Sample Handling Guidance

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Part 1: Introduction

Contents

1.1 DOCUMENT HISTORY AND CONTROL

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1.1.1 Version History

Version	Date	Description
1.0	19/02/2016	Initial Release
1.1	25/02/2016	Incorporates minor corrections
2.0	20/07/2016	Changes to reflect recommendations from review of recruitment and incorporation of the biopsy handling guidance as an addendum
2.1	26/07/2016	Updates following discussions

2.2	28/07/2016	Editorial changes
3.0	25/11/2016	Changes which includes updates to Omics collection and the addition of guidance for haematological cancers
3.1	17/01/2017	Finalised after omics consultation
4.0	25/01/2018	Guidance document restructured and updated as detailed in section 1.1.1

This is the fourth version of the sample handling guidance document. For ease of use, a summary of changes made since version 3.1 are listed below:

Section	Summary of Change
All sections	Restructuring for ease of use; removal of repetition. Part 2 for clinicians recruiting rare disease participants; Part 3 for cancer and Part 4 for processing of samples.
Parts 2,3 and 4	Guidance on avoiding DNA contamination
Part 2 section 2.1	Figure showing overview of rare disease programme
Part 2 section 2.2	Addition of summary of sample requirements for rare disease
Part 2 section 2.4	Details on use of fibroblast cultures for germline DNA
Part 2 section 2.4	Guidance for participants unable to provide blood
Part 3 section 1.2	Additional details on Human Tissue Authority (HTA) and diagram of diagnostic vs research pathways
Part 3 section 3.1.2	Figure showing overview of cancer programme
Part 3 section 3.2.1	Addition of summary of sample requirements for cancer programme
Part 3 section 3.4	Guidance for when alternative germline samples are appropriate
Part 3 section 3.4	Exceptional circumstances where submission of optimal FFPE samples are permitted
Part 3 section 3.4	Guidance on sampling small tumours
Part 3 section 3.4	Greater detail for biopsy sampling
Part 3 section 3.4	Updated haematological sampling guidance

Part 3 section 3.5.1	Extension of time between sampling to processing extended from 36 hours to 72 hours for blood collected in Streck tubes
Part 3 section 3.6	Addition of guidance on submitting multiple tumour samples
Part 3 section 3.7	New section on cold ischaemia effects
Part 3 section 3.7	New section on storing biopsy samples
Part 3 section 3.9	New section on storing frozen samples
Part 3 section 3.10	Tumour content assessment increased clarity
Part 3 section 3.12	New section on data required from pathologist
Part 3 and 4	Removal of guidance for FFPE samples to Appendix G
Part 4 section 4.1	Inclusion of summary tables of DNA requirements
Part 4 section 4.2	Addition of guidance on pre-extraction sample preparation
Part 4 section 4.3	Addition of guidance on DNA extraction
Part 4 section 4.4	Flow chart to help with decision making on optimising volume and concentration
Part 5 section 9	New Appendix E showing disease type and subtype mappings
Part 5 section 11	Optimised FFPE guidance moved to Appendix G

1.2 SCOPE

The purpose of this document is to provide guidance to National Health Service (NHS) Genomic Medicine Centres (GMCs) on sample handling and logistics for the Rare Disease and Cancer Programmes. It is intended to provide further information to the contractual requirements outlined in Annexes E, F, G, H, I and J.

It is intended for use by NHS GMC colleagues involved in any aspect of the sample collection and handling process: clinicians, laboratory staff, pathologists, informaticians and project managers.

Where the term “NHS” is used to refer to the National Health Service, where applicable this should be regarded as including Health and Social Care Northern Ireland (HSCNI) and NHS Wales, unless otherwise stated. NOTE: HSCNI is the designation of the publicly funded services providing public health and social care services in Northern Ireland. HSC is delivered by a number of organisations including the Public Health Agency (PHA) and a number of health and social care trusts (HSC Trusts).

The document is divided into different parts of more relevance to the role of particular staff groups. **Part 2** is related to the rare disease programme, in particular for clinicians enrolling patients and the laboratory staff sending samples for DNA extraction; **Part 3** focusses on the cancer programme and is for individuals responsible for enrolling patients into the cancer programme and laboratory staff sending samples for DNA extraction; **Part 4** is for central laboratories who extract DNA from samples, take overall responsibility for data submission and transport samples on to UK Biorepository (UKB).

Please note that some information may be duplicated between parts of this document. This is for ease of use and to ensure that readers of each part can access the key issues without needing to refer to other parts of the document.

1.3 GENERAL REQUIREMENTS

All samples must be collected and processed according to the specification, using the institution's approved Standard Operating Procedures (SOPs) and performed by staff with appropriate training, and who receive regular competency checking in these procedures. Institutional safety guidelines for handling of human biological materials and hazardous chemicals should also be followed.

1.3.1 Laboratory Accreditation

All designated laboratories must be currently CPA (UK) Ltd and either be accredited or working within the UKAS phasing plan for accreditation to ISO 15189 as specified in the NHS England NHS GMC contract and annexes.

All designated DNA extraction laboratories are required to participate in the UK National External Quality Assurance Schemes (UK NEQAS for Molecular Genetics/GenQA) specified in the NHS England NHS GMC contract.

If an NHS GMC does not have an accredited DNA extraction laboratory at any stage then this service may be provided by another approved NHS GMC with arrangements external to the NHS England contract.

1.3.2 Human Tissue Authority

Rare disease

All samples collected from living patients with rare disease have a diagnostic purpose. These samples can therefore be stored without a requirement to hold a HTA license.

Removal of any samples from a deceased patient must take place on licensed premises. Where samples are removed in premises other than the licensed mortuary for example on a children's ward located in a different hospital within the Trust, then a satellite licence arrangement may be required. This may take place under the authority of the coroner or under appropriate consent from next of kin. When samples are removed from a deceased child prior to the death being reported to the coroner this is unlawful as is removing material on unlicensed premises. These are offences under the Human Tissue Act, which can result in a fine, imprisonment or both.

Sample Handling Guidance:

Cancer

A patient's germline blood and tumour tissue sample are handled as part of the patient's diagnostic work up and, after validation, a report is returned to the patient's records. Up until that point these samples are not considered research samples. Samples can be kept as fresh frozen as part of the patient's diagnostic archive beyond this point. Processed blood samples for cfDNA DNA extraction and RNA extractions from tissue lysates are research samples but are acellular so do not require HTA licencing for storage.

Although samples can be taken and handled appropriately as part of the diagnostic pathway it is imperative that consent is taken before any data or samples are submitted to the 100,000 Genomes Project.

Any *cellular research* samples stored for the purposes of this project for greater than seven days in any laboratory will require them to hold a research HTA license.

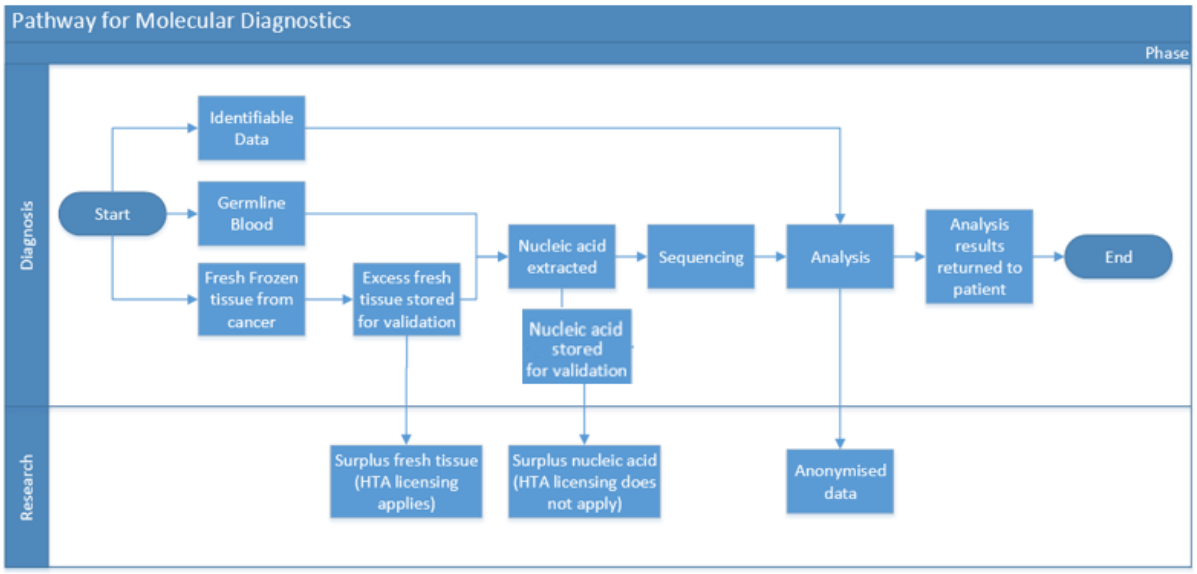


Figure 1 - Diagnostic and research arms of cancer programme

Patient consent is required before any data can be submitted (including registration data) or sample sent to the 100,000 Genomes Project.

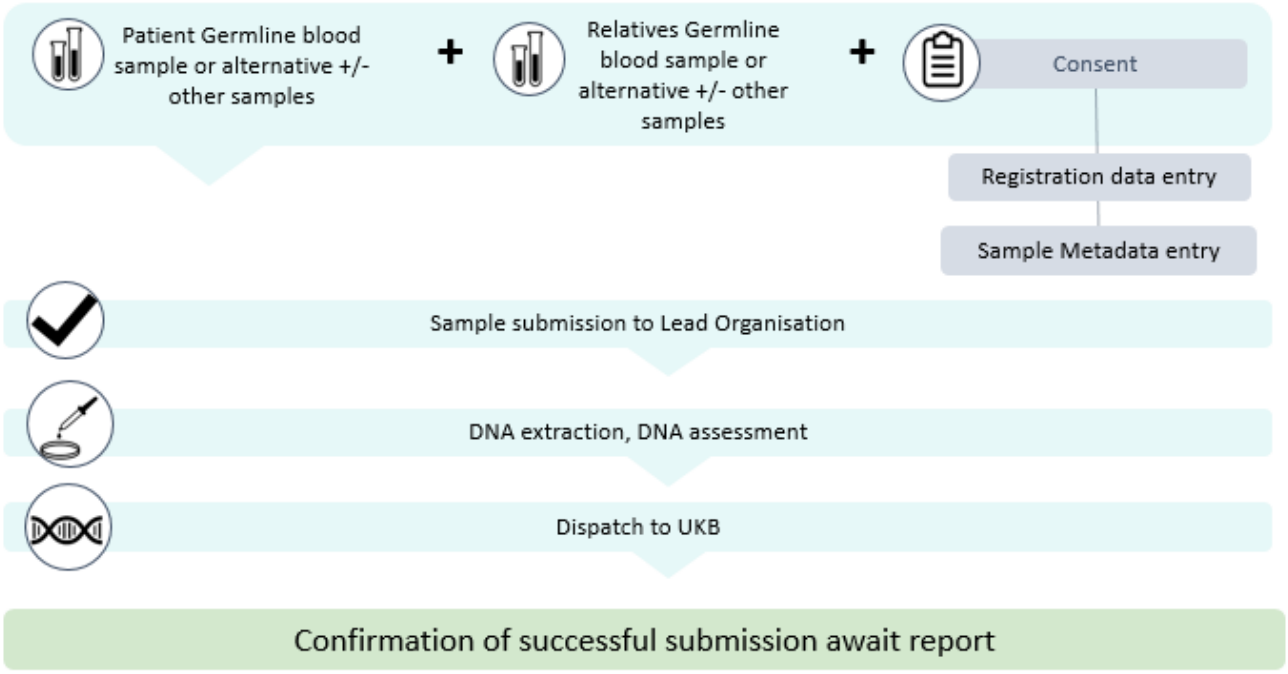
1.3.3 Precautions

Universal safety precautions should always be taken when handling biological samples to protect against infectious diseases. Local health and safety precautions should always be followed.

Part 2: Rare disease sample collection

Contents

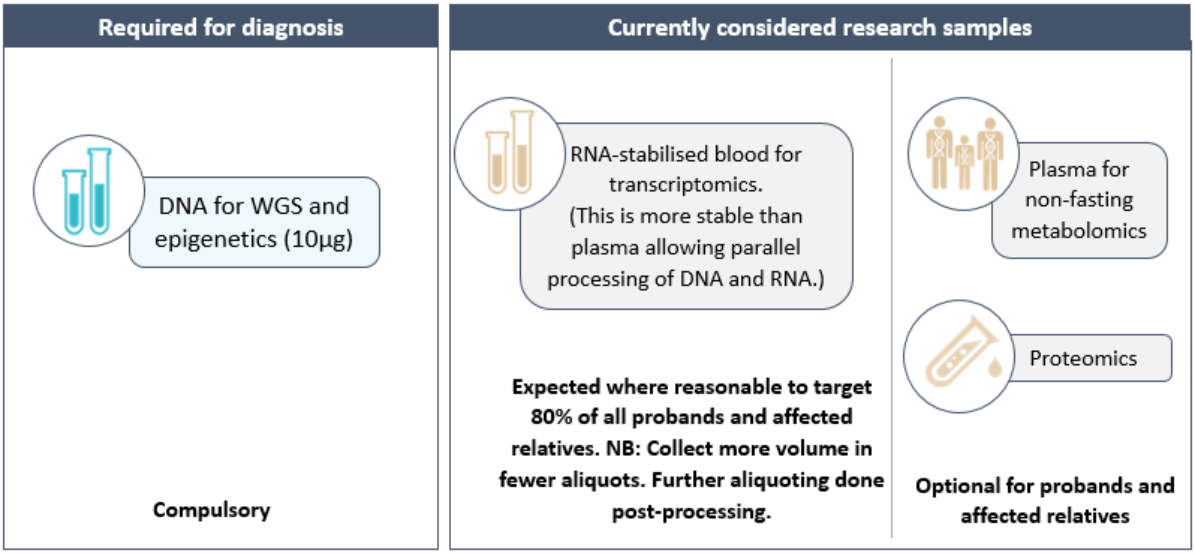
2.1 OVERVIEW OF PROGRAMME



Note: DNA extraction may not always be performed by the Lead Organisation but within an LDP. In such cases, the DNA will be transported to the central NHS GMC collection point for dispatch to UKB.

2.2 HOW MUCH TO SAMPLE

Sufficient blood needs to be taken to ensure the required quantities of DNA can be extracted (10µg to be exported to the biorepository (UKB) and 5µg to be retained locally). Two tubes, each filled with 3-5ml of blood, should be sufficient to meet these quantities in the majority of patients. This can be modified based on local evidence.



Sample Type	Purpose	Collection Strategy	Required tubes	Blood volumes
Blood for DNA extraction	WGS & excess for storage	Required for all participants	EDTA	2 x 3-5ml*
RNA - stabilised blood (PAXgene®)	Transcriptomics	Expected where reasonable for probands & affected relatives. Optional for all unaffected relatives.	PAXgene® blood RNA	2.5ml
PST collected blood for Plasma	Metabolomics	Optional for all probands & affected relatives. Not required for unaffected relatives.	PST	8ml
SST collected blood for Serum	Proteomics	Optional for all probands & affected relatives. Not required for unaffected relatives.	SST	8.5ml

Table 1 - Rare disease sample specification

Sample Handling Guidance:

2.3 BLOOD HANDLING REQUIREMENTS

2.3.1 Blood Volume requirements

The volumes given in Table 2 reflect the required blood draw volume rather than the capacity of the collection tube. Table 2 should be used in conjunction with Table 1 to define which participants require each sample type.

	EDTA	PAXgene® Blood RNA	PST***	SST***
	DNA	**RNA**	**Plasma**	**Serum**
Adult (14yrs+)	3-5ml x 2*	2.5ml	8ml	8.5ml
3-14 years**	>3ml x 2*	2.5ml	>3ml	>2.5ml
0-3 years**	1-3ml	2.5ml	1ml	1ml

Table 2 - Rare disease blood volumes

*Sufficient blood needs to be taken to ensure the required quantities of DNA can be extracted (see quantities above). Quantities can be modified based on local evidence.

**Volumes given for children and adolescents are minimum volumes, wherever possible full collection tubes (vacutainers or paediatric collection tubes) should be obtained of an appropriate size, rather than a partially filled larger tube.

***Optional for all probands and affected relatives

2.3.2 Limited blood volumes

In neonates, acutely ill children and other patients where venepuncture is challenging, clinical discretion should be applied to the volume of blood drawn.

Where only small volumes of blood are obtained and omics samples are being collected, samples should be prioritised as described in section 2.3.3. Future opportunities for blood sampling can be used to provide additional samples if not obtained at the initial venepuncture.

Where small volumes of blood for DNA extraction are obtained, a DNA aliquot containing at least 4µg should be sent to the biorepository with the remaining DNA stored locally for

validation. Further opportunities for blood sampling can be used to improve sample availability if clinically appropriate.

2.3.3 Order of Blood Draw & Prioritisation

When collecting omics sample, the order of blood draw should follow manufacturer's guidelines (typically SST -> PST -> EDTA -> PAXgene®).

In the event that the expected volume of blood that can be drawn is low, then the revised order based on the scientific value to the project is recommended:

1. EDTA for DNA extraction (at least 1ml)
2. PAXgene® for RNA extraction (2.5ml)

PAXgene® should always be drawn last due to the risk of compromising the other samples.

2.4 ALTERNATIVES TO FRESH GERMLINE SAMPLES

2.4.1 Use of stored DNA samples for individual participants

In cases where a participant has been difficult to bleed, the DNA extracted fails to meet the project requirements or telephone consent is being used but a postal sample is not possible, it is acceptable to use DNA samples that have been stored in a CPA (UK) Ltd / UKAS ISO 15189 accredited laboratory, provided they have been extracted in line with the requirements and criteria set out in Part 4.

Use of stored DNA samples has a higher risk of contamination and so the Genomics England Service Desk must be notified (ge-servicedesk@genomicsengland.co.uk). The email notifications should include the participant identifier and the LSI number (barcode ID of the tube) and the subject header should read **Stored DNA sample notification**. Once the 'stored sample option' is available in the data model then the service desk notification will no longer be required.

2.4.2 Participants unable to provide blood samples (fibroblast samples)

DNA extracted from fibroblast cultures may be submitted for patients who have undergone bone marrow transplantation or in other atypical circumstances where other samples are

unavailable, for example where no other suitable sample is available from a deceased participant (see 2.4.5 and 2.5.2). Where such samples are proposed to be taken, approval from the NHS GMC Clinical Lead must be sought and then the Genomics England Helpdesk must be notified (ge-servicedesk@genomicsengland.co.uk). For approved samples, the sample data should be submitted indicating DNA fibroblast as the sample type.

Such cultures must:

1. Have been collected, processed and stored within a CPA (UK) Ltd / UKAS ISO 15189 accredited laboratory.
2. Have been passaged to a maximum of 3 times.

2.4.3 Participants unable to provide a blood sample (saliva samples)

In exceptional circumstances, where considered clinically appropriate and no fresh or stored blood or fibroblast-derived DNA is available, DNA extracted from saliva samples may be used. Please note that DNA from saliva is less likely to produce high quality whole genome sequencing (WGS) results than a blood sample and has a higher sample failure rate. Where such samples are proposed to be taken, approval from the NHS GMC Clinical Lead must be sought and then the Genomics England Helpdesk (ge-servicedesk@genomicsengland.co.uk) must be contacted for approval on an individual basis. For approved samples, the sample data should be submitted indicating DNA saliva as the sample type. Saliva samples should be taken and processed as described in section 2.6.

2.4.4 Participants who have had blood transfusions

The timeline from last blood product transfusion to sample collection for the 100,000 Genomes Project will depend on the participant's white cell count prior to transfusion and the type of blood product transfused. It is recommended to wait at least 2 weeks after transfusion before a sample is collected for the project. If further guidance is required please contact the Genomics England Service Desk (ge-servicedesk@genomicsengland.co.uk).

2.4.5 Participants who have had a bone marrow transplant

Participants who have had a bone marrow transplant should **NOT** have peripheral blood taken for DNA extraction. The following sample types are requested instead in order of preference:

1. Pre-bone marrow transplant stored DNA extracted from blood (see 2.4.1)

2. DNA extracted from cultured fibroblasts (see 2.4.2)

2.5 SPECIAL CIRCUMSTANCES

2.5.1 Samples requirements when using telephone consent

Where there have been difficulties recruiting relatives in person, it is acceptable to use telephone consent. For these relatives, blood can be sent to the NHS GMC for extraction either by post using Registered/Tracked Royal Mail or by courier. The DNA must be extracted from blood as soon as possible and preferably within 36 hours.

RNA-stabilised blood, collected in PAXgene® RNA tubes, should also be taken along with EDTA blood and sent to the NHS GMC for aliquoting, unless there is a strong reason why this is not possible. Alternatively, a DNA sample that has been stored in a CPA (UK) Ltd / UKAS ISO15189 accredited laboratory can be used as described in section 2.4.1. The sample should have been extracted in line with the requirements and criteria set out in Part 4.

2.5.2 Deceased participants

Where deceased probands and/or relatives are eligible for inclusion in the programme, a stored DNA from accepted sample types, which passes all quality control tests, can be submitted. Guidance on stored samples given in section 2.4 should be followed.

A relative in a qualifying relationship must be available to provide appropriate consent. The consent obtained from the deceased individual at the time of sampling should not preclude use of the sample for the benefit of surviving relatives under the care of the NHS in England, and inclusion in the project must benefit surviving relatives in terms of healthcare or reproductive options if a diagnosis is made.

If the deceased individual had their own NHS number, please use this in the data submission. If the deceased individual did not have an NHS number, please use 2222222222 in the NHS number field. Please complete a data form giving details of the cause of death at the time of recruitment.

2.5.3 Foetal Samples

Foetuses can be recruited as probands or affected relatives if: there is sufficient sample available with evidence that maternal contamination is less than 5%, the sample meets all QC requirements, the parents are available for recruitment and consent, and the proband meets a set of eligibility criteria in full, whether as part of a specific foetal category or a disorder which may present antenatally or postnatally.

DNA extracted from foetal blood or foetal tissue (fresh frozen, not FFPE) are preferred. Where other sample types are proposed to be taken, including those from placenta, approval from the NHS GMC Clinical Lead must be sought and then the Genomics England Helpdesk (ge-servicedesk@genomicsengland.co.uk) must be contacted for approval. All foetal samples must have less than 5% maternal contamination. The service desk request must include the Participant ID number, confirmation that maternal contamination is less than 5% and the method used to determine this.

Foetuses should not be recruited where a pregnancy is continuing, because the turn-around time and ease of interpretation of genome sequencing are not yet suitable for use in this clinically sensitive context.

Data entry for foetuses should use the date of delivery in place of the date of birth. Forename should be entered as 'Foetus of mother's forename', for example 'Foetus of Anna'. The mother's surname should be used for the surname. The dummy NHS number 333333333 should be used. Please complete a data form giving details of the cause of death at the time of recruitment.

2.5.4 Out of area recruitment

Out of area recruitment is defined as where a proband is recruited within one GMC and an additional family member or members are recruited within a second GMC. Out of area recruitment is possible with Genomics England systems but may be restricted by the data collection systems in use at the respective GMCs. As out of area recruitment requires careful co-ordination between GMCs, it is only allowed following special approval to proceed by Genomics England.

If you have a family you would like considered for out of area recruitment, please contact the Service Desk. In order to complete a successful out of area recruitment the following steps are required (with an example following in brackets, where a proband and mother are with GMC A, and the father with GMC B).

1. Proband's GMC registers the elements of the family associated with their GMC using the group size appropriate to those people only (GMC A to register a Duo with Proband and Mother)
2. Proband's GMC submits samples for these participants. This will match the group size established and therefore pass validation (GMC A submits Proband and Mother samples)
3. Proband's GMC updates the Group Size on the Proband record to include out of area members (GMC A updates group size on proband record to Trio with Mother and Father)
4. When the update is successfully confirmed on LabKey, the out of area GMC can now register the remaining family members (GMC B registers Father)
5. The out of area GMC can then submit the final samples. As the validation checks samples submitted and previously submitted for the family, this will pass (GMC B submits sample for Father)

If your GMC is not able to conduct out of area recruitment in this manner e.g. due to local validation restrictions, samples could be collected for relatives out of the GMC area using the telephone consent and postal sample option in section 2.5.1.

2.5.5 Stored Sample Collections

Where participants are eligible, under continuing care within the NHS in England and they (or their families) could clinically benefit from WGS, then existing samples can be used providing the following applies:

- Sample numbers should not exceed 10% of contracted volumes for rare disease.
- Participants must:
    1. meet eligibility criteria outlined in contractual Annex A and Annex B
    2. meet other requirements outlined in the contract, in particular around the provision of clinical and other data
    3. have appropriate consent for inclusion in the 100,000 Genomes Project

4. have the potential to benefit or, when recruiting to relevant eligible diseases where the proband may be deceased/foetal sample, family members must have the potential to benefit (e.g. through informing reproductive choices). Foetal samples may only be included if there is an extremely strong likelihood of a heritable monogenic syndrome

- Samples must
    I. Have been collected after 1 January 2015
    II. Have been processed in line with NHS GMC contractual requirements
    III. Meet all relevant requirements in the current sample handling guidance including passing the relevant QC requirements as detailed in Table 3
    IV. Have been processed and stored in a CPA (UK) Ltd / UKAS ISO15189 accredited laboratory and be approved via the Blood DNA extraction UK NEQAS scheme
    V. Be indicated on the weekly return of samples collected to NHS England and be recorded as a stored sample.

Use of stored DNA samples has a higher risk of contamination and so the Genomics England Service Desk must be notified (ge-servicedesk@genomicsengland.co.uk). The email notifications should include the participant identifier and the LSI number (barcode ID of the tube) and the subject header should read **Stored DNA sample notification**. Once an option to indicate it is a stored sample is available in the data model, service desk notification will no longer be required.

The submission of matching omics samples are encouraged where possible.

Where collections of DNA/samples exist and consist of more than 20 individuals or were obtained before 1 January 2015 but meet other criteria outlined, permission can be sought for submission from Genomics England and NHS England for inclusion in the main programme. GMCs will need to complete the Proforma for submission of 20+ Stored Samples or outside normal criteria, Appendix C.

2.6 SALIVA SAMPLE COLLECTION REQUIREMENTS

DNA Genotek Oragene DNA Kit (OG-500) is commonly used for saliva sample collection and contains a bacteriostatic agent, but other manufacturers are available and can be used provided it is validated and fit for purpose.

It is best practice to follow manufacturer's guidelines for sample collection including the recommendation that participants should brush their teeth and use Corsodyl mouth wash 1 hour before saliva collection to reduce microbial contamination of the sample while maintaining the DNA yield. Eating and smoking should be avoided by the participant for at least 1 hour before sample collection. If the 1 hour timeline is impractical, then the use of Corsodyl mouthwash prior to sample collection is required. Saliva samples should be stored and processed as soon as possible according to the manufacturer's instructions.

2.7 SAMPLE LINKAGE AND LABELLING

A unique patient identifier, the participant ID, will be produced at the time of registration, and along with NHS number, they are the main identifiers within clinical sites.

2.7.1 Sample Linkage Forms

For those using Electronic Data Capture (EDCT), the Sample Linkage Form (SLF) can be printed once mandatory fields are completed in the EDCT, and should be printed before the blood samples are taken from the participant.

Sample ID barcodes, generated by the NHS GMC local barcoding system should be affixed to the blood collection tubes / vacutainers and duplicate copies affixed in the relevant spaces to the SLF. The SLF contains the following identifiers in text and barcode format (GS1 compliant): NHS number, Hospital number, Name, DOB, Participant ID, Family ID, Clinic ID, Disease type and Sample Type. The SLF is intended to provide a system for tracking samples from the point of blood draw / tissue collection to their recording into the DNA processing laboratory LIMS. On the left hand side are the participant identifiers rendered as 2D barcodes and also as 1D barcodes with human readable text above. The right hand side contains the different sample types that should be collected rendered as 1D barcodes. Above the sample type barcodes are

descriptions of the tube type to help the person taking the blood from the participant understand which tube to use.

The SLF is designed to provide a tool that easily links essential participant data, samples, and the processing requirements/eventual use of samples within the NHS GMC Laboratory Information Management System (LIMS). The data it contains in barcode and human readable format originates from the data that is entered into the EDCT at patient registration.

For NHS GMCs using XML to register participants, the process is different. It is expected that appropriate sample tracking including barcodes will be in place.

Several NHS GMCs print the SLF before the blood samples are taken from the participant, though NHS GMCs that have established, proven processes and infrastructure do not need to print the SLF prior to blood draw. The SLF can be downloaded and printed from the EDCT once mandatory fields are completed. The date and time should be recorded by the person taking the blood samples from the participants. Sample ID barcodes, generated by the NHS GMC local barcoding system should be affixed in the relevant spaces to the SLF. All blood tubes should be sourced by the NHS GMC including PAXgene® tubes. If multiple tubes are used please ensure that the origins of the sample are clear on labels.

2.7.2 Sample (Vacutainer®) identifiers and labelling

Vacutainers should be labelled using a local GS1 compliant barcoding system. The barcodes will be scanned into the Laboratory LIMS and used to identify the sample (i.e. Sample ID in our data model and CSV files). Vacutainer® barcode label duplicate labels should be attached to the printed SLF as a method for linking samples with patient data in addition to your local forms.

2.8 TRANSPORT OF BLOOD SAMPLES TO THE PROCESSING LABORATORY

All samples must be placed in standard specimen bags with sample request forms and transported to the NHS GMC Designated Blood Processing Laboratory. Blood samples for germline DNA extraction must be received in the Designated Blood Processing Laboratory and

all processing completed within 36 hours to meet NHS England guidance; an extension of this period can be requested via the Genomics England Service Desk (ge-servicedesk@genomicsengland.co.uk).

Omics samples will be detrimentally affected by delayed separation. It is essential that all rare disease omics samples are processed within 6 hours, except for PAXgene® stabilised blood for RNA which should be processed within 36 hours.

Planning of working practices for sample collection and optimisation of transport logistics to the NHS GMC Designated Blood Processing Laboratory from all sites of collection should be a priority with detailed process maps followed.

2.9 DATA REQUIREMENTS

Requesting clinicians need to prepare clinical data as set out in the Rare Disease Clinical Data Entry Guide and in the NHS GMC contract.

Each GMC will have their own arrangements for submission of this data to the central laboratory.

2.10 DNA REQUIREMENTS

The DNA requirement to be submitted to the biorepository for rare disease patients is 10µg. The DNA requirement is sufficient for QC, WGS and potential future analysis. In exceptional circumstances only, where only limited blood sample volumes were obtained, see section 2.3.2, 4µg - 10µg of DNA can be submitted, but this will increase the likelihood of sample QC failure.

Part 3: Cancer Sample Collection guidance

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3.1 GENERAL CONSIDERATIONS

3.1.1 Prevention of DNA contamination

In order to minimise the risk of sample to sample contamination during tissue processing it is essential that the following steps be considered standard practice:

Disposable opening knives, scalpel blades, microtome blades and forceps should be single use only.

Distel (or equivalent) should be used in conjunction with 70% ethanol for decontamination of workspaces. Please note; attempted decontamination of instruments and workspace with 70% ethanol is not sufficient to remove all traces of human tissue.

Any instruments used for dissection which are not disposable, should be soaked in 10% Decon (or equivalent) and autoclaved before next use – this includes blades and “chop up” boards.

To avoid bacterial contamination samples should not be left fresh at room temperature.

3.1.2 Overview of programme

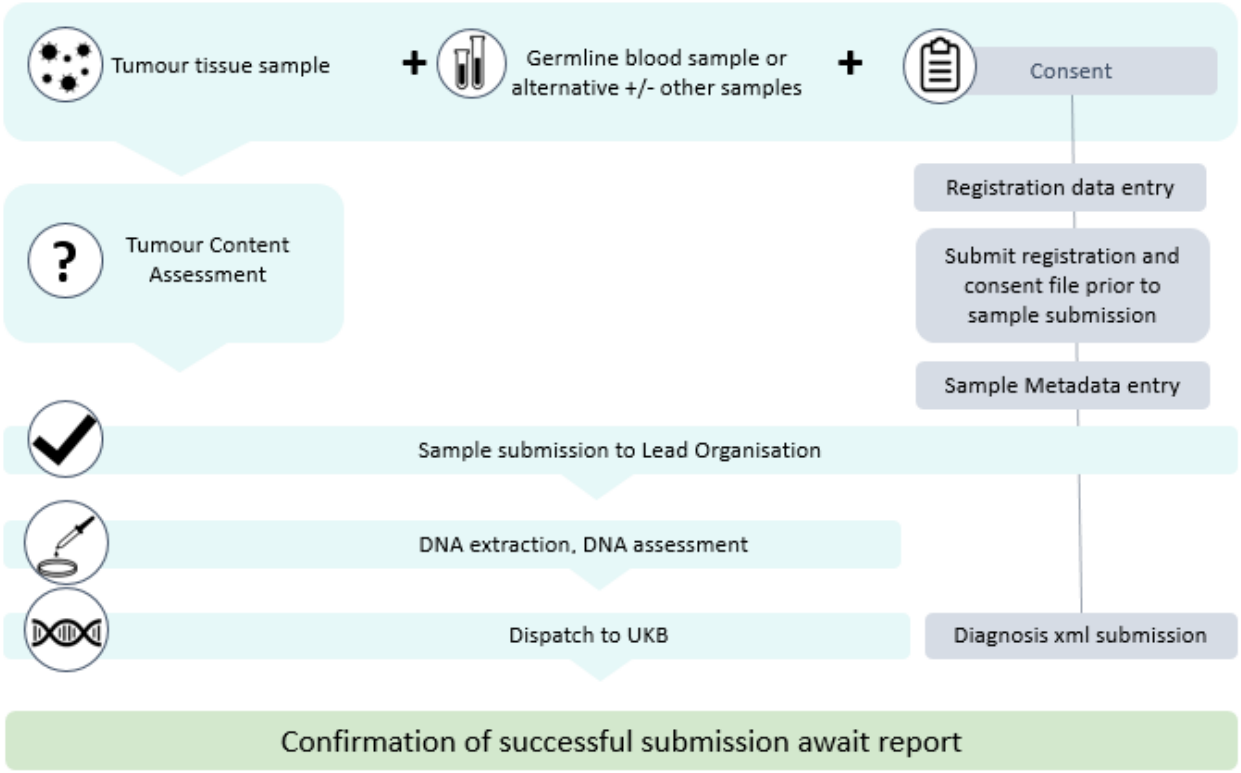

Figure 2 - Example sample and data flow for Cancer Programme

Note: DNA extraction may not always be performed by the Lead Organisation but within an LDP. In such cases, the DNA will be transported to the central NHS GMC collection point for dispatch to UKB.

3.2 HOW MUCH TO SAMPLE

3.2.1 Samples required

There are three types of samples which can be collected for the cancer programme:

1. Germline blood sample (essential)
2. Tumour sample (essential)
3. cfDNA plasma sample

A patient needs both a germline and tumour sample submitted in order to interpret the whole genome sequencing (WGS). For optimal high quality sequencing a fresh tumour sample is required. The tumour DNA sample must be submitted at the same time as the germline DNA

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sample. In addition we strongly encourage a blood sample from each patient is collected for plasma isolation for cfDNA extraction.

Tumour cells must account for at least 40% of the nucleated cells present in the tissue used for DNA extraction (see section 3.10 for details).

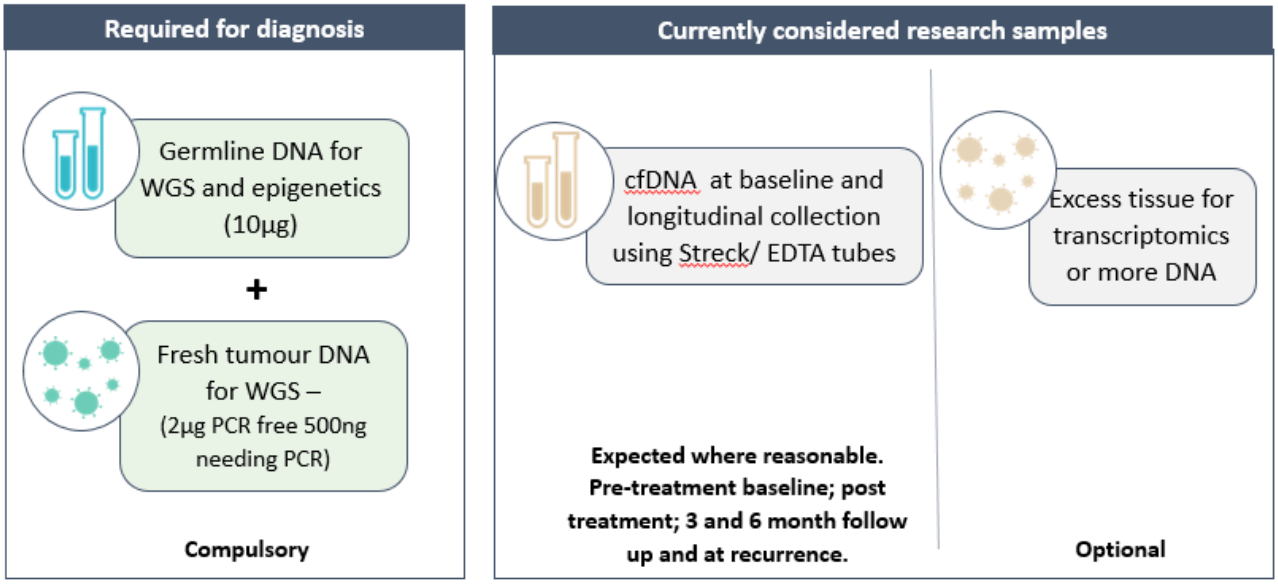

Figure 3 - Sample requirements for Cancer Programme

DNA requirements:

- 10µg of germline DNA must be submitted
- 2µg of tumour DNA must be submitted for PCR-free WGS
- Reduced requirements under special circumstances are detailed below.

The volume of blood or amount of tissue required for DNA extraction should be determined by the local processing laboratory to meet the DNA output requirements.

These sample quantities will usually be adequate to achieve the above requirements:

1. 2 x 5ml of blood for germline DNA

2. 5mm x 5mm x 2mm of tumour tissue or a 15mm x 2mm core biopsy needle for tumour DNA extraction
3. 10ml blood centrifuged to elute plasma for cfDNA extraction (Streck tubes or EDTA)

3.2.2 Low DNA quantities

The quantity of extracted DNA from the tumour tissue is variable and may be dependent on the composition of the tumour, extraction protocols as well as other variables.

Submission of at least 2µg of DNA enables utilisation of PCR-free sequencing which gives a better quality genome than if PCR is needed and allows a second library preparation to be sequenced if the first run fails. However, to enable more patients to have access to sequencing, fresh frozen tumour samples with DNA sufficient for only one library preparation will be accepted. The minimum quantity for one library preparation for PCR-free sequencing is 1.3µg.

PCR-based sequencing

Using PCR amplified sequencing libraries (Nano protocol) allows sequencing with smaller quantities of DNA. However the PCR step can introduce artifacts into the sequencing which may make it more difficult to interpret.

Despite the decrease in sequencing precision, because the use of the Nano protocol enables specimens that otherwise would not meet the acceptance criteria to be included in the 100,000 Genomes Project, the low input criteria remains open but must only be used when it is not possible to obtain sufficient DNA for PCR-free sequencing.

The minimum quantity of DNA required for a single library preparation fois 500ng.

Liquid haematological malignancies

The volume of blood or bone marrow required is entirely dependent on the nucleated cell count in the sample and as many samples from patients with haematological cancer will have an elevated cell counts it is essential to perform a nucleated cell count prior to DNA extraction. This means that in some cases even low volumes of bone marrow or peripheral blood may yield sufficient DNA for the Project.

Sample Handling Guidance:

3.3 HOW TO SAMPLE GERMLINE FOR THE CANCER PROGRAMME

Peripheral blood is the usual choice for obtaining germline DNA for the Cancer Programme and is suitable for all solid tumours. Obtaining an appropriate germline sample for bone marrow/blood based haematological cancers is more difficult and alternative sources such as saliva, cultured fibroblasts and in some instances peripheral blood (once it has been cleared of blasts) are required. The most appropriate source will vary depending on the haematological tumour type and the clinical circumstances therefore detailed guidance on selection of suitable germline material for haematological cancers is provided in section 3.4.4.

3.3.1 Blood germline

Sufficient blood needs to be taken to ensure the quantities of DNA can be extracted. Two tubes, each of 3-5ml, should be sufficient to meet these quantities in the majority of patients, but this can be modified based on local evidence.

The germline DNA requirement to be submitted to the biorepository for cancer programme participants is 10µg. The DNA requirement is sufficient for QC, WGS and potential future analysis. In exceptional circumstances, where only limited blood sample volumes were obtained 4µg - 10µg of DNA is acceptable, but this will increase the likelihood of sample QC failure. Whenever possible laboratories should store 5µg of DNA within the GMC. Samples should not be concentrated before submission to the biorepository.

Accurate volume measurement and reporting of DNA volumes is critical. Failure to accurately report volume measurement increases the chance of sample failure.

	EDTA
	Germline DNA*
Adult (14yrs+)	3-5ml x 2
Child (3-14 yrs)	>3ml x 2**
Child (0-3 yrs)	1-3ml**

Table 1 - Cancer blood volume

* The volumes given reflect the required blood draw volume rather than the capacity of the collection tube.

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** The volumes given for children and adolescents are minimum volumes. Wherever possible full collection tubes (vacutainers or paediatric collection tubes) should be obtained of an appropriate size, rather than a partially filled larger tube.

K_3EDTA or K_2EDTA are acceptable for germline samples, as long as there is local experience of using these to obtain sufficient quantity and quality of DNA. A range of volumes between 3ml and 10ml is stated to account for different local practices in Vacutainer use. The actual number and volume of tubes to be used is a local decision to ensure the provision of sufficient DNA.

Blood samples may be collected during the anaesthetic administration. Germline samples are preferred before any neoadjuvant therapy, chemotherapy or radiotherapy but if this is not possible then please wait until the white cell count has returned to normal or 4 weeks after the last treatment cycle before collection of a germline sample.

The timeline from last blood product transfusion to sample collection for the 100,000 Genomes Project will depend on the participant's white cell count prior to transfusion and the type of blood product transfused. Broadly, it is recommended to wait at least 2 weeks after transfusion before a sample is collected for the project. If further guidance is required please contact the Genomics England Service desk (ge-servicedesk@genomicsengland.co.uk).

DNA extracted from blood is the gold standard for a germline whole genome sequence. For patients with cancer cells present in the peripheral blood (e.g. leukaemia) or patients who have had a bone marrow transplant there are other options (described below) that can be considered.

3.3.2 Using saliva as a germline sample

This section is identical to Section 2.6 of the rare disease guidance but is repeated here for ease of use.

Saliva is a suitable choice of germline for several haematological malignancies but because saliva contains leucocytes as well as squamous cells it can only be collected once the peripheral blood has been cleared of blasts. Details of when saliva can be used as a source of germline for Haematological Malignancies are given in section 3.4.4 (Haematological Cancers). Any saliva

sample DNA collection kit can be used that is validated for this purpose. DNA Genotek Oragene DNA Kit (OG-500 or OG575) is commonly used and contains a bacteriostatic agent.

The manufacturer's guidelines for sample collection should be followed in particular:

1. Participants should brush their teeth and use Corsodyl mouth wash 1 hour before saliva collection to reduce microbial contamination of the sample while maintaining the DNA yield
2. Eating and smoking should be avoided by the participant for at least 1 hour before sample collection
3. If the participant has failed to comply with these guidelines, then the use of Corsodyl mouthwash prior to sample collection is required.

Saliva samples should be stored and processed as soon as possible according to the manufacturer's instructions.

3.3.3 Using skin biopsy cultured fibroblasts as a germline sample

Cultured fibroblasts are a suitable source of germline DNA for all haematological malignancies and in some instances they are the only choice (see section 3.4.4). They are also the only choice if a patient has had a bone marrow transplant.

Skin must be sampled from away from any erythema.

Cultured Fibroblasts must:

I. Have been collected, processed and stored within a laboratory with CPA (UK) Ltd / UKAS ISO 15189 accreditation for this process.

II. Have been passaged to a maximum of 3 times.

Where such samples are proposed to be taken, approval from the NHS GMC Clinical Lead must be sought and then the Genomics England Service Desk must be notified (ge-servicedesk@genomicsengland.co.uk). For approved samples, the sample data should be submitted indicating DNA fibroblast as the sample type.

3.3.4 Pre-bone marrow transplant stored DNA extracted from blood

Where a patient has had a bone marrow transplant and DNA is available from pre-transplant stored samples this can be accepted as a germline sample.

Where such samples are proposed to be taken, approval from the NHS GMC Clinical Lead must be sought and then the Genomics England Service Desk must be notified (ge-servicedesk@genomicsengland.co.uk). For approved samples, the sample data should be submitted as "DNA saliva" in the clinic sample type.

3.4 HOW TO SAMPLE TUMOUR

3.4.1 Fresh tissue requirement

Formalin fixation causes a high sequencing failure rate and if sequencing is achieved then the quality is poor. Failure rates at pre-sequencing QC are substantially higher for FFPE (in the region of 20-30%) than for fresh tissue and even optimised FFPE DNA has a high false positive variant calling rate and false negative structural variant rate. Formalin fixed tumour tissue cannot be submitted for WGS, however, under exceptional circumstances, cases may be submitted that have been processed with optimal formalin fixation. Such circumstances include inability to identify the tumour in the fresh specimen, or where the tumour is too small to sample, or if no tumour is present in the FF sample. Samples where FF tissue has not been taken due to operational reasons (e.g. late running theatre list) will not be accepted. Consent to send such cases must be sought from the Genomics England Service Desk (ge-servicedesk@genomicsengland.co.uk).

Remuneration will only be for fresh frozen samples (or exceptionally and with prior consent, optimised FFPE samples) which follow the protocols in this guidance document and will continue to be on the basis of the sample passing pre-sequencing QC until GMCs receive notification otherwise.

3.4.2 Surgical Resection Samples

3.4.2.1 Specimen macroscopic examination

Specimen cut-up should take place in a clean environment. A clean cut-up board – either a disposable cardboard board or clean plastic board, with new sterile disposable blades and clean forceps should be used for each tumour sample. Standard infection control precautions should be taken when handling fresh tissue.

The specimen should be painted, measured, weighed and opened or sliced according to tumour site-specific Royal College of Pathology Guidelines (www.rcpath.org). On opening or slicing, the location of the tumour should be determined and decision made as to which technique can be used to sample FF without compromising the histological diagnosis and staging.

3.4.2.2 Selection of fresh frozen sample

Areas of tumour that appear macroscopically necrotic or densely fibrotic should be avoided in order to maximise the chance of the sample meeting the criteria of >40% of the nuclei being malignant and <20% of the area being necrotic, required for WGS.

3.4.2.3 Sampling large tumours

The sample can be selected using a punch biopsy (5mm Skin Punch Biopsy Utensil Meditech Systems Ltd. Dorset, UK – or equivalent), or using a scalpel to select a tissue cube approximately 5 x 5 x 2mm, or slice 10 x 3 x 2mm, as appropriate to the lesion. If possible, more than one punch/slice should be taken and frozen to increase the chance of an appropriate area being selected. Whilst not mandatory, if the pathologist judges that the site(s) of sampling should be recorded, the site of selection of the block may be noted on an image: different blocks can be indicated on a photograph or diagram.

3.4.2.4 Organ specific sampling methods

The procedure for sampling may vary for different tissue types and guidance is given in Appendix B.

3.4.2.5 Small tumour sampling

When a patient only has a small tumour, pathologists are naturally concerned whether there is enough tissue for both genomic sequencing and diagnosis. It is critical that the

Sample Handling Guidance:

diagnostic pathway is not compromised in any way. What is considered small in this context varies by pathologist.

Some innovative ways to perform the sampling that can avoid impairing or compromising the diagnostic material have been identified:

1. Taking a core biopsy
2. Sampling a mirror of the full face
3. Fresh tissue blocking and punch core removal of samples

A video demonstrating these techniques is available here:

https://www.genomicsengland.co.uk/information-for-gmc-staff/cancer-programme/transforming-nhs-services/

1. Core biopsy

A 20mm length 2mm diameter core biopsy contains a similar volume of tissue to a 4mm cube but removing it from the tumour can be much less disruptive. There is no need to open the sample in order to take a core. However it may be beneficial to open a sample where this would be done anyway for fixation in order to direct where the core is taken from. For a small wide local excision of breast, for example, palpation of the tumour is enough to direct where to take the core from.

Macroscopic examination of the core may reveal some clearly benign tissue at the end of the core which can be dissected off to optimise the tumour content percentage within the sample. Where a pathologist is not available to take fresh tissue and where the surgeon and pathologist are both willing, a surgeon can take a fresh post-operative core biopsy sample and store it fresh while the remaining sample is fixed in formalin. After such a core has been taken it can be very difficult to see where in the tumour it was taken from so the effect on the diagnosis is minimal. The biopsy can then be snap frozen as described in the section on freezing.

2. Mirror block of full face

Where a full face of tumour is taken for a formalin block, a mirror block from the other half of the tumour can be taken and snap frozen. If there are any concerns about the impact on diagnosis this sample can be kept until diagnosis is complete before DNA is extracted.

3. Punches from fresh tissue blocks

Blocks of tissue can be taken from the tumour while fresh and put into cassettes. A clean punch can be used to sample well-preserved viable looking tumour from these blocks. By using a small punch e.g. 2 to 3mm the tumour can be sampled from more than one block to get enough material for WGS while leaving sufficient residual tumour in each block for diagnosis. The genomic sample can then be snap frozen. The whole sample can be sampled fresh or the fresh blocks used for punches and the remaining sample can then be fixed in formalin for a full cut up the next day.

3.4.3 Biopsy samples

This section provides guidance for NHS GMCs submitting diagnostic tumour biopsy samples to the 100,000 Genomes Project. Tissue can be treated in a genomic friendly way, as part of a standard diagnostic pathway. Once a sample suitable for DNA extraction is available in an eligible patient then a clinical decision must be taken as to the best use of that tissue for the patient. If a decision is taken to submit DNA to the 100,000 Genomes Project then patient consent should be sought at that stage. This recommendation has been agreed by the Human Tissue Authority; the Health Research Authority; the Royal College of Pathologists; NHS England; and Genomics England who produced a joint statement available here:

https://www.genomicsengland.co.uk/information-for-gmc-staff/cancer-programme/cancer-mdt-engagement-pack/

Biopsy samples can be taken either during a diagnostic procedure or at surgical resection. For example, urologists may select a sample of invasive tumour to be kept in a genomic friendly way when resecting at cystoscopy or a gynaecologist may take an endometrial pipelle to be kept fresh when performing a hysterectomy for endometrial carcinoma. Likewise where a decision is taken intra-operatively to not reset due to extensive metastases, then a genomic friendly biopsy can still be taken during the procedure before closing.

3.4.3.1 Biopsy Sample Requirements

There is an increased risk of samples yielding insufficient DNA for PCR-free WGS from biopsies compared with surgical resections. However, our current data suggests that about three

quarters of samples will be admissible. For those cases where the biopsy yields insufficient DNA there may be a second opportunity to sample at surgical resection.

As a general recommendation, in cases where risk to patients is unlikely to be increased by taking more than one biopsy, then 2-3 needle cores, or one, or more if feasible, standard endoscopic forceps biopsies are recommended. Tissue specific guidance is given below:

I. Breast cancer:
   Three-four cores (frequently 14-18G) are frequently taken at the diagnostic procedure of which at least one should be handled as a genomic biopsy. A single core biopsy will likely have sufficient tumour and DNA in many instances.

   For patients having neoadjuvant chemotherapy a core biopsy can be taken specifically for genomic sampling at the time of clip insertion.

II. Lung cancer and metastases:
   For CT-guided needle core biopsies at least one genomic biopsy is required. For bronchoscopic forceps biopsies at least 5 x 2mm forceps samples are required. For EBUS-TBNA samples at least 4-5 passes are recommended.

III. Colorectal cancer:
   At least 1 additional standard forceps genomic biopsy is recommended.

IV. Targeted prostate biopsies:
   At least one genomic biopsy is recommended.

V. Ovarian cancer biopsies:
   A single 18G genomic biopsy is likely to yield sufficient DNA.

VI. Liver tumours and metastases (of any tumour type):
   At least one genomic needle core biopsy should be taken for DNA extraction.

3.4.3.2 Biopsy sample handling

Biopsies must be kept fresh. The biopsy sample that has been kept fresh in a way that preserves DNA as well as morphology should be stored frozen pending conventional histology

on the remaining formalin-fixed tissue. If required, such as when no tumour is seen in the formalin fixed samples, the fresh sample can be fixed and used for histology.

Where a diagnosis of cancer has been confirmed, the patient can then be re-contacted and invited to participate in the 100,000 Genomes Project.

3.4.3.3 Frozen sections from biopsies

The sample should be embedded within OCT (optimal cutting temperature compound). To ensure a flat cutting surface this can be done in one of two ways:

1. Freezing onto a flat surface:

a) The core is cut into smaller pieces.

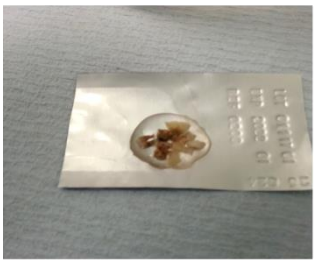
b) These are placed adjacent to each other on a smooth flat clean surface (e.g. the inside foil from a scalpel blade).

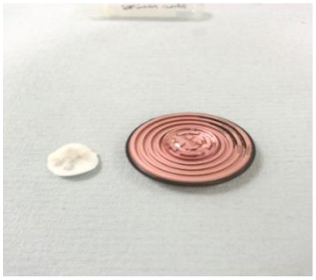
c) OCT is placed over the pieces.

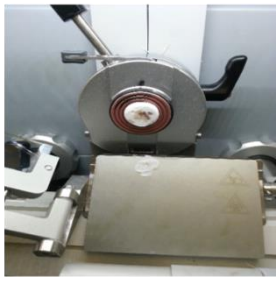
d) The foil is lowered into liquid nitrogen (LN) and frozen or sprayed with Cryospray to freeze.

e) The frozen block is inverted to reveal a flat surface for cutting.

f) It is placed in OCT on a chuck with the flat surface on top.







2) Floating onto a flat surface then freezing

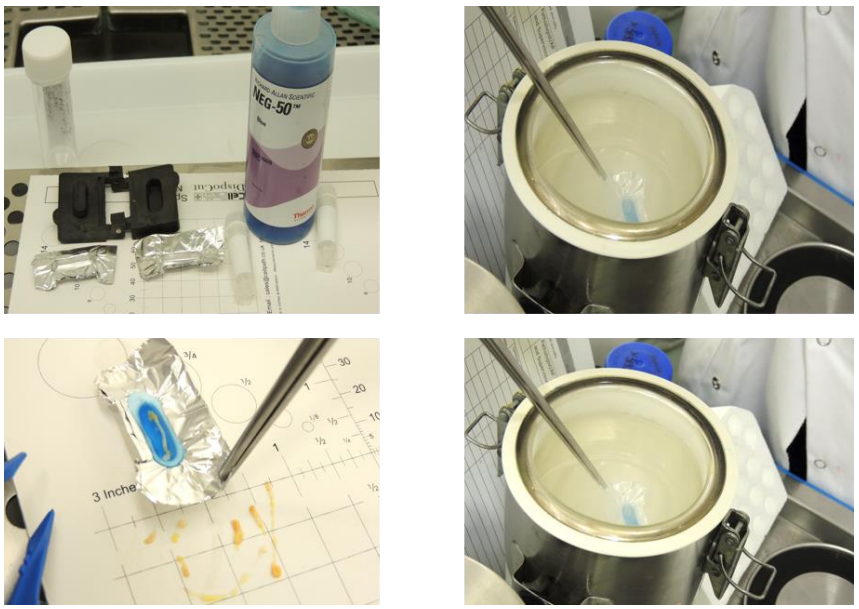
a) A foil boat is made and filled with OCT.

b) The boat is floated on LN to freeze the OCT semi-solid.

c) The biopsy is floated on to the liquid centre of the OCT which ensures it lies on a flat surface.

d) The boat is again floated on LN until the OCT is solid.

e) It is placed in OCT on a chuck with the flat surface on top.



At least one frozen section should be taken to assess for the presence of tumour (including percentage of neoplastic cell nuclei present) and cellularity (see below).

To enrich tumour DNA, if part of the biopsy/biopsies contain no tumour on the frozen section, the biopsy may be macrodissected to remove the uninvolved part of the biopsy. The whole (or remainder) of the biopsy/biopsies may then be homogenised for DNA extraction.

Alternatively, the laboratory may choose to evaluate further frozen sections for tumour assessment when cutting through the frozen block. This method has the advantage of estimating the tumour content throughout the biopsy; however, the DNA yield may be reduced as material can be left on the slide or instruments used.

3.4.4 HAEMATOLOGICAL SAMPLES

Sample Handling Guidance:

3.4.4.1 Overview

Haematological cancers are accepted in line with the Updated Haematological Malignancy Eligibility (data model v3.2.0) PAR-GUI-050 v0.1.

The following guidance describes the specific requirements for the collection of samples from haematological malignancies.

Please note sample requirements apply to both presentation and relapse samples.

Recruitment of patients also in clinical trials is both permissible and encouraged i.e. parallel recruitment; the sample requirements detailed below also apply to these patients.

Condition	GMC recruitment		
	GL	Tumour	Additional sample types (when feasible)
Acute Myeloid Leukaemia (AML)	Saliva[a] Cultured fibroblasts Others[b]	Bone marrow aspirate or peripheral blood containing >=20% blasts morphologically *or* any blast percentage if there is an AML-defining genetic abnormality as per WHO 2016 Guidelines	Tumour RNA (lysate)
Myelodysplastic Syndrome (MDS)	Saliva[a] Cultured fibroblasts Others[b]	Bone marrow aspirate or peripheral blood containing >=5% blasts morphologically	Tumour RNA (lysate)
Chronic Myeloid Leukaemia (CML) • Extreme 'Good' Responders[c]	Saliva Peripheral Blood[d]	Bone marrow aspirate or peripheral blood (no minimum blast cell percentage required)	Tumour RNA (lysate)
Chronic Myeloid Leukaemia (CML) • Extreme 'Poor' Responders[e] • Additional Cytogenetic Abnormality[f] • Accelerated or Blast Phase[g]	Cultured fibroblasts Others[b]	Bone marrow aspirate or peripheral blood (no minimum blast cell percentage required)	Tumour RNA (lysate)

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Unclassified Haematological Malignancies[h]	Saliva[a] Cultured fibroblasts Others[b]	Bone marrow aspirate or peripheral blood (no minimum blast cell percentage required)	Tumour RNA (lysate)
Acute Lymphoblastic Leukaemia	Saliva[a] Cultured fibroblasts MRD negative peripheral blood / bone marrow aspirate[i]	Bone marrow aspirate or peripheral blood containing >=40% blasts morphologically	Tumour RNA (lysate)
Lymphoproliferative Disorders[j]	Saliva Peripheral blood[k]	Fresh frozen tissue (i.e. biopsy or resection) Bone marrow aspirate or peripheral blood containing >=40% malignant cell nuclei[l] Other liquid sample containing >=40% malignant cell nuclei[m]	Tumour RNA (lysate) Plasma for cfDNA
Myeloma	Saliva Peripheral blood[n]	CD138+ sorted cells with a purity of >=40%[o]	Tumour RNA (lysate)[p]
Chronic Lymphocytic Leukaemia (CLL)	Saliva[q]	Bone marrow aspirate or peripheral blood[r] containing >=40% malignant cell nuclei	Tumour RNA (lysate) Plasma for cfDNA

Table 2 - Haematological Cancer Sample Specifications

Notes

[a] Saliva is acceptable as a germline sample in myeloid malignancies only if sufficient treatment has been given to remove all circulating myeloid cells from the peripheral blood e.g. on day 5 after administration of two doses of anthracycline chemotherapy (or equivalent) in patients receiving intensive induction in Acute Myeloid Leukaemia.

[b] Alternative germline options are being pursued in the disease types indicated to facilitate recruitment to the programme including sorted CD3+ cells (T cells) and uncultured skin biopsies. If and when these germline sample types are acceptable, supplementary guidance will be issued detailing specific requirements.

[c] Extreme 'Good' Responders in Chronic Myeloid Leukaemia are defined as those patients who, after 3 months of treatment with a tyrosine kinase inhibitor, have achieved a *BCR-ABL* transcript level (by RQ-PCR) of <1% using International Standards.

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[d] Peripheral blood is an acceptable source of germline DNA for patients who are classified as Chronic Myeloid Leukaemia Extreme 'Good' Responders providing the *BCR-ABL* transcript level (by RQ-PCR) using International Standards is <0.1%.

[e] Extreme 'Poor' Responders in Chronic Myeloid Leukaemia are defined as those patients who, after 3 months of treatment with a tyrosine kinase inhibitor have a *BCR-ABL* transcript level (by RQ-PCR) of >10% using International Standards.

[f] Refers to any additional cytogenetic abnormality detected using karyotyping in the diagnostic sample in Chronic Myeloid Leukaemia other than a variant *BCR-ABL* transcript.

[g] Patients either presenting in Accelerated or Blast Phase Chronic Myeloid Leukaemia or progressing to Accelerated or Blast Phase Chronic Myeloid Leukaemia are eligible for recruitment.

[h] The definition of this category is broad but includes disorders such as 'Triple negative' Myeloproliferative Neoplasms (defined as no variant detected in *JAK2* exon 12, exon 14 (codon 617), *CALR* exon 9 or *MPL* exon 10), and Myelodysplastic/Myeloproliferative Overlap Syndromes.

[i] In Acute Lymphoblastic Leukaemia peripheral blood or bone marrow aspirate samples which are either negative for or have a diagnostic MRD marker (e.g. BCR or TCR gene rearrangement or *BCR-ABL* transcript) detectable at a level of <0.1% are suitable for use as the source of germline DNA.

[j] Any patient with a Lymphoproliferative Disorder (high or low grade) for which treatment is planned is eligible for recruitment to the project.

[k] Peripheral blood is suitable for use as the source of germline DNA in Lymphoproliferative Disorders providing there are no circulating tumour cells in the peripheral blood. Please note it is not necessary to undertake anything beyond normal standard of care assessments to demonstrate the absence of circulating tumour cells.

[l] Peripheral blood or bone marrow aspirate samples could be used as a source of tumour DNA in Lymphoproliferative Disorders providing the malignant lymphoid cells constitute >=40% of the nucleated cells in the sample.

[m] It is appreciated that there may be situations where malignant lymphoid cells constitute >=40% of the nucleated cells in a sample of a different body fluid e.g. pleural fluid; in these rare situations these would be an acceptable source of tumour DNA.

[n] Peripheral blood is an acceptable source of germline DNA in Myeloma providing there are no circulating plasma cells in the peripheral blood.

[o] All myeloma samples should undergo enrichment for CD138+ cells even if the starting plasma cell percentage of the bone marrow aspirate smear is >=40% in order to obtain the highest possible purity of plasma cells. Laboratories carrying out CD138+ cell enrichment / sorting will need to supply verification of the sorting technique and the CD138+ sorting checklist (Part 5: Appendix D) prior to commencement.

[p] It is appreciated that most myeloma samples will not have sufficient cells for tumour lysate collection for subsequent RNA extraction.

[q] Saliva collection in Chronic Lymphocytic Leukaemia should be postponed until such a time as the peripheral blood lymphocyte count is $<25x10^9$/L.

[r] The lymphocyte count of peripheral blood samples to be used as the source of the tumour DNA in Chronic Lymphocytic Leukaemia should be $>25x10^9$/L.

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3.4.4.2 Bone Marrow Aspirate or Peripheral Blood Collection Requirements

Sufficient blood or bone marrow needs to be taken to ensure the required quantities of DNA can be extracted, exported to the biorepository and a portion stored locally for validation. Nucleated cell counts should be carried out prior to DNA extraction. Samples must be collected in EDTA tubes as described in Appendix A.

3.4.4.3 Fresh Frozen Tissue

Guidelines detailing the sampling of fresh frozen tissue should be followed for Lymphoproliferative Disorders where the tumour sample is a biopsy or resection.

3.4.4.4 Myeloma: CD138+ Sorted Cells

Prior to DNA extraction, Multiple Myeloma patients' bone marrow samples must be enriched for CD138 +ve cells. This is to ensure a sufficiently high neoplastic content for submission to the 100,000 Genomes Project cancer programme. Local practices should be used for the collection and sorting of CD138+ cells for Myeloma tumour samples but these must have been verified as fit for purpose.

Prior to commencing the collection and sorting of CD138+ cells for Myeloma tumour samples, laboratories must provide evidence of the verified protocols to be used, and show evidence that appropriate purity is being achieved. The checklist for producing CD138 +ve enriched cells from bone marrow samples (Appendix D) must be completed and submitted to england.genomics@nhs.net. Confirmation of approval must be given before any recruitment of myeloma patient commences.

3.4.4.5 RNA samples

RNA submission is detailed in "Other research samples – tissue lysates"

3.5 HOW TO SAMPLE CANCER PROGRAMME RESEARCH SAMPLES

3.5.1 CfDNA samples

Cell free DNA (cfDNA aka circulating tumour DNA or ctDNA) samples collected for the 100,000 Genomes Project are currently considered research samples. These samples are acellular so they can be stored in a laboratory without a licence under the Human Tissue Act 2004.

The volumes given reflect the required blood draw volume rather than the capacity of the collection tube.

	Streck Cell-Free DNA BCT®
	Circulating cell-free DNA
Adult (14yrs+)	10ml
Child (0-14yrs)	Not Required

Table 3 - Cancer blood volume

Timings for collection of blood for plasma for cfDNA

cfDNA blood must be drawn pre-operatively. Blood samples for plasma collection for cfDNA can be collected during the anaesthetic administration but must be taken before the first incision. When collecting multiple blood samples at the same time the order of blood draw should follow manufacturer's guidelines with EDTA being collected before the Streck Cell-Free DNA BCT® tube is used.

Storage of blood in Cell Free DNA BCT® Streck tubes

Blood collected in Cell Free DNA BCT® Streck tubes should not be stored or transported to the processing lab on ice. The samples should be stored and shipped between 15°C and 30°C and should be processed within 72 hours as described in Part 4.

Storage of blood in EDTA tubes for plasma for cfDNA

EDTA blood tubes should be kept on ice and processed as soon as possible. The samples should be processed ideally within 4 hours and always within 6 hours. EDTA blood can be centrifuged at 4°C or room temperature as detailed in section 4.2.7. Samples which fail to

comply with the manufacturer's guidelines on isolation of cfDNA in plasma from EDTA should not be submitted.

3.5.2 Haematological Malignancies

Plasma collections for cfDNA is required for participants with CLL and Non-Hodgkin Lymphoma. For these participants longitudinal sample collection is required at the following time points:

- Pre-treatment
- 3 monthly for the first year
- 6 monthly for the second year
- Recurrence (if applicable).

3.5.3 Solid tumours

Plasma collections for cfDNA are highly encouraged from all participants at recruitment and then longitudinal samples should be taken in tumour types at high risk of recurrence (detailed in Table 4).

For participants at high risk of recurrence the following longitudinal sample collection should be collected:

I. Pre-surgery
II. Post-surgery (2 weeks - 6 weeks post-surgery)
III. 3 month and/or 6 month follow-up
IV. Recurrence (if applicable).

To date, the tumour type GeCIP Leads, in consultation with their respective GeCIP members have identified the following cohorts for longitudinal cfDNA collection.

Tumour Type	Cohorts for cfDNA longitudinal collection
Ovarian	FIGO Stage III & IV undergoing primary surgery
Lung	Stage I- IV
Colorectal	All Dukes' B-D undergoing primary resection
Breast	Triple-negative (or ER and HER2-negative if PR not available) breast cancer undergoing primary surgery or ER or HER2 positive BC with >=4 axillary lymph nodes positive on imaging.
Sarcoma	All primary resections

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Renal	All primary resections
Bladder	T2-4a N0 M0 muscle invasive bladder cancer (MIBC)
Upper GI: Biliary tract	All advanced biliary tract cancers
Upper GI: Pancreatic	Advanced/metastatic pancreatic cancer. Metastatic high grade neuroendocrine tumours undergoing systemic therapy.
Upper GI: Hepatocellular (HCC)	Advanced HCC and intrahepatic/hilar cholangiocarcinomas,
Upper GI: Gastric	Locally advanced or metastatic gastric adenocarcinomas (fundus, body, distal stomach, excl Siewart type 3 or gastro-oesophageal junction).

Table 4 - High Risk of Recurrence Cohorts for Collection of Longitudinal Plasma Collection for cfDNA.

3.5.4 Excess Tissue or Lysates

It is requested that, where practical, GMCs collect and store or submit any additional tumour tissue following local procedures. Where there is sufficient tissue it is requested that this is submitted as an additional nucleic acid source for extraction of DNA or RNA for further studies. Samples can be submitted or stored as excess tissue or cell lysate from tissue or haematological samples.

It is possible to extract both DNA and RNA using a variety of extraction kits. Where these are used the excess cell lysate or RNA should be placed in a GTC containing buffer that should be stored at -80°C and submitted with the tumour and germline DNA samples. Laboratories should ensure that DNA yields are not compromised by extracting both DNA and RNA. This excess tissue and cell lysate provides an additional nucleic acid source which can be used to extract DNA or RNA for further studies.

For liquid tumours, the single bone marrow or peripheral blood sample should be used to extract both DNA and RNA. RNA should not be converted into cDNA, but stored in a GTC containing buffer at -80C.

3.6 MULTIPLE TUMOUR SAMPLE SUBMISSION

Multiple samples can be sent from one patient under these circumstances:

1. **Multi-region** Single resection sample – multiple areas sampled
2. **Multi-tumour**
3. synchronous or primary and metastasis (lymph node or other organ)
4. **Longitudinal**
    - pre- and post-chemotherapy
    - metachronous
    - second primary
    - primary and metastasis

3.6.1 Eligibility

If a cancer would be eligible for programme as a single sample then it is eligible for submission under the multi-tumour and longitudinal multiple sample submission.

There must be a sound scientific or clinical reason for submission of multiple samples from one case. The clinical reason could be to help establish number of samples needed for sensitivity for detecting variants for clinical care.

3.6.2 How to sample

Multi-region

Up to 4 samples can be taken from a single tumour sample, avoiding areas of necrosis, fibrosis and haemorrhage. Each sample must pass the tumour content assessment. Where a biopsy was submitted for WGS prior to resection of the same tumour, the resection sample can only be sent if it is sampled as a multi-region case with 3 samples sent with the biopsy acting as the fourth.

3.6.3 Request to go live for submission of multiple cancer cohorts

Permission must be sought from NHS England to submit multi-regional tumour cancer cohorts, for each new tumour pathway and by each NHS GMC.

The 'Request to *go live* for submission of multiple cancer cohorts' form can be submitted online and can be located under the 'Web forms' section at:

Sample Handling Guidance:

https://www.genomicsengland.co.uk/information-for-gmc-staff/sample-handling-guidance/

Please note that the 'Other Information' section on the form can be used to provide additional information supporting prospective cohort size where sample numbers are expected to be low, i.e. due to rarity of cancer.

NHS England will contact the NHS GMC who are seeking permission to submit multi-regional tumour samples from a new pathway, once a decision has been made.

Patients with more than one primary that would be eligible independently, which present either synchronously or separated in time, can be submitted without prior consent to open up a cohort for this cancer type. However, notification for each case through the service desk remains a requirement.

3.6.4 Data entry for patients having multiple samples submitted

Once approval has been sought and given for a specific cancer pathway, within an NHS GMC, requests must be sent for each patient having samples submitted. The 'Multiple tumour sample submission request form' should be completed, in order to request approval from Genomics England to submit specific multiple samples. This form can be submitted online and can be located under the 'Web forms' section at:

https://www.genomicsengland.co.uk/information-for-gmc-staff/sample-handling-guidance/

Longitudinal samples must be checked to ensure that the germline sample originally sequenced was sequenced using the same chemistry as the tumour. If there has been a change of chemistry then any residual stored germline DNA could be considered for re-sequencing. If this is inadequate a new germline sample would be needed.

Data must be submitted using version 3 of the data model. The tumour ID is unique for each tumour. The same tumour ID should be given for all samples submitted from multiple regions of a single tumour. Unique tumour IDs should be given for multi-tumour and longitudinal sampling.

The interpretation of a post-treatment genome is very difficult without a pre-treatment sample for comparison, so, where possible, please provide a matching pre-treatment sample; both samples must be from fresh frozen tissue.

3.7 STORAGE OF SAMPLES PRIOR TO FREEZING

3.7.1 Cold Ischaemia effects

The problems associated with cold ischaemic damage and rationale for refrigeration has been reviewed here: https://www.genomicsengland.co.uk/tissue-handling-rcp-bulletin/

Refrigerating samples over a weekend or while they await transportation to a laboratory minimizes the impact of these biological processes on protein expression, DNA degradation and cell cycling.

Tissue specimens should be delivered to Pathology without any formalin fixation and undergo sample selection within 2 hours of surgical excision unless refrigerated. Many samples can be maintained unfixed for up to 72 hours if *rapidly cooled* and kept at 4^{o}C. This can be done either by placing the dry specimen in a fridge, in a bag on wet ice or in a cold bag with ice blocks. Alternatively the sample can be placed in vacuum plastic bags, vacuum packed then placed in the fridge/ice/cold bag. Samples can be cooled in plastic pots, though rapid cooling is facilitated by placing the sample in plastic specimen bags purpose-designed for vacuum packing. These must have specimen and patient ID affixed.

3.7.2 Biopsy samples

Biopsy samples can be kept refrigerated at 4^{o}C for up to 72 hours. However where the biopsy may be needed for morphological diagnosis then the safe period of refrigeration is not known and biopsies should not be kept in saline as it is known to affect the morphology of the tissue. In all cases, to prevent drying out, the biopsy should be kept in an Eppendorf, sealed and wrapped in cling film or kept in a larger tube with adjacent damp gauze.

3.8 FREEZING

3.8.1 Snap Freezing in LN

Immediately after retrieval, the sample(s) are placed on a 'boat' of foil, which is then immersed into LN for at least 60 seconds (samples can be kept longer in LN for convenience if required). The boat is removed using forceps and the sample transferred rapidly to a labelled Eppendorf which is then kept in LN or on dry ice prior to transfer to the freezer. Samples can be kept on dry ice for 4-6 hours if required. If preferable, the sample can be placed into OCT prior to freezing.

3.8.2 Isopentane on dry ice

In a Styrofoam container of dry ice, place a stainless steel, Pyrex or polypropylene container with some pellets of dry ice. Add to this isopentane (in a fume hood). When the bubbling stops, the slurry is ready and the sample, on a foil boat, can be immersed into the liquid to freeze. Freezing takes approximately 60 seconds; the sample can then be transferred to a labelled Eppendorf and stored on dry ice prior to transfer to a freezer.

3.8.3 Freezing on dry ice

Samples can be transferred directly into labelled Eppendorfs and placed into a Styrofoam container of dry ice. The samples require at least 5 minutes for freezing but can be kept on dry ice for hours if necessary.

3.8.4 Freezing with Cryospray

Commercially available Cryospray can be used to freeze samples. The samples are sprayed with Cryospray until solid (usually ~30 seconds). The sample is then transferred to a labelled Eppendorf on dry ice until transfer to a freezer. If desired the samples can be placed onto foil and loosely covered or orientated on a support such as cork with OCT before spraying.

3.8.5 Freezing on wet ice

If no dry ice is available, samples can be placed in labelled Eppendorfs and placed on wet ice. Current data indicate that samples can be maintained on wet ice (without thawing) for up to 4 hours, allowing transfer to a laboratory where more rapid freezing can take place. Snap freezing in LN following 4 hours on wet ice leads to good quality DNA and RNA and acceptable morphology.

3.9 STORAGE OF FROZEN SAMPLES

Once frozen, samples should be stored in a freezer without defrosting during transfer. A -80°C freezer is considered the gold standard, however, samples have been kept in a -20°C freezer for months whilst in process with no discernible impact on WGS.

3.10 TUMOUR CONTENT ASSESSMENT

Invasive malignant nuclei must account for at least 40% of the nuclei present in the tissue sample submitted for WGS. In addition the sample should have less than 20% necrosis by area. Macrodissection may be required to generate a suitable sample. Personnel involved in the assessment of sample tumour content should participate in the UK NEQAS pilot on-line tumour assessment programme. This is currently an educational tool with no formal assessment of competence. This is not a requirement in order to start tumour assessment.

Tumour content may be assessed on:

1. A frozen section of the tissue to be submitted
2. A FFPE mirror block of the sample to be submitted
3. An FFPE of area surrounding small punch biopsies frozen for submission
4. Cytological preparations e.g. from EBUS samples or a touch prep of a biopsy
5. An FFPE from a representative area of tumour where the pathologist is confident that the sample taken is 100% tumour e.g. to estimate tumour content of a fine needle aspirate of the tumour:

    I. % of viable neoplastic cells as a total of all nucleated cells (including admixed inflammatory and stromal cells) to the nearest 10%.

    II. Account should be taken of the range of nuclear sizes present. A small cluster of lymphocytes will yield multiple times more DNA than an equivalent sized nest of tumour cells, in which each cell will be larger.

    III. Account should be taken of the 3D architecture of the sample. For example, if 5 lymphocytes would fit into a single tumour cell in the plane of view then in a 3D reconstruction there could be more than 20 lymphocytes within the same space as a single tumour cell.

IV. If the tissue is to be macrodissected, then tumour estimates should be in the selected area only.

The reported tumour content as estimated in the host Pathology laboratory will be defined as Low <40%; Medium = 40-60%; and High >60%.

3.11 TUMOUR IMAGING

Digital images of H&E sections from the tumour should be captured. Ideally two images should be sent for each tumour: an optimal FFPE slide representative of the tumour and the slide used for tumour content assessment. Where appropriate the area used for the assessment should be indicated.

Where possible, slides should be digitally scanned at high resolution (x40 objective resolution / 0.275 microns per pixel and x60 objective resolution / 0.1375 microns per pixel). In all cases, slides should be kept and be available for later scanning to this standard or potentially submission to a third party for centralised scanning.

Details for image data upload and/or slide submission for centralised scanning will be confirmed by Genomics England.

3.12 DATA REQUIREMENTS FROM REQUESTING PATHOLOGISTS

A registration file should be completed for every consented patient. Once a sample is available a sample metadata file needs to be completed and submitted with the sample.

In order to ensure that the tiering of the variants and the analysis of any germline predisposition is correct for the tumour submitted the disease type and subtype must be accurate for the tumour. Morphology and topography codes are also mandatory to allow for validation of the disease type and subtype. The reporting pathologist is responsible for providing the following data points:

Sample Handling Guidance:

1. Disease type and subtype as outlined in Appendix E.
   A tumour comprised of more than one subtype should be entered as follows. The predominant tumour subtype in the sample sent for WGS should be entered first. The remaining subtypes should be entered in descending order with the most prevalent subtype in the whole tumour listed second. It is helpful to include "mixed tumour type" as a subtype but this should not be entered alone.
2. Topography and morphology codes for the cancer sample

   These may be sent as ICD10 codes, SNOMED CT codes or SNOMED RT codes
3. Tumour type: Primary / Recurrence / Metastatic
4. Tumour content: Medium >40%; High >60% (Low < 40% or necrosis >20% would make sample ineligible)
5. Tumour grade (optional)
6. Tumour pathological stage (optional)

Each NHS GMC will determine how this data is collected and submitted to the Lead Organisation.

3.13 SAMPLE LABELLING AND TRACKING

A unique patient identifier, the participant ID, will be produced at the time of registration, and along with the NHS number, they are the main identifiers used in the 100,000 Genomes Project.

3.13.1 Sample Labelling

For those using Electronic Data Capture (EDCT), the Sample Linkage Form (SLF) can be printed once mandatory fields are completed in the EDCT.

Sample ID barcodes, generated by the NHS GMC local barcoding system should be affixed to the blood collection tubes / vacutainers and duplicate copies affixed in the relevant spaces to the SLF. The SLF contains the following identifiers in text and barcode format (GS1 compliant): NHS number, Hospital number, Name, DOB, Participant ID, Family ID, Clinic ID, Disease type and Sample Type. The SLF is intended to provide a system for tracking samples from the point of blood draw / tissue collection to their recording into the DNA processing laboratory LIMS. On the left hand side are the participant identifiers rendered as a 2D barcodes and also as 1D

barcodes with human readable text above. The right hand side contains the different sample types that should be collected rendered as 1D barcodes. Above the sample type barcodes are descriptions of the tube type to help the person taking the blood from the participant understand which tube to use.

The SLF is designed to provide a tool that easily links essential participant data, samples, and the processing requirements/eventual use of samples within the NHS GMC Laboratory Information Management System (LIMS). The data it contains in barcode and human readable format originates from the data that is entered into the EDCT at patient registration.

For NHS GMCs using XML to register participants, the process is different. It is expected that appropriate sample tracking including barcodes will be in place.

The date and time should be recorded by the person taking the blood samples from the participants. Sample ID barcodes, generated by the NHS GMC local barcoding system should be affixed in the relevant spaces to the SLF. All blood tubes should be sourced by the NHS GMC. If multiple tubes are used please ensure that the origins of the sample are clear on labels.

3.13.2 Transport of Samples to the Processing Laboratory

All samples must be placed in standard specimen bags with sample request forms and transported to the NHS GMC Designated Processing Laboratory.

Blood samples for germline DNA extraction must be received in the Designated Blood Processing Laboratory and all processing completed within 36 hours to meet NHS England guidance; an extension of this period can be requested via the Genomics England Service Desk (ge-servicedesk@genomicsengland.co.uk).

Planning of working practices for sample collection and optimisation of transport logistics to the NHS GMC Designated Processing Laboratory from all sites of collection should be a priority with detailed process maps followed.

Part 4: Sample Processing and Data entry at NHS GMC

Contents

4.1 DNA REQUIREMENTS

Please always try to send as much DNA and as high a volume sample as possible.

Rare disease participants require a germline DNA sample extracted from blood. Exceptionally, the sample may be from saliva instead of blood as outlined in Parts 2 and 3.

Cancer patients require a germline blood and a fresh tumour sample. For haematological cancer participants the peripheral blood sample or bone marrow aspirate will usually be the tumour rather than the germline sample and alternative germline samples may be sent as set out in Part 3.

	Germline DNA Specification (Rare disease and Cancer)
Extraction method	Automated extraction is preferable. Extraction must be carried out according to manufacturer's instructions and the laboratory SOP. Method must have been shown to produce high quality DNA suitable for WGS through the UK NEQAS EQA assessment.
Amplification	DNA must not be PCR amplified.

Sample Handling Guidance:

	Germline DNA Specification (Rare disease and Cancer)
Quantification	Quantify using a validated double stranded DNA quantification method such as Qubit, Picogreen and Glomax Quantifluor. Spectrophotometers such as Nanodrop cannot be used for DNA quantification.
DNA Purity	Assessment is not required in the NHS GMC. The biorepository A260/A280 ratio must be 1.75 - 2.04.
DNA Fragment Length	Assessment is not required in the NHS GMC. Assessment will be performed in the biorepository to ensure that samples are not degraded and more than 60% of fragments are above 23kb.
DNA Buffer	TE (10mM Tris/1mM EDTA) pH8.0 / as per manufacturers system
Total DNA quantity	10µg* (In exceptional circumstances, for limited sample volumes more than 4µg is acceptable)
Concentration	30-100ng/µl **
Volume	100-600µl Accurate measurement of the volume submitted is required. The provision of inaccurate measurement increases the likelihood of sample failure
Dilution within NHS GMC	DNA should be diluted by the NHS GMC if the DNA concentration is above the maximum specified or the DNA volume is below minimum specifications. Diluted samples should be submitted with post-dilution concentration as the QC values in the metadata files.

Table 1 - Specification for germline DNA for rare disease and cancer patients

*This accounts for all requirements, including central QC steps and storage for future research, rather than just the whole genome sequencing (WGS) requirements.

**Minimum requirement of 30ng/µl as measured by the biorepository.

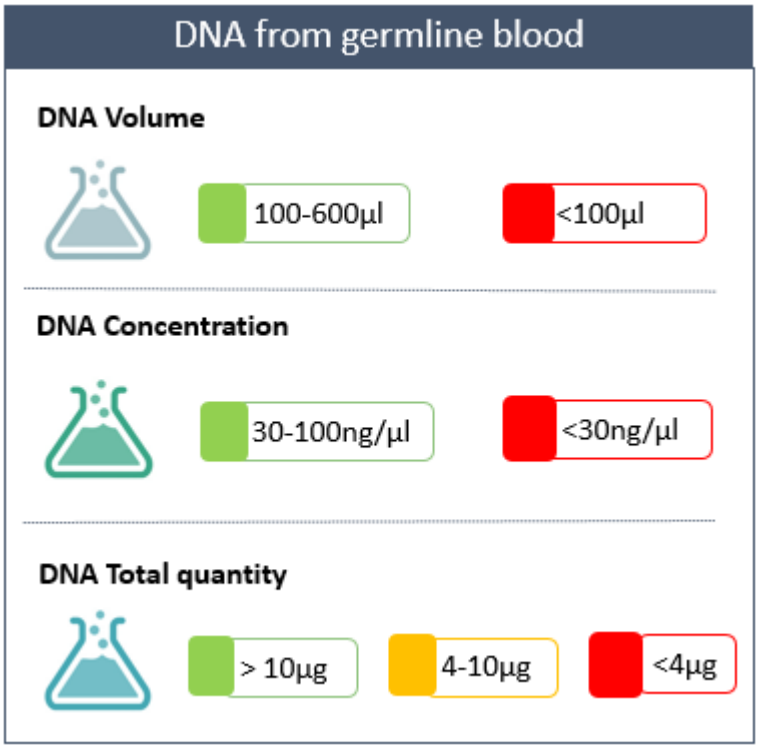
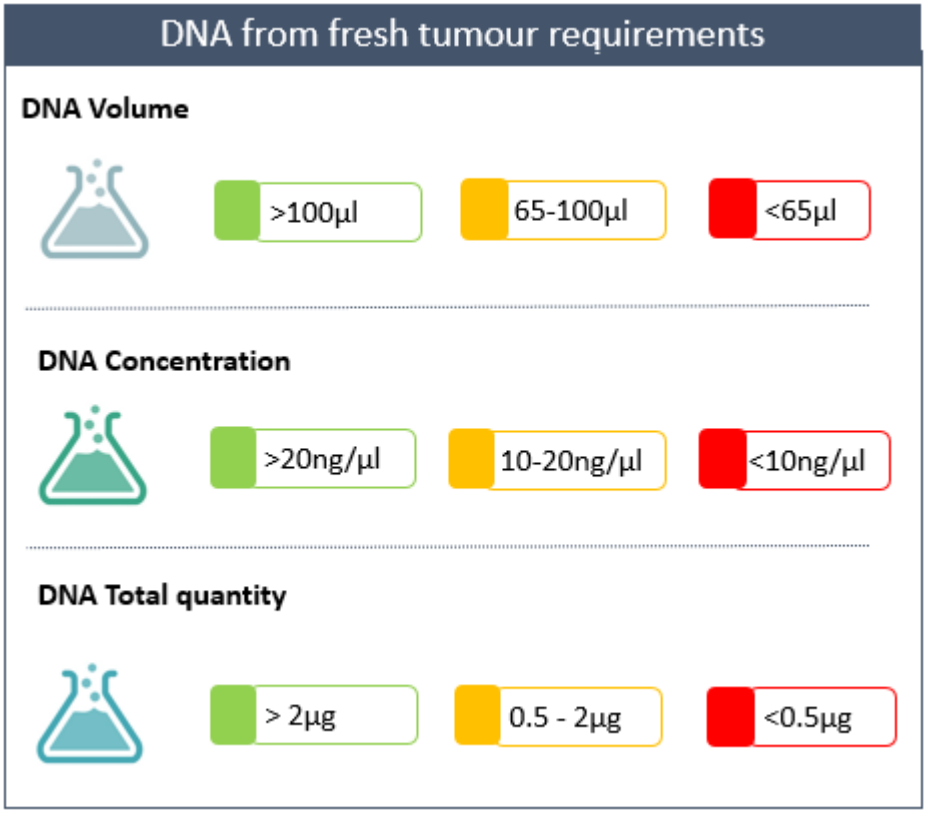


Table 2 - DNA from germline samples

DNA	Fresh Tumour sample specification (for cancer)
Extraction method	DNA should be extracted using an extraction method appropriate for fresh tissue.
Amplification	DNA must not be PCR amplified.
Quantification	Quantify using a validated double stranded DNA quantification method.
DNA Purity	Assessment is not required in the GMC. The biorepository A260/A280 ratio must be 1.75 - 2.04
DNA Fragment Length	Assessment is not required in the GMC. Assessment will be performed in the biorepository to ensure that samples are not degraded and more than 60% of fragments are above 23kb.
DNA Buffer	TE (10mM Tris/1mM EDTA) pH8.0 or as per manufacturers system.
DNA quantity for PCR-free library sequencing	1.3µg minimum* 2µg preferred wherever possible please send more.
Concentration for PCR-free library sequencing	20 - 60ng/µl** DNA should be diluted in NHS GMCs if it is over the maximum DNA concentration.
Volume for PCR-free library sequencing	65 - 600µl*** Samples submitted with less than 100µl will only have sufficient sample for one library preparation for sequencing which will increase the likelihood of sample failure. Always try to submit at least 100µl. DNA should be diluted in NHS GMCs to meet the minimum volume required. Accurate measurement of the volume submitted is required. The provision of inaccurate measurement increases the likelihood of sample failure.
DNA quantity for PCR-based library sequencing	500ng minimum▪ 1µg preferred
Concentration for PCR-based library sequencing	10 – 25 ng /µl ▪▪ DNA should be diluted in NHS GMCs if it is over the maximum DNA concentration.

Volume for PCR based library sequencing	50 – 600µl ▪▪▪ Samples submitted with less than 100µl will only have sufficient sample for one library prep for sequencing which will increase the likelihood of sample failure. Always try to submit at least 100µl. DNA should be diluted in NHS GMCs to meet the minimum volume required. Accurate measurement of the volume submitted is required. The provision of inaccurate measurement increases the likelihood of sample failure.
	Samples need to achieve both the minimum DNA total volume and concentration.

Table 3 - DNA Requirements from Tumour Samples



Green: sufficient DNA for two library preparations for PCR-free sequencing.

Amber: Single library preparation for PCR-free sequencing or sequencing after PCR library preparation which introduces artefacts.

Red: Sequencing not possible.

Table 4 - DNA from fresh tumour requirements

Sample Handling Guidance:

4.2 PRE-EXTRACTION SAMPLE PREPARATION

4.2.1 Germline Blood

Blood samples should be stored and processed as soon as possible. In the interim they should be kept refrigerated at 4°C.

4.2.2 Saliva

A specialised saliva collection kit must be used to collect saliva samples. Various commercial kits are available. Saliva samples should be collected, stored and processed according to the manufacturer's instructions.

4.2.3 Cancer: Solid Tissue

To maximise DNA yield it is advisable that the fresh tissue samples are homogenised prior to commencing the extraction procedure. Disruption of the tissue by homogenisation ensures effective disruption of the cell wall and subsequent release of the DNA. This can be achieved using a commercial homogeniser (e.g. Qiagen tissue lyser Lt) or by "mincing" with one or two scalpels. If a commercial homogeniser is used it is important to follow the manufacturer's instructions carefully as **over homogenisation can result in shearing of the DNA.**

It is important to ensure that lysis is complete i.e. the lysis solution is 'smooth' at the end of the incubation period. The incubation period required to achieve complete lysis can vary depending on the tissue type (some tissues are more resistant to lysis that others) and how well the sample has been homogenised prior to beginning the DNA extraction procedure. Lysis can be aided by mixing of the sample (intermittent or continual on a shaker) or extending the incubation time, although at the present time **we do not recommend incubation beyond 18 hours (i.e. this enables overnight incubation).**

4.2.4 Haematological cancers: Blood or bone marrow (liquid) tumour

A method appropriate for the extraction of DNA from blood should be used. It is important to perform a nucleated cell count prior to DNA extraction as many samples from patients with haematological cancer will have elevated nucleated cell counts; in these cases the sample will need dilution prior to extraction. If this is not done, there is a danger that the extraction system could be overloaded resulting in poor quality or low yield DNA. For low volume

samples (particularly bone marrow), performing a cell count is essential as these samples often have sufficient DNA for the project if the volume is adjusted for cell count.

4.2.5 Rare disease: RNA

RNA stabilised blood should be processed within 36 hours, in line with requirements for DNA requirements. RNA stabilised blood samples should be aliquoted into 1000µl FluidX tubes or 4ml FluidX tubes. The RNA stabilised blood in the FluidX tubes should then be immediately frozen and stored at -80°C until transported to the Genomics England Biorepository.

4.2.6 Rare disease: Serum and Plasma Samples for Proteomics and Metabolomics

Blood samples for proteomics and metabolomics should be processed within 6 hours and preferably within 4 hours.

Proteomics and metabolomics preparation protocol:

1. Centrifugation at 1300-2000g for 10 minutes
2. Carefully remove the plasma avoiding the separator gel and buffy coat
3. Place the plasma into 1000µl FluidX tubes. 4ml FluidX tubes should not be used
4. Immediately freeze and store at -80°C until transported to the Genomics England Biorepository

4.2.7 Cancer: Plasma for Circulating Cell-Free Tumour DNA

Plasma samples for circulating cell-free tumour DNA should be taken in EDTA or Streck Cell-Free DNA BCT® tubes. EDTA blood tubes should be kept on ice and processed as soon as possible and within 4 hours. EDTA blood can be centrifuged at 4°C or room temperature.

Blood collected in Streck tubes should **not** be stored or transported to the processing lab on ice.

The samples should be stored and shipped between 15°C and 30°C and should be processed within 72 hours as described in Part 4.

Plasma isolation for cfDNA preparation:

1. Centrifuge at 1300 – 2000 x g for 10 minutes at room temperature with the brake off
2. Immediately transfer the plasma supernatant into clean tubes taking care not to disturb the buffy coat
3. Centrifuge the plasma on a benchtop centrifuge at more than 16,000 x g for 10 minutes to remove any remaining cells
4. Aliquot the plasma supernatant into 1000µl or 4ml FluidX tubes. At least one small 1000µl tube aliquot is required for quality testing if the larger tube size is used
5. Freeze and store all aliquots immediately at -80°C until transportation to the biorepository

4.3 DNA EXTRACTION

It is critical that appropriate DNA extraction methodologies are used, in particular the method (either automated or manual) must have been validated for the sample type it is being used to extract.

4.3.1 Saliva

Saliva should be extracted using an appropriate validated method. Saliva samples can sometimes result in DNA samples with a low Absorbance 260/280nm ratio and may benefit from an ethanol precipitation clean up step.

4.3.2 Solid Tissue

NHS GMCs can use any DNA extraction method (automated or manual) suitable for fresh frozen tissue that they have validated for this tissue type. **FFPE extraction methods are NOT suitable for the DNA extraction of fresh frozen tissue.** The amount of tissue used for DNA extraction should be determined locally to meet the DNA output requirements.

Whichever kit/methodology is chosen, it is essential that the manufacturer's instructions are followed precisely. Particularly with regard to the amount of tissue extracted, insufficient could result in low yield but too much can also result in low yield/poor quality DNA due to

overloading the extraction system e.g. columns. Also, most kits come with a handbook which contains modified protocols to suit different sample types or circumstances.

4.3.3 Blood/ Bone Marrow tumour

The same method used to extract germline DNA from blood samples can be used but it is essential that a nucleated cell count is performed prior to extraction and the volume adjusted accordingly (see section 4.2.4).

4.4 DNA VOLUME AND CONCENTRATION

Please always try to send as much DNA and as high a volume sample as possible.

DNA concentration should be measured in the NHS GMC to ensure the minimum concentration and total quantity is achieved. A method that measures the double-stranded DNA content must be used.

The concentration will be re-measured at UKB and at Illumina. If Illumina's measurement of the concentration is too low, the sample will be rejected. There is variability in the DNA concentration measurements across sites and therefore caution should be used when diluting samples as over diluting could lead to having to use PCR which reduces sequencing quality or to rejection of the sample. **DNA must not be concentrated.**

NHS GMCs should dilute the DNA to ensure both the DNA concentration and volumes are met in line with Tables 1 and 3 depending on sample type. Please note that the volumes given are the minimum required and samples at or below the minimum volume requirements have increased chance of failure. If DNA is normalised then the DNA concentration measurement transmitted to Genomics England should be the post-dilution quantification result.

Whichever of the extraction methods is used it is important to adjust the final sample volume with elution buffer to ensure:

a) The minimum sample submission volume of 100µl is met (105µl or above preferred)

b) There is sufficient DNA volume to allow for local QC

c) The DNA concentration is within the permissible range.

The DNA volume, once measured in the NHS GMC is not re-measured. It is therefore **essential** that the volume given to Genomics England accurately reflects the volume of the submitted sample. Inaccurate volume measurements increase the likelihood of sample failure and DNA samples failing to meet the sample volume requirements will be returned to the NHS GMC.

The absolute minimum volume requirement is 100µl; 105µl or above is preferred, to compensate for possible evaporation during transport to UKB.

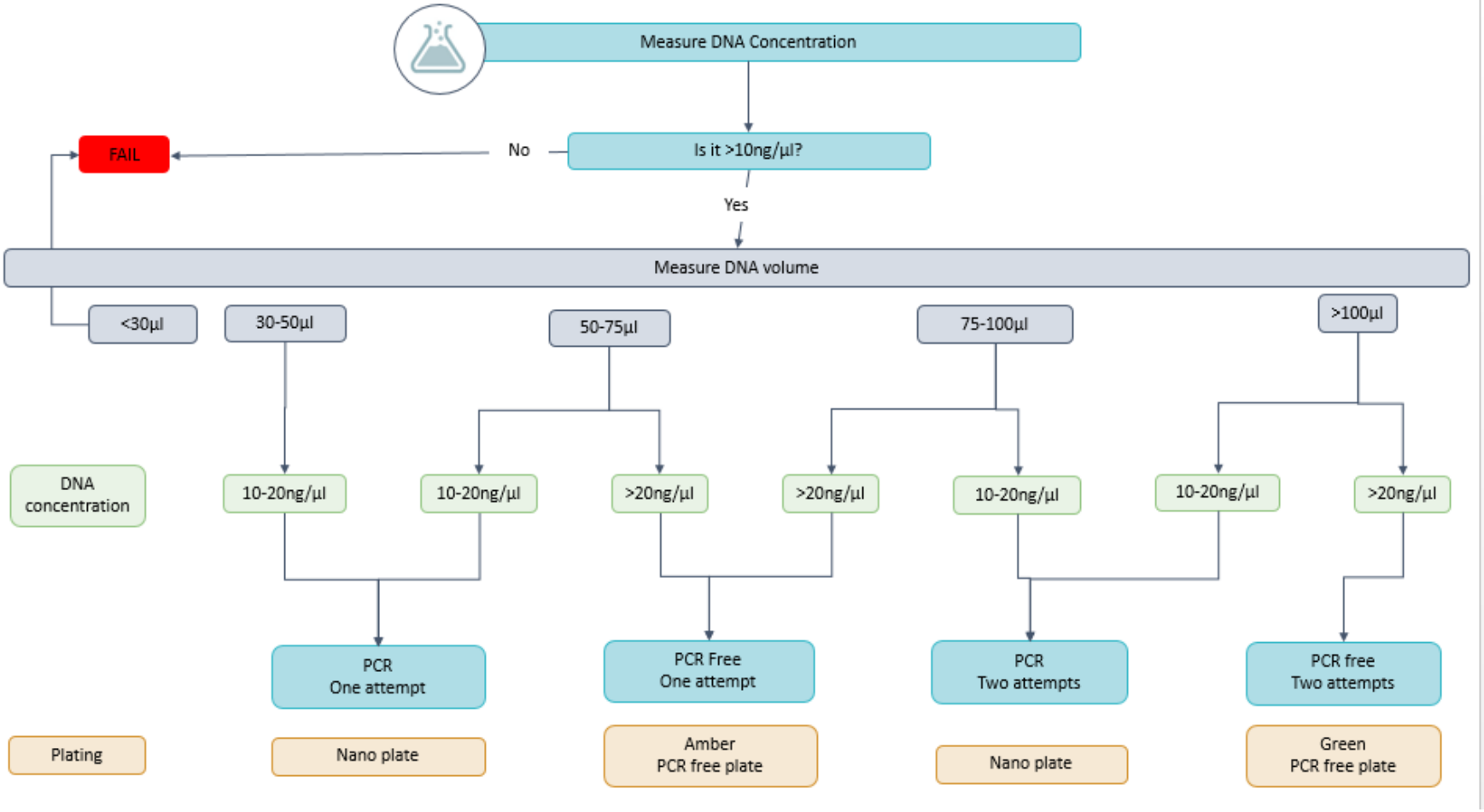


Figure 1 - Decision Tree for volume and concentration requirements

Sample Handling Guidance:

Document Key	PAR-GUI-023	Version Number	4.0

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4.5 DNA FOR VALIDATION

DNA should be stored at the designated NHS GMC Delivery Entity for local testing and validation.

For germline bloods two blood samples are taken for DNA extraction and these should be extracted separately: One DNA sample should be sent to the Genomics England Central Biorepository for QC, plating and transporting to Illumina for WGS and the other stored for validation.

For tumour DNA samples any residual DNA beyond that needed for sequencing should be stored for validation.

4.6 DNA STORAGE

DNA should be eluted into FluidX® 0.7ml external thread screw-cap tubes with 2D data matrix barcodes and stored in the FluidX® racks.

The individual FluidX® racks can be recycled and, where possible, racks are returned to NHS GMCs along with the polystyrene transport boxes. The boxes will be returned to any of the nominated NHS GMC collection points not necessarily the location from which they originated.

It is requested that, where practical, GMCs collect and submit any additional tumour tissue following local procedures. Both DNA and RNA can be extracted using a variety of extraction kits, where these are used the excess cell lysate or RNA in GTC buffer can be stored in 1000µl FluidX tubes at -80°C but laboratories should ensure that DNA yields are not compromised. Excess tissue or cell lysate should be stored at -80°C and submitted with the other omics samples.

DNA samples should be kept at 4°C until all relevant DNA samples are collected, but storage at 4°C should not exceed six weeks. If matched DNA samples will take longer than six weeks then storage should be at -20°C. Any DNA samples which have been stored at -20°C can be shipped on dry ice with the omics samples but DNA samples should be in a separate DNA rack

or defrosted (if required for QC) and shipped with other DNA samples. The aim is to minimise freeze-thaw cycles.

4.7 SAMPLE TUBES

All samples need to be stored and sent to the biorepository in 2D barcoded FluidX® tubes. During shipping to the biorepository the tubes must be stored in FluidX® racks as in Figure 4.

Figure 4 - FluidX® rack showing 2-D barcodes.

DNA and cell lysate samples should be stored in 700µl FluidX® tubes, as in Figure 5.

Figure 5 - FluidX® 700µl tube.

For all omics samples (RNA stabilised blood, serum, plasma, and plasma for circulating cell-free tumour DNA) 1000µl FluidX® tubes may be used (functional capacity 920µl), as in Figure 6.

Figure 6 - FluidX® 1000µl tube

All aliquots should be filled to full functional capacity where there is sufficient sample volume to do so. All aliquots should be sent to the biorepository.

Additionally, 4ml FluidX® tubes can now be used for cancer plasma samples for cfDNA and for RNA stabilised blood to reduce aliquoting. If 4ml tubes are utilised, at least one aliquot should be in a 1000µl tube to allow for quality testing. FluidX® 4ml tubes (65-7511) require 48-well racks (65-7542) rather than the normal 96-well rack used for other tube types.

Figure 7 - FluidX® 4ml tubes

Excess fresh frozen tissue samples should be placed in FluidX® tissue tubes (68-4000-11) and shipped in 24 place racks.

Figure 8 - FluidX® tissue pots

*Collection tube type	Processed sample type	Expected aliquots	Comments – only use FluidX® labware
Rare disease patient			
EDTA	DNA - central	1	Elute DNA into a maximum of 600µl buffer. FluidX® labware system must be used
PST	Plasma	4-5	All aliquots

Sample Handling Guidance:

PAXgene®	RNA stabilised blood	2-9	All aliquots using 4ml or 1000µl Fluid X tubes
SST	Serum	3-4	All aliquots
Cancer patient			
EDTA	DNA	1	Elute DNA into a maximum of 600µl buffer. FluidX® labware system must be used
EDTA/ Streck Cell-Free DNA BCT® tubes (10ml)	Plasma for circulating cell free DNA	5-6	All plasma to be aliquoted using 4ml or 1000µl FluidX® tubes
Tissue	Excess tissue	1	FluidX® tissue tubes
Tissue	Cell Lysate	1	Aliquot into 1000µl FluidX® tube

Table 5 - Expected sample aliquot numbers from adult participants.

4.8 SAMPLE IDENTIFICATION AND DATA FILES

4.8.1 Sample tracking

Before samples can be sent to the biorepository, local systems must be validated to ensure tracking of samples from the point of collection, to arrival at the designated blood processing laboratory and entry into the Laboratory Information Management System (LIMS). All samples should be entered onto a LIMS and processed as soon as they reach the NHS GMC Designated Blood Processing Laboratory. All LIMS systems must be able to:

- Create unique GS1 compliant barcodes/identifiers.
- For each participant, create codes for the different sample types based on the guidance provided from time to time.
- Manage and track samples through an internal barcode system for sample collection vessels and a specified 2D barcoding system on FluidX® consumables for aliquoted samples.
- Be customisable to incorporate all required identifiers for the study, and link and export this data into a csv file and post it to your SFTP folder.
- Record a consignment number for dispatch via NHSBT.

- Link and export information to provide required data to Genomics England and/or NHS England.

4.8.2 Creation of CSV files to send to Genomics England

Sample data must be successfully submitted by the lead GMC to Genomics England along with the accompanying XML registration file or completed OpenClinica registration CRF 24 hours before sample submission to the biorepository. Data should have been successfully received by **12 noon on a Tuesday** for sample collection on a Wednesday. Failure to successfully deliver data by 12 noon will result in shipment cancellation and will delay sample flow. Any fast track cases with delayed data delivery will be transferred to the main programme.

Please refer to sample tracking guidance for details about the data submission.

4.8.3 Sample QC Data

Please refer to Rare Disease Data Model and Cancer Data Model for details.

4.8.4 Sample Metadata

Sample metadata should be linked to all samples and submitted to Genomics England in the form of a .csv file (see example below) prior to dispatch of the samples. A NHSBT consignment number must be included to track the samples and enable the Biorepository to be informed of the samples being dispatched.

Please refer to sample tracking guidance and data models for details.

4.8.5 Sample Manifest

A hard copy of the csv metadata manifest should be printed and included in the sample transport box.

4.8.6 Timings of Data Submissions

All data submissions must have been successfully received by **noon on Tuesday** for pick up by NHSBT on a Wednesday. Failure to do this may result in the sample shipment being cancelled and the sample submission being delayed.

4.9 SAMPLE TRANSPORT TO BIOREPOSITORY

4.9.1 Grouping samples

DNA should be forwarded to the Genomics England Biorepository only when paired with the matching tumour sample (for cancer) or family samples (for rare diseases); samples should be locally stored (and tracked on the LIMS system) until that time. Where the clinical team identify that it is no longer likely that a sample will be collected for a particular family member of a proband, they must update the family size in the data transmitted to Genomics England before forwarding locally stored samples for that family. Should a sample from the family member become available at a later date this should still be collected and should be reported via the service desk before forwarding to the Genomics England Biorepository. The addition of a new family member needs to be assessed on a case by case basis as approval has dependencies on where the rest of the family members are in the sequencing and interpretation pipelines.

4.9.2 Transportation

DNA samples can be transported in any quantities using 96 tube FluidX® racks. However, they must not have hand-applied labels at the time of shipment as the biorepository's automated freezers are unable to process these. If labels are used within the GMC, then please remove prior to shipment.

DNA can be submitted in suitable packaging at ambient temperature. However, given the variable time frame for transportation and to enable ease of shipment of DNA with other samples NHS GMCs may choose to submit all samples frozen. All omics samples must be shipped frozen.

Packaging materials with thermo-regulating capabilities will be provided by Genomics England (supplied by NHSBT Supply Chain) along with further guidance on requirements for packaging. Dry ice will need to be provided by the NHS GMC Lead Organisation.

Collections of (frozen / unfrozen) DNA samples will occur weekly. Packages will be collected from the agreed NHSBT pick up point, unless agreed otherwise, on the same day and time window each week. The NHS GMC lead organisation should endeavour to combine samples from individual LDPs into a single FluidX® rack prior to transportation to the Biorepository.

The NHS GMC lead organization will also ensure the FluidX® micro tubes and Rack ID are rescanned to confirm the new positions of all the samples in the rack before the dispatch manifest is created in LIMS and the samples packaged for dispatch.

4.9.3 Other Blood Samples Transportation

Packages will be collected from an agreed NHSBT pick up point (unless agreed otherwise) on the same day and time window each week.

All samples with the same storage requirements (typically storage at -80°C can be racked together). These samples must be sent in full FluidX® 96 tube racks in multiples of five. An individual participant's omics samples can be split across different racks, and even different consignments. The biorepository cannot safely store FluidX® tubes with hand-applied labels at this time, therefore, any hand-applied labels must be removed from the FluidX tubes prior to shipment.

Omics samples (plasma, serum and RNA stabilised blood aliquots) should be transported frozen on dry ice. Packaging materials with thermo-regulating capabilities for this will be supplied by Genomics England (supplied by NHSBT Supply Chain) along with further guidance on requirements for packaging and returned for re-use. Dry ice will need to be provided by the NHS GMC Lead Organisation.

4.9.4 Frequency and Timing of Sample Collections

Collections of DNA samples will occur weekly on Wednesdays. **Collections must be booked by 12 noon on Tuesday for collection on Wednesday. Please update your local NHSBT collection manager if a collection is not required that week.** Packages will be collected from an agreed NHSBT collection point on the same day and time window each week.

Collections of Fast Track DNA samples will occur weekly on Wednesdays **but will occur independently to Main Pipeline collections.** The collection time slot is managed locally but must be a morning collection so that **samples must reach the Biorepository by 3pm on Wednesday. Please refer to the Fast Track SHG document for full details.**

The collection time slot is managed locally, so please consult with your local NHSBT Supply Chain representative directly. Empty FluidX® racks and transport boxes will be returned to NHS GMCs for recycling (if the NHS GMC system allows), after transport to the Biorepository

is complete*. Please note that it will not be possible to return boxes and racks to the NHS GMC they originated from. If your systems will not allow recycling please inform Genomics England who will stop rack and box returns to your NHS GMC.

*Please note that FluidX® Rack IDs are not unique once recycled and are for transport purposes only.

4.9.5 Consignment Registration with NHSBT

NHSBT is the transportation service for the project. The notification and tracking sheet (provided by NHSBT) should be populated as follows:

- Tab 1: NHS GMC name and collection date;
- Tab 2: Number of Sample and other sample boxes (please note, if none, zero should be entered e.g. if no frozen samples); and number of damaged boxes destroyed at NHS GMC. Once tabs 1 and 2 are populated, the spreadsheet should be emailed to the address generated on Tab. For Fast Track i**t is imperative that the "Fast Track" drop down menu is toggled to "yes" – Failure to do so, may result in the shipment being shipped via the main pipeline;**
- Tab 3: Sample label to be printed;
- Tab 4: Other sample label to be printed.

The Ambient or Frozen labels as printed out from the 'Notification and tracking sheet' are to be placed in the clear plastic document label on the side of the box prior to collection. Consignment ID and Package ID will be allocated by this mechanism.

Responsibility lies with the GMC to ensure that the correct consignment goes with the correct transportation service – along with the correct Notification and Tracking sheet.

4.9.6 Collection Point

Collection is from a transfusion/NHSBT laboratory unless otherwise agreed. When the NHSBT driver arrives; they will have two copies of the notification and tracking sheet in order to check that they are physically collecting the correct number of each type of box (ambient and or dry-ice) and the consignment number labelled on the box against the notification and tracking sheet. When details have been confirmed, both the driver and a member of NHS GMC staff

will put their signature, name, date and time on both copies of the document. One will be left at the NHS GMC and one retained by NHSBT Supply Chain for tracking purposes.

4.9.7 NHSBT communication and process for managing stock levels of transport boxes.

Following delivery of boxes to the biorepository; NHSBT will collect empty used boxes and FluidX® racks and re-distribute the same number and type back to NHS GMCs to ensure levels at each GMC are constant. NHSBT Supply Chain will also remove and destroy old and damaged boxes from the transport cycle and replace with new boxes.

Please notify NHSBT Supply Chain if you destroy a damaged box by completing the relevant fields in the notification and tracking sheet.

4.9.8 NHSBT communication and process for issues with transportation

The first point of contact should be your local NHSBT delivery manager. If an issue requires escalation, please contact the Genomics England Service Desk.

4.10 FAILED SAMPLES

The confirmation of sample receipt at the biorepository is sent out via the weekly 'GMC Reports' distributed by the Genomics England Service Desk team. Details of any samples failing QC at the biorepository or Illumina will be communicated via the Service Desk to a nominated individual in the lead organisation of the NHS GMC for further appropriate distribution. This step enables rapid resolution of issues i.e. obtaining a further aliquot of DNA from the stock sample, or obtaining further samples from the participant as required.

Part 5: Appendices

Contents

5 Appendix A: Sample Collection Consumables

5.1.1 Blood Collection Consumables

The following blood collection tubes are listed to provide indicative blood volume(s) and type(s). The specific tubes used are up to the discretion of the NHS GMC provided that the additive and volume are equivalent.

Multiple tubes for a single sample type can be used to meet total volumes if preferred, although this then needs to be accounted for in sample collection and tracking documentation locally.

5.1.2 Blood EDTA tube

K_3EDTA or K_2EDTA are acceptable for DNA extraction, as long as there is local experience of using these to obtain sufficient quantity and quality of DNA for the project. For DNA extraction a range of volumes between 3ml and 10ml is stated to account for different local practices in Vacutainer use and automated extraction volumes, actual number and volume of tubes to be used is a local decision to ensure the provision of sufficient DNA.

10ml – 16 x 100mm x 10.0ml BD Vacutainer® Plus plastic whole blood tube (K_2EDTA). Lavender BD Hemogard™ closure. Cat # 367525. NHS Code KFK367

5ml – 13 x 100mm x 5.0ml BD Vacutainer® plastic molecular diagnostics tube. Pearlescent white BD Hemogard™ closure. Cat # 362788

4.5ml – 13 x 75mm 4.5ml BD Vacutainer® Glass EDTA tube with Lavender BD Hemogard™ closure. Cat # 367654

4ml – 13 x 75mm x 4.0ml BD Vacutainer® Plus plastic whole blood tube. Lavender BD Hemogard™ closure. Cat # 367839. NHS Code KFK171

3ml – 13 x 75mm x 3.0ml BD Vacutainer® Plus plastic whole blood tube. Lavender BD Hemogard™ closure. Cat # 367838. NHS Code KFK233

5.1.3 PAXgene® for RNA-Stabilised Blood

2.5ml – 16 x 100mm 2.5 ml BD Vacutainer® Plastic RNA tube with Clear BD Hemogard™ closure. Cat # 762165

5.1.4 PST for Plasma Separation

8ml – 16 x 100mm x 8.0 ml BD Vacutainer® Plus plastic PST™ II plasma tube. Light green closure. Cat # 367377. NHS Code KFK128

5.1.5 SST for Serum Separation

- 8.5ml – 16 x 100mm x 8.5ml BD Vacutainer® SST II Advance plastic serum tube. Cat # 367958. NHS Code KFK127

5.1.6 Streck for Plasma for cfDNA

- Cell free DNA BCT tubes, Streck

5.1.7 Saliva Collection

- Oragene DNA OG500, DNA Genotek
- Oragene DNA OG575 for assisted collection, DNA Genotek

5.2 Appendix B: Guidance on sampling by specific organ

Breast Cancer Sampling

Sampling process:

- The fresh breast specimen should be weighed, painted, measured and sliced as per local protocols and RCPath guidelines
- If the tumour is identified a full face with the maximum diameter should be left intact to retain ability to measure microscopically, samples for freezing should be taken from a parallel slice
- The fresh specimen may then be fixed as per usual with formalin for standard examination as per RCPath guidelines

Colorectal Cancer Sampling

Background

- RCPath guidelines on dissection and macroscopic examination of colorectal samples are clear in their requirement for samples to be fixed in formalin for at least 24-48 hours before slicing of the tumour segment
- To avoid contravention of this, sampling of the fresh tumour can be carried out as described below, with subsequent fixation and slicing as per RCPath guidelines

Sampling process:

- For tumours proximal to the peritoneal reflection open the colon as per RCPath guidelines, to within approximately 1cm proximal and distal to the tumour (leaving the tumour segment intact). The tumour is then sampled from the luminal aspect (everting the tumour can facilitate this.)
- For tumours distal to the peritoneal reflection the fresh specimen should be inked as per local protocol. The anterior opening of the specimen may then be extended through and distal to the peritoneal reflection to within 1-2cm of the tumour. The tumour can then be sampled from the luminal aspect (everting the tumour to facilitate this)
- For all colorectal tumours, the very edge of the tumour should be avoided, as this often comprises adenoma
- Frozen sections are necessary not just for tumour content assessment but also to ensure invasion (and not just adenoma) is represented and for accurate image of the tumour

sample (colorectal adenocarcinomas can show great variation in short distances in terms of both proportion of tumour cell nuclei and necrosis). A number of centres take up to three fresh samples for potential frozen section to ensure invasive tumour is represented

Lung Cancer Sampling

Sampling process:

- Fresh lung specimens should preferably be handled in a safety hood (cabinet often used for frozen sections and / or handling fresh tissue.)
- The relationship of the lung mass to the pleura should be assessed and recorded before any incision for fresh sampling
- The tumour can then be incised from the pleural surface avoiding any areas suspicious for pleural invasion and the sample(s) from the fresh tumour obtained under direct visualisation
- Areas of cavitation and suspicious for necrosis should be avoided in order to increase the likelihood of an eligible sample

Ovarian Cancer Sampling

Background:

- Sampling strategies for ovarian cancer may be stage-dependent
- Low stage (I/II) disease (~25% of patients) has a greater distribution of histotypes (of both clinical and academic interest) but may be more challenging to sample fresh
- High stage (III/IV) disease (~75% of patients) comprises almost exclusively (>85%) high grade serous carcinoma but may be less challenging to sample fresh

Sampling process:

- For low stage disease (where tumour is confined to or predominantly involves only the ovary) the specimen should be examined as per RCPath guidelines, with weighing, measuring and careful capsular and tubal examination before sampling, as findings contribute to final stage
- The fresh tumour may be sampled in one of two ways:

    1. After careful external examination of the specimen, the specimen can be sliced at 1cm intervals (as per RCPath guidelines) and the tumour sampled with a clean blade and forceps (to avoid contamination with non-tumour DNA), taking care to

avoid areas of haemorrhage or necrosis to increase the chance of an eligible sample;

2. In centres where frozen sections are carried out on ovarian tumours arriving in the laboratory, local protocols for this may be followed, with sampling conducted in a similar way.

- In either case, sampling should be documented, preferably in the final pathology report (as per the view of the BAGP [British Association of Gynaecological Pathologists] working group)
- For high stage disease, samples of the primary tumour may be taken as described above. Samples of metastatic tumour can be taken from omental or peritoneal deposits (avoiding the need to sample the primary ovarian mass), either at primary surgery (by the surgeon) or in the laboratory post-surgery (by the pathologist). Ultrasound-guided biopsies of intra-abdominal deposits may also be taken for DNA extraction

Prostate Cancer Sampling

Background:

- References used for the process below:

Gill PS, Roberts IS, Browning L, Perera R, Warren AY, Hamdy FC and Verrill C. (2012) 'The handling and sampling of radical prostatectomy specimens for reporting and research: the Oxford approach'. *J Clin Pathol*. 65(12), pp1057-61.

- A video demonstrating these techniques is available here:

https://www.genomicsengland.co.uk/information-for-gmc-staff/cancer-programme/transforming-nhs-services/

Sampling process:

- Punches should be selected systematically from a prostate slice near the apex
- The location of the punches should be indicated on an image or using coloured inks to correlate with the whole mount slide
- Tumour content can be assessed on FFPE slides from the tissue at the periphery of each fresh sampled block

Renal Cancer Sampling

Sampling process:

- Orientate and macroscopically examine the specimen as per RCPath guidelines
- From the bi-valved specimen a fresh tumour sample may then be taken, ensuring this sample does not come from any area where staging could be impacted

Sarcoma Sampling

Background:

- In many cases, sarcoma diagnosis and prognosis is based on more than morphological assessment alone, with genetic information and molecular diagnostic techniques playing an increasingly important role, with some investigations requiring unfixed material
- The RCPath dataset for soft tissue sarcoma states that 'where feasible, arrangements should therefore be made for specimens to be submitted to the laboratory in the fresh state and without delay so that a suitable sample of tissue can be frozen in liquid nitrogen and stored at -80°C in an appropriate facility.'

Sampling process:

- After inking (according to local protocols) and slicing of the fresh mass, the sample should be examined as per RCPath guidelines
- A fresh sample should be collected away from the resection margin, avoiding cystic, haemorrhagic and necrotic areas if possible

Bladder (& Urinary Tract) Cancer Sampling

Background:

- As stated in the updated (August 2016) Annex B, for the purposes of this project, bladder cancer includes similar (urothelial carcinoma) of the renal pelvis, ureter urinary bladder and urethra
- For TUR specimens (sampling process described below), communication between Urologist and Pathologist is key, and SOPs for this should be a collaborative effort

Sampling process:

- For transurethral resection (TUR) specimens the preferred method for sample selection is for the Urologist to identify and sample an area of malignancy to be kept as a separate fresh 'genomic' specimen with the remaining sample put into formalin for conventional diagnosis. This facilitates more reliable sampling of tumour (as opposed to uninvolved urinary tract wall) and mitigates the risk of fresh tissue sampling interfering with staging on the FFPE diagnostic series
- The 'genomic' specimen can then be kept fresh and transported to the laboratory
- From this 'genomic' specimen, tissue can be taken for formal freezing with frozen section for tissue assessment
- For resection specimens fresh tissue should be collected according to agreed protocols by, or under the guidance of, a pathologist
- As per RCPath guidance, protocols should be designed such that diagnosis, staging and margin assessment are not compromised (as for all tumours)
- For cystectomy specimens, after inking (or not, as per local protocols) if a tumour of the bladder wall is visible, a shave of the macroscopic tumour can be taken, avoiding incision into the bladder wall itself
- Beyond this, as types of resection specimens from the urinary tract are wide-ranging and varied, more explicit instructions are outside the scope of this document, and specimens should be assessed by the pathologist on a case-by-case basis

Endometrial Cancer

Background:

- As stated in the RCPath dataset, endometrial cancers are particularly susceptible to autolysis, therefore the fresh specimen should be refrigerated immediately (if necessary) before transport to the Pathology lab as soon as possible

Sampling process:

- The uterus should be opened (after any inking and parametrial sampling, as per protocol) with a clean knife as soon after receipt as possible

- Tumour should be sampled away from the deepest depth of myometrial invasion (if macroscopically assessable), or sampling only intra-cavity tumour, to avoid impacting on tumour staging

Melanoma

Background:

- Many primary melanomas (in particular, cutaneous melanomas) are difficult to sample fresh without potentially compromising assessment. Where there are lymph node metastases, satellite nodules or more distant metastases, these may be sampled fresh instead.

Testes

Background:

- Many germ cell tumours are poorly cohesive, and become more so, with delays in incision and fixation. These specimens should therefore be refrigerated immediately (if necessary) before transport to the Pathology lab as soon as possible to avoid compromising assessment, particularly tumour subtyping
- All subtypes of germ cell tumour (not just classical seminoma) are of interest, therefore, areas with variable macroscopic appearance, including haemorrhagic areas, may be sampled as these often signify areas of non-seminomatous germ cell tumour

Sampling process:

- For orchidectomy specimens the fresh specimen should be opened as per local protocols and with a clean knife as soon as possible after receipt in the laboratory.
- The lesion should be sampled away from the rete and cord to avoid compromising accurate staging

Upper GI Tumours – Gastric Tumours

Background:

- As per RCPath guidelines, specimens should be received fresh as soon as possible after resection, refrigerating before transport if this will be delayed

- Most gastric resections for carcinoma contain a macroscopically visible and/or palpable tumour
- However, increasingly with the use of neoadjuvant therapy, tumour may not be macroscopically obvious, in which case, fresh sampling may not be possible

Sample processing:

- The gastric specimen should be opened as per RCPath guidelines
- For macroscopically visible tumours, the tumour should be sampled from the luminal aspect parallel to the wall away from any serosal abnormalities and without extending deep into the gastric wall to prevent compromising assessment of depth of invasion and serosal involvement

Pancreatobiliary Tumours

Sample processing:

- The margins of the specimen should be examined and inked (the latter according to local protocols) before being sampled, as per RCPath guidelines
- For pancreatic resections the fresh specimen may be taken after the first slice through the pancreas using the axial slicing technique, as described in the RCPath guidelines
- The remainder of the specimen can then be fixed in formalin before completing slicing and block selection for the diagnostic series

Hepatic Tumours

Background:

- Liver resections may be carried out for the excision of hepatocellular carcinoma (HCC), cholangiocarcinoma (CC), colorectal cancer liver metastases (CRCLM) and more rarely, gallbladder cancer
- Unless stated, guidance is generic for all of the above scenarios

Sample processing:

- Fresh tumour may be obtained by slicing the specimen fresh after inking the resection margin, or, if identifiable from the capsular surface, by excising a portion through the capsule

If the capsule appears involved, or has adherent adipose tissue (which may indicate underlying capsular breach) the tumour may be sampled under direct vision through adjacent uninvolved capsule.

5.3 Appendix C: Proforma for submission of 20+ Stored Samples or outside normal criteria

For NHS Genomic Medicine Centres who wish to submit stored samples of DNA that consist of more than 20 individuals or were collected prior to 1 January 2015, but meet all other criteria outlined as stated below, permission on a case by case basis can be given by Genomics England and NHS England for inclusion in the main programme. GMCs need to complete this proforma and submit to ge-servicedesk@genomicsengland.co.uk with 'stored samples proforma' in the subject bar.

Sample Information

1.	**Rationale for inclusion**	Cohort of samples greater than 20	Yes/No
		Are *all* samples collected after 1 January 2015 (if no please provide specific numbers in point 4)	Yes/No
		Exemption from other stored sample requirements (see point 2)	Yes/No
2.	**If you are seeking an exemption from other requirements please specify** (Note: exceptions may require approval by the Genomics England Scientific Advisory Committee)		
3.	**If the sample(s) were collected before 1 January 2015, will the patient(s) be re-consented using the Genomics England consent?**	Yes/No	
	If no to the above, please provide the consent you propose using		
4.	**How many Stored samples are you proposing submitting (please confirm in which disease groups and indicate if collected pre/post 1 January 2015)**		
5.	**For cancer please confirm if any of these samples are linked to any prospective samples being submitted for sequencing**		
6.	**Please confirm family structures of samples (if applicable)**		
7.	**Please detail in which state the samples have been stored (DNA, Frozen tissue, buffy coat etc)**		

Sample Handling Guidance:

8.	**I confirm that DNA has been extracted from these samples using the standard GEL protocol**	Yes/No
	If no to the above, please describe the extraction method used:	
9.	**I confirm that these samples have been extracted and stored in an ISO accredited/ NEQAS approved lab**	Yes/No
	If no to the above, please give details of the storage facility and any relevant accreditation:	
10.	**I confirm that only DNA samples passing current QC standards will be submitted**	Yes/No
11.	**Please provide any other relevant information**	

NHS GMC Information

By completing this you are confirming that, other than detailed above, these stored samples meet all other criteria outlined below.

Name of GMC:	
Contact name for any queries on this proforma:	
Job title:	
Email:	
Tel:	
Clinical Director approval given:	
Name:	
Date:	

Stored Samples Criteria for Inclusion

1. Stored samples should not exceed 10% of contracted volumes for cancer or rare disease.

2. Participants must:

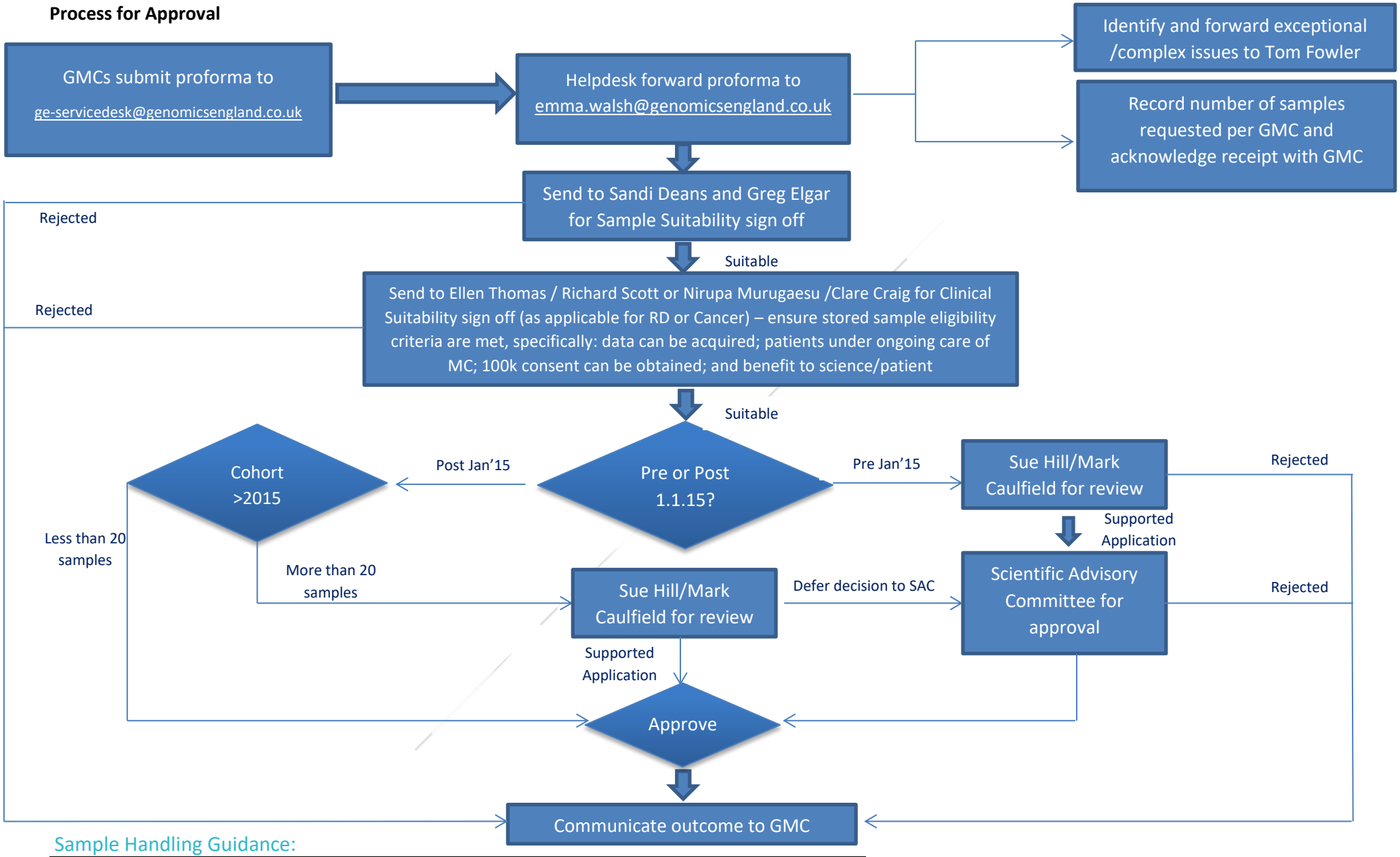
- meet eligibility criteria outlined in Annex A or Annex B
- meet other requirements outlined in the contract, in particular around the provision of clinical and other data
- have appropriate consent for inclusion in the 100,000 Genomes Project
- have the potential to benefit or, when recruiting to relevant eligible diseases where the proband may be deceased/foetal sample, family members must have the potential to benefit (e.g. through informing reproductive choices). Foetal samples may only be included if there is an extremely strong likelihood of a heritable monogenic syndrome

Sample Handling Guidance:

3. Samples must:
   - have been collected after 1 January 2015 (or specific approval obtained for samples collected prior to this date)
   - have been processed in line with NHS GMC contractual requirements for sample handling and have passed the relevant QC requirements
   - for tumour samples, the DNA should be extracted from fresh frozen tissue
   - be indicated on the weekly return of samples collected to NHS England and recorded as a stored sample (initially through notification to the Genomics England helpdesk and as the relevant data models are updated via standard submission of data)

4. Omics samples are currently optional but encouraged.

Process for Approval



Sample Handling Guidance:

5.4 Appendix D: Checklist for producing CD138+ve enriched cells from bone marrow samples

Prior to commencing the collection and sorting of CD138+ cells for Myeloma tumour samples, laboratories must provide evidence of the verified protocols to be used, and show evidence that appropriate purity is being achieved. The checklist for producing CD138+ve enriched cells from bone marrow samples (below) must be completed and submitted to england.genomics@nhs.net. Confirmation of approval must be given before any recruitment of a patient with myeloma commences.

Checklist for producing CD138 +ve enriched cells from bone marrow samples	
Rational ***Prior to DNA extraction, Multiple Myeloma patients' bone marrow samples must be enriched for CD138 +ve cells. This is to ensure a sufficiently high neoplastic content for submission to the 100,000 Genomes Project cancer programme.***	
Checklist item	**GMC response**
Process in place for rapid transfer of the bone marrow sample to the cell sorting laboratory. Please provide details and target timelines.	
Confirmation that in-house verification of CD138 cell sorting has been performed. Please submit the verification report.	
Confirmation that the cell sorting will be performed as soon as possible after sample collection. Preferably on the day of collection but certainly within 24 hours. Please provide evidence that this is routinely met within the laboratory.	
Methodology is in place for the measurement of CD138 +ve cell purity, pre and post sort. Please submit SOP.	
Methodology is in place for isolating (sorting) highly purified CD138 +ve cells from bone marrow. Please submit SOP.	
Confirmation that bone marrow samples will only be processed if the pre-sort sample contains > 5% CD138 +ve cells.	
Confirmation that post-sort samples will only proceed to DNA extraction and submission to Genomics England if they have a purity of >40% CD138 +ve cells. Samples with <40% purity must not be submitted.	
DNA extraction method is in place for extracting DNA from purified CD138 +ve cells. Please submit SOP.	
Methods are in place for the measurement of quantity & quality of DNA extracted from CD138 +ve cells. Please provide details of methods performed.	

5.5 Appendix E: Disease type and subtype mappings

Breast	Lung	Renal	Testicular
Ductal	Adenocarcinoma	Chromophobe	Choriocarcinoma
Lobular	Large cell	Clear cell	Classical seminoma
Medullary	Mesothelioma	Collecting duct	Embryonal carcinoma
Mesenchymal	Mixed tumour type	Non specified renal carcinoma	Germ cell tumour
Mixed tumour type	Neuroendocrine carcinoma	Not available	Granulosa cell tumour
Mucinous carcinoma	Not available	Oncocytic	Leydig cell tumour
Neuroendocrine	Other	Other	Not available
Not available	Small cell	Papillary Type 1	Other
Other	Squamous cell	Papillary Type 2	Sertoli cell tumour
Papillary	Unknown	Unknown	Sertoli-Leydig
Tubular / cribriform		Urothelial (in situ)	Spermatocytic Seminoma
Unknown		Urothelial (invasive)	Teratoma differentiated
		Neuroendocrine carcinoma	Teratoma undifferentiated
			Unknown
			Yolk sac tumour
			Neuroendocrine carcinoma

Prostate	Bladder	Upper GI	Colorectal
Adenocarcinoma	Adenocarcinoma	Gastric adenocarcinoma	Adenocarcinoma
Not available	Not available	Gastric carcinoma with lymphoid stroma	Not available
Small cell	Other	Hepatoid adenocarcinoma	Unknown
Unknown	Sarcomatoid carcinoma	Not available	Neuroendocrine carcinoma
Neuroendocrine carcinoma	Small cell	Other	Other
Other	Squamous cell	Poorly cohesive including signet ring cell type	
	Unknown	Unknown	
	Urothelial (in situ)	Adenocarcinoma	
	Urothelial (invasive)	Neuroendocrine carcinoma	
	Neuroendocrine carcinoma	Squamous cell	

Hepatopancreatobiliary	Adult Glioma	Adult Glioma cont.	Childhood
Acinar cell carcinoma	Anaplastic astrocytoma	Pilomyxoid astrocytoma	Atypical teratoid rhabdoid tumours
Biliary adenocarcinoma	Anaplastic ependymoma	Pineal tumour of intermediate differentiation	Childhood other
Biliary adenosquamous carcinoma	Anaplastic ganglioglioma	Pineoblastoma	CNS primitive neuroectodermal tumors
Biliary squamous cell carcinoma	Anaplastic oligodendroglioma	Pineocytoma	Embryonal tumour with multilayered rosettes
Cholangiocarcinoma	Anaplastic oligoastrocytoma	Pleomorphic xanthoastrocytoma	Extracranial rhabdoid tumour
Combined HCC-cholangiocarcinoma	Central neurocytoma	Primitive neuroectodermal tumour	Medulloblastoma (CLA)
Fibrolamellar HCC	Diffuse astrocytoma	Protoplasmic astrocytoma	Medulloblastoma (DN)
HCC acinar	Ependymoma	Subependymal giant cell astrocytoma	Medulloblastoma (LCA)
HCC compact	Extraventricular neurocytoma	Subependymoma	Medulloblastoma (MBEN)
HCC trabecular	Fibrillary astrocytoma	Teratoma (immature)	Medulloblastoma (medullomyoblastoma)
Hepatocellular carcinoma	Gangliocytoma	Teratoma (mature)	Medulloblastoma (melanotic)
IPMN with invasive carcinoma	Ganglioglioma	Teratoma with malignant transformation	Medulloblastoma (NOS)
Lymphoepithelioma-like HCC	Gemistocytic astrocytoma	Unknown	Neuroblastoma (INPC)
MCN with invasive carcinoma	Germinoma		Neuroblastoma (NOS)
Neuroendocrine	Giant cell glioblastoma		Not available
Neuroendocrine carcinoma	Glioblastoma		Other
Not available	Gliomatosis cerebri		Paediatric malignant glioma
Other	Gliosarcoma		Unknown
Pancreatic adenocarcinoma	Mixed germ cell tumour		Neuroendocrine carcinoma
Pancreatic adenosquamous carcinoma	Myxopapillary ependymoma		
Pancreatic neuroendocrine carcinoma	Not available		
Sarcomatoid HCC	Oligoastrocytoma		
Schirrous HCC	Oligodendroglioma		
Serous cystadenocarcinoma	Other		
Undifferentiated HCC	Papillary tumour of the pineal region		
Unknown	Pilocytic astrocytoma		

Sample Handling Guidance:

Haematological Oncology	Cancer of unknown primary	Sarcoma	Sarcoma cont.
(ALL) Acute lymphoblastic leukaemia	Adenocarcinoma	Adamantinoma of bone	Malignant peripheral nerve sheath tumour
(AML) Acute myeloid Leukaemia	Cancer not confirmed (pathology indeterminate)	Alveolar soft part sarcoma	Malignant rhabdoid tumour
Chronic lymphocytic leukaemia	Pathology indeterminate – cancer NOS (non-carcinoma, lymphoma , sarcoma etc)	Angiomatoid fibrous histiocytoma	Myoepithelioma / Malignant myoepithelioma
Burkitt lymphoma	Pathology indeterminate – carcinoma NOS (Not otherwise specified)	Angiosarcoma	Myxofibrosarcoma
Chronic myeloid leukaemia	Melanoma	Chordoma	Myxoid liposarcoma
Diffuse large B-cell lymphoma	Neuroendocrine	Clear cell	Myxoinflammatory fibroblastic sarcoma
Haematological malignancy unclassified	Neuroendocrine carcinoma	Conventional chondrosarcoma	Not available
High grade lymphoma NOS	Not available	Dedifferentiated chondrosarcoma	Ossifying fibromyxoid tumour
Mediastinal B-cell lymphoma	Other	Dedifferentiated liposarcoma	Other
Multiple myeloma	Small cell	Dermal sarcoma	Pleomorphic sarcoma
Myelodysplastic syndrome (high risk)	Squamous cell	Desmoplastic round cell sarcoma	Primary conventional osteosarcoma
Not available	Unknown	Epithelioid angiosarcoma	Pseudomyogenic haemangioendothelioma
Other		Extraskeletal chondrosarcoma	Rare sarcoma entities
Unknown		Extraskeletal chordoma	Rhabdomyosarcoma
Non-Hodgkins B cell lymphoma low / moderate grade		Gnathic bone	Sarcoma NOS
Classical Hodgkins		Intimal sarcoma	Sclerosing epithelioid fibrosarcoma
Nodular Lymphocyte Predominant Hodgkins		Leiomyosarcoma from all sites	Synovial sarcoma - monophasic and biphasic
T-cell lymphoma		Low grade fibromyxoid sarcoma	Unknown

Sinonasal
Adenocarcinoma
Adenoid cystic carcinoma
Inverted papilloma
Melanoma
Neuroendocrine
Not available
Squamous cell
Undifferentiated carcinoma
Unknown
Neuroendocrine carcinoma
Other

Nasopharyngeal
Nasopharyngeal carcinoma Type I
Nasopharyngeal carcinoma Type II
Nasopharyngeal carcinoma Type III
Not available
Unknown
Neuroendocrine carcinoma
Other

Oral /Oropharyngeal
Betel nut-induced oral cancer
HPV negative oropharyngeal cancer
HPV positive oropharyngeal cancer
Not available
Unknown
Neuroendocrine carcinoma
Other

Endometrium
Carcinosarcoma
Clear cell
Endometrioid adenocarcinoma
High grade endometrial stromal sarcoma
Low grade endometrial stromal sarcoma'
Mucinous carcinoma
Not available
Other
Serous carcinoma
Small cell
Transitional cell carcinoma
Undifferentiated carcinoma
Undifferentiated uterine sarcoma
Unknown
Neuroendocrine carcinoma

Melanoma
Acral lentiginous
Anorectal mucosal melanoma
Conjunctival melanoma
Desmoplastic melanoma
Genital mucosal melanoma
Lentigo maligna
Nodular
Not available
Orbital
Oromucosal melanoma
Other
Primary CNS
Spitz
Superficial spreading
Unknown
Uveal melanoma

Ovarian
Carcinosarcoma
Choriocarcinoma
Clear cell
Clear cell adenocarcinoma
Dysgerminomas
Embryonal carcinoma
Endodermal sinus
Endometrioid adenocarcinoma
Germ cell tumour
Granulosa cell tumour
Granulosa theca
High grade serous carcinoma
Leydig cell tumour
Low grade serous adenocarcinoma
Mucinous carcinoma
Not available
Other
Sertoli cell tumour
Sertoli-Leydig
Small cell carcinoma (including hypercalcaemic type)
Stromal tumour
Teratoma (immature)
Teratoma (mature)
Teratoma with malignant transformation
Transitional cell carcinoma
Unknown
Yolk sac tumour
Neuroendocrine carcinoma

Sample Handling Guidance:

5.6 Appendix F: Summary Table of Cancer DNA requirement

Blood			Sequencing information	Risk
Optimal DNA sample requirements	**DNA Quantity**	>10µg For limited blood sample volumes >4µg is acceptable	PCR-free WGS, sufficient DNA for additional sequencing attempt if required.	Low risk of sample rejection and loss of sequencing quality
	Concentration	30-100ng/µl		
	Volume	100-600µl		
	A260/A280 ratio	1.75-2.04		
FF Tissue (including biopsy material)				
Optimal DNA sample requirements	**DNA Quantity**	Minimum 2µg	PCR-free WGS, sufficient DNA for additional sequencing attempt if required.	Low risk of sample rejection and loss of sequencing quality
	Concentration	20-60ng/µl		
	Volume	100-600µl		
	A260/A280 ratio	1.75-2.04		
Alternative options	**If DNA Quantity**	1.3-2.5 µg, concentration >25ng/µl	PCR-free WGS, sufficient DNA for only 1 sequencing attempt.	Moderate risk of sample rejection and loss of sequencing quality
	If DNA Quantity	1.0-1.3µg, concentration >10ng/µl	PCR-based WGS, sufficient DNA for additional sequencing attempt if required.	Moderate risk of sample rejection and loss of sequencing quality
	If DNA Quantity	0.5-1.0µg, concentration >10ng/µl	PCR-based WGS, sufficient DNA for only 1 sequencing attempt.	Moderate risk of sample rejection and loss of sequencing quality
	If Concentration	10-25ng/µl	PCR-based WGS	Moderate risk of sample rejection and loss of sequencing quality
	If Volume	50-100µl	Sufficient DNA for only 1 sequencing attempt.	Moderate risk of sample rejection and loss of sequencing quality
FFPE Tissue				
Optimised FFPE DNA sample requirements	**DNA Quantity**	2µg	PCR free WGS allows 2x sequencing attempts	Moderate risk of sample rejection and loss of sequencing quality
	Concentration	20-100ng/µl		
	Volume	100-600µl		
	A260/A280 ratio	1.75-2.04		
	ΔCq assay	≤2.5		

5.7 Appendix G: Formalin Fixed tissue preparation and processing

Under exceptional circumstances, cases may be submitted that have been processed with optimal formalin fixation. Such circumstances include inability to identify the tumour in the fresh specimen, or where the tumour is too small to sample, or if no tumour is present in the FF sample. Samples where FF tissue has not been taken due to operational reasons (e.g. late running theatre list) will not be accepted. Consent to send such cases must be sought from the Genomics England Service Desk (ge-servicedesk@genomicsengland.co.uk).

5.7.1 Controlled Sample Fixation

Controlled fixation is essential to minimise damage to nucleic acid, thus selection of appropriate blocks from the fresh sample should be undertaken and placed into fixative. Evidence indicates that damage is reduced using 10% Neutral Buffered Formalin (NBF), pH 7. ***All FFPE samples must be fixed using Neutral Buffered Formalin.*** The time of placing the specimen or genomic block into fixative should be recorded.

5.7.2 FFPE Sample Selection

Immediate Selection of FFPE

If the tumour is identifiable, but too small to take FF samples, or the likely area of abnormality is evident but not sufficiently targetable to take FF samples, genomic blocks may be selected from the fresh sample and placed into 10% NBF. This allows optimal and controlled fixation. The selected tissue should be standard size (not megablock) and be fixed for 12-24 hours, (including fixation time on the processor). Fixation for more than 24 hours adversely affects the quality of WGS. Selection of this 'genomic block' circumvents the need to cut-up the entire specimen within 24 hours.

The remainder of the specimen can then be placed in a large volume of formalin (10-20 x specimen volume), using tissues to interleave between slices.

FFPE sample selection post-fixation

Where the tumour is too diffuse to select blocks on the fresh specimen, a fixed genomic block should be selected within 24 hours fixation, having cut or sliced the specimen as outlined above. The genomic block should be selected and placed in a cassette for separate processing even if the rest of the specimen is not ready for cut-up. If no separate block can be selected (in the case of a macroscopically unidentifiable tumour) then the specimen should be cut-up for

diagnostic purposes, within a maximum of 36 hours fixation (including fixation time on the processor).

The length of time of fixation of genomic block and diagnostic blocks should be recorded and submitted as applicable. The genomic block should be labelled with the unique Genomics England participant ID (though it may also form part of the diagnostic block set).

5.7.3 FFPE Tissue Processing and Embedding

Prolonged incubation in formalin or in hot wax is deleterious to tissue nucleic acid, therefore 'weekend' processing schedules is not permitted. The total time in formalin for the genomic blocks should not exceed 24 hours (with a maximum of 36 hours in certain situations, as indicated above). Tissue blocks should be embedded according to standard procedures.

5.7.4 DNA Extraction from FFPE Tissue

Two FFPE DNA extraction protocols are currently permissible. They have been developed from the best available evidence and must be followed whenever DNA extracted from FFPE tissue is to be submitted to the cancer programme. NHS GMCs may select either method and will be expected to follow the outlined protocol for their selected method. The experimental phase is still ongoing and it is envisaged that as new evidence becomes available, these protocols will be superseded, at which time new procedures will be circulated by Genomics England. The protocols are provided in full in below.

FFPE sample type and preparation

Three types of samples are routinely taken from FFPE blocks for DNA extraction (scrolls, mounted sections or punch cores); at the present time, there is no clear evidence that any of these sample types is superior therefore all are currently acceptable. However each sample type has specific requirements so it is very important that the following recommendations are observed.

Slide mounted sections

Five to 10 sections (5-10 microns thick) should be cut and mounted onto slides. The greater the number of sections used (or the bigger the tissue area) the higher the DNA yield is likely to be. However it is very important not to overload the extraction system as this could have a detrimental effect on downstream DNA yield and purity. ***Therefore when using the maximum thickness of 10 microns with a surface area of up to 250mm^2 per DNA extraction, do not exceed 8 sections for DNA extraction.*** The use of mounted sections will allow for macro-dissection (scraping with a scalpel) of the neoplastic area (using an accompanying marked H&E

slide as a guide) into a tube, this will help to ensure the material going forward into the DNA extraction has >40% neoplastic content.

Scrolls

Between 5-10 scrolls at 5-10 microns thick should be cut and placed into a tube. The greater the number of scrolls used (or the bigger the tissue area) the higher the DNA yield is likely to be; however, it is very important not to overload the extraction system as this could have a detrimental effect on downstream DNA yield and purity. ***Therefore, blocks must be trimmed of excess wax before the scrolls are cut. When using the maximum thickness of 10 microns with a surface area of up to 250mm^2 per DNA extraction, do not exceed 8 scrolls for DNA extraction.*** The use of scrolls will not allow macro-dissection of the samples prior to DNA extraction so it is very important to ensure that each scroll has >40% neoplastic content.

Punched cores

A disposable 1mm puncher (e.g. 1mm Miltex disposable biopsy punch with plunger (or equivalent) can be used to obtain punch core samples from the FFPE block. The block is lined up with an H&E slide on which the tumour area has been marked. The puncher is stabbed into an area of the block corresponding to that marked on the slide. It is then twisted, pulled out of the block and the core dispensed (by pressing the puncher button) into a 1.5ml tube. It is recommended that x2 cores are used per DNA extraction. Using punched cores allows a constant and uniform amount of FFPE material to go forward for DNA extraction for every patient, however it is important to ensure that both ends of the core have >40% neoplastic content.

5.7.5 DNA extraction from FFPE sections using the QIAamp® FFPE Tissue kit

The QIAamp® DNA FFPE Tissue Kit (manufactured by QIAGEN) is optimized for purification of DNA from FFPE tissue sections. Paraffin is removed from the sample using solvents and incubation at an elevated temperature after proteinase K digestion partially removes formalin crosslinking of the released DNA. After sample lysis, the QIAamp® procedure uses a spin column to yield pure DNA bound to a silica-based membrane which is eluted in buffer or water and is immediately ready for use.

5.7.5.1 Protocol Summary

Please note this protocol will require local COSHH assessment, staff training and may require adaption to fit local guidelines on labelling and sample transfers.

The procedure consists of 6 steps:

I. Remove paraffin: paraffin is removed using xylene.

II. Lyse: Sample is lysed under denaturing conditions with Proteinase K at 56°C.

III. Heat: Incubation at 90°C reverses formalin crosslinking.

IV. Bind: DNA binds to the membrane and contaminants flow through.

V. Wash: Residual contaminants are washed away.

VI. Elute: Pure, concentrated DNA is eluted from the membrane.

5.7.5.2 Preparation of Kit Buffers

Preparing Buffer AW1

Add 25 ml ethanol (96–100%) to the bottle containing 19ml Buffer AW1 concentrate. Tick the check box on the bottle label to indicate that ethanol has been added and add the date of addition. Reconstituted Buffer AW1 can be stored at room temperature (15–25°C) for up to 1 year.

Note: Before starting the procedure, mix reconstituted Buffer AW1 by shaking.

Preparing Buffer AW2

Add 30 ml ethanol (96–100%) to the bottle containing 13ml Buffer AW2 concentrate. Write the date of addition of ethanol on the bottle label. Reconstituted Buffer AW2 can be stored at room temperature (15 - 25°C) for up to 1 year.

Note: Before starting the procedure, mix reconstituted Buffer AW2 by shaking.

5.7.5.3 Deparaffinisation

Before proceeding to DNA extraction the paraffin wax needs to be removed from the FFPE sample. If scrolls are used then this should be done using the xylene tube based method (below). Punch core samples can also be deparaffinised using this method but because of their low surface area it is recommended that they undergo disruption prior to de-wax e.g. using the Qiagen TissueLyser.

If mounted sections are used then there is a choice of either deparaffinisation after macro-dissection (using the tube based method below) or they can be dewaxed whilst still on the slide (using the slide based method below) and macrodissected afterwards.

Whichever protocol is used, it is important to ensure the wax has been thoroughly removed from the sample so that it does not interfere with the downstream DNA extraction processes.

Xylene tube based method

1ml of xylene is added to FFPE scrolls or macro-dissected scrapings in a 1.5ml tube. Close the lid and vortex vigorously for 10 seconds. Centrifuge at high speed for 2 minutes (room temperature), remove the supernatant by pipetting. If there is still paraffin in the pellet it may be necessary to repeat this step with 1ml of fresh xylene. Add 1ml of ethanol (96-100%) and mix by vortexing, centrifuge at full speed for 2 minutes and remove the supernatant (this will remove the residual xylene). Incubate the tube at 37°C for 10 minutes to allow the ethanol to evaporate.

Xylene slide based method

In this protocol the paraffin is removed from mounted sections prior to macro-dissection. Add the slides to a slide staining dish with sufficient xylene to cover the tissue and soak for 5 minutes. Transfer the slides to a staining dish with sufficient ethanol (100%) to cover tissue and soak for 5 minutes. Remove the slides and allow to air dry completely (5 to 10 minutes). The tumour area from the slides can then be macrodissected (using an accompanying marked H&E slide as a guide) into a 1.5ml tube containing the lysis buffer (see below) which will be used in the first step of DNA extraction.

5.7.5.4 DNA Extraction

I. Add 180µl Buffer ATL and 20µl proteinase K to each labelled tube containing the FFPE sample (or macrodissect the de-waxed sample into the lysis mixture).

II. Incubate the tubes on a heating block at 56°C for between 1 - 18 hours. If a shaking heating block is available the solution can be mixed at 1000 rpm. If not, manual mixing by vortexing several times during the incubation period will help to digest and disperse clumps of tissue. To obtain a good yield of DNA it is important to ensure lysis is complete before proceeding to the next step, however prolonged digestion (>overnight) is not recommended as it could potentially cause DNA degradation which could influence the quality of the downstream WGS.

III. De-crosslink by incubating at 90°C on a heating block for a further 1 hour (ensuring the tissue is covered in solution). If a shaking heating block is available the solution can be mixed at 1000 rpm.

IV. Briefly centrifuge the 1.5ml tube to remove drops from the inside of the lid.

V. Add 200µl Buffer AL to the sample, and mix thoroughly by vortexing. Then add 200µl ethanol (96– 100%), and mix again thoroughly by vortexing. Buffer AL and ethanol can be premixed and added together in one step to save time when processing multiple samples.

VI. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.

5.7.5.5 DNA purification

I. Label the lid of a QIAamp® MinElute column for each sample.

II. Carefully transfer the entire lysate to the QIAamp® MinElute column (in a 2ml collection tube) without wetting the rim, close the lid, and centrifuge at 6000 x g for 1 minute. Place the QIAamp® MinElute column in a clean 2ml collection tube. Pour off the flow-through and discard the collection tube. If the lysate has not completely passed through the membrane after centrifugation, centrifuge again at a higher speed until the QIAamp® MinElute column is empty.

III. Carefully open the QIAamp® MinElute column and add 500µl Buffer AW1 (ensuring that the ethanol has been previously added – see 5.1) without wetting the rim. Close the lid and centrifuge at 6000 x g for 1 minute. Place the QIAamp® MinElute column in a clean 2ml collection tube, and discard the collection tube containing the flow-through.

IV. Carefully open the QIAamp® MinElute column and add 500µl Buffer AW2 (ensuring that the ethanol has been previously added see 5.2) without wetting the rim. Close the lid and centrifuge at 6000 x g for 1 minute. Place the QIAamp® MinElute column in a clean 2ml collection tube, and discard the collection tube containing the flow-through.

V. Centrifuge at full speed (20,000 x g) for 3 minutes to dry the membrane completely.

VI. Place the QIAamp® MinElute column in a clean de-lidded 1.5ml microcentrifuge tube and discard the collection tube containing the flow-through. Carefully open the lid of the QIAamp MinElute column and apply 50µl Buffer ATE to the centre of the membrane.

VII. Close the lid and incubate at room temperature (15 - 25°C) for 5 minutes. Then centrifuge at full speed (20,000 x g) for 1 minute.

VIII. Carefully open the lid of the QIAamp® MinElute column and apply an additional 50µl Buffer ATE to the centre of the membrane. Close the lid and incubate again at room temperature (15 - 25°C) for 5 minutes. Then centrifuge at full speed (20,000 x g) for 1 minute.

IX. It is important to adjust the final sample volume with elution buffer to ensure: a) the minimum sample submission volume of 100µl is met b) there is sufficient DNA volume to allow for local QC c) the DNA concentration is within the permissible range.

X. Transfer the eluted DNA to a FluidX® tube.

5.7.6 DNA Extraction from FFPE tissue cores using the Covaris truXTRAC FFPE DNA kit

The truXTRAC FFPE DNA Kit (Manufactured by Covaris) is designed for the extraction of DNA from formalin-fixed, paraffin-embedded (FFPE) tissue samples with Adaptive Focused Acoustics (AFA™). AFA enables the removal of paraffin from FFPE tissue samples without the use of organic solvents or high temperature.

Sample Handling Guidance:

5.7.6.1 Storage

The truXTRAC FFPE DNA Kit should be stored at room temperature (18 - 25°C).

5.7.6.2 Procedure Summary

DNA is extracted using the manufacturer's protocol (option C) to extract high molecular weight genomic DNA (summarised below). The use of the Covaris M220 Focused-ultrasonicator is described in this protocol, however it is acceptable to use other Covaris ultrasonicator instruments but it will be necessary to refer to the manufacturer's guidelines for differences in instrument set up and operation. Please note this protocol will require local COSHH assessment, staff training and may require adaption to fit local guidelines on labelling and sample transfers.

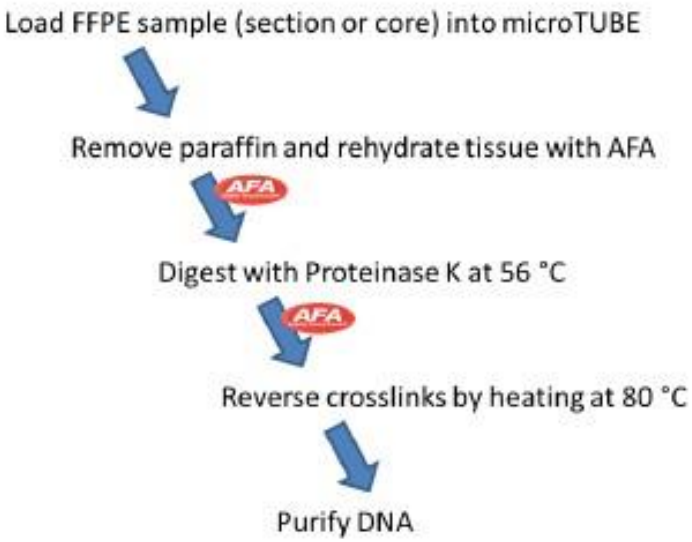


Figure 9 - Covaris truXTRAC FFPE DNA Kit Protocol Summary

5.7.6.3 Sample Preparation

FFPE Tissue Cores

It is currently recommended that punch core samples are used as alternative FFPE sample types (scrolls or sections) have not yet been assessed for the Covaris protocol. Two x 1mm punches should be used per extraction. Deparaffinisation of the punch cores is achieved by focussed acoustics during the course of the procedure.

5.7.6.4 Preparation of Kit Buffers and Reagents

Preparation of Buffer B5

Add 28ml of ethanol (>96%) to Buffer B5 concentrate and mark label on the cap to indicate its position. After preparation, Buffer B5 can then be stored for one year at ambient temperature.

Sample Handling Guidance:

Preparation of Proteinase K

Add 1.35ml Buffer PB to the lyophilized Proteinase K vial. The re-suspended Proteinase K solution should be stored in the fridge.

Check Buffer B1 and Tissue SDS Buffer

A white precipitate may form during storage. If this has occurred Incubate the bottles at 50 - 70°C before use to dissolve any precipitate.

5.7.6.5 Preparation of equipment

For M220 Focused-ultrasonicator, fill the water bath with the Holder-XT microTUBE in place until the water reaches the top of the holder (AFA grade water must be used).

Preheat the heat block to 56°C.

5.7.6.6 Extraction of Genomic DNA

I. Open microTUBE Screw cap and load FFPE tissue into micro tube, add 80µl Tissue SDS Buffer and affix Screw-Cap back in place.

II. Process the sample using the settings provided below (truXTRAC FFPE programme) to dissociate the paraffin while simultaneously rehydrating the tissue. During the AFA process it is normal for the solution to turn milky white as the paraffin is emulsified.

System	Duty Factor	Peak Incident Power	Cycles per burst	Treatment Time	Temperature (Instrument)
M220	20%	75 Watts	200	300 sec	20°C

III. Open Screw-Cap microTUBE, add 20µl of Proteinase K solution to the sample and affix Screw-Cap back in place.

IV. Process the sample using the settings below (truXTRAC FFPE 10s programme) to thoroughly mix Proteinase K with the sample.

System	Duty Factor	Peak Incident Power	Cycles per burst	Time	Temperature (Instrument)

M220	20%	75 Watts	200	10 sec	20 °C

Protein digestion at 56°C

I. Insert the required number of Heat Block microTUBE Adapters into the Heat Block.

II. Load the microTUBE into the adapter once the heat block has reached its set point of 56°C

III. Incubation overnight at 56°C (with shaking if possible).

Incubate the samples at 80°C for 1 hour to reverse formaldehyde crosslinks

I. Insert the required number of Heat Block microTUBE Adapters into a Heat Block and set the temperature to 80°C.

II. Load the microTUBE into the adapter once the heat block has reached its set point.

III. If you are using the same heat block for both the 56°C & 80°C incubations, the microTUBE should be stored at room temperature until the heat block equilibrates at 80°C.

Using a pipette transfer the sample to a clean 1.5ml microcentrifuge tube and proceed to DNA purification.

5.7.6.7 DNA Purification

Set heat block (or water bath) to 70°C and preheat the required volume of Buffer in a 1.5ml microfuge tube:

I. Add 140µl Buffer B1 to your sample and vortex thoroughly.

II. Add 160µl ethanol (>96%) to the sample and vortex thoroughly.

III. Centrifuge at 10,000 x g for 2 minutes at room temperature. After centrifugation much of the paraffin will have formed a white layer, floating on top of the liquid.

IV. Place a Purification Column into a provided Collection Tube.

V. While holding the sample tube at about the same angle as in the rotor, use a pipette to slowly recover the liquid layer, and transfer to the column. Transfer of a small amount of paraffin particles to the column is acceptable and will not interfere with the DNA purification.

VI. Spin the assembly at 11,000 g for 1 minute.

VII. Discard the flow-through and place the Column back in the Collection Tube.

VIII. 1st wash: Add 500µl Buffer BW. Spin the assembly at 11,000 g for 1 minute.

IX. Discard the flow-through and place the Column back in the Collection Tube.

X. 2nd wash: Add 600µl Buffer B5. Spin the assembly at 11,000 g for 1 minute.

XI. Discard the flow-through and place the column in a new Collection Tube (provided in the kit).

XII. Dry column: Spin the assembly at 11,000 g for 1 minute.

XIII. Elute DNA - Place the Purification Column into a new labelled 1.5ml micro centrifuge tube and add a minimum of 50µl pre-warmed Buffer BE (70°C) to the centre of the column. Incubate at room temperature for 3 minutes. Spin the assembly at 11,000 g for 1 minute.

XIV. It is important to adjust the final sample volume with elution buffer to ensure: a) the minimum sample submission volume of 100µl is met b) there is sufficient DNA volume to allow for local QC c) the DNA concentration is within the permissible range.

XV. Transfer the eluted DNA to a FluidX® tube.

5.7.7 Eluted DNA Handling

Whichever of the two FFPE DNA extraction methods is used it is important to adjust the final sample volume with elution buffer to ensure:

a) The minimum sample submission volume of 100µl is met (105µl or above preferred)

b) There is sufficient DNA volume to allow for local QC

c) The DNA concentration is within the permissible range

Whenever possible a proportion of the extracted DNA can be retained at the NHS GMC for potential validation of the project's findings.

The eluted DNA can be stored for up to 6 weeks at 4°C pending transport to the Genomics England Biorepository. Longer periods than this will require storage at -20°C.

5.7.8 Optimised FFPE DNA Requirements

DNA	Specification
Extraction method	FFPE DNA should be extracted using protocols detailed in section 11.
Amplification	DNA must not be PCR amplified.
Quantification	Quantify using a validated double stranded DNA quantification method.
DNA Purity	Assessment is not required in the GMC. The biorepository A260/A280 ratio must be 1.75 - 2.04.
DNA Fragment Length	FFPE samples: No assessment of fragment length is required.
FFPE QC	For FFPE samples only: Delta Cq ≤ 2.5*

Sample Handling Guidance:

DNA Buffer	FFPE samples: The buffers used to elute the DNA in the two recommended FFPE DNA extraction protocols are acceptable. Other buffers should not be used.
Total DNA quantity	2µg minimum**
Concentration	20 - 100ng/µl DNA should be diluted in NHS GMCs to meet the minimum volume required, or if it is over the maximum DNA concentration.
Volume	100-600µl Samples need to achieve both the minimum DNA total volume and concentration. Accurate measurement of the volume submitted is required. The provision of inaccurate measurement increases the likelihood of sample failure.