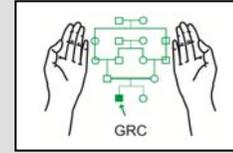


Setting-up a PCR Lab

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Since the applications of PCR range very widely it is advisable to establish separate sections for genetic, malignant and infectious disorders. The forensic DNA lab in any case needs to be established separately. PCR is a relatively new technique for most diagnostic pathology laboratories. Therefore more than often one is required to create a space for PCR facility in an already established lab. No matter how small the PCR setup would be it should be housed in a separate room. The size of the lab depends on the range of applications and the quantum of workload.

Whether the PCR lab is small, medium or large or is aimed to cater for genetic, malignant, or infectious disorders the basic format remains the same. Considering the extreme sensitivity of PCR it is essential to divide or at least restrict the flow of work to three separate areas. These include areas for specimen preparation, PCR setup and post PCR processing. A suggested layout for a medium sized PCR lab is shown in Fig 12.1.

Specimen preparation area

The specimen preparation area is dedicated for receiving and processing all specimens. Special precautions are also required if any of the infectious samples are being received. No handling of amplified product should be done in this area. The equipment used in this area especially the pipettes etc. should not be used in other areas of the lab.

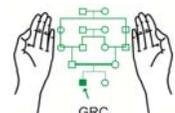
PCR setup area

This is an area where PCR is setup. It should be maintained free of contamination from sample preparation and post PCR processing areas. The pipettes etc. should not be used at other areas. If possible each worker should have its own bench space and set of pipettes etc. This area is also used for reagent preparation and storage.

Post PCR processing area

This area is used for PCR amplification and processing of the amplified products. The thermal cyclers may also be placed in this area. This is potentially the most dangerous area that may spread contamination to the rest of the work areas. The pipettes used in this area must be marked “amplified DNA only”.

In a small lab the entire procedure of PCR can be done in a single room. The areas may be demarcated or physically separated by creating partitions. Whether PCR is done in separate rooms or separate areas in a single room the flow of work should remain uni-directional.



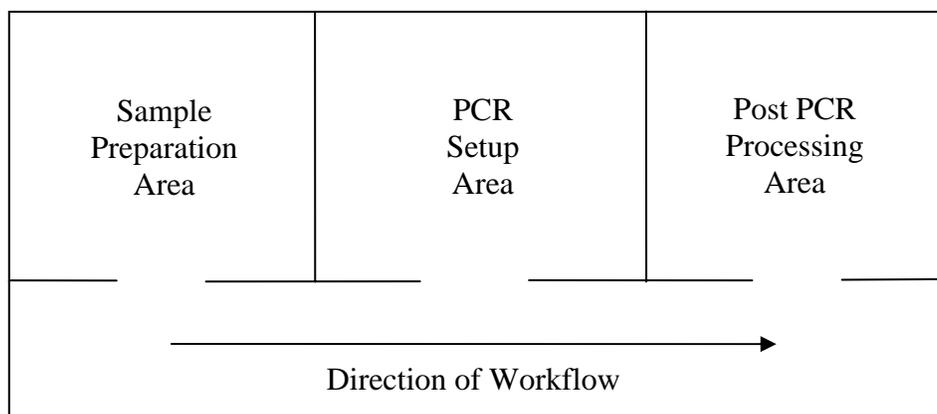


Fig 12.1 Suggested layout of a PCR laboratory.

Equipment required for a PCR lab

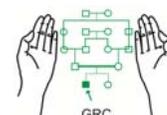
The basic equipment required for a PCR lab is listed in Table 12.1.

Table 12.1 List of basic equipment for a medium sized PCR lab.

Equipment	Quantity
DNA Thermal cycler	2
Micro-centrifuge (up to 15000 rpm)	2
Vortex mixer	2
Analytical balance (0.000g)	1
Adjustable pipettes (full range)	4 sets
Poly-acrylamide gel electrophoresis apparatus	2
Submarine agarose gel electrophoresis tanks	2
DC Power Supply (100-300 volts)	2
Water bath	1
Gel dryer with vacuum pump	1
Gel documentation system	1
Refrigerator (-20°C)	2
Real time PCR machine (optional)	1

Thermal cycler

It is the most important piece of equipment in a PCR lab. A wide variety of instruments are available with widely ranging prices. In general machines that can accommodate greater numbers of tubes and have higher ramping rates (ability to rapidly cool or heat)



are more expensive. Such machines are usually suitable for labs with very large quantum of work.

One should buy a machine that best suits the requirement. If the daily work load of a lab is between 10-20 samples there is no point in buying an expensive machine with capacity to hold 96 samples or to have very high ramping rates. In a diagnostic lab a spare PCR machine should be available as backup.

Real time PCR machine

A fairly good number of manufacturers are making real time PCR machines. A machine with capacity to detect larger number of fluorescent dyes (colours) is more expensive. While choosing a real time PCR machine one should keep the requirements in mind. A diagnostic lab that is mostly involved in real time PCR of infectious disorders should buy a machine with two colour detection capability. It is good enough for doing TaqMan® probe based real time PCR.

Measures to protect against power breakdowns

1. Frequent power breakdowns can adversely affect the overall functioning of the lab. All sensitive equipment especially the thermal cyclers must have a backup electric supply. Un-interrupted Power Supply (UPS) that generates square wave electric current can be harmful for the equipment with moving parts. All such equipment should have a sine wave UPS. The UPS should be of appropriate power. Lab equipment like incubators, water baths, and water distillation plants that use maximum electric power should not be placed on UPS.
2. Loose power connections can be a source of instrument malfunction. Adequate attention must be paid to electric wiring. Loose connections and inappropriate electric wiring can also be a fire hazard in a lab.
3. In a large or a medium sized lab a central generator supply with UPSs for individual instruments can be a good combination to tackle power breakdowns. Refrigerators used for storage of reagents/kits should also be put on generator supply. The generator used should have the capacity well in excess of the total power load. It may be advisable to keep the high power consuming equipment like air conditioners and water baths etc. off the generator supply.

