

Considerations for Selecting PCR Consumables

Introduction

PCR (or polymerase chain reaction) is a technique used to amplify small amounts of DNA. Since its advent in 1983 by Kary Mullis it has become an essential part of research laboratories worldwide. The theory behind PCR is simple, but there are many components in the process which are critical for proper execution. PCR setup involves the combination of template DNA, DNA polymerase, primers, nucleotides, Mg²⁺ and buffer in a small vessel, such as a tube or plate. This vessel is then subjected to multiple rounds of heating and cooling in a thermal cycler which results in the amplification of the DNA. PCR assays can be qualitative (used to detect the presence or absence of a specific DNA product) or quantitative (used to detect how much of a specific DNA or gene is in a sample).¹ Reverse transcriptase PCR is a variation of PCR in which a purified RNA sample is enzymatically reverse transcribed into complementary DNA prior to amplification. This article discusses key considerations for a successful PCR assay with regards to the selection of laboratory equipment and consumables.

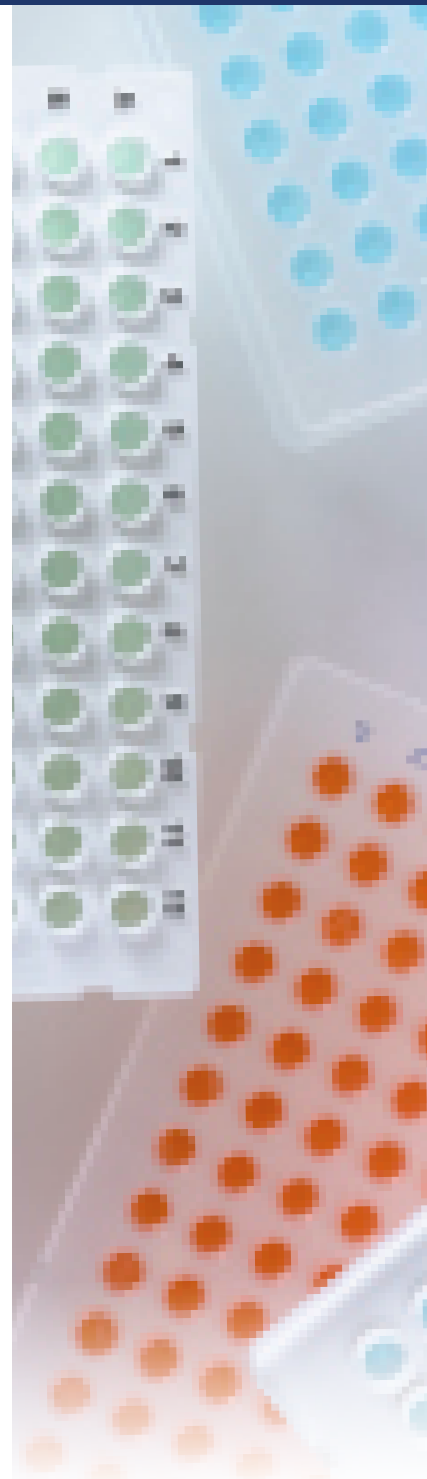
Thermal Cycler

The first consideration for a successful PCR assay is the selection of the thermal cycler in which your assays will be run. This is crucial to all future assays as it will dictate which PCR consumables you will need with regards to throughput (the number and volume of samples you can process at one time) and the type of PCR assay (qualitative or quantitative). There are many high-quality manufacturers of thermal cyclers including Applied Biosystems®, Bio-Rad®, Eppendorf®, and Roche® to name a few. The thermal cycler itself will have heating blocks with wells to hold your samples. The shape, number and volume of wells within the heating block will dictate which consumables can be used. Often thermal cyclers will have interchangeable blocks allowing for an instrument with great versatility. The quality of the heating block is crucial in the prevention of hot spots and edge effects which can result in inconsistent amplification. For quantitative (or real-time) PCR assays, a thermal cycler with a high level of sensitivity and wide dynamic range should be selected for the optimal detection of the amplified fluorescent signal.

Throughput and Volume

Thermal cycler heating blocks can have anywhere between 24-wells and 384-wells for sample processing. In addition, the wells are also sized for vessels that can hold sample volumes from 0.1 mL to 0.5 mL. It is important to select a consumable (be it tube, tube strip, or plate) that is the correct volume and will fit within the heating block. Assays are most commonly conducted at volumes of 20 µL to 50 µL. Using a smaller sample volume can result in quicker assays, as well as reduce the amount of limiting and expensive reagents required to run the assay.

Individual tubes can be used in all thermal cyclers as long as the volume of the tube matches the volume allowed by the heating block. Tube strips are available in 8-strips or 12-strips and will also fit into most thermal cyclers, with the caveat that the strip tube volume must match what is accepted by the heating block. Plates are available in 24-, 32-, 48-, 96-, and 384-well configurations at volumes of 0.1 mL (low-profile) and 0.2 mL (standard profile). Quality plates will be alphanumeric coded allowing for easy identification and tracking of samples. Plates are available in unskirted, semi-skirted, and skirted configurations. Unskirted plates are the most versatile as they can be used in the widest array of instruments. Semi-skirted or full skirted plates offer a more rigid structure for easier handling and less warping but may be limited to use in only specific thermal cyclers. Thermal cycler heating blocks will often have different cut corners (e.g. A12, H12, A24, P24) that dictate which type of skirted consumable will fit. Care should be taken to select semi-skirted and skirted plates with similar cut corners as the heating block. For reproducible results, choose tubes, strips and plates from quality suppliers. Quality consumables are manufactured under clean room conditions to ensure no foreign nucleic acid contamination. Additionally, vessel walls need to be as consistently thin as possible for maximum and even heat transfer, but still need to provide structural integrity.



Assay Type

Once you have selected the consumable type with the correct number of wells and the correct volume, it is important to select the correct consumable color. Colored or clear consumables are perfectly fine for qualitative PCR but white PCR products are recommended for use with quantitative (or real-time) PCR. This is due to the detection of fluorescent signals during quantitative PCR which allows for the relative calculation of the amount of DNA during amplification. The uniform coloring provides a more effective reflection of the fluorescent signal for more reliable results.

Sealing Methods

A final consideration for the selection of PCR consumables is the method in which the PCR vessel is sealed. The PCR process involves heating steps which can lead to evaporation of the liquid sample. Correctly sealing the vessel will reduce the amount of liquid that may evaporate from the reaction mixture. Individual tubes will have individually attached caps with either a dome or flat shape. Whether to select a dome or flat cap is often up to the end user, but dome caps may provide a better fit in the heating block and may allow for more efficient heat transfer. Tube strips can be effectively sealed with attached strip caps, separate strip caps, as well as sealing film strips. PCR plates can also be effectively sealed with sealing film sheets, or sealing film strips, as well as strip caps. It is important to note, that for quantitative PCR, clear flat caps and optical sealing films are recommended for the proper detection of fluorescent signal. If using strip caps with either strip tubes or plates, it is important to use strip caps from the same manufacturer matched to the tubes and plates to provide the best seal.

Summary

The selection of the correct PCR equipment and consumables is crucial to the fidelity of PCR assays. In addition to selecting the correct PCR vessel with respect to well number, vessel type, volume, and color, it is important to select a high-quality plastic. During the heating and cooling process, it is possible for contaminants to leach into the sample. It is also possible for some products to harbor unwanted DNA or RNA which could lead to erroneous data. Therefore, select a PCR product from a trusted manufacturer that does not use additives such as di (2-hydroxyethyl) methyl dodecyl ammonium (DiHEMDA) and 9-octadecenamide (oleamide), and certifies their products as DNA, RNase, and endotoxin free. This should also be a consideration for any other plastic consumables, such as pipette tips used to transfer reagents, or microtubes that hold a master mix.

References

1. Garibyan, Lilit, and Nidhi Avashia. "Polymerase chain reaction." *The Journal of investigative dermatology* vol. 133,3 (2013): 1-4. doi:10.1038/jid.2013.1

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