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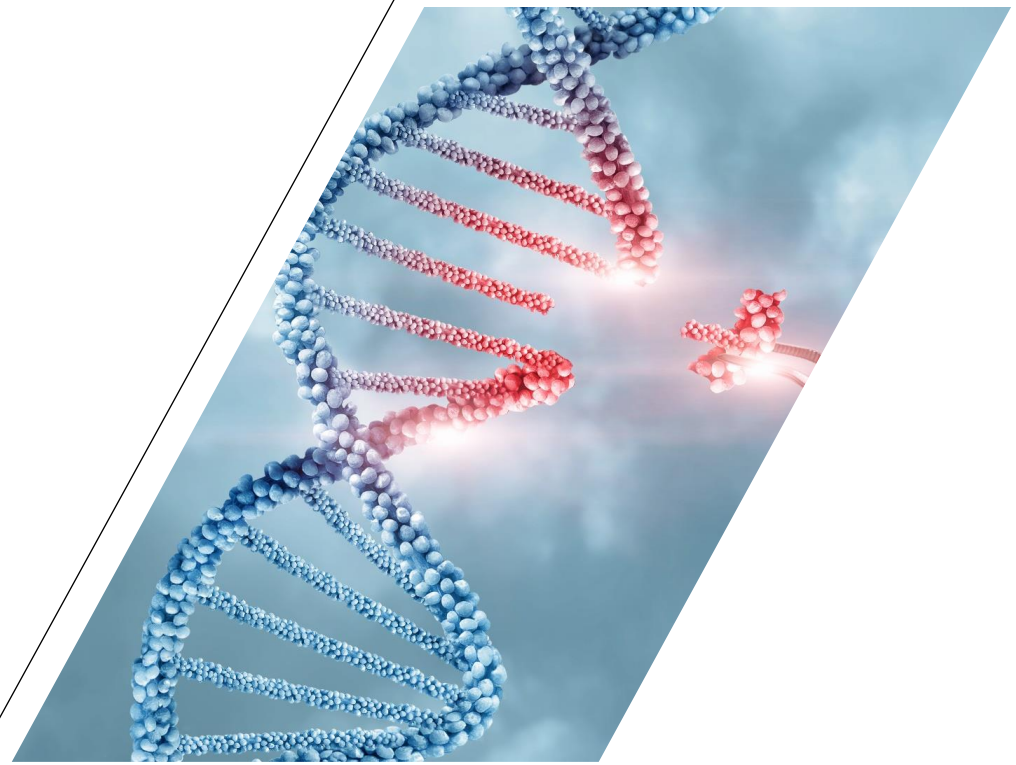
Subject: Genetic engineering

(Lecture 11)

RECOMBINANT DNA AND HEREDITARY DISEASES. GENE THERAPY. ITS BASIC PRINCIPLES.

AIM

- To study the application of genetic engineering methods in the treatment of hereditary diseases.
- To analyze the methods used in gene therapy.



Key Words
Recombinant DNA, hereditary diseases, gene therapy, somatic gene therapy, germline gene therapy, viral vectors, non-viral vectors, insertional therapy, ex vivo therapy, in vivo therapy, regulatory elements, transgene expression.

TASKS

T1 — Understand what hereditary diseases are

T2 — Understand the types of mutations

T3 — To study the main hereditary disease

T4 — Understand gene therapy methods

SUMMARY

1) Recombinant DNA and hereditary diseases

Recombinant DNA technology allows the isolation, modification, and delivery of genes responsible for hereditary disorders.

Enables development of therapeutic strategies targeting the genetic cause of disease rather than only symptoms.

2) Gene therapy concept

Introduction of functional genes into a patient's cells to correct or compensate for defective genes.

Can be **somatic** (affecting body cells, not heritable) or **germline** (affecting reproductive cells, heritable).

Delivery methods

3) Viral vectors: adenovirus, lentivirus, AAV, retrovirus — high efficiency, can integrate or remain episomal.

Non-viral vectors: plasmids, liposomes, nanoparticles — safer, lower efficiency.

4) Ex vivo vs. in vivo therapy

Ex vivo: patient cells are modified outside the body, then transplanted back.

In vivo: genes delivered directly into patient tissues.

5) Regulatory elements in gene therapy

Promoters, enhancers, and terminators control transgene expression.

Tissue-specific or inducible elements ensure safe and targeted expression.

6) Challenges and ethical considerations

Immune reactions, insertional mutagenesis, long-term expression stability.

Ethical issues, especially in germline modifications.

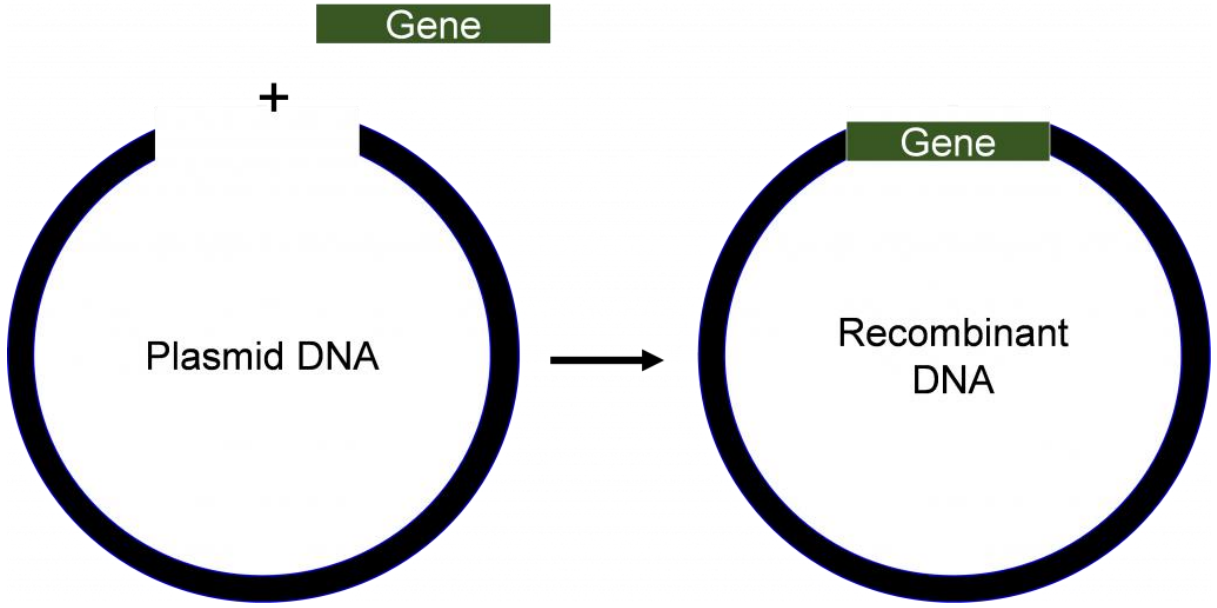
KEY QUESTIONS

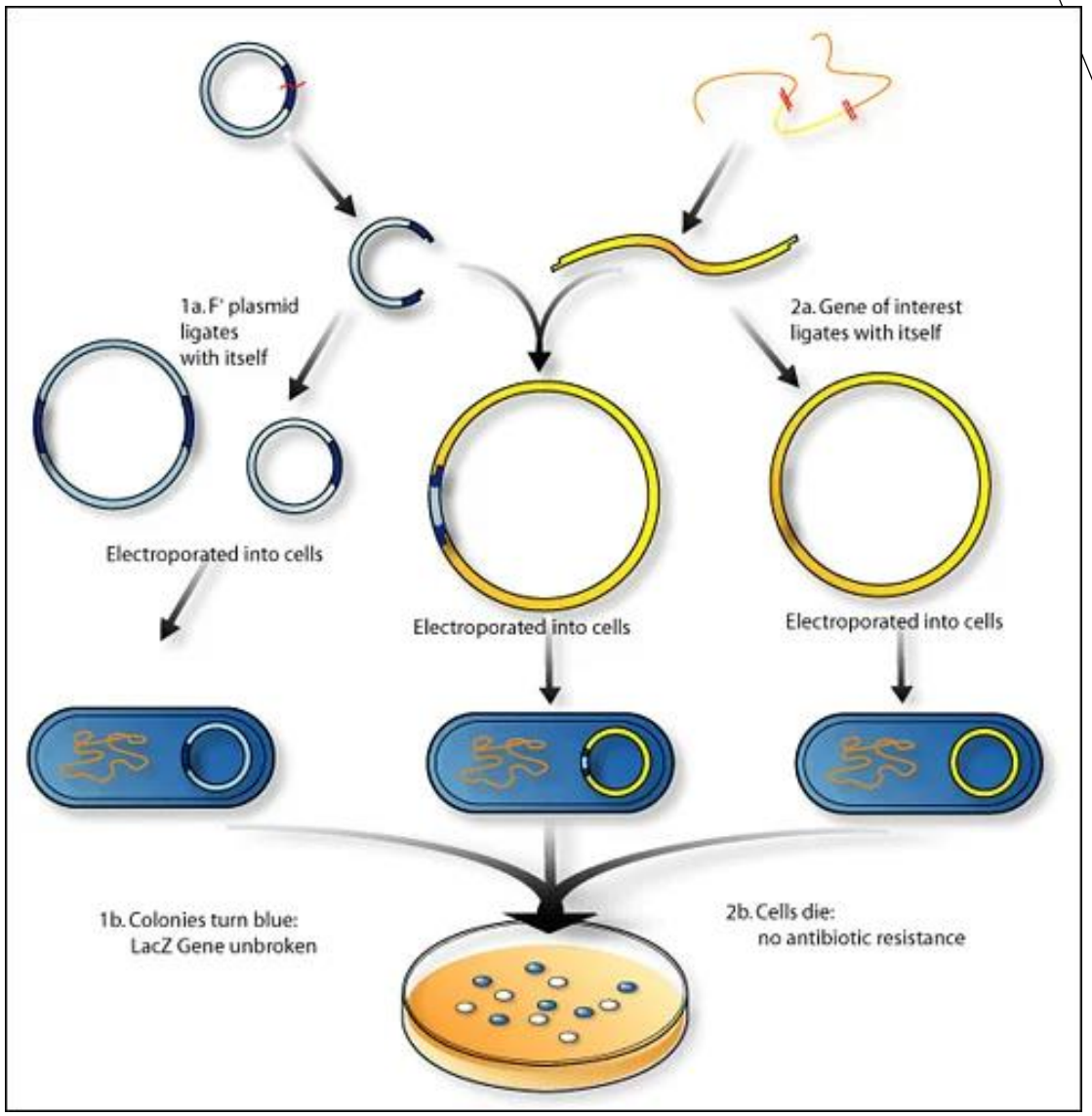
1. What is the principle of gene therapy and how does it differ from conventional treatments?
2. What are the main types of gene therapy (somatic vs germline)?
3. Name the main viral and non-viral vectors used in gene therapy.
4. What is the difference between ex vivo and in vivo gene therapy?
5. How do regulatory elements control transgene expression in gene therapy?
6. What are the main challenges and ethical concerns associated with gene therapy?

RECOMBINANT DNA TECHNOLOGY

Recombinant DNA technology involves the manipulation of DNA molecules in a way that allows for the creation of new combinations of genetic material.

This technology has been used to develop new therapies and treatments for various diseases, including some hereditary diseases.

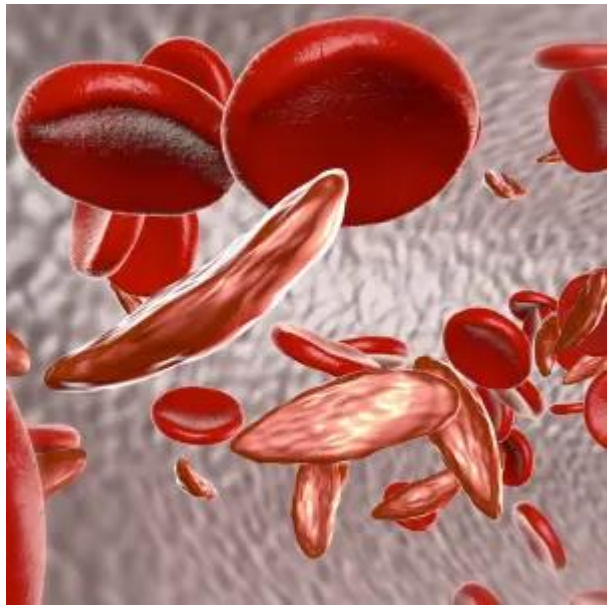




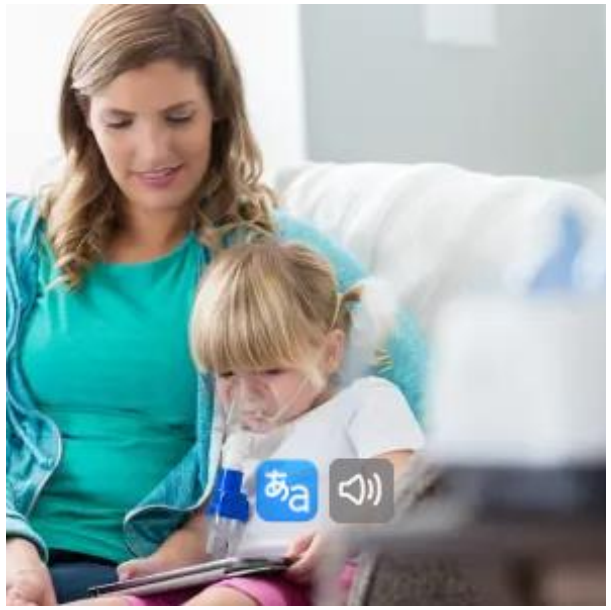
HEREDITARY DISEASES

Hereditary diseases are caused by **genetic mutations** that are passed down from parents to their offspring. In some cases, these mutations can be corrected or replaced using **recombinant DNA technology**.

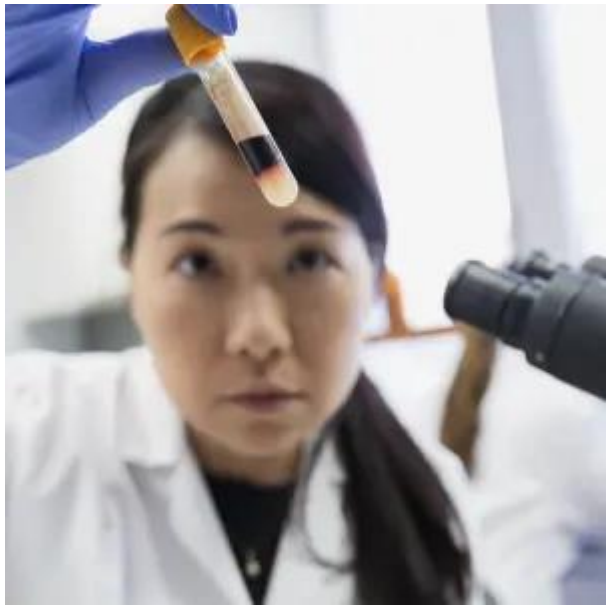
Hereditary diseases are diseases caused by gene or chromosomal mutations.
Humans have between **20,000 and 25,000** genes.



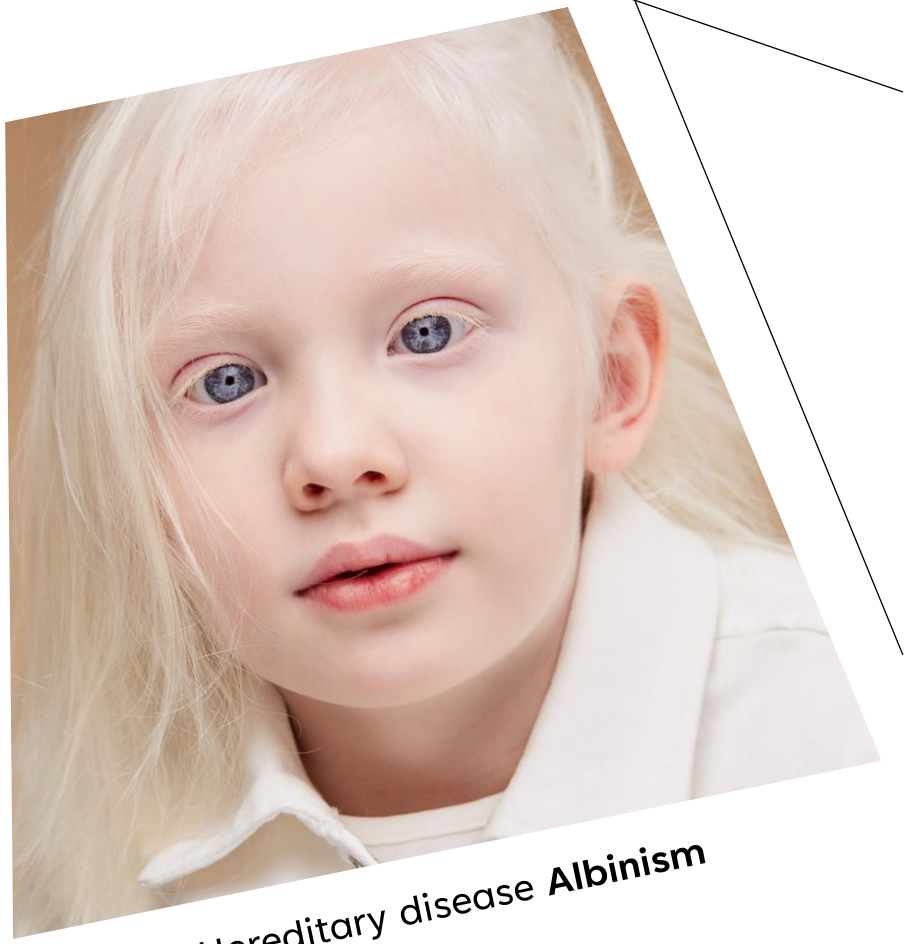
Sickle Cell Disease



Cystic Fibrosis



Hemophilia

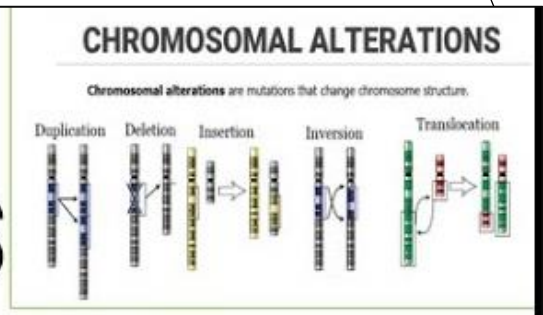


Hereditary disease **Albinism**

Hereditary diseases are based on violations of hereditary information - **mutations**: *chromosomal*, gene and *mitochondrial* mutations.

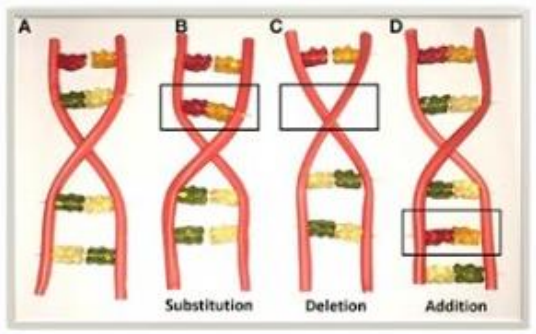
According to statistics, out of 1000 newborns, 35-40 have a hereditary pathology!

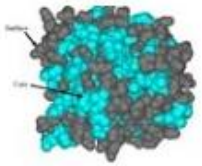
TYPES OF MUTATIONS



At the DNA level

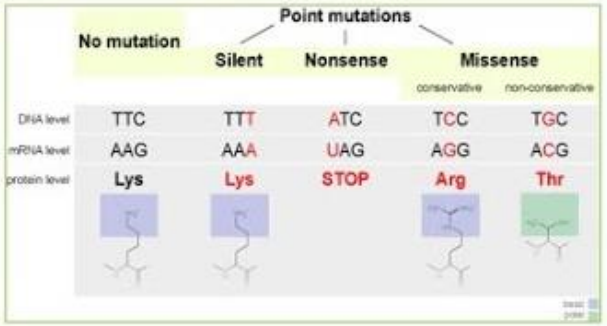
- ❑ Substitutions
- ❑ Deletions
- ❑ Insertions
- ❑ Inversions

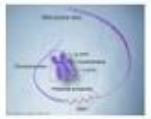




At Protein level/ Point Mutations

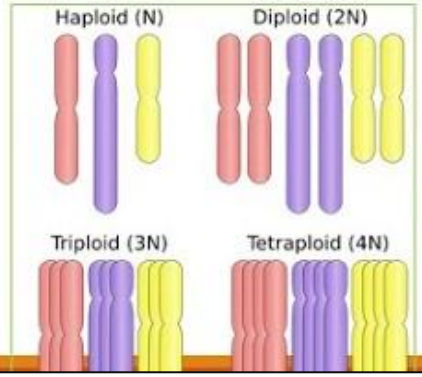
- ❑ Silent mutations
- ❑ Missense mutations
- ❑ Nonsense mutations
- ❑ Frameshift deletion mutations
- ❑ Frameshift insertion mutations





In molecular genetics

- ❑ Neutral
- ❑ Non-neutral



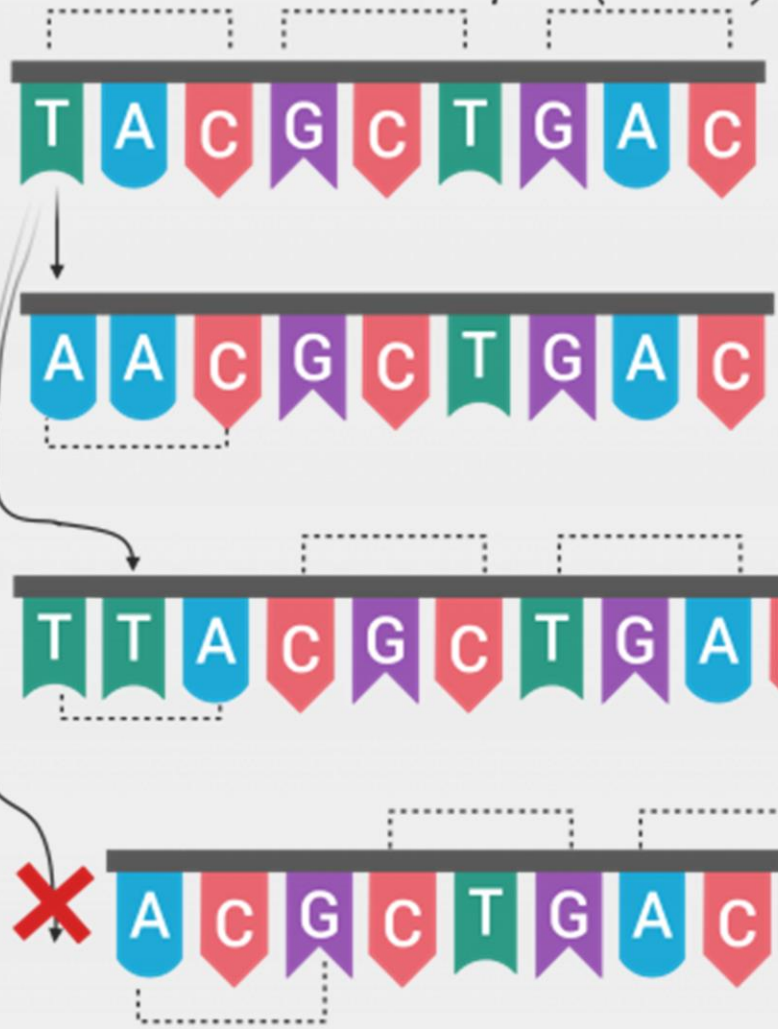
Chromosome mutations

- ❑ **Structural mutations**
  - Deletions
  - Duplications
  - Inversions
  - Insertions
  - Translocations
- ❑ **Numerical mutations**
  - Polyploidy
  - Aneuploidy

ANY SOMATIC HUMAN PATHOLOGY IS ALWAYS ASSOCIATED WITH HEREDITY.

The different type of mutations

Genetic code is read in triplets (3 bases)



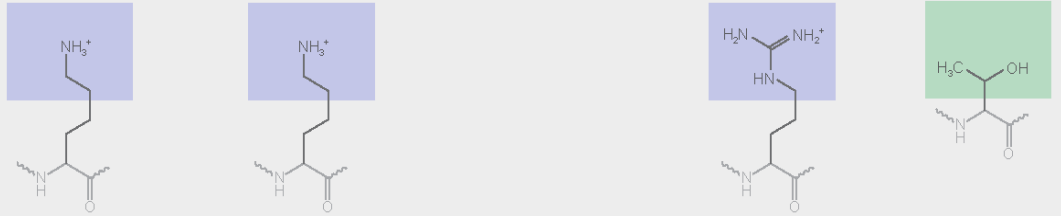
Original copy of the gene sequence, with **TAC** being the correct triplet code

Substituion of the first base, where 'T' has become 'A', and therefore the first triplet is incorrectly **AAC** instead of TAC. Changes the code of a single triplet.

Insertion of an extra 'T' within the genetic sequence, where the original TAC triplet has incorrectly become **TTA**. Changes the genetic code of all triplets following the mutation.

Deletion of the first 'T' in the genetic sequence, where the original TAC has incorrectly been changed to **ACG.** Changes the genetic code of all triplets following the mutation.

	No mutation	Point mutations			
		Silent	Nonsense	Missense	
				conservative	non-conservative
DNA level	TTC	TTT	ATC	TCC	TGC
mRNA level	AAG	AAA	UAG	AGG	ACG
protein level	Lys	Lys	STOP	Arg	Thr



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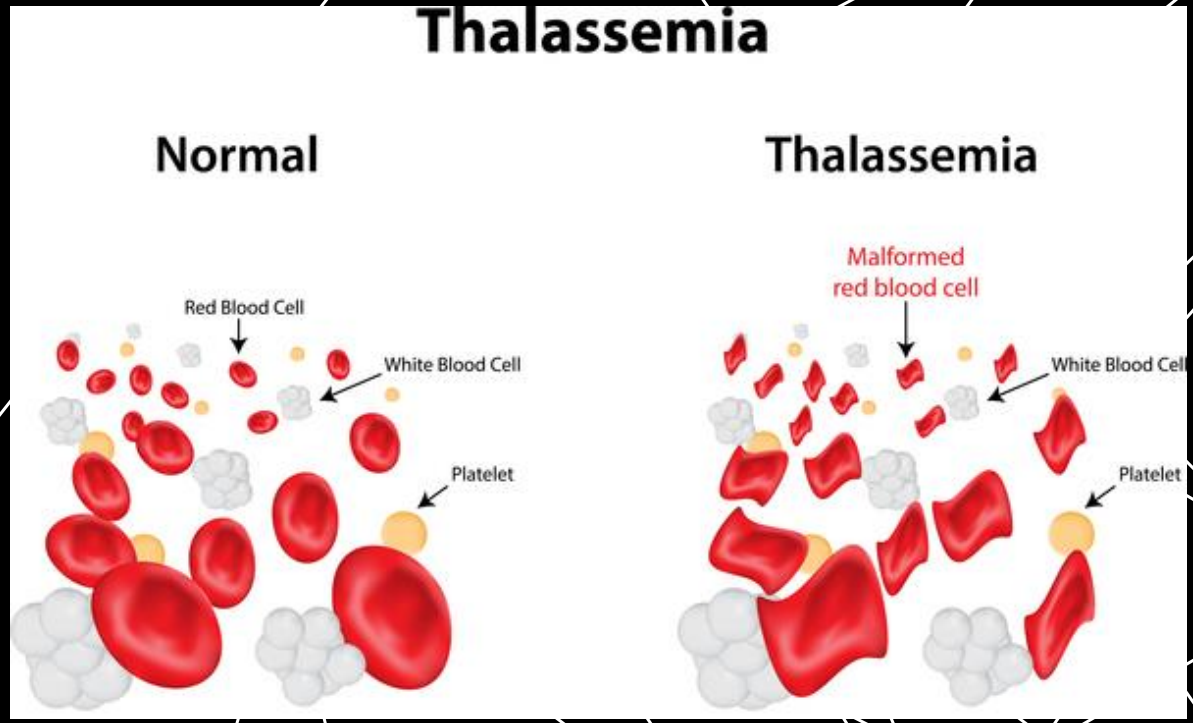
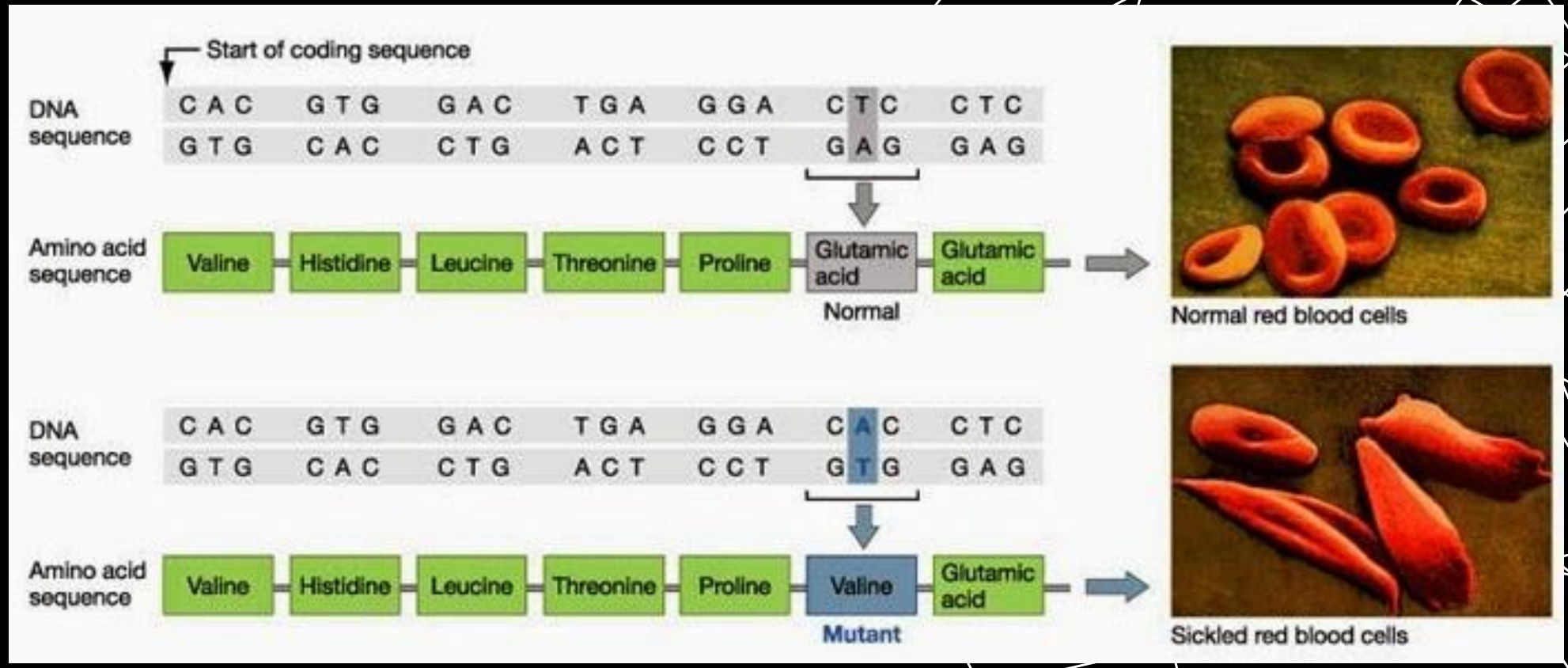


It has been shown that β-thalassemia, in which β-globin is not synthesized at all, is caused by a point mutation in the 17th or 39th codons of the gene encoding this protein.

SICKLE CELL ANEMIA

Sickle cell anemia results from a mutation that results in the replacement of the glutamic acid residue encoded by the GAG triplet at position 6 of the β-globin hemoglobin chain with a valine residue encoded by GTG.



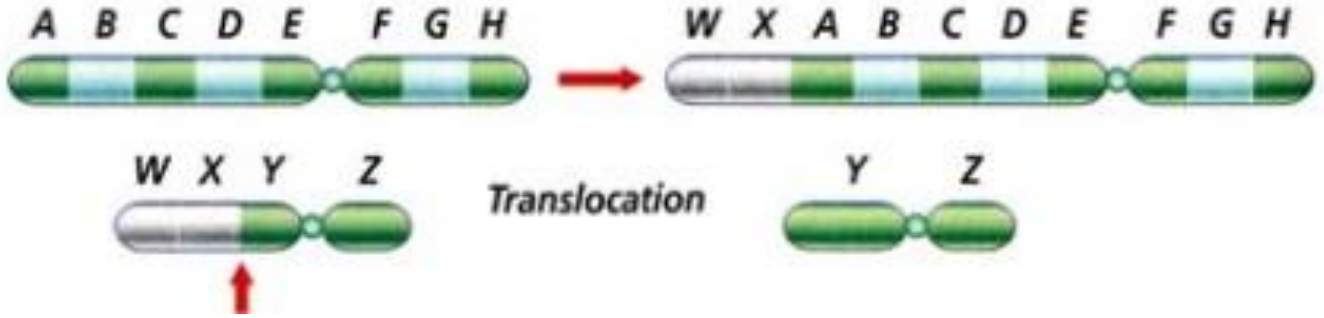
In normal DNA, two fragments with a length of 201 and 175 base pairs are detected, and in mutant DNA, a single fragment with a size of 376 nucleotide pairs (DdeI).

A When a part of a chromosome is left out, a deletion occurs.

B When part of a chromatid breaks off and attaches to its sister chromatid, an insertion occurs. The result is a duplication of genes on the same chromosome.

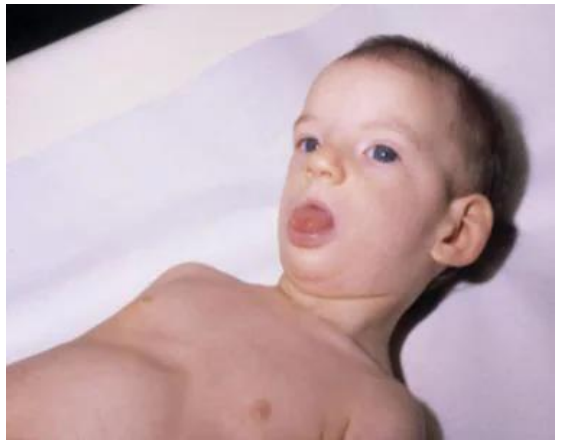
C When part of a chromosome breaks off and reattaches backwards, an inversion occurs.

D When part of one chromosome breaks off and is added to a different chromosome, a translocation occurs.



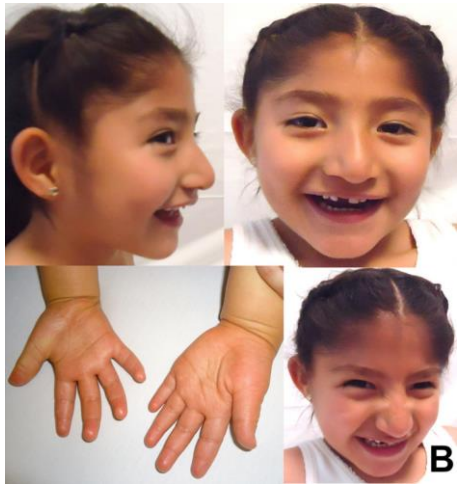
E This is when part of a chromosome is duplicated

EXAMPLES OF CHROMOSOMAL STRUCTURAL ABNORMALITIES



Cri-du-chat syndrome:

Babies with this condition have a cry that sounds like a cat. They also may have intellectual disability and congenital heart defects.



Angelman syndrome

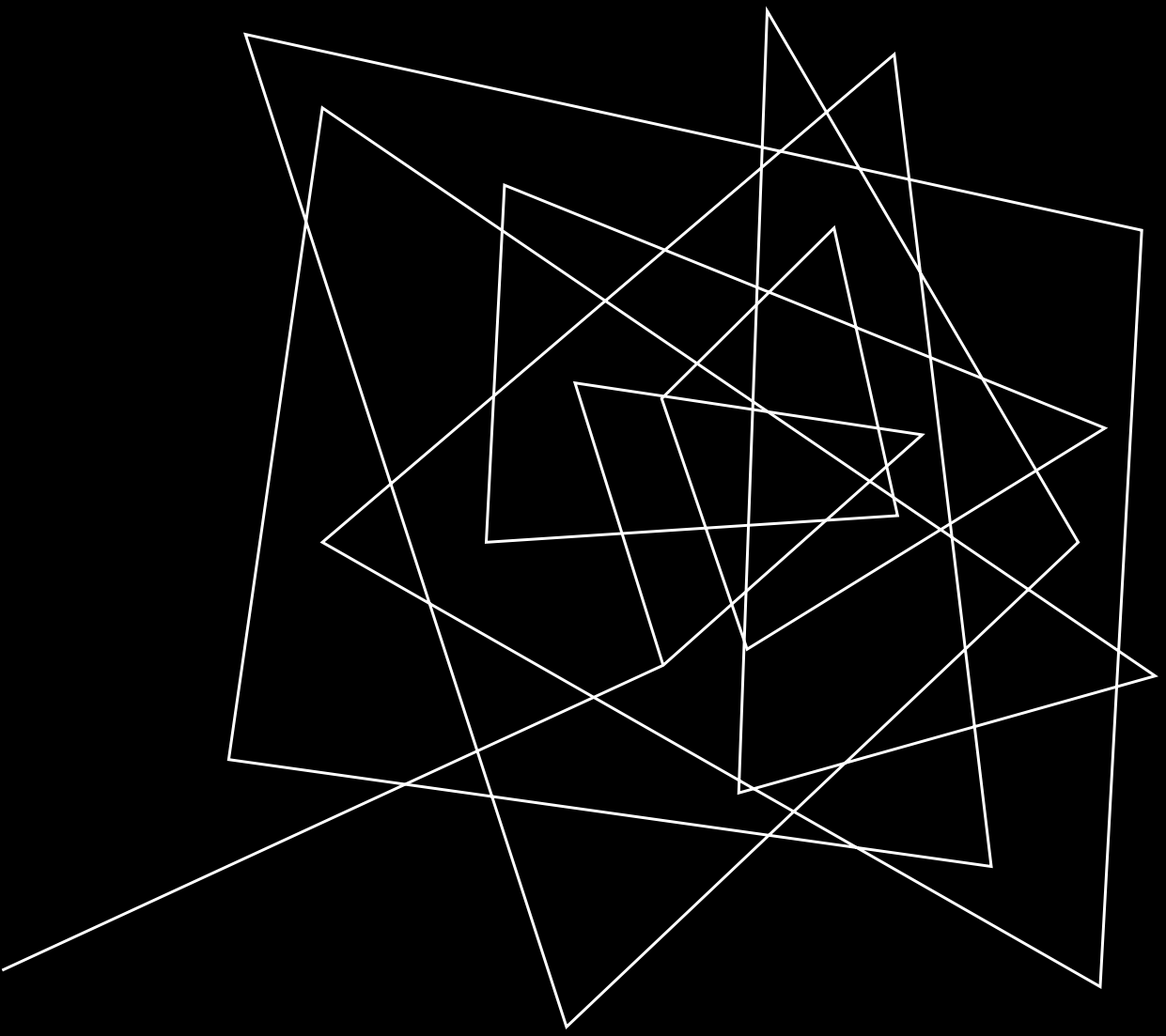
Infants with Angelman syndrome have intellectual disability, cannot speak, and have problems with their motor development.

Prader-Willi syndrome

This condition causes obesity, intellectual disability, lower than normal amounts of testosterone in boys, testes that do not descend properly into the scrotum, and muscles that are too relaxed in tone.

Fragile X syndrome

This is the second most common chromosomal cause of severe intellectual disability, after Down syndrome. Other characteristic features include an elongated face, prominent jaw, large ears, and, in boys, enlargement of the testicles.



GENE THERAPY

Its basic principles

AFTER THE MOLECULAR BASIS OF BACTERIAL TRANSFORMATION (THE TRANSFER OF GENES FROM ONE STRAIN TO ANOTHER) WAS ESTABLISHED, SCIENTISTS BEGAN TO HOPE THAT A SIMILAR MECHANISM—**THE INTRODUCTION OF NORMAL GENES INTO DEFECTIVE SOMATIC CELLS**—COULD BE USED TO TREAT HUMAN HEREDITARY DISEASES.

Genetic engineering technologies — the construction of functionally active genetic structures, their introduction into the human body, integration into the genome — make it possible to develop new, in some cases, unique genetic, biochemical, and physiological properties.

IN 1990, THE FIRST ATTEMPT WAS MADE TO USE GENE THERAPY FOR THE TREATMENT OF SCID (TABLE 21.1) IN TWO GIRLS.

Gene Product	Disease and Symptoms	Frequency	Treatment	Prognosis
Adenosine deaminase	Severe combined immunodeficiency (SCID) Loss of T- and B-lymphocytes	1:1,000,000	Bone marrow transplant; adenosine deaminase administration	Without treatment: fatal outcome by age two With treatment: improvement in patient condition
Low-Density Lipoprotein Receptor	Familial hypercholesterolemia Elevated blood cholesterol level, ischemic heart disease	1:500 (heterozygotes)	Diet, medication, liver transplant	Improvement in patient condition
Glucocerebrosidase	Gaucher's disease Accumulation of glucocerebrosides in macrophages, leading to liver, spleen, and bone involvement	1:2,500 (among Jews); rare in non-Jews	Symptomatic treatment: splenectomy, antibiotic administration, bone disease treatment, bone marrow transplant, glucocerebrosidase administration	Improvement in patient condition
Factor VIII of Coagulation System	Hemophilia A Alteration of factor VIII, leading to impaired blood clotting, chronic joint bleeding, severe bleeding from trauma	1:10,000 (in men)	Increasing factor VIII concentration through plasma transfusions	Increased life expectancy with constant treatment; risk of viral infection with transfusions
Phenylalanine hydroxylase	Phenylketonuria Excess phenylalanine in newborns' blood, mental retardation	1:10,000	Diet excluding phenylalanine	With early diagnosis and continuous treatment — generally favorable
α1-Antitrypsin	Emphysema Serum protease inhibitor deficiency, lung involvement, liver cirrhosis	1:3,500	Replacement therapy; reducing environmental risk factors	Disease progression slows

IN ACCORDANCE WITH US LAW, BEFORE A NEW DRUG IS APPROVED FOR USE, IT MUST PASS FOUR STRICTLY DEFINED STAGES OF TESTING.

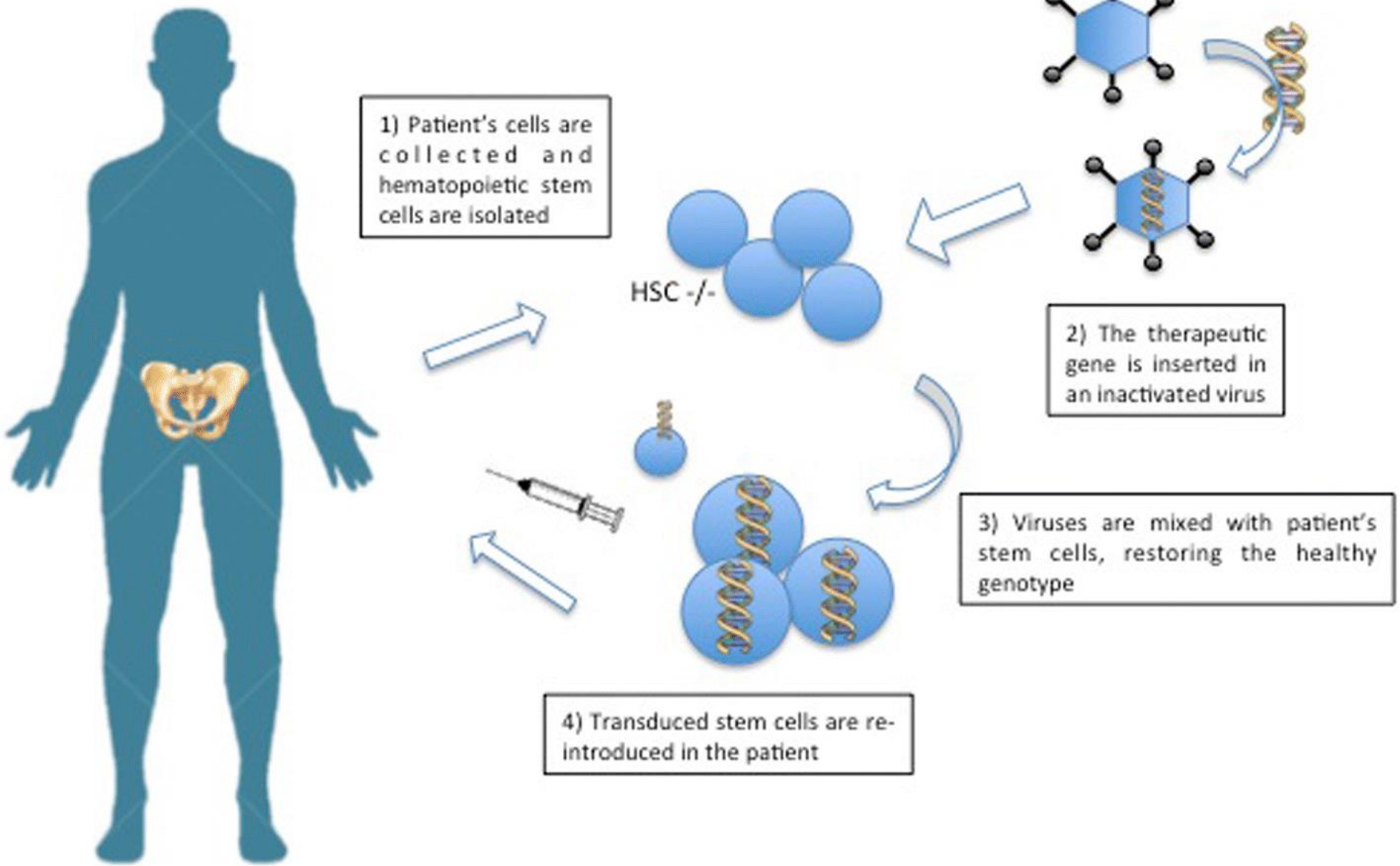
1. **Preclinical trials**, which include numerous experiments conducted in vitro and on laboratory animals.
2. **Phase I** clinical trials are conducted on a small number (from 6 to 10) of patients and are often aimed at testing the safety of the drug.
3. **Phase II** clinical trials are being conducted on a larger number of patients and are intended to test the effectiveness of the drug.
4. **Phase III** clinical trials are conducted in a large number of subjects and include a comprehensive analysis of the reliability and efficacy of the drug, while using information obtained in the previous stages.

Ex vivo gene therapy

➢ ***Ex vivo* gene** therapy typically includes the following steps:

1. Obtaining cells from a patient.
2. Correction of a genetic defect by transferring the desired gene into isolated cells.
3. Selection and growth of genetically "corrected" cells.
4. Infusion or transplantation of these cells into the patient.

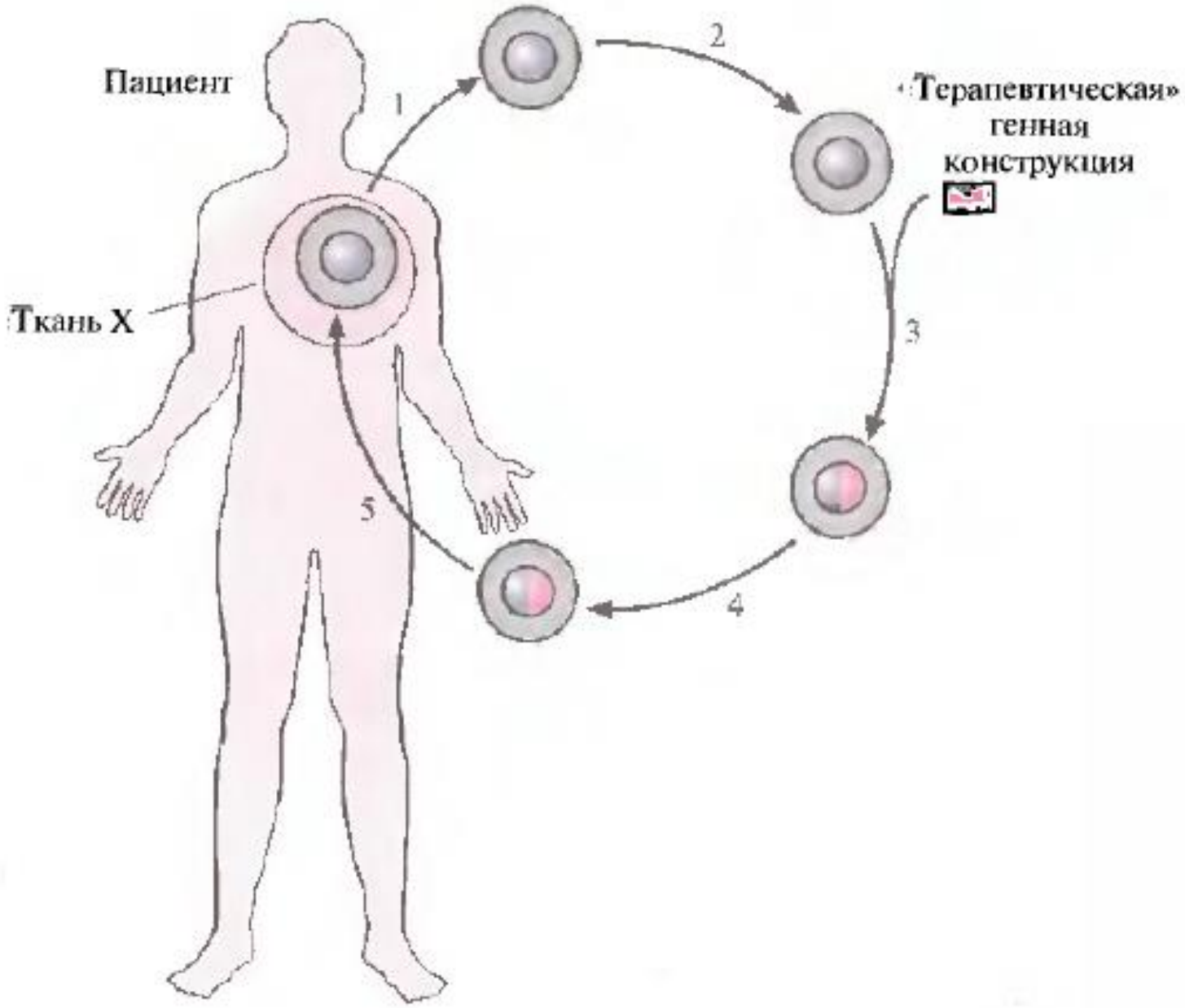
Ex Vivo Gene therapy



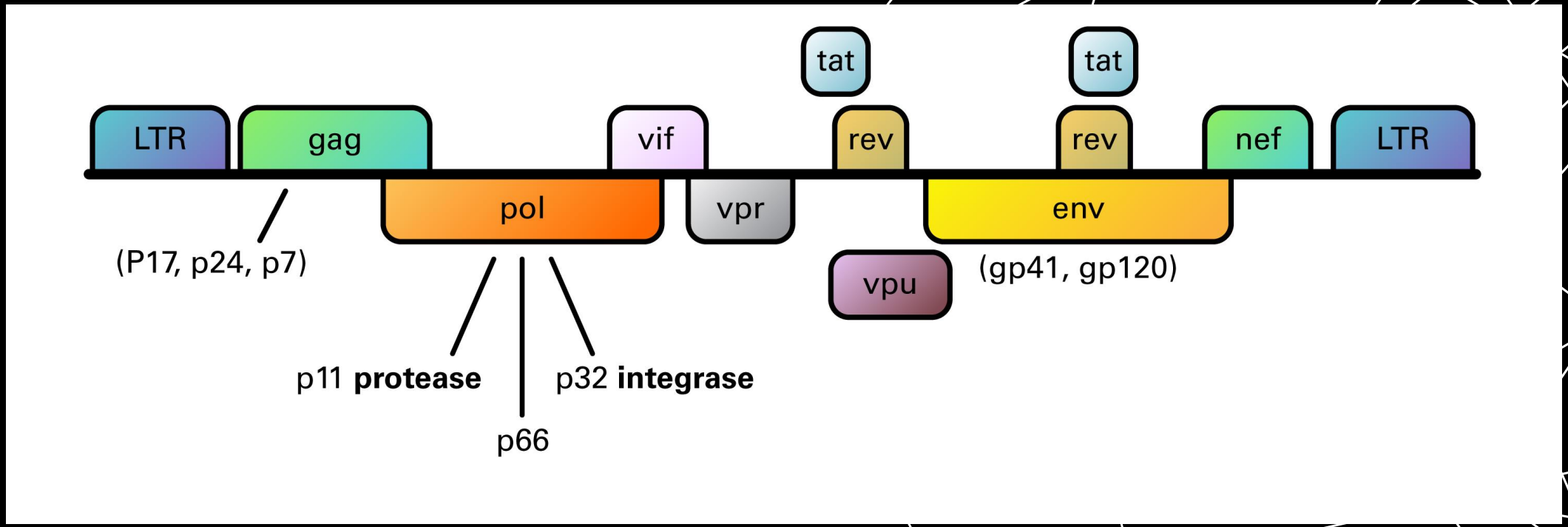
Schematic representation of ex vivo Gene Therapy.

The procedure includes:

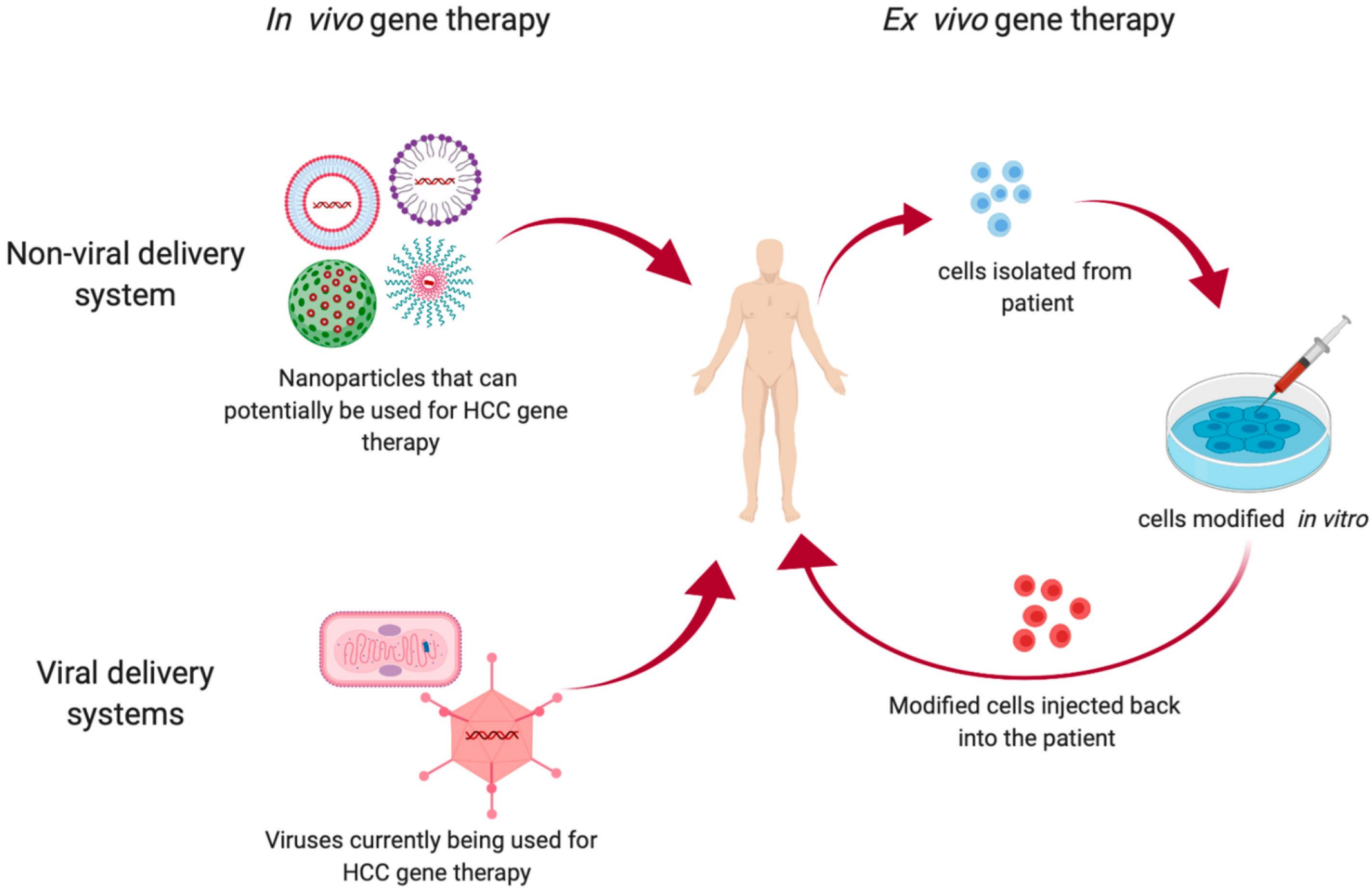
1) obtaining cells with a gene defect from a patient;
2) cultivation of isolated cells;
3) transfection of a "therapeutic" gene construct into isolated cells;
4) selection, cultivation and testing of transfected cells:
5) transplantation or transfusion of the transfected cells to the patient.



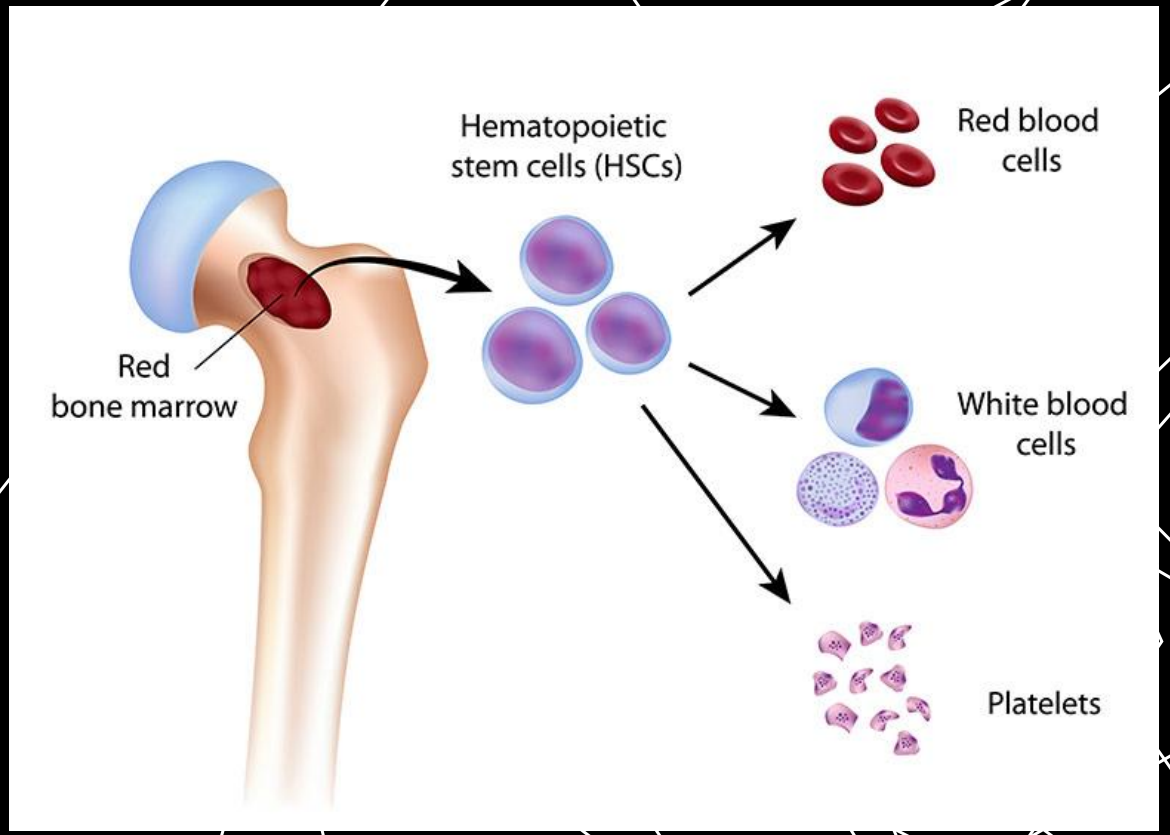
Retroviruses belong to a family of enveloped RNA viruses (Retroviridae) that infect vertebrates. Retroviruses are divided into five sub genera, with two members (Delta retroviruses HTLV-1 and HTLV-2 and Lentiviruses, HIV-1 and HIV-2) causing human disease. They can be exogenous, transmitted horizontally among hosts, or endogenous, inherited vertically through the genomes of their hosts. The virus requires a reverse transcriptase to convert the viral RNA genome into DNA, which integrates into host chromosomes and utilizes host proteins for gene expression and replication. Retroviruses can also be oncogenic through insertional activation of host genes. In humans, HIV is responsible for causing the acquired immunodeficiency syndrome (AIDS) and HTLV-1 and HTLV-2 are the retroviruses that cause human T-cell leukemia/lymphomas.



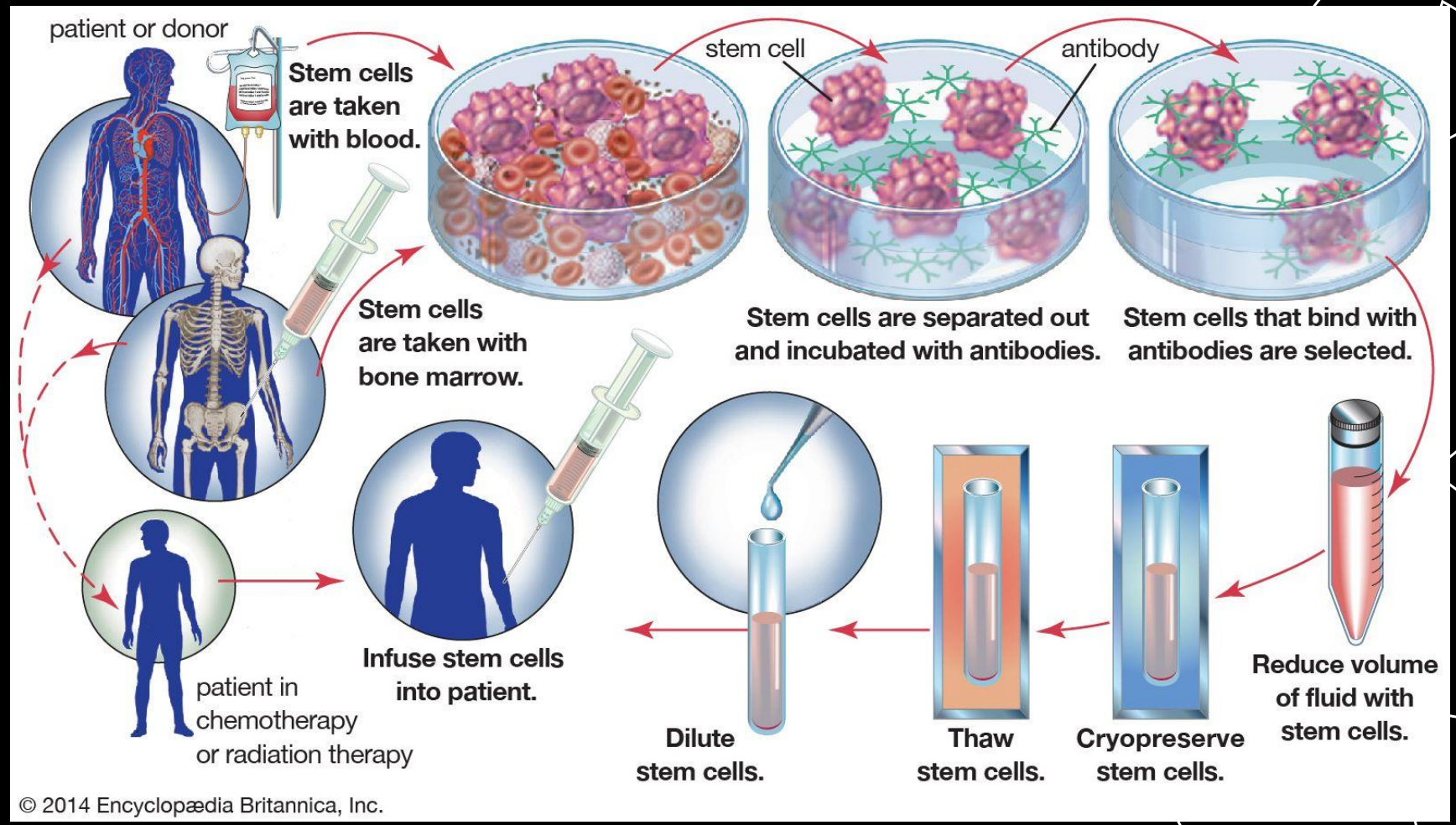
Organization of the HIV-1 genome showing the reading frames of the genes coding for structural and regulatory proteins: LTR = long terminal repeat; gag = group-specific antigen; pol = polymerase; env = envelope. The gag, pol, and env genes are expressed as precursor polyproteins, which are then cleaved to yield mature viral proteins. In the case of the regulator genes, the proteins of tat and rev are composed of two gene regions. The genome consists of 9,200-9,600 nucleotides in HIV-1.


In vivo gene therapy
Ex vivo gene therapy
Non-viral delivery system
Nanoparticles that can potentially be used for HCC gene therapy
cells isolated from patient
cells modified *in vitro*
Modified cells injected back into the patient
Viral delivery systems
Viruses currently being used for HCC gene therapy

- The most likely candidates for **ex vivo gene therapy** are patients with hereditary diseases treated with bone marrow transplantation.
- The therapeutic effect of bone marrow transplantation in relation to a number of diseases is associated with the presence of **totipotent embryonic stem cells** in it, which occur at a frequency of 10^{-4}-10^{-5}, can proliferate and differentiate into various cell types, such as B- and T-lymphocytes (B cells and T cells), macrophages, erythrocytes, platelets and osteoclasts.
- For example, when a gene mutation impairs macrophage function, bone marrow transplantation provides the recipient with a constant supply of competent macrophages derived from a population of totipotent stem cells.



Hereditary diseases for treatment resulting in bone marrow transplantation.

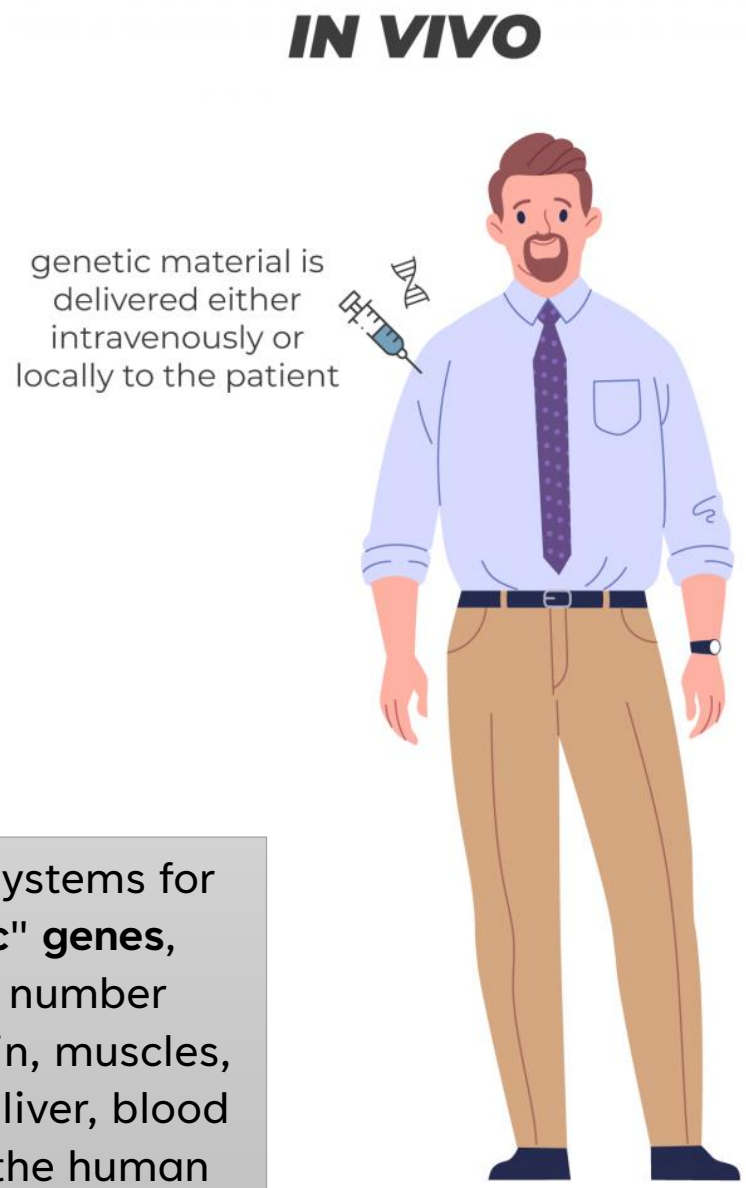


1. ADA-dependent Severe Combined Immunodeficiency
2. Adrenoleukodystrophy
3. Chediak–Higashi Syndrome
4. Chronic Granulomatosis
5. Fanconi Anemia
6. Gaucher's Disease
7. gpI-115 Deficiency
8. Actin Deficiency in Granulocytes
9. Hunter's Disease
10. Hurler Syndrome
11. Neonatal Agranulocytosis (Infant Agranulocytosis)
12. Maroteaux–Lamy Disease (Mucopolysaccharidosis Type VI)
13. Metachromatic Leukodystrophy
14. ADA-independent Severe Combined Immunodeficiency
15. Osteoporosis
16. Purine Nucleoside Phosphorylase Deficiency
17. Reticular Dysplasia
18. Sanfilippo Syndrome
19. Sickle Cell Anemia
20. Thalassemia
21. Wiskott–Aldrich Syndrome
22. X-linked Agammaglobulinemia

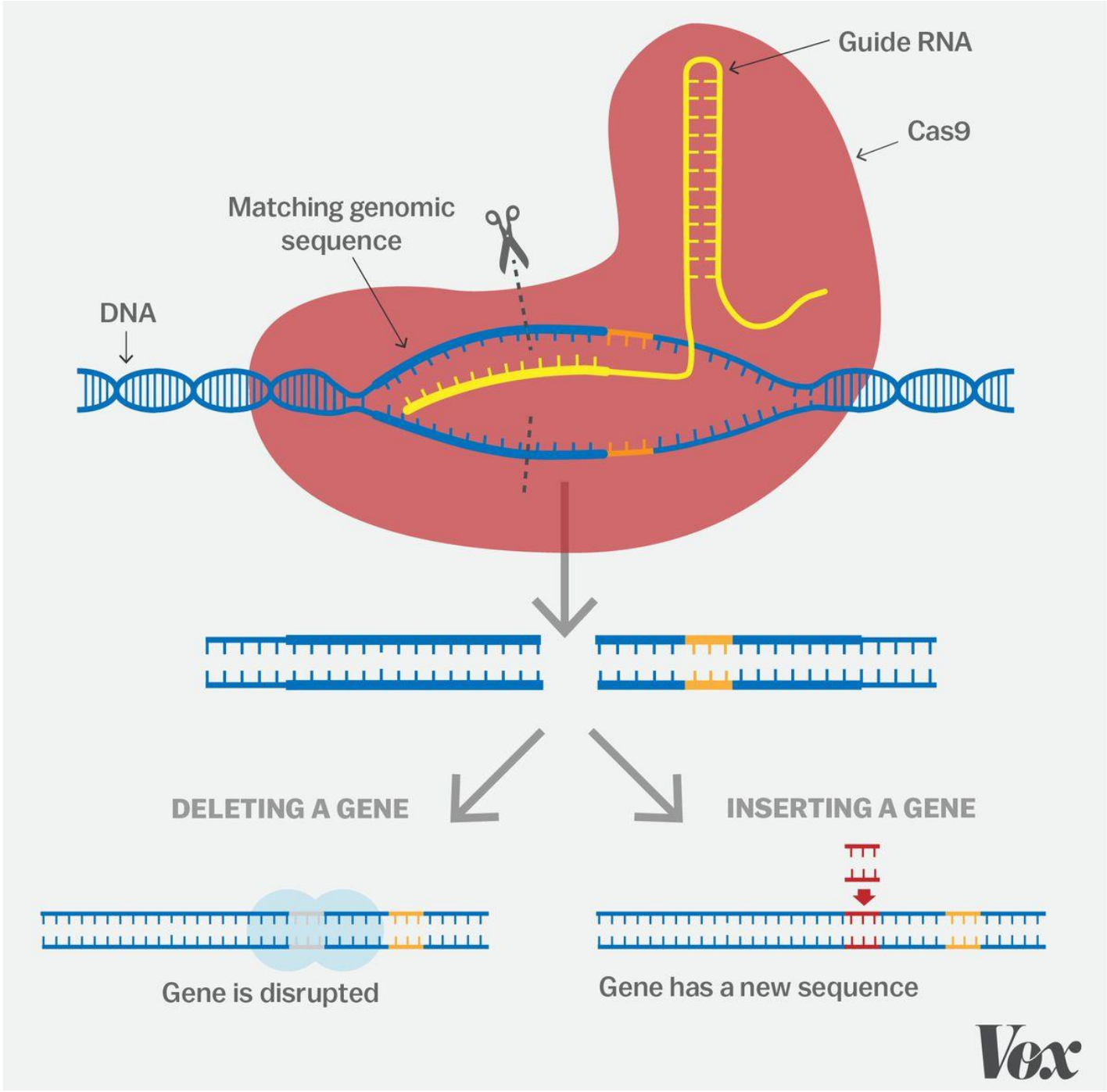
IN VIVO GENE

***In vivo* gene** therapy in vivo gene therapy involves the delivery of a "therapeutic" gene directly into the cells of a specific patient tissue.

Viral and non-viral vector systems for the delivery of "**therapeutic**" **genes**, taking into account a large number potential target tissues (skin, muscles, lungs, brain, colon, spleen, liver, blood cells) and their location in the human body.



At least 6,000 human diseases are caused by heritable genetic mutations[1]. A long-time dream of physicians and patients alike has been to specifically treat these diseases by manipulating the genetic code in affected patients. This dream became one step closer to reality when last month, researchers injected CRISPR, the most promising of existing genetic engineering tools, into a live adult for the first time[2].



THE PROCEDURE IS RELATIVELY SIMPLE

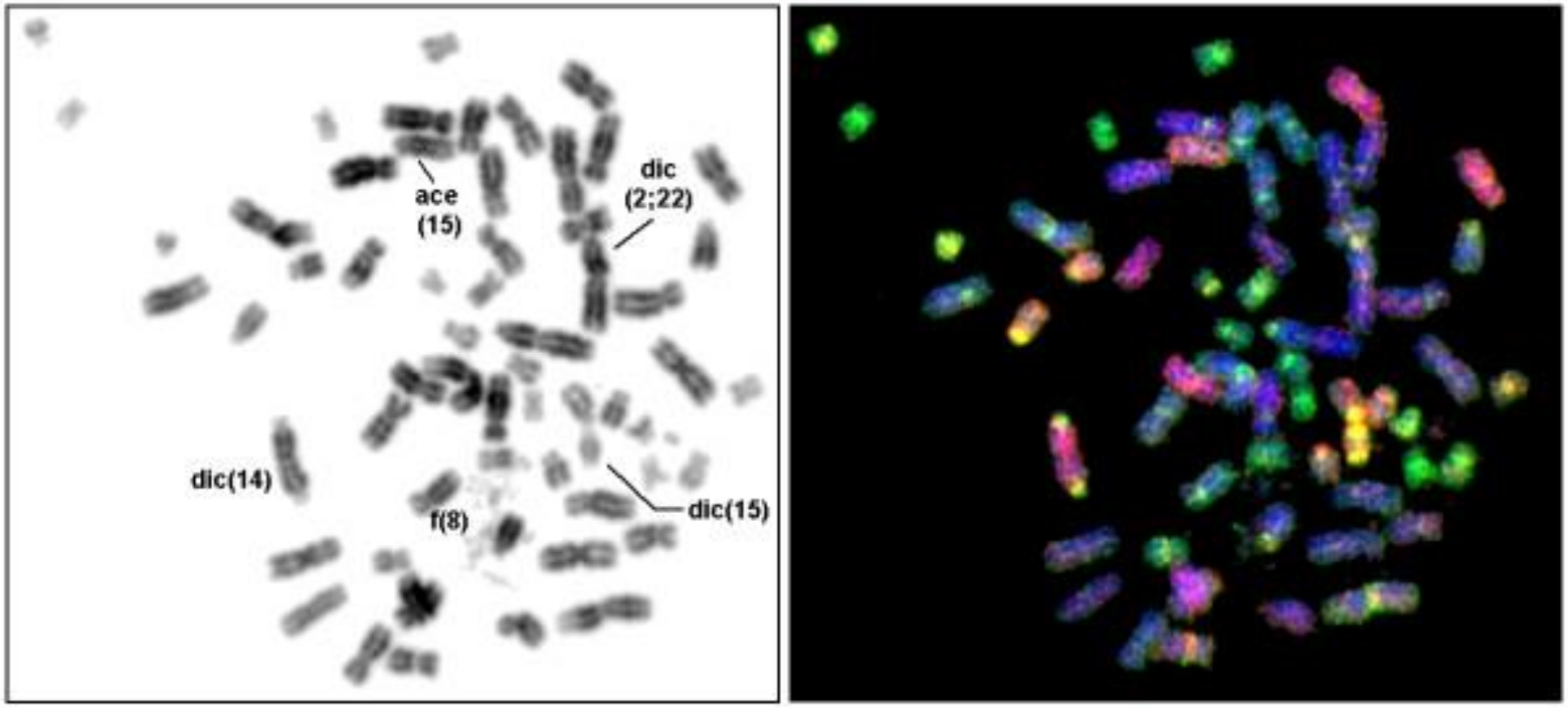
First, a surgeon creates an incision in the eye. Next, through that incision a few droplets of the crispr-equipped adenoviruses are injected onto the patient's retina[2].

The crispr-cas9 system itself is adapted from a natural antiviral defense mechanism found in bacteria (see figure 1). A short guide RNA sequence is generated that complements a target DNA sequence. Once the guide RNA attaches to the correct site, the enzyme cas9 cuts the DNA. This cut is then repaired by the cell's endogenous DNA repair machinery[7].

Often times, a "repair template" DNA sequence is also added so that the repaired genomic DNA has a specific, desired sequence[8]. Using this technology, researchers hope to fix the *CEP290* mutation and produce a properly spliced RNA transcript.

This should result in a fully functioning CEP290 protein and hopefully restore vision for these patients. It is too early to tell if this treatment will be effective however, researchers plan to monitor patient progress over the next few months[2].

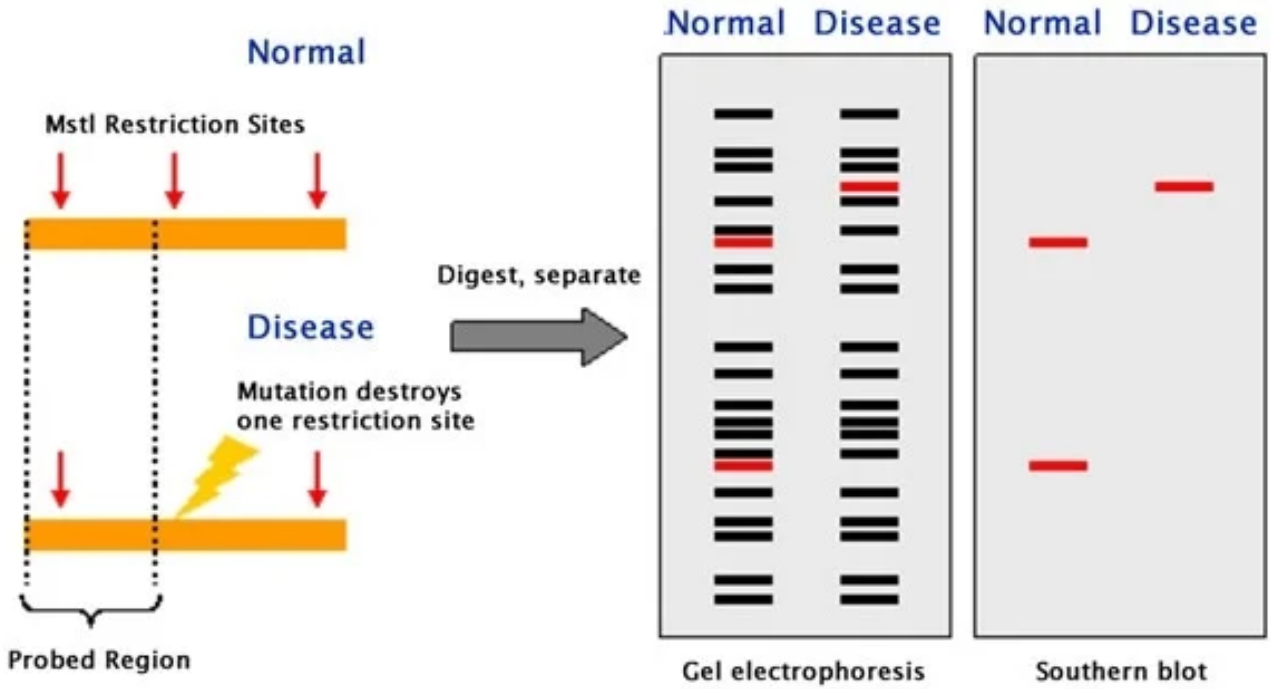
DIAGNOSTIC METHODS
(FLUORESCENT IN SITU HYBRIDIZATION (FISH))



Chromosome aberrations studied by Multiplex-FISH (M-FISH) method applying whole chromosome painting probes. Result is depicted in inverted DAPI-banding on the left and after M-FISH on the right. The near-triploid (hypotriploid) metaphase carried 60 chromosomes and multiple aberrations including fragmented chromatin belonging to chromosome 8 (= f(8)), dicentric chromosomes like a dic(14;14), a dic(15;15), and a dic(2;22), accompanied by acentric fragments (e.g. ace(15)).

• RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) TECHNIQUE

Restriction fragment length polymorphism (RFLP) is a technique invented in 1984 by the English scientist Alec Jeffreys during research into hereditary diseases. It is used for the analysis of unique patterns in DNA fragments in order to genetically differentiate between organisms – these patterns are called Variable Number of Tandem Repeats (VNTRs).
Genetic polymorphism is defined as the inherited genetic differences among individuals in over 1% of normal population. The RFLP technique exploits these differences in DNA sequences to recognize and study both intraspecies and interspecies variation.



Restriction Fragment Length Polymorphism (RFLP)

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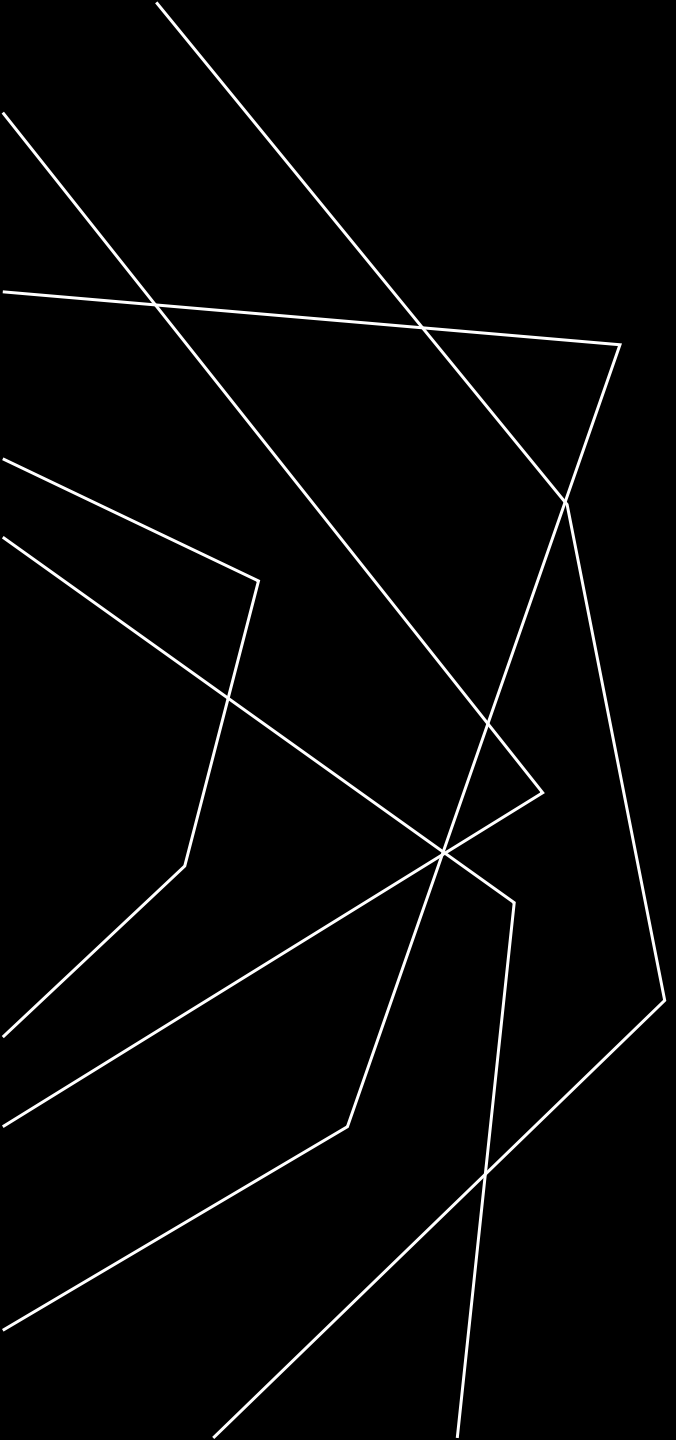

THANK YOU

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