Genomics Training Academy (GTAC)

NHS England

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

Model answers document

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About this document

This document has been designed to support the non-invasive prenatal diagnosis (NIPD) virtual reality resource in training sessions. The questions and model answers below provide a reference point for discussions that will have happened during the session, and can be kept by learners for future reference, or used by trainers to aid in session planning.

Model answers

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

1. What happens during library preparation?

Library preparation ensures that DNA fragments are the correct size for sequencing. It then adds universal adaptors and individual indexes to the fragments in preparation for massively parallel sequencing.

2. Why do we fragment the genomic DNA?

Short read sequencing technologies cannot readily sequence long DNA strands, so genomic DNA samples are fragmented into shorter, uniform pieces about 150 base pairs long to prepare them for sequencing.

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?

When DNA is fragmented, the complementary strands don’t usually break at exactly the same point – one strand will be slightly longer than the other. End repair turns these overhanging ends into blunt ends that are the same length.

A-tailing is crucial to allow the ligation of adaptors. 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 – these are small partially double-stranded DNA adaptors that ligate to double-stranded DNA fragments. They contain sequencing primer binding sites and allow for the incorporation of unique dual-index adapters via polymerase chain reaction (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 for the library fragments to attach to the flow cell surface. Unique dual-indexes allow for many patients to be pooled into a single prep which can then be loaded onto a DNA sequencer. Each sequenced DNA fragment can then be sorted and assigned to the correct patient via bioinformatics processing due to these unique sequences.

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

Magnetic beads bind and release DNA. Whether the DNA binds to the beads or is released from the beads is based on 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 have any impurities removed. 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?

They are 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 the sufficient concentration of DNA and correct fragment size.

A Qubit and TapeStation are used to check the quality of the samples. Both measure concentration, sample integrity and quality, but the TapeStation also measures the fragment sizes in the sample.

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 fragments. 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?

You would expect two DNA peak sizes due to the presence of the genomic DNA fragments and the cfDNA fragments of approximately 300 and 470 base pairs, respectively.

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.

We do this by 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 – some laboratories, such as in this video, have different lab coats for pre- and post-PCR.
    - Gloves – new gloves must always be used when moving between and pre- and post-PCR.
- 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 – pre-PCR rooms will usually operate under positive pressure so that air from other laboratories does not flow into it.
- Workflows – if possible, workflows should be designed such that people work from pre- to post-PCR during the day.
- 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?

When samples or reagents have been mixed, they are centrifuged to ensure all of the liquid is at the bottom of the tube. This for two reasons: it is safer, as liquid is much less likely to splash, and also 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. They also have 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).