Genomics Training Academy (GTAC)

NHS England

Virtual reality resource supporting material: Multiplex ligation-dependent probe amplification (MLPA)

Model answers document

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About this document

This document has been designed to support the multiplex ligation-dependent probe amplification (MLPA) 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 (6:44)

1. **What information regarding the patient samples and MLPA procedure is required before we begin?**

We need to know which patient samples are to be processed, along with how many and which normal controls to use, which positive control to use and which MLPA probe kit to use. We also need to know the concentration of all the DNA samples (patient samples and controls).

2. **Why are DNA samples stored in a fridge? Why are some reagents stored 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.

3. **Why are DNA samples mixed with a vortex and then centrifuged?**

Each DNA sample is vortexed to ensure rapid and efficient mixing so that a representative aliquot can be pipetted up and used in testing.

Each DNA sample is centrifuged to collect the sample to the bottom of the tube, enabling it to be accurately pipetted. Centrifuging the sample also helps to prevent contamination through the release of aerosols and splash contamination from the tube when opened.

4. **Why do we need to dilute the patient's DNA?**

DNA is diluted to ensure that the input concentration, which will be specific to the procedure, is suitable for the PCR reaction. If too little DNA is added to the PCR reaction, it may not be possible to determine an accurate result as the signal intensity on the analyser will be too

low. If too much DNA is added, then the signal intensity on the analyser will be too high, causing saturation, which can also impact on the accuracy of the result.

5. **What do we use to dilute the DNA and why?**

DNA is usually diluted with molecular grade water or a buffer such as Tris EDTA (TE). It is important that the diluent is molecular-grade to ensure that no contaminants are introduced into the PCR reaction and that there are no nucleases that could degrade the DNA.

6. **How do we know where to place each DNA sample for the liquid handling robot?**

Locations of each patient sample tube have been pre-specified by supporting paperwork created for the MLPA set-up.

7. **What is used as the negative control?**

TE buffer. A liquid with no contaminants and no DNA present.

8. **When are transfer checks performed?**

When there is any transfer of genetic material between two locations.

9. **What other checks by a supporting technician are also required and how are these recorded?**

Other checks are required to make sure that the locations of patient samples/reagents are correct, and that key reagents are correct.

The supporting technician signs the relevant paperwork which is an important internal quality control measure.

Bookmark 2: Biomek (10:26)

10. **Why is the Biomek liquid handler used rather than manual pipetting?**

Liquid handlers enable precise, accurate pipetting in a reproducible manner; it is also easier and faster to process large numbers of samples. Automation frees up time for technical colleagues to perform other tasks and thereby increases efficiency. The robots are also flexible – scripts can be written for various different tasks that instruct the robot what to do and when. There is also a record of the steps completed by the robot and so these enable monitoring and analysis of liquid handling actions.

11. **What checks are made prior to using the Biomek?**

We check that the waste bottle has enough capacity (is over half empty), that the reservoir bottle is over half full, and that the biobin has enough capacity for waste.

12. **What steps are needed to set up the Biomek? What is the purpose of each of these?**

- Home all axes – to ensure that the robot is navigating the deck correctly and going to the correct place for each action.
- Free the tubes of air bubbles – to ensure correct pipetting and dispensing of liquids.
- Bring in the script – ensure the correct liquid handling actions are selected for the task (as set out in the SOP).
- Set up Biomek deck – to ensure components are in the pre-determined locations for the liquid handler to navigate to.
- Label the plates – to ensure correct patient sample handling and identification of different plates in the task.
- Unscrew the DNA tubes – to allow the liquid handler access to the samples.

13. **What checks are performed by a supporting technician for the Biomek set up and why?**

They check the orientation and layout of the deck, and that the correct script has been loaded. This is an internal quality control step to reduce the risk of error and help ensure correct sample processing.

Bookmark 3: Hybridisation (14:45)

14. **What are thermocyclers used for?**

They hold liquids at specific temperatures for specific times to allow heat-based reactions to occur. Sometimes a reaction requires a set temperature; for others, the temperature fluctuates in a cycle with varying time durations at various temperatures.

15. **Why is a compression pad used in the thermocycler?**

It helps reduce the possibility of evaporation occurring.

16. **What happens during the denaturing step?**

The sample is heated to 98°C for five minutes. It separates the DNA strands – from double-stranded into single strands.

17. **Why are MLPA probe mixes prepared in a clean room?**

A clean room is a space that maintains a low concentration of airborne particles and therefore potential contaminants to minimise the risk of contamination of the stock reagents.

18. **Why are probe mixes not centrifuged?**

Probes must be transferred to the sample in equal amounts which will be the case if they are adequately mixed; centrifuging the mixes would separate the probes according to size.

19. What is pipette mixing?

Gentle pipetting up and down before expelling the last of the liquid from the pipette tip.

20. What happens in the probe hybridisation step?

The sample is heated to 95°C for one minute and then held at 60°C for 16 hours. This allows the probes to anneal to their complementary targets. The probe mix can contain up to 60 MLPA probes. The left and right oligo probes for each target bind to immediately adjacent locations on the target DNA.

21. Why are lot numbers recorded?

This is an essential component of internal quality control and helps us to identify which components of a test might be causing an issue in the event of a test failing to meet the specific expected quality metrics for that assay.

Bookmark 4: Ligation (16:50)

22. What is in the ligation master mix?

Ligation buffer and Ligase 65

23. What happens during the ligation step?

Ligase enzyme joins (ligates) the probes together. Specifically, the enzyme (ligase 65) catalyses the creation of a covalent bond between the left and right oligo. The enzyme is then inactivated by heating the reaction.

24. Why must the ligase enzyme be kept on ice?

To ensure the enzyme's ability to function is maintained. It is very sensitive to temperature.

25. Why are the probes designed as left and right probes that need ligating?

For specificity: only if the specific target site is present in the sample DNA will the left and right probe pair be able to bind to it. Once bound, the ligase enzyme joins them together to then amplify in the next step. If the probe was a single probe, it would result in non-specific amplification without the need for target DNA recognition.

End of film

26. What is being amplified during the PCR step?

The ligated probes.

27. What is happening at each of the different temperatures of the PCR cycle?

Denaturation, primer annealing, extension.

28. **What is the purpose of capillary electrophoresis in the MLPA process?**

It enables separation of the amplified fragments by size using capillary electrophoresis.

A laser excites the fluorescent dye incorporated in the amplified fragments and fragments are detected. Fragments move through the separation polymer towards the detector; smaller fragments travel faster than larger fragments.

29. **What is the purpose of the size standard in the reaction?**

The size standard consists of fluorescently labelled DNA fragments of known length which function as a molecular ruler. The size standard is labelled with a different fluorescent dye to that of the MLPA probe products. The fluorescence of the size standard and MLPA amplicons are detected by the detector in the capillary electrophoresis machine as the fragments migrate through based on size - small fragments pass through quicker than larger fragments. The migration of each MLPA amplicon is compared to the migration of each fragment of the size standard to determine the size and therefore the identity of the MLPA amplicon.

30. **What are the good laboratory practice steps for using the capillary electrophoresis machine for fragment separation?**

Check the maintenance is up to date, and visually check there is enough polymer for the run.

31. **Why do the samples need to be denatured prior to loading on the capillary electrophoresis machine?**

To ensure the DNA fragments are single-stranded to enable accurate size separation.

32. **How is the data visualised and QC checked?**

It is loaded into the specialist analysis software.

33. **Can you summarise the quality measures that help to minimise errors and are important in monitoring metrics and identifying issues?**

PPE – lab coat (pre- and post-PCR), gloves.

- Separating pre- and post-PCR techniques.
- Bristol lab have a clean room for making up master mixes in a DNA-free environment – minimise risk of reagent contamination with DNA.
- Transfer checks by an independent supporting technician.
- Checking programs and scripts before use.

- Machine set up checks – check by an independent supporting technician, check that the machine is ready to be used.
- Recording lot numbers.
- Centrifuging plates and using seals to minimise contamination through aerosols.
- Keeping reagents and patient samples at the optimal temperature to preserve integrity.
- Setting up work area in a way that minimises contamination when manual pipetting performed - being mindful of where clean pipette tips are located and ensuring that you don’t have to reach over them to get to the waste bin.