MOLECULAR BIOLOGY RESEARCH UNIT

SETTING UP A PCR LABORATORY

By

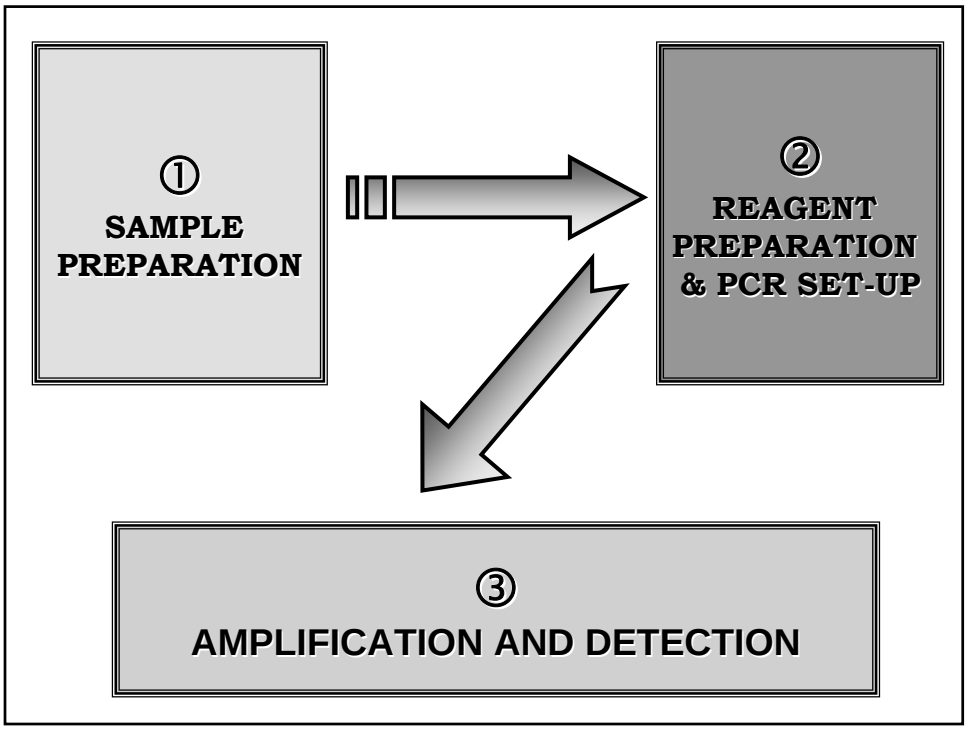
Prof. Dr. Asmaa Hussein

Prof. of Zoonoses & Director of the MBRU

Assiut University

The PCR **laboratory should consist of three distinct work areas. In order to avoid the contamination problems, each area should be dedicated to a single procedure.**

Specimen preparation occurs in the first area, reagent preparation and PCR set-up in the second area, and amplification and detection in the third area.

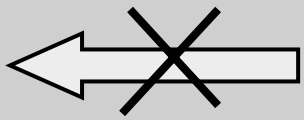


AREA 1:SAMPLE PREPARATION:

- **Positive-displacement pipettes or pipettors with aerosol-resistant tips.**
- **Gloves & laboratory coat.**
- **Refrigerator, freezer, water bath or dry – heat block laminar flow biosafety cabinet.**
- **Cell lysis reagents.**

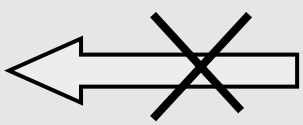
AREA 2:REAGENT PREPARTION & PCR SET-UP:

- Amplification reagents & supplies.
- Positive-displacement pipettes or pipettors with aerosol-resistant tips.
- Laminar-flow biosafety cabinet or dead air box.
- Gloves & laboratory coat.
- Refrigerator & freezer.
- Water bath or dry –heat block.



AREA 3: AMPLIFICATION & DETECTION:

- Thermal cycler.
- Pipettors with aerosol-resistant tips.
- Detection equipment (electrophoresis unit, incubator, plate washer, plate reader, water bath).
- Refrigerator & freezer.
- Reagents and supplies for detection.



<u>The following practices will diminish the potential for contamination:</u>

☑ Each area should have dedicated supplies and reagents.

☑ Color coding of reagents and supplies identifies those that belong to a particular area.

☑ Reagents, supplies and equipment should never be taken from one area to another, three sets of pipettors are therefore essential.

☑ The workflow must be unidirectional from "clean" (pre-PCR) to "dirty" (post-PCR).

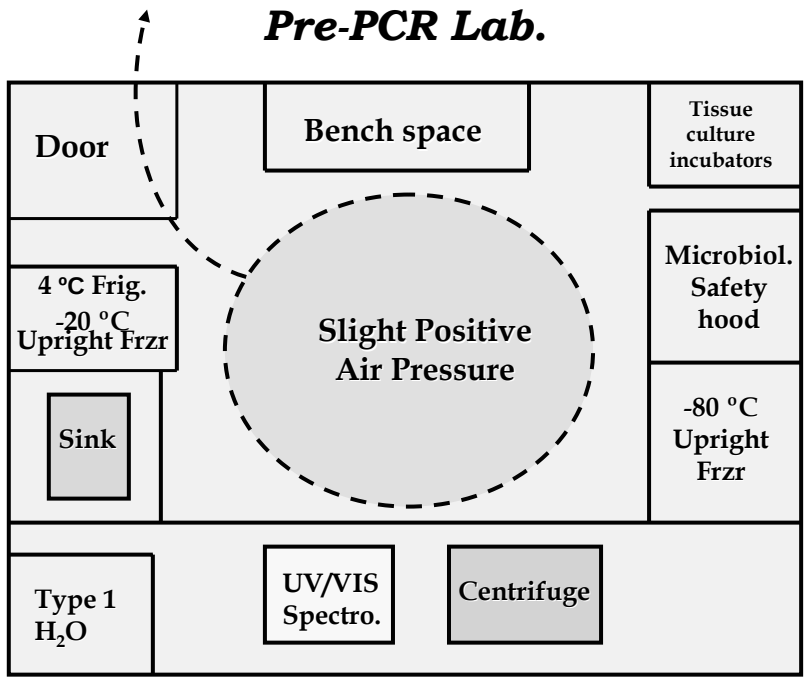
☑ Dedicated labcoats and gloves should be worn at each work site; when moving to a new area, workers should put on new gloves and labcoats.

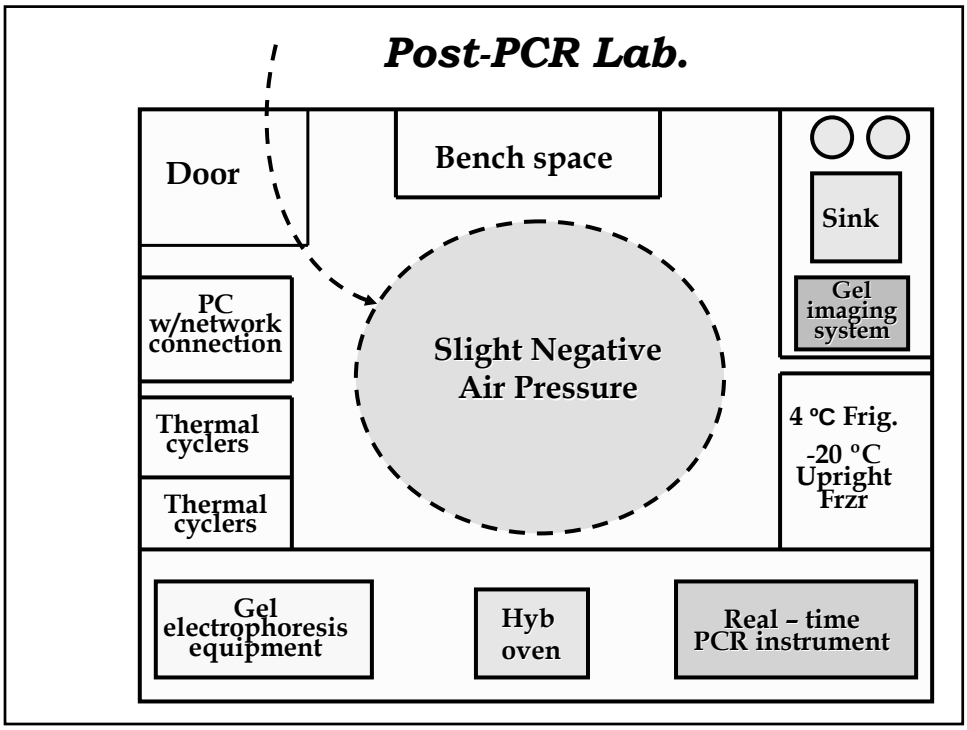
PCR LABORATORY ORGANIZAITON

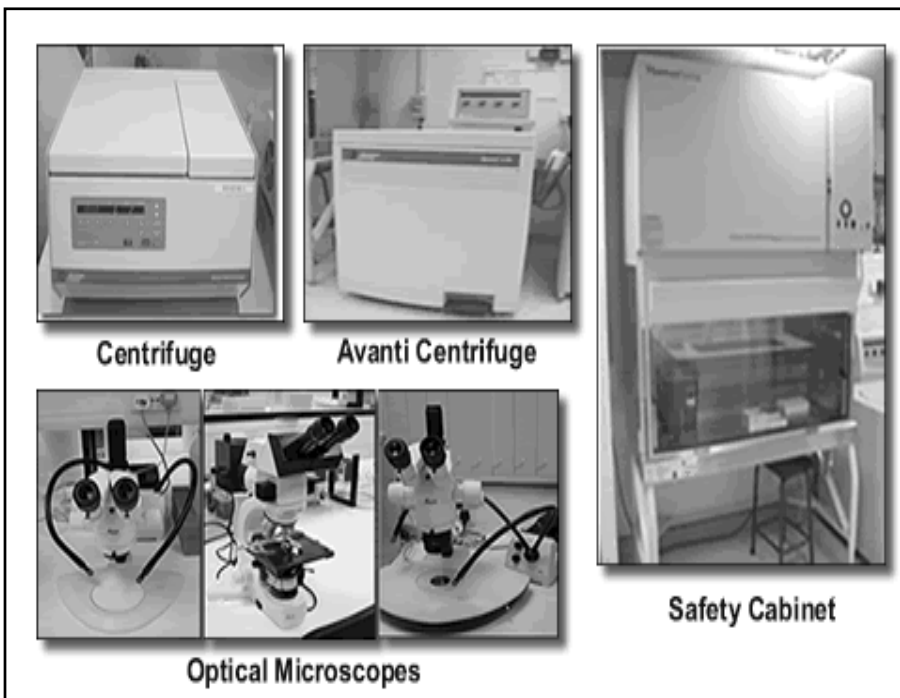
Flow of samples for PCR analysis

PRE-PCR LAB → **POST-PCR LAB**

Pre-PCR **is the protocols and equipment required for the isolation of nucleic acid and the assembly of the reaction to amplify the samples**



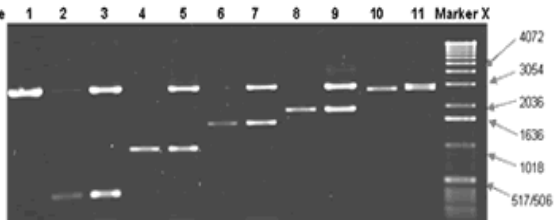

Post-PCR Lab.
Door
Bench space
Sink
Gel imaging system
PC w/network connection
Slight Negative Air Pressure
Thermal cyclers
Thermal cyclers
4 °C Frig.
-20 °C Upright Frzr
Gel electrophoresis equipment
Hyb oven
Real – time PCR instrument


Centrifuge
Avanti Centrifuge
Safety Cabinet
Optical Microscopes

Gel tank (to place the gel mold and run electrophoresis)

Gel documentation (capture and print the image of agarose gel)

UV transluminator (basic device for visualizing fluorescence-stained gels)



Example of an image captured by gel documentation and UV transluminator of the gel agarose electrophoresis

Thermal Cycler

PCR microcentrifuge tubes (0.5µl)




The main forensic research laboratory contains all equipment needed to conduct lab members' experiments. There are centrifuges, autoclave, digital gel photography, microfuges, PCR hoods, fume hoods, water purification, computers, etc.



The PCR and genetic analysis laboratory features ABI 310 and Beckman CEQ 8000 genetic analyzers, electrophoresis equipment, digital photography, and multiple thermo-cyclers.

Strict adherence to proper laboratory technique:

- **Physically isolate PCR preparations and products**
- **Autoclave solution.**
- **Aliquot reagents.**
- **Use disposable gloves and change gloves often during set-up**
- **Avoid splashes.**

- **Use positive-displacement pipettes or aerosol resistant tips on air-displacement pipettes**
- **" Premix" reagents.**
- **Add DNA last.**
- **Choose positive and negative controls carefully.**

Thank you for your attention!