

Workshop Long-term storage of DNA material

Systematics 2009, Leiden (NL), Wednesday 12th August, 14:00 to 17:00

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Chair: Gerhard Haszprunar

Summary

The workshop aimed at addressing both institutional as well as technical efforts for long-term storage of nonhuman DNA and tissue samples. The necessity to establish standards and Best Practices to manage these new types of biospecimen repositories was stressed by all participants.

Institutional strategies for long-term DNA and tissue storage

Living in the age of unprecedented accelerated species loss, speakers argued that the time to preserve at least the genome diversity is now. The current status of long-term DNA and tissue storage and sample exchange policies of 18 biospecimen collections in Europe has been summarised and discussed by the key note speaker Ole Seberg based on the outcome of a SYNTHESYS NA E study (www.synthesys.info/network_activities_e.htm). While the advantage of centralized facilities vs. individual laboratory storage is obvious (e.g. costs, expertise, standardization, disaster plan, availability) they are not common practice yet. Plans for establishing centralized DNA and tissue storage facilities have been presented by scientists of the NHM London, the Smithsonian NMNH, and the NHM Denmark; while the DNA Bank Network is already been working for 2 years. It was emphasized that international coordination of collection responsibilities to amend and complement collection efforts on an international scale should be coordinated. At present, the DNA Bank Network focuses on DNA and tissue samples from Central Europe and the Mediterranean region. Additionally, the Smithsonian NMNH (The Planetary Genome Project) pointed out to consider major gaps of taxonomic coverage and knowledge on a global scale. Differences in DNA sample distribution policies among institutions (restricted vs. free access, free of charge vs. at cost fee) were controversially discussed.

Specimen and tissue collections as well as documentation, DNA isolation and storage are traditional tasks of research collections. A key problem for most institutions is to upgrade their bioinformatic infrastructure to guarantee the availability of DNA samples and documentation data. Here, the established data architecture and software of the DNA Bank Network (www.dnabank-network.org/Infrastructure.php) offers an open access solution currently only available as beta version upon request. The data architecture is based upon GBIF tools bundling all relevant data of deposited DNA samples via one webportal with the ability to link out to external data provider (e.g. GenBank).

Technical strategies for long-term DNA storage

The two general rules to prevent DNA damage during storage are "low temperature" and "as dry as possible". Aliquotation of DNA samples (lyophilized masterstock and ready-to-use-aliquots) to prevent repeated freeze-thaw cycles as well as to reduce contamination (e.g. nucleases) or cross contamination has been proclaimed. A literature review and storage tests carried out by the DNA Bank Network revealed that long-term storage of DNA samples in buffer should be carried out at -80°C or below. Lyophilized DNA must be stored at low relative humidity to avoid DNA aggregation. Since secondary compounds and heavy metal ions can result in highly reactive intermediates causing all sorts of DNA damage, high purity of extracted DNA must be ensured. The impact of fast and careful tissue fixation and tissue preparation prior to DNA extraction to receive high quality DNA

has yet been underestimated. Degradation due to wrong sample collection and preparation is much higher than the influences during to long-term storage.

Energy cost and - in consequence environmental cost - is the main argument for dry storage at ambient temperature. The characteristics of four commercial dry storage systems at ambient temperature were presented (GenPlate™, QIAsafe™/SampleMatrix™, GenTegra™, DNAshell™). The reliability of these products has been debated based on performance tests conducted by the manufacturer (accelerated aging, PCR downstream applications, cloning, secondary structure).

Preliminary results of DNA storage experiments based on q-RT PCR data were presented by the DNA Bank Network. Thus, freezing-thawing is not as relevant for DNA degradation as suggested in the present literature. QIAsafe™ shows good storage performance at ambient temperature but did better at -20 and -80°C. Unbuffered DNA in water (RT, 4°C and -20°C) was subject to fast degradation. Storage in liquid nitrogen performed not significantly better than by -80°C or low temperature if QIAsafe™ was used, even the recommended theoretical best practice is storage in liquid nitrogen.

Outlook

Most of the participants agreed to foster cooperation among nonhuman DNA and tissue banks in research and management on a European and global level. Therefore, a proposal for an ESF grant (Research Networking Programmes) for a European Network of DNA and tissue banks will be adduced. The network activity aims at supporting nationally funded research activities for four to five years, to address a major scientific issue or a science-driven topic of research infrastructure at the European level with the aim of advancing the frontiers of science. Proposals with a global dimension which intend to create links to networks of scientists in non-ESF Member Organisation countries, (e.g. Australia, Canada, China, Israel, Japan, Korea, Russia, USA and others) with funding through agencies in those countries are also encouraged.

Key objectives include:

- creating interdisciplinary fora
- sharing knowledge and expertise
- developing new techniques
- training young scientists

Scientists being interested to join this activity are welcome to contact b.gemeinholzer@bgbm.org. An upcoming workshop to organise the proposal will be held by end of September/beginning of October 2009 in London.

Workshop schedule

14:00 – 14:30

O. Seberg: Towards a European network of DNA and tissue banks

14:30 – 14:50

J. Mackenzie-Dodds & R. Huxley: A Molecular Collections Facility for the Natural History Museum

14:50 – 15:10

S. Bready: Planetary Genome Project: Preserving a synoptic sample of genomes representing life on Earth

15:10 – 15:30

B. Gemeinholzer: DNA Bank Network – Recent and upcoming activities

15:30 – 15:50

H. Zetzsche: DNA storage for the long term: basics, practice and challenges

15:50 – 16:10

T. Knebelsberger: Preliminary results of DNA storage experiments

16:10 – 16:30

T. Doedt: QIASafe™ DNA - An innovative Anhydrobiosis-based technology for long-term storage of DNA at room temperature

Video: GenTegra™ Overview – Room temperature DNA storage in a mineral matrix

16:30 – 17:00

Discussion

Listing of workshop abstracts

Towards a European network of DNA and tissue banks

Ole Seberg & Gitte Petersen (Natural History Museum of Denmark, Copenhagen)

The use of DNA data is routine in many research areas and massive amounts of data from genomics and proteomics have changed the scientific landscape radically, changes that will become even more profound with the spread of next-generation sequencing technology.

Although many institutions over the last decades have accumulated considerable collections of DNA and tissue samples, these are often stored decentralized under suboptimal conditions usually in mechanical freezers at -20° – -80°C and are thus gradually degrading. Such samples represent a considerable investment in both time and money, but when individual research projects finish their fate is frequently uncertain.

However, conservation and preservation of samples derived from biological specimens (e.g. DNA extracts or tissue samples) and their associated passport data are essential to ensure compatibility and reproducibility in all areas of biological research and many Natural History Collections have established or are planning to establish centralized DNA and tissue banks on their premises.

Data collected through efforts of SYNTHESYS has given us an assessment of the status of DNA and tissue banks across Europe. This status will be presented and discussed here and future collaborative efforts will be outlined.

A Molecular Collections Facility for the Natural History Museum, London

Jacqueline Mackenzie-Dodds & Robert Huxley (Natural History Museum, London)

The Natural History Museum, London is planning a centralised facility to store its rapidly expanding collections of, animal and plant tissues for DNA extraction, extracted DNA, RNA and whole cells.

The facility will be a repository for material used by or generated by current NHM research but also be actively developed through acquisition to be a resource for the future in parallel with the Museum's traditional collections.

A number of questions were addressed in designing the facility:

- Future proofing – how much investment should be made in liquid nitrogen storage when ambient storage of DNA may become a viable option in the future?
- NHM is working with GE Healthcare in developing an ambient method of storage of DNA/tissues 'on paper' using "FTA cards"; how will this affect future storage needs and what are its limitations?
- What is the optimum distribution of material between the various storage conditions (liquid N₂, ambient, -80, -20, + 4, freeze dried etc)
- What will future users need? – e.g. more demand for whole tissues, proteins, RNA and how can we factor this into the facility'?
- How much DNA should we store relative to tissues?

- What effect will new technologies such as new generation sequencing and genomics have on a future facility?

Alongside development of the facility, policies and procedures for molecular collections are being revised to ensure best practice in management and use of these collections.

Planetary Genome Project: Preserving a synoptic sample of genomes representing life on Earth

Sean Bready (Smithsonian NMNH, Washington)

The National Museum of Natural History (NMNH) is steward for the largest collection of biological specimens in the Western Hemisphere and houses research staff and collections from several United States government agencies such as the Departments of Agriculture, Commerce, Defense, and Interior. Like all major biorepositories, NMNH's collections of frozen tissue and DNA are expanding rapidly, and a major new collection facility will be opened to house them in 2010. As plans for this facility develop, NMNH seeks to build long-term partnerships through a global consortium of leading cryo-repositories as part of its Planetary Genome Project (PGP). The goals of PGP center on supporting future generations of research on the most urgent questions related to the evolution and conservation of biological diversity. Specific goals of PGP are to: (1) preserve a dense, synoptic sample of genomes representing life on Earth; (2) create a state-of-the-art NMNH facility reflecting the best practices of the cryopreservation community; (3) promote the development and application of data standards and informatics tools to maximize interoperability among biodiversity cryo-repositories around the world; (4) develop a global information resource that provides free and instant access to information on the species represented in cryo-repositories; and (5) design sampling schemes and conduct collecting expeditions that fill the important gaps in taxonomic coverage and knowledge. Plans for PGP begin with the formation of a global consortium of leading cryo-repositories. The PGP Consortium would host workshops on topics of shared importance including taxonomic coverage, cryo-repository design and management, legal and ethical issues, collaborative planning of international collecting expeditions, and raising awareness of the impact of frozen tissue and DNA collections on research and conservation among researchers, government officials, students and the public.

DNA Bank Network – Recent and upcoming activities

Birgit Gemeinholzer (Botanic Garden and Botanical Museum Berlin-Dahlem, Berlin)

The DNA Bank Network was established in spring 2007 and is currently funded by the German Science Foundation (DFG). The network is maintained by four partner institutions of complement collections and expertise.

The main focus of the network is to enhance taxonomic, systematic, genetic, conservation and evolutionary studies by providing:

- at-cost availability of DNA material,
- high quality, long-term storage of DNA material on which molecular studies have been performed, so that results can be verified, extended, and complemented,
- complete online documentation of each sample, including provenance of the original material, the place of voucher deposit, information about DNA quality and extraction methodology, digital images of vouchers and links to published molecular data if available.

At present the infrastructure has been established at all partner institutions and 9400 DNA samples from 5350 taxa are currently being stored. DNA bank databases decentrally administered at each network partner and accessible via a central Web portal

(<http://www.dnabank-network.org>) have been developed. Standards for the online exchange of DNA data have been proposed (ABCDDNA).

We now aim to optimize, standardize and record the workflows concerning information technology, documentation and laboratory handling. DNA storage experiments are further being developed; tests to propose best practice in the field are envisaged, as well as generating DNA barcodes for quality standards. Furthermore, quality and yield of high-throughput DNA extraction techniques as well as whole genome amplification to reduce storage capacity will be tested. Standardization procedures will allow for quality assurance and expansion of the network in the future.

Long term DNA storage: basics, practice and challenges

Holger Zetsche (Botanic Garden and Botanical Museum Berlin-Dahlem, Berlin)

The analysis of DNA extracted from all kinds of biological samples has become common practice in organismic research. This includes DNA from museum specimens, tissue collections, rare organisms or non-invasively collected samples of potentially high value. Although DNA is routinely stored for longer periods and the chemical nature of DNA degradation is well understood, procedures and methods for their optimal long term storage remain inadequately documented. This talk reviews the current state of knowledge about storage of DNA samples, applied methods and new approaches such as dry DNA storage at ambient temperature. It also highlights the need of a fully integrated DNA curation: from collection of the DNA containing material, sample preparation, DNA extraction and DNA storage.

First results of DNA storage experiments

Thomas Knebelsberger (Bavarian State Collection of Zoology, München)

In molecular systematics an enormous amount of DNA samples are currently being processed. While DNA isolation methods, sequencing technologies and data analysis methods improve continually the influence of different storage conditions on the quality of DNA samples is still insufficiently investigated.

To optimize the storage conditions in our DNA banks we investigated DNA degradation in sample storage at five temperatures (room temperature, +4°C, -20°C, -80°C and liquid nitrogen) and three different agents (TE buffer, water and QIA safe DNA tubes (QIAGEN)). The influence of sample dehydration and protective additives (trehalose) was also examined. DNA degradation was measured by quantitative real-time polymerase chain reaction (PCR) method which has the ability to estimate accurately the amount of targeted fragments in a sample.

After 6 months DNA quality was best preserved at -20°C or -80°C. Minimal quality loss occurred in dried samples in the presence of trehalose and in QIA safe DNA tubes (QIAGEN).

QIA safe™ DNA - An innovative Anhydrobiosis-based technology for long-term storage of DNA at room temperature

Thomas Doedt, QIAGEN GmbH

GenTegra™ Overview- Room temperature DNA storage in a mineral matrix

Video (<http://www.youtube.com/watch?v=1VgvduUyQAg>), GenVault Corp.