

Thymidine
DNA

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DNA

- Polymerase DNA/E. coli
- dATP, dCTP, dGTP, dTTP

+ddGTP

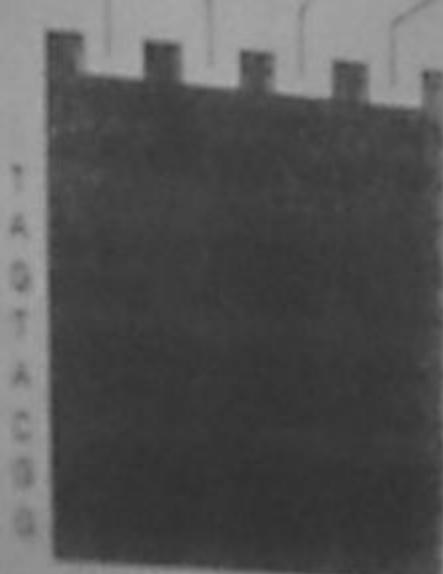
+ddATP

+ddTTP

+ddCTP

- GGCAT_{ddd}
- G_{ddd}
- ddG

- GGCATG_{ddd}
- GGC_{ddd}
- GGCATG_{ddT}
- GGCATG_{ddT}
- GG_{ddC}



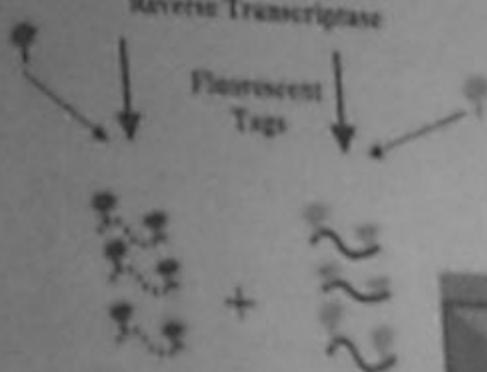
Rys. 1 Schematyczna DNA metoda „ściskowej” Sangera



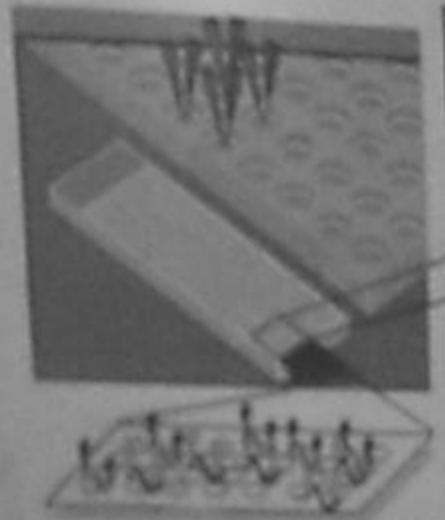
B. cDNA Generation
C. Labeling of Probe

Reverse Transcriptase

Fluorescent Tags



D. Hybridization
to Array

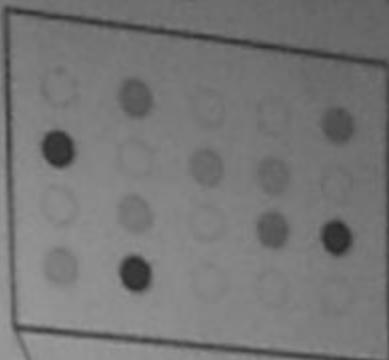


E. Imaging

● Sample A > B

● Sample B > A

○ Sample A = B



Fluorescence in Situ Hybridization

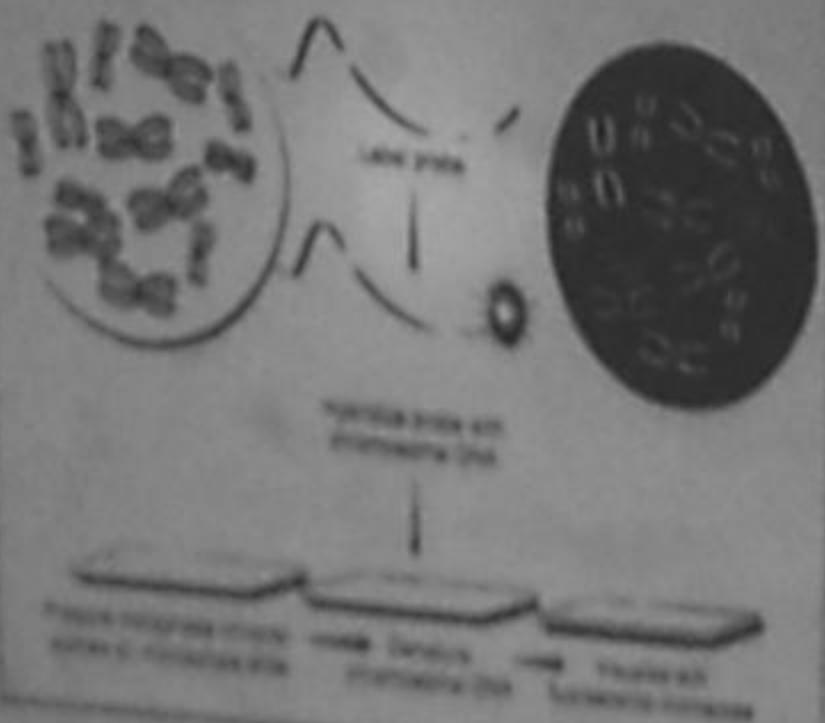
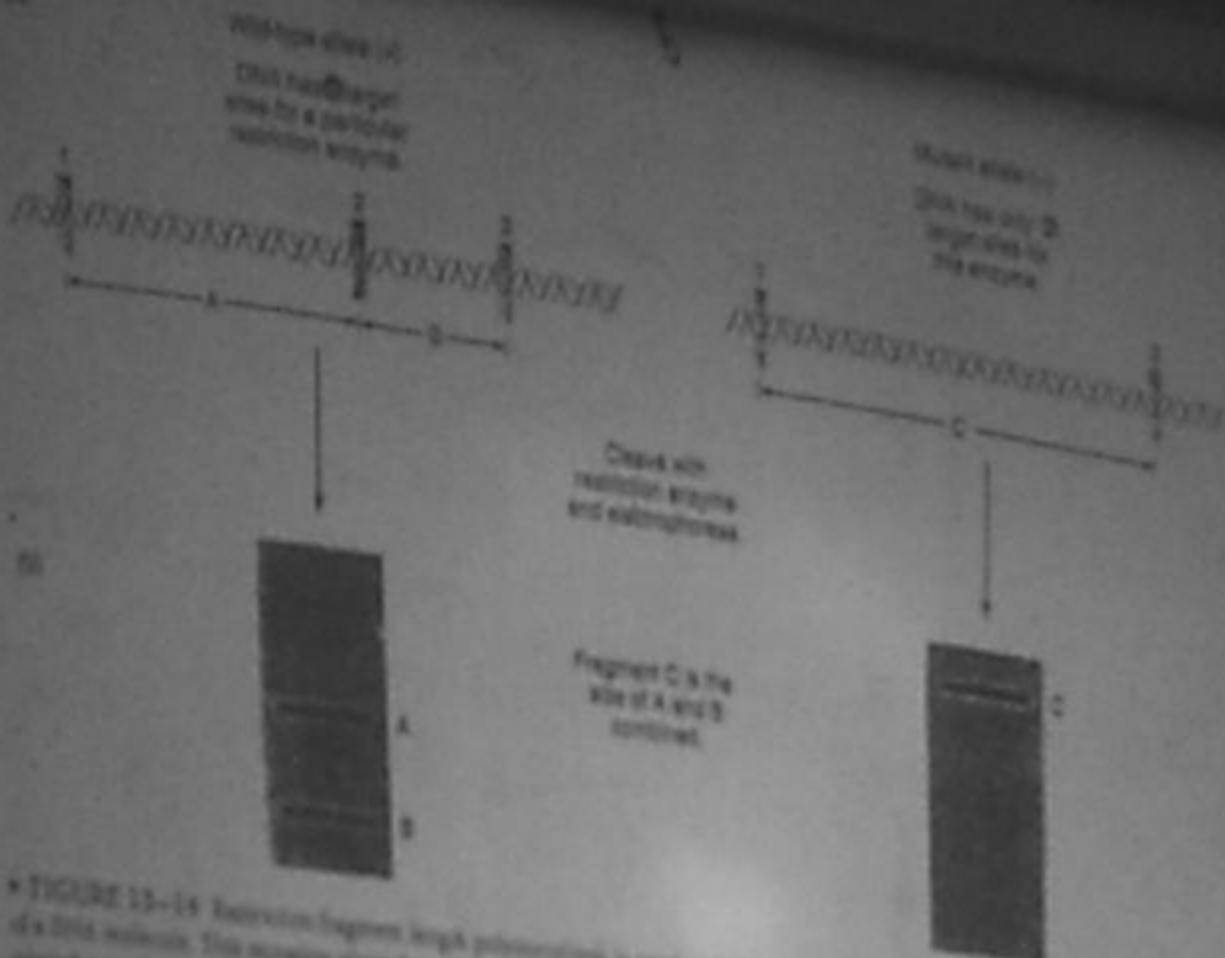


Figure 3. Three solutions of Minerva Diagnos.

The upper row shows nucleic acids with clusters of centromeres of human genes. In the middle, a synthetic probe (fluorescent DNA) is hybridized with the chromosomes. The results are shown below. Each row has three samples: normal fibroblasts and two patients with chromosomal abnormalities. The first row shows a normal pattern of fluorescence. The second row shows a normal pattern of fluorescence, but the patient with chromosomal abnormalities shows a different pattern. The third row shows a normal pattern of fluorescence, but the patient with chromosomal abnormalities shows a different pattern.

The lower row shows the results of fluorescence in situ hybridization. The first row shows normal chromosomes and no fluorescence. The second row shows normal chromosomes and some fluorescence. The third row shows normal chromosomes and a lot of fluorescence. These results indicate that the patient with chromosomal abnormalities has a different pattern of fluorescence than the normal cells.



*FIGURE 13-14. **Restriction fragment length polymorphisms (RFLPs).** If a nucleic acid has a restriction site along a section of a DNA molecule, the nucleic acid is cleaved at that position. The number gives the pattern of fragments needed to distinguish RFLPs would also serve to measure genetic variation in a nucleic acid sample (see Fig. 13-15).

Sample

DNAs from various
normal tissues

100 ng

Sample

DNAs from various
normal tissues

100 ng

Amplification

DNA with
restriction enzymes

Electrophoresis
through 1% agarose gel



Wild-Type Sequence

Wild-type primer

ATGGGACTTCACTTC

TACCCGTTTGCGTAC

Mutant primer

ATGGGACTTCACTTC

TACCCGTTTGCGTAC

Downstream primer

Mutant sequence

ATGGGACTTCACTTC

TACCCGTTTGCGTAC

No PCR

ATGGGACTTCACTTC

TACCCGTTTGCGTAC

No PCR

c

WT

HET

MUT

WT

MUT

WT

MUT

WT

MUT

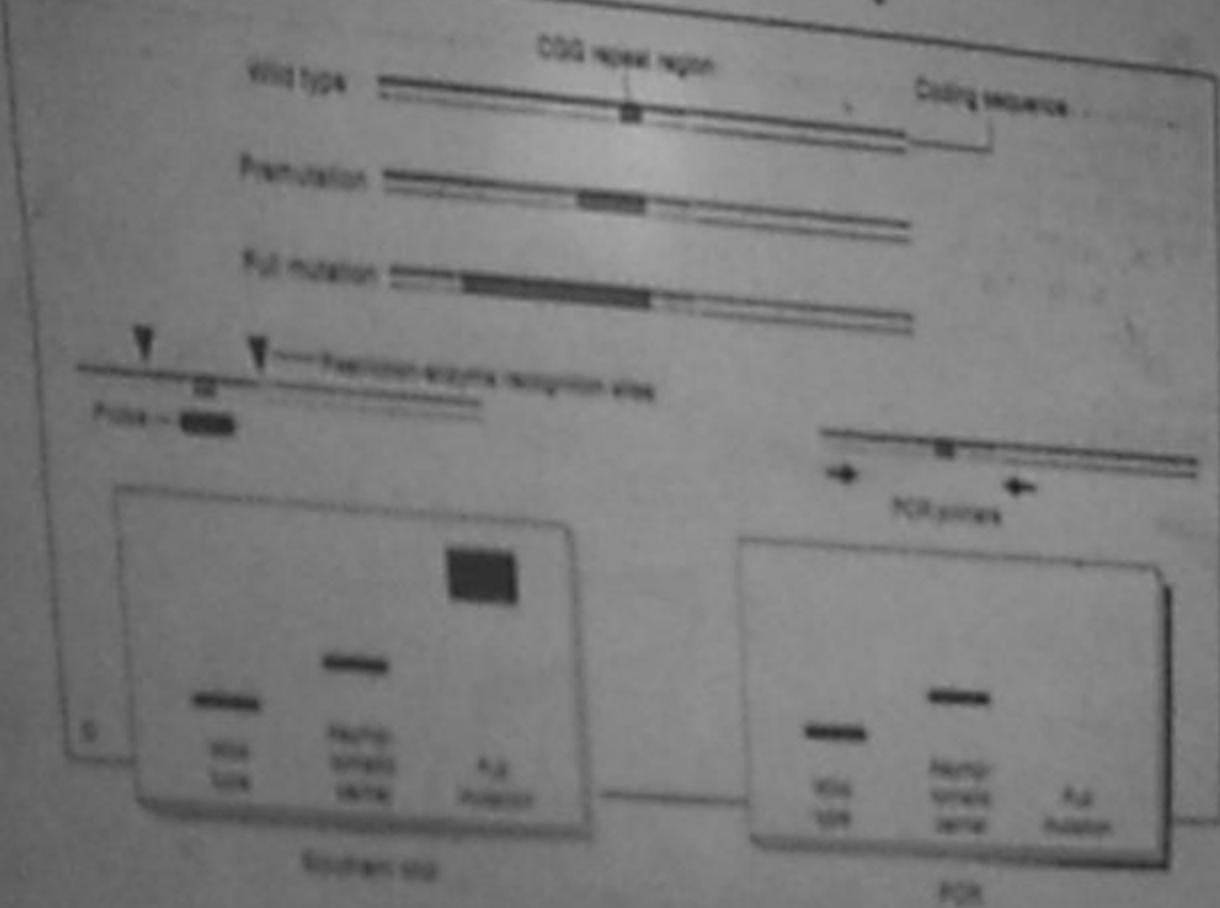
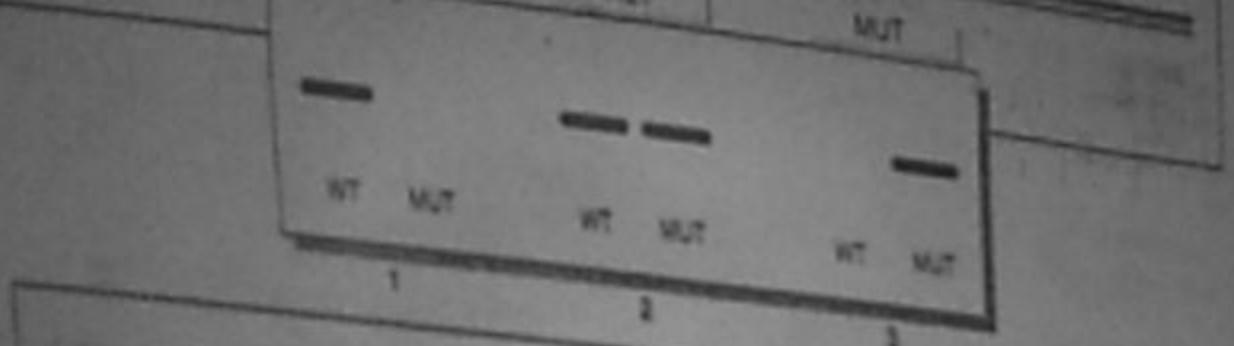
Wild type

CGG normal region

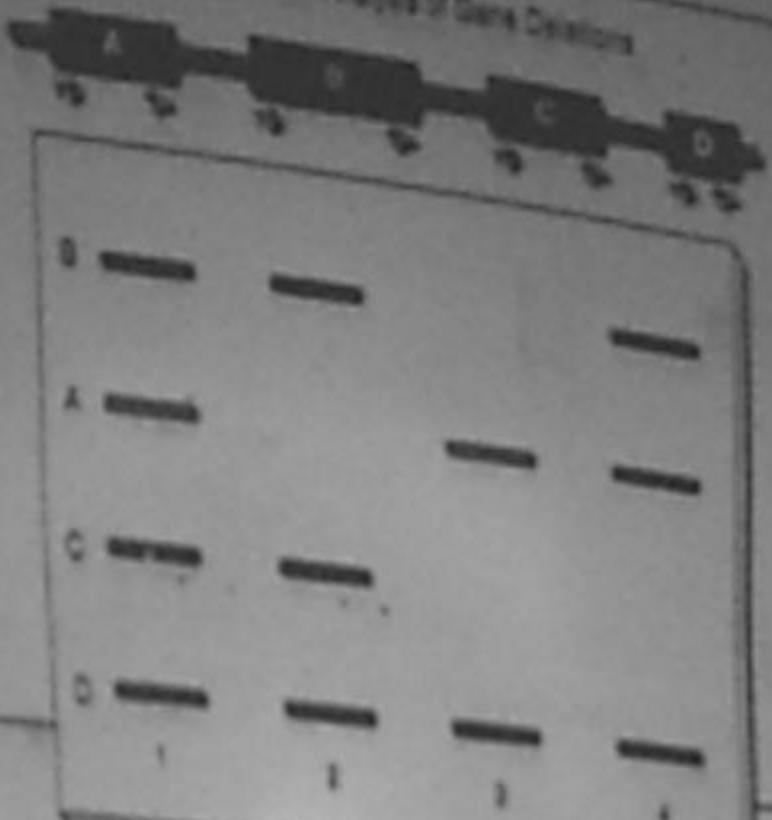
Coding sequence

Premature

Termination



Multiplex PCR Analysis of Gene Deletions



Fluorescence in situ hybridization

RT-PCR



Fig. 4 Expression of IFN- γ gene in peripheral blood T cells of renal allograft recipients. MW denotes the molecular weight marker (100 bp ladder, Invitrogen). RAK-S: lanes 2,5; RAK-V: lane 1; Control: lanes 3,4.

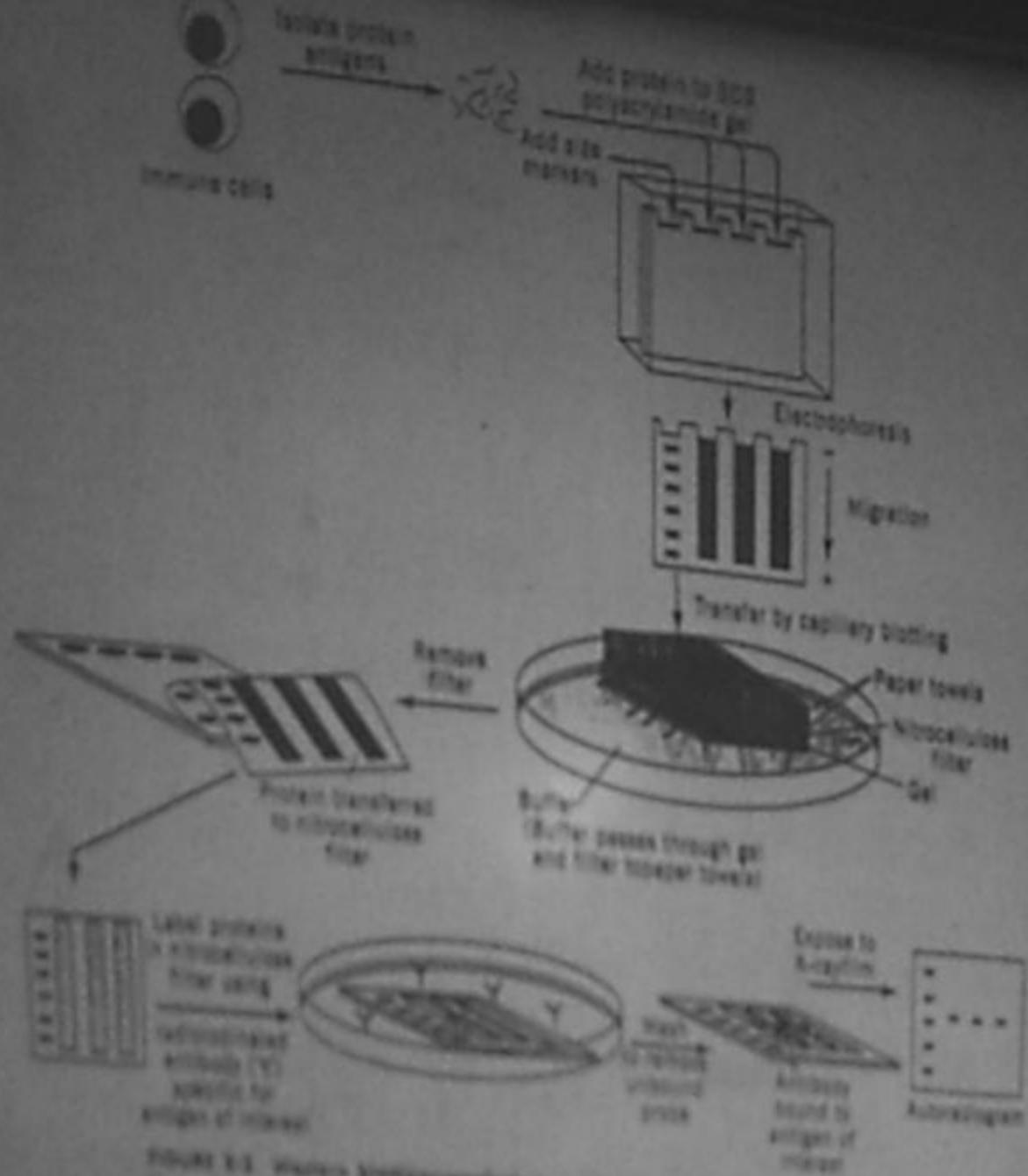


FIGURE 3-3. Western blotting—separating proteins by gel electrophoresis and blotted to an insoluble membrane filter. The protein is applied to a polyacrylamide gel and separated by size and isozyme. Proteins from lysing various components are loaded onto a protein blotter. The blotter passes through gel and filter toward towels. The protein is transferred to a nitrocellulose filter. The process is completed (see Fig. 3-2). The protein immunologically reacts on the nitrocellulose filter. A radioactive antibody specific for protein of interest is incubated with the filter for 1 h. The antibody binds to the specific protein. In the final detection procedure a solution of I^{32} is added and the filter is exposed to X-ray film. An autoradiogram will appear with bands on the film if the antibody is specific to the specific protein.