

Virtual reality resource supporting material: SNP microarray

Model answers document

About this document

This document has been designed to support the 'SNP microarray' 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: DNA normalisation and amplification (6:04)

1. What are SNPs?

Single nucleotide polymorphisms: benign single base changes common throughout the genome.

2. What is normalisation?

DNA normalisation uses a buffer to dilute the patient samples to the same correct starting concentration.

Not all samples, however, will be of a high enough concentration, especially in prenatal situations. Samples that aren't of high enough concentration are vacuum-evaporated which removes water from them to increase their concentration instead of diluting them.

3. Why do we vortex and then centrifuge the plate?

Vortexing mixes the contents of a tube or wells in a plate. Centrifugation then ensures that all liquids are at the bottom of the tube or wells.

4. What is the purpose of amplification?

Amplification makes many more copies of the DNA so that there is enough to use in the assay. To do this, the DNA is denatured and then incubated with multi-amplification reagents overnight for 20 to 24 hours. It is important to consider what time the incubation will end due to the onward processing and sufficient time will need to be allowed for this.

5. Why is the DNA transfer checked, but not the buffer?

The DNA transfer is checked by a supporting technician to ensure that the correct patient DNA is being added to the correct well on the plate. Although adding the wrong amount of buffer may lead to a poor result, it is not a risk that would cause an incorrect result.

6. Why do we record batch numbers for reagents on receipt of them?

This is part of the quality management system and is in line with UKAS ISO 15189 accreditation. It allows the tracking of reagents through the laboratory and identifies any batches that are not performing as expected. These would then be followed up with the company and/or a different batch would be used.

7. What chemicals are used during whole genome amplification and what are their roles?

- **Sodium hydroxide** is added to each well to denature double-stranded DNA.
- **Mineral oil** is added on top of the mix to prevent evaporation during the overnight incubation.
- **dNTPs**, which are essential for PCR amplification, are added after denaturation. There are four different types of dNTPs: dATP, dCTP, dGTP, dTTP. They are the single bases which go into the DNA during the amplification.
- **DNA polymerase** is added at the same time as the dNTPs. This enzyme helps amplify the DNA by adding the nucleotides to the strands of DNA as they are copied.
- **Uracil bases** are incorporated into the new copies of DNA randomly instead of dNTPs for end-point fragmentation.

Bookmark 2: DNA fragmentation and precipitation (10:49)

8. How and why is the DNA fragmented?

The DNA is fragmented by a solution containing an enzyme that cleaves at the modified uracil bases that were incorporated during amplification. It is fragmented into short sections to optimise binding to the probes which are located on the BeadChip later on in the process. This is called end-point fragmentation and prevents over-fragmentation of the DNA.

9. Why would you use a liquid handling robot like the Tecan in this film?

The accuracy of pipetting is higher using robots like this, which means more consistent results. They also reduce the amount of pipetting that needs to be done by technicians, therefore reducing the risk of a repetitive strain injury (RSI). The robot can also perform faster than humans – this is particularly crucial as 24 patients can be run on one BeadChip and each run will have four BeadChips (a total of 96 patients).

10. Why is cleaning the Tecan robot important for quality control?

This helps to reduce the chance of contamination. It is important that all equipment is kept clean to remove any potential DNA contamination on the robot which may then be inadvertently transferred to other samples or reactions, causing an incorrect result.

11. How and why is the DNA precipitated?

The precipitation process purifies the DNA, removing contaminants, to get the best quality results. Isopropanol is used to displace the DNA out of the solution – the displaced DNA is then collected at the bottom of each well when centrifuged. The precipitation buffer contains an inert blue dye that forms a blue pellet of DNA once the precipitation procedure is completed.

12. What is the impact of over-drying the pellets at the DNA precipitation stage?

The pellets can be difficult to resuspend, which then will affect the data quality at analysis.

Bookmark 3: DNA resuspension and denaturation (16:31)

13. Why does the excess resuspension solution get discarded in a specialist manner?

The solution contains formamide which is embryotoxic and therefore poses a risk to pregnant individuals.

14. What is the role of formamide?

Formamide lowers the melting temperature of DNA and stabilises it in its denatured state.

15. What health and safety considerations are there when handling formamide?

Formamide is embryotoxic and so any pregnant staff should be warned that the reagent is in use within the laboratory. Waste is discarded into chemical waste and placed in a chemical cabinet, therefore minimising risks to anyone within the laboratory area.

16. What is DNA denaturation and how is it performed?

DNA denaturation separates double-stranded DNA into single strands. The DNA is heated on the heat block at 95°C. This is the temperature where the hydrogen bonds are disrupted, and the DNA becomes single-stranded.

17. What is the role of the 16-to-24-hour incubation?

This incubation hybridises the fragmented single-stranded DNA to complementary probes on the BeadChip.

Single-stranded DNA leaves bases available to hybridise with complementary DNA sequences. In this case, probes on the BeadChip are designed to be complementary and this tethers the DNA being tested to the BeadChip. Incubating the single-stranded DNA with the BeadChip under optimal conditions (48°C with humidifying buffer) means as much complementary DNA as possible is hybridised to the probes on the BeadChip.

18. Why is the dilution plate stored until the assay has been completed?

This is in case of any failures. Rather than repeating the process again from the beginning, it can be picked up from this point instead. However, if there is a suspicion that the dilution is the problem, the process would need to be repeated from the start of Day 1.

19. Why is it important for quality control that the BeadChips are checked for correct positioning and order, and that the barcode numbers match the worksheet?

If the BeadChips are not in the correct position and barcode numbers don't match the worksheet, this will result in samples being incorrectly assigned to a BeadChip and the wrong result being issued.

Bookmark 4: Single-base extension and stain (23:29)

20. Why is it important that there are no air bubbles in the TeFlow chamber?

Bubbles would alter the temperature of the TeFlow chamber, or create areas that are not at the correct temperature, which would affect the incubation.

21. Why are the BeadChips washed?

The wash removes any unbound sample and reagents.

22. Why are goggles worn when washing the BeadChips?

There is a risk of formamide being in the buffer after it is washed off the BeadChips. Therefore, goggles should be worn to reduce the risk of eye splashes.

23. What happens during extension and stain?

A complementary probe hybridises immediately adjacent to each loci of interest. The single base extension process then incorporates a fluorescently labelled chain terminating ddNTP (dideoxynucleotide) into the sequence. The extension process ends with this ddNTP – no more nucleotides are incorporated into the sequence.

When excited by a laser, the fluorescently labelled nucleotide will emit a signal. The downward analysis of this data looks at which alleles are present and the intensity of the signal, providing an allelic ratio at the locus of interest.

24. Why does the location of the reagents have to be checked during this process?

The reagents need to be in the correct location on the Tecan to ensure that they added correctly to the BeadChip.

25. How should the BeadChips be handled?

BeadChips are easily damaged so should always be handled carefully by the edges or the barcoded end, making sure not to touch the arrays.

End of film

26. Why is it important that the BeadChips don't dry out?

Until the last step of the procedure, it is important that the BeadChips do not dry out to ensure the controlled entrapment of the reagents over the surface.

27. What does the iScan do?

Within the iScan, there is a laser that is directed at each of the array subsections and excites the labelled nucleotides. The signal emitted from each nucleotide label provides the allele ratio at that position; the iScan records and interprets this hybridisation signal.

28. What data processing is carried out by the wet lab team?

Once the iScan has finished scanning, the wet lab team carry out initial data processing. This involves checking a number of quality control parameters. If any samples don't meet these criteria, the duty scientist is informed and samples may need to be re-run. The data is then sent to the scientist team for analysis and interpretation.