SETTING UP A PCR LABORATORY

كيفية تأسيس معمل بيولوجيا جزيئية

By

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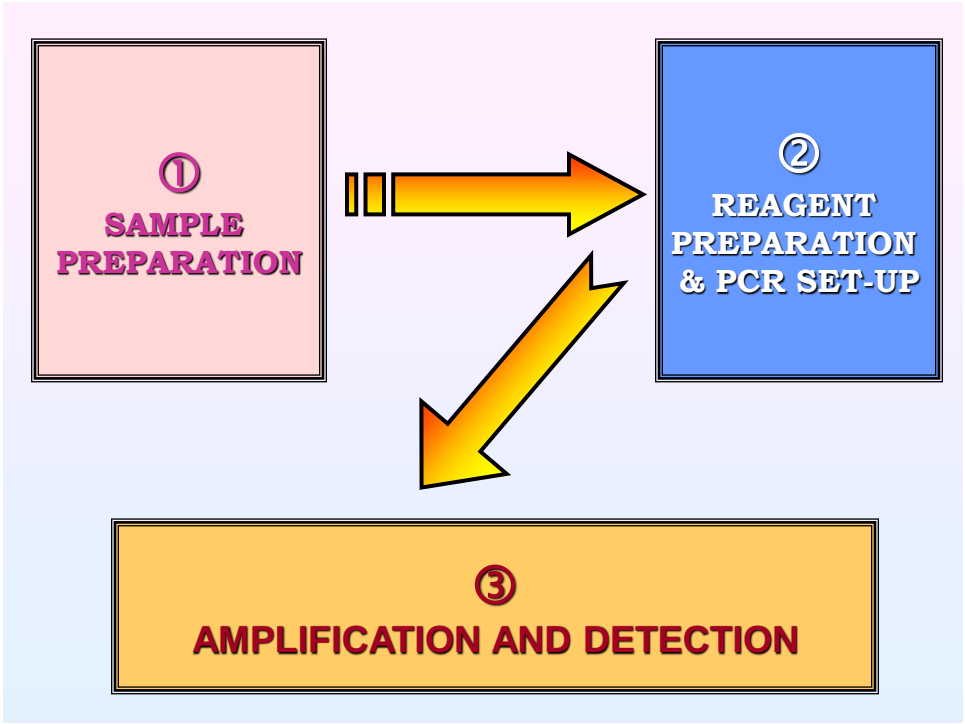
The PCR **laboratory should consist of three distinct work areas. In order to avoid the contamination problems, each area should be dedicated to a single procedure**

First area ⇒ **Specimen preparation occurs**

Second area ⇒ **Reagent preparation & PCR set-up**

Third area ⇒ **Amplification & detection**

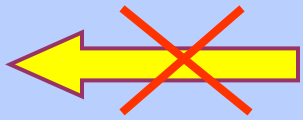


AREA 1:SAMPLE PREPARATION:

- 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



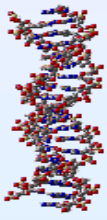
AREA 3: AMPLIFICATION & DETECTION:

- ☑ 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 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**



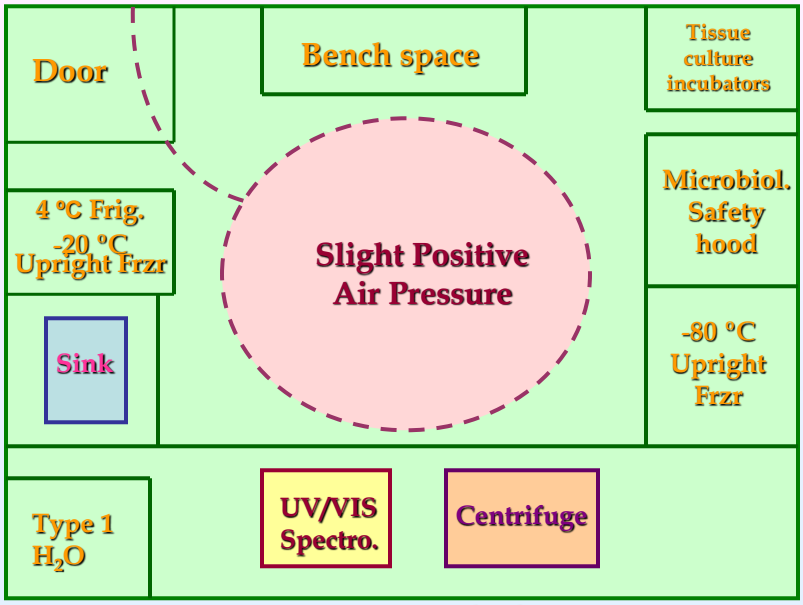
PCR LABORATORY ORGANIZAITON

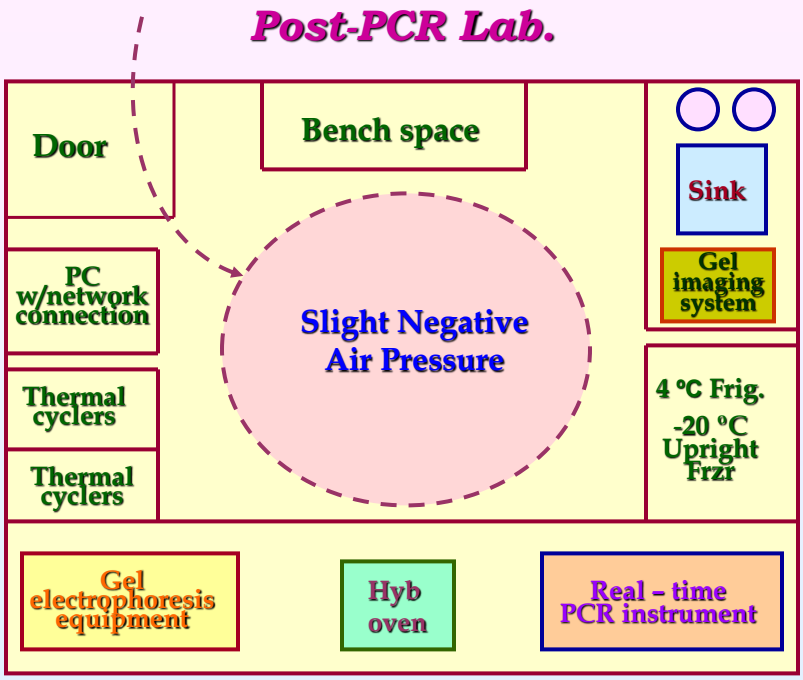
Flow of samples for PCR analysis

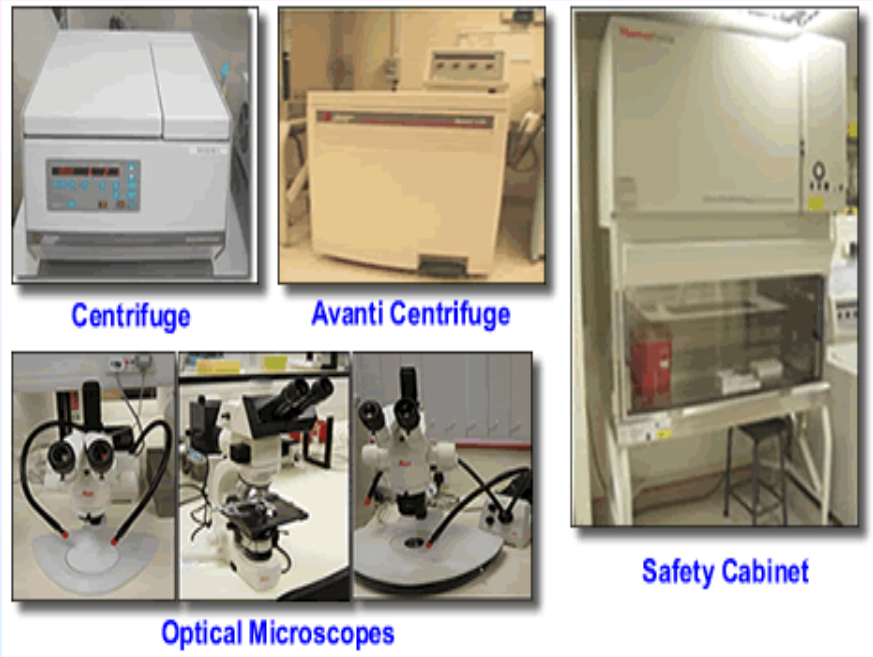
PRE-PCR LAB → **POST-PCR LAB**

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

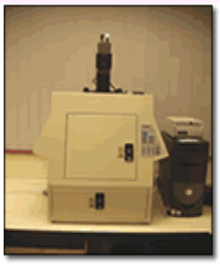
Pre-PCR Lab.





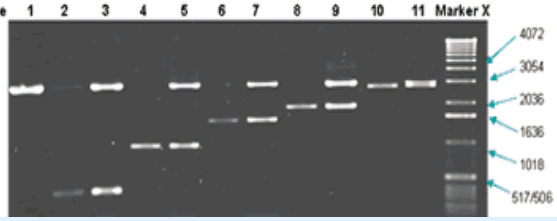


Gel tank (to place the gel mold and run electrophoresis)



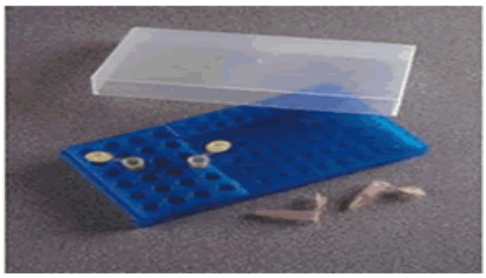
Gel documentation (capture and print the image of agarose gel)

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



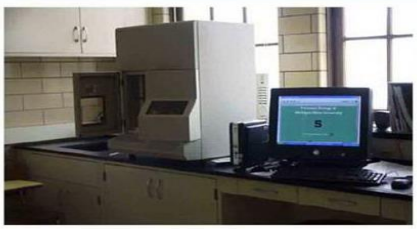
Example of an image captured by gel documentation and UV transluminator of the gel agarose electrophoresis

Thermal Cycler



PCR microcentrifuge tubes (0.5µl)

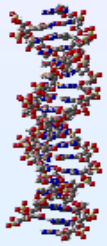



Strict adherence to proper laboratory technique:

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



Thank you for your attention!