Genomics Training Academy (GTAC)

NHS England

Virtual reality resource supporting material: Polymerase chain reaction (PCR)

Trainer pack

V3 26/09/24

About the resource

The virtual reality resource has been developed to show the PCR laboratory 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.

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.

[1] https://forms.office.com/e/azueZLUS4w

Pre-learning

Before beginning the VR training, it is important that learners understand the principles of PCR. We recommend that all learners look at this resource[2] in advance of either their taught session or working through the VR film on their own.

Learning objectives

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

- identify the principle components of a PCR reaction;
- outline the quality control procedures in PCR reactions;
- explain the key steps in the PCR procedure; and
- describe how PCR is used in a laboratory environment.

Assessment

There is a short assessment[3] 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.

[2] https://www.yourgenome.org/theme/what-is-pcr-polymerase-chain-reaction/
[3] https://pgvle.co.uk/course/view.php?id=600

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.

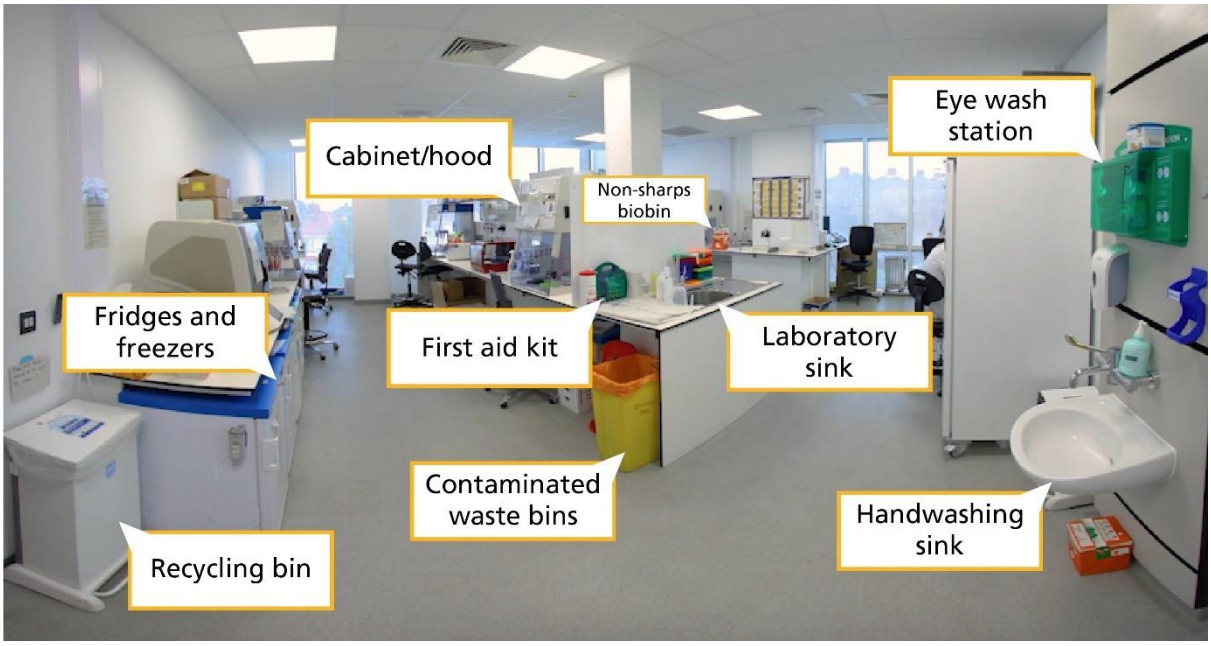
At each of these bookmarks, as well as at the end of the film, there are questions to ask and discuss, as outlined in this pack. Your role is to ensure that the group sufficiently covers each of the points. Each question is in **bold**. This is not a script – you may wish to discuss additional things, 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: Laboratory overview (1:59)

Learners will see an image of the lab on screen (without the labels).



1. What items used in the PCR procedure can you see?

Note to trainer: make sure learners understand what each item is used for.

- Fridges and freezers
- Cabinets and hoods
- Bins. Many different types (see image, below)

2. What other laboratory items can you see?

- Handwashing and laboratory sinks
    - What different purposes do they serve?
- First aid kit and eye wash station
- Cleaning materials
    - Why is it important to keep the lab clean?

3. What other generic laboratory items would you expect to see?

Some examples include:

- Centrifuges/microfuge/mini-spin
- Robots
- Vortex
- Heat block
- Pipettes
- Spill kit
- Gloves
- Lab coats

Bookmark 2: Preparation (2:40)

4. Why are the reagents and DNA kept refrigerated or frozen?

- Stability
- UKAS accreditation
- False negatives and positives

5. Where is the storage information for each reagent kept?

- SOP
- Manufacturers material data sheet

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

- Safer, less likely to splash liquid
- Ensures no material is lost

Bookmark 3: PCR set-up (5:09)

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

- Needs to be at input concentration, specific to procedure. Too little/too much DNA can cause problems, especially in qualitative methods (e.g. real-time qPCR)

8. What do we use to dilute the DNA?

- Molecular-grade water or buffer (e.g. Tris EDTA (TE))

9. What reagents go into a PCR master mix and what do they do?

- Polymerase
- Nucleotides
- Buffer
- Primers
- Quencher (not included in all types of PCR)
    - Note: use of commercial mixes means components may not all be added individually.

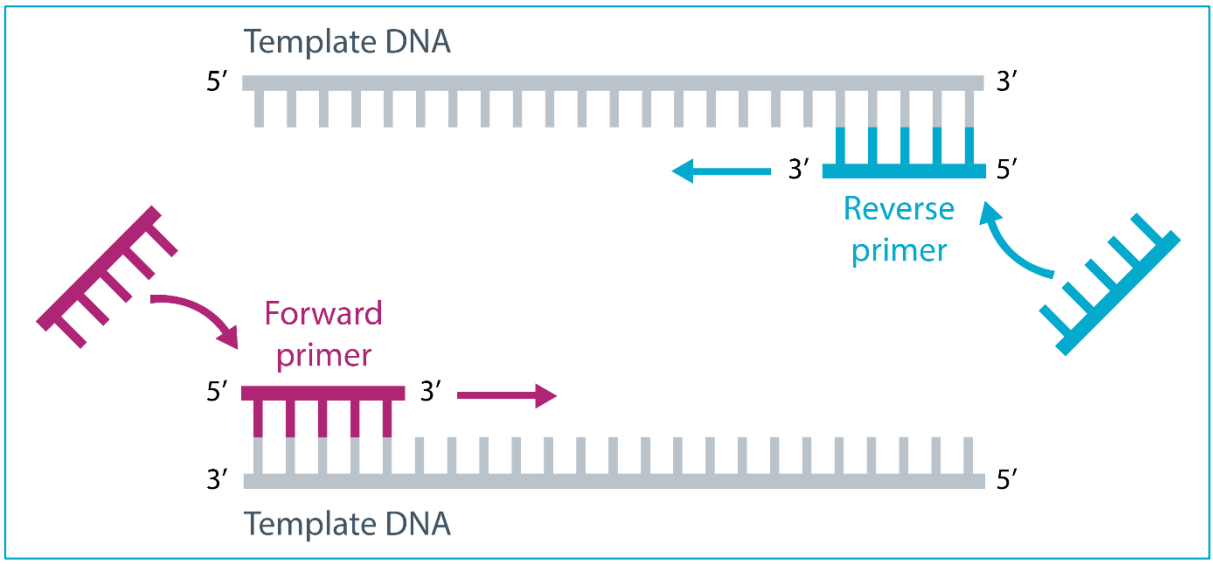


Figure 1 - Reference image of primer annealing process

10. What are the key considerations when designing primers?

- They need to be unique to the region of interest you are looking at.
- Need to amplify a product which can be analysed in your laboratory.
- Need to avoid common SNPs which could stop the primers from binding.
- Not have a high GC content.
- Have an annealing temperature which is standard for your laboratory.

11. What controls would you use in a routine PCR? Why?

- Non-template/water blank negative
- Negative/normal control
- Positive control
    - Purpose of normal/positive controls will be assay-specific (e.g. QF-PCR: to ensure that PCR conditions enable detection of a trisomic ratio).

End of film

12. What factors pertaining to the DNA sample may affect the reaction?

- Contamination; poor quality; suboptimal samples

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

- Organisation/cleanliness
- Checks
- Lack of distractions

- Training and competence
- SOPs/worksheets

13b. (sub question) How is competency assessed in your laboratory?

14. When are witnessed transfer checks used?

14b. (sub question) How are checks performed in your laboratory?

15. The person completing the check is described as 'trained' – why is this important?

16. What steps have been taken to reduce contamination and why?

- PCR cabinet
- Organisation/cleanliness
- Items kept inside cabinet
- PPE
- Changing pipette tips
- Pre- and post-PCR

17. What other quality control measures might be taken as part of this procedure?

- Lab coats
- Gloves

18. Why is it important to set the cycle number and annealing temperature?

- If the annealing temperature is not correct, the primers will remain unbound, causing the PCR to fail.
- The number of cycles is optimised. We don't want non-target products, but we need enough cycles that there is enough target product for analysis.

19. How do you ensure that the PCR machine has run correctly?

- Machine is at holding temperature
- Machine indicates it is at end of run
- Check for error messages on the screen, investigate them if there are any.

20. What happens if the controls fail?

- Fail the run and investigate why they failed.

21. Why was the plate put in a different waste bin to the gloves?

- Plate has reagents in it so can't go in domestic waste.
- Gloves can go in domestic waste if they haven't come into contact with DNA or contaminated waste; this reduces costs.

22. When would you put the gloves into the contaminated waste bin?

- Where gloves have been contaminated, they need to be changed immediately and disposed of in the contaminated waste bin.