

RECOMBINANT DNA TECHNOLOGY IN MEDICINE

50 years of recombinant drugs

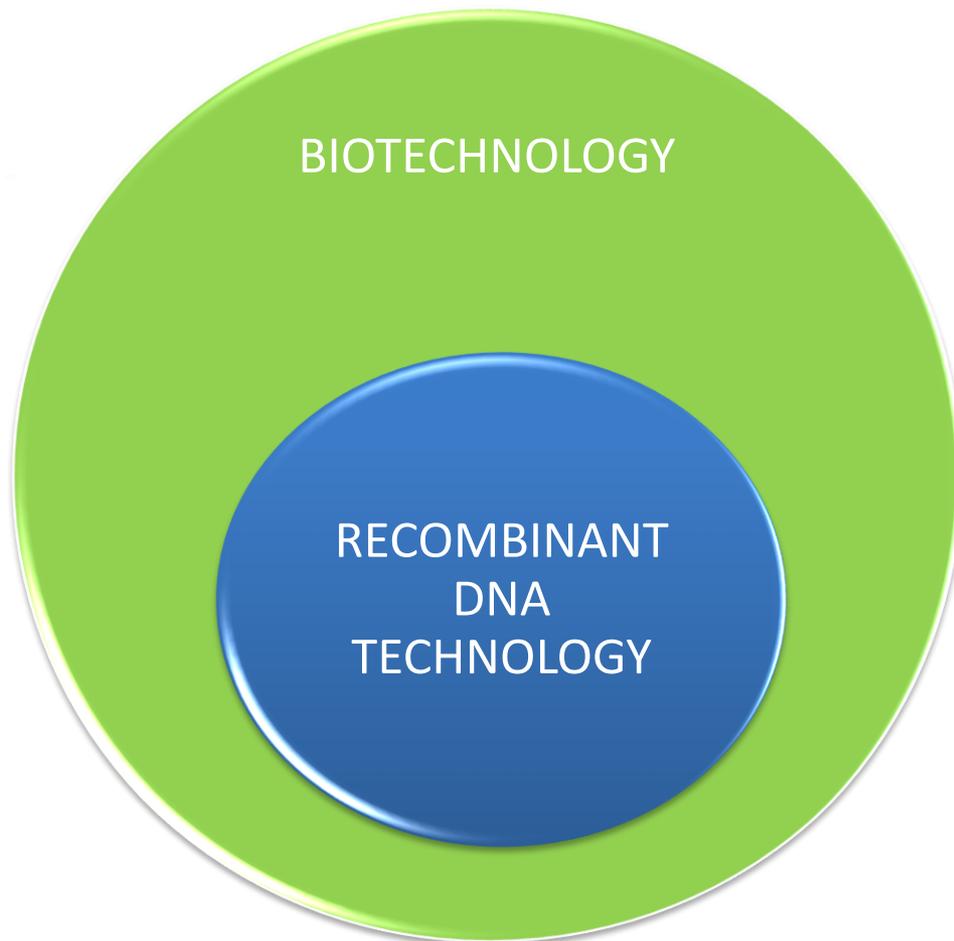


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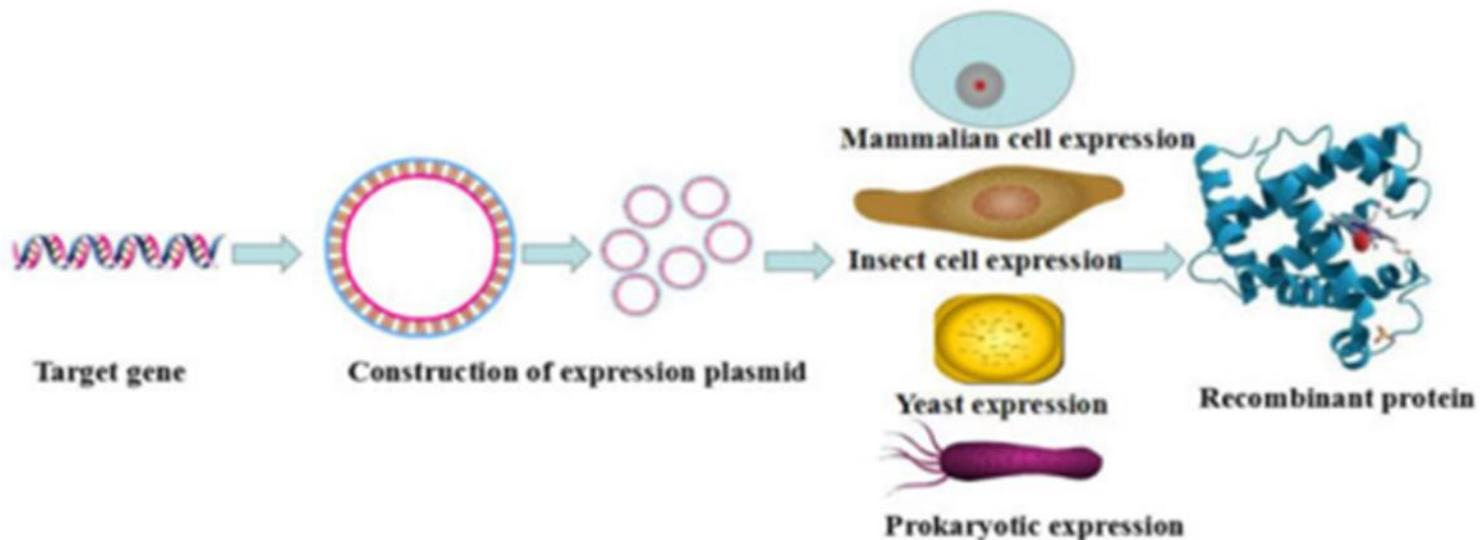
RECOMBINANT DNA TECHNOLOGY





RECOMBINANT DNA TECHNOLOGY

Recombinant DNA (rDNA) technology refers to the process of joining DNA molecules from two different sources and inserting them into a host organism, to generate products for human use.





APPLICATIONS OF RECOMBINANT DNA TECHNOLOGY

Therapeutic products

Vaccines
Recombinant proteins
Vectors
Growth hormones
Anticancer drugs

Genetically modified products

GM vegetables
GM crops
GM microbes
GM animals

Recombinant DNA Technology

Diagnosis

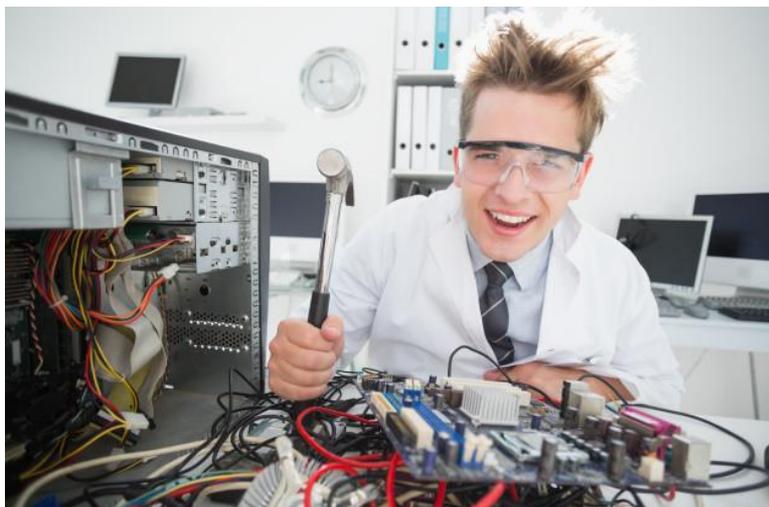
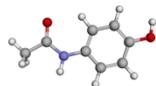
Gene therapy
CRISPR
Monitoring devices
Therapeutic strategies

Energy applications

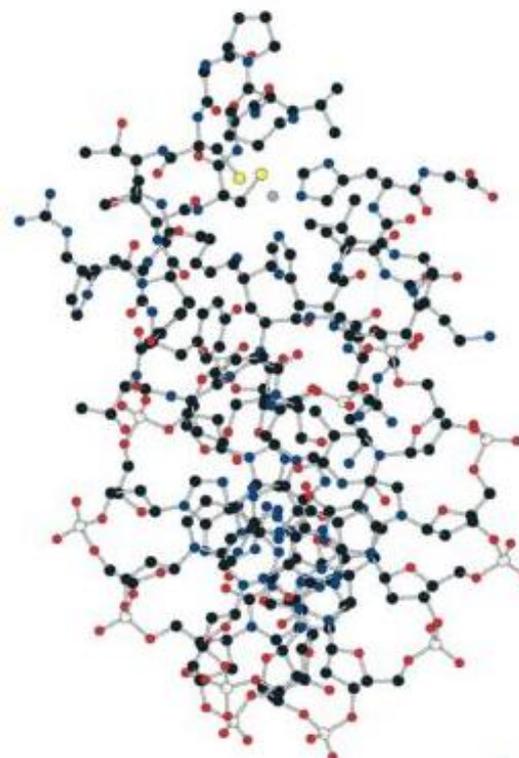
Biohydrogen
Bioethanol
Biomethanol
Biobutanol



SMALL MOLECULES AND PROTEINS



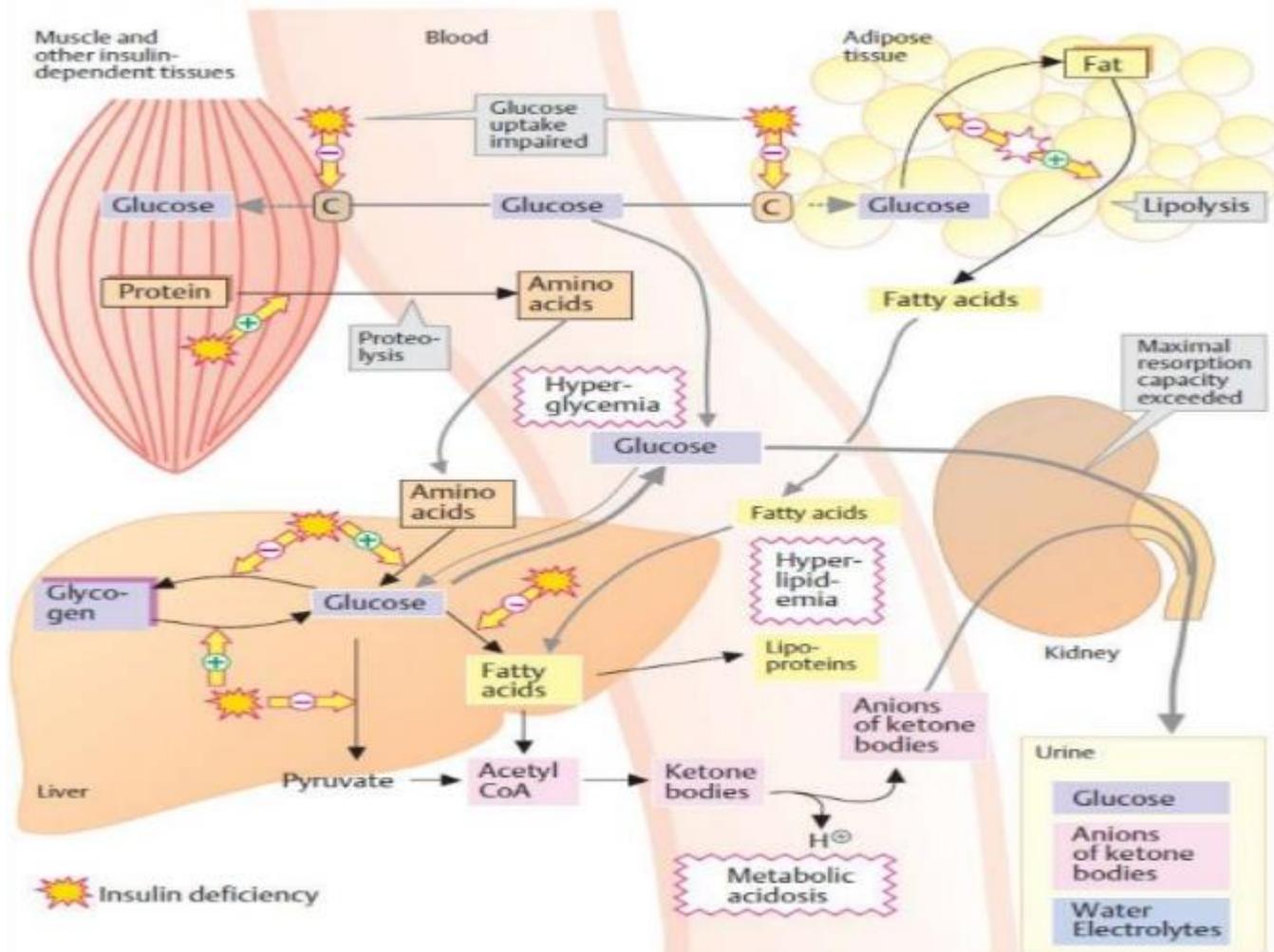
Discovered by chance



Very similar to proteins in the body



DIABETES (Type 1)

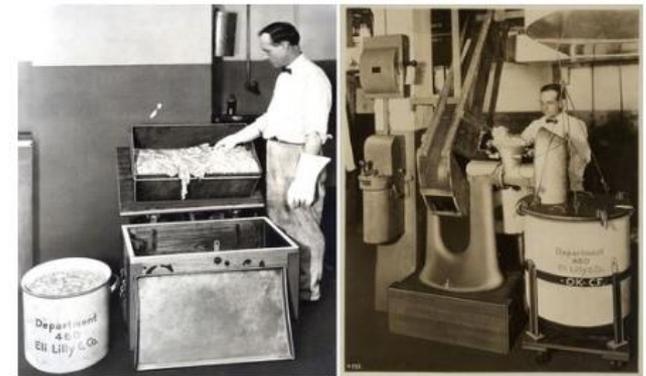
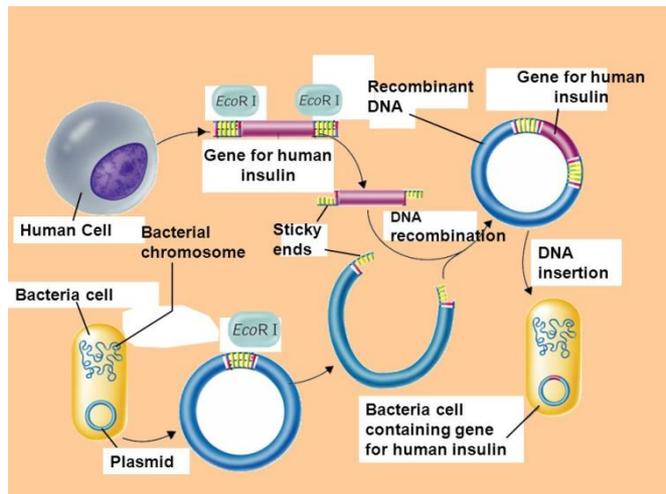




History of Diabetes treatment

- Until 1920s deadly disease with no cure
- Porcine and bovine insulin (allergic reactions)
- 2 tones of pig pancreatic tissue for 250 ml of Insulin

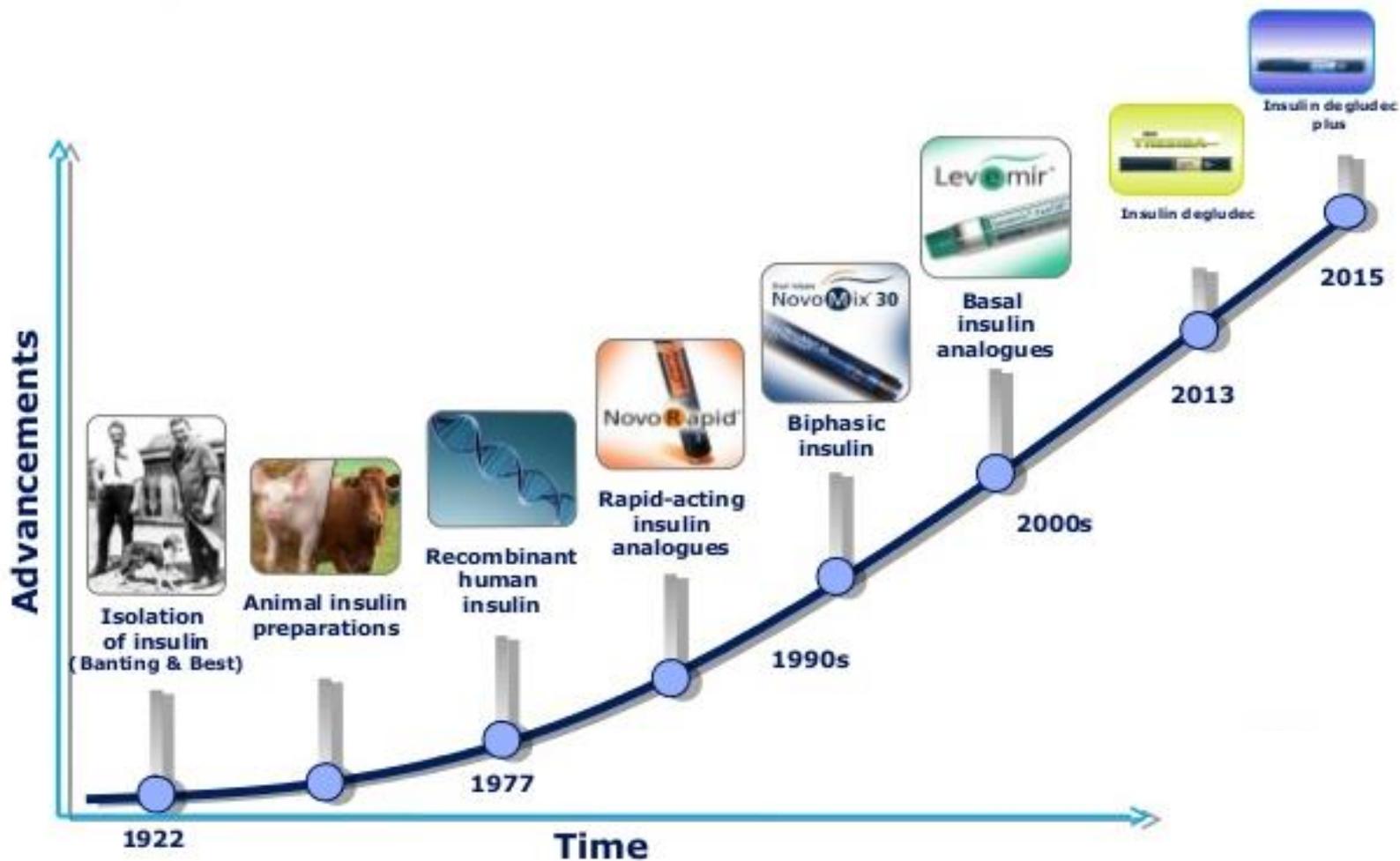
- Recombinant (human) insulin (1980s)



On the left, pancreas glands are examined as they arrive from the meatpacking house. On the right, the glands are run through grinders before the next step in the process, insulin extraction.

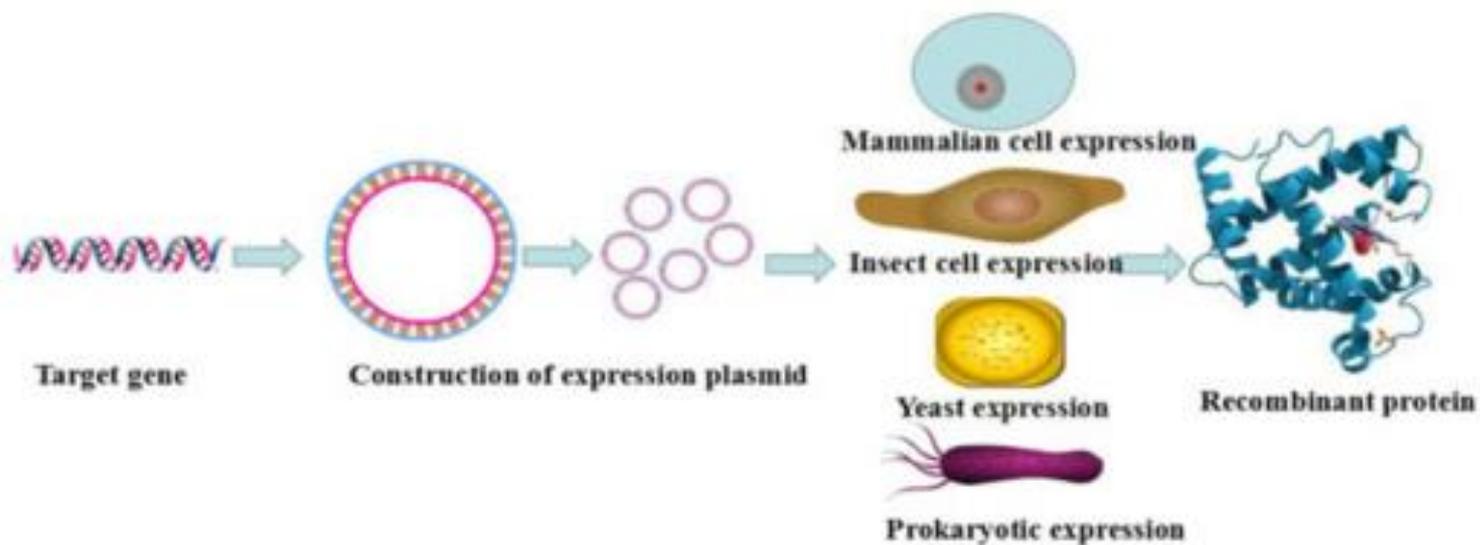


Advancements in Insulin therapy



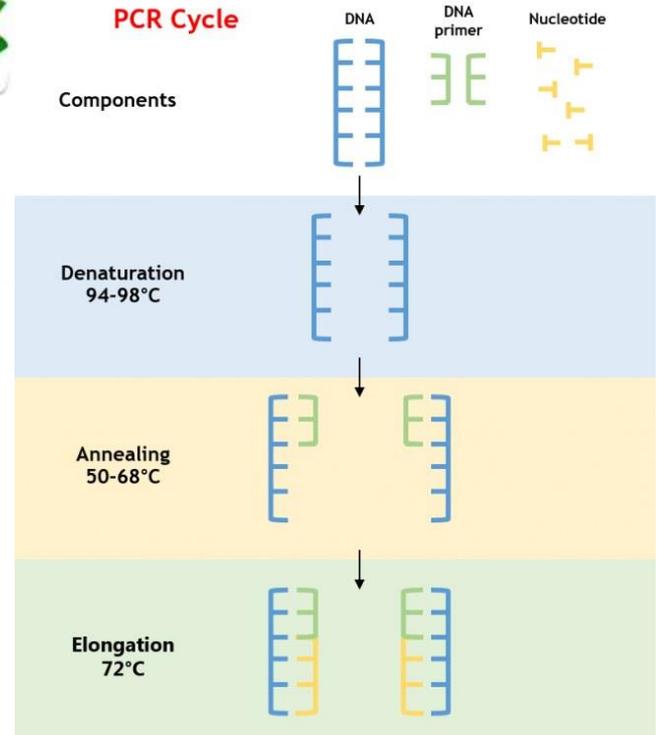
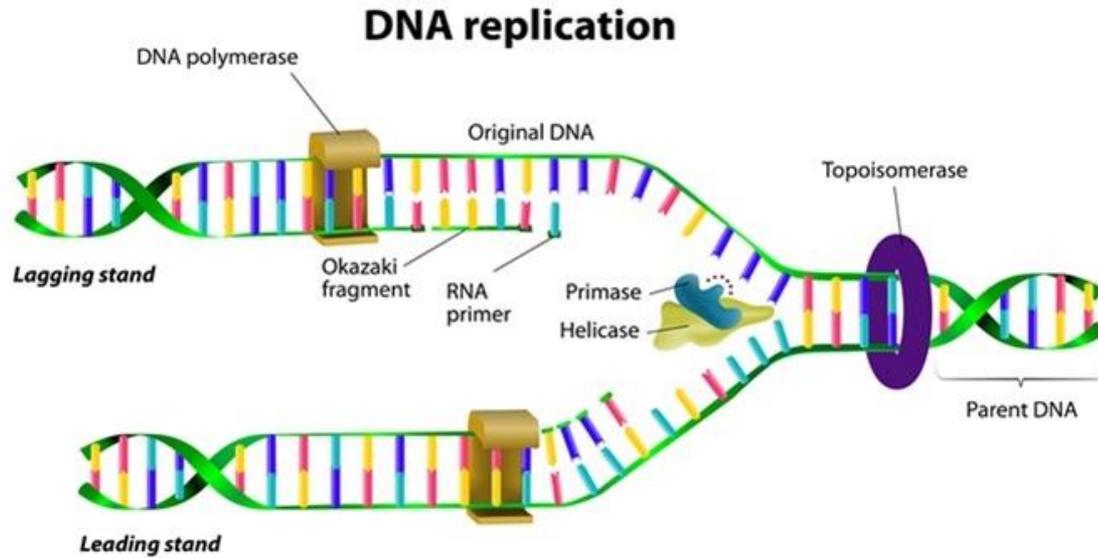


From Gene to Protein





Polymerase chain reaction





Polymerase chain reaction

- Kary Mullis 1983
- thermo-stabile polymerase

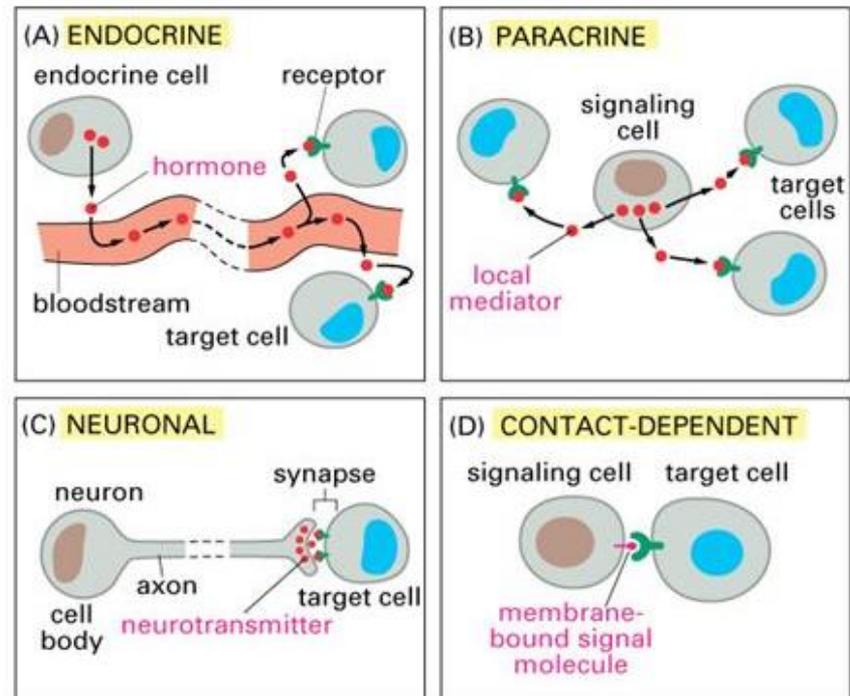
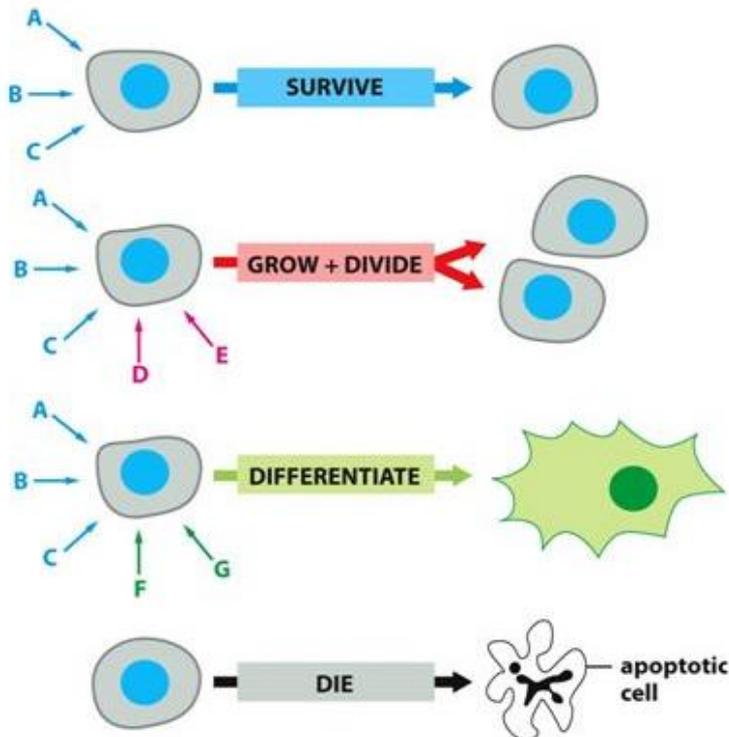
<https://www.youtube.com/watch?v=iQsu3Kz9NYo>



Principles of drug discovery

Signaling mechanisms

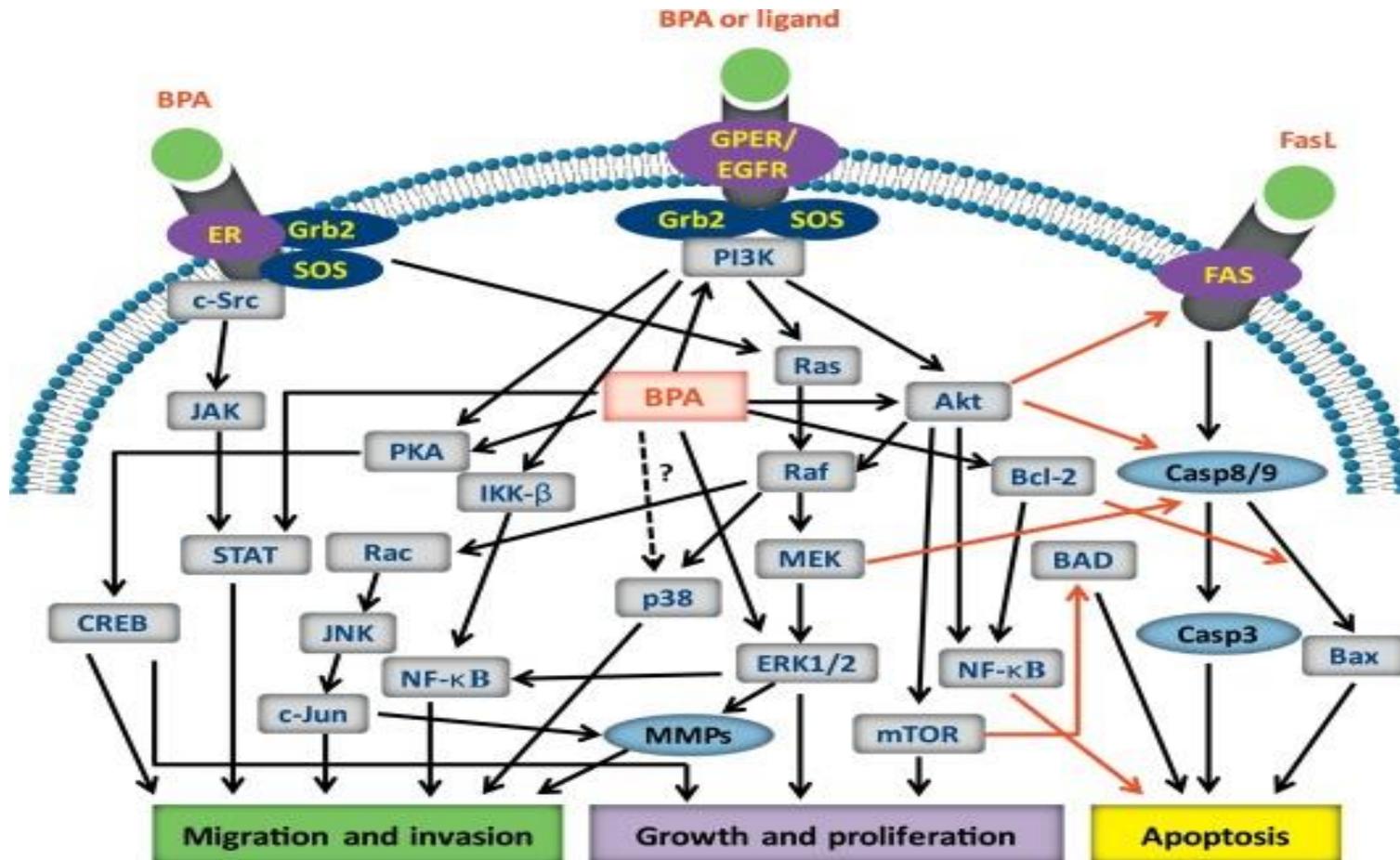
Signaling responses



Receptors & ligands!

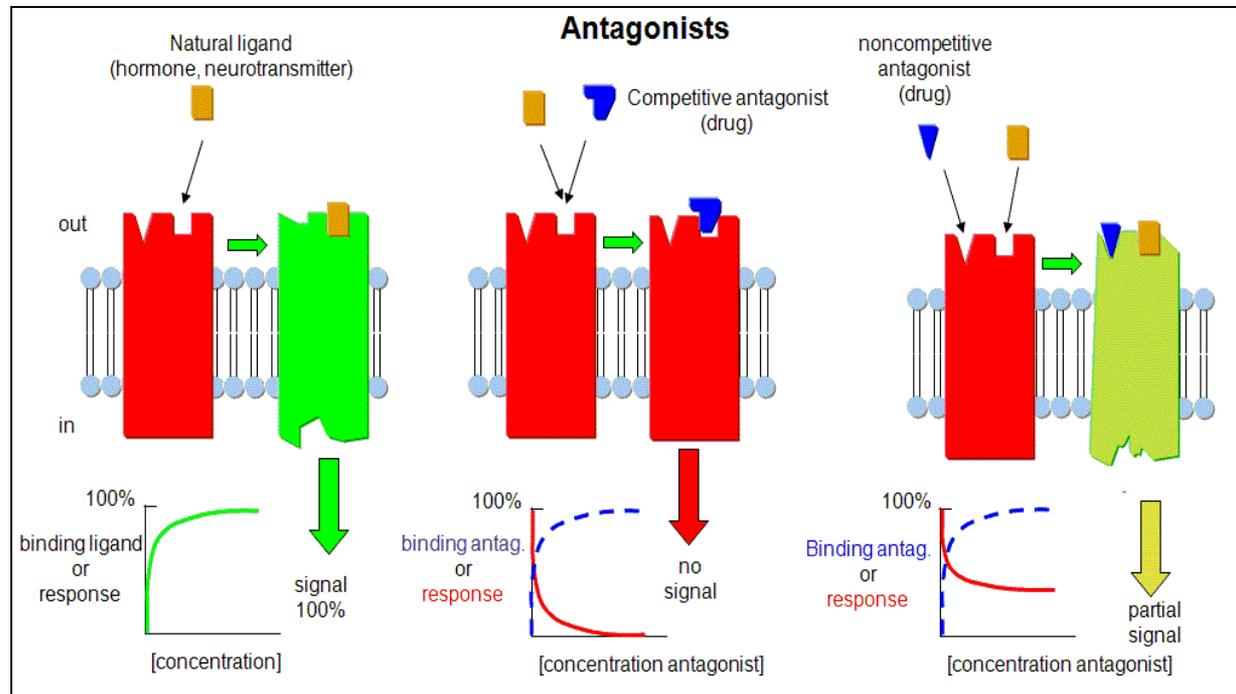
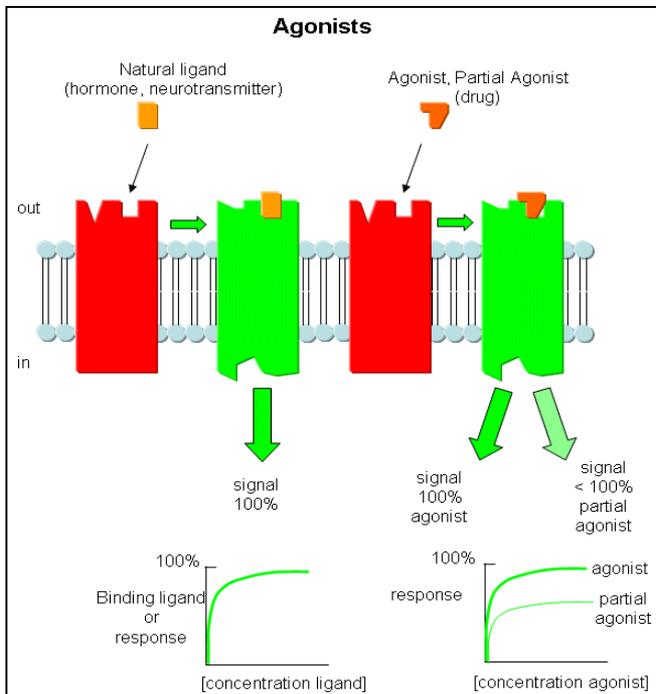


Cell communication & physiological response





Agonists and antagonists



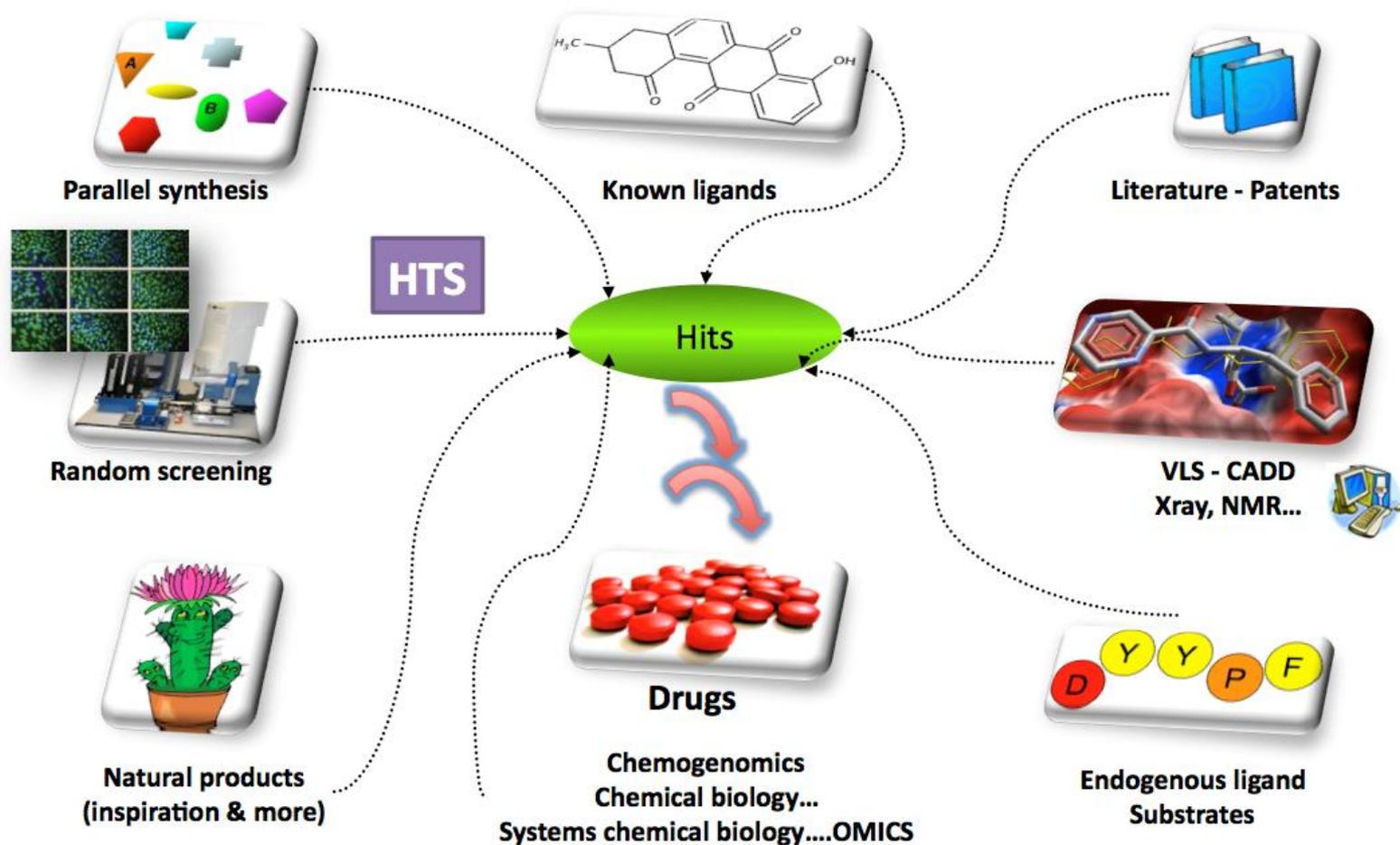


PROCESS OF RECOMBINANT DRUG PRODUCTION





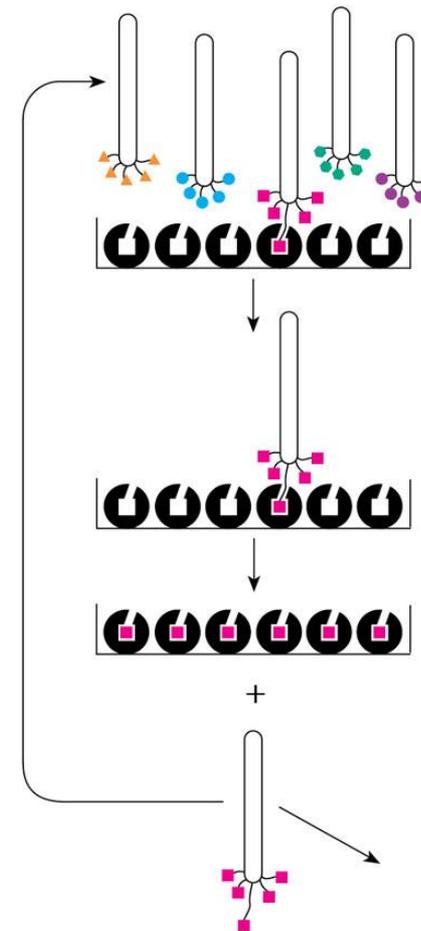
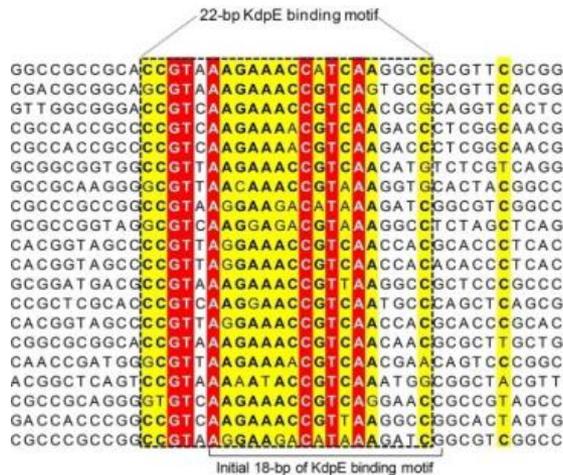
Searching for new drug candidates





Peptide candidates screening

- Phage Display
- Bacteriophages
- Libraries of peptides
- Up to 10^{12} combinations



A library of phage, each displaying a different peptide sequence, is exposed to a plate coated with the target.

Unbound phage are washed away.

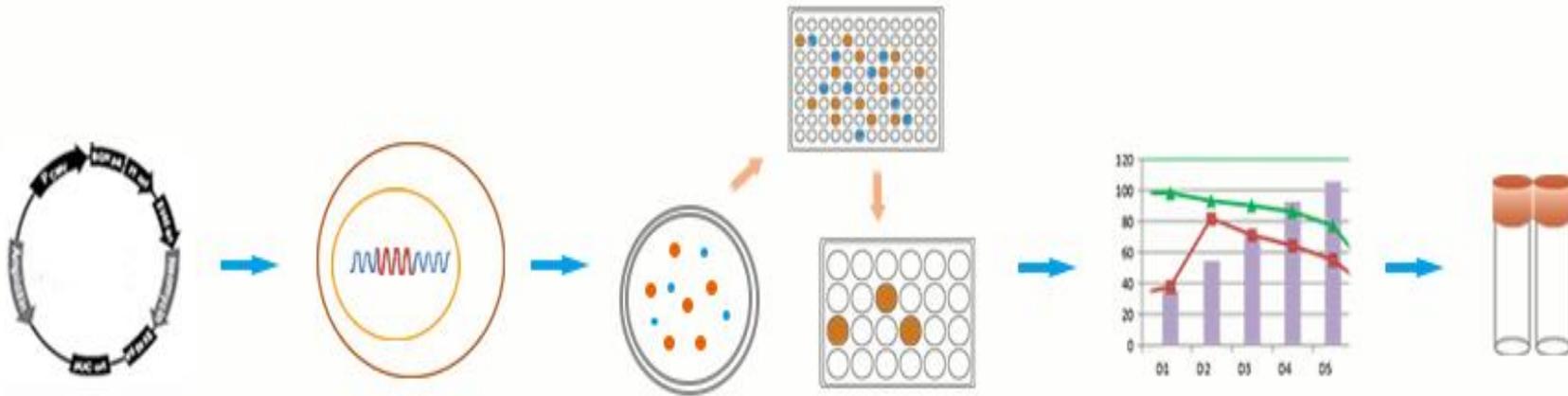
Specifically-bound phage are eluted with an excess of a known ligand for the target, or by lowering pH.

After 3 rounds, individual clones are isolated and sequenced.

- Sequencing of positive clones



Development of recombinant cell line



Gene Synthesis

- gene optimization
- plasmid construction

Cell Transfection

- Max-Prep
- transient evaluation
- stable transfection

Single Clones Screening

- cell pool screening
- MTX/MSX amplification
- subcloning & single clones screening

QC

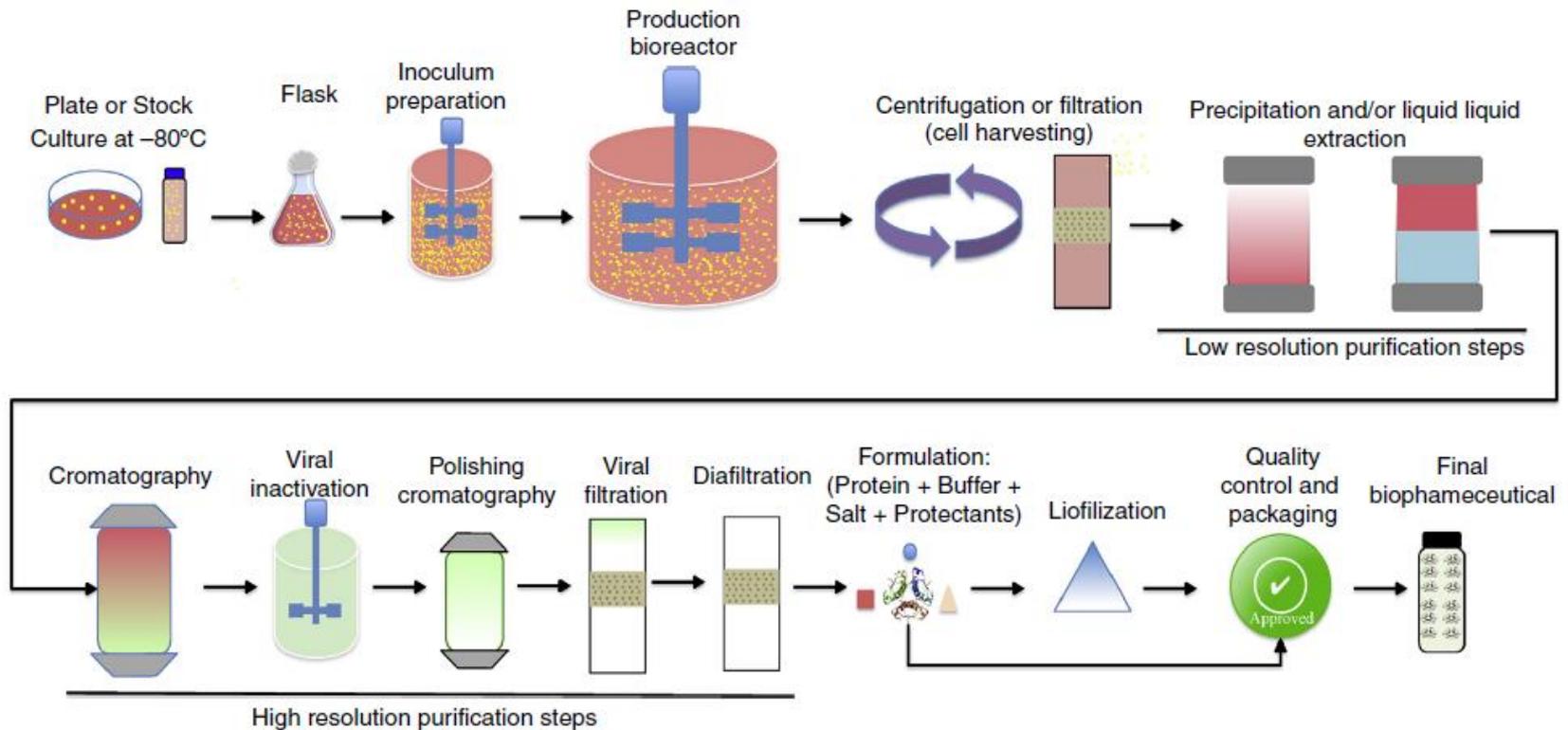
- batch culture
- stability test
- mycoplasma test

Delivery

- identify and freeze
- packing & delivery



Expression and purification of recombinant protein





RECOMBINANT MOLECULES IN CLINICAL USE

-Enzymes and regulatory proteins

(Human growth hormone, Insulin,...)

-Antibodies

(Cancer therapy)

-Vaccines

(HPV virus)

-Proteins for diagnostics

(affinity molecules)

-Gene therapy

(nucleic acids, recombinant vectors)





Pros and Cons of recombinant proteins

Pros:

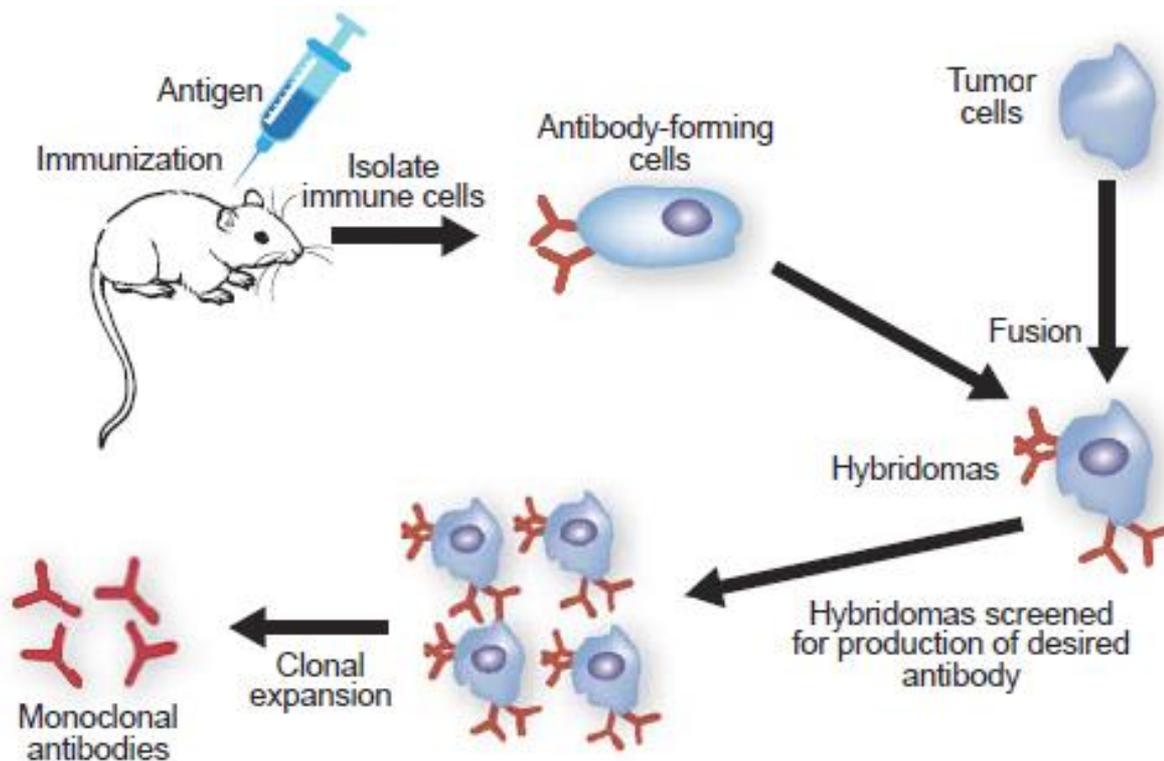
- high efficiency
- homology to endogenous proteins in our body
- few side effects
- new therapy options

Cons:

- expensive to produce
- protein structure usually requires parenteral application
- extensive purification, sterility required
- systemic effect



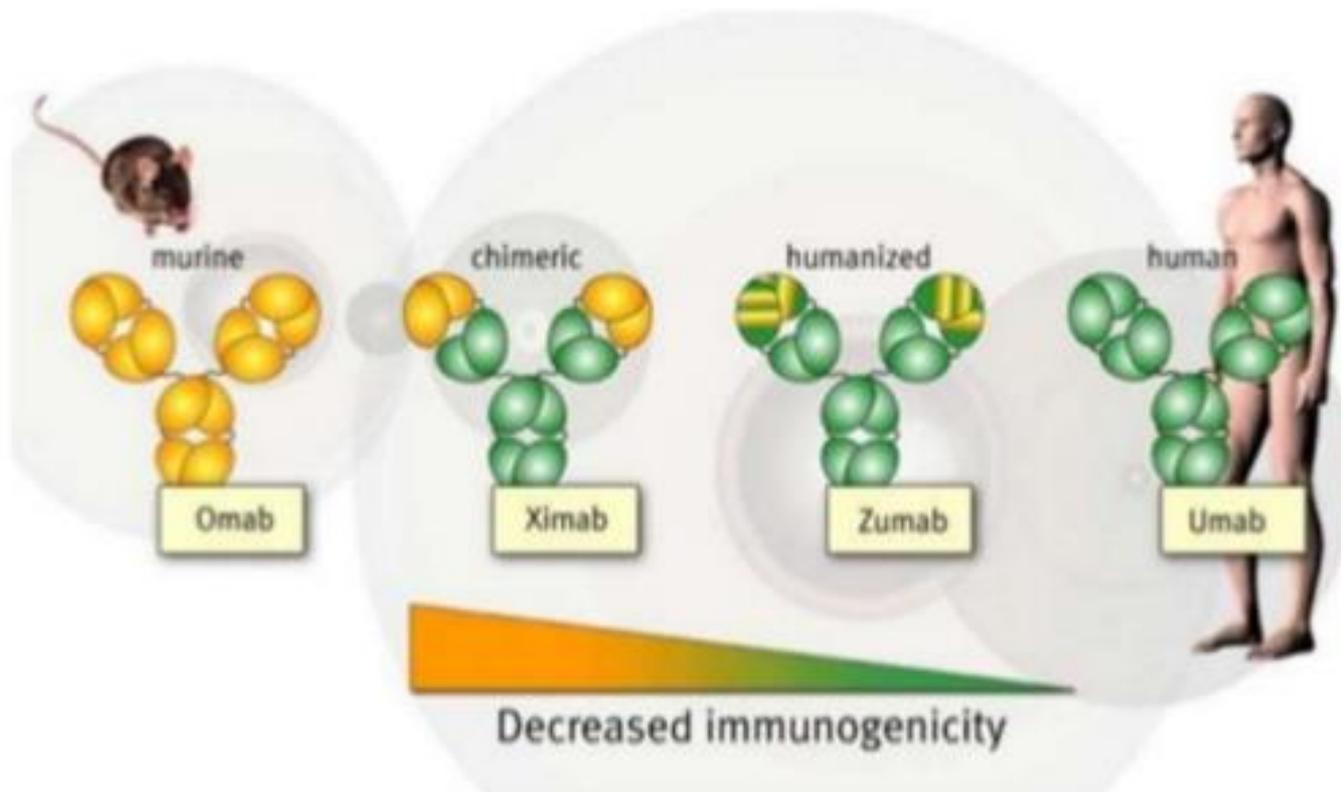
ANTIBODIES





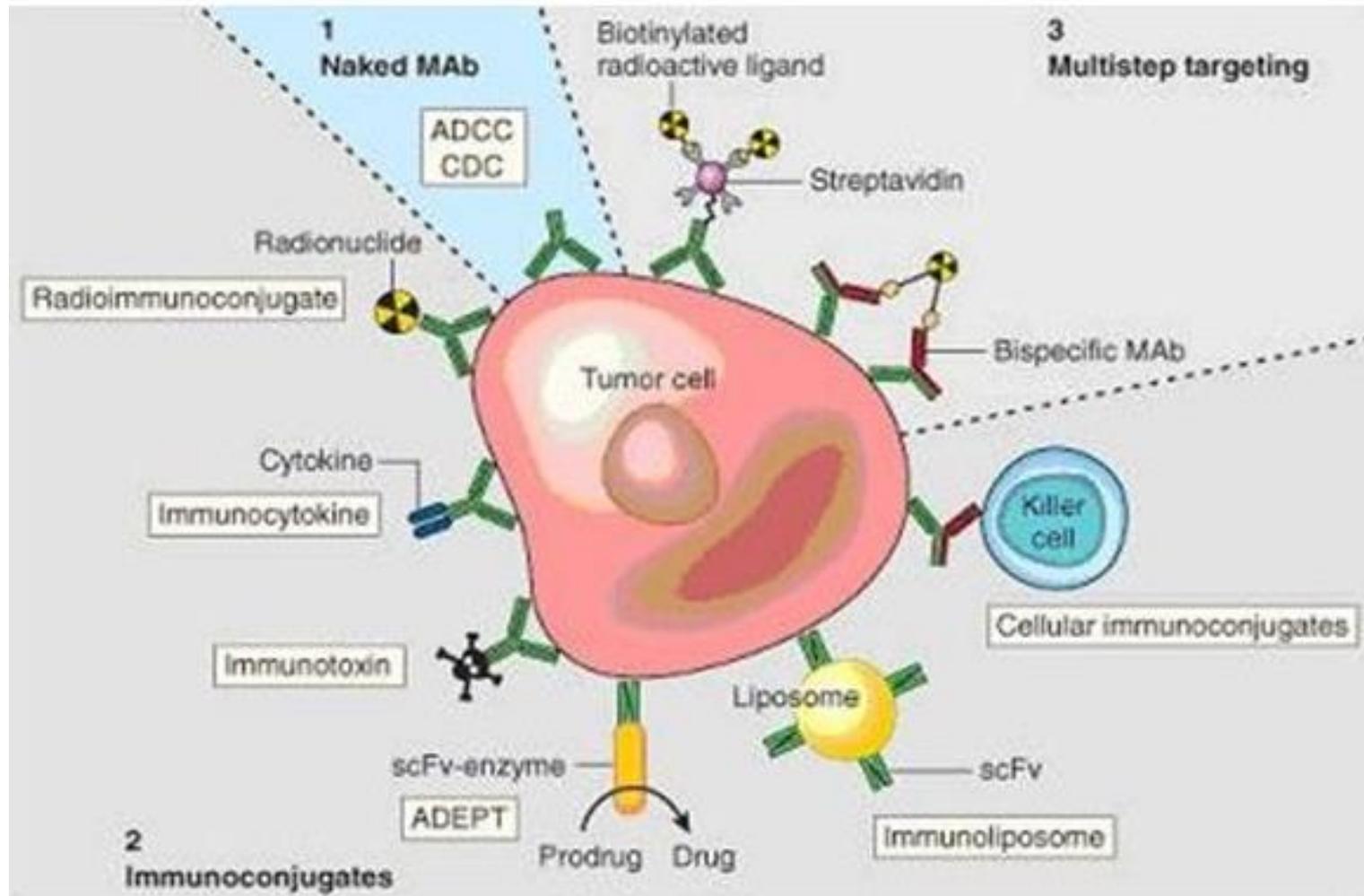
Types of monoclonal antibodies

- mAb's can be raised from a number of species
- Being **protein based**, they will be recognised by our immune system





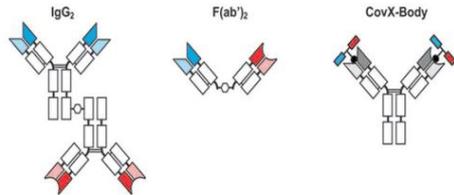
Antibodies targeting cancer cells



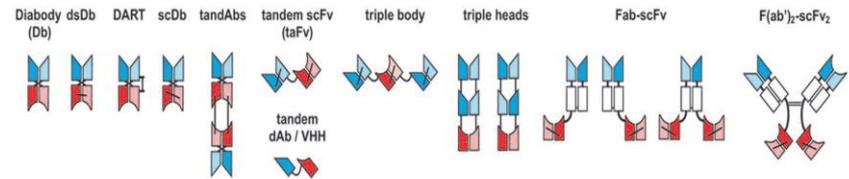


Varieties of antibody fragments

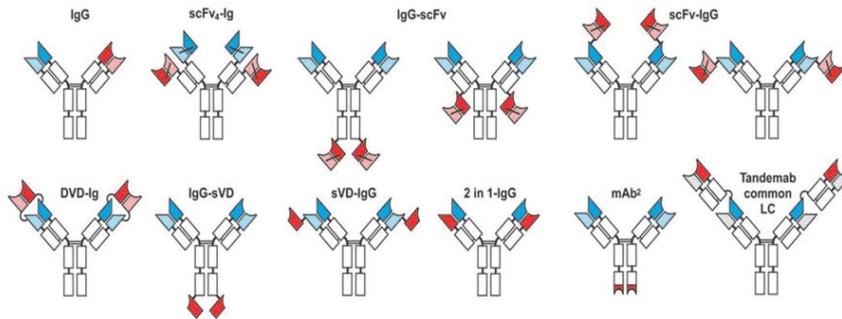
bispecific antibody conjugates



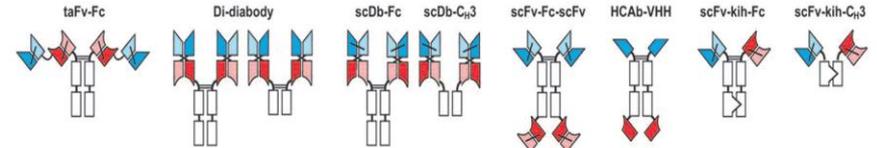
small bispecific antibody molecules



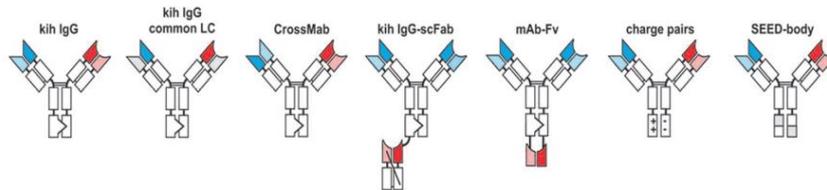
bispecific IgG and IgG-like molecules



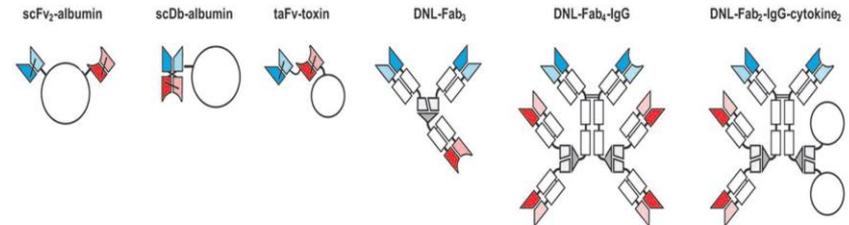
bispecific Fc and C_μ3 fusion proteins



asymmetric bispecific IgG and IgG-like molecules

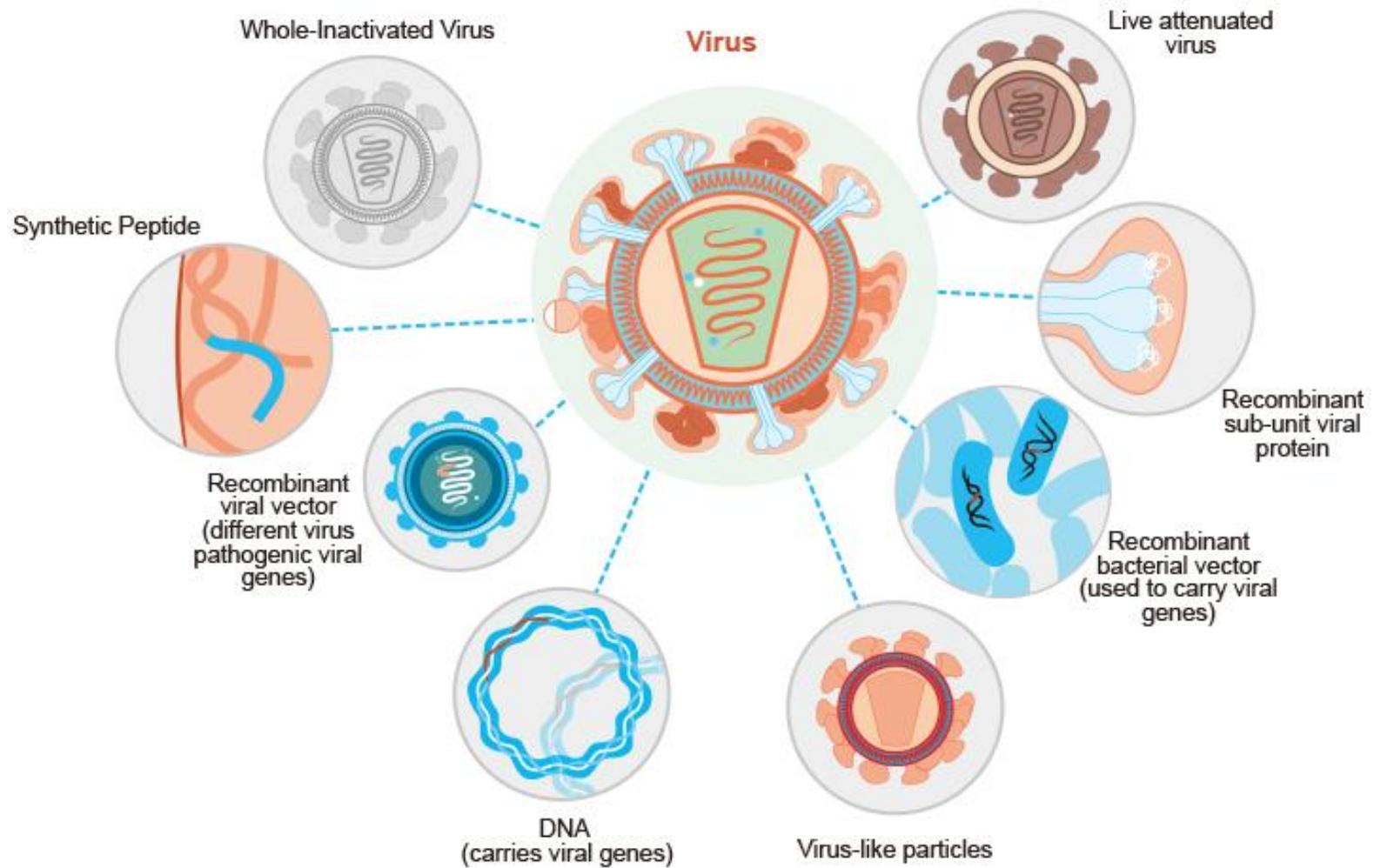


bispecific fusion proteins





Recombinant vaccines



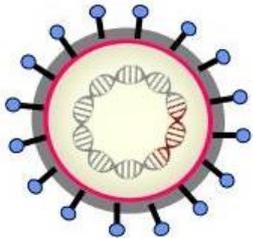


DNA vaccines

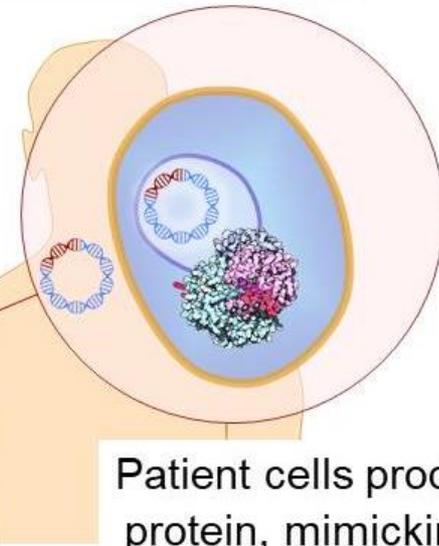
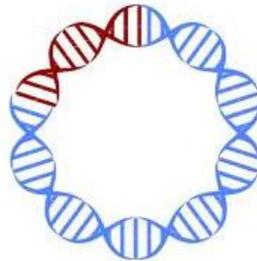


Plasmid is a short circular piece of DNA commonly used to shuttle genes into cells in the lab.

Transfer viral protein gene into a plasmid



Deliver plasmid into patient skin or muscle cells



Patient cells produce viral protein, mimicking a viral infection and inducing a strong immune response



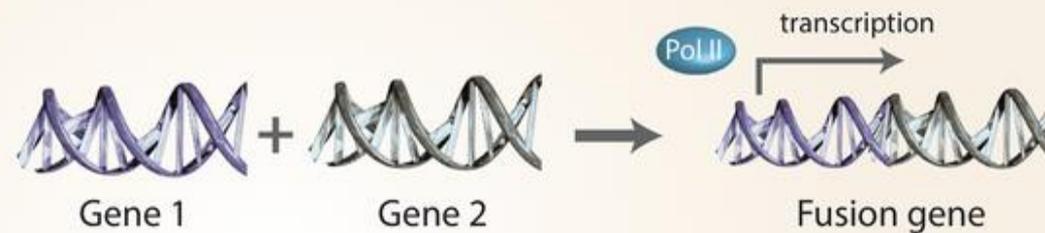
FUSION PROTEINS

-Chemical fusion

-Recombinant fusion

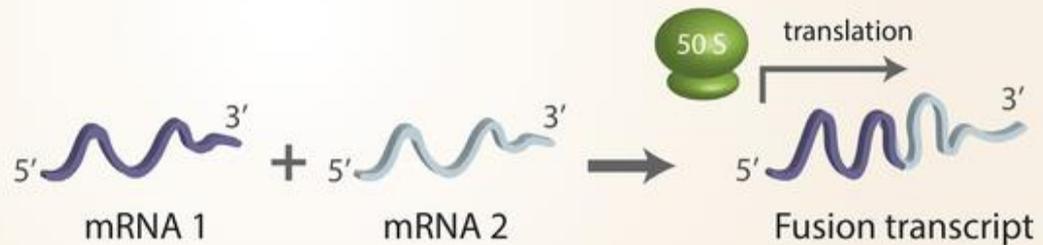
A Fusion by structural rearrangements

Translocations, inversions, deletions and insertions



B Fusion by transcription or splicing

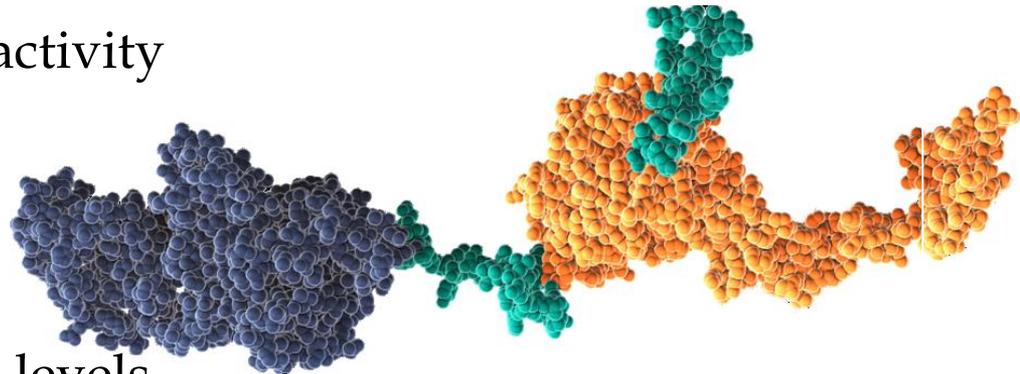
Transcription read-through, mRNA *trans*-splicing or *cis*-splicing





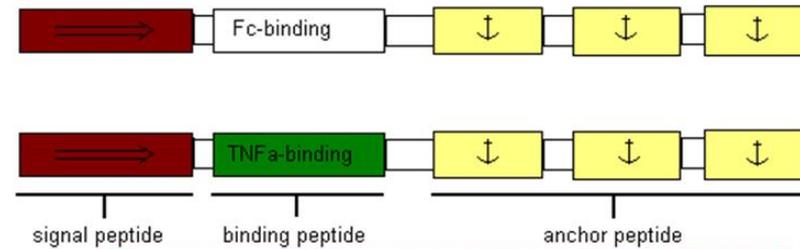
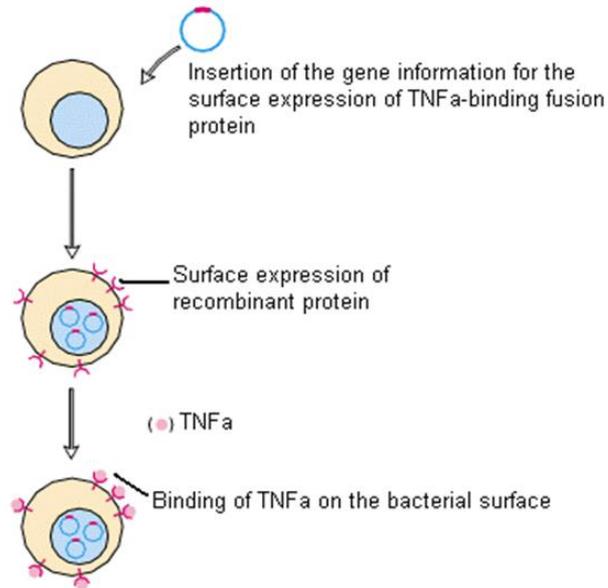
Fusion proteins advantages

- Easier cleaning (affinity tags)
- Directed expression (signaling peptide, extracellular)
- Visualization (fluorescent peptides)
- Enhancing catalytic activity
- Enhancing stability
- Enhancing solubility
- Improving expression levels
- ...





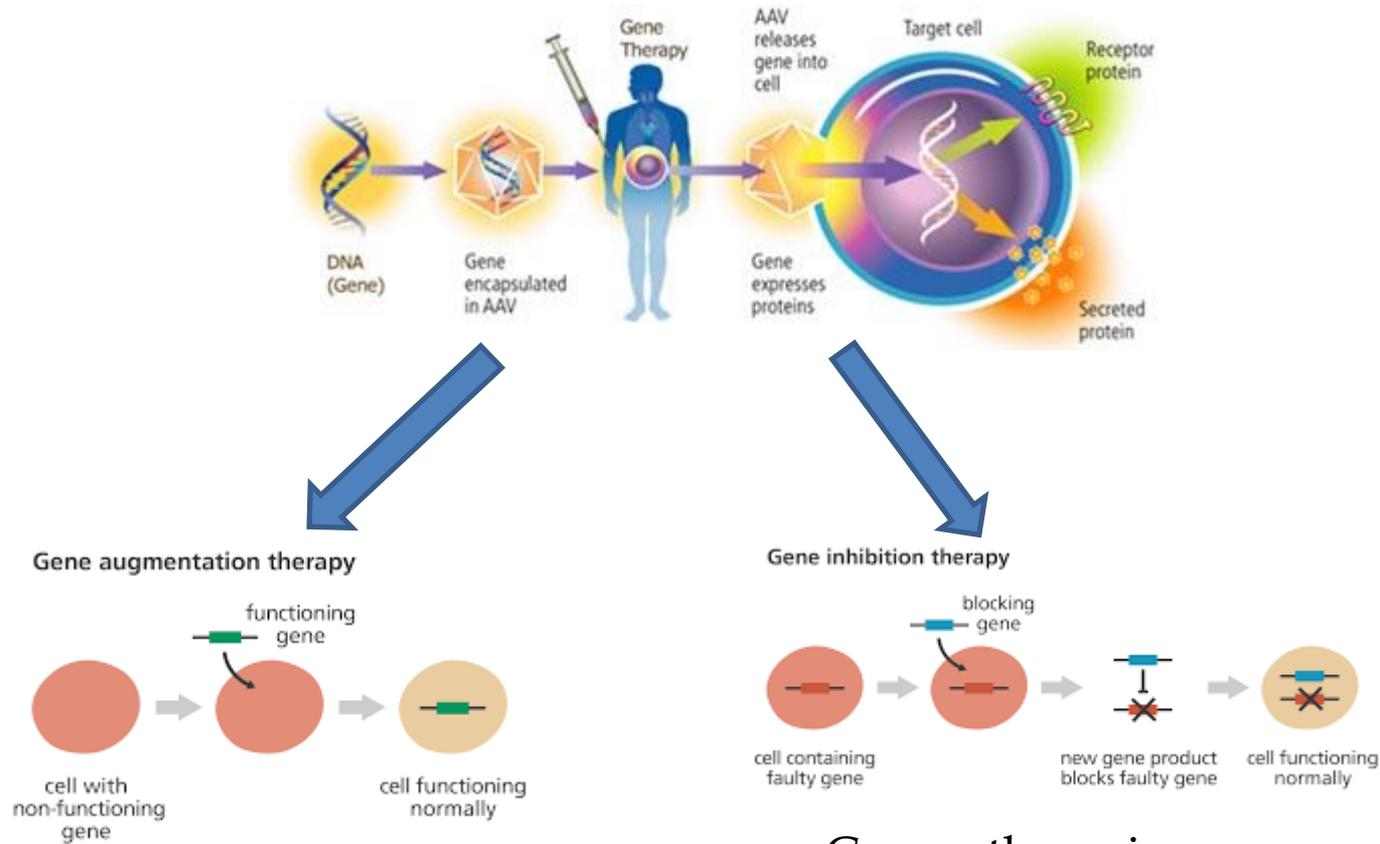
Example: design and preparation of recombinant probiotic





GENE THERAPY

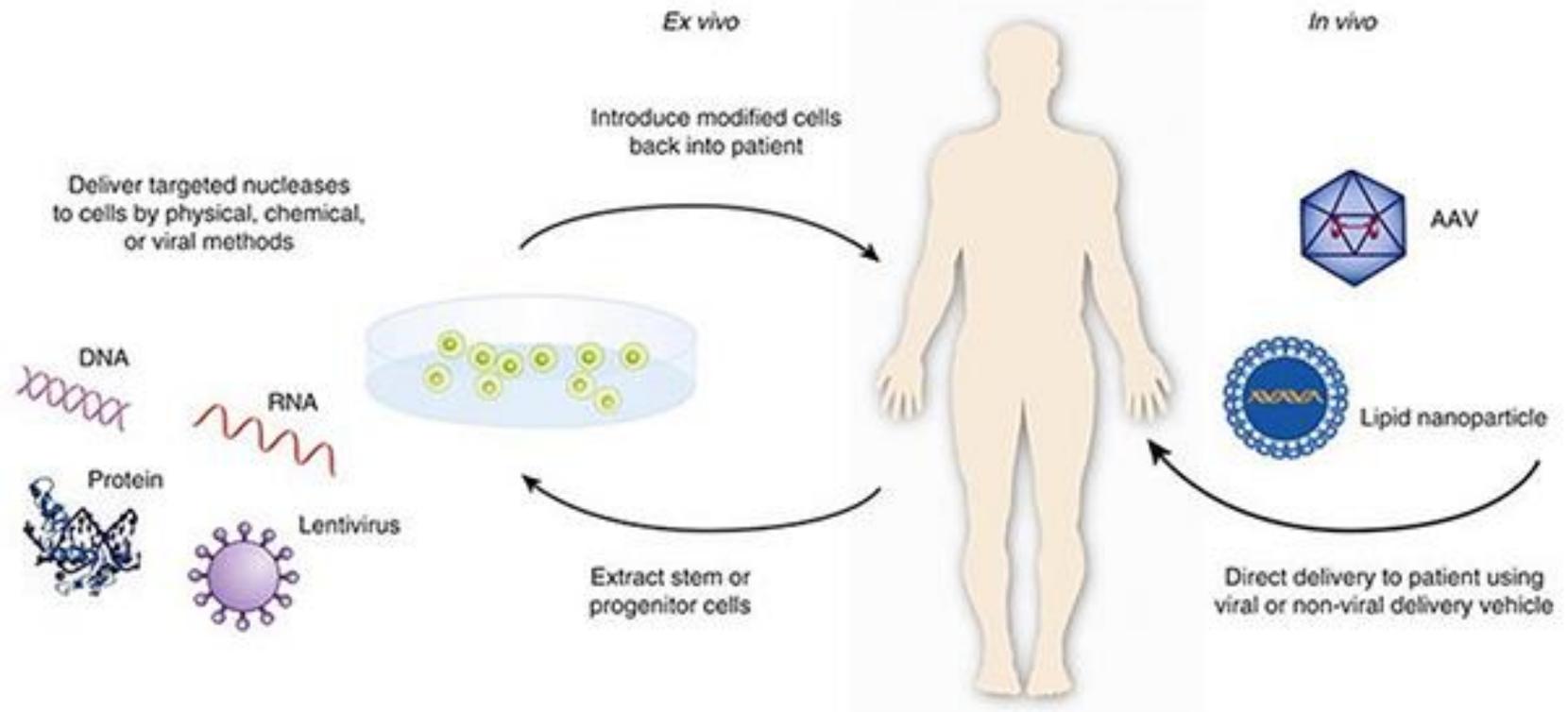
(fixing genetic problem at its source)



Cancer therapies



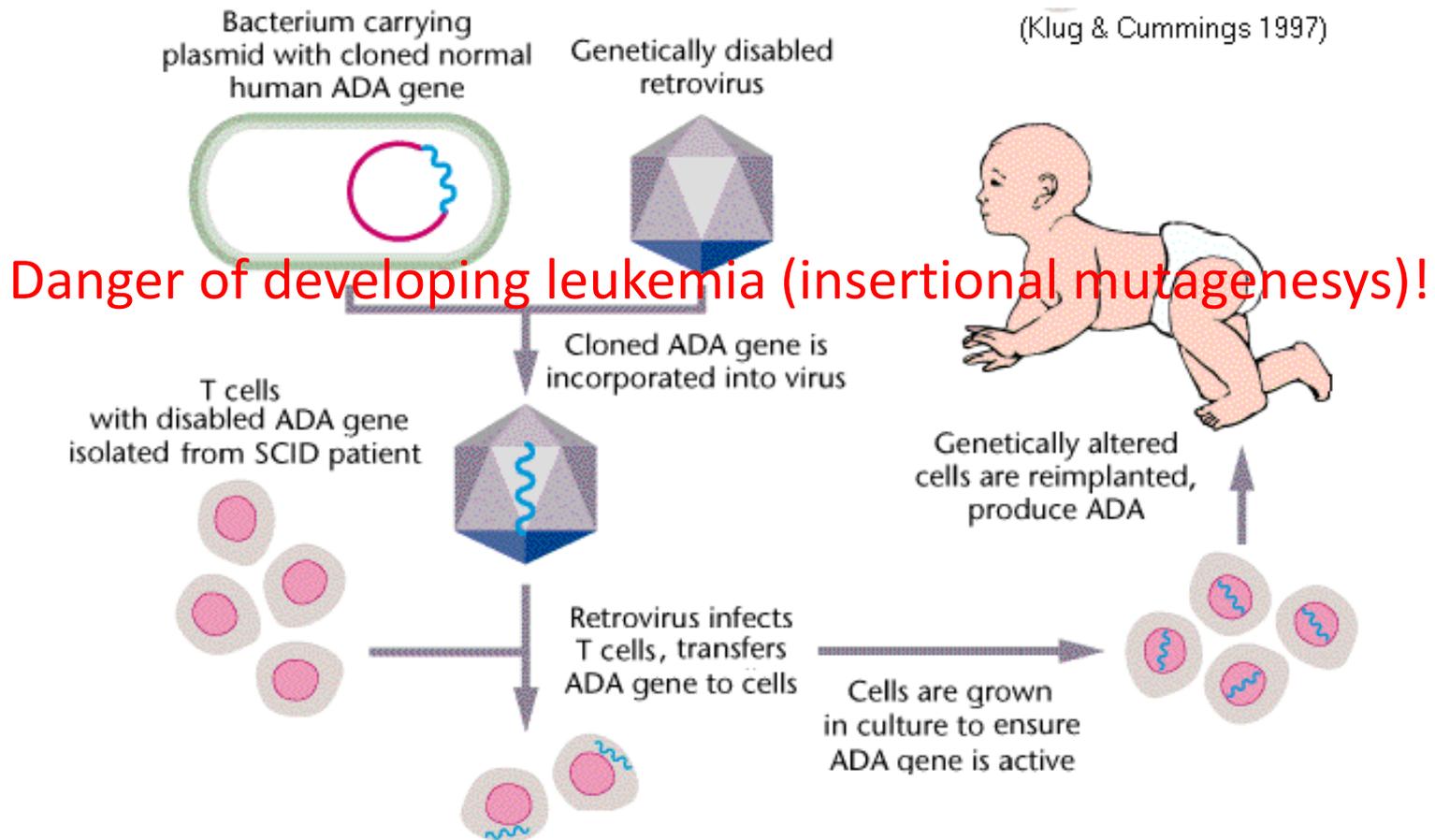
In-vivo and Ex-vivo gene transfer





First trials

-Adenosine deaminase deficiency leads to immunodeficiency ("Bubble kids")





Drawbacks of early gene therapies

-can damage vital genes

-can trigger lethal immune response to viral carrier

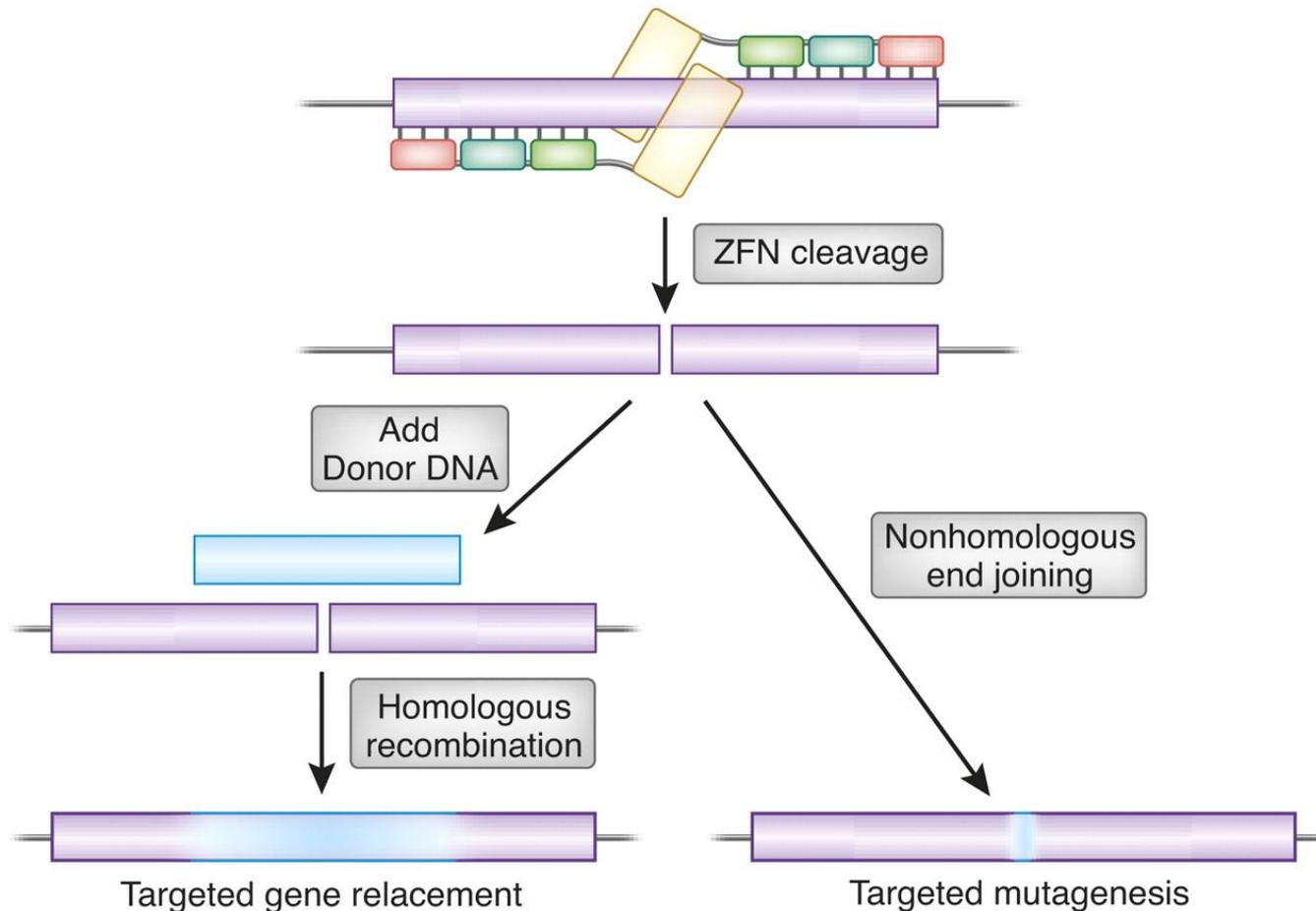
However...

-In therapy: Zolgensma[®] (1.8MEur) - Spinal muscular atrophy (AAV)



"Zinc finger" nucleases

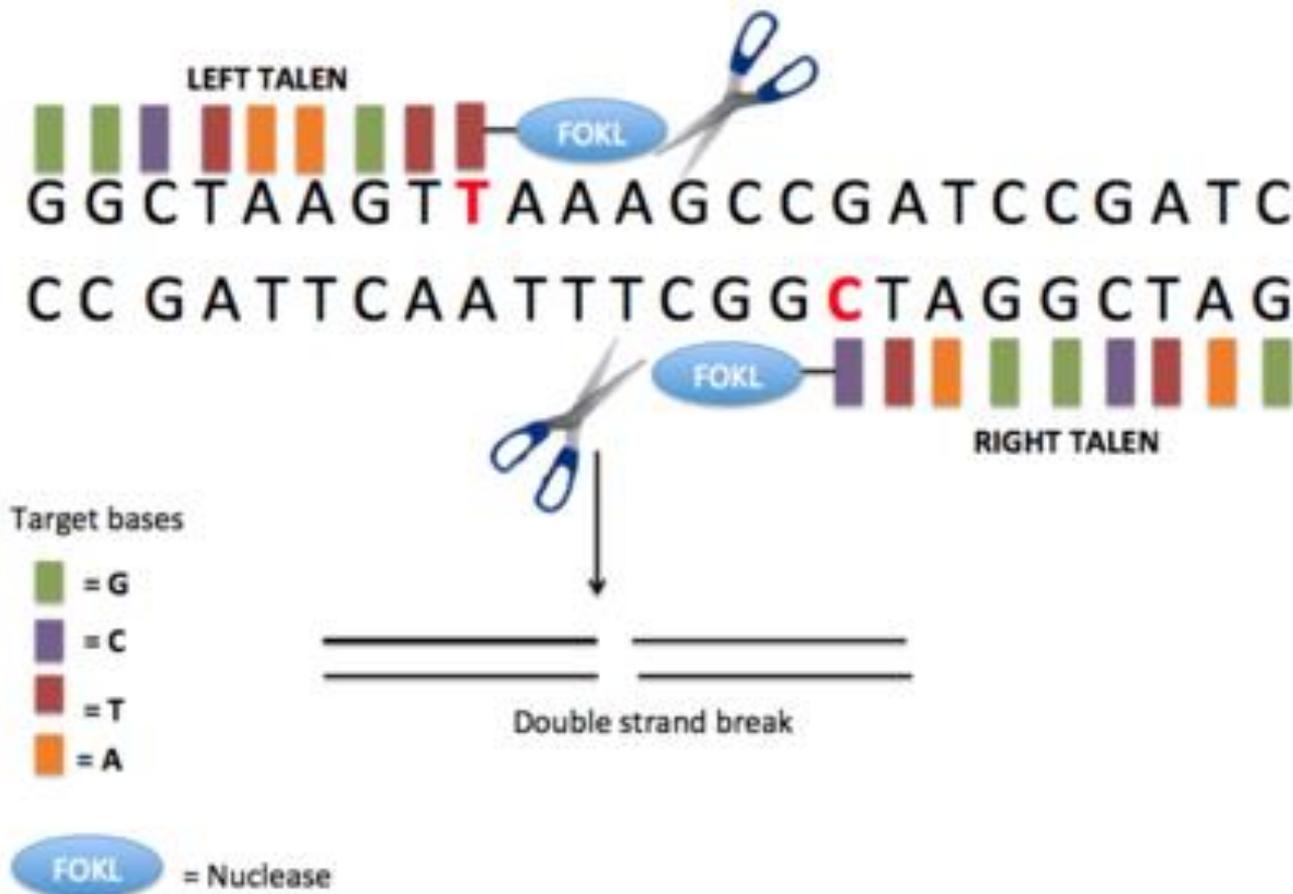
Artificial nucleases, capable of recognizing specific DNA sequences





TALENs (Transcription Activator-Like Effector Nucleases)

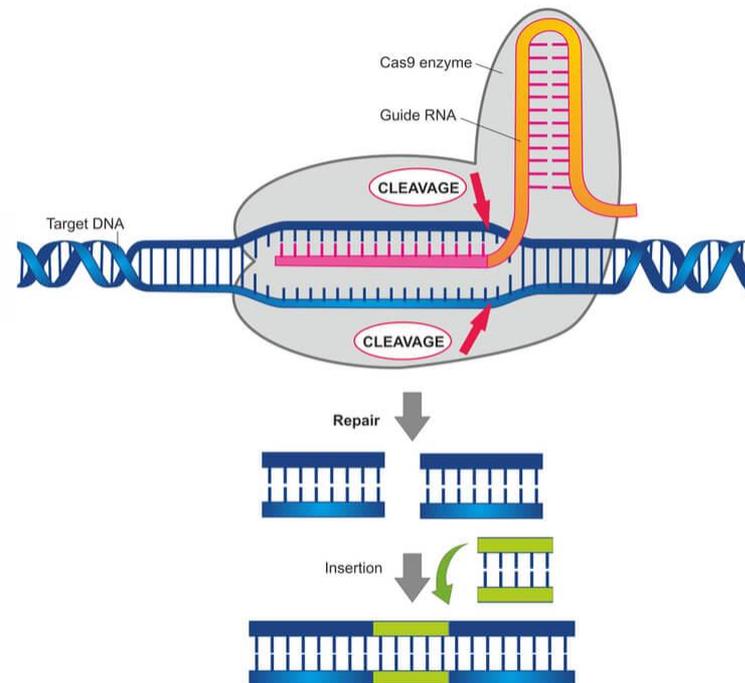
-tandem repeats of 34 amino acids, containing 2 variable AA





CRISPR CAS

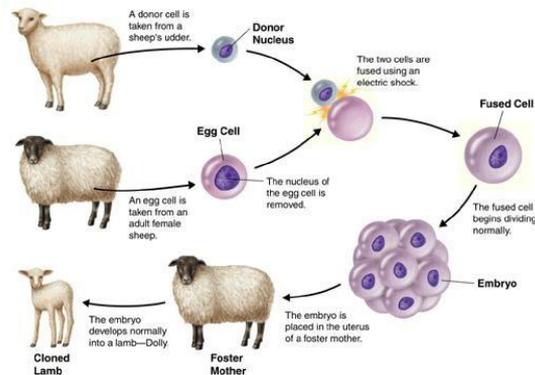
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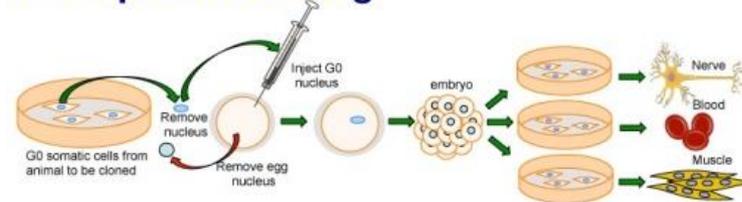


Future of recombinant DNA technology in medicine and ethical concerns

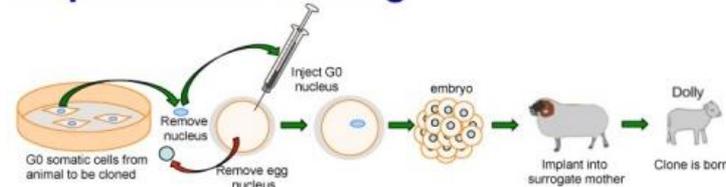
- Reproductive cloning: mice, cattle, pigs, goats, rabbits, and cats



Therapeutic cloning



Reproductive cloning



- From repairing to enhancing human performance?

Thank you for your attention!



