Polymerase Chain Reaction (PCR)

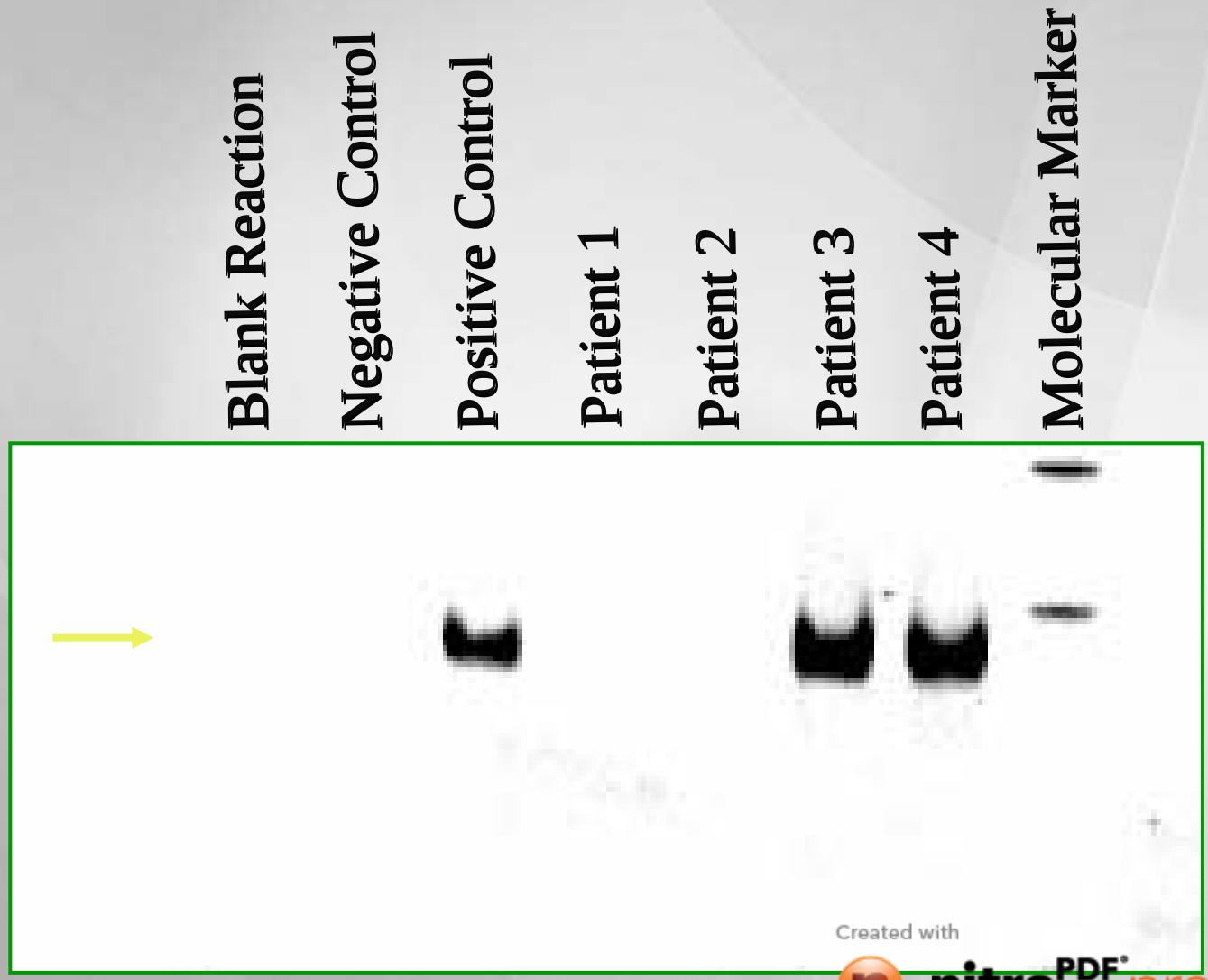
PCR Troubleshooting

Prof. Dr. Hamdy M. El- Aref
Assiut University, Faculty of Agriculture
Genetics Department

Experiment design

- **Blank reaction**
  - **Controls for contamination**
  - **Contains all reagents except DNA template**
- **Negative control reaction**
  - **Controls for specificity of the amplification reaction**
  - **Contains all reagents and a DNA template lacking the target sequence**
- **Positive control reaction**
  - **Controls for sensitivity**
  - **Contains all reagents and a known target-containing DNA template**

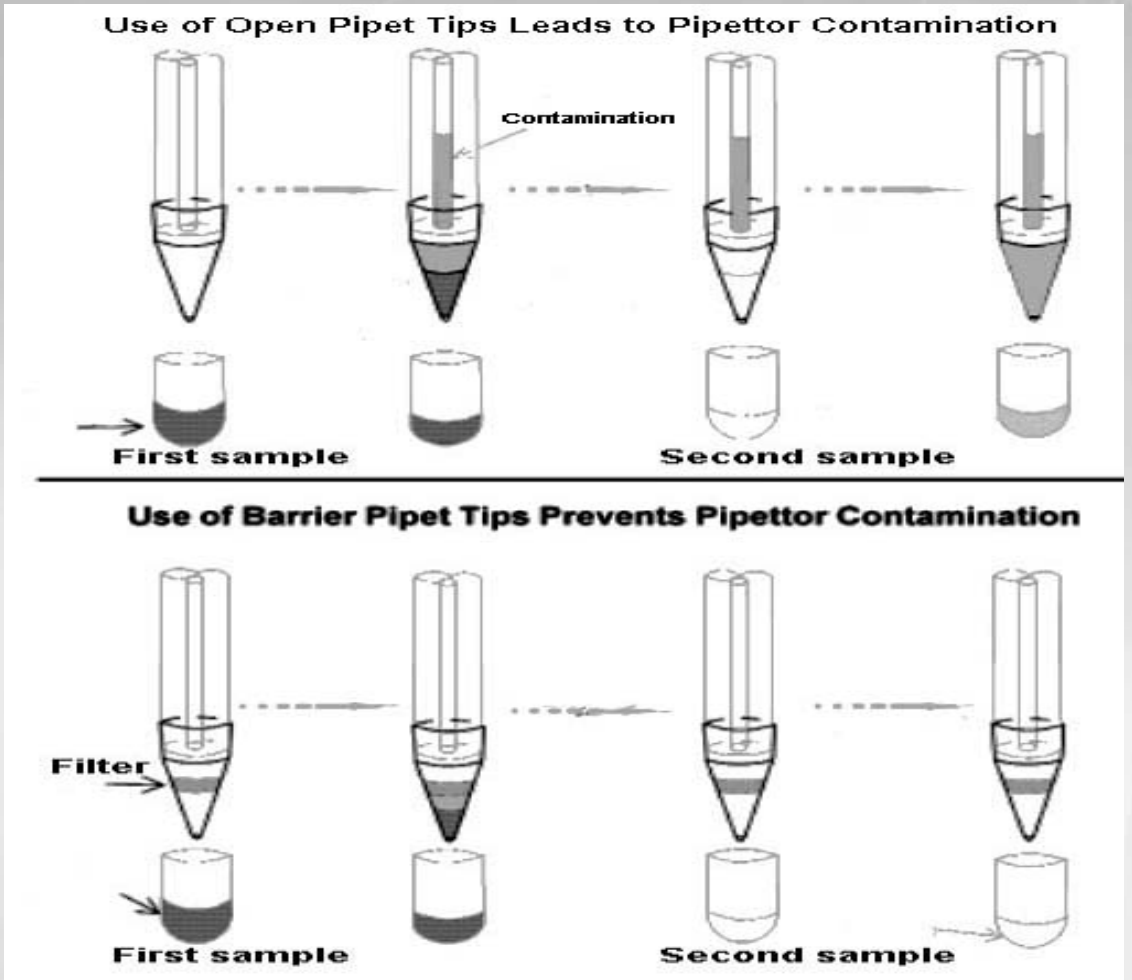
Experimental design



Avoiding Contamination

- DNA sample preparation, reaction mixture assemblage should be performed in separate areas.
  i.e. Separate pre and post PCR facilities.
- A Laminar Flow Cabinet with a UV lamp is recommended for preparing the reaction mixture.
- New gloves should be used for DNA purification.

The use of tips with aerosol filters for both DNA sample and reaction mixture preparation, is strongly recommended.



Autoclaving of all solutions, except dNTPs, primers and *Taq* DNA Polymerase is recommended.

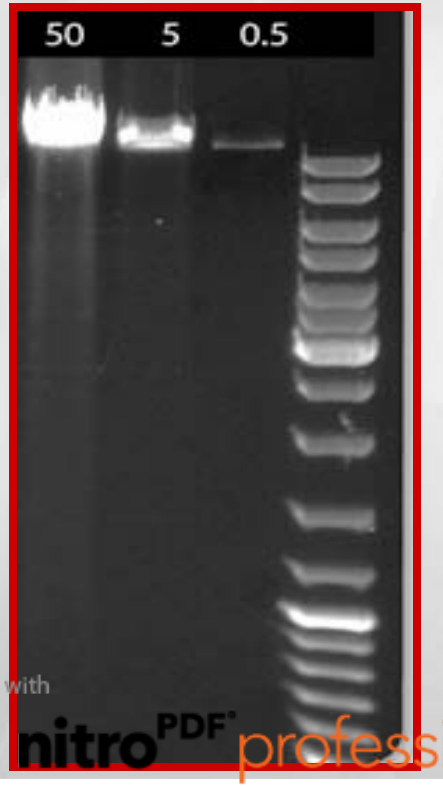
Troubleshooting PCR

- **No PCR product**
  - No marker + product
  - No marker
  - Marker +ve but no product
  - No positive control

  - **Verify that all components were added to the reaction.**
  - **Check pipetors and reagents.**
  - **Check detection method.**
  - **Temp. (annealing / extension)**

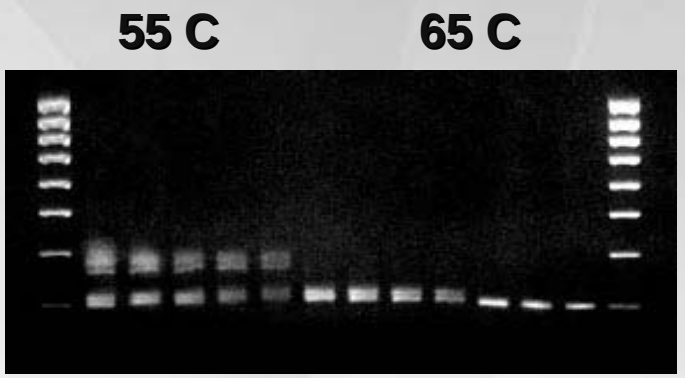
Troubleshooting PCR

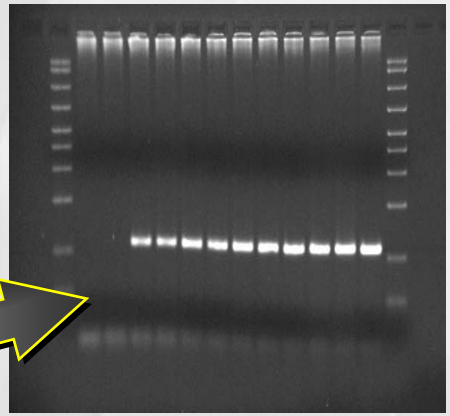
- **Too many bands**
  - Specificity of primers.
  - Annealing temp too low, excessive Mg^{++} or cycles.
- **Low quantity**
  - Increase DNA
  - Increase cycle
  - Decrease annealing temp (2-5)
  - PCR inhibitors
  - Change enzyme



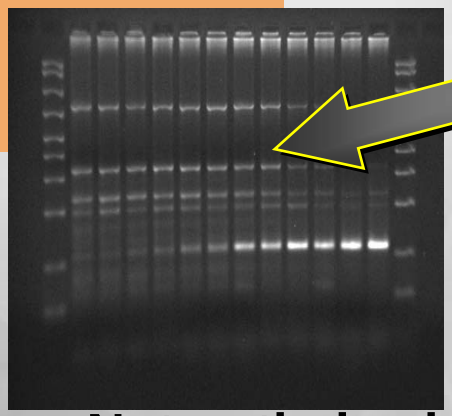
Troubleshooting PCR

- **Non specific bands**
  - **Increase annealing temp**
  - **Contamination**
  - **Primer Conc. & design**
  - **Decrease cycles**
  - **High $MgCl_2$ concentration**
  - **Pre PCR mispriming**
  - **Hot start**





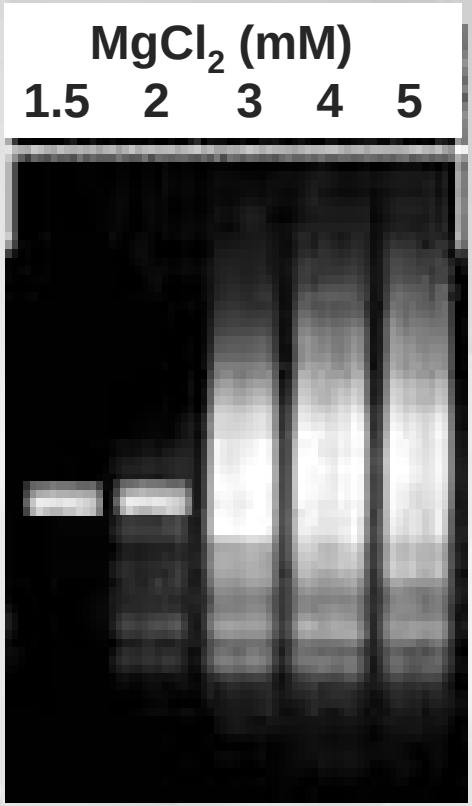
Well optimized



Not optimized

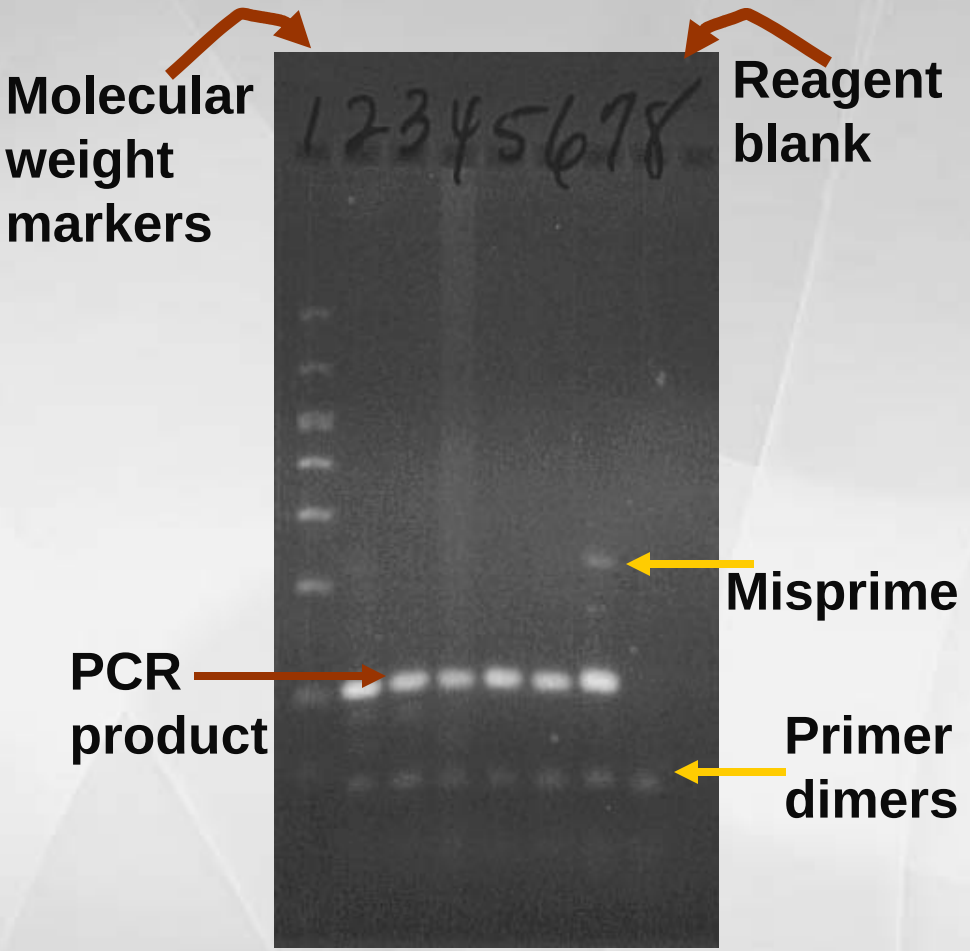
Troubleshooting PCR

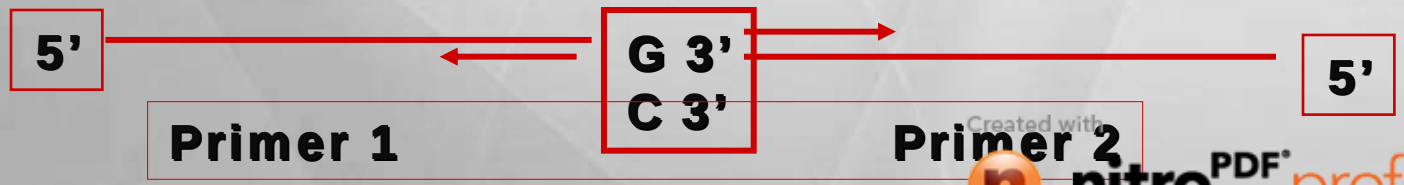
- **Diffuse smearing**
  - DNA degradation
  - Decrease:
  - DNA
  - $MgCl_2$
  - Taq polymerase



Troubleshooting PCR

- **Primer dimers and misprime:**
  - Annealing temp. too low (dimers) or too high (misprime).
  - excess primers
  - Design primers carefully
  - Hot start
  - Size is the sum of two primer lengths.





PCR Inhibitors

- Detergent
- Phenol
- Heparin
- Heme
- Dyes (bromphenol blue)
- urine, paraffin

Common problem during PCR

- **Template DNA:**

  Larger template DNA amounts usually increase the yield of non-specific PCR products.

- **Primers.**

  - The primer should not be self-complementary or complementary to any other primer in the reaction mixture, to prevent primer-dimers and hairpin formation.

MgCl concentration.

* It forms complexes with dNTPs, primers and DNA templates
  - Too few Mg2+ ions result in a low yield of PCR product
  - Too many will increase the yield of non-specific products.

Taq DNA polymerase.

- Higher Taq polymerase concentrations than needed may cause synthesis of non-specific products.

dNTPs.

The concentration of 4 dNTPs (dATP, dCTP, dGTP, dTTP) should be equal in the reaction mixture.