SETTING UP A PCR LAB

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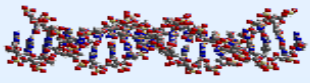
PCR lab should consist of 3 distinct work areas

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



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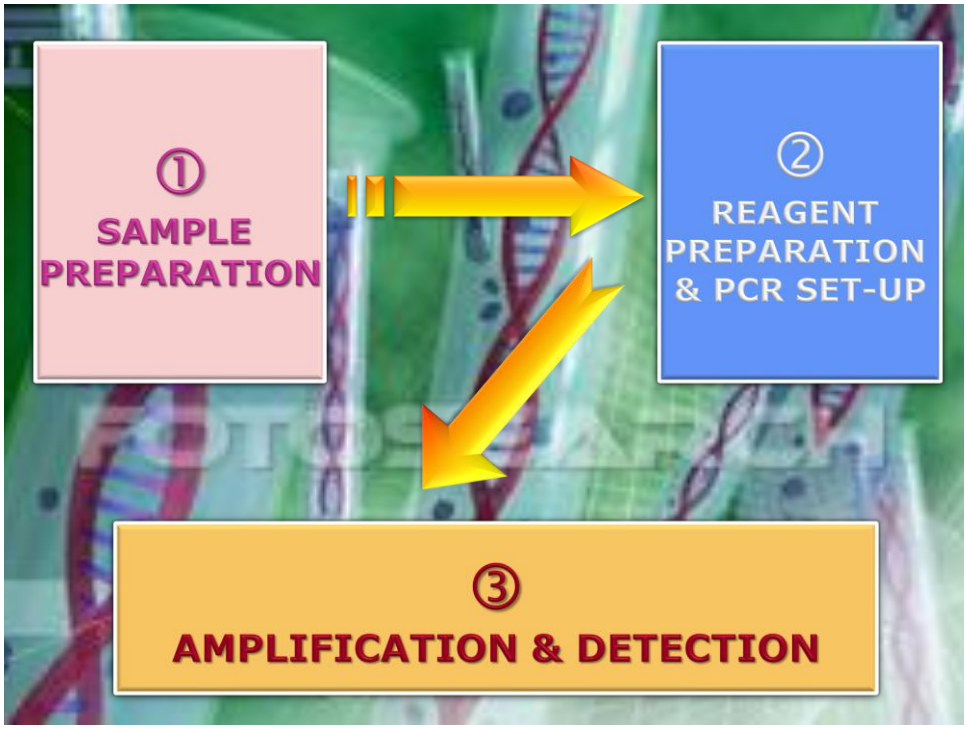

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AREA 1:SAMPLE PREPARATION:

- 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

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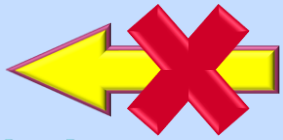
AREA 2: REAGENT PREPARTION & PCR SET-UP:

- 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



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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

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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

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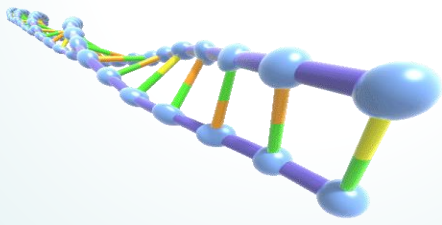
- The workflow must be unidirectional from "clean" (pre-PCR) to "dirty" (post-PCR)
- Dedicated labcoats & gloves should be worn at each work site
- When moving to a new area, workers should put on new gloves & labcoats



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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

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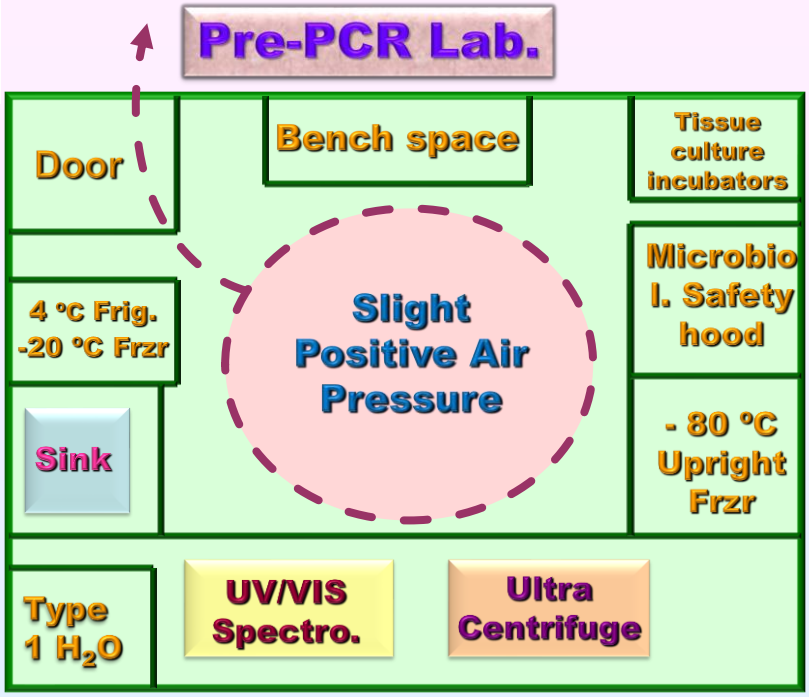

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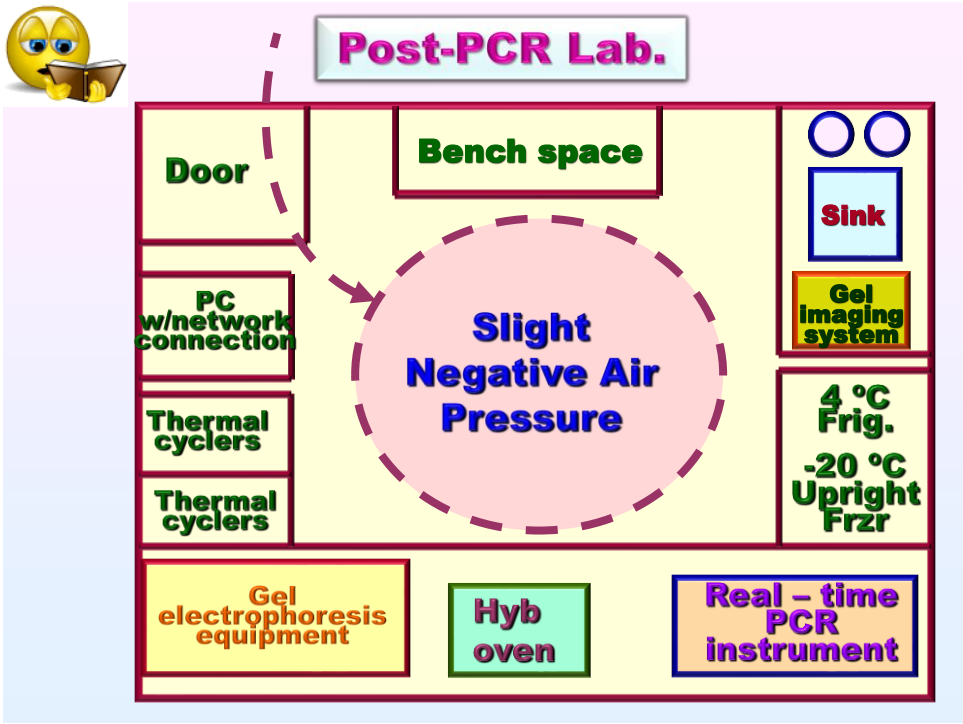
Post-PCR Lab.
Door
Bench space
Sink
Gel imaging system
PC w/network connection
Slight Negative Air Pressure
Thermal cyclers
Thermal cyclers
4 °C Frig.
-20 °C Upright Frzr
Gel electrophoresis equipment
Hyb oven
Real – time PCR instrument

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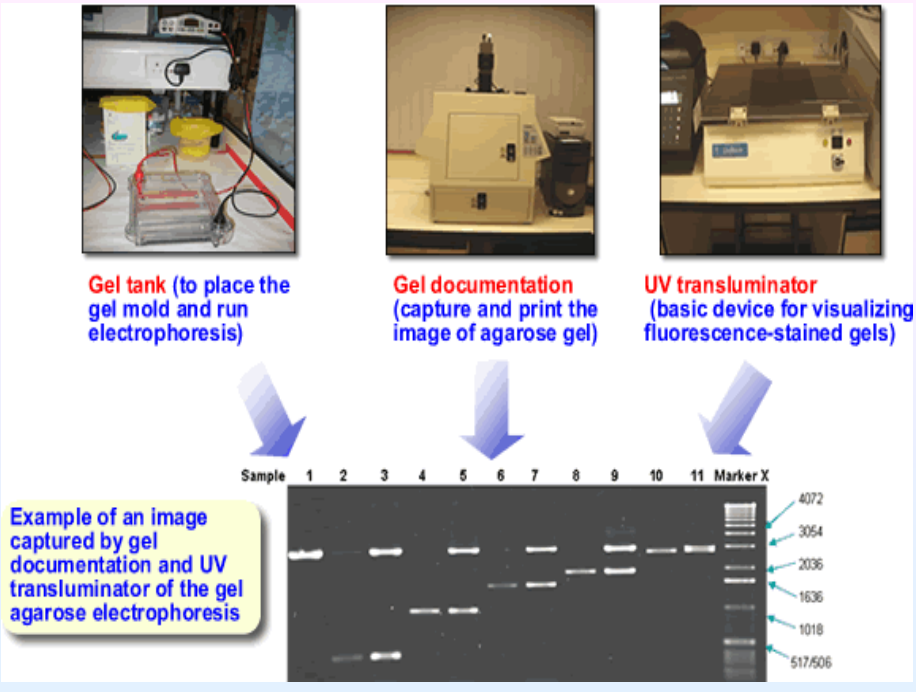
Gel tank (to place the gel mold and run electrophoresis)
Gel documentation (capture and print the image of agarose gel)
UV transluminator (basic device for visualizing fluorescence-stained gels)
Example of an image captured by gel documentation and UV transluminator of the gel agarose electrophoresis
Sample 1 2 3 4 5 6 7 8 9 10 11 Marker X
4072
3054
2036
1636
1018
517/506

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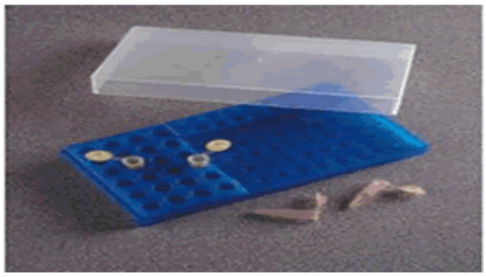
Thermal Cycler



PCR microcentrifuge tubes (0.5µl)

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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

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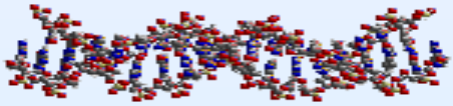
- Use positive-displacement pipettes or aerosol resistant tips on air-displacement pipettes
- " Premix" reagents
- Add DNA last
- Choose positive & negative controls carefully



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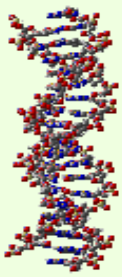

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