Biotechnology Overview
DNA and RNA

DNA - A string of bases A, C, G, and T arranged in an order that makes up the genetic code

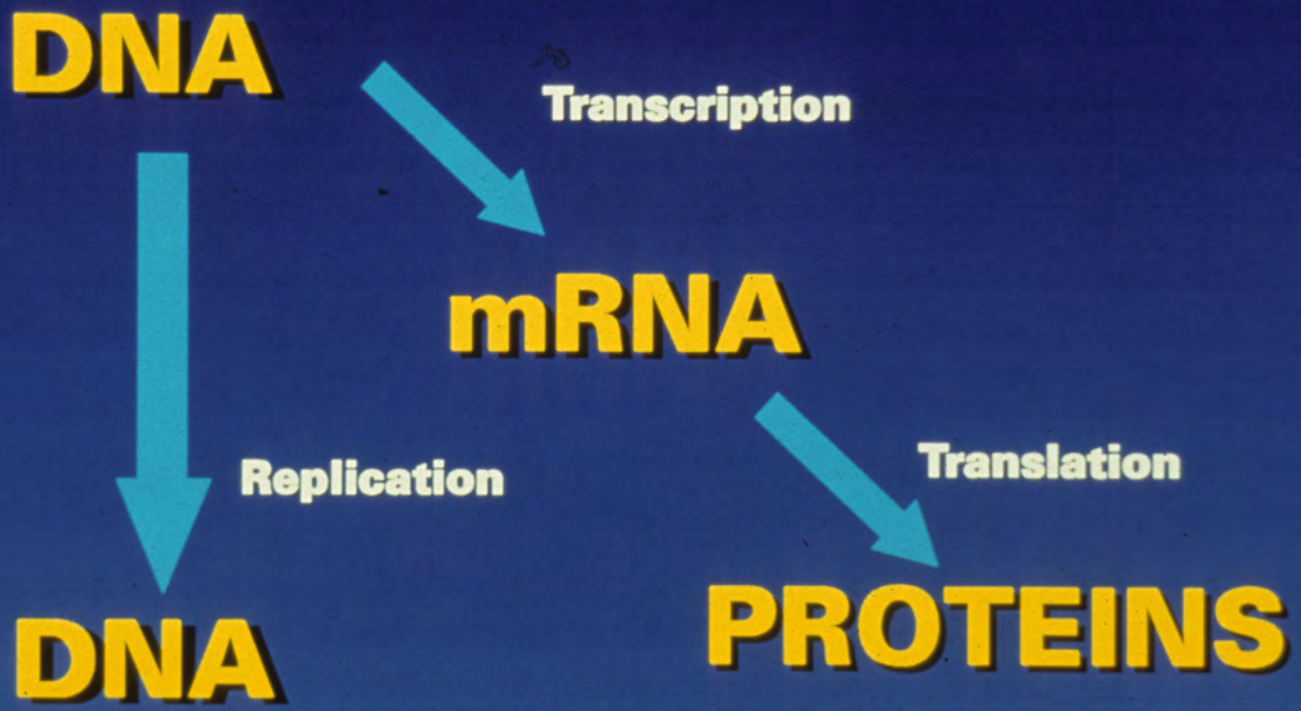
- It is double stranded, A pairs with T and C pairs with G

5'--AGCTGACGTATCAT--3' Sense strand
3'--TCGACTGCATAGTA--5' Antisense or minus-strand

RNA - Similar to DNA but instead of T it contains a U and is usually single stranded.

5'--AGCUGACGUAUCAU--3'

The Flow of Genetic Information in a Cell



Restriction Enzymes

- Cuts double stranded DNA at specific sequences called recognition sites

- Named after the bacterium from which they were isolated
  EcoRI= E. coli restriction enzyme #1
  Recognition site= –GAATTC–
  –CTTAAG–

Nucleic acid Modifying Enzymes

Ligase – Links 2 pieces of double stranded DNA together

DNA Polymerase – Uses DNA to synthesize a new complementary strand of DNA

Reverse Transcriptase – Uses RNA to synthesize a new complementary strand of DNA

Nucleic Acid Cloning

The purpose of cloning is to isolate a gene and synthesize many copies of it in bacteria so that it can be further manipulated.

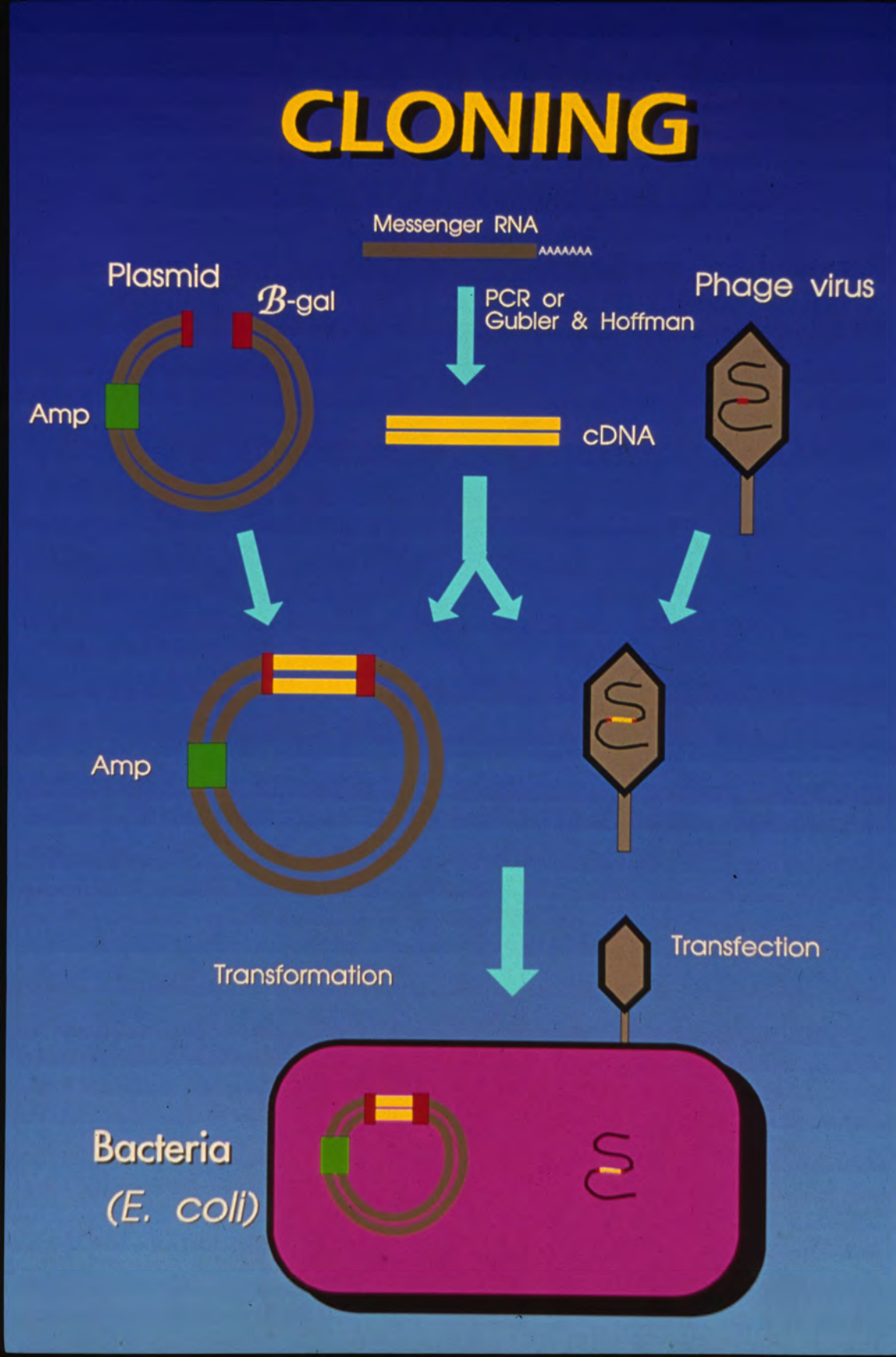
Definition – Insertion of a foreign gene into a bacterial plasmid or phage virus, and propagation of that DNA in bacteria.

Plasmid = small circular DNA separate from chromosomal DNA in bacteria.

Phage virus = a virus that infects bacteria

Nucleic Acid Cloning (Recombinant DNA Production)

- Purify the gene of interest (mRNA)
- Make double stranded DNA
- Prepare the cloning vector (plasmid or phage virus)
- Insert (ligate) the DNA into the vector
- Transform competent *E. coli* cells
- Grow the *E. coli* containing the recombinant DNA (clones)
- Purify the recombinant DNA from the bacteria


CLONING
Messenger RNA
AAAAAAA
Plasmid
B-gal
Amp
PCR or
Gubler & Hoffman
cDNA
Phage virus
Amp
Transformation
Transfection
Bacteria
(E. coli)

Polymerase Chain Reaction (PCR)

- A rapid method for synthesizing large amounts of a specific piece of DNA

- One copy of DNA can be amplified to 10^6 copies in several hours

Two Innovations Lead to the Automation of PCR

- Taq polymerase – an enzyme that survives the high temperatures required to denature DNA

- Thermal cycler – a computerized temperature block that can be programmed to change temperatures automatically

PCR Reaction Mixture

- The following ingredients are combined in a test tube and placed in a thermal cycler;

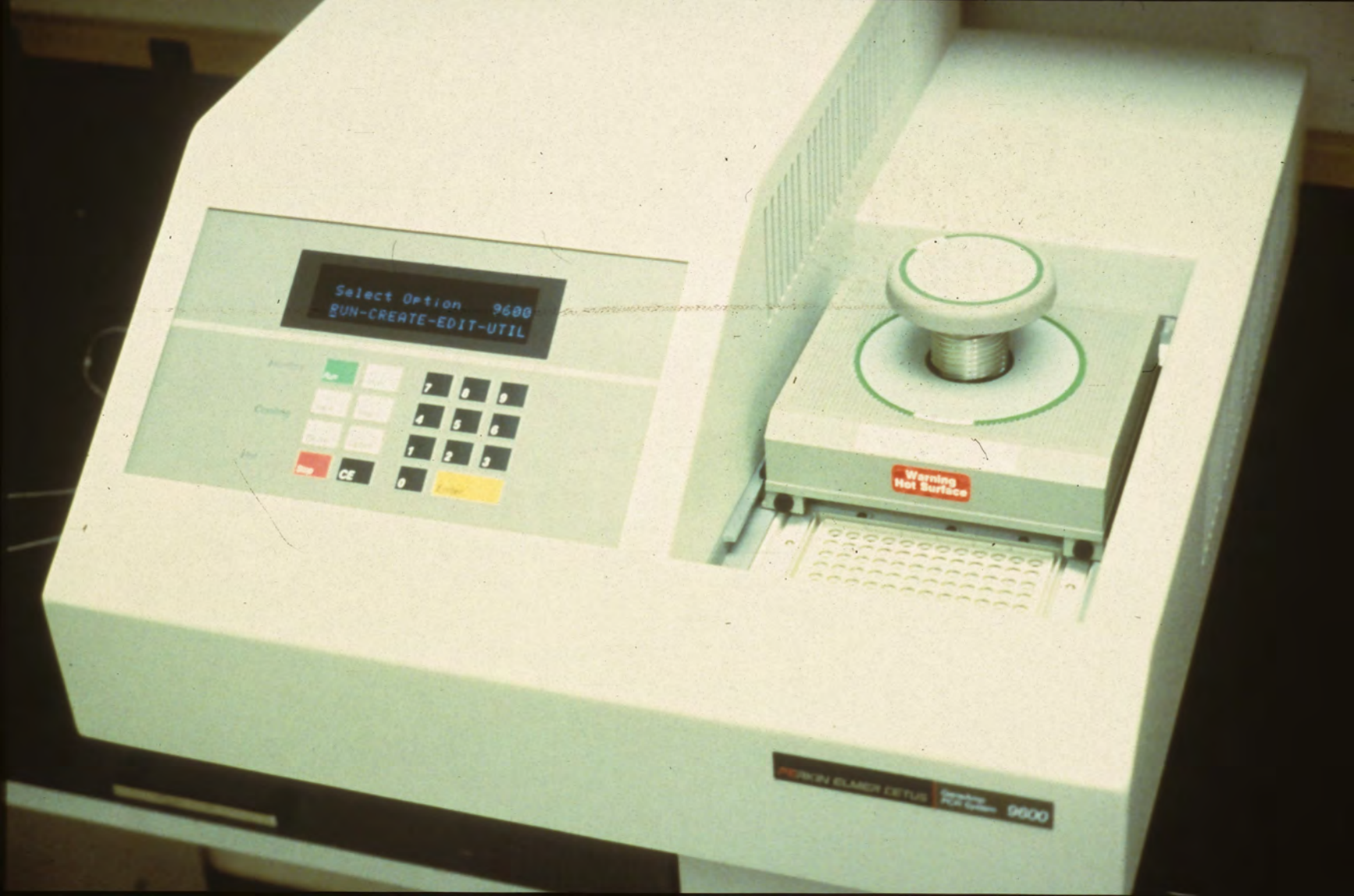
  Taq polymerase

  Nucleotides A, C, G, and T

  Primers

  Reaction buffer (containing $MgCl_2$)

  Template DNA


Select Option 9600
RUN-CREATE-EDIT-UTIL
CE
Warning
Hot Surface
9600

The PCR Cycle

- 3 steps make up a cycle which is repeated 30 to 40 times

1. Denaturation (95 C)
2. Primer annealing (30 C to 56 C)
3. Polymerization (72 C)

POLYMERASE CHAIN REACTION

Template DNA

Denature (95C)

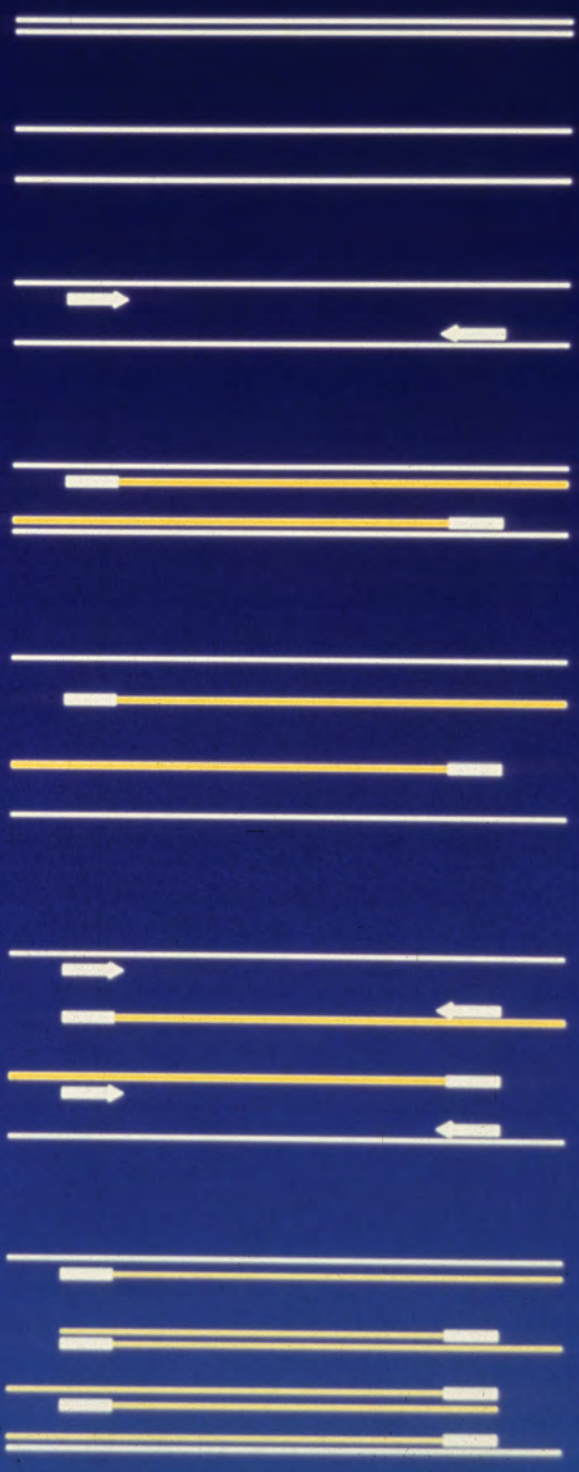
Anneal primers (37C to 56C)

Synthesize new DNA strand (75C)

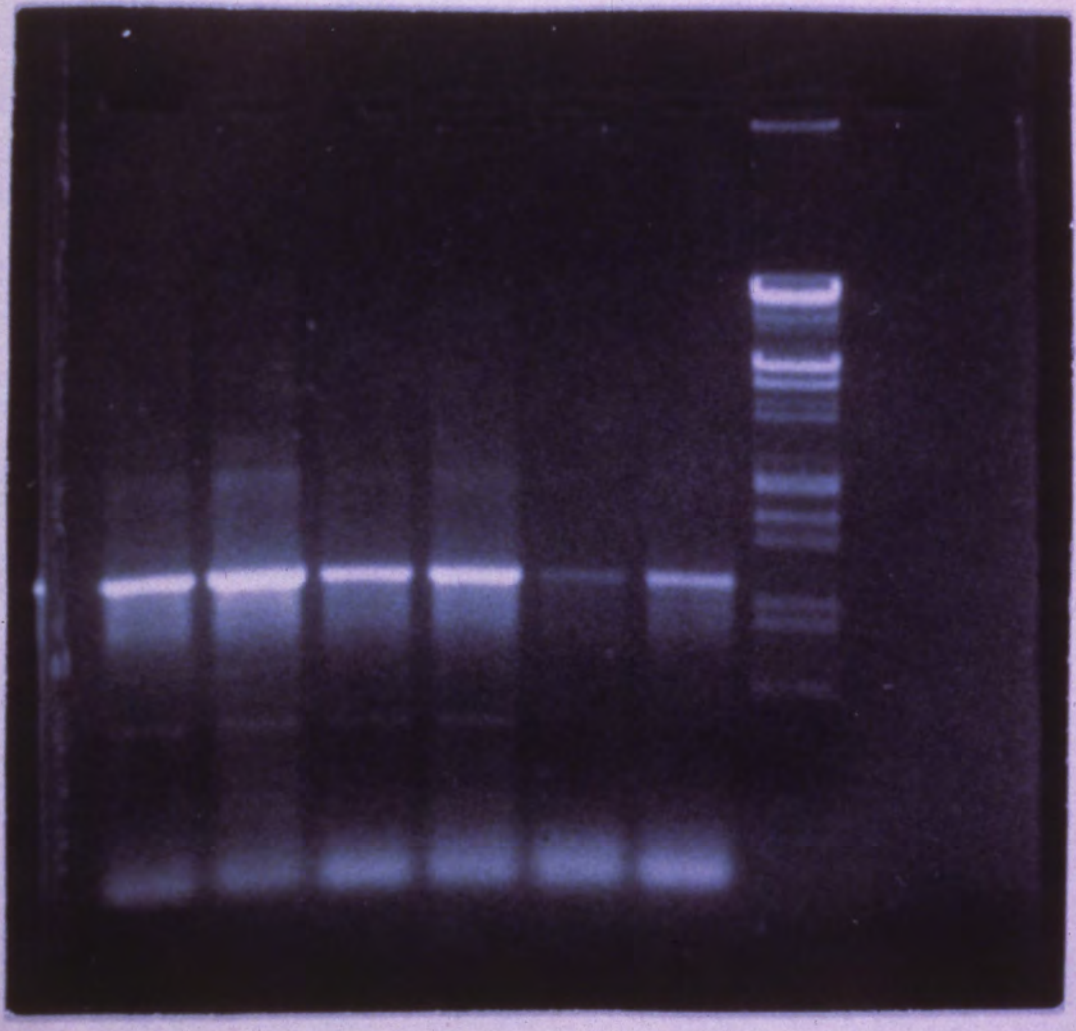
Denature (95C)

Anneal primers (37C to 56C)

Synthesize new DNA strand (75C)



PCR



Types of Nucleic Acid Probe Labels

Radioactivity -- ^{32}P or ^{35}S

Colorimetric -- Horseradish Peroxidase (HRP)* or Alkaline Phosphatase (AP)*

Fluorescence -- Fluorescein* or Rhodamine*

Chemiluminescence -- AP/1,2-Dioxetane* or HRP/Luminol*

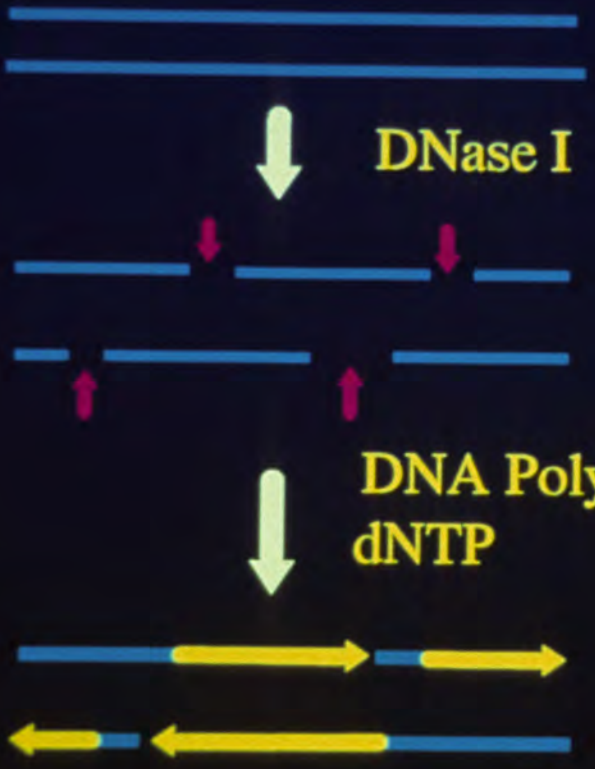
*These labels are often attached to nucleic acids using biotin/streptavidin or haptin/antibody bridiges.

Haptin -- Digoxygenin or Sulphonated cytosine

Methods of Labeling Nucleic Acid Probes

- Incorporation of labeled nucleotide triphosphates
  - Nick Translation
  - Oligonucleotide Primed Synthesis
  - Kinase End Labeling
  - RNA Polymerase Transcription

Probe Synthesis
Nick Translation

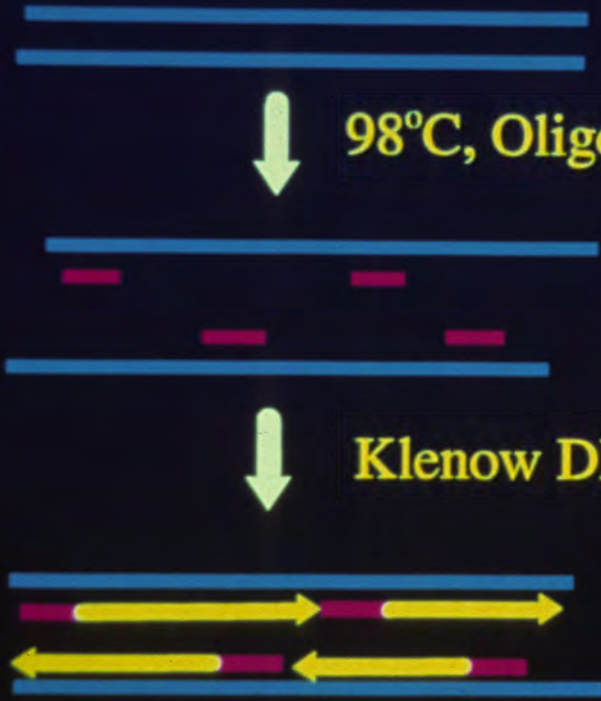


Double stranded DNA

DNase I nicks the double stranded DNA

DNA polymerase I begins synthesis of the DNA probe at the site of the nick using labeled dNTP.

Probe Synthesis
Oligonucleotide Primed Synthesis



Double stranded DNA

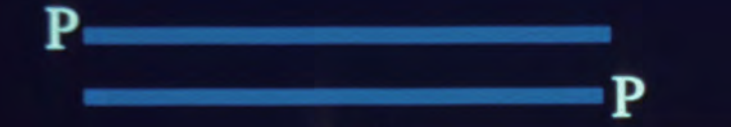
98°C, Oligonucleotide primers

DNA is denatured to form single-stranded DNA and oligonucleotides are annealed

Klenow DNA Polymerase I

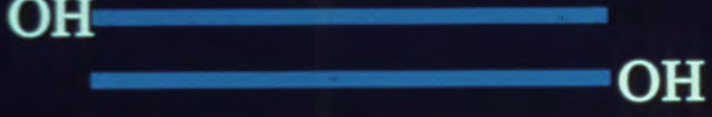
Klenow fragment of DNA polymerase I is used to synthesize probe using labeled dNTP.

Probe Synthesis
Kinase End Labeling



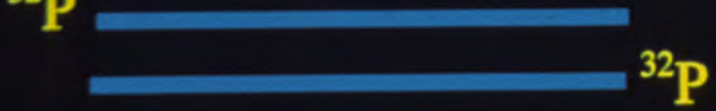
dsDNA, ssDNA, or RNA

Alkaline Phosphatase



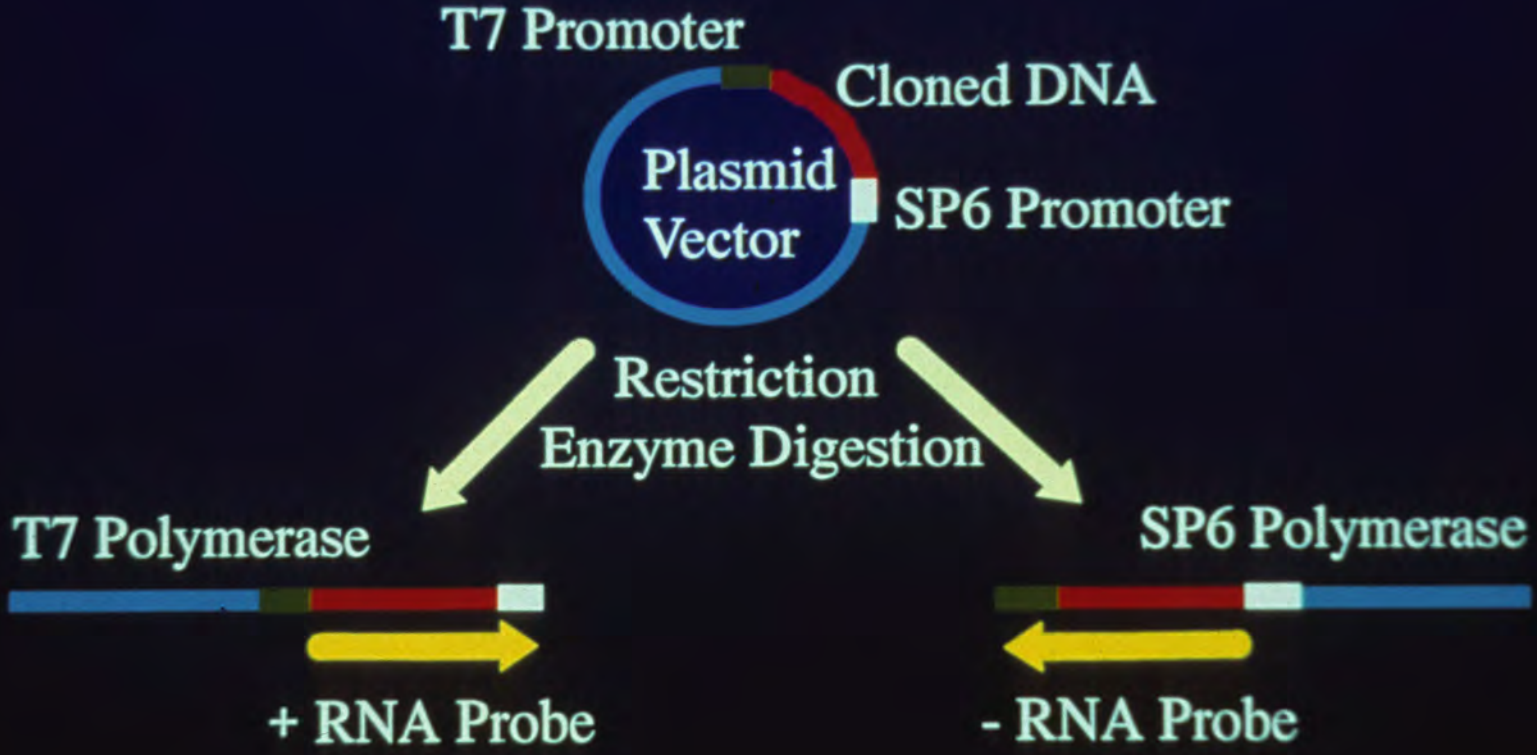
Dephosphorylation:
The 5′ phosphates are removed.

T4 Polynucleotide Kinase
[Gamma-^{32}P]ATP



Polynucleotide kinase from the T4 bacteriophage removes the ^{32}P from ATP and adds it to the 5′ -terminus of DNA or RNA.

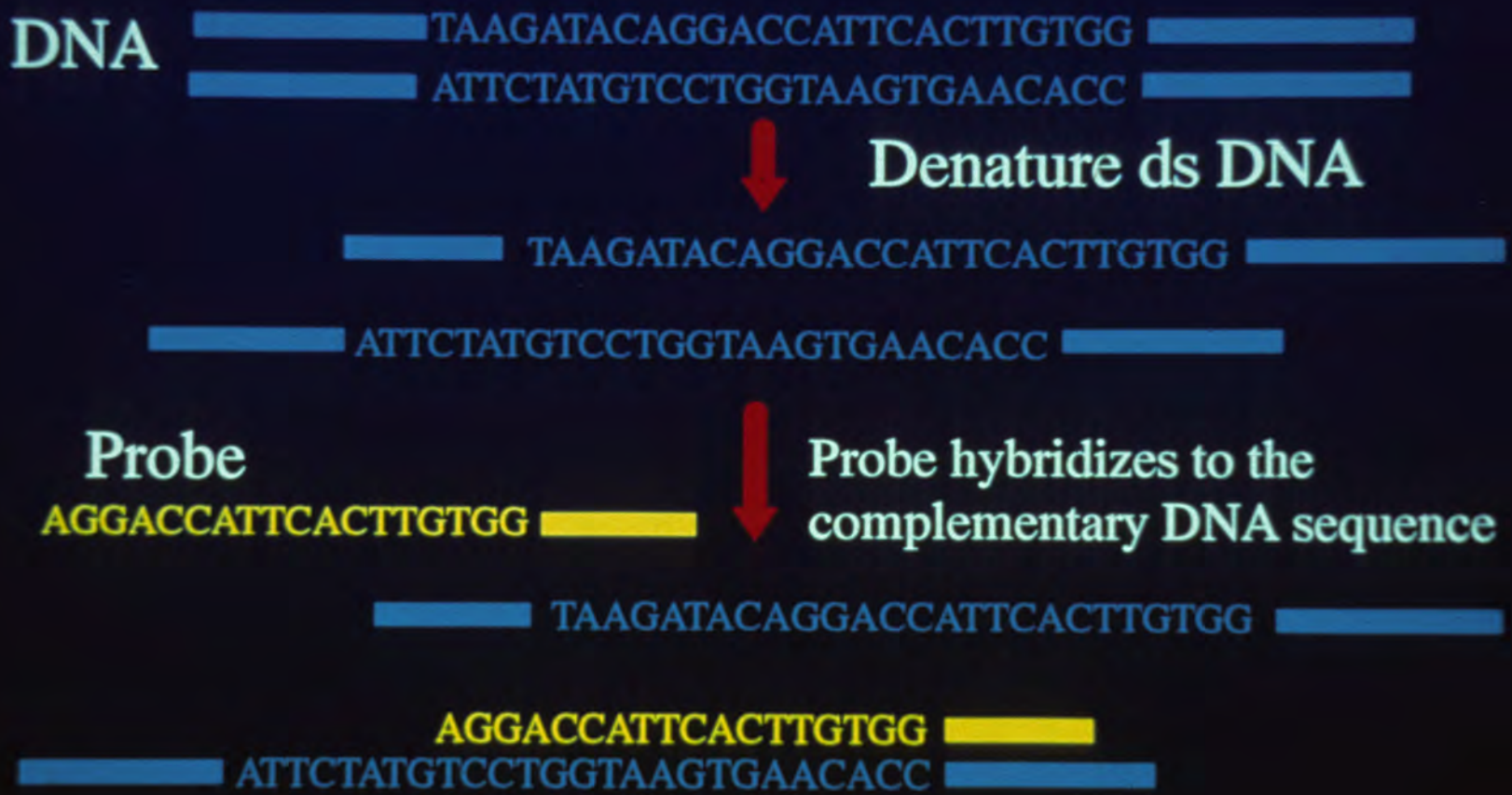
Probe Synthesis
RNA Polymerase Transcription



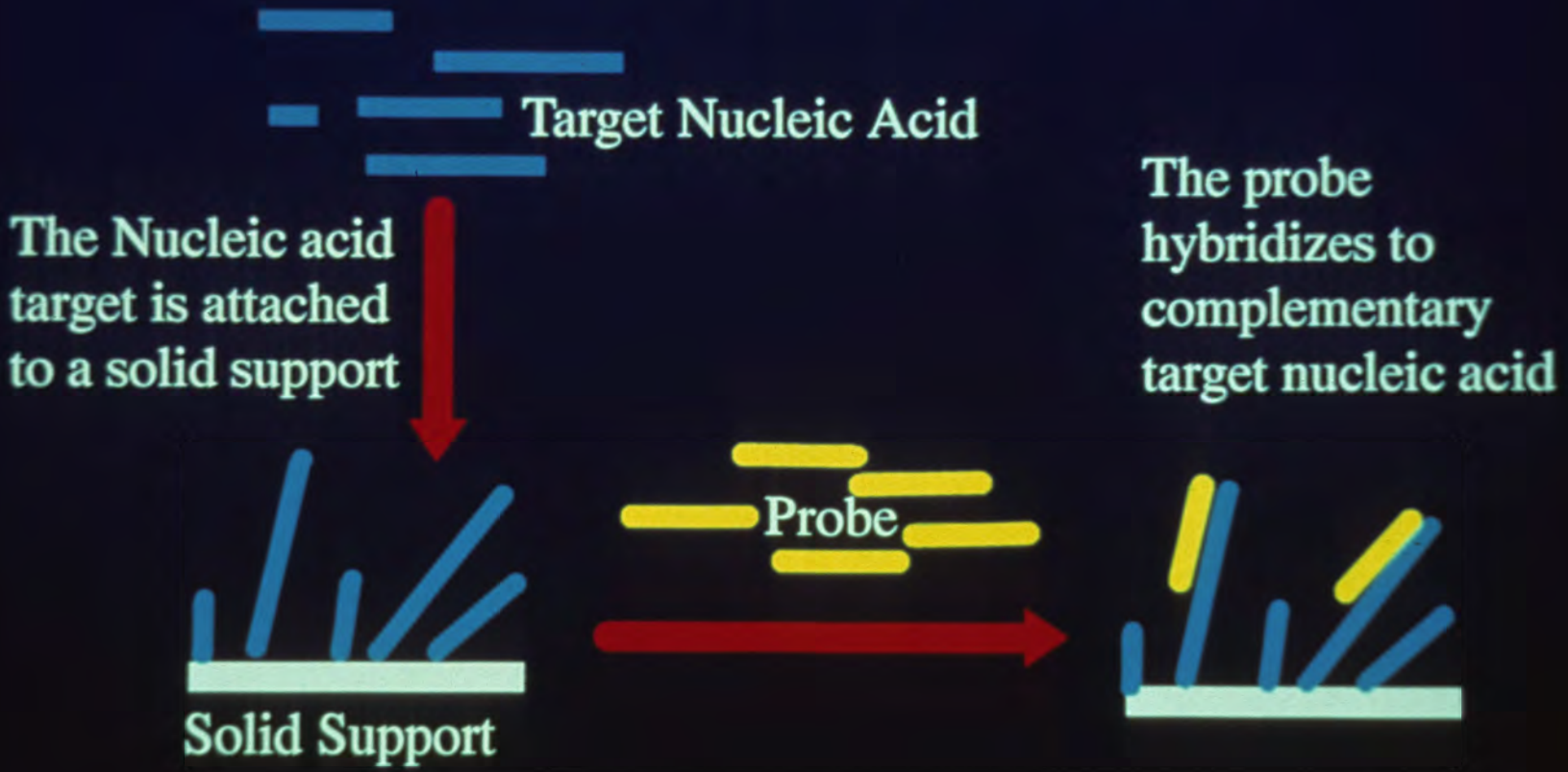
Methods of Labeling Nucleic Acid Probes

- Direct Conjugation of Nucleic Acids
  - Photobiotin
  - Photodigoxigenin
  - Horseradish peroxidase /polyethyleneimine

Hybridization Analysis



Hybridization Analysis
Immobilized Nucleic Acids



Hybridization Analysis
Immobilized Nucleic Acids

Dot or Slot Blot
(DNA or RNA)

Southern Blot
(DNA)

Northern Blot
(RNA)

Restriction Enzyme Digestion

Electrophoresis

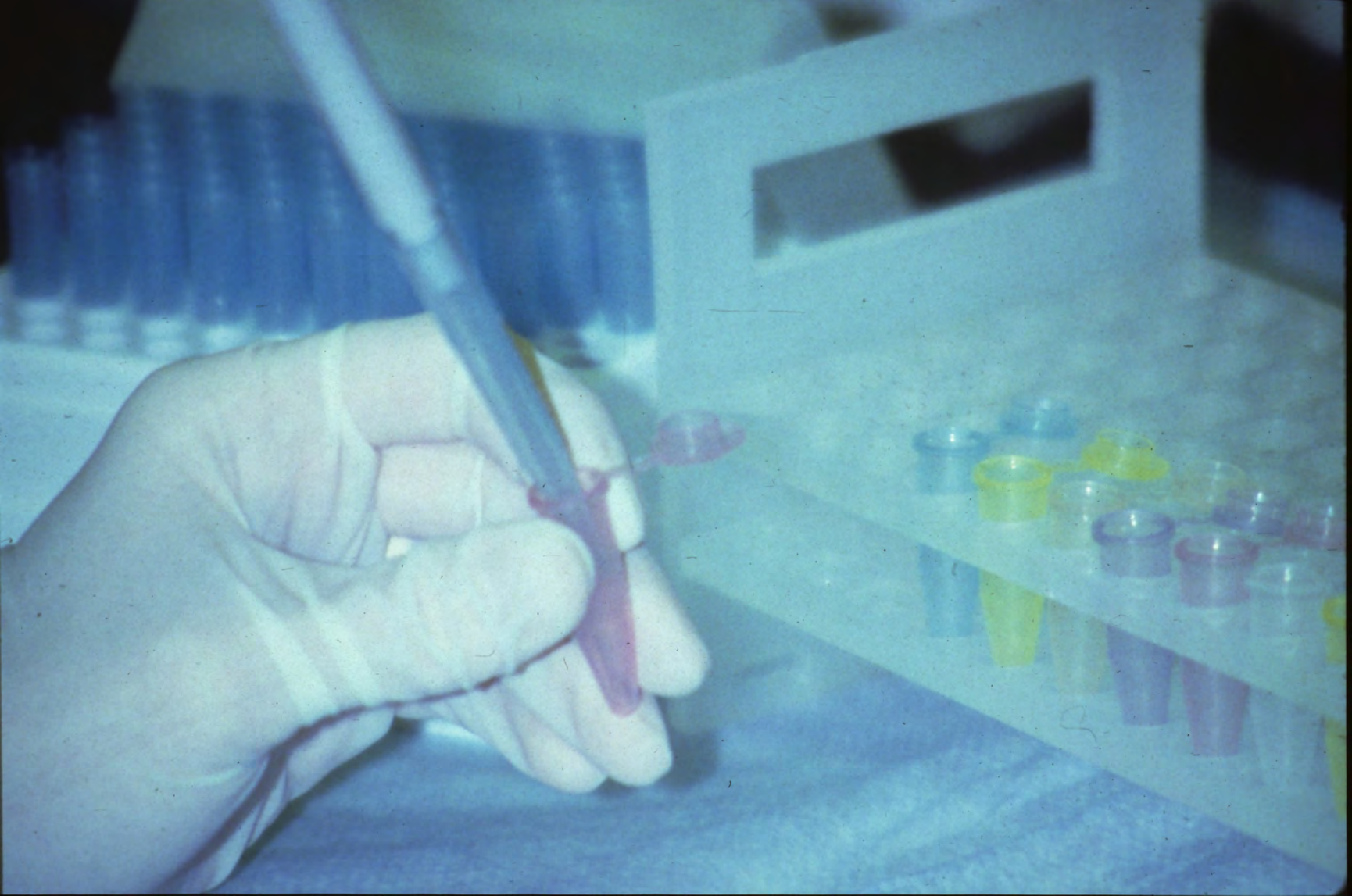
Electrophoresis

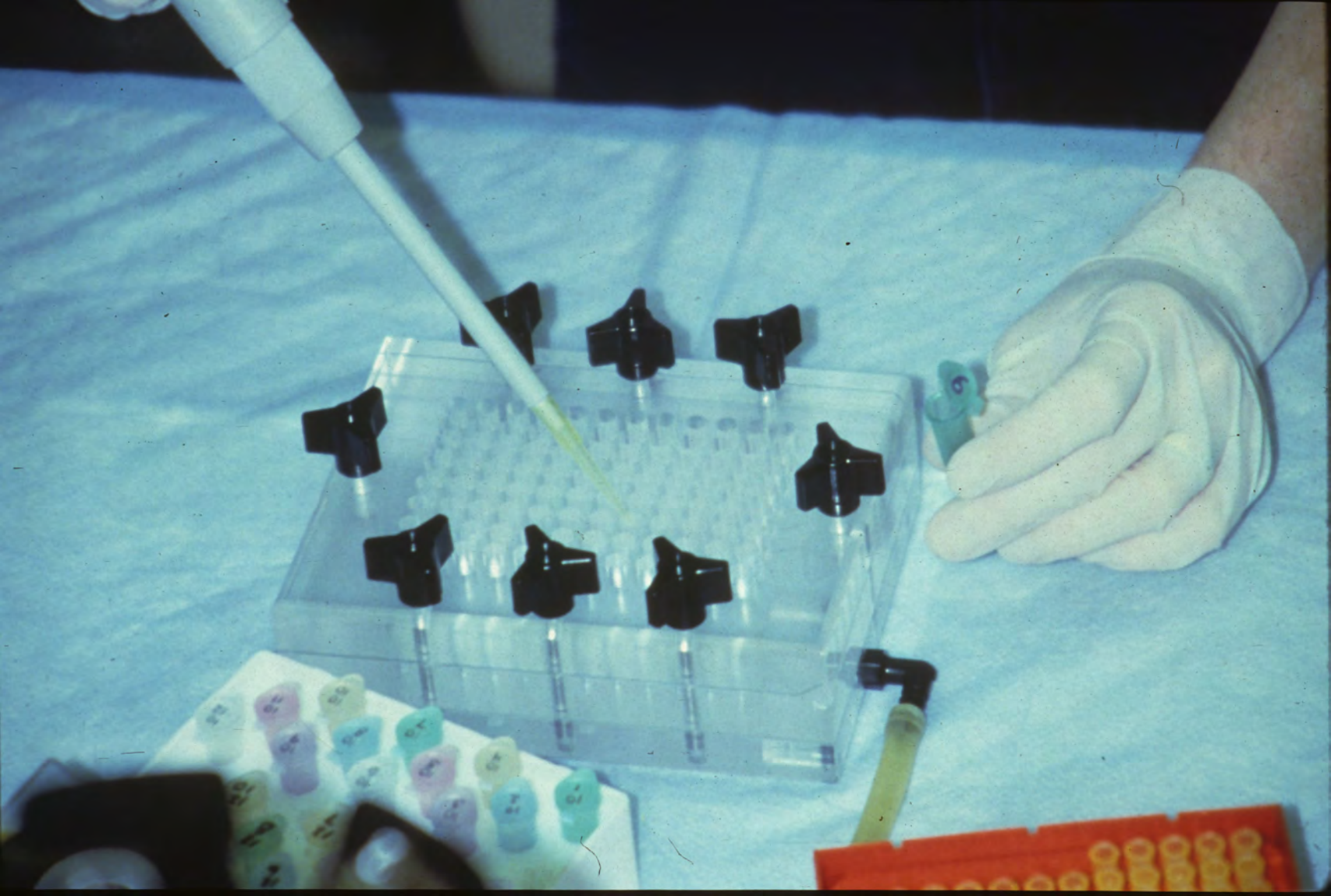
Transfer to Solid Support

Transfer to Solid Support

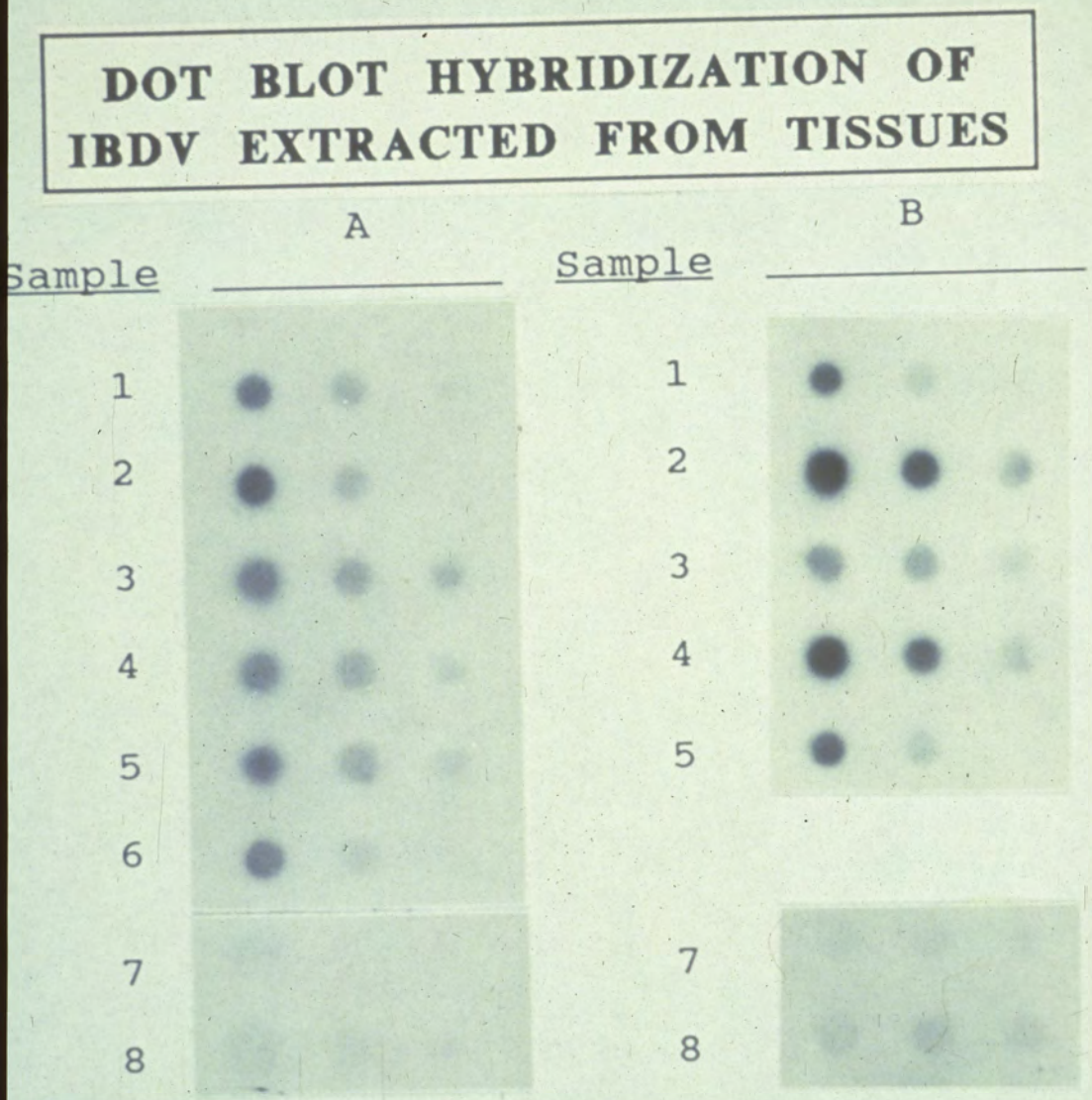
Transfer to Solid Support

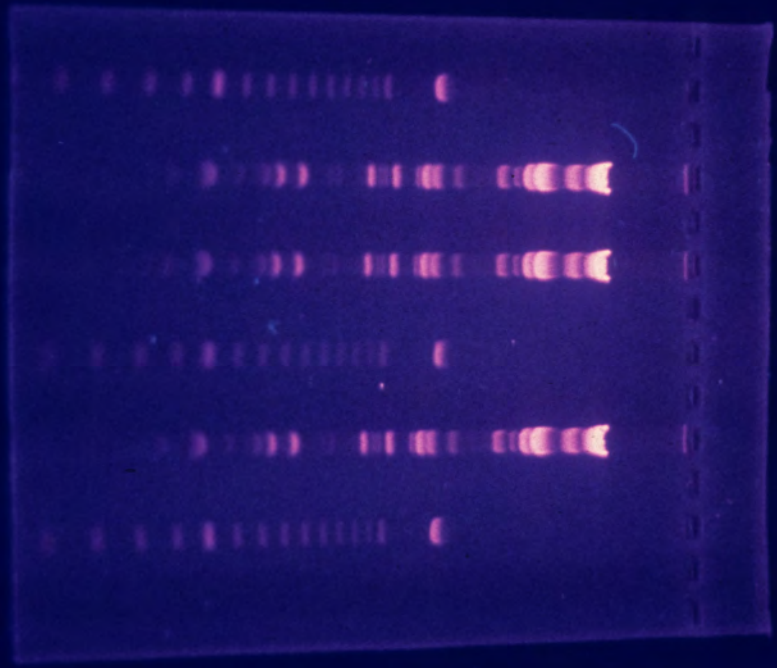
Hybridization

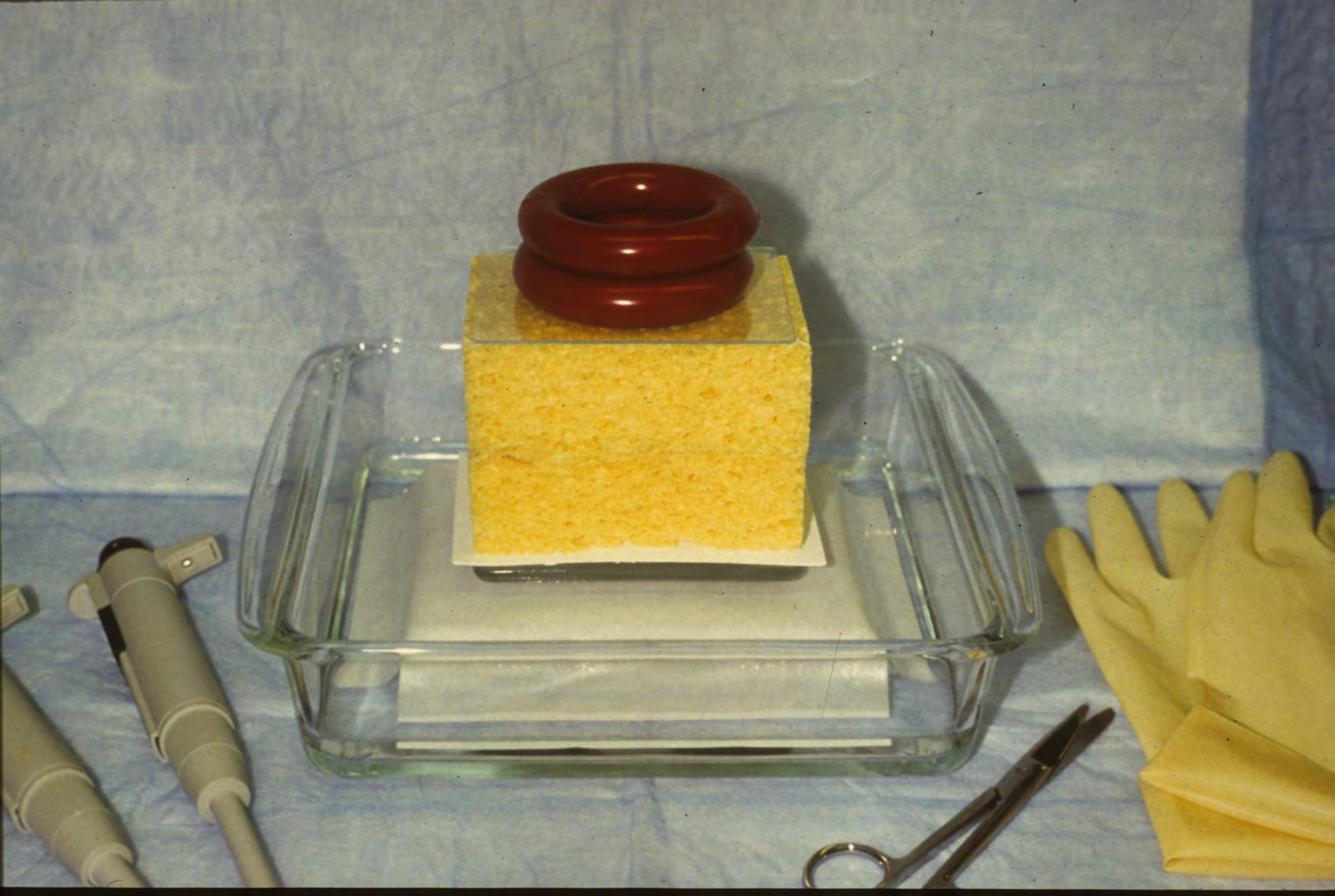




DOT BLOT HYBRIDIZATION OF IBDV EXTRACTED FROM TISSUES







NORTHERN BLOT HYBRIDIZATION
IBDV BB-15 PROBE