DNA preservation

DNA is thought to be one of the more inherently stable molecules that we use in our research. Formation of the double helix is typically fully reversible as long as secondary and tertiary structures are maintained. Nuclease contamination must be avoided but the main threat to DNA preservation if usually chemical degradation.

EDTA is the anticoagulant of choice for blood collection for DNA extractions because it inhibits DNase activity and does not introduce volume changes. It does affect magnesium concentrations in downstream applications. Tubes with ACD (Acid Citrate Dextrose) can be used for blood collection, but it is important to fill them to the mark to avoid causing changes in osmolarity, which can affect cell lysis. It does however provide good preservation of RNA and DNA. Heparin should be avoided, as it can bind to DNA during purification and can inhibit Taq polymerase used for PCR. Irrespective of the anticoagulant, the Vacutainer tube should be inverted several times to mix the blood. Blood can be shipped at ambient temperature, but if the delay between collection and extraction is >3 d, there will be some degradation of DNA and the yield will be lower than that from fresh blood.

For high quality DNA care must be taken in sample handling. Breakdown of cell integrity releases degrading enzymes which degrade the DNA. In a comparison of 28 extraction techniques silica membrane based kits were found to be superior for routine extractions.

Saliva and urine – both are becoming more popular as easy to obtain sources of DNA. Cells should be washed in a Tris-HCl/EDTA buffer and frozen if not extracting immediately. Importantly a large proportion of DNA extracted from buccal cells is non-human (50-90%) with a proportion being bacterial.

Formalin affects DNA quality therefore Formalin-fixed paraffin embedded (FFPE) tissue is very variable as a source of DNA. Fixation conditions and length of time are important. Formalin reacts with nucleic acids. The formaldehyde reacts with the bases to form mono-methylol groups but these can be reversed by heating in buffer. The N-methylol in formalin can also form a methylene bridge between bases. Formaldehyde can generate apurinic and apyrimidinic site and also slowly hydrolyses phosphodiester bonds which gives rise to strand breaks. Much of this is irreversible and it is necessary to establish how much useable DNA is present in a sample. Real time PCR is good for this and kits are available to repair damaged DNA prior to this application. Typical yields from FFPE tissue are in the sub-mg range which precludes the use of some techniques like ChiP.

The most common method of storage of DNA is as a suspension in ethanol at -80oC, but obviously this then has to be further prepared for before use. Storage in aqueous solution long term is not recommended as DNA is sensitive to depyrimidination, deamination, and hydrolytic cleavage. Alkaline conditions have been suggested to be of use as this inhibits the mentioned reactions. Also ionic strength influences depurination and beta-elimination so the use of saline is very effective. When preps are stored this way at pH 8.5 then the main threat is oxidation. The demetalation of the buffer will drastically reduce this too. If this is not possible (and it’s very difficult to fully achieve) then chelators (such as 200uM EDTA) are the next best option but these can interfere with downstream reactions. An alternative is to add an antioxidant/scavenger such as 1% ethanol. DNA stored in buffer is much less stable if denatured prior to storage i.e. single stranded is less stable.

As a general rule isolated DNA can be stored at 4oC for several weeks, at -20oC for several months and at -80oC for several years. Obviously samples should be aliquoted and frost-free freezers avoided as this causes drying out of the sample. Little is known about the effects of freezing long term and thawing on the DNA. There is a phenomenon known as cryolysis where DNA degradation is dependent on the rate of cooling, with rapid being bad but this has been contested more recently (so make your own mind up on that one!). The presence of sugars may protect the DNA by forming a ‘glass’ structure.
Drying is a more practical alternative and also removes the water that takes part in any hydrolytic reactions. Very long strands of DNA are susceptible to shear induced damage so most methods avoid spraying now. There is a lack of information on the effectiveness of drying as a storage method. Lyophilisation does cause breakdown of helical structure and therefore leaves the molecule open to risk of oxidation. Again the addition of sugars such as trehalose may aid preservation.
Cards are becoming a popular method of storage due to space and cost issues. Cards contain a pretreatment (usually methanol) which inactivates inhibitors of PCR found in whole blood. There are 2 prevalent forms – FTA elute and FTA Classic. These were compared head-to-head. In this study time, temperature and EDTA had no effect on storage but complete drying prior to further extraction of the DNA was essential, it was recommended no less than one week. Also good washing to remove haemoglobin

was recommended. The major benefit of collecting onto these cards is that finger prick can be used to collect samples. The major drawback is much less DNA is recovered than would be from blood as about 40% of the DNA is retained on the card. The yield can be increased by re-isolation techniques. The FTA classic cards gave a better yield, took longer but had less haemoglobin contamination.

Storage vessel is also a matter of note. Plastic microtubes have gained bad press in the past for spoiling or binding samples. DNA is charged and hydrophilic. Polypropylene is hydrophobic which should preclude any binding. However is has been noted that interactions do occur, which leads to conformational change. This interaction is particularly strong at high ionic strength. Even siliconised tubes were still able to bind significant amounts. Polyethylene tubes bind DNA even more strongly. Polyallomer tubes are the best but are quite costly. If the DNA solution is of a lower ionic strength then binding is reduced. There are now specialised tubes on the market that claim lower binding levels.

Refs

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Good luck!
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