DNA IMMUNIZATION

Week 13

AŞILAR

KONVANSİYONEL

1.İnaktif aşılar

2.Canlı aşılar

- attenue aşılar
  - kültür attenue
  - konak attenue
  - doğal attenue (mutant)
- heterolog aşılar
- tam virulent aşılar

3.Toksoid aşılar

4.Subunit aşılar

BİYOTEKNOLOJİK

1.Sentetik peptidler

2.Anti-idiotip aşılar

3.Genetik mühendisliği teknikleri

- rekombinant antijenler (kategori I)
- genetik-attenue organizmalar (kategori II)
- canlı rekombinant organizmalar (kategori III)

4.DNA aşıları

DNA Immunization

Instead of delivering the agent to the body, an antigen belonging to the agent, the plasmid containing the DNA sequence (gene) encoding the antigen is administered using controlled conditions, appropriate methods and methods, and the target (protective) antigen is produced and presented to the immune system in cells receiving the plasmid or plasmid transfected. It is called DNA IMMUNIZATION.

Gene cloning

- Obtaining identical copies of a gene
- The gene encoding the synthesis of an important product (or protein) is excised from the genome (or chromosome) of the cell (prokaryotic or eukaryotic) to which it belongs, by special methods, it is combined with a carrier vector DNA and transferred to a recipient cell (prokaryotic or eukaryotic) is the expression of the gene in the cell.

Important steps in gene cloning

Obtaining pure gene carrying DNA (or RNA),

Determining the location of the gene,

Removal of the gene

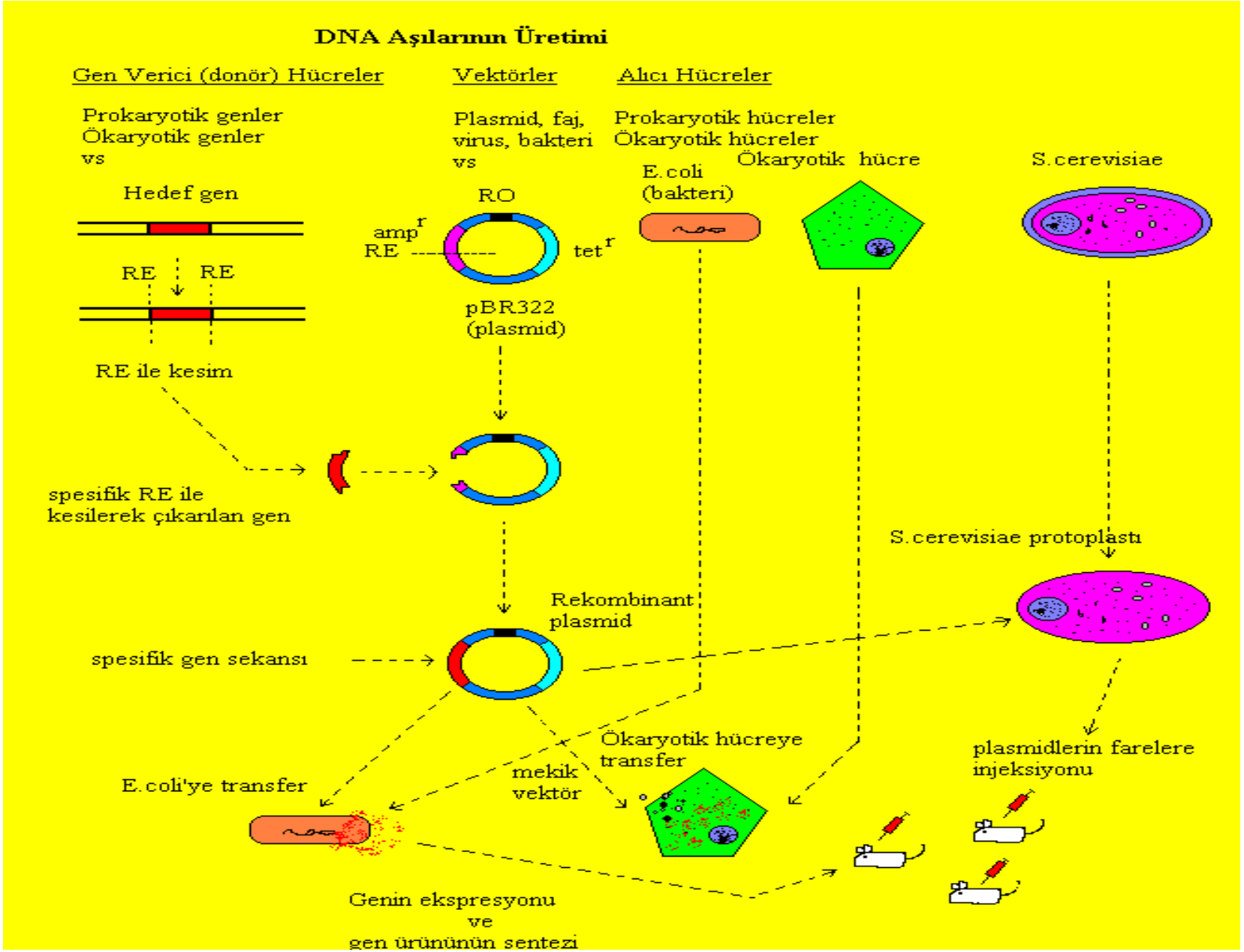
Obtaining the carrier (vector) DNA,

Combining gene DNA with vector DNA,

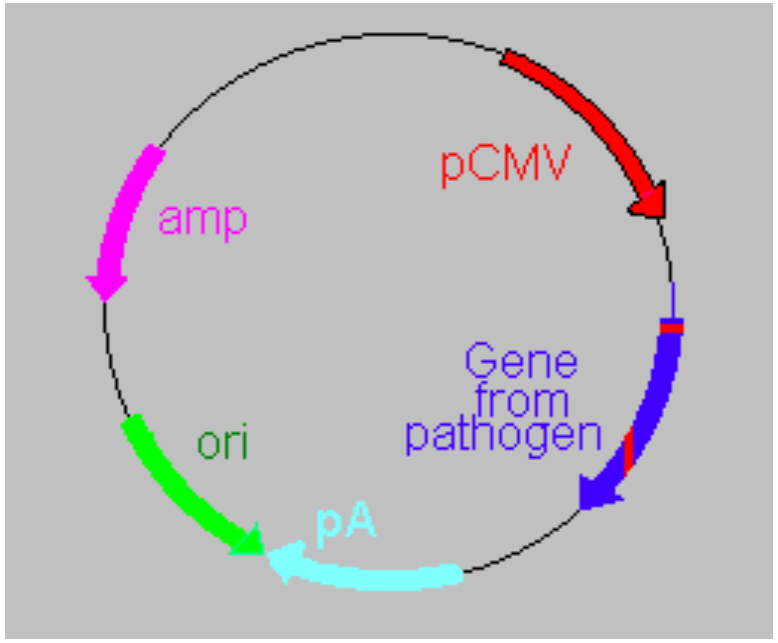
Transferring the resulting recombinant vector DNA to the recipient cell,

Selection,

Checking the gene product


DNA Aşılarının Üretimi
Gen Verici (donör) Hücreler
Vektörler
Alıcı Hücreler
Prokaryotik genler
Ökaryotik genler
vs
Plasmid, faj,
virus, bakteri
vs
Prokaryotik hücreler
Ökaryotik hücreler
Ökaryotik hücre
S.cerevisiae
E.coli
(bakteri)
Hedef gen
RO
amp r
RE
tet r
RE
RE
pBR322
(plasmid)
RE ile kesim
spesifik RE ile
kesilerek çıkarılan gen
S.cerevisiae protoplastı
Rekombinant
plasmid
spesifik gen sekansı
Ökaryotik hücreye
transfer
plasmidlerin farelere
injeksiyonu
E.coli'ye transfer
mekik
vektör
Genin ekspresyonu
ve
gen ürününün sentezi

Components of an Ideal Plasmid

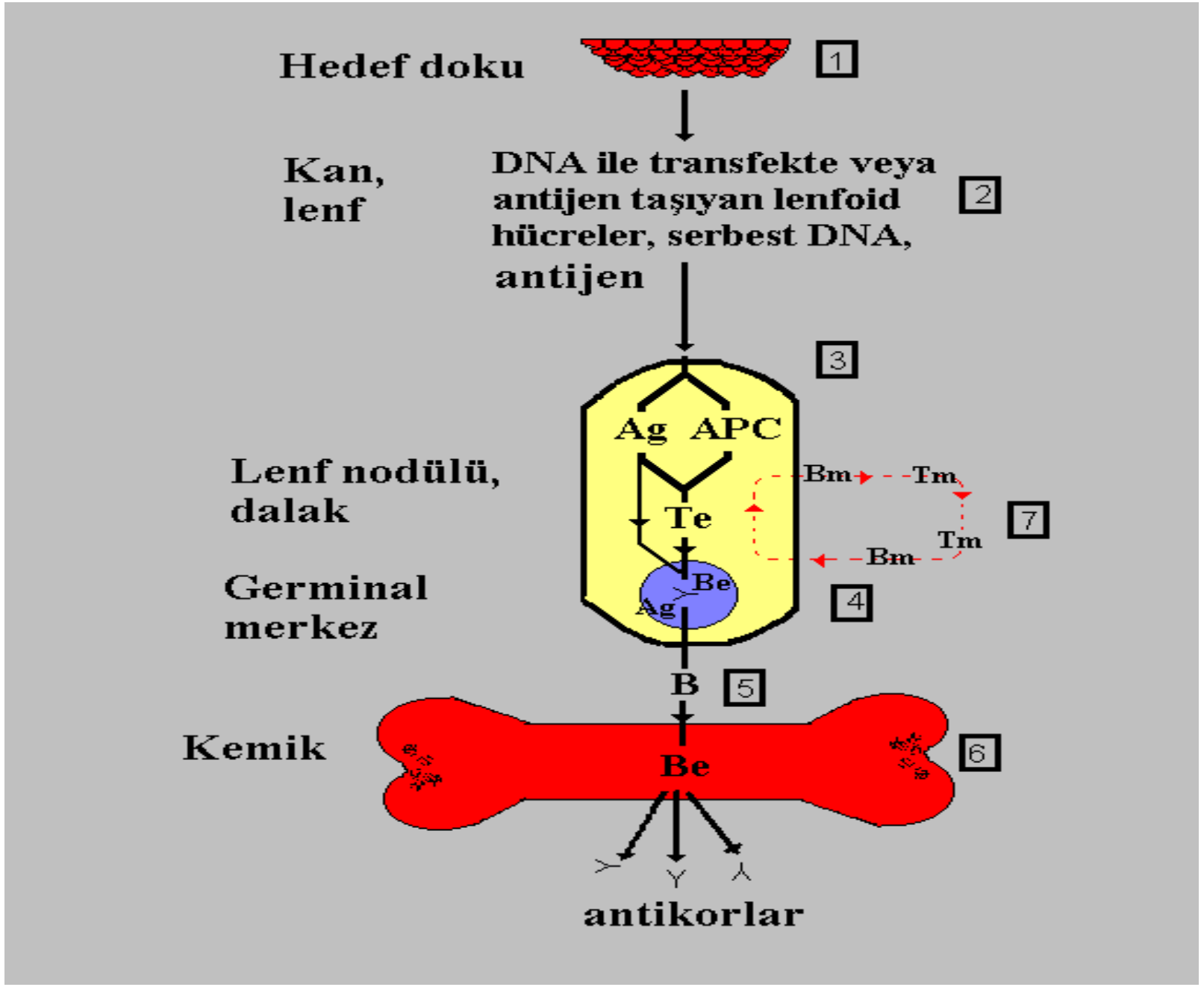


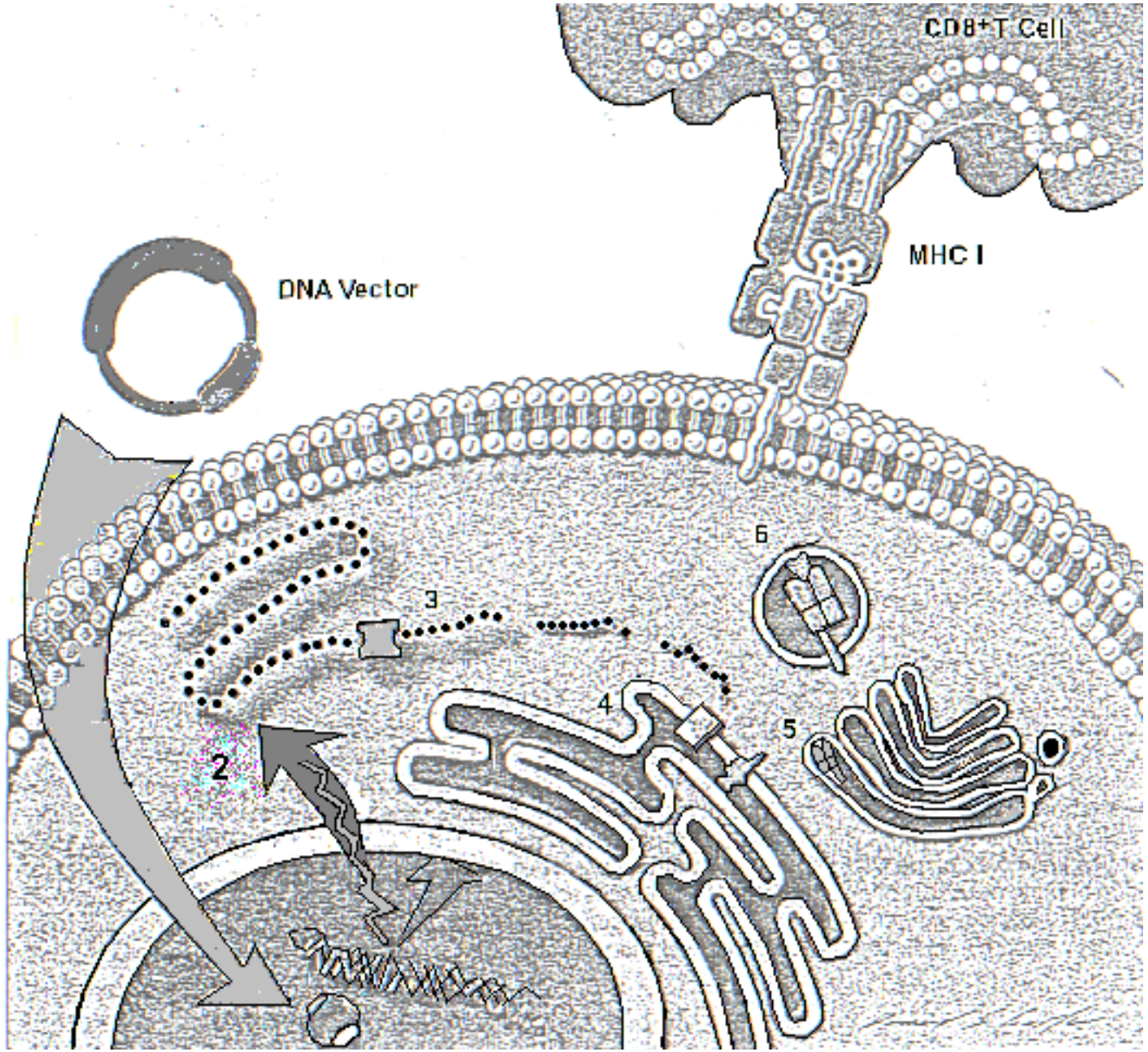
- A strong eukaryotic promoter,
- A cloning site for the insertion of the gene belonging to the pathogen,
- A polyadenylation-termination sequence,
- A prokaryotic origin of replication,
- A marker enabling selection, such as an ampicillin-resistance gene (amp)

Model	Antijenler	Hayvanlar	Şekillenen hücreler
HSV-1 zosteriform	gB	Fare/BALB/c	T hücreleri
HSV-1 zosteriform	ICP27	Fare/BALB/c	T hücreleri
HSV-1-CTL in vitro	gB and ICP27	Fare/BALB/c/ C57.B46	T hücreleri
HSV-2 vaginitis	gD	Fare/BALB/c	B hücreleri
HSV-2 vaginitis	gD	Guinea pig	B hücreleri
Bovine herpes virus	gIV	Fare/BALB/c	B hücreleri
Influenza lung	NP	Fare/BALB/c	T ve B hücreleri
Influenza lung	HA	Tavuklar	B hücreleri
Influenza lung	NP	Fare/BALB/c	T ve B hücreleri
Influenza lung	HA	Tavuklar	B hücreleri
Influenza	NP	Ferretler ve Afrika yeşil maymunları	B hücreleri
Influenza lung	HA	Fare/BALB/c	B hücreleri
Rabies-I/M	G-protein	Fare/BALB/C3 H/ HEN	T ve B hücreleri
Rabies-I/M	G-protein	Fare/C3H/HEN	T ve B hücreleri
LCMV I/C	NP	Fare/BALB/c	T ve B hücreleri
LCMV I/C	NP	Fare/C57BL/6	T ve B

Model	Antijenler	Hayvanlar	Şekillenen hücreler
Hepatitis C virus	Core protein	Fare/BALB/c	T ve B hücreleri
Hepatitis B virus	HBs Ag	Fare/BALB/c	T ve B hücreleri
Hepatitis B virus	HBs Ag	Fare/BALB/c C57BL/6	T ve B hücreleri
Hepatitis B virus	HBc Ag	C57BL/6J	T ve B hücreleri
HIV	gp 160	Fare/BALB/c	T ve B hücreleri
HIV	gp 120	Fare/BALB/c	T hücreleri
SIV	env ve gag	Rhesus maymunları	T hücreleri
SV-40	Tümör antijeni	Fare/BALB/c	T ve B hücreleri
Plasmodium yoelii	Circumsporozoit protein	Fare/BALB/c	T ve B hücreleri
Plasmodium yoelii	CSP, PyHep17	Fare/BALB/c	T ve B hücreleri
Leishmania major	gp 63	Fare/BALB/c	T hücreleri
Mycoplasma pulmonis	Bütün antijenler ELI	Fare	T ve B hücreleri
Mycobacterium tuberculosis	M. leprae HSP 65	Fare/BALB/c	T hücreleri

TABLO1 DNA Aşılarında Kullanılan Deney Hayvanı Modelleri


Hedef doku
1
Kan,
lenf
DNA ile transfekte veya
antijen taşıyan lenfoid
hücreler, serbest DNA,
antijen
2
3
Ag APC
Lenf nodülü,
dalak
Bm
Tm
Te
Tm
7
Bm
Germinal
merkez
Be
Ag
4
B
5
Kemik
Be
6
antikorlar


CD8+T Cell
MHC I
DNA Vector
6
3
4
5
2

Comparison of DNA vaccines with others

- Plasmid DNA purity, ease of production, physico-chemical stability,
- Various combinations of immunogens in a single dose
- Cheaper in vaccine production and distribution than subunit vaccines and recombinant proteins,
- Expression of vaccine antigens in natural form in DNA-provided transfer,
- Both CD4 + helper T cell and CD8 + CTL response
- Repeatability of plasmids without being affected by the existing vector specific immunity,
- Immunity formation in very young animals even in the presence of maternal antibodies,
- Production of vaccine encoded proteins in vivo, continuous and low levels, high affinity T and B cells

Advantages of DNA Vaccines

- Any DNA sequence, even those containing long inserts, can be inserted into the plasmid.
- Plasmids can be lyophilized for long periods of time at room temperature when they are produced and purified in large quantities, the transport of vaccines is easy and cheap,
- The most reliable way to prepare immunogen against harmful agents such as Ebola virus,
- Long-term antigen expression provides long-term T cell response and immunological memory formation,
- The potential to encode multiple antigens, including molecules that may affect the nature of the immune response.
- DNA vaccines encoding multiple epitopes,
- T cell response generation associated with protection rather than tissue damage

Disadvantages of DNA Vaccines

- The necessity of antigens to have protein character and difficulties in ensuring their glucosylation
- Important possibilities regarding DNA vaccines
- Integration of plasmid DNA into the host genome leading to insertional mutations and tumor formations
- autoimmune responses, including anti-DNA antibodies
- Cessation of tolerance to self proteins due to tolerance formation or heavy antigen expression

Points to Consider in the Development of DNA Vaccines

- Absence or insufficient vaccines available (eg HIV, hepatitis C, influenza, tuberculosis, leishmaniosis, schistosomiosis, malaria)
- Nowadays, the costs of vaccination strategies reach prohibitive levels.
- The need for immunity in newborns

Promising Results of Recent DNA Vaccines

- CTL-based protection formation in MHC haplotype in mice vaccinated against malaria (malaria) multigene
- Reports of high protection against agents with antigenic variability such as influenza virus
- Protective immunity formation in chimpanzees against infection shaped by heterologous strain of HIV
- Elimination of persistent mycoplasmal pneumonia in mice with vaccination after exposure to the agent.

Uses of DNA Vaccines

- Bacterial Infections
- Viral Infections
- Parasitic Infections
- Tumors

Bacterial Infections

- Brucellosis
- Lyme Disease
- Mycoplasmosis
- Salmonellosis
- Tetanus
- Tuberculosis

Other DNA Vaccines

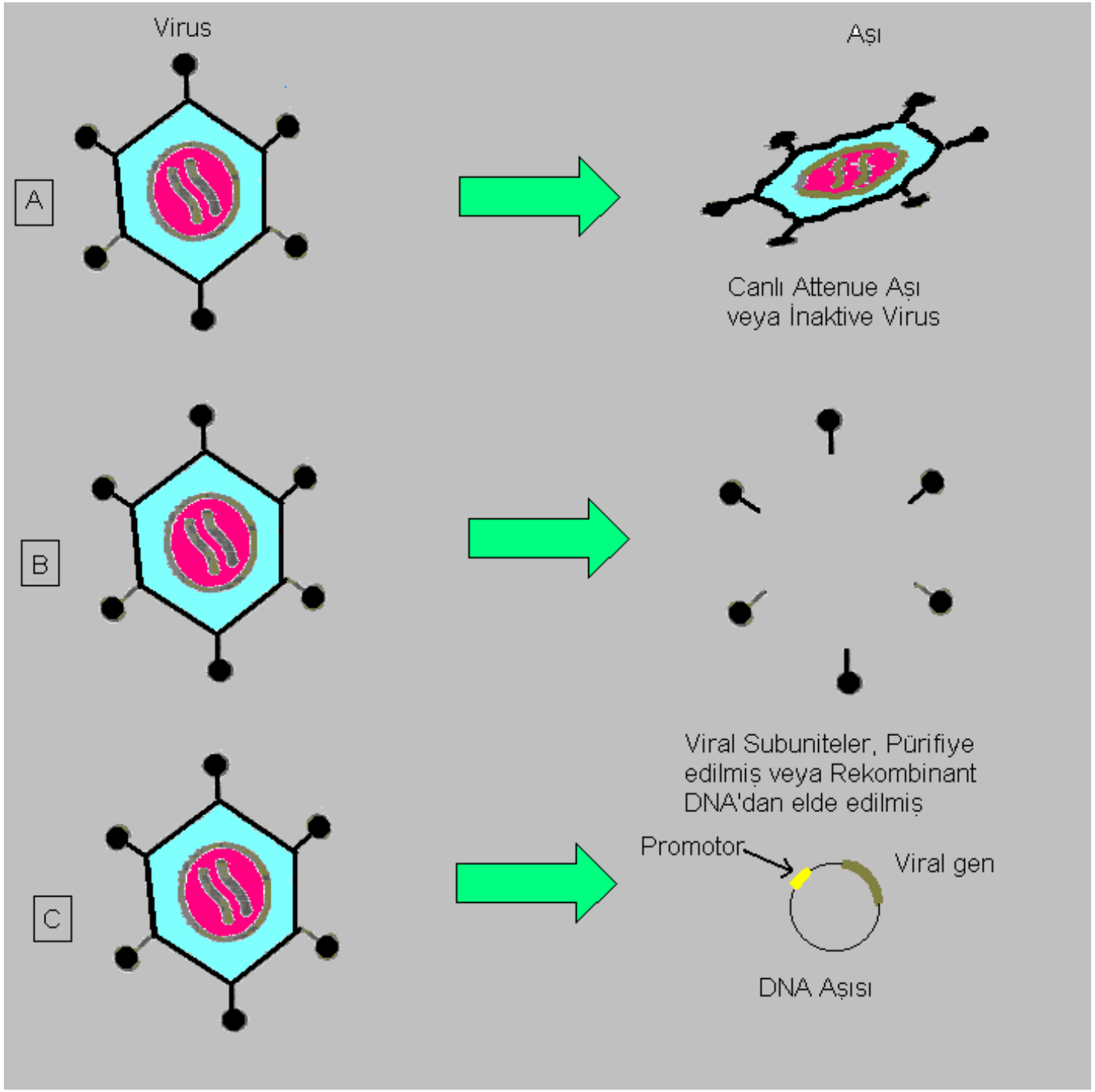
Viral Infections

- Influenza virus
- bovine herpes virus
- Human herpes simplex virus
- rabies virus
- lymphocytic choriomeningitis virus
- cottontail rabbit papilloma virus
- hepatitis B virus
- HIV virus

Parasitic Infections

- Schistosoma japonicum
- Leishmania major
- Plasmodium yoelii

Tumors


Virus
Aşı
A
Canlı Attenue Aşı
veya İnaktive Virus
B
Viral Subuniteler, Pürifiye
edilmiş veya Rekombinant
DNA'dan elde edilmiş
C
Promotor
Viral gen
DNA Aşısı