DNA storage methodologies: Principles and Protocols

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Conservation of DNA that is amenable to downstream processes after storage, forms the basis of conservation of genomic resources. DNA is one of the most stable molecules known owing to its structural features-the double stranded alpha helical molecule with a deoxy ribose sugar as the backbone has been isolated from samples millions of years old . The concept of DNA banking and conservation has found ground in the post PCR era owing to the ease in analysis of nanogram quantities of DNA. Contamination with nucleases and chemical degradation however are the main threat to DNA preservation. Thus DNA storage methodologies depend on various factors viz. the type of DNA to be stored, the duration of storage, the storage temperatures and conditions and the downstream applications for which the DNA is to be used. The procedures for DNA isolation and it's purity at the time of storage also determine the stability of the stored sample.

There are four temperature based strategies for long term DNA conservation. These are storage at:

- -20° C
- -80° C
- -196° C
- Dried, at room temperature

When stored at -196° C, DNA is maintained in a vitreous state wherein the molecules do not diffuse and hence nuclease and chemical degradation does not occur. When the temperature is raised above the glass transition temperature of water, movement and reactivity of molecules is re-established and DNA degradation is initiated (Yuanzheng and Angell, 2005) .

Storage of DNA in the dry state is a practical alternative to long term storage. Dehydration reduces molecular mobility and inhibits the depurination, depyrimidination, deamination and

hydrolytic reactions that damage DNA. It is important to store dried DNA samples at low relative humidity. Several methods for dry preparations of DNA include spray drying, spray freeze drying and air drying or lyophilisation which is the cheapest and most popular method. Storage of dried DNA on FTA Cards (Whatman) is another option for storage at room temperature. The DNA immobilized on FDA cards is amenable to PCR after 17 years storage. This is particularly useful for storage of DNA from blood, saliva etc.

Storage of DNA for medium term is done at -20° C or -80° C depending on the duration .Since acidic conditions cause hydrolysis of DNA, DNA in the aqueous phase is stored under slightly basic conditions in Tris: EDTA buffers or as a precipitate under ethanol(at -80° C). Often used samples of DNA can be stored in aliquots at 4° C to avoid repeated freeze-thaw cycles. In addition to being sensitive to nucleases and hydrolysis, DNA is sensitive to oxidation reactions due to the presence of trace amounts of metals.

DNA conservation for eternity is exemplified by its recovery from archaeological samples, fossilized plants and bacteria, mummified samples and permafrosts etc. despite that these samples were not processed for maintaining DNA in a stable state. A study on the decay kinetics of DNA shows that mitochondrial DNA degrades exponentially at the rate of one base pair after 6,830,000 years at −5 °C (Allentoft et al.2012). Today, the development of plasticware and reagents for long term storage and management of DNA samples under different conditions is an upcoming industry of its own. For practical use in clinical and surveillance diagnostics storage of DNA in 50% glycerol double distilled water was found satisfactory based on real-time PCR assays (Roder et al 2010). However, experiments on DNA storage methodologies are yet to come up with a single best method that would suit the wide applications. Studies on the relative importance of various storage temperatures viz a viz the storage buffers and their combinations however show that ‘the drier the better’ is one thumb rule by which most DNA samples can be stored as hydrolysis is one of the major factors contributing to DNA degradation. Contrary to belief, repeated freeze thaw cycles do not affect DNA quality (Schuster and Appleby, 1983) provided the DNA is of good purity.

The ease of PCR based DNA analysis has made possible the efficient utilization of stored DNA samples, thus leading to a surge in the field of DNA banking and genomic resource conservation. Efficient DNA conservation depends on storage temperatures, composition of storage buffer, ionic strength, purity of DNA, length of DNA, presence of metal ions etc. Although considered as the most simple and routine activity of 'keeping DNA in a refrigerator' methodologies for long term storage of DNA samples need to evolve across laboratories and DNA storage facilities to enable the conservation of genomic resources for eternity.

References:

1. Allentoft ME, Collins M, Harker D, Haile J, Oskam CL, Hale ML, Campos PF, Samaniego JA, Gilbert MTP, Willerslev E, Zhang G, Scofield RP, Holdaway RN, Bunce M (2012).The half-life of DNA in bone: measuringdecay kinetics in 158 dated fossils. Proceedings of the Royal Society of Biological Sciences **279**: 4724–33.
2. Roder B, Fruhwirth K, Vogl C, Wagner M and Rossmanith P (2010).Impact of long term storage on stability of standard DNA for nucleic acid based methods Journal of Clinical Microbiology48:4260-4262
3. Schuster D and Appleby D (1983).Does freezing and thawing of DNA solutions insert nicks in the double helix. Focus5(2): 10
4. Yuanzheng Y and Angell C (2005).Clarifying the glass transition behaviour of water by comparison with hyperquenched inorganic glasses. Nature 427,717