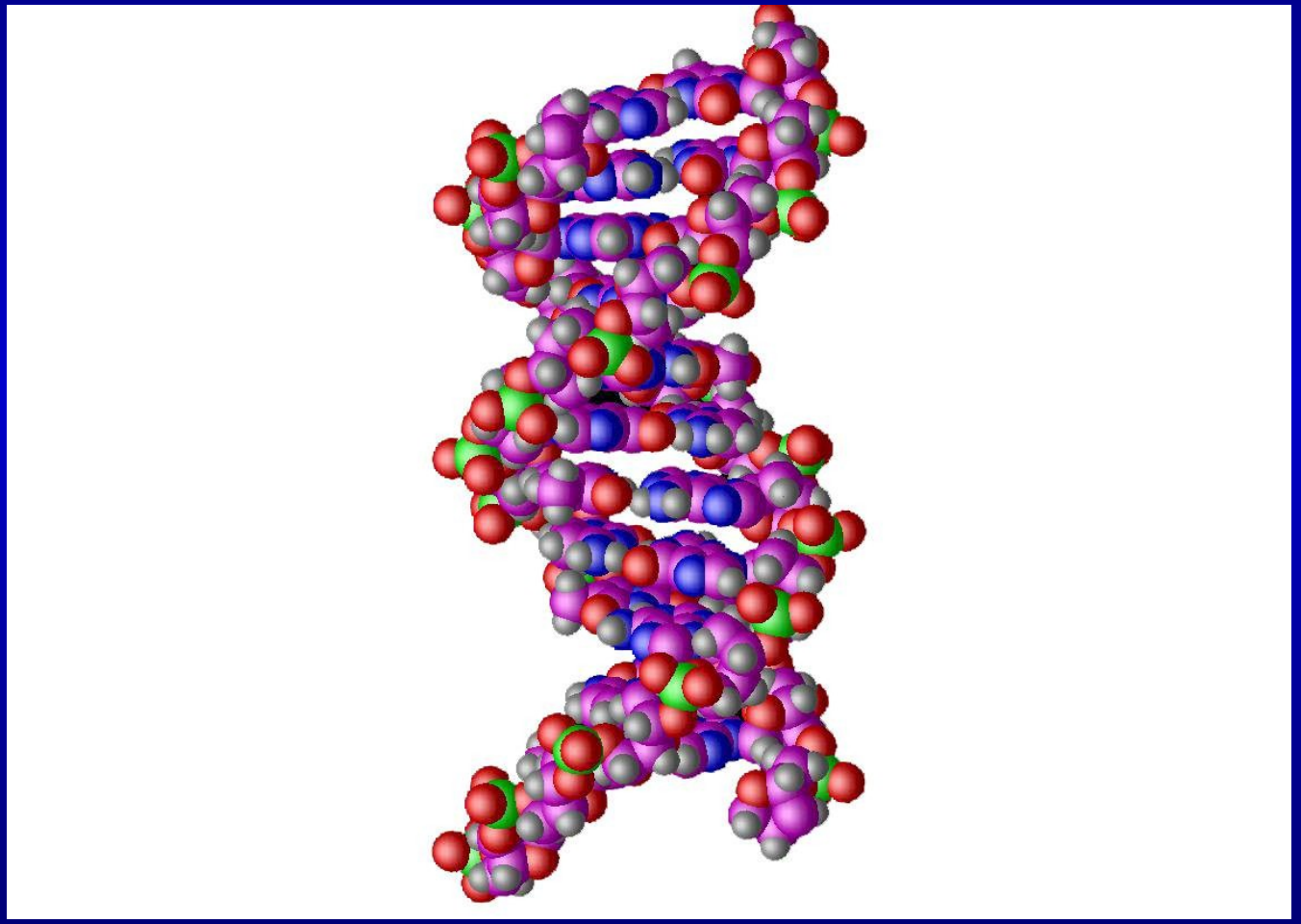
# Rapid antigen testing and molecular methods in virology



# Molecular methods

- Dot blot
- Southern blot
- Northern blot
- In situ hybridization
- PCR
- DNA Sequencing

# Molecular Methods

| Technique | Purpose | Clinical Examples |
|---|---|---|
| RFLP | Comparison of DNA | Molecular epidemiology, HSV-1 strains |
| DNA electrophoresis | Comparison of DNA | Viral strain differences (up to 20,000 bases) |
| Pulsed-field gel electrophoresis | Comparison of DNA (large pieces of DNA) | Streptococcal strain comparisons |
| In situ hybridization | Detection and localization of DNA sequences in tissue | Detection of nonreplicating DNA virus (e.g., cytomegalovirus, human papillomavirus) |
| Dot blot | Detection of DNA sequences in solution | Detection of viral DNA |
| Southern blot | Detection and characterization of DNA sequences by size | Identification of specific viral strains |
| Northern blot | Detection and characterization of RNA sequences by size | Identification of specific viral strains |
| PCR | Amplification of very dilute DNA samples | Detection of DNA viruses |
| RT-PCR | Amplification of very dilute RNA samples | Detection of RNA viruses |
| Real-time PCR | Quantification of very dilute DNA and RNA samples | Quantitation of HIV genome: virus load |
| Branched-chain DNA | Amplification of very dilute DNA or RNA samples | Quantitation of DNA and RNA viruses |
| Antibody capture solution hybridization DNA assay | Amplification of very dilute DNA or RNA samples | Quantitation of DNA and RNA viruses |
| SDS-PAGE | Separation of proteins by molecular weight | Molecular epidemiology of HSV |

*DNA*, Deoxyribonucleic acid; *HIV*, human immunodeficiency virus; *HSV-1*, herpes simplex virus-1; *PCR*, polymerase chain reaction; *RFLP*, restriction fragment length polymorphism; *RNA*, ribonucleic acid; *RT-PCR*, reverse transcriptase polymerase chain reaction; *SDS-PAGE*, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

# Molecular methods

- A **Southern blot** is a method used in molecular biology for detection of a specific DNA sequence in DNA samples. Southern blotting combines transfer of electrophoresis-separated DNA fragments to a filter membrane and subsequent fragment detection by probe hybridization.

- The method is named after its inventor, the British biologist Edwin Southern.

- Other blotting methods (i.e., western blot, northern blot, eastern blot, southwestern blot) that employ similar principles, but using RNA or protein, have later been named in reference to Edwin Southern's name. As the technique was eponymously named, Southern blot is capitalized as is conventional for proper nouns. The names for other blotting methods may follow this convention, by analogy.

# Molecular methods

- **Nucleic acid hybridization is a method for identifying closely related nucleic acid molecules within two populations, a complex target population and a comparatively homogeneous probe population**

# A wide variety of nucleic acid hybridization assays can be used

- Numerous applications in molecular genetics involve taking an individual DNA clone and using it as a hybridization probe to screen for the presence of related sequences within a complex target of uncloned DNA or RNA.

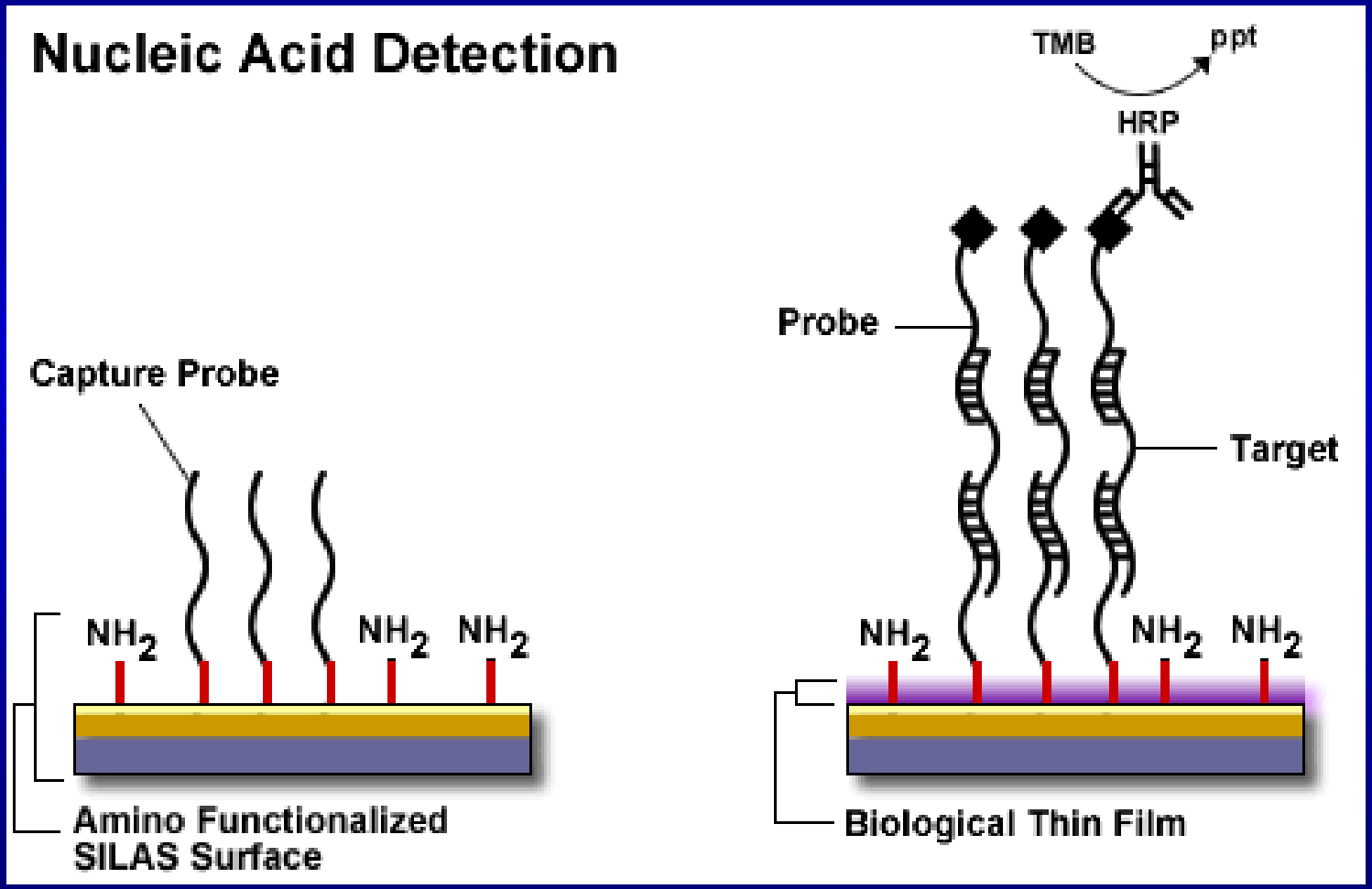
# Molecular methods

- **Dot-blot hybridization is a rapid screening method which often employs allele-specific oligonucleotide (ASO) probes to discriminate between alleles differing at a single nucleotide position**
- **Southern and Northern blot hybridizations detect target DNA and RNA fragments that have been size-fractionated by gel electrophoresis**
- **In situ hybridization usually involves hybridizing a nucleic acid probe to the denatured DNA of a chromosome preparation or the RNA of a tissue section fixed on a glass slide**

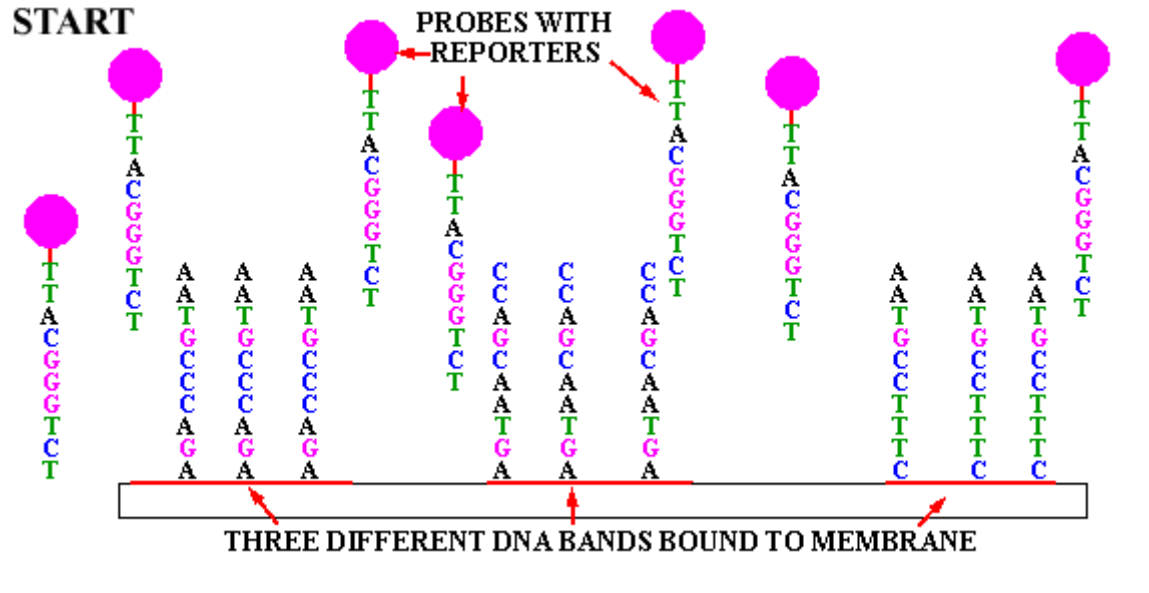
# Molecular methods

- **Nucleic acid hybridization is a method for identifying closely related nucleic acid molecules within two populations, a complex target population and a comparatively homogeneous probe population**

# Dot blot

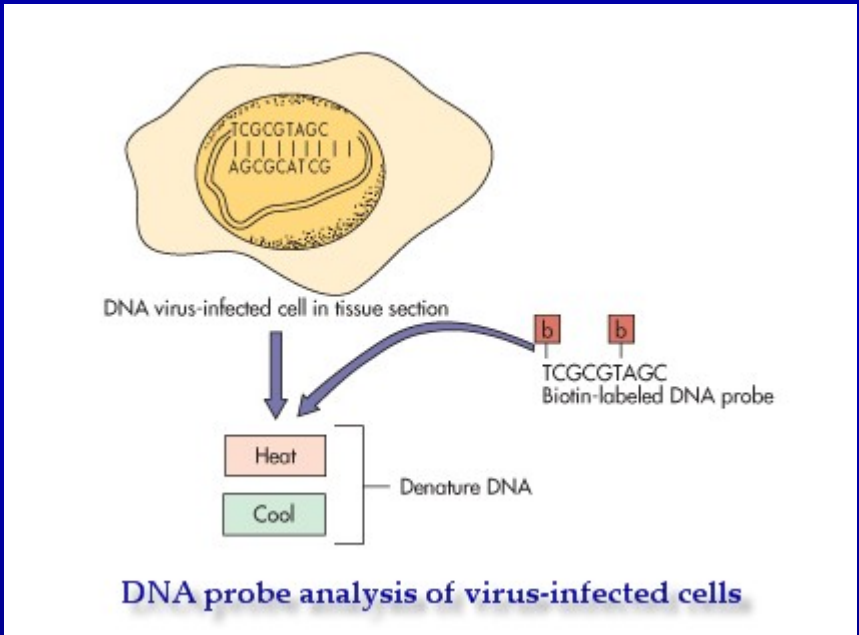

Nucleic Acid Detection
TMB
ppt
HRP
Probe
Target
Capture Probe
NH2
NH2
NH2
NH2
NH2
NH2
Amino Functionalized
SILAS Surface
Biological Thin Film

# Southern blot

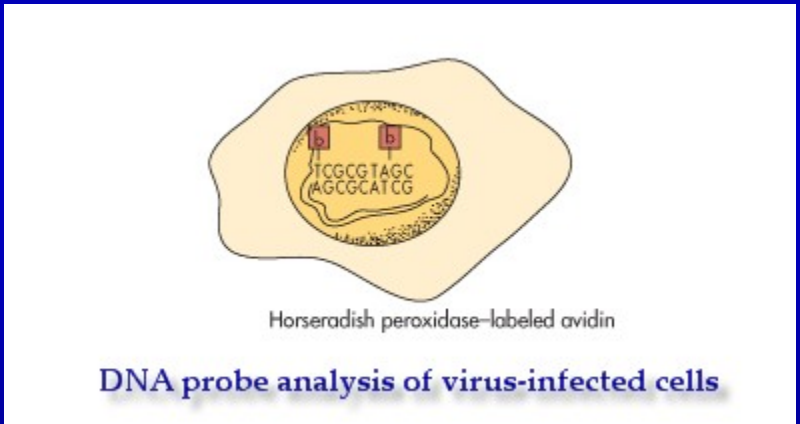


# DNA Hybridization

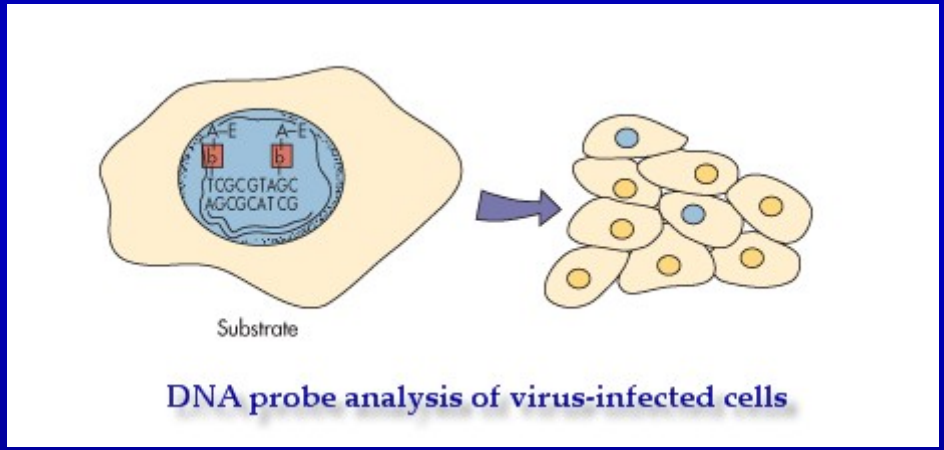
# In situ hybridization *(1)*



# In situ hybridization *(2)*

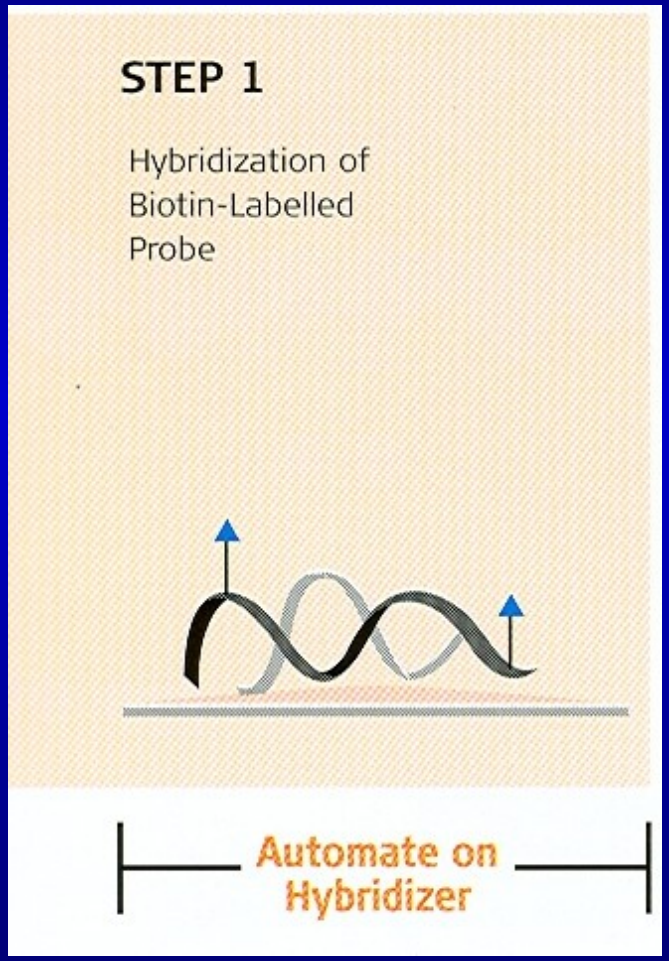


# In situ hybridization *(3)*

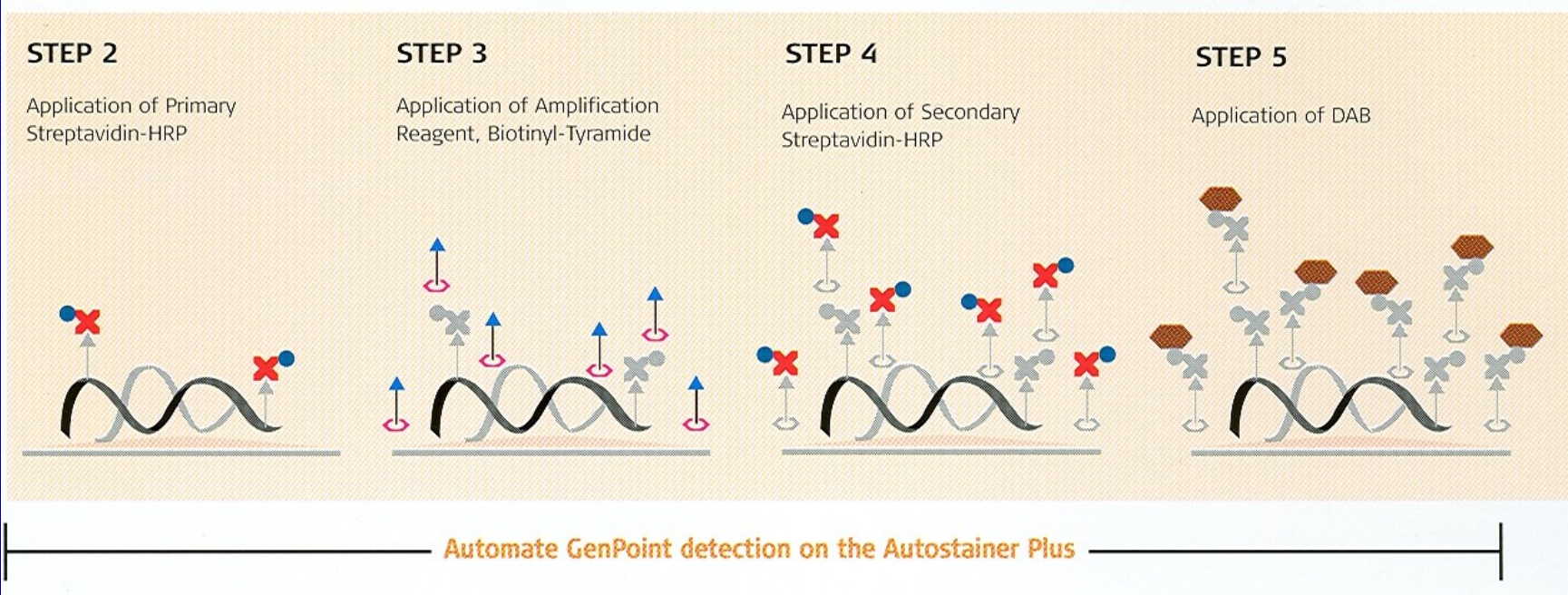



DNA probe analysis of virus-infected cells

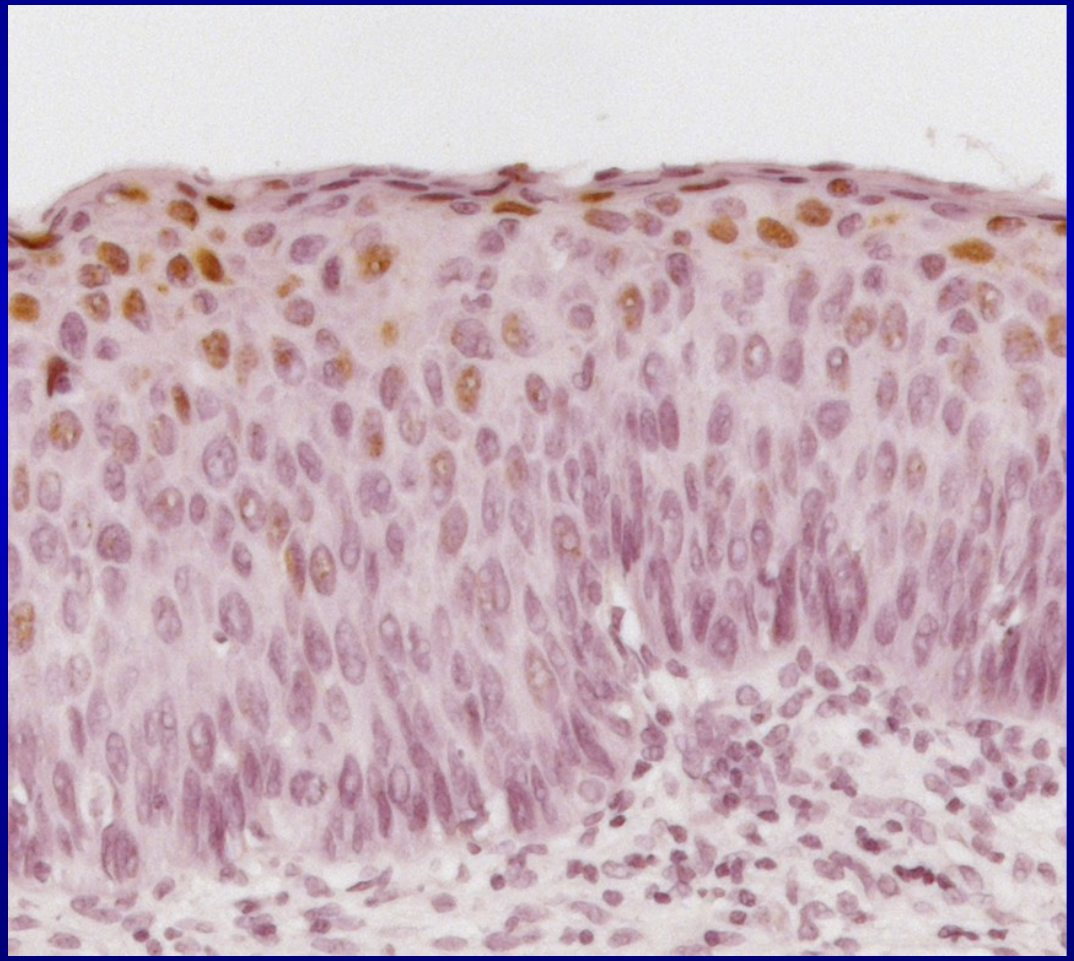
# In situ hybridization



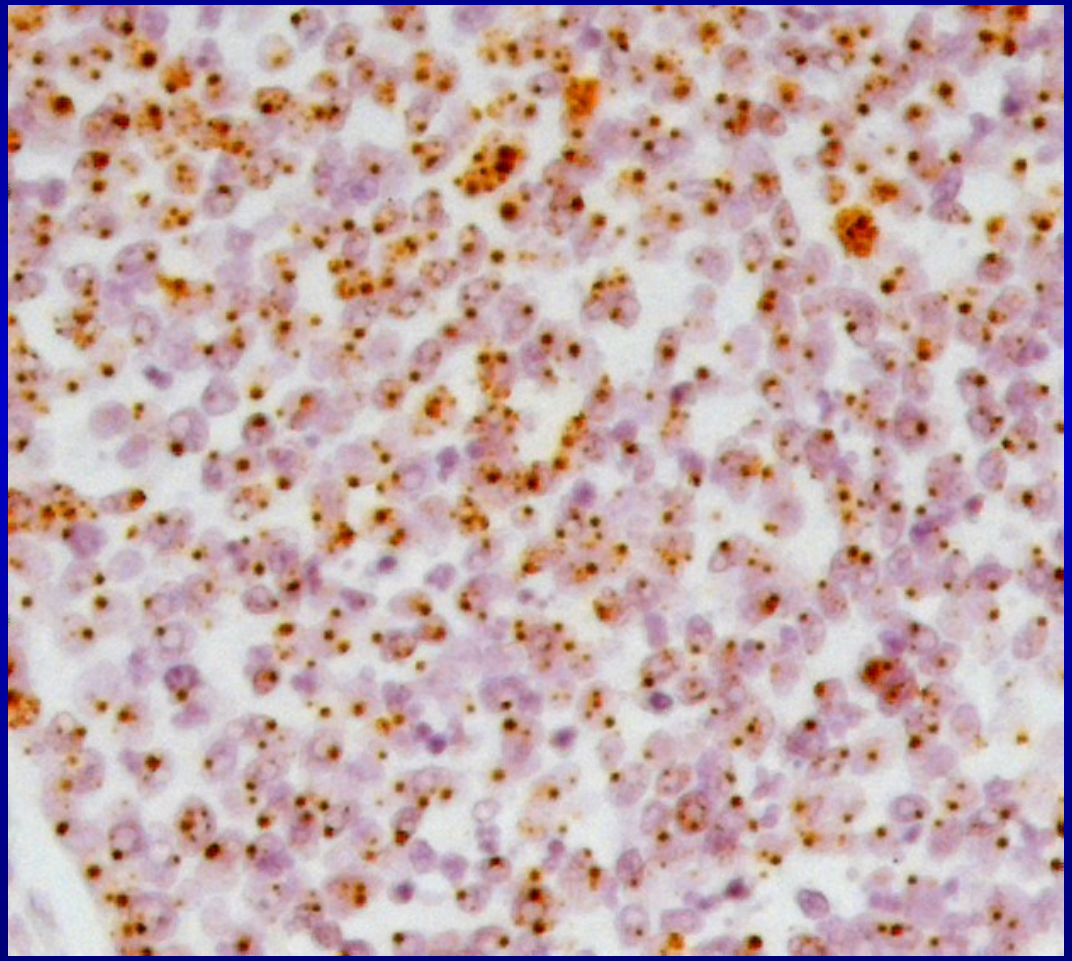
# In situ hybridization



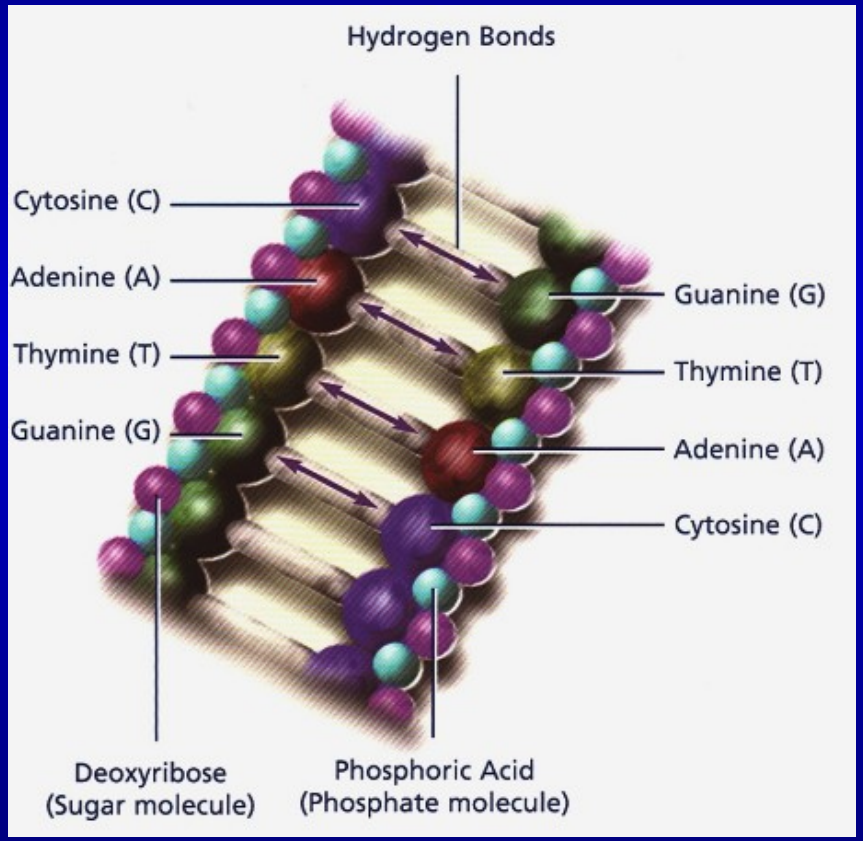
# In situ hybridization



# In situ hybridization

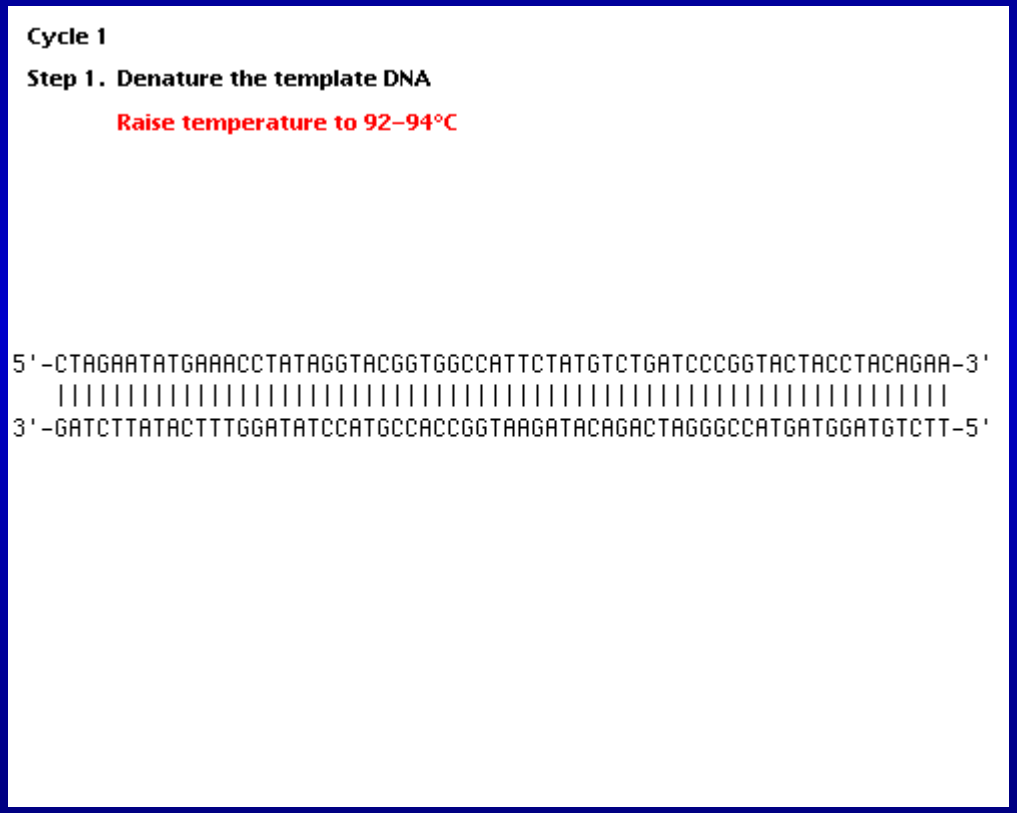


# Polymerase chain reaction

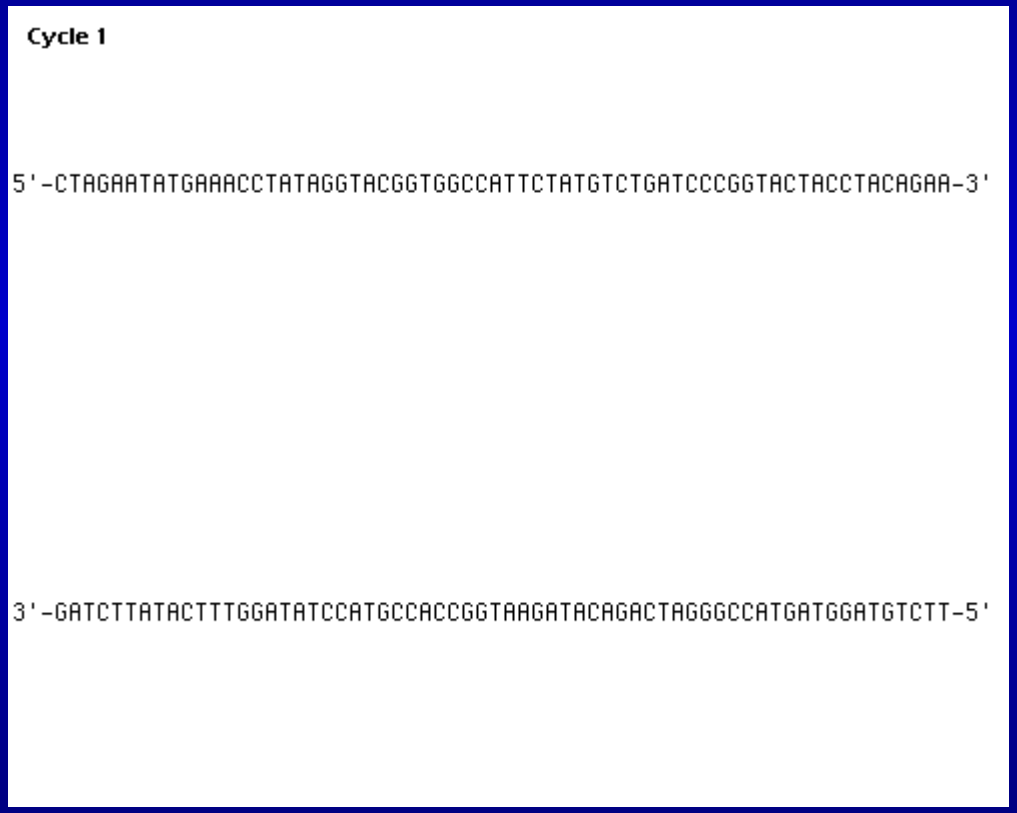



- PCR
- RT-PCR

# Polymerase chain reaction



# Polymerase chain reaction



# Polymerase chain reaction

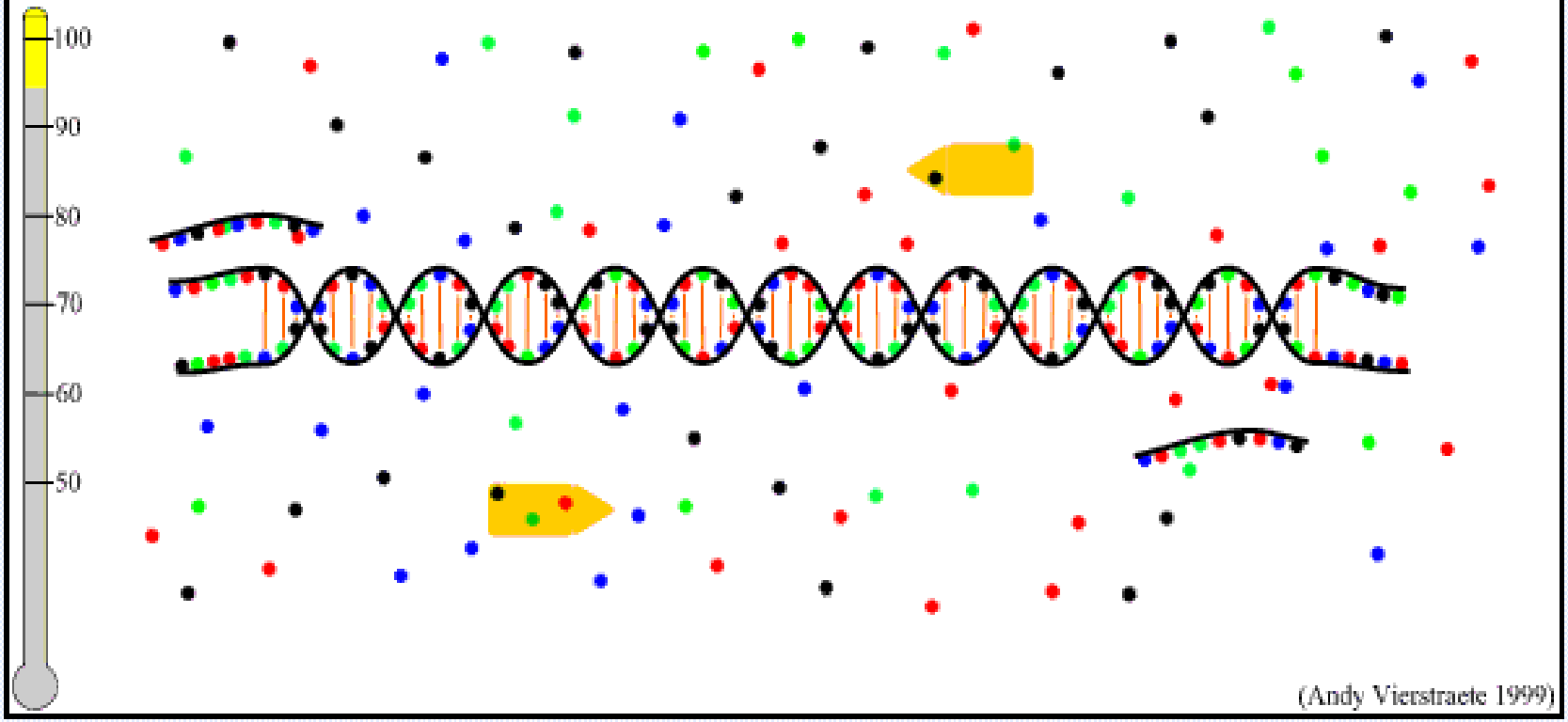
# Polymerase chain reaction

**Choosing primers**

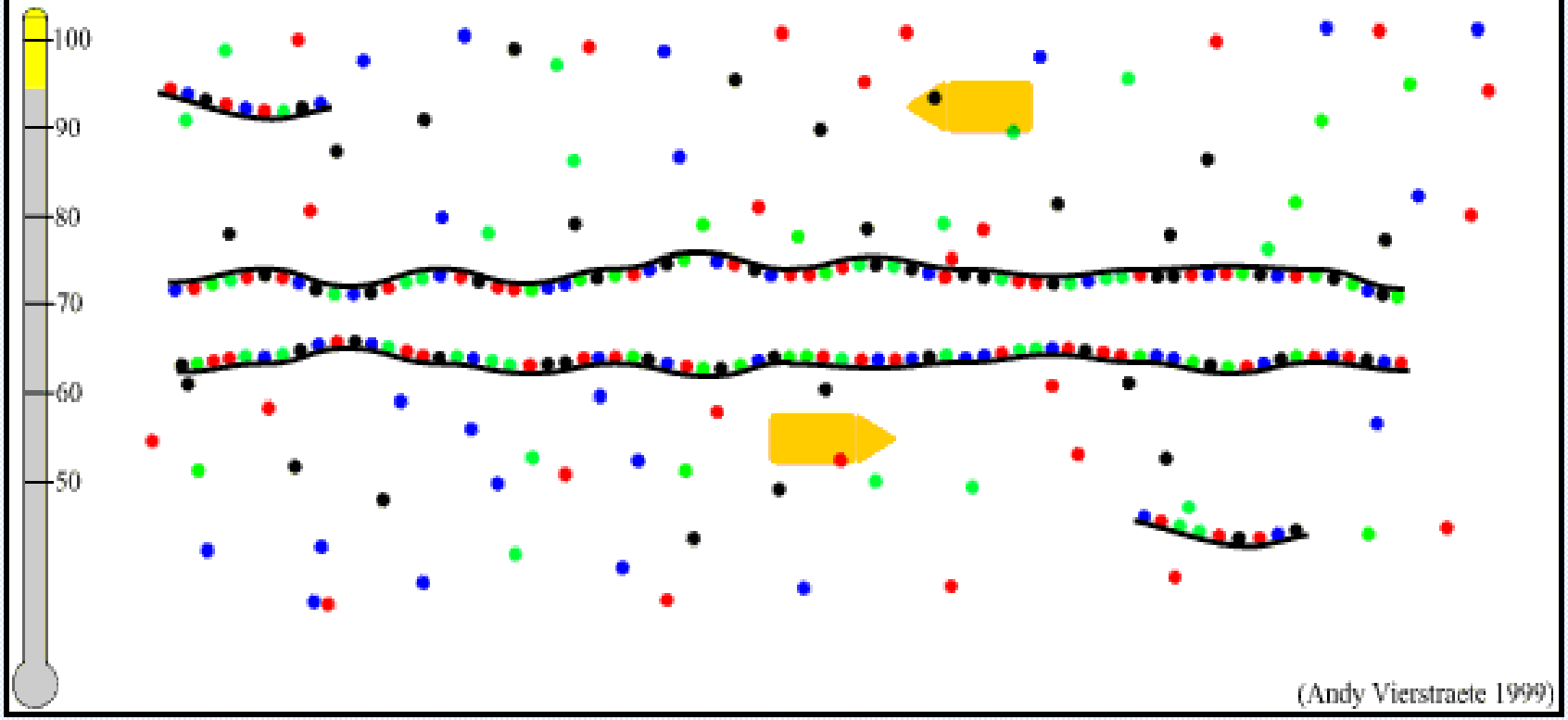
Because DNA polymerase can add subunits (nucleotides) only to the 3' end of the primer, the primer has to be situated "upstream" – i.e., more 5' than – the sequence to be copied:

```
5'-CTAGAATATGAAACCTATAGGTACGGTGGCCATTCTATGTCTGATCCCGGTACTACCTACAGAA-3'
   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
3'-GATCTTATACTTTGGATATCCATGCCACCGGTAAGATACAGACTAGGGCCATGATGGATGTCTT-5'
```

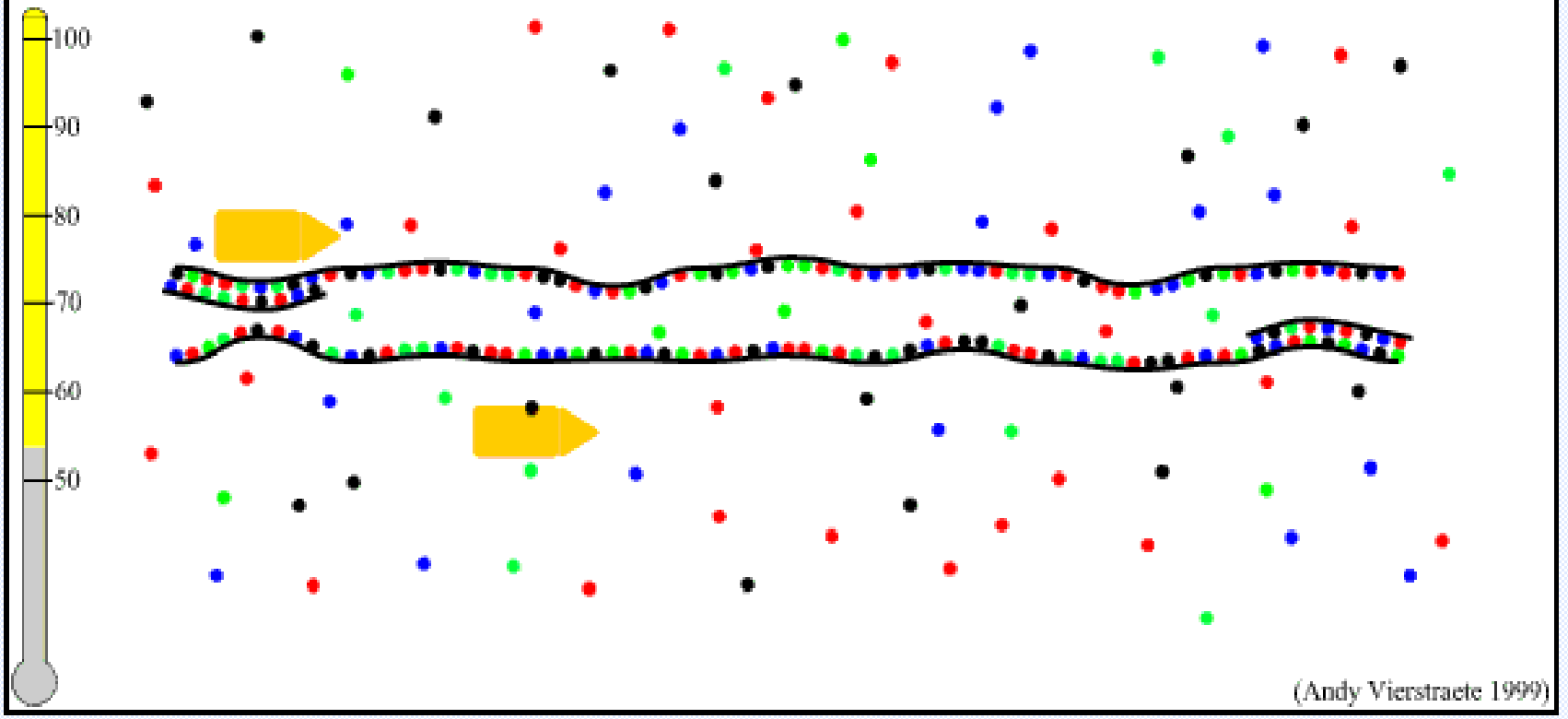
# PCR : Denaturation 94°C



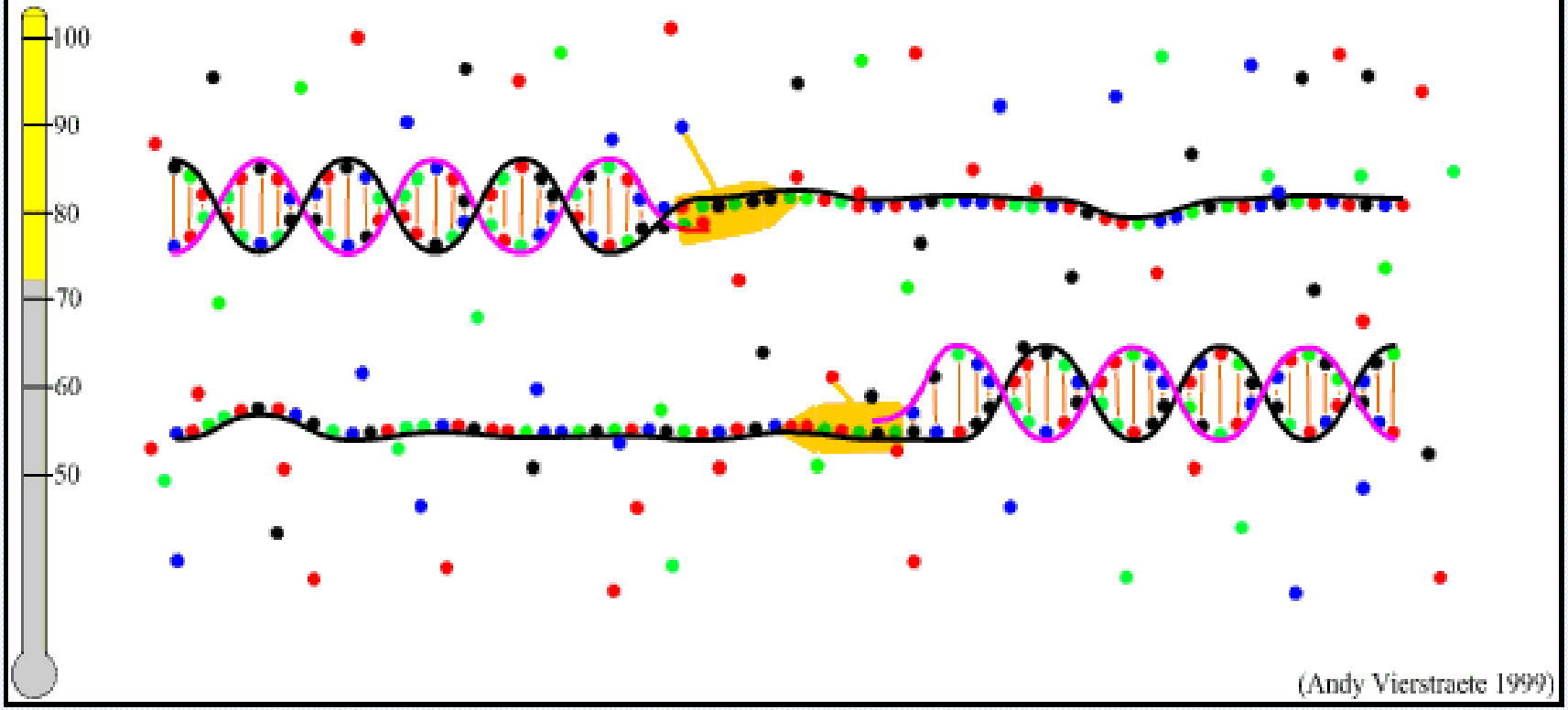
# PCR : Denaturation 94°C



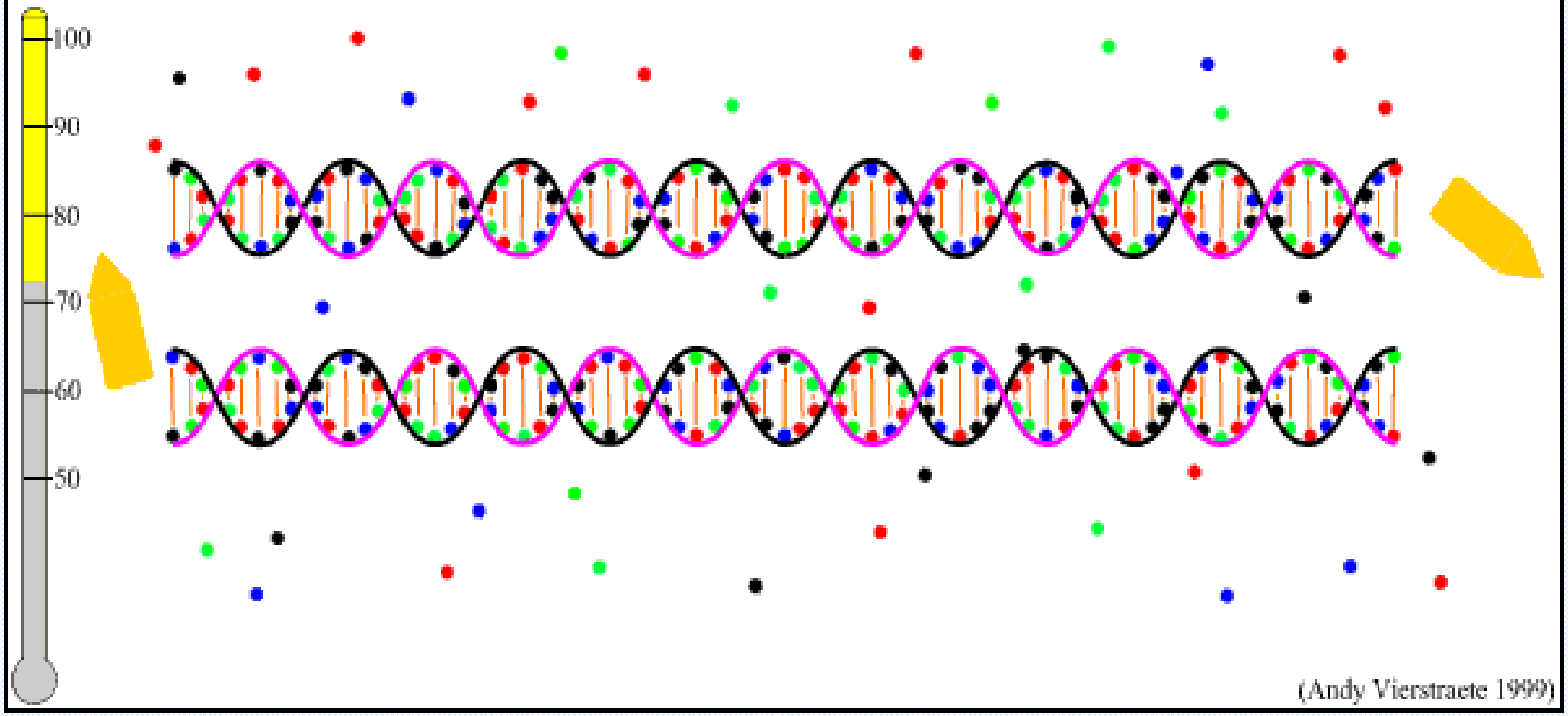
# PCR : Annealing 54°C

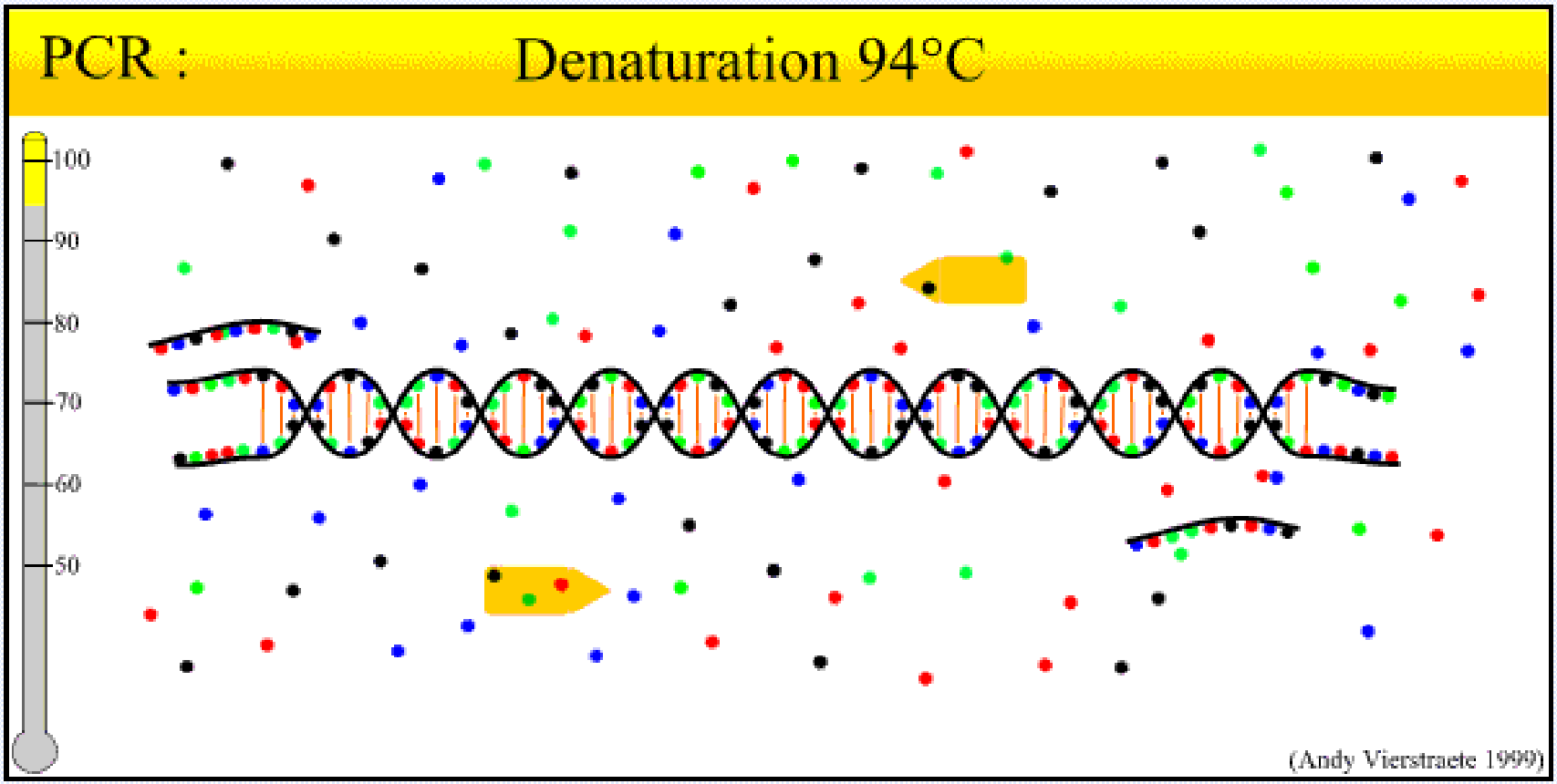


# PCR : Extension 72°C

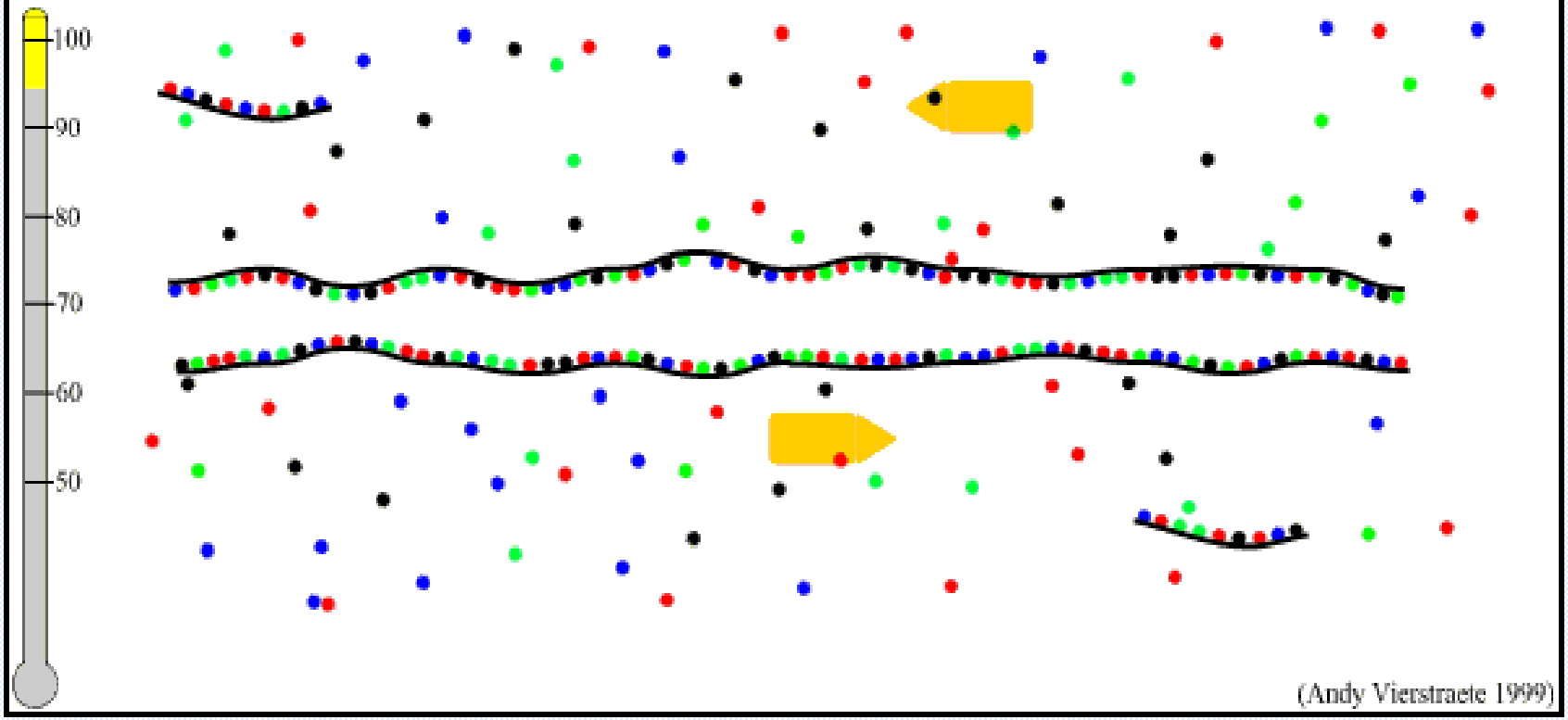


# PCR : Extension 72°C




PCR : Denaturation 94°C
100
90
80
70
60
50
(Andy Vierstraete 1999)

# PCR : Denaturation 94°C



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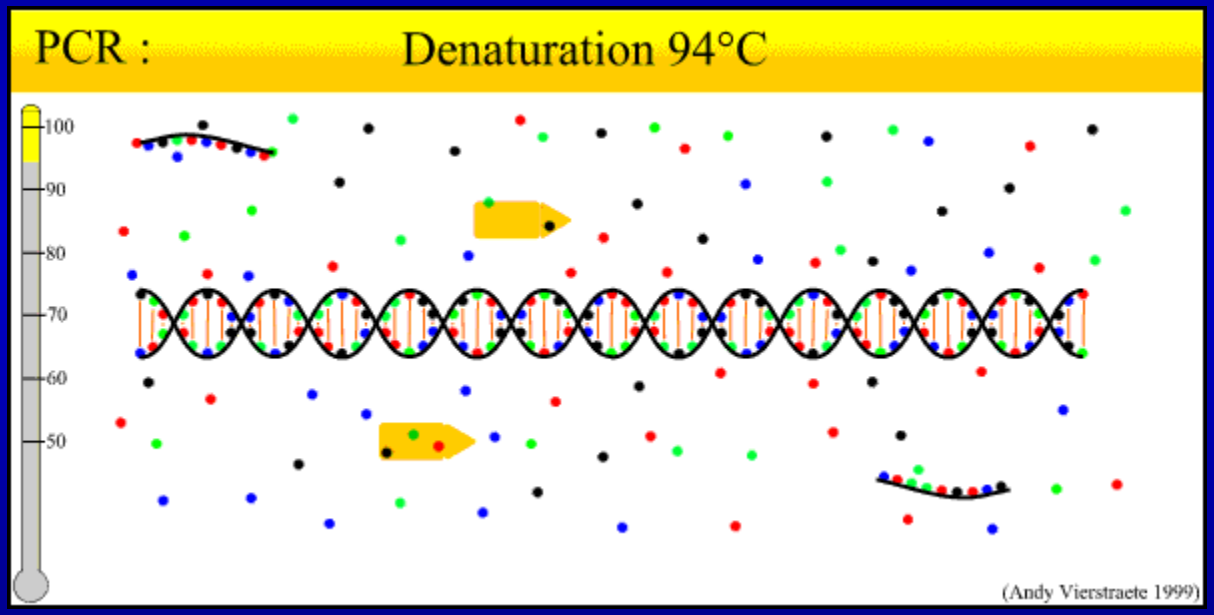
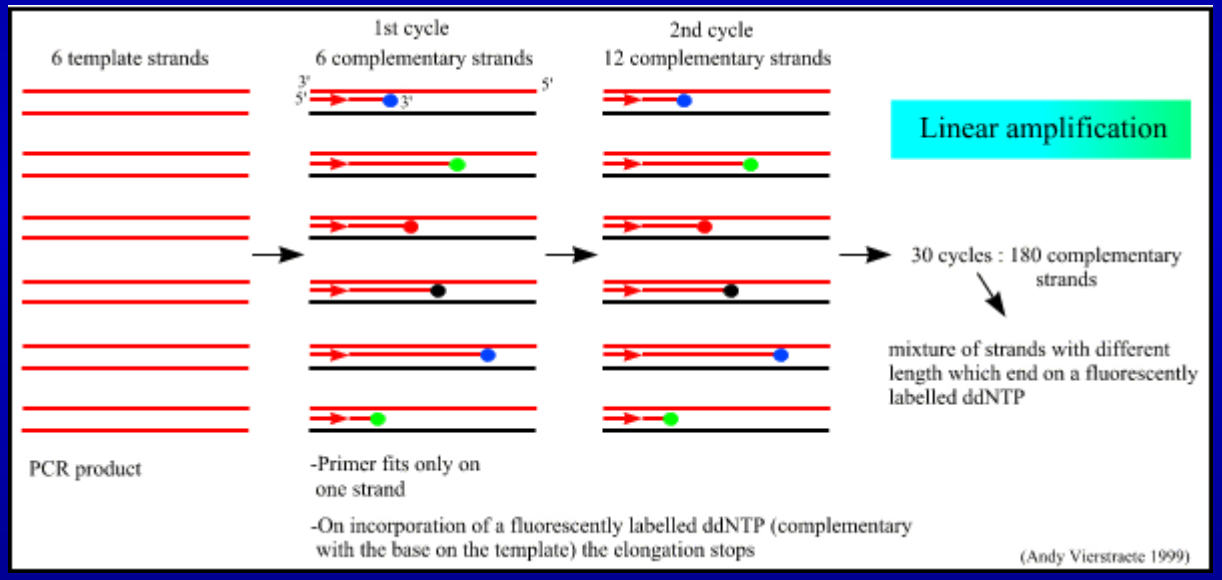


# DNA Sequencing



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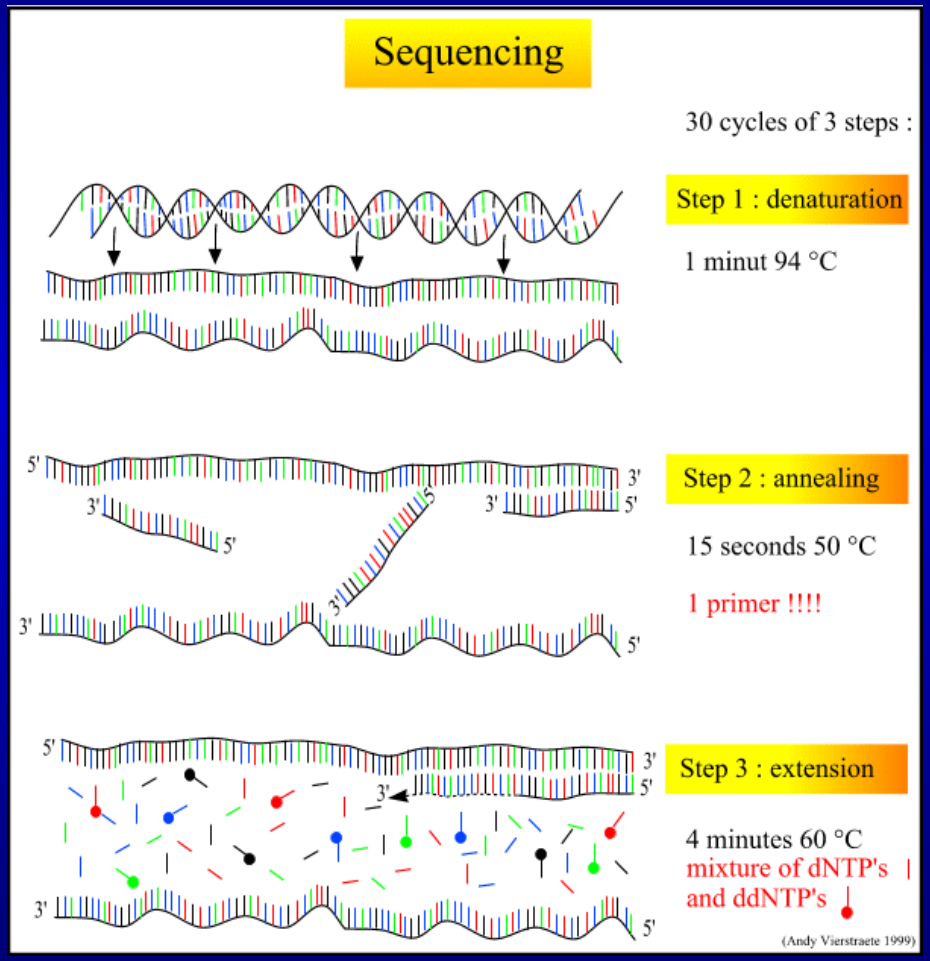
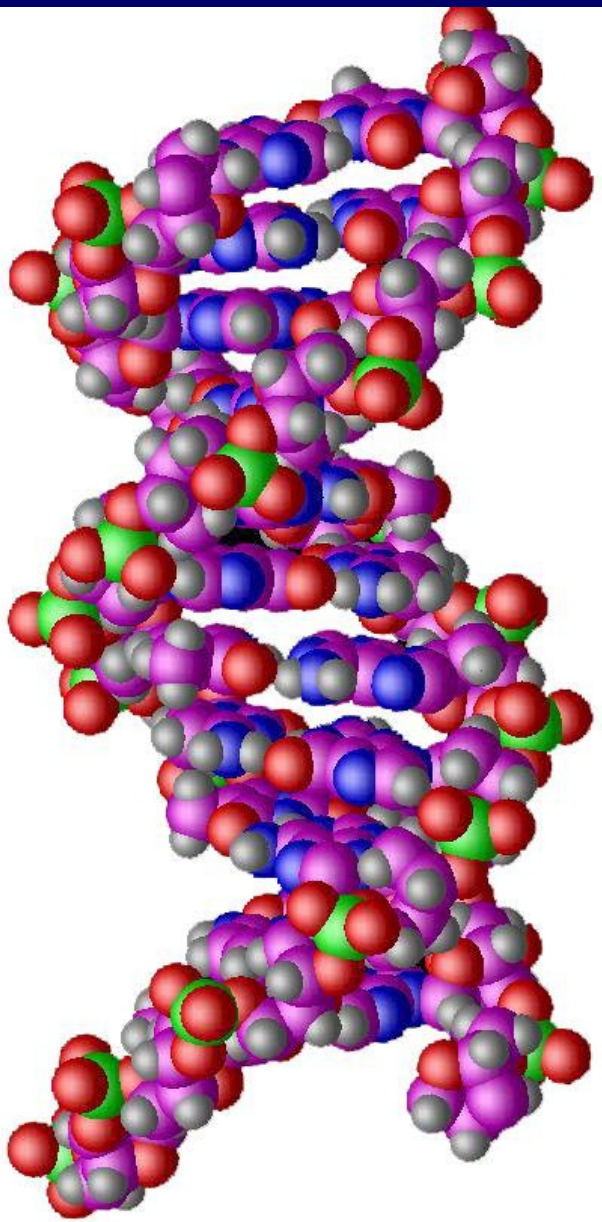



Prä - PCR
Biogefährdung
Biohazard
Biorisque

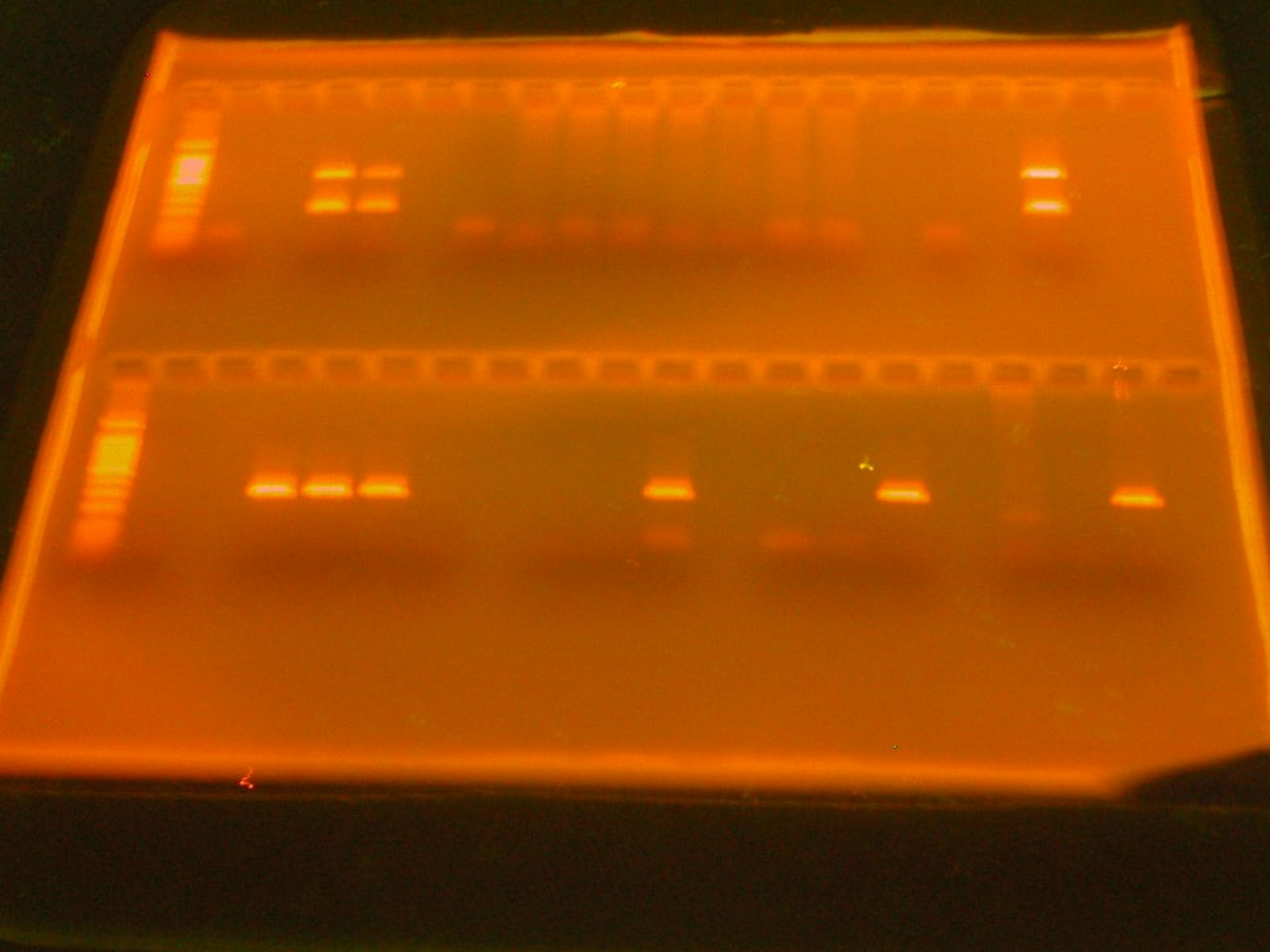



Reinst- Raum
kein Zutritt!

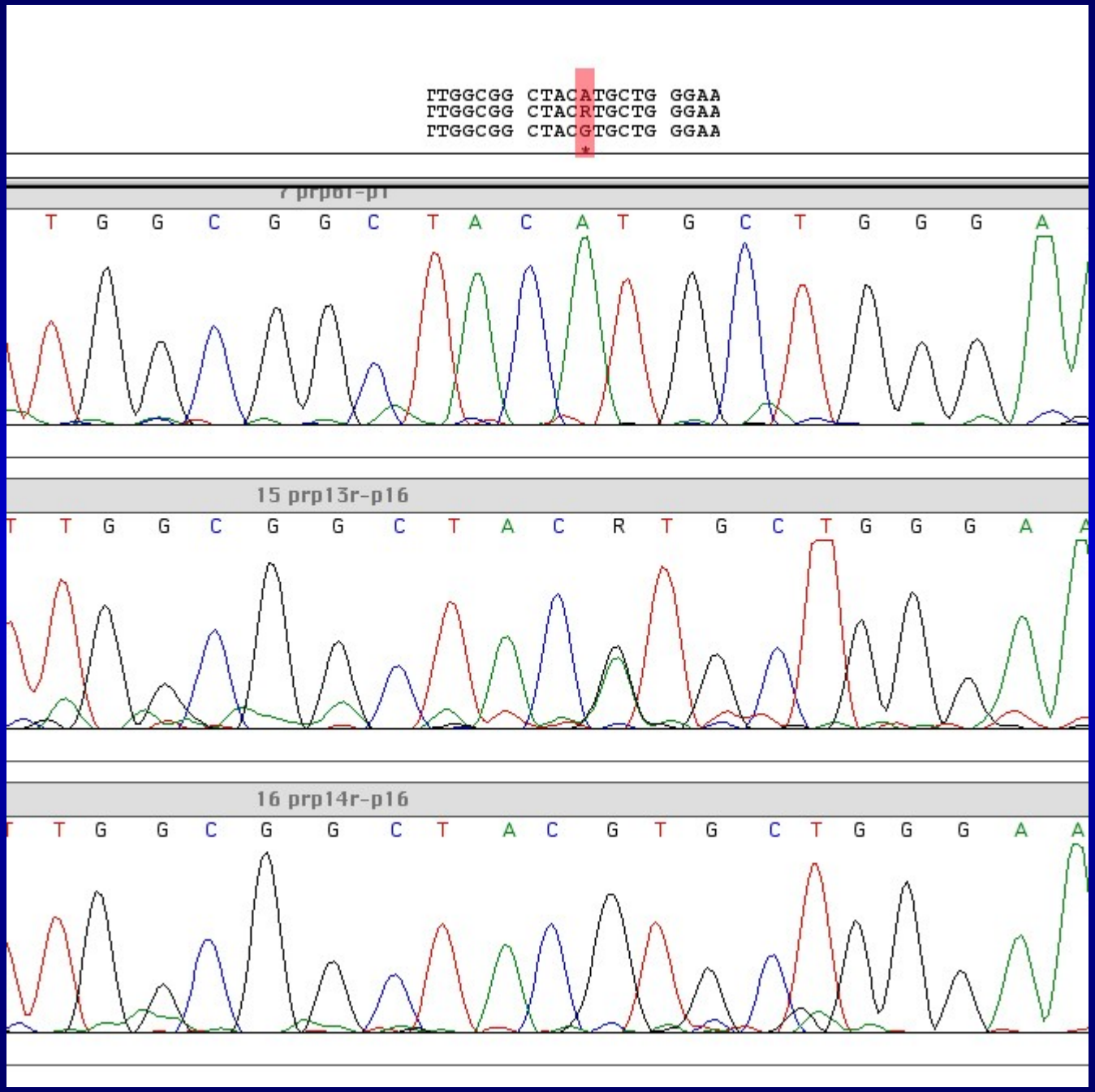

Amplifikation
Sequenzierung


ritips
UNO II
Biometra


eppendorf
Biometra
PCR

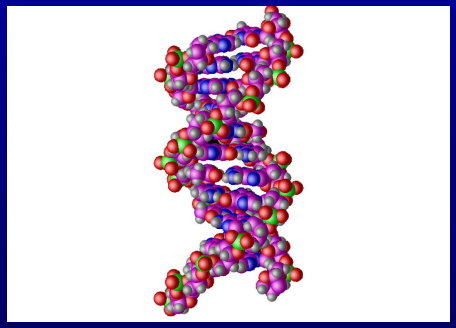



PHILIPS
power on
cycling
run completed
HEWLETT PACKARD
HEWLETT PACKARD


TTGGCGG CTACATGCTG GGAA
TTGGCGG CTACRTGCTG GGAA
TTGGCGG CTACGTGCTG GGAA
*
7 prp6r-p1
T G G C G G C T A C A T G C T G G G A
15 prp13r-p16
T T G G C G G C T A C R T G C T G G G A A
16 prp14r-p16
T T G G C G G C T A C G T G C T G G G A A

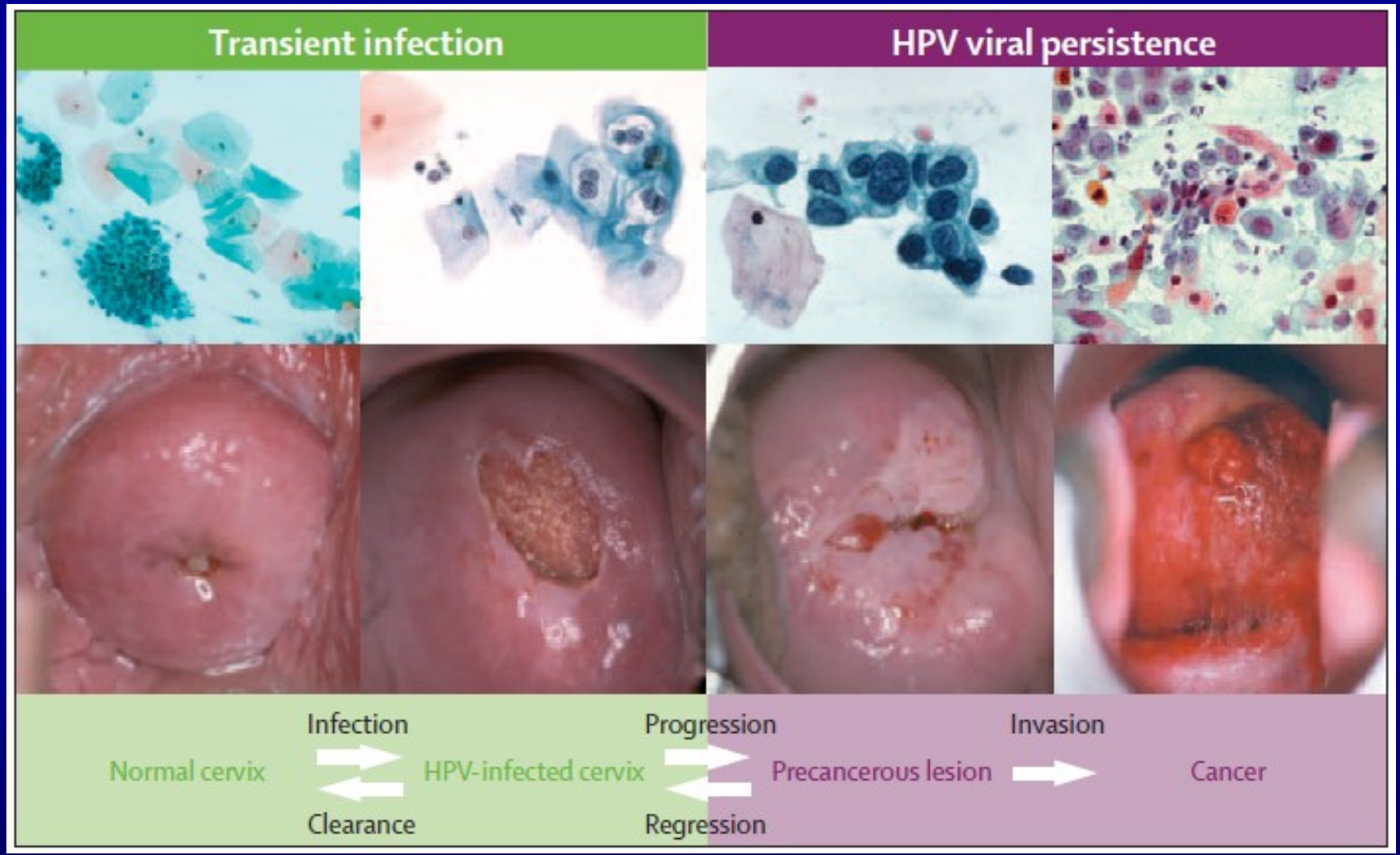
# Rapid antigen testing and molecular methods in virology

## In situ hybridization



**Exercise 3**

# Major steps in the development of cervical cancer



# HPV affinity

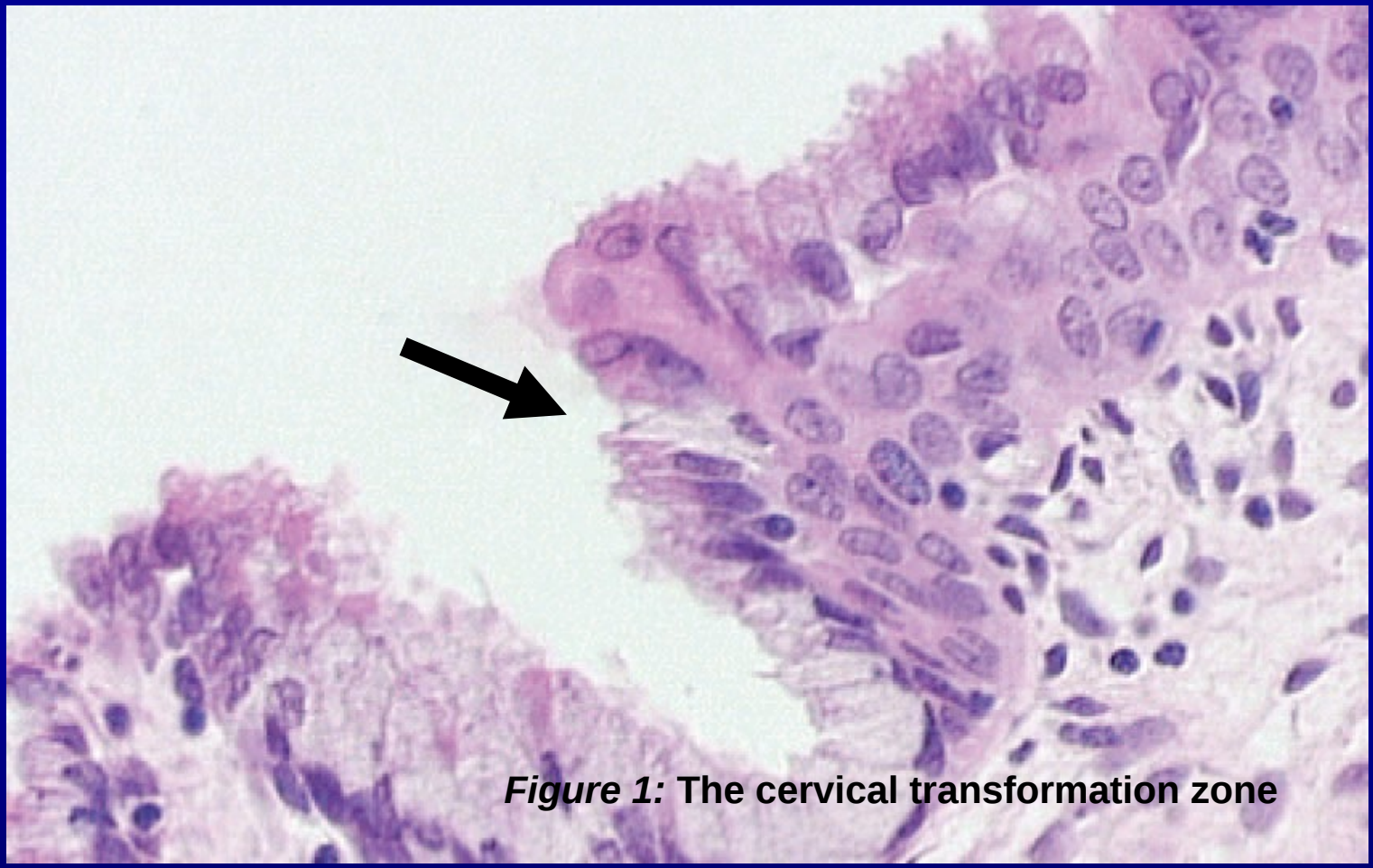
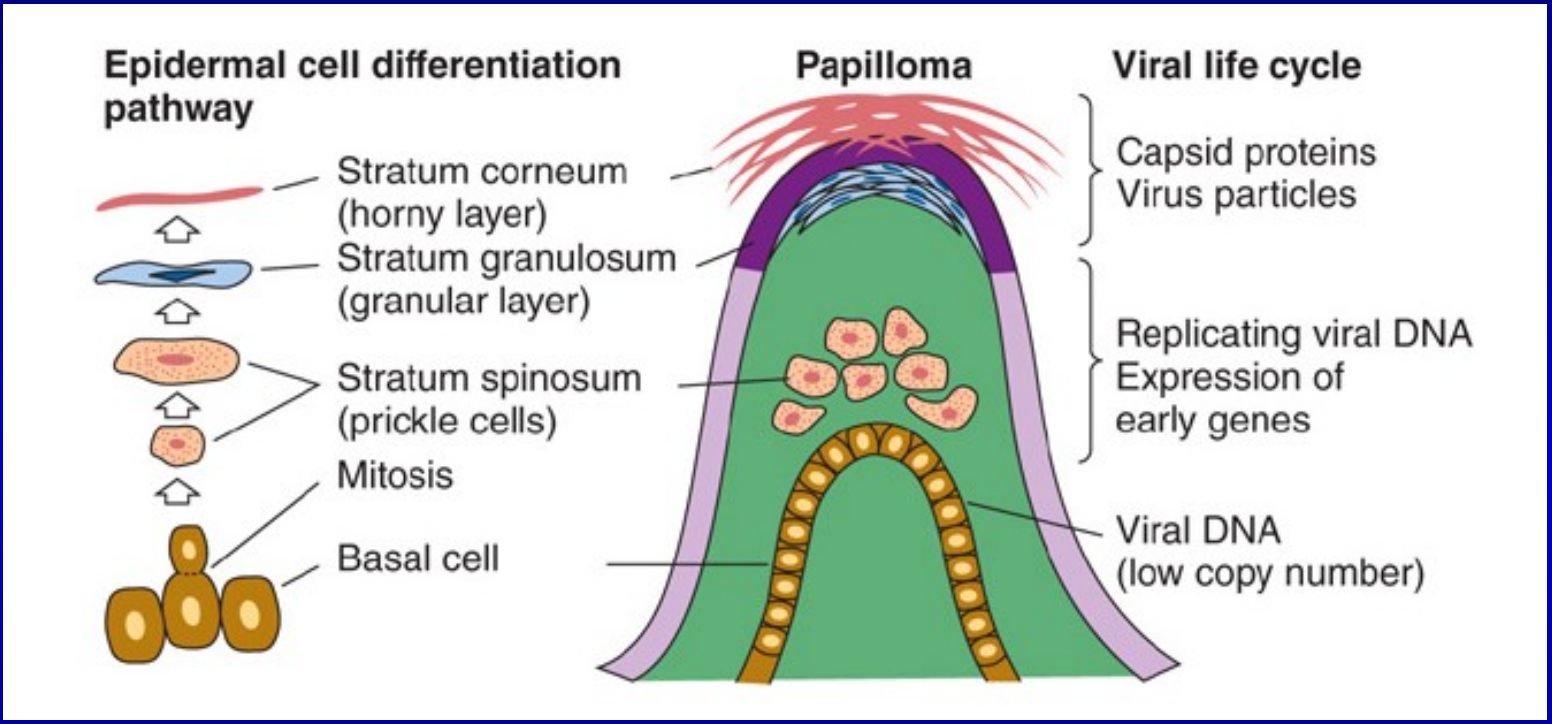


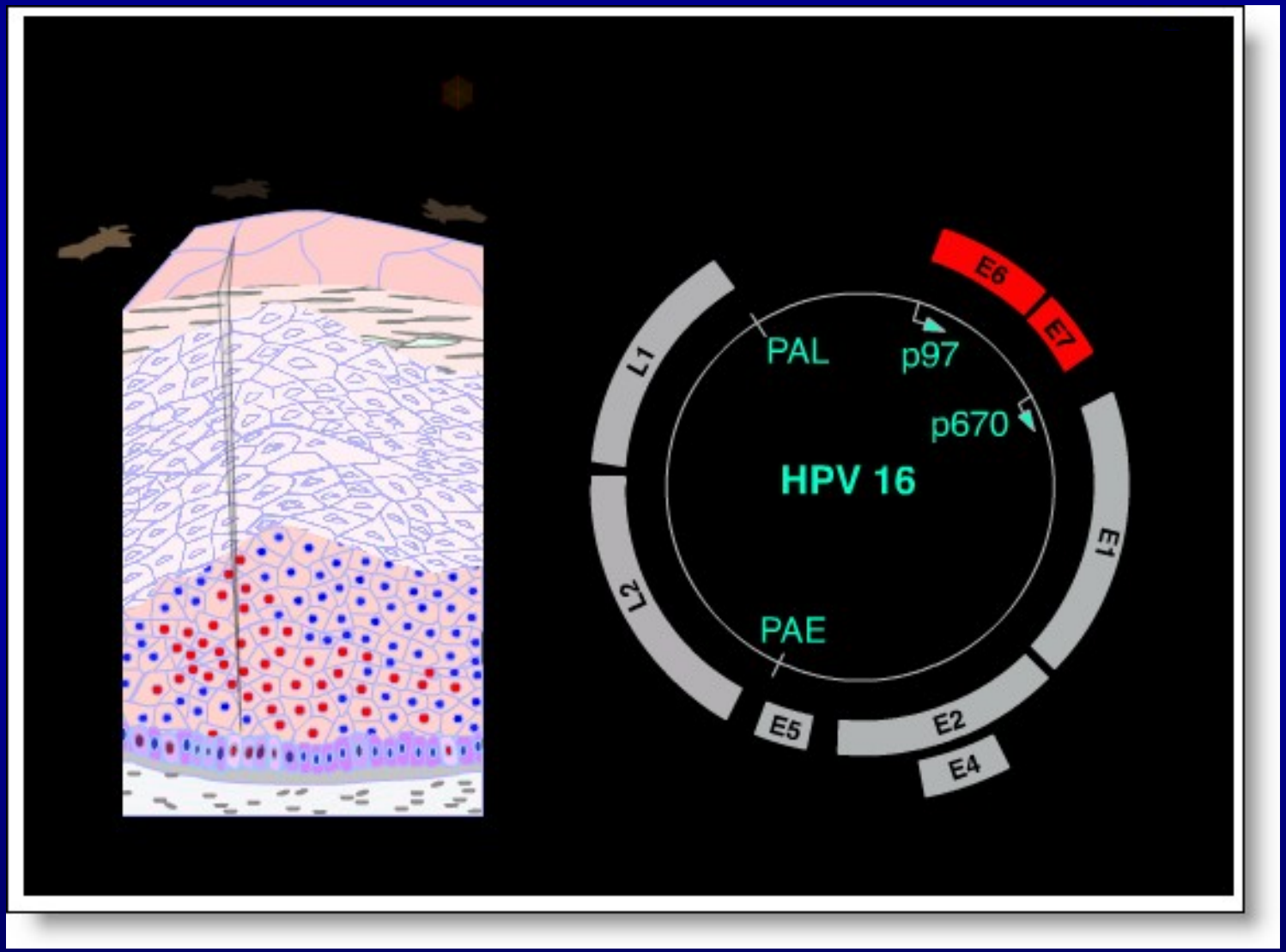
***Figure 1:*** **The cervical transformation zone**

# Papillomavirus Replication

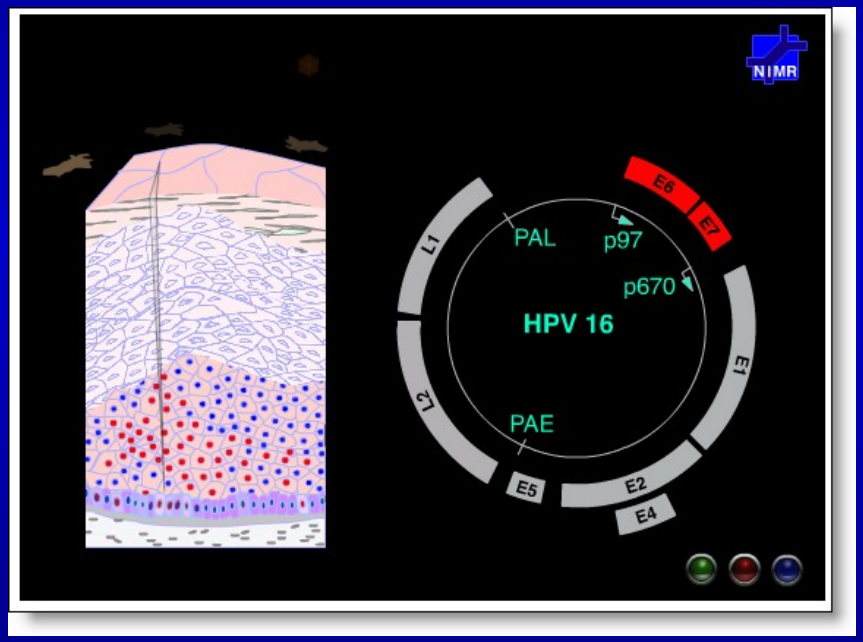



- The papillomavirus life cycle is tied to epithelial cell differentiation.
- Late events in viral replication (capsid protein synthesis and virion morphogenesis) occur only in terminally differentiated cells.

# Map of the HPV genome

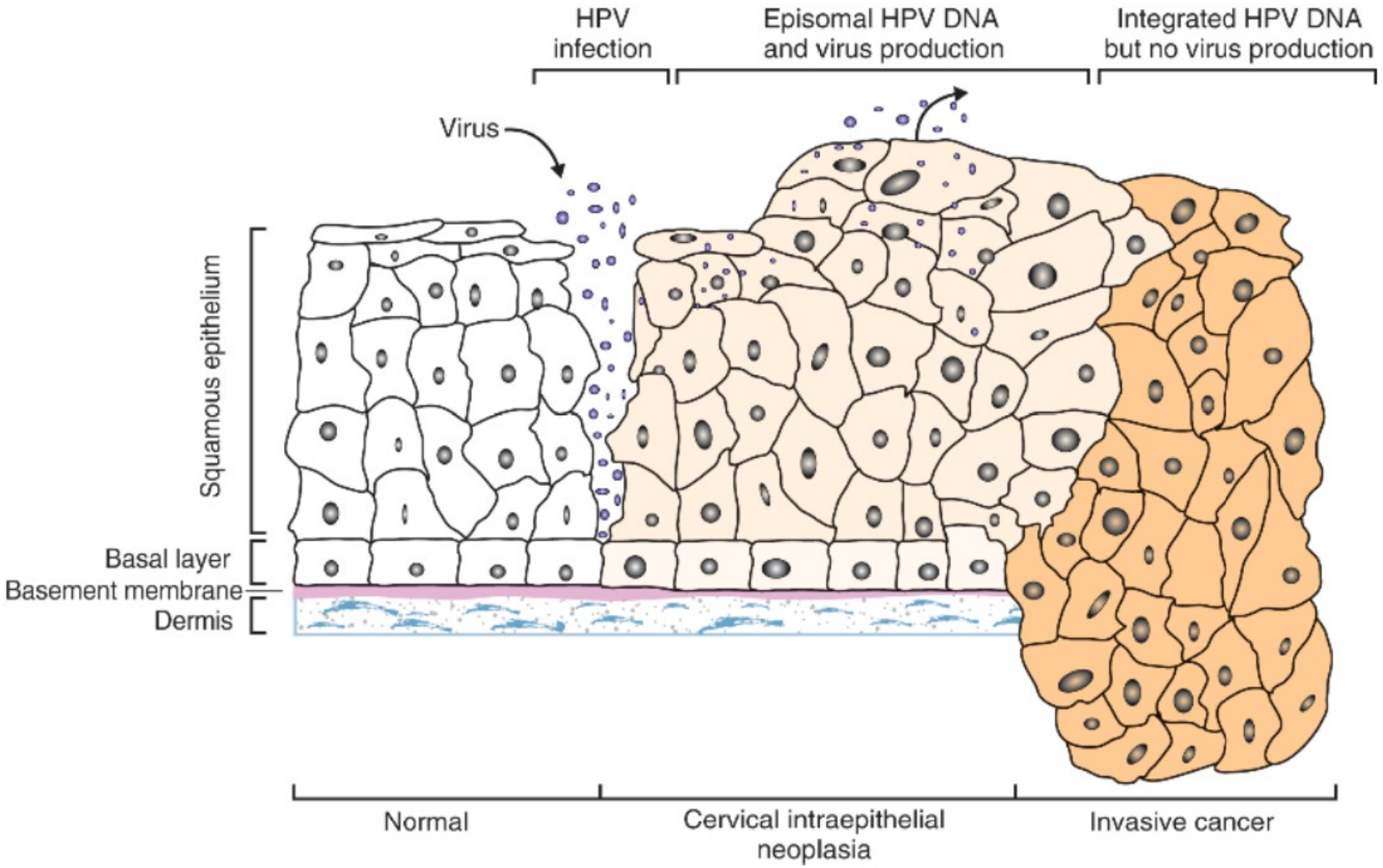


# HPV Oncogenesis

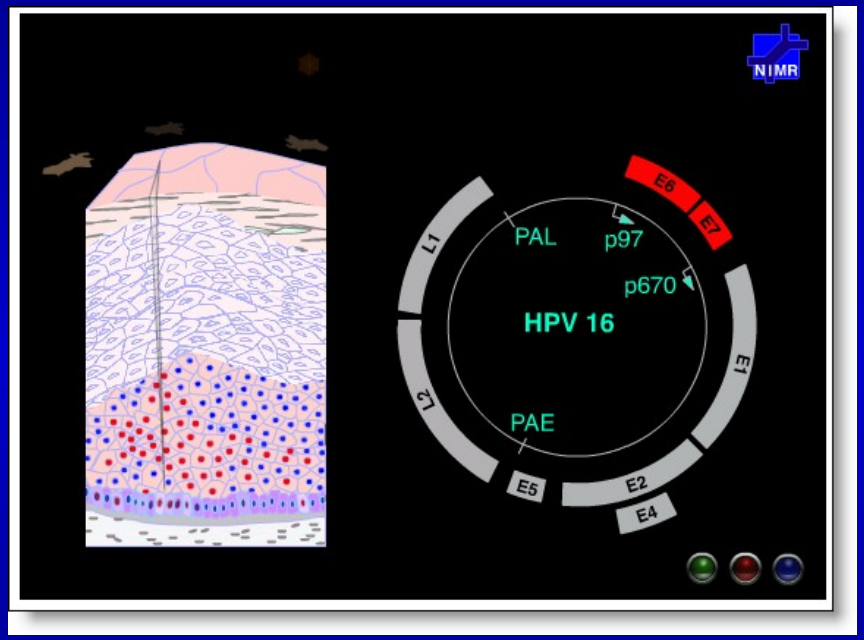



- Virusni se genom prije ugradnje u genom stanice uvijek najprije otvori, a potom izravna.
- Prekid genoma nastaje u području E2 (područje važno za inhibiciju E6 i E7).
- Posljedica prekidanja E2 je gomilanje E6 i E7 u inficiranoj stanici i njihovo vezanje na proteine tumorsupresorskih gena (p53 i RB)

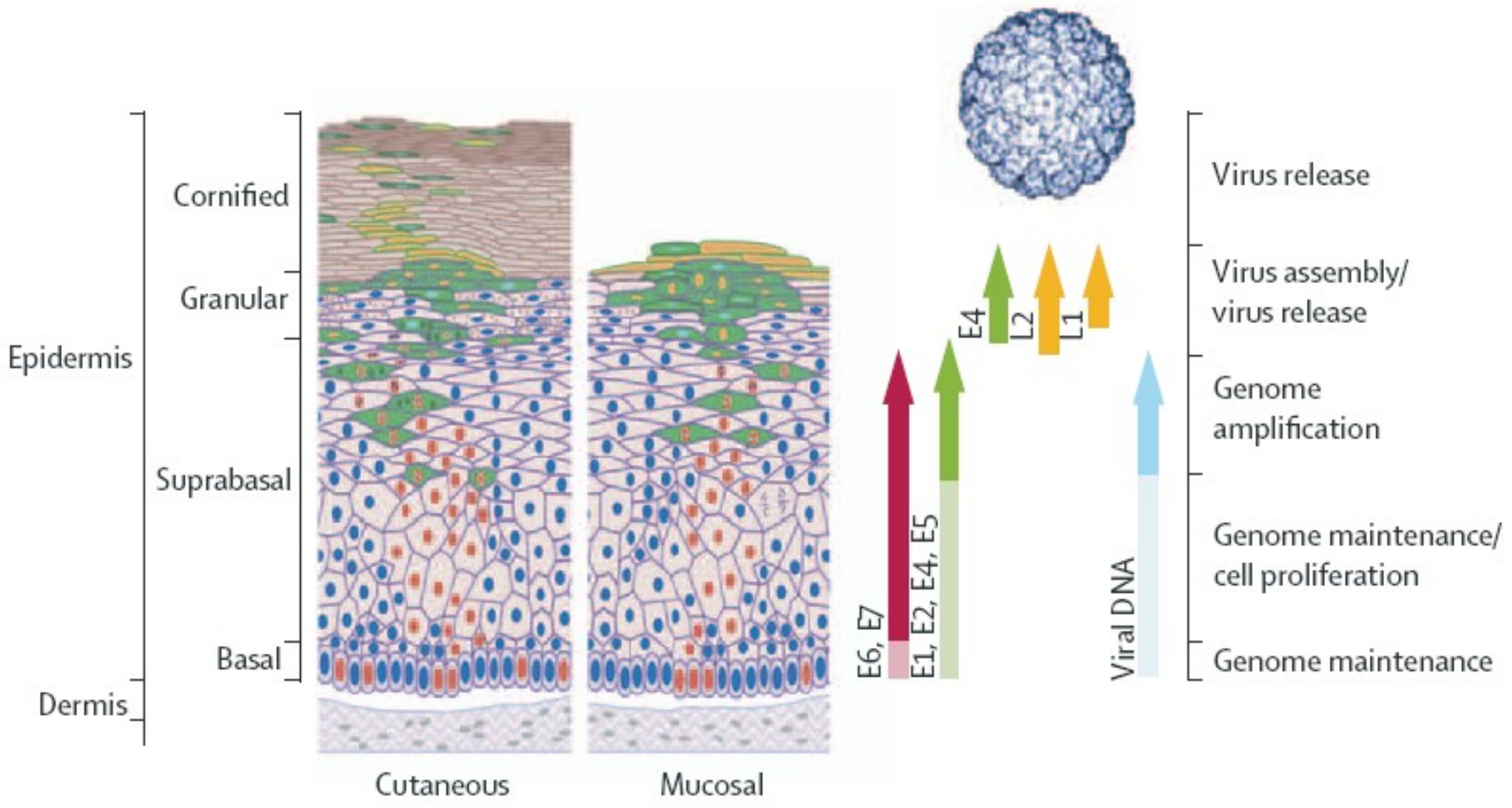
# Progression of human papilloomavirus *(HPV)*-mediated cervical carcinoma



# HPV Oncogenesis

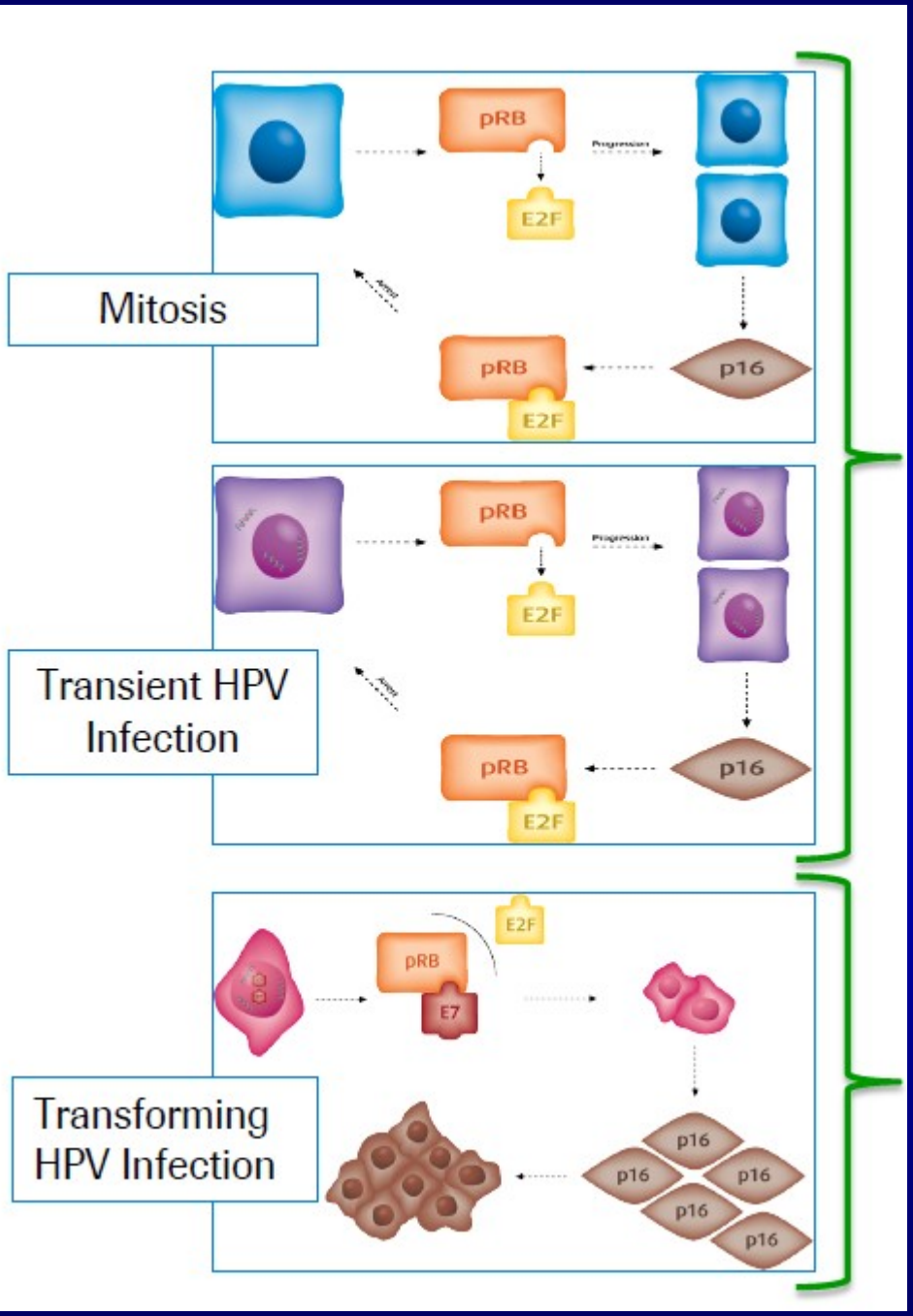



- Osim proteina E6 i E7 visokorizičnih genotipova, sposobnost spajanja na proteine p53 i RB imaju i E6 i E7 niskorizičnih tipova, ali je njihova učinkovitost stotinu puta slabija



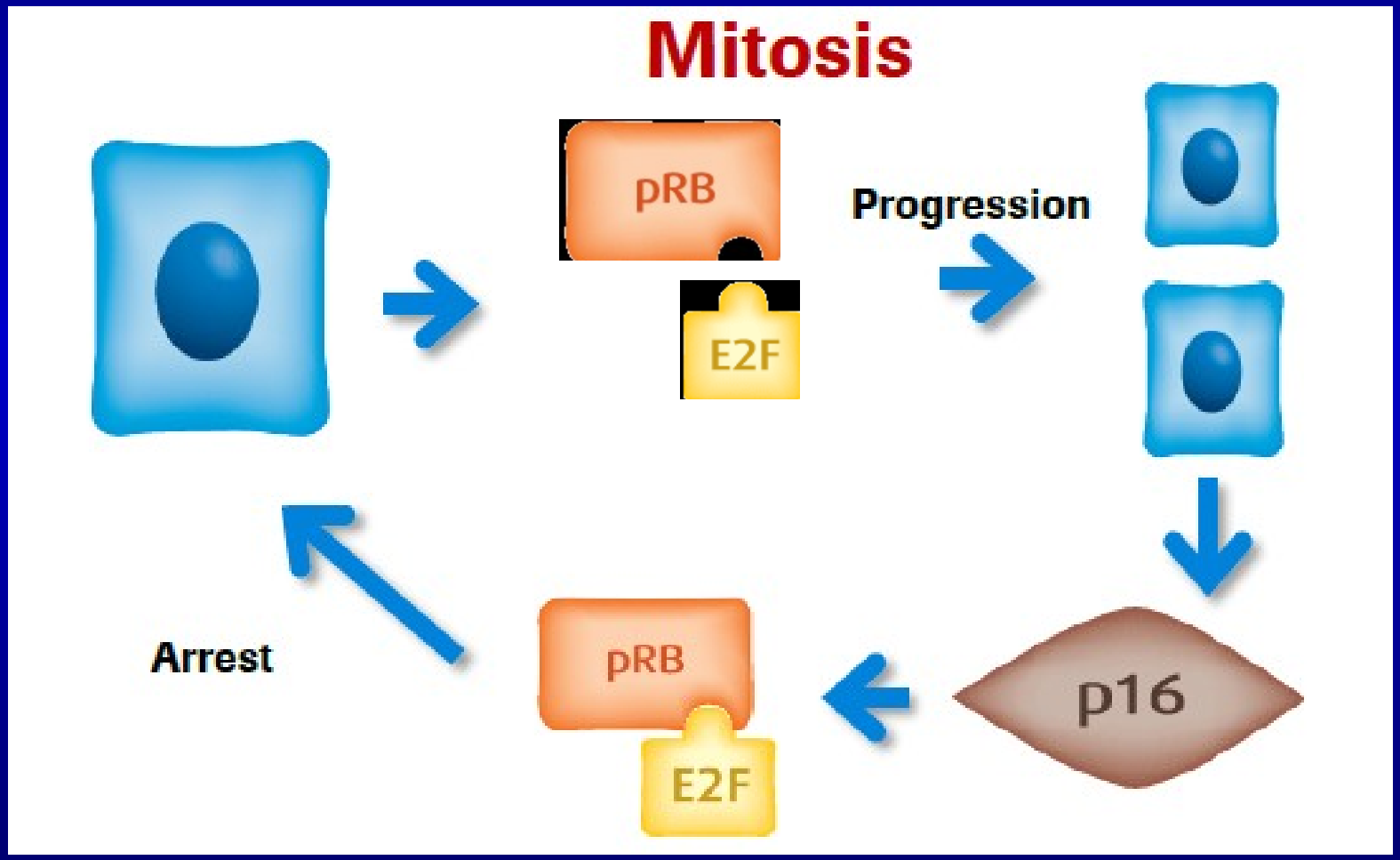

E6 and E7 are the primary HPV oncoproteins,
and E7 is the primary transforming protein

Adapted to: Doorbar J. *Clin Sci* (Lond) 2006; 110: 525–41.

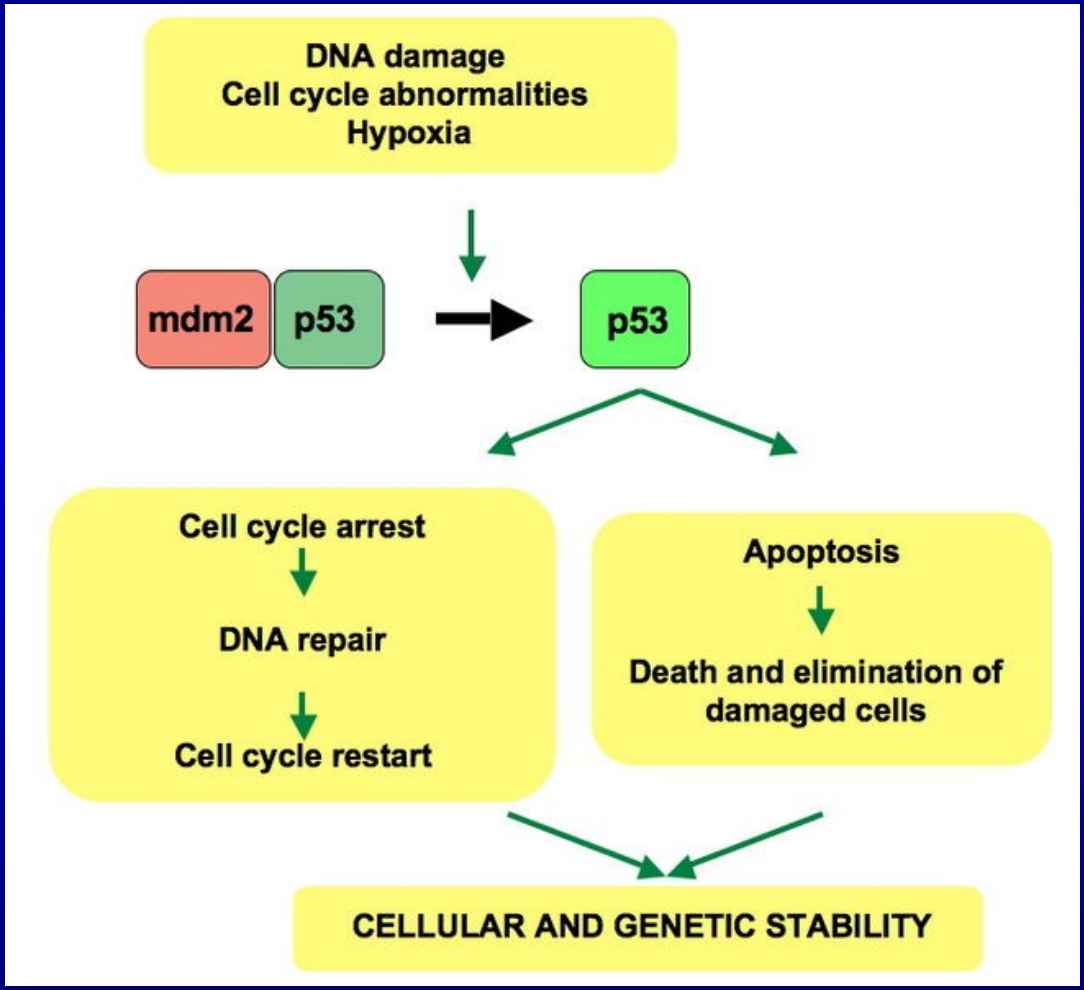



- E6 and E7 interact with the cellular tumor suppressor gene products, p53 and pRb family members.

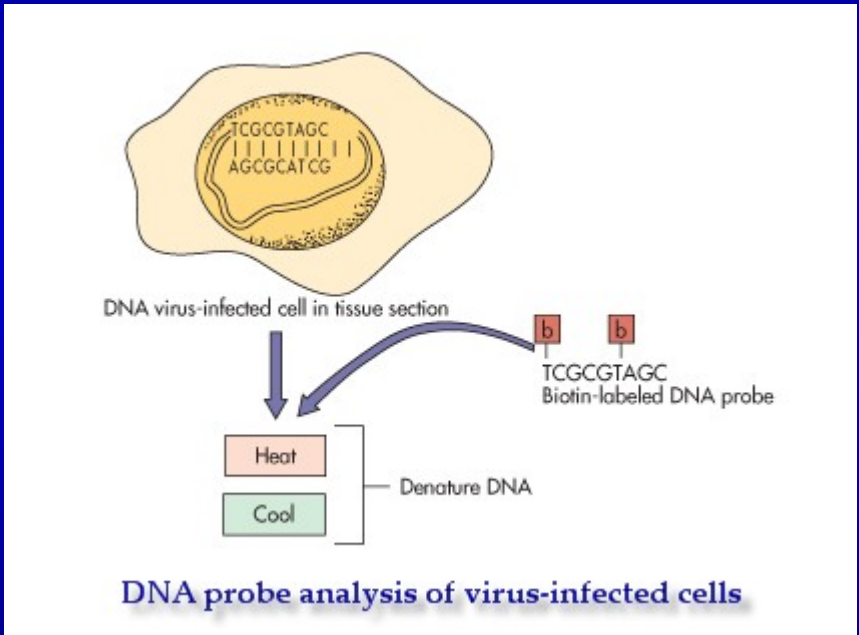
# Retinoblastoma protein



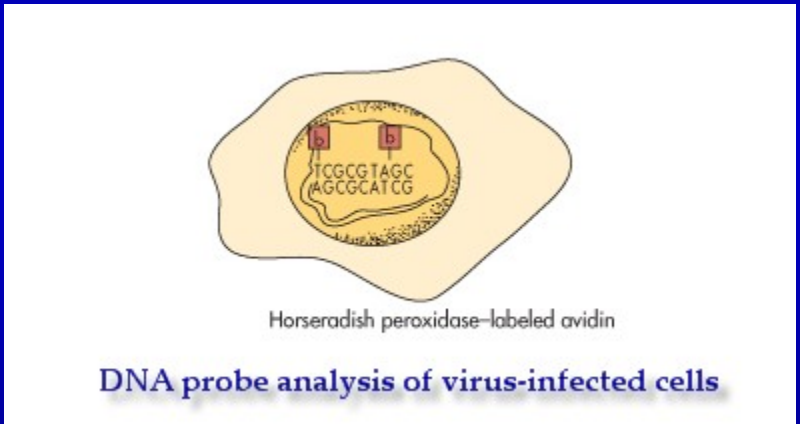
# Functions of p53



# In situ hybridization *(1)*

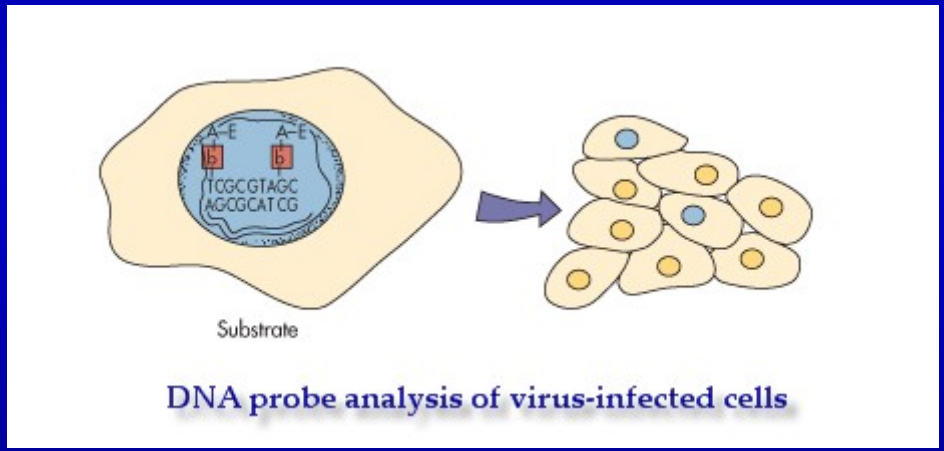


# In situ hybridization *(2)*



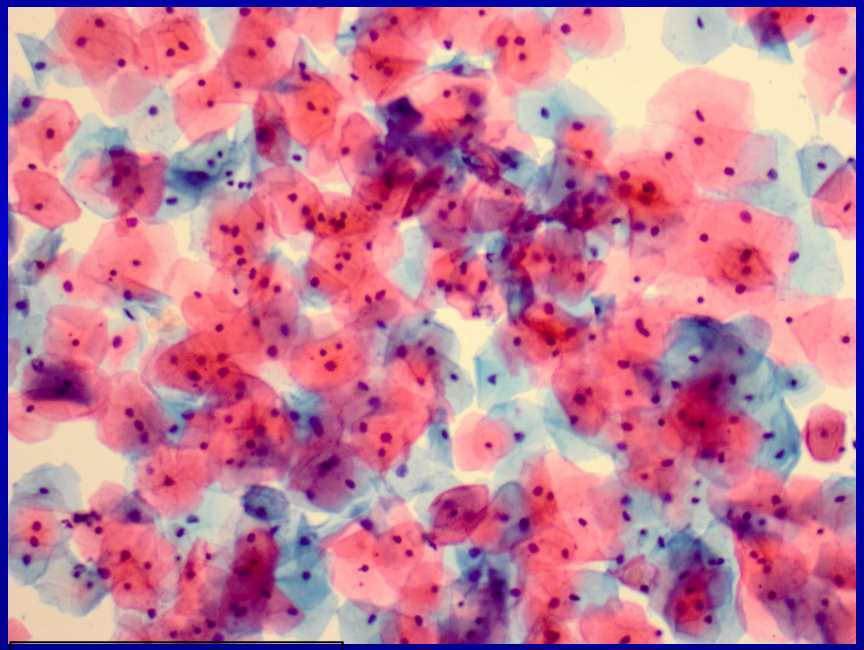

DNA probe analysis of virus-infected cells

# In situ hybridization *(3)*

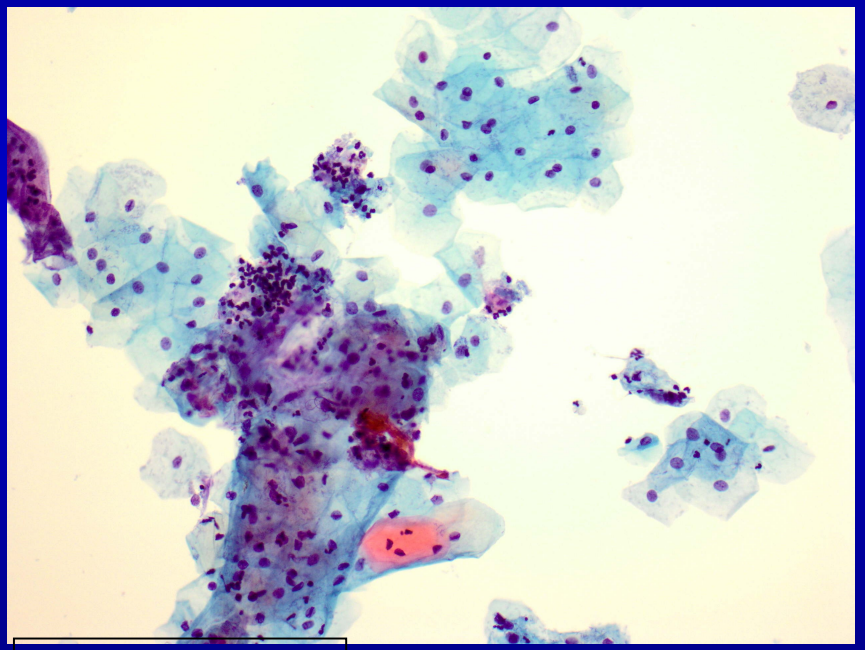



DNA probe analysis of virus-infected cells

# Normal cells

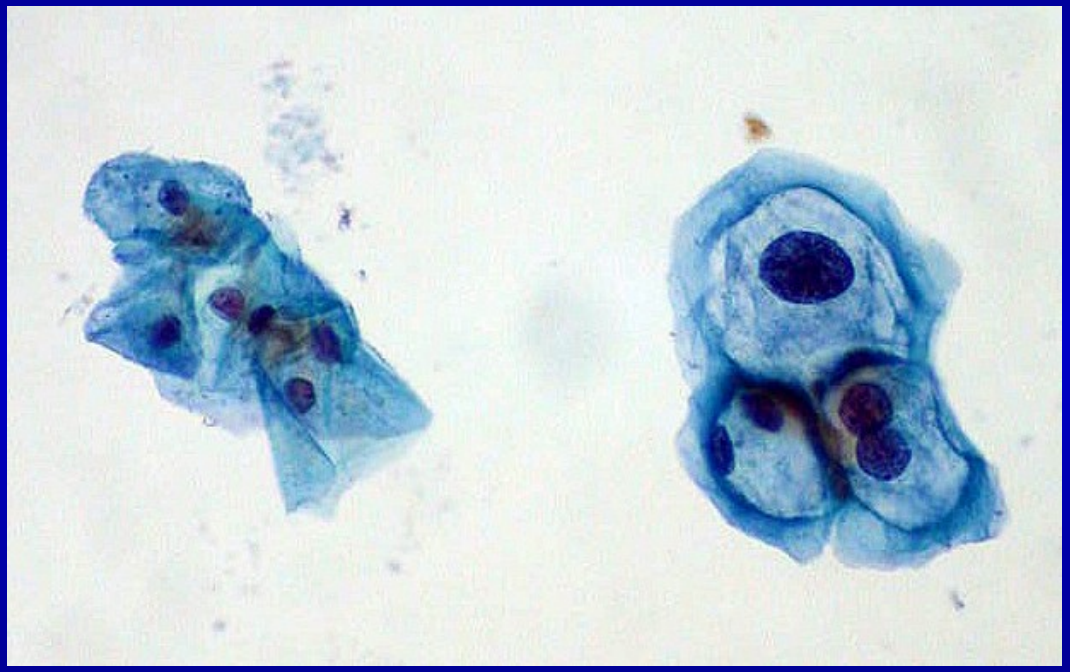


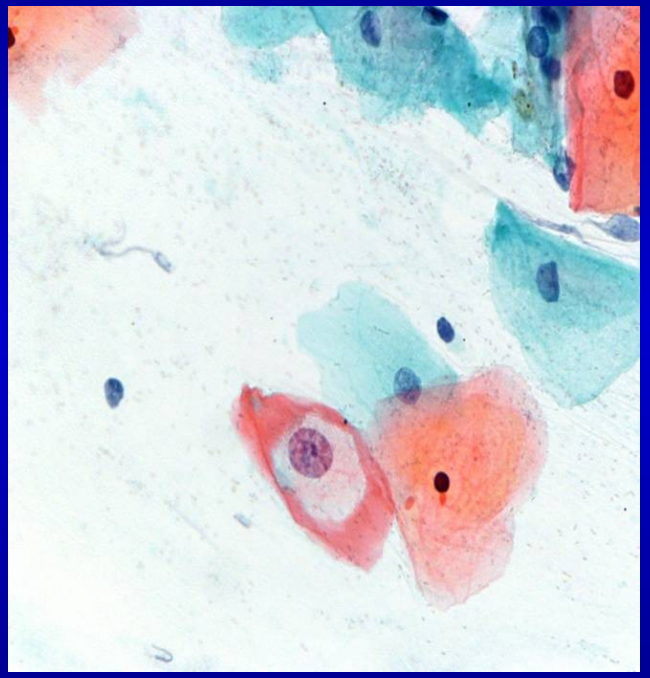
Prvi dio ciklusa



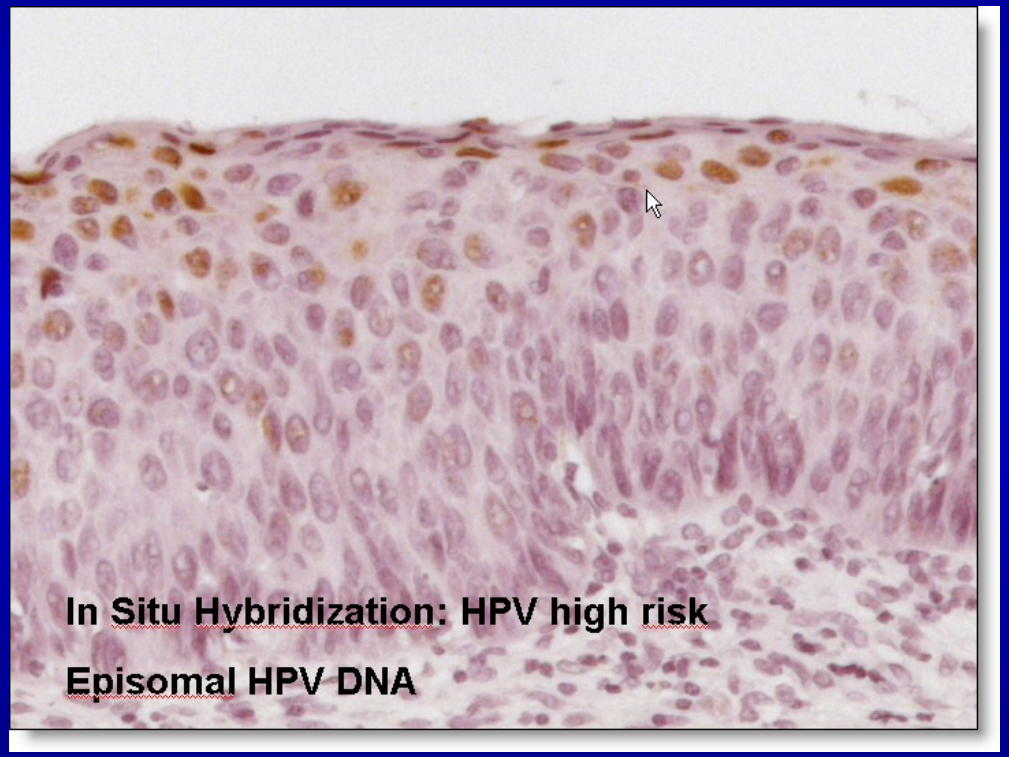
Drugi dio ciklusa

# Koilocytes

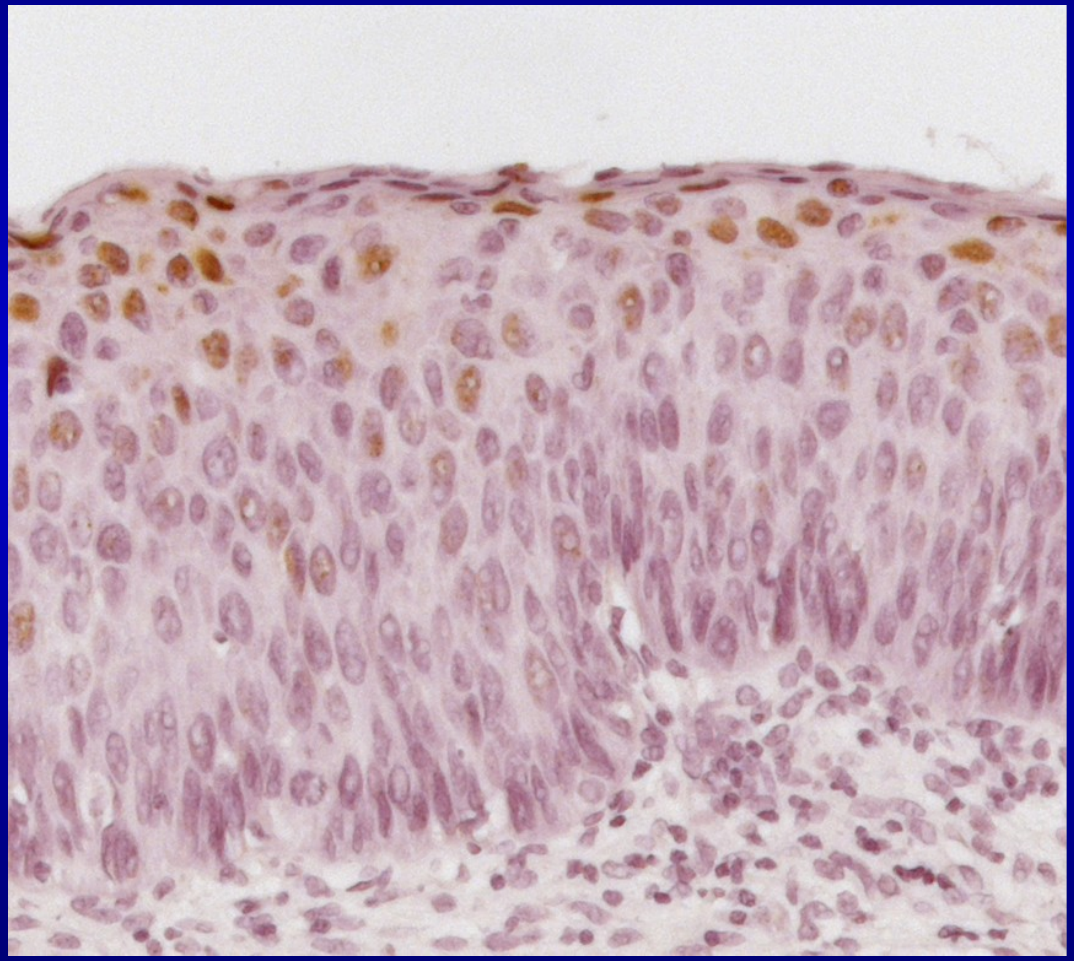




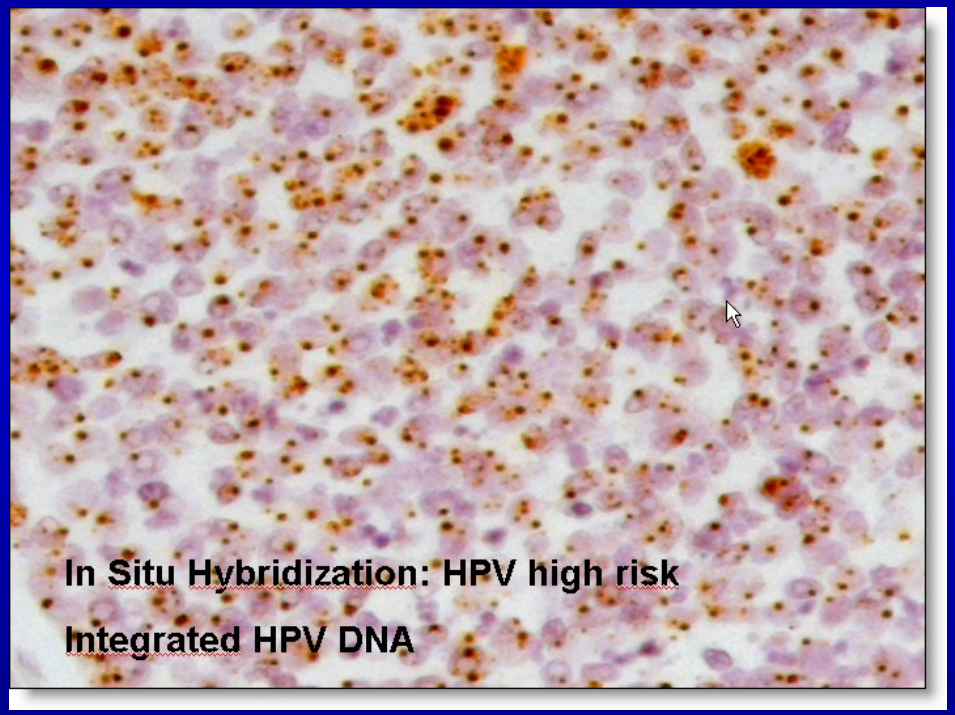
# *Papillomavirus* - Laboratory Diagnosis



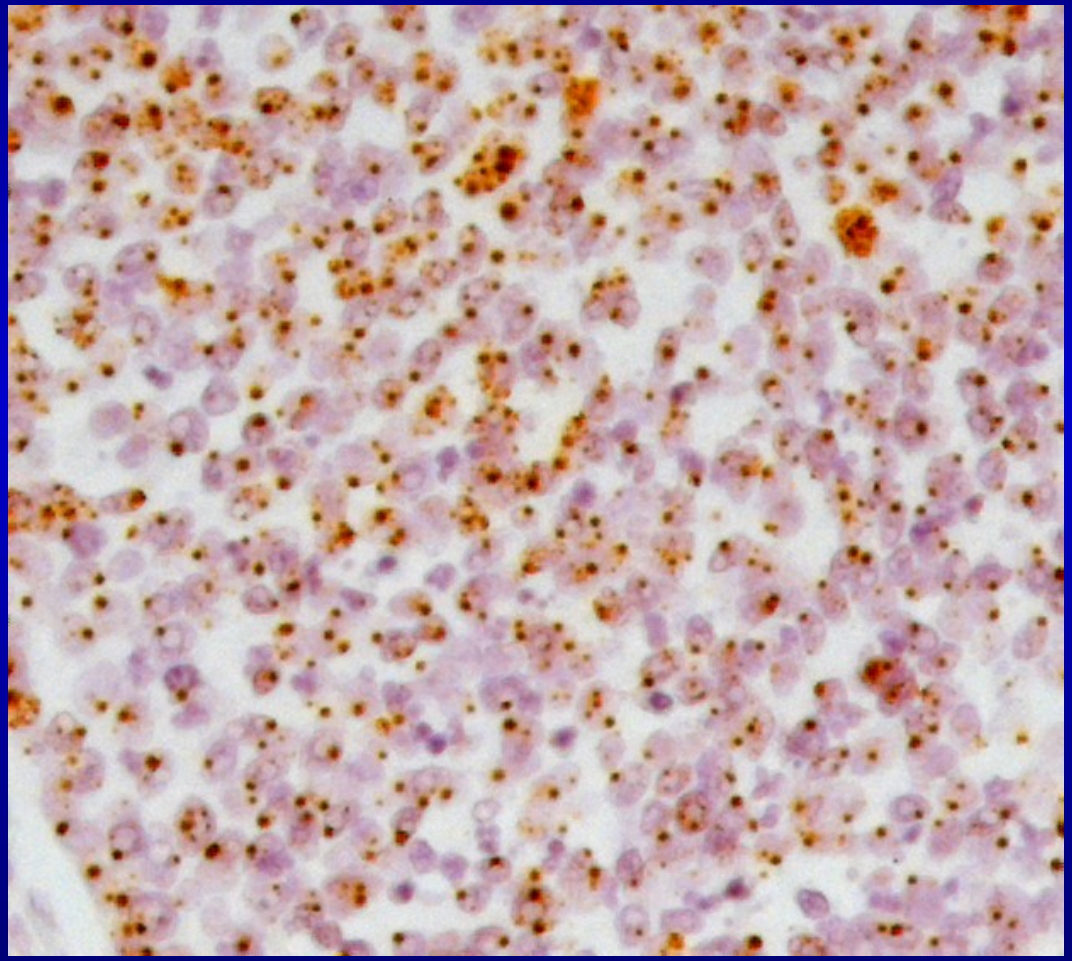
# HPV 6 (Wart)



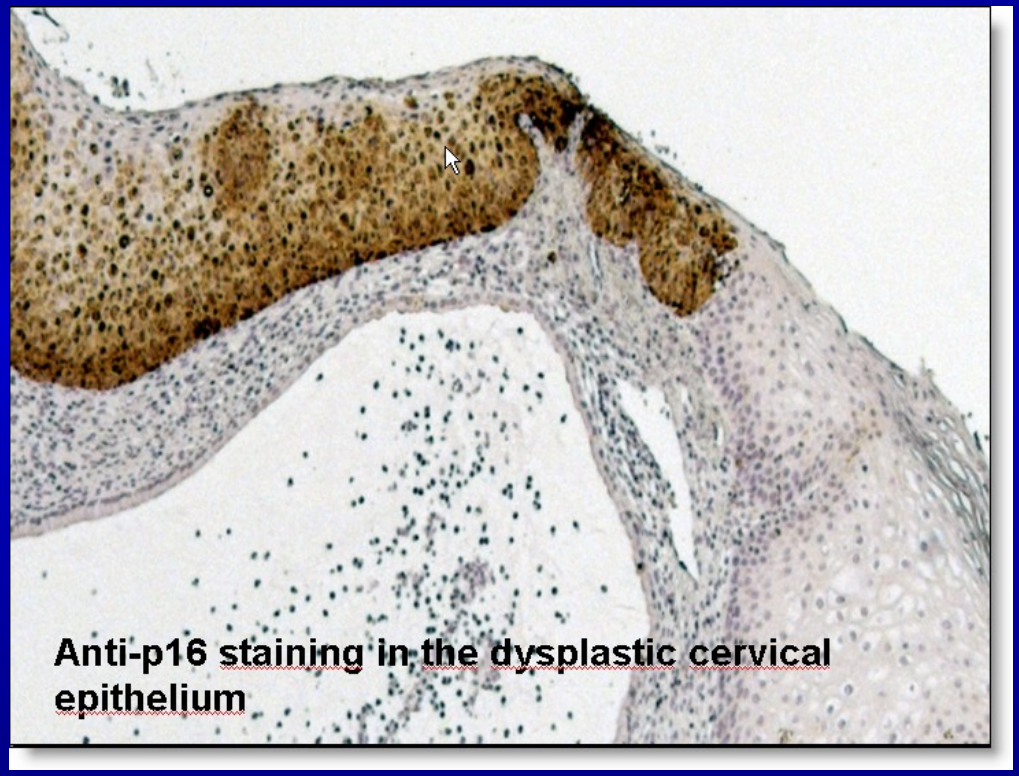
# *Papillomavirus*
# - Laboratory Diagnosis



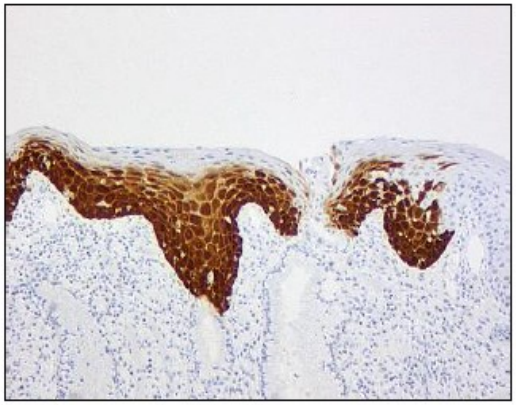
# HPV 16 (Cervix)

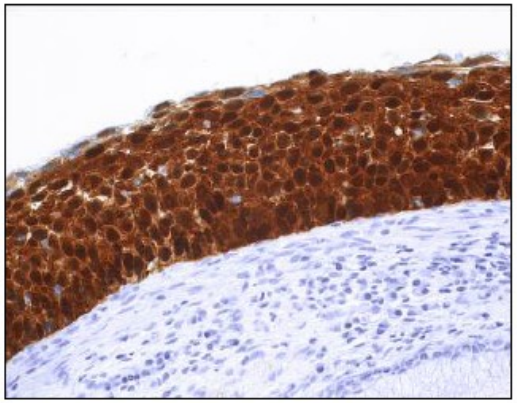


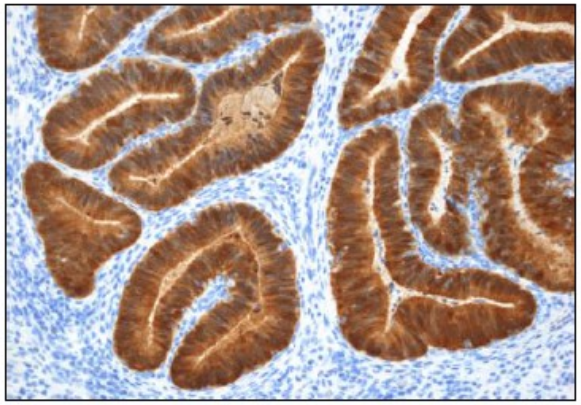
# Anti-p16



# Anti-p16





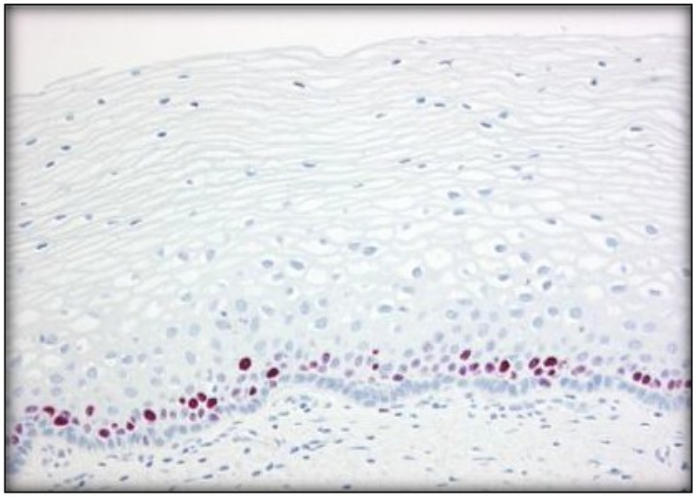


- **Diffuse staining**

# Ki-67 – proliferation marker

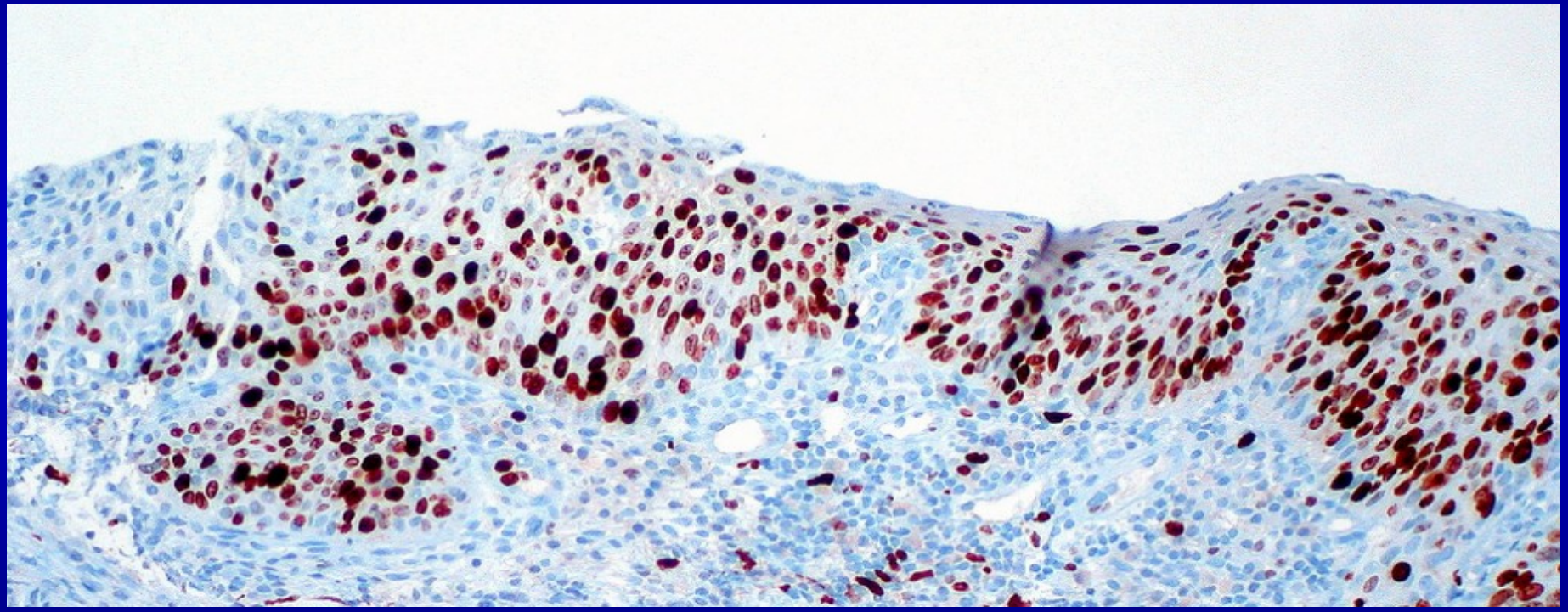
- Ki-67 protein is expressed in proliferating cells within the nucleus, e.g. in parabasal cells of normal cervical epithelium

- Ki-67 over expression indicates cellular proliferation

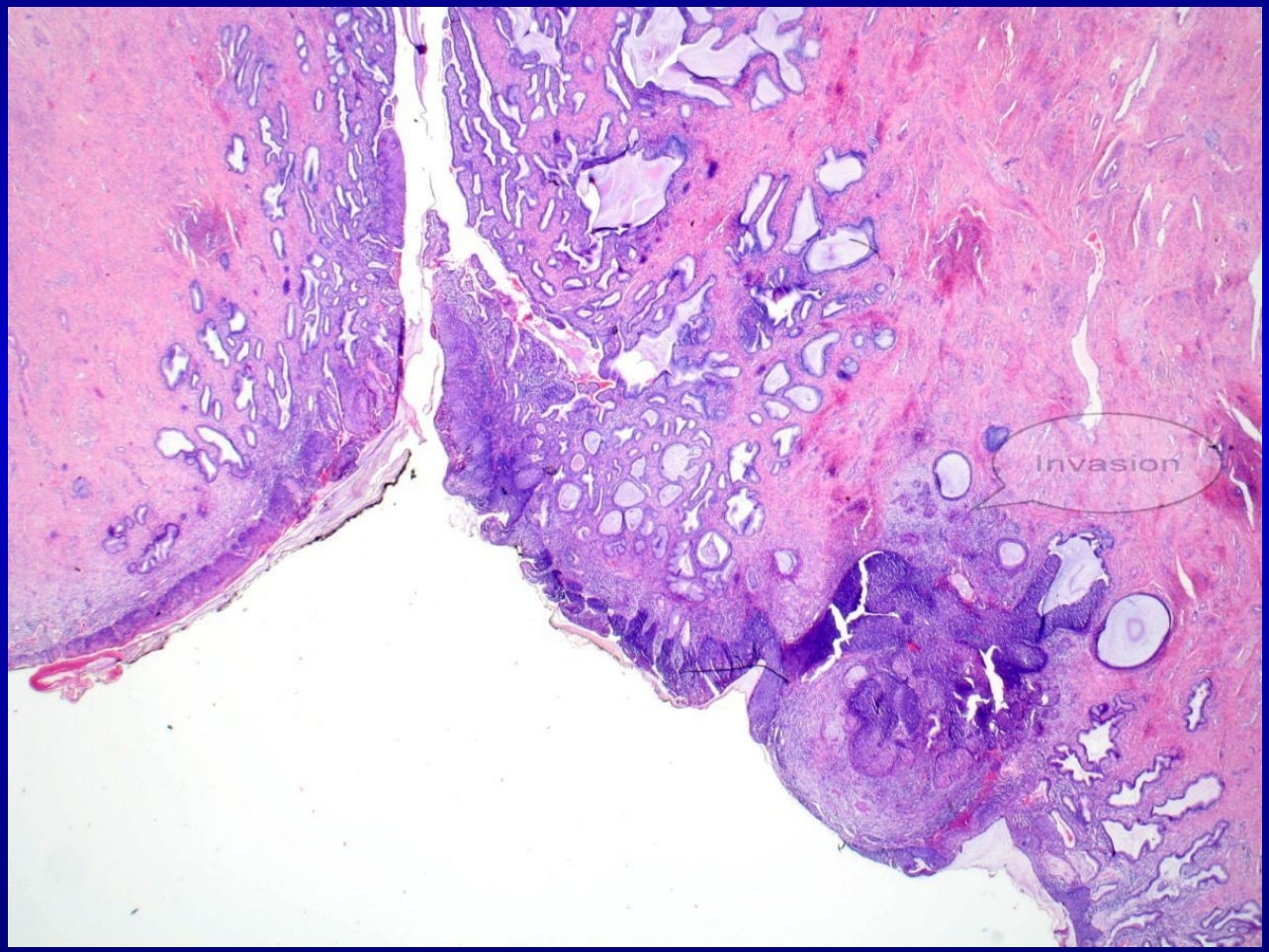


Normal squamous epithelium

# Ki-67 – proliferation marker



# Metastatic spread



# Cervical cancer

# Cervical cancer