Gene modification techniques

PhD course

- Transient
- Permanent

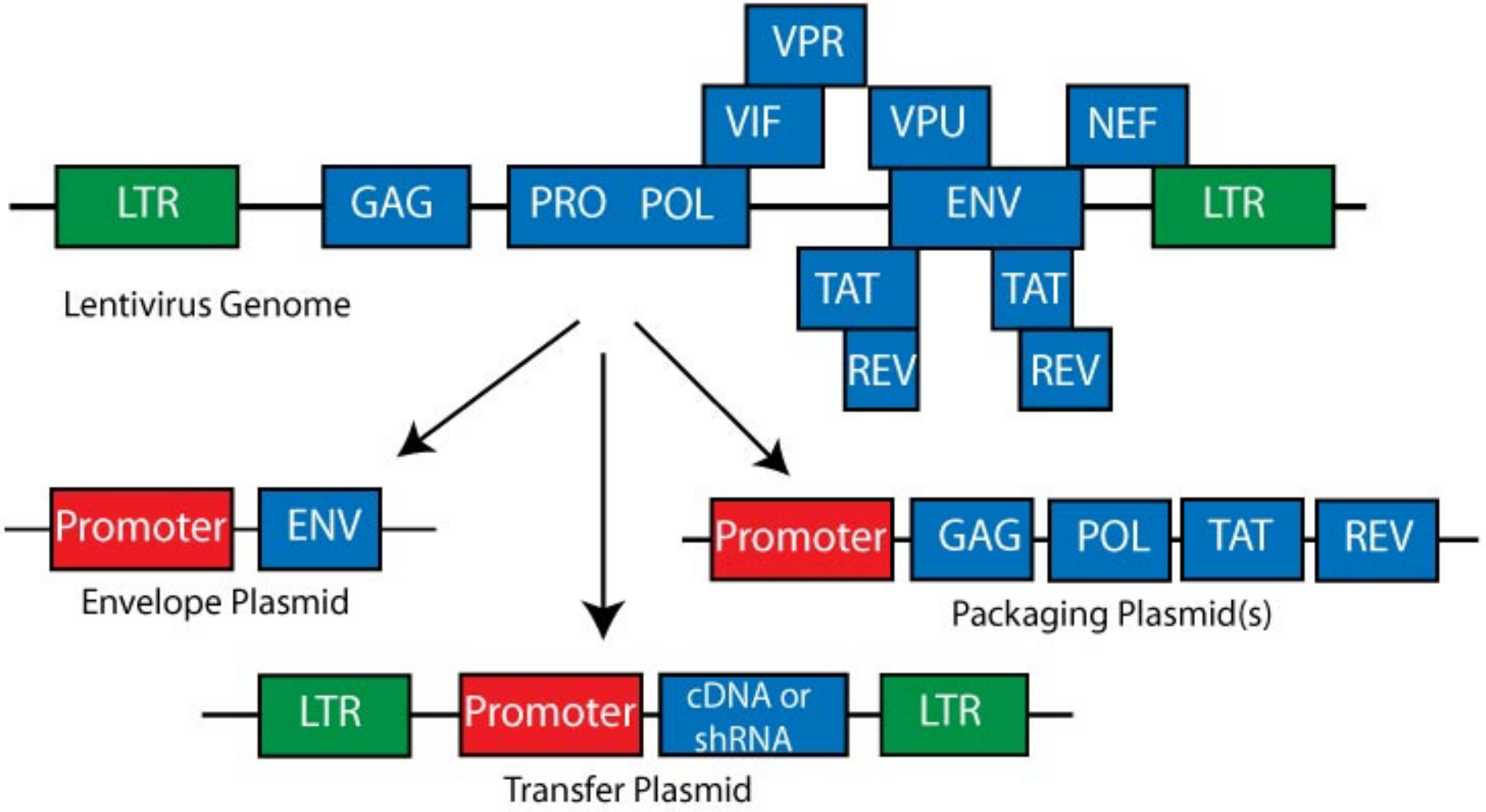
Transient methods

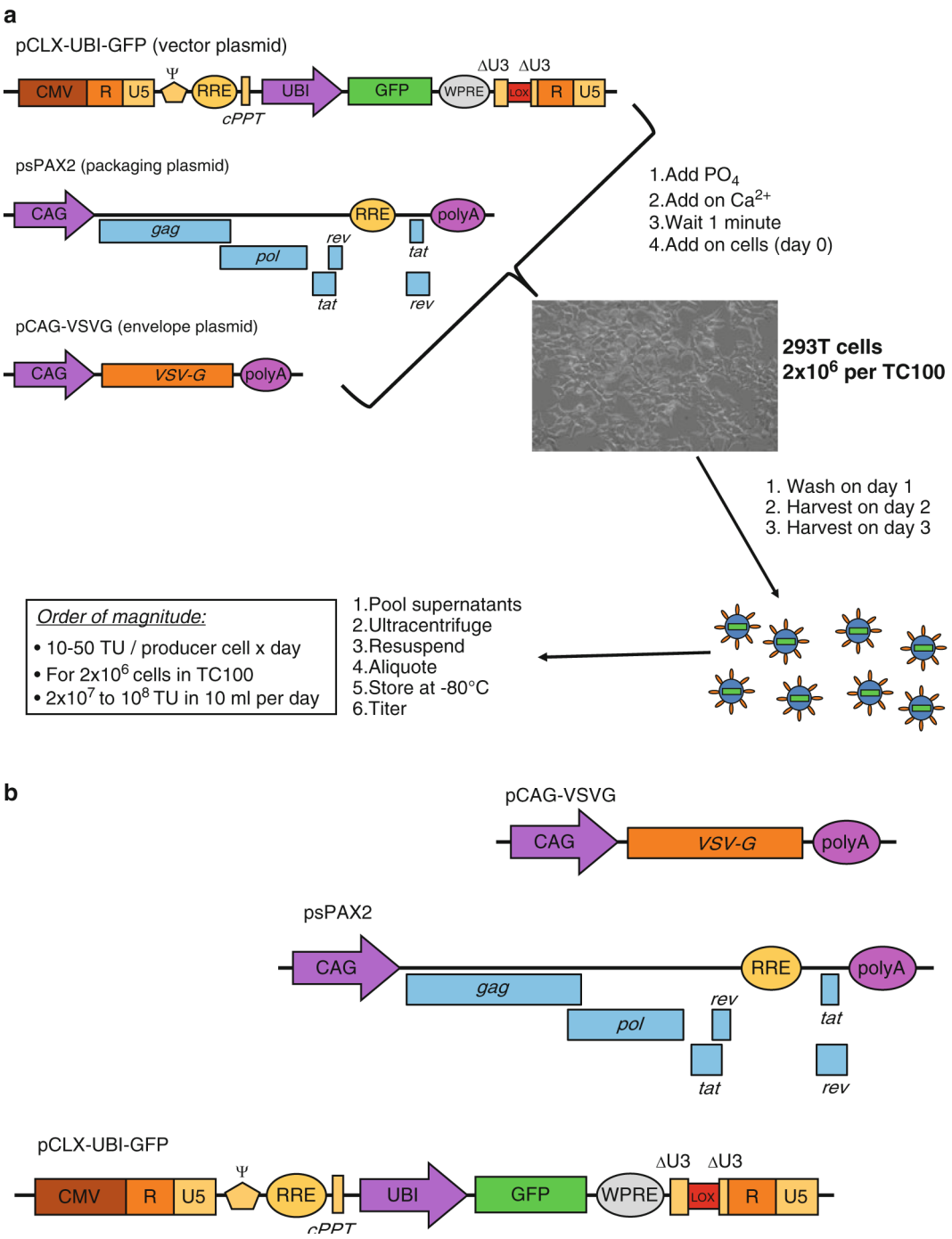
- Calcium-phosphate precipitate
- Electroporation
- Lipofectamin
- “Gene-gun”
- Adenovirus

Permanent methods

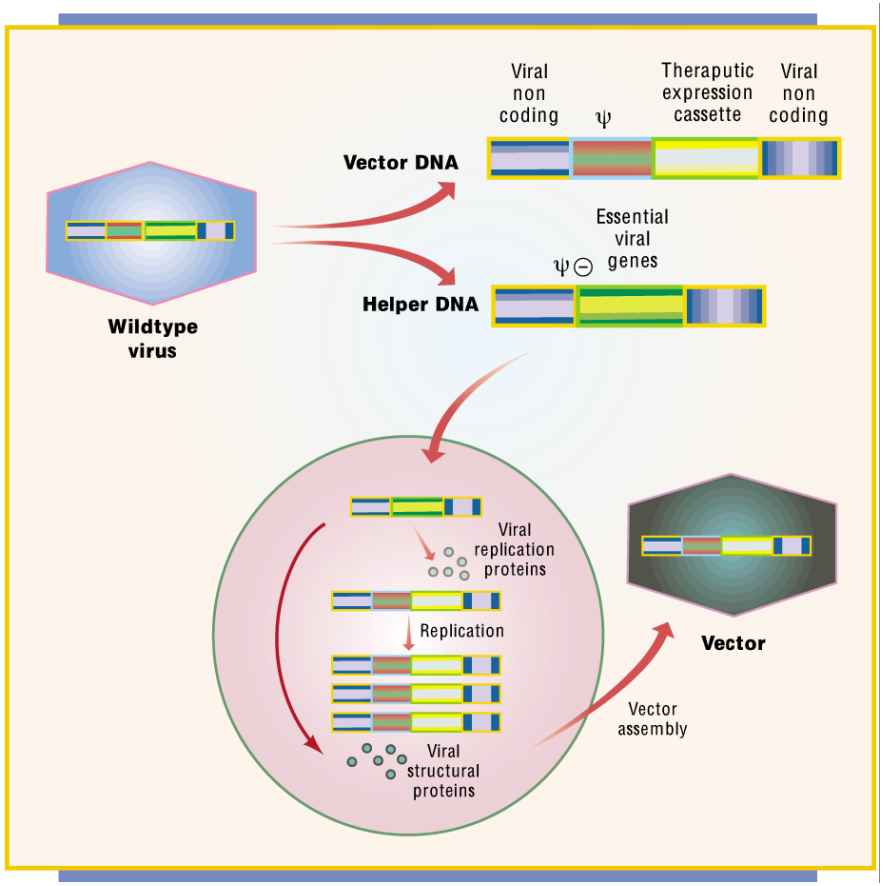
- Retrovirus
- Lentivirus
- Adeno-associated virus

Lentiviral system

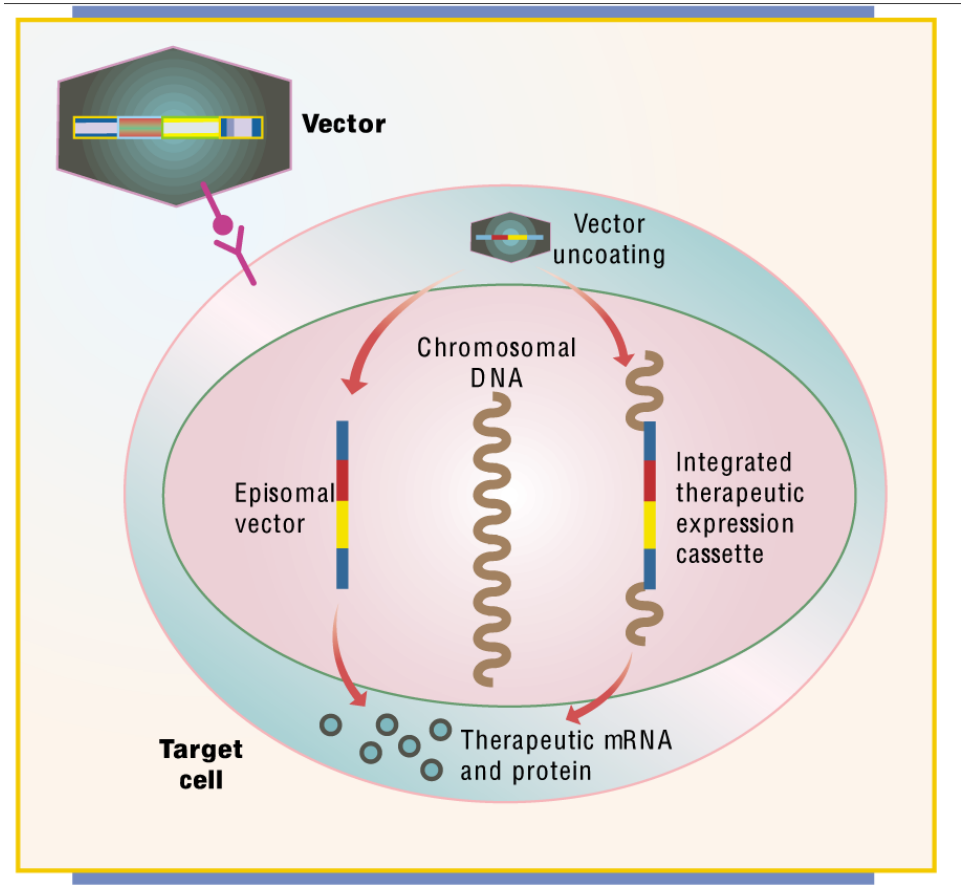



a
pCLX-UBI-GFP (vector plasmid)
CMV
R
U5
Ψ
RRE
cPPT
UBI
GFP
WPRE
ΔU3
Lox
ΔU3
R
U5
psPAX2 (packaging plasmid)
CAG
gag
pol
RRE
rev
tat
tat
rev
polyA
pCAG-VSVG (envelope plasmid)
CAG
VSV-G
polyA
1.Add PO4
2.Add on Ca2+
3.Wait 1 minute
4.Add on cells (day 0)
293T cells
2x10^6 per TC100
1. Wash on day 1
2. Harvest on day 2
3. Harvest on day 3
Order of magnitude:
• 10-50 TU / producer cell x day
• For 2x10^6 cells in TC100
• 2x10^7 to 10^8 TU in 10 ml per day
1.Pool supernatants
2.Ultracentrifuge
3.Resuspend
4.Aliquote
5.Store at -80°C
6.Titer
b
pCAG-VSVG
CAG
VSV-G
polyA
psPAX2
CAG
gag
pol
RRE
rev
tat
tat
rev
polyA
pCLX-UBI-GFP
CMV
R
U5
Ψ
RRE
cPPT
UBI
GFP
WPRE
ΔU3
Lox
ΔU3
R
U5

Viral gene transfer

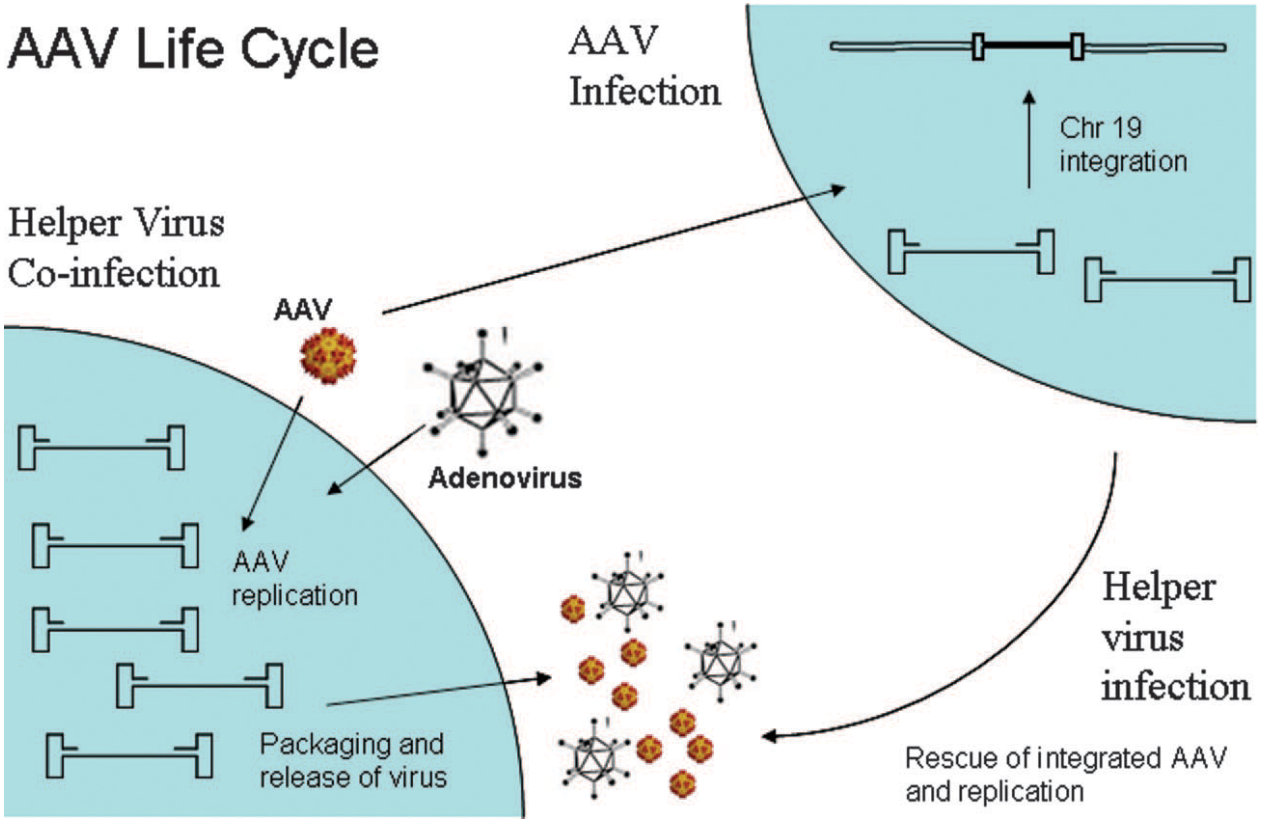


Virus production



Transfection

AAV


AAV Life Cycle
AAV
Infection
Chr 19
integration
Helper Virus
Co-infection
AAV
Adenovirus
AAV
replication
Packaging and
release of virus
Helper
virus
infection
Rescue of integrated AAV
and replication

AAV

TABLE 2. Clinical trials involving AAV vectors

Condition	Gene product(s)	Phase
CF	CFTR	I/II
Canavan's disease	Aspartoacylase	I
Parkinson's disease	GAD65, GAD65, AADC, neurturin	I
Alzheimer's disease	Beta nerve growth factor	I
Alpha-1-antitrypsin deficiency	AAT	I
Arthritis	TNFR:Fc	I
Leber congenital amaurosis	RPE65	I
Hemophilia B	Factor IX	I
Late infantile neuronal lipofuscinosis	CLN2	I
Muscular dystrophy	Minidystrophin, sarcoglycan	I
Heart failure	SERCA-2a	I
Prostate cancer	Granulocyte-macrophage colony-stimulating factory	I/II/III
Epilepsy	Neuropeptide Y	I

Monoclonal antibodies for therapy

1. Murine Abs
2. Chimera Abs
3. Humanized Abs
4. Human Abs

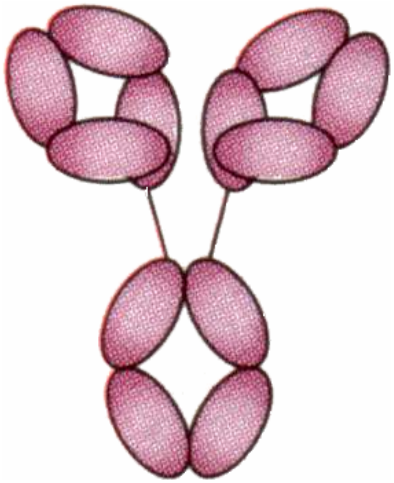
Mostly IgG molecules are used for therapy.

- **Long biological half-life (~21 days)**
- **induces ADCC & CDC**

Main disease groups

- Immunological (autoimmune, immunosuppression)
- Tumors

Murine antibodies



The first therapeutic monoclonal antibody registered by FDA.

OKT3 – mouse monoclonal anti-CD3 (1986).

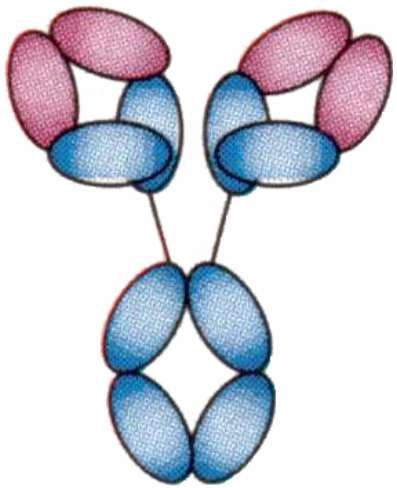
This therapy is quite effective but its repeated use is accompanied with severe immunological side effects:

HAMA (human anti-mouse-antibodies)

The constant part of an Ig is conserved, but there are some differences between human and mouse Igs.

HAMA can be detected after 8-12 days of treatment, the peak concentration is after 25-30 days.

Chimeric antibodies



The Fv region of the chosen monoclonal antibody gene is fused to Fc part of a human Ig gene.

Approximately 75 of a chimera Ig % is of human origin.

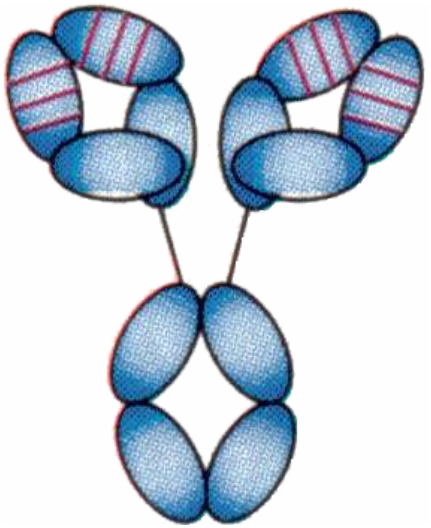
The specificity of the antibody is similar to that of the original mouse antibody.

The in vivo half life and effector functions of the Ig are similar to those of the original human antibody.

HACA (human anti-chimeric-antibodies)

Less immunogeneicity, but sometimes HACA can be detected.

Humanised antibodies



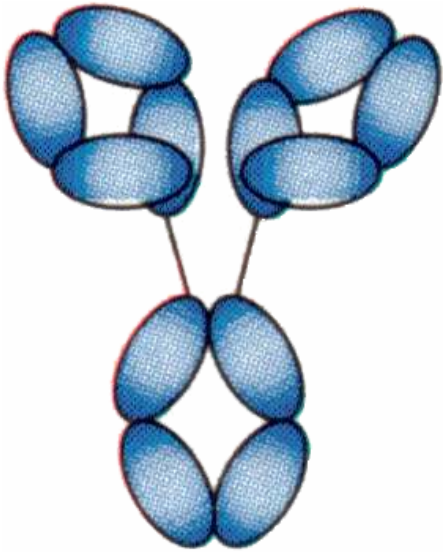
The genes of the CDR regions of a mouse monoclonal antibody are implanted into the genes of human antibody.

More than 90% of the antibody is of human origin.

The specificity of the humanized antibody is similar to that of the original mouse antibody.

The in vivo half life and effector functions of the humanized antibody are similar to those of the original human antibody.

Human antibodies



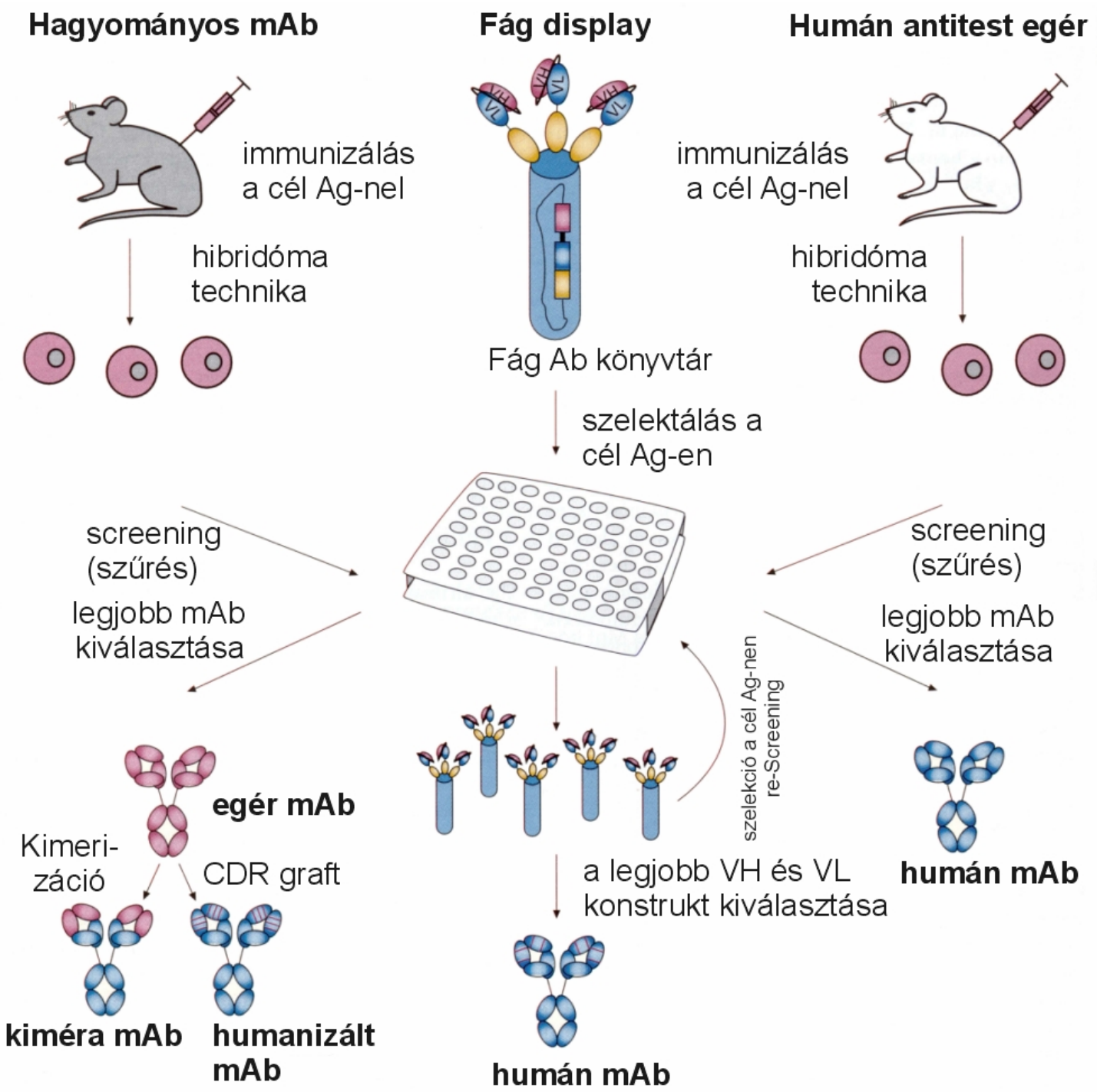
The ultimate aim is to use totally biocompatible monoclonal Abs in therapy.

Difficulties when producing human hybridomas.

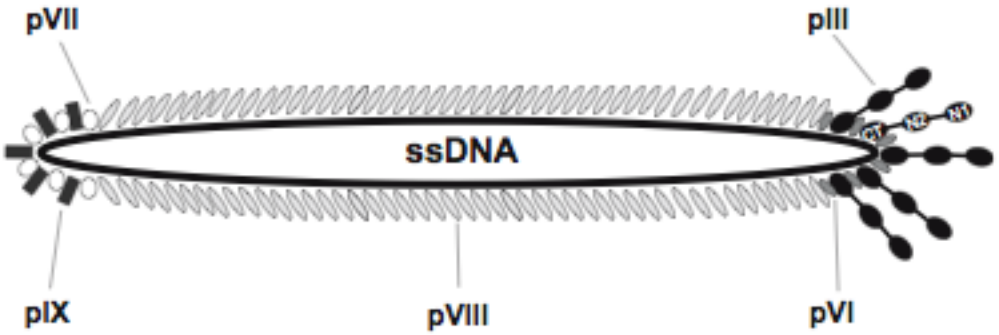
Eg. the antigens are often human proteins, which are tolerated by the immune system.

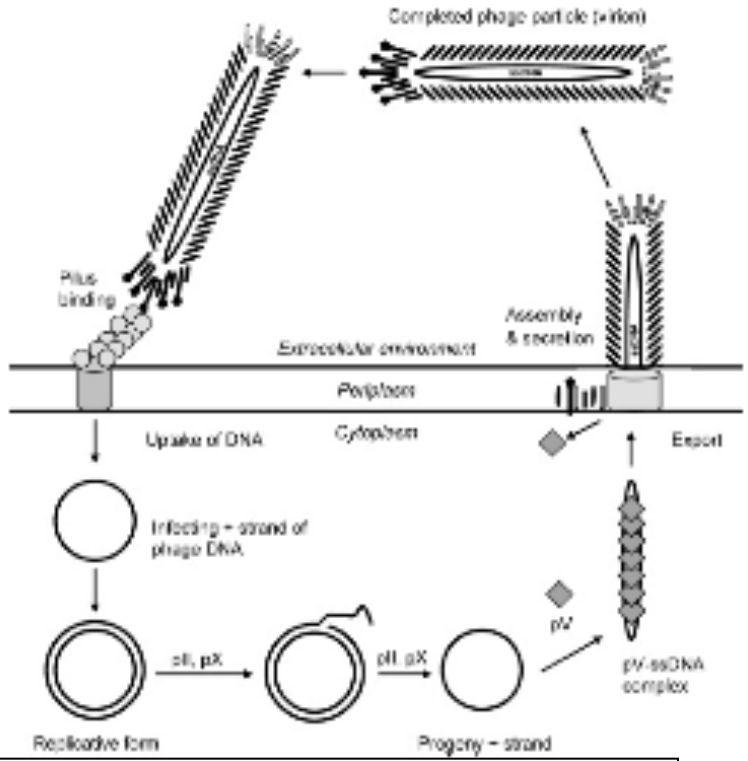
The following methods are feesible to produce human antibodies:

- **Phage display**
- **genetically modified mice**


Hagyományos mAb
Fág display
Humán antitest egér
immunizálás
a cél Ag-nel
hibridóma
technika
immunizálás
a cél Ag-nel
hibridóma
technika
Fág Ab könyvtár
szelektálás a
cél Ag-en
screening
(szűrés)
legjobb mAb
kiválasztása
screening
(szűrés)
legjobb mAb
kiválasztása
szelekció a cél Ag-nen
re-Screening
egér mAb
Kimeri-
záció
CDR graft
kiméra mAb
humanizált
mAb
a legjobb VH és VL
konstrukt kiválasztása
humán mAb
humán mAb

M13





Science. 1985 Jun 14;228(4705):1315-7.

Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface.

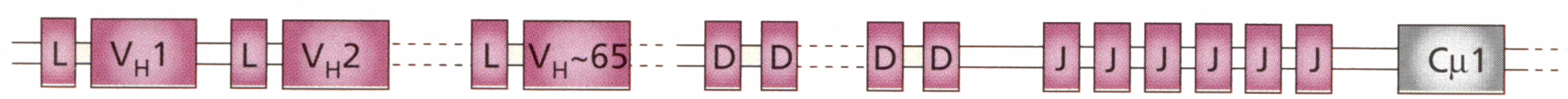
Smith GP.

Foreign DNA fragments can be inserted into filamentous phage gene III to create a fusion protein with the foreign sequence in the middle. The fusion protein is incorporated into the virion, which retains infectivity and displays the foreign amino acids in immunologically accessible form. These "fusion phage" can be enriched more than 1000-fold over ordinary phage by affinity for antibody directed against the foreign sequence. Fusion phage may provide a simple way of cloning a gene when an antibody against the product of that gene is available.

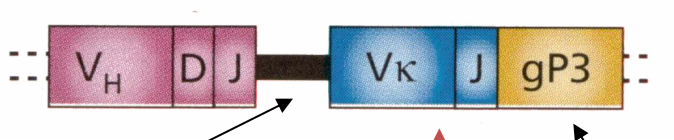
PMID: 4001944 [PubMed - indexed for MEDLINE]

Phage display

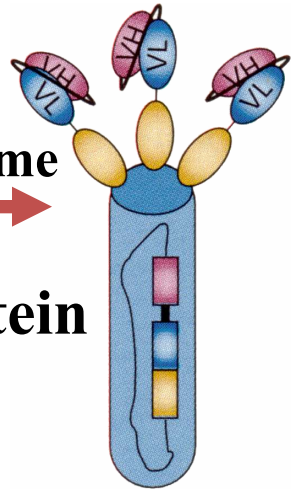
H chain



1) Amplification of V_H (~65), D_H (27) & J_H (6) genes by PCR
2) Ligation (~ 10.000 variations)



Cloning into the phage genome



Linker
correct orientation of the
H- & L chains

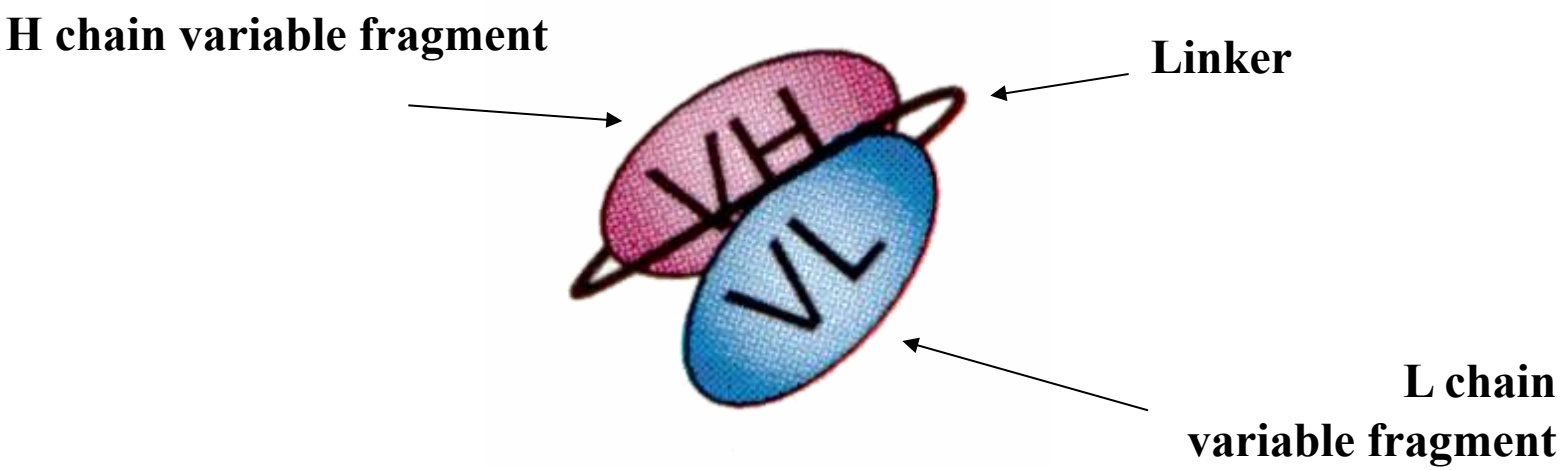
Bacteriophage capsid protein
binds the „mini-Ab to the
bacteriophage

2) Ligation (~ 200 variations)
1) Amplification of Vκ (~40) & Jκ (5) genes by PCR

L chain

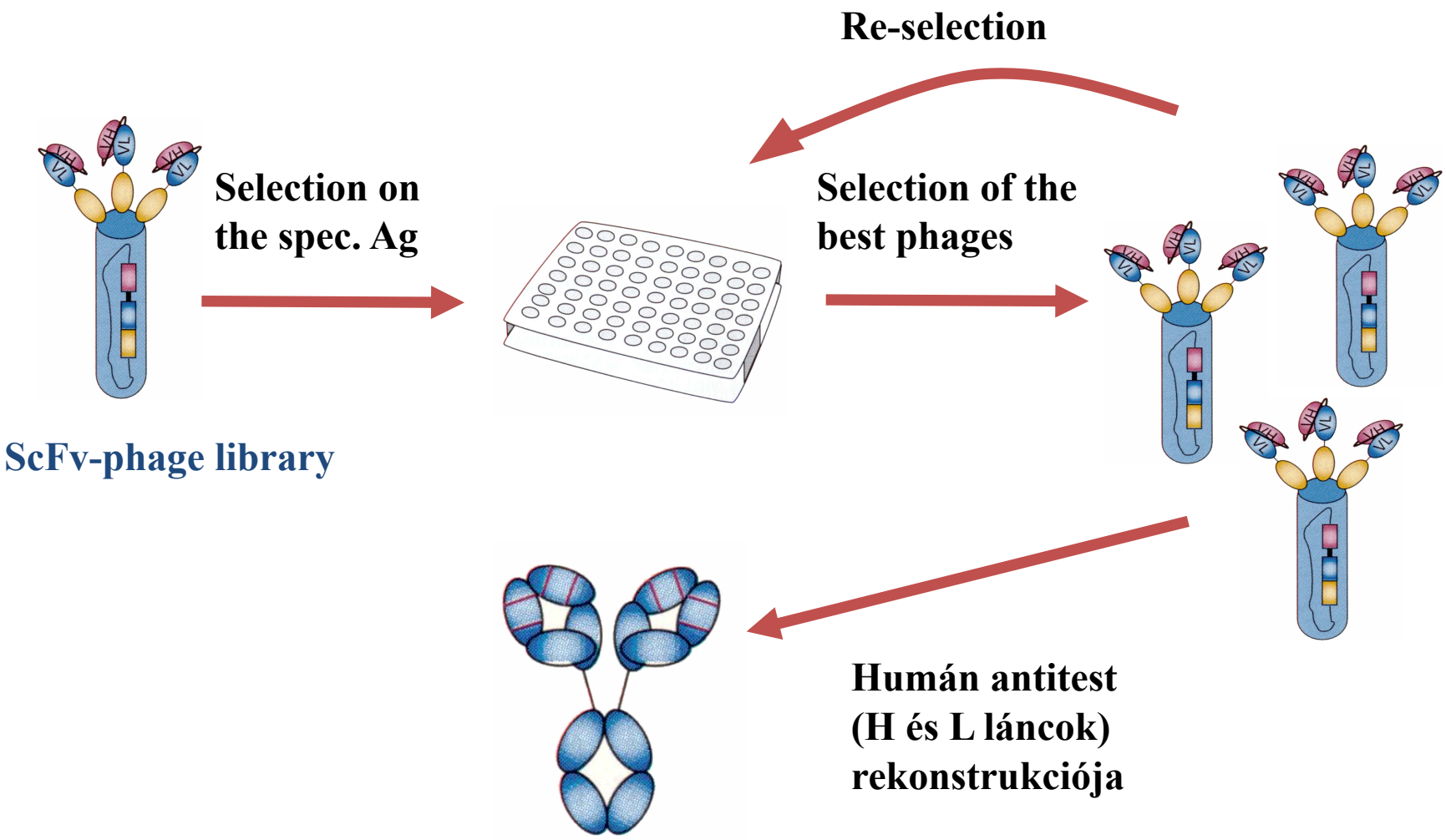
ScFv

Single chain variable fragments

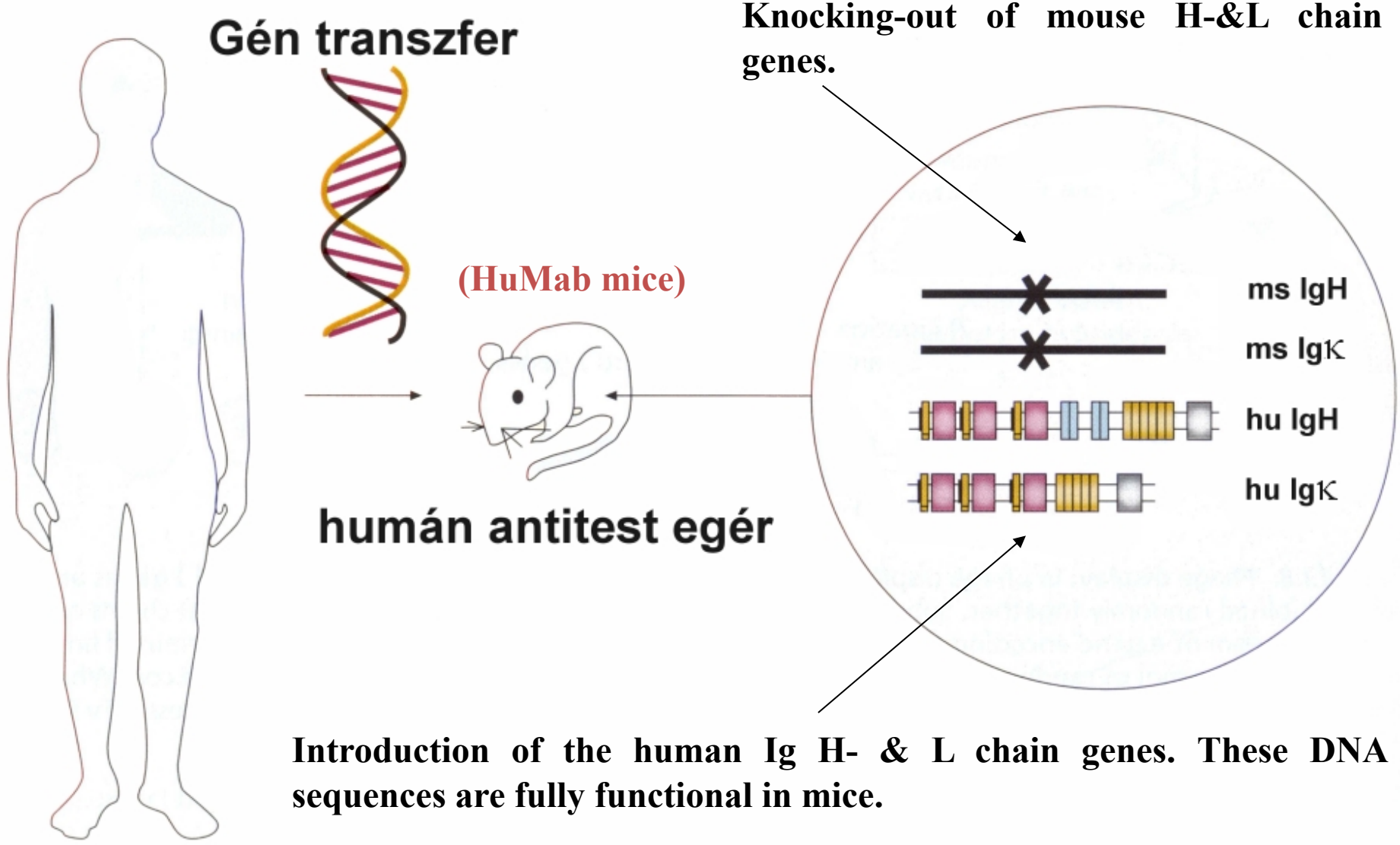


This „mini Ab” is able to bind the antigen!

Screening of the ScFv-phage library



Genetically-modified mice



Knocking-out of mouse H-&L chain genes.

Introduction of the human Ig H- & L chain genes. These DNA sequences are fully functional in mice.

Trans-chromosomal mouse

Besides the normal chromosomes such mice contain a „human mini-chromosome”, which was constructed from the human Chr. 14 (H chain) and 2 (κ L chain).

The mini chromosome contains all germ line H-& L chain gene clusters.

In these mice the human antibody response can be mimiced almost perfectly.

Diagnostic mAbs

Generic name	Commercial name	Indication
Technécium-99m-acitumomab	CEA-scan	Metastatic colorectal cancer
Imicromab penetrate	MyoScint	Myocardial infarction
Satumomab pendetide	OncoScint CR/OV	Colorectal and ovary cancer
Capromab	ProstataScint	Prostata adenocarcinoma
Nofetumomab	Verluma	Small cell lung cancer

Termék neve	Specificitása	Típusa	Alkalmazási terület	Év
Orthoclone OKT3	CD3	egér	Transzplantátum rejekció	1986
ReoPro	GpIIb/gpIIa	kiméra Fab	Kardiovaszkuláris betegségek	1994
Rituxan (mabthera)	CD20	kiméra	Non-Hodgkin lymphoma	1997
Zenapax	CD25	humanizált	Transzplantátum rejekció	1997
Remicade	TNFα	kiméra	Crohn betegség, rheumatoid arthritis	1998
Simulect	CD25	kiméra	Transzplantátum rejekció	1998
Synagis	RSV	humanizált	Respiratorikus synctitium vírusfertőzés	1998
Herceptin	Her-2	humanizált	Metasztatikus emlőrák	1998
Mylotarg	CD33	humanizált	Akut myeloid leukémia (AML)	2000
Campath	CD52	huamnizált	B sejtes krónikus limfoid leukémia (B-CLL)	2001
Zevalin	CD20	egér	B sejtes non-Hodgkin-lymphoma	2002
Erbitux	EGFR	kiméra	Colorectális carcinoma (EGFR+ tumorok?)	2004
Avastin	VEGF	humanizált	Colorectális carcinoma	2004
Tysabri	α4β1/7 integrin	humanizált	Sclerosis multiplex	2004