Genomics Training Academy (GTAC)

NHS England

Virtual reality resource supporting material: non-invasive prenatal diagnosis (NIPD)

Trainer pack

V1 07/03/25

About the resource

The virtual reality resource has been developed to show the non-invasive prenatal diagnosis (NIPD) procedure to learners without them having to go into a physical laboratory. This means that more learners can view a process at once than would otherwise be possible within limited laboratory space.

It is important to note that this resource is not designed to fully train someone to competency; learners who will ultimately be carrying out the procedure will need additional hands-on training within the laboratory. For some learners, however, observing and understanding the procedure is all that is required for their training pathway.

Note around laboratory processes

Please note that this is labelled non-invasive prenatal diagnosis (NIPD). It is important to note that the technique demonstrated is NIPD by relative haplotype dosage (RHDO), which is used for NIPD of cystic fibrosis, spinal muscular atrophy, Duchenne muscular dystrophy and congenital adrenal hyperplasia. This is different from NIPD by PCR and massively parallel sequencing. The massively parallel sequencing-based techniques are used for bespoke familial tests and *FGFR3* screening.

This resource demonstrates the key processes in the laboratory. There will be differences in the finer details depending on the local laboratory. Please refer to local laboratory policies and SOPs.

For trainer-led sessions

The virtual reality film is not designed to be used standalone. To support trainers and learners, there are two sets of supporting material that should be referenced and used during and after a training session.

- Trainer pack (this document) – for trainers leading a session with any number of learners.
- Model answers document – for learners who are attending a trainer-led session. We suggest that learners are directed to this material for their reference after their session, as it contains model answers to the formative questions that will be discussed.

For self-led sessions

- For learners working through the resource without a trainer present, please follow this link[1] to access the appropriate resource, which includes formative questions and model answers.

This material has been developed by practice educators and NHS England's Genomics Education Programme team to ensure that learners get the most out of the session.

Learning objectives

By the end of the session, learners will be able to:

- Identify the principal components of NIPD.
- Outline the quality control procedures used during NIPD.
- Explain the key steps in the NIPD procedure.

Assessment

There is a short assessment available for learners to complete after watching the VR film. We recommend that learners complete this assessment to assure themselves that they have met the learning objectives.

To get access to the assessment, learners should:

1. Register for a VLE account, using this form[2].
2. Wait for an email providing instructions on how to finish setting up their account (Note: applications are processed manually by the GTAC team and it may take time to receive this email).
3. Navigate to the VR assessment using this link[3]. Then click 'self-enrol' to access the assessment.

[1] https://forms.office.com/e/XfGWC15RpE
[2] https://forms.office.com/e/tPaF1kLFcE
[3] https://pgvle.co.uk/course/view.php?id=667

Pre-course reading

Understand the basic principles of NIPD by:

- reading this article: ‘non-invasive prenatal diagnosis (NIPD)’;
- watching two other VR films in this series: ‘Polymerase chain reaction (PCR)’ and ‘Massively parallel sequencing’ (log-in to the VLE for more information);
- reading the following information (below) around types of NIPD testing:

The technique demonstrated is NIPD by relative haplotype dosage (RHDO) which is used for NIPD of cystic fibrosis, spinal muscular atrophy, Duchenne muscular dystrophy and congenital adrenal hyperplasia. This is different from NIPD by PCR and massively parallel sequencing. The massively parallel sequencing-based techniques are used for bespoke familial tests and *FGFR3* screening.

Trainer pack

There are a number of ‘bookmarks’ throughout the VR film – points that can be set to automatically pause the footage to allow for discussion to take place.

Important: when you first receive your VR kit, bookmarks will not be preloaded and will have to be added manually. We recommend that this is done as part of familiarising yourself with the equipment. After doing so once, they will be remain for future sessions. For help in adding bookmarks, please scan the QR code inside the kit box to be directed to supporting materials.

At each of these bookmarks, as well as at the end of the film, there are questions to ask and discuss, as outlined in this pack. Your role is to ensure that the group sufficiently covers each of the points. Each question is in **bold**. This is not a script – you may wish to discuss additional things, or additional questions may arise, however, these are the key points to cover.

Note: You may wish to look at the detailed information in the ‘model answers document’ in advance of leading a session; the answers provided here are intentionally brief and are designed to act as prompts rather than prescriptive responses.

After discussing the points below, finish the VR session with your group and direct them to the assessment so that they can test their knowledge.

Bookmark 1: Preparation of sample library (15:12)

1. **What happens during library preparation?**

- DNA fragmentation.
- Universal adaptors and individual indexes are added.

2. **Why do we fragment the genomic DNA?**

- Short read sequencing technologies can’t sequence long DNA strands.
- Uniform pieces about 150 base pairs long are needed.

3. **Why don’t we fragment cell-free DNA (cfDNA)?**

- cfDNA is already fragmented and naturally made up of shorter fragments.

4. **What is end repair and A-tailing?**

End repair:

- DNA fragmentation results in overhanging ends.

- End repair turns these overhanging ends into blunt ends that are the same length.

A tailing:

- A single adenine base is added to form an overhang by an A-tailing reaction. This A overhang allows adapters containing a single thymine overhanging base to pair with the DNA fragments.

5. **Once A-tailing and end repair are completed, why do we need to move on to the next step straight away?**

- If not done quickly, fragments can start to ligate to each other.

6. **What happens during indexing?**

- Indexing gives each sample its own unique molecular tag. This allows us to pool multiple sample libraries into one sequencing reaction, and to be able to know which DNA sequences came from which sample during analysis.
- Universal adaptors are added first. They contain sequencing primer binding sites and allow for the incorporation of unique dual-index adapters via PCR.
- Unique dual-index adapters are then incorporated. They have distinct sequences, so each patient library has its own unique molecular tag. They also contain oligo binding site sequences which allow the library fragments to attach to the flow cell surface.

7. **How do magnetic beads work in the clean-up steps?**

- Magnetic beads bind and release DNA.
- Binding and releasing is due to the presence of either a specific buffer or water.
- By binding the DNA to beads and placing the tube in a magnetic rack, we can remove the supernatant from the tube but not the DNA. This allows the DNA to be washed and remove any impurities.
- Once the DNA is washed, it is then released from the beads and by placing the tube in a magnetic rack the supernatant which now contains the DNA can be transferred to a clean tube.

8. **Why do we air dry the plate after pipetting off the ethanol?**

- This allows any remaining ethanol to evaporate as any remaining will be detrimental to downstream processes.

- It is important not to allow beads to dry for too long as it can lead to loss in DNA yield.

Bookmark 2: Pre-capture library amplification and clean-up (19:17)

9. What is the purpose of the sample sheet?

- The sample sheet allows us to keep track of which sample has which index.
- It is used to tell the sequencer which indexes are associated with which sample in the final pooled sample library and allows sequence data to be allocated back to each sample during analysis, ensuring there is no sample mix up.

10. How are the index primers stored?

- Stored in 96-well plates in the fridge.
- They need to be hydrated before use.

Bookmark 3: Library quality control and DNA measurement; pre-capture pooling of sample libraries and hybridisation of capture probes (23:51)

11. What is the purpose of quality checks? How are they done?

- To ensure samples have sufficient concentration of DNA and correct fragment size.
- A Qubit and Tapestation are used.

12. How does the TapeStation work?

- The TapeStation is an electrophoresis platform.
- Samples are loaded at one end of a lane and an electrical current is applied with the DNA fragments moving towards the negative charge – smaller fragments travel faster than bigger ones.
- The machine and software calculate the average base pair length and outputs a distribution curve.

13. Why do we compare the TapeStation output with examples in the SOP?

- Genomic DNA and cfDNA samples have optimal profiles for successful sequencing.
- Comparing sample profiles to optimal profiles helps to identify any irregularities and if any additional bead clean-up or repeating library preparation steps are required.

14. What do capture probes do?

- Hybridise to parts of the genome we want to sequence, allowing us to keep these fragments and discard those we don't need, which makes sequencing and analysis easier.

Bookmark 4: Probe capture and washes; post-capture library amplification and clean-up; pooled library quality control and DNA measurement (30:31)

15. **Why is it important to complete probe capture and washes quickly?**

    - So that the beads don't dry out and to keep temperature as close to 55°C as possible, while still being careful and accurate.

16. **How do we bind the probes, and their associated hybridised DNA, to the capture beads?**

    - By incubating them at 55°C for 15 minutes.

17. **What does library amplification do?**

    - Makes many more copies of DNA using PCR.

18. **What are the expected sizes (in base pairs) from the TapeStation once we have pooled the libraries?**

    - Genomic DNA: approximately 300 base pairs.
    - cfDNA fragments: approximately 470 base pairs.

End of film

19. **Why do we denature the DNA before sequencing? How is this done?**

    - DNA must be single-stranded for the sequencer. Denaturing the double-stranded DNA separates it into single strands.
    - Incubating it with sodium hydroxide for five minutes. It is important not to incubate it for longer than this because the DNA will degrade.

20. **What steps have been taken to reduce contamination throughout the procedure and why?**

    - The use of PPE:
        - Lab coats.
        - Gloves.

- Separation of pre- and post-PCR set up.
- Equipment – most laboratories will have equipment specific to each area or methods of cleaning equipment before being used in pre-PCR rooms.
- Air pressure.
- Workflows.
- The use of filter tips.
- It is important to minimise contamination as cell-free fetal DNA can account for as little as 1% of the total cell-free DNA sample tested. As we are looking for very low levels, it is a much more sensitive assay.

21. Why are the reagents and DNA kept in the fridge or frozen?

- Manufacturers will provide information regarding how reagents need to be stored, and these guidelines must be followed to ensure that they remain stable.
- Under UKAS accreditation, we are unable to use reagents that are not stored correctly in case of a false negative or false positive result, or a run failure.
- DNA samples being kept long-term should be stored in the freezer (-20ºC/-80ºC) to preserve their stability; in the short term, they can be stored in the fridge.

22. Why is it important to centrifuge samples and reagents after vortexing?

- It ensures all of the liquid is at the bottom of the tube.
- It is safer as liquid is much less likely to splash.
- It ensures that no material is lost.

23. What steps do we take to ensure that no mistakes are made with the patient samples?

You will see several steps taken during the procedure to ensure there are no mistakes:

Organisation

- The work area is free from unnecessary items and only the reagents and patient samples used in the assay are present.
- As the operator pipettes a reagent or sample, they move it in the rack or to a different rack so that they can keep track of where they are in the process.
- The operator will only have one reagent or sample open at one time to reduce the chance of using it incorrectly.

Checks

- The operator gets a second person to check that they have set up the samples correctly before transferring them.
- Transfer checks are carried out when patient material moved from one vessel to another. Transfer checks require presence of two individuals to verify that vessels are labelled correctly. This reduces the risk of sample mix-up.
- Transfer checks may also be used in some cases to confirm that the correct chemical/reagent being used particularly when the indexes are being added to the samples
- Transfer checks are recorded on worksheet for audit purposes.

Lack of distractions

- It is important that the operator is not disturbed during set up of the assay.

Training and competence

- The operator must be trained and competent in the procedure. All laboratories will have procedures for training and assessing competency of staff.

SOPs/worksheets

- The operator will be following a SOP to ensure that everything is set up in the same way each time.
- There may also be a worksheet that details the samples that are to be used and may also contain information about which reagents to use and in what volume.
- Worksheets are often created by or using the LIMS (Laboratory Information Management System).