A Warmer Reception

Omoshile Clement, Rolf Müller and Judy Müller-Cohn at Biomatrica Inc discuss the background and current state of the technology for storage, preservation, archiving, shipping and management of DNA samples at ambient conditions

It is often critical to maintain the integrity and quality of nucleic acids for use in downstream analysis. For more than 50 years, researchers have defaulted to freezing biological samples at -20ºC or -80ºC as a means of preservation and storage. Technologies for analysing nucleic acids have evolved through the years, although information about new storage technologies of these bio-samples has not garnered as much attention within the life science and research communities. Storing and preserving the quality of nucleic acids at ambient room temperature therefore represents a paradigm shift with significant savings in cost, environmental impact and ensured sample integrity.

In the life sciences, the research landscape is changing dramatically; this is largely due to significant innovations in genomics research that have continued to drive down costs while delivering innovative tools for genome sequencing (human and other species), and the potential realisation of the concept of personalised medicine. The genomics-based tools drive new scientific discoveries, enabling researchers to explore complex biological processes and diseases in an integrative and quantitative manner via 'systems biology' approaches (1). These advances are concomitantly driving the increased collection, storage and analysis of large amounts of biological samples. The global drive to perform large population-based studies, afforded by the low-cost technologies in genomics and translational research, is creating a huge need for warehousing and managing such large collections in both commercial and academic institutions.

In the US alone, there are more than 40,000 individual research laboratories located within university campuses, research institutes and commercial organisations. Researchers within these laboratories have assembled very large collections of biological samples from clinical and field studies – some irreplaceable, all representing enormous scientific and financial value for the researcher and the organisation. The collection and storage costs per sample can be as little as a few dollars to as high as $10,000, and there are over a billion of these samples stored globally, for example DNA and RNA, cells, clones, tissue organs, blood and buccal swabs (2). These samples are of high value to researchers and current research trends are increasing the growth of these collections at an escalating rate.

Unfortunately, the publicity generated by the significant innovations in genomics research in the last decade has not included new technologies for collecting, shipping, storing, archiving and managing these biological samples. The lack of publicity for these new tools however, has not stopped innovations; rather it has spurred new ideas and their adoption.

STORAGE OF BIO-SAMPLES: COLD FREEZING

Nucleic acid degradation is a major concern in biomedical research. Degradation can occur by various means such as multiple freeze/thaw cycles, hydrolysis, UV light, elevated temperatures, oxidation or alkylation (3). While DNA shows greater stability at room temperature, the more labile RNA is far more fragile to preserve and stabilise, even at extremely low temperatures.

In a recent article the current default mode of preservation, shipping and storing biological samples, predominantly by cold freezing/refrigeration (4ºC, -20ºC and -80ºC) was described (4). However, this approach is fraught with problems:

- Multiple freeze-thaw cycles can lead to sample quality degradation
- Cold freezing, especially of bio-tissues, can lead to cell membrane damage
- Cold refrigeration/freezers produce hydrofluorocarbons, a potent greenhouse gas pollutant in the environment. A report in

The Economist highlights that the consumption of a typical ultra-low temperature freezer at about 7,665kWh per year releasing 54,805 pounds of carbon dioxide is equivalent to the emission from about four cars

- Cold packing and shipping produces a large amount of waste materials
- Power failure or freezer failure can place samples at risk of degradation or loss

Despite these deleterious effects of cold freezing on the quality of preserved biological assets, it remains the medium of choice for a large proportion of biomedical researchers. Alternative storage technologies are available, but they have not garnered as much publicity or attention from the biomedical research community.

ROOM TEMPERATURE-BASED STORAGE TECHNOLOGIES

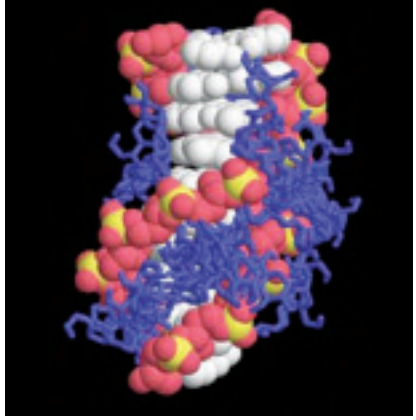
In an article published in *The Scientist* the frequently-told stories of large amounts of legacy biological samples collected over 20 years and stored in freezers and refrigerators in research laboratories were associated with the type of problems described above (5). Therefore, alternative approaches and techniques are required that can offer better management of the legacy samples currently warehoused in cold freezers in laboratories all over the world, as well as support the increasingly large growth in sample collections being undertaken today and that will be, in the future. Hence, a low-cost, energy efficient and robust nucleic acid storage method based on ambient room temperature that offers long-term stabilisation and preservation of these biological assets is critical.

Room temperature-based technologies for long-term storage and preservation of bio-samples represent a paradigm shift in stabilising and preserving these fragile biological materials. In 1988, Leigh Burgoyne and Craig Fowler at Flinders University reported the development of chemicals that, when infused into a cotton paper, protects DNA from nucleases, oxidative and UV damage (6). This was termed Flinders Technology Associates (FTA) and the technology was commercially licensed and globally distributed. This approach enjoyed wide adoption as an alternative method of preserving DNA in blood or buccal cells at room temperature. Over the years, a number of drawbacks to using FTA cards have been observed in terms of the quality and quantity of recovered nucleic acids after storage in this medium (7).

A new approach based on mimicry of natural biological processes of DNA preservation has led to the development of novel synthetic formulations that have been shown to offer better stabilisation, storage and shipping options for a wide range of nucleic acids. It mimics the natural biostability process of extremophile organisms in how they preserve their tissues and cells in a dry state for longer than 100 years and are still able to resume their normal function upon hydration. The original observation of this natural phenomenon was made as far back as 275 years ago by Antony van Leeuwenhoek, a Dutch microbiologist, who first coined the term 'anhydrobiosis', or life without water, to describe this natural phenomenon. In a series of studies demonstrating this ability in natural organisms, separate teams led by Clegg and Crowe described trehalose, a sugar-based compound, as offering significant protection of the double-helical structure of DNA from degradation (see Figure 1) (8,9). A comprehensive review of anhydrobiosis and trehalose stabilisation of nucleic acids in various organisms has also been described by Professor John H Crowe (10).

The principle of anhydrobiosis has now been applied to develop commercial reagents for stabilising, preserving and shipping nucleic acids at ambient room temperature. The technology has also subsequently been extended to the storage and shipping of RNA and bacteria clones at room temperature. In general, protection is afforded by synthetic formulations that create a protective thermo-stable glass-like barrier around individual nucleotide bases of the double-helical DNA structure. This concept of anhydrobiosis has been adopted and concomitant products for RNA or DNA stabilisation are available in the market today. Other non-cold methods of nucleic acid storage involving the use of an inert

Figure 1: DNA protection by trehalose



environment (in the absence of water and oxygen) have been advocated as providing long-term preservation for DNA samples at ambient or elevated temperatures.

FURTHER RESEARCH

Significant testing and validation of the various room temperature-based technologies have been conducted, and accounts of these research studies are increasing in the public domain. For example, Hernandez *et al* at The Scripps Research Institute in San Diego, US applied gene expression array-based tools to evaluate the quality of RNA samples (11). These were stored for four weeks at room temperature and compared to RNA samples preserved at -80ºC for the same time period. The results show that the sample quality (Bioanalyser RIN scores) and the number of expressed genes are similar for both storage conditions, suggesting that storage and preservation of these fragile nucleic acids can also be achieved at ambient conditions. Bonnet *et al* provided research data supporting work on technology for stabilising DNA in vacuum-sealed containers that affords long-term stabilisation even at extremely high temperatures (12). Other major studies of ambient room temperature-based stabilisation of nucleic acids have been performed by forensic scientists in evaluating how to better preserve forensic DNA samples, chains of evidence and retain sample quality all at reduced costs. Results conclude that these technologies indeed offer significant DNA sample integrity, with a quantitative yield equal to or better than samples stored at -20ºC (13).

CONCLUSION

Ambient temperature-based nucleic acid storage and stabilisation technologies potentially offer significant savings in cost and energy consumption compared to current ultra-low temperature storage methods. A pilot study at Stanford University provided an initial evaluation of this technology and the cost savings that could be accrued upon the adoption of the technology to approximately 10 million biological sample collections in cold storage campus-wide (14). The projected 10-year cost savings were about $16 million (in total costs), greater than 200,000 million BTUs in energy savings, and more than 18,000 tons of reduced carbon dioxide emission. The affordability and ease of use of this technology has also been promoted by some genomic service labs, as well as government agencies, as a viable alternative to RNA or DNA shipping in dry-ice boxes (15). The result? Room temperature-preserved RNA or DNA samples can be shipped via standard postage envelopes without the worry of potential degradation or loss of sample quality.

About the authors

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