranded and involved in protein synthesis. The mitochondria of the eukaryotic cell, which are responsible for the energy metabolism of the organism, contain DNA, which is almost exclusively inherited maternally. Prokaryotic cells such as bacteria and yeast contain circular DNA and RNA in their cytoplasmic compartment. Viruses finally contain a great variety of nucleic acids. They can consist of either double- or single-stranded DNA, which may be linear or circular and is packed into a protein capsid. Some types of viruses consist of RNA only, which may be double stranded.
Both DNA and RNA show nucleotides as subunits, which are joined together by phosphodiester linkages to form the macromolecule. The nucleotides in turn are composed of a base (adenine, cytosine, guanine, thymine, or uracil), a sugar molecule, and a phosphate group.

2. States of Preservation of aDNA

Immediately after death autolysis sets in, i.e., the organism’s own enzymes start to decompose the organic substances of the body. Usually this first phase of decomposition is followed by the decay of soft tissues under involvement of microorganism and fungi and also at an advanced stage, of higher organisms such as insects and vertebrates. This process regularly leads to the complete disappearance of soft tissues; only the mainly inorganic exo- or endoskeletons and keratinic appendices like hair and feathers (cf. Chapter 15) remain. However, the endoskeletal remains at least from vertebrates are known not to lose their organic components. Besides collagen, fatty acids, etc., there are still cellular structures left, as histological cross-sections of bones show (cf. Chapter 3). In contrast to soft tissues, the organic structures in bones and teeth persist even under normal burial conditions (cf. Chapter 13). This can be explained by the comparatively low water and enzyme content of hard tissues. Each osteocyte or cementoblast seems to undergo a process of individual mummification. Moreover, these cells are protected by their topology against physical and biochemical decay by microorganisms: situated in little caves, they are completely surrounded by protective hard tissue.

A special case of long-term preserved genetic information are plant seeds (cf. Chapter 16). When stored under proper conditions—for example, in the clay walls of ancient buildings—they keep their ability to sprout, in some instances for centuries.

Another form of individual mummification of single cells is observed in dried body fluids, such as evidence blood stains, sperm, or saliva (cf. Chapter 9). In these cases the chance for DNA to persist occurs when the fluid spreads, favoring evaporation.

The destruction of soft tissues may be slowed down or interrupted at any stage of decomposition by either environmental factors or artificial treatment. Examples for conservation of a body by environmental factors are natural mummies and bog bodies. Natural mummification, as can be observed, for example, in most South American mummies, takes place when decomposition due to enzymatic autolysis and decay due to physical and chemical factors are arrested by rapid loss of humidity. This may happen in dry, hot, and draughty climates (cf. section on Dried Samples: Soft Tissues), but also in saline environments. Herbarium specimens may be considered naturally mummified in a broader sense, since no artificial fixatives have been applied (cf. section on Dried Samples: Soft Tissues).