Automation and validation of DNA-banking systems

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DNA banking is one of the central capabilities on which modern genetic research rests. The DNA-banking system plays an essential role in the flow of genetic data from patients and genetics researchers to the application of genetic research in the clinic. Until relatively recently, large collections of DNA samples were not common in human genetics. Now, collections of hundreds of thousands of samples are common in academic institutions and private companies. Automation of DNA banking can dramatically increase throughput, eliminate manual errors and improve the productivity of genetics research. An increased emphasis on pharmacogenetics and personalized medicine has highlighted the need for genetics laboratories to operate within the principles of a recognized quality system such as good laboratory practice (GLP). Automated systems are suitable for such laboratories but require a level of validation that might be unfamiliar to many genetics researchers. In this article, we use the AstraZeneca automated DNA archive and reformatting system (DART) as a case study of how such a system can be successfully developed and validated within the principles of GLP.

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Genetics research has increased in scale and application in recent years. Such research is used to discover genes involved in disease as well as those involved in response to drug treatment. Methods of genotyping and sequencing have now increased in efficiency so that up to half a million genotypes can, in some cases, be delivered in one day [1]. At the same time, the size and complexity of DNA collections has risen dramatically. These collections have become some of the most precious resources in academic and private institutions for the discovery of new and improved medical treatments. Hence, genetics researchers can no longer rely on manual storage, retrieval and reformatting of samples. There is a need to automate DNA banking to increase throughput, eliminate manual errors and improve the productivity of genetics research.

Another recent change in genetics research is that, as genetics data becomes more well established in pharmaceutical R&D, regulatory authorities are becoming increasingly interested in the nature and quality of data submitted to them. The FDA, in particular, has become what it terms a 'proactive and thoughtful advocate' of pharmacogenetics in the translation of genetics and genomics data from bench to bedside [2]. This new emphasis challenges the genetics community to develop appropriate quality standards to all aspects of genetics research, including DNA banking. The ability of academic and private institutions to demonstrate quality standards

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BOX 1

Types of human genetics studies and typical numbers of DNA samples

• Linkage studies of single gene disorders	(50–100 samples)
• Linkage studies of complex diseases	(1000–20,000 samples)
• Genetic association (linkage disequilibrium mapping)	(1000–5000 samples)
• Genetic association (candidate genes)	(1000–5000 samples)
• Pharmacogenetics (pharmacokinetics)	(10–100 samples)
• Pharmacogenetics (efficacy)	(1000–5000 samples)
• Pharmacogenetics (safety)	(100–500 samples)

in their storage and handling of volunteers' DNA should also reassure the public that DNA banks can be developed and maintained to the highest possible standards.

Why and how is DNA stored?

Until relatively recently, large collections of DNA samples were not common in human genetics and it was not generally necessary to have specialized systems for storage, retrieval or formatting of DNA. Sample numbers were sufficiently low that tubes of isolated genomic DNA could be stored in laboratory fridges or freezers and, because it would take many years to complete genotyping or sequencing of these samples, tubes could be accessed manually whenever more DNA was needed (see for example a linkage study reported in 1991 on the rare disease tuberous sclerosis, using samples from 22 families [3]).

Now, however, DNA is used for many different types of study, some of which use thousands of different samples. Box 1 shows some of the main types of human genetics studies and the typical numbers of DNA samples used in them [1].

The increased demands for sample numbers and the technology available to generate genotyping data quickly and easily [4] have led to immense interest in the banking of genetic samples, with many biobanks being set up in the public and private sectors [5,6]. A variety of biological material is now banked for the purpose of genetic analysis such as isolated genomic DNA, whole blood, dried whole blood spots, buffy coats, buccal epithelial cells and immortalized lymphocytes [5–8].

A survey carried out in 2002 [5] looked at the types and numbers of samples that are being stored in biobanks in the European Union (EU). Isolated DNA samples formed the majority with a median number of 1000 samples stored in over 95 biobanks. National biobanks include the Medical Biobank in Sweden (www.biobanks.se/medicalbiobank.htm), Estonia (www.genomics.ee/), where samples from a million Estonian adults will be stored, and Japan (www.src.riken.go.jp/eng/src/project/person.html). Most of the systems surveyed are based on frozen genomic DNA samples accessed by a combination of manual and automated systems. The Japanese biobank incorporates a custom-built automated storage and retrieval system of 300,000 samples stored at 4°C.

Other examples include the UK national biobank (www.ukbiobank.ac.uk) where approximately half a million whole-blood samples intended for DNA extraction will be spotted onto filter paper in a 384-well configuration for storage. The UK National DNA Database® (www.forensic.gov.uk), the world's first national intelligence DNA database, goes one step further and only data generated from DNA samples (taken from individuals arrested for recordable offences) is stored. The samples themselves are destroyed.

Details of sample collections owned by private companies are more difficult to obtain. The best-known example is perhaps DeCode Genetics (www.decode.com), a public–private collaboration that has collected over 100,000 samples from Icelandic volunteers. Other biotech companies such as Oxagen (www.oxagen.co.uk), Genizon (www.galileogenomics.com/) and First Genetic Trust (www.firstgenetic.net) aim to make DNA samples available for disease and pharmacogenetic research. There is an increasing number of small companies with more specialized collections of DNA and material from which genetic material can be extracted.

Most pharmaceutical companies also have collections of DNA samples acquired either through research collaborations or from subjects enrolled in clinical trials. These collections can include hundreds of thousands of samples and, depending on the informed consent, data generated from them can be used in exploratory research and to support regulatory submissions of new drugs [9,10].

Genomics Collaborative Inc. (GCI) (www.genomicsinc.com) have recently moved from a manual system to an automated one [8]. Formerly, manual picking and plating a study of 1000 samples could take up to a month but their new automated system, co-developed with RTS Life Science (Irlam, UK), can pick and reformat over 4000 samples per day with minimal intervention. The GCI–RTS Life Science system requires the intervention of a laboratory worker to de-cap and re-cap tubes using a semi-automated system. A mixing system is not incorporated, even though tubes are stored at 4°C and –20°C. We will discuss the implications of this further, in this article.

As the size of DNA collections grows, and there is an increased need to access more samples quickly and accurately, genetics researchers can no longer rely on manual storage, retrieval and reformatting of samples. Locating

BOX 2

Key principles of GLP

- Test facility organization and personnel.
- Quality assurance programme.
- Adequate test facilities.
- Control of apparatus, materials and reagents.
- Test systems: conditions to ensure quality of the data.
- Test and reference items: handling, characterisation, stability.
- Rules: protocols and written standard operating procedures.
- Performance of the study: study plan and conduct.
- Reporting of study results.
- Storage and retention of records and materials.
- Documentation: raw data, final report and archives.

and retrieving the correct samples from crowded freezers and then aliquotting small volumes into 96- or 384-well microtitre plates manually is time-consuming and particularly open to human error. Automation of these processes should enable improved tracking, accuracy and consistency, as well as dramatically increasing throughput.

What guidelines and regulations cover DNA banking?

Regulations and guidelines that are relevant to the storage, retrieval and use of DNA samples vary among the USA, Europe and Japan. Most of these regulations are focused on the ethical and legal aspects of DNA sampling and research [11] but some also include aspects of organization and sample coding.

A recent example is the Swedish biobank act (www.sweden.gov.se/sb/d/3873/a/23126), which contains guidance for all identifiable (coded) biological samples collected under the Swedish healthcare system. As one of the key recommendations of this law is that these Swedish samples should be returned for storage in a registered biobank within Sweden, even if they are analyzed abroad, traceability of each sample is obviously of prime importance.

The quality standards for pharmacogenetics data used in drug development have been addressed in the FDA's recent Guidance for Industry: Pharmacogenomic Data Submissions (www.fda.gov/cder/guidance/6400fnl.pdf). Although the question of appropriate technical quality standards has not yet been addressed [12], data can be used for decision making or to support regulatory submissions (i.e. used as a valid or probable-valid biomarker) only if 'it is measured in an analytical test system with well-established performance characteristics'. Likewise, the recent FDA guidance document, Drug Metabolizing Enzyme Genotyping System (www.fda.gov/cdrh/oivd/guidance/1551.html) states that 'Consideration of pre-analytical factors is critical for high-quality genetic tests…You should evaluate the accuracy and precision of your assay, including DNA extraction, from all the sources of DNA that you recommend…You should also evaluate all sample collection and storage options you recommend.'

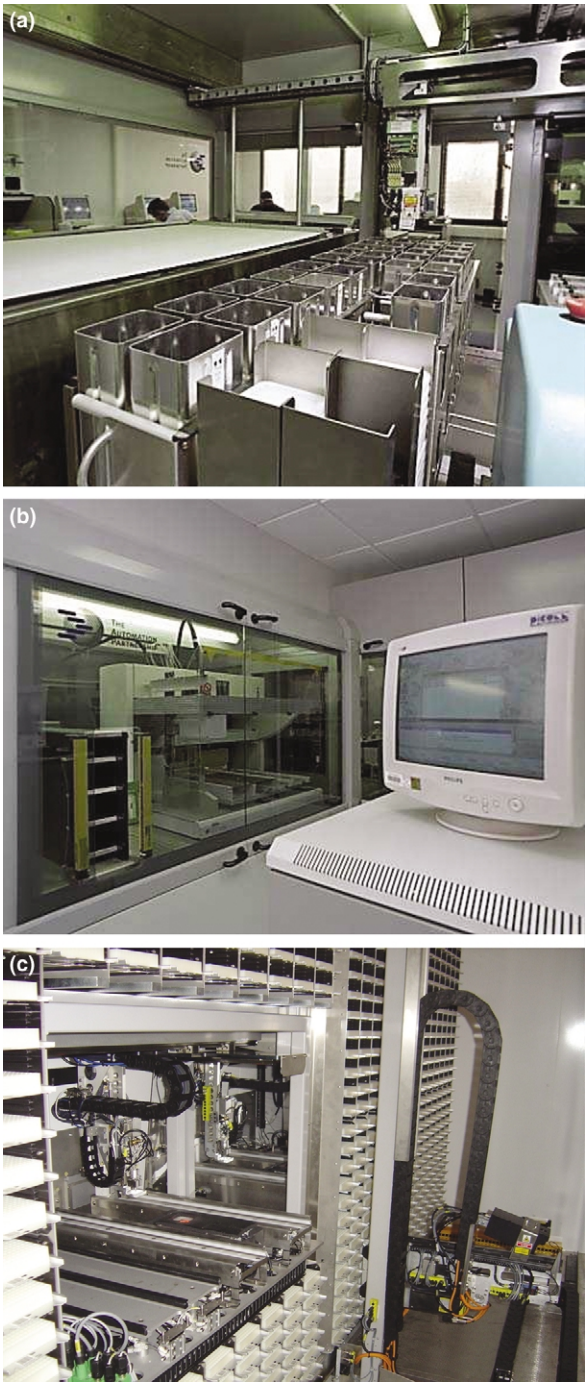

FIGURE 1

The DART system. These pictures show **(a)** the DART plate handling module, **(b)** the DART liquid handling module and **(c)** the DART tube store.

In most countries, the most relevant quality standards for such data are believed to be those of good laboratory practice (GLP) (Box 2) [13]. These regulations do not contain specific guidance to developers of DNA-banking systems but the key principles highlighted can easily be adapted to specific applications.

How should a DNA storage system be validated?

International Conference on Harmonization (ICH) guidance states that the objective of validating an analytical

procedure is to demonstrate that it is suitable for its intended purpose (www.fda.gov/cder/guidance/ichq2a.pdf). In the case of DNA banking, factors that are most likely to affect the intended purpose (i.e. providing material for downstream processes such as genotyping and sequencing) are those affecting sample integrity. For example, DNA samples can be contaminated by neighbouring samples (either by sample carry-over on pipette tips or by aerosols created during sample handling). This contamination could be detected during sensitive downstream processes. Other factors affecting sample integrity are conditions of storage, that might lead to degradation of the sample (temperature, evaporation, microbial growth, shearing) or that could affect the capability to reformat DNA samples accurately for downstream processing (evaporation, mixing, pipetting accuracy).

There is little available in the literature on the validation of DNA storage and reformatting systems. In this article, we will use the AstraZeneca automated DNA archive and reformatting system (DART) (Figure 1) as a case study of how such a system can be developed successfully and validated within the principles of GLP.

Development and validation of AstraZeneca's DART system

The development and validation of a regulated system starts with the selection of the parameters required and the supplier(s) who can develop them. AstraZeneca, like most pharmaceutical companies, uses genetics to support the development of new and improved medicines throughout the R&D process. The DNA-banking system plays an essential role in the flow of genetic data from patients and genetics researchers to the application of genetic research in the clinic (Figure 2). Therefore, the banking system must fulfil certain minimum requirements, which are listed in Box 3.

The system that could meet some of AstraZeneca's requirements was The Automation Partnership's (TAP) archiving and liquid handling system, Solar. This system is normally used for storage and retrieval of compounds in dimethyl sulfoxide (DMSO). Consequently TAP custom built a version of Solar to handle DNA for AstraZeneca. This process involved a lengthy period of planning and development when many major decisions were made. This included the overall design, capacity and throughput of the system, the choice of barcoded vessels for DNA storage and output, and the selection of a mixing method.

BOX 3

Requirements for AstraZeneca's DNA banking system

- Fully automated storage, retrieval and reformatting system.
- Tracking of operators and access permissions.
- Liquid, genomic DNA stored at –20°C.
- Storage of >400,000 tubes.
- Ability to 'cherry pick' up to 650 tubes per hour.
- Storage of >650 tubes per hour.
- Output of >5000 samples per day reformatted into microtitre plates.
- Accessing of tubes via split-septum caps.
- Pipetting accuracy error >5%.
- Pipetting precision error >3%.
- Individual DNA samples tracked via 2D data matrix barcoded tubes.
- Thawing DNA without risk of microbial growth.
- No significant degradation of DNA during maximum freeze–thaw cycles.
- Tip washing regime sufficient to show no significant sample carry-over.
- Mixing regime to restore DNA homogeneity before reformatting.
- No significant evaporation during maximum life of the cap.

We decided to store DNA within the DART system as isolated liquid genomic DNA at –20°C. This is in contrast to the GCI biobank [8] that stores some of its DNA at 4°C and the UK biobank, which is expected to store the majority of its DNA as whole blood on filter paper. Previous studies [14–16] have shown that DNA stored at –20°C is more stable than at 4°C, and liquid genomic DNA is easily accessible compared with DNA stored on filter paper.

The DART system incorporates a Tecan Genesis RSP100 eight-tip liquid-handling robot, which accesses DNA through a SepraSeal™ cap into a TrakMates® tube (Matrix Technologies, Wilmslow, UK). The maximum volume of DNA that could be stored in these tubes is 800 µl. The minimum volume of DNA that could be pipetted within the required accuracy and precision parameters was expected to be 10 µl. Therefore, every TrakMates® tube can be accessed a maximum of 80 times.

As part of the installation and handover of the system by the manufacturer, DART underwent a series of acceptance tests. An overview of these tests, and the evidence recorded that shows DART can meet its requirements, is given in Table 1.

Sample storage and use within DART is controlled and tracked by a fully-integrated genotyping laboratory information management system (LIMS). The genotyping LIMS is a customized version of the LabVantage Sapphire™ LIMS (Bridgewater, New Jersey, USA) and is a fully validated,

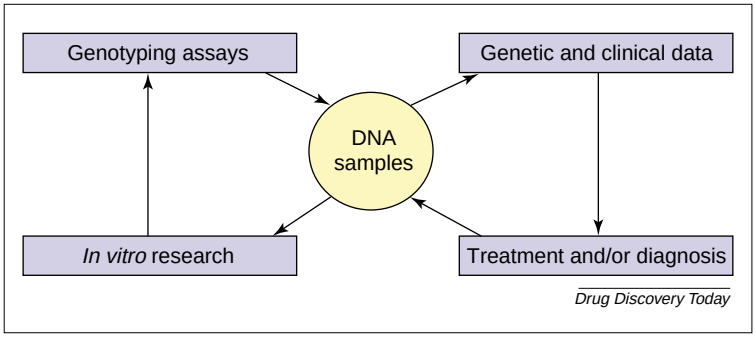

FIGURE 2

The central place of DNA banking in genetics research. This picture illustrates the flow of genetic data from patients and genetics researchers to the application of genetic research in the clinic.

TABLE 1

DART requirements, nature of acceptance tests and results

Requirement	Means of measurement	Result
Storage of >400,000 tubes	Counting of tubes in archive	464,100 tubes stored
Individual DNA samples tracked via 2D data matrix barcoded tubes	Position of all tubes tracked by barcode. Tubes that have been deliberately misplaced are rejected.	Position of individual tubes checked. On finding the wrong tube DART rejects the tube rack
Conditions established to thaw DNA without risk of microbial growth	Time taken to thaw rack of tubes at 25°C	2.5 h
Ability to 'cherry pick' up to 650 tubes per hour	Tubes containing coloured dye correctly picked from different storage zones	176 tubes accessed in 14 min 18 s
Storage of >650 tubes per hour	Count tubes stored	658 tubes stored in 53 min 15 s
Output of >5000 samples per 24 h	Time taken to reformat samples into 384-well plate	384 samples reformatted in 38 min
Tracking of operators and access permissions	All operators have login system.	All users logged. No users can log in with missing or incorrect passwords.
Pipetting accuracy error <5%	Bias over a range of pipetting 10 µl to 100 µl	Bias recorded 1.0% to 3.4%
Pipetting precision error <3%	CV over plate when pipetting 10 µl to 100 µl	CV recorded 0.8% to 2.8%

good clinical practice (GCP) compliant system that handles all aspects of DNA sample handling from DNA extraction through to genotyping. The genotyping LIMS enables full tracking of all the required information relating to each sample, including records of the concentration and volume of DNA available and audit trails recording the entire usage history of each individual tube. In addition, special consideration was given to aspects of DART's development that were not covered in the normal operation of the Solar system.

Assess cross contamination from tips and sample carryover

To avoid the possibility of DNA being carried across to other samples, leading to ambiguous genotyping results, we optimized a reliable wash regime that removes detectable DNA contamination from the Teflon-coated pipetting tip after each tube is accessed. The wash regime selected for operational use washed 5 ml water through the inside followed by 5 ml water over the outside of each pipetting tip. To ensure there was no build up of DNA after multiple samples were accessed, the tips were tested for contamination after pipetting 2496 samples. No contamination was detected.

As explained already, the anticipated maximum number of times that any individual tube could be accessed in DART is 80. To check whether or not cross-contamination could occur over 80 cycles of plate creation, two racks of alternate columns of tubes containing DNA or water were accessed 80 times. Samples from the water tubes were tested periodically for contamination by amplification in a standard PCR.

We were able to detect weak contamination in a small number of water samples between 50 and 80 cycles of plate creation. However, it is important to realize that this level of contamination was unlikely to have been detected in the normal operating situation because it is easier to amplify traces of DNA from water than from stock DNA. We concluded that the risk of compromising downstream genetic data in this way is negligible.

Tip washing is crucial for maintaining sample integrity, and it is possible that a build up of contamination could occur on the tips after large numbers of samples have been pipetted. We decided to use a 1% sodium hypochlorite tip wash at the start of each day when plates were created and after every 2496 samples have been processed.

Evaluate DNA shearing

Passing DNA through a small-bore needle and applying pressure to DNA can be used as shearing methods [17], therefore, we designed an experiment to check that multiple cycles of pipetting followed by freezing to –20°C and thawing at 25°C [18] would not shear DNA stored inside DART. DNA samples were taken through 80 cycles of freeze–thawing, during which the integrity of the DNA was tested at intervals by amplifying a large fragment of DNA by PCR. After 80 cycles all samples amplified successfully, demonstrating that thawing and mixing of DNA over 80 cycles does not significantly compromise DNA integrity.

Control Evaporation

We did a series of experiments to check whether evaporation would occur from tubes with split-septum caps. Evaporation could reduce the volume of the samples, thus, increasing their concentrations and potentially affecting genotyping results.

We weighed a set of tubes filled with water, with caps that had been accessed different numbers of times (up to a maximum of 80), alongside a set of empty tubes with unpierced caps as controls. The tubes were subjected to 80 freeze–thaw cycles, re-weighed and the weight difference calculated. The greatest weight loss occurred in one of the eight tubes that had been accessed 80 times, but this was only 2.3%, and overall evaporation loss from the tubes was not statistically significant ($p>0.05$). We concluded that evaporation is not a significant problem in DART.

Ensure DNA mixing

DNA frozen at –20°C tends to form a concentration gradient on thawing. Hence, it is normal laboratory practice to mix thawed tubes before removing aliquots of DNA, to prevent smaller or larger amounts of DNA being transferred for downstream processing (such as genotyping) with potentially misleading results.

During the development of DART, it was proposed that the Tecan Genesis RSP100 eight-tip liquid-handling robot would be used to mix samples. However, this was found to be unsuitable after DNA contamination was detected in the system fluid as a result of pipetting large volumes of DNA.

We explored other options, including an automated magnetic mixing system using the vertical movement of magnetic stirrer balls (www.vp-scientific.com/magnetic_levitation.htm). With the collaboration of V&P Scientific Inc. (San Diego, USA) and TAP, a system was designed so that four racks of 96 tubes (each containing two 2.47 mm magnetic stirrer balls) could be mixed simultaneously by passing between fixed magnets on a platform.

We designed experiments to find the minimum speed and number of cycles required to mix the DNA thoroughly within DART. A concentration gradient was created in two sets of tubes by freezing and thawing the tubes several times without mixing. Each set of tubes was then subjected to a range of mixing repetitions at two different tray-movement speeds. We carefully removed DNA aliquots from different heights within the tubes and determined the concentration of each aliquot. Control samples were mixed by vortexing. To have confidence that the mixing regime chosen was effective at mixing the DNA sample, data were only accepted if the standard error (SE) of the concentration of the aliquots taken from one tube was within the 95% confidence interval (CI) of the SE of the concentration of the aliquots taken from the vortexed tubes (95% CI = 1.30). At 400 mm/s with 20 mixing cycles mixing was observed to be sufficient (mean SE = 1.03) and we chose these operating conditions for the DART system.

Determine the accuracy and precision of the liquid handling robot

In early experiments we found that good pipetting accuracy is difficult to achieve when aspirating DNA from septum sealed tubes, because of the viscosity of the DNA and the pressure differences between the interior of the tube and the external atmosphere. Slow cap piercing and slow aspiration and dispensing speeds are essential to pipette DNA accurately out of tubes sealed with split septum caps.

To validate the accuracy and precision of the DART liquid-handling system, a set of tubes was weighed before and after various volumes of DNA were pipetted into them using optimized liquid handling parameters. Accuracy error (bias from expected volume) and precision were calculated by statistical analysis of the results.

The liquid-handling system achieved an overall accuracy error of standard deviation <5% and precision error with a coefficient of variance <3%. This is in line with the manufacturer's expectations and with published accuracy and precision levels for this type of robot [19]. The small amount of variability between the volumes pipetted into plates is unlikely to affect downstream genotyping activities, so we concluded that pipetting accuracy and precision in the DART system is within acceptable limits.

Ongoing QA, QC and maintenance

As discussed, the main concerns for GLP compliance within DART are sample traceability and the maintenance of sample integrity. Regular maintenance and periodic quality assurance (QA) and quality control (QC) procedures have been implemented that will help ensure sample traceability and integrity are preserved. QA is assessed by procedural review and by full system and facility audits. QC procedures monitor sample traceability by tracing stock DNA samples back to their original blood samples using micro-satellite profiling. Further QC testing is carried out on a specific set of QC DNA samples to check for contamination through repeated access of tubes during plating operations and to check for degradation as a result of repeated freeze–thaw cycles.

Summary and prospective

In recent years dramatic improvements in the throughput of genetic technologies have meant that manual storage, retrieval and reformatting of DNA samples are no longer adequate. Automation of DNA banking can dramatically increase throughput, eliminate manual errors and improve the productivity of genetics research.

Companies that have automated their DNA banking systems, such as GCI and AstraZeneca, can demonstrate a marked improvement in throughput and productivity. In the case of GCI [8], the RTS Life Science system enabled the plating out of 1000 samples in one day, with minimal manual intervention, which would have taken one month with the manual system.

In the case of AstraZeneca's DART system, which is fully automated, a recent pharmacogenetics study of 2000 samples took a single operator less than two days to format into plates. Accessing and processing these samples in a manual archiving system within the principles of GLP would previously have taken up to a month.

A result of this automation is that retrieval and reformatting of DNA samples is no longer a rate-limiting step in genetic studies. Samples can be stored in secure systems within the principles of GLP, tracked through a barcoded system and accessed in a controlled manner. In the future, we hope that the increased availability of such automated systems will allow researchers to use this precious resource as intended by the volunteers who provide such samples, to discover genes involved in common disease and to help researchers develop new and improved treatments.

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