Validation of shaken biopsy sampling

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Contents

1 Document History and Control

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1.1 Version History

Version	Date	Description
1.0	18/05/18	First version

2 Purpose & Scope

Proof of principle experiments have demonstrated that gentle agitation of a core biopsy sample releases cells into suspension resulting in high quality and high purity DNA for PCR free sequencing in the majority of cases. To validate this method of sampling for whole genome sequencing (WGS) 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.

3 Roles & Responsibilities

Role	***Responsibility***
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.
Radiologist or clinician taking sample	The NHS GMC radiologist or other clinician taking the core biopsy is responsible for sampling the core biopsy and placing into phosphate buffered saline as well as overseeing the shaking of the biopsy before transfer to a formalin filled pot for conventional fixation.
Pathologist or Scientist	Tumour content assessment and ensuring material is appropriately prioritised to best answer the diagnostic questions for the patient.

4 Background to validation cohort

A proof of principle experiment has demonstrated sufficient yields for whole genome sequencing (see Appendix A) from shaken pseudo biopsies cut from surgical resections. Subsequent work on additional single diagnostic core biopsies from patients with breast cancer showed less generous DNA yields but it is proposed that if more than one core biopsy is shaken then adequate DNA for PCR free WGS could be obtained in 75% of cases.

Where adequate DNA is extracted from the shake fluid this can be submitted for whole genome sequencing. DNA yield from shaken biopsy samples must be sufficient for PCR free Whole Genome Sequencing i.e. 1.3µg. Samples with only enough DNA for PCR based library preparation cannot be submitted as part of the validation cohort. Where this is inadequate then alternative fresh samples should be submitted where possible.

The two pilot cases showed a higher tumour purity in the shaken samples (87%-97%) compared to the control fresh frozen biopsies (44-79%). Tumour purity is calculated by the copy number caller based on tumour depth and B-allele ratios. Higher purity samples allow for more confident variant calling particularly of low frequency variants.

Validation of this sampling method will require approximately 100 cases across tumour types. The number required may increase during the course of the pilot if initial data reveals unexpected findings that require further work to fully understand.

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 how the data is collected and submitted are impacted as well as how tumour DNA is sampled. 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-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 whether there is sufficient material for WGS will take place after DNA extraction of the shaken sample cell suspension in the laboratory. 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

Cell suspensions must be from fresh tissue and there must have been no contact with formalin. 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

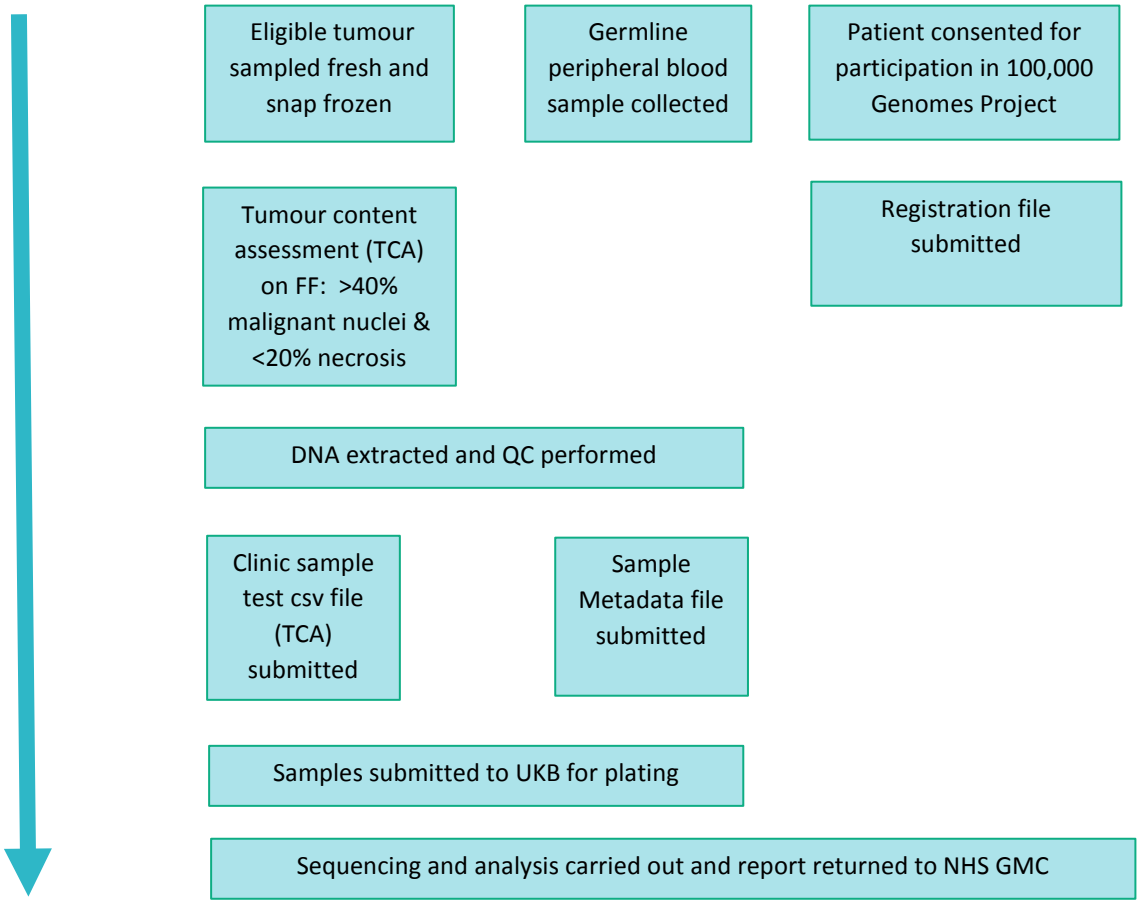
After DNA extraction, samples must be submitted to UKB following the recommendations of the current Sample Handling Guidance. Samples from this pilot should not be a clinical priority for fast or medium track slots, however, if they meet the volume and concentration requirements for fast track cases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

5.6 Return of Results

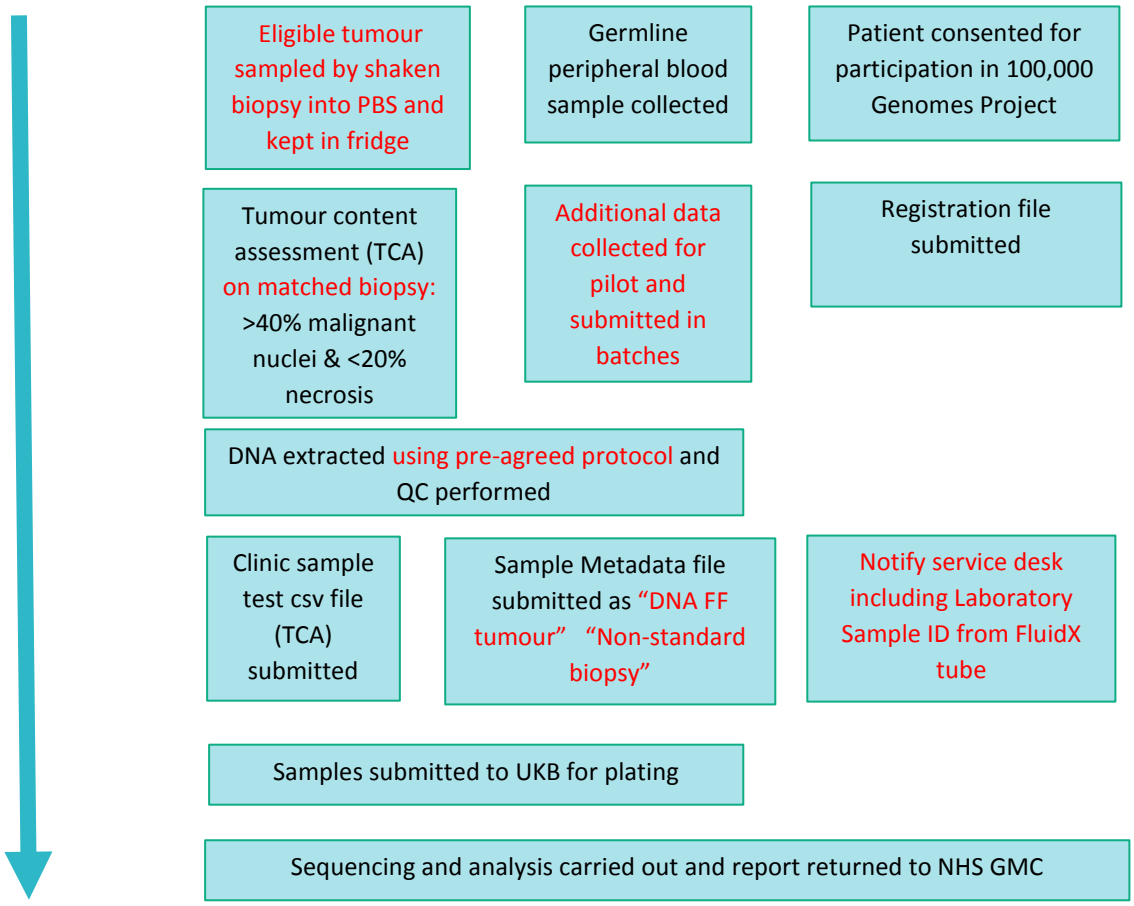
Samples submitted as part of the validation cohort will be processed through the main programme and results will be returned to NHS GMCs via the usual route (cancer interpretation portal). As with all 100,000 Genomes Project cancer cases results should be interpreted and reported in accordance with the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

5.7 Where pilot cases differ from normal practice

Conventional process

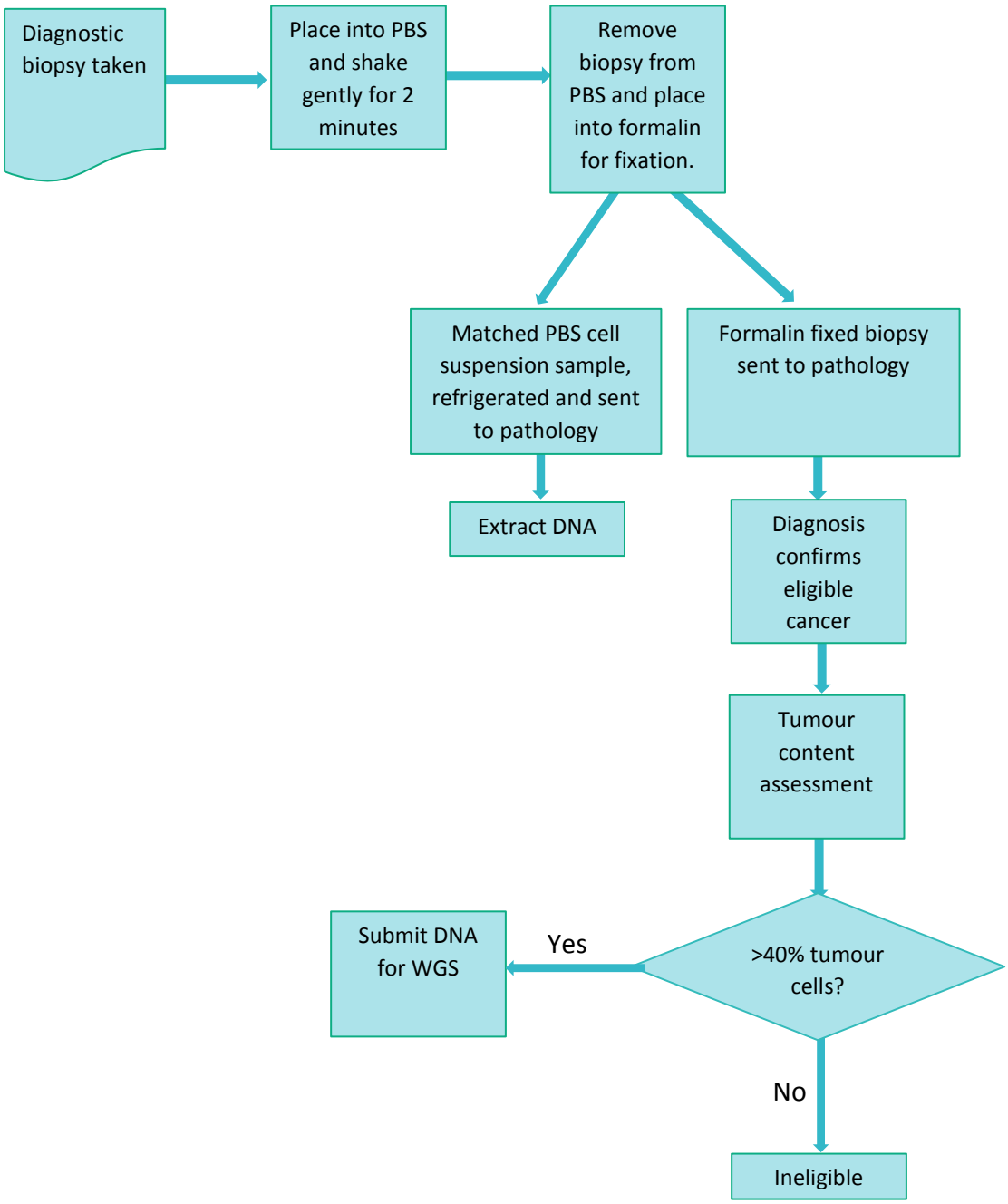


Validation pilot process



6 Procedure

6.1 Process Flow



6.2 Procedural Steps

Equipment needed:

1.5ml Eppendorf or similar tube for shaking.

PBS of sufficient volume to submerge tissue once placed into tube

Sampling

1. Place PBS into empty sterile tube.
2. Core biopsy taken and placed into tube containing sufficient PBS to cover the tissue
3. Needle can be rinsed into PBS at this point
4. Tube should be inverted repeatedly for at least 2 minutes at a rate of two inversions per second.
5. The tube should be opened and the tissue removed with clean, sterile forceps.
6. The tissue should be placed into a formalin sample pot.
7. Care should be taken to not contaminate the forceps with formalin if they are to be used again.
8. Any further core biopsies taken can be shaken in a similar fashion and the samples collated.

Sample storage

9. The cell suspension must be kept refrigerated at 4^{o}C until DNA is extracted to avoid DNA contamination and to inactivate nucleases.

Diagnosis

10. The formalin fixed tissue can be used to confirm the presence of an eligible cancer.
11. Tumour content assessment should be done on this matched tissue.

7 Outcomes

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

1. Participant ID
2. Disease type and subtype
3. Diagnostic topography and morphology code
4. Number of biopsies shaken
5. Time refrigerated before extraction
6. Tumour content assessment
7. DNA yield
8. 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 shaken biopsy 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 shaken biopsy sampling validation cohort at Genomics England, or after every 10 cases have been sampled whichever is soonest. Genomics England will then assess the following outcomes for these samples:

1. DNA yield
2. Tumour purity
3. Coverage
4. Variant calling rate (proportion of cases where specific variants were detected to be compared to sequences from FF cases with same diagnosis)
5. Pick up rate for known mutations

8 Definitions & Abbreviations

Abbreviation / Term	Description
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

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 shaken biopsy samples.

Clinic Sample Type should be entered as DNA FF Tumour since there is no DNA_fluid_tumour sample enumeration in the current data model. This will be added in release 3.16 at the end of April. Laboratory Sample ID from the FluidX tube for the FNA DNA sample should be documented.

Tissue Source should be entered according to the type of biopsy sampled e.g. endoscopic_biopsy or ct_guided_biopsy etc.

10 DNA Extraction Protocol

It is important that the homogenisation and lysate steps are completed as for any fresh tumour sample.

The DNA extraction technique used is critical to achieving adequate DNA yield and quality. Extraction must be performed using a kit and protocol which has been recommended by the manufacturer as appropriate for cells pelleted from suspension and using a suitable volume. QC requirements of the resulting DNA are the same as for a fresh frozen tissue sample.

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

Prior to commencing collection of shaken biopsy samples, NHS GMCs will be required to submit a completed checklist (see appendix) and review procedural aspects of the shaken biopsy 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 carefully track and monitor the metadata and sequencing metrics on these samples the Genomics England Helpdesk must be notified of each case (ssd.genomics@hscic.gov.uk) with the subject title 'Shaken Biopsy Sample', the extraction technique used and both the Participant ID and Laboratory Sample ID (for the FF Tumour 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

Shaken Biopsy sampling allows genomic sequencing from cancer patients who otherwise could not get a sequence. The standard that these samples need to reach in order to be of diagnostic benefit therefore needn't be as high as the gold standard. For example, if purity or coverage were not as good as for fresh frozen samples but the samples were still able to pick up mutational signatures with an acceptable degree of sensitivity then there will still be Validation of Shaken Biopsy sampling

diagnostic value in sequencing these samples. On the other hand if shaken biopsy sampling improved any of the QC metrics it may become the gold standard sampling technique.

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 A

15.1 Experiment 1

15.1.1 Rationale

This experiment was designed in order to establish whether shaking tissue in solution would release sufficient cells into solution for whole genome sequencing (WGS).

15.1.2 Outcomes

- Morphological assessment of the tissue to ensure it remains suitable for full histological analysis for diagnosis.

- Examination of the cells from the suspension to quantify the proportion of malignant cells among those harvested and assess the state of preservation.

- DNA yield from the shaken fluid.

15.1.3 Methods

A total of eight patients, consented for research and having surgical resections for breast cancer, were recruited. For six of these patients three diagnostic pseudo-biopsy measuring approximately 15 x 2 x 2 mm were cut from the tumour tissue. The seventh patient had a smaller tumour and only one pseudo-biopsy could be cut which was handled as for group B. For the eighth patient no tumour tissue was evident and the three pseudo-biopsies were cut from benign tissue as a control.

Three pseudo-biopsies were cut and handled in one of three ways, groups A, B and C as set out in Figure 1. Each pseudo-biopsy was placed into a 1.5ml Eppendorf tube containing 1ml of phosphate buffered saline solution. The tube was then gently inverted and righted at a rate of once per second for one minute. The tissue was then removed from the solution.

The tissue from group A was removed and formalin fixed and paraffin embedded and a section cut for haematoxylin and eosin staining and examination of the morphology of the remaining tissue after shaking. The solution was cytospun onto a glass slide for microscopy to examine the type and quality of cells that were removed by shaking. The tissue from group B was snap-frozen in liquid nitrogen for WGS.

The solution from group B was centrifuged at 10,000 rpm for 10 minutes to form a cell pellet. DNA extraction was performed using QIAGEN DNeasy Blood and Tissue Kit, using a modification of the manufacturer's recommended protocol for DNA extraction from Animal Blood or Cells (Spin-Column).

The tissue from group C was also snap-frozen in liquid nitrogen and the solution was kept in the refrigerator for 72 hours at 4^{o}C before being processed identically to group B.

Each patient had a blood sample collected in an EDTA tube for germline sequencing alongside their tumour.

The extracted DNA from the shake solutions and the tissue from groups B and C underwent library preparation using TruSeq PCR free protocol. Whole genome sequencing was carried out on the tumour using three lanes of an Illumina HiSeqX. The matching germline blood sample was sequenced using one lane.

15.1.4 Results

Tissue morphology

The tissue biopsies were intact and there was no evidence of the tissue shaking having had an impact on the appearance of the tissue. See Figure 2.

Cell suspension morphology

The cytological preparations had a high cellularity and contained predominantly intact malignant cells with an appearance and morphological quality similar to that seen in routine diagnostic fine needle aspiration samples.

DNA yield and quality

The mean DNA yield from the cell suspensions was 4.0 micrograms with a range of 1.1 – 11.6 micrograms as shown in Figure 3. The DNA integrity number for these samples was over 6 for all samples and had a mean of 8 which shows minimal DNA degradation in these samples. The DNA contamination was assessed and the 260/280 ratio fell between 1.74 and 2.04 for all cases, which was the predetermined range for eligibility to be included for whole genome sequencing in the forthcoming NHS Genomic Medicine Service.

The DNA yield from suspensions in group C was reduced compared to group B in five of the six cases. The mean yield from group B was 4.5 micrograms whereas the mean yield from group C was 3.3 micrograms. The difference was not statistically significant.

The cell suspensions from the shakes of three pseudo-biopsies cut from benign tissue each yielded less than 1 microgram of DNA.

Whole genome sequencing results

Overall the genome quality metrics are comparable between the tissue and shaken biopsy samples, with no negative impact in variant calling in shaken biopsies. For the two analysed cases, the same variants in known cancer genes show higher variant read frequency in shaken biopsy samples compared to FF due to higher purity. See figure 4.

15.1.5 Discussion

This proof of principle experiment demonstrates that intact cells can be retrieved from a small volume of tissue without deleteriously disrupting the architecture of that tissue. The cells freed into suspension were predominantly intact tumour cells with retained morphology suitable for investigations or testing requiring whole cells. The high tumour content observed microscopically is in keeping with the high genomic tumour content estimated by copy

number caller based on tumour depth and B-allele ratios. The DNA retrieved from the tissue suspension of group B and C were all of sufficient yield and quality for whole genome sequencing demonstrating one particular utility of this means of harvesting cellular material.

15.2 Experiment 2

15.2.1 Rationale

To demonstrate that the shaken biopsy technique could successfully retrieve cells from 'real-life' diagnostic biopsy tissue, which are smaller and more fragile than pseudo-biopsies and likely to contain a smaller proportion of tumour than the pseudo-biopsy tissue used in Experiment 1.

15.2.2 Outcomes

DNA yield and quality from shaken cells was assessed and compared with yield and quality from the matched biopsy, and also related to the underlying diagnosis. Frozen sections of the tissue were assessed for diagnosis, tumour content and morphology.

15.2.3 Methods

Thirty patients having radiologically guided breast core biopsy samples in patients with a lesion suspicious for breast cancer were consented for research use of their tissue. Breast cores were taken using a 14 G core biopsy needle. The first core was placed into neutral buffered formalin for fixation. Subsequent cores were placed into a 1.5ml Eppendorf tube containing 1ml of phosphate buffered saline. The vial was then inverted and righted at a rate of once per second for a period of 1 minute.

The tissue was then removed and snap frozen. The cell suspension was centrifuged at 10,000 rpm for 10 minutes to form a cell pellet. DNA extraction was performed using QIAGEN DNeasy Blood and Tissue Kit, using a modification of the manufacturer's recommended protocol for DNA extraction from Animal Blood or Cells (Spin-Column). The frozen tissue was prepared as a frozen section on a glass slide and stained with haematoxylin and eosin for morphological assessment.

15.2.4 Results

Of the 30 biopsies 23 contained invasive malignancy (one of which was in situ DCIS only) and 7 were benign. Three of the seven cell suspensions from benign tissue yielded over 1 microgram of DNA whereas the other four cell suspensions yielded less than 500 nanograms.

17 of the 23 cell suspensions (74%) from malignant tissue yielded over 500 nanograms which is sufficient for whole genome sequencing in the forthcoming NHS Genomic Medicine Service. The mean DNA yield from the malignant cell suspensions was 1.9 micrograms with a range of 0.08-6.1 micrograms (see Figure 5).

The DNA integrity number for these samples was over 6 for all samples with over 500 nanograms of DNA and had a mean of 8 which shows minimal DNA degradation in these samples. The DNA contamination was assessed and the 260/280 ratio was outside the 1.74 and 2.04 range for two of the cases with more than 500 nanograms of DNA. Of the total of 23 malignant cases 13 had DNA of sufficient yield and quality for whole genome sequencing based on a single shake (once per second for 1 minute) of a single biopsy.

The frozen sections of the biopsy tissue showed intact architecture with no evidence of the shaking having had a deleterious effect on morphology.

15.2.5 Discussion

This experiment demonstrates that intact cells can be retrieved from biopsy tissue without deleteriously disrupting the architecture of that tissue. The cells freed into suspension were predominantly intact tumour cells with retained morphology suitable for investigations or testing requiring whole cells.

Although this work was carried out on breast cancer samples, it is reasonable to assume the results can be extrapolated to other tumour types. It is an inherent feature of malignant cells that they lose their cell-cell adhesion molecules meaning they are more freely released from the surrounding tissue. This accounts for the difference between the benign and malignant breast tissue cell yield in this experimental work. This is a biological phenomenon that is not tissue type specific.

If greater yield is required, cell suspensions from two or more biopsies could be pooled. Preliminary work on extending shaking time shows that this too would increase yield. If shaking two biopsies or extended shaking doubles yields this would have resulted in sufficient yield for whole genome sequencing from 19 of the 23 patients.

Figure 1

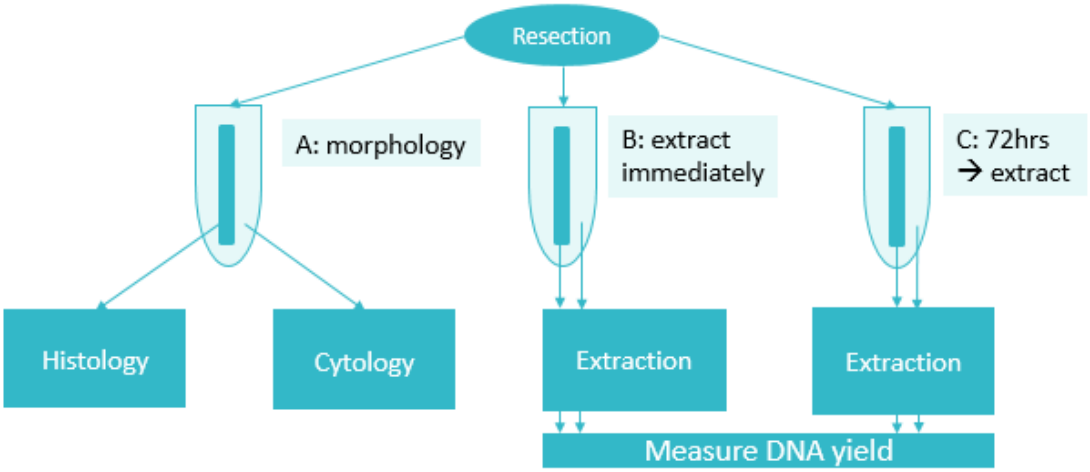


Figure 1: Schematic overview of handling of tissue and cell suspensions from Experiment 1.

Figure 2

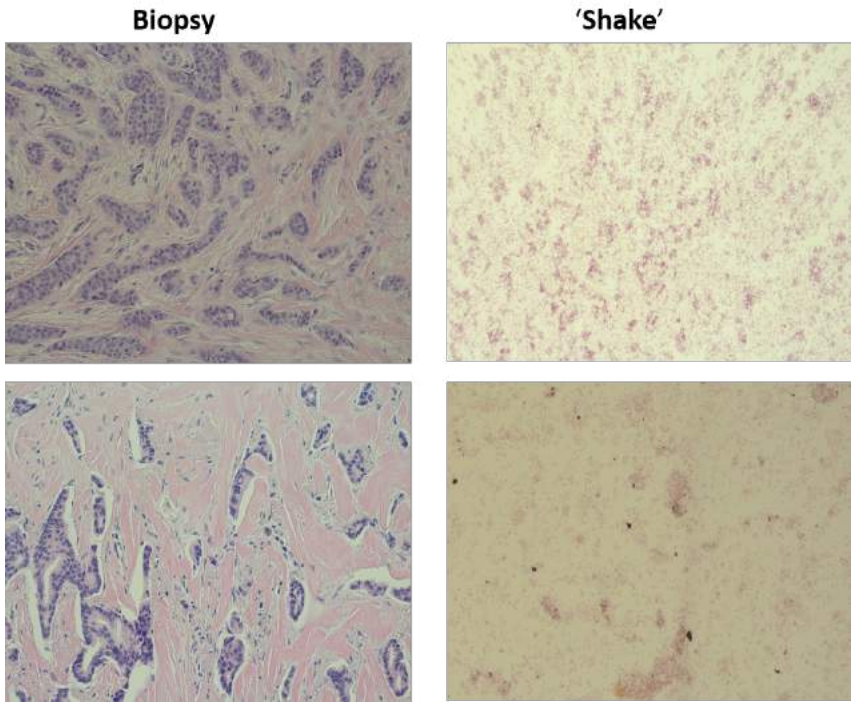


Figure 2 H&E stained sections from the biopsy samples after shaking and from the corresponding cells in suspension.

Figure 3

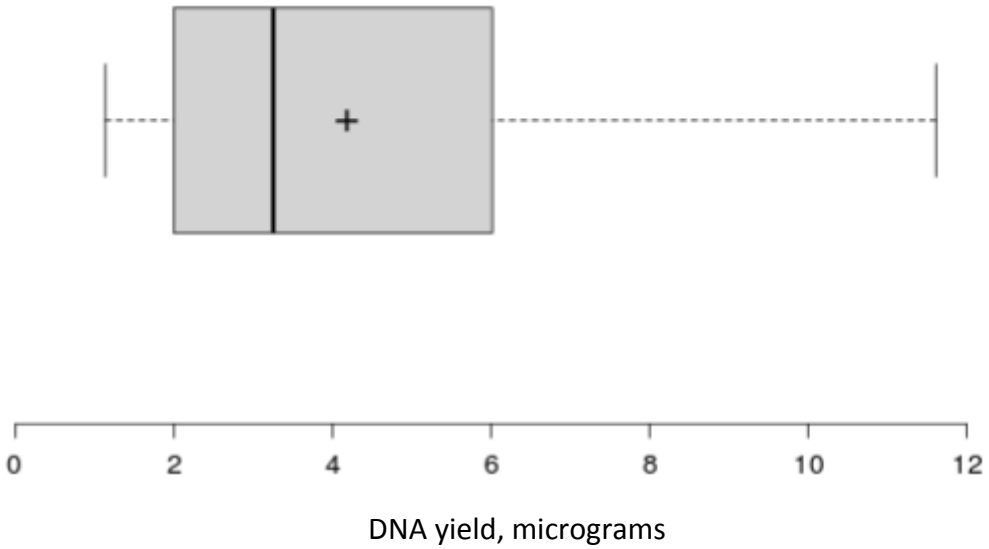


Figure 3: DNA yield from Experiment 1. The X axis shows DNA yield from single pseudo-biopsies with invasive ductal carcinoma of the breast after a single 1 minute shake (inverting once per second for one minute). Box shows 2nd and 3rd quartiles with black line at median. Plus shows mean. Whiskers show range.

Figure 4: Case 1

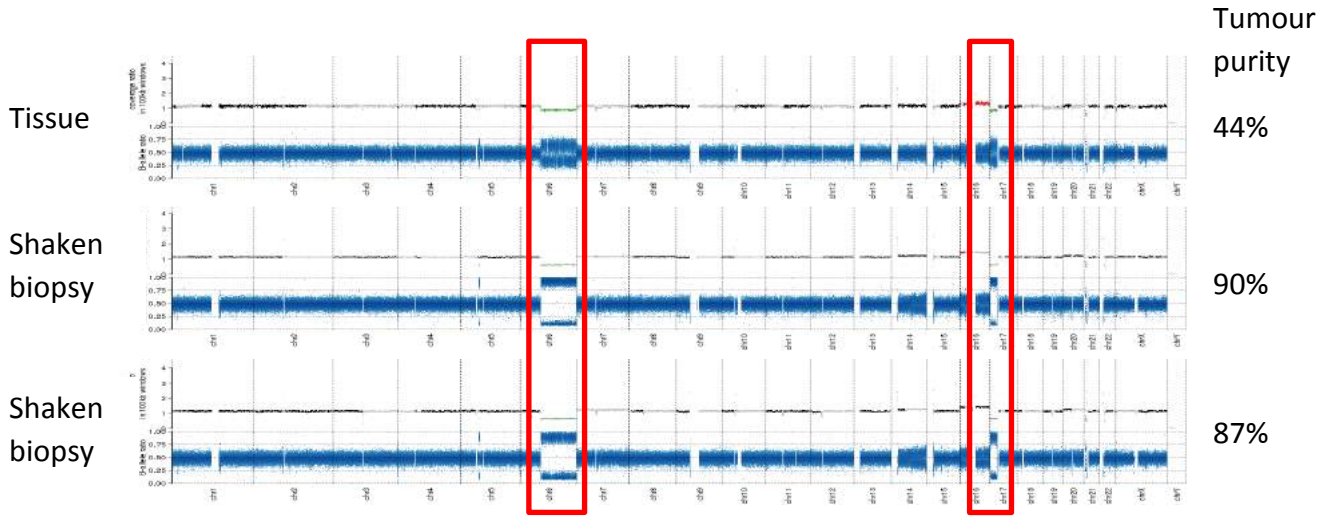


Figure 4: In this example, shaken biopsy samples have higher purity compared to the associated tissue sample, as demonstrated by the greater separation of B-allele ratio bands on chr6 and chr17.

Figure 5

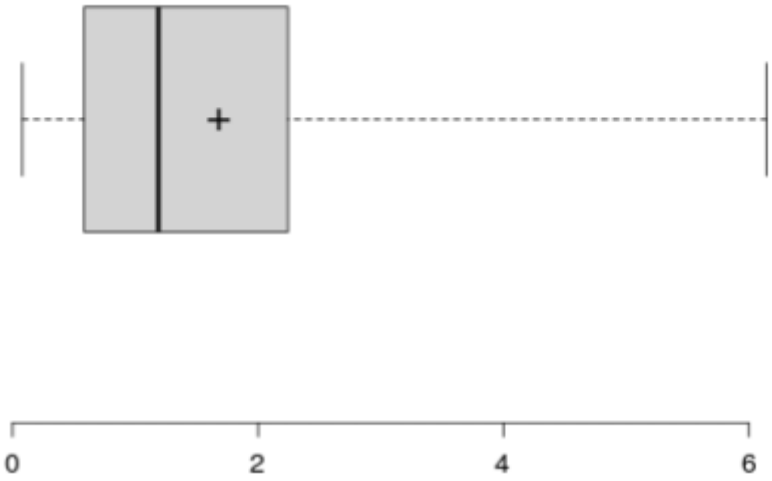


Figure 5: DNA yield Experiment 2. The X axis shows DNA yield from single diagnostic biopsies with ductal carcinoma of the breast after a single 1 minute shake (inverting once per second for one minute). Box shows 2nd and 3rd quartiles with black line at median. Whiskers show range.

16 Appendix B

Checklist for participation in Shaken Biopsy validation pilot	
Sample Handling	
Number of cases expected	
Confirmation of which laboratories will process the samples	
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 **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 intended sampling methods	
Clinical Scientist to talk through DNA extraction methods proposed	