

Fine Needle Aspiration for sampling surgical resections of tumour for Whole Genome Sequencing

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1 Document History and Control

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1.1 Version History

Version	Date	Description
0.1	03/11/2017	First draft
0.2	09/11/2017	Kay Lawson
0.3	15/11/2017	Shirley's edits
0.4	16/11/2017	Final draft inc Jane Moorhead comments and comments from experimental
0.5	12/01/2017	Final draft following feedback from Cancer Transformation Working Group
0.6	15/01/2018	Changed lysate to PBS and added external video links
0.7	17/01/2018	Including Sue Hill's requests after cancer transformation meeting
0.8	29/01/2018	Incorporating Sandi Dean's edits and feedback from pathologists
0.9	5/2/18	New title

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2 Purpose & Scope

Preliminary experiments, undertaken by Dr Lisa Browning, using fine needle aspiration (FNA) as a sampling method in surgically resected renal tumours have demonstrated that the quality and quantity of DNA extracted from FNA samples are sufficient to act as a potential source of DNA for whole genome sequencing (WGS). To validate this method of sampling in other tumour types and to demonstrate it is transferable across NHS GMCs, this protocol has been devised.

NHS GMCs can opt to take part in a pilot of this technique and the requirements for this are outlined in this document.

Fine needle aspiration can be used for genomic sampling of solid tumours. Any solid tumour can be sampled in this way and it will be of particular use for sampling small tumours as there is no disruption to the tissue architecture ensuring routine histopathological procedures are not compromised. Necrotic, cystic and haemorrhagic areas should not be sampled with this technique. Areas of dense fibrosis should only be sampled if there is no alternative and the process repeated in these cases to ensure adequate DNA yield.

3 Roles & Responsibilities

<i>Role</i>	<i>Responsibility</i>
Clinical Lead for validation cohort	Nominated NHS GMC individual responsible for ensuring protocol is adhered to; samples are tracked and data is collected and submitted correctly.
Pathologist	The reporting pathologist is responsible for taking the decision to sample a case and the choice of sampling method.
Pathologist or Scientist	Sampling of the tissue for DNA extraction; sampling for tumour content assessment and ensuring the sample is prepared adequately for histopathological diagnosis.

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4 Background to validation cohort

Using fine needle aspiration enables sampling of tumours with minimal disruption and creates a liquid sample to allow efficient DNA extraction. A proof of principle experiment (WGS of five renal tumours) demonstrated that these particular FNA samples had higher purity than their matched fresh frozen samples. The tissue had similar degrees of tumour heterogeneity to the fresh frozen solid tissue samples and WGS data demonstrated an evenness of sequencing coverage and comparable variant calling. A validation project to analyse a pilot cohort has now been initiated and NHS GMCs are invited to participate.

Validation of this sampling method will require approximately 50 cases. The number required may increase during the course of the pilot if initial data reveals unexpected findings that require further work to fully understand.

It is hoped that this technique will increase access to genomic testing for patients with small tumours; patients where there is already great demand on the availability of tissue for clinical trials or other research and for patients with tumours rich in stroma where it is difficult to sample with an area with adequate tumour content e.g. pancreatic cancer resection samples.

Only surgical resection samples aspirated within the laboratory will be accepted as part of this pilot. Samples will be submitted as part of the main programme with a Whole Genome Analysis report returned to the GMC and these samples will count towards the recruitment trajectory and be eligible for funding.

5 General Information

When a sample is submitted as part of this validation cohort then the way the tumour is sampled will be affected as well as how the data is collected and submitted. There is a requirement to track these cases through the Genomics England service desk as outlined below.

For all other aspects the usual guidance applies to how patients should be approached for consenting and how sampling should be carried out. The current sample handling guidance can be found here: <https://www.genomicsengland.co.uk/information-for-gmc-staff/sample->

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[handling-guidance/](#) and guidance on consenting can be found as part of the Genomics Education Programme here:

<https://www.genomicseducation.hee.nhs.uk/courses/courses/consent-ethics/>

5.1 Consenting

This work is considered service development work. The samples are therefore diagnostic samples. The decision on how best to sample a tumour will take place after arrival of the specimen in the pathology department. The decision on the best use of any DNA retrieved will be taken once the diagnosis and yield of DNA are known. Sampling for DNA can take place in the patient's best interests prior to specific consent for participation in the 100,000 Genomes Project in accordance with the Consensus Statement, which can be accessed here: <https://www.genomicsengland.co.uk/information-for-gmc-staff/cancer-programme/cancer-mdt-engagement-pack/>

5.2 Germline DNA sampling

A germline peripheral blood sample must be taken in an EDTA tube and the extracted germline DNA must be submitted with the tumour DNA sample to UKB for plating as set out in the current Sample Handling Guidance.

5.3 Tumour sampling

Only tumour that has been collected fresh can be submitted for WGS. The tumours must fulfil the eligibility criteria detailed here: <https://www.genomicsengland.co.uk/information-for-gmc-staff/cancer-programme/eligibility>

5.4 Data entry

The patient must have a completed Registration file including consent information and a completed sample metadata file and Clinical Sample Test csv file (for tumour content assessment) prior to sample submission to UKB.

5.5 Sample submission

After DNA extraction, samples must be submitted to UKB following the recommendations of the current Sample Handling Guidance. Samples from this pilot should not be a clinical priority for fast or medium track slots, however, if they meet the volume and concentration requirements for fast track cases then they can be included on a fast track plate. Where possible samples submitted as part of the pilot should fulfil the fast track criteria in order to expedite the validation work.

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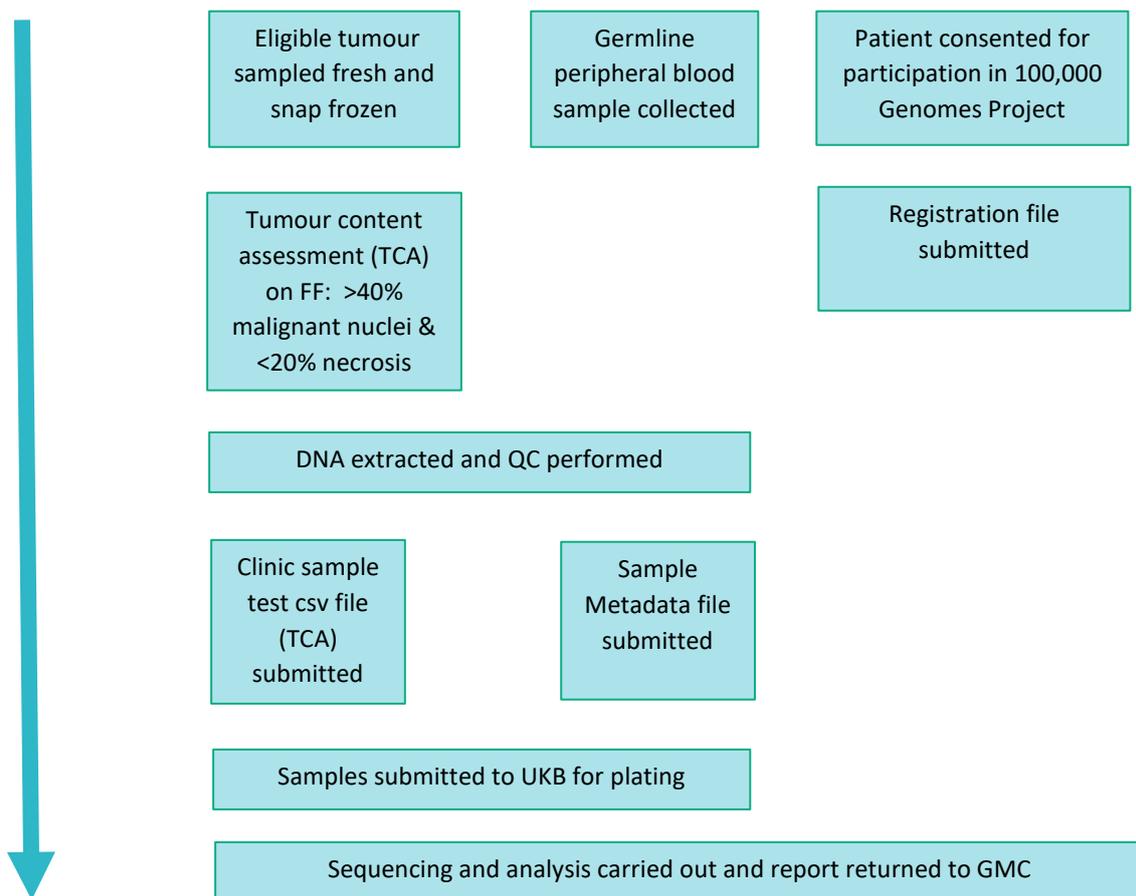
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5.6 Return of Results

Samples submitted as part of the validation cohort will be processed through the main programme and results will be returned to NHS GMCs via the usual route (cancer interpretation portal). As with all 100,000 Genomes Project cancer cases results should be interpreted and reported in accordance with the NHSE Cancer Validation and Reporting working group guidance document (in preparation).

5.7 Where pilot cases differ from normal practice

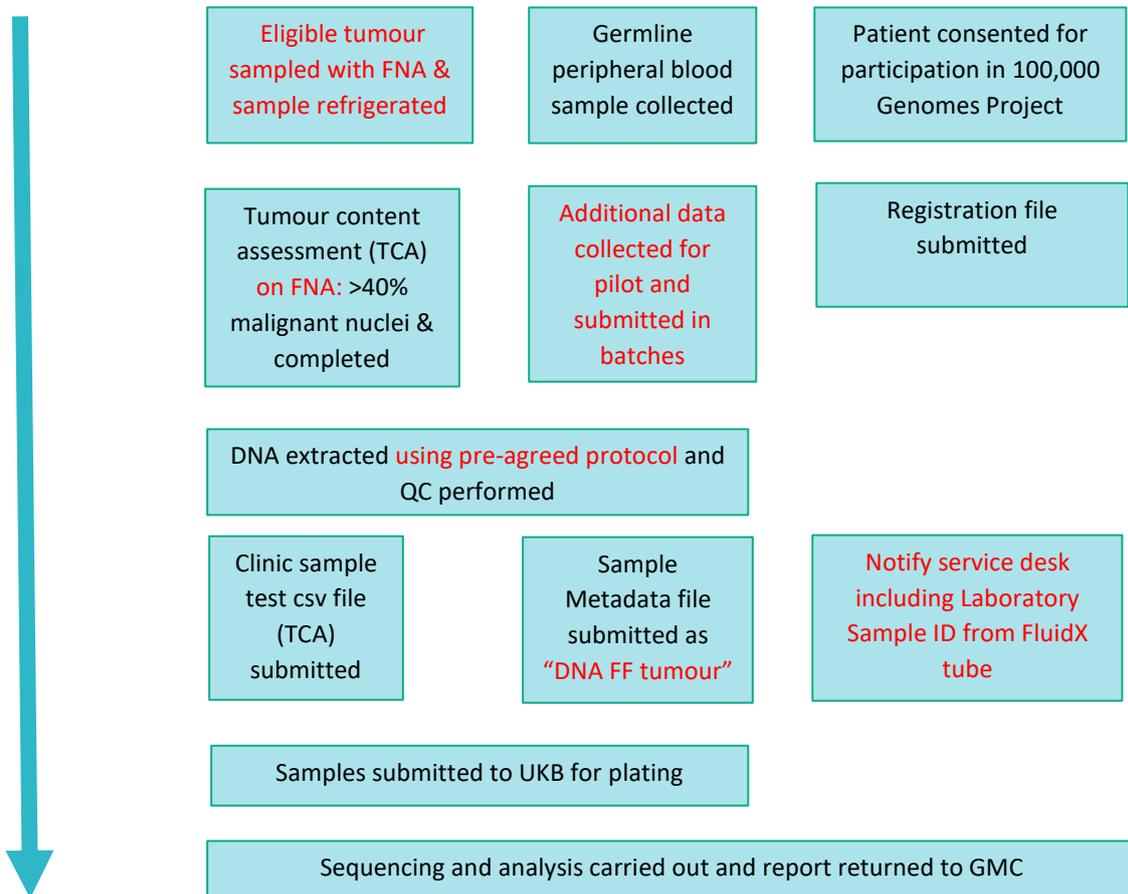
Conventional process



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Validation pilot process



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6 Procedure

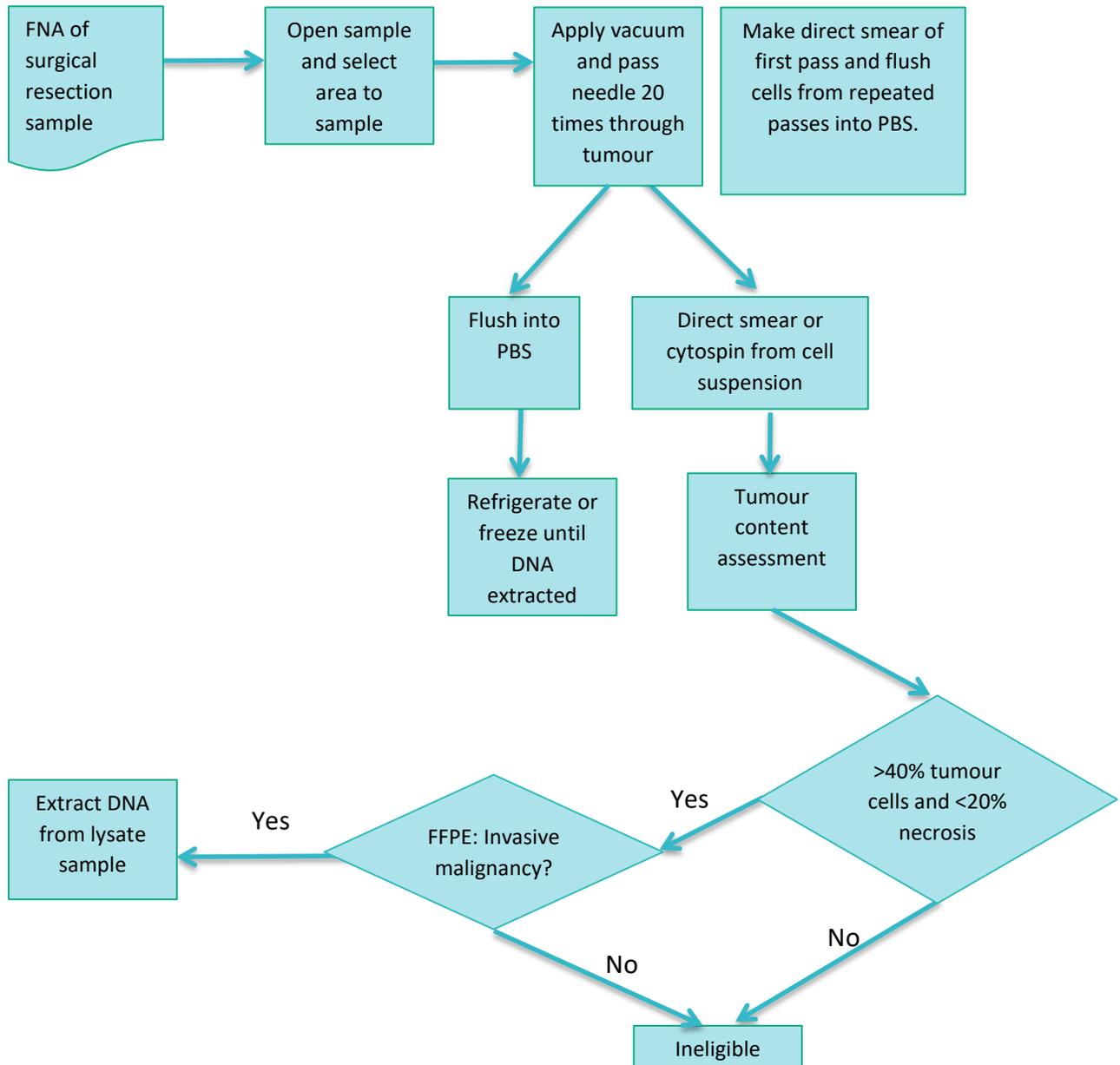
6.1 Introduction

Whenever possible a piece of fresh tissue should be frozen and stored in parallel with the FNA sample so that if the aspirate sample fails tumour content assessment or has a poor DNA yield the frozen tissue can be submitted as a substitute. For smaller samples where it is not possible to obtain a piece of fresh tissue that could be submitted to the 100,000 Genomes Cancer Project then it is acceptable to take an FNA sample alone.

DNA yield must be sufficient for PCR free Whole Genome Sequencing. Samples with only enough DNA for PCR based library preparation cannot be submitted as part of the validation cohort.

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6.2 Process Flow



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6.3 Procedural Steps

The procedure below describes in outline how to obtain an FNA sample and demonstrations of the technique can be found in these video links:

[Introduction](#)

[Aspiration technique](#)

[Expelling the material](#)

[Making a smear.](#)

This is a specialist technique and may require individuals to practice in order to acquire the skills. It is advised that individuals inexperienced in performing this technique should liaise with local cytopathologists or radiologists to obtain training. Alternatively if required, it will be possible to arrange a visit from a cytopathologist to train individuals in how to perform the sampling technique.

6.3.1 Equipment needed

- 22 gauge needle
- 10 or 20ml syringe
- Labelled glass slide
- Second slide for smearing
- 30ml Universal tube (or equivalent) containing 5-10mls of PBS

6.3.2 Preparation Steps and Equipment needed

1. Surgical resection samples should be kept refrigerated to enable fresh sampling in the laboratory.
2. Ensure the workbench and tools are clean and any disposable instruments are fresh. Distel (or equivalent) should be used in conjunction with 70% ethanol for decontamination of workspaces. Any instruments used for dissection which are not disposable, should be soaked in 10% Decon (or equivalent) before next use.

6.3.3 Opening and sampling

3. If necessary and according to the sample type, the specimen should be described, measured, weighed and inked prior to opening in keeping with Royal College of Pathologist guidelines.
4. Once opened, examine the tumour to identify areas of necrosis, haemorrhage and fibrosis.

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5. Before attaching the needle and syringe ensure that the seal on the syringe has been broken by moving the plunger up and down.
6. Having identified a suitable area to sample, insert the needle into the tumour and apply a vacuum.
7. Pass the needle through the tumour and back 20 times.
8. Hold the tumour at the edges to steady it ensuring there is no opportunity for a needle stick injury.
9. Try to avoid the bevel of the needle exiting the tumour as this breaks the vacuum and the sample will be sprayed higher into the barrel of the needle. If this does happen the cells can still be retrieved through needle washing but the vacuum should be reapplied and the procedure repeated.

6.3.4 Smear preparation and needle washings

10. Prepare a direct smear as follows: Remove syringe. Fill syringe with air. Reattach needle. Hold needle at base of labelled slide with needle bevel pointing down. Rapidly depress syringe plunger to expel contents of needle. Put down syringe and pick up second slide. Let it fall flat on to the first allowing any fluid to momentarily spread laterally. Keep bottom slide still and slide top slide over bottom steadily and while keeping the full flat face in contact with lower slide. Place into alcohol immediately to fix or air dry depending on staining preference of the pathologist.
11. Flush the needle into the PBS solution.
12. Repeat the procedure ensuring the same area is sampled and flush the second sample into the PBS solution.
13. The procedure can be repeated again ensuring the same area is sampled and the third sample flushed into the PBS if there are any concerns about adequacy.

6.3.5 After aspiration is completed

14. Carry out any other fresh sampling that may be required then place the sample into formalin for fixation. Where the tumour is large enough take a conventional fresh tissue sample and freeze following the guidance set out in the Sample Handling Guidance. This will allow a backup in case the FNA sample has insufficient tumour content or yields insufficient DNA
15. The direct smear is stained with Giemsa or H&E (or the favoured stain for evaluation) and used for tumour content assessment. This is recommended as a way of minimising the work of the laboratory staff. If a pathologist has a preference for

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preparing a cytospin from a collated cell suspension sample this would be an excellent sample for tumour content assessment. The pathologist must ensure the sample contains greater than 40% tumour cells to ensure eligibility.

16. The sample in PBS can be kept refrigerated for up to 72 hours prior to DNA extraction.
17. When sampling the fixed tumour, ensure a formalin fixed block is taken from the region aspirated to ensure that the aspirate is from an eligible tumour. This block can also be used, in conjunction with the aspirate, to assess the tumour content of the FNA sample. Where this is desired inking the FNA needle prior to aspiration can indicate later the position of the needle track.

7 Additional data

In addition to the submission of sample metadata the following data must be collected on each case within the pilot.

1. Participant ID
2. Organ sampled
3. Disease subtype
4. Diagnostic morphology code
5. Operator sampling
6. Impact on residual tissue
7. Time refrigerated before freezing / extraction
8. Was the sample frozen?
9. Tumour content assessment
10. DNA yield
11. Local DNA QC metrics

Any NHS GMCs participating in the pilot must submit Standard Operating Procedures for the DNA extraction method they propose to use for cytology samples prior to commencing the pilot. This method must be used for all cases in the pilot. These methods must be submitted to the Clinical Lead for the validation cohort when requesting permission to participate in the pilot.

This data must be sent to the Clinical Lead for the FNA sampling validation cohort at Genomics England, monthly or after every 10 cases have been sampled whichever is soonest. Genomics England will then assess the following outcomes for these samples:

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1. DNA yield
2. Tumour purity
3. Coverage
4. Variant calling rate
5. Pick up rate for known mutations

8 Definitions & Abbreviations

Abbreviation / Term	Description
FNA	Fine Needle Aspirate
DNA	Deoxyribonucleic Acid
PBS	Phosphate Buffered Saline
QC	Quality Control
UKB	United Kingdom Biobank
WGS	Whole Genome Sequencing
NHS GMC	National Health Service Genomic Medicine Centre
FF	Fresh Frozen
FFPE	Formalin Fixed Paraffin embedded

9 Data Model Requirements

There are a number of specific requirements around entering sample metadata on these samples since the current data model does not fully support FNA Tumour samples.

Clinic Sample Type should be entered as DNA FF Tumour since there is no DNA FNA Tumour enumeration in the current data model. Laboratory Sample ID from the FluidX tube for the FNA DNA sample should be documented.

Tissue Source should be entered as 'Surgical Resection'. Further guidance will be issued for submission of diagnostic FNA samples.

10 DNA Extraction Protocol

The DNA extraction technique used is critical to achieving adequate DNA yield and quality. In the proof of principle experiments DNA extraction was carried out using the Qiagen

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Allprep DNA/RNA Mini Kit. Extraction must be performed using a kit and protocol which has been recommended by the manufacturer as appropriate for cells pelleted from suspension. QC requirements of the resulting DNA are the same as for a fresh frozen tissue sample.

11 Permission to Commence Collection of FNA samples for the 100,000 genomes cancer programme

Prior to commencing collection of FNA samples, NHS GMCs will be required to have submitted a completed checklist (see appendix) and have reviewed procedural aspects of the FNA sample collection and the details of the DNA extraction method they plan to use with Genomics England and NHS England (usually via a teleconference).

12 Notification

Whilst early experimental outcomes for this technique have been very encouraging, this has only been on a limited number of tumours. In order that we may monitor the metadata and sequencing metrics on these samples the Genomics England Helpdesk must be notified (ssd.genomics@hscic.gov.uk) with the subject title 'FNA Sample', the DNA extraction technique used and both the Participant ID and Laboratory Sample ID (for the FNA DNA sample) should be included in the text of the notification.

13 Related Documents, References & Procedures

Sampling of tumours with this technique should adhere to the guidance set out in the current [Sample Handling Guidance](#).

14 Requirements for success

There are two tiers to the findings of a successful validation cohort:

1. Whole genome sequencing of an FNA sample provides results which are equivalent or an improvement on those obtained from fresh frozen sampling.
2. Whole genome sequencing of an FNA sample provides results which are adequate for detecting diagnostic changes but not as sensitive as fresh frozen sampling.

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The outcome may vary by tumour type.

Where FNA sampling is shown to be better than fresh frozen sampling (for example enabling higher tumour purity) then it can be rolled out for sampling of both large and small surgical resection samples.

Where FNA sampling is adequate but not as good as fresh frozen sampling then fresh frozen will remain the gold standard for sampling of large tumours but FNA sampling will provide an option for sampling small tumours that otherwise could not be sampled.

Where FNA sampling is found to be inadequate and should not be used for the collection of samples for WGS.

Results of the validation from each GMC cohort will be shared with the participating GMC once they are available.

Once results are available for the validation cohort as a whole these will be shared with all GMCs.

15 Appendix:

Checklist for participation in FNA sampling of surgical resections pilot	
Sample Handling	
Proposed tumour types that will be sampled	
Number of cases expected	
Pathologists or Scientists who will be carrying out procedure	
Confirmation that individuals carrying out the FNA procedure have received appropriate training in FNA sampling. Please provide brief details.	
Confirmation of which laboratories will process the biopsy samples for (i) tissue processing and (ii) DNA extraction e.g. tissue bank or pathology laboratory	
Storage and transportation requirements prior to DNA extraction	
Details of proposed DNA extraction method and SOP	
Data capture	
Confirmation of how Pathology Department will record cases including data on ineligible cases and those that fail QC <ul style="list-style-type: none"> i) Tissue sampling details ii) DNA extraction details 	
Designated responsibility for collecting the meta-data	
Sample tracking	
Process for tracking when these patients have been diagnosed, consented to GEL and sample has been retrieved, including responsibility to complete this action	
Confirmed process to notify GEL service desk that a sample has been submitted and associated patient identifier, including responsibility to complete this action	
Teleconferences	
Proposed operators to talk through procedural aspects of tissue sampling	
Clinical Scientist to talk through DNA extraction methods proposed	

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Checklist for participation in FNA sampling of surgical resections pilot

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