# SETTING UP A PCR LAB

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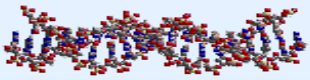
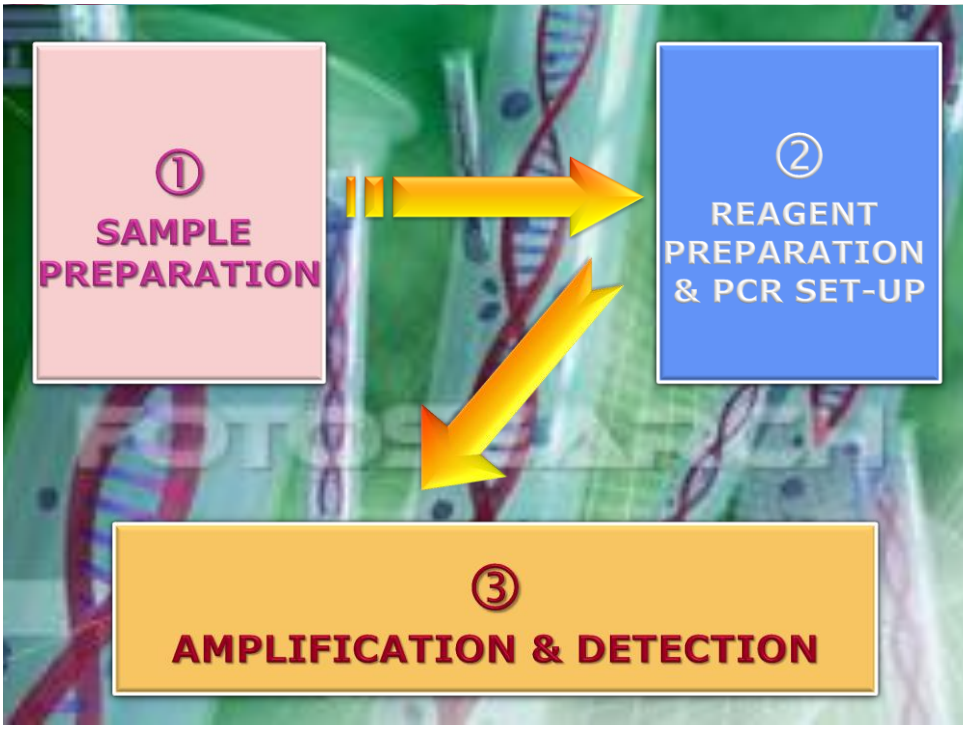
**PCR lab should consist of 3 distinct work areas**

**In order to avoid the contamination problems, each area should be dedicated to a single procedure**

- **First area** → **Specimen preparation**
- **Second area** → **Reagent preparation & PCR set-up**
- **Third area** → **Amplification & detection**



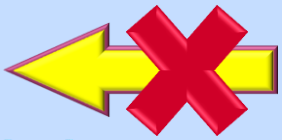



## AREA 1:SAMPLE PREPARATION:

- 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 & supplies for detection

## The following practices will diminish the potential for contamination:

- Each area should have dedicated supplies & reagents
- Color coding of reagents & supplies identifies those that belong to a particular area
- Reagents, supplies & equipment should never be taken from one area to another, three sets of pipettors are essential

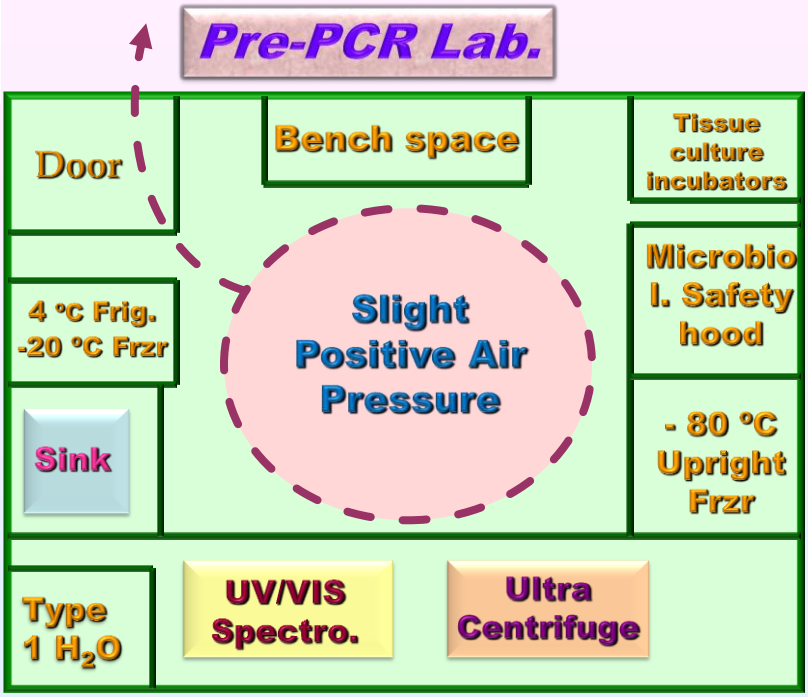
- The workflow must be unidirectional from "clean" (pre-PCR) to "dirty" (post-PCR)
- Dedicated labcoats & gloves should be worn at each work site
- When moving to a new area, workers should put on new gloves & labcoats

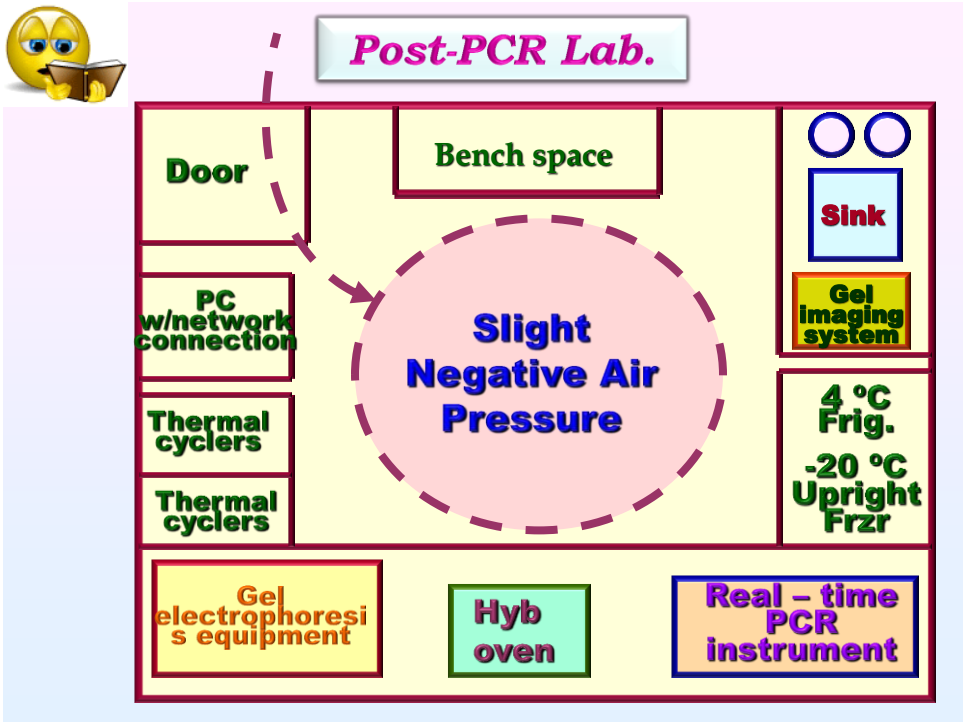
# PCR LABORATORY ORGANIZAITON

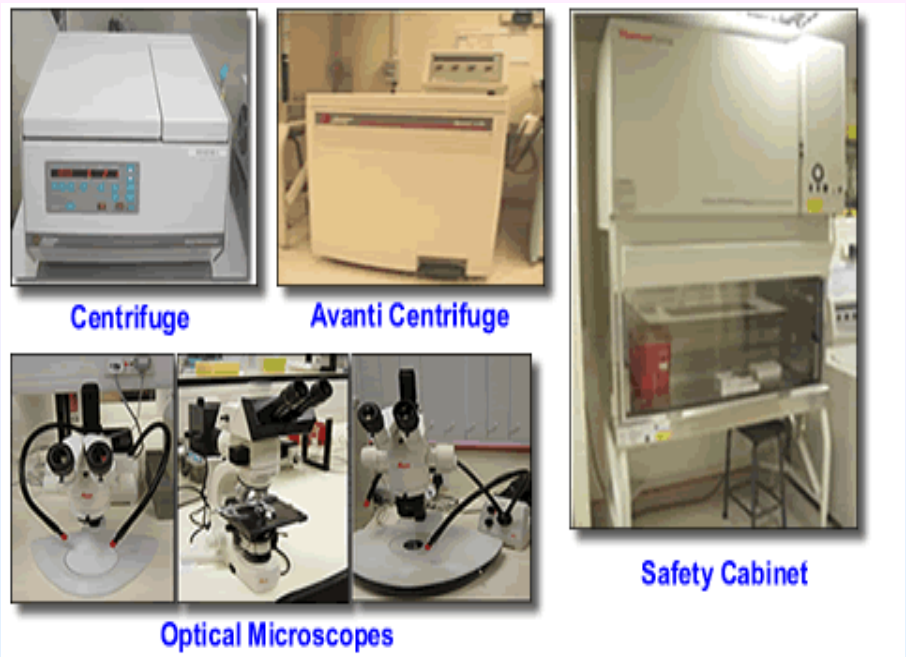
Flow of samples
for PCR analysis

PRE-PCR LAB → POST-PCR LAB

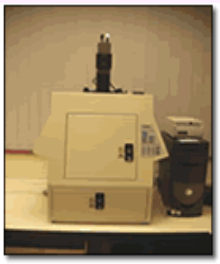
***Pre-PCR*** **is the protocols & equipment required for the isolation of nucleic acid & assembly of the reaction to amplify the samples**




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


Centrifuge
Avanti Centrifuge
Safety Cabinet
Optical Microscopes

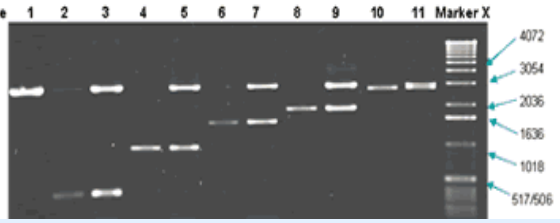
Gel tank (to place the gel mold and run electrophoresis)



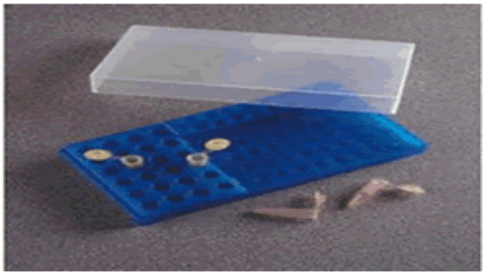
Gel documentation (capture and print the image of agarose gel)

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

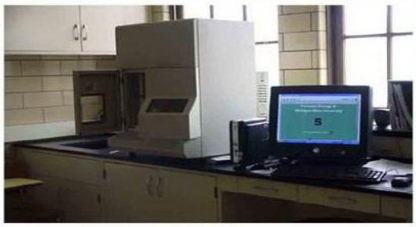



Thermal Cycler



PCR microcentrifuge tubes (0.5µl)

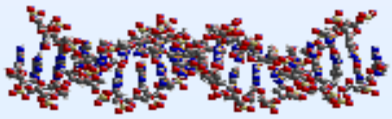


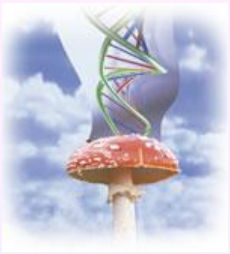




## Strict adherence to proper laboratory technique:

- Physically isolate PCR preparations & products
- Autoclave solution
- Aliquot reagents
- Use disposable gloves & 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 & negative controls carefully





*Thank you for your attention!*