Banking on Results – Biobanks Maintain Sample Viability

Human biological material has been collected, stored and used for a variety of purposes almost since the dawn of modern medicine. However, the combination of high-throughput molecular genetic analysis and the explosion in computing power makes possible for the first time the mining of this physical medium for data to link human health or disease to molecular characteristics. Enter the Biobank – a potentially vital resource for identifying the causes and mechanisms of diseases, both hereditary and environmental.

Cancer Banks, as the name suggests, are collections of tissue and blood collected from patients where cancer is a possible diagnosis. Given that many research studies are aimed at increasing our understanding of the molecular mechanisms involved in tumour initiation, metastatis, and response to treatment, the cancer bank holds out the hope of accelerating the development of more effective, targeted treatments through the availability of high-quality patient samples.

With the collection of samples starting in January 2005, the Wales Cancer Bank (WCB) was the first population-based collection of tumour and control tissue samples to be established in the UK. A collaborative project involving Higher Education establishments, various NHS Trusts in Wales, funded by the Wales Assembly Government Office of Research and Development, and Cancer Research Wales, the WCB aims to collect voluntary samples from all patients in Wales who undergo an operation to remove tissue where cancer is a possible diagnosis.

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SAMPLE COLLECTION

Overall, Wales is a relatively sparsely populated country with just 140 people per square kilometre. The population is relatively stable, and the Welsh clinical database for patients with cancer (CANISC) makes it feasible to collect both initial and follow-up. This coupled with results from research studies using the Wales Cancer Bank, where access to material is conditional upon return of results on a case-by-case basis, provides an excellent opportunity to link molecular biological studies with clinical outcome.

A large proportion of the population of Wales lives and works in a small area bounded by the Severn Bridges to the East, the end of the M40 to the west and the Brecon Beacons to the north, containing the major conurbations of Cardiff, Swansea, and Newport. The WCB currently works through five hospitals to service this area – Cardiff (2), Swansea (2) and Newport - with Bangor and Haverfordwest currently supporting areas of North and West Wales. Through specialist nurses in these hospitals, the WCB approaches patients with proven or suspected cancer to request tissue and blood samples.

To date, 2676 patients have donated biological material (*Figure 1*). The Wales Cancer Bank collects a variety of different types of samples: EDTA blood and serum from patients and EDTA blood from a non-blood related control (spouse, partner or friend); and paired samples of frozen and paraffin embedded tumour and normal tissue.

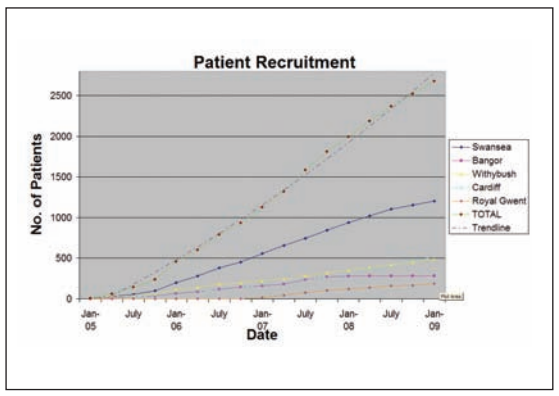


Figure 1. Patient recruitment from January 2005 until present

This amounts to several thousand individual samples that can be used in different research projects.

Not all tumours are collected in every hospital yet, but each collection centre aims to gradually expand the tumour types collected until all are covered. With the recent inclusion of cervical cancer, seventeen different tumour types are currently being collected (*Figure 2*). Three types of material account for almost three-quarters of the samples: breast samples (37%) are collected from Swansea and Haverfordwest, colorectal samples (20%) are collected from all sites and prostate (16%) in Cardiff, Bangor and Swansea.

Importantly, donation to the Wales Cancer Bank does not alter the treatment of the patient in any way. The tissue collected is that which is removed during the patient's routine surgery and remains after all the necessary diagnostic tests have been concluded and would normally be discarded. Where possible, blood samples are taken at routine pre-operative sampling, but may occasionally be at another convenient time. And the anonymity of donors is safeguarded through the use of an individual patient identification number unrelated to any other patient identifier (for example, NHS number and histology number).

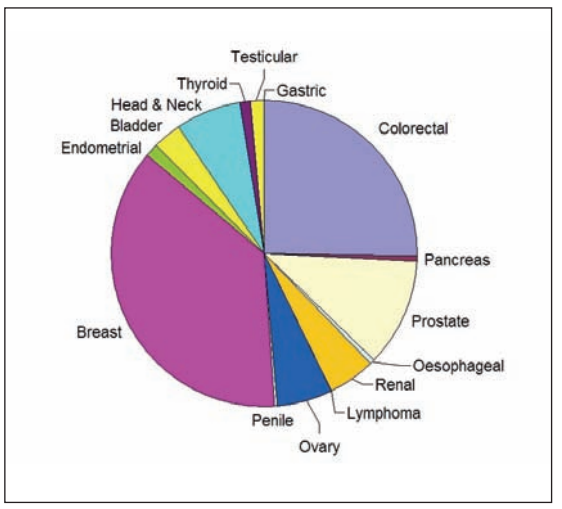


Figure 2. Collection by tumour type

SAMPLE PROCESSING

After collection, initial processing, such as freezing or embedding in paraffin, takes place in the Histopathology departments of the seven participating hospitals where the tissue is then stored. When material is requested by a an individual researcher or research team, the WCB searches its databank for a relevant sample or samples and further processing takes place to provide samples in the format that suits the researchers needs. Samples are supplied with a basic minimum dataset of gender, age, type and grade of tumour and sub-type (where applicable) and appropriate diagnostic information, such as hormone receptor status for breast cancers (*Table 1*) (below).

DNA and RNA are extracted from frozen tissue at Swansea. Frozen blocks of tissue are sectioned in a cryostat (Carl Zeiss) prior to processing to assess the amount of tumour present. RNA and DNA are extracted from the same tissue block to enable direct comparison of changes in gene regulation at the DNA and RNA level.

From FROZEN blocks	**Size**	**Quantity**
Single tumour slides	5 micron	5/box
Matched tumour and normal slides (one box of each)	5 micron	5/box
Single tumour sections (in eppendorfs)	30 micron	5
Matched tumour and normal sections (in eppendorfs)	30 micron	5
Extracted nucleic acid – all associated QA data		
Extracted DNA single tumour	3 microgram	1
Extracted RNA single tumour	5 micrograms	1
Extracted DNA matched tumour and normal	3 microgram	1
Extracted RNA matched tumour and normal	5 micrograms	1
From PARAFFIN blocks		
Single tumour slides	5 micron	5/box
Matched tumour and normal slides (one box of each)	5 micron	5/box
From BLOOD		
Genomic DNA	5 micrograms	1
Serum	500 microlitres	1

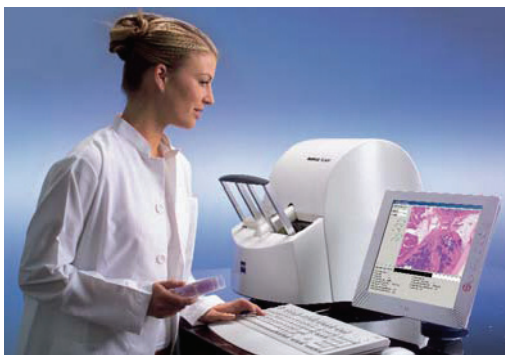


Figure 3. Initial review of digital slides from Mirax Scan on screen

This method ensures extraction of high quality DNA, for use in techniques such as array CGH, and high quality RNA, for use in expression microarray systems. DNA is extracted using a DNA kit (Qiagen) and routinely supplied as three microgram in 50 microlitres, while RNA is supplied as 5 micrograms in 50 microlitres after extraction with a kit (Qiagen). RNA quality is assessed by 260/230 and 260/280 ratio using a nanospectrophotometer and then subjected to QA by Agilent Bioanalyser. DNA quality is assessed by spectrophotometry and by gel electrophoresis.

DNA from blood is extracted at Cardiff Gene Park, using an automated Gentra system. This DNA is routinely available in one microgram aliquots. Tissue Microarrays are made at Cardiff from samples embedded in paraffin.

DIGITAL QUALITY ASSURANCE

"The sheer quantity of samples that the WCB makes available from individual tissue samples is a huge advance," said Professor Gerry Thomas, Director of Scientific Services. "It allows different research groups to directly compare their findings across a variety of sample types and analytical techniques and should pay dividends as a 'Systems Biology' approach to cancer research.

"However, it is crucial that a complete record of the tissue sample is documented before processing begins and that all tissue samples from tumours have its presence verified by a pathologist and that a permanent record of the H & E (hematoxylin and eosin) slide is available for review. Given that collection takes place currently at seven sites, with the possibility of further sites in the future, coping with increasing workloads while maintaining the accuracy, precision and quality of results was a key requirement.

"After looking at various solutions, we chose the Mirax range of fully-automated slide scanning systems from Carl Zeiss to digitise the raw samples and to verify and archive the presence of tumour in relevant sections (*Figure 3*). The WCB now has two Mirax Scan installed at Cardiff and Swansea, with a pair of Mirax Desk units at Bangor and Haverfordwest."

Rather than using a conventional microscope the optics and optics technology from Zeiss is integrated with the digital archiving experience of IBM to produce a high resolution 'digital slide' of each specimen – up

Figure 4. Simple loading of up to 300 slides

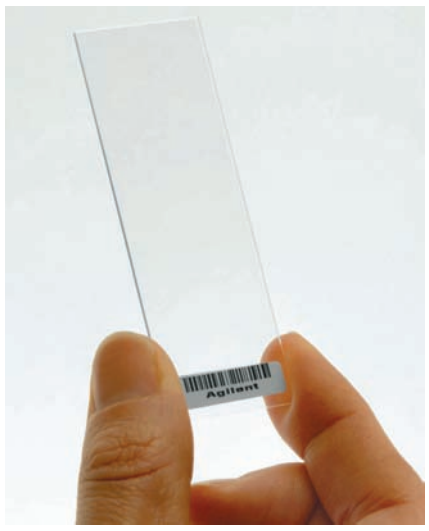
to 300 slides at a time in fluorescence, phase contrast or brightfield mode. .

The instruments' operation is controlled by the Mirax View software package that displays the virtual slides (*Figure 4*), which can be viewed either individually or in groups. A teleconsulting module allows virtual slides to be viewed and evaluated by more than one pathologist in real time.

"Image quality, while important, is only part of what we gained when we opted for the Zeiss Mirax systems and Cryostats" said Thomas. "Comprehensive training and support across all our sites is the hidden advantage that has underpinned our image recording process."

More information on Mirax is available on the Zeiss website (www.zeiss.de/mirax).

Custom miRNA Capability to Online Microarray Design Tool



Agilent Technologies, Inc recently announced the release of eArray 5.4 online microarray design tool, which enables users to design custom microRNA (miRNA) microarrays. miRNA is a rapidly emerging field of study because researchers are increasingly associating these abundant molecules with some types of cancers, heart disease, other disorders and stem-cell differentiation.

Researchers can use Agilent's pre-designed human, mouse or rat miRNA probes, or access miRNA sequences for all 87 species in Sanger 12.0 in the designs of their miRNA microarrays.

Agilent also announced updates to its human and mouse microarrays, miRNA Release 12.0, which contain the latest probes from Sanger miRBASE 12.0 database. Like all Agilent miRNA microarrays, these include about 15,000 features and are printed eight-arrays-per-slide.

"The miRNA community is eager to profile more miRNAs as they become available in the public domain, and we're enabling researchers to do this rapidly on a proven microarray platform with robust, user-friendly protocols," said Sangita Parikh, Agilent miRNA Product Manager.

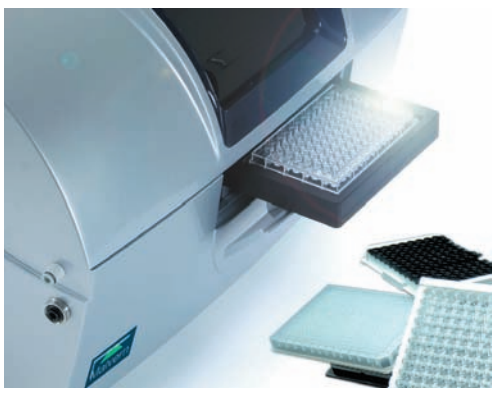
eArray enables users to design custom microarrays by choosing from Agilent-optimised probes, uploading their own sequences or designing new probes using eArray tools. When the array design is complete, the file is uploaded to Agilent where the ink-jet-based SurePrint fabrication can print any probes at any location.

Sixty mer oligos provide a high level of sensitivity. Delivery of the finished microarrays can be made in two weeks. eArray also enables researchers to collaborate on designs from remote locations.

Circle no. 245

Standard Microwell Plates Deliver Fully Automated DLS Measurement

Malvern's new Zetasizer Auto Plate Sampler (APS) is designed primarily for protein specialists, delivering fully automated protein and nanoparticle size measurements using dynamic light scattering (DLS), for improved productivity. Unlike other systems, the Zetasizer APS does not require micro well plates to be flat-bottomed or have specific optical properties. A unique sample handling process makes it compatible with virtually all commercially available microwell plates.



Automated measurement procedures using industry standard 96- or 384-well plates enable users to simply insert a sample plate and press 'start'. The Zetasizer APS delivers accurate and repeatable protein size and molecular weight data with no compromise in data quality due to automating the measurements. Only 20µl of sample is used, and this is returned to the same well for use for other characterization techniques. Programmable dual temperature controls maintain optimal conditions for samples in the well plate while enabling automated temperature trend measurements for melting point determination, protein purity or shelf-life investigations for example. DLS data can be used to solve application problems in bioprocessing, drug target development and protein therapeutics. DLS measures the thermal diffusion of proteins and nanoparticles in solution, and uses this property to determine an absolute hydrodynamic size. This technique is especially sensitive to the presence of aggregates, and their proportion as a function of solvent and buffer conditions can also be determined, speeding development of the protein's optimum environmental conditions.

The Zetasizer APS from Malvern Instruments allows quick and easy investigation of a protein's condition at different stages of the purification process, and at later stages of formulation under a range of environmental conditions. User-friendly software and a worldwide customer support network complete the package, making this system an ideal solution for protein characterisation.

Circle no. 246