MANCHESTER 1824

The University of Manchester

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From the sample to the researcher
Types of samples, sample preparation, manipulation and archiving.

Centre for Integrated Genomic Medical Research

Sample handling and storage has improved dramatically! Though not for everyone

Then

Circa 1976

Now

2007

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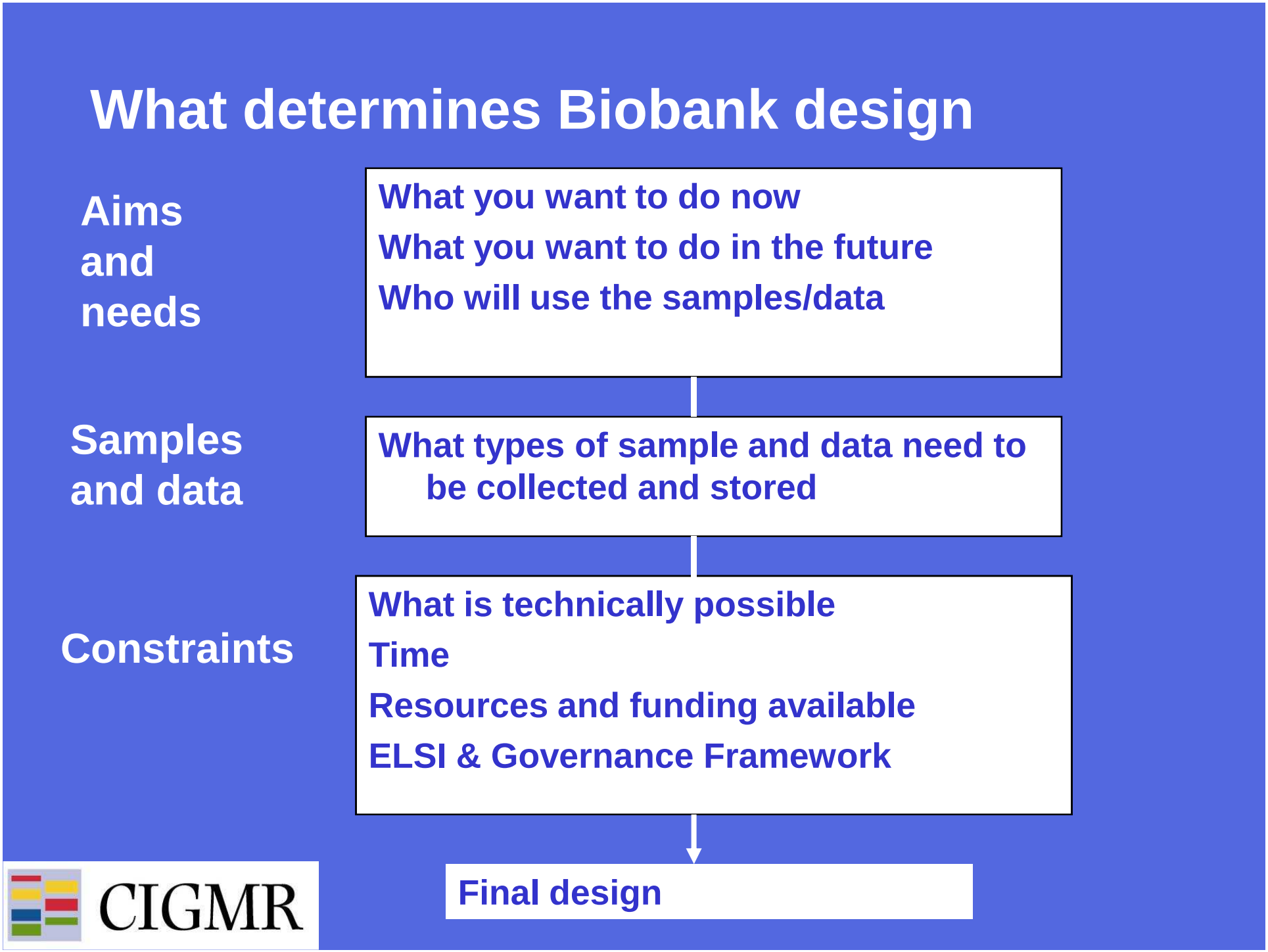
What determines Biobank design



Types of samples

- Determined by what down-stream analysis applications are planned
- Bio-banks are largely either focussed around “liquid” or “solid” samples (some store both)
- Solid and liquid archives have some infrastructure requirements in common (e.g. LIMS) but have may distinct needs and characteristics

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Types of samples- Tissues

- Tissues
  - biopsy
  - surgical and clinical "waste"
  - post- mortem

- Uses
  - proteomics
  - gene expression
  - culture
  - localisation, immuno / insitu hybridisation
  - histological, toxicity and functional studies

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Types of samples- Tissues

- Wide range of processing and storage needs/conditions e.g.

- Formalin fixed paraffin embedded pathology samples stored at room temperature and less need for immediate processing
- Tissues for proteomics require immediate processing / snap freezing and storage in LN2
- Culture samples may require complex processing (e.g. cell suspensions) , and specialist cryopreservation

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Types of samples – Hair and nails

- **Uses**
  - **DNA extraction (nails and hair follicles)**
  - **environmental and drug exposure levels (some complications !)**

- **Storage**
  - **relatively straight forward and usually in room temperature and dry conditions**

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Types of samples - Urine

- Collection – usually as a mid stream sample
- Uses
  - metabolomics
  - clinical chemistry

- Storage
  - with or with out boric acid and stored at
    - 80 C

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Types of samples - Saliva

- Collection either into dry tube or Oragene pot
- Uses
  - DNA and RNA (Oragene)
  - Cortisol levels (HPA axis)
  - metabolomics
  - secretary IgA
  - smoking exposure / cotanine assays

- Storage at – 80 C for whole saliva or various for processed saliva components (RT for Oragene)

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Types of samples- viable blood cells

- Uses – for immortalised cell line transformation or functional studies
- Collected as ACD or preservative free heparinised blood sample
  - processing required for PBL and Buffy coat (Ficol gradient and /or centrifugation)
  - no processing needs for whole blood
  - specific cryopreservation to ensure viablity
- Storage in LN2

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Types of samples- Plasma

- Uses
  - proteomics and metabolomics
  - antibody profiles
  - inflammatory and other biomarkers
  - clinical chemistry and clotting studies

- Usually collected as a by product of EDTA blood sample taken for DNA
  - requires chilled transport and rapid processing for proteomic studies
  - storage at - 80 C or LN2 in multiple aliquots
  - new RT storage methods becoming available

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Types of samples- Serum

- **Uses**

  similar to that for plasma but less applications

- **Collection as a dry tube or clot- activating gel tubes**

- **Storage- at -80 C or LN2 as multiple aliquots (RT storage solutions being developed)**

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Types of samples- DNA

- Uses
  - wide range of genetic and genomic applications
  - methylation and epigenetics
  - exposure / DNA adduct formation
- Collected from a range of samples
  - mouth swabs (small amounts)
  - saliva (reasonable amounts)
  - EDTA blood

All need down-stream processing (wide range of methods)

- Storage in TE from + 4 to -80 C

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Types of samples- RNA

- Uses
  - transcriptomic and gene expression
- Collection
  - usually extracted from anti-coagulated blood but can also be derived from tissue and saliva
  - requires expensive processing
- Storage – at either – 80 C or LN2 (RT alternatives becoming available)

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Sample preparation and manipulation

- A wide range of procedures may be required
  - these include sample login and tracking, processing , extraction, measurement, normalisation, aliquotting, cherry picking and distribution
- Dependent on need, throughput and budget you will need to decide on the level of automation required and protocols used
- All should be conducted under a Quality assured environment

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Sample logging and tracking

- Why? – Collection management
  how many samples?
  what kind of samples?
  where are the samples?
  what's happened to the samples?

- How?
  LIMS – laboratory information management system
  Relational database which records an audit trail for each of your samples. E.g. Nautilus from ThermoElectron Ltd.

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Choosing your robots

- **Examples:**
- **Liquid handling robotics - Generalists**

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Specialists

BioMicroLab Cherrypicking Robot

PSS DNA Extraction Machine

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Estimation of DNA concentration

- Picogreen on Tecan robotic work station
- Nanodrop single and multichannel

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DNA normalisation

- Hamilton Star Robotic Work Station
- Normalised and stock aliquots made in 2D bar coded tubes for secure sample tracking
- Normalised aliquots stored on two geographical sites

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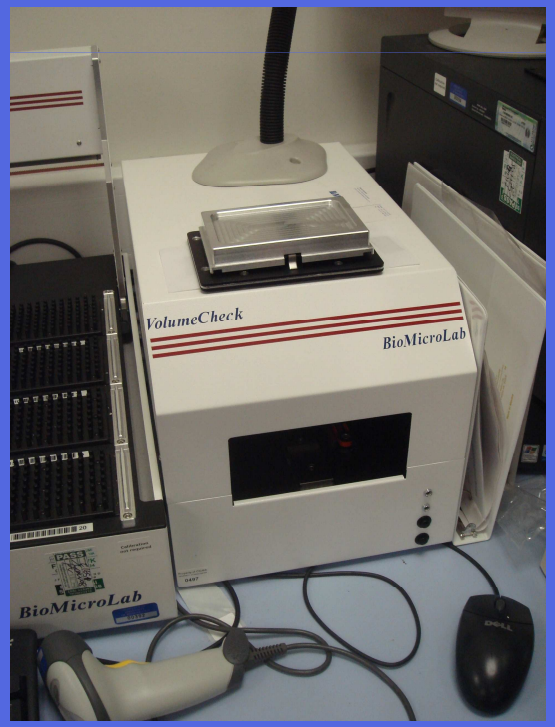
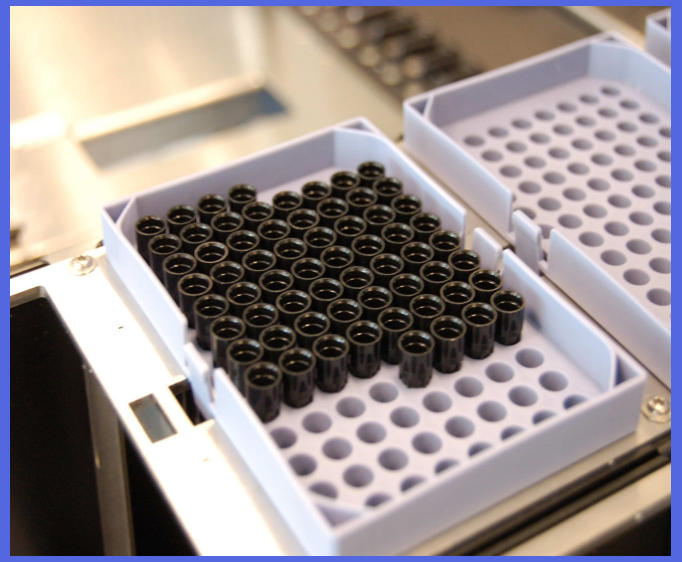
DNA Aliquoting

- Tecan Genesis Freedom
- Ensure LIMS stores the correct volume of sample





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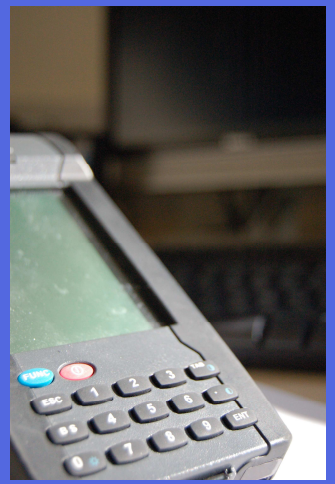
DNA storage

- All DNA is stored at -80^{O}C in freezers which are monitored by automated telemetry systems and have CO_2 back-ups
- Freezers have bar coded compartments
- Staff ‘scan’ racks of samples in and out of freezer locations using hand held PDAs
- The PDA uploads the location info into LIMS when it is docked



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Sample archiving

- There are many solutions which need to considered in the context of type of sample, biobank size, expected with-drawl and budget

- Where ever possible all samples should be tracked using 2D bar code labels and have complete audit trail

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2D bar-coded tubes

- The code, in a 14 x 14 array, provides 3.6 quadrillion (3.6 x 1015) unique codes assuring an endless supply of code combinations.
- Individually labelled 2D barcode labelled micro tubes in 96 array
- Sealed with robot split septum's
- Enables cherry picking of ordered tubes from the rack
- Minimising free/thaw cycles

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Automated sample stores now available – range of sizes and temperatures (-20 to -80) and also range of costs !

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Large scale LN2 storage facilities

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Freezer back-ups and second sites

Minimizing risk of catastrophic loss

- **Freezer alarm system, audio alarm and automated e-mail and text alerts**
- **$C0_2$ back-up system for -80^{0}C freezers**

Second Site storage

- **Geographically separate from primary site**

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Energy costs are now a primary consideration in large scale biobanks

- Running costs for large -80 C freezer farms and large scale LN2 facilities are becoming prohibitive and difficult to justify as energy cost increase
- Low temperature storage facilities present problems for automated rapid sample retrieval
- Health and safety considerations with low temperature solutions
- The above is driving increased interest in RT storage solutions

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DNA storage media

- ISOCODE DNA Storage Cards
- Blood or DNA applied directly to the card
- DNA eluted off
- Cost effective storage media
- No DNA purification required
- No refrigeration required
- Sample only eluted as required for investigate
- Very high storage density possible

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DNA safe ISOCODE storage

PDA

Bespoke program:

- **Audits user**
- **Validates bar codes**

LIMS

Safes:

- **Fire proof**
- **Flood Proof**
- **Store 12-15,000 cards**
- **No running costs**

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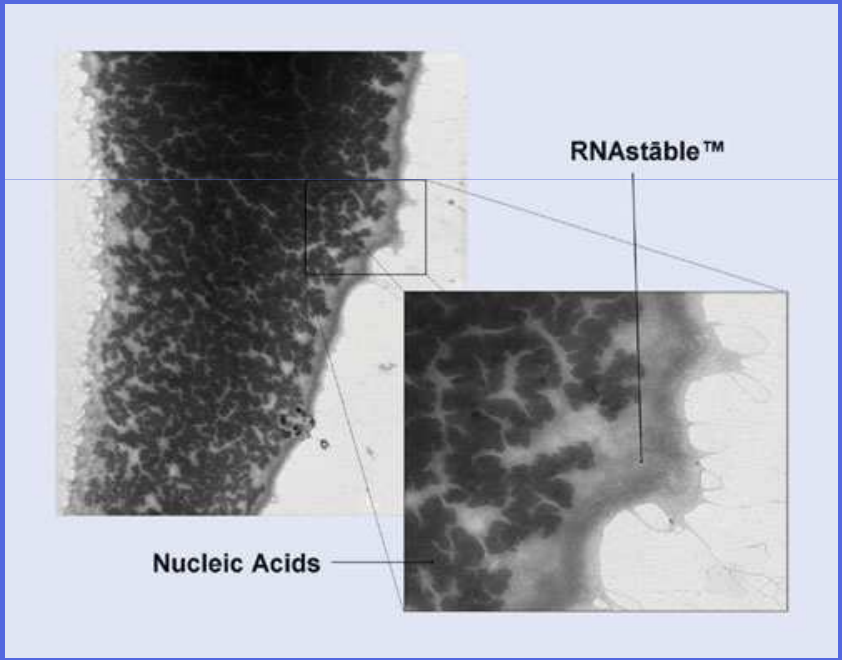
Room temperature storage of DNA



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Some banks are storing oragene samples at RT

- Samples can maintained at RT before extraction of DNA or can be frozen
- Samples could be transferred into matrix 2D tubes for easy manipulation

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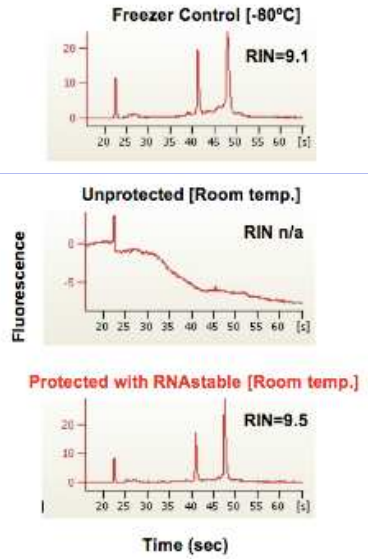
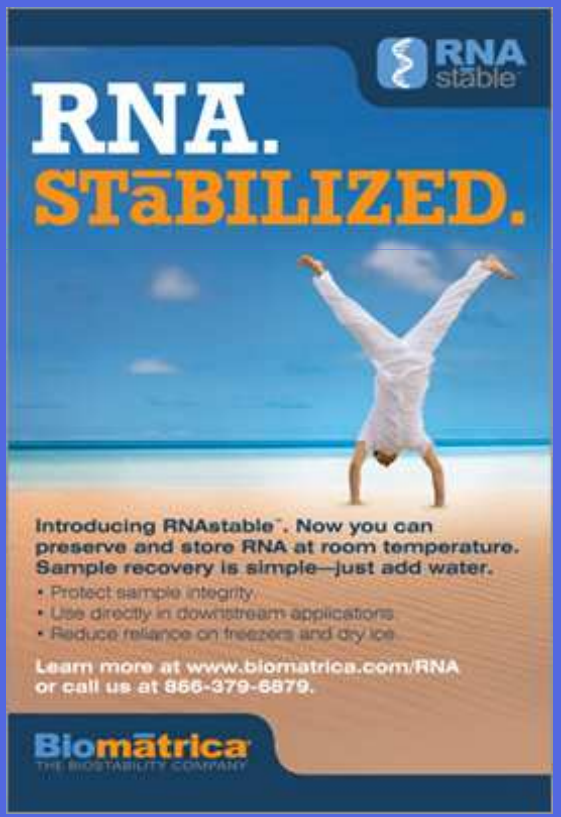
Room temperature storage of RNA

RNAstable protects RNA sample integrity at room temperature



Agilent 2100 Bioanalyzer: Samples stored for 10 months

Each total RNA sample (100 ng) was analyzed on the Agilent 2100 Bioanalyzer. The 28S rRNA:18S rRNA ratio is equivalent in all tested temperatures (-80°C and room temp.) at 10 months after storage.



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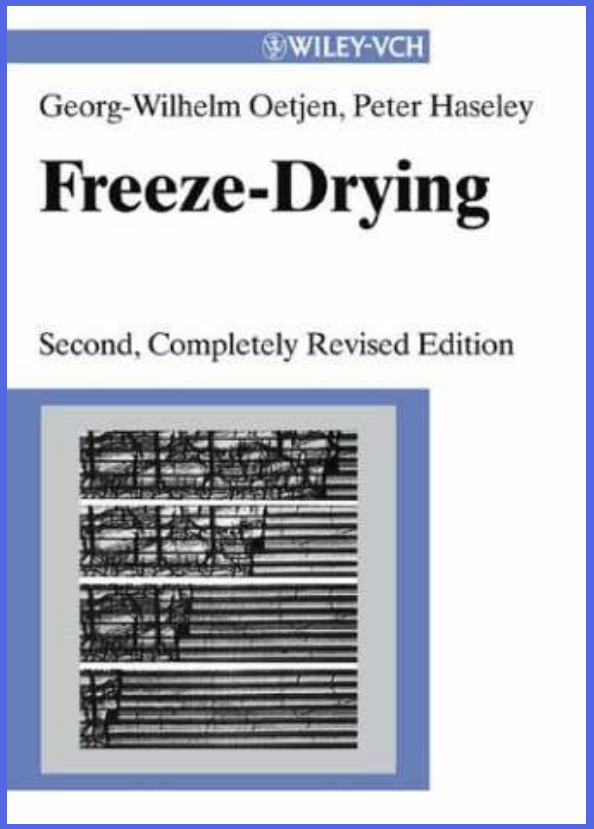
Freeze drying may still have a role to play in sample storage



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