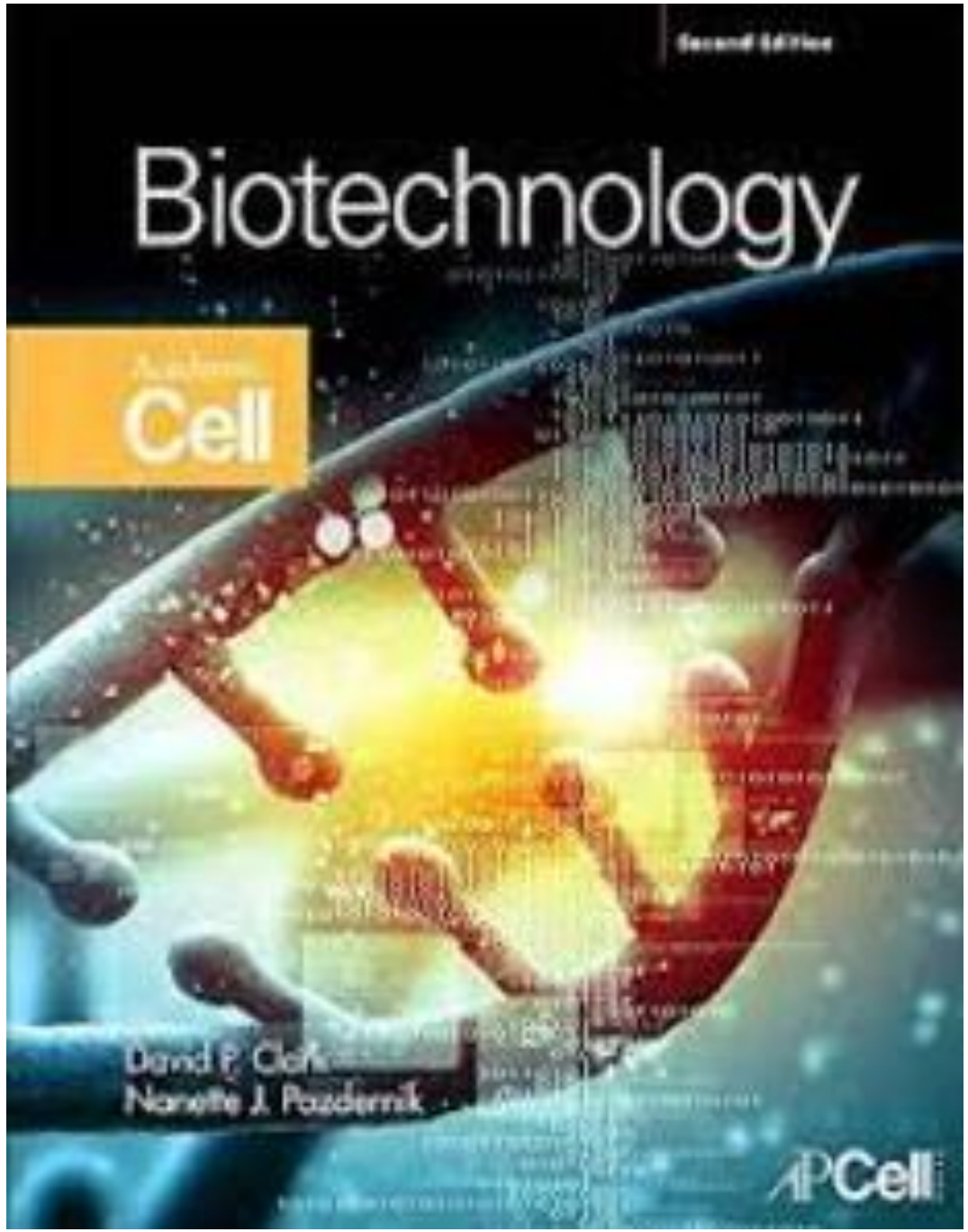
- CHAPTER 6
- Immune Technology
- Compiler:samaneh fashi
- Relevant professors:

✓Dr.bahrami

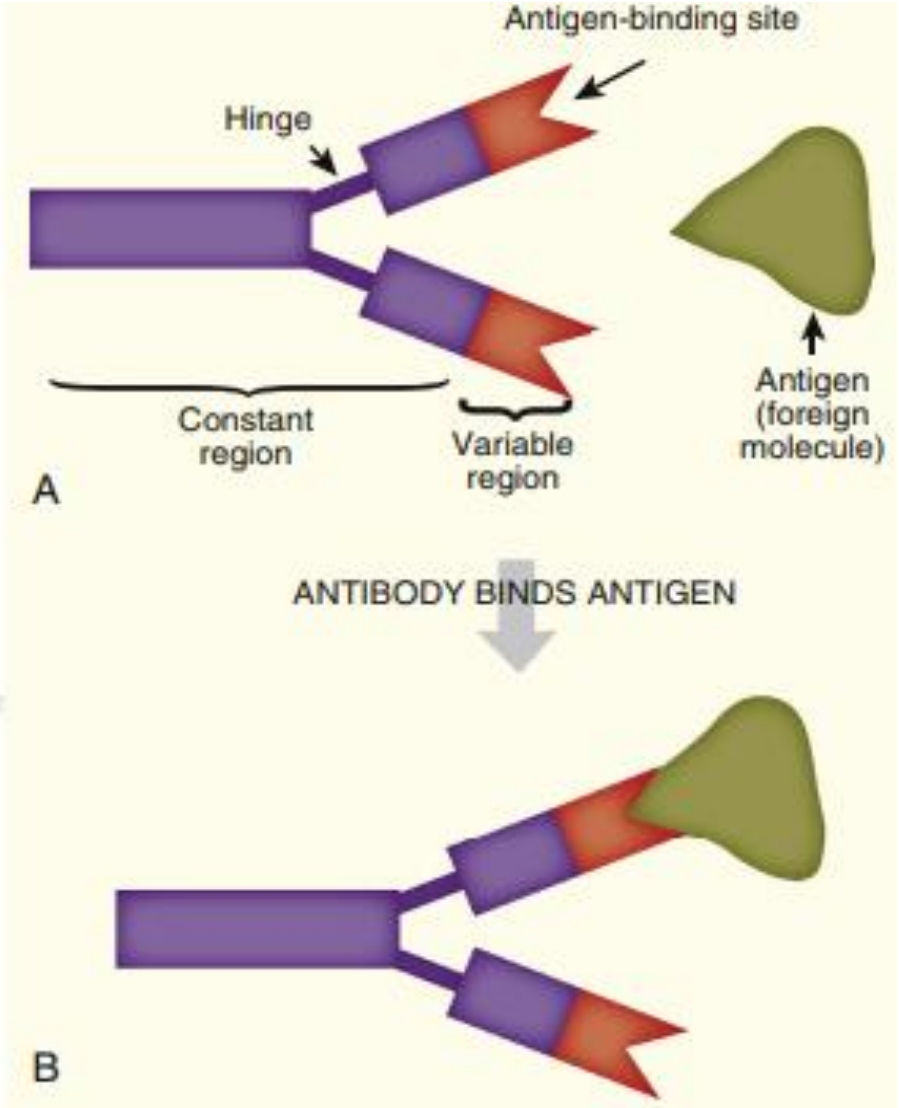
✓Dr.mohammad zadeh



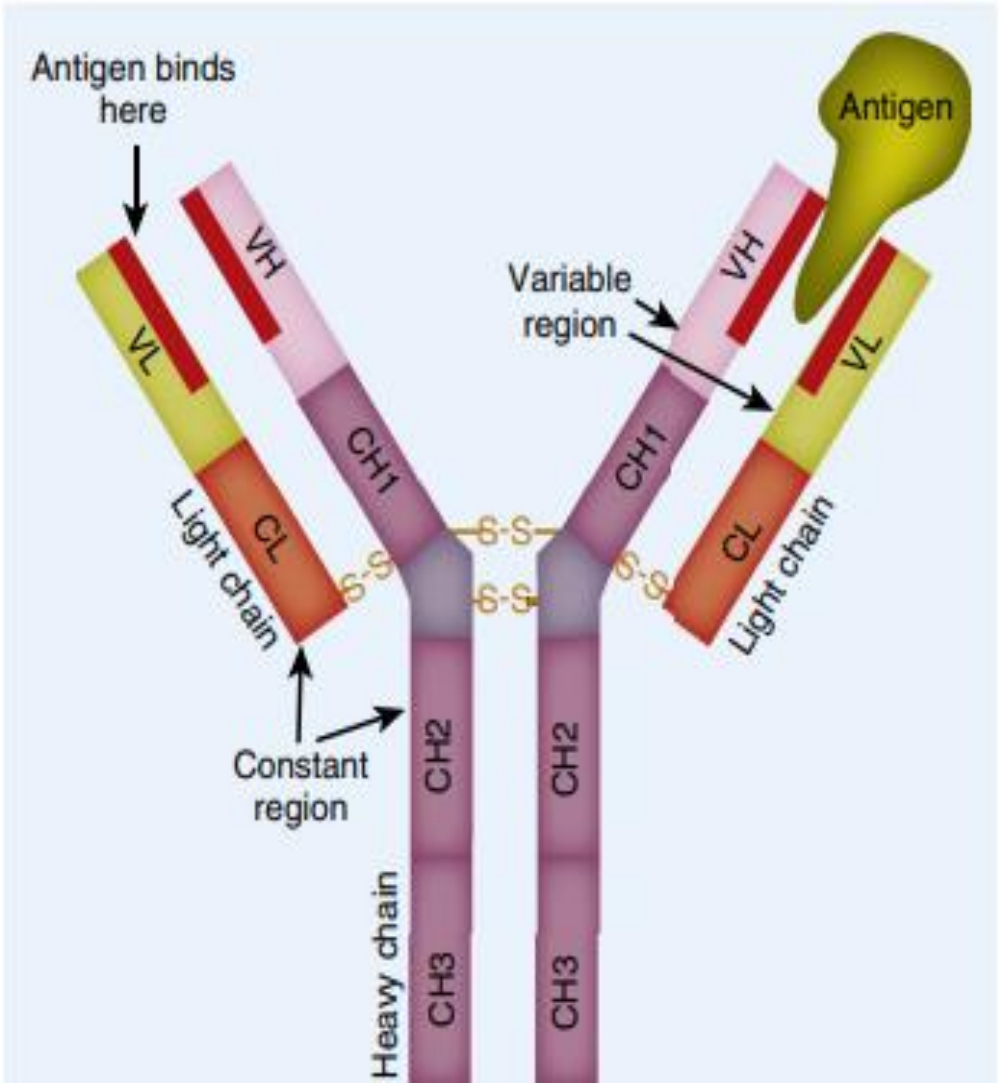
INTRODUCTION

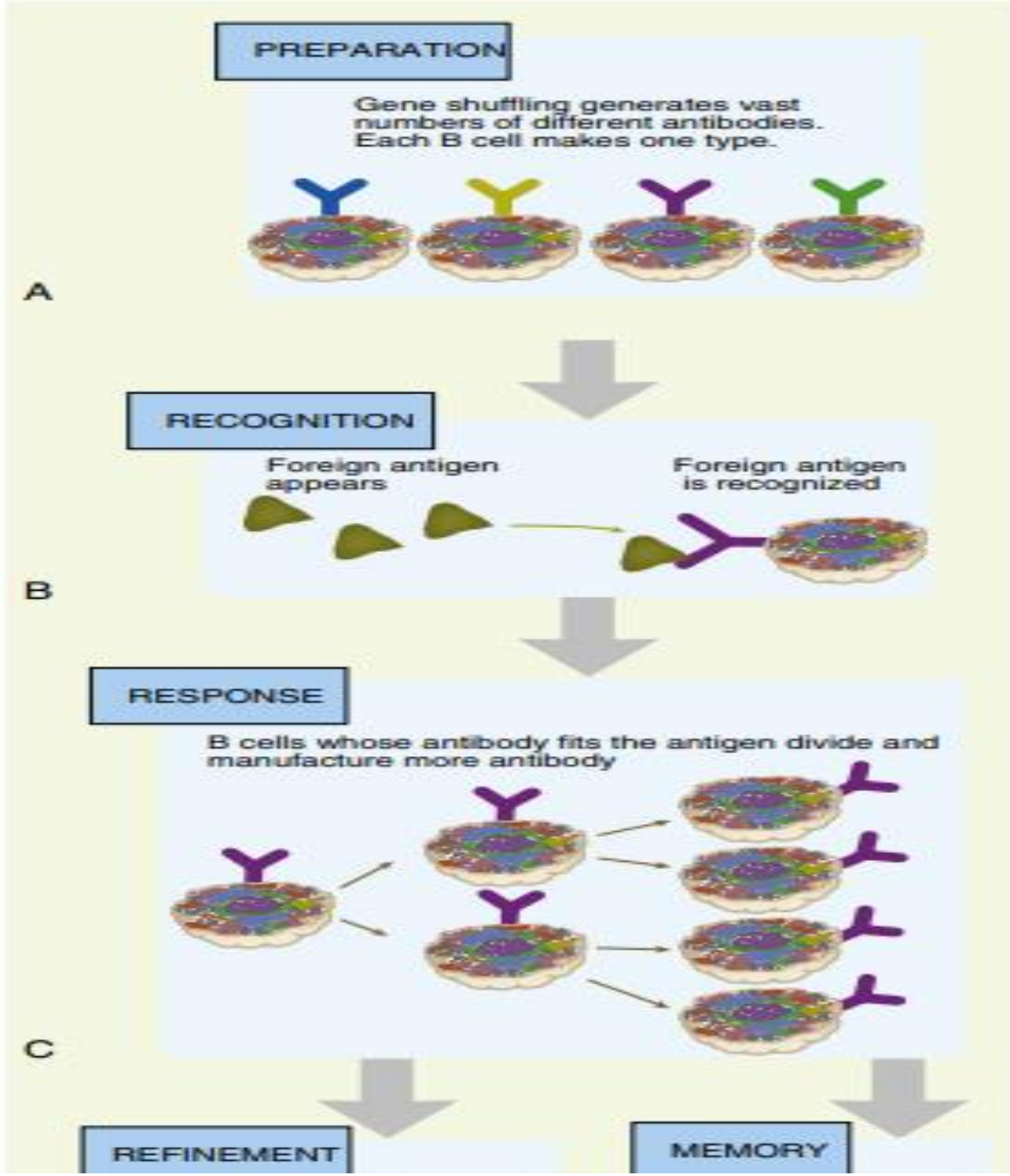
- The world is full of infectious microorganisms, all looking for a suitable host to infect. Bacteria, viruses, and protozoans are constantly attempting to gain entry into our tissues. If nothing prevented these attempts at invasion, no human could survive. Fortunately, cells of the immune system patrol the organism, protecting the entire body from attack. Any foreign macromolecules that are not recognized as being “self” are regarded as signs of an intrusion and trigger an immune response

- Antigens
- Antibodies
- EPITOPES
- paratopes



THE STRUCTURE OF ANTIBODY

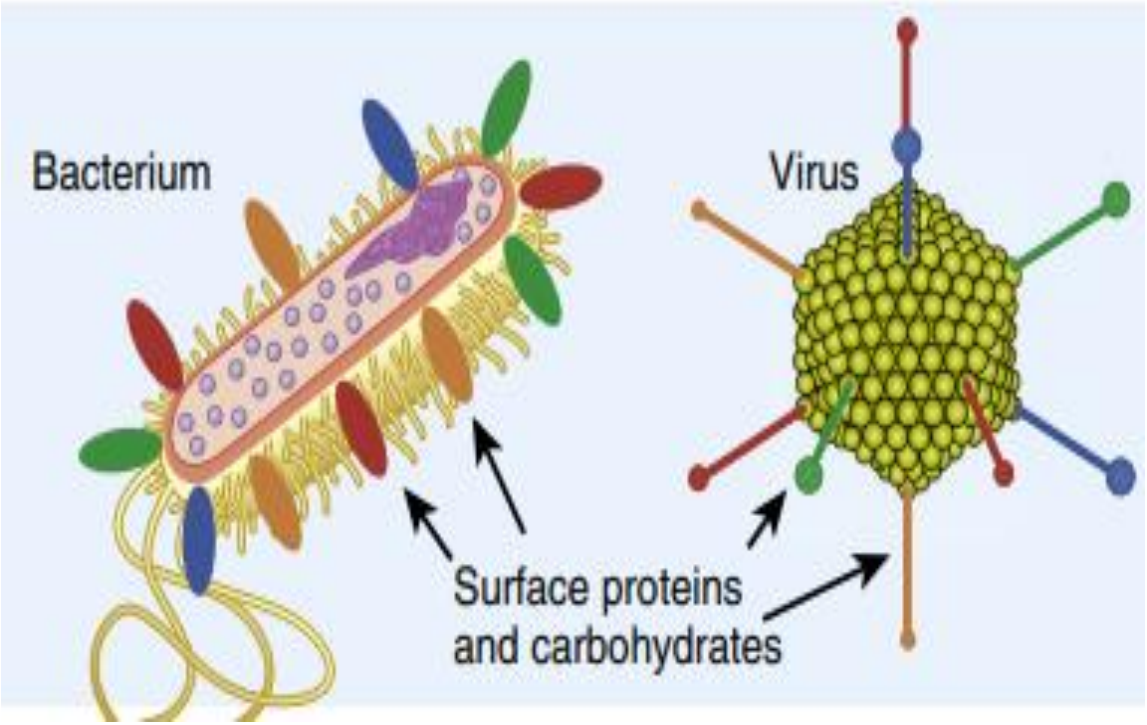


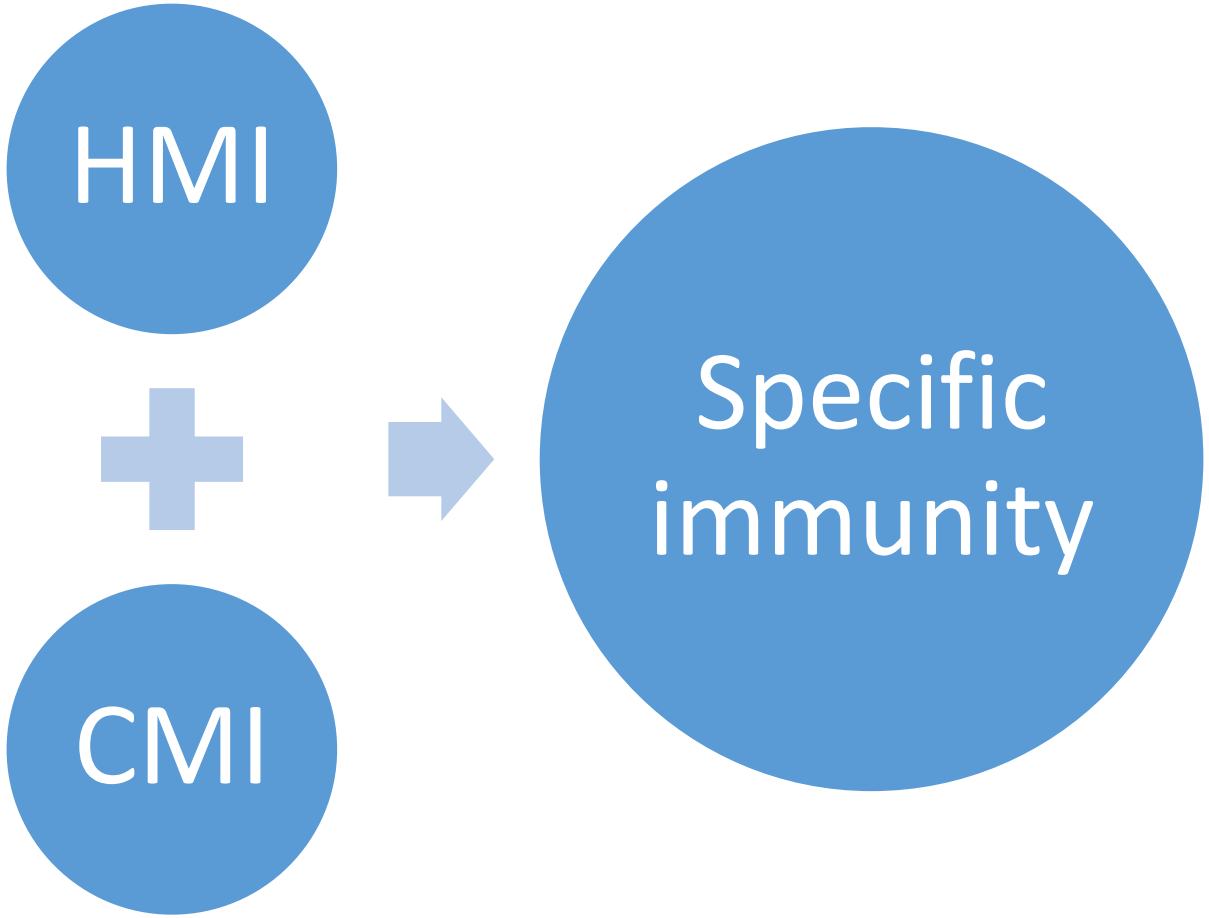

PREPARATION
Gene shuffling generates vast numbers of different antibodies. Each B cell makes one type.
A
RECOGNITION
Foreign antigen appears
Foreign antigen is recognized
B
RESPONSE
B cells whose antibody fits the antigen divide and manufacture more antibody
C
REFINEMENT
MEMORY

Clonal expansion

- To be prepared for any possible invasion, the B cells of the adaptive immune system generate billions of different antibodies. Most antibodies are secreted into the lymph, but some remain bound to the cell surface and are called B-cell receptors (BCR). Eventually, when a foreign antigen appears, a few of the billions of predesigned antibodies will fit the antigen reasonably well .Those B cells that make antibodies that recognize the antigen now divide rapidly and go into mass production. Thus, the antigen determines which antibody is amplified and produced. Once a matching antibody has bound invading antigens, the immune system brings other mechanisms into play to destroy the invaders.

- BCR
- TCR

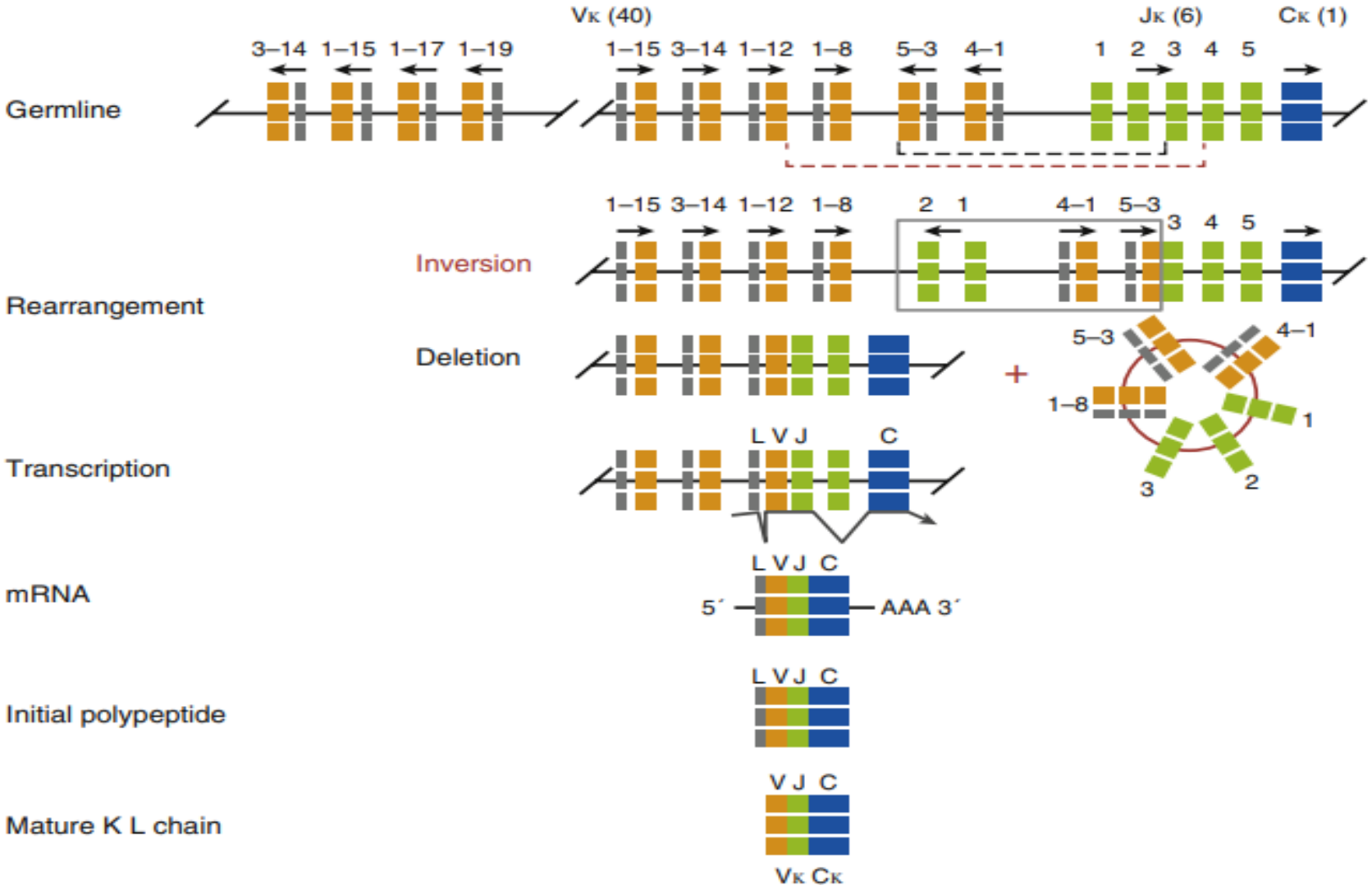



HMI
CMI
Specific immunity

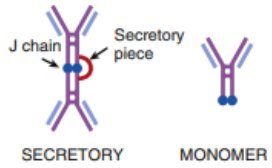
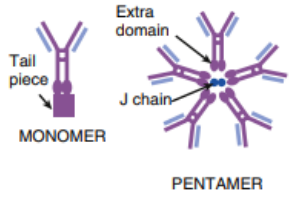
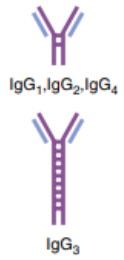
THE GREAT DIVERSITY OF ANTIBODIES

- Since there is an almost infinite variety of possible antigens, a correspondingly vast number of different antibody molecules are needed.
- If a separate gene encoded each antibody, this would require a gigantic number of genes and a vast amount of DNA.

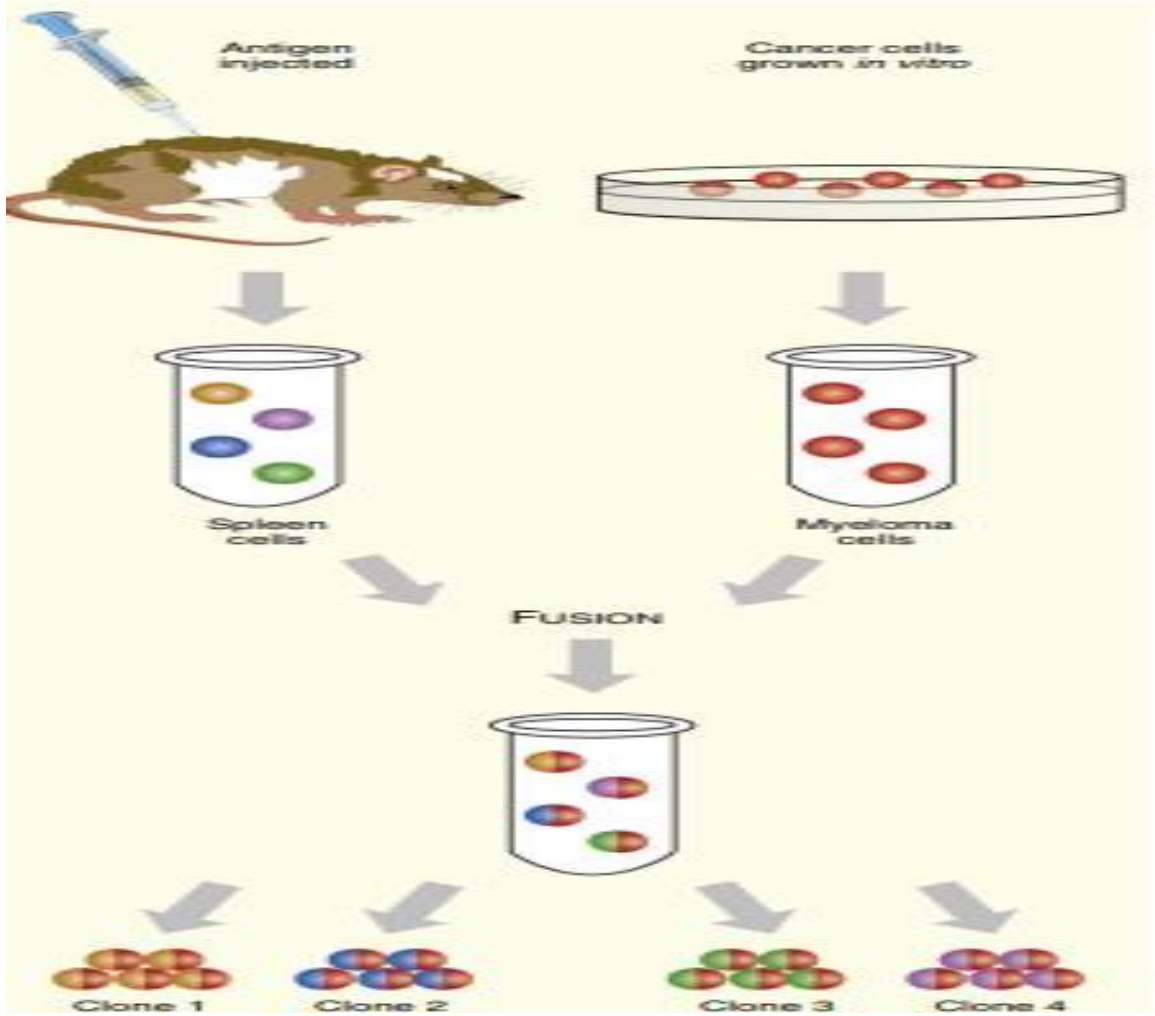
V(D)J recombination



STRUCTURE AND FUNCTION OF IMMUNOGLOBULINS

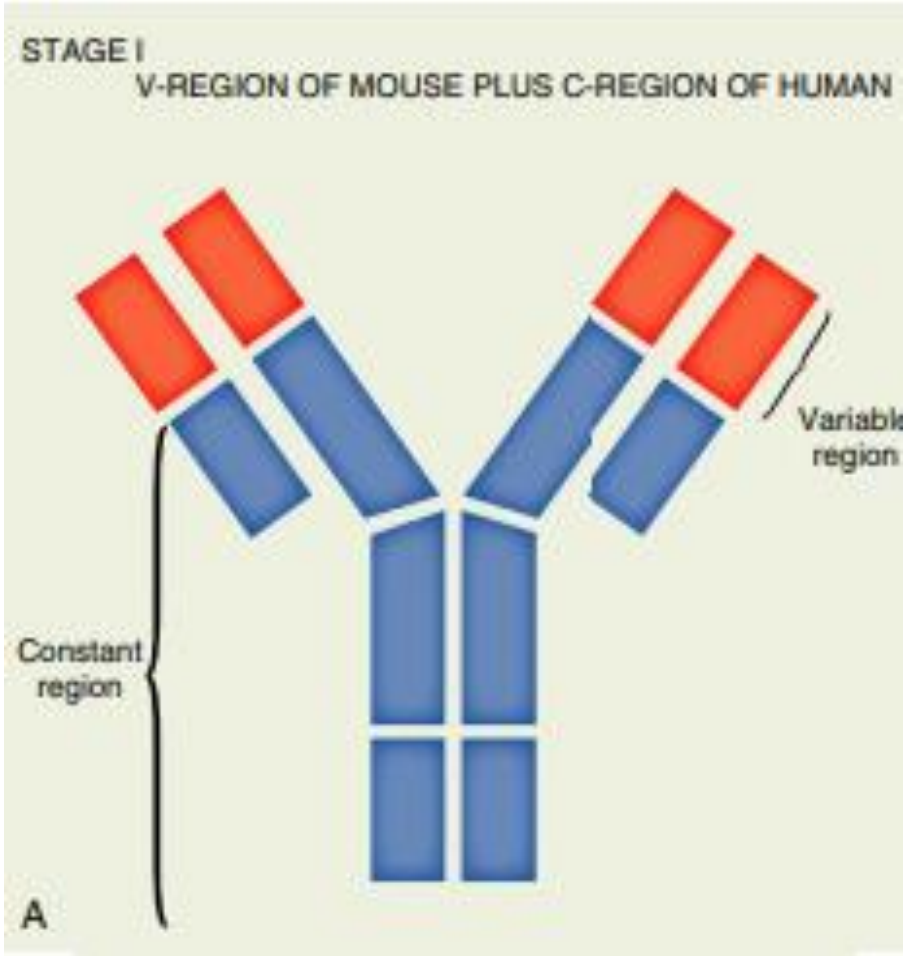
Antibody	Subtype	Light Chain	Heavy Chain	Function	Structure
IgA	IgA_1 IgA_2	κ or λ	α_1 α_2	Prevents pathogen attachment	
IgE	none	κ or λ	ε	Allergic reaction; inhibits parasites	
IgD	none	κ or λ	δ	Activates lymphocytes	
IgM	none	κ or λ	μ	Clumps microbes and activates complement	
IgG	IgG_1 IgG_2 IgG_3 IgG_4	κ or λ κ or λ κ or λ κ or λ	γ_1 γ_2 γ_3 γ_4	Activates complement, activates other immune cells	

MONOCLONAL ANTIBODIES FOR CLINICAL USE



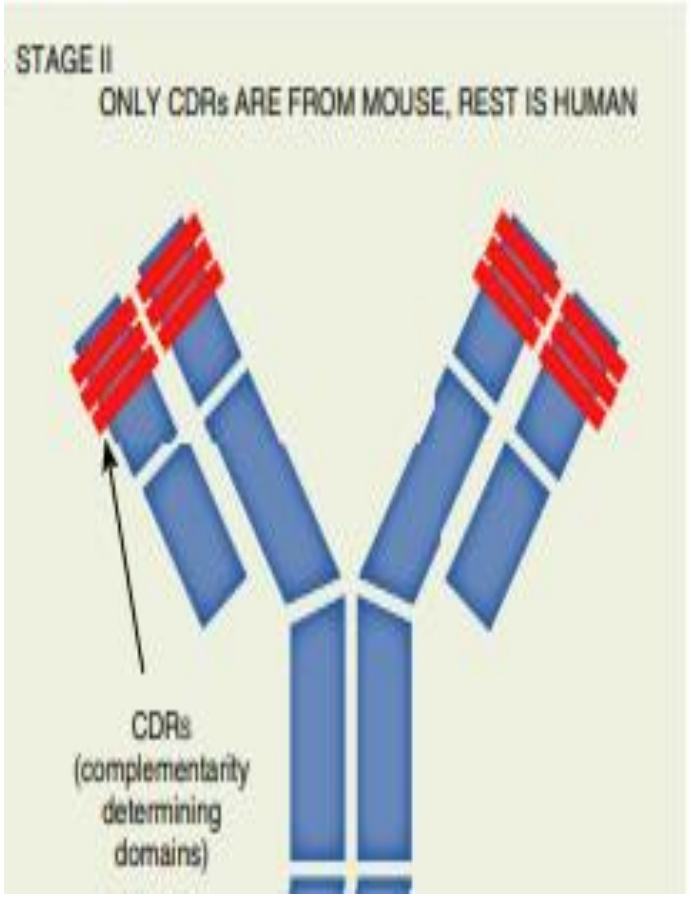
HUMANIZATION OF MONOCLONAL ANTIBODIES

Since the variable, or V-region, of the antibody recognizes the antigen, the constant, or C-region, may therefore be replaced with a humanized version. To accomplish this, scientists isolate and culture the first-generation hybridoma, generally using mouse B cells. Then the DNA encoding the mouse monoclonal antibody is isolated and cloned. The DNA for the constant region of the mouse antibody is then replaced with the corresponding human DNA sequence. The V-region is left alone. The human/mouse hybrid gene is then put back into a second mouse myeloma cell for production of antibody in culture



HUMANIZATION OF MONOCLONAL ANTIBODIES CONT

These are known as hypervariable regions or as complementarity determining regions (CDRs). Overall, each antigen-binding site consists of six CDRs—three from the light chain and three from the heavy chain. Full humanization of an antibody involves cutting out the coding regions for these six CDRs from the original antibody and splicing them into the genes for human light and heavy chains



EXAMPLES

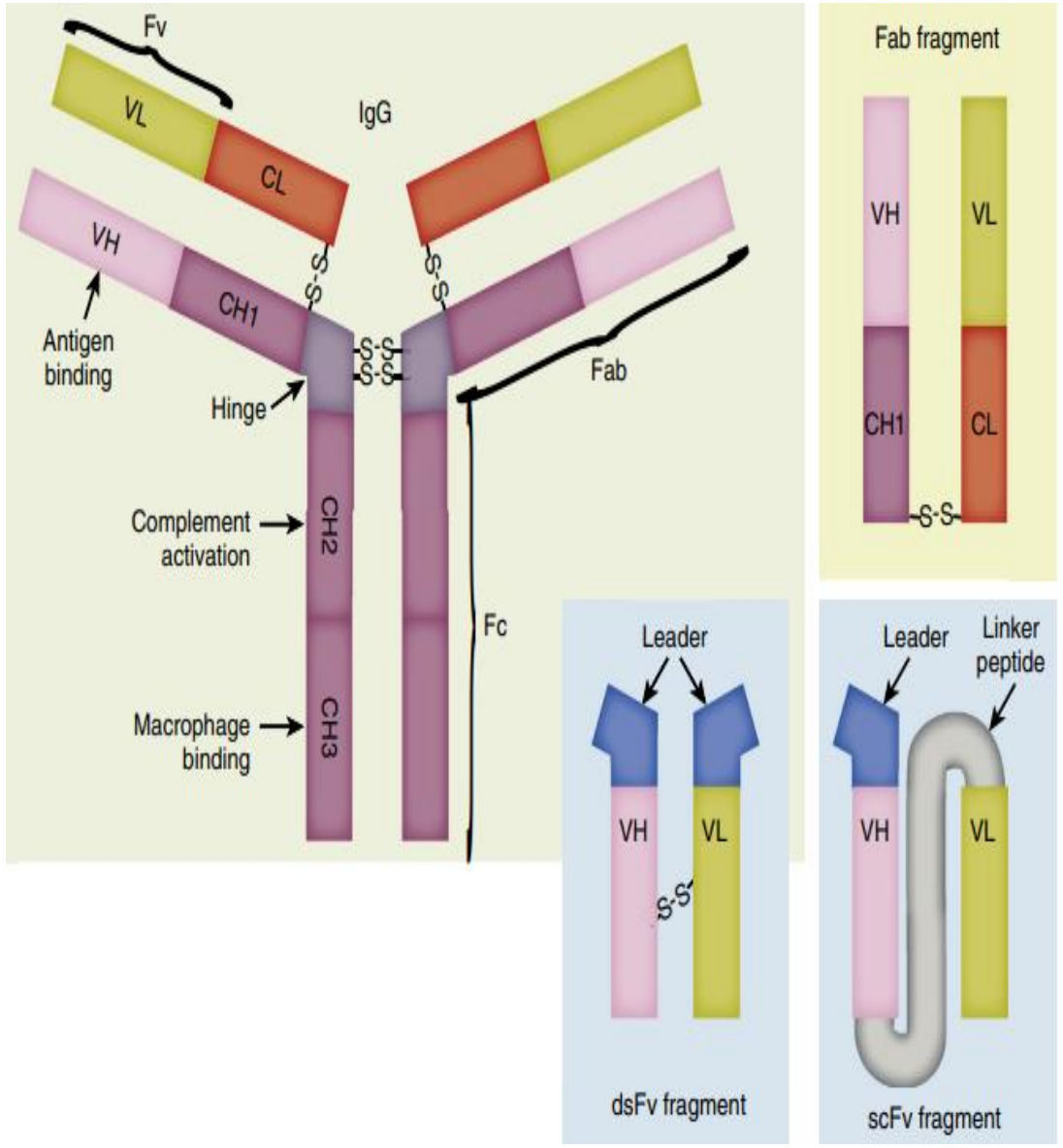
- Herceptin:Herceptin recognizes a cell surface receptor called human epidermal growth factor receptor type 2 (HER2).
- These receptors control whether a cell proliferates, differentiates, or undergoes programmed suicide by signaling a variety of intracellular proteins that modulate gene expression
- Infliximab:is used to treat rheumatoid arthritis (RA).
- The antibody targets tumor necrosis factor alpha (TNFα)
- Antibodies to TNFα inhibit inflammation in RA by blocking the release of IL-1, a pro-inflammatory cytokine

ANTIBODY ENGINEERING

- the incredibly high specificity with which antibodies bind to a target protein is useful for a variety of purposes. Consequently, antibody engineering uses the antigen-binding region of the antibody. These antibodies are manipulated and are attached to other molecular fragments.
- To separate an antigen-binding site from the rest of the antibody, scientists subclone gene segments encoding portions of the variable antibody chains and express them in bacterial cells.

single-chain Fv (scFv)

In a Fab fragment, an interchain disulfide bond holds the two chains together. However, the Fv fragment lacks this region of the antibody chains and thus is less stable. This led to development of the single-chain Fv fragment in which the VH and VL domains are linked together by a short peptide chain

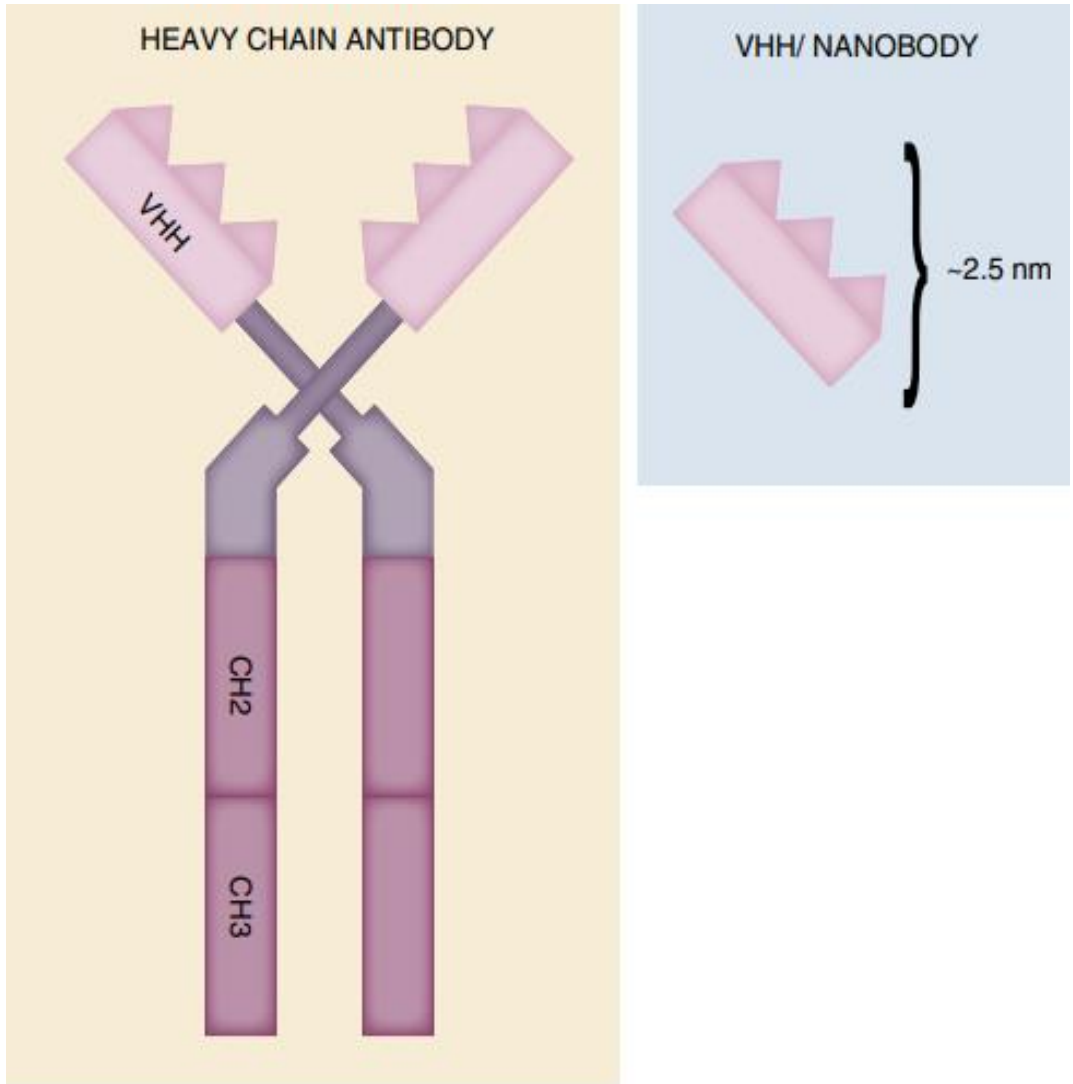


single-chain Fv (scFv)cont

- Such scFv fragments are attached to various other molecules by genetic engineering.
- role of the scFv fragment:
- recognize some target molecule, perhaps a protein expressed only on the surface of a virus-infected cell or a cancer cell.
- provide the active portion of the final recombinant antibody.
- provides a way of delivering a therapeutic agent in an extremely specific manner

heavy-chain antibodies (hcAb)

- Recent work studying camel antibody structure has elucidated a new structure of an antibody not seen in any other model organisms studied to date. Antibodies in camels and their relatives (llamas and alpacas) have only the heavy chain and no light chains.
- The ends of the heavy chain have the binding sites for the foreign antigens or paratopes.
- for therapeutic purposes.
- The variable domain of the single heavychain antibody called VHH.
- a recombinant protein containing only this domain is called a nanobody (Nb)


HEAVY CHAIN ANTIBODY
VHH
CH2
CH3
VHH/ NANOBODY
~2.5 nm

ADVANTAGES OF nanobody

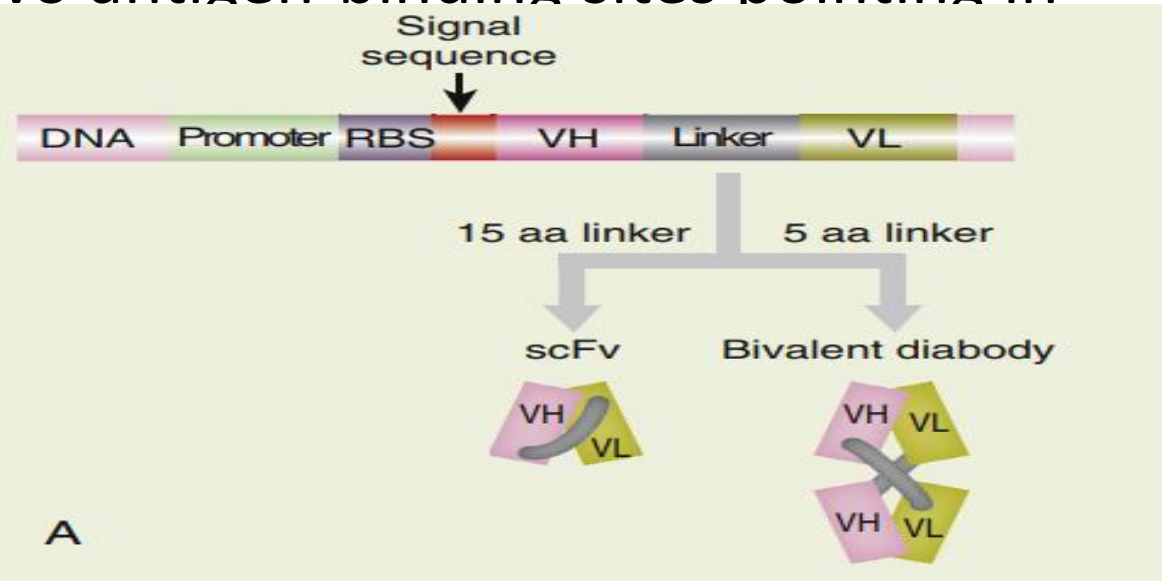
- are small, work as monomers
- have no disulfide bonds
- and are very stable , even maintaining their structure in high heat or denaturing conditions
- They have a very high affinity for the antigen
- They can recognize epitopes that protrude as regular antibodies, and they can recognize epitopes that are dimples or concave in shape
- can easily pass through the kidney, so they are rapidly cleared from the body

ADVANTAGES OF nanobody

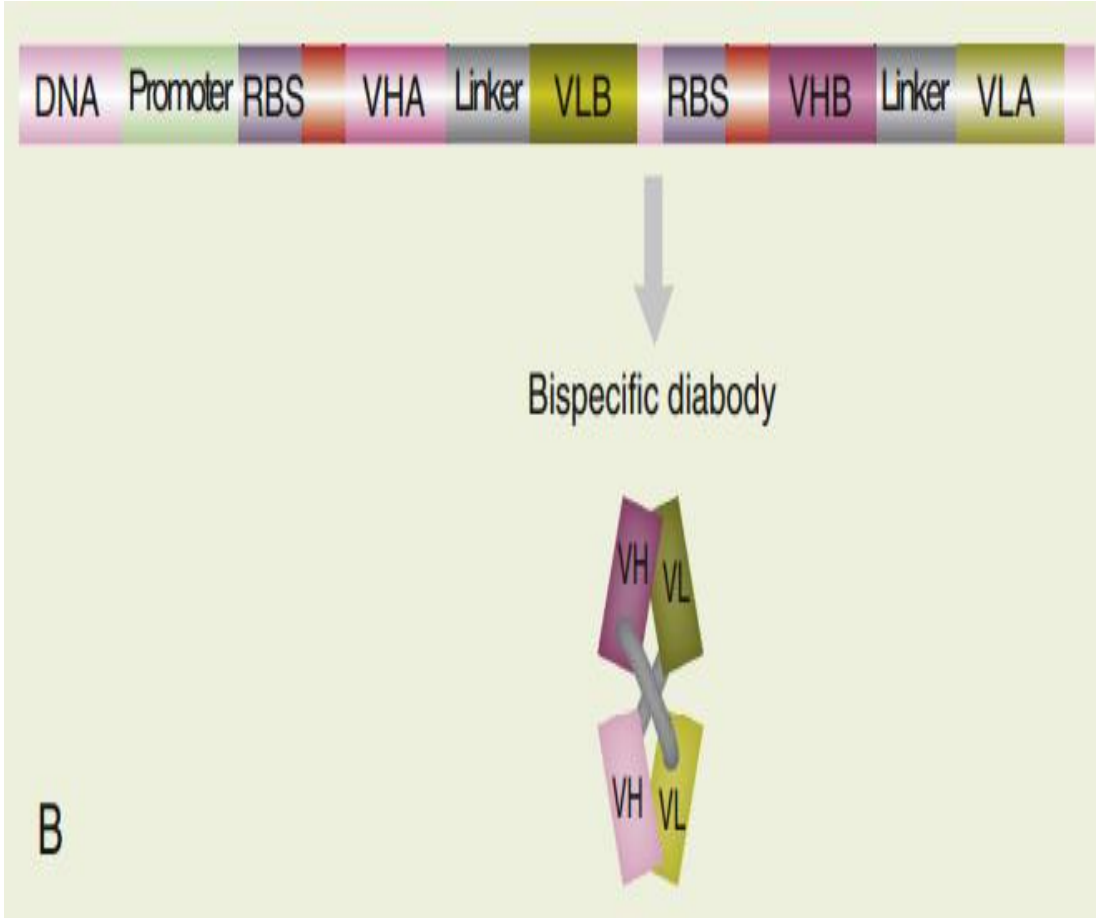
- They can pass through the blood–brain barrier to target regions of the brain
- Nbs can also be humanized and conjugated to different small molecule therapeutics just as scFvs

DIABODIES AND BISPECIFIC ANTIBODY CONSTRUCTS

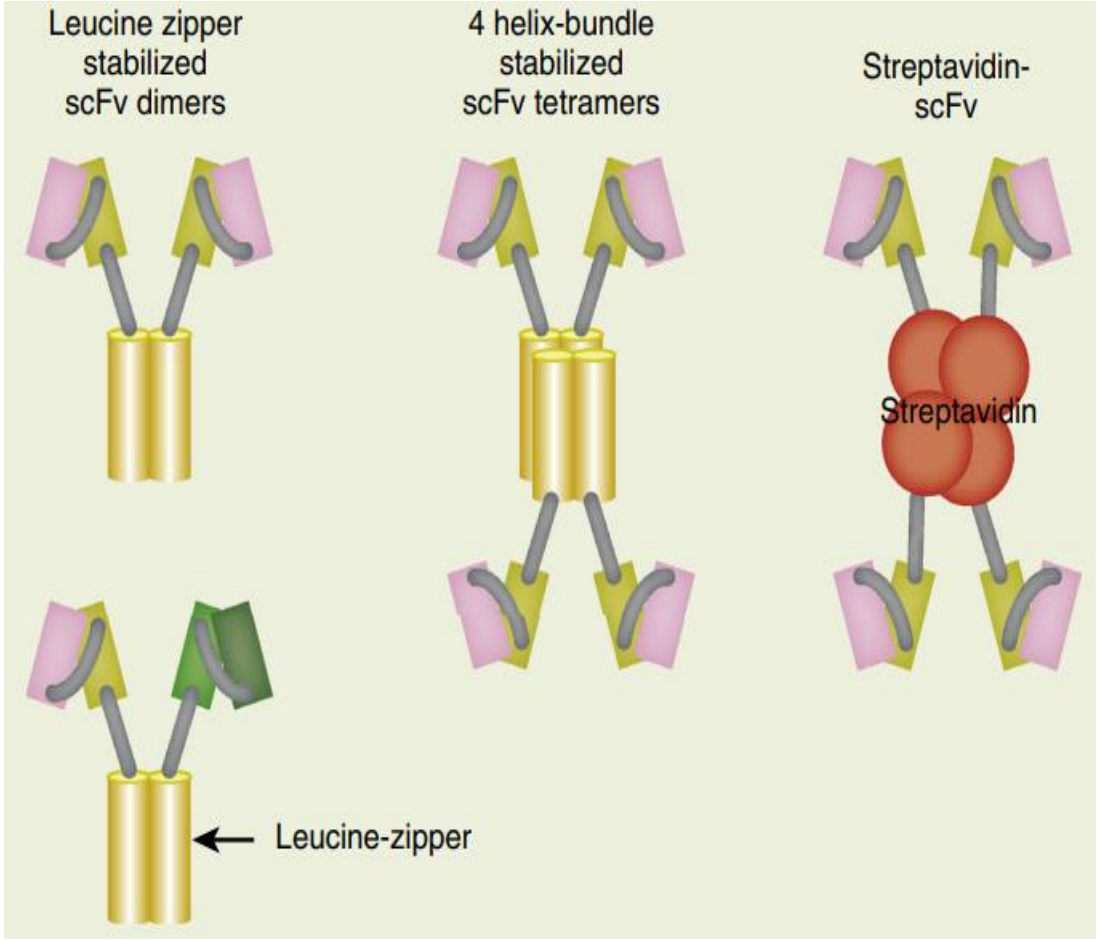
- A diabody consists of two single-chain Fv (scFv) fragments assembled together
- Shortening the linker from 15 to 5 amino acids drives dimerization of two scFv chains
- The resulting diabody has two antigen-binding sites pointing in opposite directions.



- If two different scFv fragments are used, the result is a bispecific diabody that will bind to two different target proteins simultaneously
- of course, possible to engineer both sets of VH and VL regions onto a single polypeptide chain encoded by a single recombinant gene



- Instead of genetic linkers to hold diabodies, various proteins can also hold scFv fragments together.
- when scFv genes are genetically fused to these proteins, the scFv domains come together .
- Like Proteins with a leucine zipper domain and streptavidin



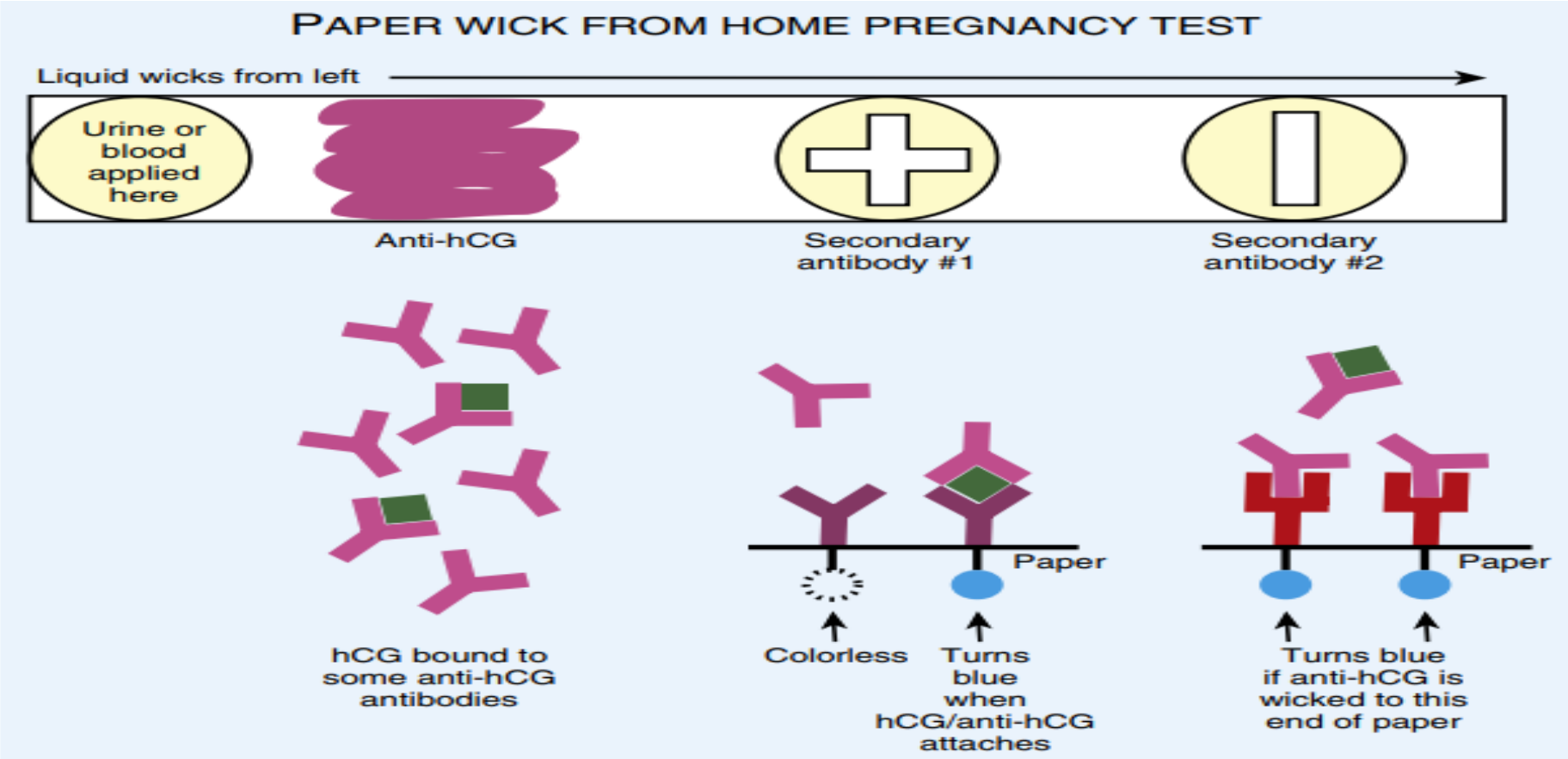
ELISA ASSAY

- The enzyme-linked immunosorbent assay (ELISA) is widely used to detect and estimate the concentration of a protein in a sample.
- The protein to be detected is regarded as the antigen. Therefore, the first step is to make an antibody specific for the target protein. A detection system is then attached to the rear of the antibody.
- Often, binding and detection are done in two stages, using two different antibodies.

THE ELISA AS A DIAGNOSTIC TOOL

- Diagnostic kits that rely on the ELISA are produced for clinical diagnosis of human disease.
- ELISA kits can detect the presence of minute amounts of pathogenic viruses or bacteria, even before the pathogen has a chance to cause major damage.

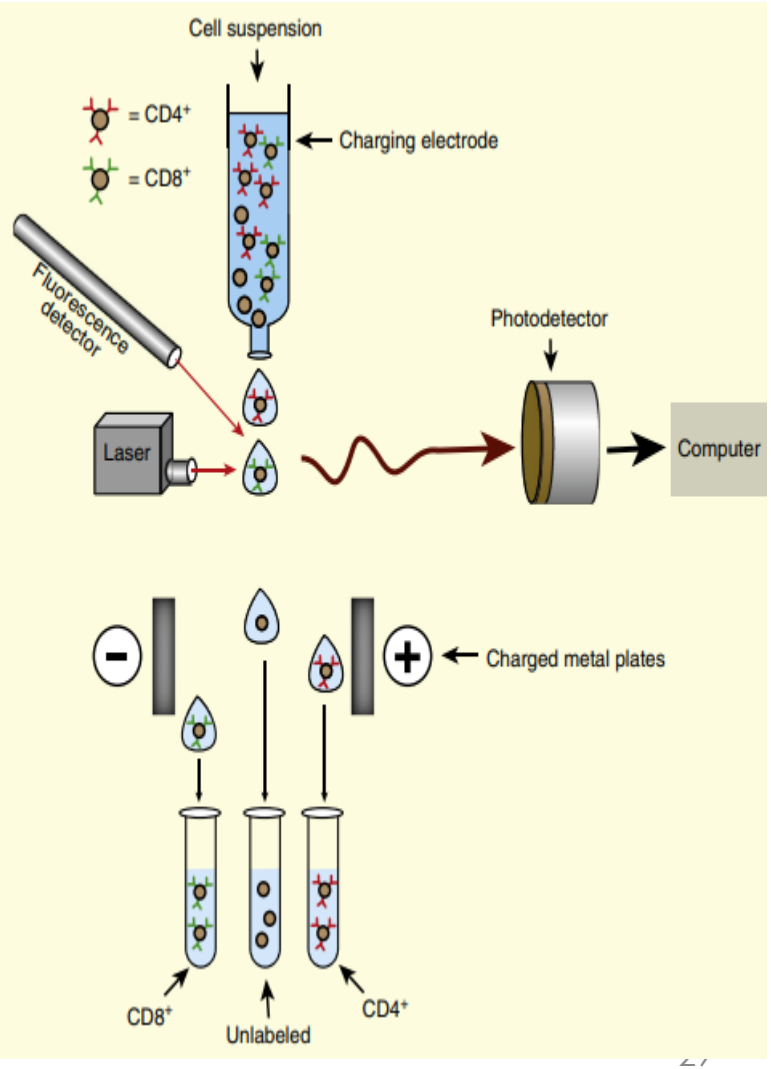
human chorionic gonadotropin (hGC).



Fluorescence-activated cell sorting (FACS)

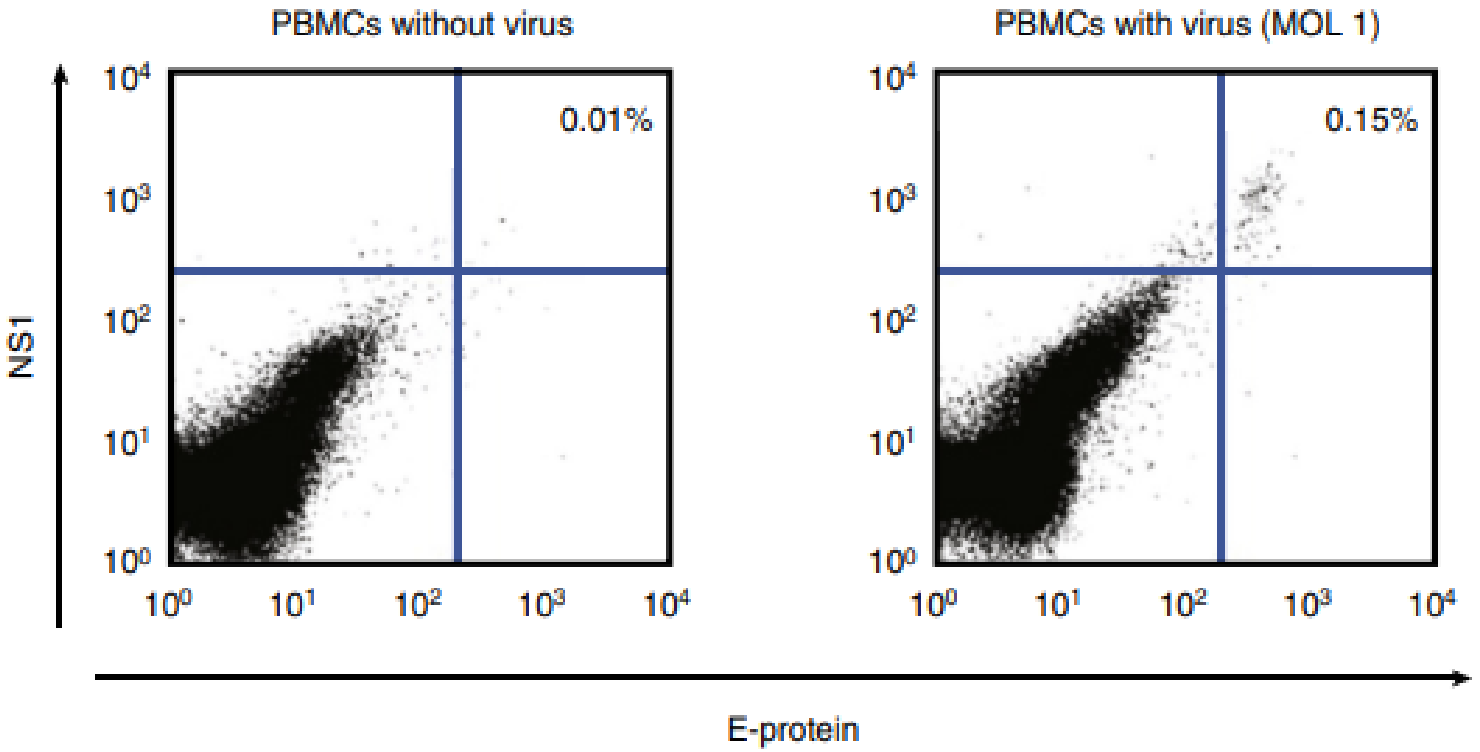
-involves the mechanical separation of a mixture
of cells into different tubes based on their surfa
Antigens.

-helper T cells and killer T cells can be separated
other white blood cells based on the presence o
CD4 or CD8 surface antigens



Flow cytometry

- Flow cytometry is a related technique to analyze fluorescently labeled cells.



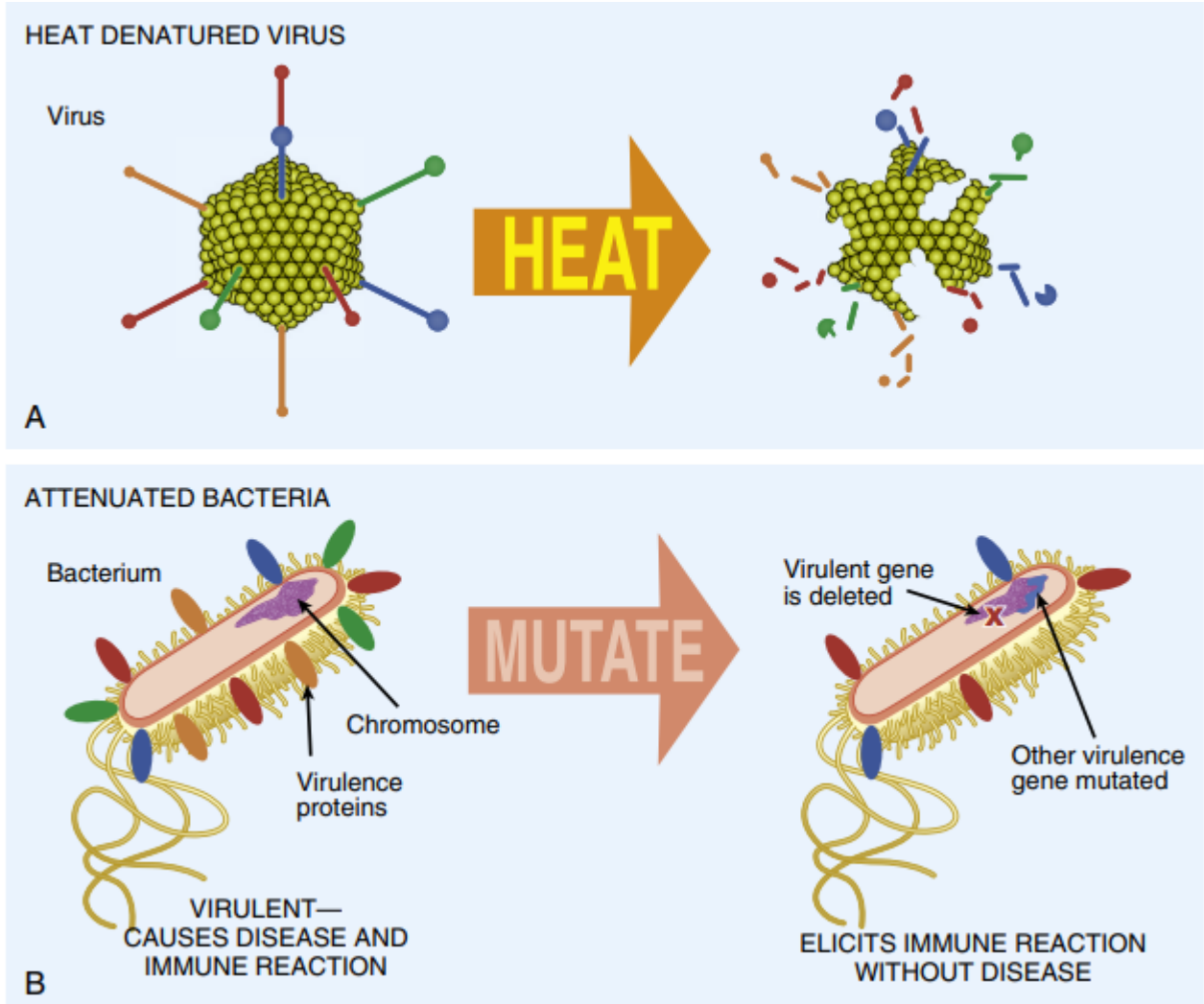
IMMUNE MEMORY AND VACCINATION

- Individuals who survive an infection normally become immune to that particular disease, although not to other diseases. The reason is that the immune system “remembers” foreign antigens, a process called immune memory.
- virgin B cells are triggered to divide if they encounter an antigen that matches their own individual antibody.
- a few active B cells become memory cells, and instead of making antibodies, they simply wait. If one day the antigen that they recognize appears again, most of the memory cells switch over very rapidly to antibody production.

CREATING A VACCINE

- whole vaccines

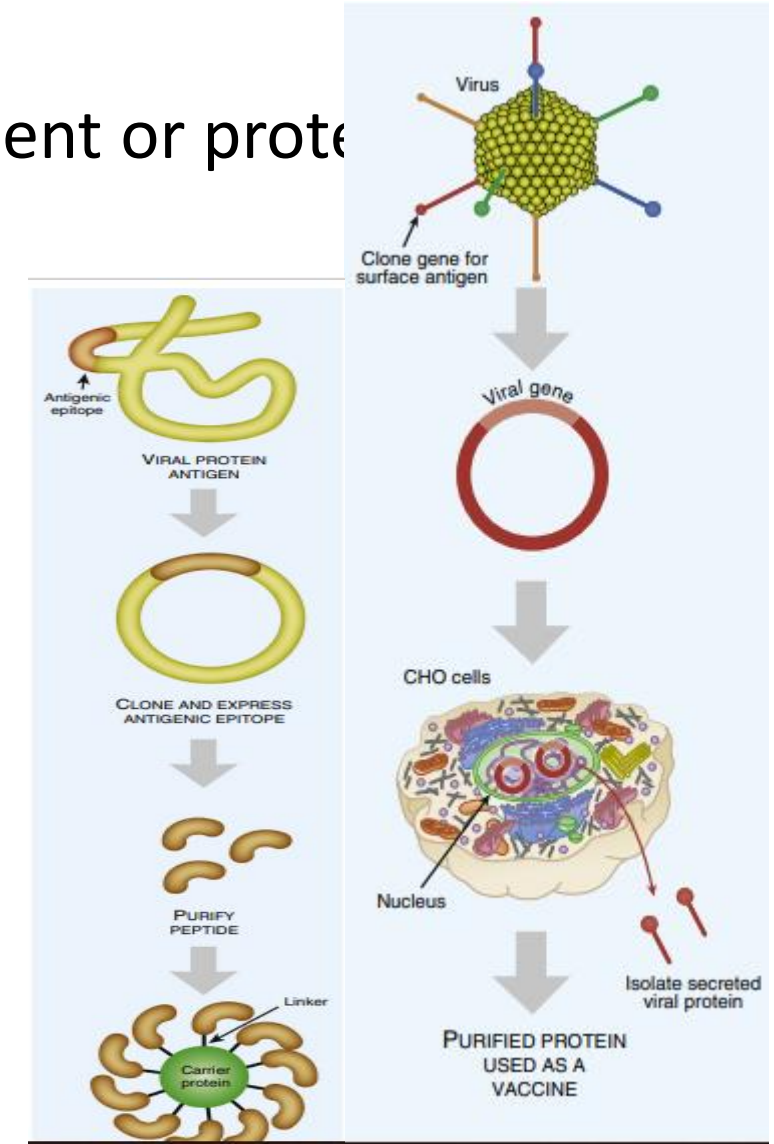
1. Killed
2. Attenuated



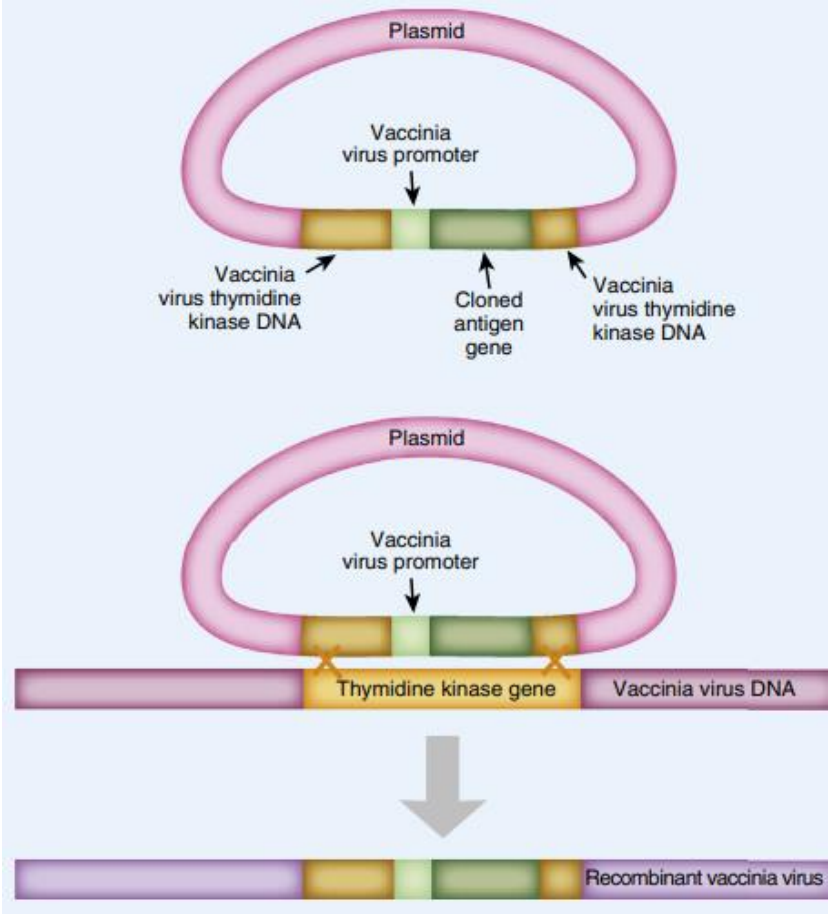
Subunit vaccines

- Subunit vaccines are effective against one component or prote
  the disease agent, rather than the whole diseas.
- peptide vaccines.
- Multivalent:

1. Flu
2. MMR vaccine (measles, mumps, and rubella)

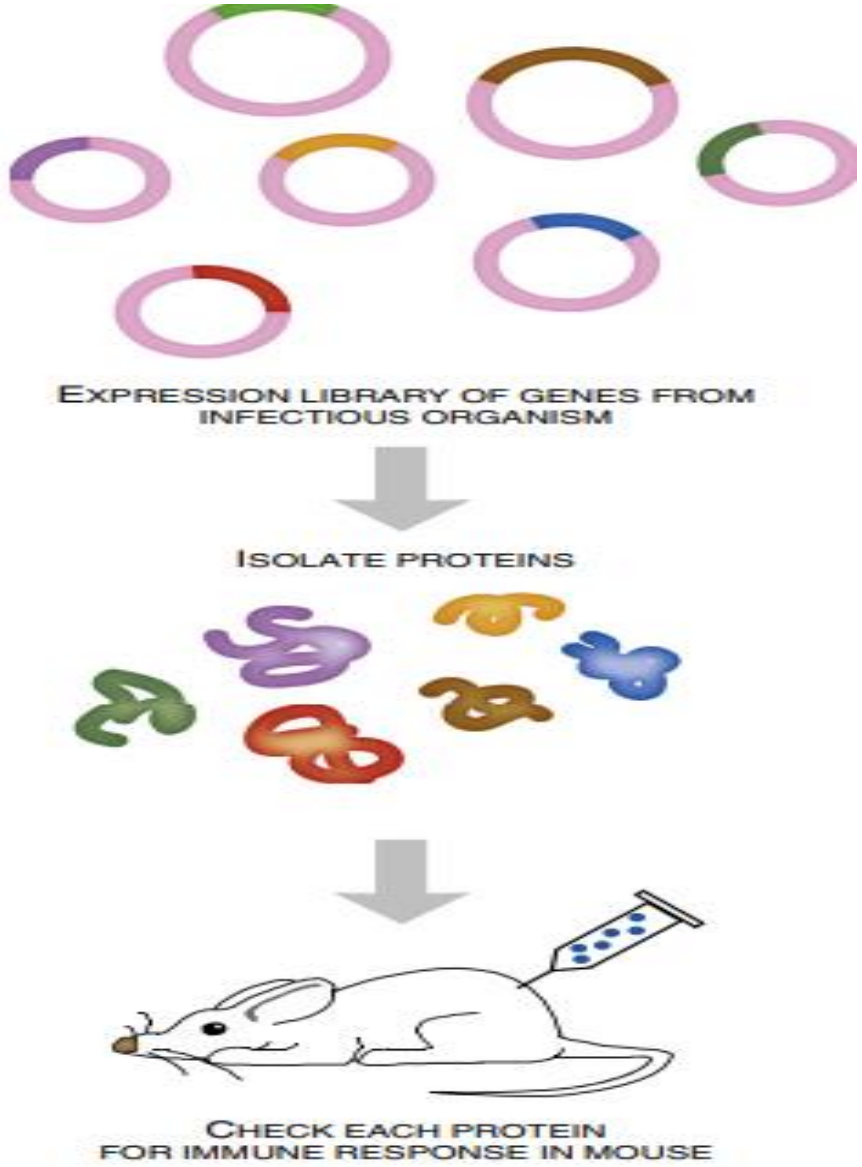


MAKING VECTOR VACCINES USING HOMOLOGOUS RECOMBINATION



REVERSE VACCINOLOGY

- Neisseria meningitidis

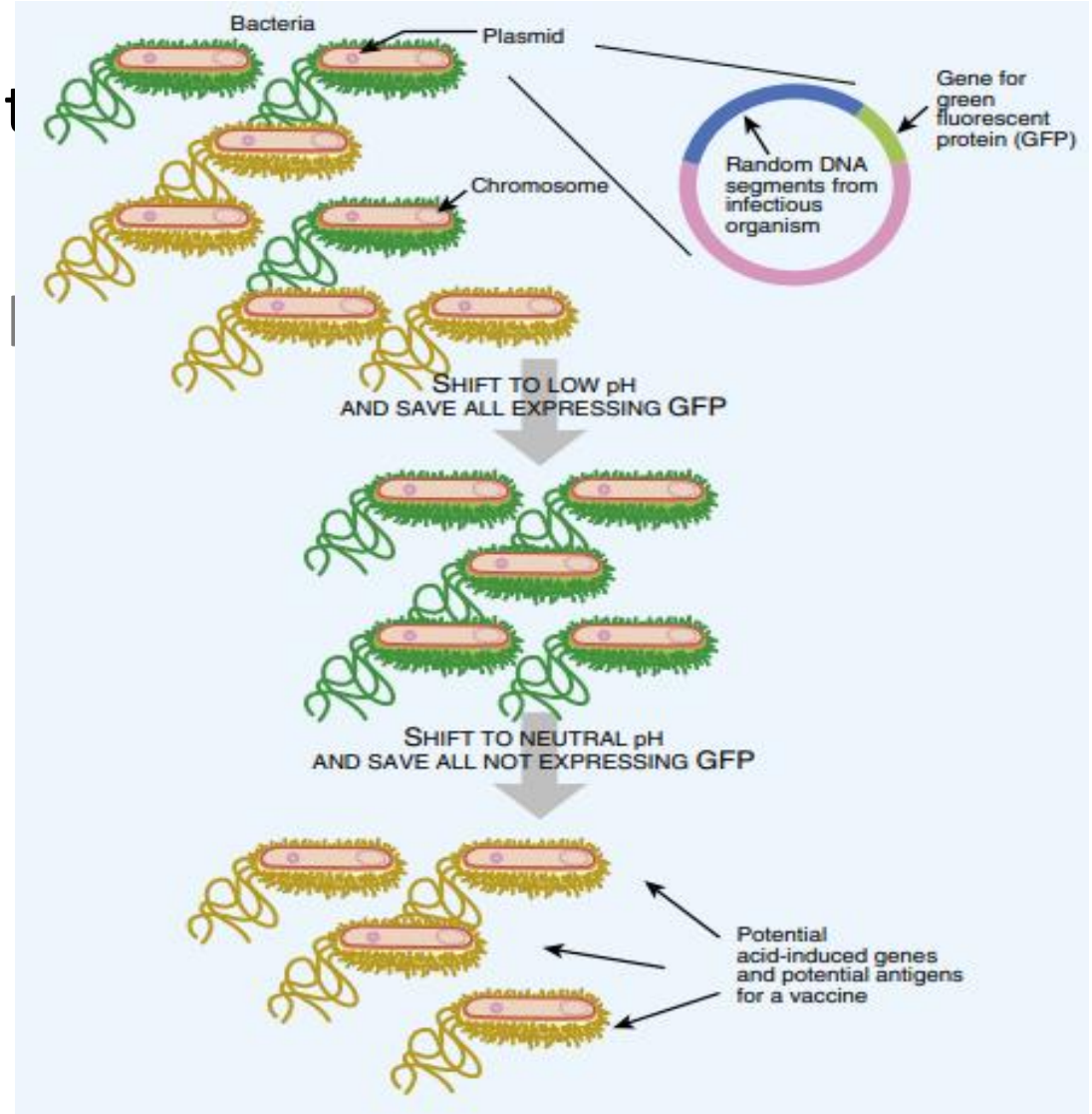


IDENTIFYING NEW ANTIGENS FOR VACCINES

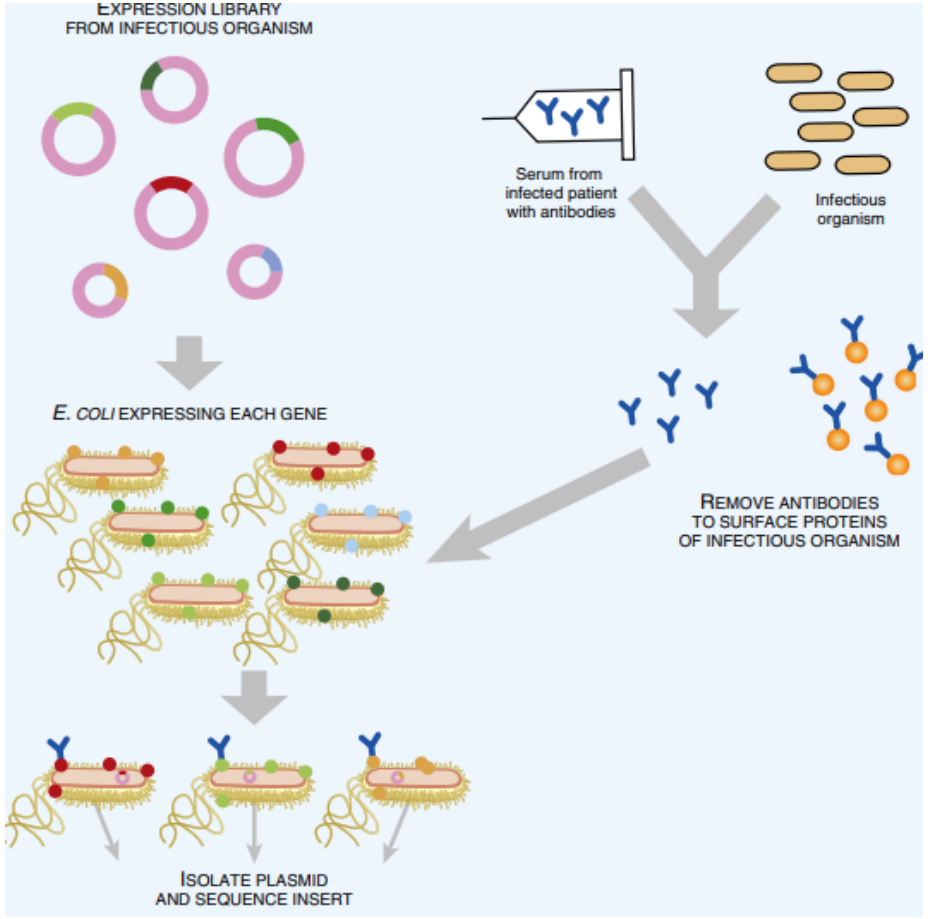
1. differential fluorescence induction (DFI)
2. in vivo induced antigen technology (IVIAT)

differential fluorescence induction (DFI)

-Another approach to creating vaccines is t
identify bacterial pathogen genes that are
expressed when the pathogen enters the



in vivo induced antigen technology (IVIAT)



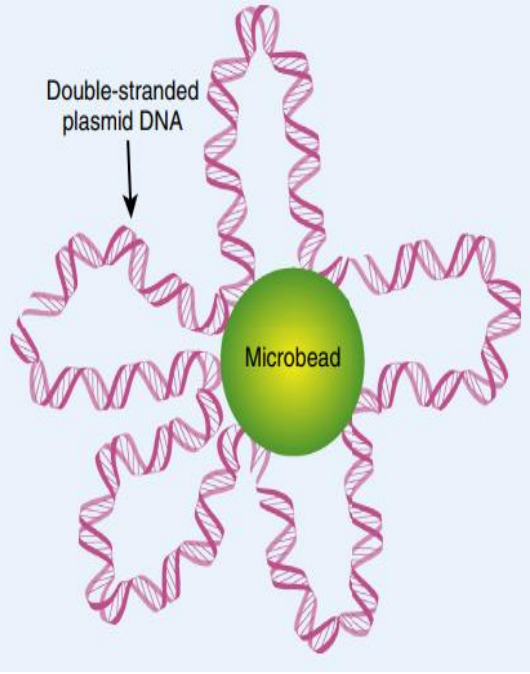
DNA VACCINES BYPASS THE NEED TO PURIFY ANTIGENS

Advantages:

1. immune response is localized to the chosen muscle, whic
   avoid side effects.
2. purified DNA is much cheaper to prepare than purified pr
3. stored dry at room temperature, avoiding the need for re

- Disadvantages:

1. may cause the body to target its own DNA, thus generatir
   autoimmune response



EDIBLE VACCINES

- Why are they made?

Thanks for your attention