

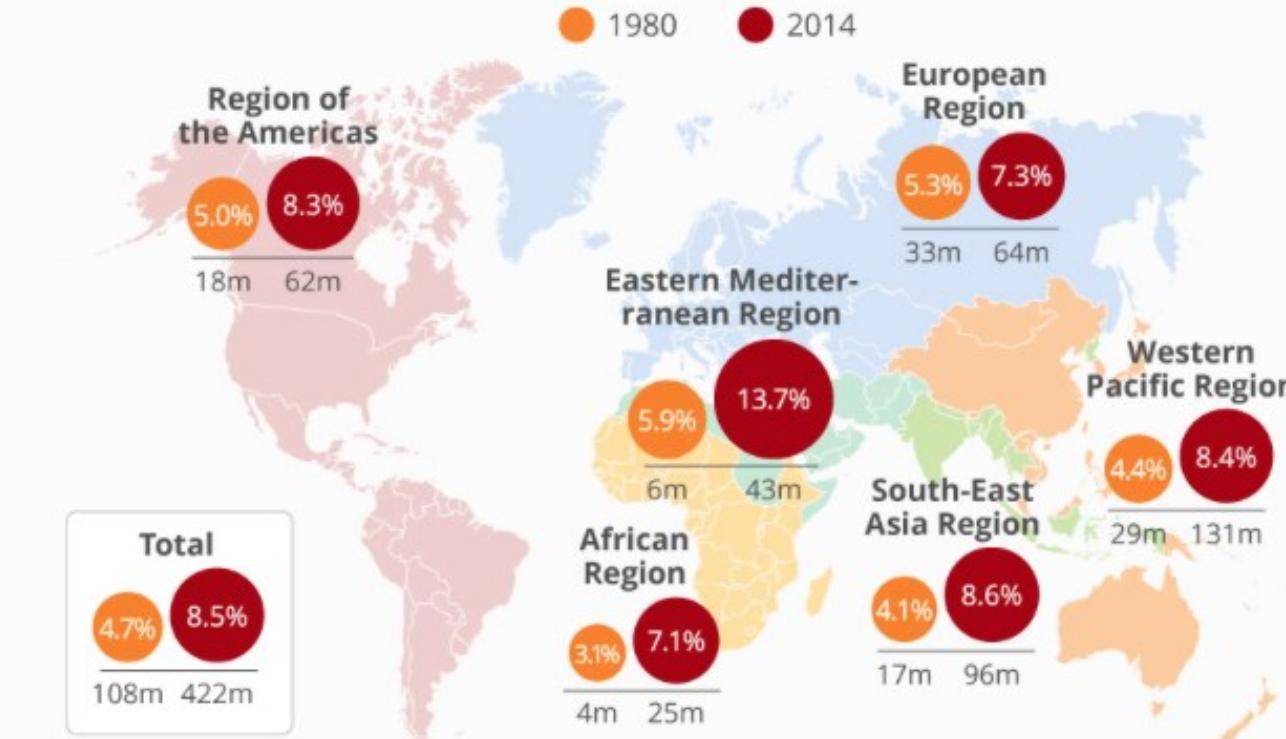
# BIOTEHNOLOGIJA

PRIMJENA BIOLOŠKIH ORGANIZAMA, SUSTAVA I PROCESA U  
PROIZVODNJI (AGRIKULTURA, PREHRAMBENA, TEKSTILNA  
INDUSTRija, FARMAKOLOGIJA, MEDICINA, ZAŠTITA OKOLIŠA)

# PRIČA 1 – DIJABETES I INZULIN

## The Unrelenting March Of Diabetes

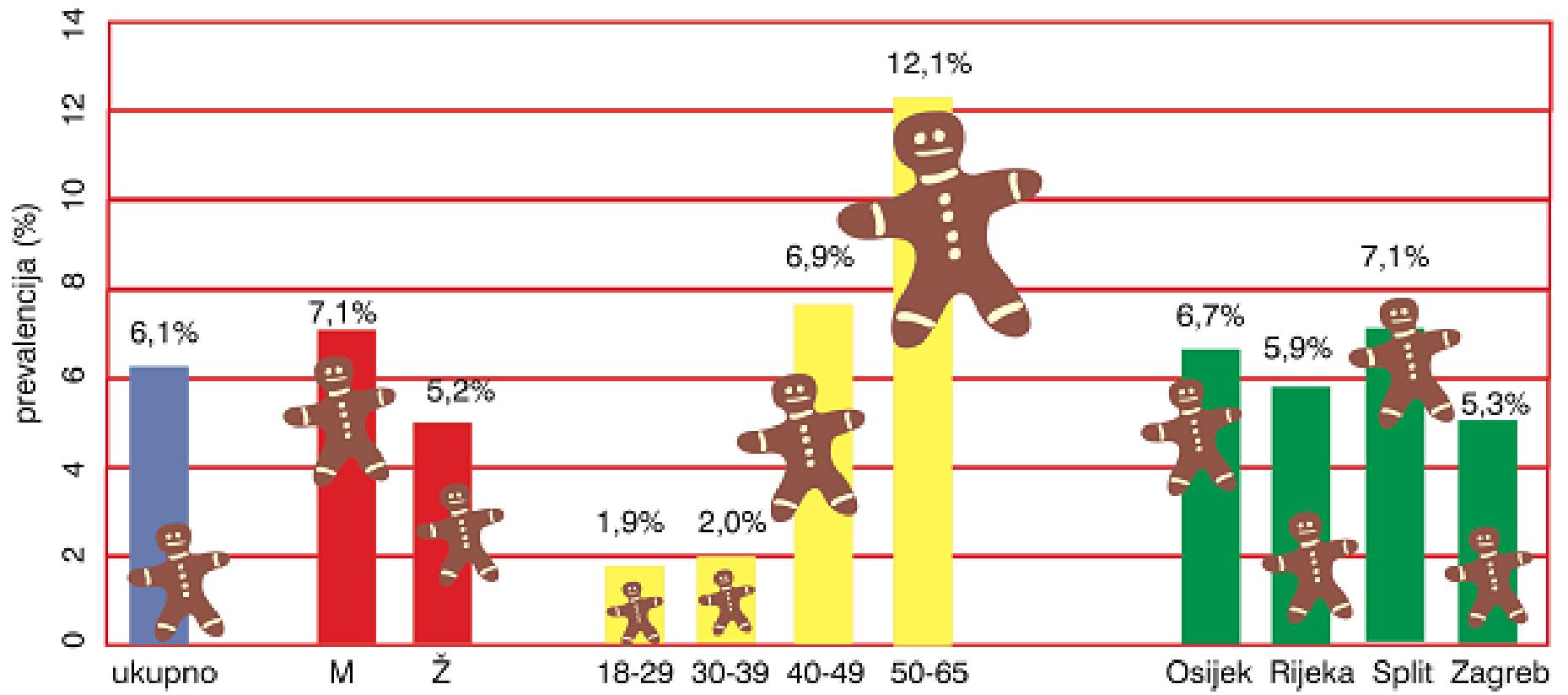
% prevalence and number of adults with diabetes by WHO region in 1980 and 2014\*



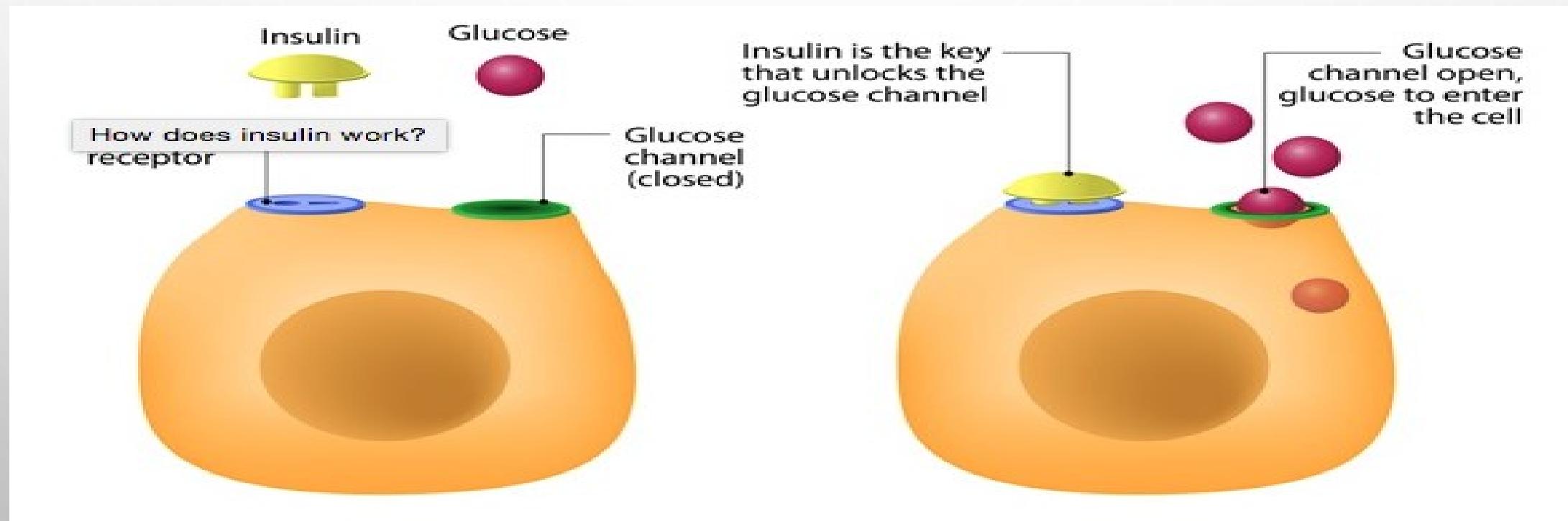
\* Millions of people and % of total regional population  
Source: World Health Organization

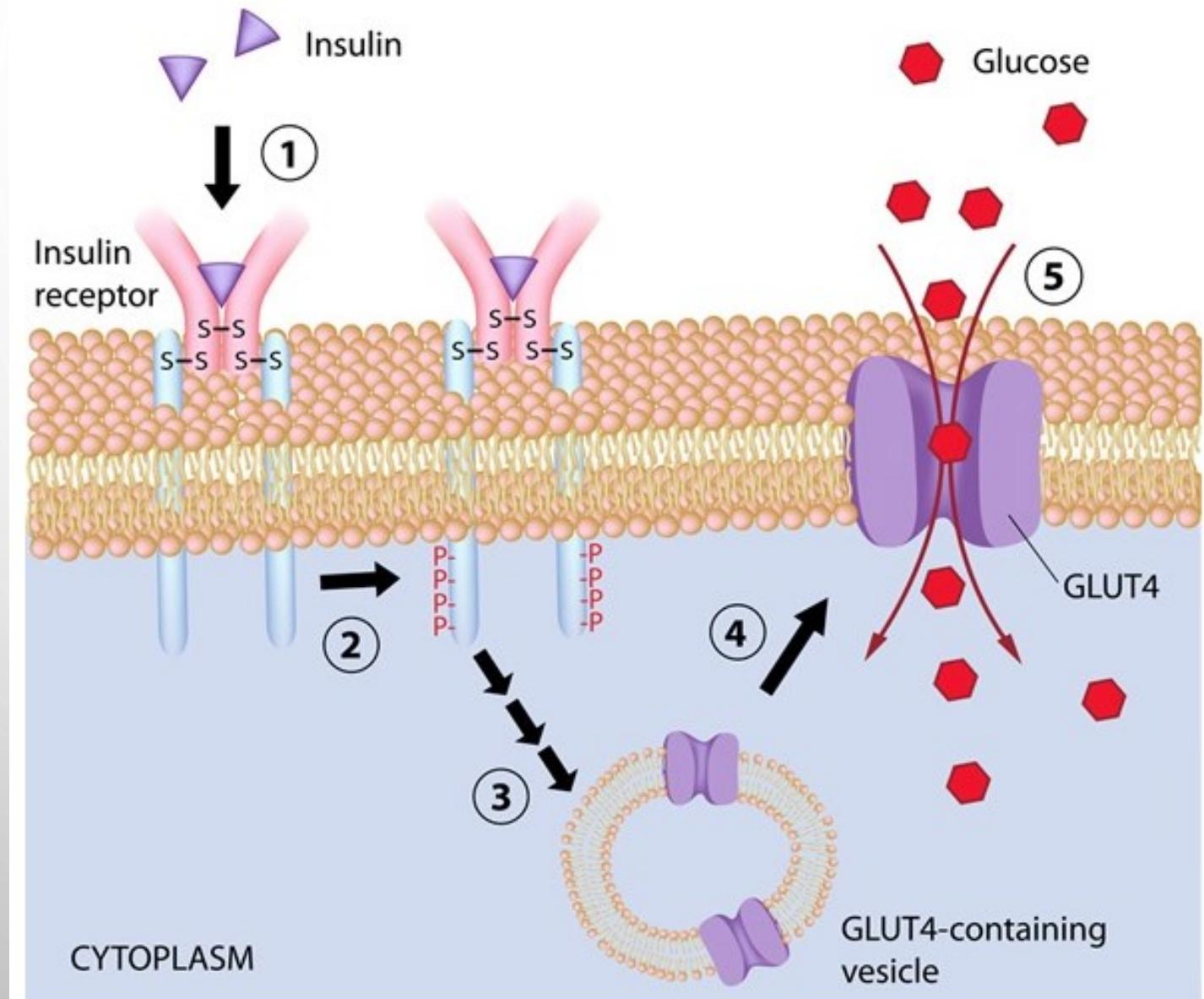
statista

Prevalencija šećerne bolesti u RH, prema spolu, dobi i regiji, za dob od 18 do 65 godina



# ULOGA INZULINA

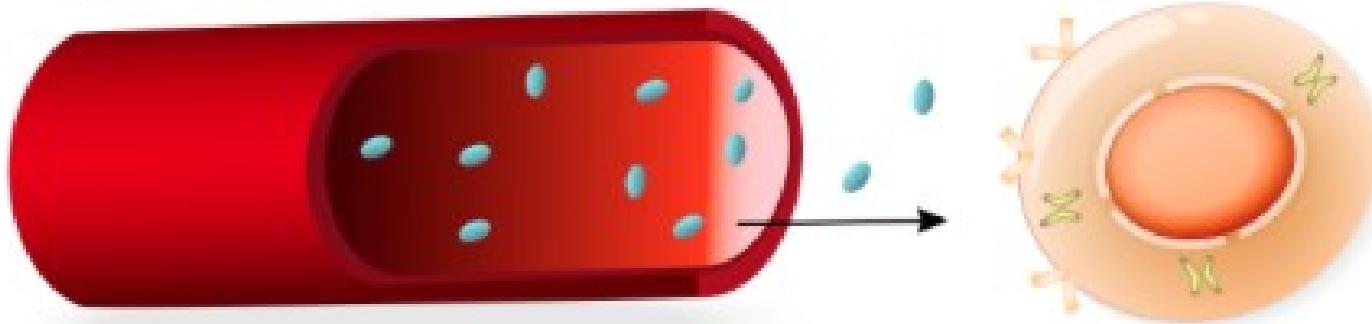




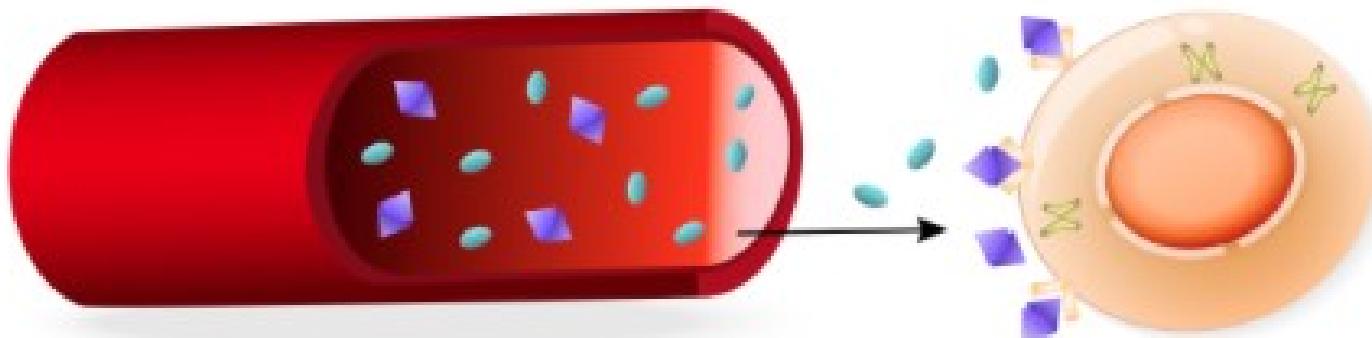
*Image Copyright: Alila Medical Media, Image ID:114645271 via Shutterstock.com*

# TYPES OF DIABETES

## Type I diabetes



## Type II diabetes



● Glucose

X Glut-4

■ Insulin

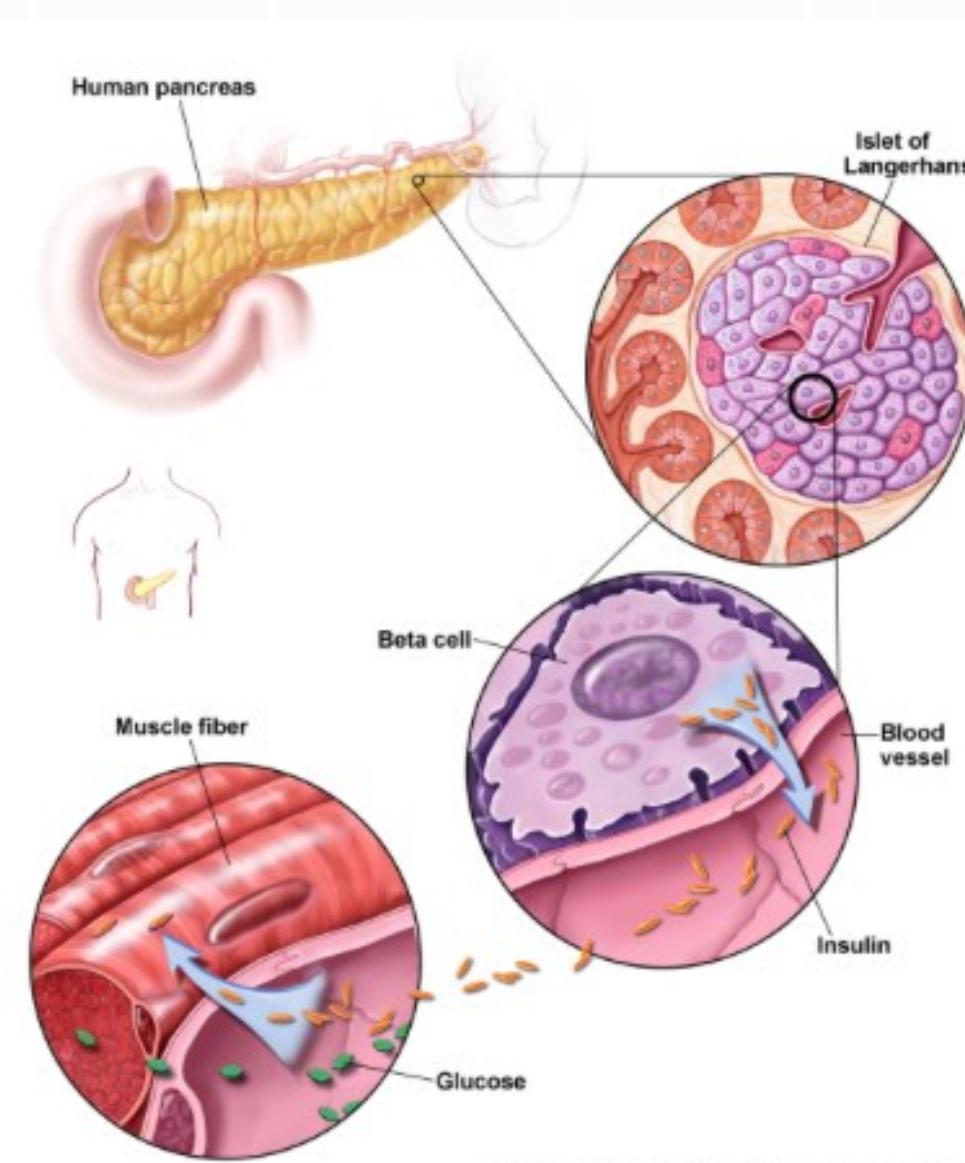
◆ Insulin receptor

# ULOGE INSULINA

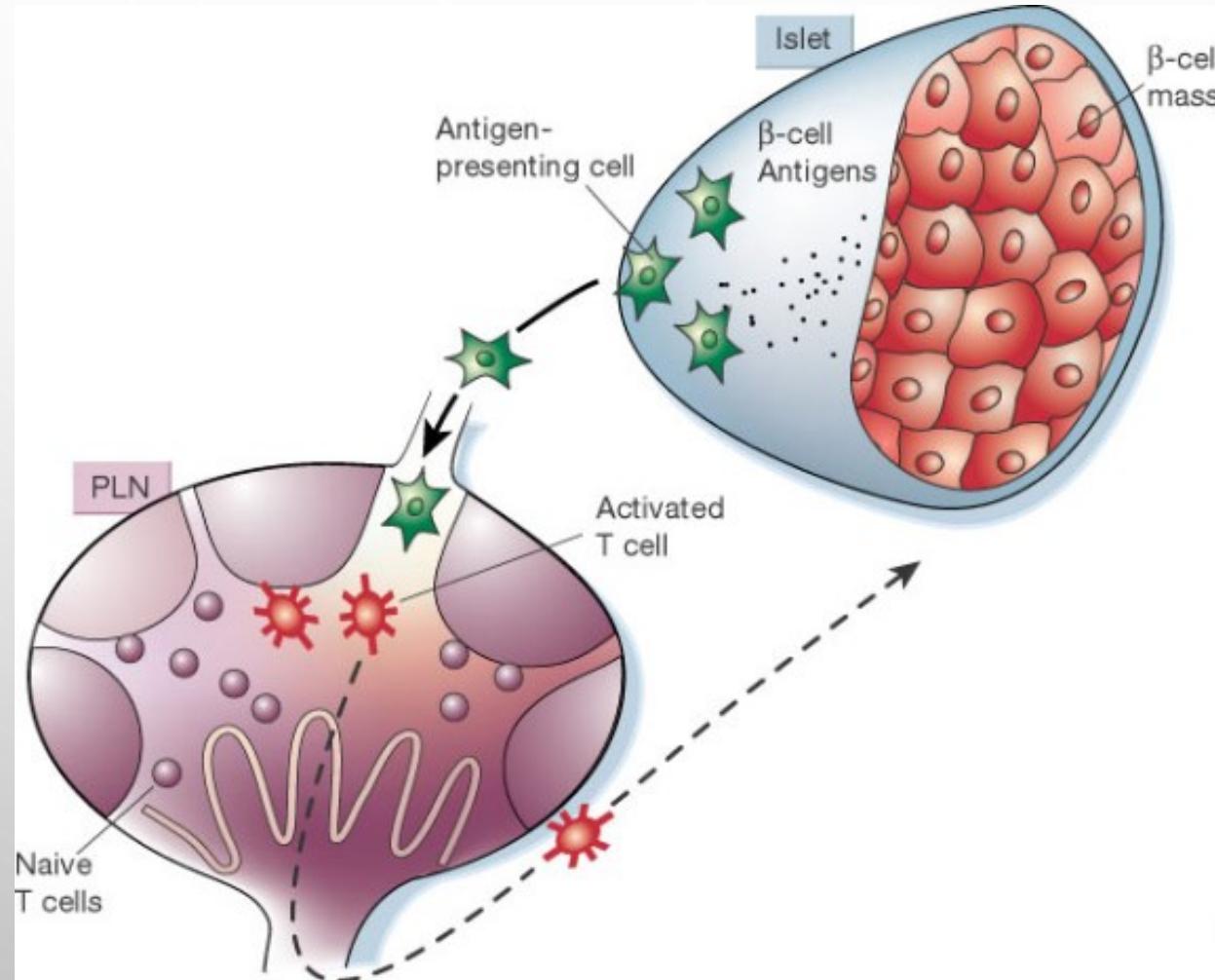
REGULACIJA RAZINE ŠEĆERA U KRVI:

1. ULAZAK U STANICE
2. POSPREMANJE VIŠKA GLUKOZE U GLIKOGEN
3. STIMULACIJE SINTEZE GLIKOGENA

# GUŠTERAČA



# DIJABETES TIP 1



# KAKO PRISTUPITI LIJEČENJU?

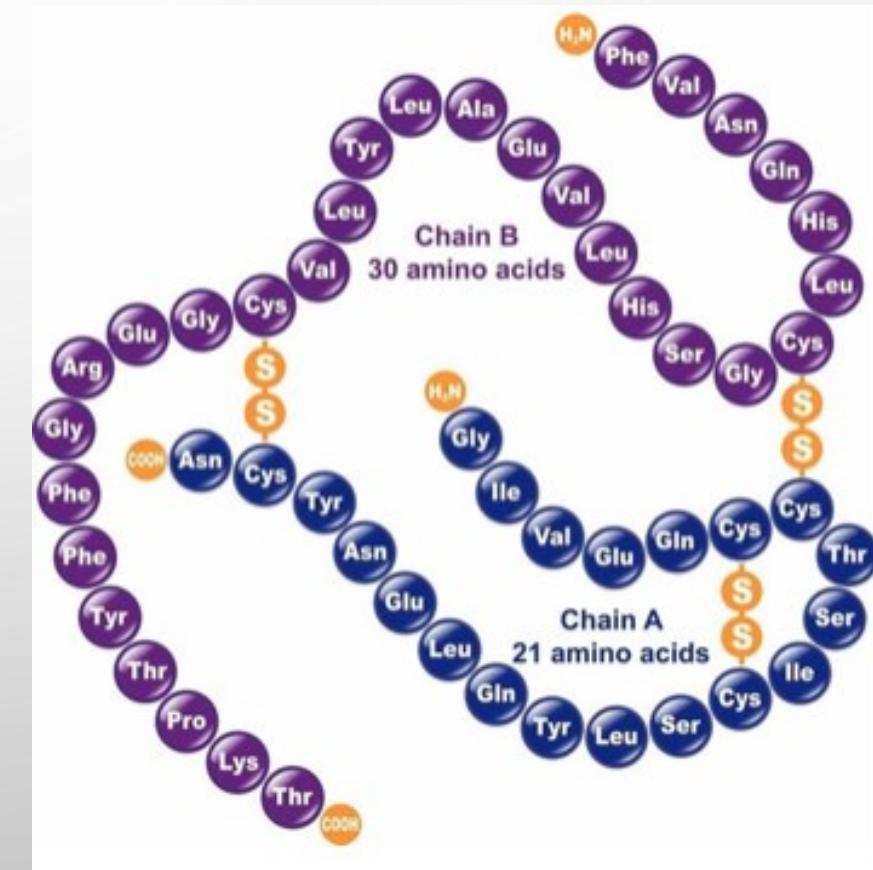
- DODATI INZULIN
- ZAUSTAVITI UNIŠTAVANJE STANICA KOJE LUČE INZULIN
- DODATI STANICE KOJE LUČE INZULIN

# PRVE INZULINSKE TERAPIJE

- 1920. – IZOLACIJA IZ ŽIVOTINJSKIH GUŠTERAČA
- ZA 1 PACIJENTA KROZ 1 GODINU POTREBNO ŽRTVOVATI 50 SVINJA
- PROIZVOĐAČ HOECHST: 100000 ŽIVOTINJA DNEVNO ZA 11 TONA GUŠTERAČA

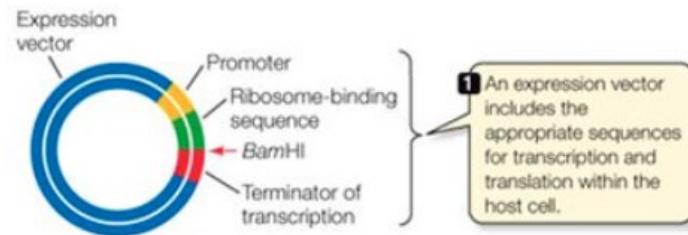
# OTKRIĆE STRUKTURE INZULINA

- 1955. FRED SANGER – 1. NOBELOVA NAGRADA



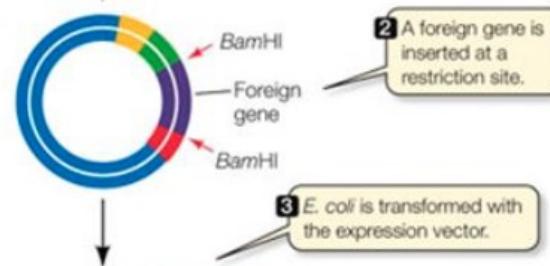
# PROIZVODNJA U BAKTERIJI

**Vector**



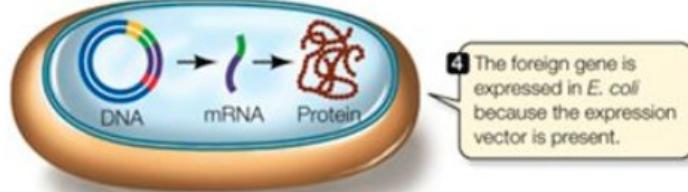
**Plasmid**

**Gene**



**Insulin**

**Host**



***E. coli***

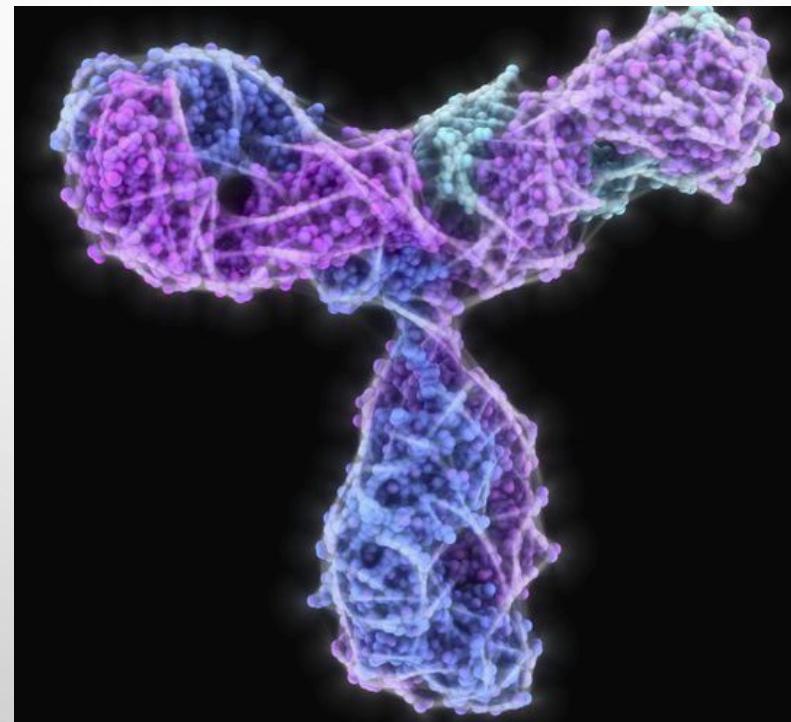
1 An expression vector includes the appropriate sequences for transcription and translation within the host cell.

2 A foreign gene is inserted at a restriction site.

3 *E. coli* is transformed with the expression vector.

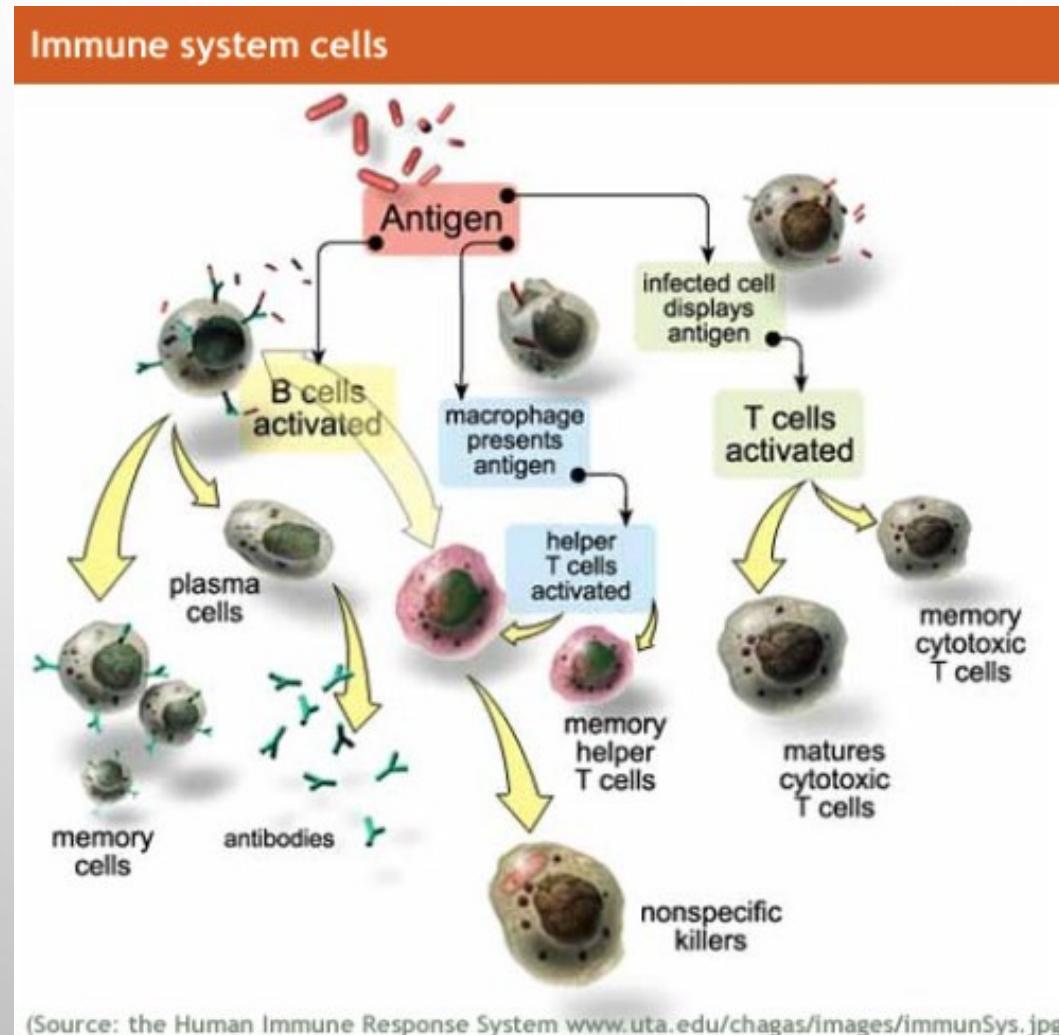
4 The foreign gene is expressed in *E. coli* because the expression vector is present.

# PRIČA 2 – ANTITIJELA

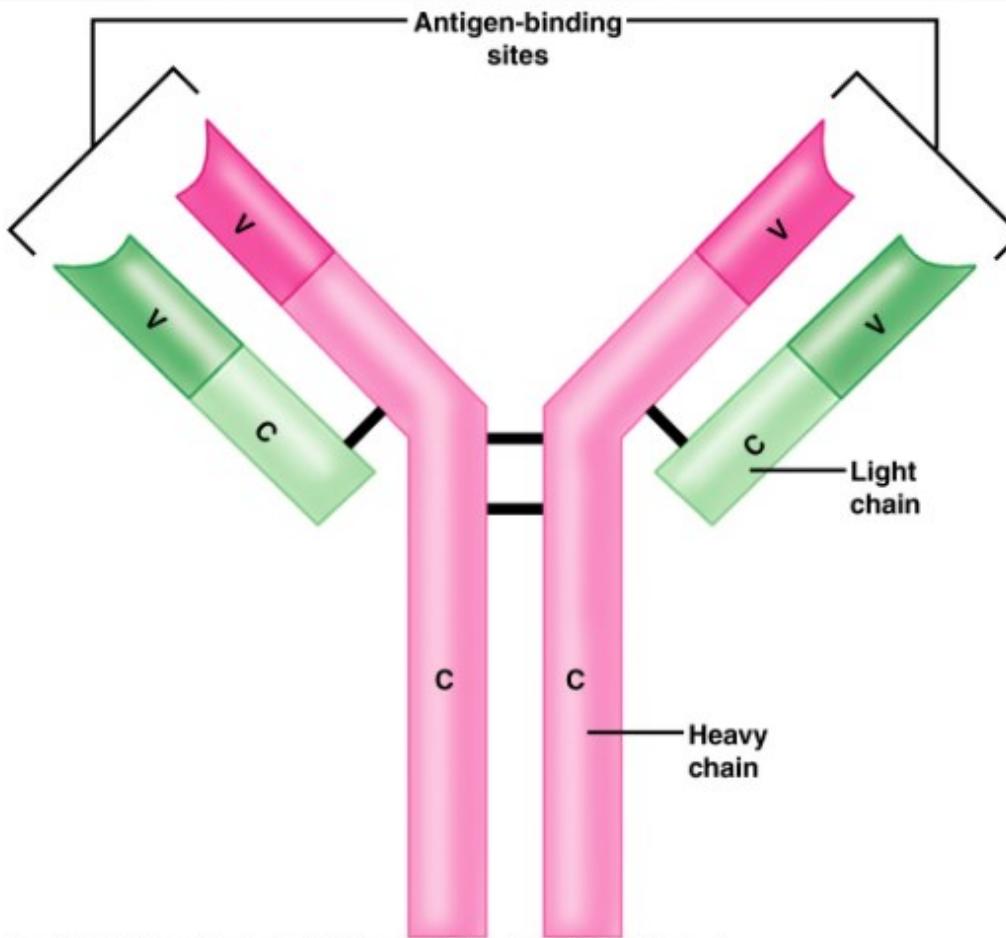


# IMUNOLOŠKI SUSTAV

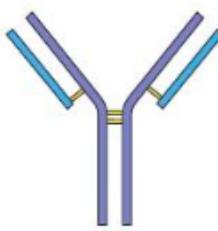
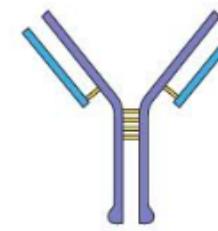
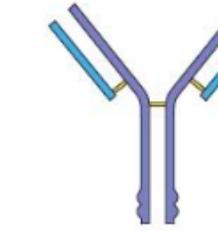
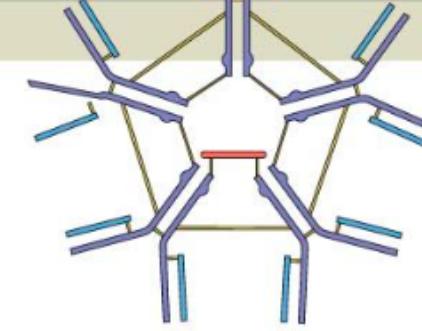
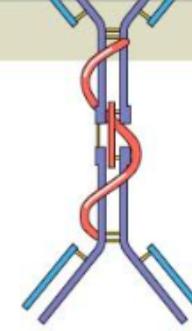
[HTTPS://WWW.YOUTUBE.COM/WATCH?V=MI-BLAJ5NFK](https://www.youtube.com/watch?v=MI-BLAJ5NFK)



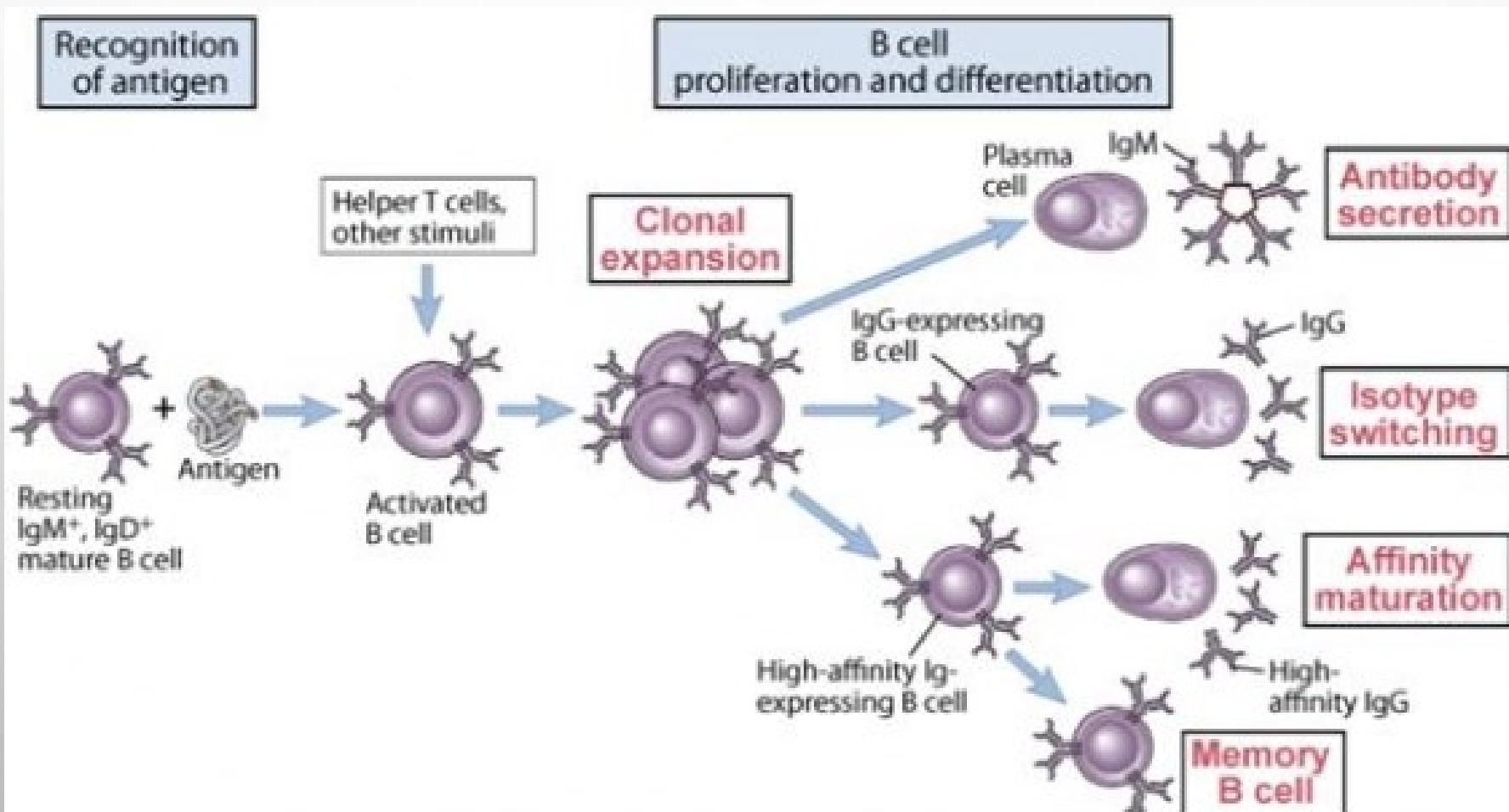
# GRAĐA ANTITIJELA



# VRSTE ANTITIJELA

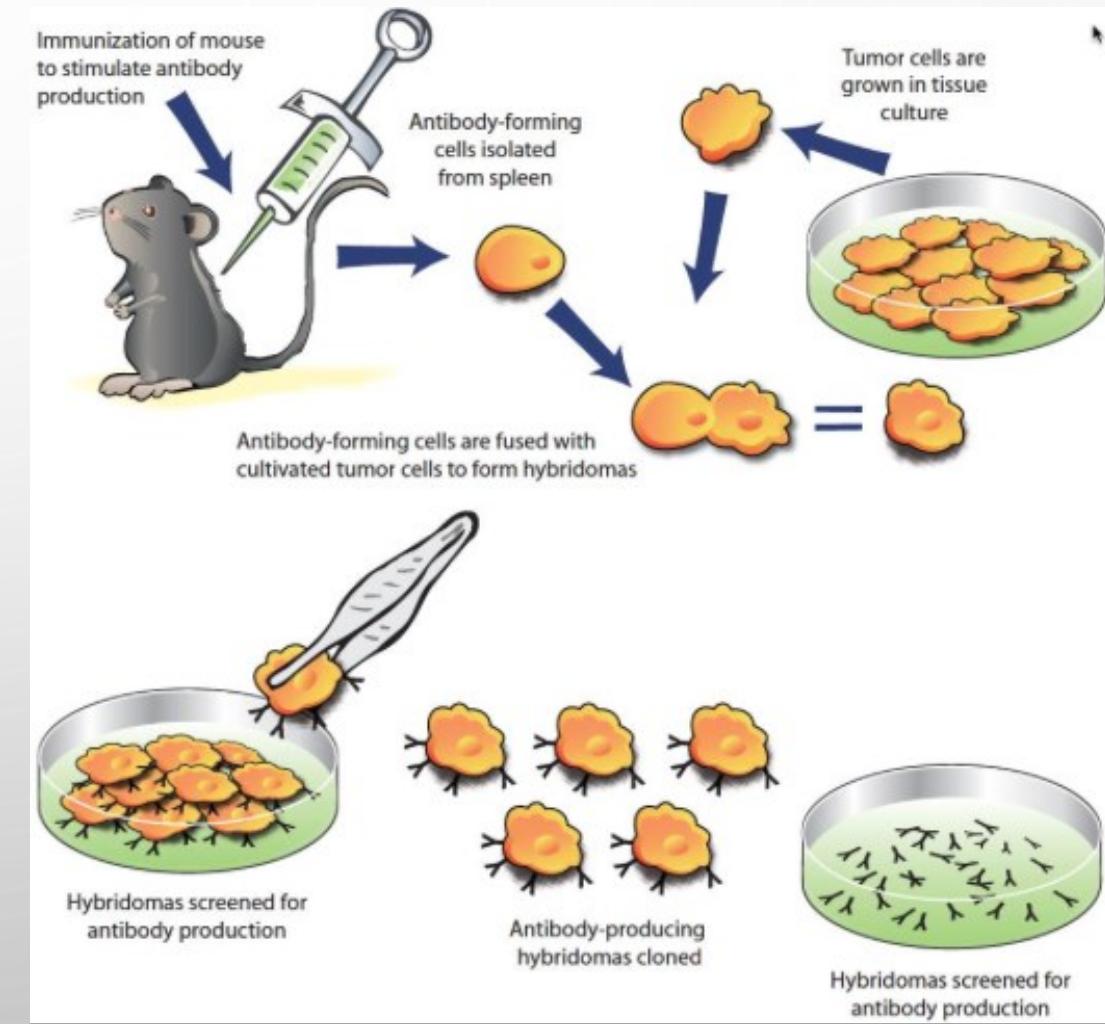
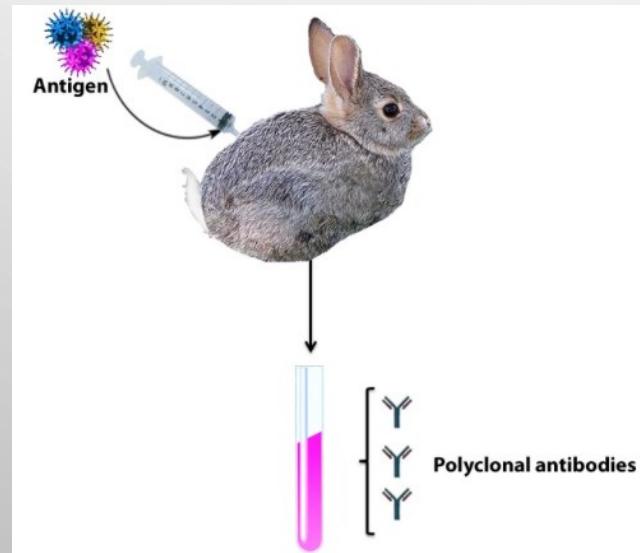
Classes of Antibodies				
				
<b>IgG</b> antibodies account for 80 percent of all antibodies. IgG antibodies are responsible for resistance against many viruses, bacteria, and bacterial toxins.	<b>IgE</b> attaches as an individual molecule to the exposed surfaces of basophils and mast cells.	<b>IgD</b> is an individual molecule on the surfaces of B cells, where it can bind antigens in the extracellular fluid. This binding can play a role in the sensitization of the B cell involved.	<b>IgM</b> is the first class of antibody secreted after an antigen is encountered. IgM concentration declines as IgG production accelerates. The anti-A and anti-B antibodies responsible for the agglutination of incompatible blood types are IgM antibodies.	<b>IgA</b> is found primarily in glandular secretions such as mucus, tears, saliva, and semen. These antibodies attack pathogens before they gain access to internal tissues.

# AFINITET ANTITIJELA



# PROIZVODNJA ANTITIJELA

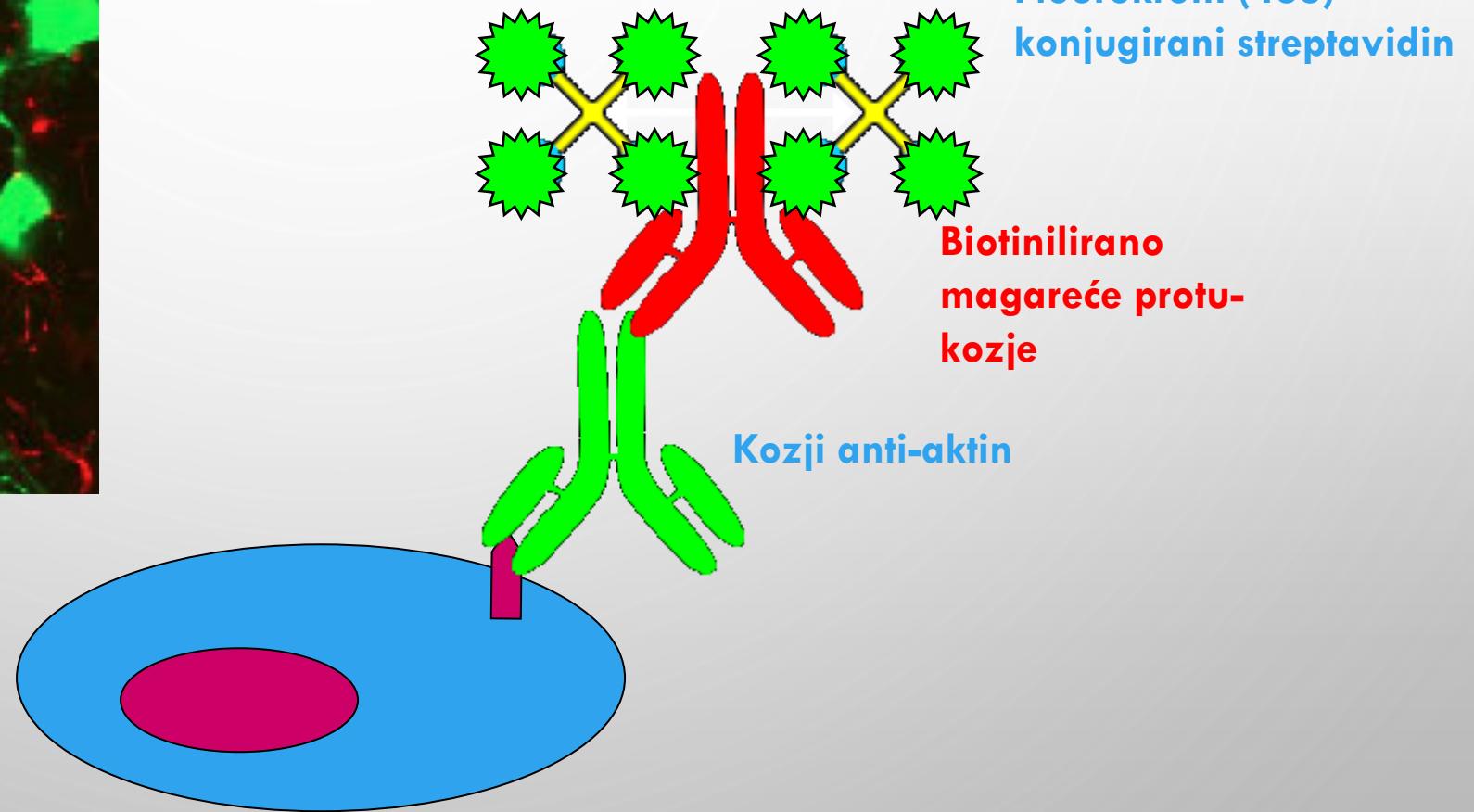
- POLIKLONSKA
- MONOKLONSKA
- REKOMBINANTNA



# UPOTREBA ANTITIJELA

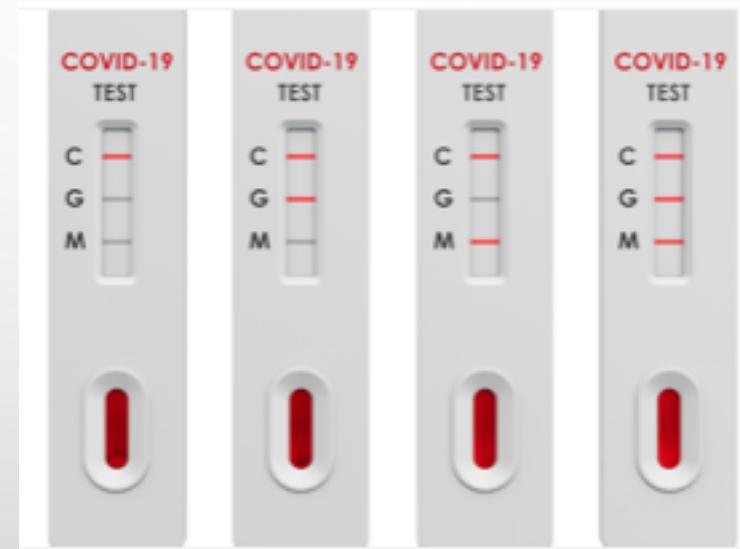
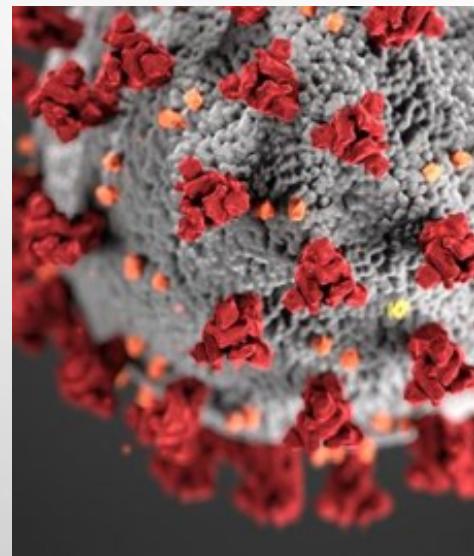
- ISTRAŽIVANJE
- DIJAGNOSTIKA
- CJEPIVA I TERAPIJE

# ISTRAŽIVANJE

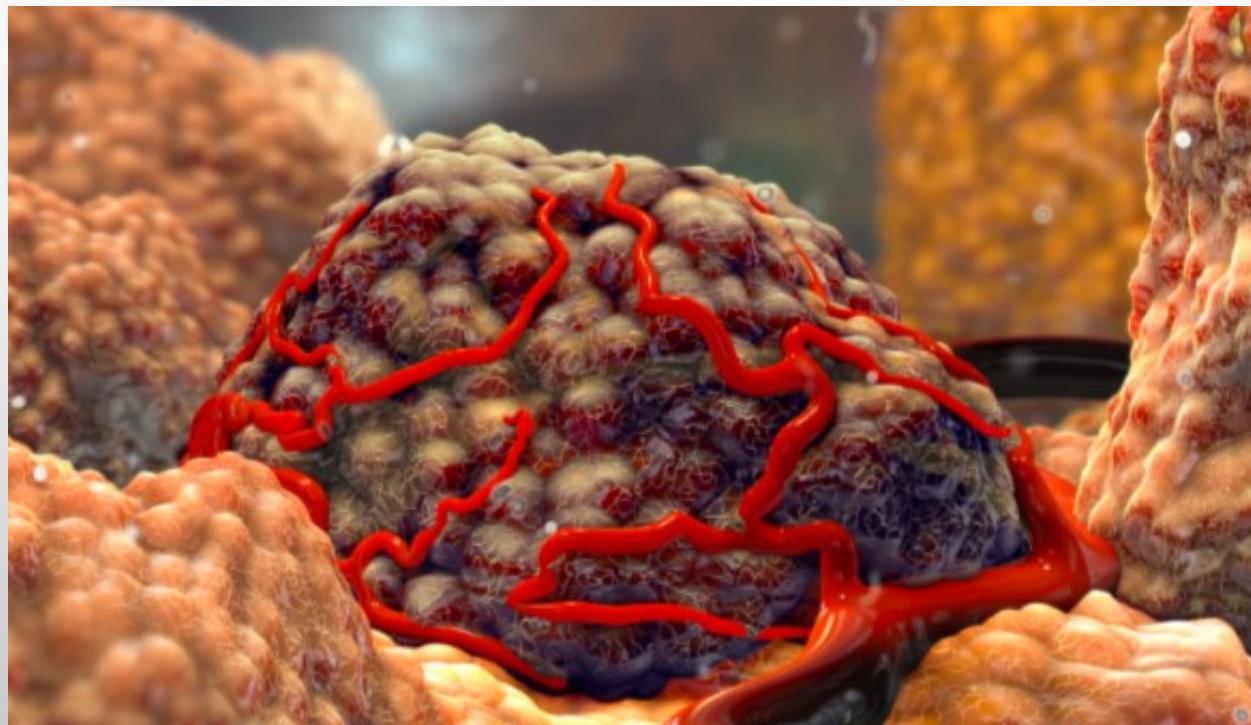


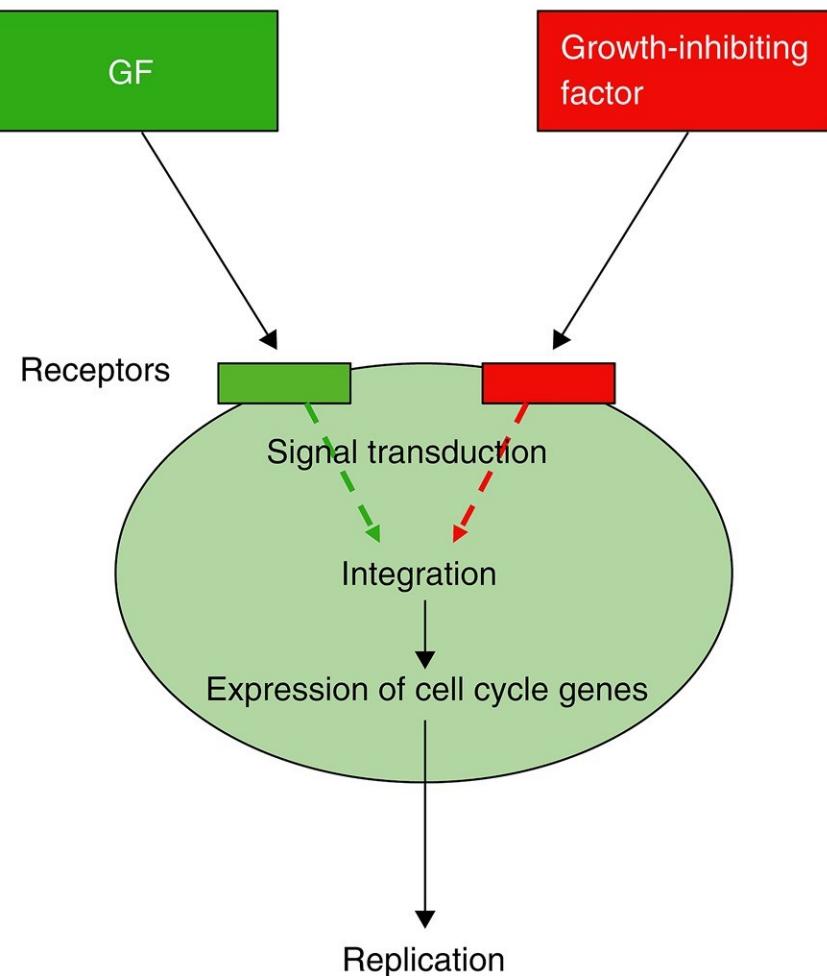
# DIJAGNOSTIKA

[HTTPS://WWW.YOUTUBE.COM/WATCH?V=VMQMY-QYWS](https://www.youtube.com/watch?v=VMQMY-QYWS)

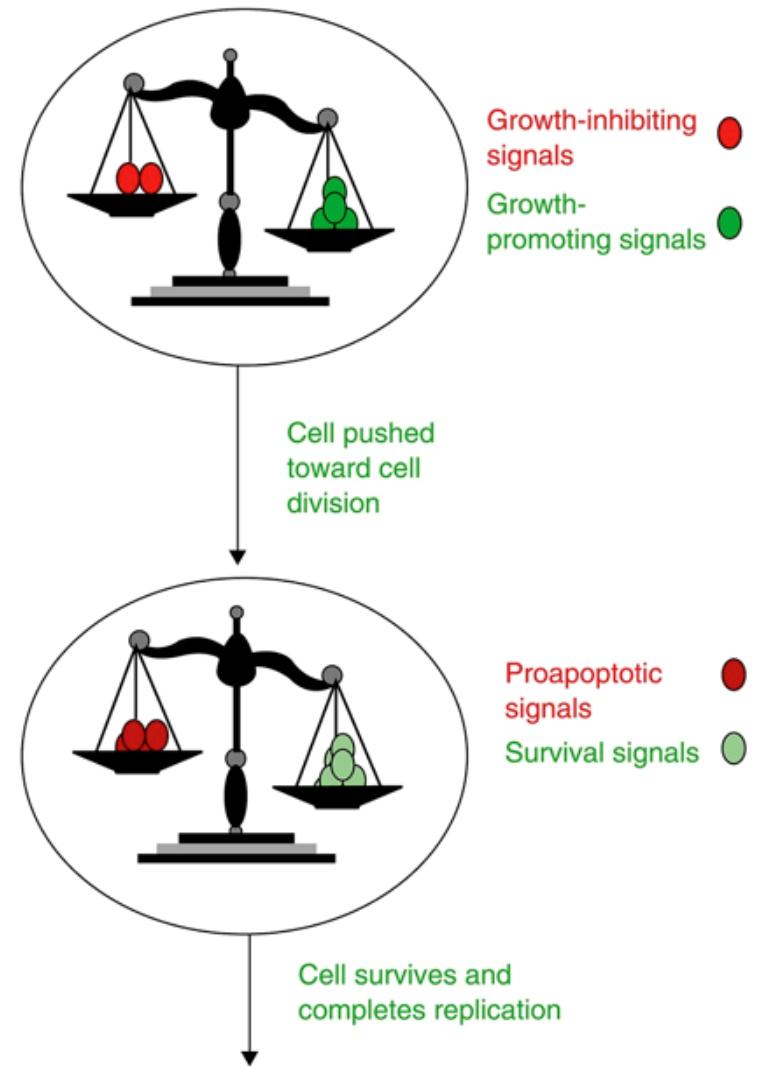


# TERAPIJE



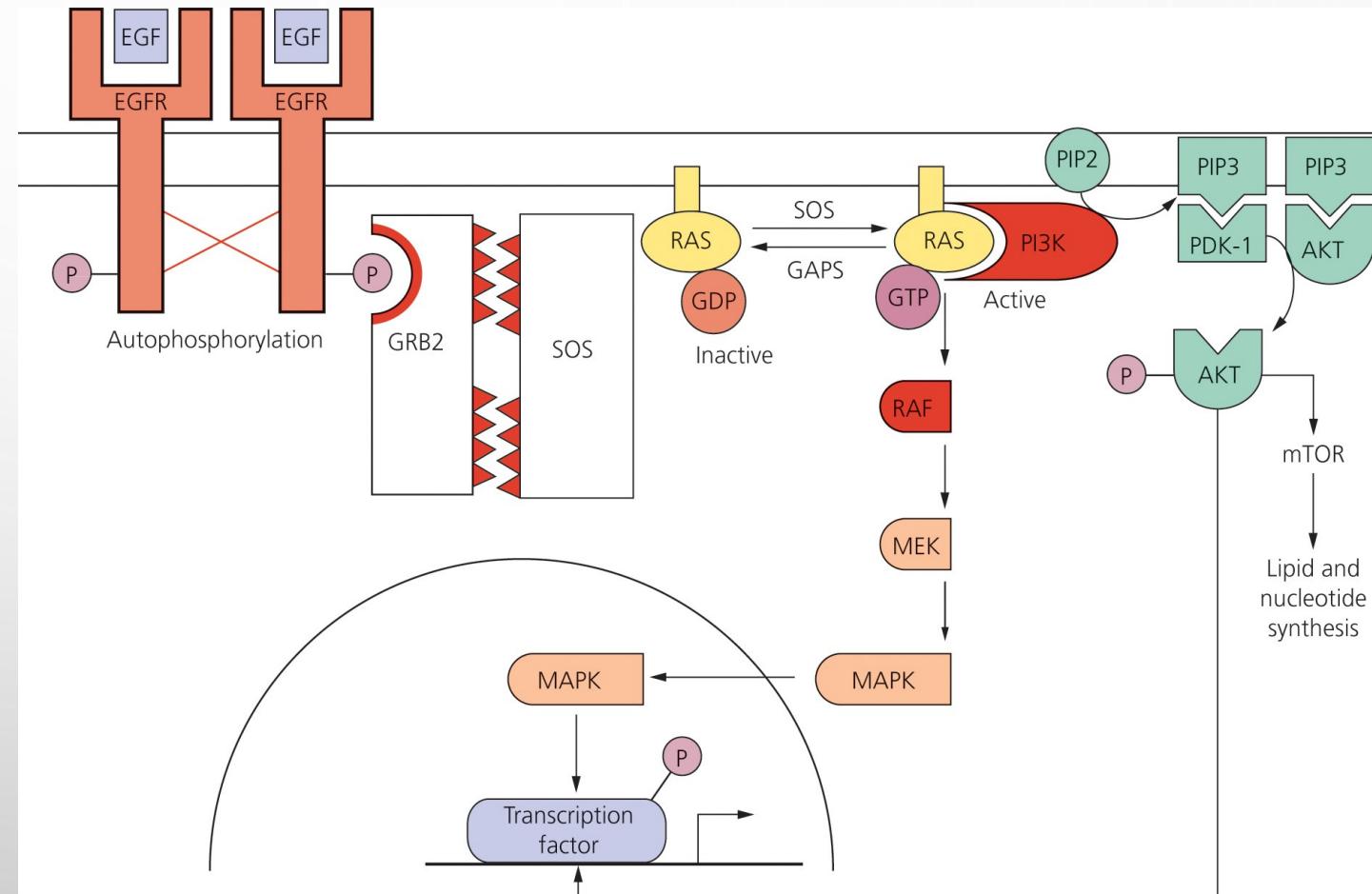


**Figure 5.11** Signaling through membrane-bound growth factor receptors. Positive and negative signals are integrated at various levels before a decision on whether to respond or not is completed.



# EGF SIGNALNI PUT

- VEZANJE FAKTORA RASTA
- DIMERIZACIJA RECEPTORA
- AUTOFOSFORILACIJA  
RECEPTORA
- AKTIVACIJA UNUTARSTANIČNE  
KINAZNE KASKADE
- AKTIVACIJA  
TRANSKRIPCIJSKIH FAKTORA
- REGULACIJA GENSKE  
EKSPRESIJE

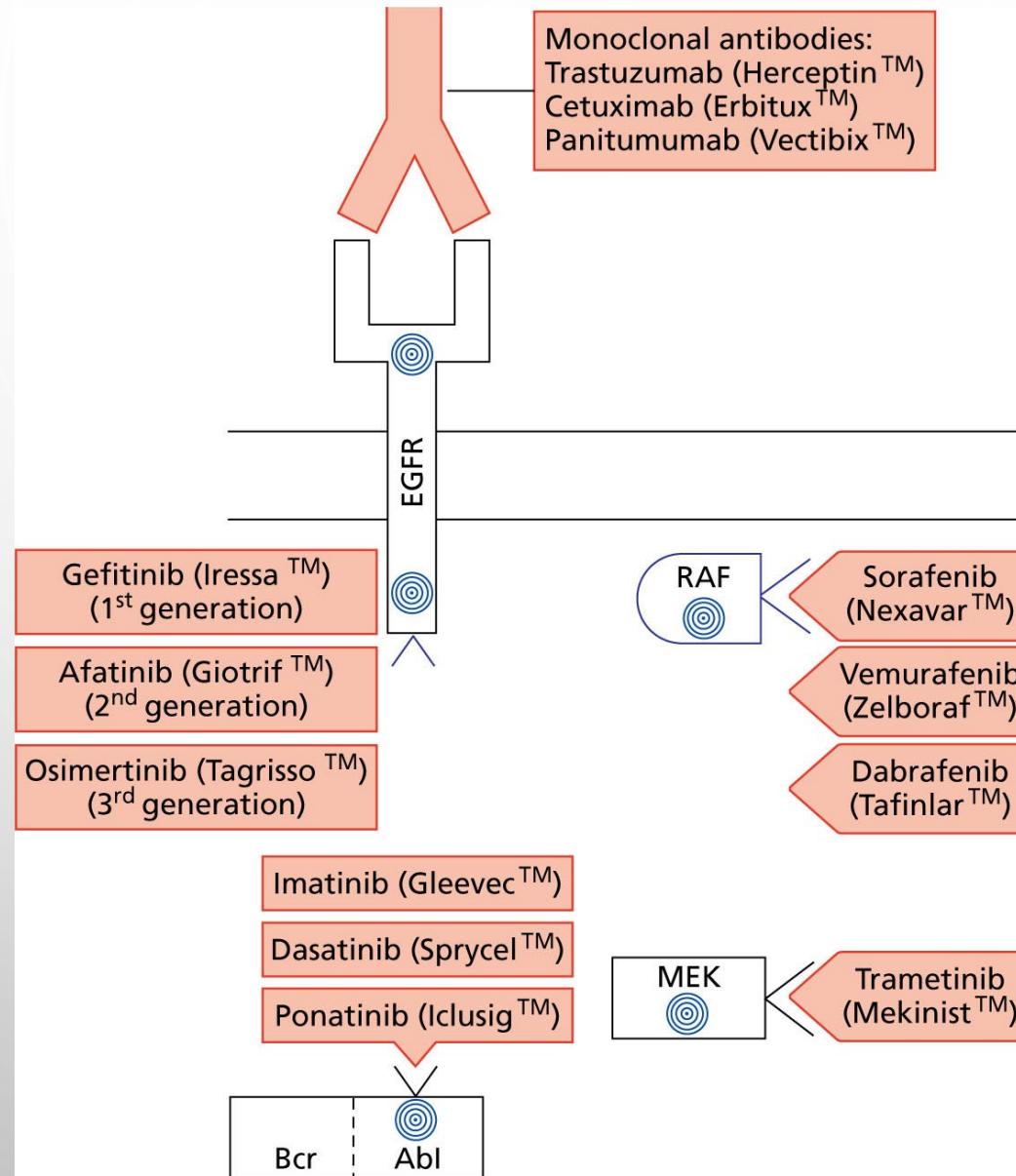


# EGFR

Tumor Type	% of Tumors Over-Expressing EGFR	X
Head and Neck	80-100	
Renal Cell	50-90	
Non-small-cell Lung	40-80	
Glioma	40-50	
Ovarian	35-70	
Bladder	31-48	
Pancreatic	30-50	
Colon	25-77	
Breast	14-91	

Table 1. Frequency of elevated EGFR expression in different types of epithelial tumors<sup>1</sup>.

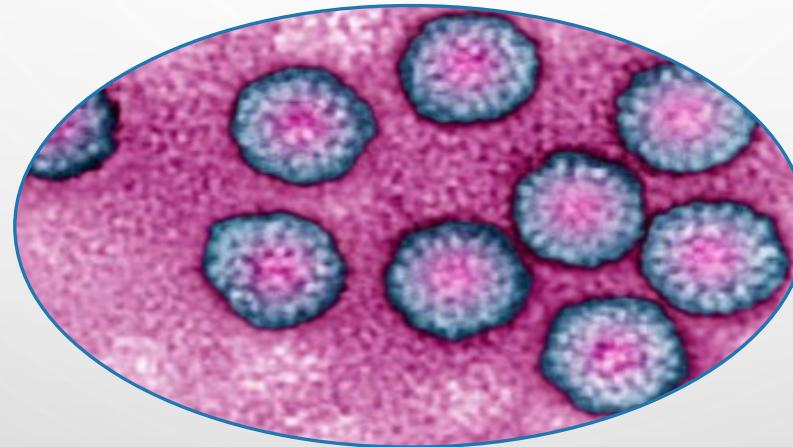
- KONSTITUTIVNA AKTIVACIJA
- AMPLIFIKACIJA C-ERBB



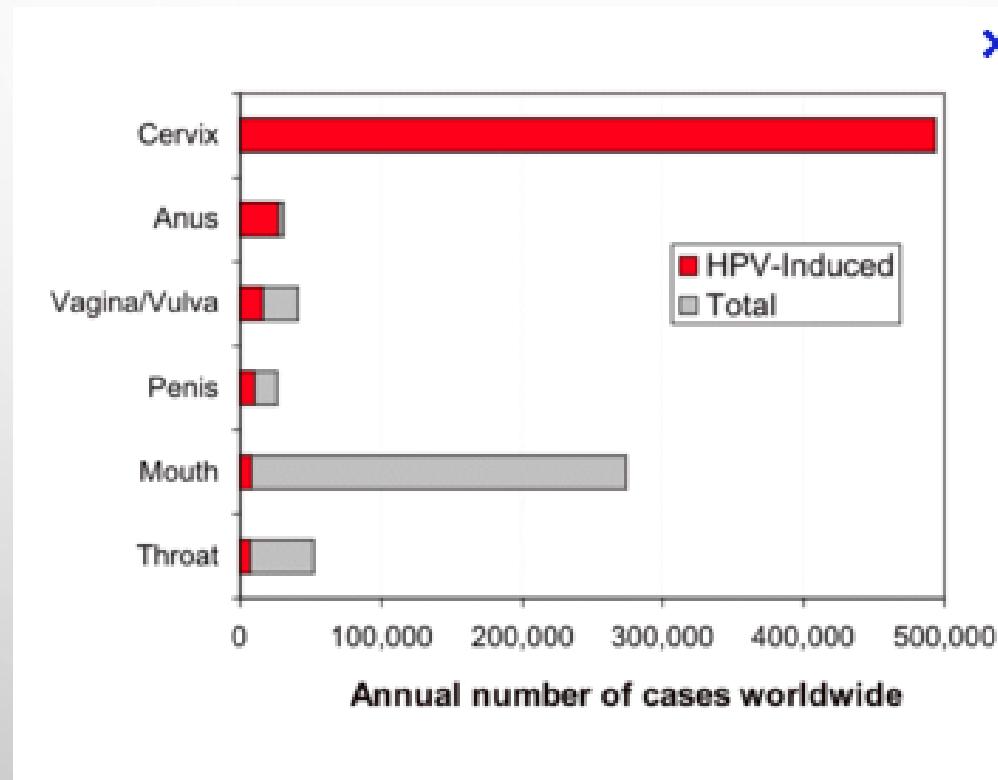
# ERBITUX

- [HTTPS://WWW.YOUTUBE.COM/WATCH?V=HCLJ-TIZX1M](https://www.youtube.com/watch?v=HCLJ-TIZX1M)

# PRIČA 3 - DETEKCIJA INFKECIJE

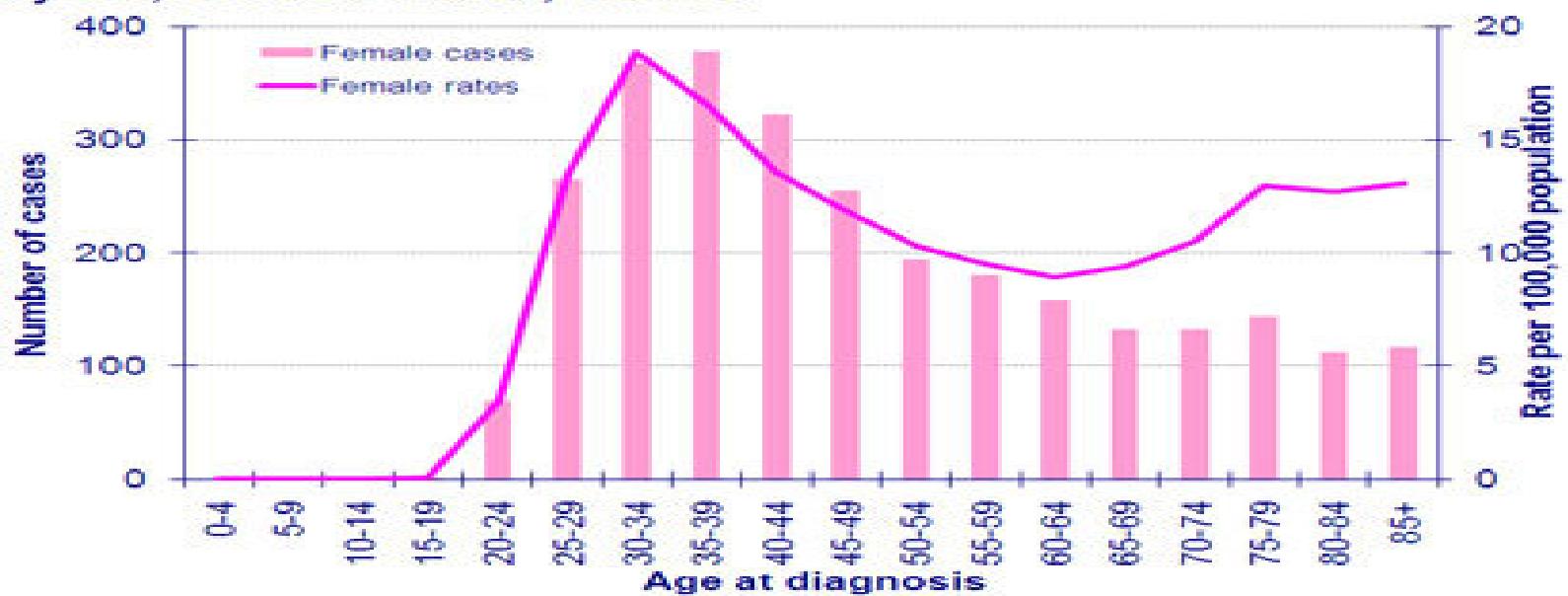


# HUMANI PAPILOMA VIRUS

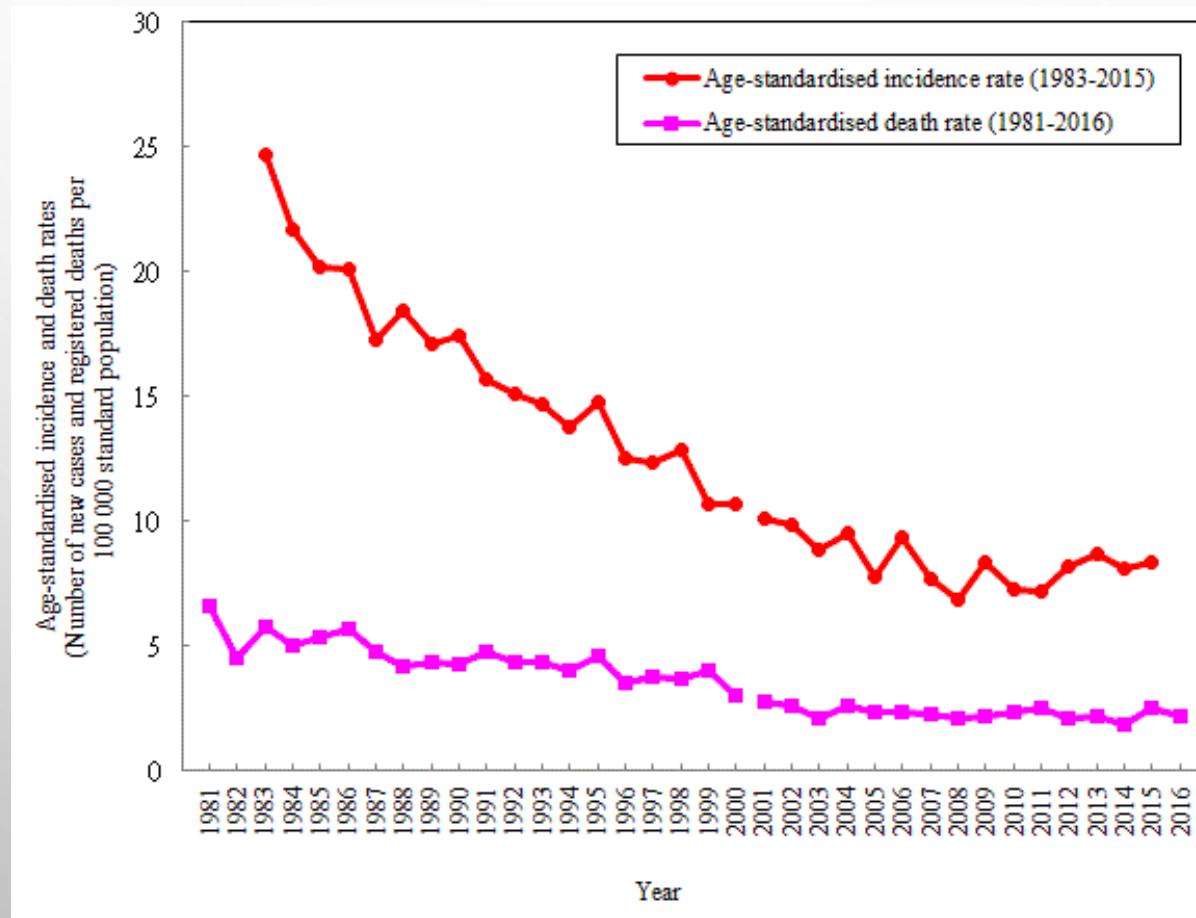


# RAK GRLIĆA MATERNICE

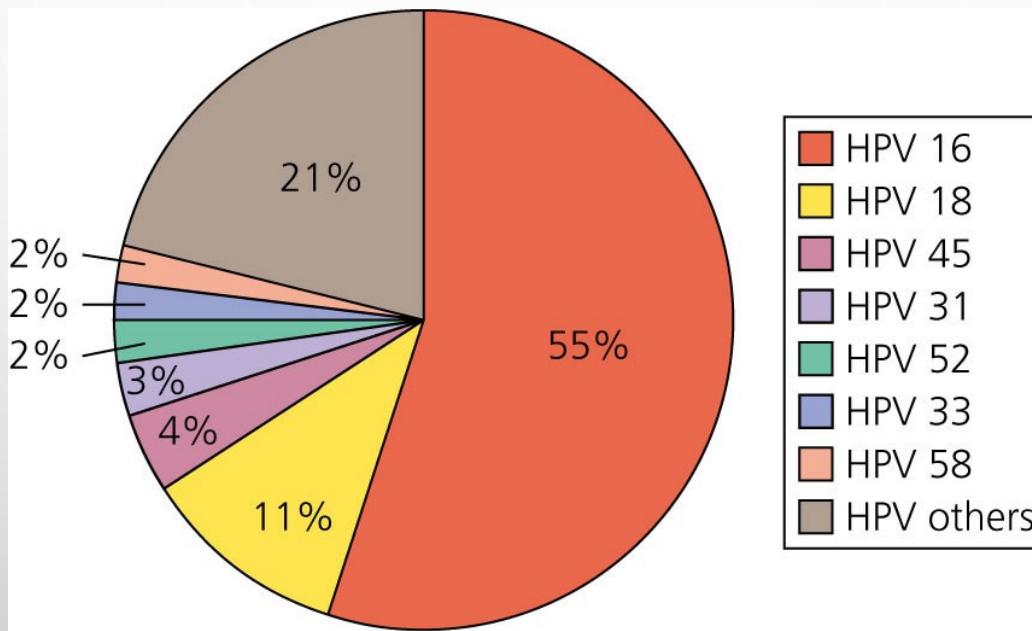
**Figure 1.1: Numbers of new cases and age specific Incidence rate by sex, cervical cancer, UK 2007**



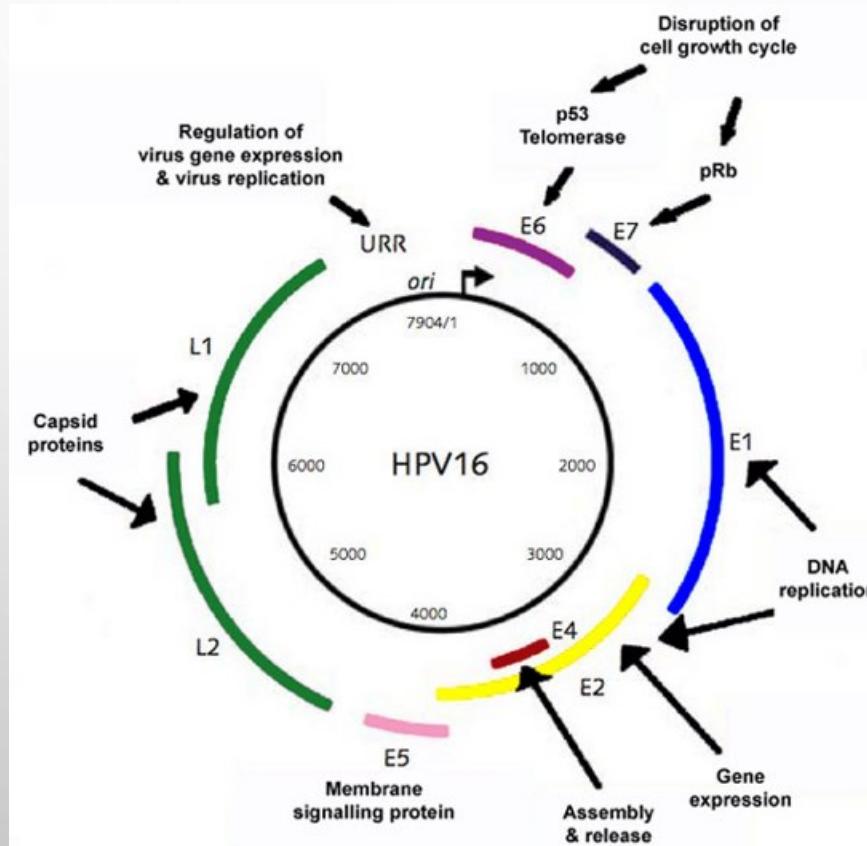
# RAK GRLIĆA MATERNICE

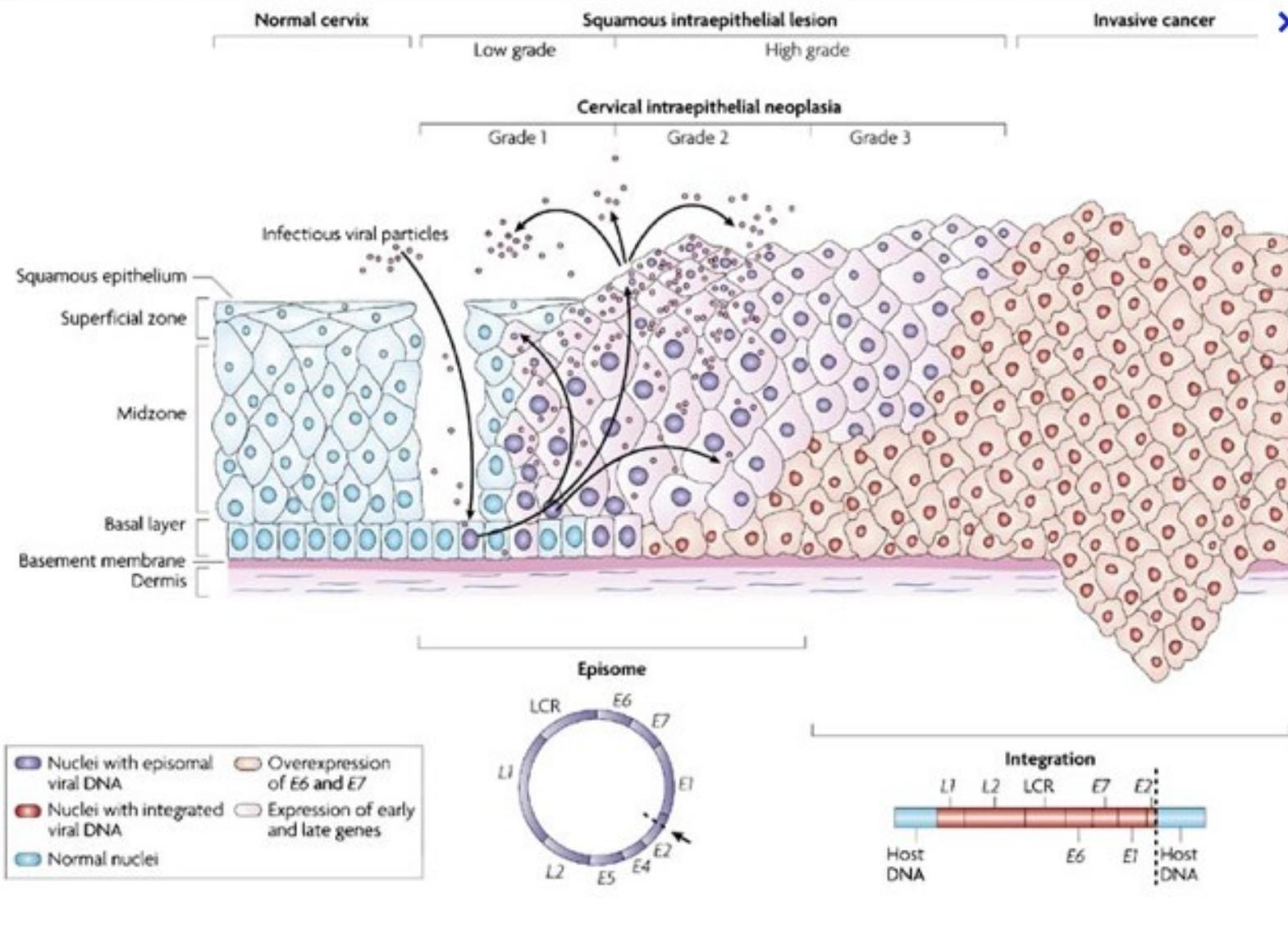


# HPV I RAK GRLIĆA MATERINICE

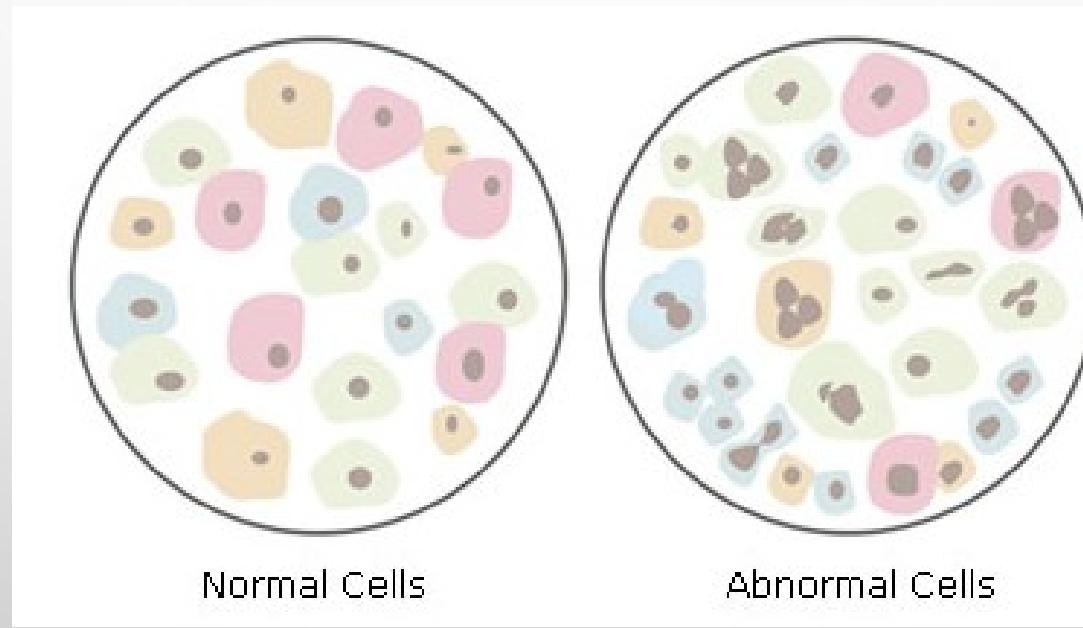


# GENOM HPVA





# PAPA TEST



# PAPA TEST



## HPV INFEKCIJA

- [HTTPS://WWW.YOUTUBE.COM/WATCH?V=UKCHCSXWQBM](https://www.youtube.com/watch?v=UKCHCSXWQBM)

# PRIČA 4 - PRIMJENA DNA ANALIZE U SUDSKOJ MEDICINI

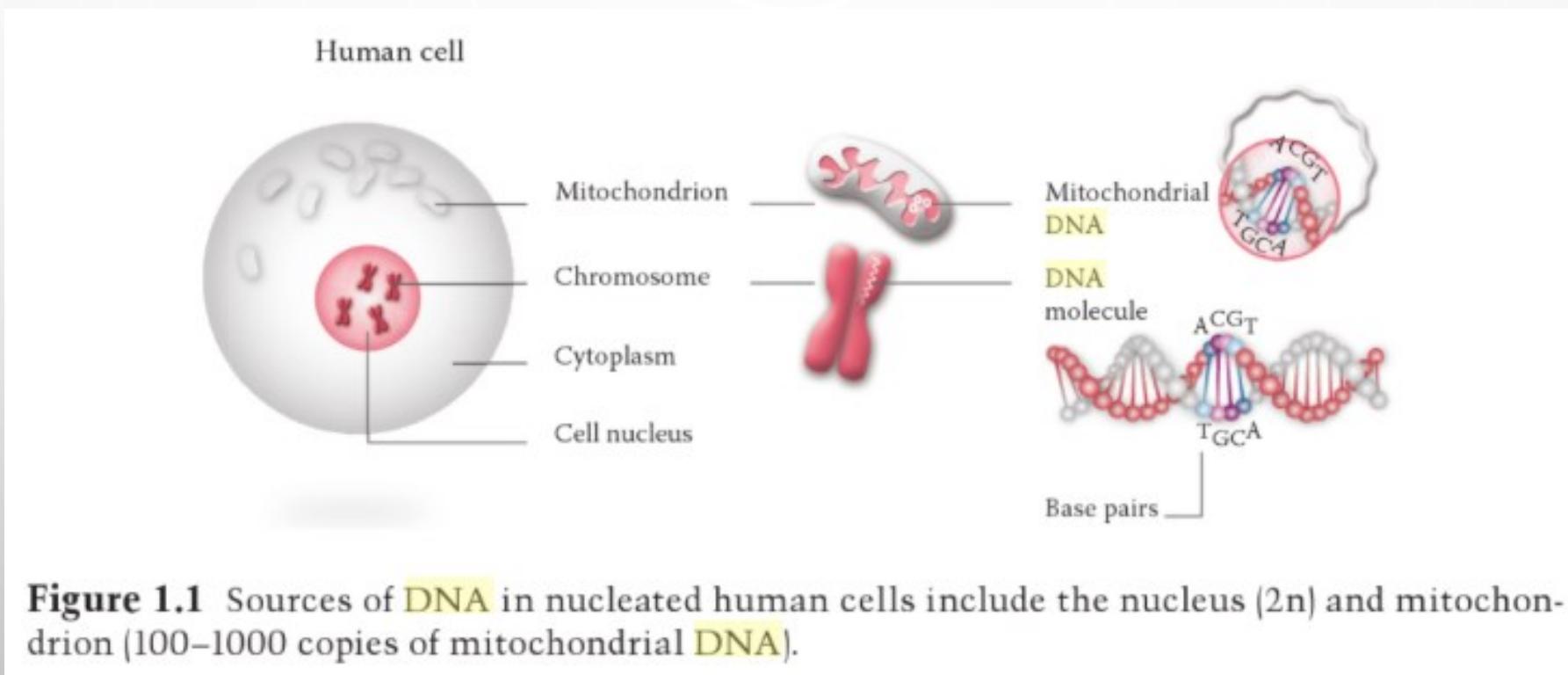
- UTVRĐIVANJE IDENTITETA
- DOKAZIVANJE SRODSTVA



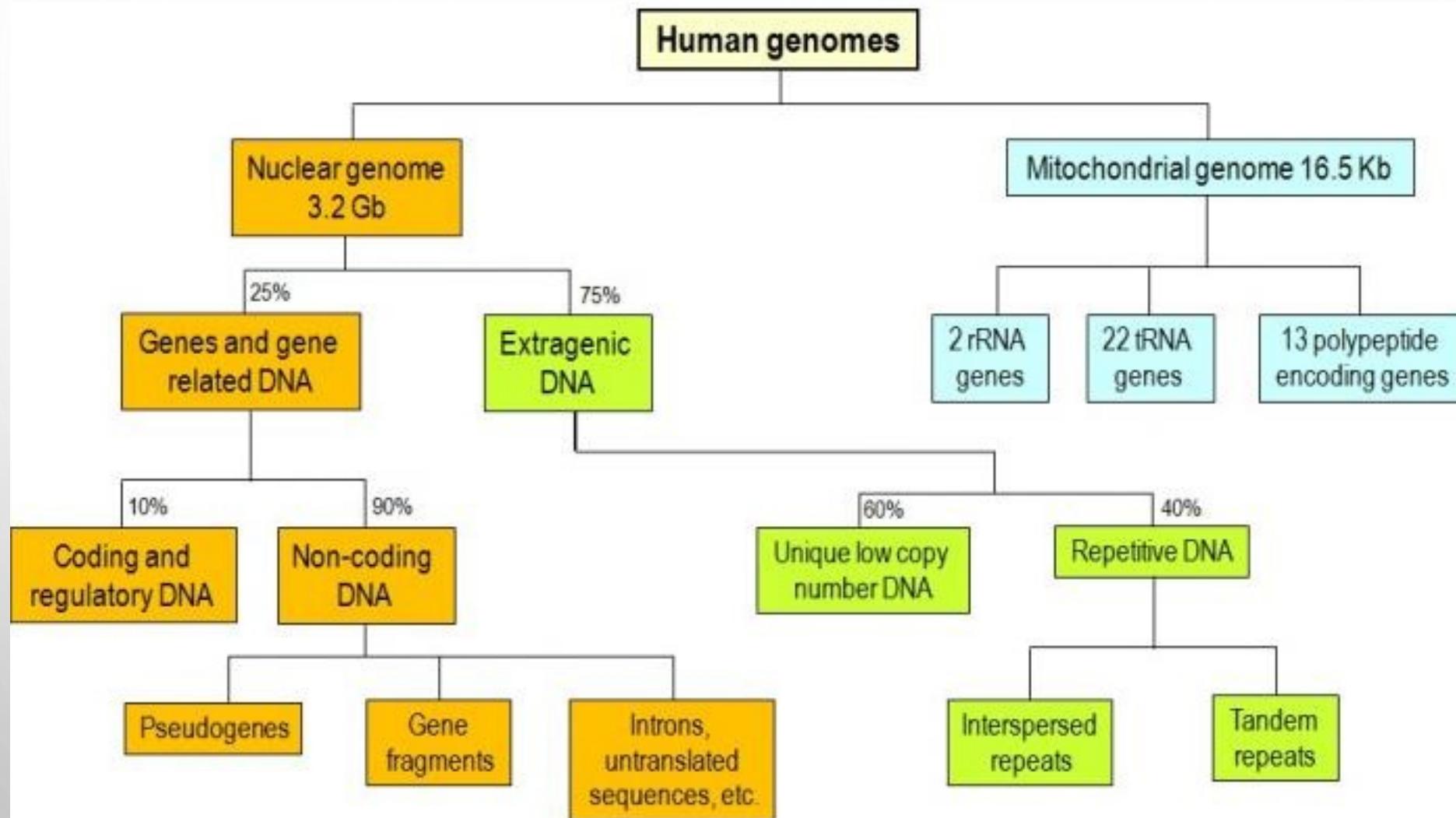
# IZVORI DNA

Biološki	Kemijski	Fizikalni	Ostali
krv	Vlakna	Otisci prstiju	Marka odjeće
sperma	kemikalije	oružje	Analiza glasa
slina	staklo	rukopis	poligraf
Ostale izlučine	zemlja	crtež	fotografije
kosa	barut	otisci	
Biljni tragovi	metal		
kosti	minerali		
tkivo	droga		

# STANIČNA DNA

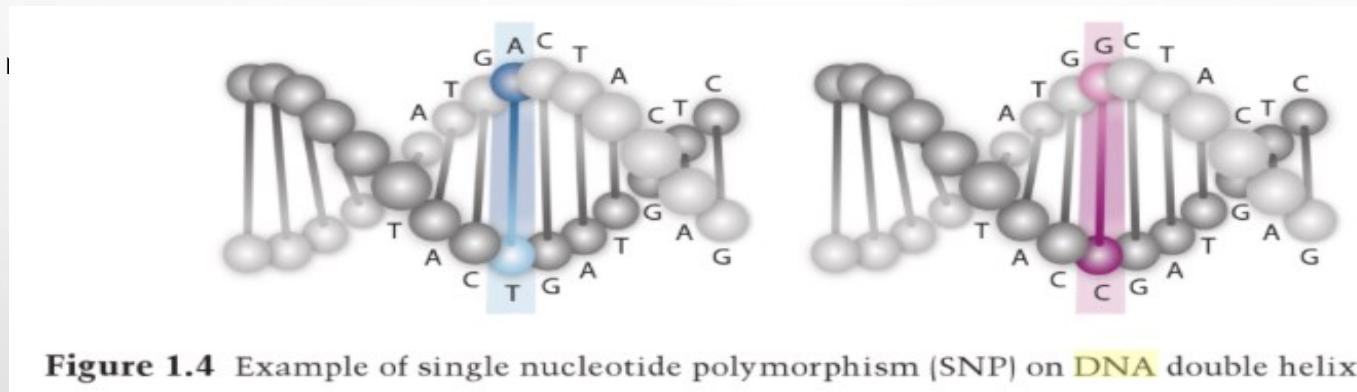


**Figure 1.1** Sources of DNA in nucleated human cells include the nucleus ( $2n$ ) and mitochondrion (100–1000 copies of mitochondrial DNA).



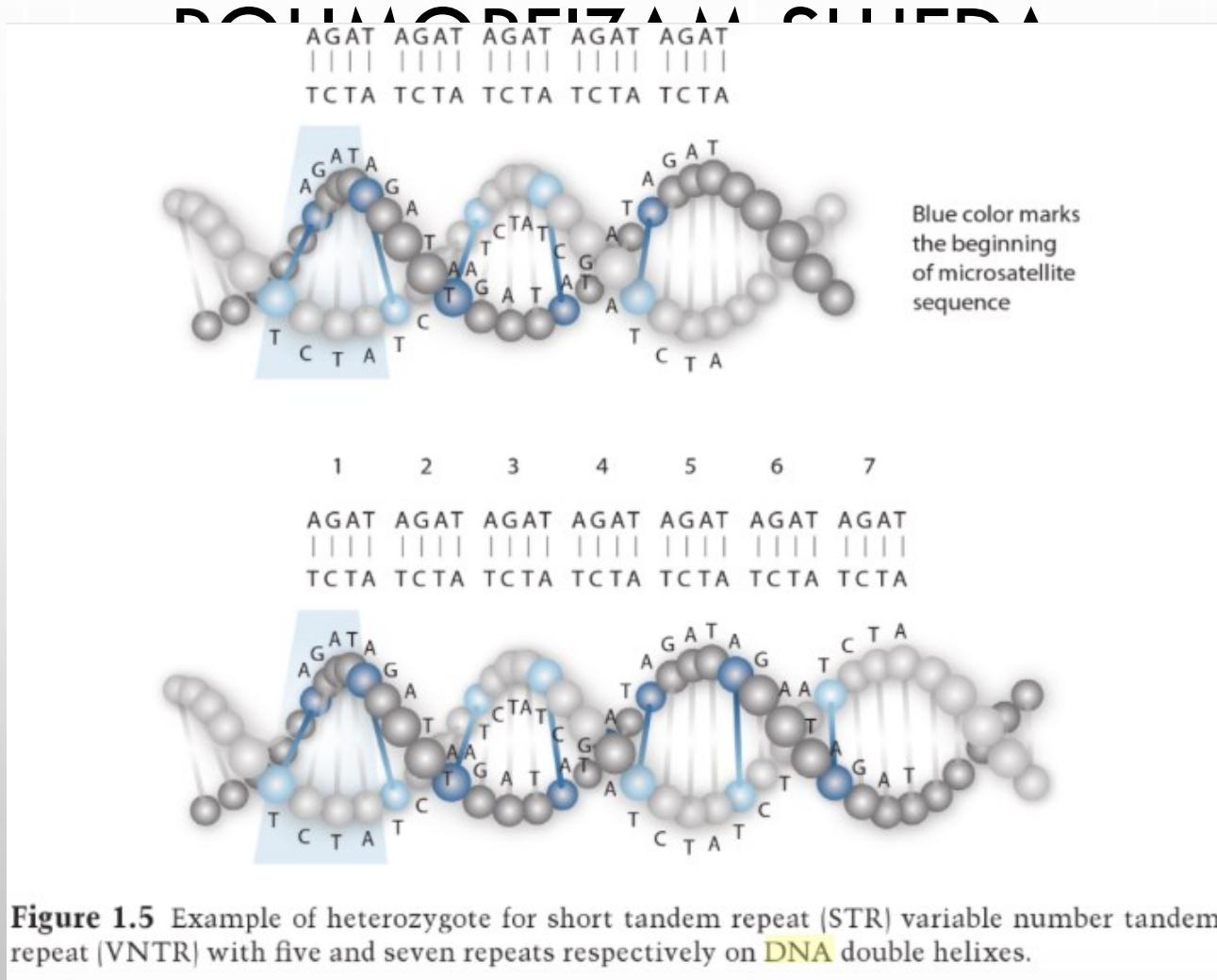
# VARIJACIJE U DNA = POLIMORFIZMI

- RAZLIKE U SLIJEDU I



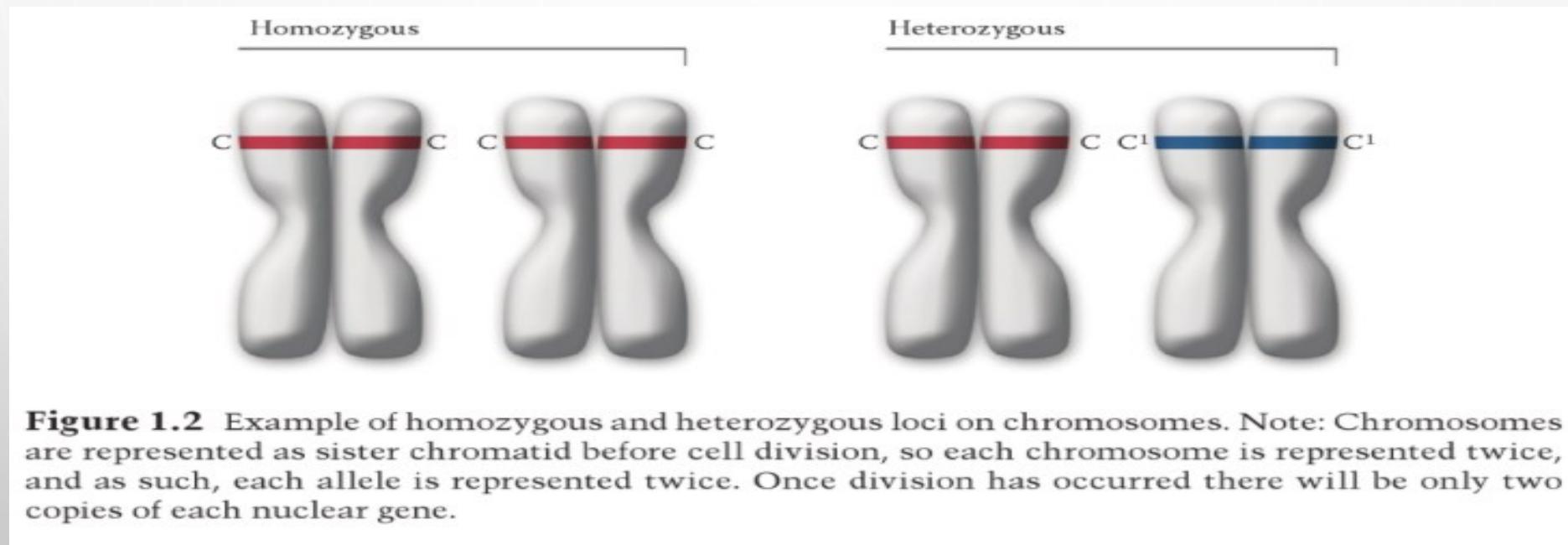
**Figure 1.4** Example of single nucleotide polymorphism (SNP) on DNA double helix.

- INSERCIJE I DELECJI - INDEL
- RAZLIKE U DULJINI NUKLEOTIDNOG SLIJEDA
  - MAKROSATELITI (100-6500BP)
  - MINISATELITI (10-20BP)
  - MIKROSATELITI (2-4BP)



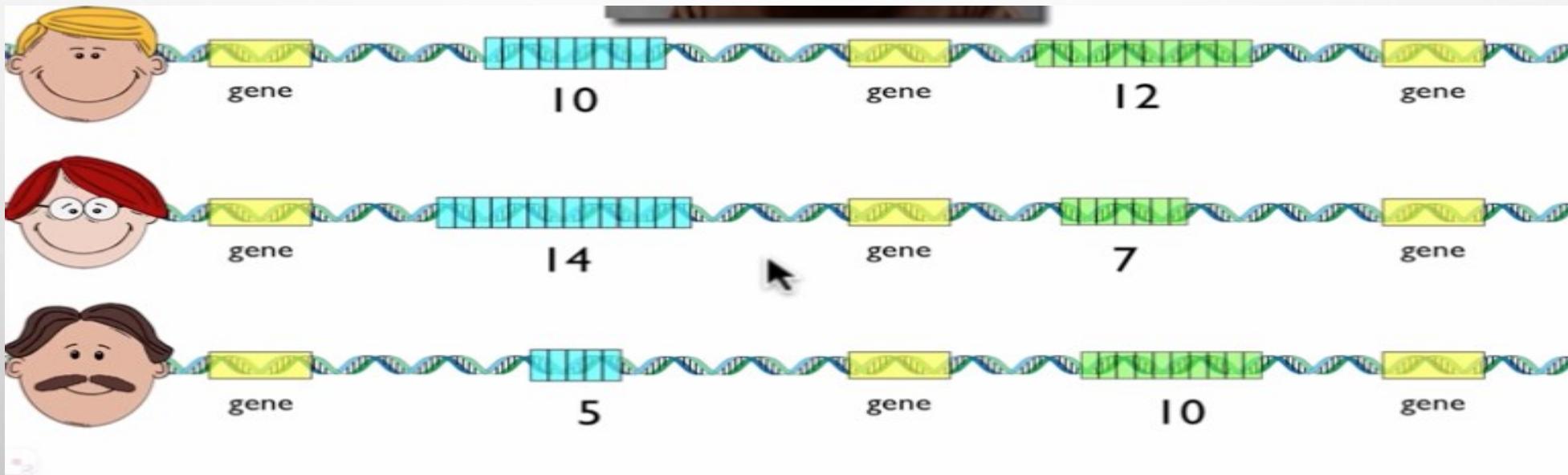
**Figure 1.5** Example of heterozygote for short tandem repeat (STR) variable number tandem repeat (VNTR) with five and seven repeats respectively on **DNA** double helixes.

# HOMOZIGOTNI I HETEROZIGOTNI LOKUSI

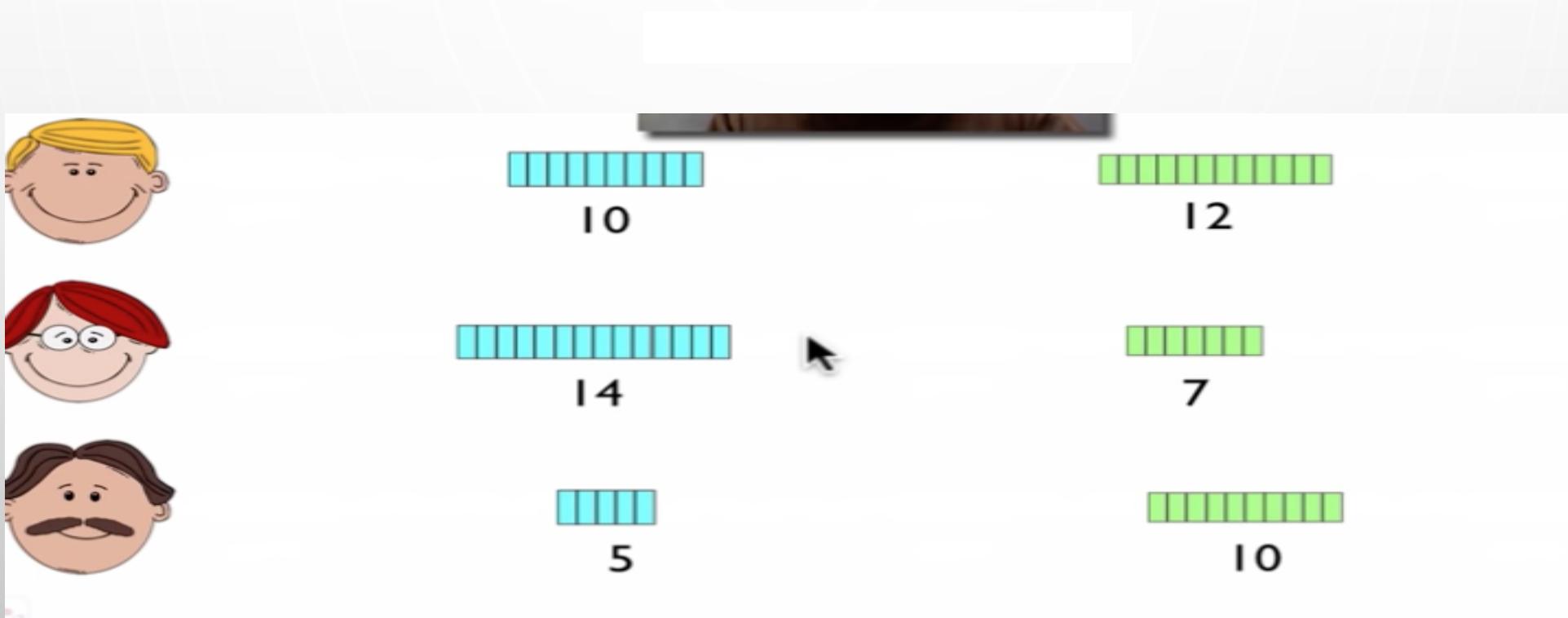


**Figure 1.2** Example of homozygous and heterozygous loci on chromosomes. Note: Chromosomes are represented as sister chromatid before cell division, so each chromosome is represented twice, and as such, each allele is represented twice. Once division has occurred there will be only two copies of each nuclear gene.

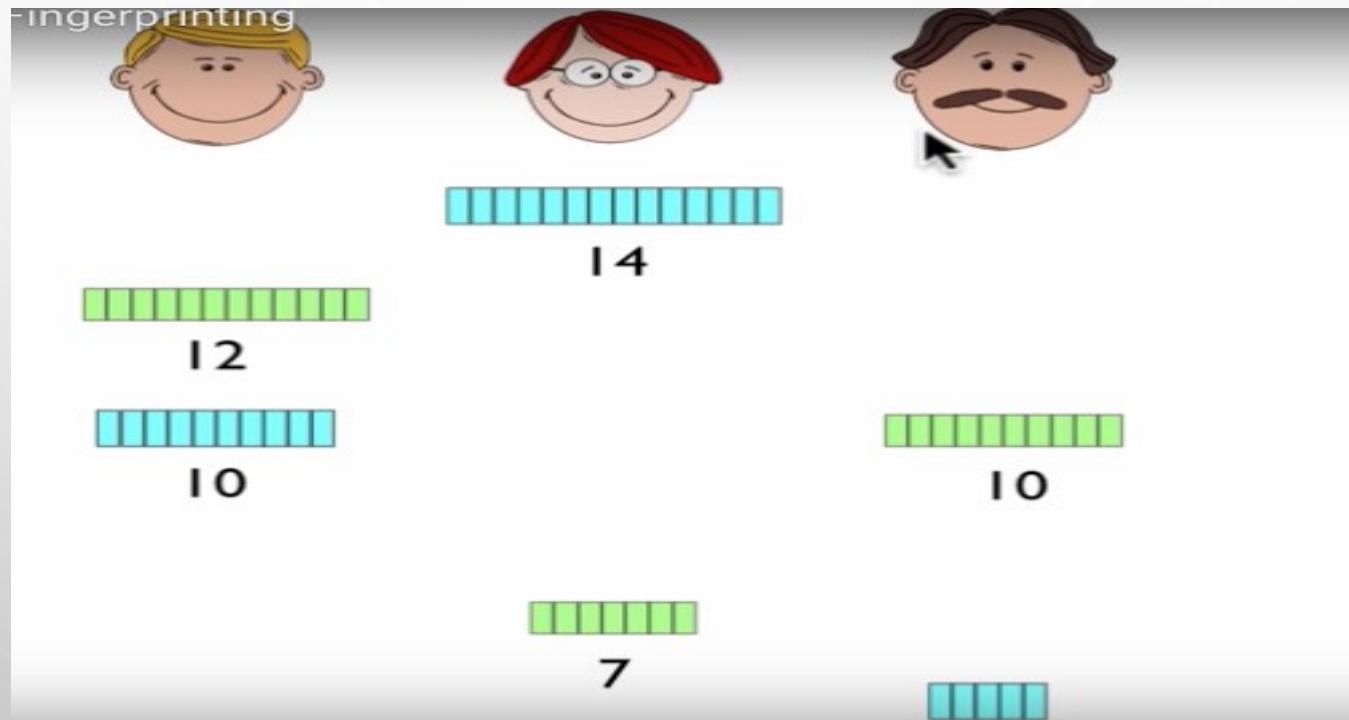
# VNTR (STR)



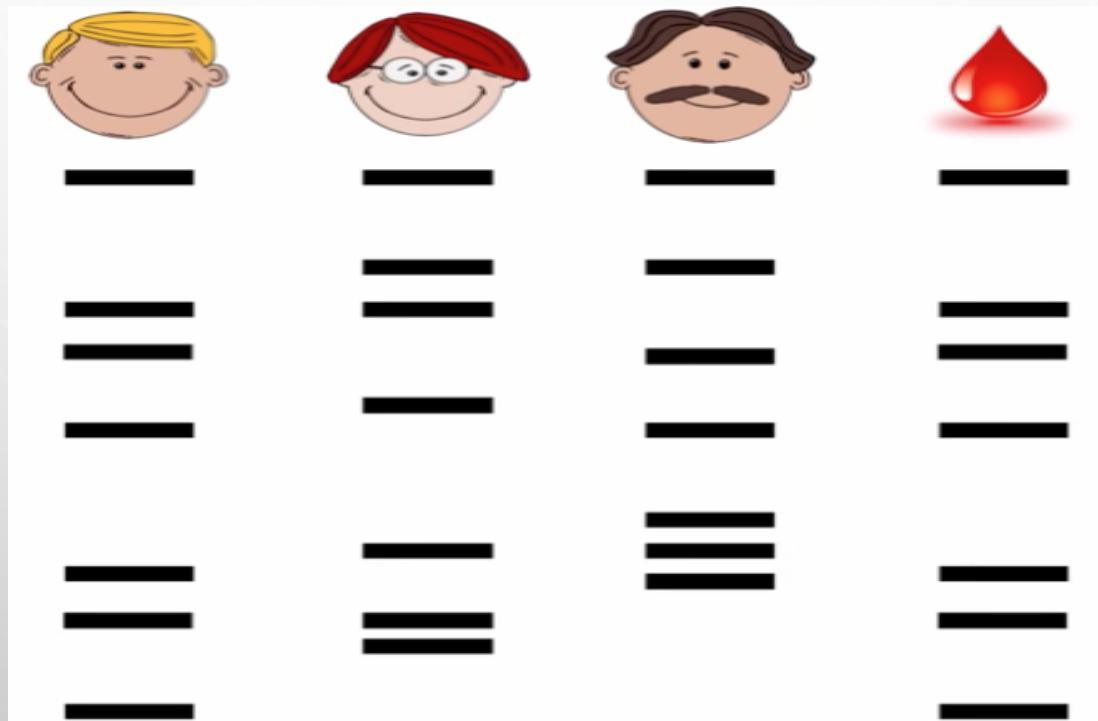
# REZANJE ILI UMNAŽANJE ŽELJENIH FRAGMENATA



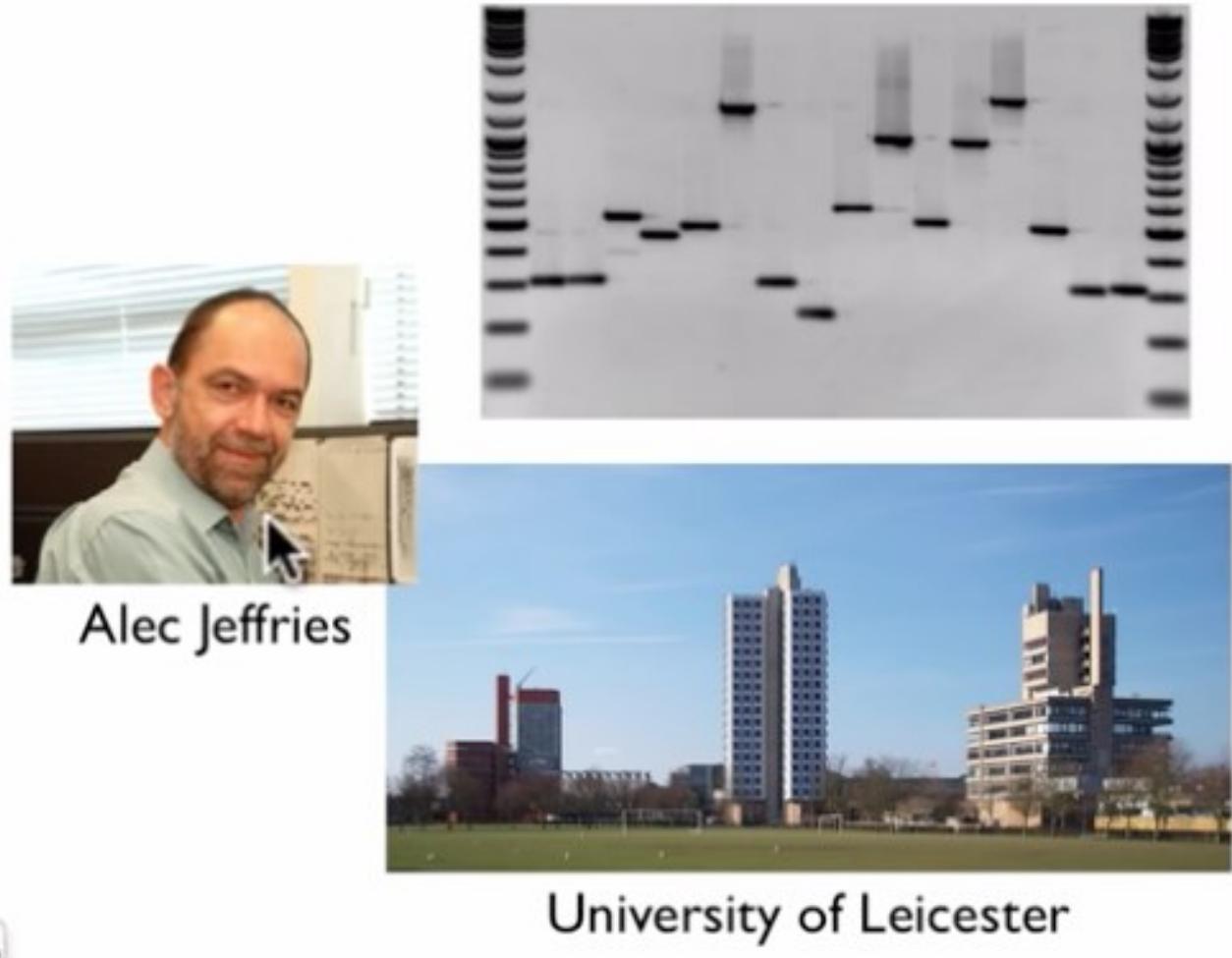
# ELEKTROFOREZA FRAGMENATA



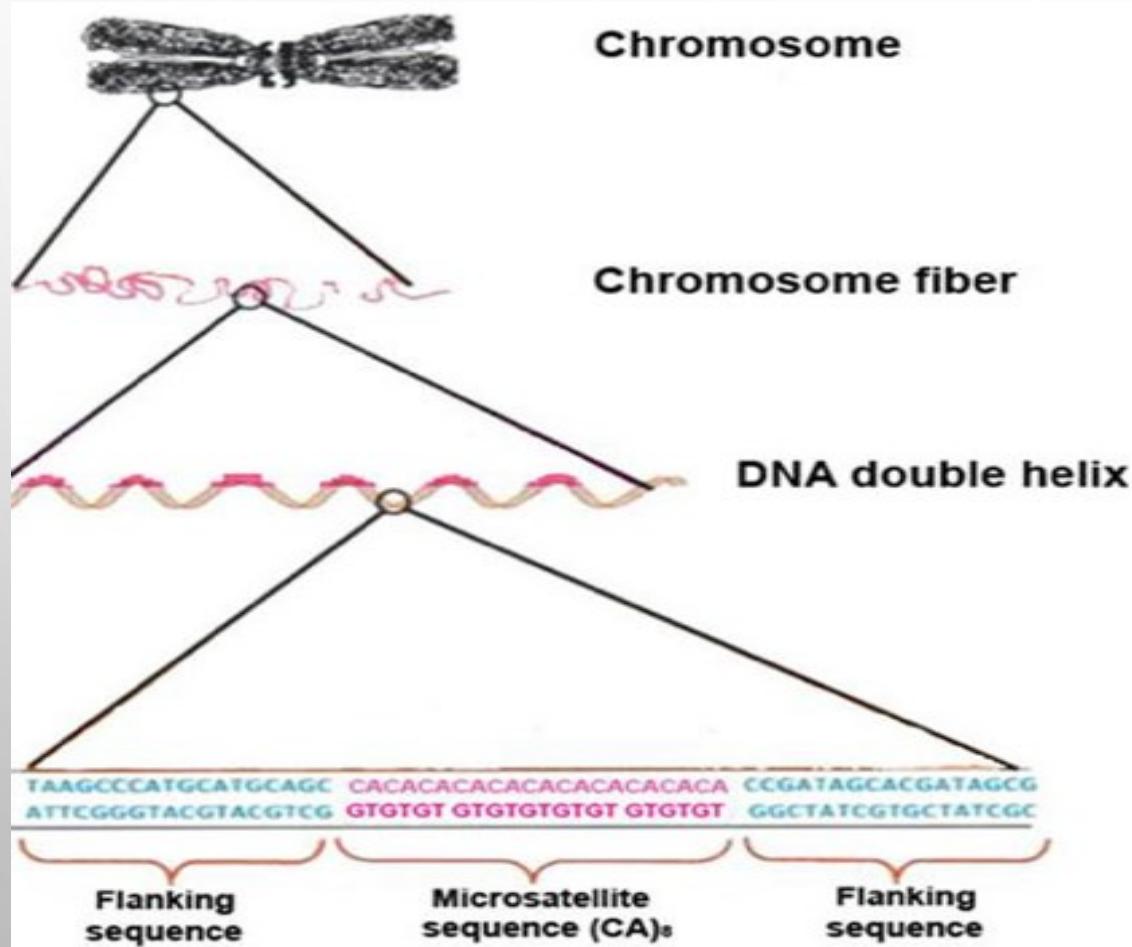
# IDENTIFIKACIJA



# ANALIZA VNTR - 1985



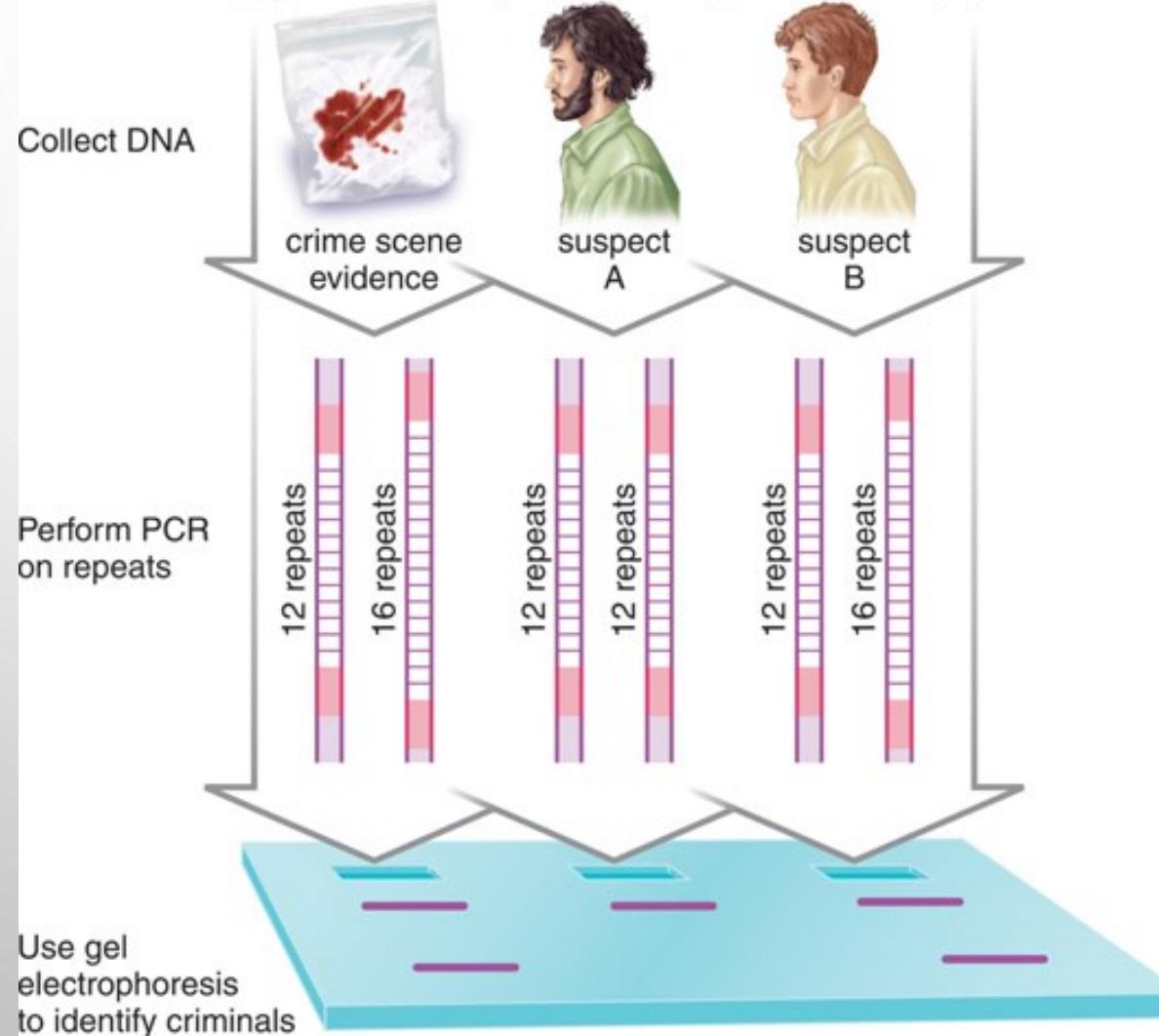
# STR ANALIZA



- VISOKA VARIJABILNOST
- DEFINIRAN BROJ PONAVLJANJA
- DEFINIRANE AELNE VARIJANTE
- POUZDANA AMPLIFIKACIJA

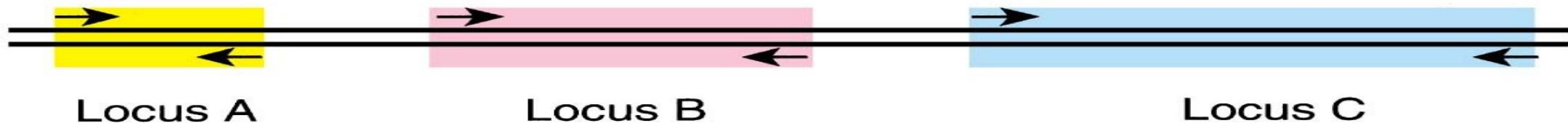
# STR ANALYSIS

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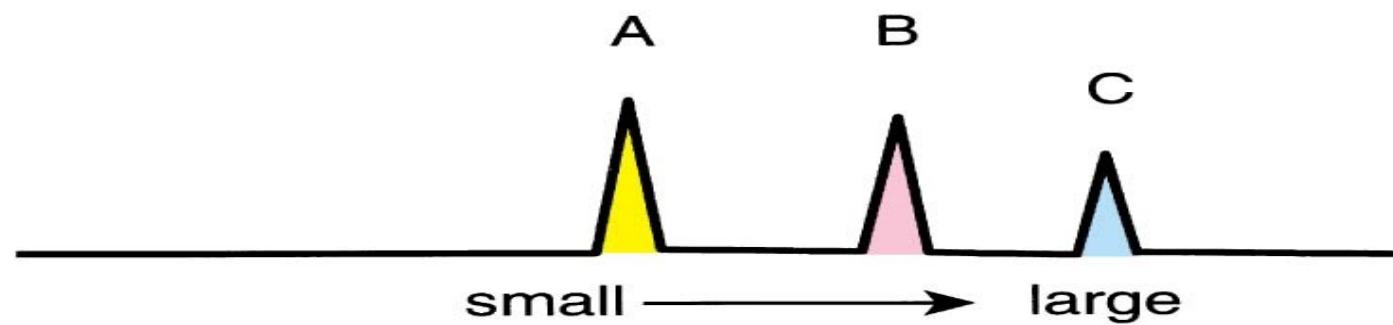


# MULTIPLEX PCR

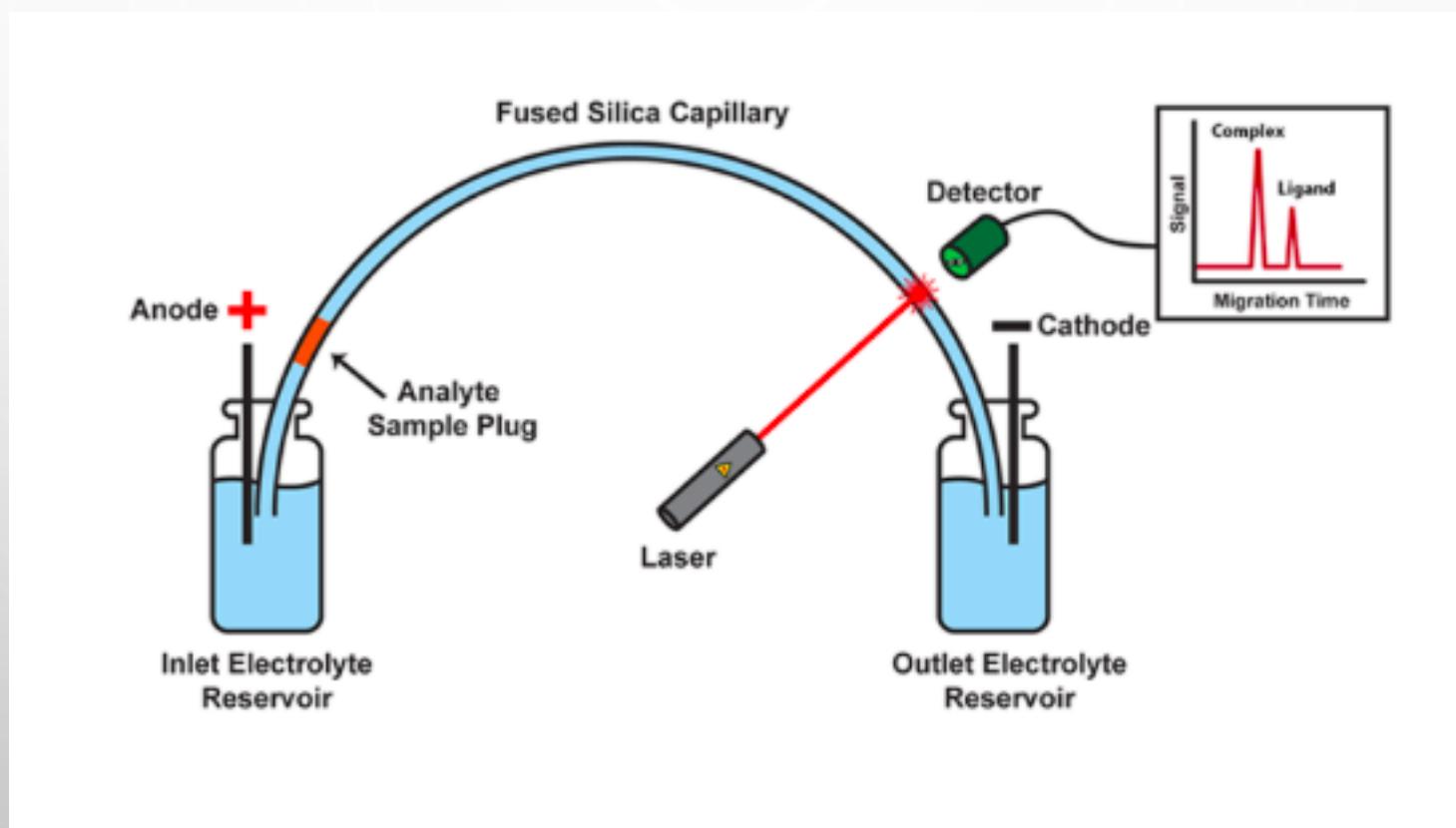
(a)



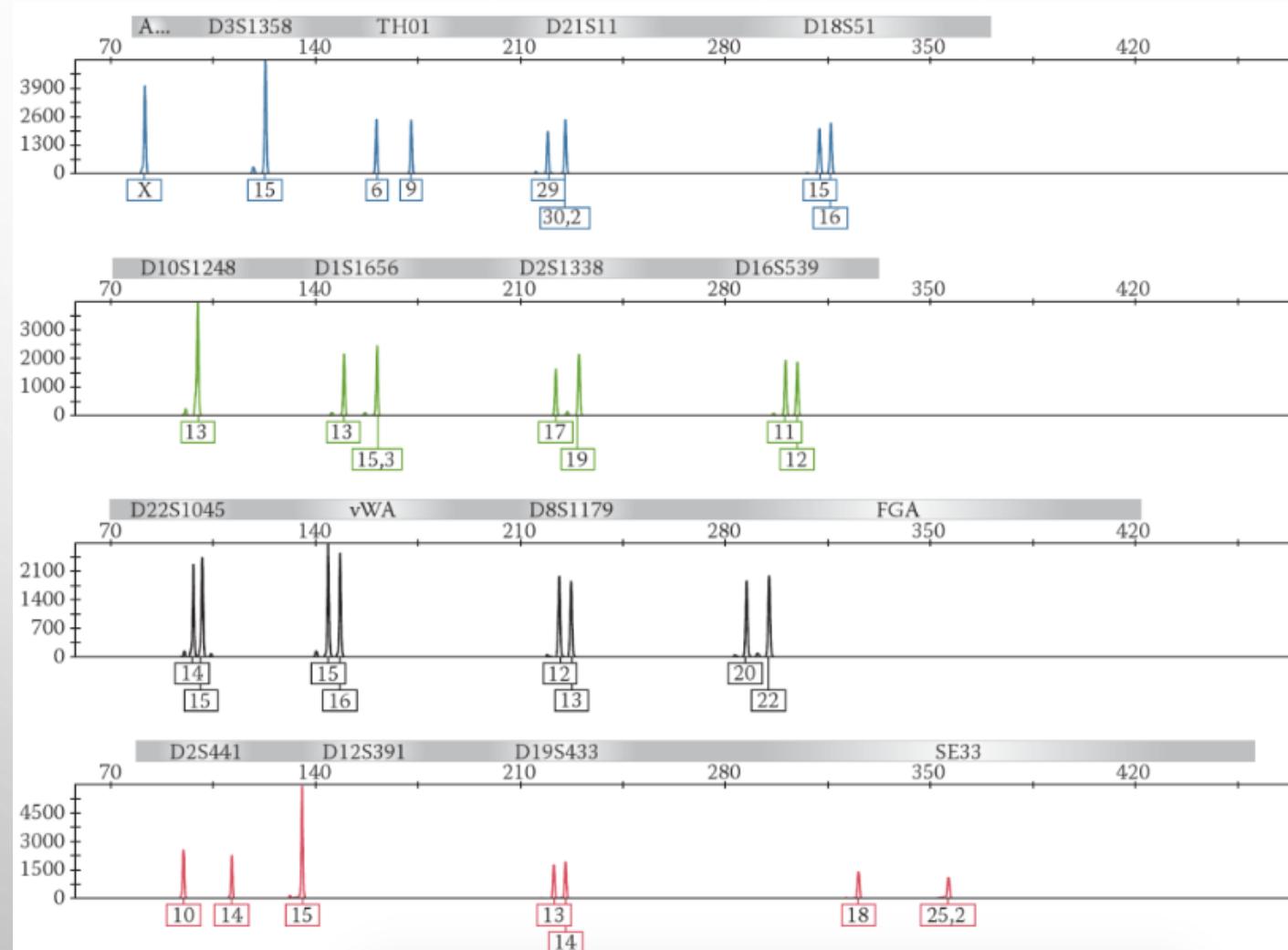
(b)



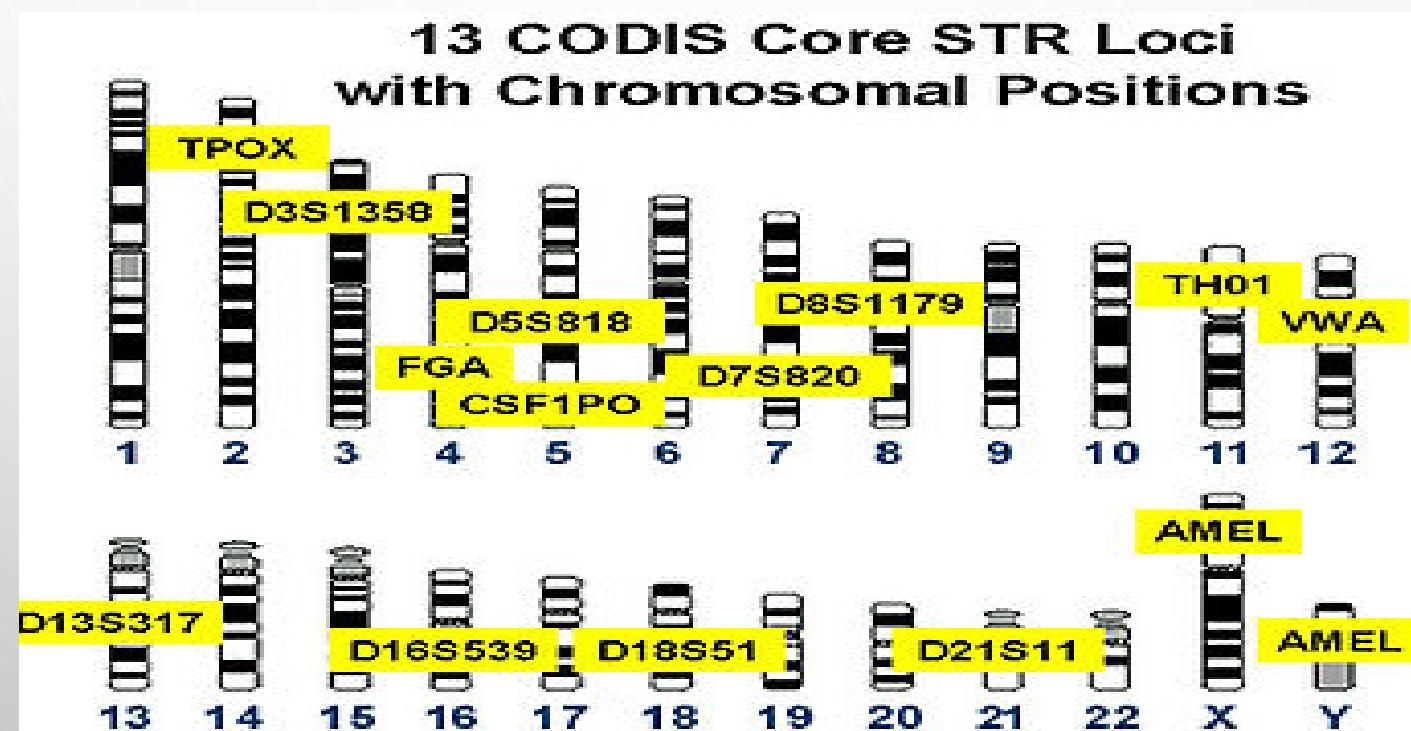
# KAPILARNA ELEKTROFOREZA



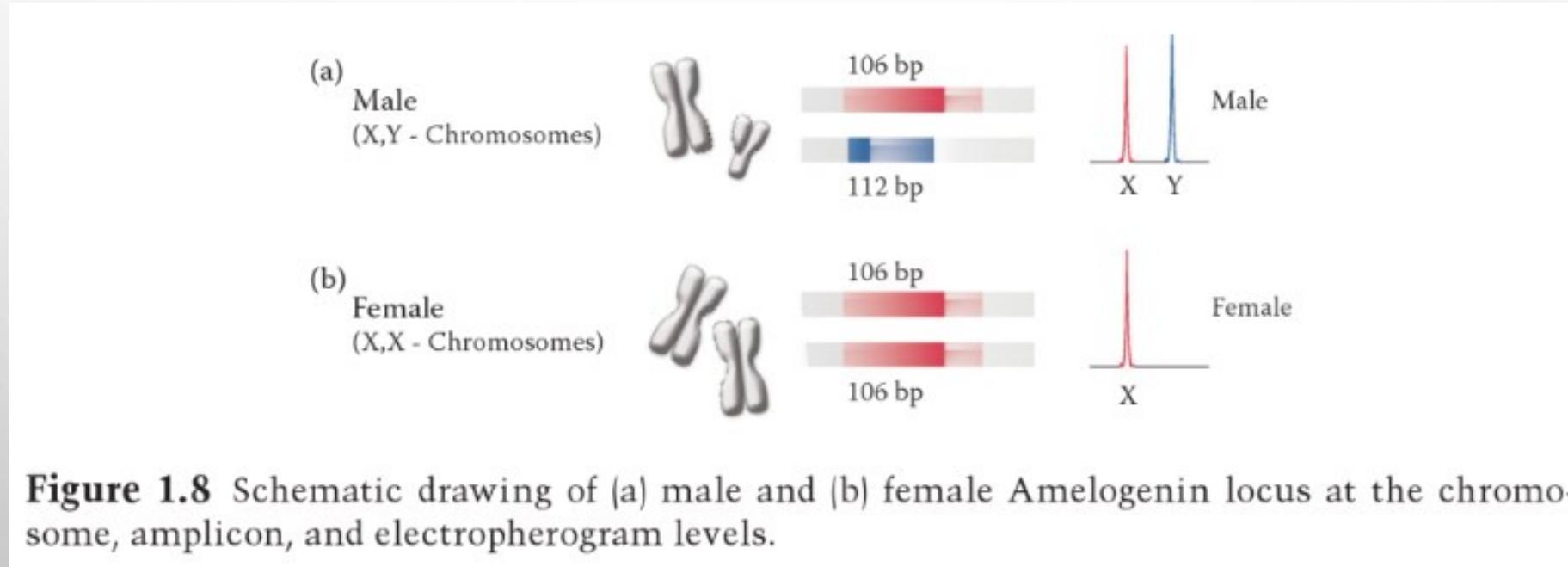
# ELEKTROFEROGRAM



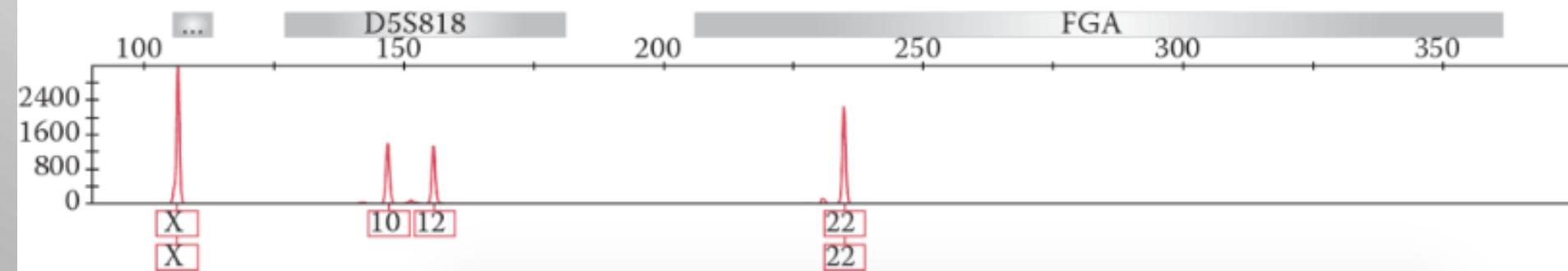
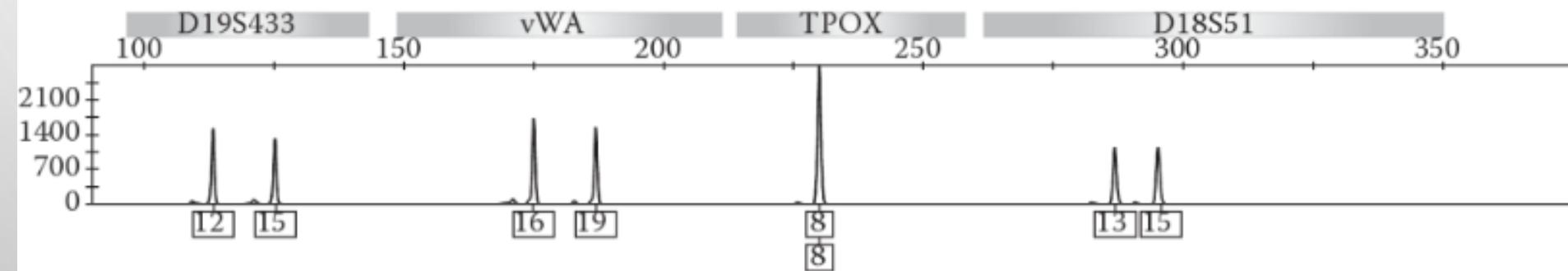
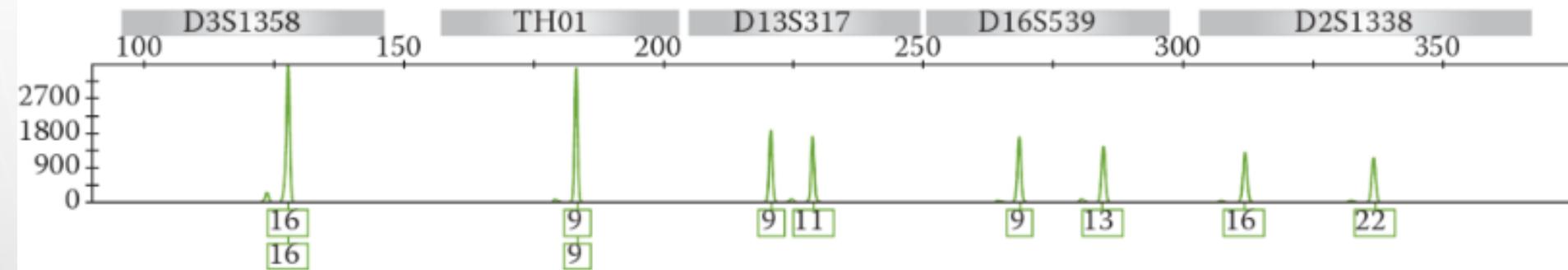
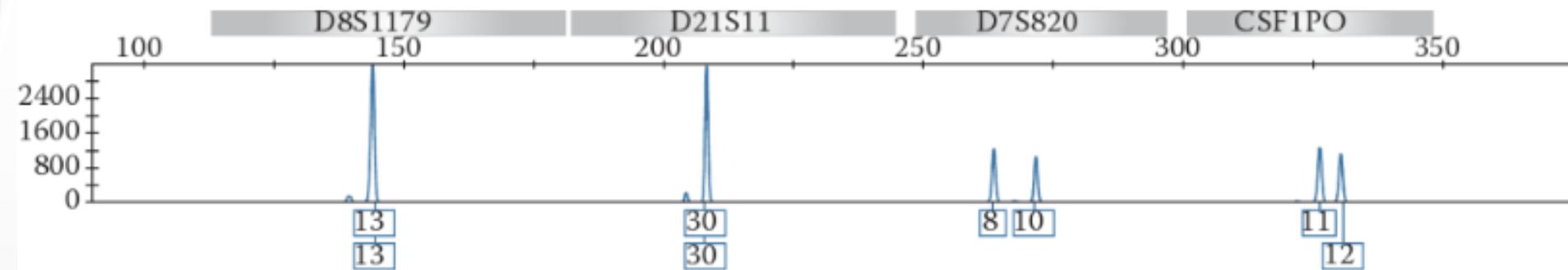
# MULTIPLEX STR ANALIZA



# UTVRĐIVANJE SPOLA – LOKUS ZA AMELOGENIN



**Figure 1.8** Schematic drawing of (a) male and (b) female Amelogenin locus at the chromosome, amplicon, and electropherogram levels.



# NAČELA GENETIKE I STATISTIKE

- NASLJEĐIVANJE PO MENDELU – MONOHIBRIDNA KRIŽANJA
- HARDY-WEINBERGOVA RAVNOTEŽA
- KOLIKA JE VJEROJATNOST POSTOJANJA OSOBE S ISTOM KOMBINACIJOM ALELA?

# VRIJEDNOST PM (RANDOM MATCH PROBABILITY)

Tablica 1-7. Primjer forenzične identifikacije prijepornoga traga krvi analizom 15 STR-lokusa						
STR-LOKUS	Nesporni trag osumnjičene osobe	Sporni trag krvi	p	q	Formula	Učestalost
D3S1358	16, 18	16, 18	0,265	0,155	2pq	0,1352
TH01	6, 9, 3	6, 9, 3	0,225	0,330	2pq	0,1485
D21S11	28, 32, 2	28, 32, 2	0,165	0,085	2pq	0,0281
D18S51	12, 17	12, 17	0,080	0,100	2pq	0,0160
PENTA E	7, 13	7, 13	0,190	0,155	2pq	0,0589
D5S818	12, 12	12, 12	0,340	0,340	$p^2$	0,1156
D13S317	12, 13	12, 13	0,270	0,075	2pq	0,0405
D7S820	10, 11	10, 11	0,290	0,185	2pq	0,1073
D16S539	11, 12	11, 12	0,340	0,280	2pq	0,1904
CSF1P0	11, 12	11, 12	0,245	0,345	2pq	0,1691
PENTA D	9, 9	9, 9	0,245	0,245	$p^2$	0,0600
VWA	16, 19	16, 19	0,205	0,065	2pq	0,0267
D8S1179	12, 15	12, 15	0,165	0,080	2pq	0,0264
TPOX	8, 8	8, 8	0,570	0,570	$p^2$	0,3249
FGA	21, 22	21, 22	0,155	0,190	2pq	0,0589
Amelogenin	XY	XY	////	////	////	////

Zajednička učestalost (ZU)

$6,9598 \times 10^{-18}$

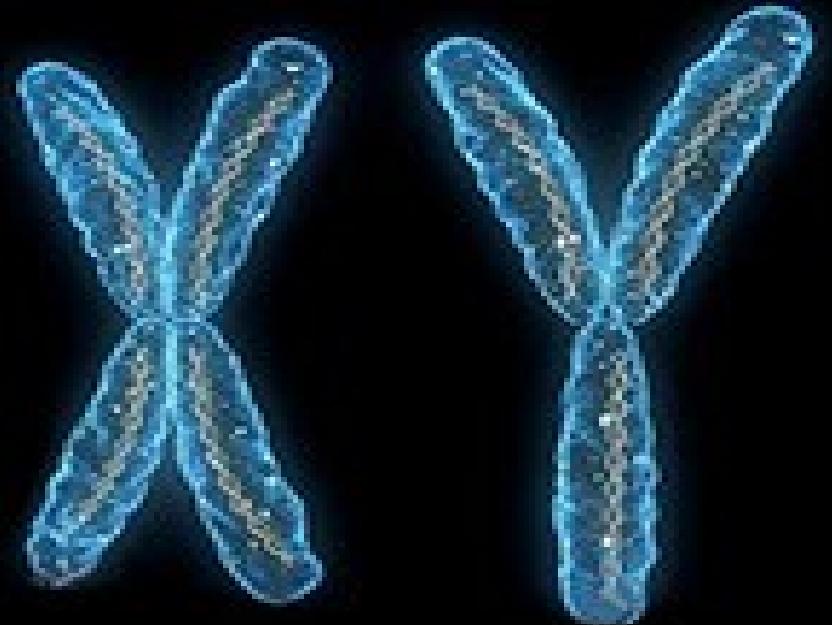
Vjerojatnost (*likelihood*) 1/ZU

143 681 443 246 475 686

# ZADATAK

Locus	Woman	Foetus	Brother	Father	Paternal grandfather	Maternal grandfather
D21S11	28,29	29,30	30	29,30	27,29	28,31.2
D7S820	10	10	10,12	8,10	8	10,12
CSF1PO	11,12	11	10,11	11	11	10,12
D3S1358	15	15	15	15	15,16	16,18
TH01	8,9	8	8,9	9	6,9	6,8
D13S317	11,12	12	12,13	11,13	9,11	12
D16S539	9,11	9,14	9,14	11,14	11	9,12
D2S1338	17,20	17,18	18,20	20	20,24	18,20
D19S433	16,16.2	14,16.2	14,16.2	14,16	12,14	13,15
vWA	14,17	17	17,18	14,18	14,17	17,19
TPOX	8,9	8,11	8,11	8,9	9,10	8,11

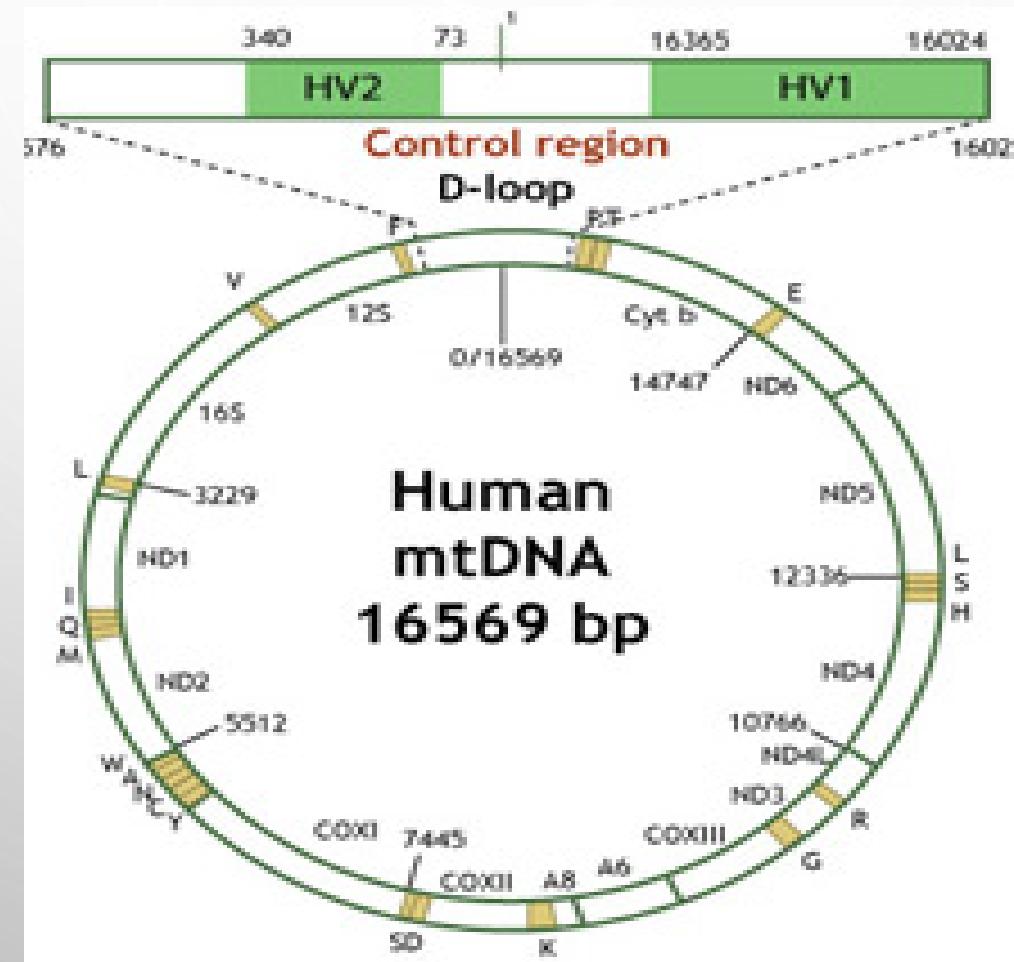
# Y-STR ANALIZA



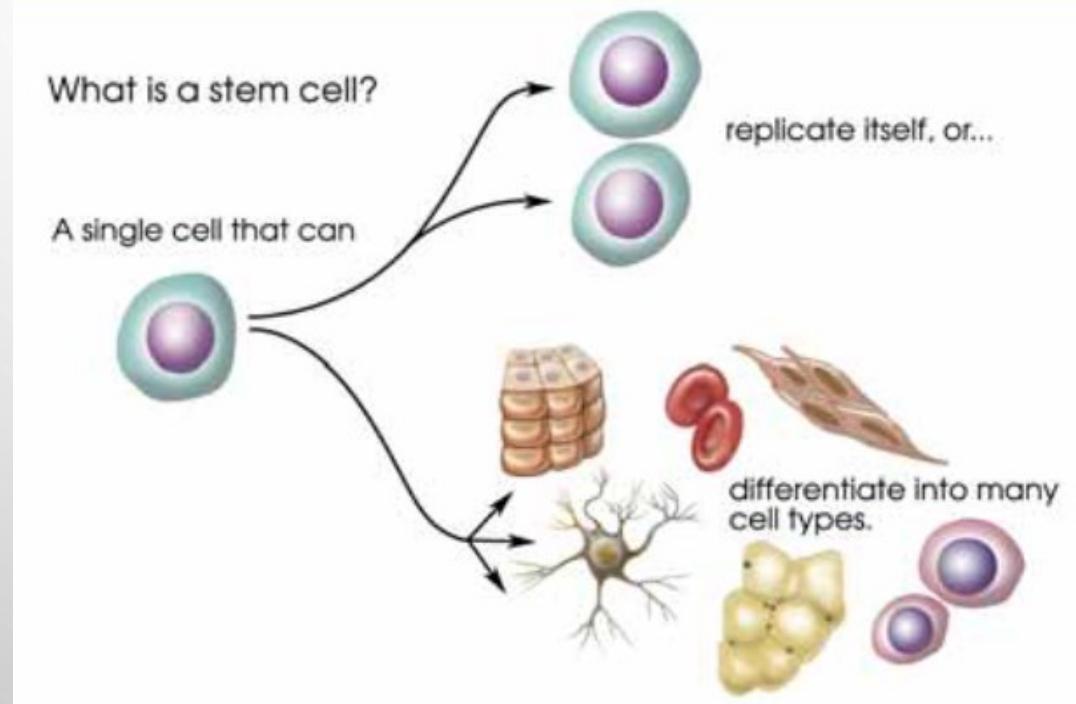
investigating sexual assault cases  
**why use Y?**

- SILOVANJA
- DOKAZIVANJE RODITELJSTVA
- MIGRACIJE

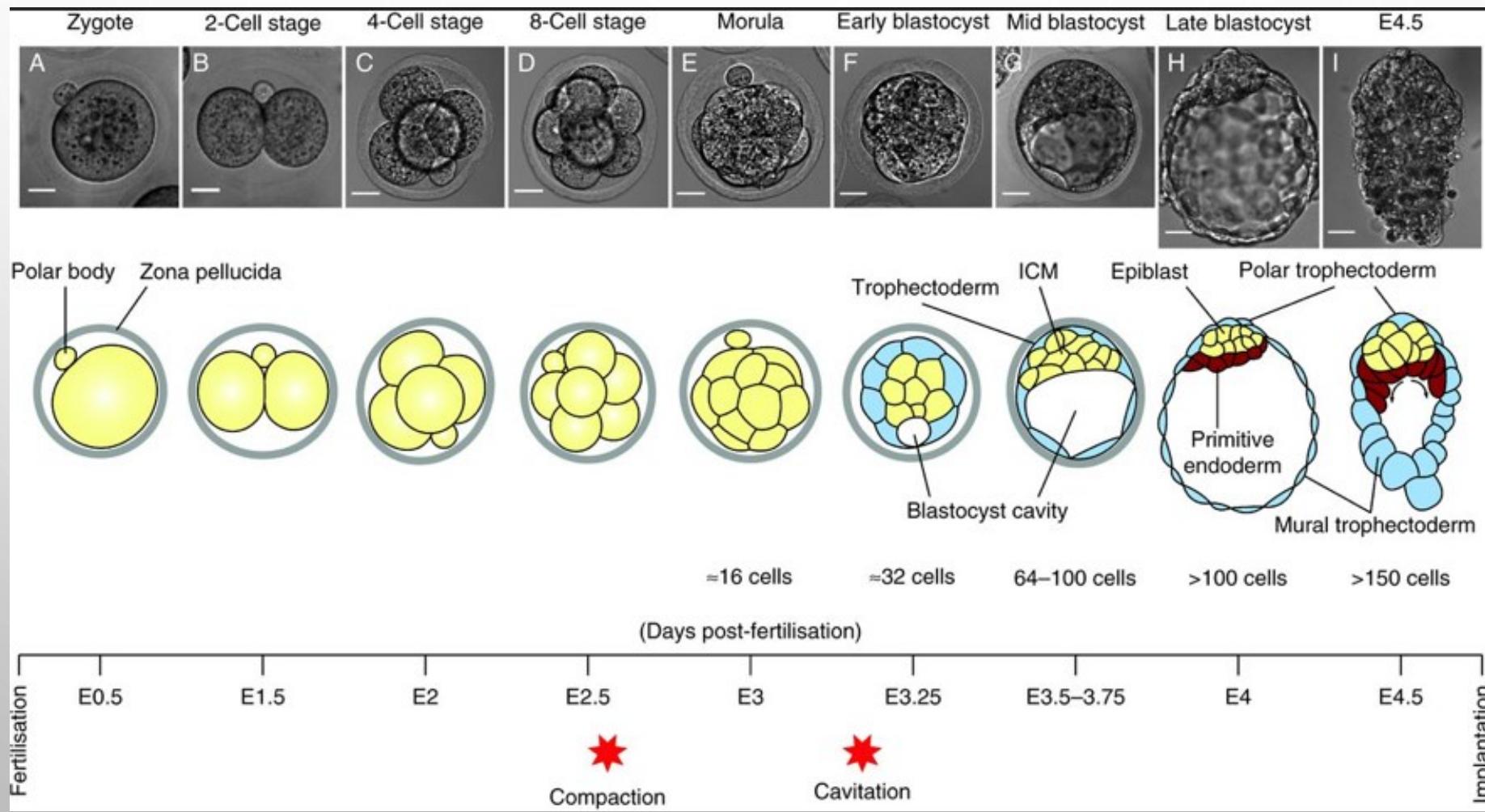
# ANALIZA MTDNA



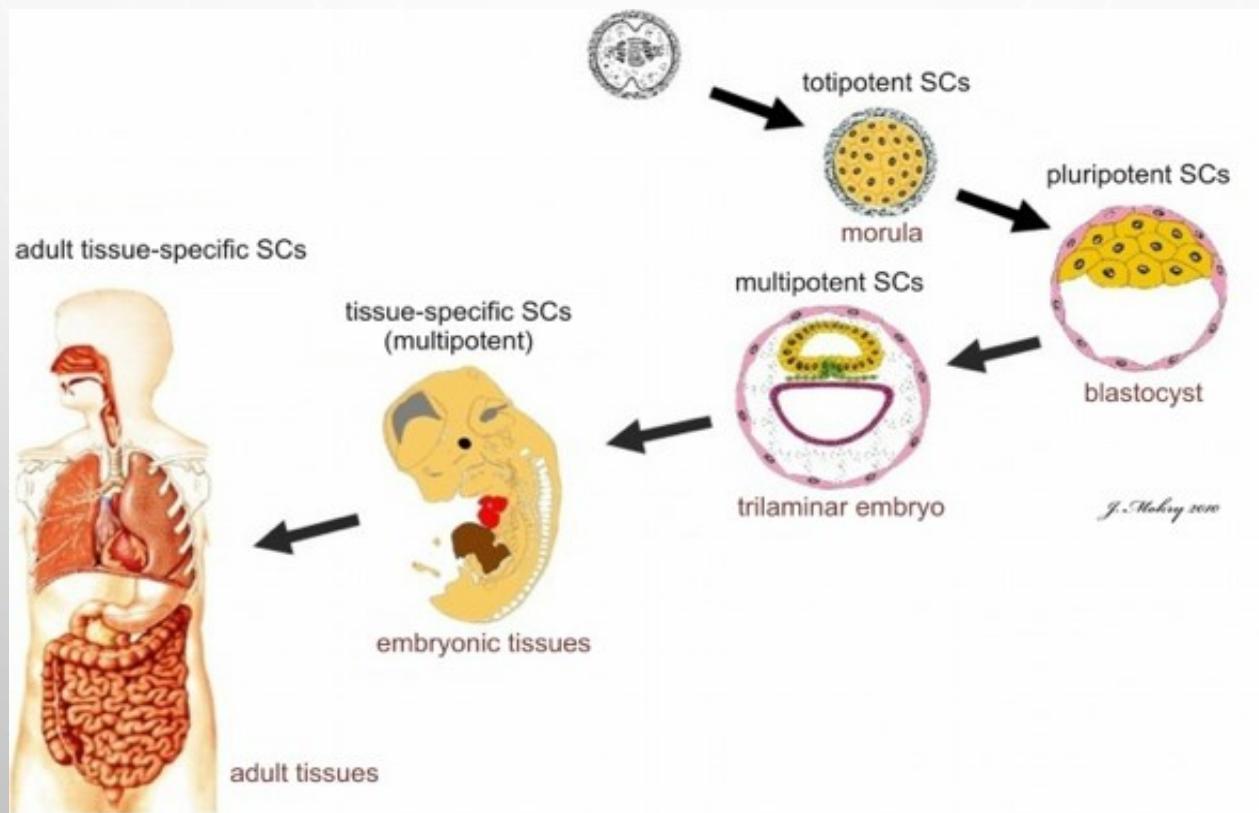
# PRIČA 5 - MATIČNE STANICE



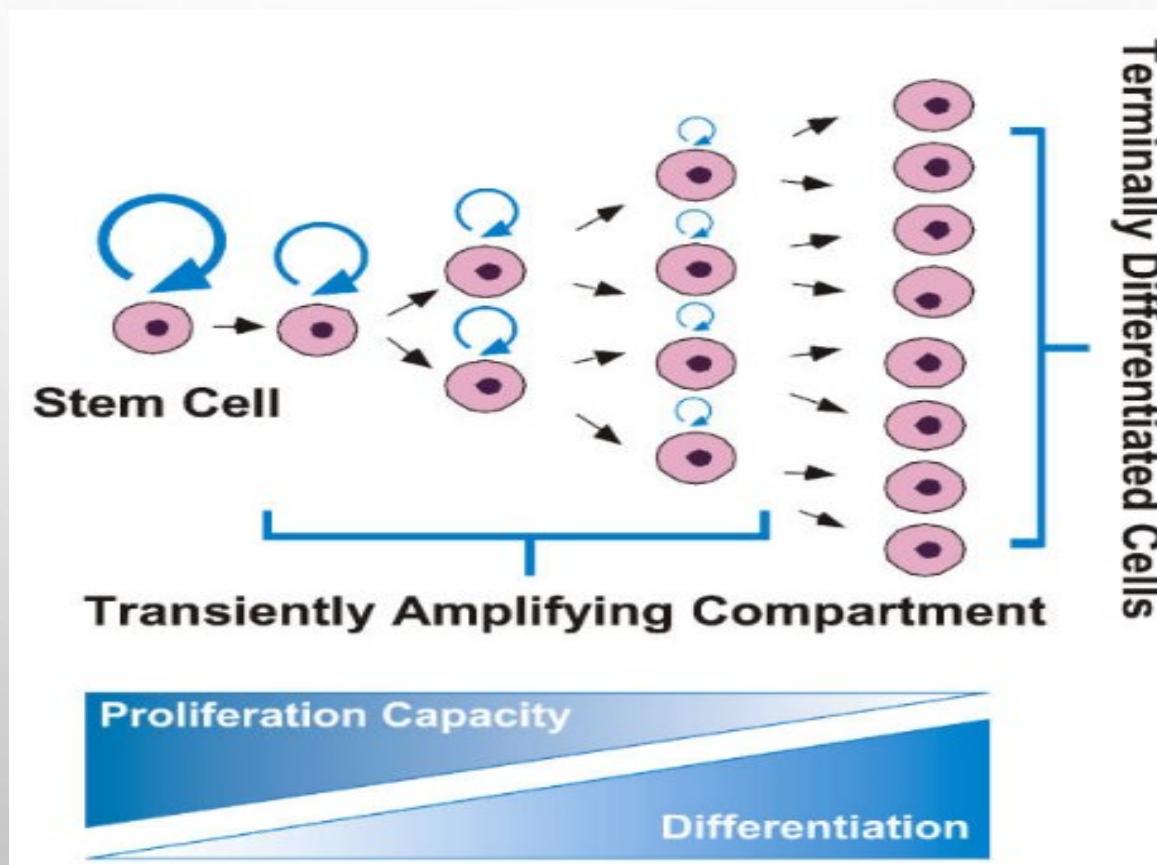
# PREIMPLANTATION DEVELOPMENT



# STEM CELLS DURING DEVELOPMENT



# TISSUE COMPARTMENT

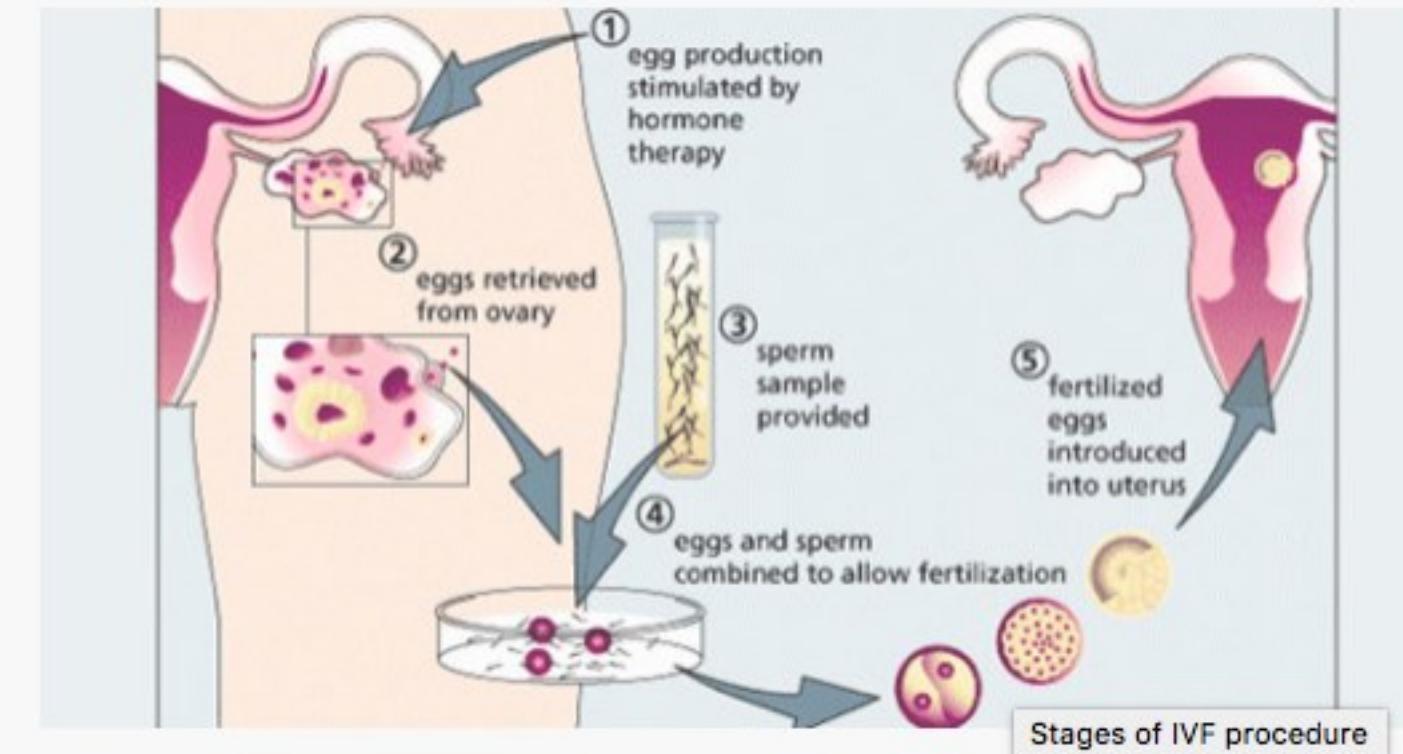


# STEM CELL POTENCY

Type of Cell	Potential
Zygote	Totipotent
Embryonic Stem Cell	Pluripotent
Adult Stem Cell	Multipotent
Progenitor	Unipotent
Differentiated	No further differentiation potential

 = Self-Renewing

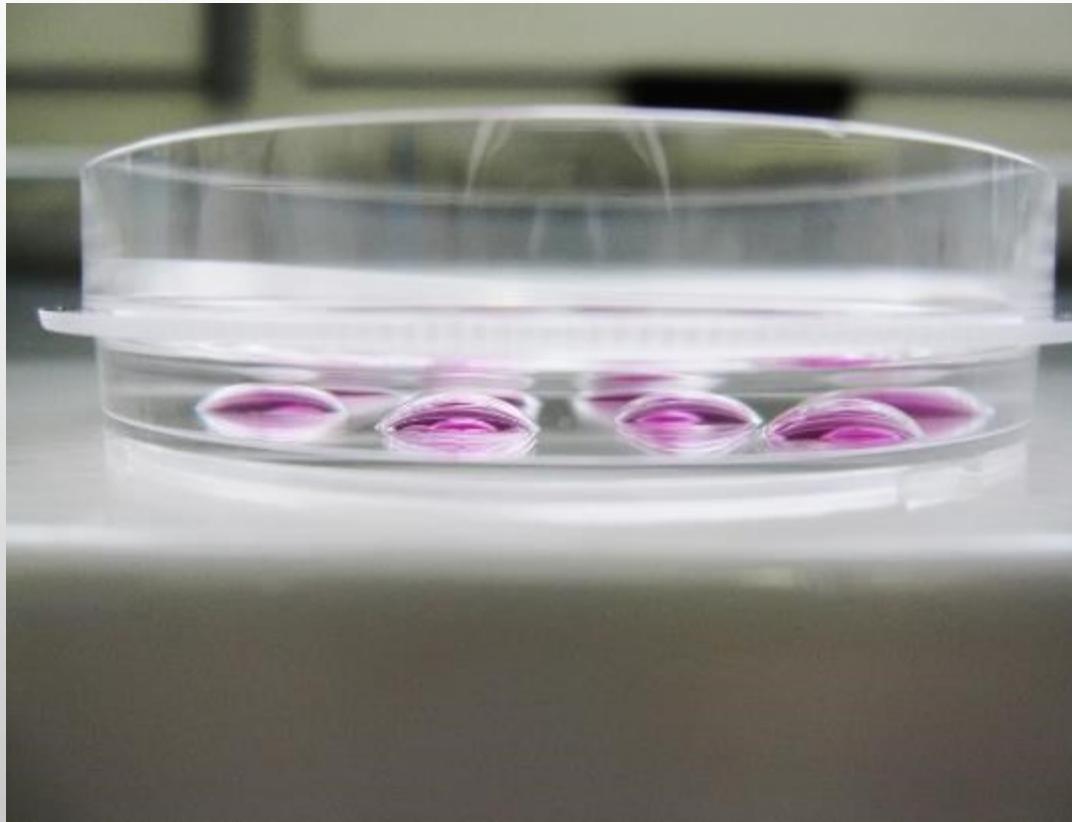
# EMBRYONIC STEM CELLS

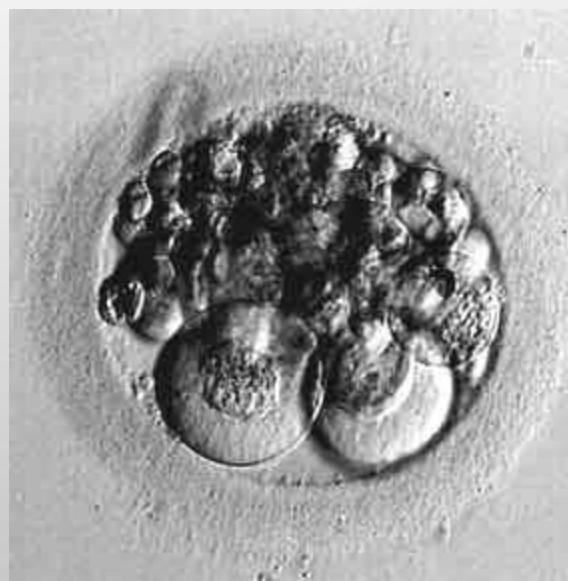
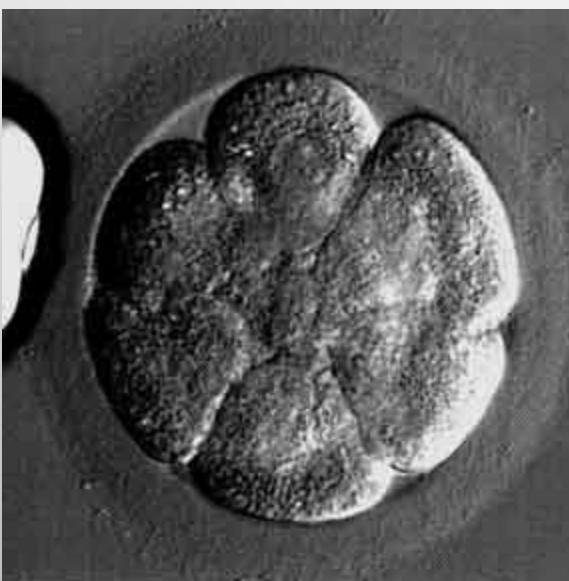
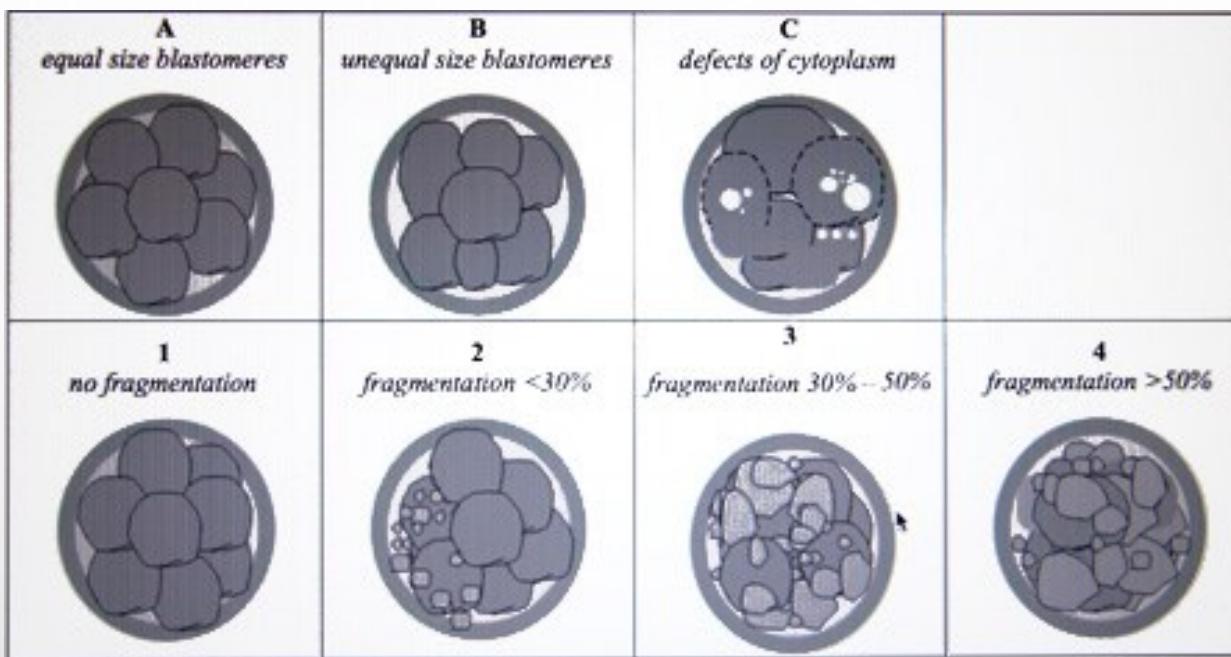


Stages of IVF procedure

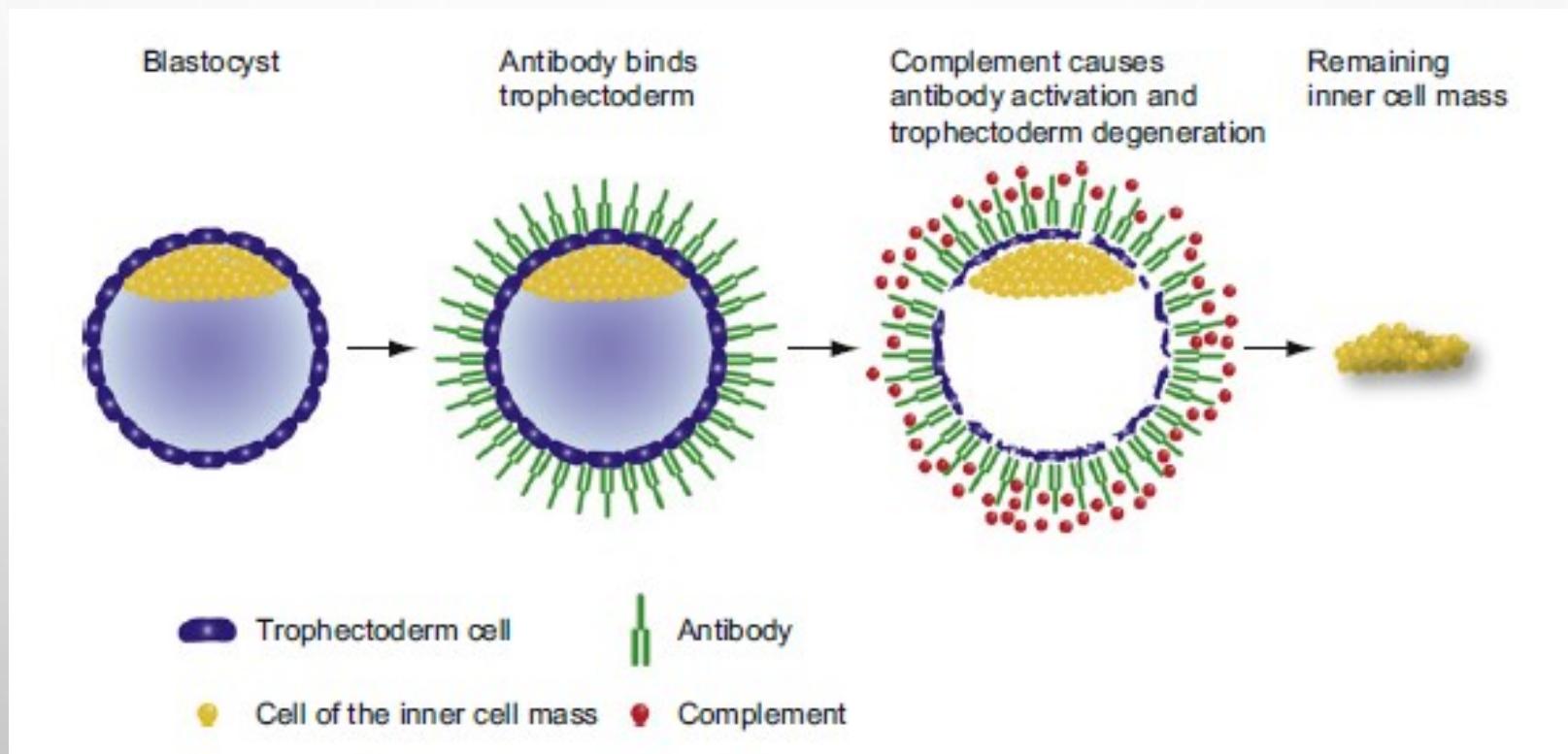
# IVF

<HTTP://WWW.GINEMED.CO.UK/ASSISTED-REPRODUCTION/LABORATORY-TECHNIQUES/ICSI-SPERM-MICROINJECTION>



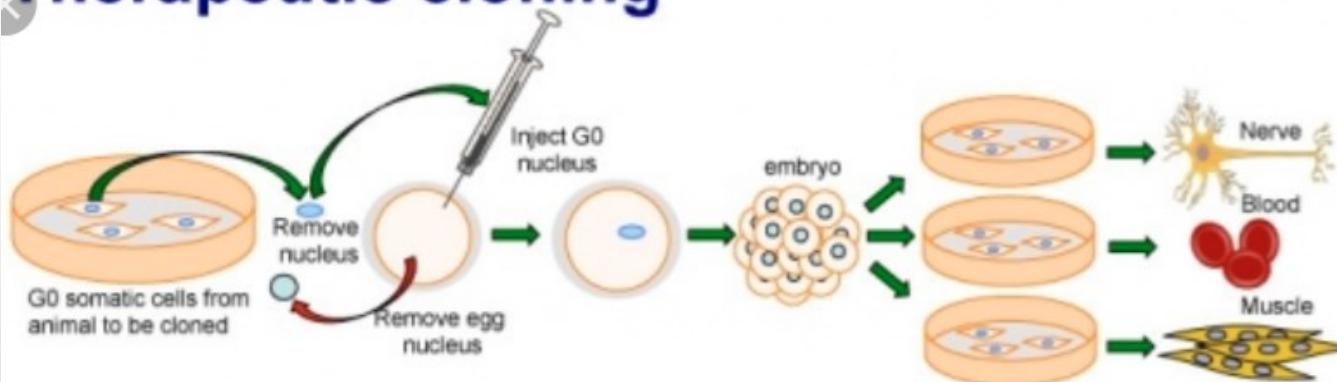


# ISOLATION OF ICM

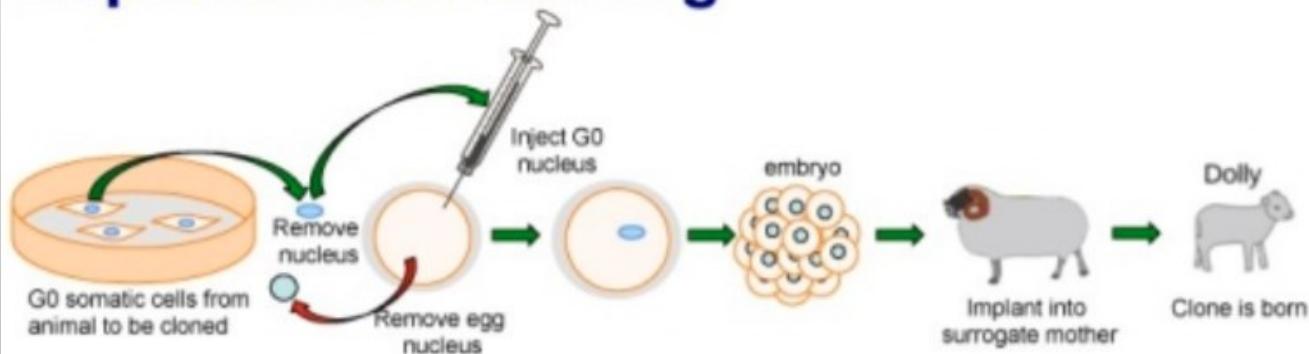


# REPRODUCTIVE VS. THERAPEUTIC CLONING

## Therapeutic cloning



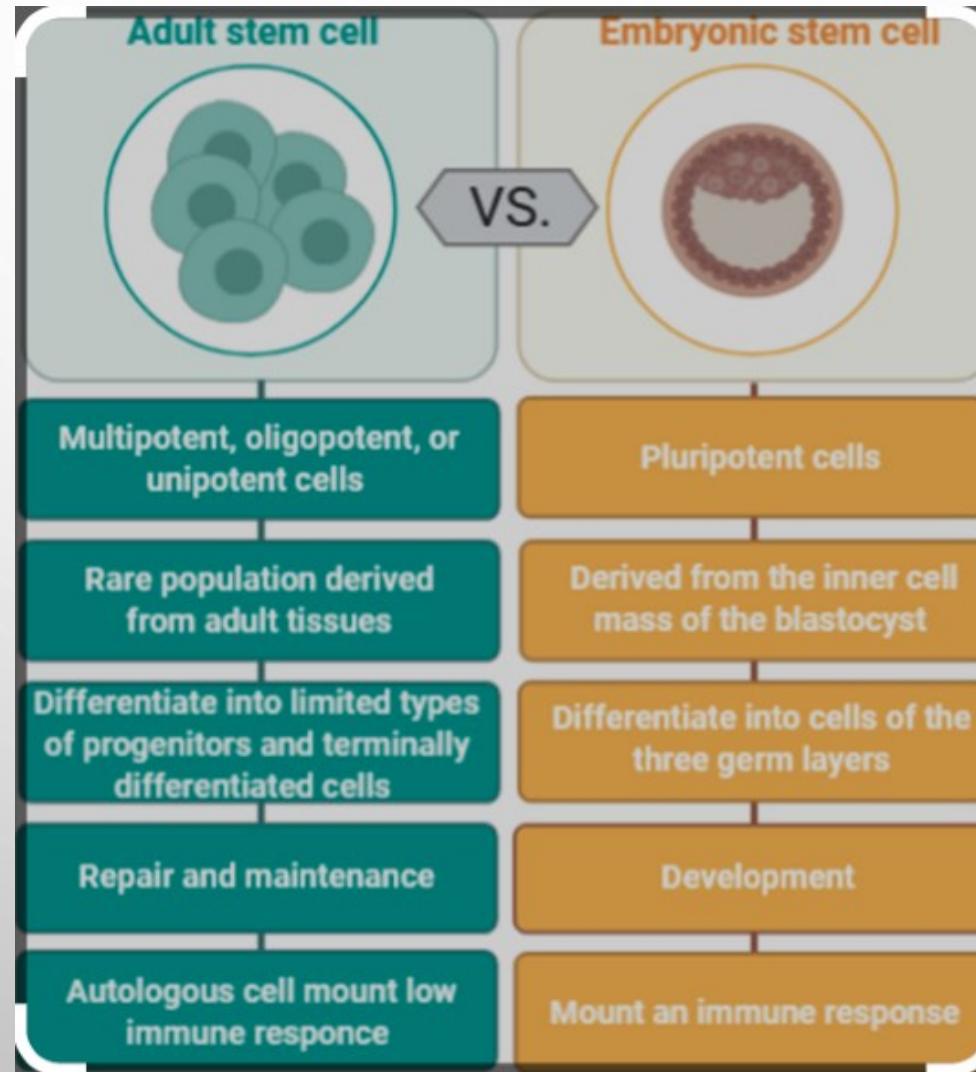
## Reproductive cloning



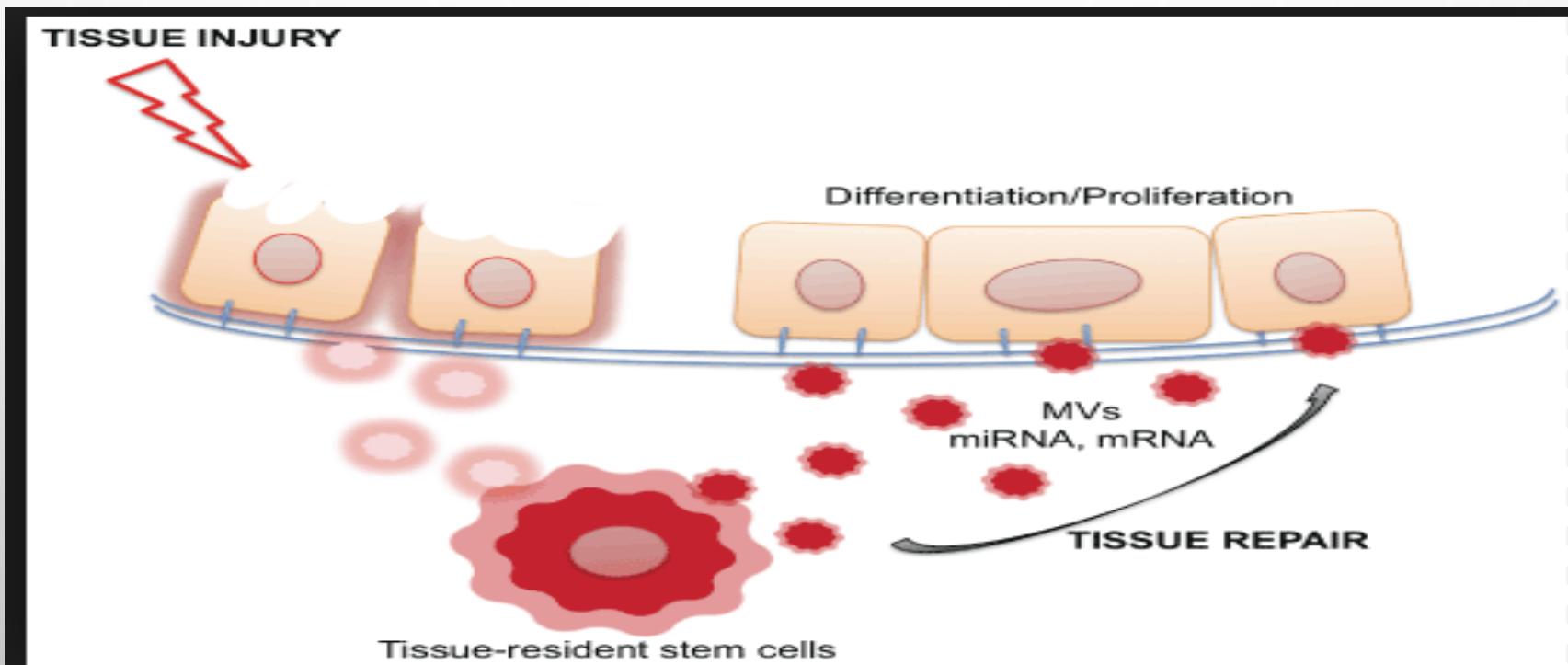
- ▣ Permissive approach with respect to hESC research derivation (IVF, SCNT)
- ▣ Intermediate approach (restrictions in place for hESC research and derivation)
- ▣ Restrictive approach (prohibitions on embryo research or on derivation and use of hESC embryos, or research limited to imported hESC lines)
- ▢ No specific legislation in place regarding embryo or hESC research
- Federated country where hESC and derivation are both a matter of federal and state law. Policy approaches range from permissive ▣, to restrictive ▣.



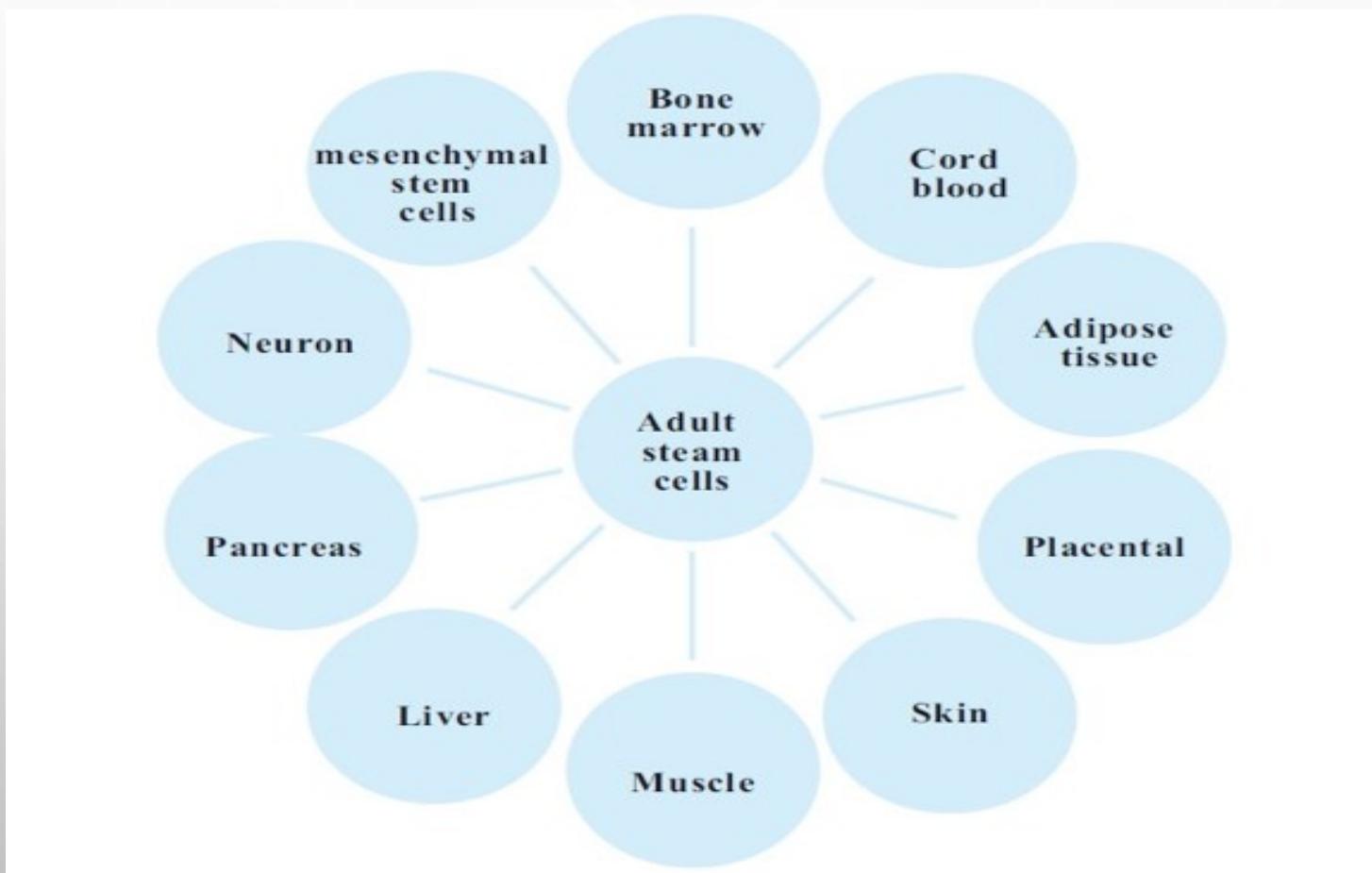
# ADULT STEM CELLS



# THE ROLE OF STEM CELLS



# ADULT STEM CELL TYPES

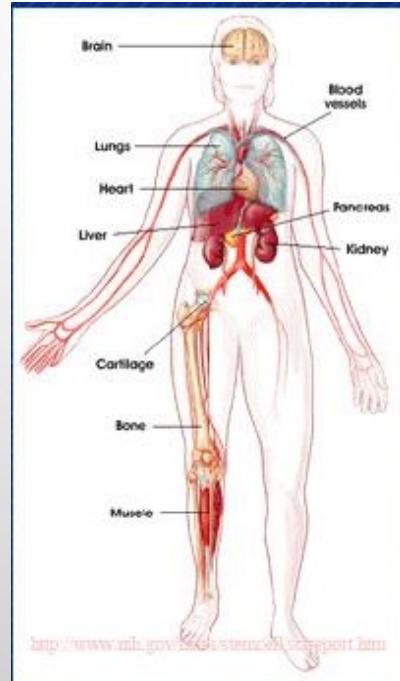


# AXOLOTL (MEKSIČKI DAŽDEVNJAK)



	Animal	What they regenerate
Invertebrates	Flatworm	Any part of their bodies, including their heads!
	Sea star	Limbs and even their whole bodies if their central nerve ring is intact.
Vertebrates	Axolotl	Limbs and spine.
	Frog	Tadpoles can regenerate limbs but lose this ability in adulthood.
	Human	Adult human regeneration is largely limited to skin and liver cells.

# REGENERATIVE MEDICINE



Repair  
Replace  
Restore  
Regenerate

# Why not simply transplant donated organs?

- Need greatly exceeds supply



US Transplant Waiting List  
as of 2 November 2006

Total Transplants  
In 2005

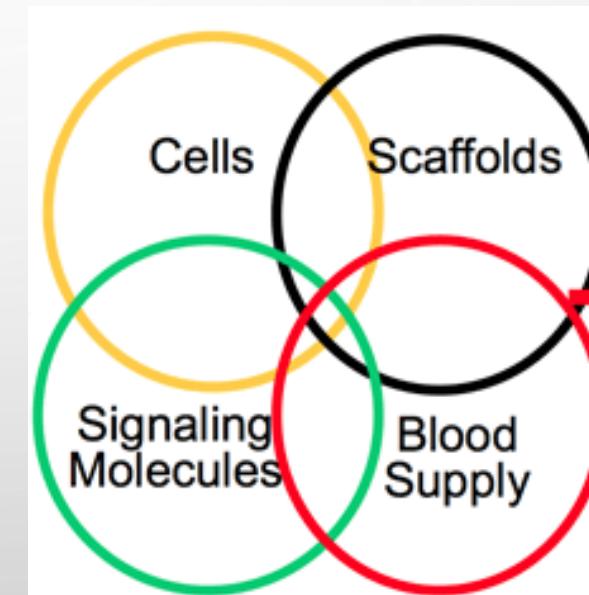
All	<b>93,725</b>	<b>28,108</b>
Kidney	68,476	16,477
Pancreas	1,729	541
Kidney/Pancreas	2,407	903
Liver	17,115	6,443
Intestine	240	178
Heart	2,835	2,125
Lung	2,878	1,406
Heart/Lung	144	35

# WHAT IS MEDICAL REALITY?

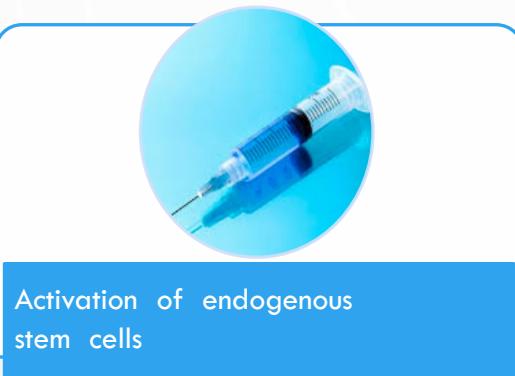
- TRANSPLANTATION OF HEMATOPOIETIC STEM CELLS
- TRANSPLANTATION OF TISSUE-ENGINEERED SKIN
- BONE AND CARTILAGE REPAIR

# THE REASONS FOR SLOW PROGRESS?

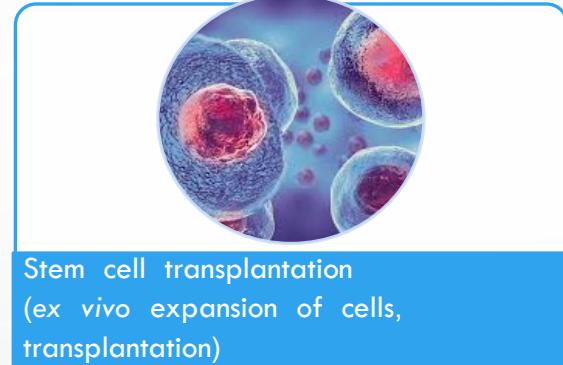
- PROBLEM WITH CELL EXPANSION IN VITRO
- INADEQUATE BIOMATERIALS
- POOR VASCULARIZATION
- DIFFERENTIATION



# REGENERATIVE MEDICINE APPROACHES



Activation of endogenous  
stem cells



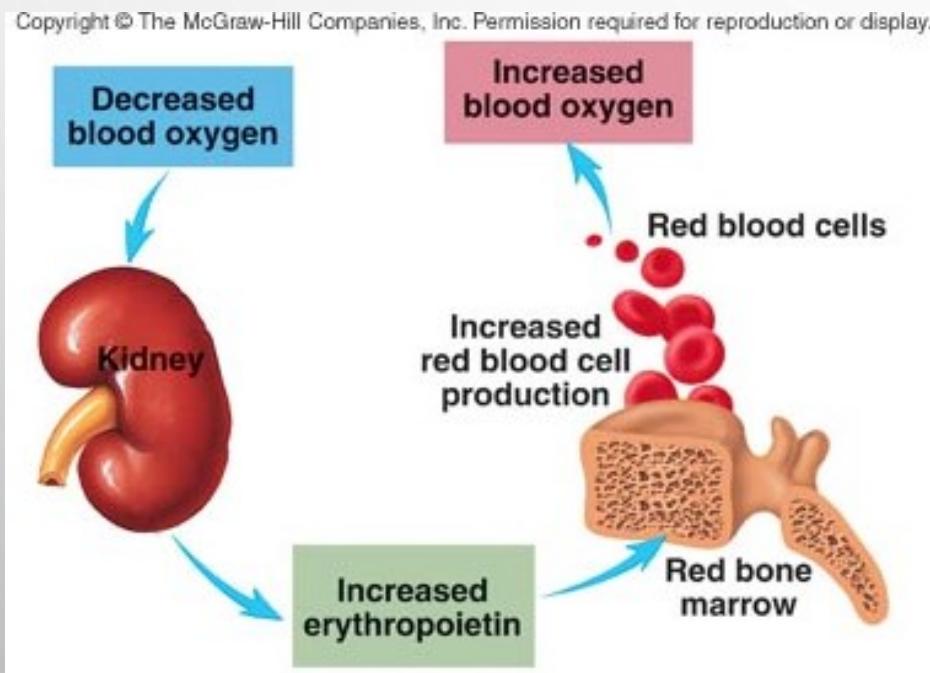
Stem cell transplantation  
(ex vivo expansion of cells,  
transplantation)



Tissue engineering (organ  
and tissue ex vivo  
formation, transplantation)

# 1. ACTIVATION OF ENDOGENOUS STEM CELLS

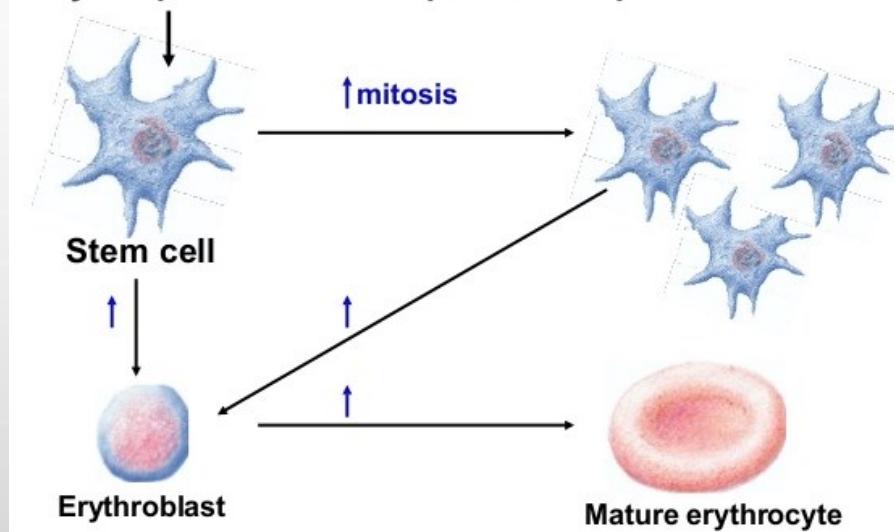
## PRIMJER 1



## ERITROPOETIN

### Mechanism of action:

Erythropoietin acts on specific receptors on stem cells



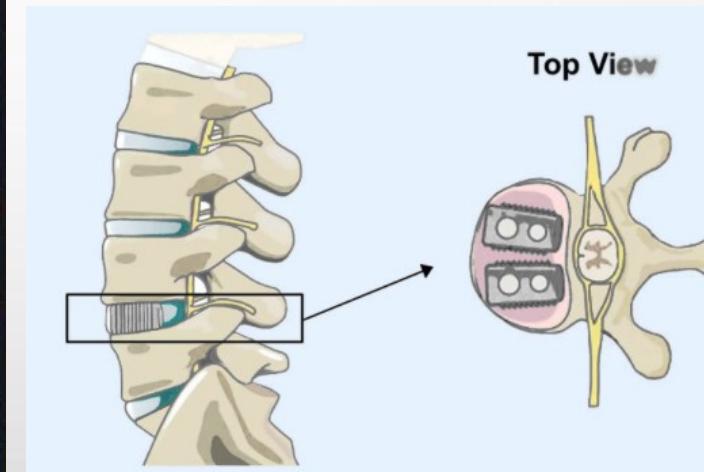
Erythropoietin causing speeding up of all the stages of development of proerythroblasts into mature erythrocytes

# 1. ACTIVATION OF ENDOGENOUS STEM CELLS

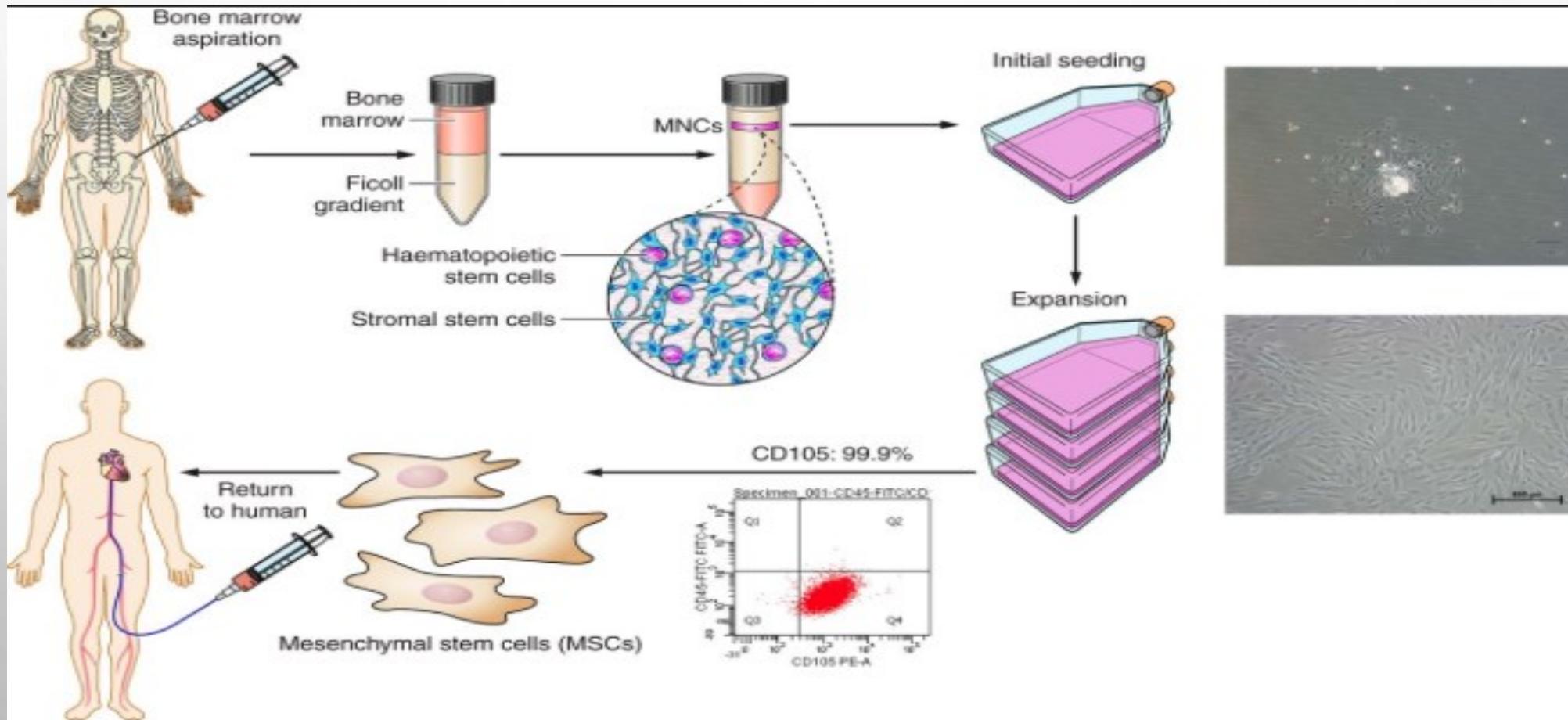
**PRIMER 2**



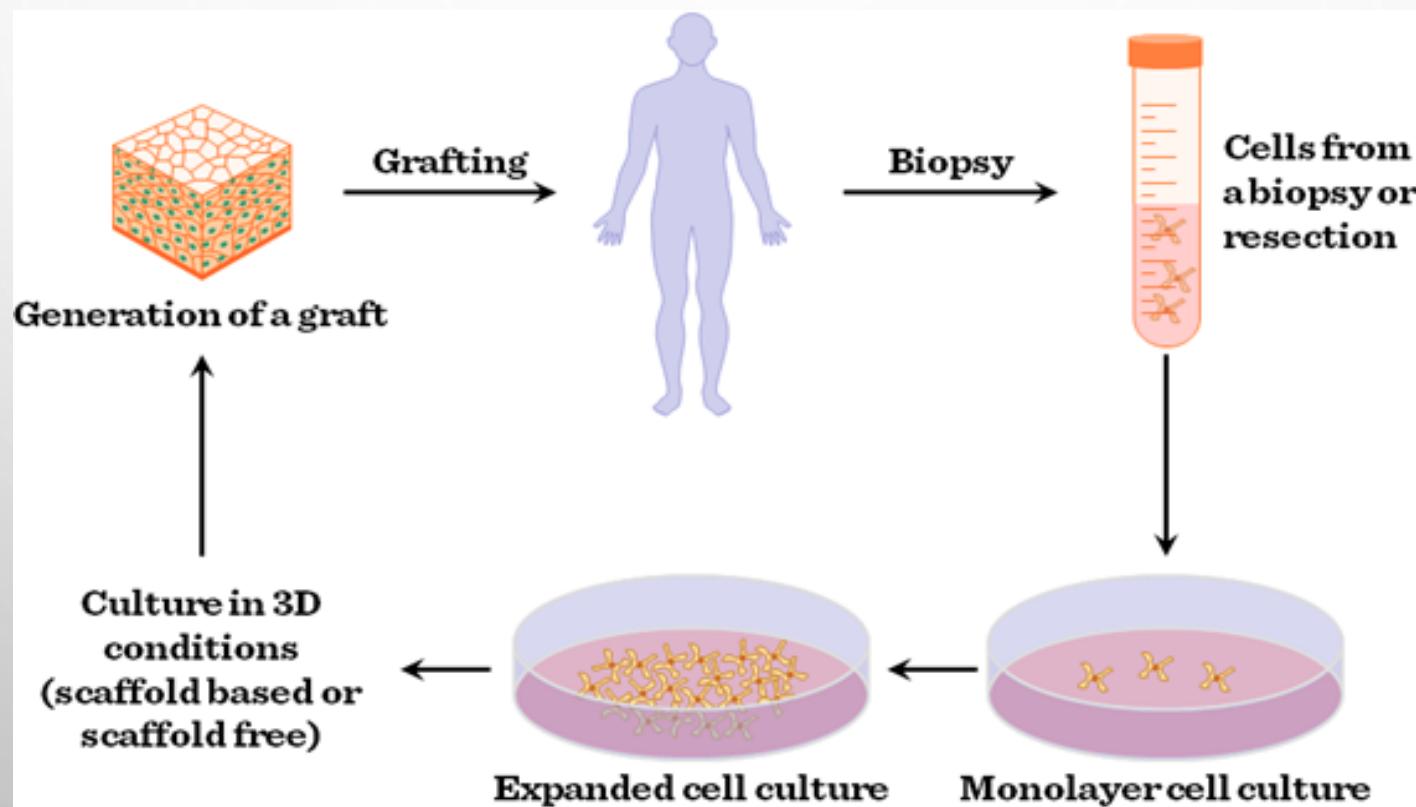
**INFUSE BONE GRAFT**



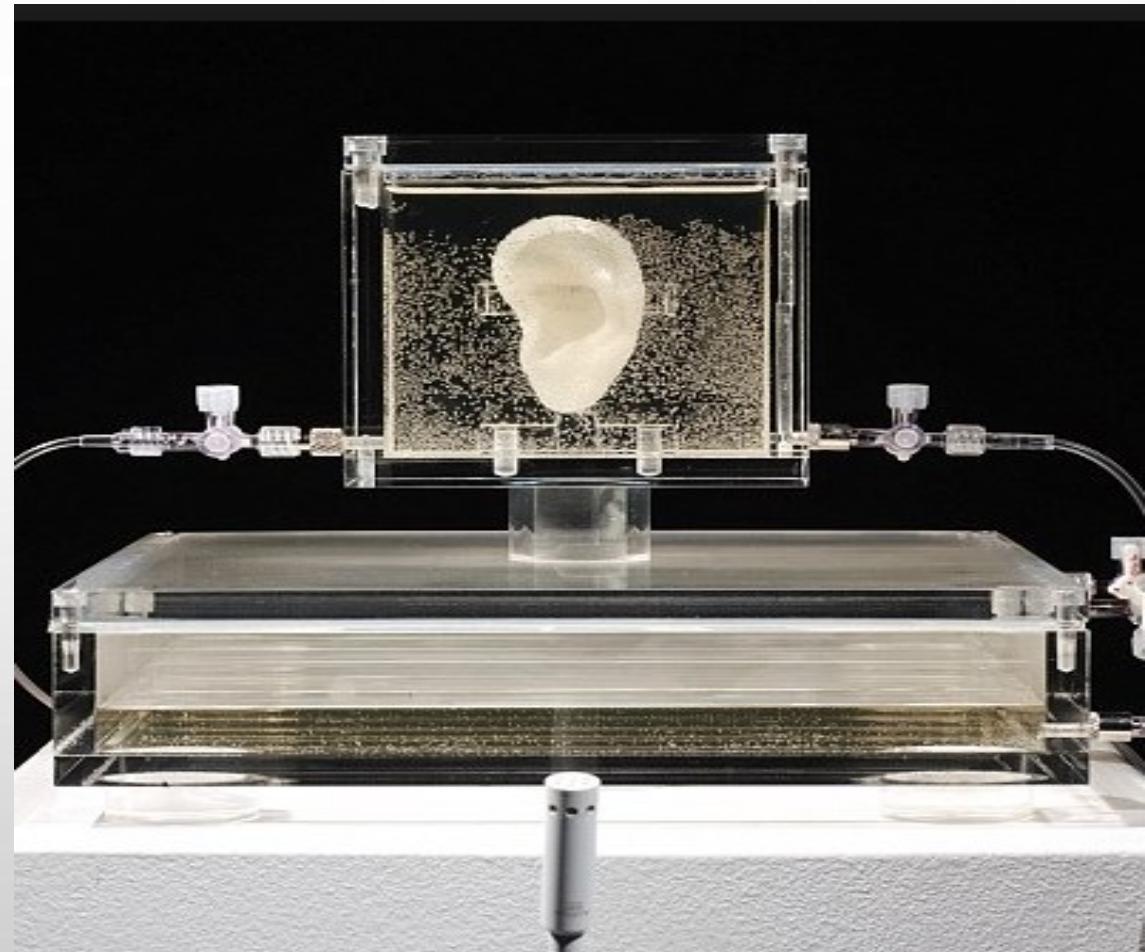
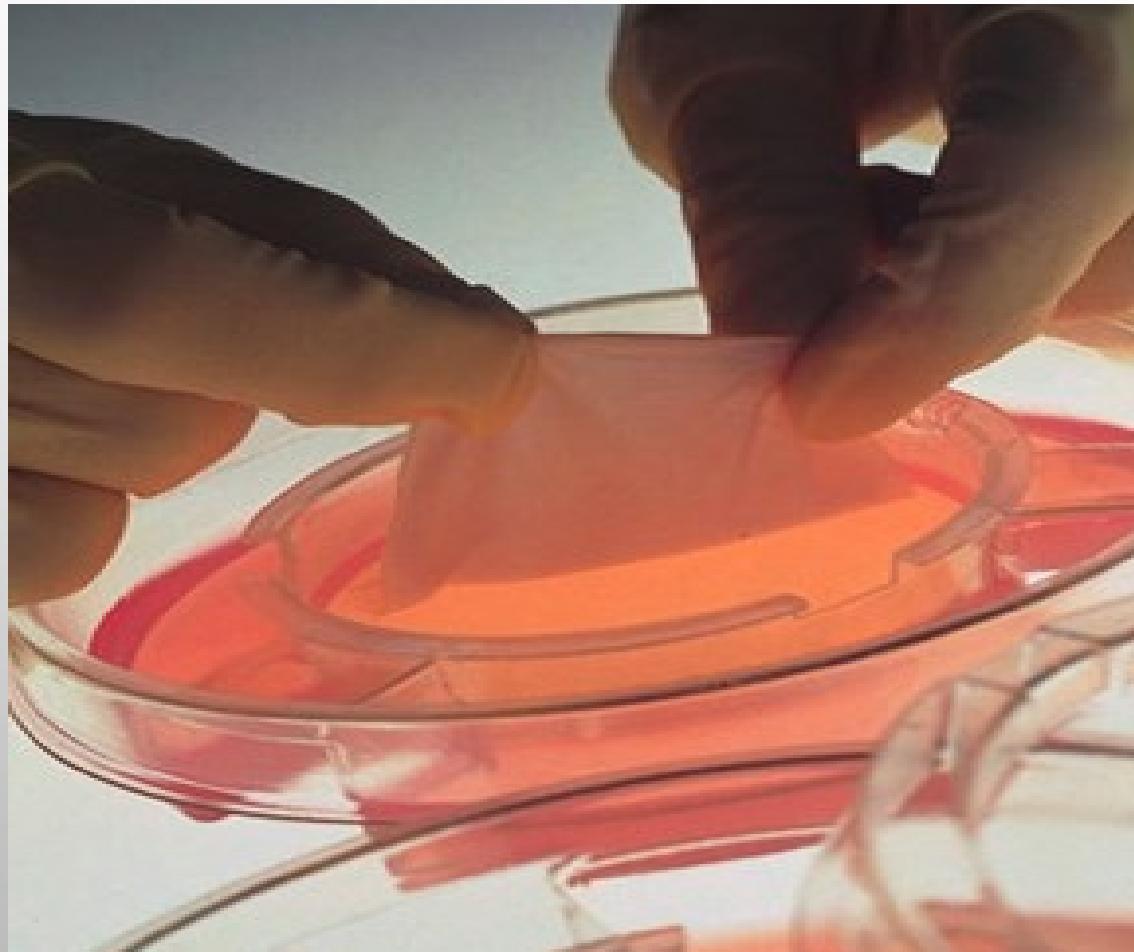
## 2. STEM CELL TRANSPLANTATION



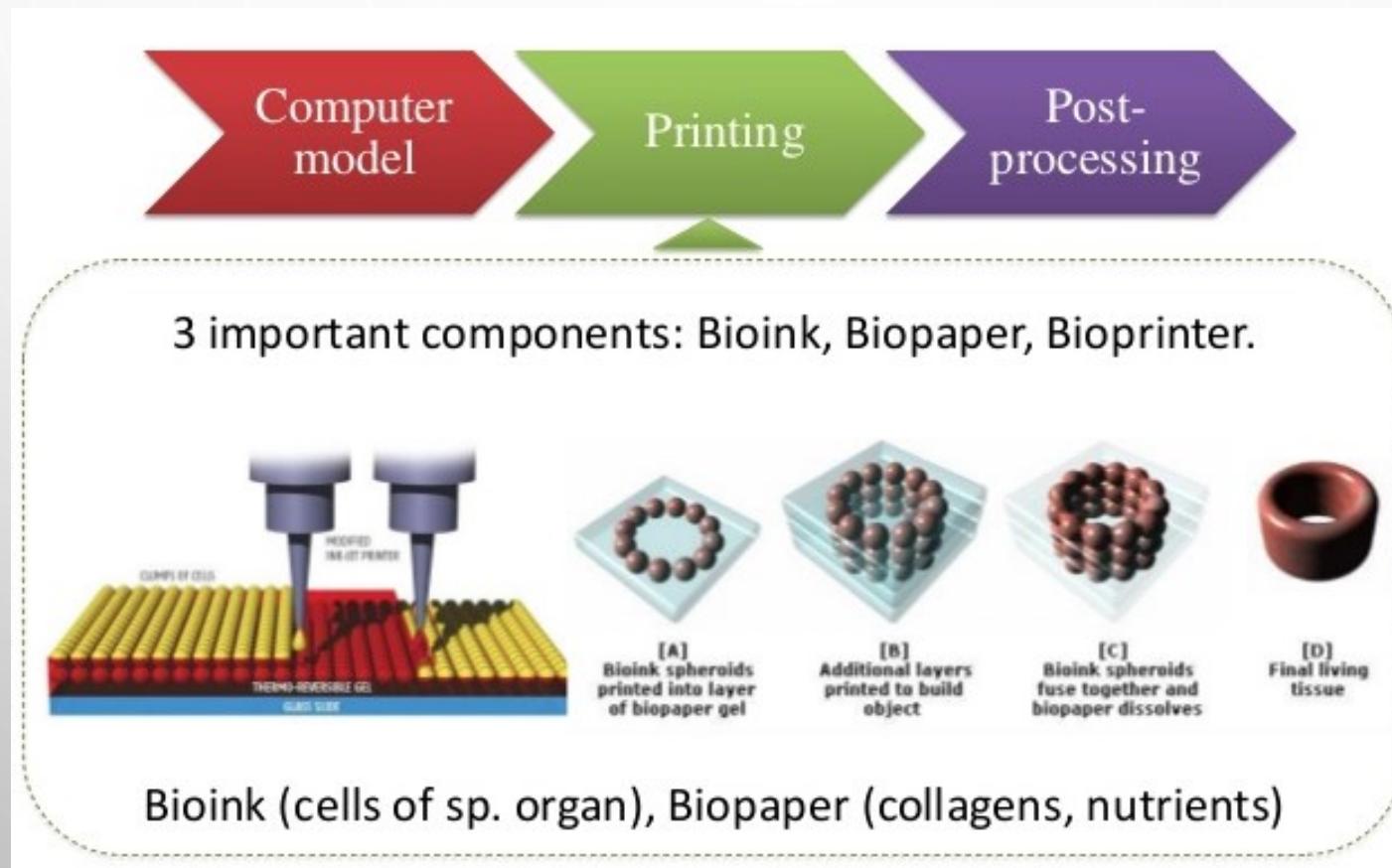
### 3. TISSUE ENGINEERING

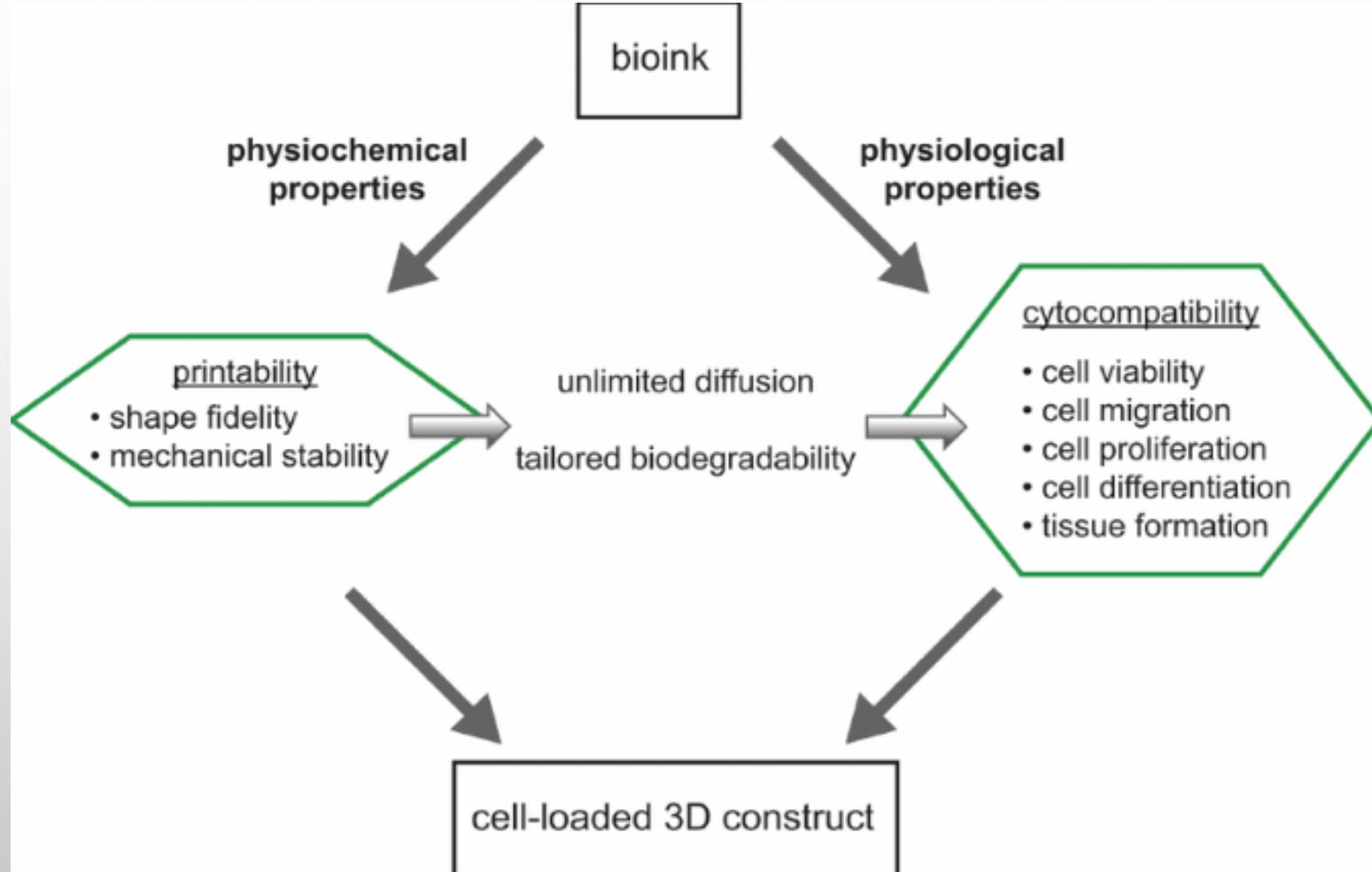


### 3. TISSUE ENGINEERING

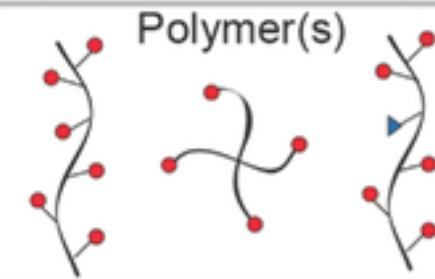


# BIOPRINTING





### 1 Bioink Formulation



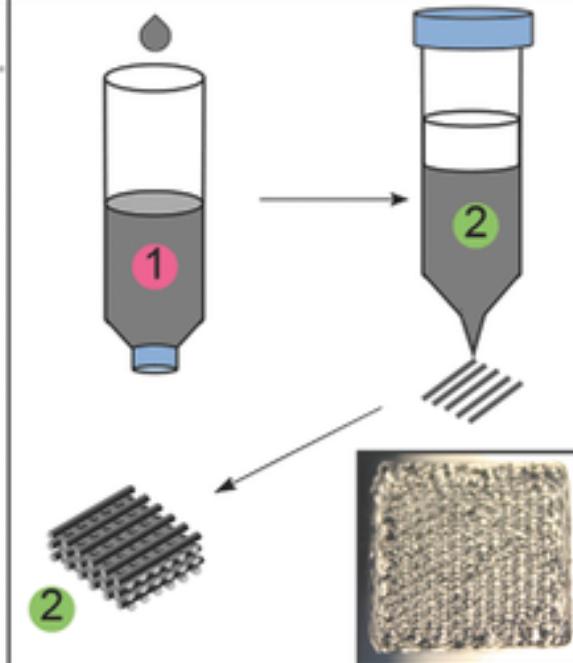
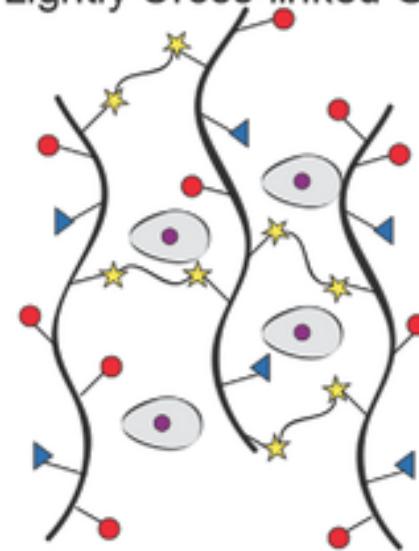
PEG Cross-linker (PEGX)



Cells

### 2 Bioink

Lightly Cross-linked Gel

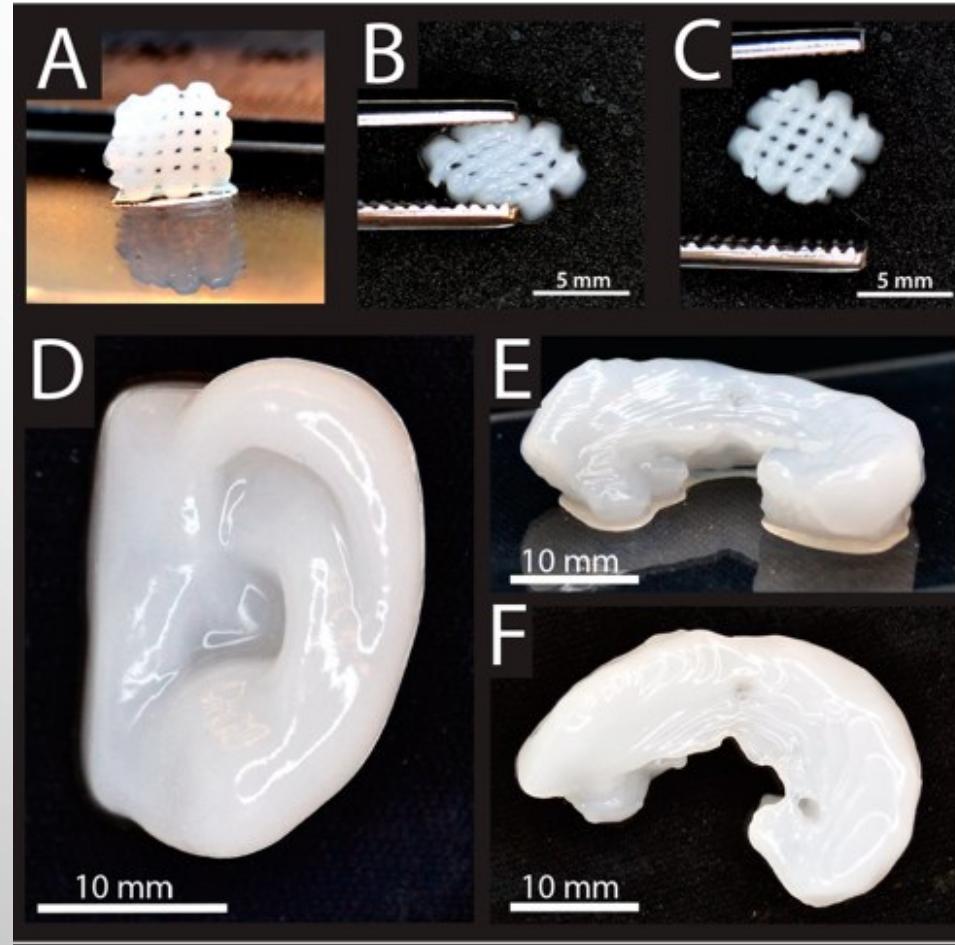




### Bioreactor

- Supply nutrients for further cell growth
- Physiological environment for tissue maturation.
- Mechanical and bio. testing.





## *Case Study on Bioprinting of Kidney*



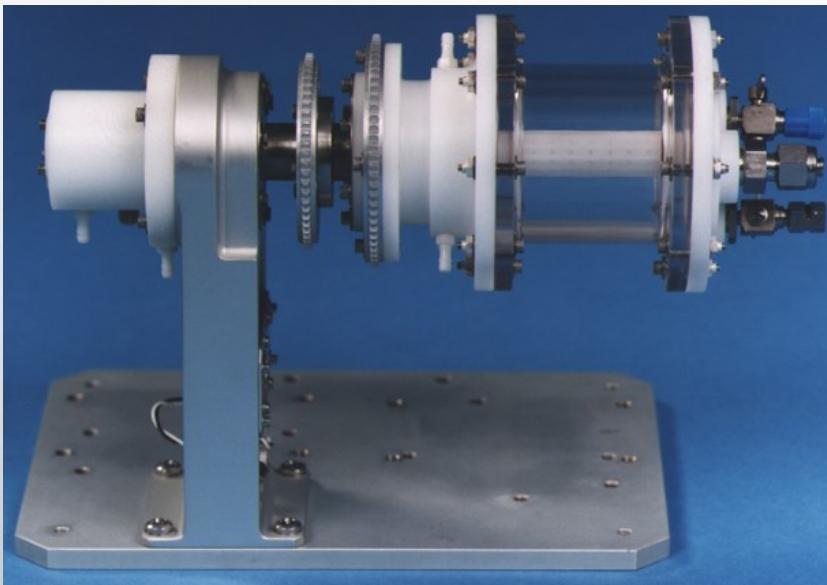
- Cost of Kidney Transplant : \$ 80,000 USD
- Cost for Bioprinting of Kidney : \$ 280,000 USD\*

\* Projected cost for bioprinted kidney 2013

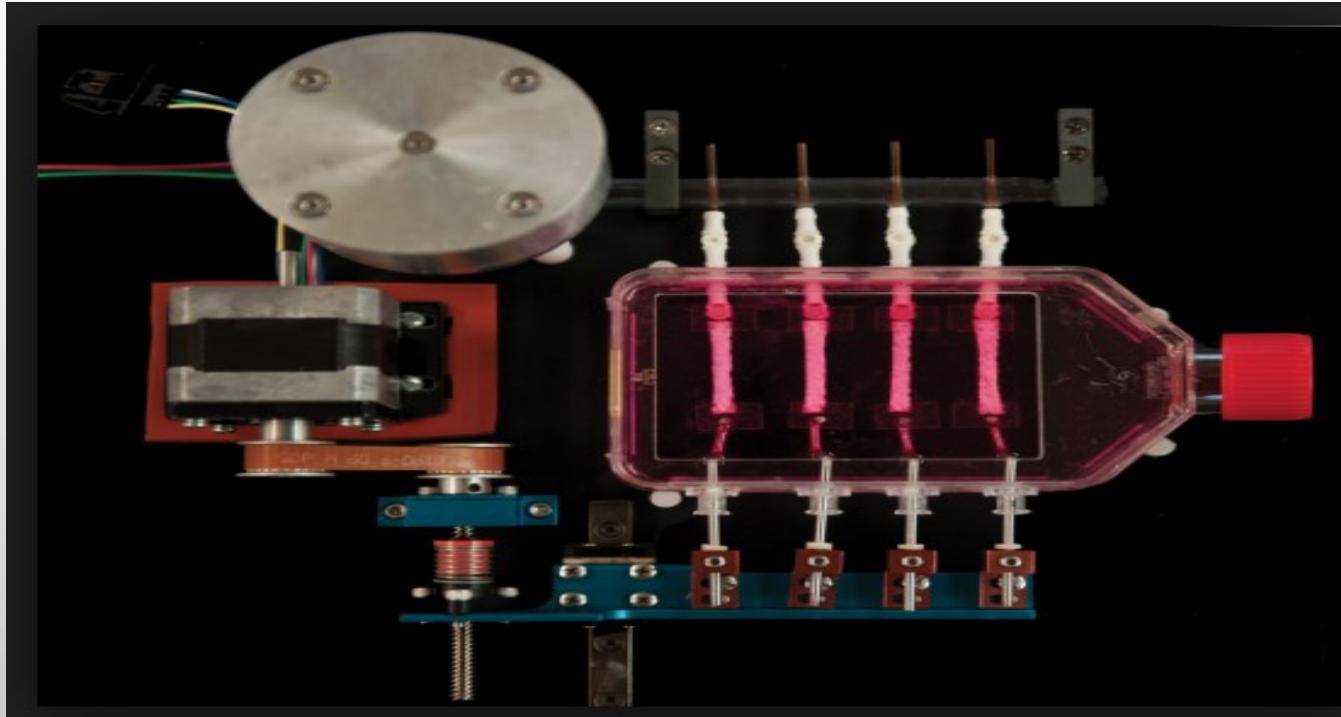
- Dialysis treatment costs **\$55,000-\$75,000** per patient per year.
- Treatments for diabetes costs around **\$6,000** per year per patient.
- Total cost of **\$245 billion** per year has been spent in the United States for diabetes treatment.

Takes around 10 hours to bioprint a Kidney\*

# BIOREACTORS

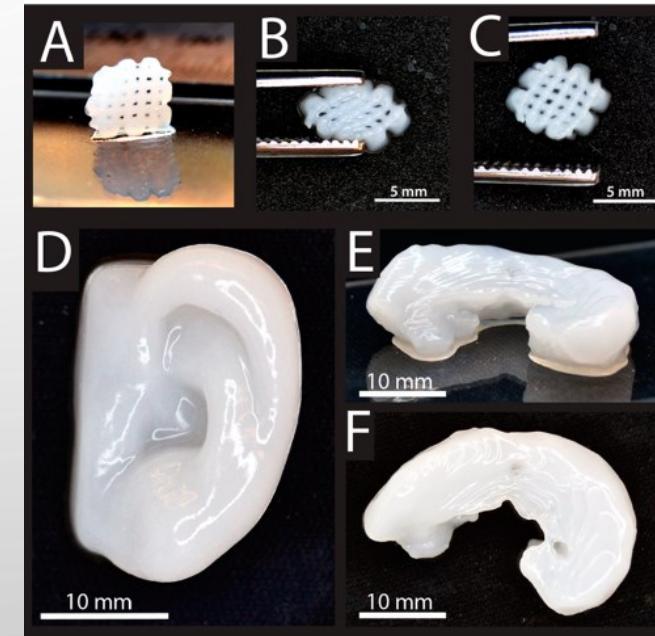
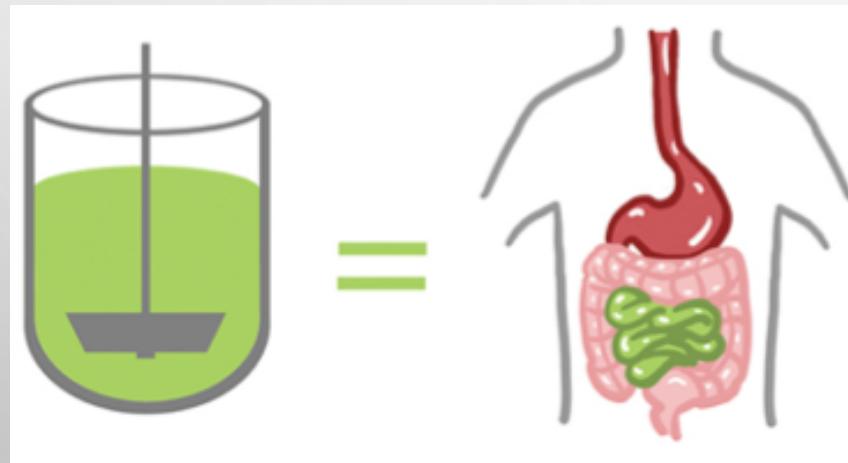


# SPECIJALNI SUSTAVI BIOREAKTORA VIBRACIJSKI BIOREAKTOR ZA GLASNICE

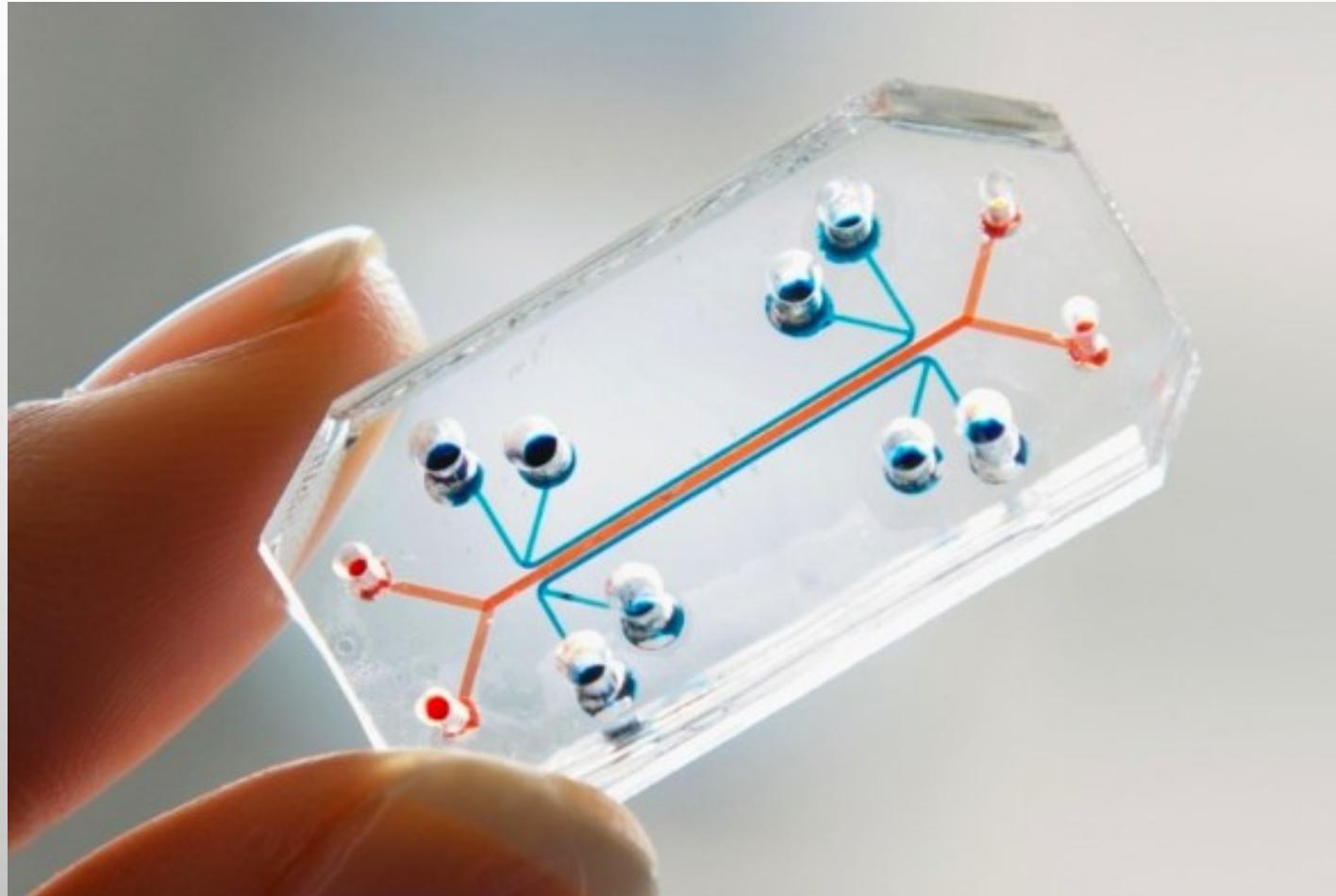


# PURPOSE

- MIMIC *IN VIVO* ENVIRONMENT
- ENABLE MONITORING AND CONTROL OF THE ENVIRONMENT

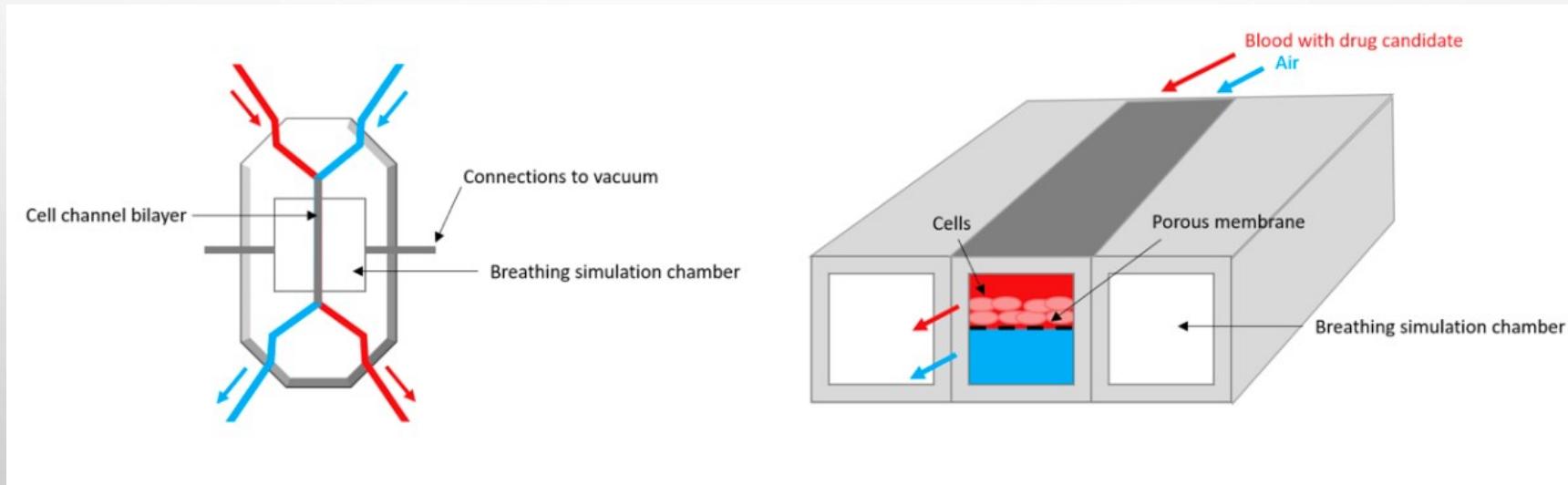


# ORGAN-ON-A-CHIP



# LUNG-ON-A-CHIP

<https://wyss.harvard.edu/media-post/human-organs-on-chips/>



# DRUG DEVELOPMENT PIPELINE

