



# PROJECT PRAKASH

Programmed Approach to Knowledge and Sensitization on Hepatitis

## HEPATITIS INDUCTION PROGRAM FOR LAB TECHNICIANS

# Molecular Methods in Viral Hepatitis Testing

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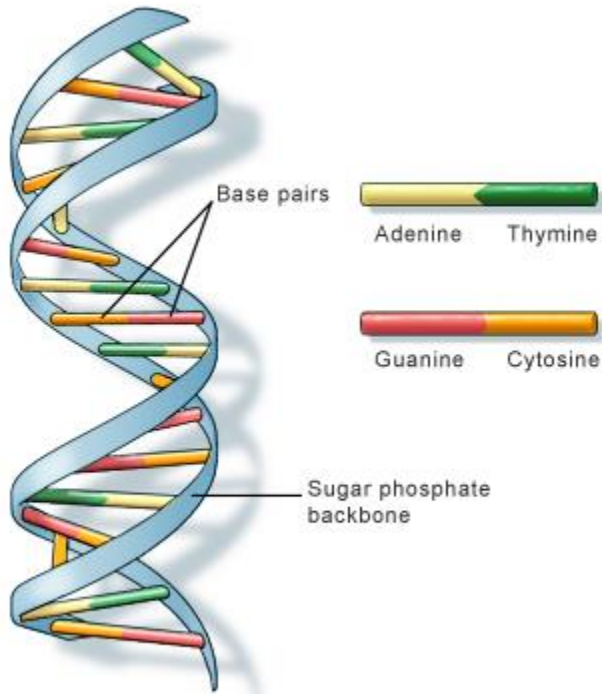
[www.ilbs.in](http://www.ilbs.in)

# INSTITUTE OF LIVER & BILIARY SCIENCES, NEW DELHI

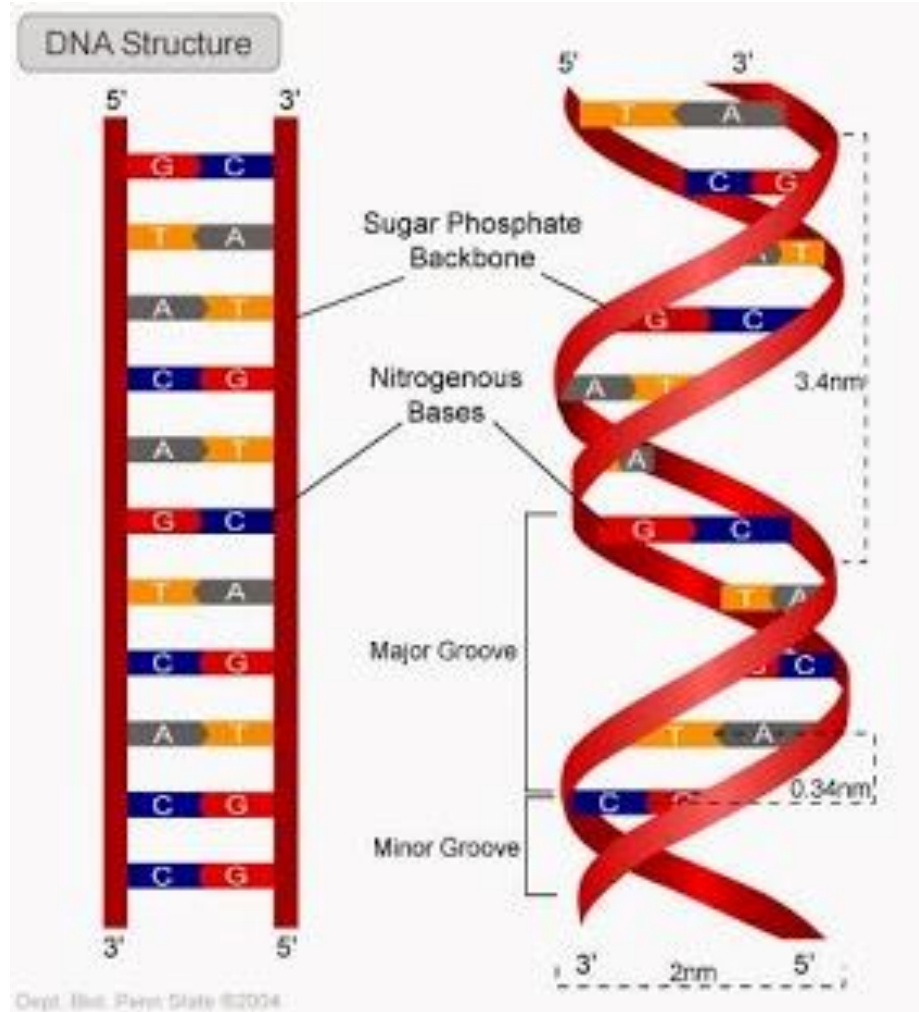




# DNA Structure



U.S. National Library of Medicine

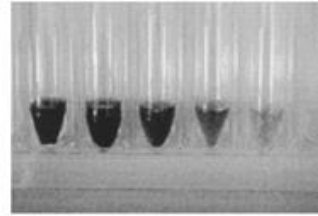




### 1. Sampling



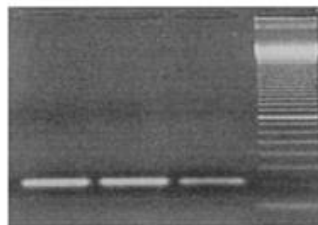
### 2. Sample preparation



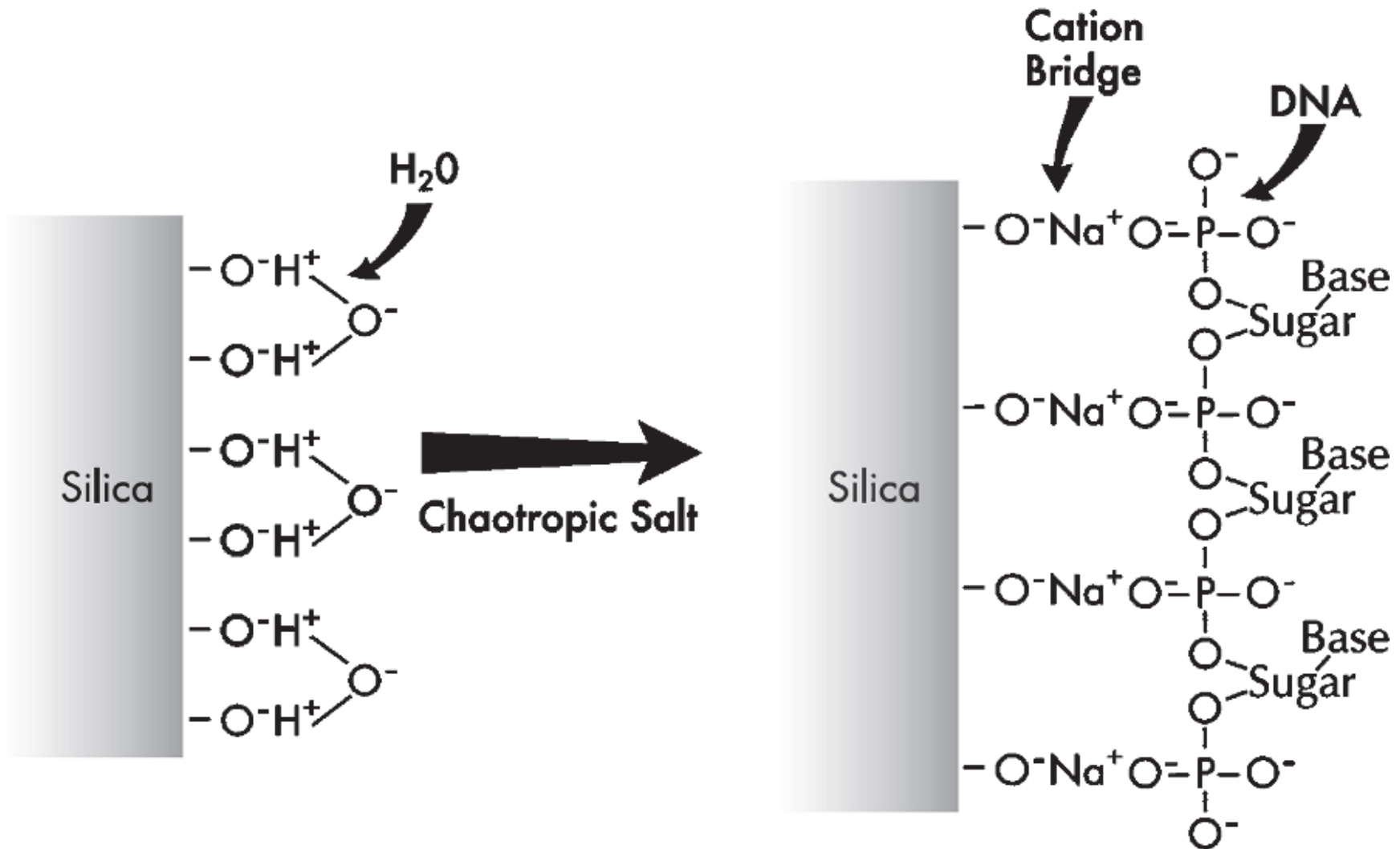
### 3. DNA/RNA Amplification



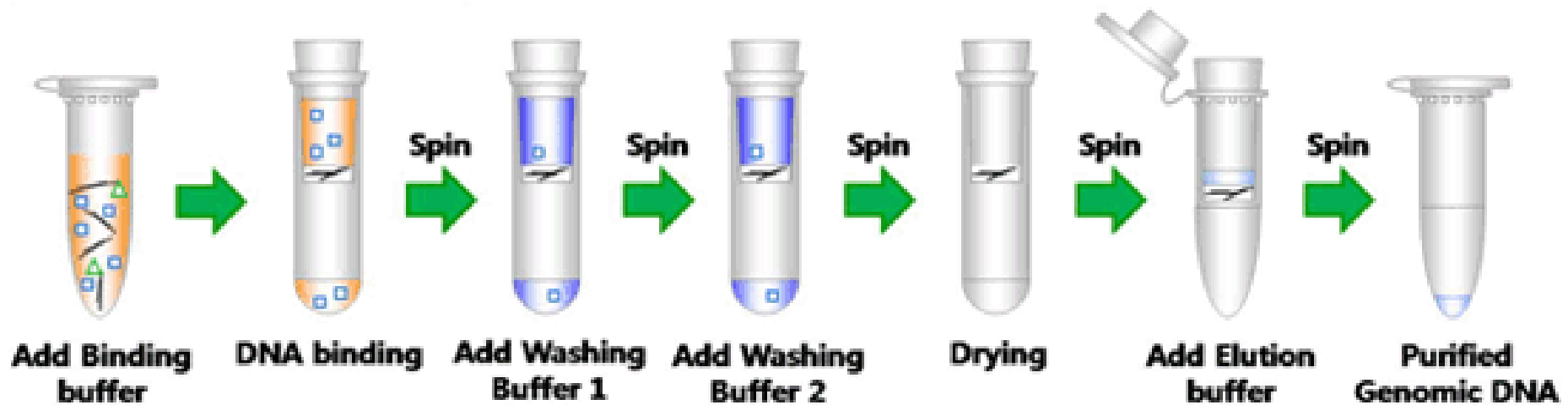
### 4. Detection of PCR product



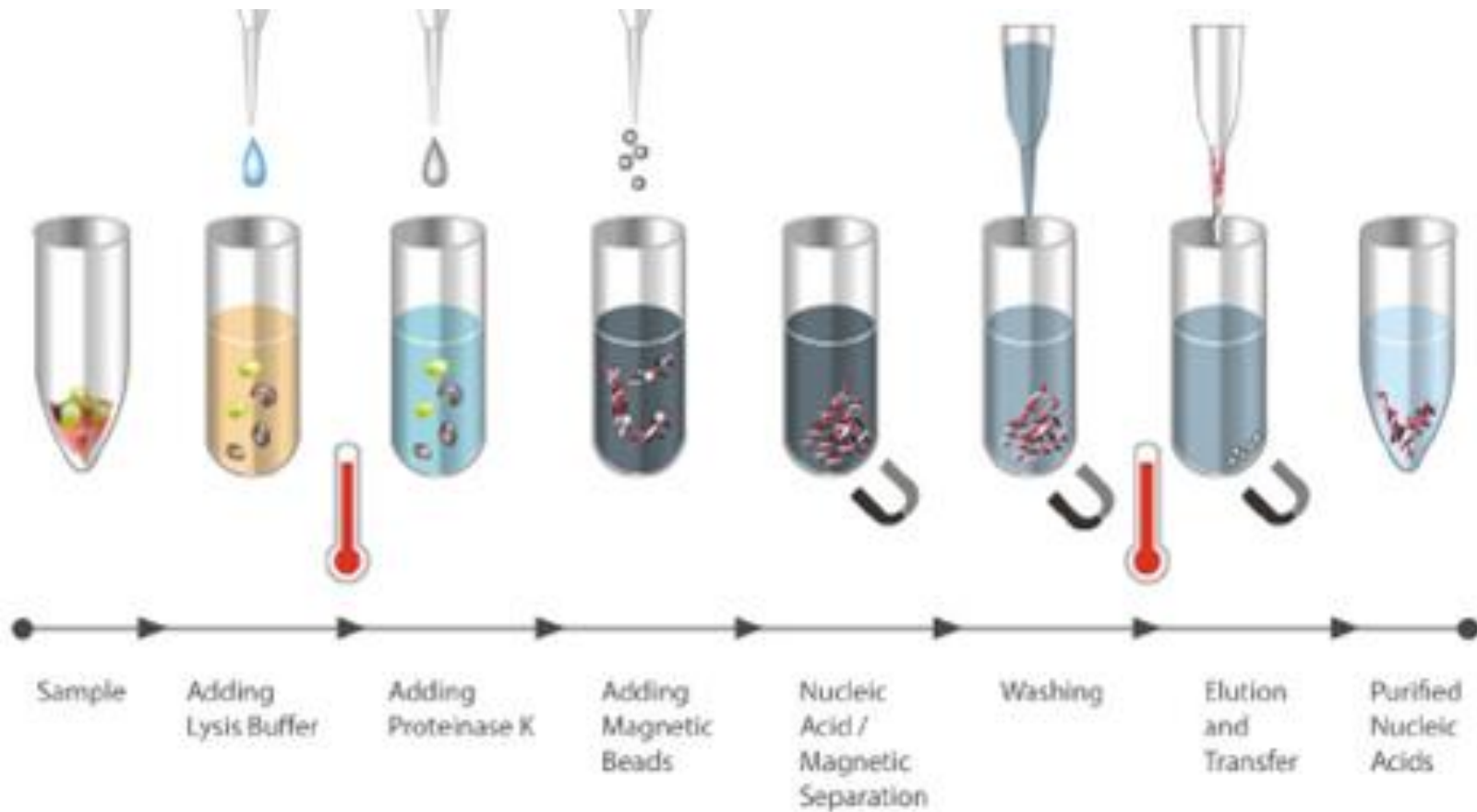
# Immobilization of nucleic acids using silica

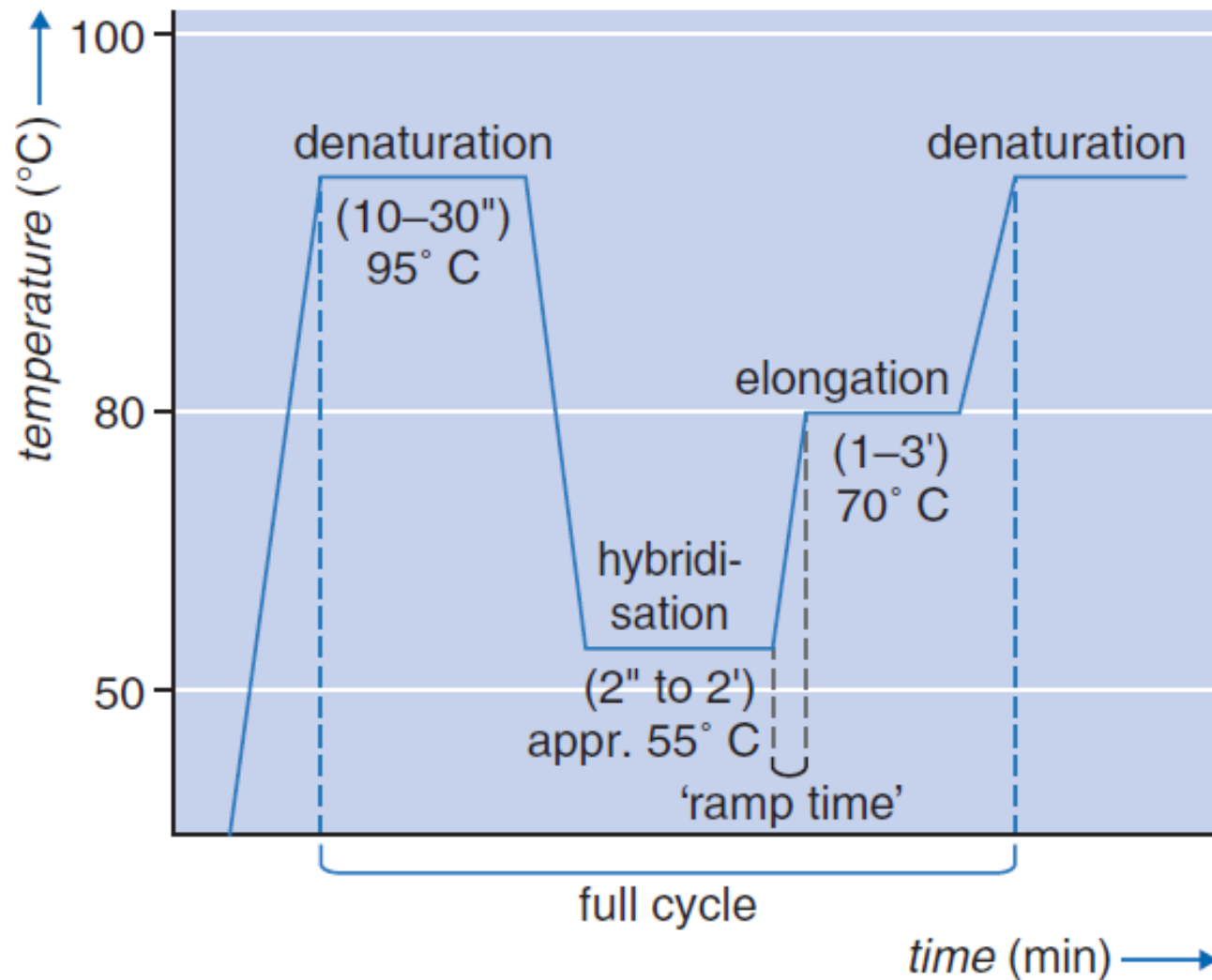


# Spin column based extraction



# Magnetic beads based extraction

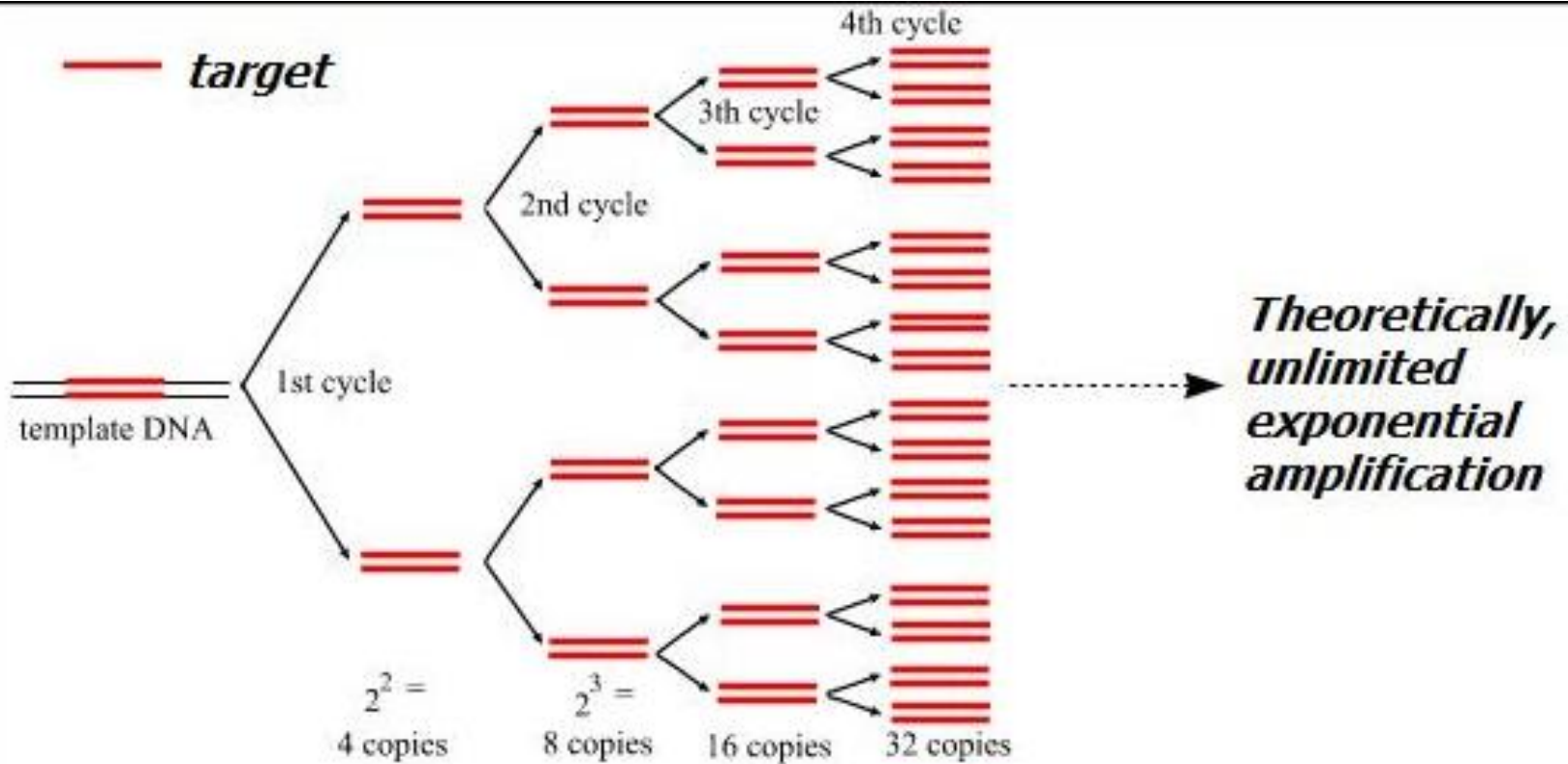




A typical three step PCR cycle



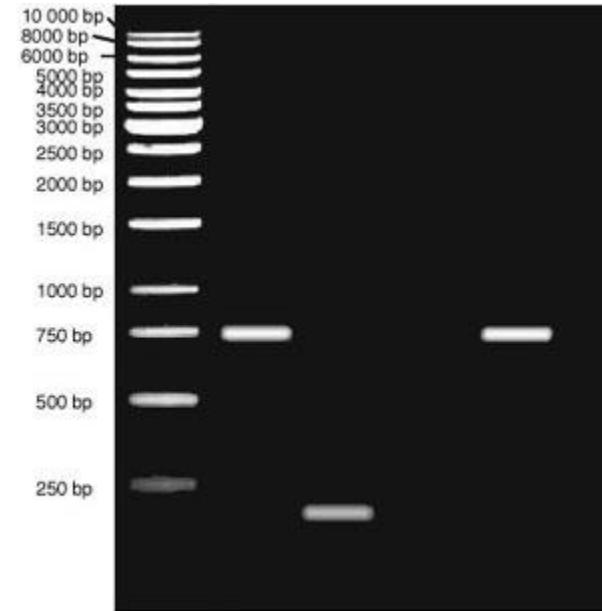
# Amplification by PCR



# Types of Molecular Diagnostic Tests

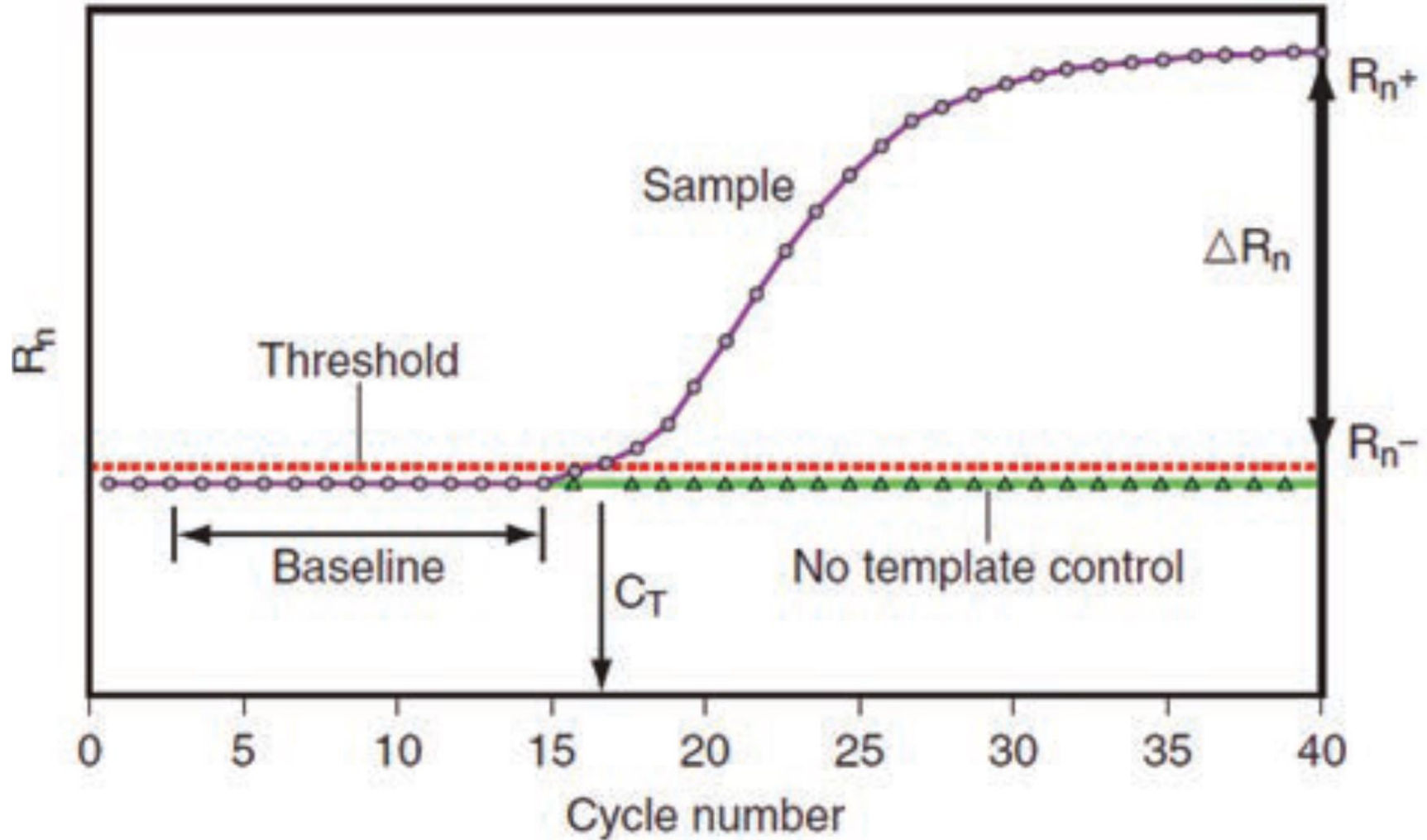
- Polymerase Chain Reaction
  - Conventional PCR
  - RT PCR
  - Nested PCR
  - Multiplex PCR
  - Real-Time PCR

# Conventional PCR



# Real-time PCR – Post amplification analysis

- Qualitative
- Quantitative



# Unidirectional Workflow

- Restricted access
- Both working personnel and cleaning personnel
- Amplification product-free to product-rich
- Remove PPE before leaving one area
- Avoid or limit reverse direction



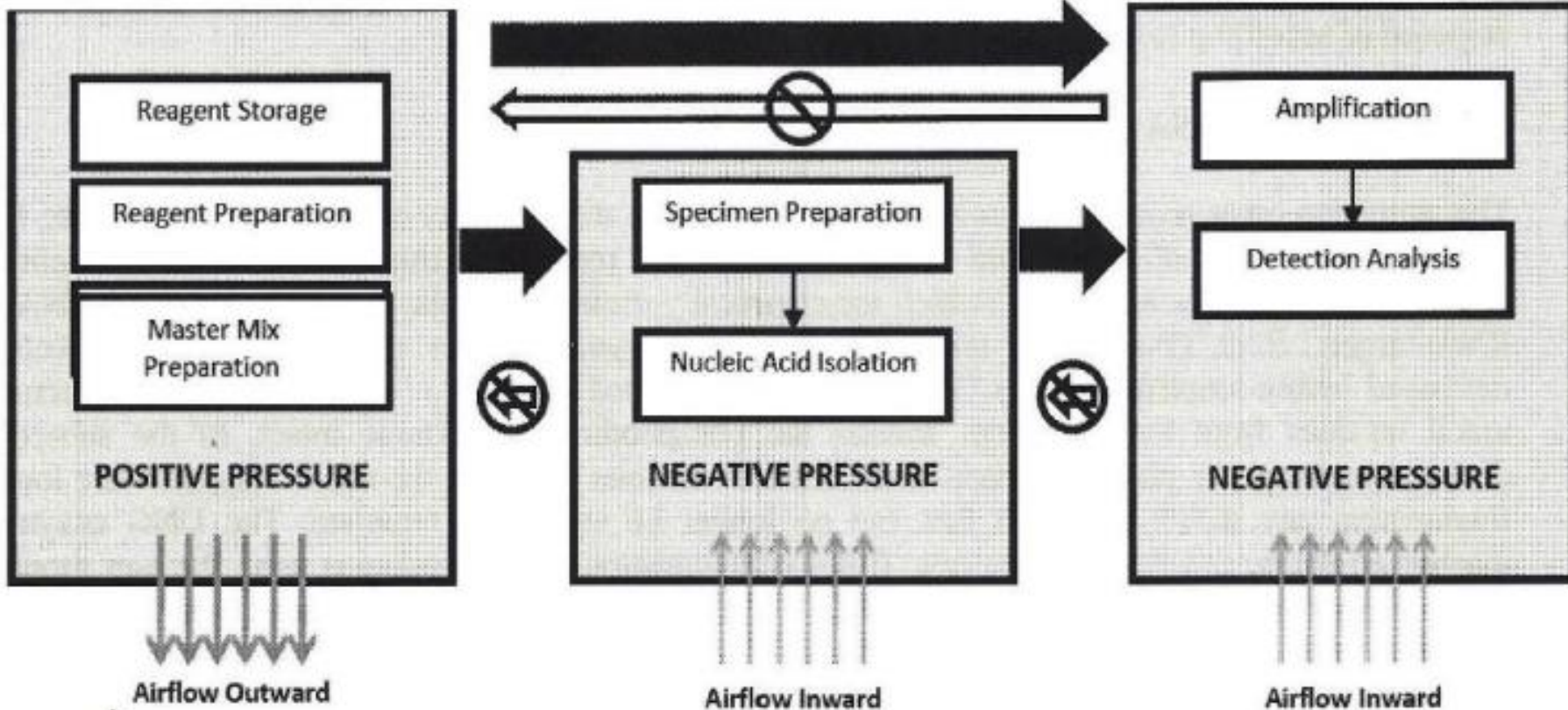
# Unidirectional Workflow

Backflow traffic – must be restricted to minimum!

## Reagent Preparation Room/Area

## Sample Preparation Room/Area

## Amplification Room/Area



Permitted workflow



Restricted workflow

# Newer Methods in Molecular Diagnostics

- Automated nucleic acid extraction
- Fully automated – Extraction + Amplification + Detection

# Automated nucleic acid extraction platforms



Abbott m2000sp



COBAS Ampliprep



COBAS Taqman48

# Fully automated PCR platform



COBAS® AmpliPrep/COBAS® TaqMan

- Near POC
- CBNAAT



GeneXpert HCV Viral Load

# Qualitative v/s Quantitative

- Qualitative – Detection of infection (Diagnosis)
- Quantitative –
  - Diagnosis
  - Monitoring
  - Treatment response
  - Treatment failure
  - Relapse
- For drug resistance testing – Qualitative PCR followed by sequencing



# Steps in Testing

- Pre-analytical
- Analytical
- Post-analytical

# Pre-analytical Considerations

- Sample collection
- Transport of sample
- Acceptability of sample
- Adequacy of sample
- Separation
- Proper storage



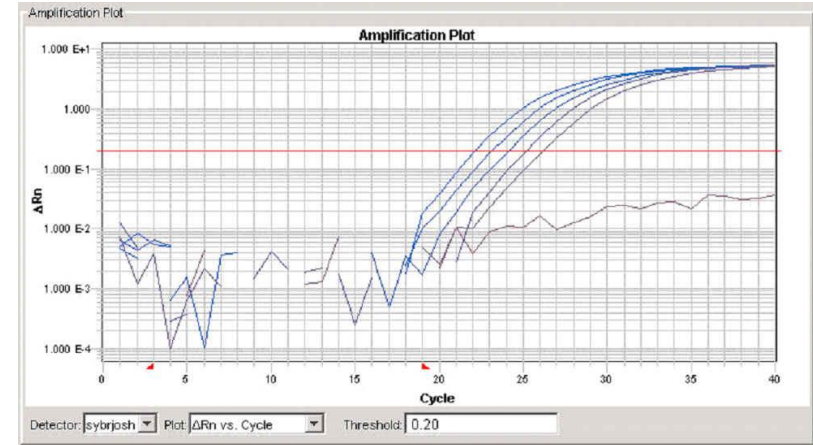
# Analytical considerations

- Planning the assay
  - TAT of the assay
  - Kit controls
  - Standards
  - Internal controls
- Performance of the assay
  - Thawing of samples and reagents
  - NA Extraction
  - Setting up PCR



# Post-analytical considerations

- Recording of results
- Validity of assay
- Calculations
- Interpretation of results
- Validation of results
- Reporting



**Thank you!**