Sample Handling Guidance for Whole Genome Sequencing of Germline Samples

Document name:	Sample Handling Guidance for Whole Genome Sequencing of Germline Samples
Document number:	WGS-LAB-001
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Version	1.0
Date:	July 2021
Review period	Annually

Document management

Revision history

This document was previously issued for use during User Acceptance Testing (UAT) and Live Clinical Testing (LCT) phases for whole genome sequencing (WGS). This current version is the first issue following these phases.

Version	Date	Author	Summary of changes
1.0 UAT	10/06/19	Sandra Hing	Initial circulation for GLH feedback
2.0 UAT	14/08/19	Sandra Hing	Incorporation of GLH feedback where appropriate
1.0	July 2021	Rachael Mein	Incorporation of GLH and other relevant feedback where appropriate

Reviewers

This document was reviewed by the following people:

Reviewer name	Title/responsibility	Date	Version
Sandra Hing	Senior Scientific Advisor	July 2021	1.0

Approved by

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Name	Title	Date	Version
Prof Dame Sue Hill	SRO for Genomics, NHS England and NHS Improvement	July 2021	1.0
Prof Sandi Deans	Deputy Director (Laboratory & Scientific), Genomics Unit, NHS England and NHS Improvement	July 2021	1.0

Related documents

Title	Document number	Owner	Location
Sample Handling Guidance for Whole Genome Sequencing of Solid Tumour Samples	WGS-LAB-002	Sandra Hing	NHS Futures
Sample Handling Guidance for Whole Genome Sequencing of Haematological Malignancies	WGS-LAB-003	Polly Talley	NHS Futures
DNA extraction and Quality Control for Whole Genome Sequencing	WGS-LAB-005	Sandra Hing	NHS Futures

Document control

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Contents

1 Summary

The purpose of this document is to provide Guidance to NHS Genomic Laboratory Hubs (GLHs) on the collection, processing and transportation of germline material for Whole Genome Sequencing (WGS) for rare disease and cancer. The document covers the process from sample collection to transportation of samples to the GLH WGS DNA extraction laboratory. The document does not set out local standard operating procedures (SOPs) and it will be necessary for GLHs to produce detailed SOPs outlining local practice.

2 Introduction

Germline DNA can be extracted from many different sources, and peripheral blood is the easiest sample type to obtain and hence is the most common choice for obtaining germline DNA for both rare disease and solid tumour referrals. Obtaining an appropriate germline sample for bone marrow/blood based haematological cancers is more challenging and alternative sources such as cultured fibroblasts, saliva 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. Please refer to the *Sample Handling Guidance for Whole Genome Sequencing of Haematological malignancies,* document for further details on obtaining germline samples for haematological malignancies.

3 Eligibility for WGS

The rare disease and cancer clinical indications eligible for whole genome sequencing are listed in the test directory published on the NHS England website https://www.england.nhs.uk/publication/national-genomic-test-directories/.

If a patient and family members, if applicable, have been consented for WGS then samples should be submitted for DNA extraction and WGS wherever possible.

4 Sample Requirements for WGS

Sufficient blood needs to be taken to ensure the required quantities of DNA can be extracted. A minimum of 2µg of DNA is required to be exported to the plating GLH and sufficient DNA should be retained locally for any required technical validation. Please refer to *DNA extraction and Quality Control for Whole Genome Sequencing* guidance document for DNA requirements.

For patients who have received a blood transfusion, the timeline from last blood product transfusion to WGS blood sample collection will be dependent upon the patient's white cell count prior to transfusion and the type of blood product transfused. As a guide, it is recommended to wait at least 2 weeks following a transfusion before a blood sample is collected. The referral form should clearly state that the patient has received a transfusion and the date when this occurred.

For patients who have received a bone marrow transplant a peripheral blood sample shall not be taken for DNA extraction. Suitable alternative sample types are either pre-bone marrow transplant stored DNA which had been extracted from blood or DNA extracted from cultured fibroblasts (section 3.2.1).

4.1 Blood Volume and collection tubes

The volumes given in Table 1 reflect the recommended blood volumes required to obtain the DNA quantities for whole genome sequencing and to enable DNA to be retained locally for technical validation or further testing if required. This blood volume can be modified based on local evidence and individual circumstances.

The volume of blood taken for germline testing must be stated on the appropriate test request form used for blood collection.

In neonates, acutely ill children and other patients where venepuncture is challenging, clinical discretion should be applied to the volume of blood drawn. Where small volumes of blood for DNA extraction are obtained, and a small yield of DNA is obtained then a reduced amount of DNA (1µg) can be submitted, in exceptional circumstances, via the low volume pathway. See *DNA extraction and Quality Control for Whole Genome Sequencing* guidance document for DNA requirements for this **Low Volume** pathway.

In extreme circumstances when it is not possible to obtain a further sample, but where the concentration of the DNA sample obtained is below low it may be possible to submit a low concentration DNA sample if there is sufficient volume. See *DNA extraction and Quality Control for Whole Genome Sequencing* guidance document for DNA requirements for this **Low Concentration** pathway.

Where local validation is required following WGS, and no stored DNA is available another blood sample can be taken for DNA extraction and testing with appropriate sample identity checks performed.

Table 1 – Recommended Blood volumes

Referral	Volume of Blood	Blood tube Preservative Type
Adult	3-5ml	EDTA
Paediatric	>3ml	EDTA
Infant	1-3ml	EDTA

4.2 Alternatives to fresh blood samples for germline testing

For individuals unable to provide blood samples the following alternative sources of DNA can be used.

4.2.1 Fibroblast samples

DNA extracted from fibroblast cultures may be submitted for individuals who have undergone bone marrow transplantation or circumstances where other options such as stored DNA are unavailable. The sample type indicating DNA extracted from

fibroblasts must be stated on the cancer or rare disease test request form. Refer to section 6 for further sample processing details and to the *Sample Handling Guidance for Whole Genome Sequencing of Haematological Malignancies* for detailed requirements for these requests.

4.2.2 Saliva samples

In exceptional circumstances, where considered clinically appropriate and if no fresh or stored blood or fibroblast derived DNA is available, DNA extracted from saliva samples may be used.
These samples should be submitted indicating DNA extracted from saliva as the sample type in the rare disease or cancer test request form.

4.2.3 Stored DNA samples

In cases where an individual is difficult to bleed, has had a bone marrow transplant, is deceased or it is not possible or clinically appropriate to obtain a new sample then it is acceptable to use stored DNA samples which have been extracted and stored in a UKAS ISO 15189:2012 accredited laboratory provided the DNA meets the WGS DNA sample requirements.

5 Sample Prioritisation

All germline samples submitted for either rare disease or cancer have specified turnaround times at the WGS Provider. All samples will be processed as per this specification. If a case is flagged as urgent on the Test Order form (Rare Disease only as urgency field is not present on the Cancer Test Order form as all Cancer cases are presumed urgent) this indicates that the test should be prioritised by the GLH laboratory prior to sequencing, and again once results have been returned to the laboratory via the interpretation portal. The priority will be taken into account by the Plating GLH, so the samples will always be plated for the next shipment to the WGS provider.

There will be occasions when, despite every effort being made to ensure efficient usage of all available material, there will not be sufficient material for all indicated tests. In this scenario priority should be given to those tests that will inform immediate management at the discretion of the treating clinician. However, it is envisaged that these cases will constitute a minority and in order to maximise potential future benefit to patients and to ensure equity of access to WGS, samples should be submitted for WGS whenever possible.

6 Sample Processing

6.1 Peripheral blood

Peripheral blood and bone marrow samples should be collected in EDTA tubes stored at 4ºC and DNA extraction carried out within 72 hours of collection. Samples

should ideally be transported at 4°C but it is acceptable to send samples at ambient temperature if the total time from collection to extraction does not exceed 72 hours.

6.2 Fibroblast Cultures

Cultured fibroblasts must have been collected, processed and stored within a laboratory with UKAS ISO 15189:2012 accreditation for this process. Cultured fibroblasts can be transported in flasks containing transport media or the cells stripped and sent in tubes. Samples should ideally be transported refrigerated or in cool boxes, but it is acceptable to send samples at ambient temperature if the transportation time does not exceed 24 hours.

Please refer to the *Sample Handling Guidance for Whole Genome Sequencing of Haematological Malignancies* for detailed requirements for these requests.

6.3 Saliva Samples

A specialised saliva sample DNA collection kit must be used. A variety of commercial saliva sample DNA collection kits are available. Any can be used but local validation of performance must be performed. Saliva samples should be collected, stored and transported according to the kit manufacturer's instructions. DNA extraction from collected saliva samples will be performed at the GLH WGS DNA extraction laboratory.

Please refer to the *Sample Handling Guidance for Whole Genome Sequencing of Haematological Malignancies* for detailed requirements for these requests.

7 Transport of Samples

Local and inter GLH transport is as per local arrangements. It is the responsibility of the Home GLH of the proband, whom is to be referred for WGS, to liaise with the GLHs where family members are located in order to ensure all family members are submitted at the same time as the proband for WGS. The rare disease GMS test order form details the family members to be submitted. The probands Home GLH has the responsibility for completing the form and for ensuring that requests for family member samples is in place in an appropriate timeframe.

Out of GLH area family members shall be phenotyped and consented at the Proband's Home GLH and arrangement for the collection of the sample from the family member should also be arranged by the probands Home GLH. DNA extraction can be performed at either the family member or proband GLH, depending on the most practical sample collection and transport option, to ensure the sample integrity is not compromised and DNA extraction is not delayed. If DNA extraction is performed at the family members GLH then that GLH should arrange for the DNA sample to be sent to the probands Home GLH as soon as possible. The probands Home GLH is responsible for ensuring all appropriate forms are completed and for the submission of samples from all family members.

For solid tumour referrals it is the responsibility of the patient's Home GLH to ensure that the germline sample has been taken, and then transported to the Home GLH WGS DNA extraction laboratory. Alternatively, where the tumour sample has been sent to

another GLH for histopathology processing then the fresh tissue must be transported and extracted at that GLH WGS DNA extraction laboratory.

All samples must be placed in standard specimen bags with a Genomic Medicine Service test order form and transported to the GLH WGS DNA extraction Laboratory. Blood samples for germline DNA extraction must be received in the WGS DNA extraction Laboratory and all processing completed within 72 hours.

Planning of working practices for sample collection and optimisation of transport logistics to the GLH WGS DNA extraction Laboratory from all sites of collection should be a priority with detailed process maps followed.

8 Roles and responsibilities

It is the responsibility of the patient's managing Clinicians to ensure that whenever possible appropriate samples are obtained and submitted for WGS from eligible patients.

It is the responsibility of the GLH to ensure that appropriate local pathways and SOPs are in place to facilitate the submission of samples to the GLH WGS DNA extraction laboratory.

It is the responsibility of the GLH senior management team to ensure that all appropriate clinical and laboratory teams across the geography of the GLH are fully aware of which patients are eligible for WGS and that the teams understand the local clinical pathways and processes that have been put into place to obtain and process samples from these patients for WGS.