Troubleshooting of PCR

By
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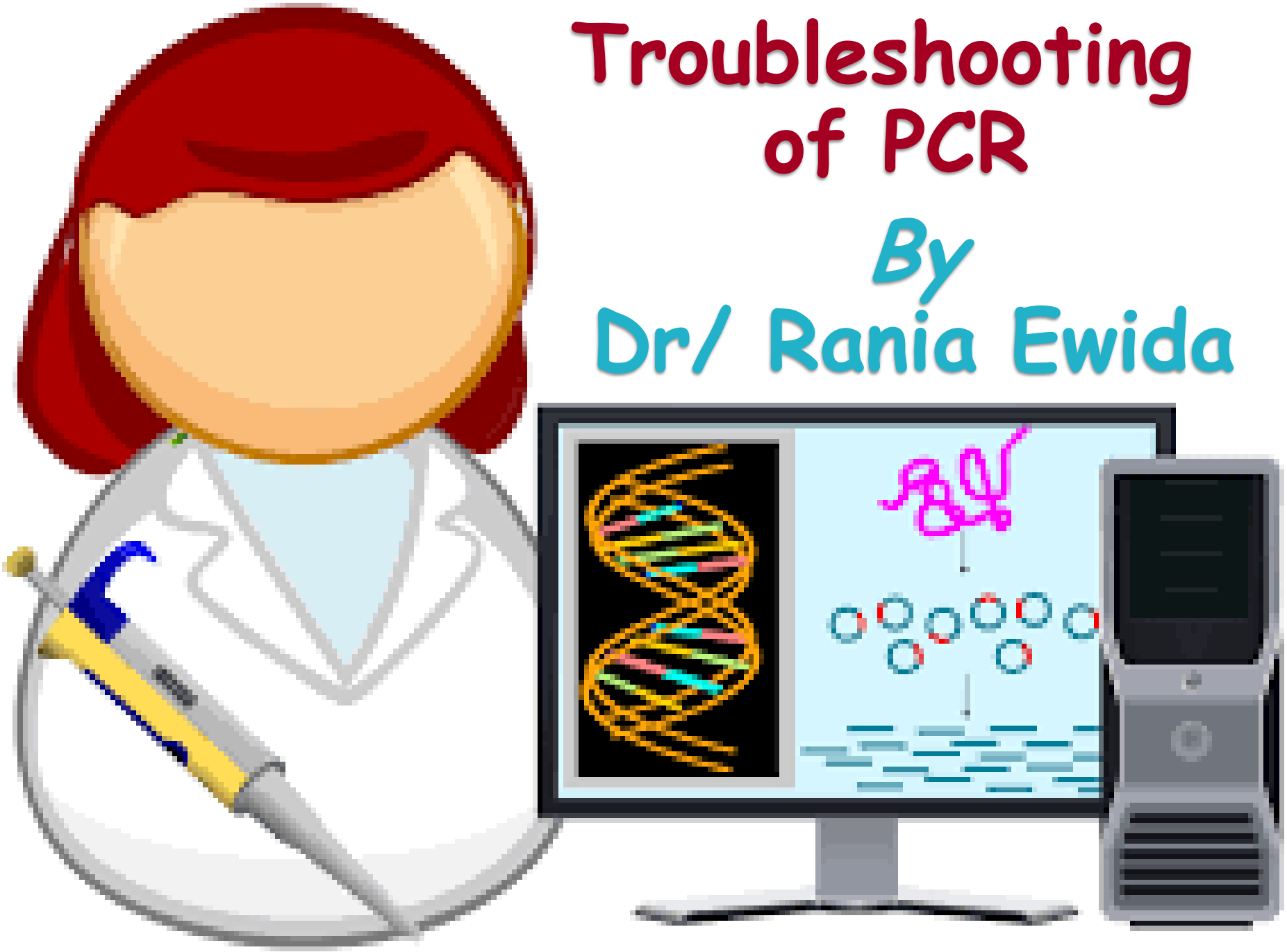
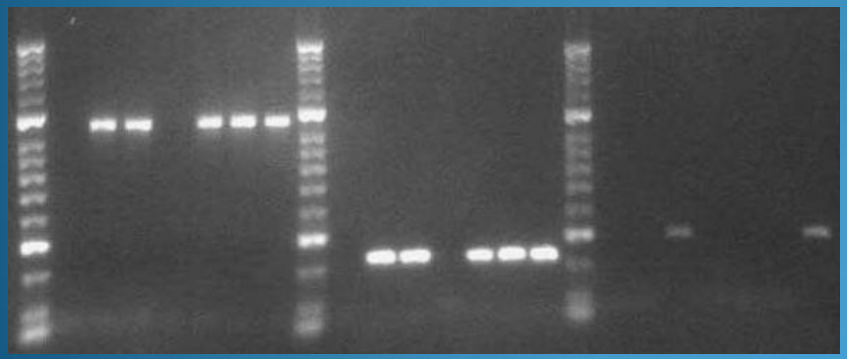
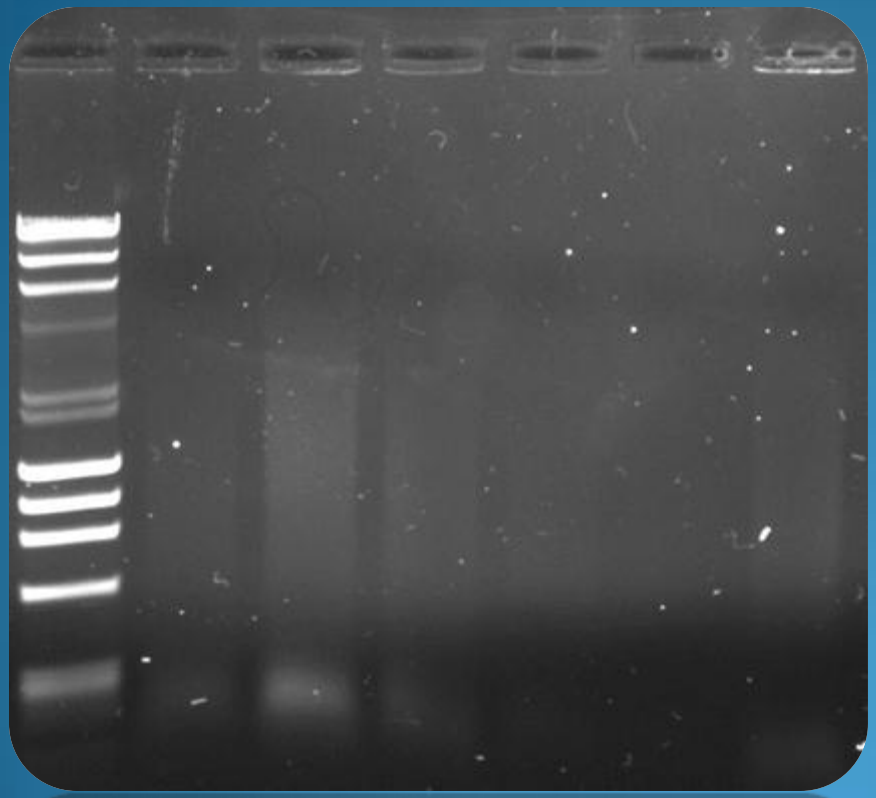



BNA-mid I



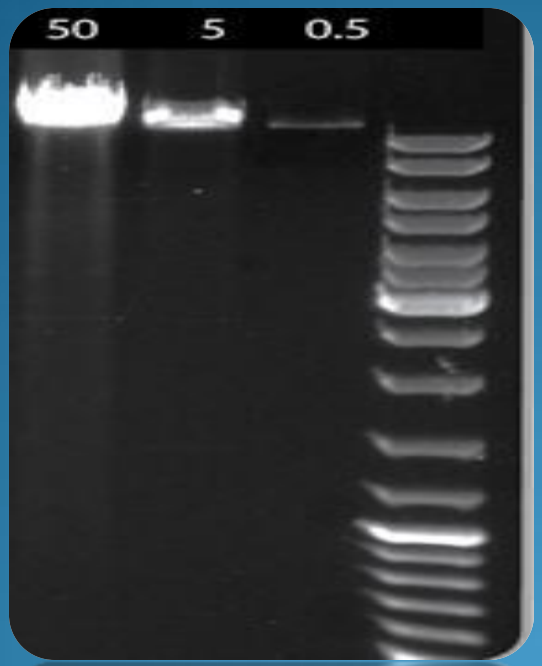
BUT what if you don't…

No products



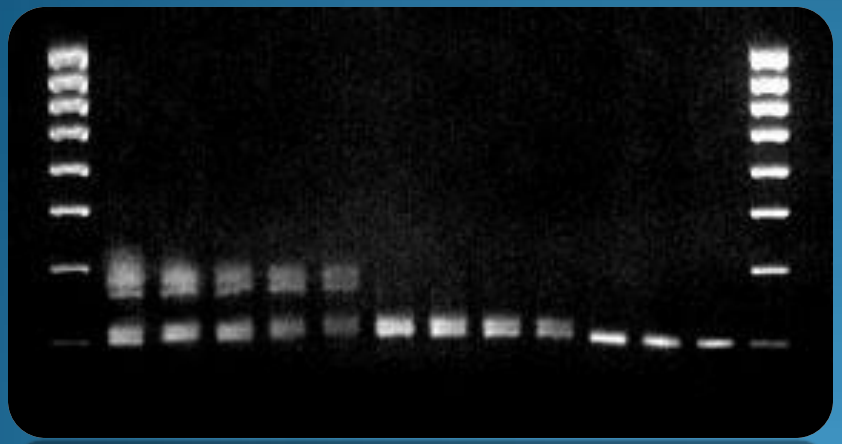
- ✓ No marker
- ✓Marker positive but no product
- ✓No positive control
- ✓Positive control

Low quantity



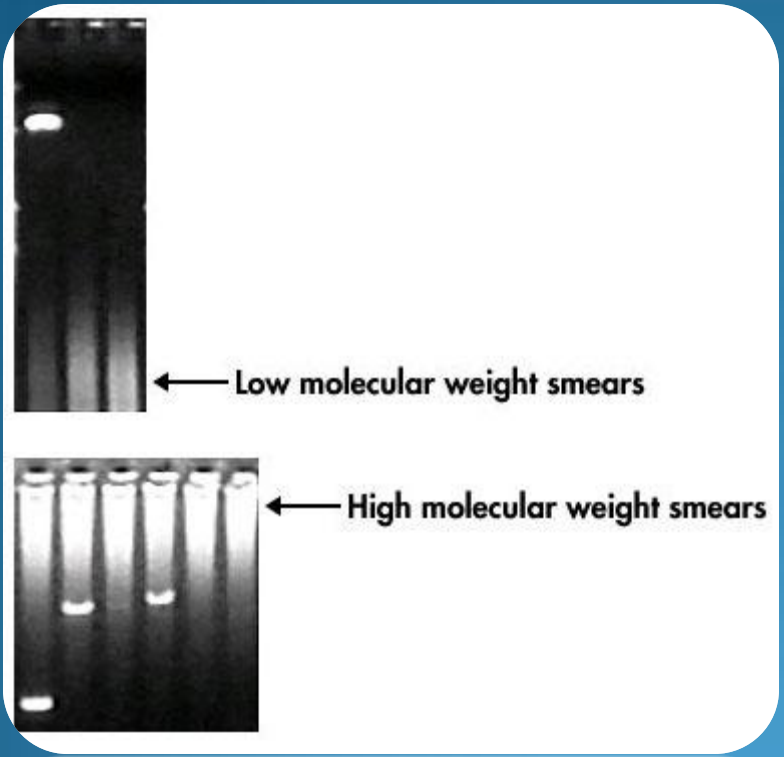
- ✓ DNA amount
- ✓Cycling Number
- ✓Enzyme
- ✓Annealing Temp.
- ✓PCR inhibitors

Non specific bands



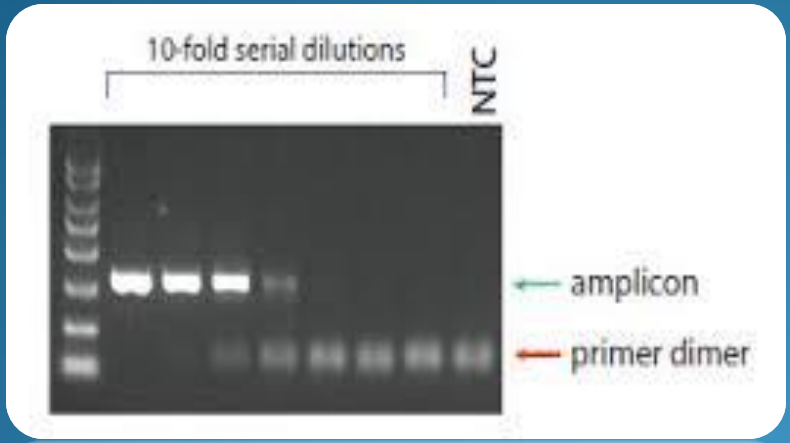
- ✓ Annealing Temp.
- ✓Cycling Number
- ✓Primer
- ✓Contamination
- ✓Hot start
- ✓Using ice

Diffuse smearing



- ✓Conc. $MgCl_2$
- ✓Conc. DNA
- ✓Polymerase

Primer dimers



- ✓Design
- ✓Conc.

PCR Inhibitors

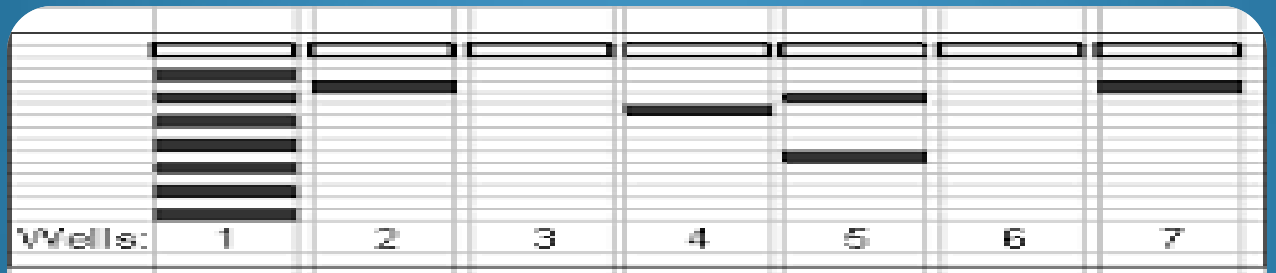
- ✓Detergent
- ✓Phenol
- ✓Heparin
- ✓ Urine
- ✓Lipids

Basic Experimental Design

Blank reaction

Negative control

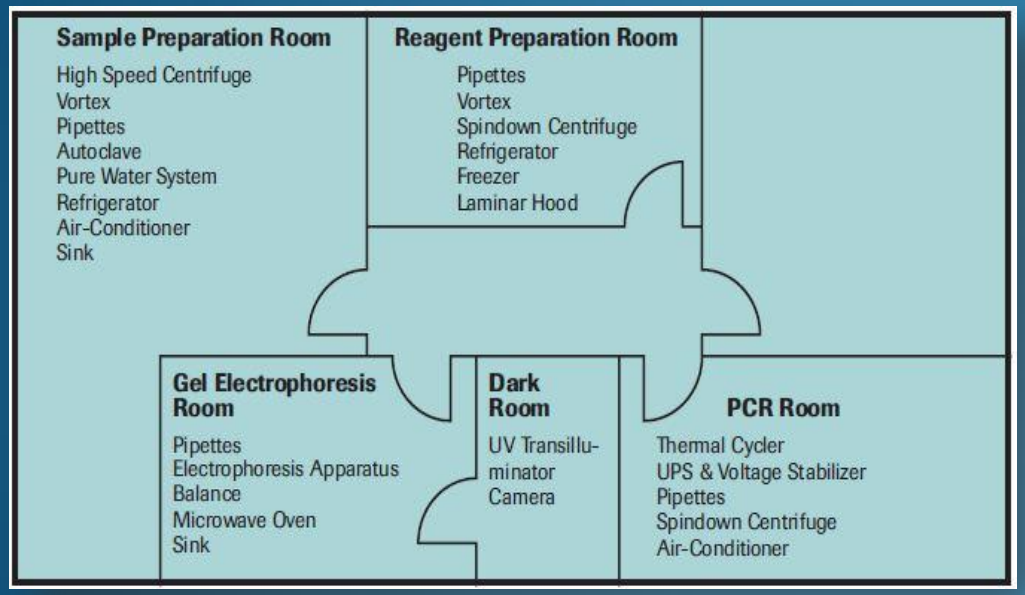
Positive control

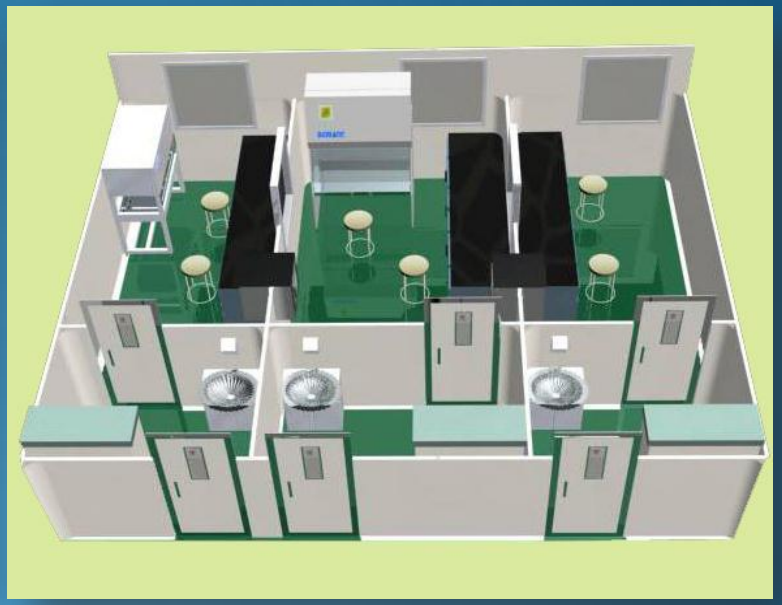


Well 1:	DNA Marker
Well 2:	Positive Control (Bacteria)
Well 3:	Negative Control (Water Only)
Well 4:	Student 1 Results
Well 5:	Student 2 Results
Well 6:	Student 3 Results
Well 7:	Student 4 Results

Avoid contamination

- DNA sample preparation, reaction mixture assemblage should be performed in separate areas. i.e. Separate pre and post- PCR facilities.





Avoid contamination

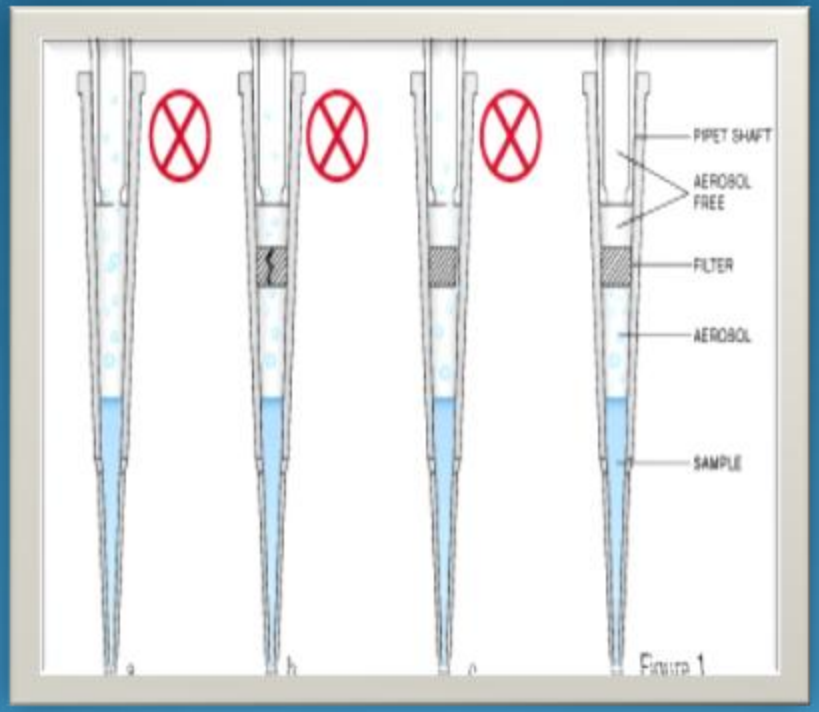
- A Laminar Flow Cabinet with a UV lamp is recommended for preparing the reaction mixture.

Avoid contamination

- New gloves should be used for DNA purification.

Avoid contamination

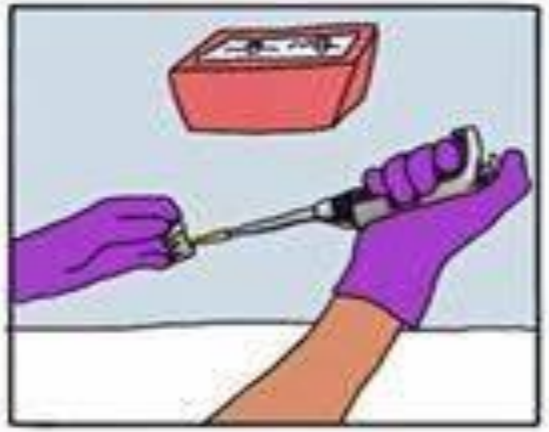
- Using filter tips.



PCR Protocol

Get the reagents

Prepare the mix



Set up conditions

Analyze the gel

Negative result

Cry

Sketching Science


Thank You!