Polymerase Chain Reaction (PCR)

2- PCR Lab setup

Prof. Dr. Hamdy M. El-Aref

Assiut University, Faculty of Agriculture

Genetics Department

Organization of PCR lab.

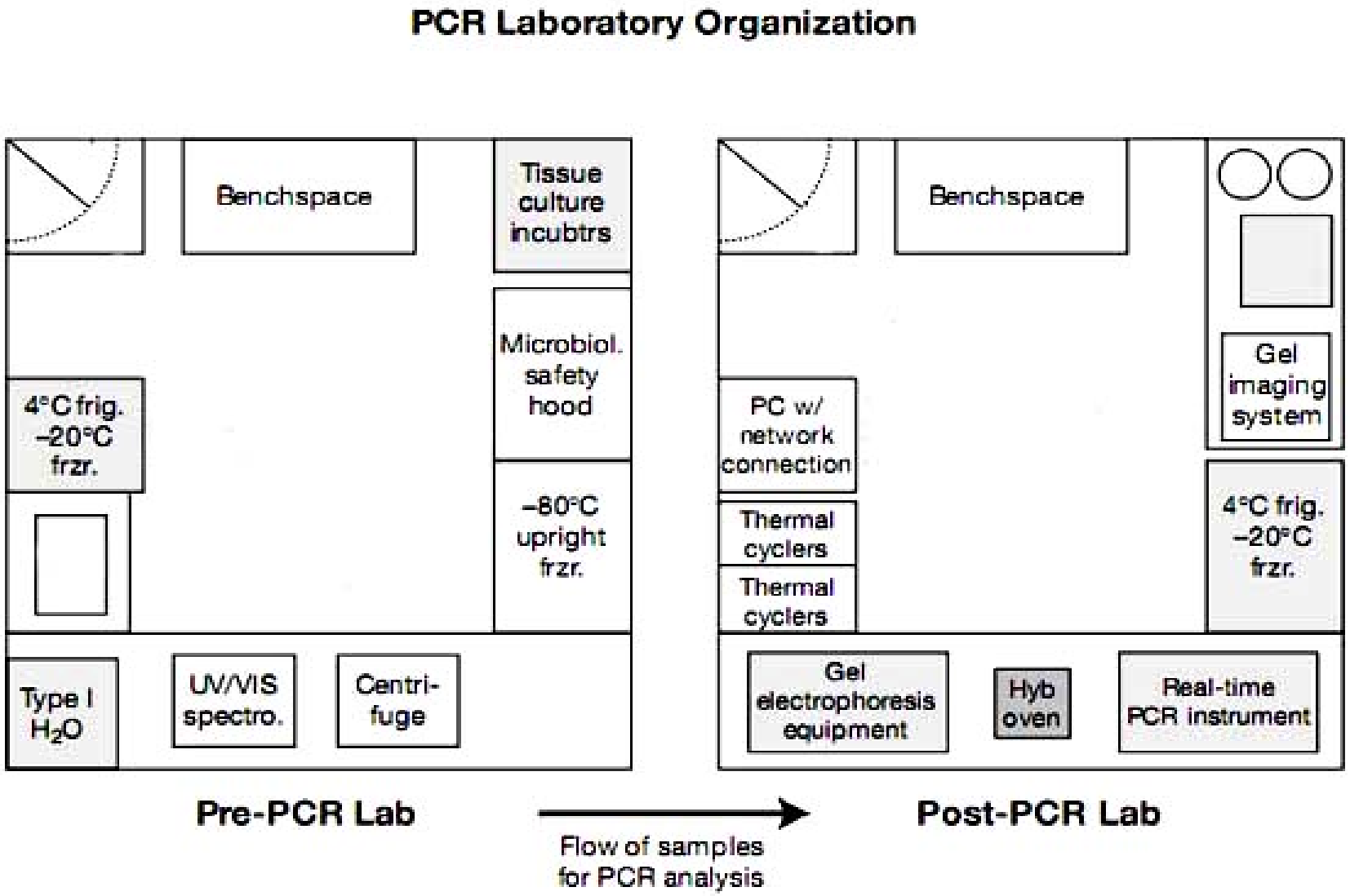

FIGURE 2. Organization of a PCR laboratory with separate pre- and post-PCR rooms.

Setting up PCR technique

Materials:

- **Template (Sample) DNA**

  0.01 - 1.0 ng for plasmid or phage DNA

  0.10 - 1.0 µg for genomic DNA,

  for a total reaction mixture of 50 µl.

- **PCR Mastermix**

PCR buffer: (50mM KCl, 10mM Tris-HCl, pH 8.3)

25mM $MgCl_2$

2mM dNTPs mix.

***Taq* Polymerase.**

Primers. (Primer I, Primer II)

ddH_2O

Materials: Cont.

- 20 ul micro-pipette
- Pipette tips
- 1.5 ml Eppendorf tubes
- Marker Pen
- Ice box

Procedure:

1- Label your tube

2- In 1.5 ml Eppendorf tube add the master mix containing water, PCR buffer, dNTPs, primers, and Taq DNA Polymerase.

3- Then add MgCl2 and template DNA solutions.

Components of PCR for one PCR reactions

Component	Amount of one PCR reaction
ddH2O	11.0 µl
10X PCR reaction buffer	3.0 µl
2mM dNTP's mix	3.0 µl
Primer I	1.0 µl
Primer II	1.0 µl
Taq DNA polymerase	1.0 µl
25mM $MgCl_2$	4.0 µl
Template DNA	1.0 µl
Total volume	25.0 µl

Procedure: Cont.

4- Mix by pipetting up and down 2-3x.
Cap the PCR tube tightly.

5- Take 25 ul of the PCR reaction mix and add to your PCR tube.

6- Load your samples in the PCR machine

PCR (Thermal Cycler) Program:

Cycle	Step	Function	Temperature	Time
1	Step1 Repeat 1	Pre-denaturation	94° C	2 minutes
2	Step 1 Step 2 Step 3 Repeat 40	Denature Anneal Extend	94° C 60° C 72° C	1 minute 1 minute 2 minutes
3	Step 1 Repeat 1	Final extention	72° C	10 minutes

- **Hold at 4°C until use**

Initial Denaturation Step.

- Needed for complete denaturation of the DNA template.
- **Incomplete denaturation of DNA results in the inefficient utilization of template in the first amplification cycle and in a poor yield of PCR product.**
- The initial denaturation should be performed for 2-3 min at 95°C if the GC content is 50% or less.
- **It should be extended up to 10 min for GC-rich templates.**

Denaturation Step.

- Usually denaturation for 0.5-2 min at 94-95°C is sufficient, since the PCR product is significantly shorter than the template DNA.

Primer Annealing Step.

- Optimal annealing temperature is 5°C lower than the melting temperature of primer.
- Incubation for 0.5-2 min is usually sufficient.

Extension Step.

- Usually the extending step is performed at 72°C for 1 – 3 min.
- The rate of DNA synthesis by *Taq* DNA Polymerase is 1 kb/min.

No. of Cycles.

- **Depends on :**
  1- the amount of template DNA in the reaction mix
  2- the expected yield of the PCR product.

- For less than 10 copies of template DNA, 40 cycles should be performed.

- If the initial quantity of template DNA is higher, 25-35 cycles are usually sufficient.

Final Extension Step.

- Performed at 72°C for 5-15 min.
- to fill-in the protruding ends of newly synthesized PCR products.
- Also, the terminal transferase activity of *Taq* DNA Polymerase adds extra A nucleotides to the 3'-ends of PCR products.
- Therefore, if PCR fragments are to be cloned into T/A vectors, this step can be prolonged to up to 30 min


Thank you
Prof. Dr. Hamdy El-Aref