

# Virtual reality resource supporting material: SNP microarray

## Trainer pack

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## About the resource

The virtual reality resource has been developed to show the SNP microarray 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

The general procedure for SNP microarray will be the same across all NHS genomic laboratories, however, there may be local variations depending on individual lab set-ups, including different types of equipment. In addition, some laboratories will have witnessed checks in specific areas. Please also refer to your local COSHH, risk assessments and SOPs for further information.

### 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](#)<sup>1</sup> 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.*

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<sup>1</sup> <https://forms.office.com/e/tYYxU2uWjZ>

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## Learning objectives

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

- Identify the principal components of SNP microarray processing.
- Outline the quality control procedures in SNP microarray processing.
- Explain the key steps in SNP microarray processing.

## 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 Moodle account, using [this form](#)<sup>2</sup>.
2. Wait for an email from Moodle 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](#)<sup>3</sup>. Then click 'self-enrol' to access the assessment.

## Pre-course reading

Understand the basic principles of microarray by:

- reading this article: GeNotes: [Microarray \(array CGH\)](#)
- understanding the types of referrals that can be received for microarray processing:
  - microarray testing is relevant for postnatal and prenatal referrals;
  - postnatal referrals can be from blood samples, products of conception or skin samples;
  - microarray testing from postnatal blood samples includes testing for congenital malformation and dysmorphism syndromes when clinical features strongly suggest a chromosomal cause, confirming uniparental disomy, and

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<sup>2</sup> <https://forms.office.com/e/tPaF1kLFcE>

<sup>3</sup> <https://pgyle.co.uk/course/view.php?id=659>

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- unexplained global developmental delay or unexplained intellectual disability;  
and
- for prenatal referrals, DNA is extracted from amniotic fluid or chorionic villi. This tests a fetus with a likely chromosome abnormality, including ongoing pregnancies or pregnancy losses with features of a chromosome abnormality, or a third trimester intrauterine death or stillbirth in the absence of other likely causes; and
  - consider watching this video: GENie: [SNP and CGH Arrays](#) (via genomic laboratory training videos module). Note that this film is behind a log-in, but is accessible to those working in NHS England Genomic Laboratory Hubs (GLHs).

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## 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 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: DNA normalisation and amplification (6:04)

#### 1. What are SNPs?

- Single nucleotide polymorphisms.
- Benign single base changes that are common throughout the genome.

#### 2. What is normalisation?

- DNA normalisation uses a buffer to dilute the patient samples to the same correct starting concentration.
- However, not all samples 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 ensures that all liquids are at the bottom of the tube or wells.

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#### 4. What is the purpose of amplification?

- It 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 to ensure that the correct patient sample 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?

- It 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 which 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;
- mineral oil;
- dNTPs;
- DNA polymerase; and
- uracil bases.

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

#### 8. How and why is the DNA fragmented?

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

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## 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.
- Less pipetting to be done by the technicians and therefore lower risk of a repetitive strain injury (RSI).
- The robot is faster than humans. This is particularly important as 24 patients can be run on one BeadChip and each run will have four Beadchips (for a total of 96 patients).

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

- Helps to reduce the chance of contamination.
- It is important that all equipment is kept clean – any potential DNA contamination on the robot 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, affecting 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, which poses a risk to pregnant individuals.

## 14. What is the role of formamide?

- Formamide lowers the melting temperature of DNA and stabilises it in the 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.
- The waste is discarded into chemical waste and placed in a chemical cabinet therefore minimising risks to anyone within the laboratory area.

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## 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.
- This tethers the DNA we are testing 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, the process 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 affecting 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, to reduce the risk of eye splashes, goggles should be worn.

## 23. What happens during extension and stain?

- A complementary probe hybridises immediately adjacent to each loci of interest.



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- The single base extension process then incorporates a fluorescently labelled chain terminating ddNTP (dideoxynucleotide) 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 they are 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?**

- Its laser is directed at each of the array subsections which 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?**

- The wet lab team carry out initial data processing.
- They check 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.