

തന്മാത്രാ സാങ്കേതിക വിദ്യയുടെ അത്ഭുത ലോകം



പ്രപഞ്ചവും ജീവനും
സയൻസ് ക്ലാസുകൾ



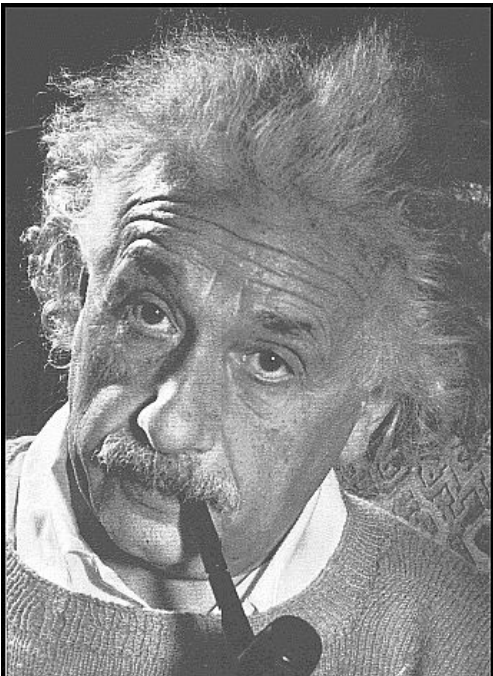
കേരള ശാസ്ത്രസാഹിത്യ പരിഷത്ത്

നവ ജീവശാസ്ത്രം

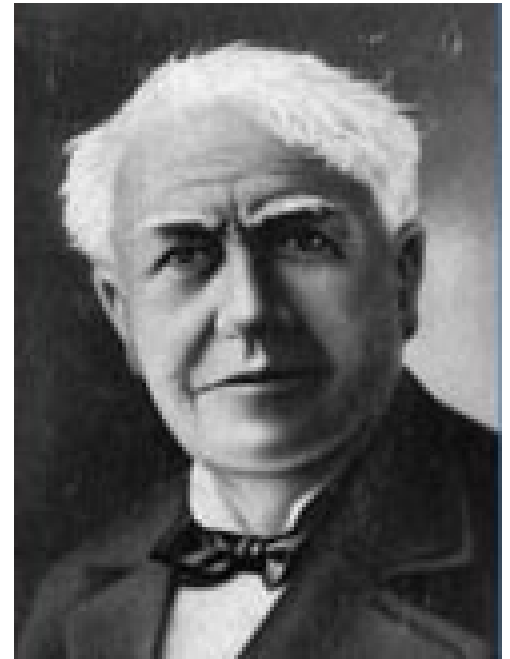


ശാസ്ത്രം

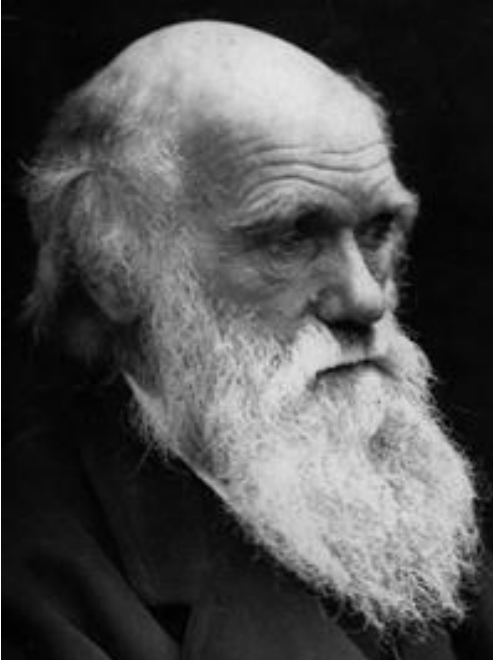
സാങ്കേതിക വിദ്യ



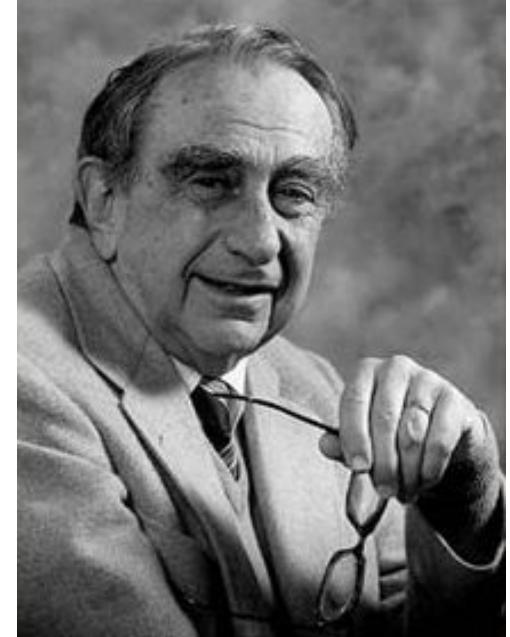
Einstein



Edison

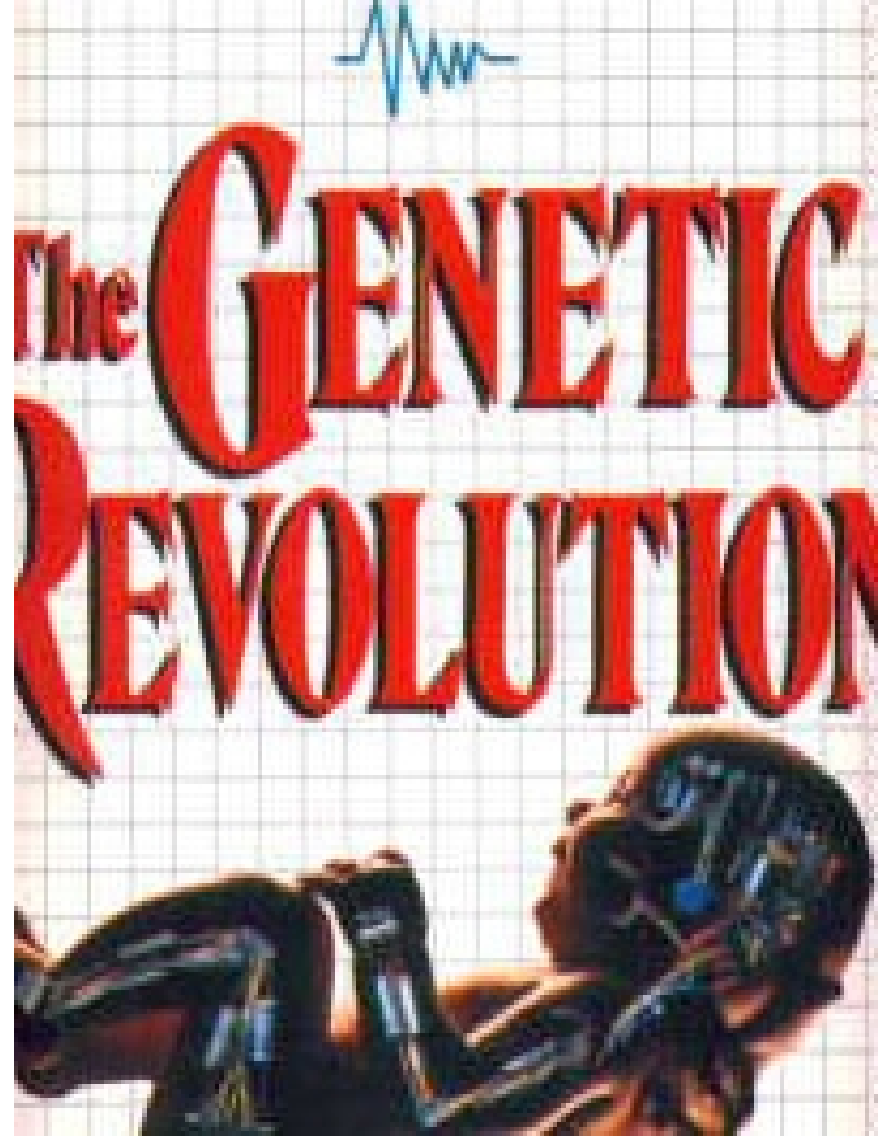
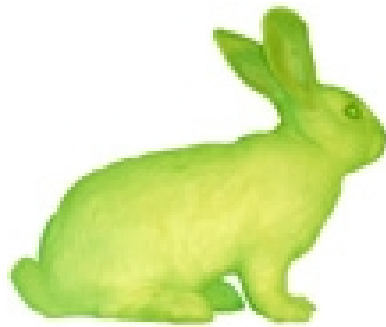


Darwin

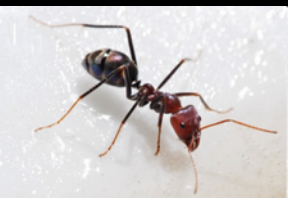
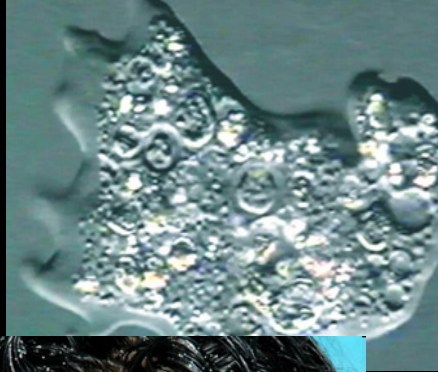


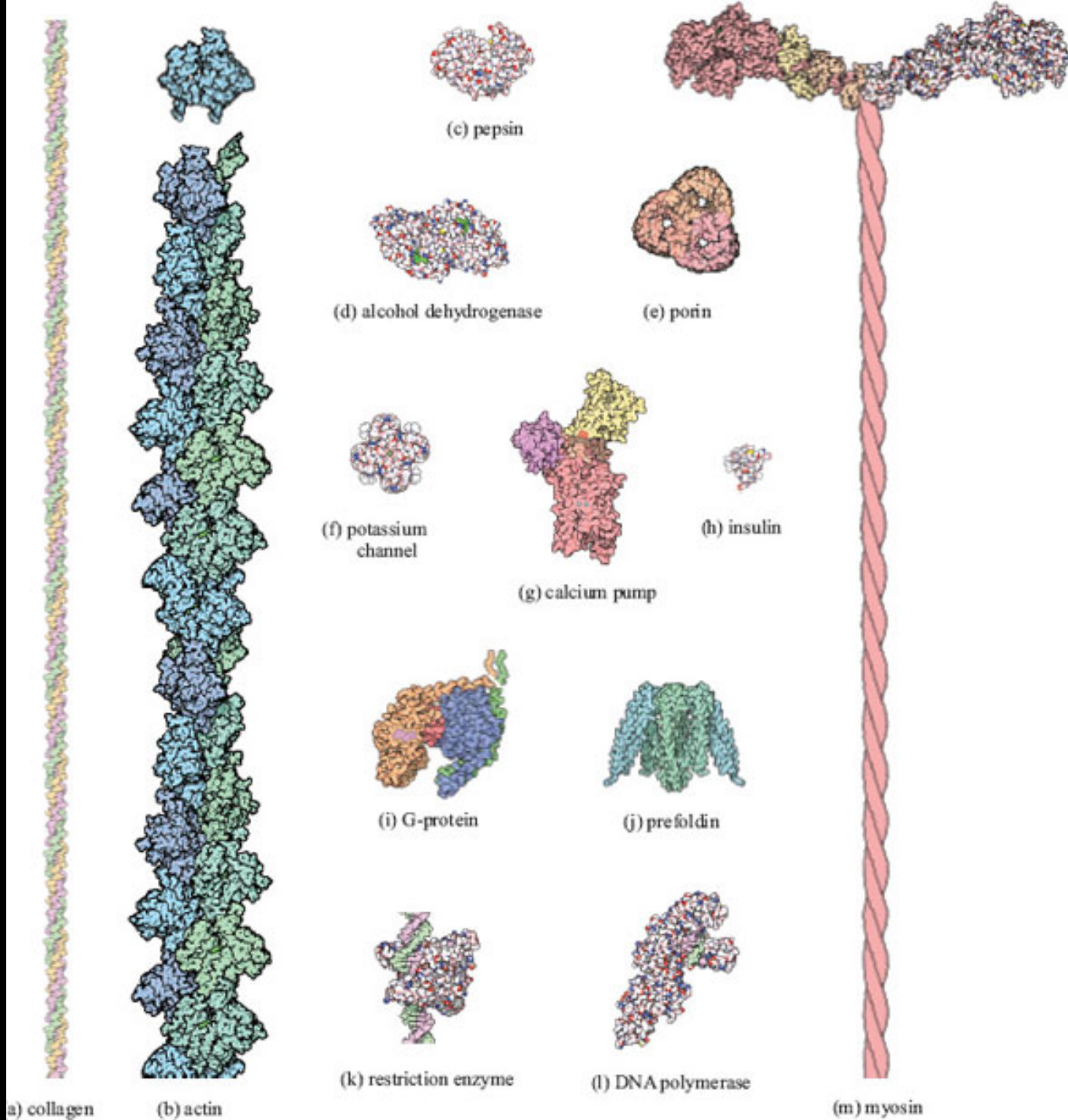
Teller

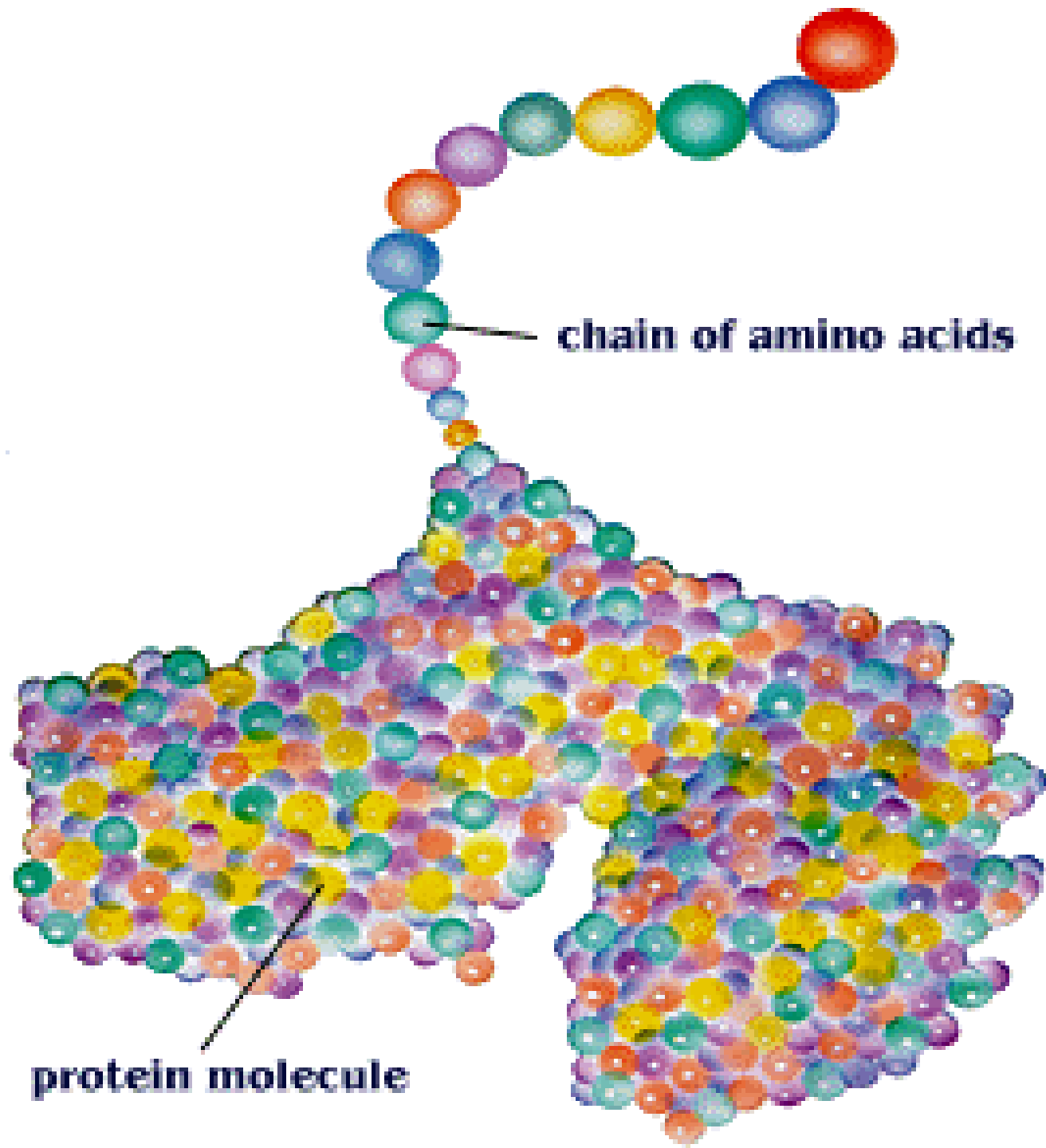
ശാസ്ത്രം → സാങ്കേതിക വിദ്യ



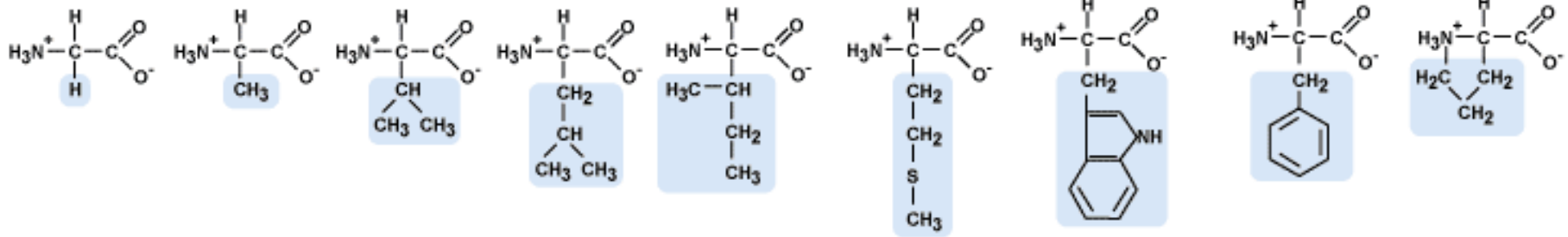
ശാസ്ത്രം \rightleftharpoons സാങ്കേതിക വിദ്യ





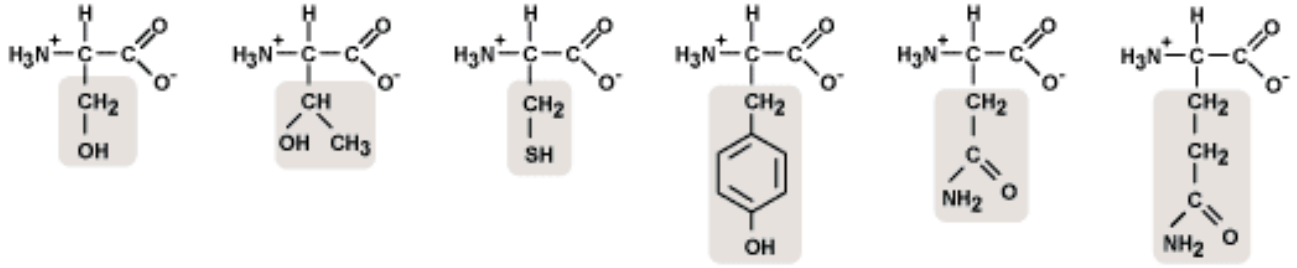


NONPOLAR



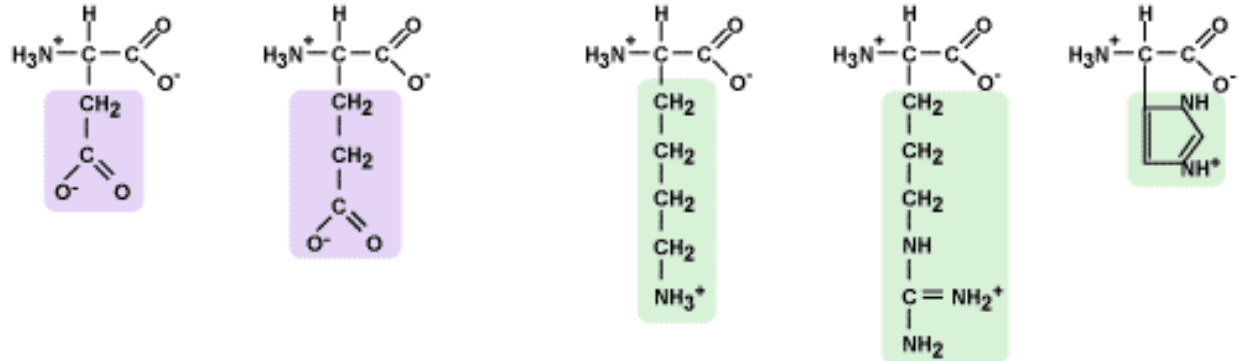
Glycine (Gly) Alanine (Ala) Valine (Val) Leucine (Leu) Isoleucine (Ile) Methionine (Met) Tryptophan (Trp) Phenylalanine (Phe) Proline (Pro)

POLAR



Serine (Ser) Threonine (Thr) Cysteine (Cys) Tyrosine (Tyr) Asparagine (Asn) Glutamine (Gln)

Electrically Charged

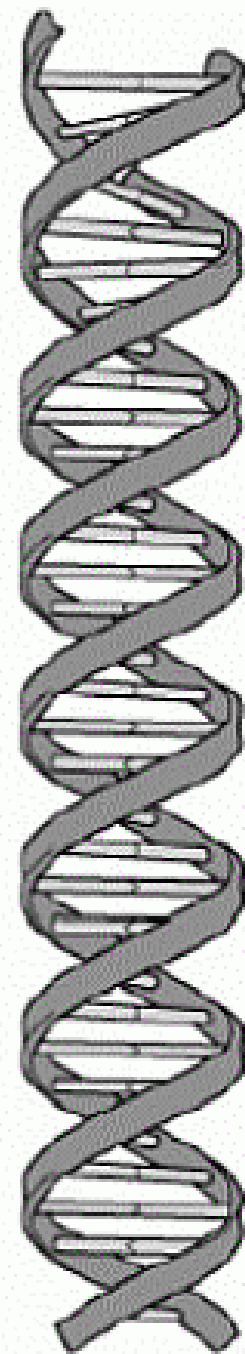
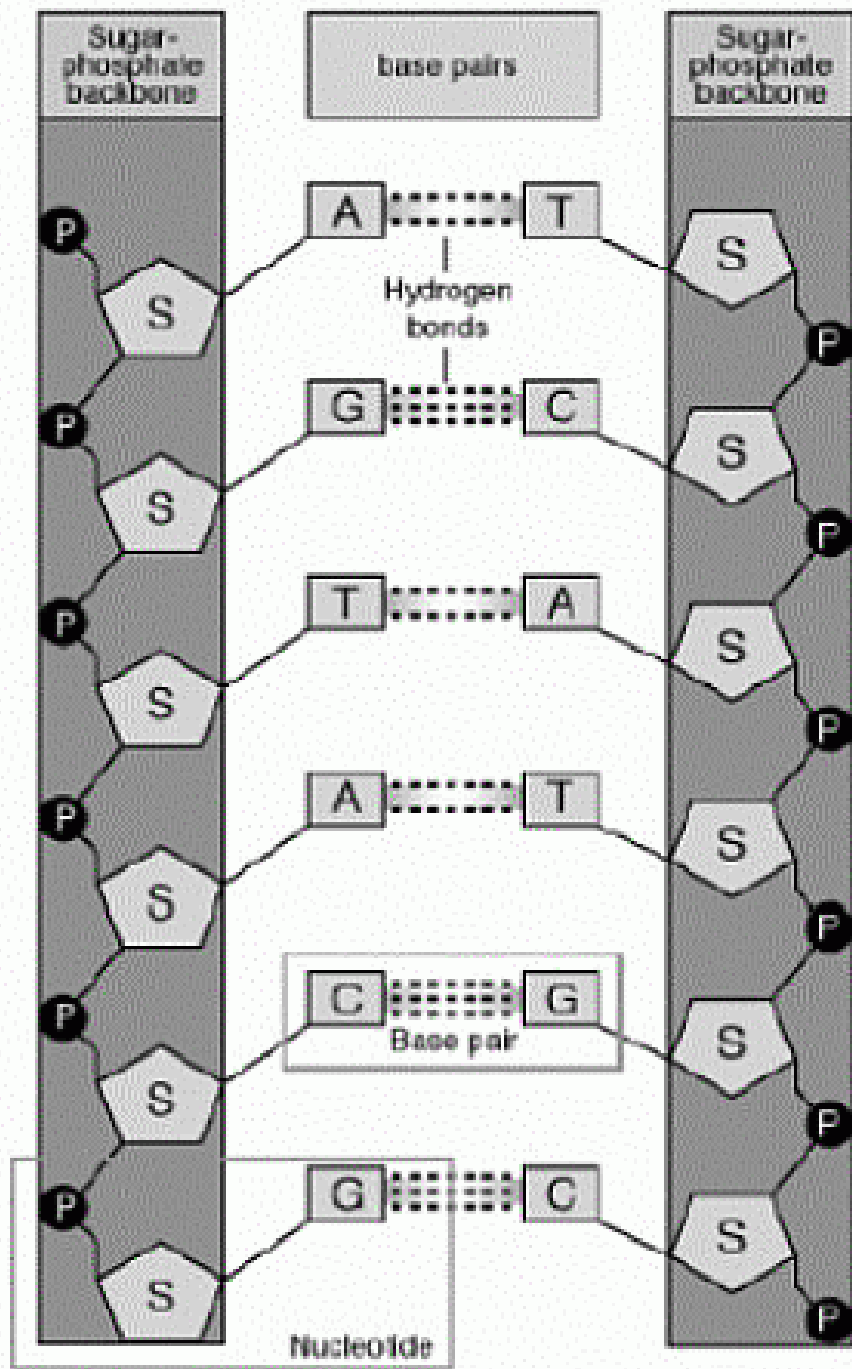


Acidic

Aspartic Acid (Asp) Glutamic Acid (Glu)

Basic

Lysine (Lys) Arginine (Arg) Histidine (His)



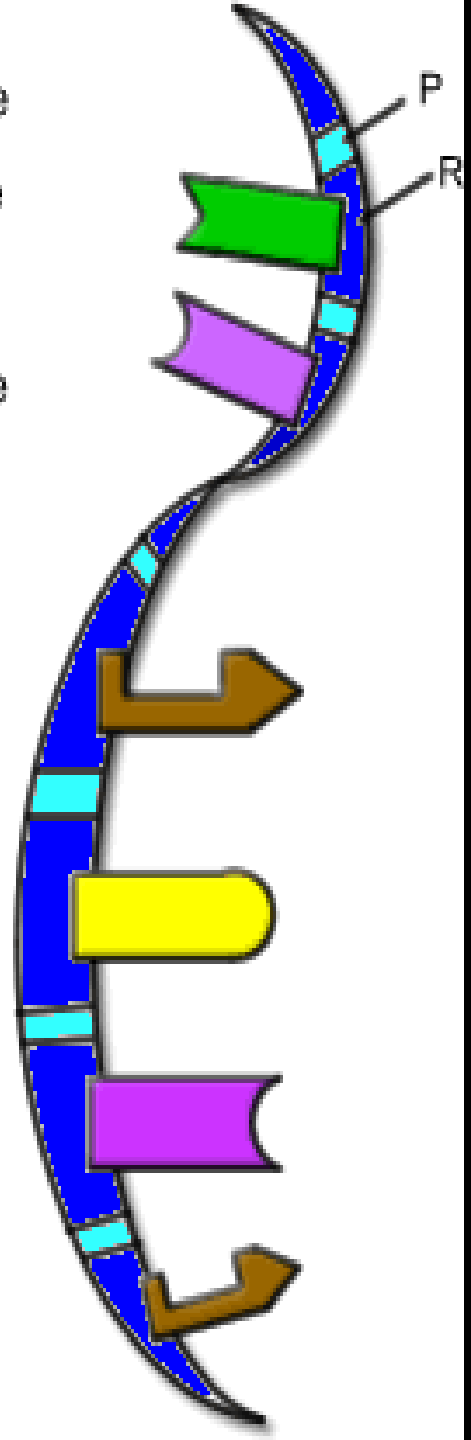
Second Letter

		T	C	A	G				
First Letter	T	TTT } Phe TTC } TTA } Leu TTG }	TCT } TCC } Ser TCA } TCG }	TAT } Tyr TAC } TAA } Stop TAG } Stop	TGT } Cys TGC } TGA } Stop TGG } Trp	T	C	A	G
	C	CTT } CTC } Leu CTA } CTG }	CCT } CCC } Pro CCA } CCG }	CAT } His CAC } CAA } Gln CAG }	CGT } CGC } Arg CGA } CGG }	T	C	A	G
	A	ATT } ATC } Ile ATA } ATG } Met	ACT } ACC } Thr ACA } ACG }	AAT } Asn AAC } AAA } Lys AAG }	AGT } Ser AGC } AGA } Arg AGG }	T	C	A	G
	G	GTT } GTC } Val GTA } GTG }	GCT } GCC } Ala GCA } GCG }	GAT } Asp GAC } GAA } Glu GAG }	GGT } GGC } Gly GGA } GGG }	T	C	A	G

Third Letter

- Adenine
- Guanine
- Uracil
- Cytosine

P= phosphate
R= Ribose



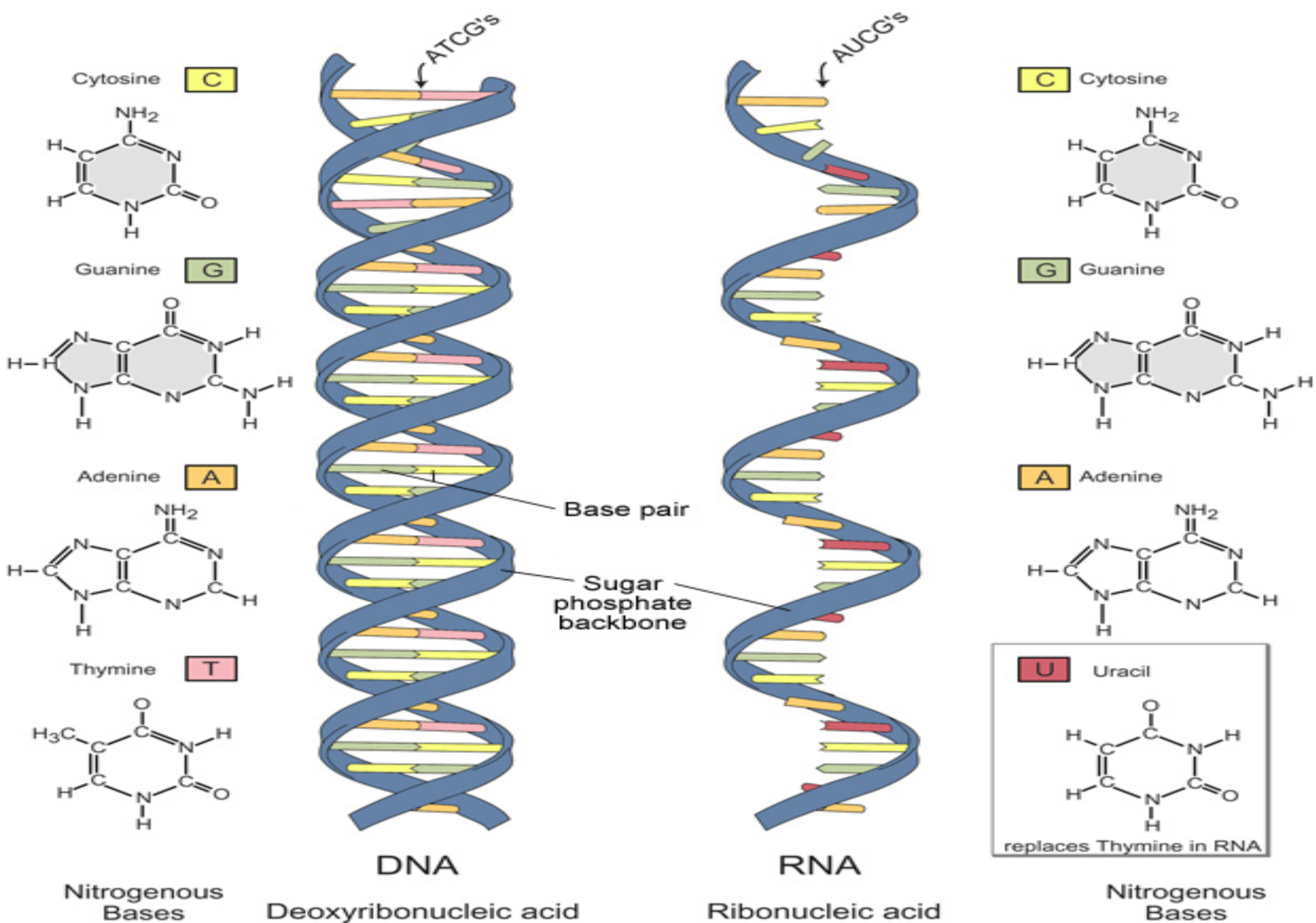
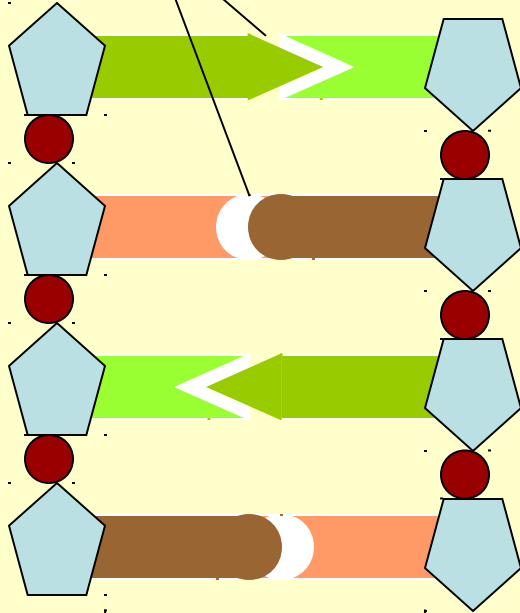


Image adapted from: National Human Genome Research Institute.

DNA Structure

Hydrogen bond
(H-bonds)



Nitrogenous base "rung"

Sugar / phosphate "strand"



thymine



adenine



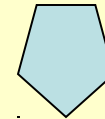
cytosine



guanine



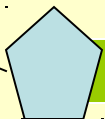
phosphate



deoxyribose sugar

DNA nucleotide

Deoxyribose sugar



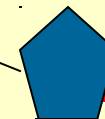
phosphate



Nitrogenous base
(guanine)

RNA nucleotide

ribose sugar

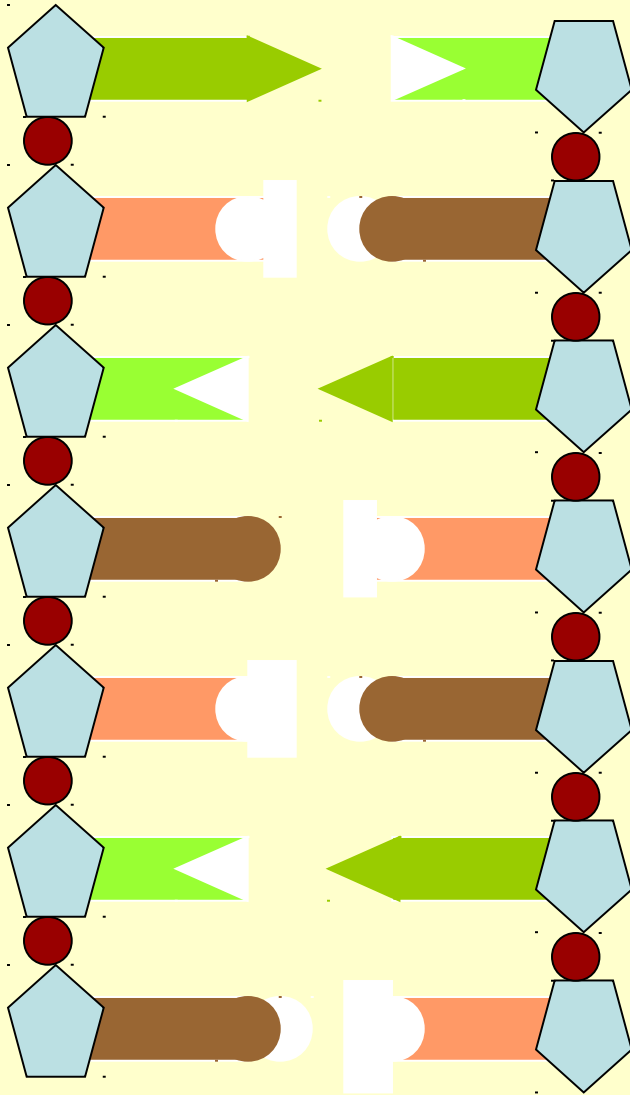


phosphate



Nitrogenous base
(uracil)

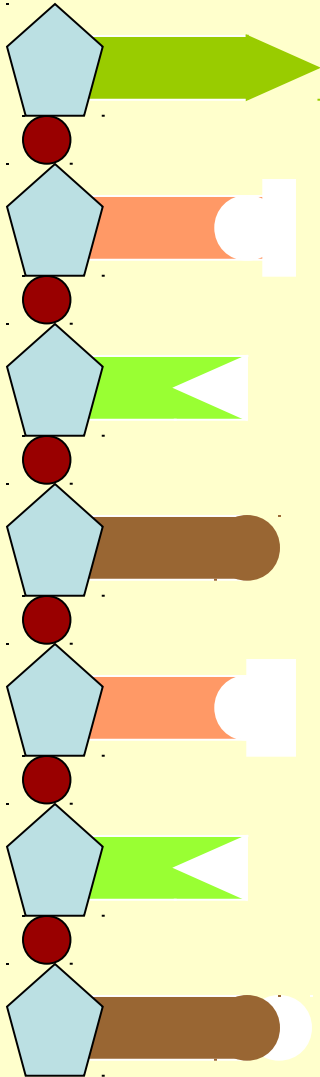
DNA Replication



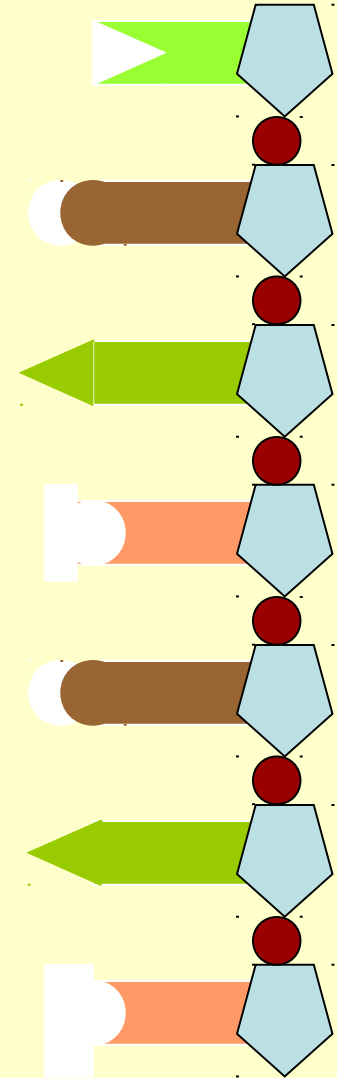
Step 1: Hydrogen bonds between complimentary bases break

DNA “unzips”

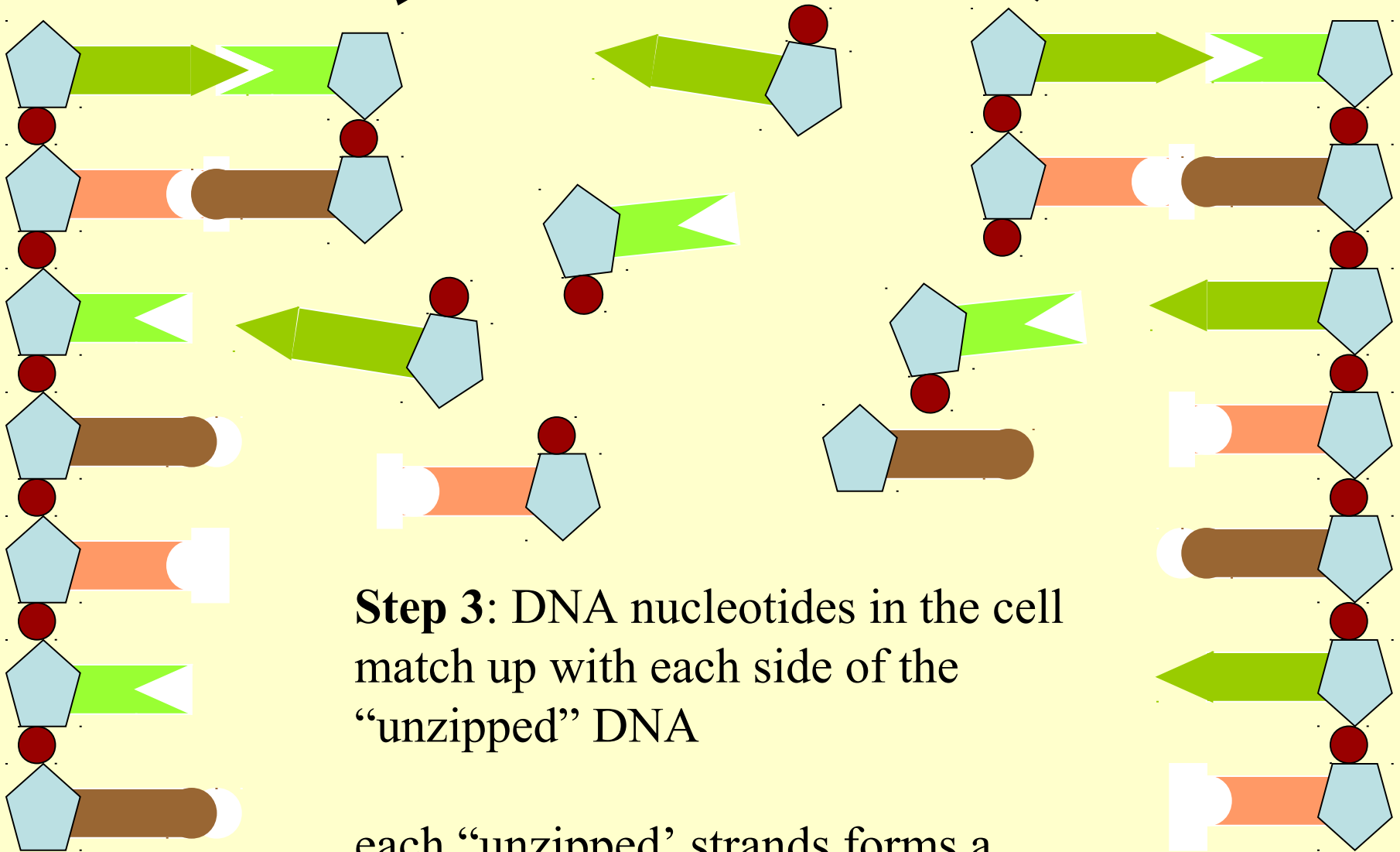
DNA Replication



Step 2: DNA strands
pull apart from each other



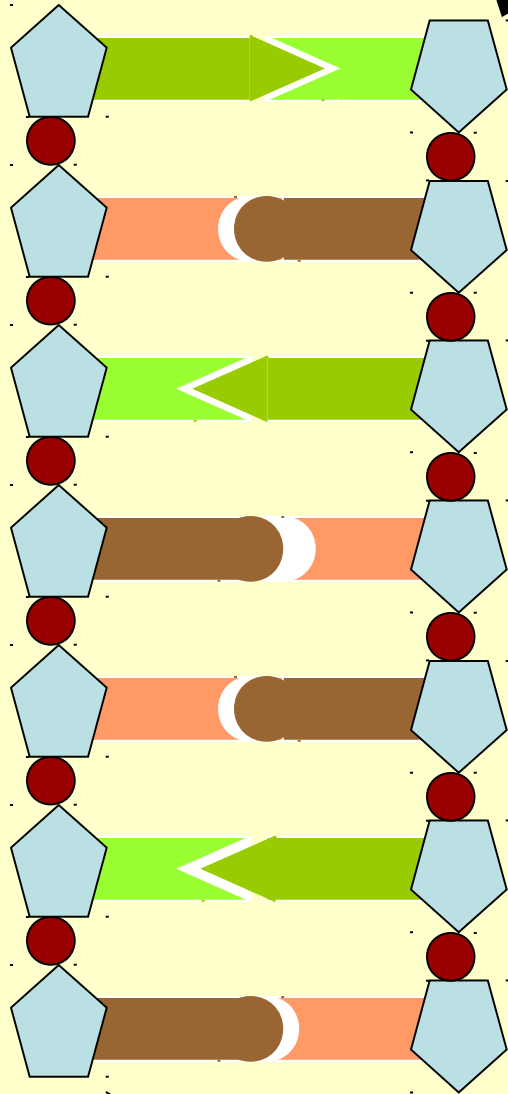
DNA Replication



Step 3: DNA nucleotides in the cell match up with each side of the “unzipped” DNA

each “unzipped” strands forms a template for a new strand

DNA Replication

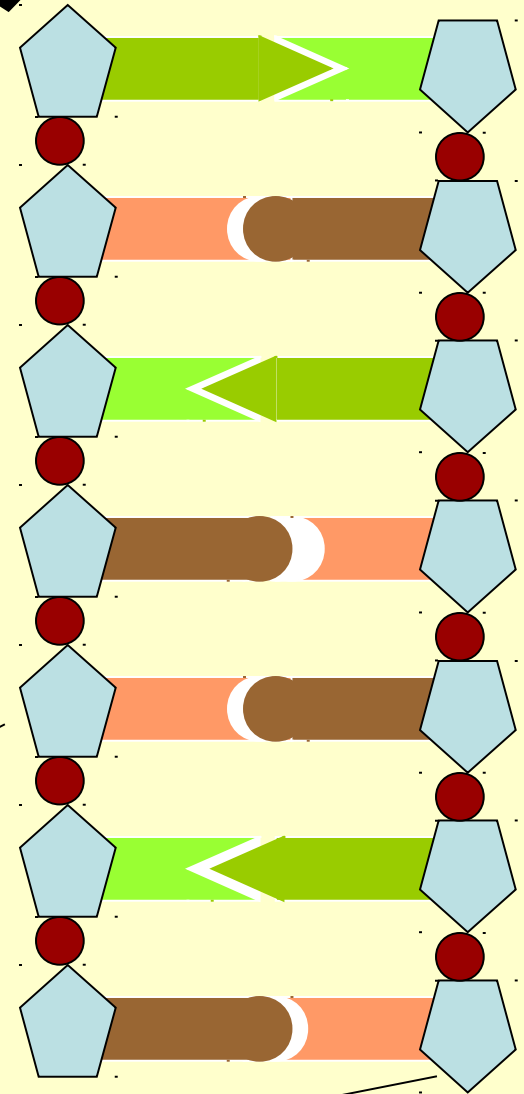


Step 4: Each “old” strand forms a template for a “new” strand

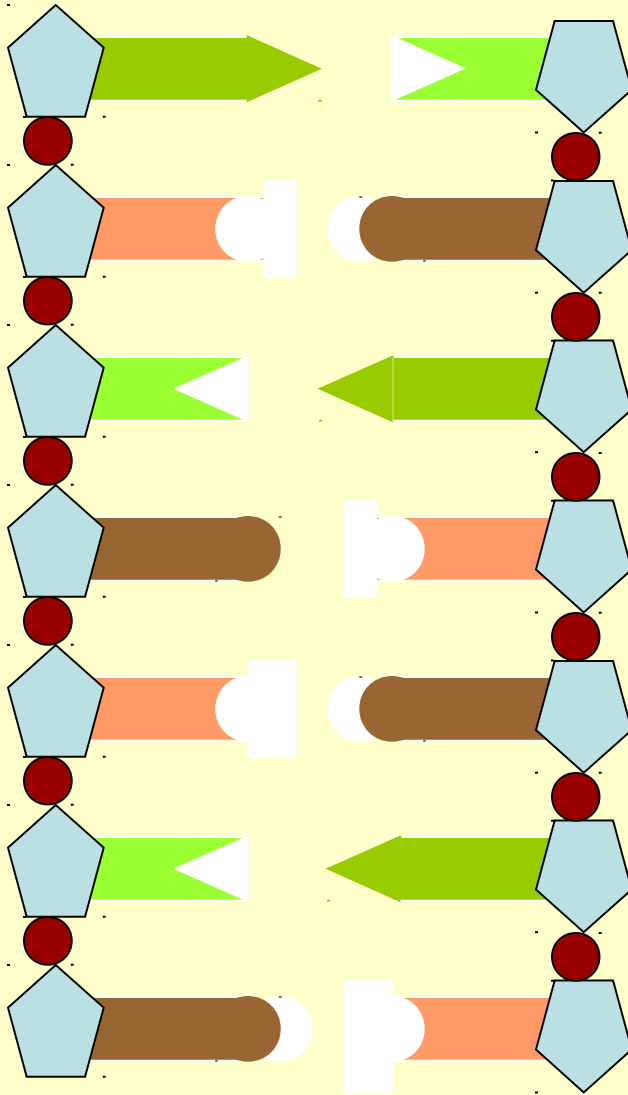
two identical DNA molecules form

“new” strand, identical sequence to the original

“old” (original) strand



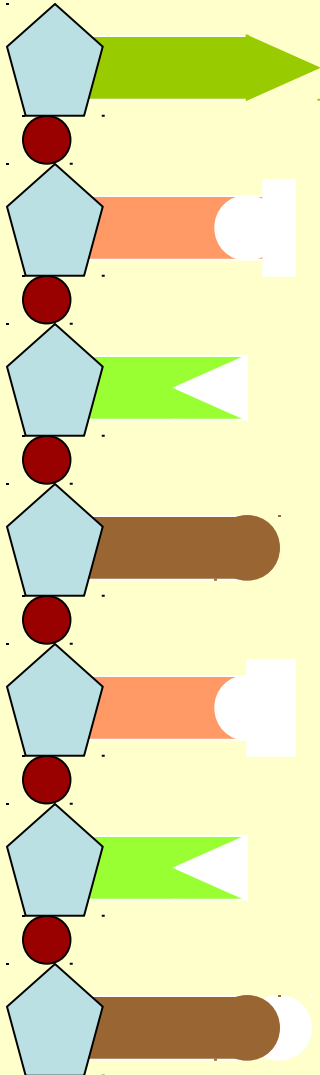
RNA Transcription



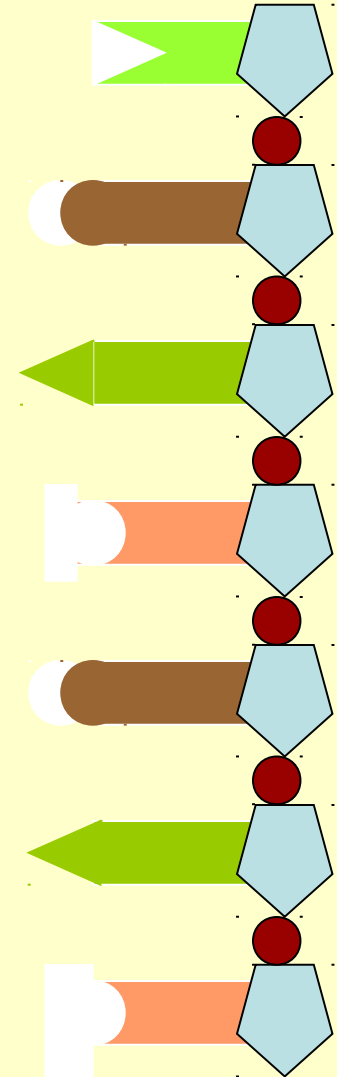
Step 1: Hydrogen bonds between complimentary bases break

DNA “unzips”

RNA Transcription



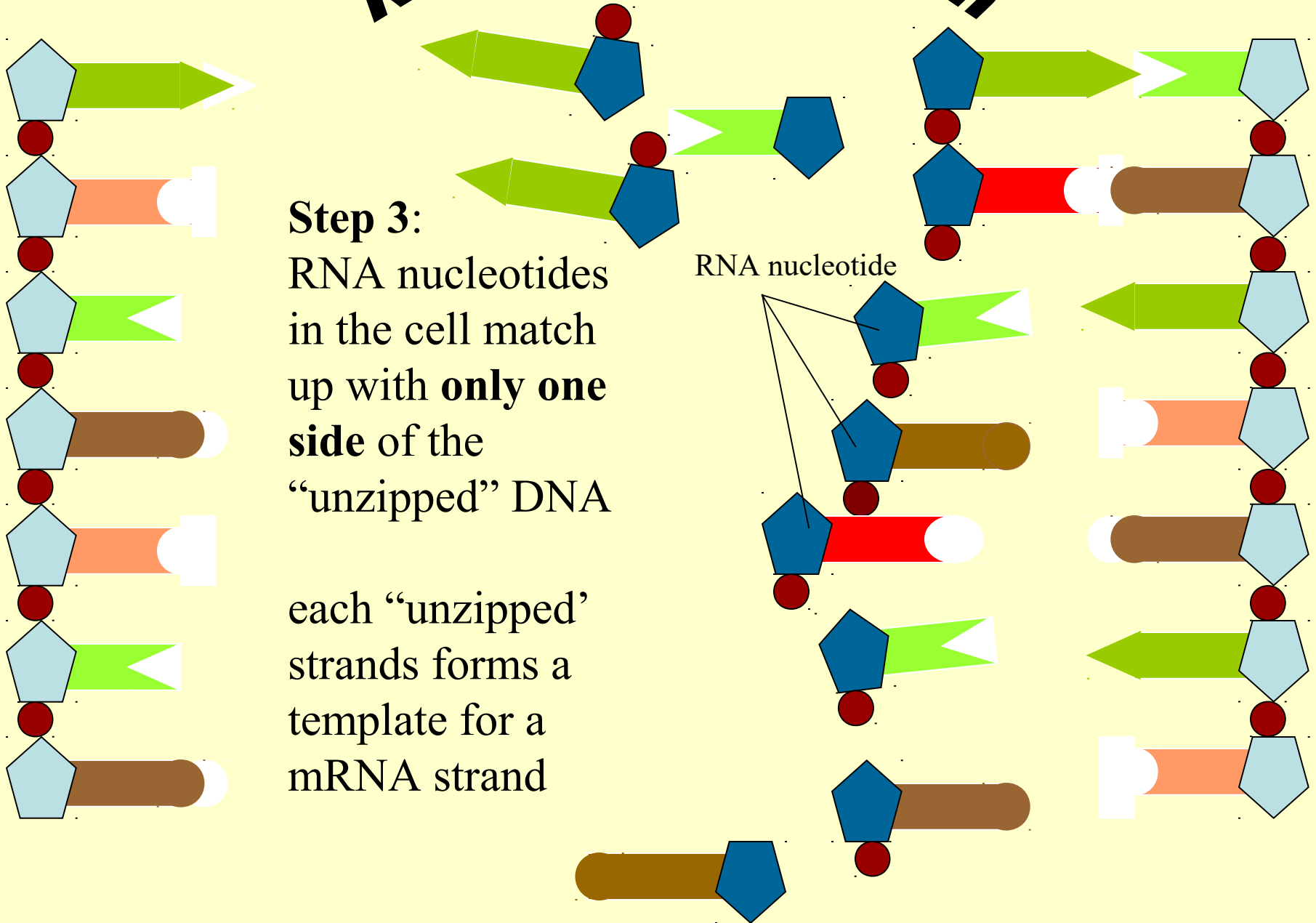
Step 2: DNA strands
pull apart from each other



RNA Transcription

Step 3:
RNA nucleotides
in the cell match
up with **only one**
side of the
“unzipped” DNA

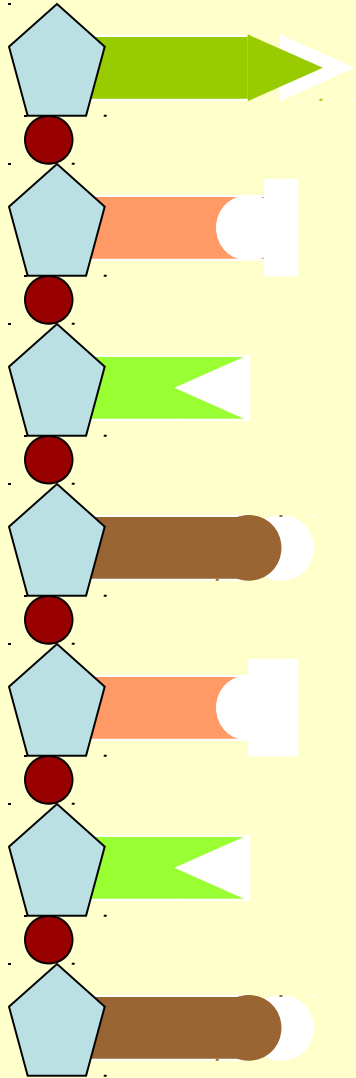
each “unzipped”
strands forms a
template for a
mRNA strand



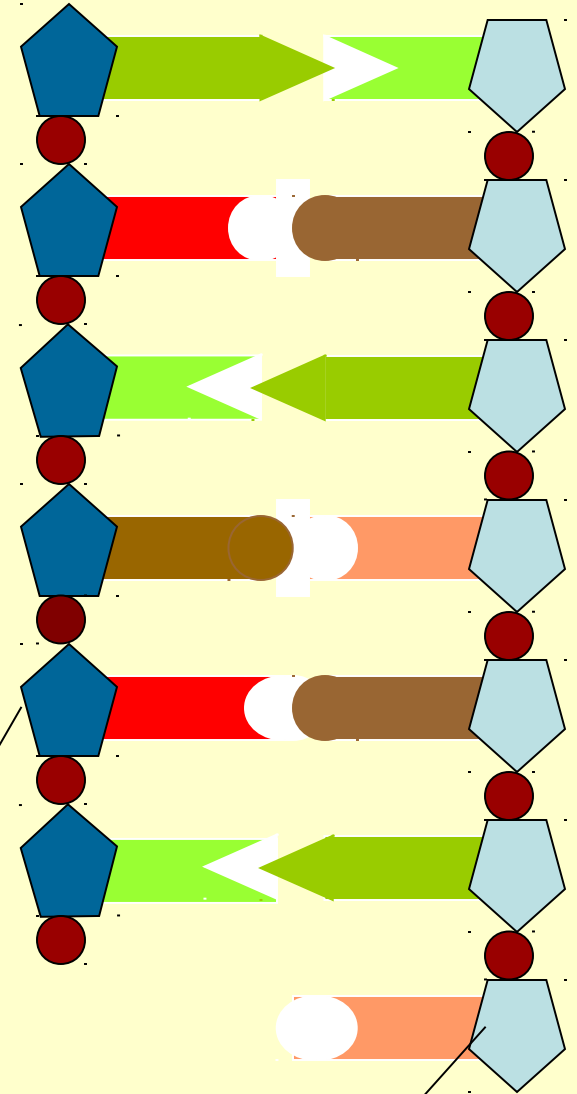
RNA Transcription

Step 4:
RNA nucleotides
continue to match
up with
“unzipped” DNA

until the message
is completely
transcribed

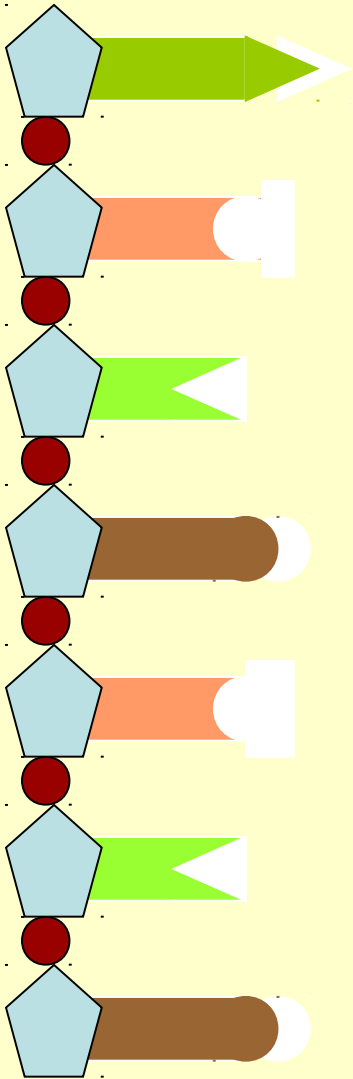


mRNA strand

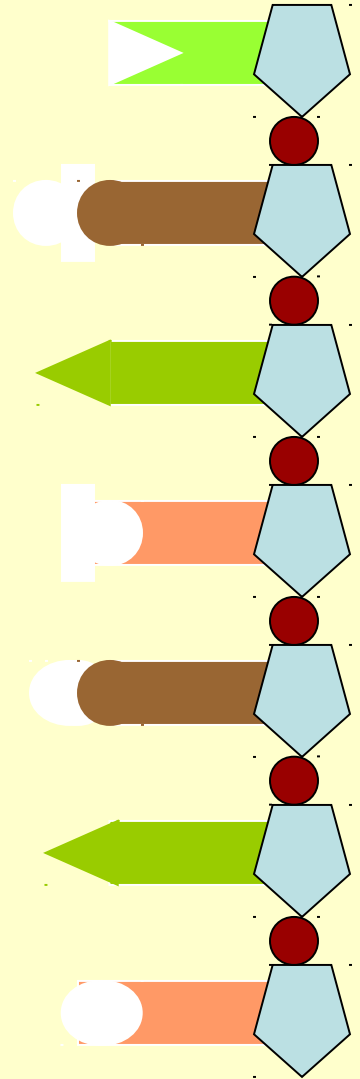
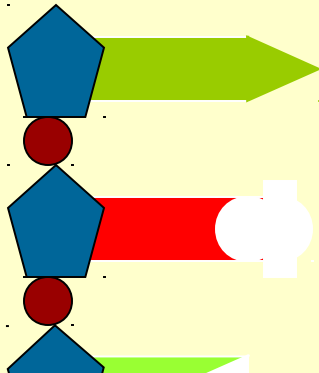


One side of DNA strand

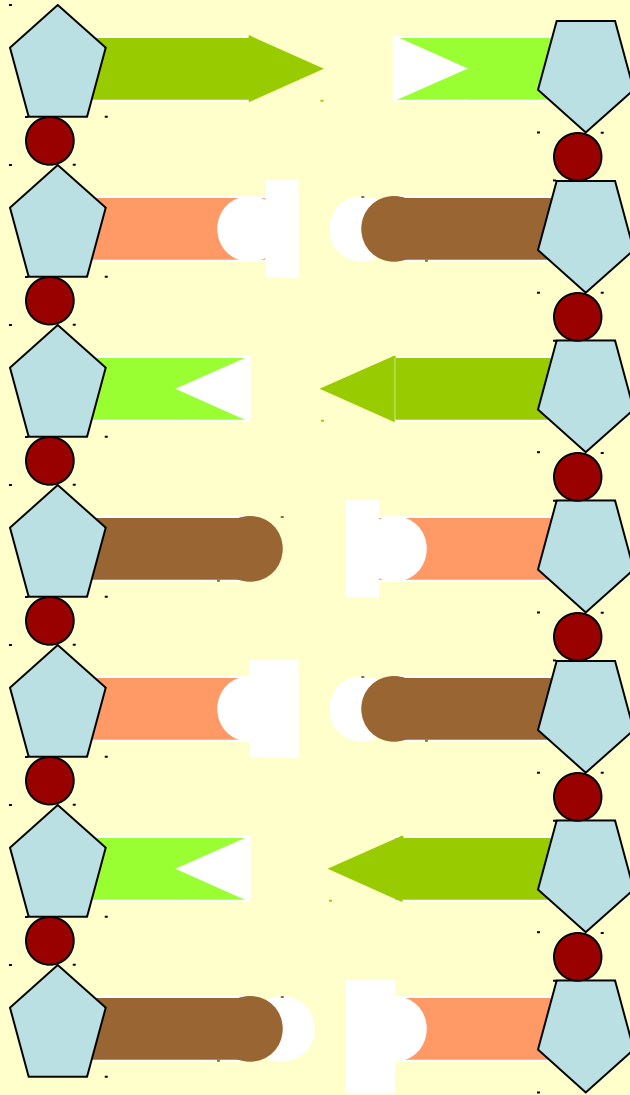
RNA Transcription



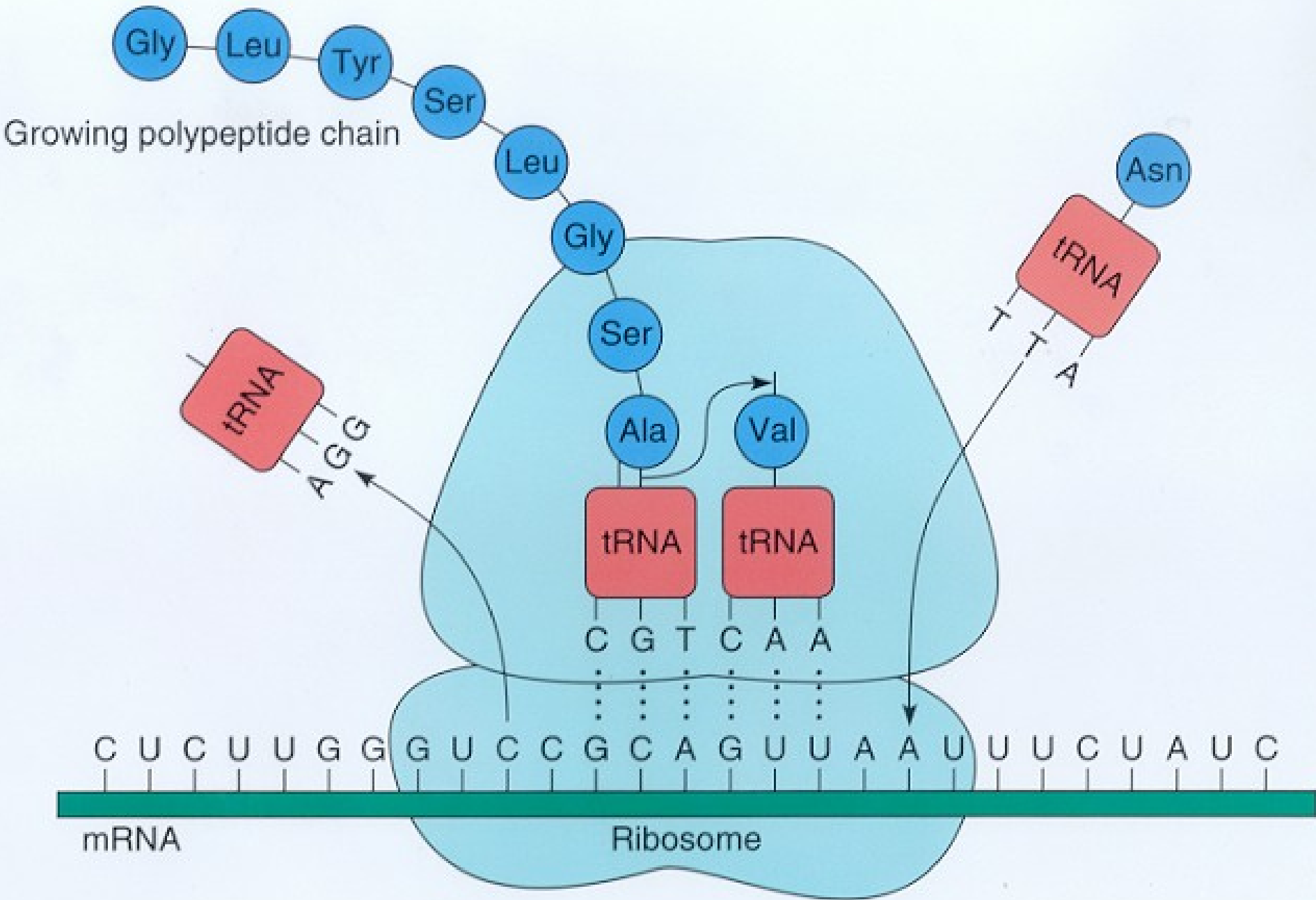
Step 5:
mRNA strand
leaves the
nucleus for
the ribosome



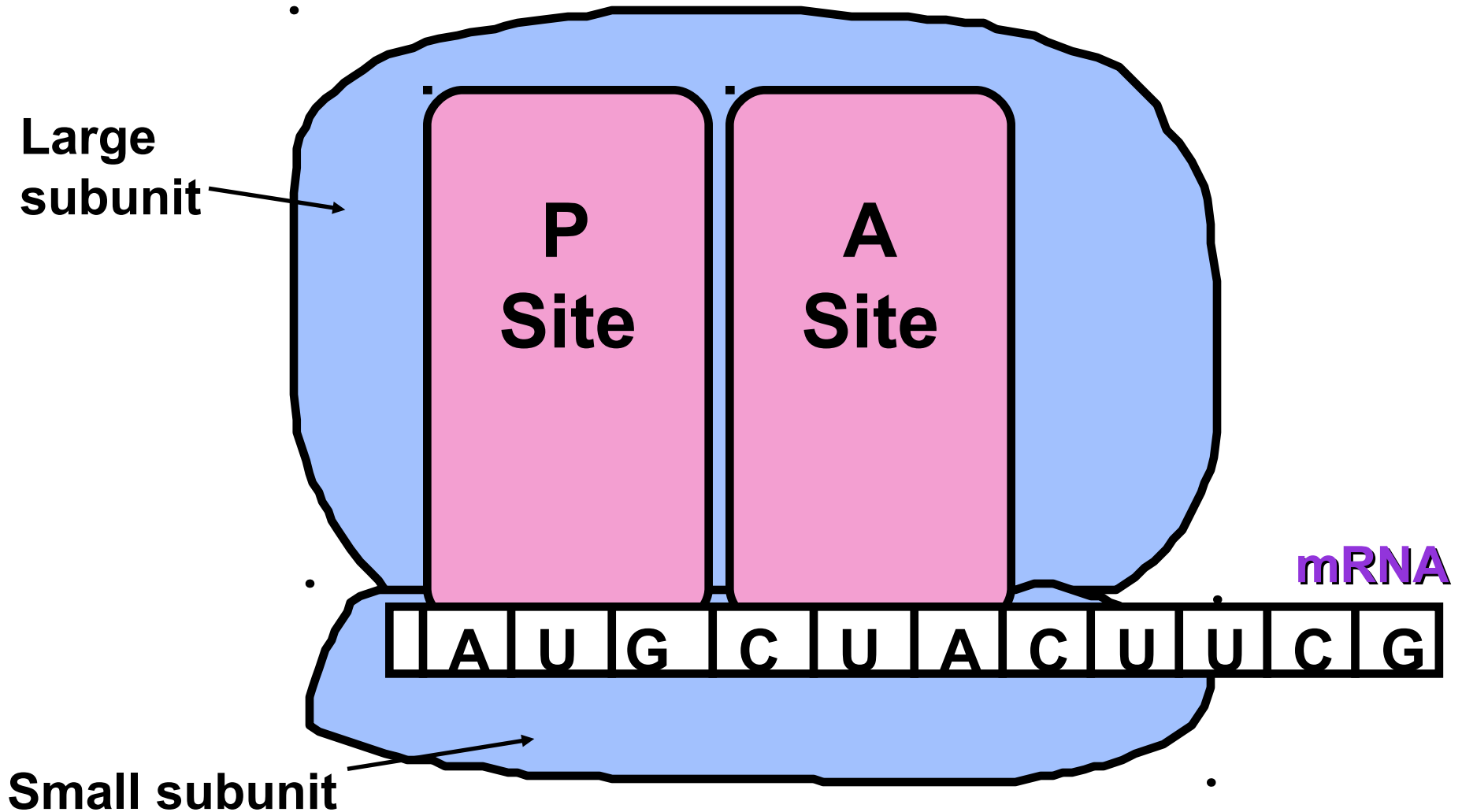
RNA Transcription



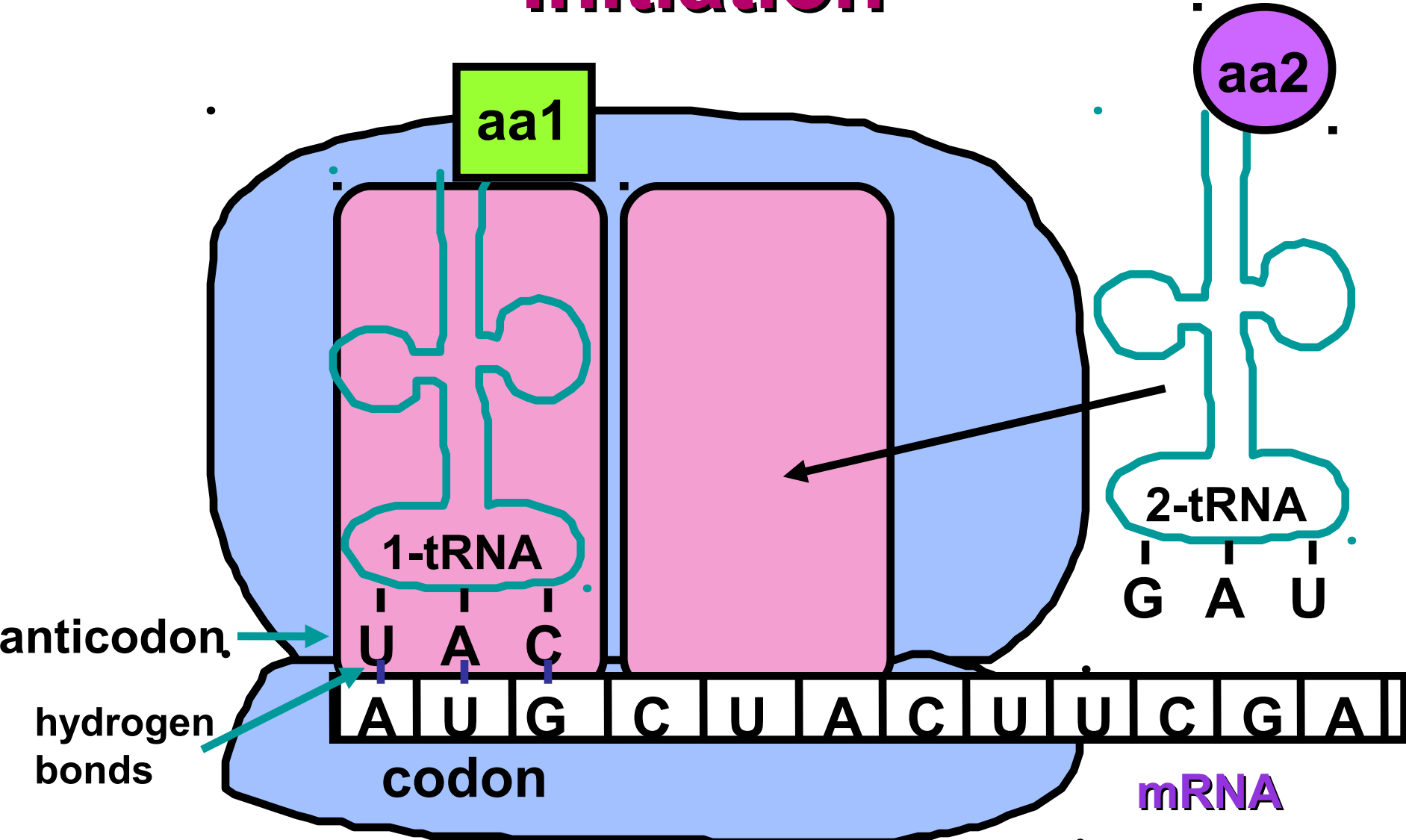
Step 6: Once the mRNA leaves, the DNA “zips” back together



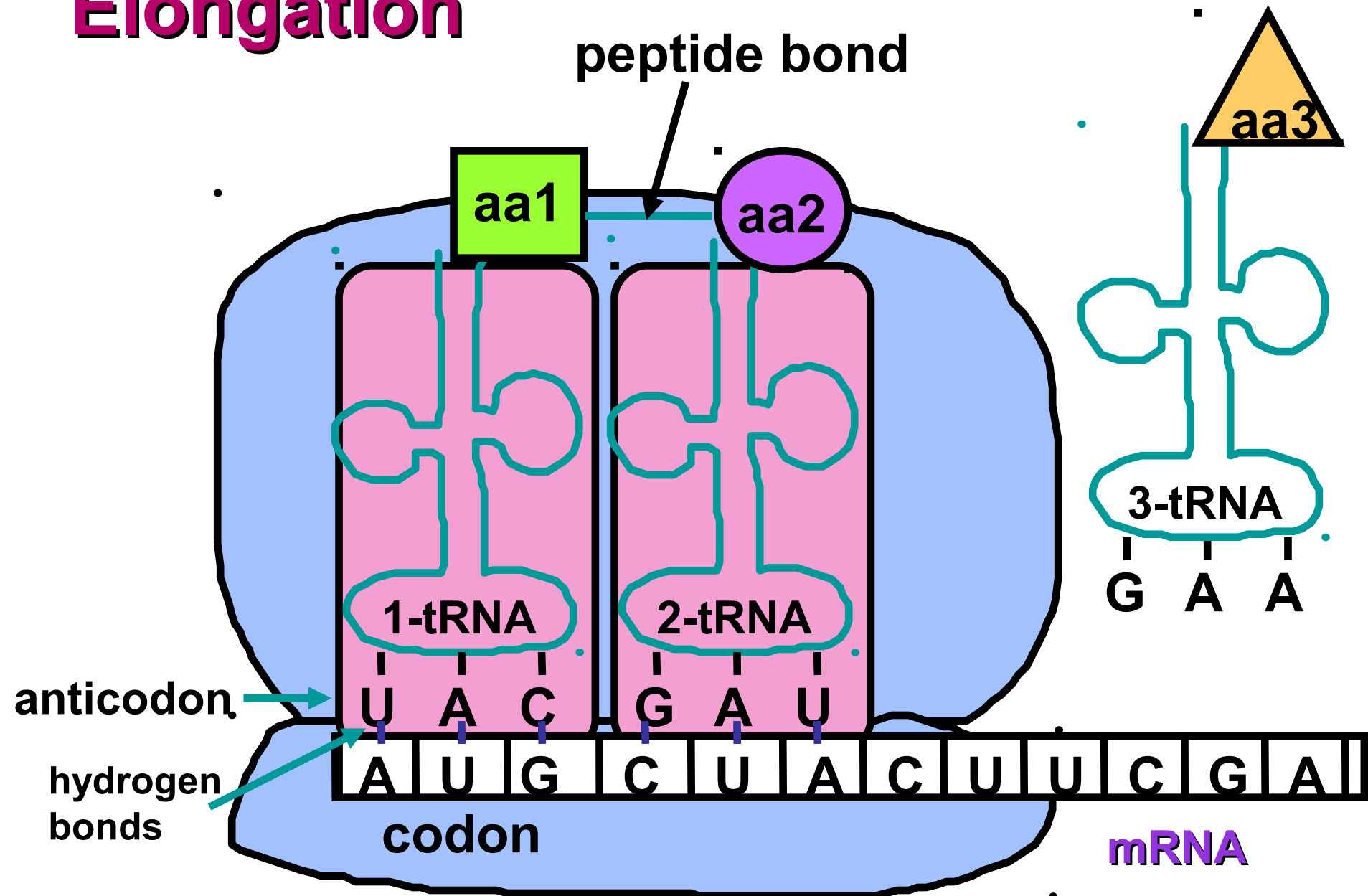
3. Translation

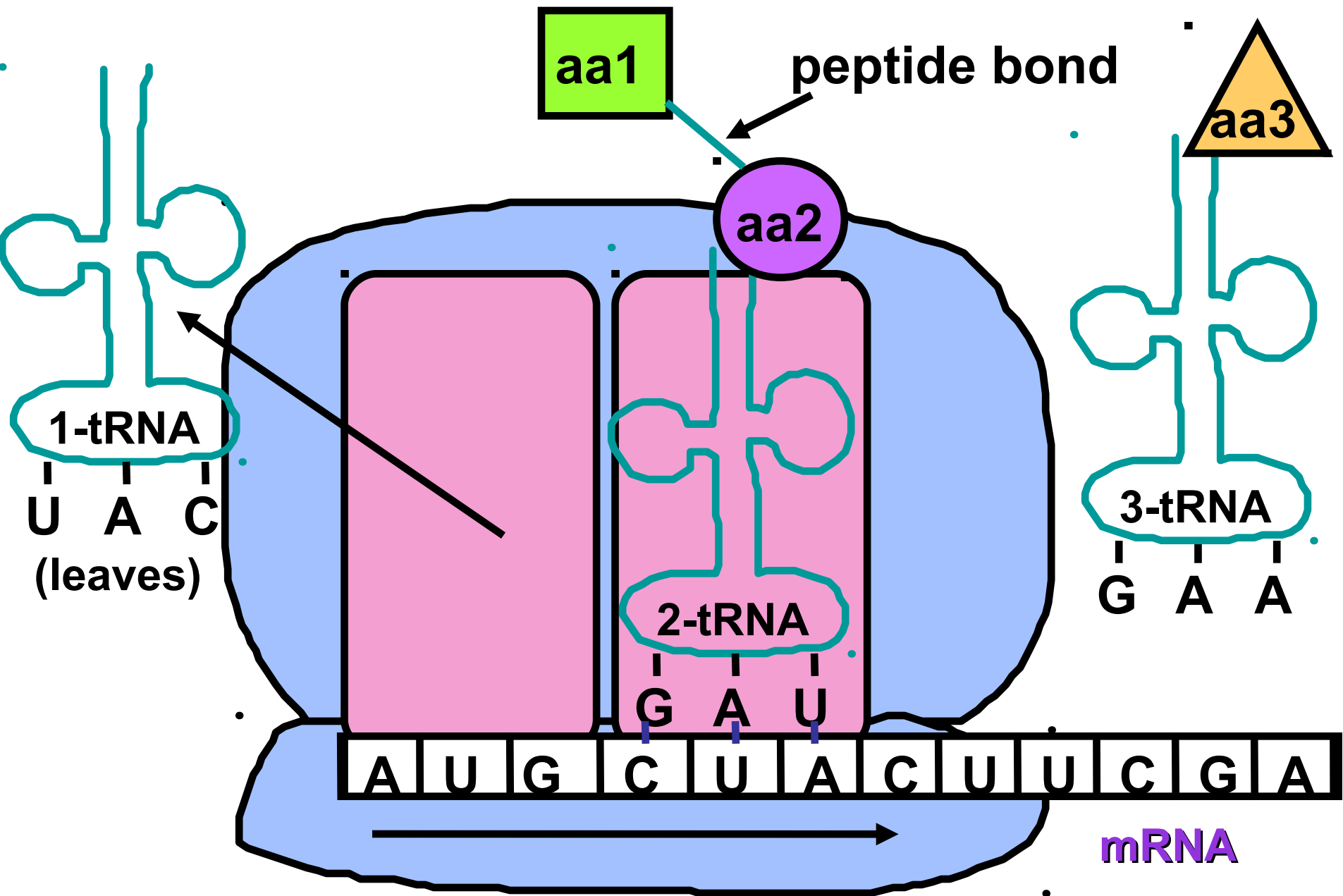


Initiation

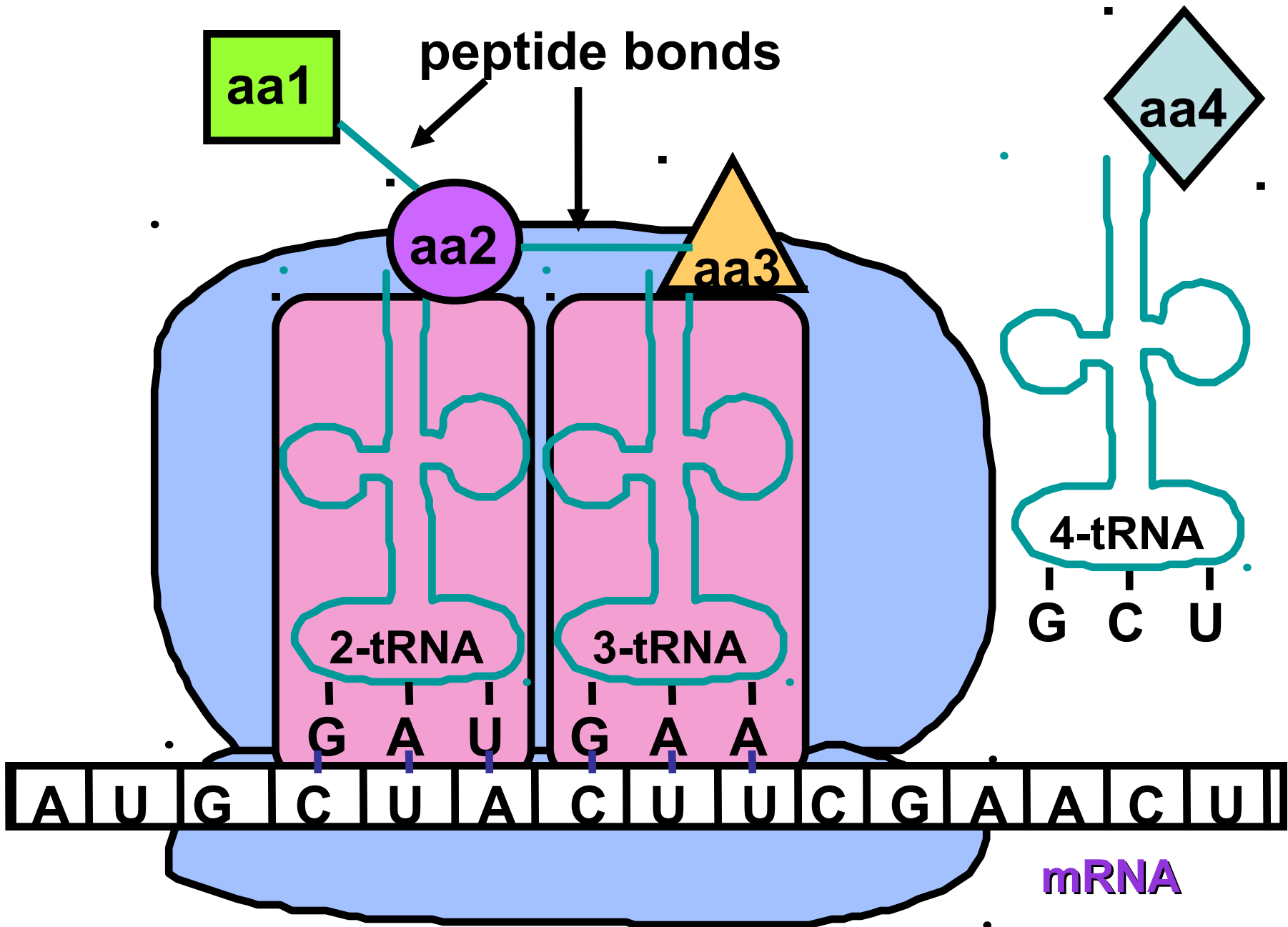


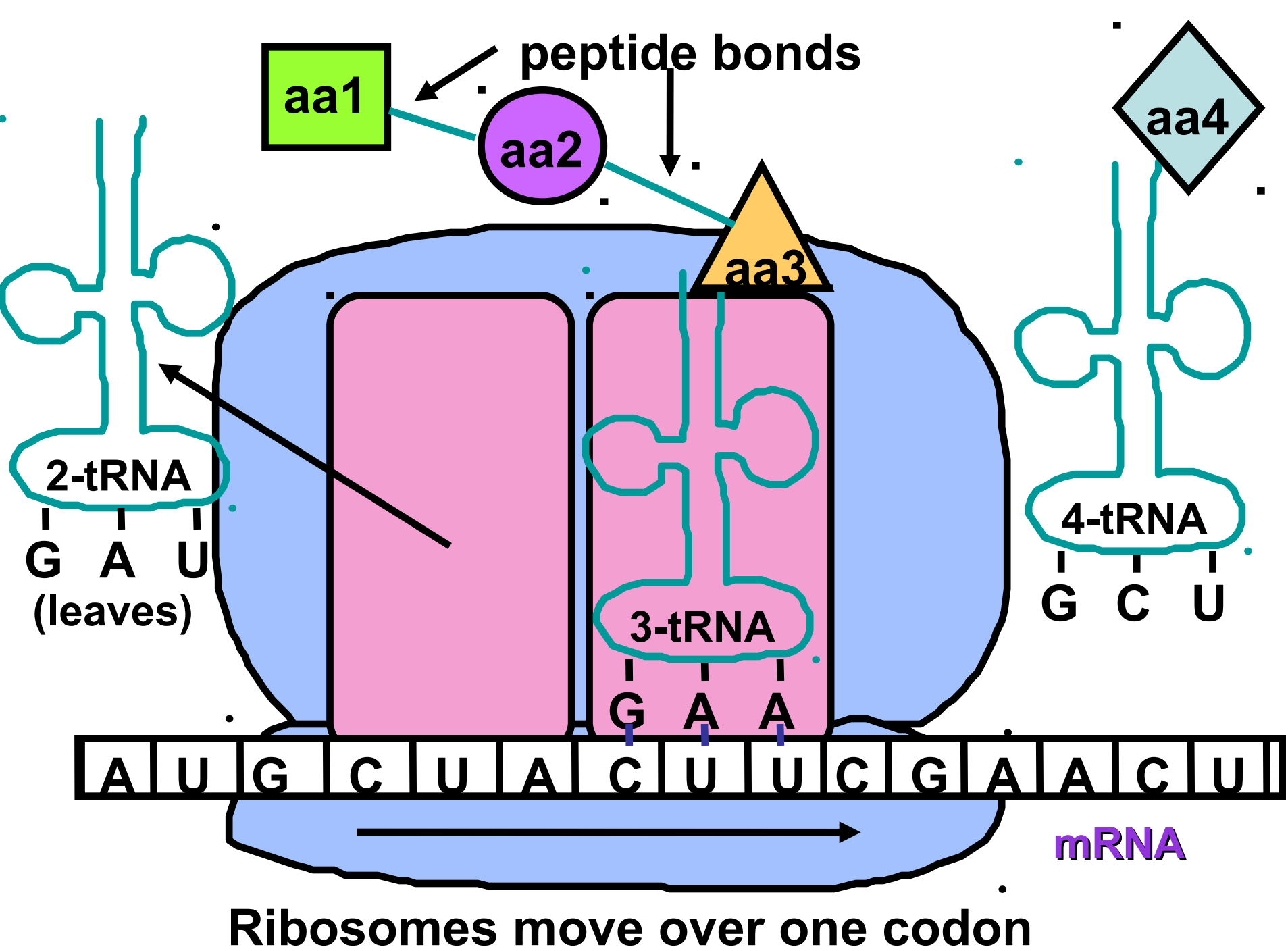
Elongation

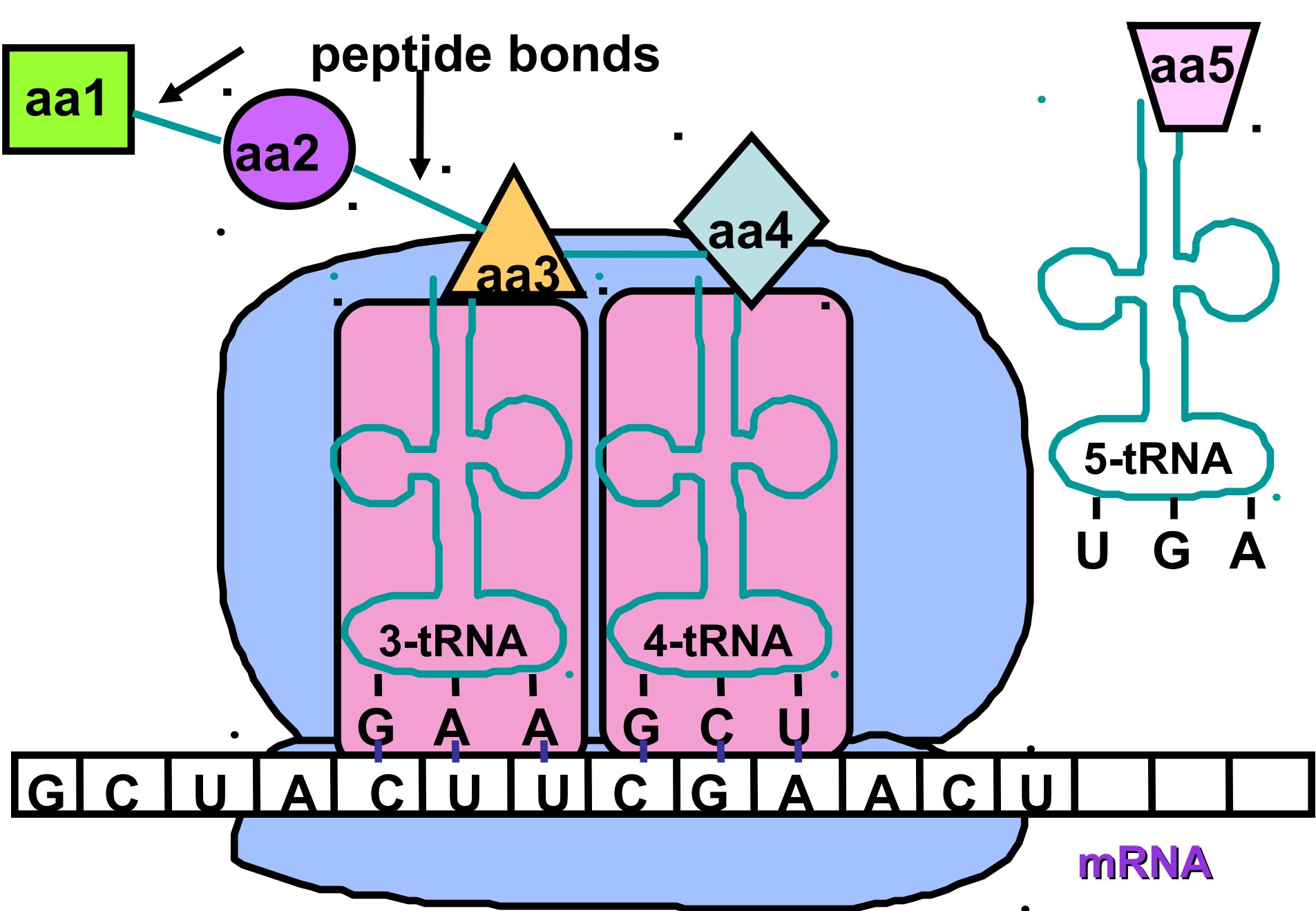


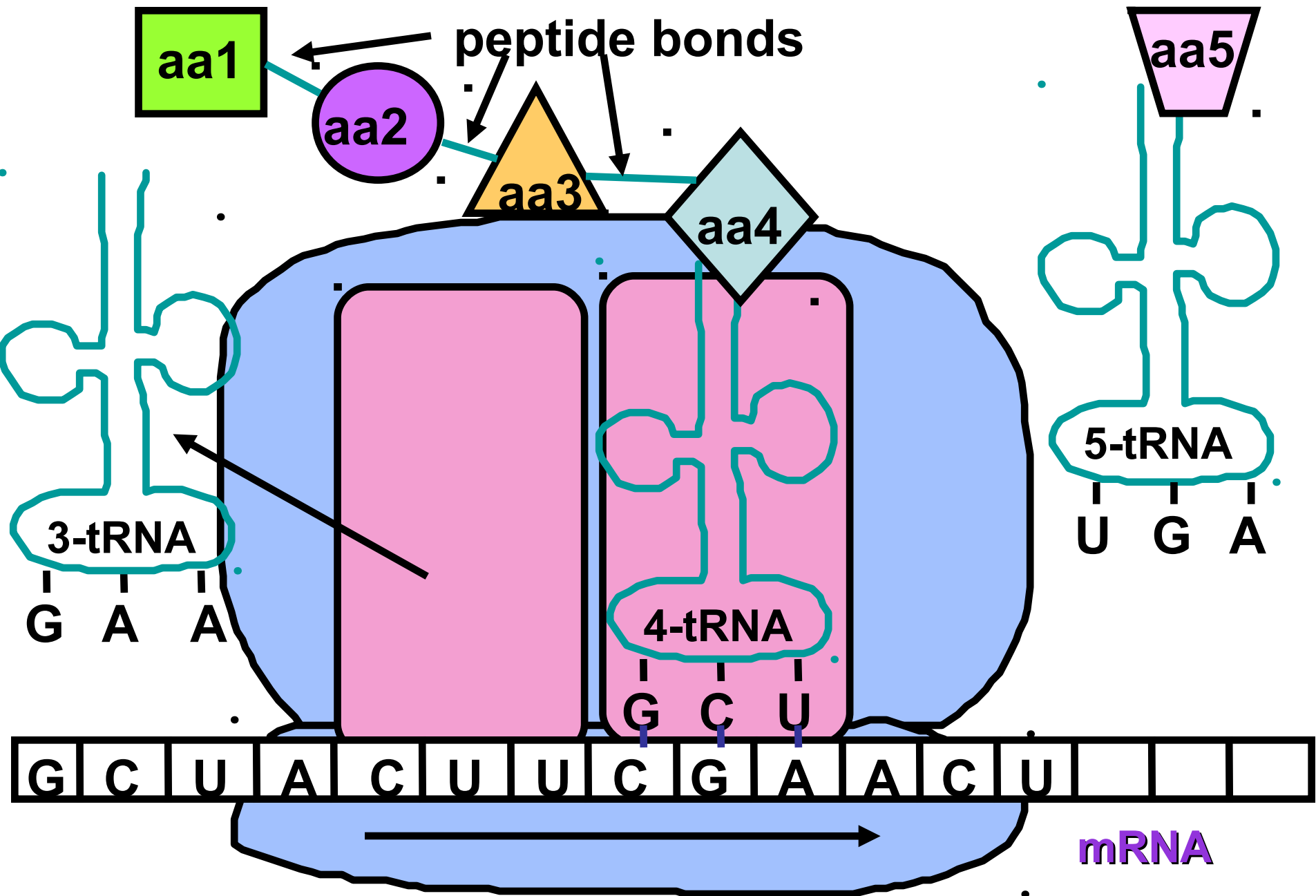


Ribosomes move over one codon

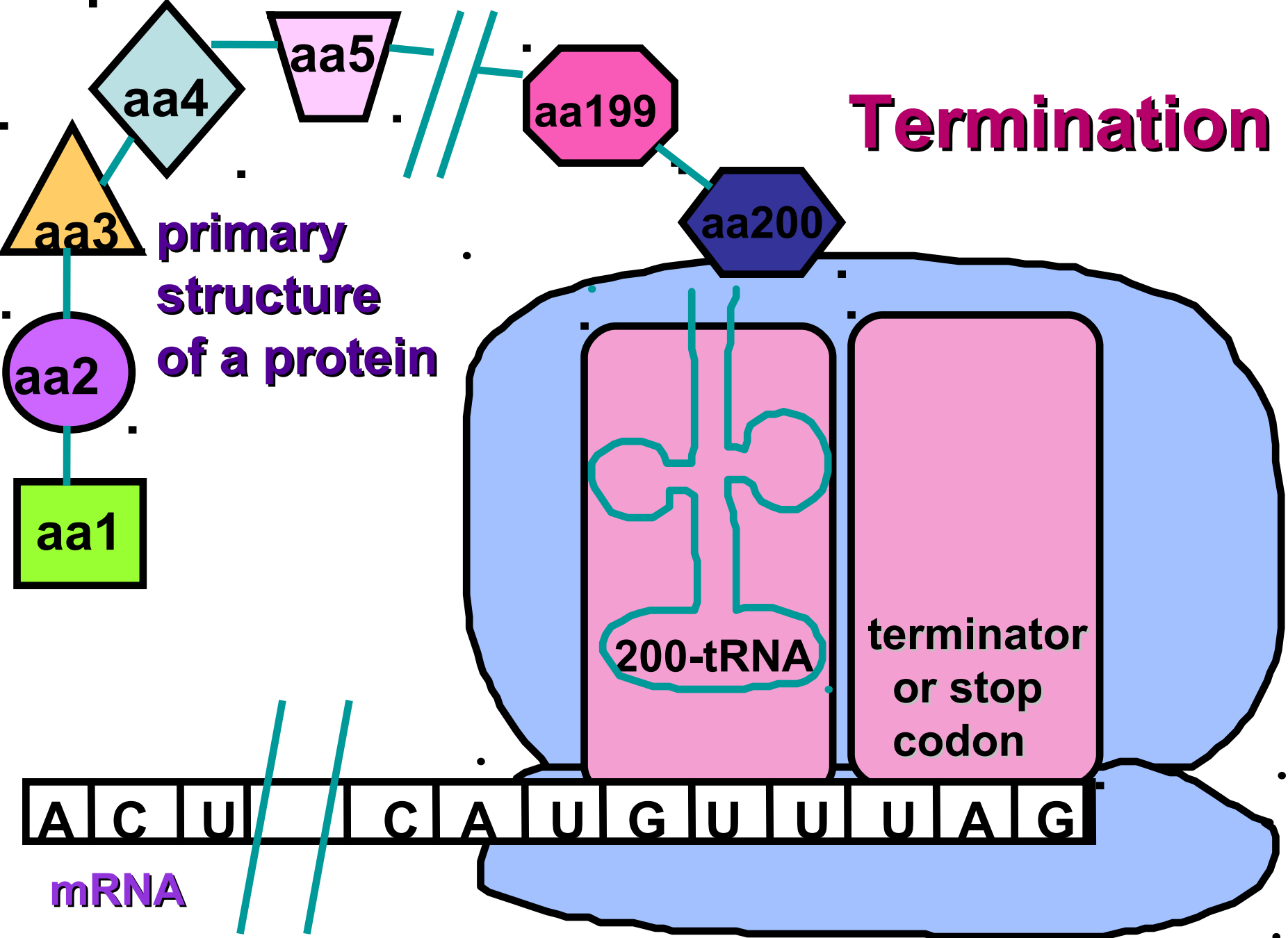




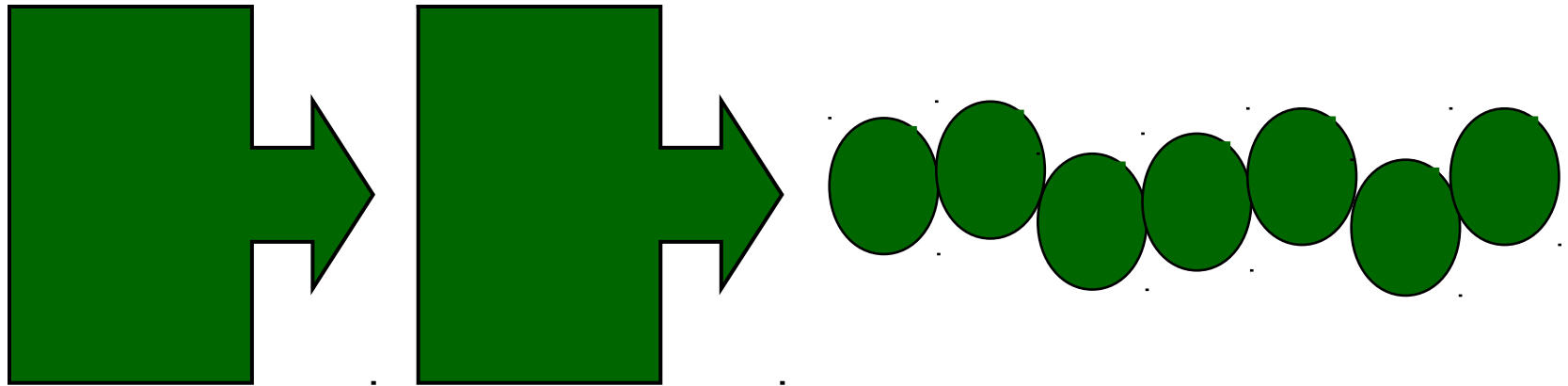




Ribosomes move over one codon



The Central Dogma



Central Dogma - Exceptions

- **Reverse transcriptase**
 - RNA >> DNA
- **Retrotransposons**

ഡിഎൻഎ ഇരട്ടിക്കൽ, ട്രാൻസ്ക്രിപ്ഷൻ, പരിഭാഷ

- എല്ലാ ജീവികളിലും ഒന്നു തന്നെ
- പരിണാമത്തിന്റെ ശക്തമായ തെളിവ്
- തന്മാത്രാ ജൈവ-സാങ്കേതിക വിദ്യയുടെ അടിസ്ഥാനം

ജൈവസാങ്കേതിക വിദ്യ

ജീവികളെ ഉപയോഗിച്ചു കൊണ്ടുള്ള
ഉൽപ്പാദന പ്രക്രിയകൾ





ജൈവസാങ്കേതിക വിദ്യ

- പരമ്പരാഗത ജൈവസാങ്കേതിക വിദ്യ
- ജനിതക സാങ്കേതിക വിദ്യ



ജനിതക സാങ്കേതിക വിദ്യ

- ജീവികളുടെ ജനിതക പദാർഥങ്ങൾ (**DNA**) ഉപയോഗിച്ചുള്ള ഉൽപ്പാദനം



ജനിതക സാങ്കേതിക വിദ്യ

- സ്പീഷീസുകൾ തമ്മിലുള്ള പ്രതിബന്ധങ്ങൾ ഇല്ലാതാവുന്നു
- ഏതു സ്പീഷീസിൽ നിന്നും ഏതിലേക്കും ജീനുകൾ കൈമാറ്റം ചെയ്യാനുള്ള കഴിവ്
- *Genetic Engineering*

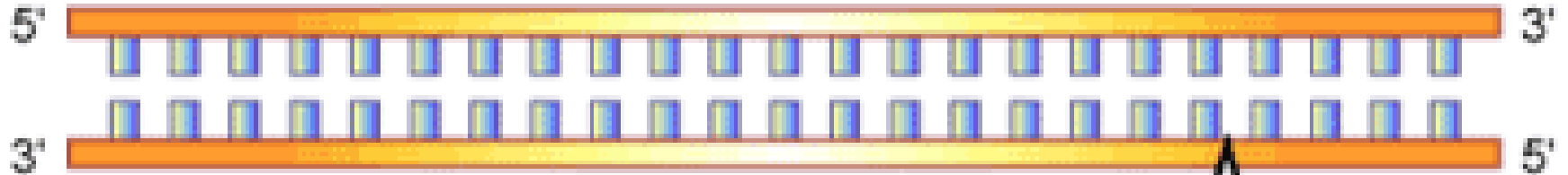
ജനിതക സാങ്കേതിക വിദ്യകൾ

- ഡി ന്റെ എ പുനർസംയോജന വിദ്യ
 - *Recombinant DNA technology*
 - *(Genetic engineering)*
- മറ്റു ഡി ന്റെ എ സാങ്കേതിക വിദ്യകൾ
 - *Hybridization, amplification, sequencing*
- പുതിയ പ്രത്യുൽപ്പാദന സാങ്കേതിക വിദ്യകൾ
 - *New Reproductive technologies*

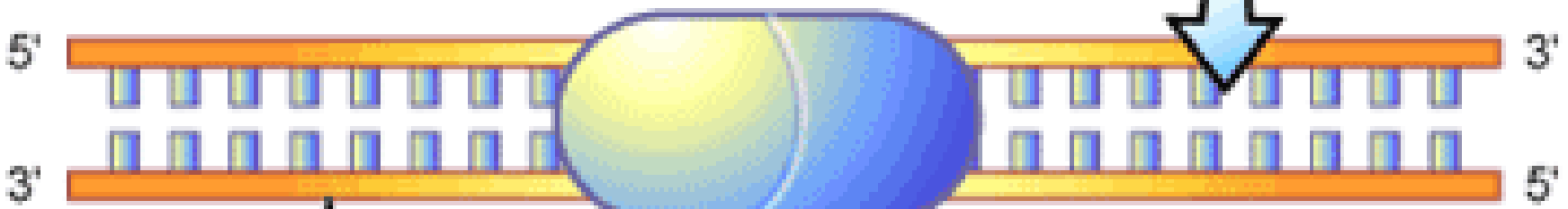
Technologies in Molecular biology

- **Cutting up and joining DNA**
- **Cloning**
- **Hybridization**
- **Amplification**
- **Sequencing**
- **Microarrays and expression profiling**

G A A T T C



C T T A A G



EcoRI, restriction endonuclease

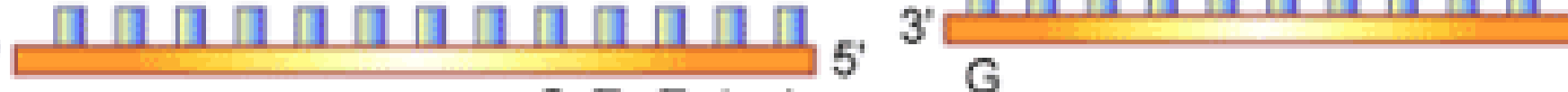
G

A A T T C



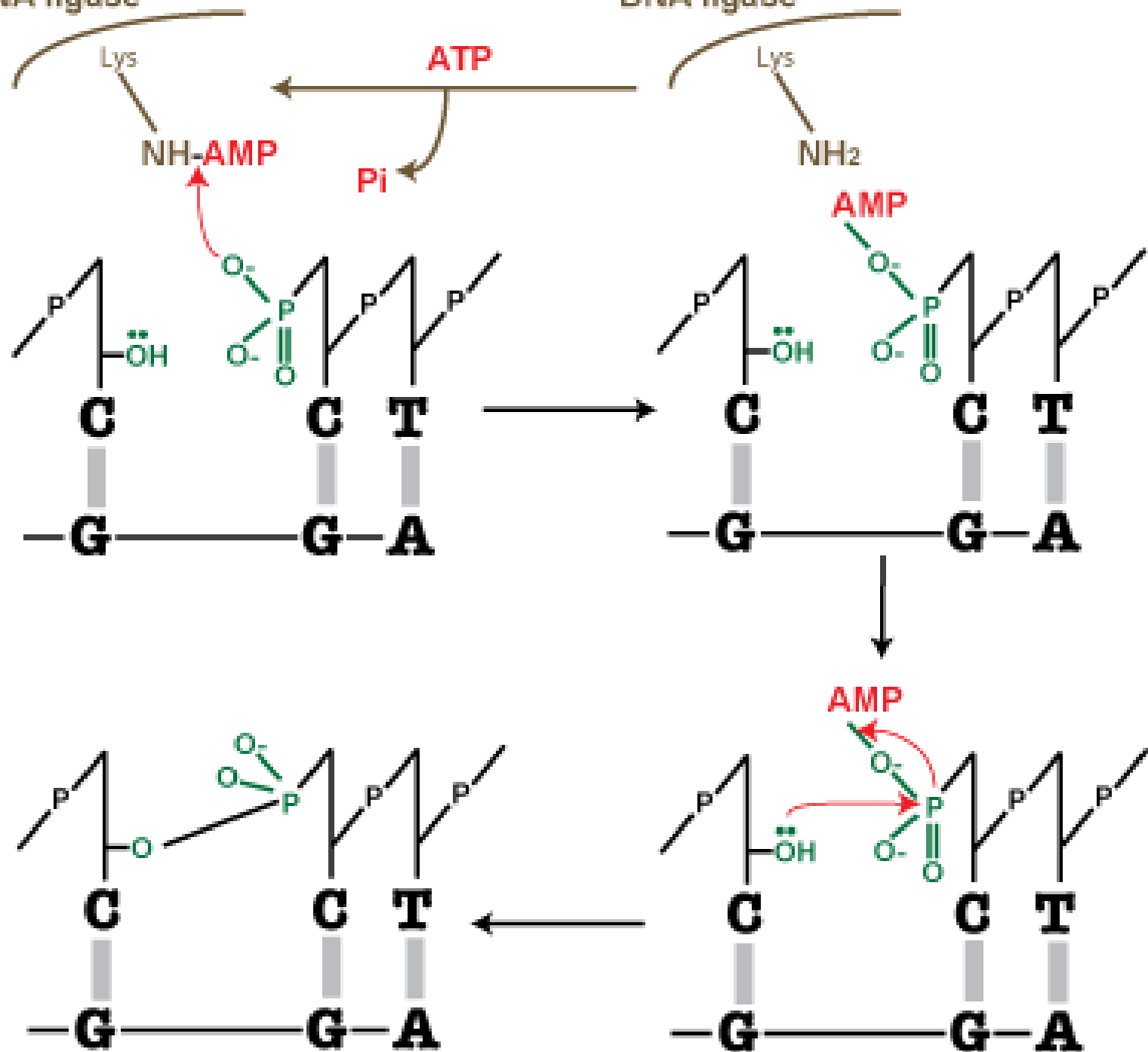
C T T A A

G



DNA ligase

DNA ligase



EcoRI



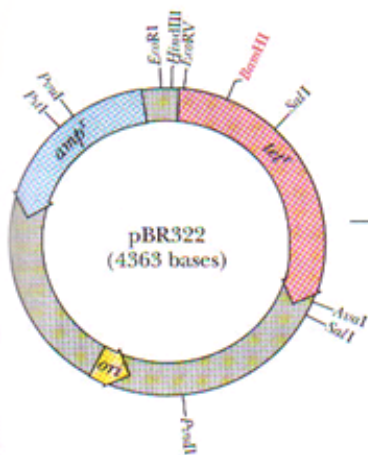
EcoRI



Mixing together and annealing



Recombinant DNA

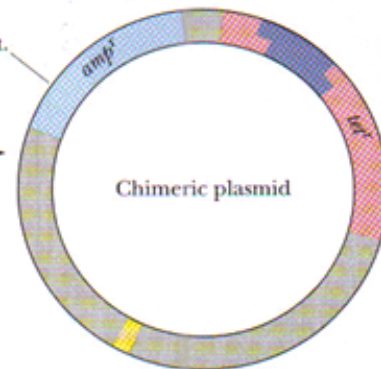


- 1 A plasmid with genes for ampicillin resistance (*amp^r*) and tetracycline resistance (*tet^r*). A *Bam*HI restriction site is located within the *tet^r* gene.

- 2 *Bam*HI restriction fragment of DNA to be cloned is inserted into the *Bam*HI site of *tet^r*.

amp^r gene remains intact.

- 3 *tet^r* gene is split by the insertion of DNA fragment. *amp^r* gene remains intact.

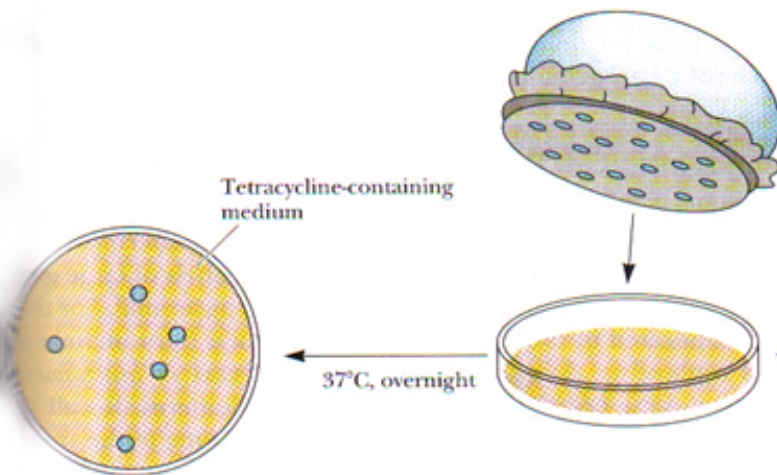


- 4 Suspend 20 ng plasmid DNA + 10^7 *E. coli* cells in CaCl_2 solution.

42°C, 2 min

- 5 Plate bacteria on ampicillin media.

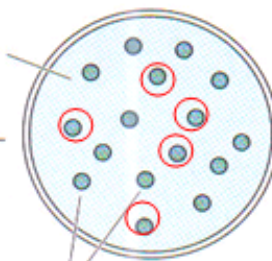
37°C, overnight



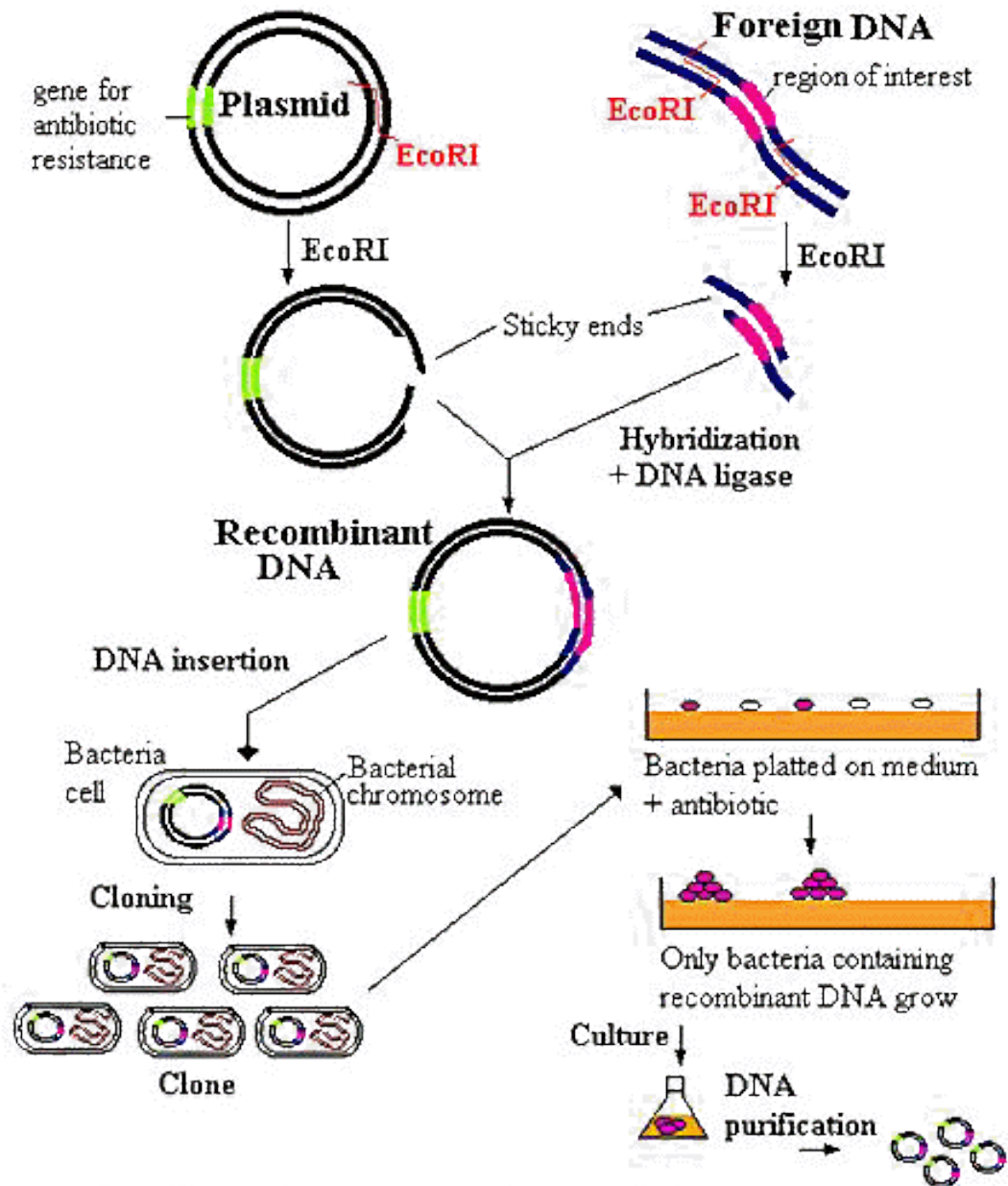
Only *tet^r* colonies appear; *tet^r* colonies can be recovered from *amp^r* plate by comparing two plates.

- 7 Using velvet-covered disc, bacterial colonies are lifted from surface of agar *amp^r* plate and pressed briefly to surface of plate containing tetracycline media.

Ampicillin-containing medium



- 6 Only ampicillin-resistant (*amp^r*) bacterial colonies grow.



Cloning into a plasmid

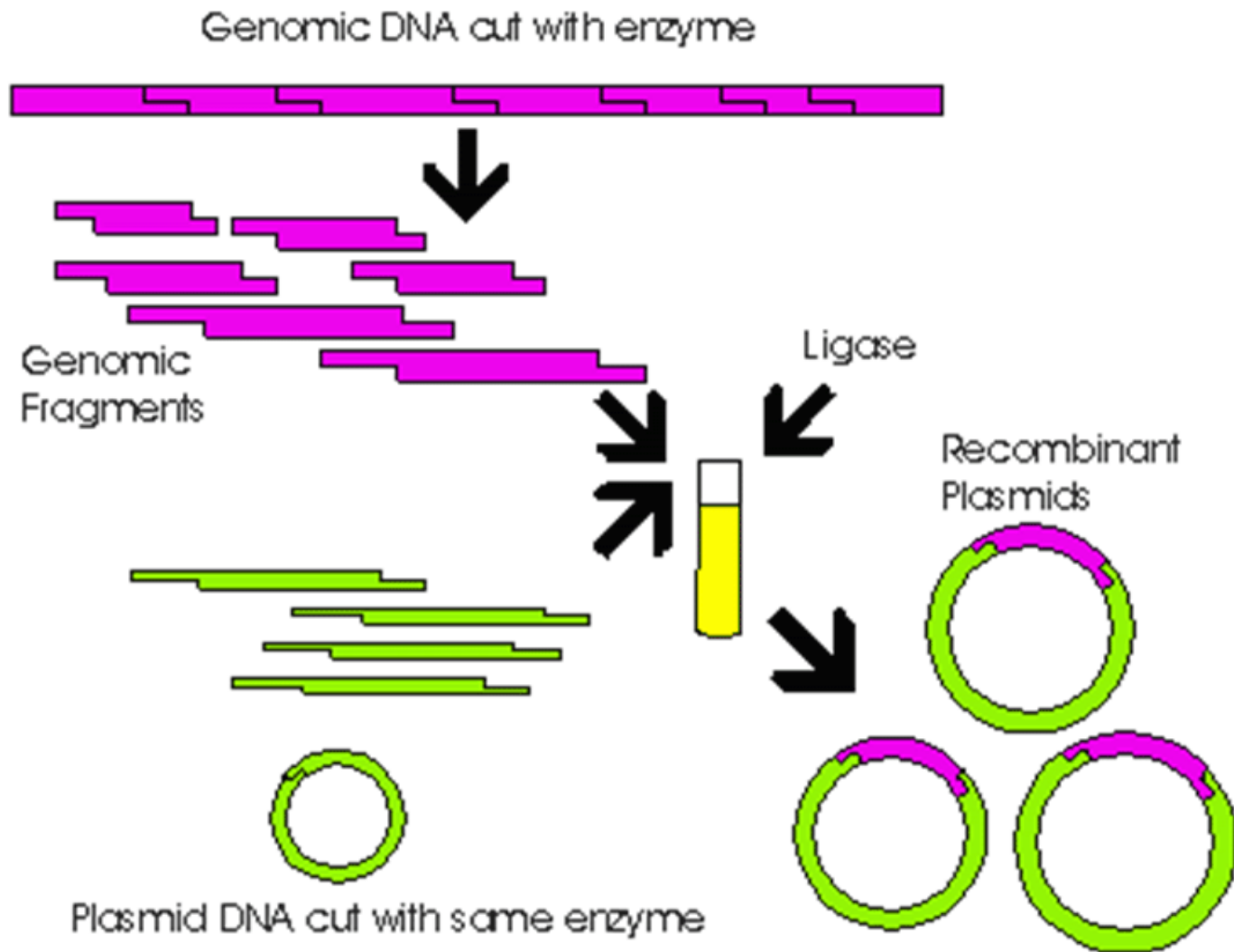


Herbert Boyer

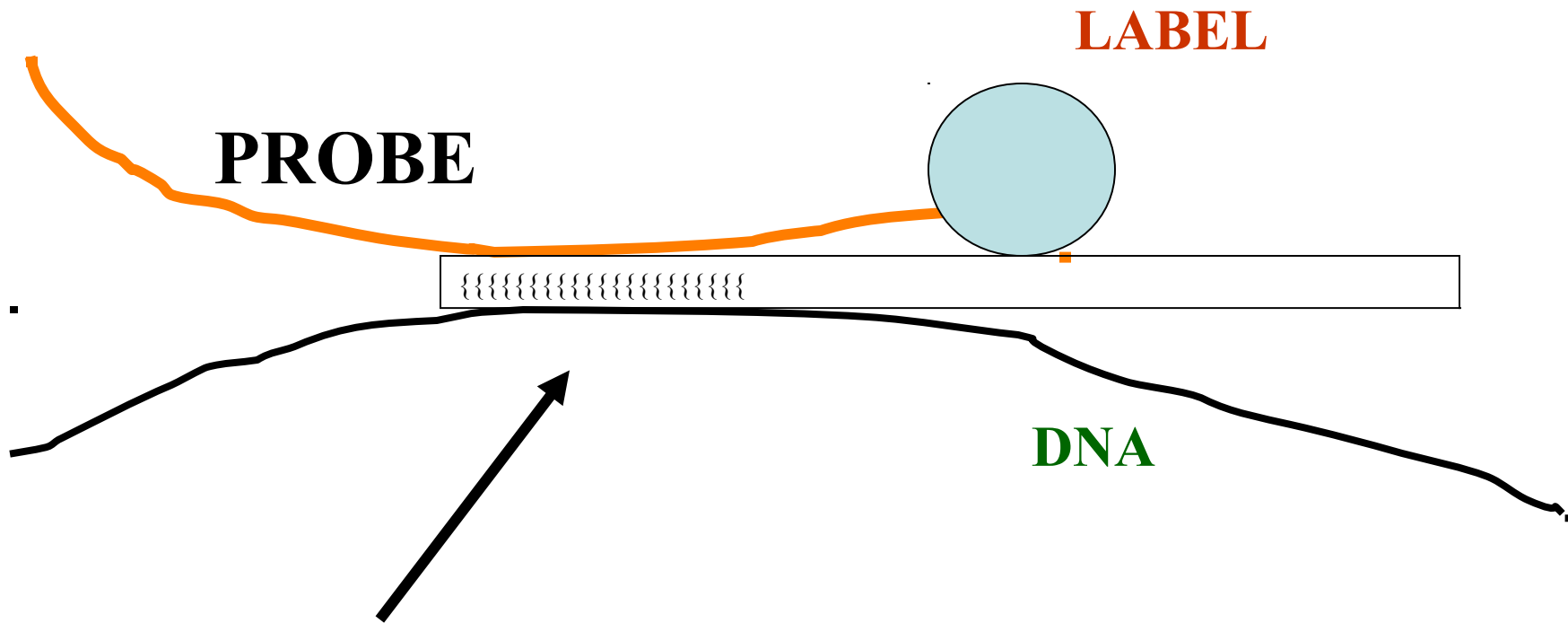


Stanley Cohen

മനുഷ്യ ഇൻസുലിൻ ഉൽപ്പാദനം



Hybridization methods



CGGCAACGA
GCCGTTGCT

Specificity from sequence

Southern blotting procedure

human genomic DNA (isolated from many cells)

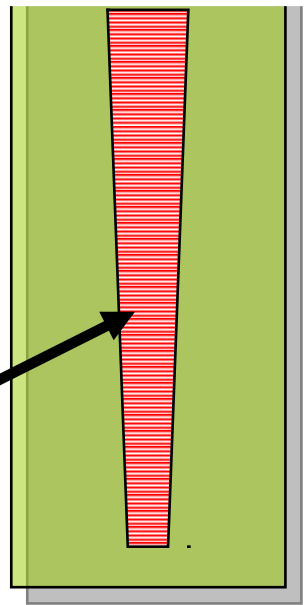
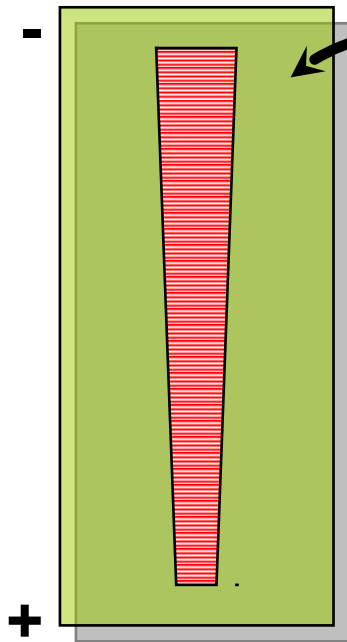


• restriction enzyme digestion

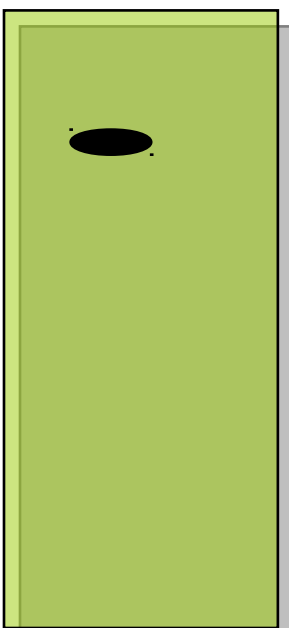


• millions of DNA fragments

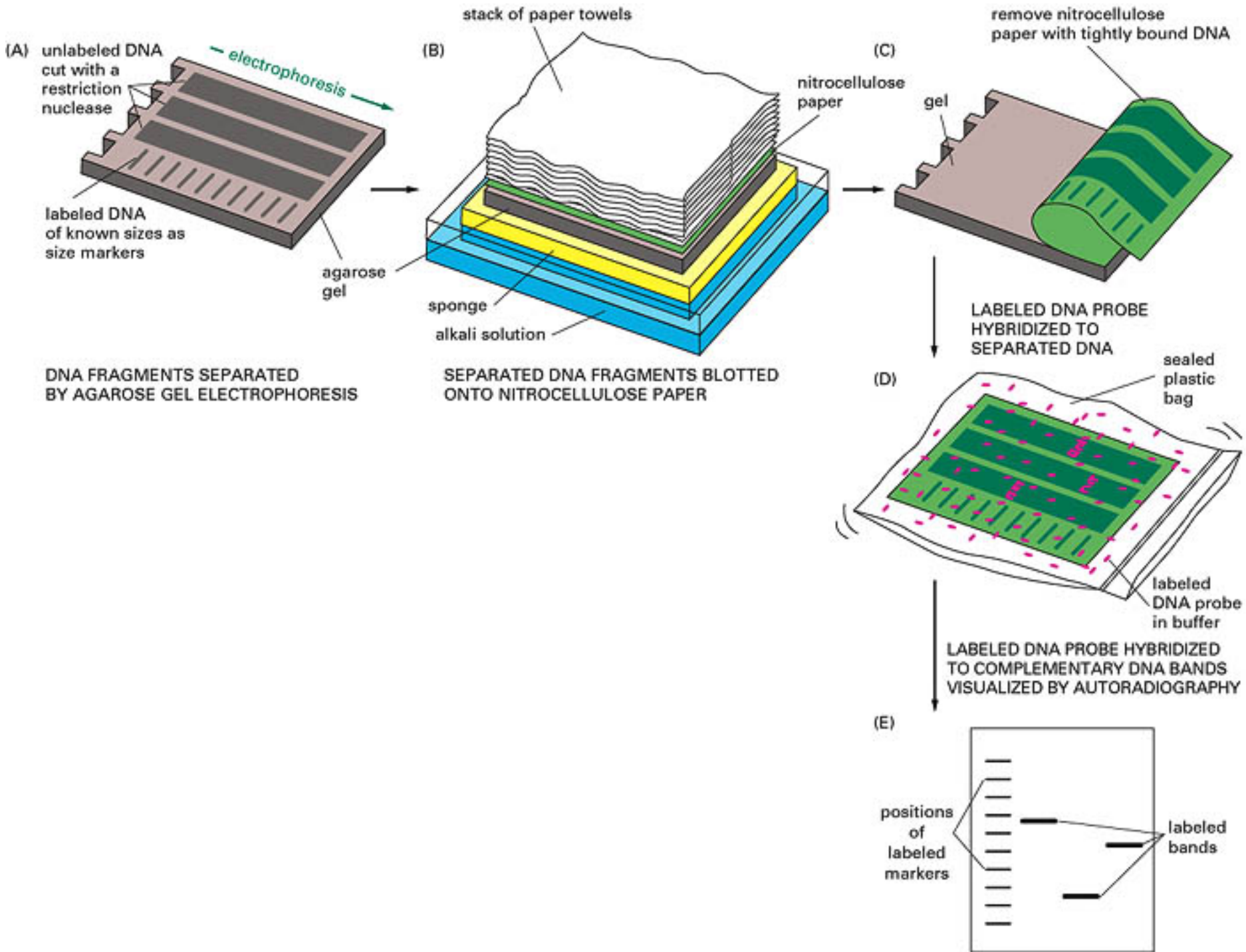
- gel electrophoresis of the DNA fragments
- gel will separate DNAs according to size

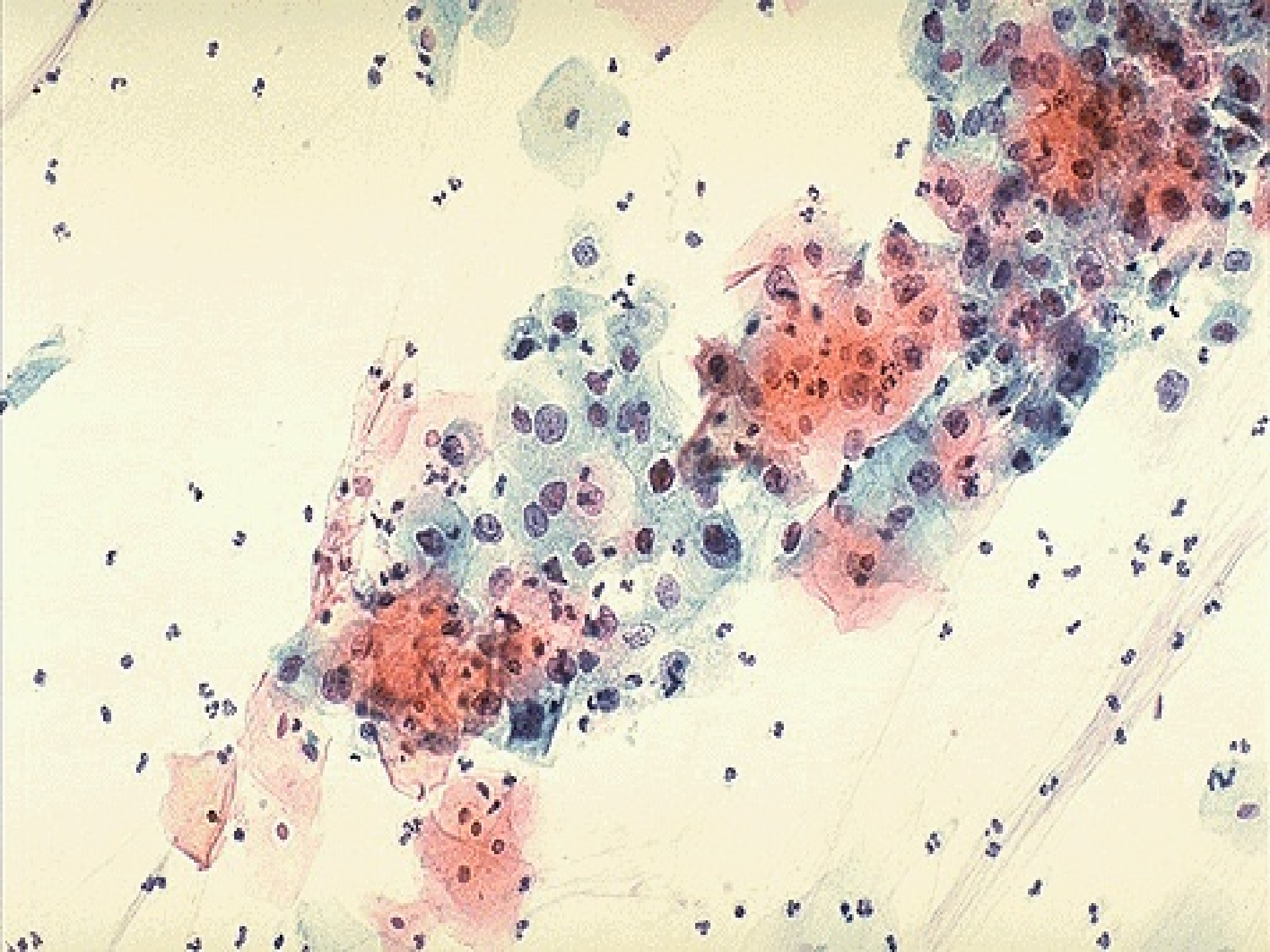


PROBE WITH RADIOACTIVE TAG



AUTORADIOGRAPHY





1

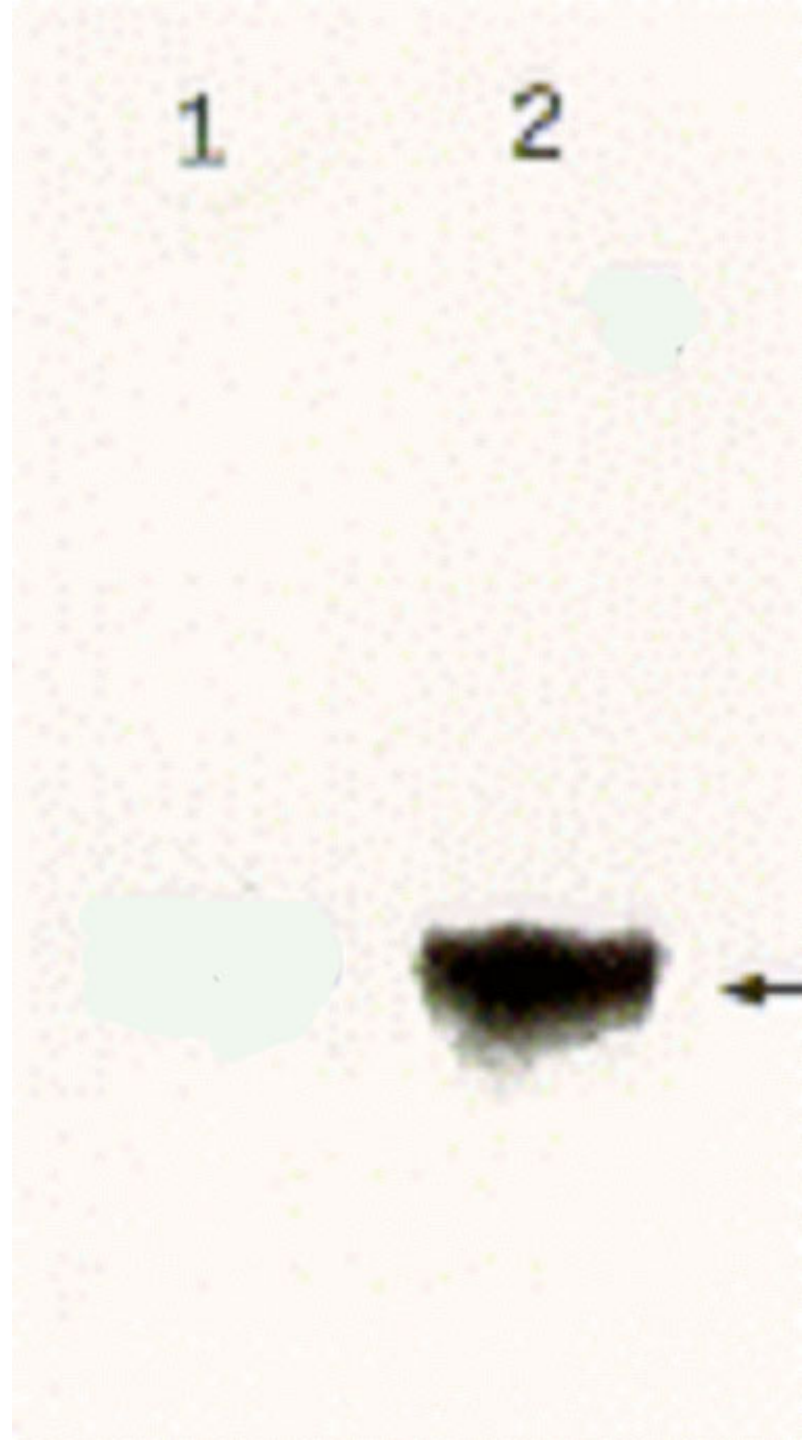
2

Southern Blot

HPV 18 Probe

Patient 1 negative

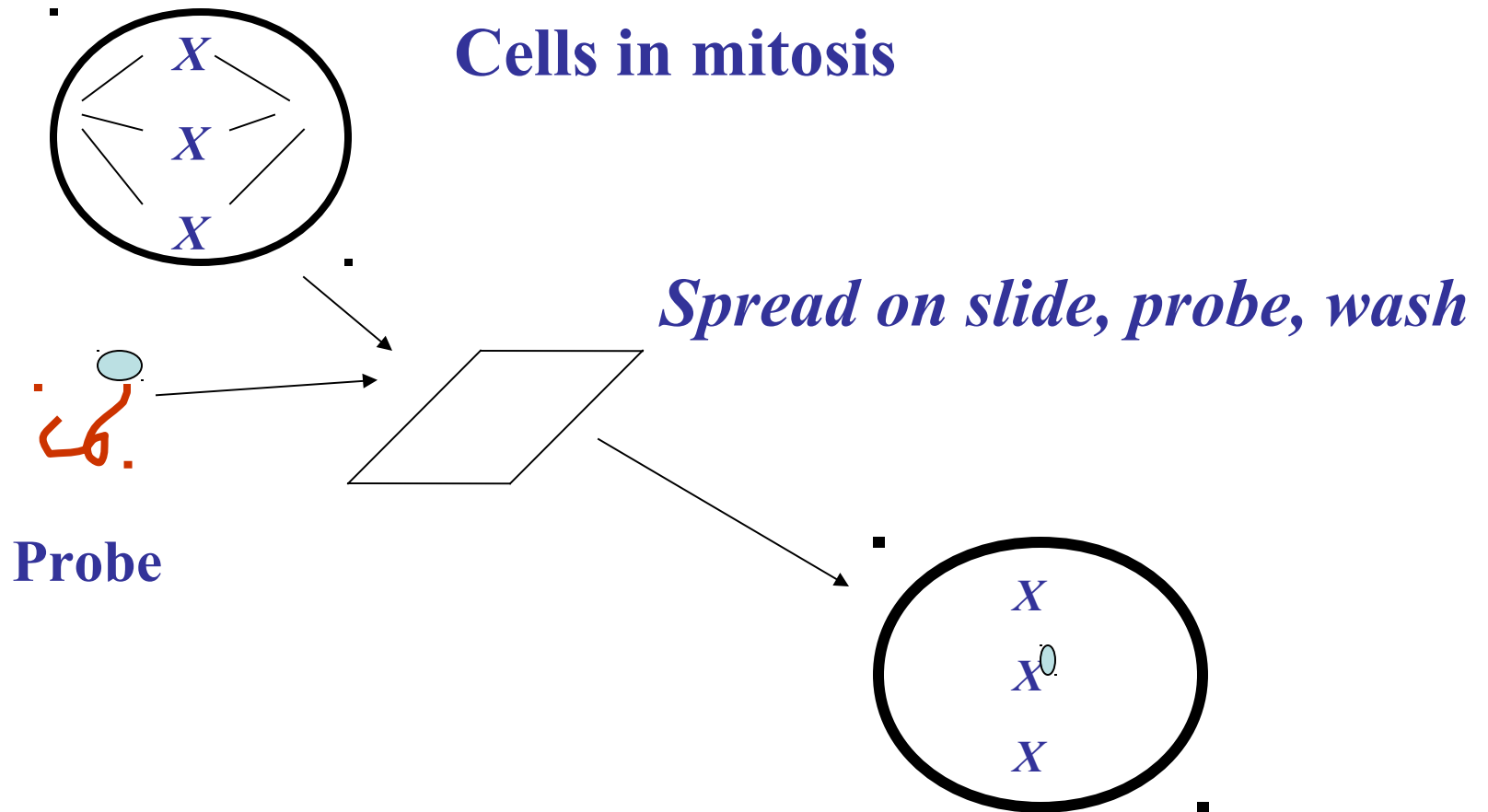
Patient 2 positive



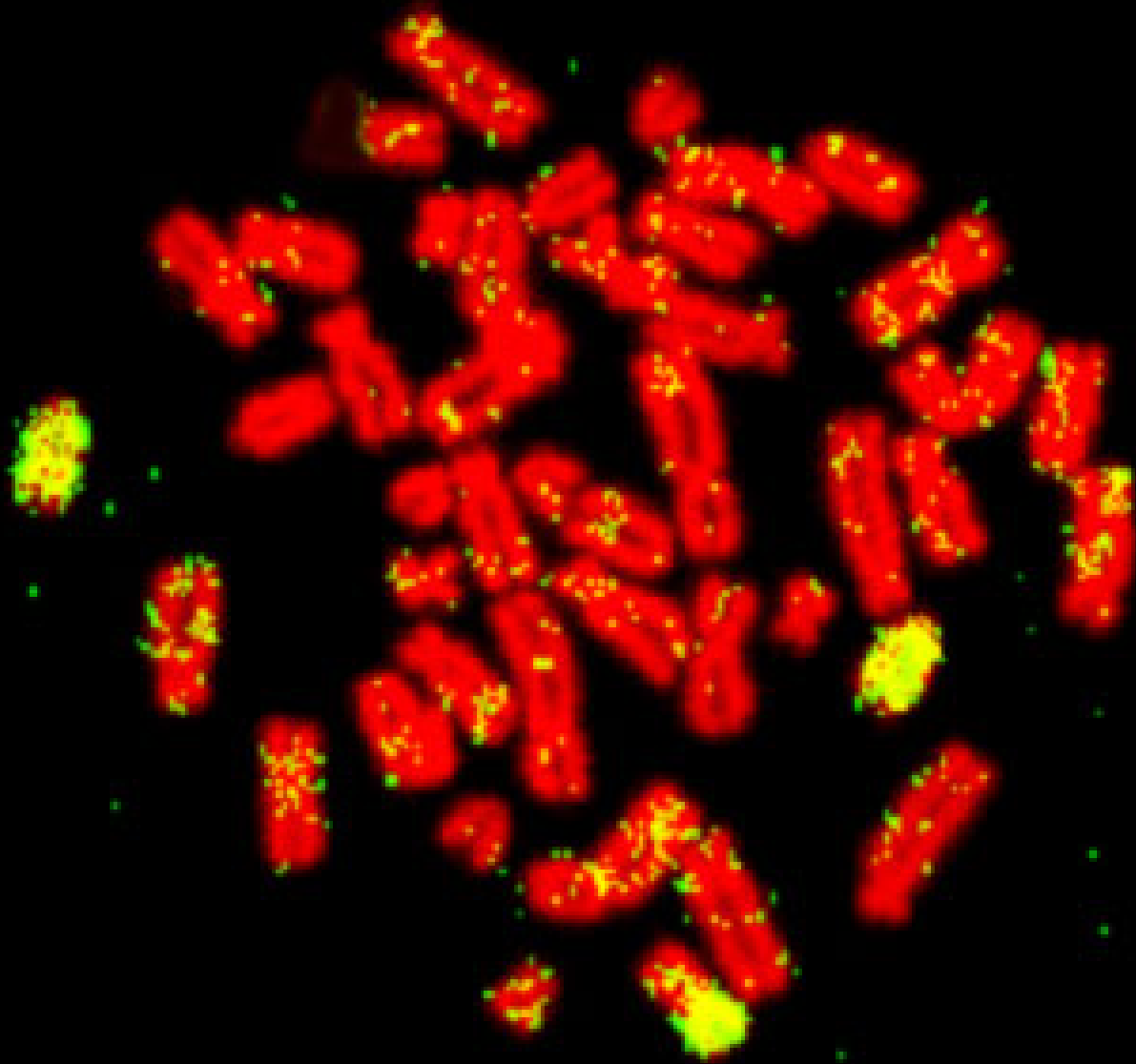
In situ hybridization

- **Specific probe directed against a sequence of interest in**
 - **Chromosome spreads**
 - **Tissue sections**
- **The probe can be tagged with**
 - **Fluorescent marker (FISH)**
 - **Enzyme marker**

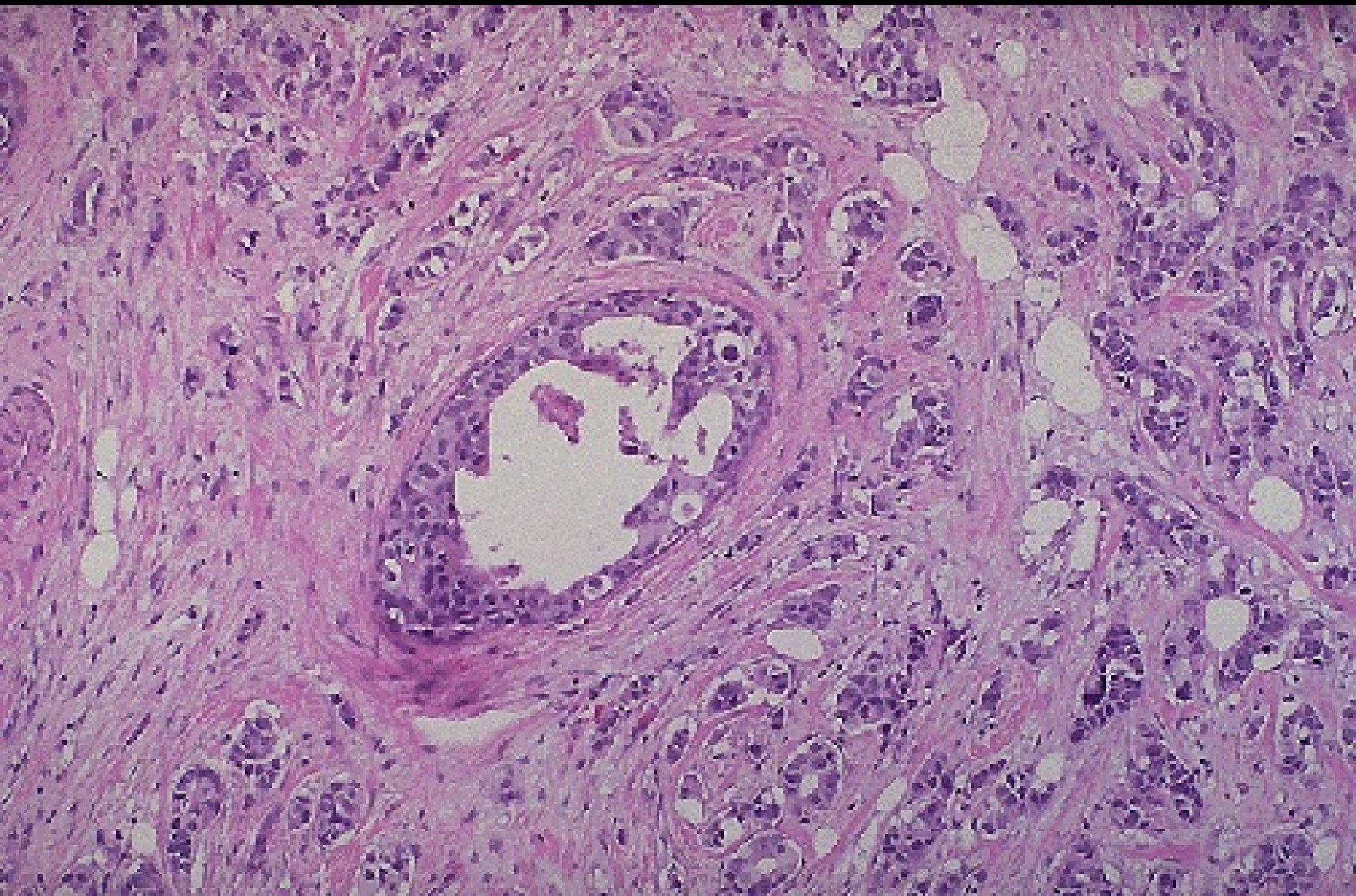
In situ hybridisation for DNA (FISH)



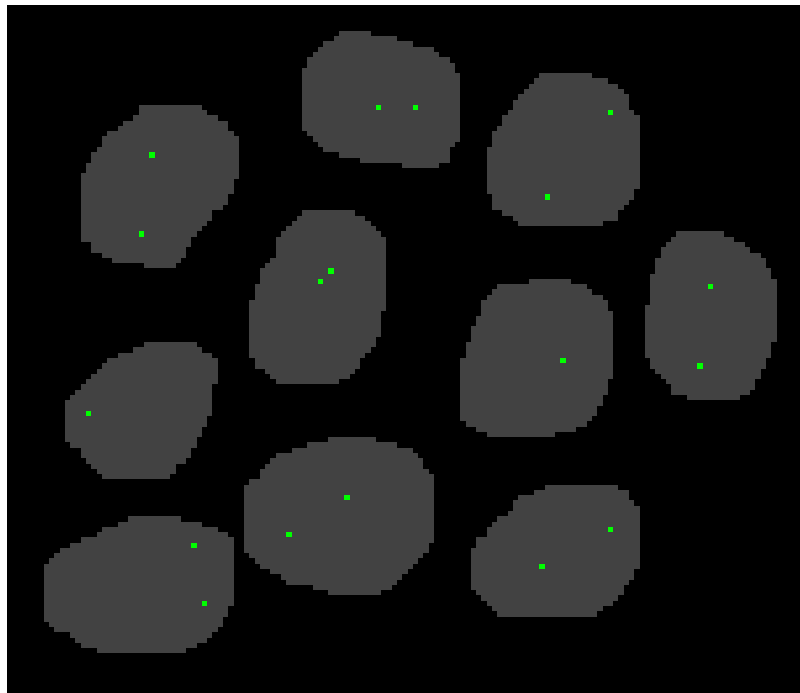
Probe against specific sequence on chromosome 21



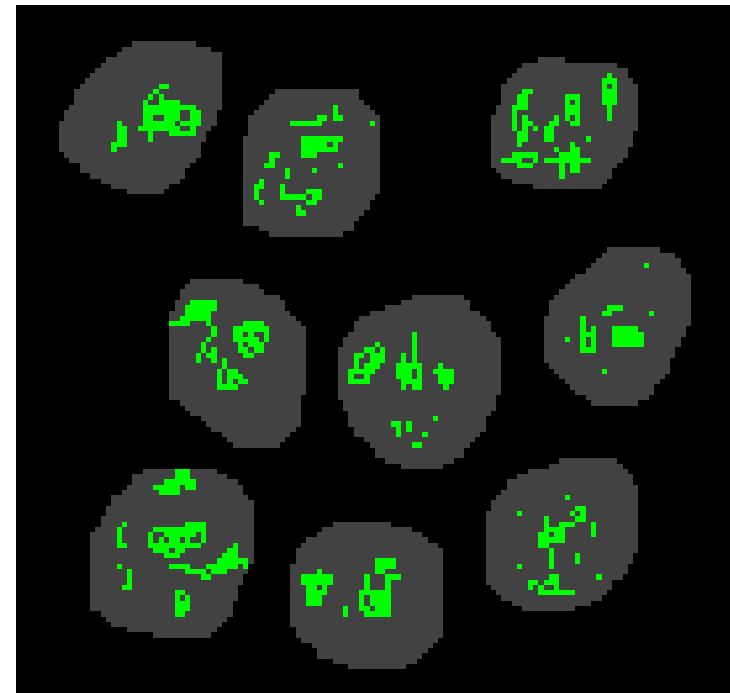
FISH



Fluorescent In-Situ Hybridization Detecting Amplified *neu*

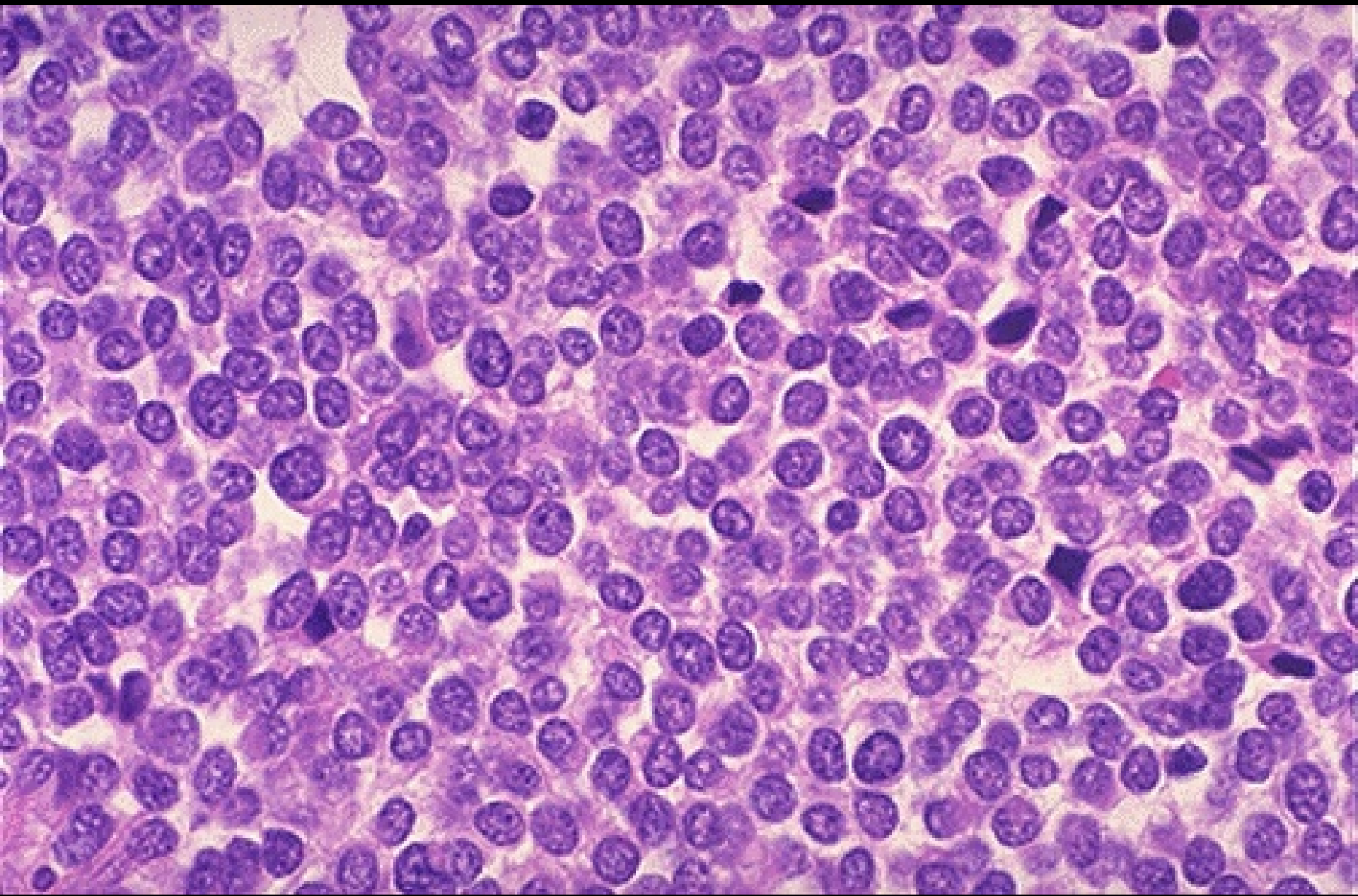


Not Amplified

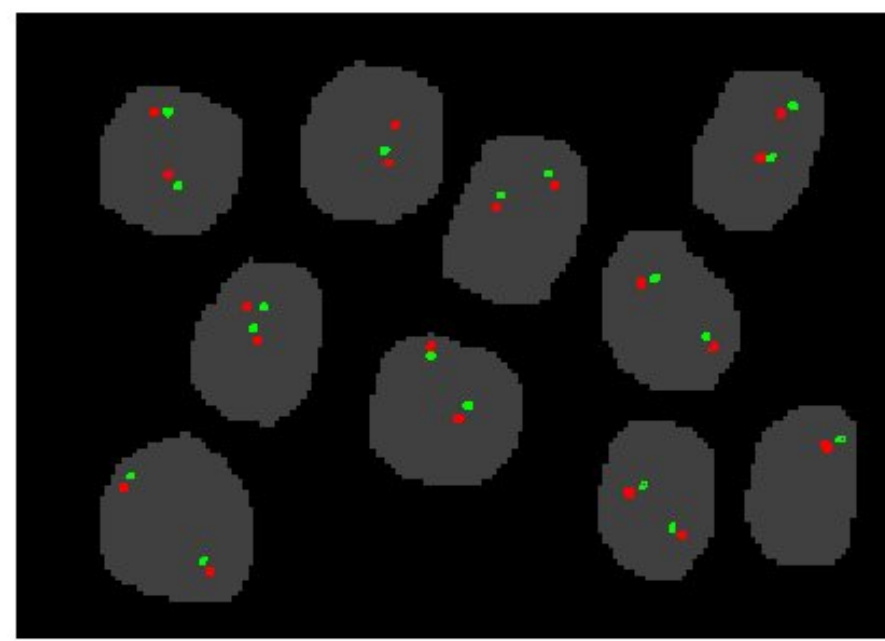


Amplified

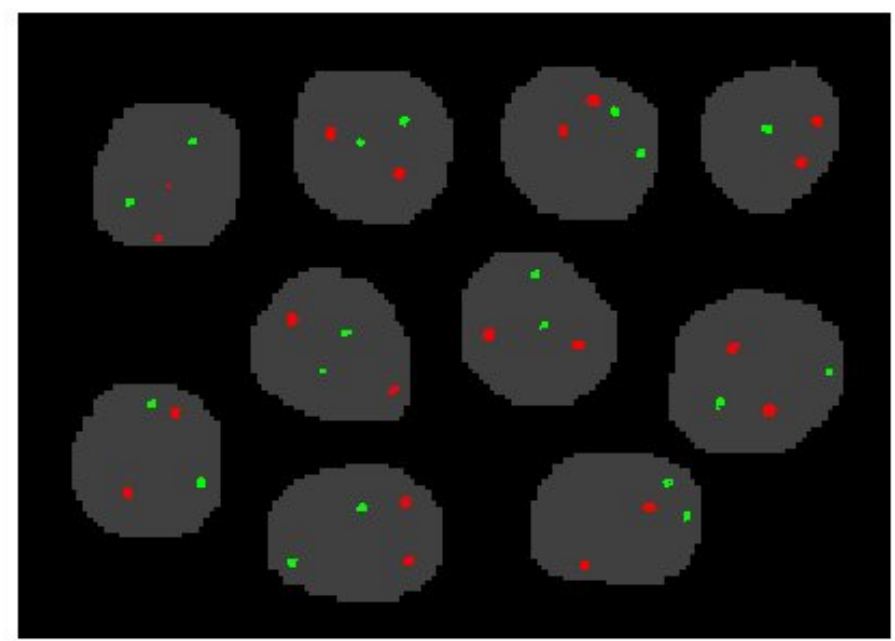
**Breast Cancer Cell
Nuclei in Paraffin**



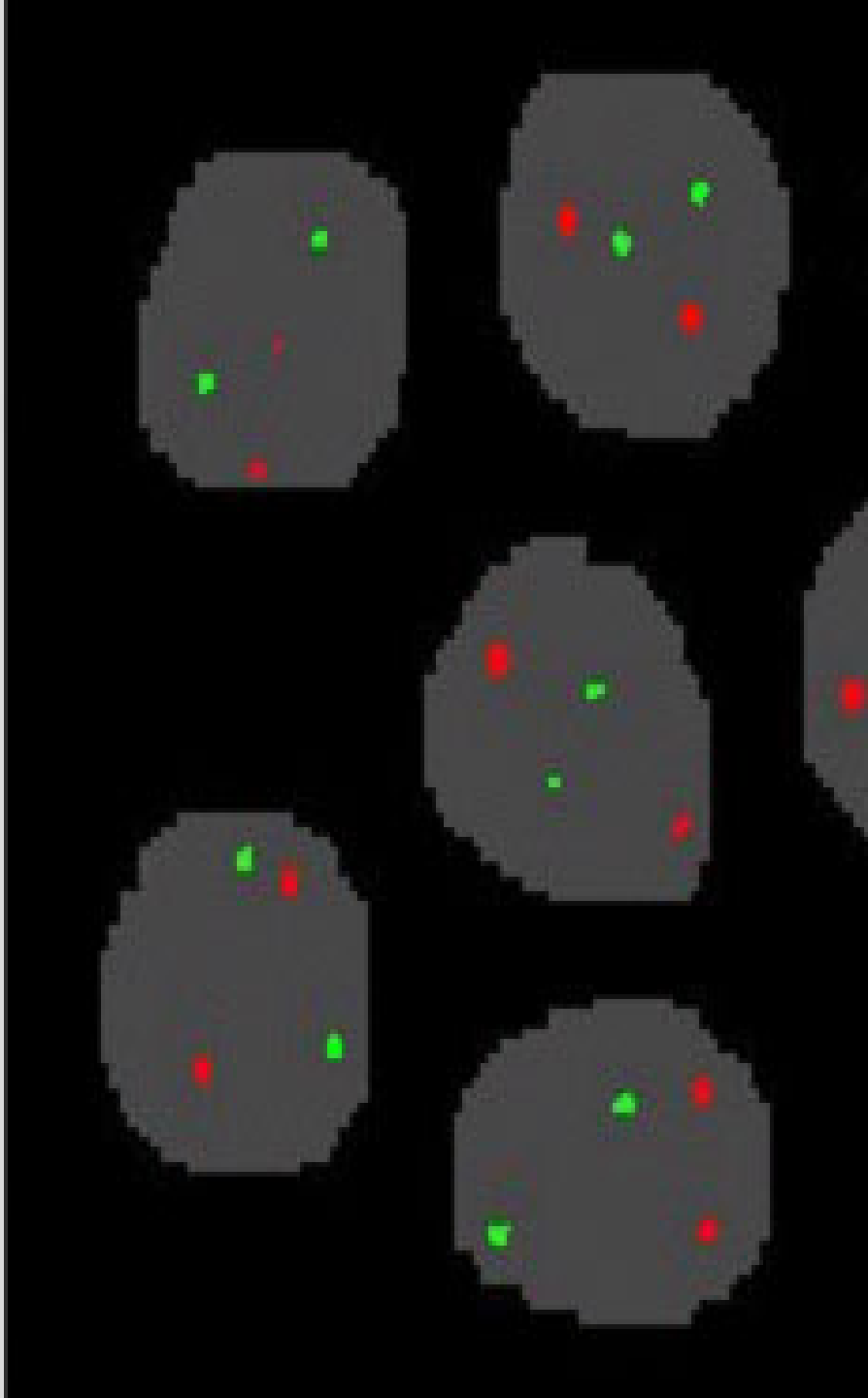
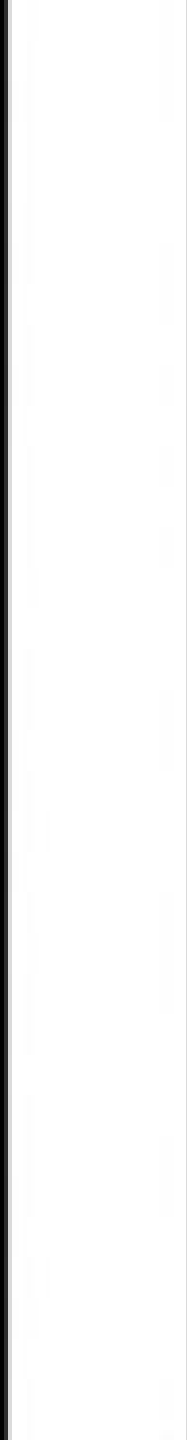
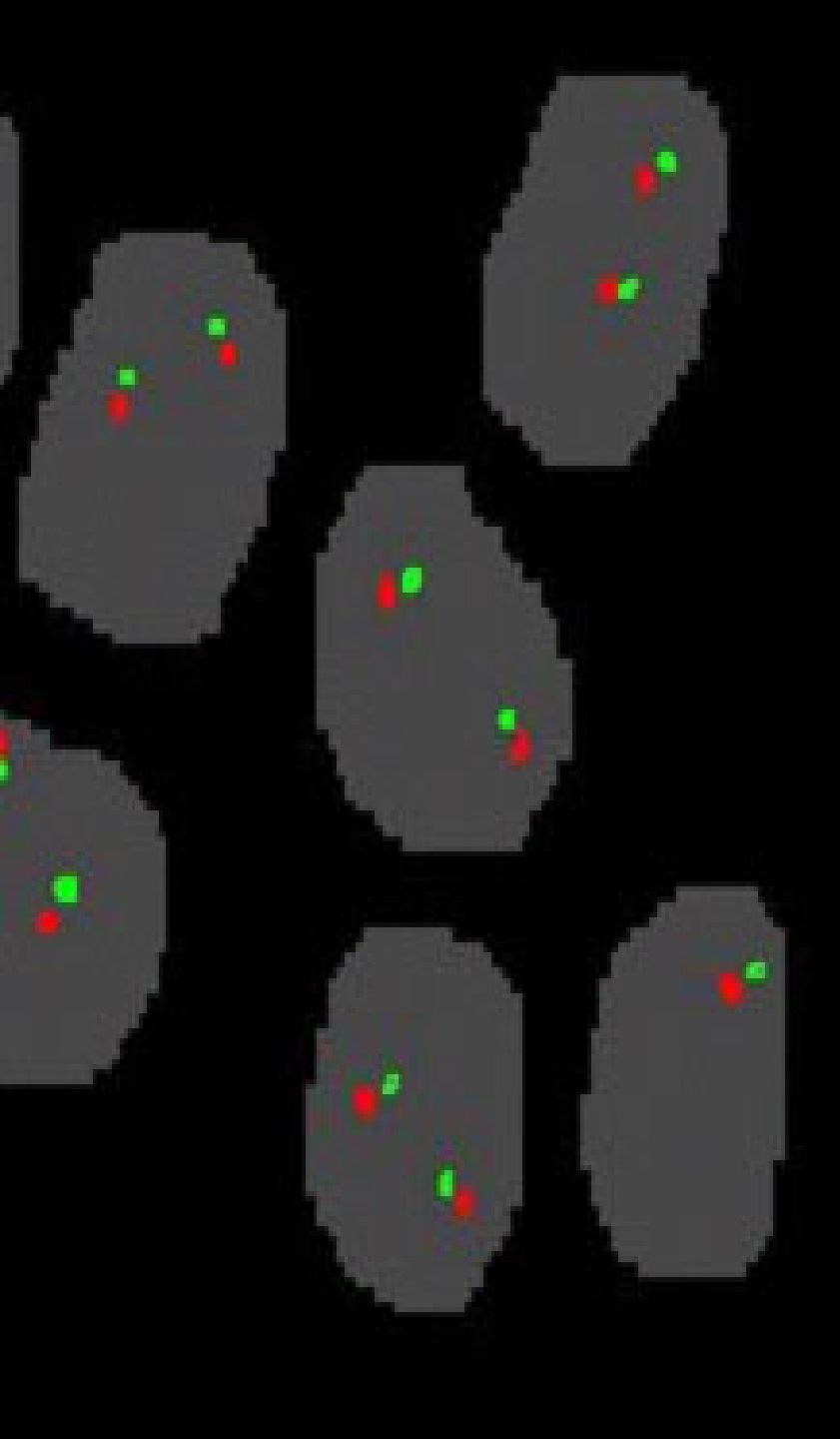
Fluorescent In-Situ Hybridization Detecting *ews* Translocations



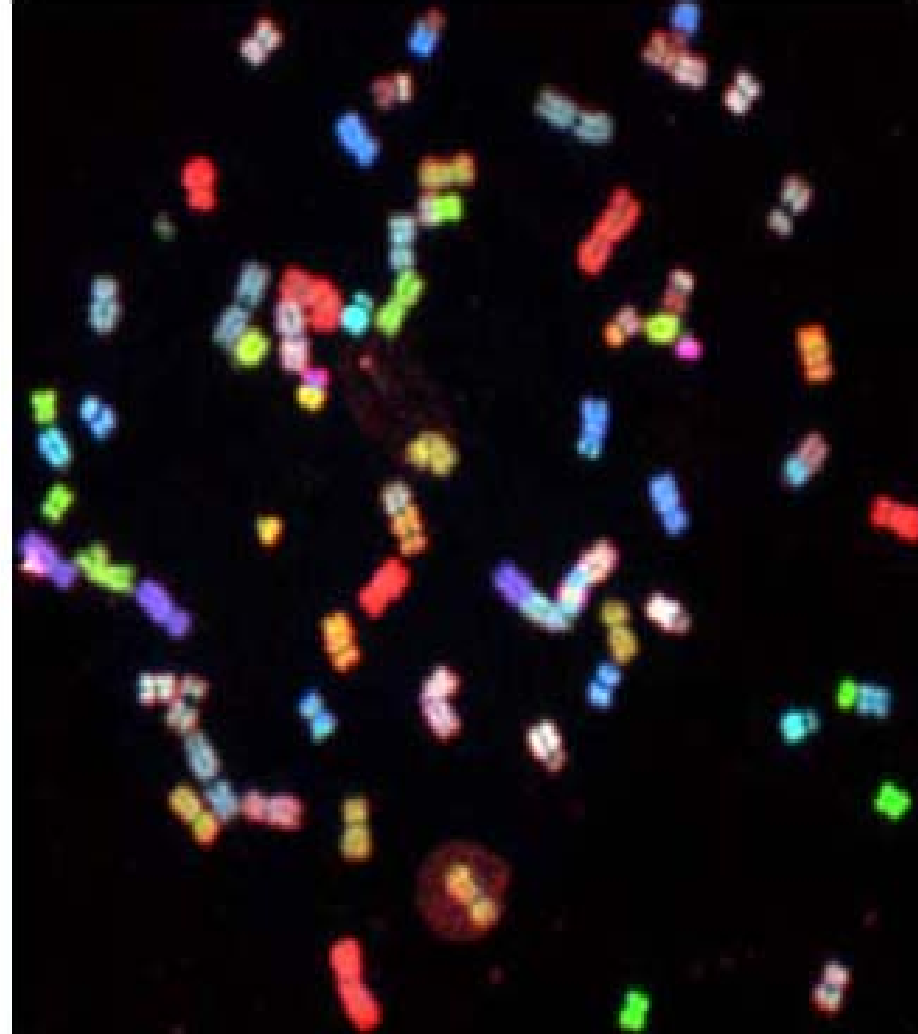
No Translocation



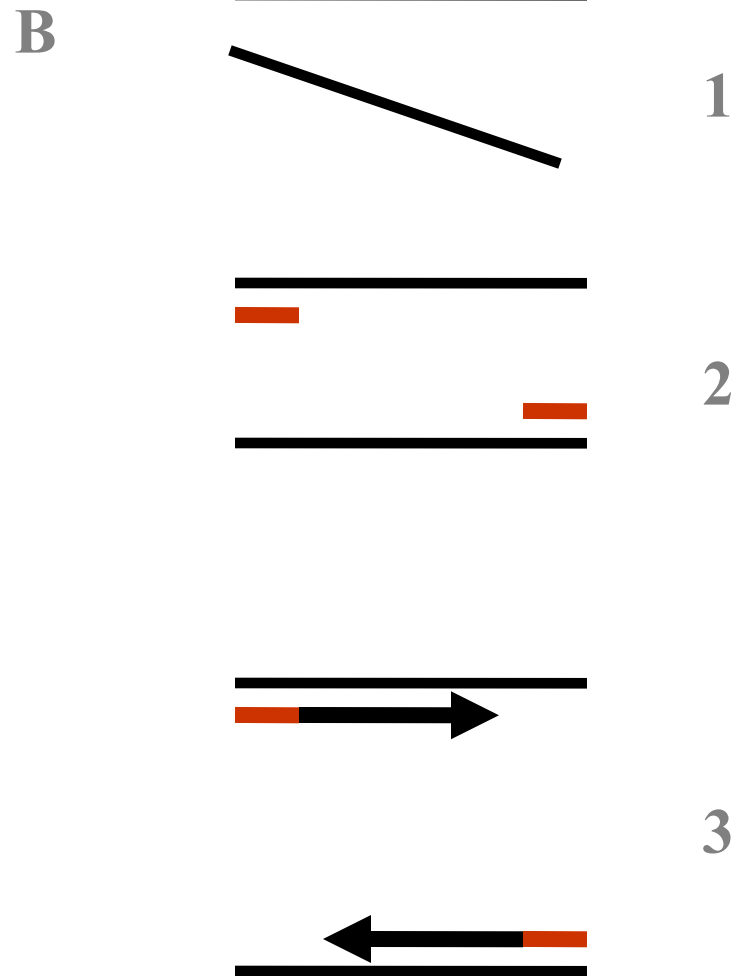
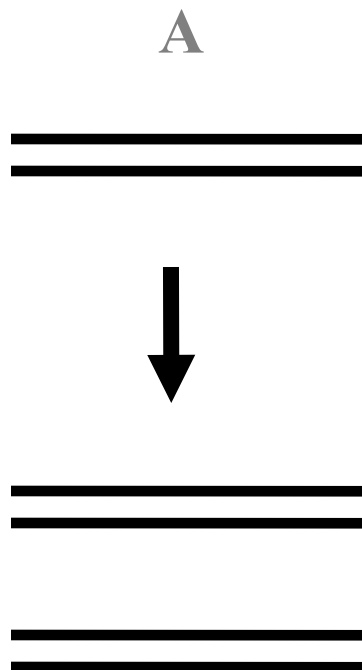
Translocation



Spectral karyotyping (SKY)

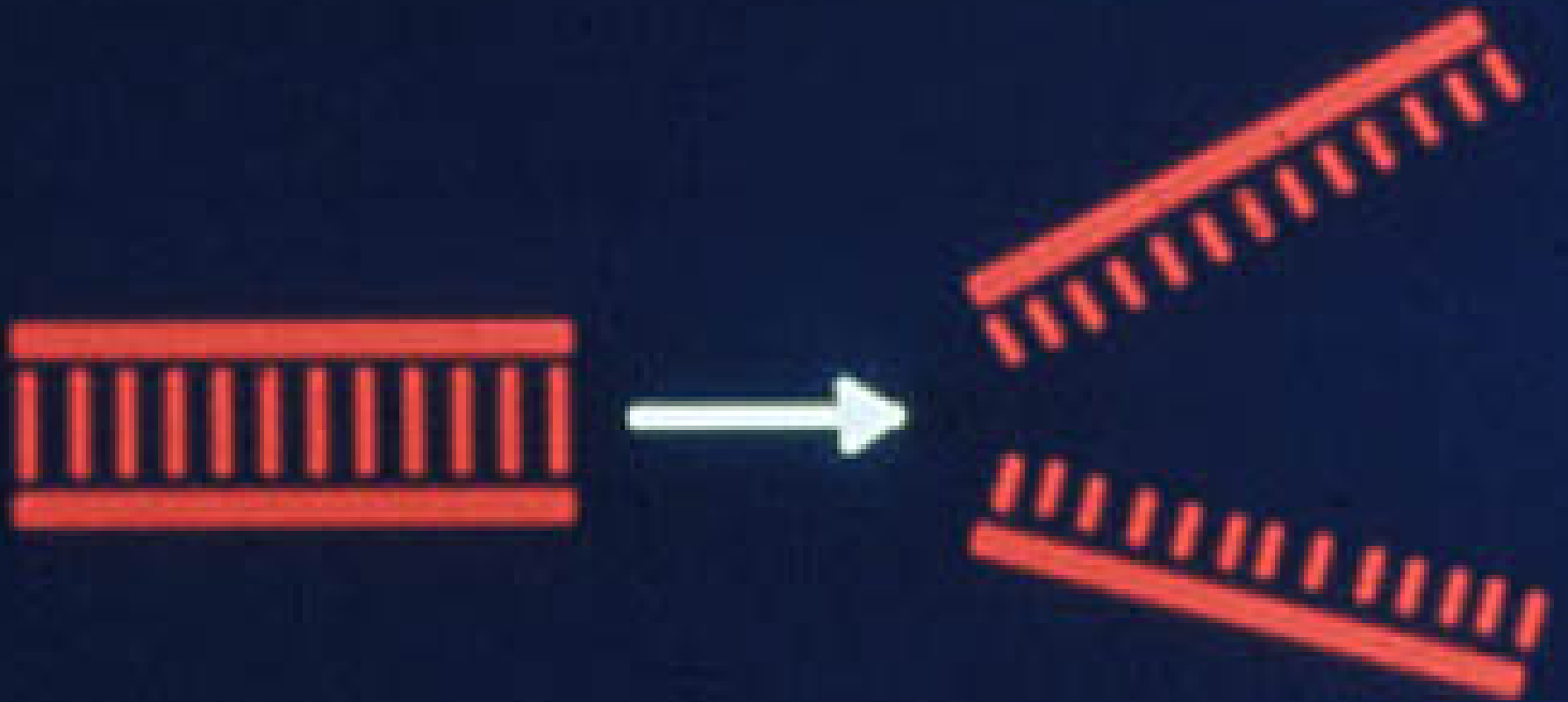


PCR

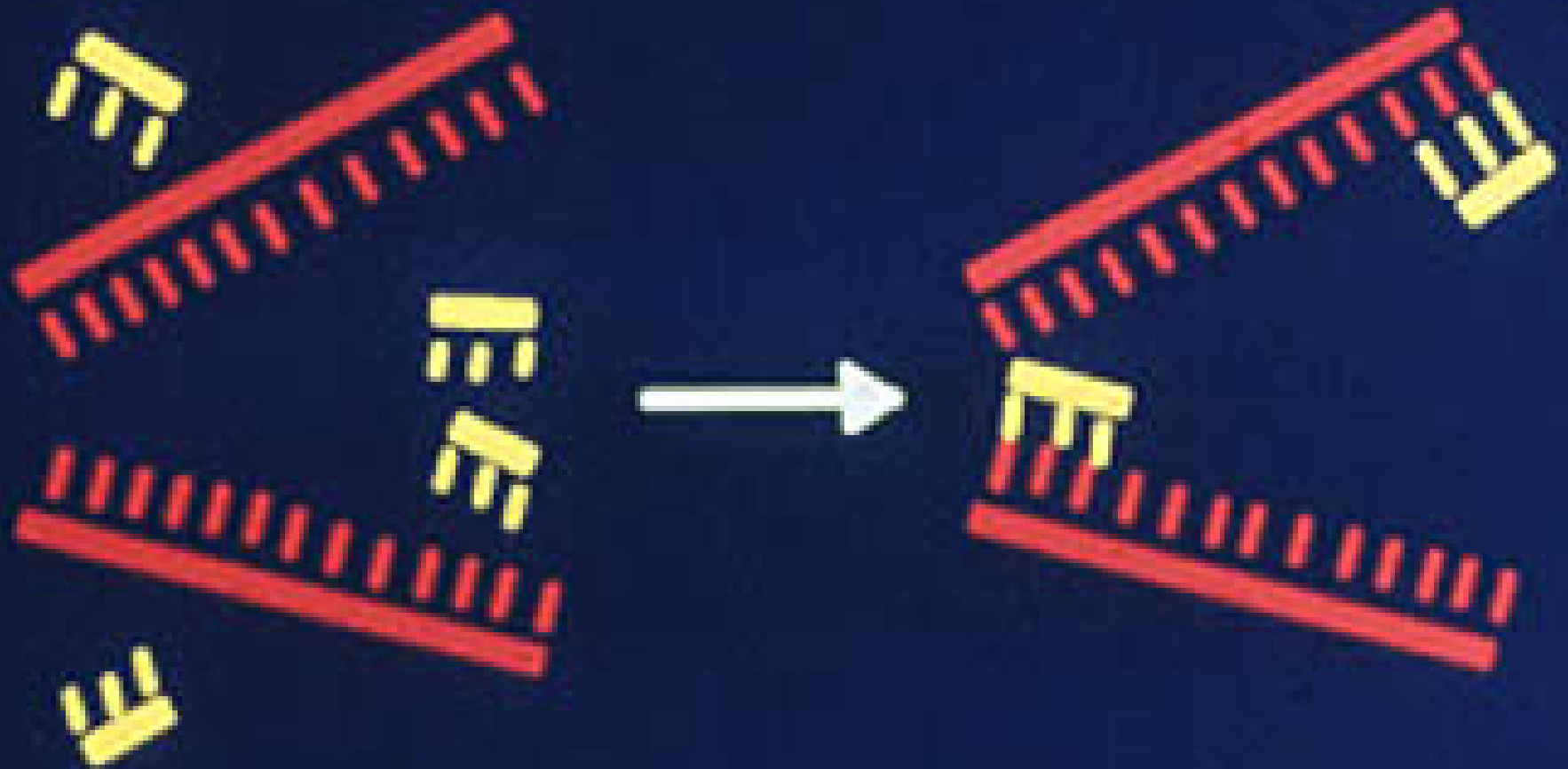




Denature DNA



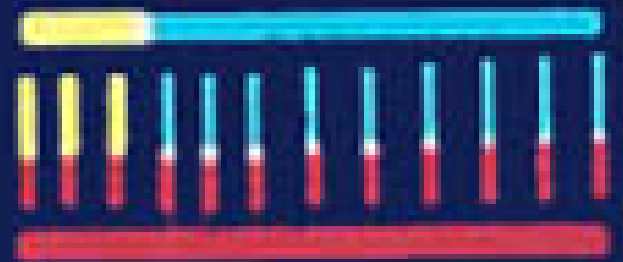
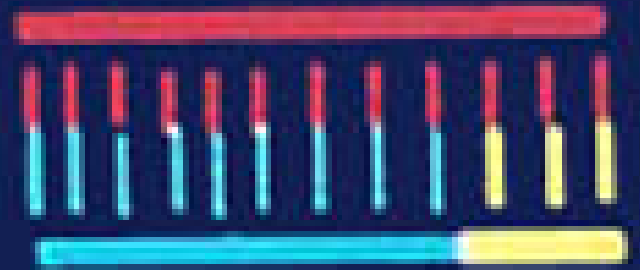
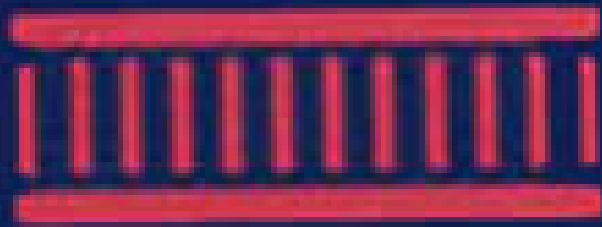
Anneal primers



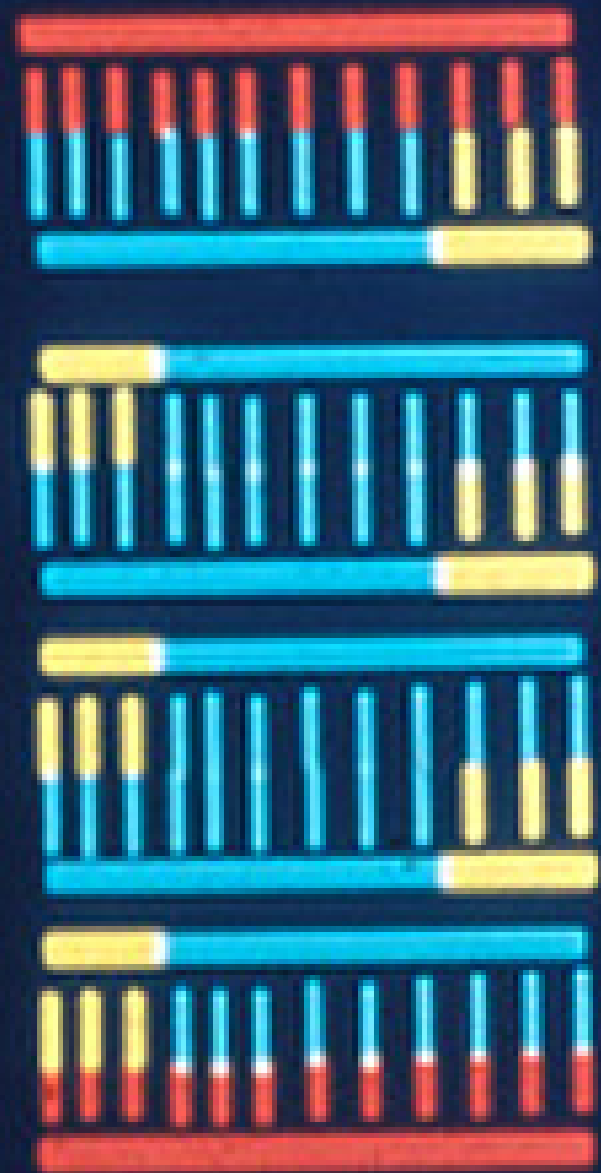
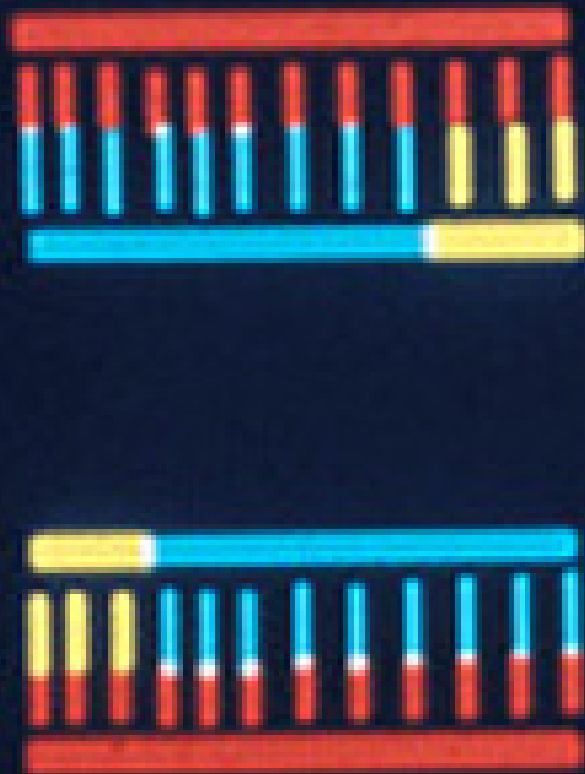
Extend with polymerase (▼)



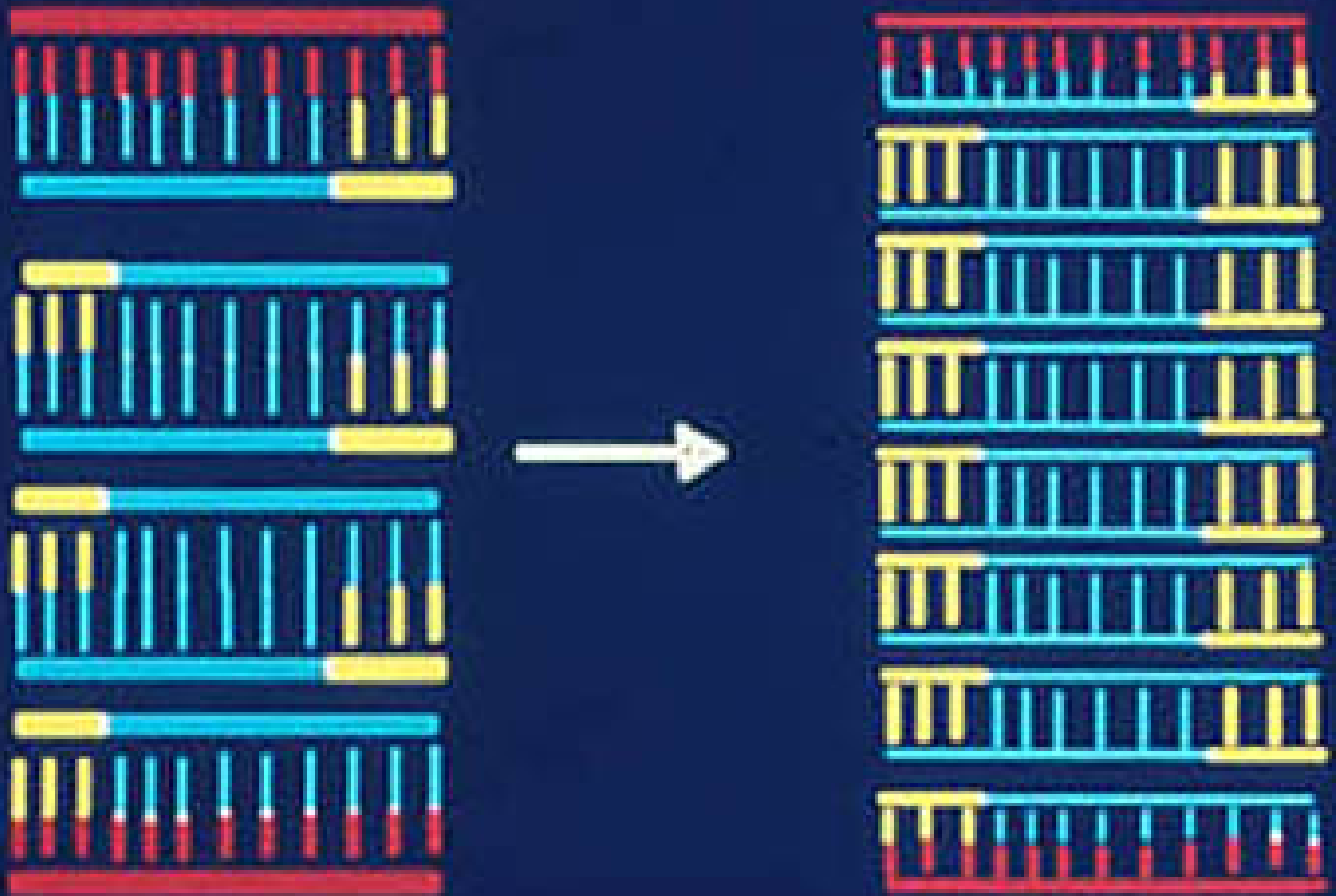
One Cycle



Two Cycles

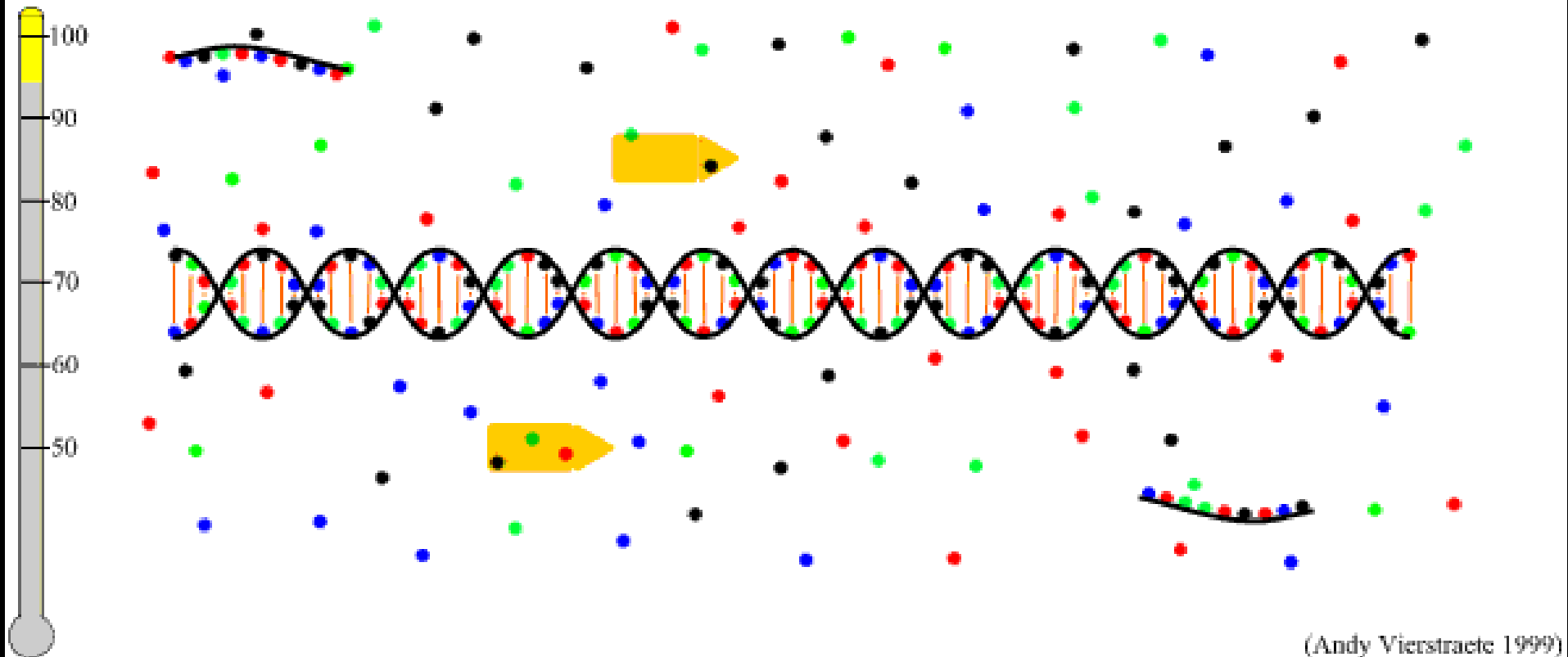


Three Cycles



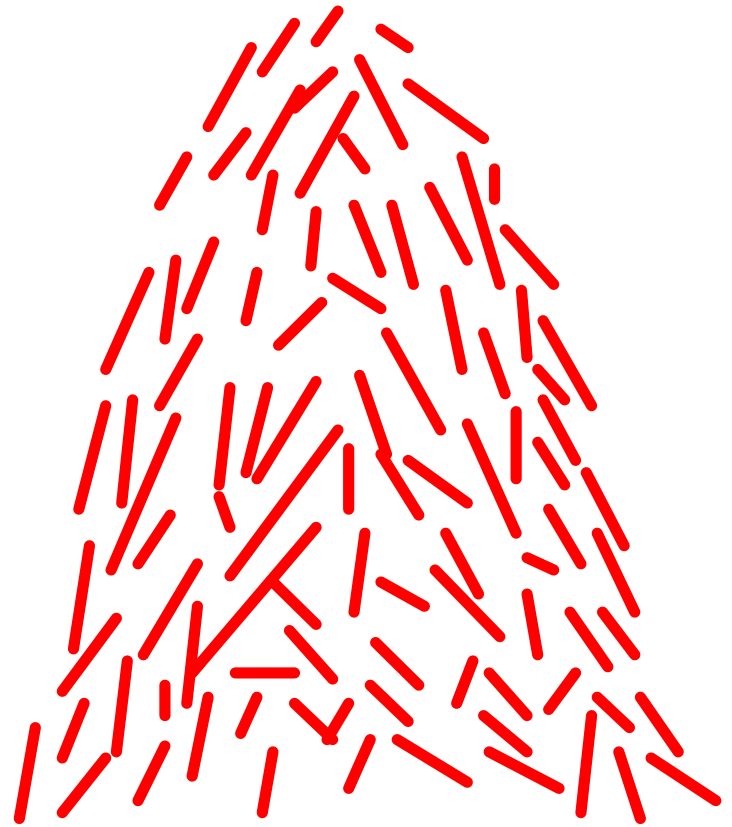
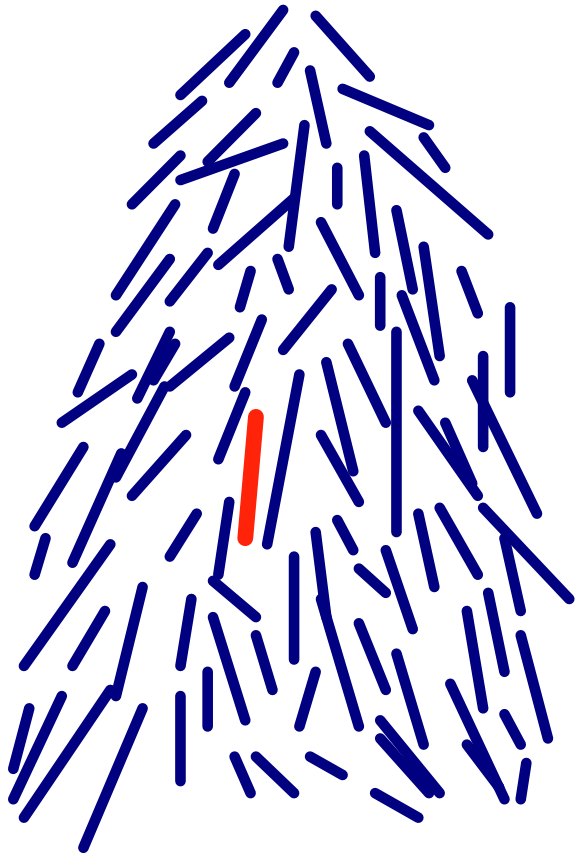
PCR :

Denaturation 94°C

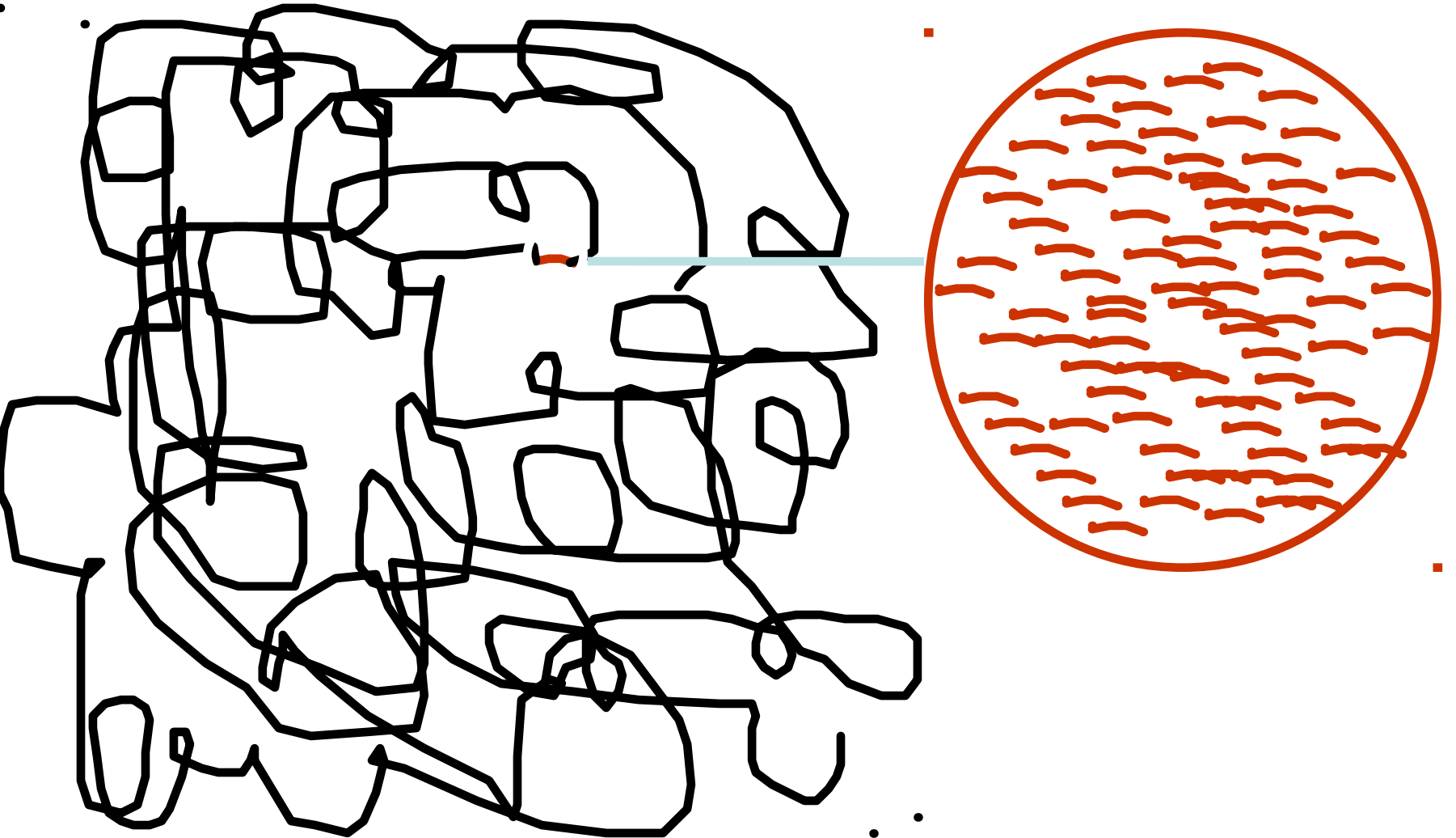


(Andy Vierstraete 1999)

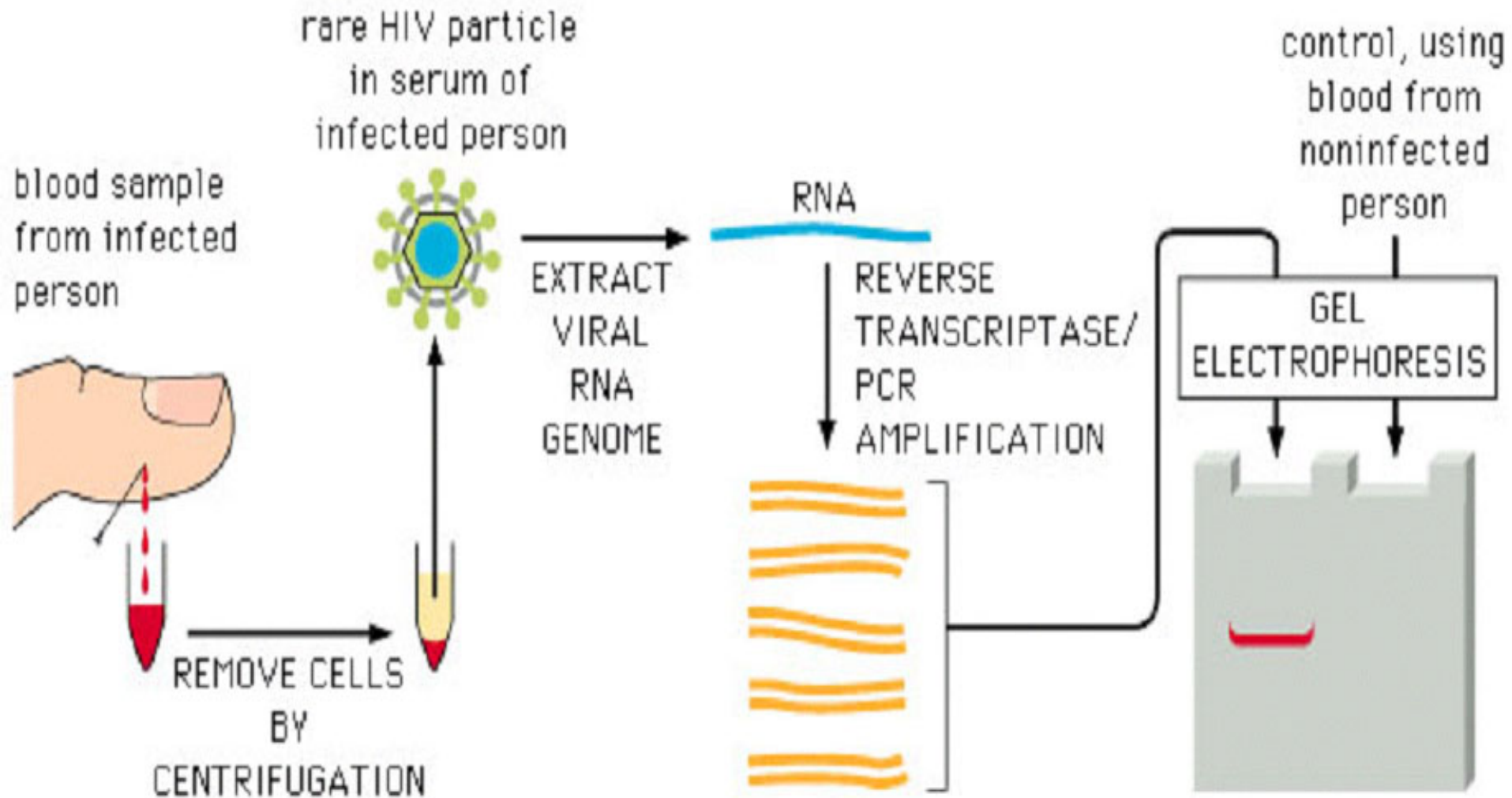
PCR



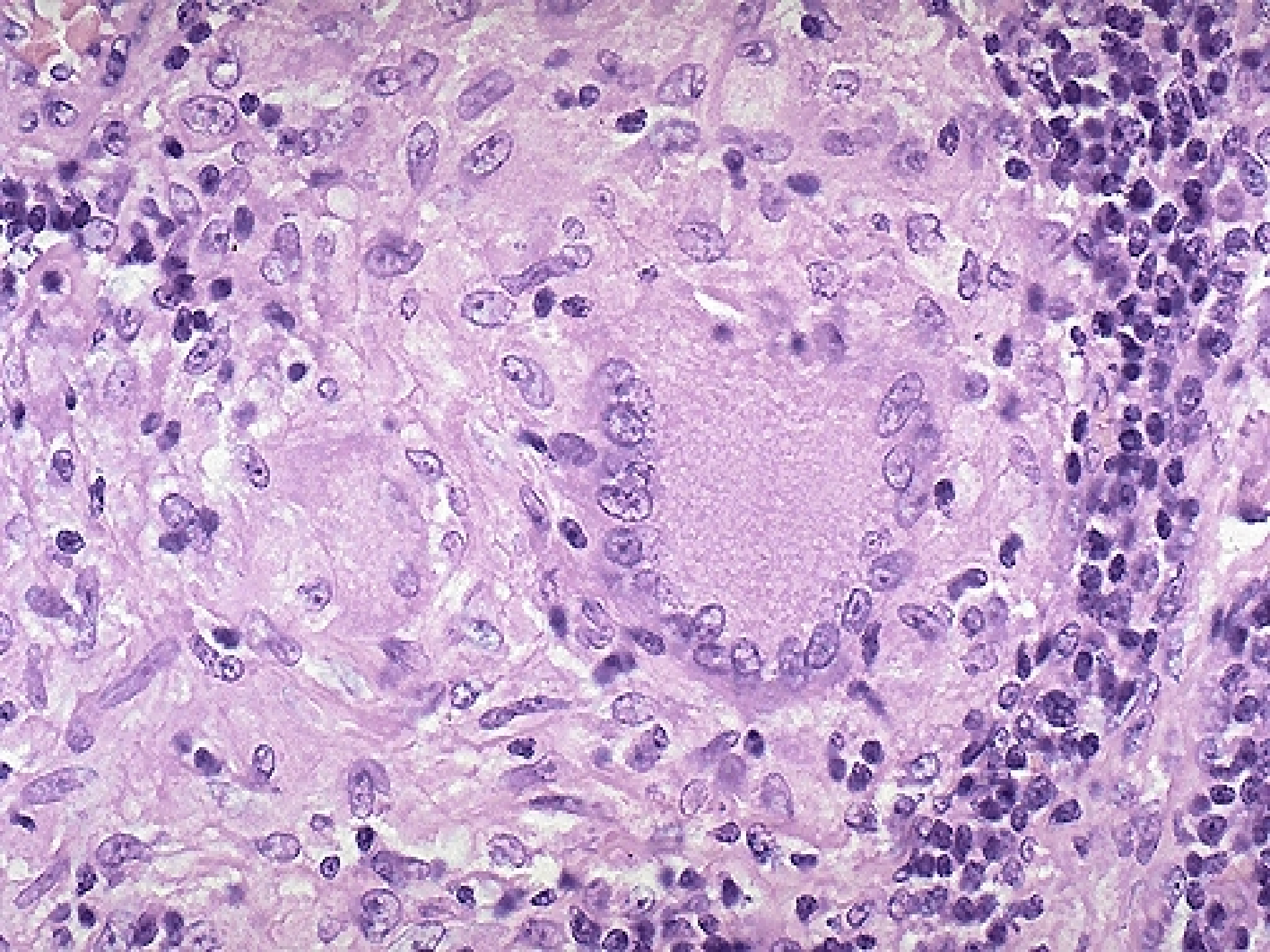
SPECIFIC PRIMERS TARGET ANY GENE

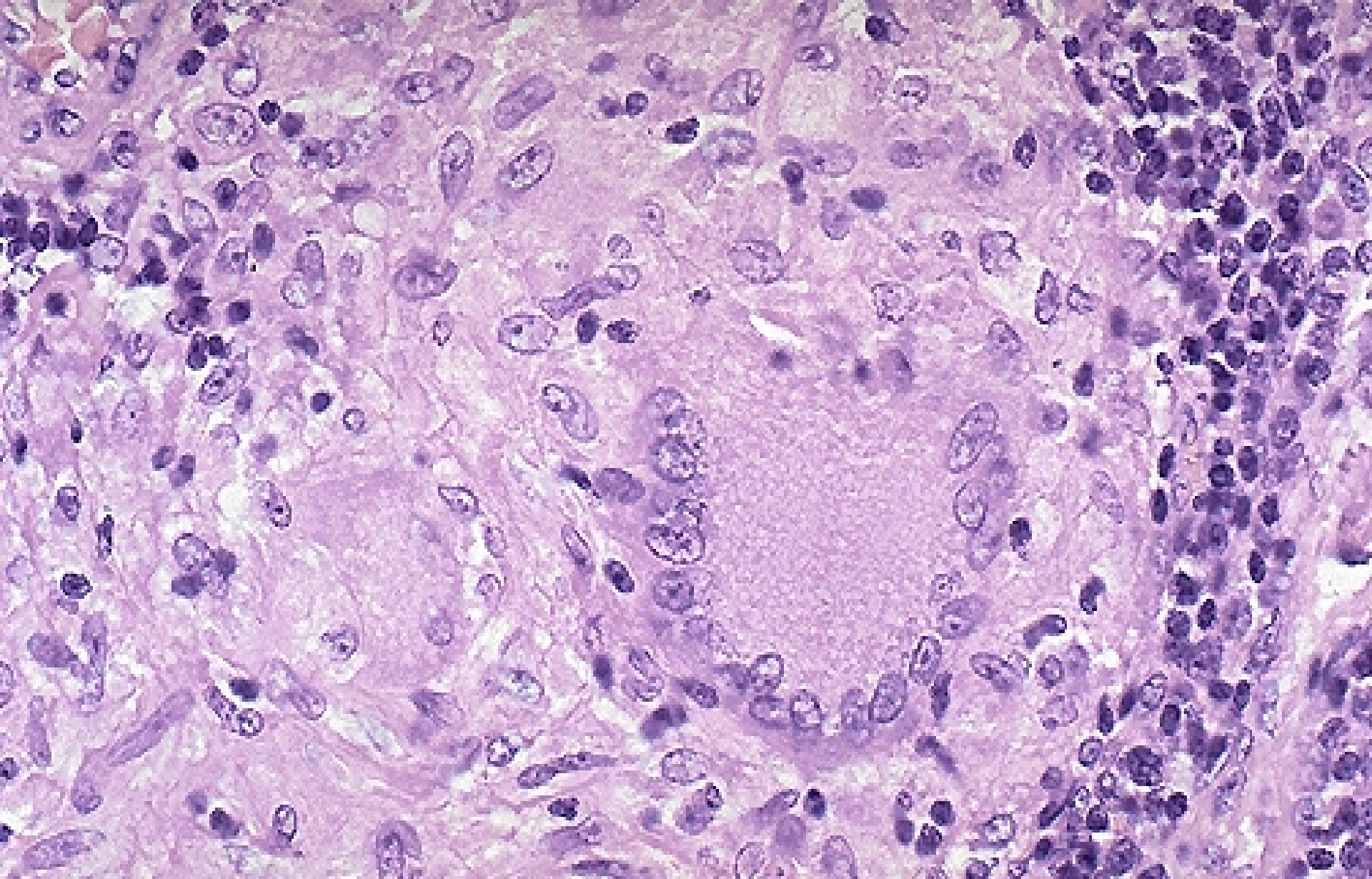


PCR for Medical Diagnostics

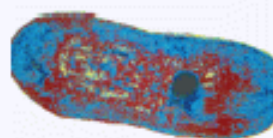








Tuberculosis or Crohn disease?



Mycobacterium tuberculosis

[Projects Home](#)

[M.tuberculosis](#)

[Blast Search](#)

[Sequence FTP](#)

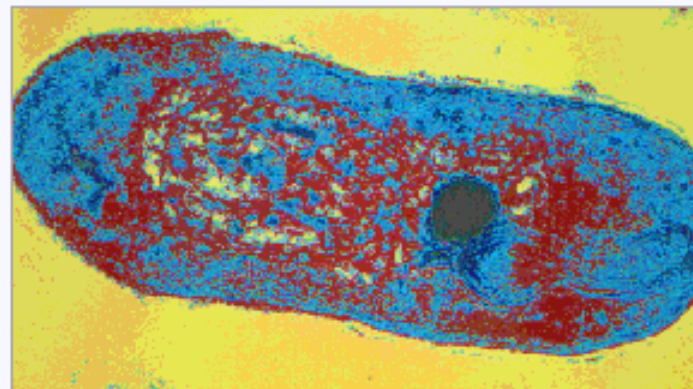
[Artemis](#)

Related Projects

[M.bovis](#)

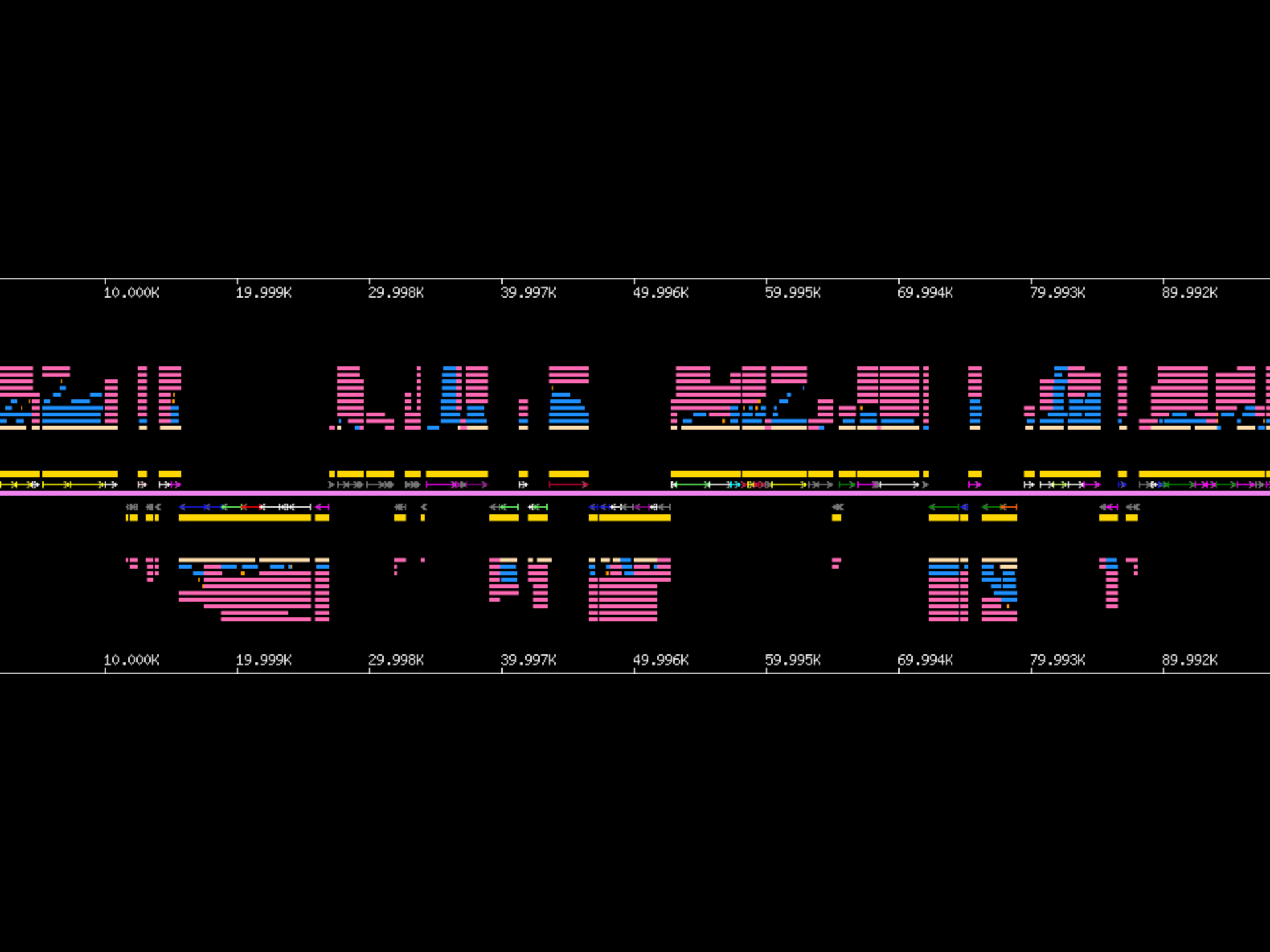
[M.leprae](#)

Mycobacterium tuberculosis



Electron micrograph of *M. tuberculosis* courtesy of the Institut Pasteur image library

The recently re-annotated genome of Mycobacterium tuberculosis has been published in *Microbiology*



375K

41.408K

41.441K

41.474K

41.507K

41.540K

41.573K

41.606K

41.639K

41.672K

375K

41.408K

41.441K

41.474K

41.507K

41.540K

41.573K

41.606K

41.639K

41.672K

DNA Sequence

>

```
GTGGTGGCGCCGCACGAAGACCCCGAGGACCATGTCGCACCCGCCGCACAACGGGTGCGA
GCGGGCACCTTATTGTTGGCCAAACACCGATCTCCTTGAACCGACATTTTCGCCGCAGTGTG
ATCTACATCGTGGAGCACAACGACGGCGGCACCCTCGGTGTGGTCCTCAATCGGCCCAGC
GAAAACCGCGGTCTACAACGTGTTGCCGCAGTGGGGCCAAACTCGCGGCCAAGCCAAAAGACA
ATGTTTCATCGGTGGGCCCGGTGAAGCGCGACGCGGCGCTGTGTCTGGCGGTATTGCGGGTT
GGCGCTGACCCGGAAGGCGTGCCGGGCCTAAGGCATGTCGCGGGCAGGCTGGTGATGGTC
GATCTGGATGCCGACCCGAGGTGCTCGCAGCGGCGGTGGAAGGGGTGCGCATCTACGCC
GGGTA CTCCGGCTGGACCATCGGTCAGCTCGAAGGTGAAATCGAGCGCGACGACTGGATT
GTGTTGTGCGGCGTTGCCATCTGACGTTTTGGTGGGGCCGAGAGCCGACCTGTGGGGGCAG
GTGCTGCGACGGCAGCCGCTGCCGCTGTCGCTGCTGGCCACCCACCCGATCGATCTGAGC
CGGAAC
```

Protein Sequence

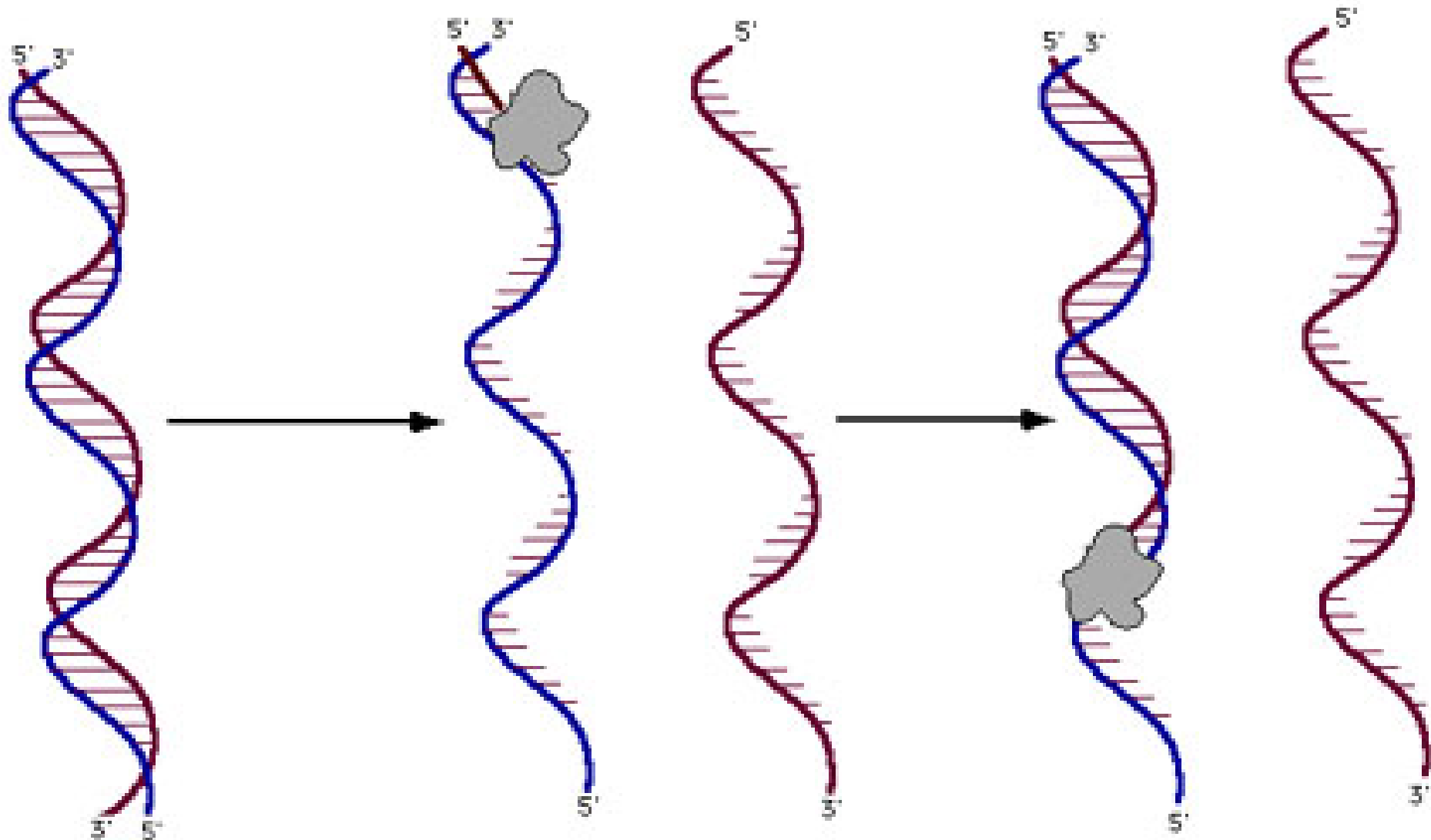
>

```
MVAPHEDPEDHVAPAAQRVRAGTLLLANTDLEPTFRRSVIYIVEHNDGGTLGVVLNRPS
ETAVYNVLPQWAKLAAKPKTMFIGGPVKRDAALCLAVLRVGADPEGVPLRHVAGRLVMV
DLADPEVLAAAVEGVRIYAGYSWGTIGQLEGEIERDDWIVLSALPSDVLVGPRADLWQ
VLRQPLPLSLLATHPIDLSRN
```

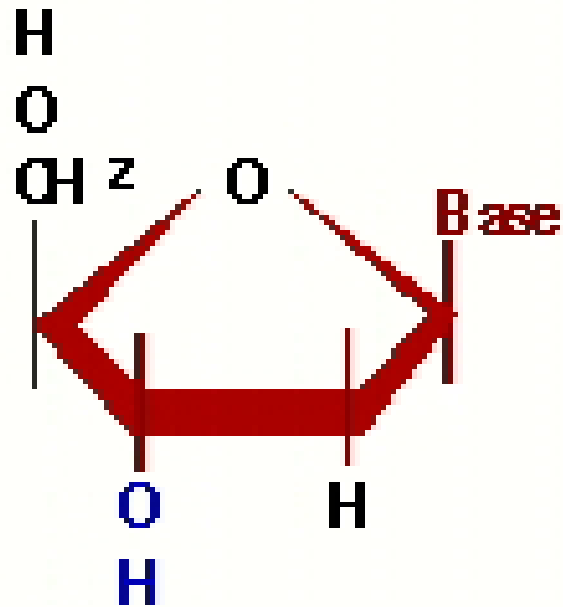
- 1 Sample**
- 2 Positive control**
- 3 Negative control**



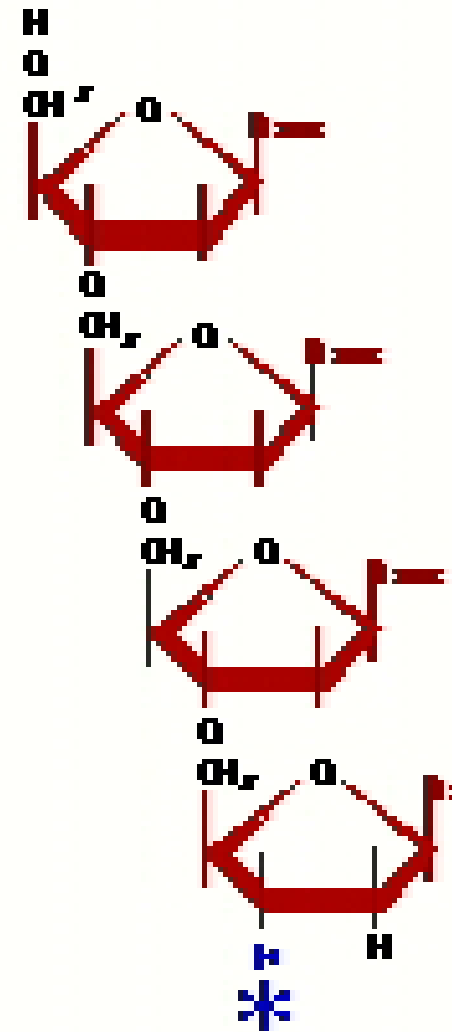
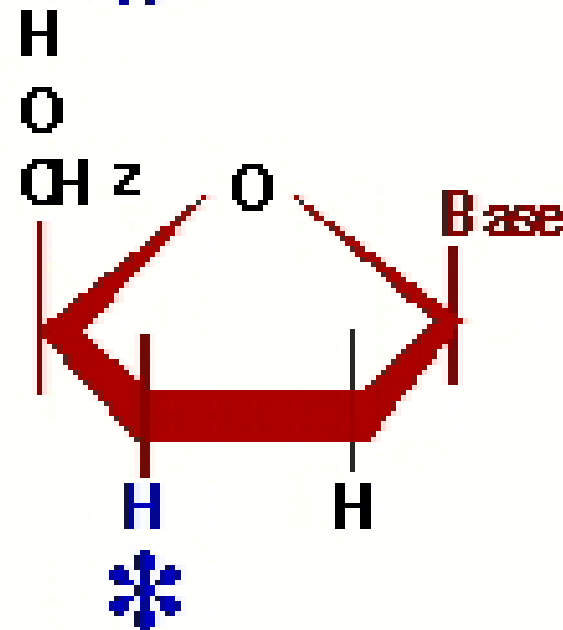
DNA Sequencing



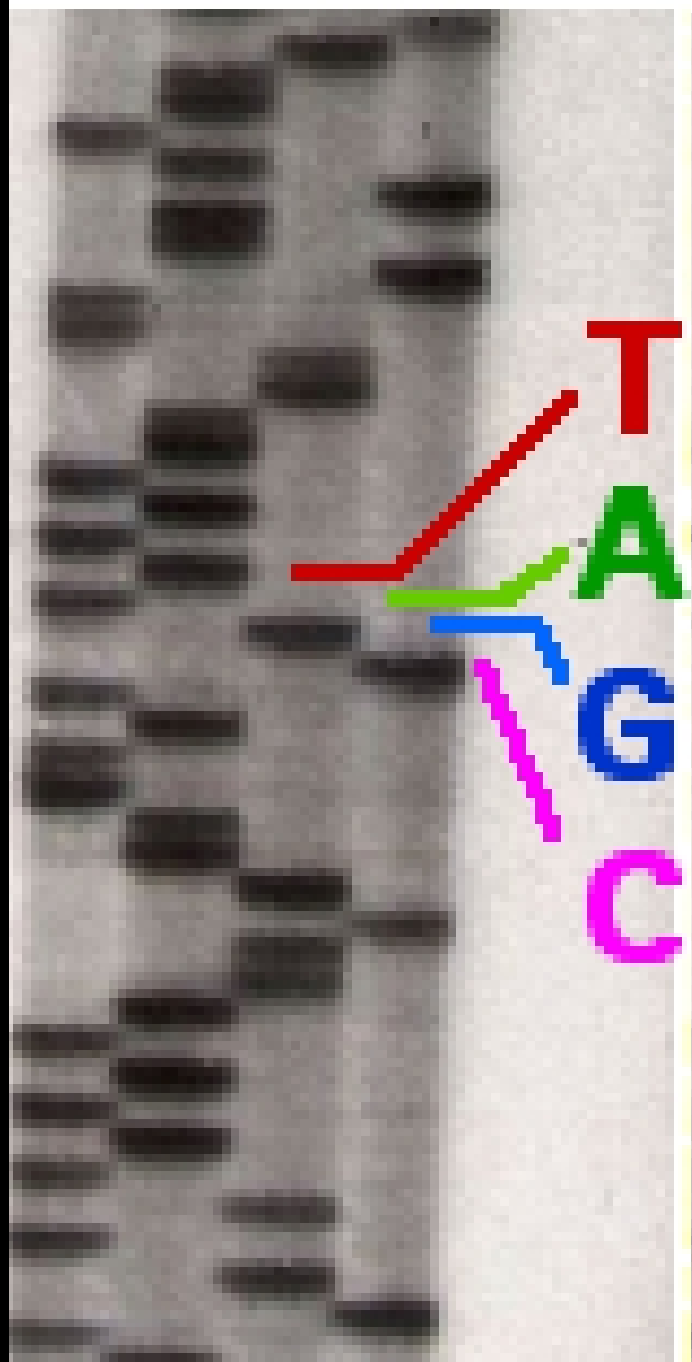
Normal nucleotides:



Dideoxy Chain Terminators:



A T G C

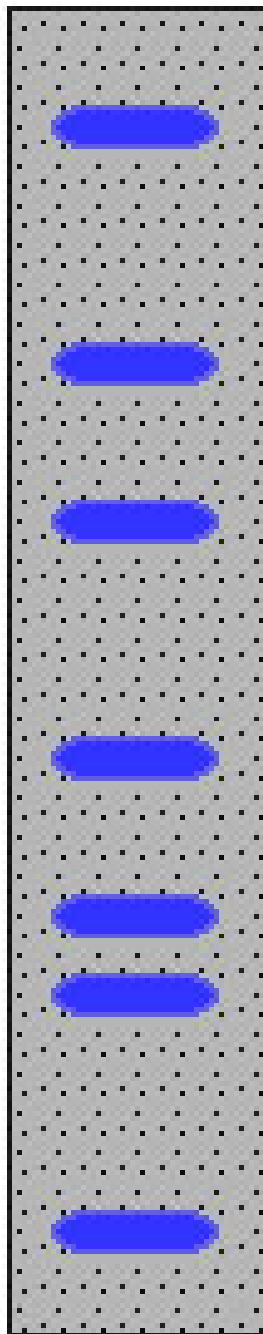


T

A

G

C



GCGAATGCGTCCACCAACGCTAC

GCGAATGCGTCCACCAACGC

GCGAATGCGTCCACCAAC























GCGAATGCGTCCAC

GCGAATGCGTCC

GCGAATGCGTC

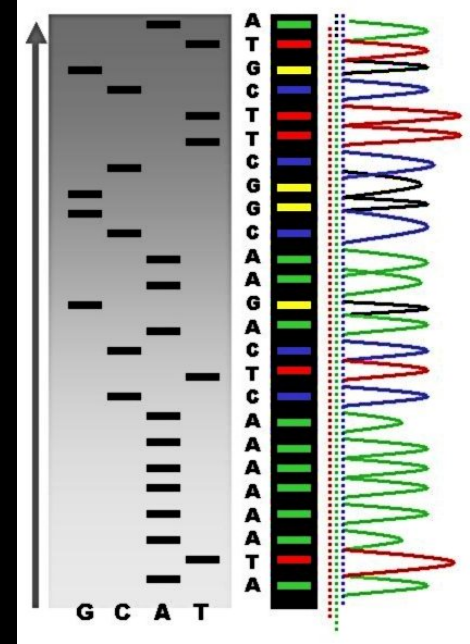
GCGAATGC

Gel:

	G	GCGAATGCGTCCACAACGCTACAGGTG
	T	GCGAATGCGTCCACAACGCTACAGGT
	G	GCGAATGCGTCCACAACGCTACAGG
	G	GCGAATGCGTCCACAACGCTACAG
	A	GCGAATGCGTCCACAACGCTACA
	C	GCGAATGCGTCCACAACGCTAC
	A	GCGAATGCGTCCACAACGCTA
	T	GCGAATGCGTCCACAACGCT
	C	GCGAATGCGTCCACAACGC
	G	GCGAATGCGTCCACAACG
	C	GCGAATGCGTCCACAAC
	A	GCGAATGCGTCCACAA
	A	GCGAATGCGTCCACA
	C	GCGAATGCGTCCAC
	A	GCGAATGCGTCCA
	C	GCGAATGCGTCC
	C	GCGAATGCGTC
	T	GCGAATGCGT
	G	GCGAATGCG
	C	GCGAATGC
	G	GCGAATG
	T	GCGAAT



Frederick Sanger



"just a chap who messed about in a lab"



Science

Volume 295
Number 5511
November 12, 2002
\$10.00

THE HUMAN GENOME

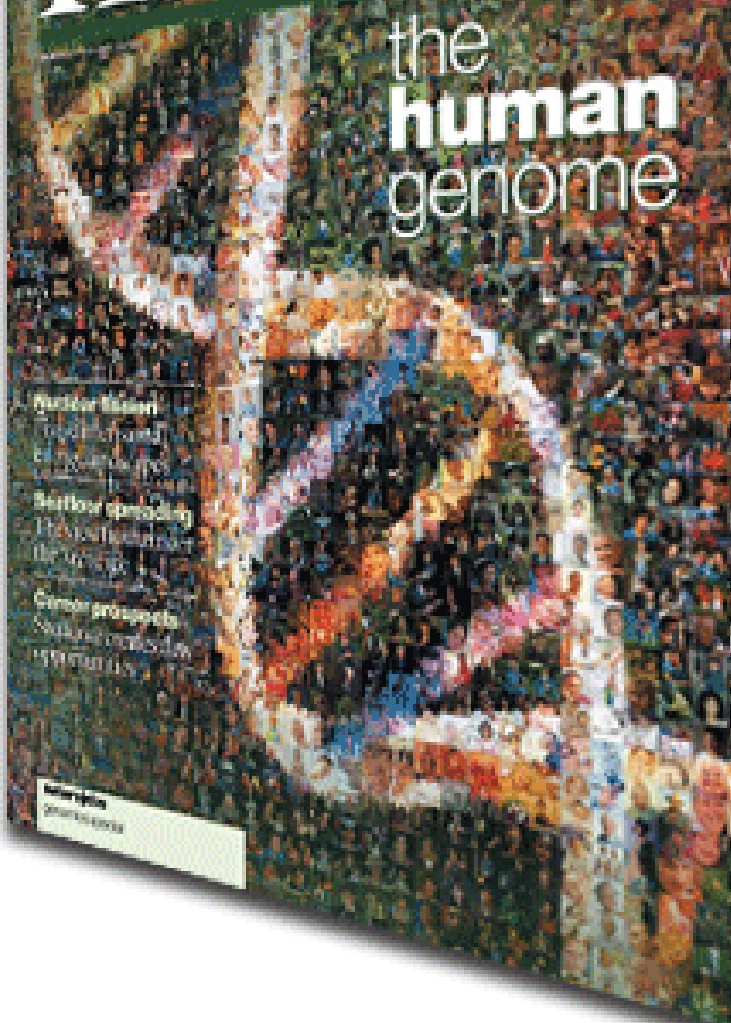


 AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

nature

www.nature.com

the human genome



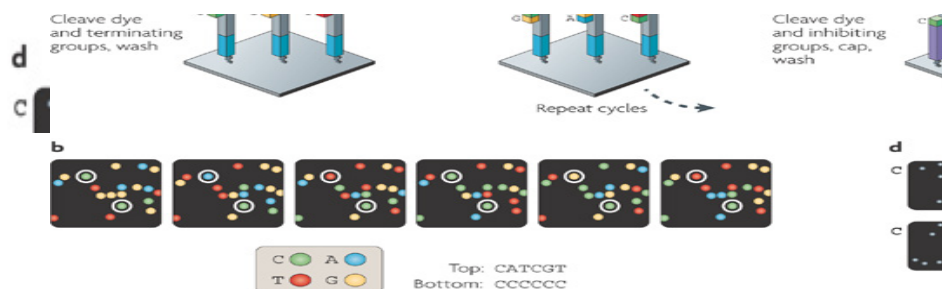
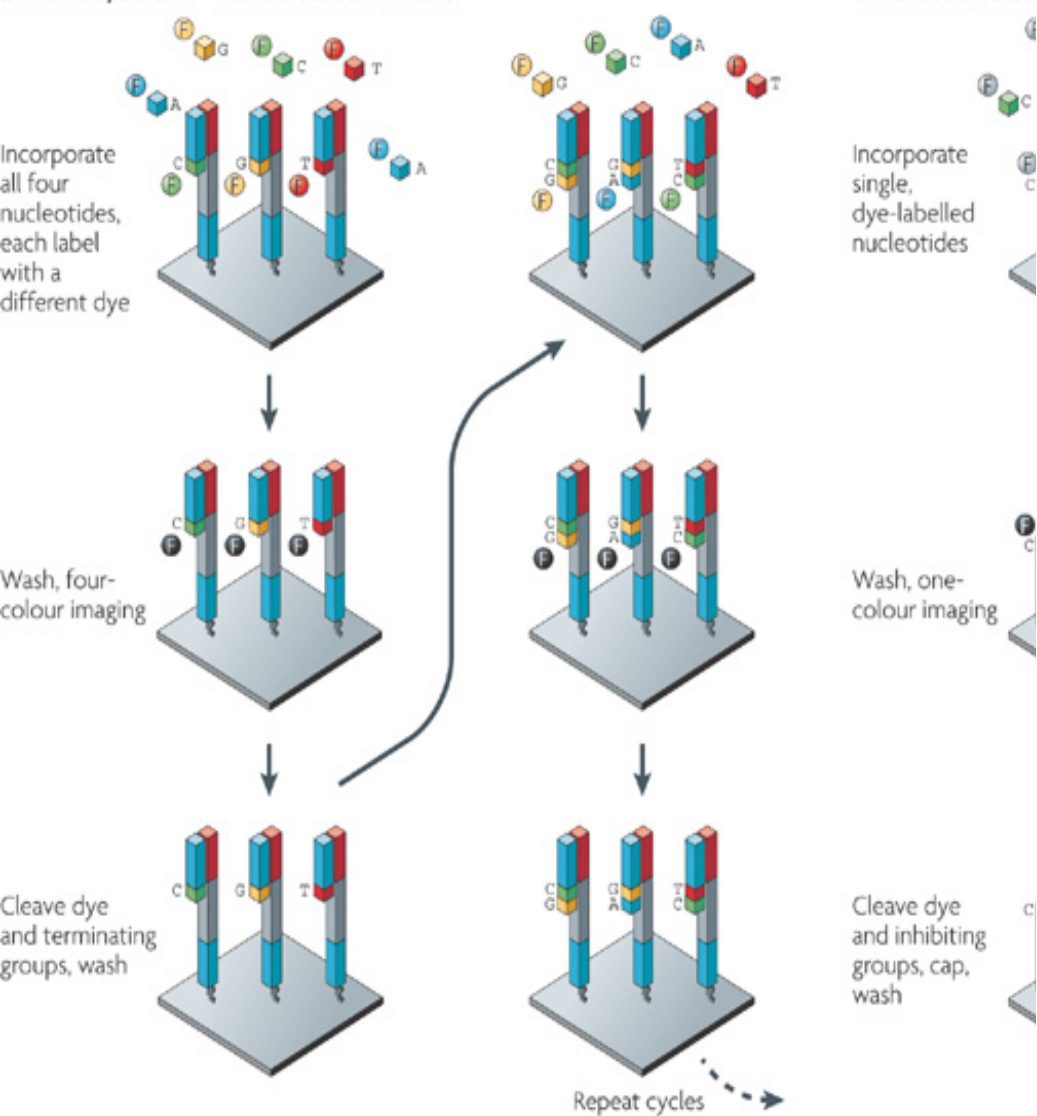
Wanted: Men!
Scientists find
a way to
clone
babies

Bird flu spreading
The deadly
H5N1 virus
is on the
move

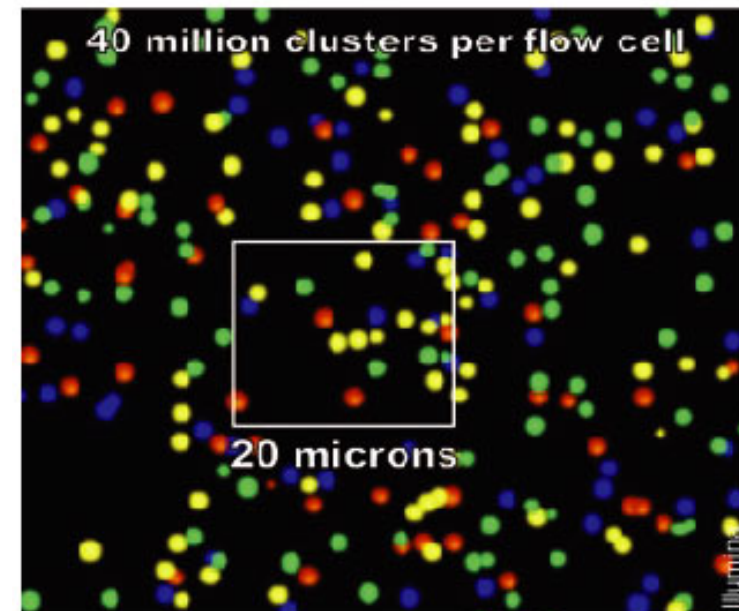
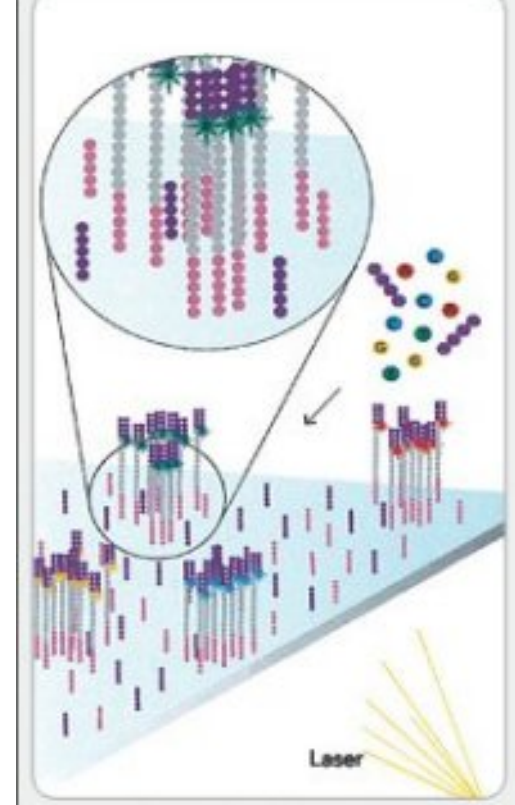
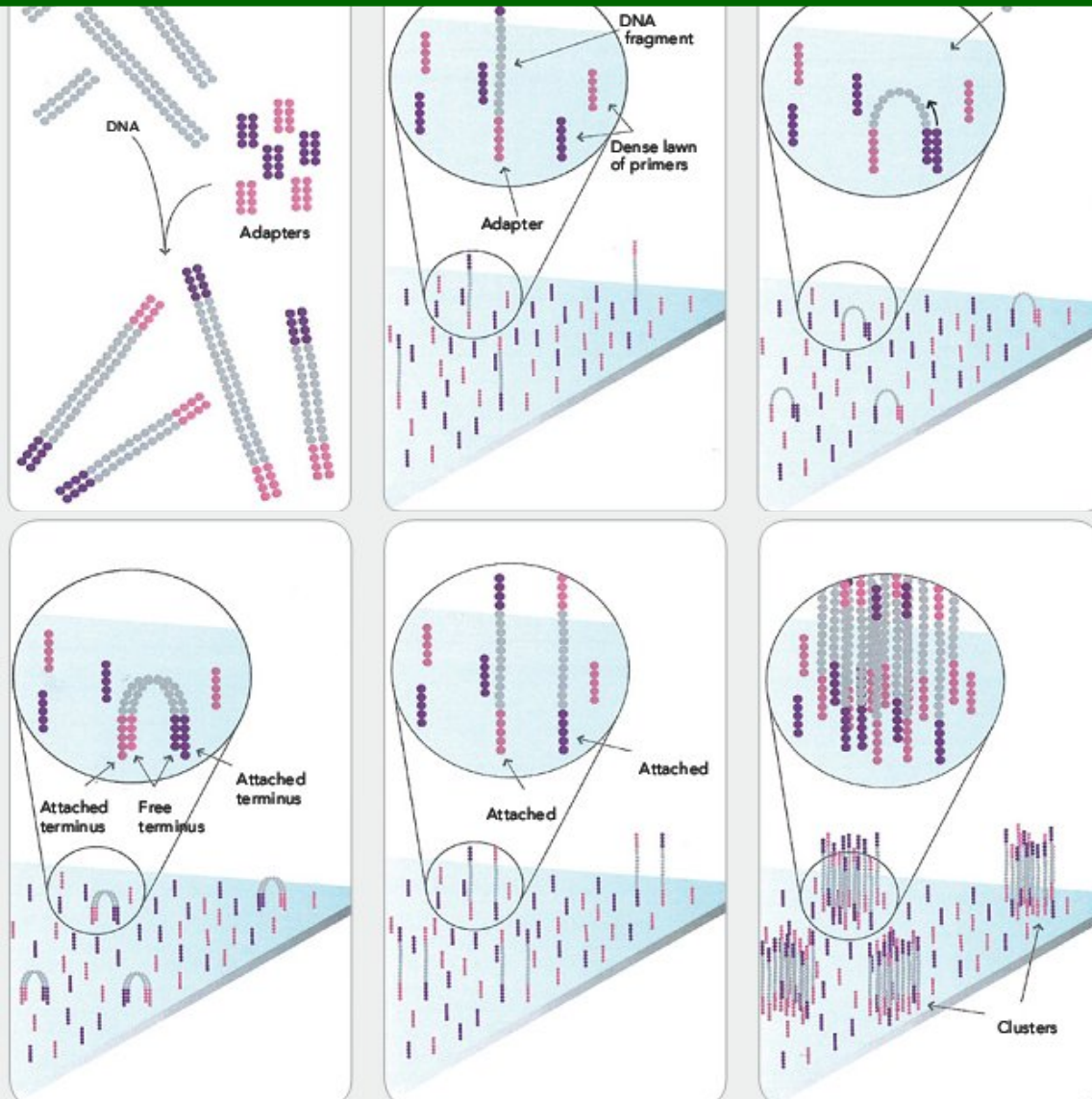
Cancer prognosis
New tests
could
predict
survival

Genetics
The human genome

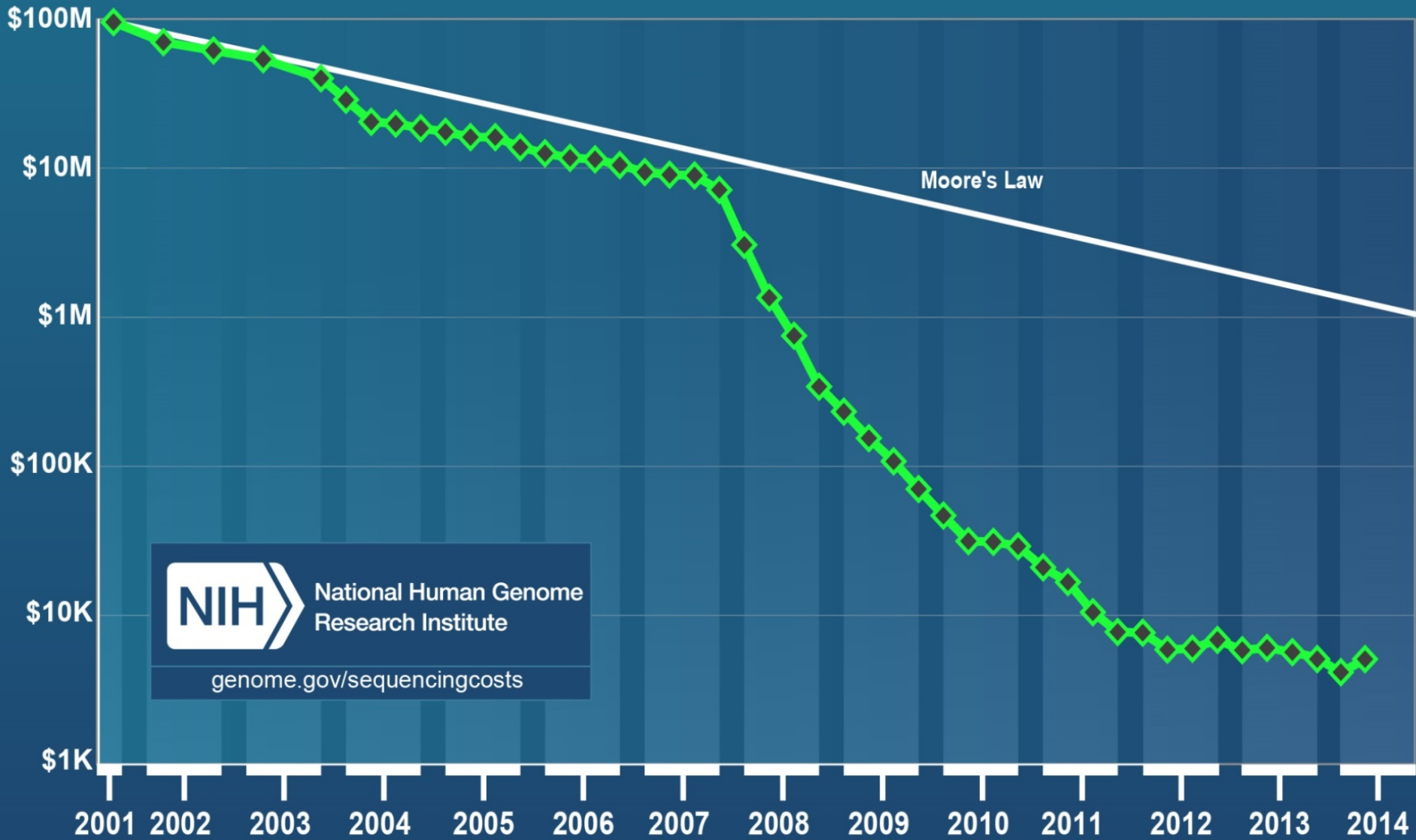




Next Generation Sequencing

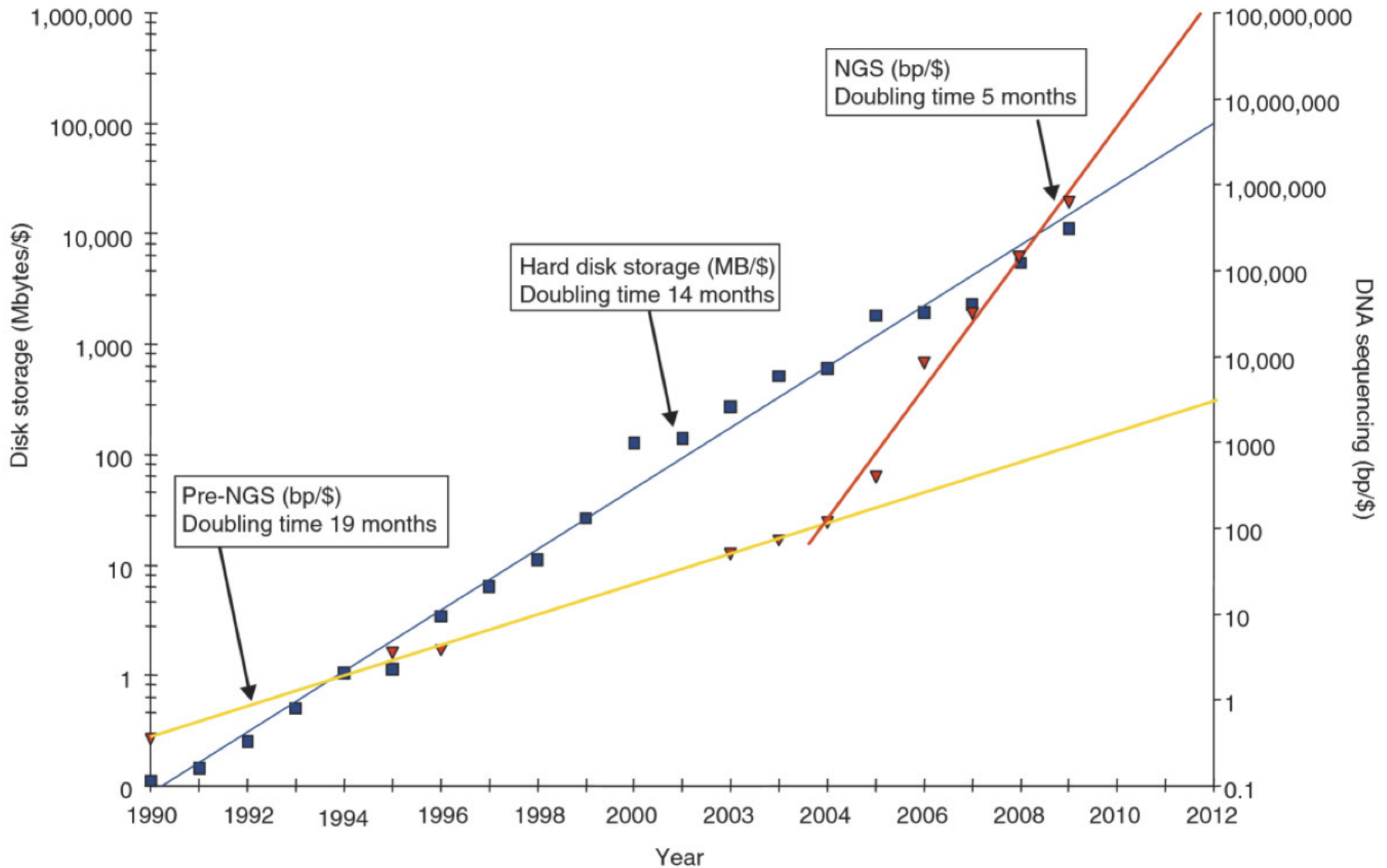


Cost per Genome



NIH National Human Genome Research Institute
genome.gov/sequencingcosts

NextGen Sequencing a Game-Changer



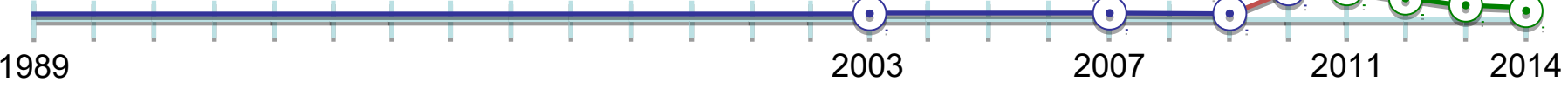
Economics of Genome Sequencing

Cost Per Genome

2003: \$2,300,000,000 / 15 years

2014: \$2,300 / 15 days

Number of Whole Genomes Sequenced



Next Generation Sequencing

Next Generation = Massively Parallel

“Read Depth”

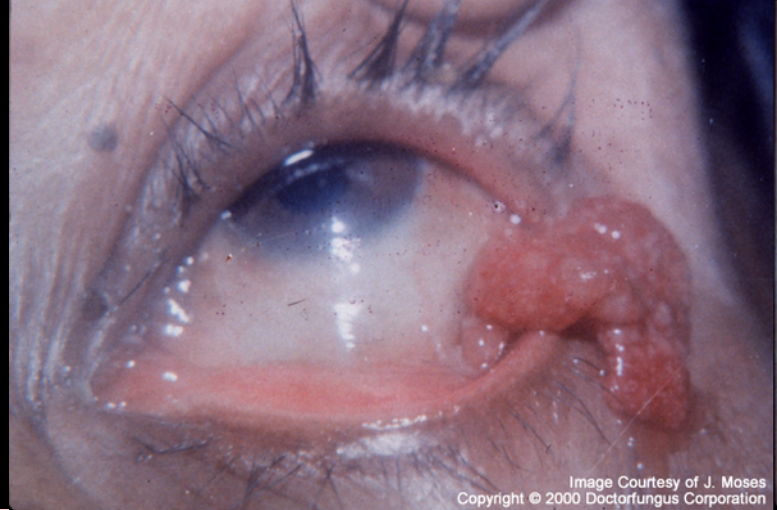
“Depth of Coverage”

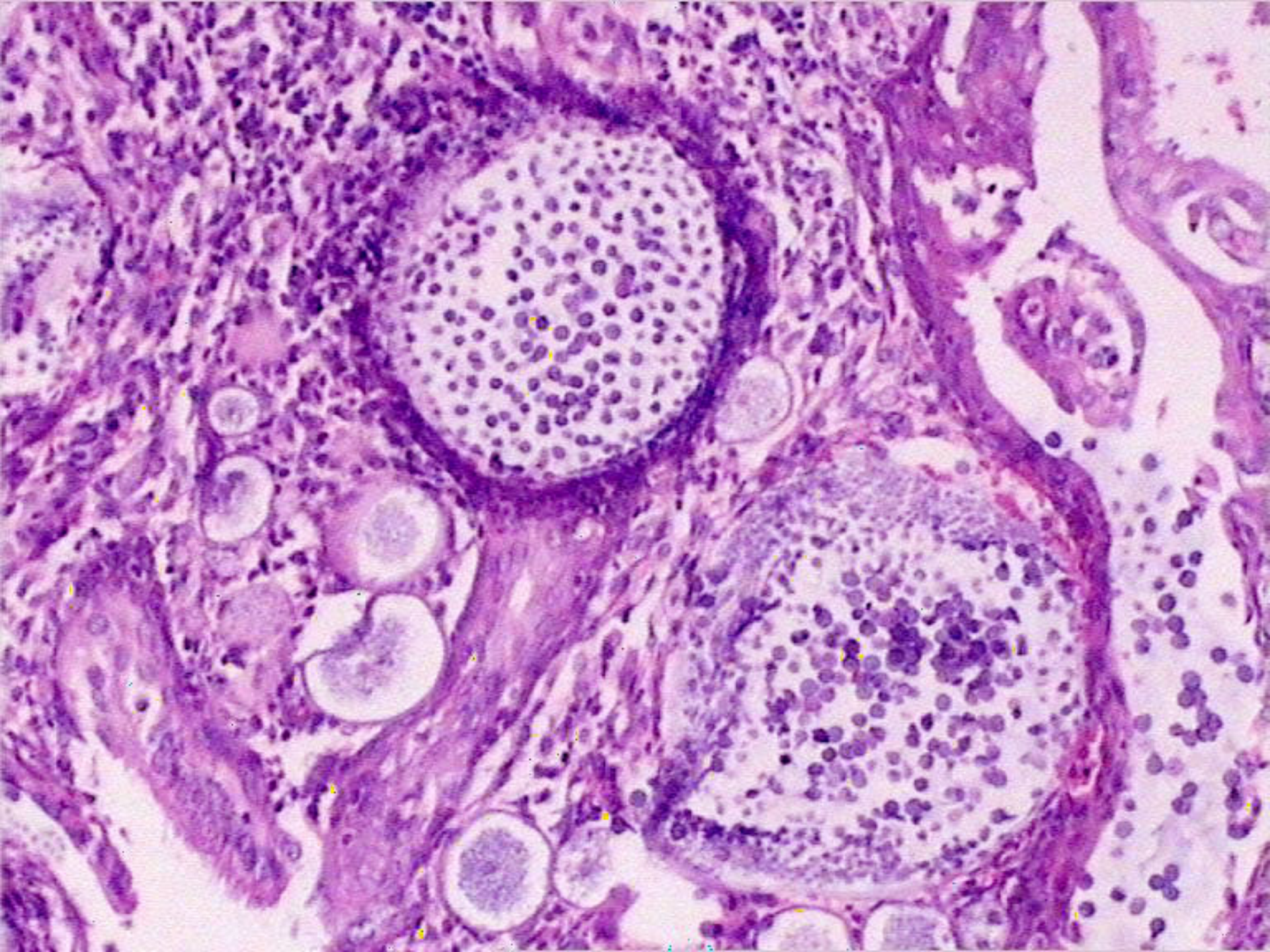


CGATGAACTAGTCTC
CTCGGACCCGTAATG
TTTCGATGAACTAGT GACCCGTAATGGTCT
ATTTCGATGAACTAG
CTAGTCTCGGACCCG
GTGCCATTTTCGATGA
ACTAGTCTCGGACCC
TCGATGAACTAGTCT
GATGAACTAGTCTCG
CCATTTTCGATGAACT

REFERENCE 5'-

AGTGCCATTTTCGATGAAC**AT**GTCTCGGACCCGTAATGGTCTCTTGGGTCTGAA - 3'





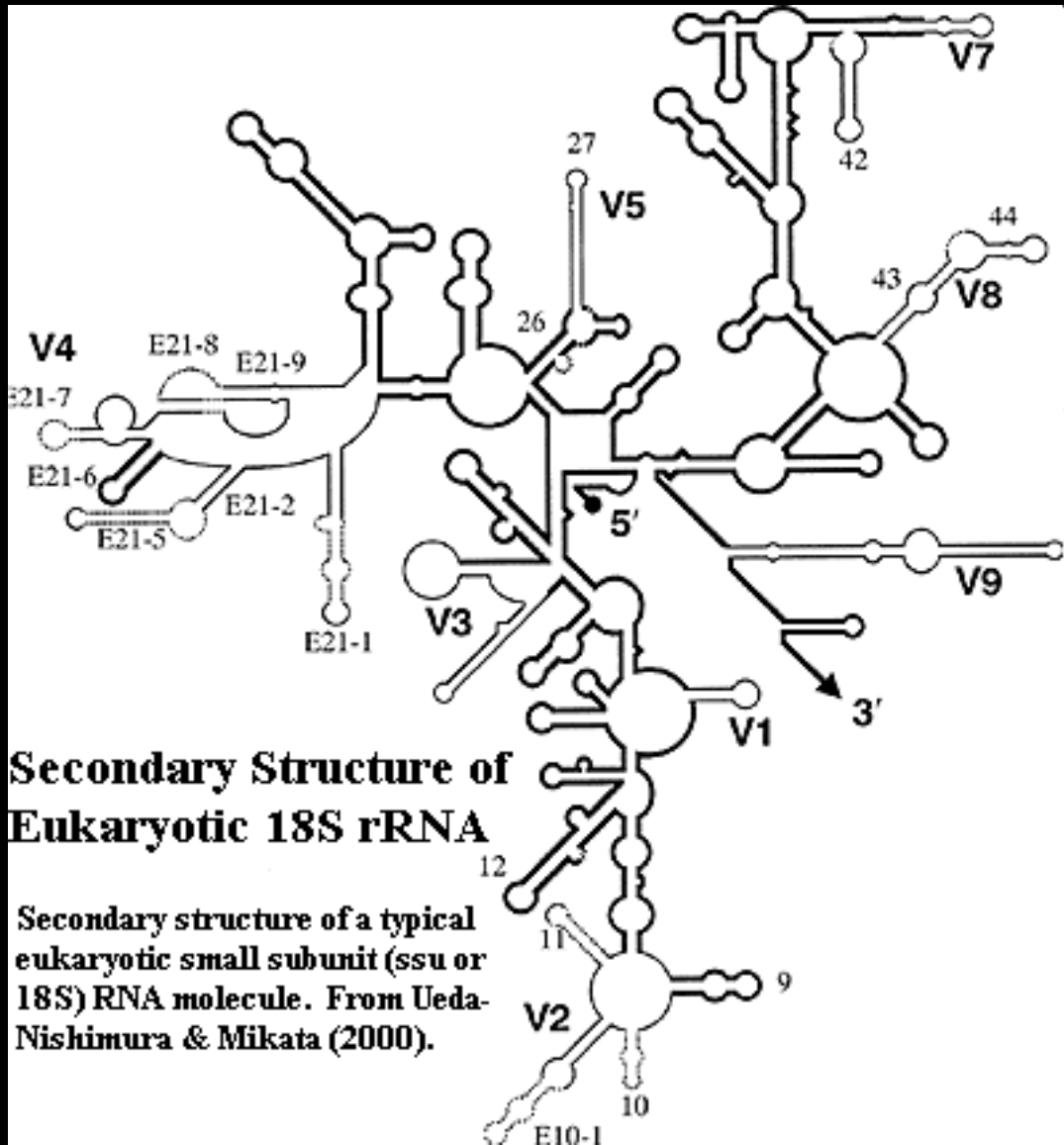
റൈനോസ് പൊരിഡിയോസിസ്

- ഫൻഗസ് എന്ന് കരുതിയിരുന്നു
- തെളിവൊന്നുമില്ല
- ലബോറട്ടറിയിൽ വളർത്താനായിട്ടില്ല





hybrid
MEDICAL ANIMATION



Secondary Structure of Eukaryotic 18S rRNA

Secondary structure of a typical eukaryotic small subunit (ssu or 18S) RNA molecule. From Ueda-Nishimura & Mikata (2000).

Phylogenetic Analysis of *Rhinosporidium seeberi*'s 18S Small-Subunit Ribosomal DNA Groups This Pathogen among Members of the Protoctistan Mesomycetozoa Clade

ROGER A. HERR,¹ LIBERO AJELLO,² JOHN W. TAYLOR,³ SARATH N. ARSECULERATNE,⁴
AND LEONEL MENDOZA^{1*}

Medical Technology Program, Department of Microbiology, Michigan State University, East Lansing, Michigan 48824-1031¹; Department of Ophthalmology, Emory University School of Medicine, Atlanta, Georgia 30322²; Department of Plant and Microbial Biology, University of California, Berkeley, California 94720-3102³; and Department of Microbiology, Faculty of Medicine, University of Peradeniya, Peradeniya 20400, Sri Lanka⁴

Received 26 February 1999/Returned for modification 4 May 1999/Accepted 25 May 1999

For the past 100 years the phylogenetic affinities of *Rhinosporidium seeberi* have been controversial. Based on its morphological features, it has been classified as a protozoan or as a member of the kingdom Fungi. We have amplified and sequenced nearly a full-length 18S small-subunit (SSU) ribosomal DNA (rDNA) sequence from *R. seeberi*. Using phylogenetic analysis, by parsimony and distance methods, of *R. seeberi*'s 18S SSU rDNA and that of other eukaryotes, we found that this enigmatic pathogen of humans and animals clusters with a novel group of fish parasites referred to as the DRIP clade (*Dermocystidium*, rossete agent, *Ichthyophonus*, and *Psorospermium*), near the animal-fungal divergence. Our phylogenetic analyses also indicate that *R. seeberi* is the sister taxon of the two *Dermocystidium* species used in this study. This molecular affinity is remarkable since

***Rhinosporidium seeberi*: A Human Pathogen from a Novel Group of Aquatic Protistan Parasites**

**David N. Fredricks,*† Jennifer A. Jolley,* Paul W. Lepp,*
Jon C. Kosek,† and David A. Relman*†**

*Stanford University, Stanford, California, USA; and †Veterans Affairs,
Palo Alto Health Care System, Palo Alto, California, USA

Rhinosporidium seeberi, a microorganism that can infect the mucosal surfaces of humans and animals, has been classified as a fungus on the basis of morphologic and histochemical characteristics. Using consensus polymerase chain reaction (PCR), we amplified a portion of the *R. seeberi* 18S rRNA gene directly from infected tissue. Analysis of the aligned sequence and inference of phylogenetic relationships showed that *R. seeberi* is a protist from a novel clade of parasites that infect fish and amphibians. Fluorescence in situ hybridization and *R. seeberi*-specific PCR showed that this unique 18S rRNA sequence is also present in other tissues infected with *R. seeberi*. Our data support the *R. seeberi* phylogeny recently suggested by another group. *R. seeberi* is not a classic fungus, but rather the first known human pathogen from the DRIPs clade, a novel clade of aquatic protistan parasites (Ichthyosporidia).

Rhinosporidiosis manifests as slow-growing, tumorlike masses, usually of the nasal mucosa or ocular conjunctivae of humans and animals.

that has been difficult to classify. Recently, *R. seeberi* has been considered a fungus, but it was originally thought to be a protozoan parasite (?).

1. POLYMERASE CHAIN REACTION

F1-fw b CAAGTCTGGTGCCAGCAGCC 554-573

F2-revc GATTTCTCGTAAGGTGCCGA 1068-1087

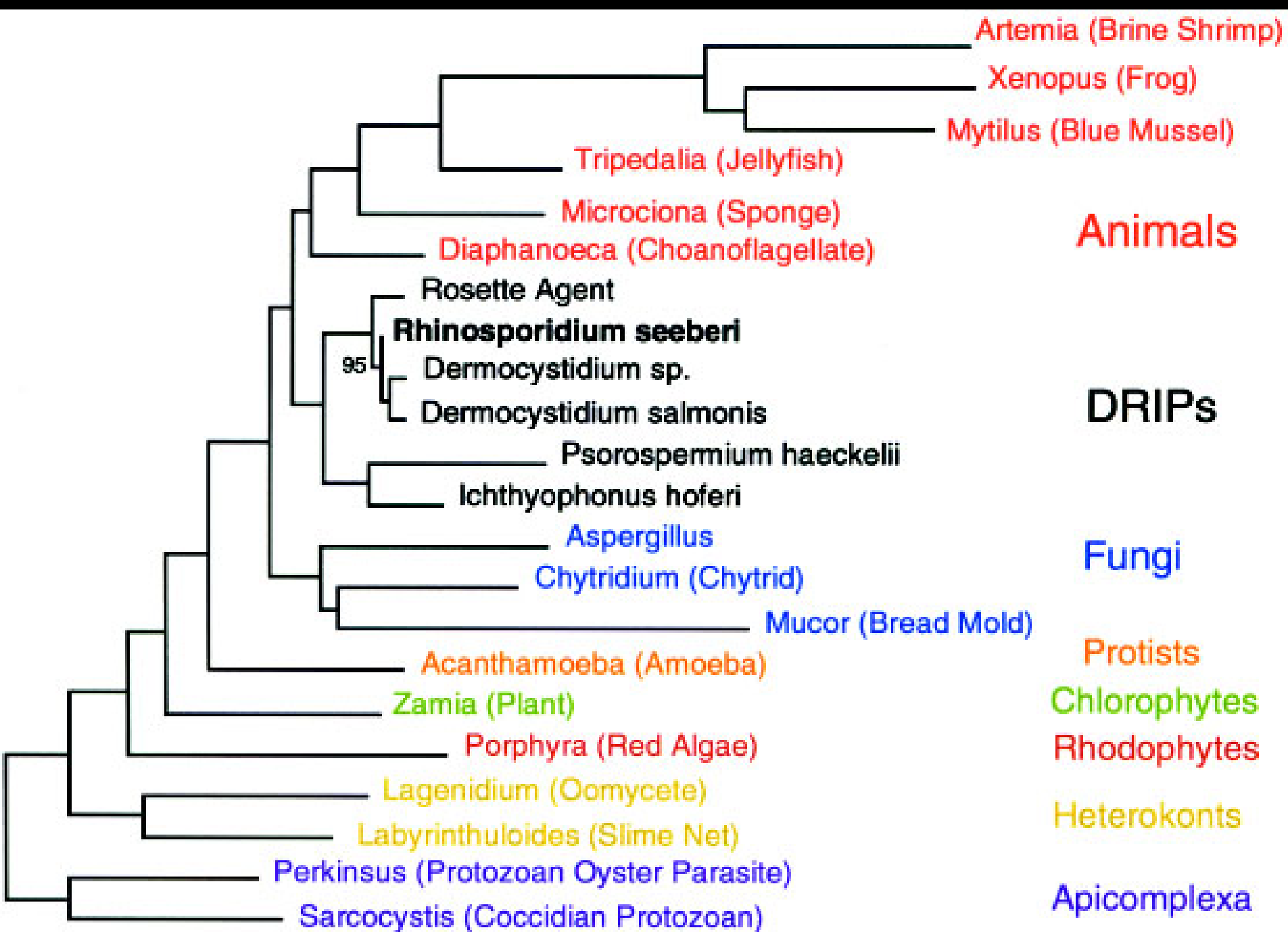
2. CLONING

3. SEQUENCING

4. RHINOSPORIDIUM PROBES

Rhino FISH probe  BTGCTGATAGAGTCATTGAATTAACATCTACB 

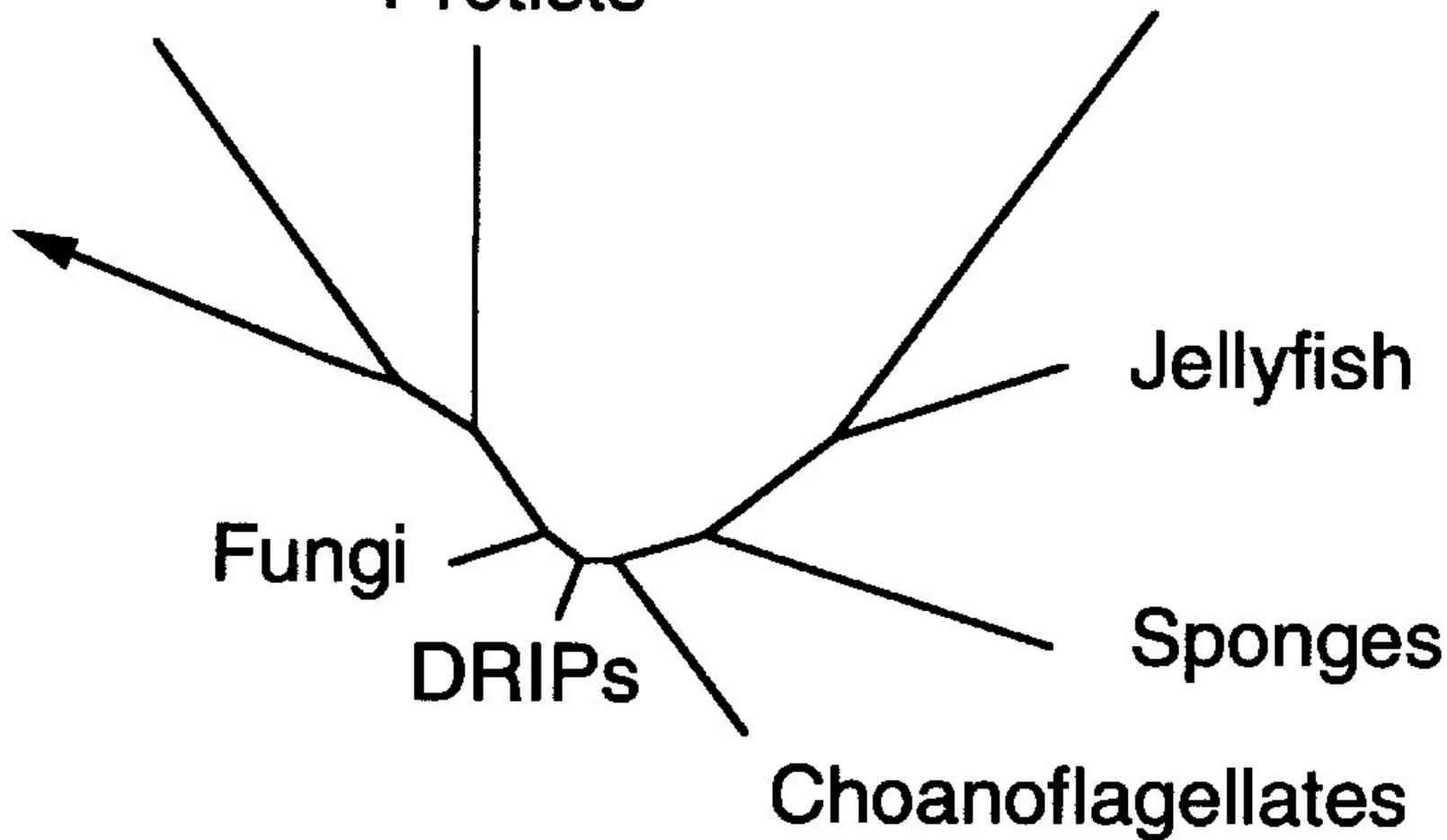
Control FISH probe  BACGACTATCTCAGTAACTTAATTGTAGATGB 



Plants

Protists

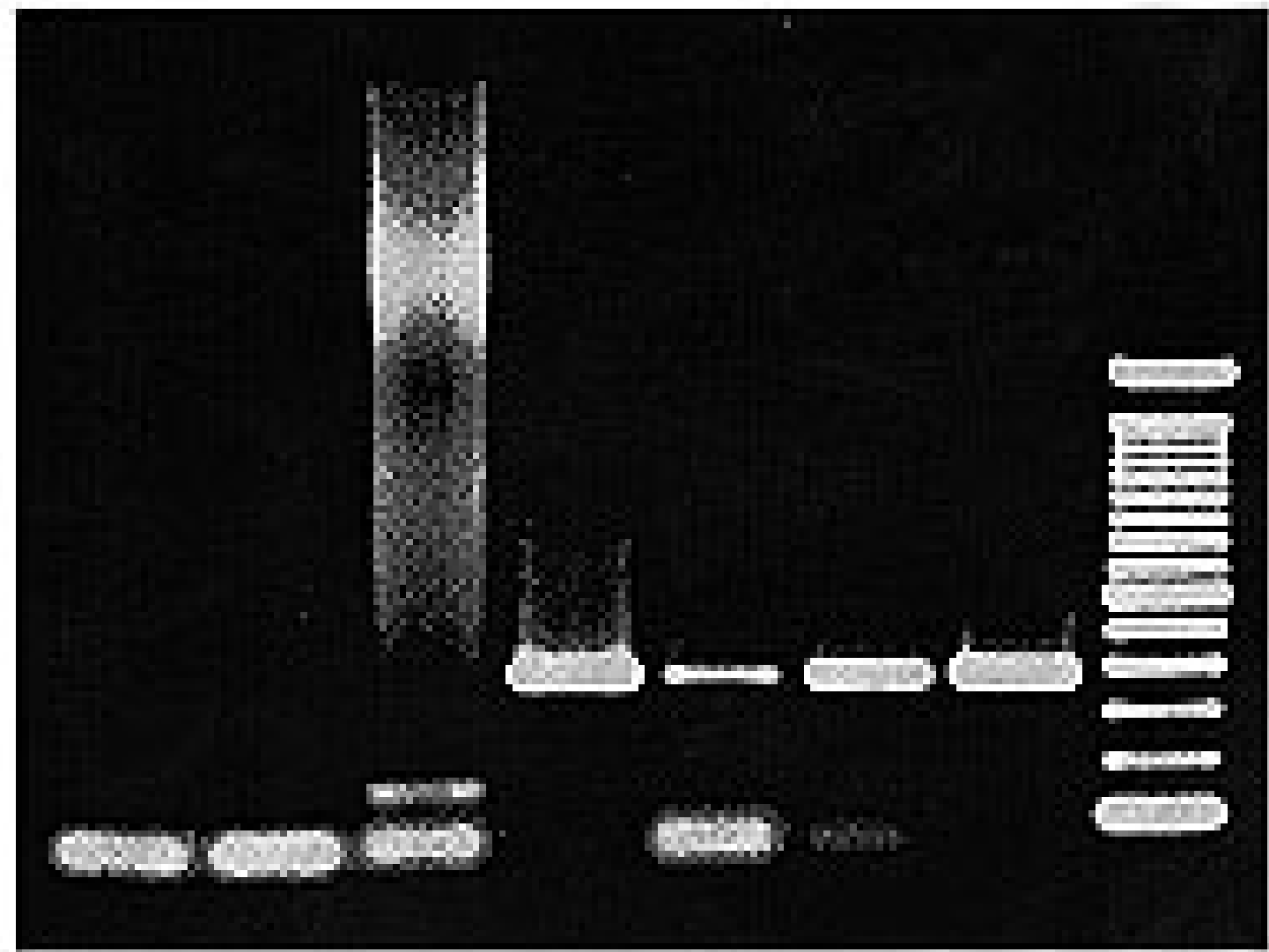
Other Animals



—————

0.1

Neg Controls			EDuman Rhinov			Canine	100 bp
						Polyp	ladder
						+ Cnt	
H ₂ O	DB	Tis Cnt	R51	R52	R53		



← 400 bp

