Medical Biotechnology

Insulin - první gen biotech 1982

Recombinant proteins for human use

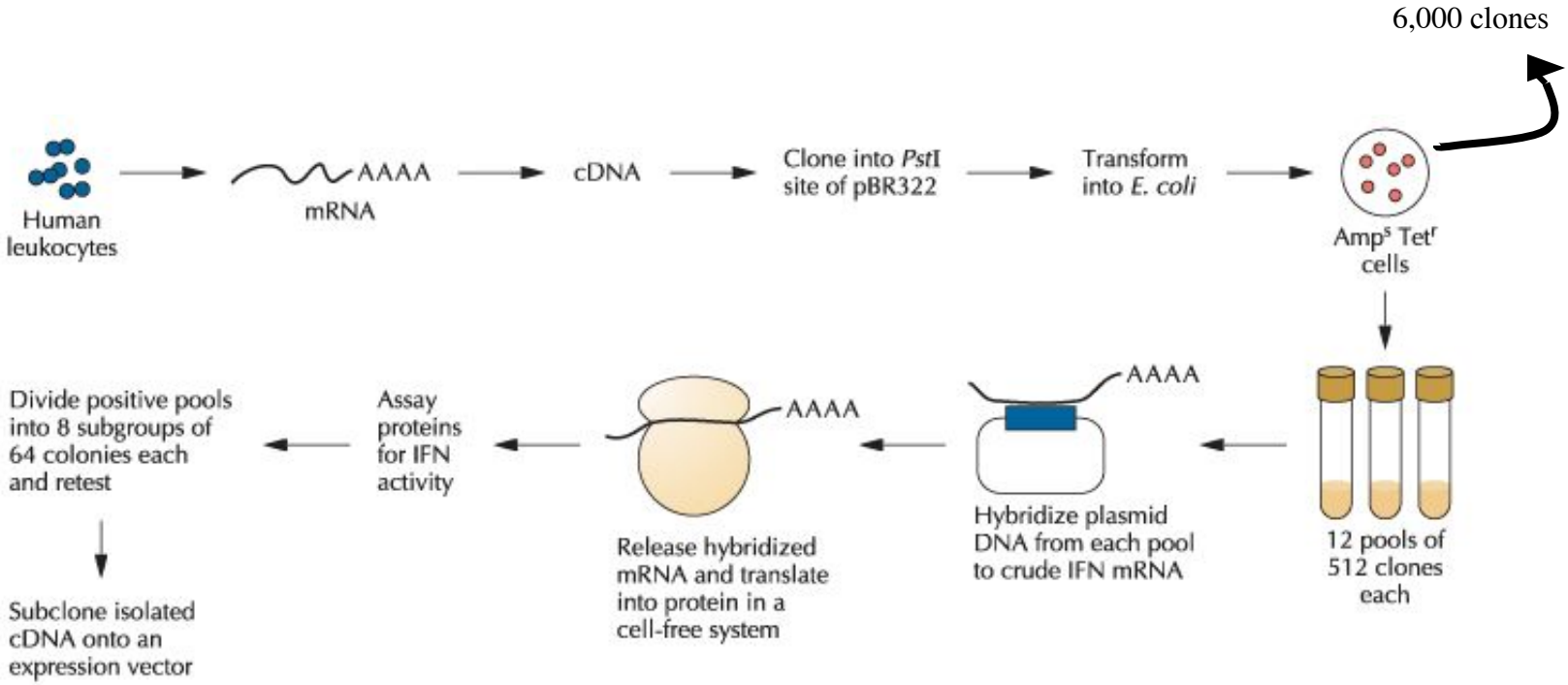
Table 10.1 Some recombinant proteins that have been approved for human use in either the United States or the European Union

Compound	Company	Disorder
Factor VIII	Baxter Healthcare, Genetics Institute, Centeon, Bayer	Hemophilia A
Factor VIIa	Novo Nordisk	Some forms of hemophilia
Factor IX	Genetics Institute	Hemophilia B
Hirudin	Ciba Novartis, Europharm, Hoechst Marion Roussel	Venous thrombosis, heparin-associated thrombocytopenia
Tissue plasminogen activator	Genentech	Acute myocardial infarction
Truncated tissue plasminogen activator	Galenus Mannheim, Boehringer Mannheim/Centocor	Acute myocardial infarction
Insulin	Eli Lilly, Novo Nordisk, Hoechst AG	Diabetes mellitus
Insulin analogues	Eli Lilly, Novo Nordisk, Aventis	Diabetes mellitus
Human growth hormone	Eli Lilly, Genentech, Biotechnology General, Pharmacia, Upjohn, Novo Nordisk, Serono Laboratories	Growth hormone deficiency in children
Human growth hormone analogue	Genentech	Growth hormone deficiency in children
Human growth hormone	Serono Laboratories	AIDS-associated catabolism and wasting
Glucagon	Novo Nordisk	Hypoglycemia
Thyrotrophin-α	Genzyme	Thyroid cancer
Follicle-stimulating hormone	Ares-Serono, Organon	Anovulation and superovulation
Erythropoietin	Amgen, Ortho Biotech, Boehringer-Mannheim	Anemia
Platelet-derived growth factor	Ortho-McNeil Pharmaceuticals, Janssen-Cilag	Lower-extremity diabetic neuropathic ulcers
DNase I	Genentech	Cystic fibrosis
β-Glucocerebrosidase analogue	Genzyme	Gaucher disease
IFN-α_{2a}	Hoffmann-La Roche, Schering-Plough	Hairy cell leukemia, hepatitis B and C
Synthetic type 1 IFN-α	Amgen, Yamanouchi Europe	Chronic hepatitis C
IFN-α_{2b}	Schering-Plough	Hairy cell leukemia, genital warts, hepatitis B and C
IFN-β_{1b} analogues	Schering AG, Berlex Laboratories, Chiron	Multiple sclerosis
IFN-β_{1a}	Biogen, Ares-Serono	Relapsing multiple sclerosis
IFN-γ_{1b}	Genentech	Chronic granulomatous disease
IL-2 analogue	Chiron	Renal cell carcinoma
IL-11 analogue	Genetics Institute	Prevention of chemotherapy-induced thrombocytopenia

Abbreviations: IFN, interferon; IL, interleukin.

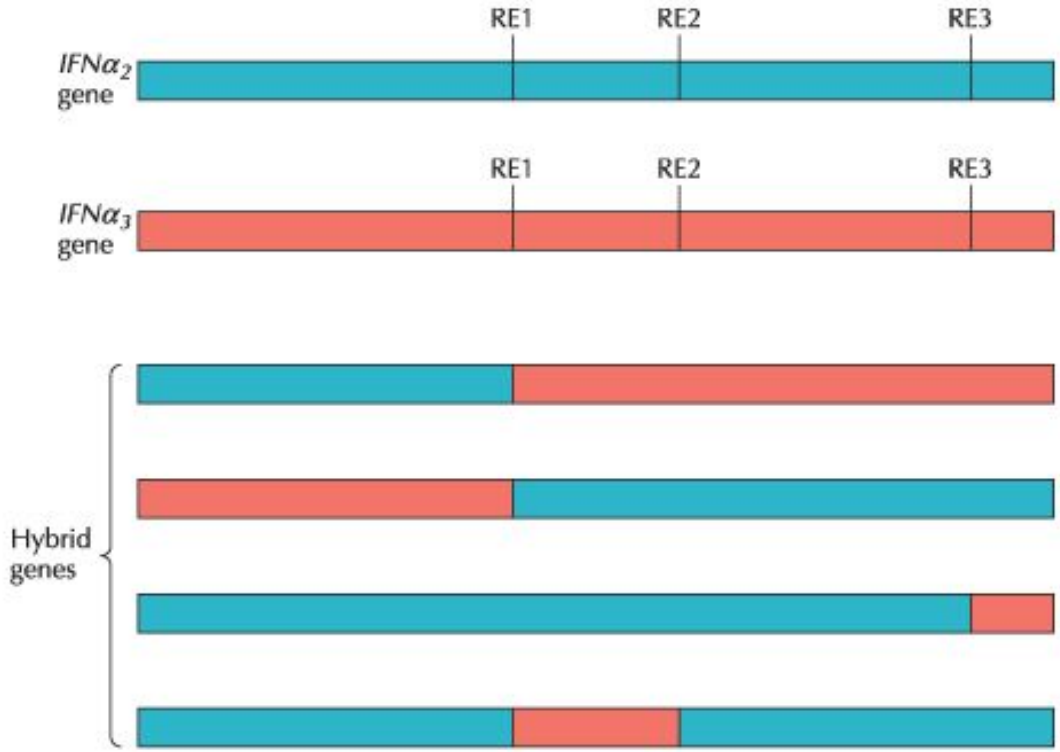
- ~2003
- Approved in US or EU

Recombinant interferon: isolation of cDNA



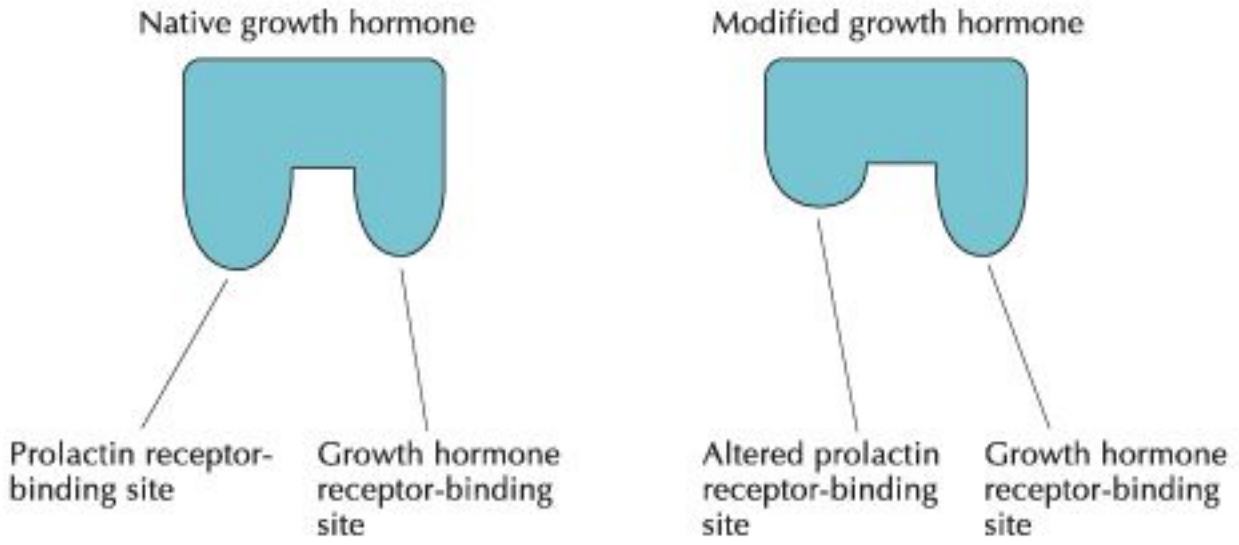
- Strategies for isolating either the genes or cDNAs for human proteins
- 1) Isolate target protein and determine partial AAc sequence
  - Synthesize oligo as probe to screen cDNA library
- 2) Generate Ab against purified proteins
  - Screen gene library
- Interferon strategy above, **pre-human genome sequence**

Hybrid products: INF



- Interferons assist the immune response by **inhibiting viral replication** within host cells, activating natural killer cells, increasing antigen presentation to lymphocytes, and inducing the resistance of host cells to viral infection
- IFN cDNA isolated early 80s
- Now, three groups of IFN genes identified: α, β, γ
  - IFNα family of 13 genes; IFNβ family of 2 genes; IFNγ of 1 genes
- IFN α1 and α2 have common RE sites
- Hybrid INFs demonstrate potential therapeutics by combining functional domains
- Some (2003)- successful clinical trials, approved for use as human therapeutic agents

Site-specific directed mutagenesis: hGH



- hGH: 191 AAc, 22,1 kDa
  - One of first therapeutic proteins approved for human use
  - Recombinant form produced in E. coli, identical to native pituitary-derived hGH
  - Native binds to growth hormone receptor and prolactin receptor
- Side effects
- Prolactin receptor binding function of Zn^{++} binding
  - Domain: His-18, His-21, Glu-174
- 2003, testing mutants

Optimizing gene expression

Table 10.2 Levels of interleukin-3 synthesis achieved in different host systems

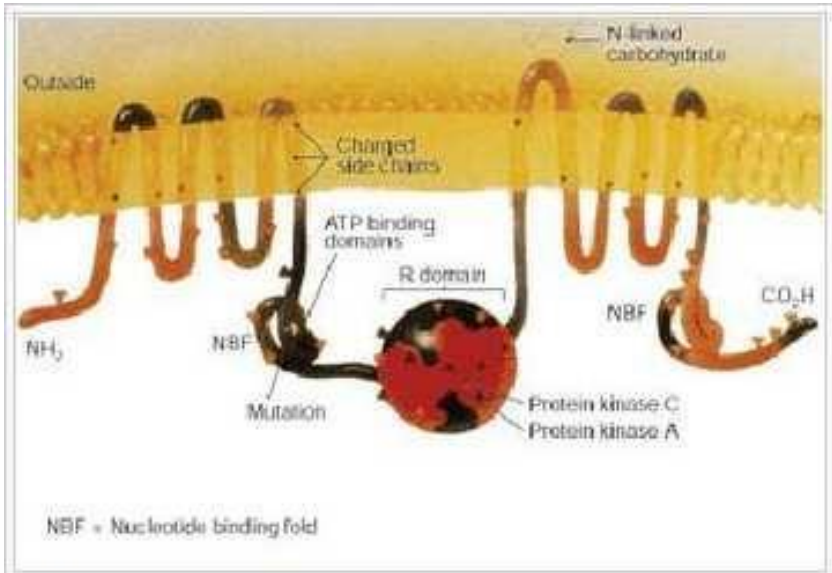
Host system	Promoter	Expression level (units)	Protein form
B. licheniformis	Amylase	300	15 kDa (mature)
E. coli	*lacZ*	500	20 kDa (fusion)
E. coli	*lacZ*	20	15 kDa (mature)
Human cells	Metallothionein	2	20–40 kDa
K. lactis	Lactase	20	20–100 kDa
S. cerevisiae	Mating factor α	20	20–100 kDa

Adapted from van Leen et al., *BioTechnology* 9:47–52, 1991.
In each case, one of the strongest available promoters for that system was used.

- Multistep process:
  - Design a protein, construct a recombinant molecule, express and characterize
- Need to optimize expression
- First, either prokaryote or eukaryote host
  - Comparative analysis of host and expression
- ex., interleukin-3 expression
- Best in Bacillus licheniformis
- Balance with glycosylation in eukaryotic hosts
- But, glycosylation is not essential for interleukin-3 activity

Cystic fibrosis

Mutation	Frequency worldwide
ΔF508	66.0%
G542X	2.4%
G551D	1.6%
N1303K	1.3%
W1282X	1.2%



CFTR protein - Molecular structure of the CFTR protein

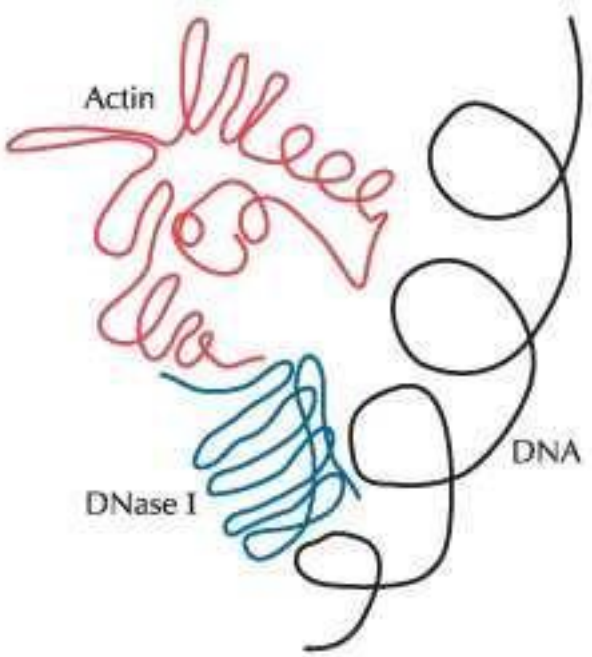
•Genetic disease affecting lungs and digestive system
•Average life span 37 years, extended and extending
•In US, ~1/3,900; 1/22 are carriers
•Most common in Europeans and Ashkenazi Jews
•Cystic fibrosis transmembrane conductance regulator (CFTR)
•Chloride ion channel, sweat, digestive juices and mucus
• thick, sticky mucus to build up in the lungs and digestive tract
•7q31.2 -> 180,000 bp gene, 1,480 AAc
•Most common mutation DF508; 1,400 other mutations
•DF508: missense, not folded correctly
•Lungs susceptible to bacterial infection
•Antibiotics treatment results in resistance and
combination with DNA from bacteria and leukocytes causes pulmonary problems (mucus)

•wikipedia

Treatment

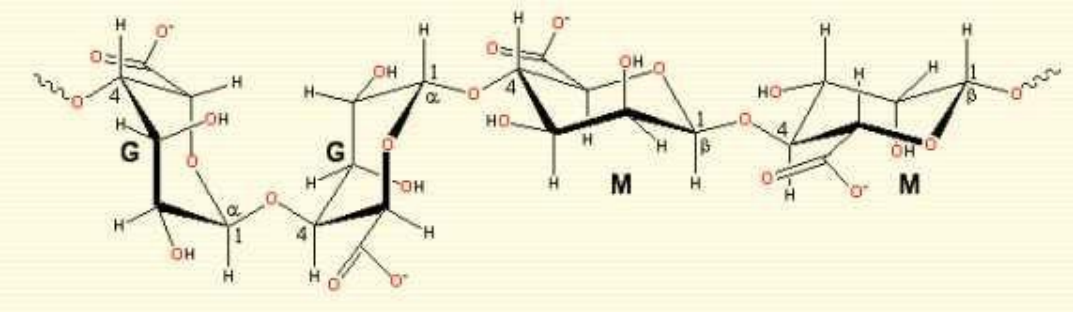
- Genentech: hDNase I in CHO cells
- Not a cure, but alleviates symptoms
- Purified protein delivered via aerosol mist to lungs of CF^-
- Approved by FDA in 1994

Optimizing treatment



- Another symptom,
- In response to bacteria in lungs,
  leukocytes cluster and lyse bacteria (and leukocytes)
    - Lysed leukocytes release actin
    - Monomeric actin binds DNase I very tightly and inhibits
    - Limits effectiveness
- X-ray structure data suggested Ala-144 required for binding
  or Tyr-65
- Changing either to Arg decreases actin binding by 10,000x
- Clinical efficacy of mutants to be determined (2003)

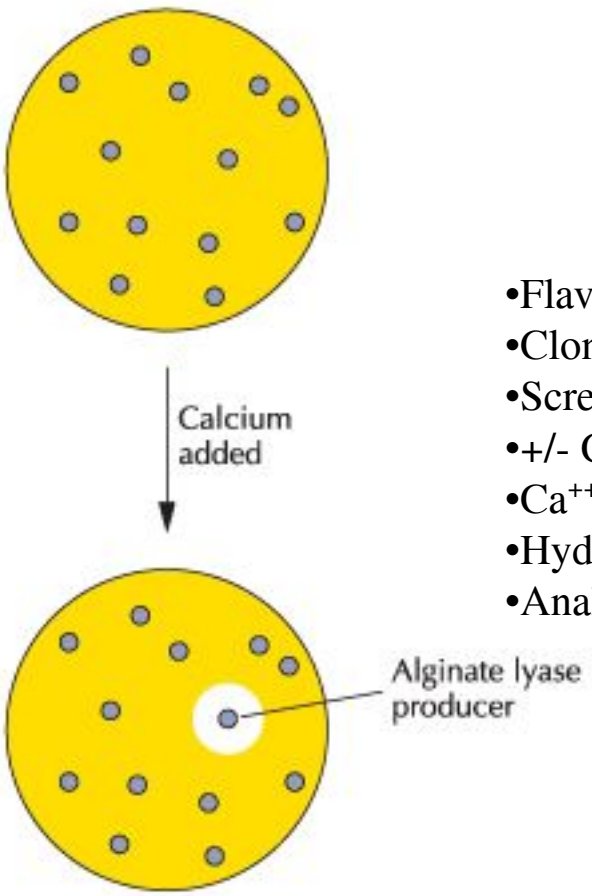
Clearing the lungs 2 with alginate lyase



- Alginate produced by seaweeds, soil and marine bacteria
- P. aeruginosa excretion in lungs contributes to viscosity of mucus
- In addition to DNase I treatment, alginate lysate can be used as therapeutic agent

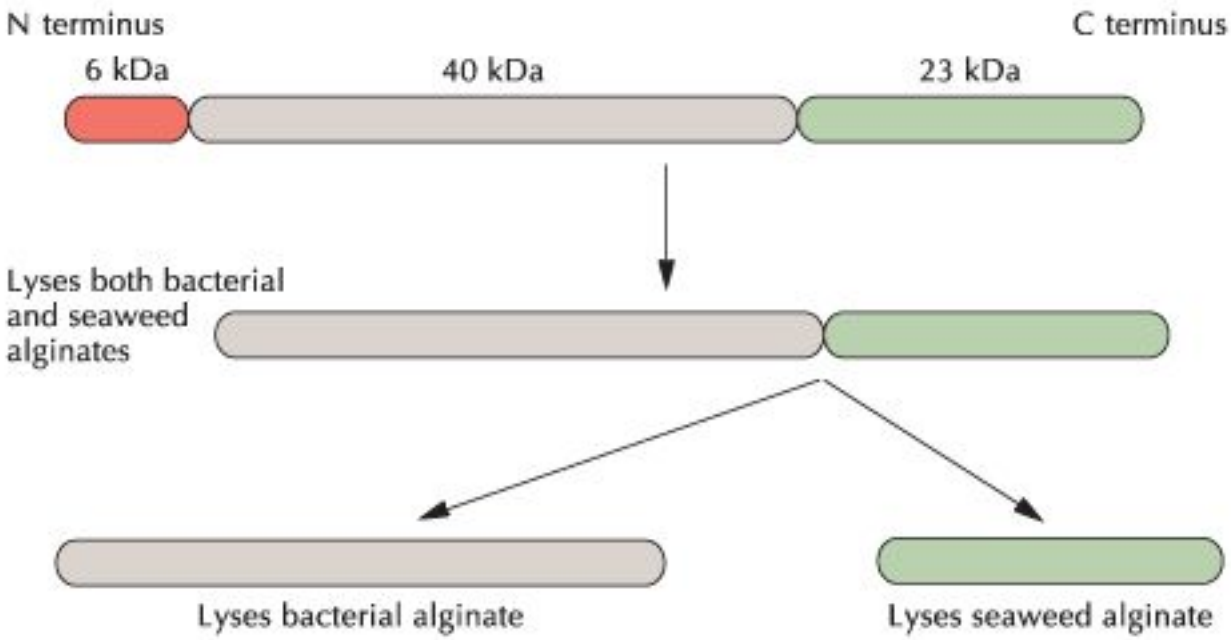
- http://www.lsbu.ac.uk/water/hyalg.html

Cloning alginate lyase



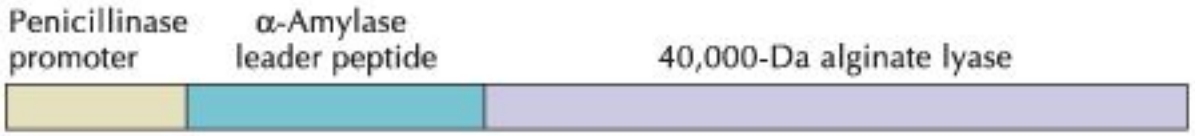
- Flavobacterium sp.
- Clone bank in E. coli
- Screen by plating onto medium plus alginate
- +/- Ca^{++}
- Ca^{++} + alginate = cross-linked opaque
- Hydrolyzed alginate does not cross-link
- Analysis and characterization of clones and alginate lyase

Alginate lyase[s]



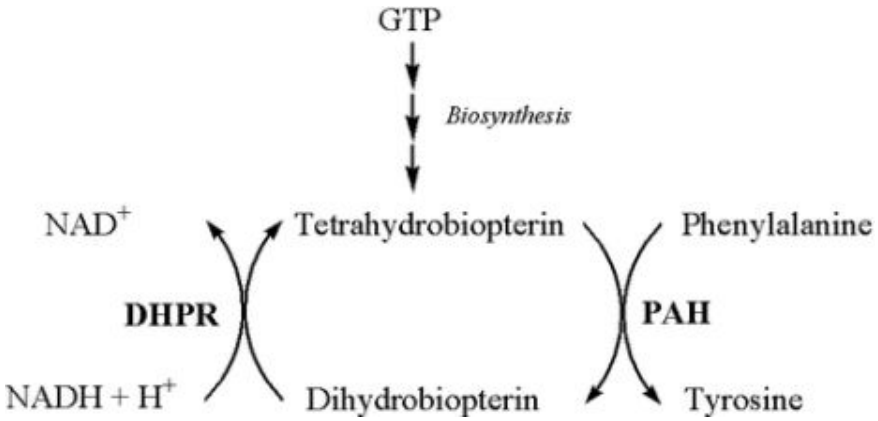
- ORF 69,000 Da
- Precursor of three alginate lyases
- -> 3,000 Da + 63,000 Da
- 63,000 Da lyses both bacterial and seaweed alginates
- 63,000 Da -> 23,000 Da seaweed effective+ 40,000 Da bacterial effective
- Clone bacterial activity portion

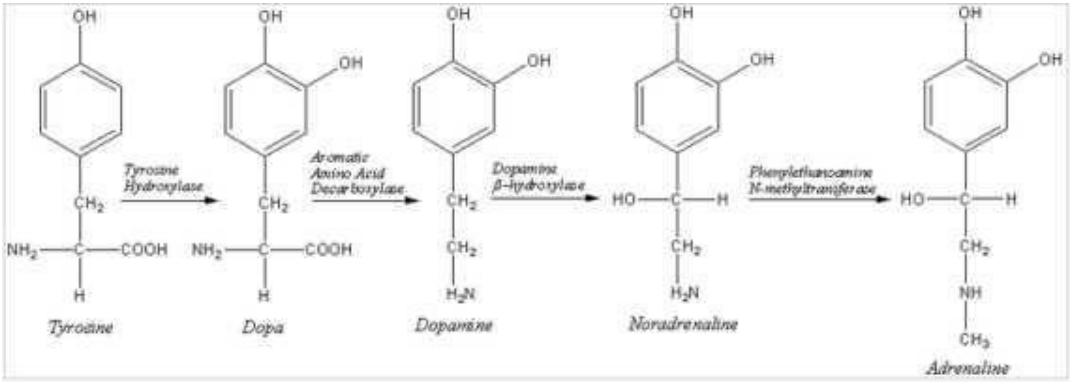
Optimization of activity

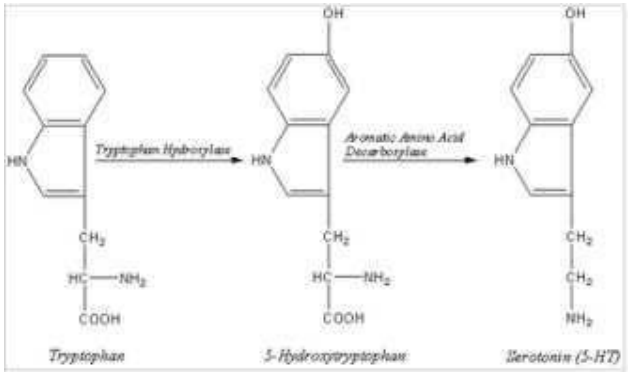


- Increase expression of 40,000 Da protein
- PCR amplify and insertion behind strong promoter
- B. subtilis plasmid, fused to a B. subtilis a-amylase leader peptide, directs secretion and penicillinase gene promoter
- Expressed and assayed for halo phenotype
- Liquifies alginates produced by P. aeruginosa isolated from lungs of CF patients
- 2003, additional trials to determine if effective therapeutic agent

Phenylketonuria (PKU)



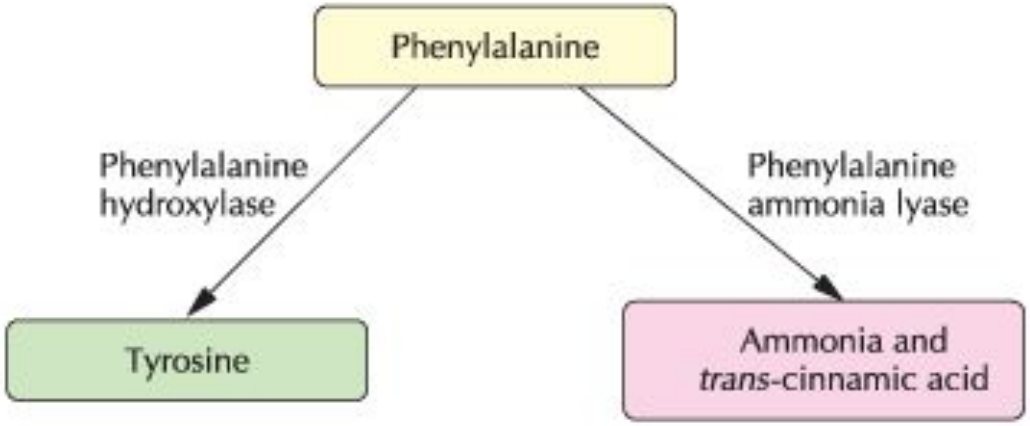




- Autosomal recessive genetic disorder in phenylalaniine hydroxylase
- Phe accumulation, decreases other 'large, neutral AAc' in brain, needed for protein and neurotransmitter synthesis
- Brain development; progressive mental retardation and seizures
- Incidence ~1/15,000; varies: 1/4,500 Ireland and 1/100,000 Finland
- 12q22-q24.1
- Macaque genome: PAH gene sequence identical to a human PKU mutation

•wikipedia

Phenylketonuria treatment[s]



- Traditional treatment: diagnosis at birth or prenatal
- Controlled semi-synthetic diet with low levels of Phe
- Possible treatment: metabolism of Phe
  - PAH multienzyme complex, requiring cofactor
- Phe ammonia lyase (PAL) converts Phe as well
  - Stable and does not require cofactor
- To test concept, yPAL cloned and overexpressed in E. coli
  - Preclinical studies (2003) with mice deficient in PAL
  - See lower plasma levels of Phe when PAL injected or administered as oral encapsulated enzyme

Monoclonal antibodies (mAb) as therapeutic agents

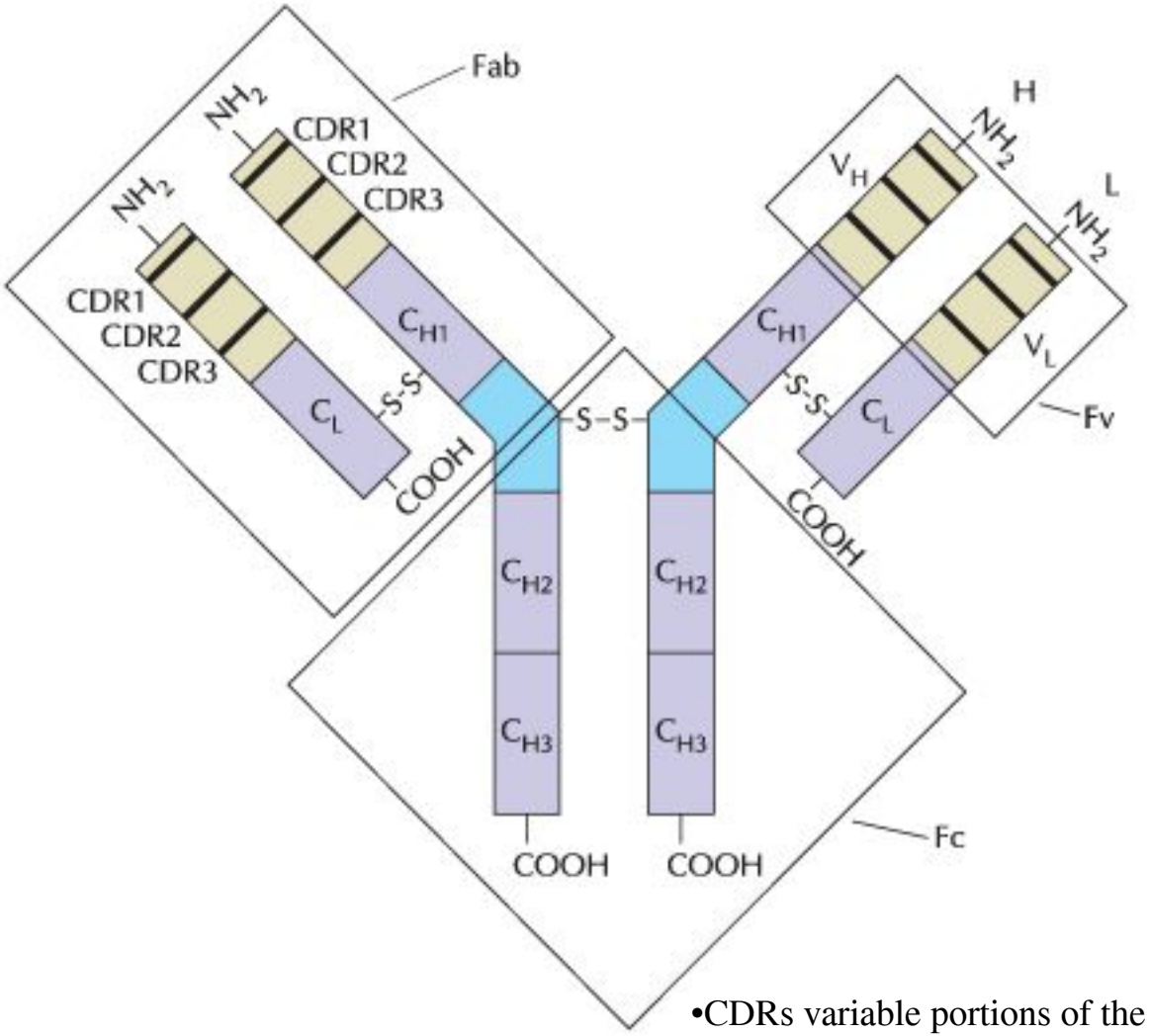
Table 10.3 Some therapeutic monoclonal antibodies that have been approved for human use in either the United States or the European Union

Date of approval	Type of antibody	Company	Therapeutic use
1986	Mouse	Ortho Biotech	Prevention of acute kidney transplant rejection
1994	Chimeric	Centocor	Prevention of blood clots
1997	Chimeric	Genentech, Idec Pharmaceuticals	Non-Hodgkin lymphoma
1997	Humanized	Protein Design Labs, Hoffmann-La Roche	Prevention of acute kidney transplant rejection
1998	Chimeric	Centocor, Schering-Plough	Crohn disease and rheumatoid arthritis
1998	Chimeric	Novartis	Prevention of acute kidney transplant rejection
1998	Humanized	Genentech	HER2-positive breast cancers
1998	Humanized	Medimmune	Respiratory syncytial virus infection in children
1998	Chimeric	Hoffmann-La Roche	Non-Hodgkin lymphoma
2000	Humanized	American Home Products, Celltech	Relapsed acute myeloid leukemia
2001	Humanized	Millennium Pharmaceuticals, Schering	Chronic lymphocytic leukemia
2001 pending	Humanized	Genentech, Novartis, Tanox	Asthma

In addition to the antibodies listed here, 11 have been approved for diagnostic purposes.

- Mouse mAb OKT3 first to be approved by FDA
- Immunosuppressive agent after organ transplant in humans

Antibody molecular structure



- CDRs variable portions of the protein, both H and L
- Fc elicits immunological responses after Ag-Ab
- Complement cascade

Polyclonal antibodies (Ab)

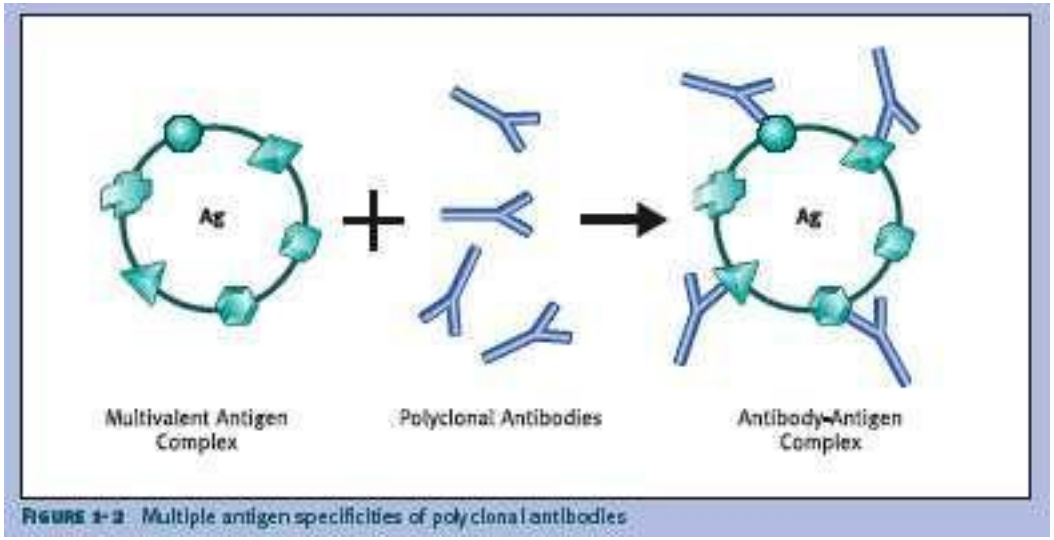


FIGURE 1-2 Multiple antigen specificities of polyclonal antibodies

•www.abbottdiagnostics.com/Science/pdf/learning_immunoassay_01.pdf

Monoclonal antibodies (mAb)

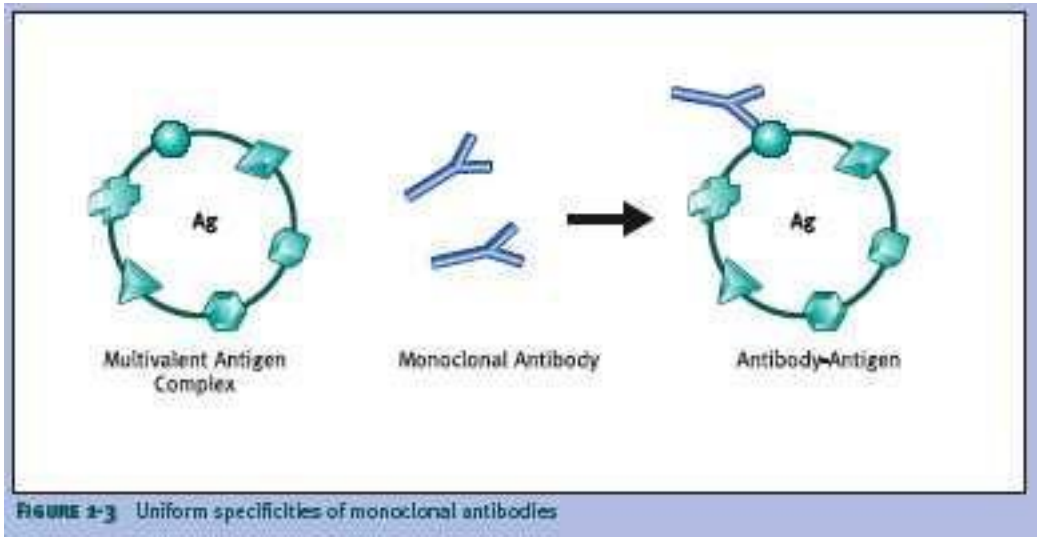


FIGURE 1-3 Uniform specificities of monoclonal antibodies

•www.abbottdiagnostics.com/Science/pdf/learning_immunoassay_01.pdf

Monoclonal antibodies (theoretical)

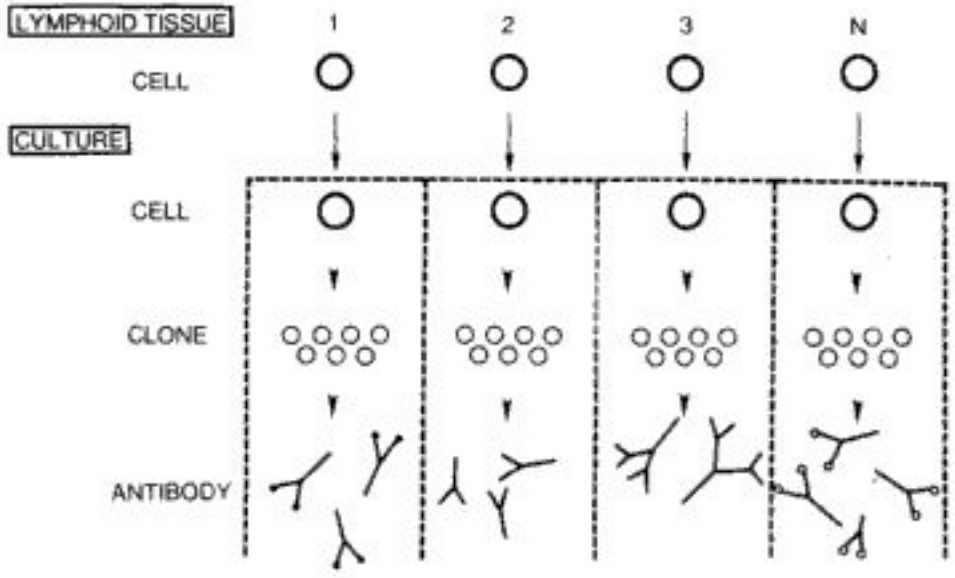


FIGURE 2. Keeping the cells separated yields pure antibody: if the stimulated B lymphocytes can be kept separated and made to form antibody-secreting clones in separate cultures in vitro, we can harvest pure antibody. Compare with Figure 1.

•Monoclonal Antibodies: A Manual of Techniques. HZola

Monoclonal antibodies (mAb) protocol

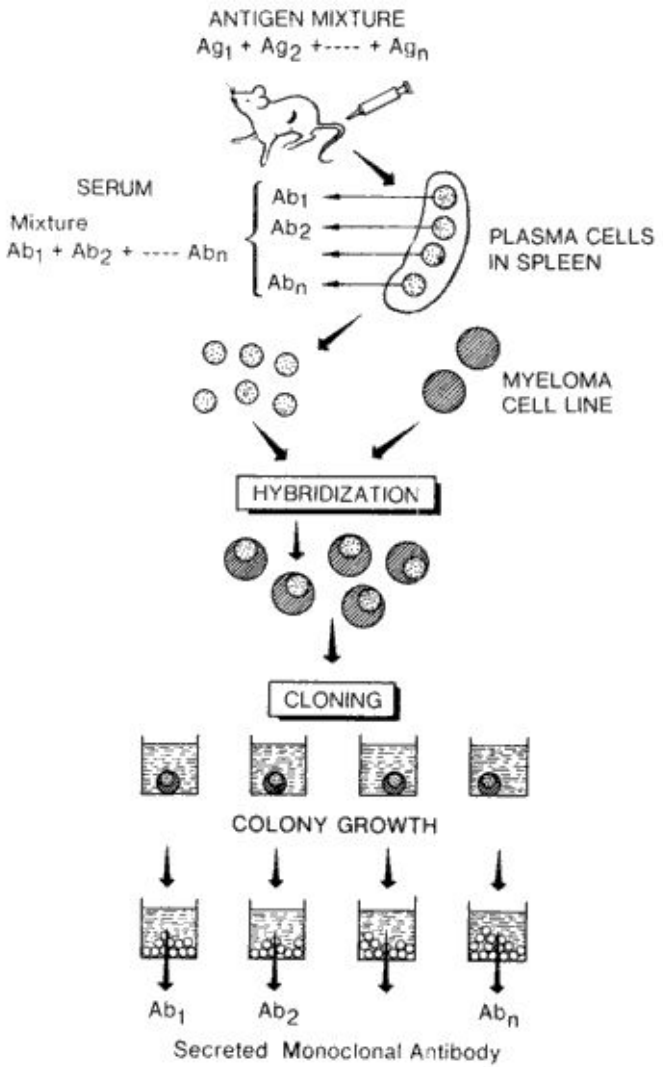


FIGURE 4. Schematic representation of the process of immortalizing an antibody-producing clone by hybridization, cloning, and selection of clones producing the desired antibodies.

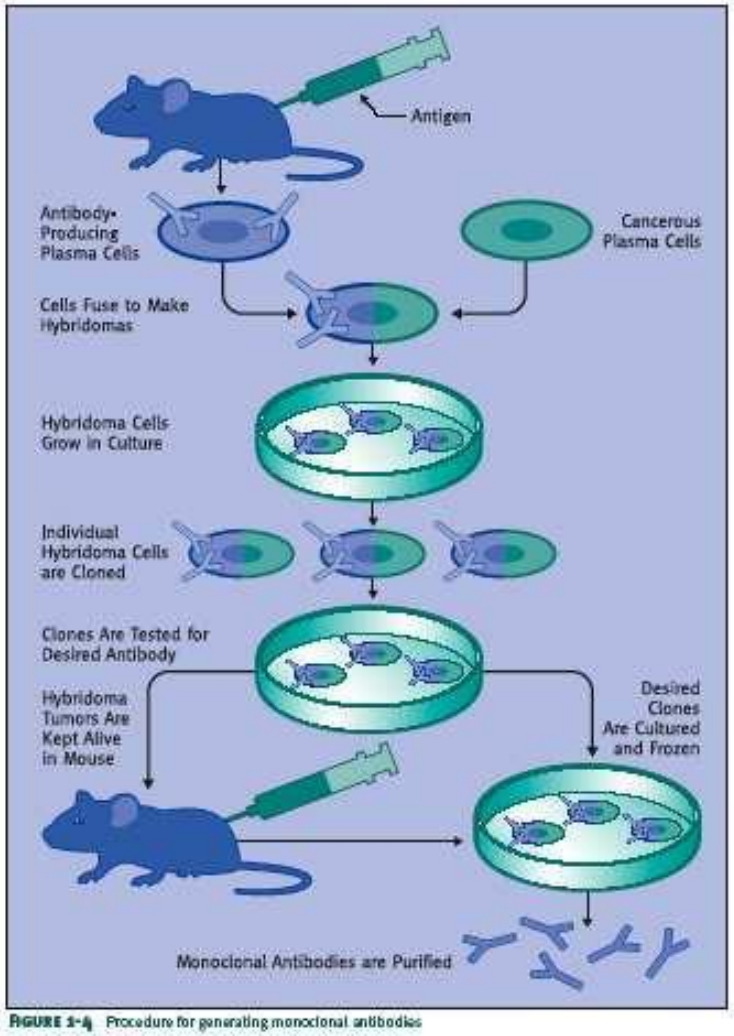
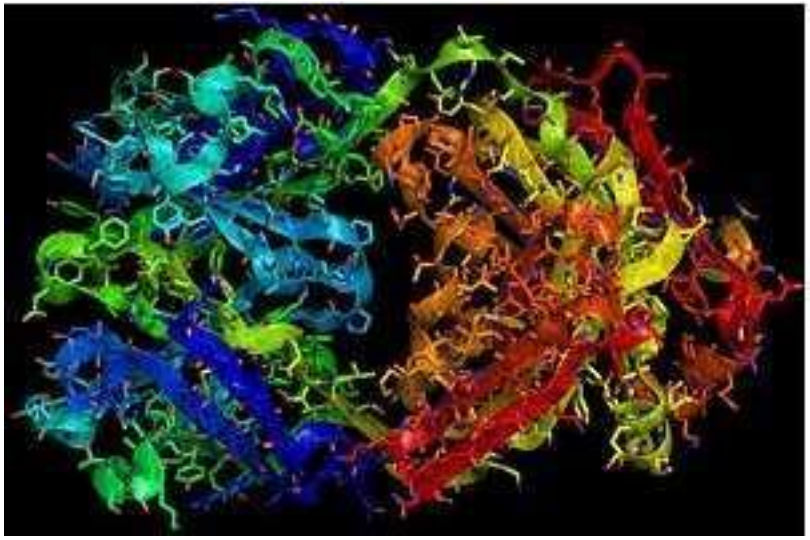


FIGURE 1-4 Procedure for generating monoclonal antibodies

- www.abbottdiagnostics.com/Science/pdf/learning_immunoassay_01.pdf
- Monoclonal Antibodies: A Manual of Techniques. HZola

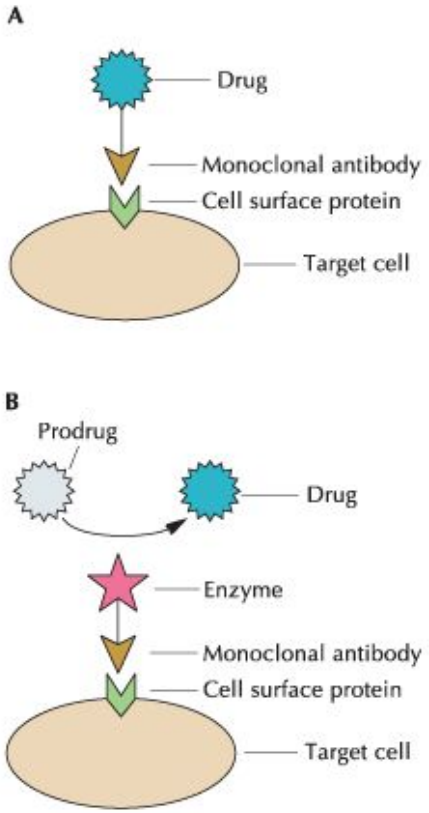
Herceptin®



•"Magic bullet"
•Genentech. FDA 9/98; Aullrich/Genentech and DSlamon/UCLA Jonsson Cancer Ctr
•Trastuzumab (trade name Herceptin)
•Humanized monoclonal antibody
•Target is HER2/neu receptor (erbB2)
•HER2-positive metastatic breast cancer
•Anti-cancer therapy in breast cancer, over-expressing erbB2 receptor
•ErbB2 receptor amplification occurs in 25-30% of early-stage breast cancers
•Transmembrane Tyr kinase, activating PI3K/Akt pathway and MAP pathway
•Overexpression promotes invasion, survival and angiogenesis of cells
•Also confers therapeutic resistance to cancer therapies
•Herceptin binds to extracellular domain of erbB2 receptor,
•Arresting cell at G1 phase

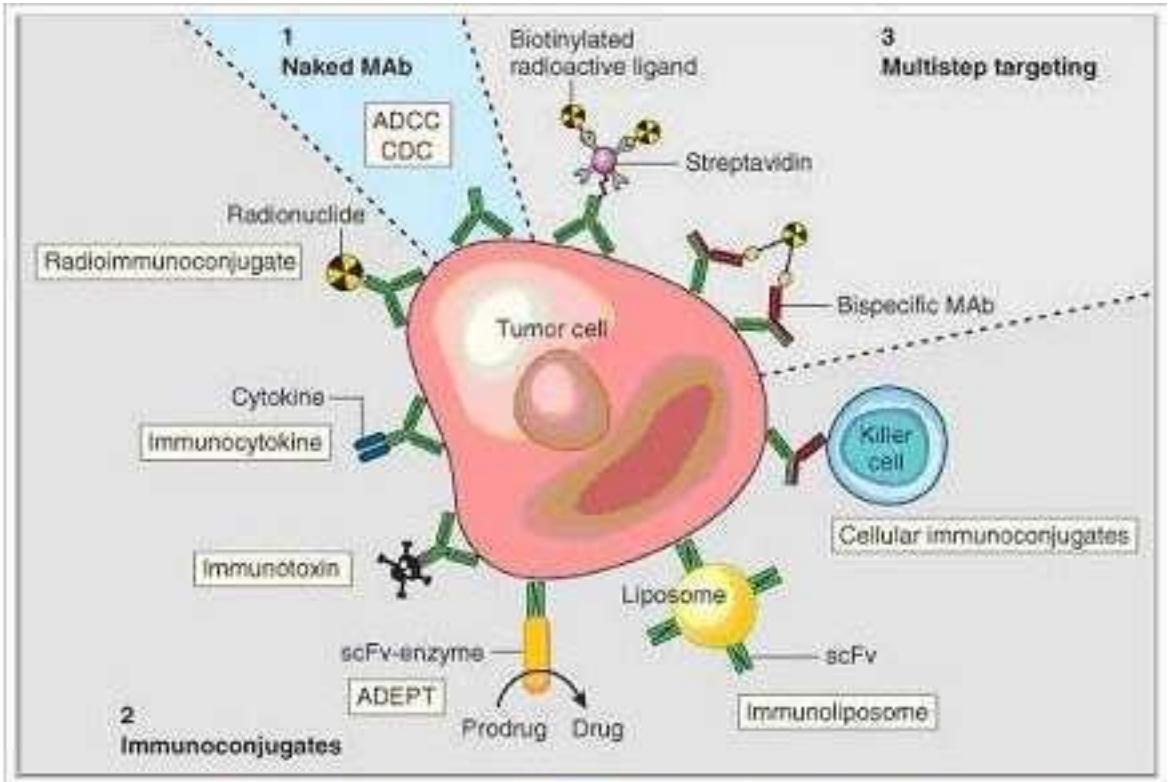
•wikipedia

Magic bullet: delivery of drug to site



- Binding of mAb requires second step
- 1) delivery of drug
- 2) delivery of enzyme to convert pro-drug

Magic bullet: delivery of active agent to site



Monoclonal antibodies for cancer. ADEPT, antibody directed enzyme prodrug therapy; ADCC, antibody dependent cell-mediated cytotoxicity; CDC, complement dependent cytotoxicity; MAb, monoclonal antibody; scFv, single-chain Fv fragment.[8]

- Binding of mAb requires second step
- variations

- Priklad ANTISENSE delivery

Human mAb problem

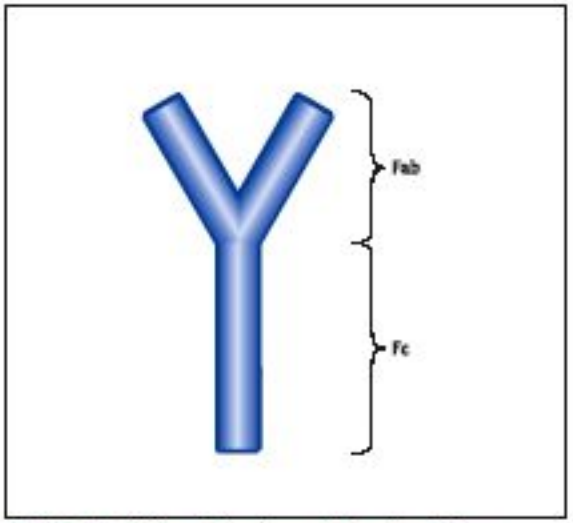


FIGURE 1-1 Antibody Structure and Functional Sites

Drawbacks to immunotherapeutic agents use

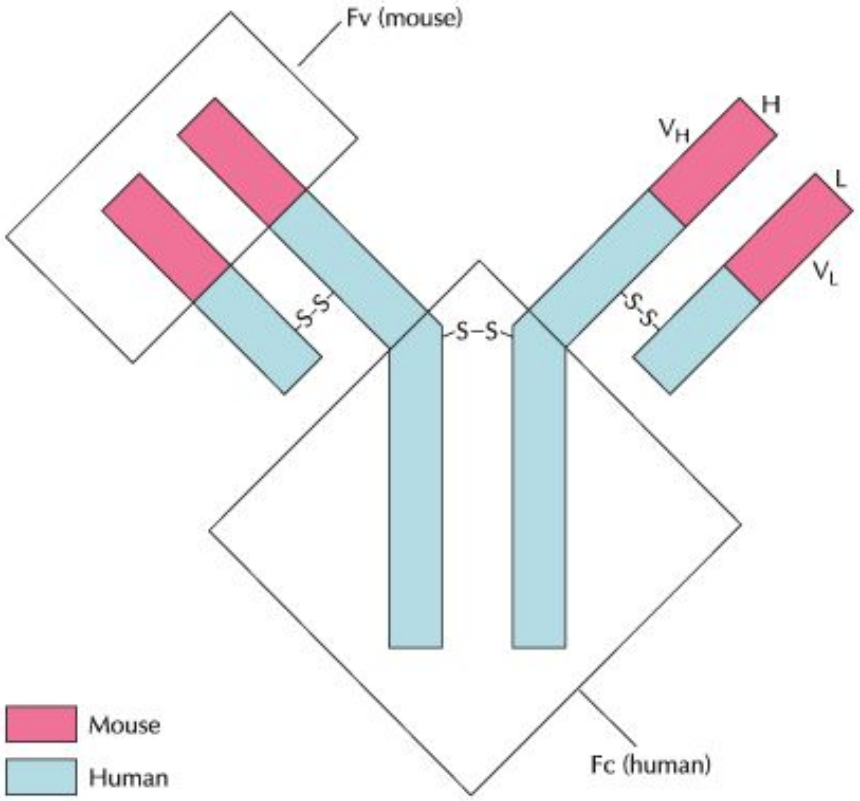
- Chemical couplings problem
  - Yields low; coupling at random sites; chemical portion may inactive attached enzyme
- Nonhuman mAb
  - If condition requires multiple treatments, nonhuman mAb causes immune response

Human mAb

- Human chromosomes of fused human lymphocyte-mouse myeloma cells are unstable
- No human myeloma cell line can replace mouse myeloma cell line
- Ethics of injecting human subject to generate Ab-producing cells and doing partial splenectomy
- to collect Ab-producing cell

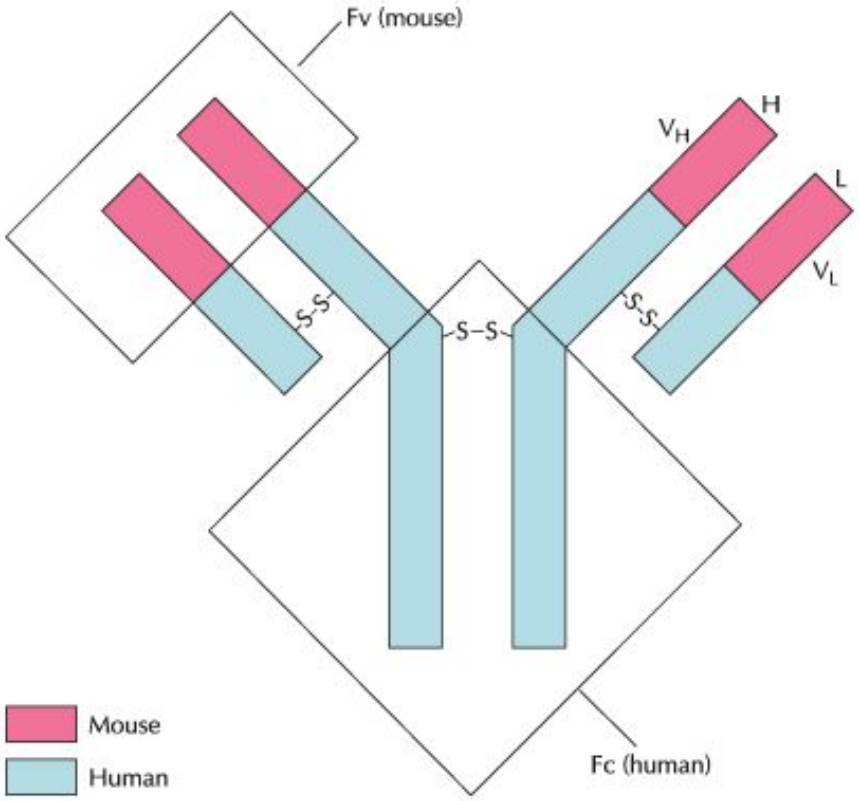
•www.abbottdiagnostics.com/Science/pdf/learning_immunoassay_01.pdf

Hybrid human-mouse mAb: chimeric



- Genetic engineering to convert mouse mAb into a hybrid
- Exchange Fc portions
- Using oligonucleotides and in vitro DNA replication or cloned segments
- Construct in expression vector; transfect into cultured B lymphocytes
- Chimeric Abs are 70% human/30% mouse

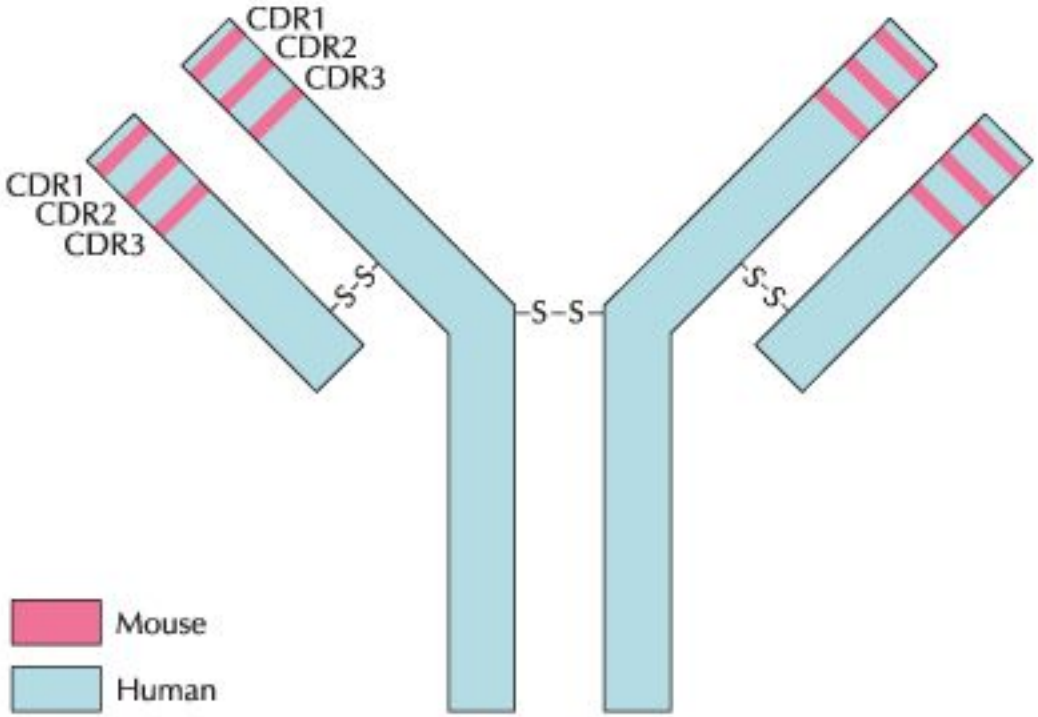
Hybrid human-mouse mAb: chimeric



- Ex., chimera of mouse mAb against surface of human colon cancer cells
- Tested in patients with colorectal cancer
- Half-life in blood system 6x longer
- 1/10 patients developed mild response against chimera
- But, no anti-tumor activity observed (2003)
- Low dosage and/or advanced state of the cancer?

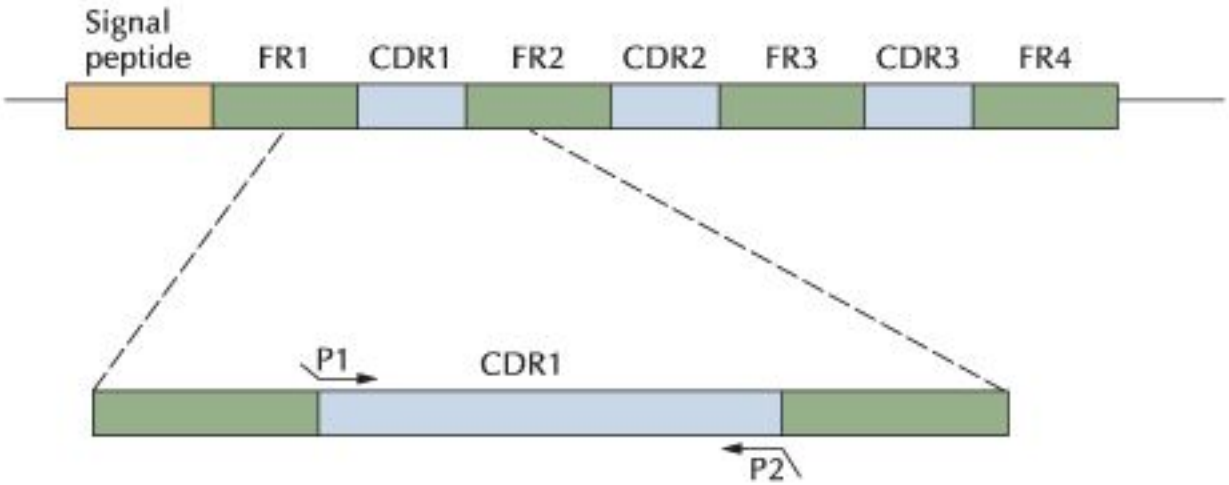
Jak je to delano

Hybrid human-mouse mAb: humanized



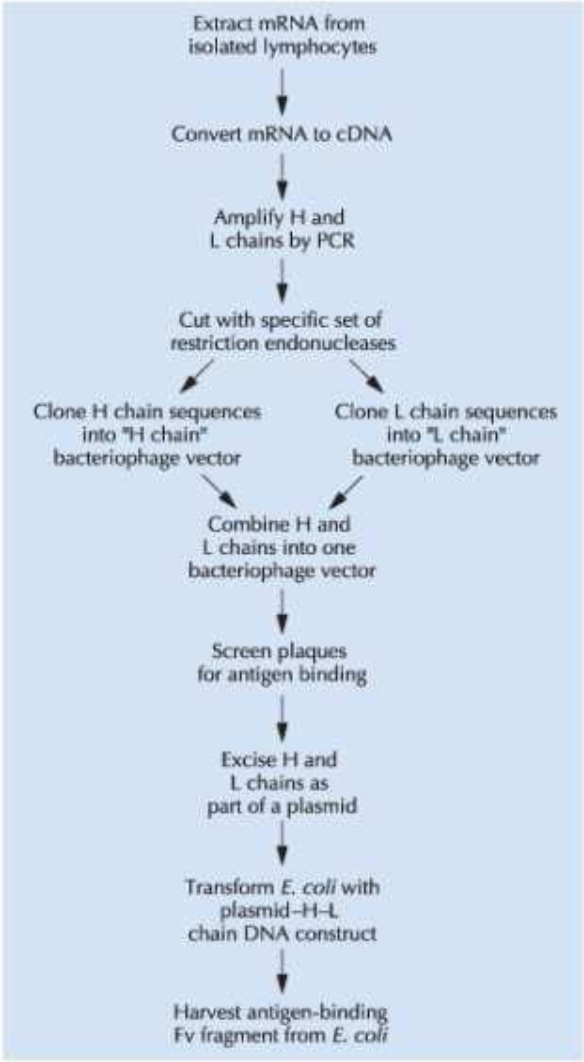
- Humanized Ab
- Substitute CDRs into human Ab
- 95 %human/5 % mouse
- Construction by isolating cDNAs for L and H chains
- Amplify variable regions using PCR protocol
- Primers are complementary to ends of variable regions, conserved
- CDRs are highly variable sequences

PCR amplification of CDR



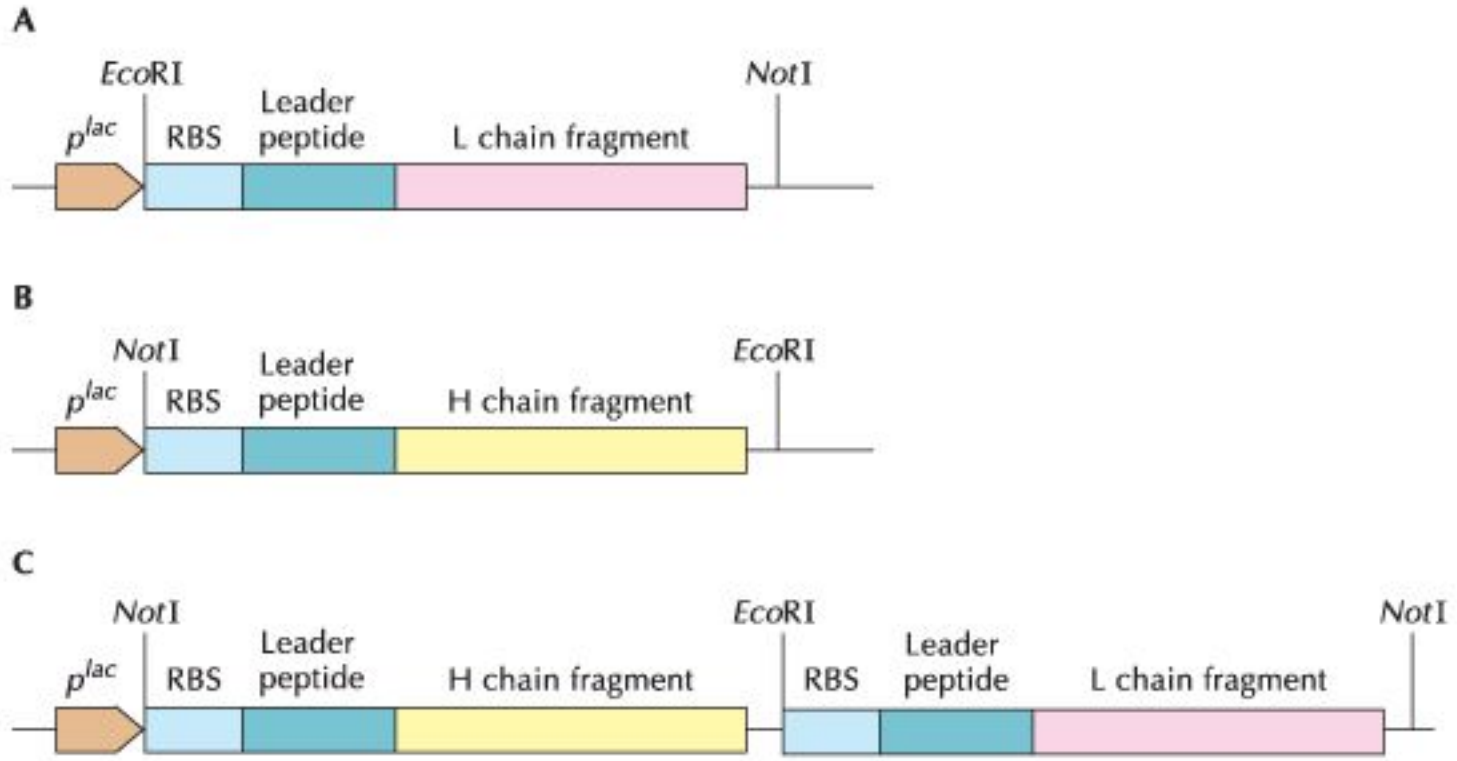
- Primers are hybrids, with
- 12 bases at ends corresponding to human mAb L chain cDNAs
- Six pairs of primers: 3 for V_L and 3 for V_H
- PCR protocol to splice these segments into human Ab, replacing CDRs
- 2003. 50 different mAbs have been humanized
- Technology is effective and widely applicable
- Time-consuming and expensive procedure

E. coli production of mAb: phage display



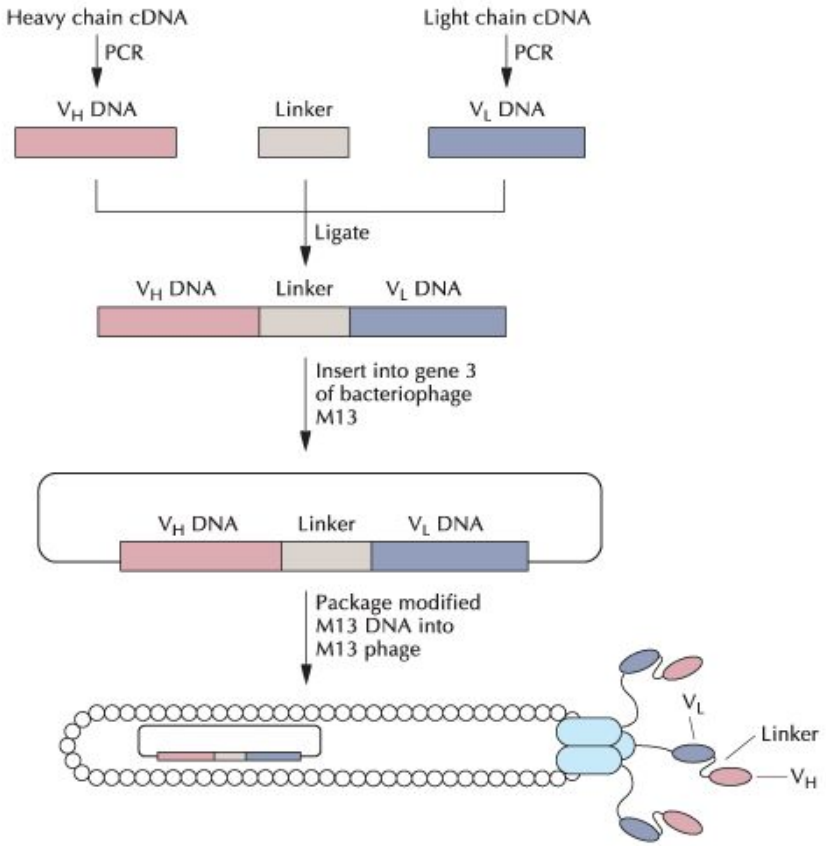
- Protocol for creating phage combinatorial libraries
- Hybridoma cells grow slowly, do not reach high cell densities, expensive to maintain
- Bioreactors: bacteria, plants and animals

DNA constructs of Fv combinatorial gene library



- Lambda phage
- Clones each L and H into two separate libraries
- Cut with common RE
- Directionally clone into third library: H -> L
- Combinations random
- RBS= ribosome binding site

Combinatorial library in M13



- PCR amplify VH and VL separately
- Add linker
- Ligate into M13 genome
- Displays on surface

Phage Display

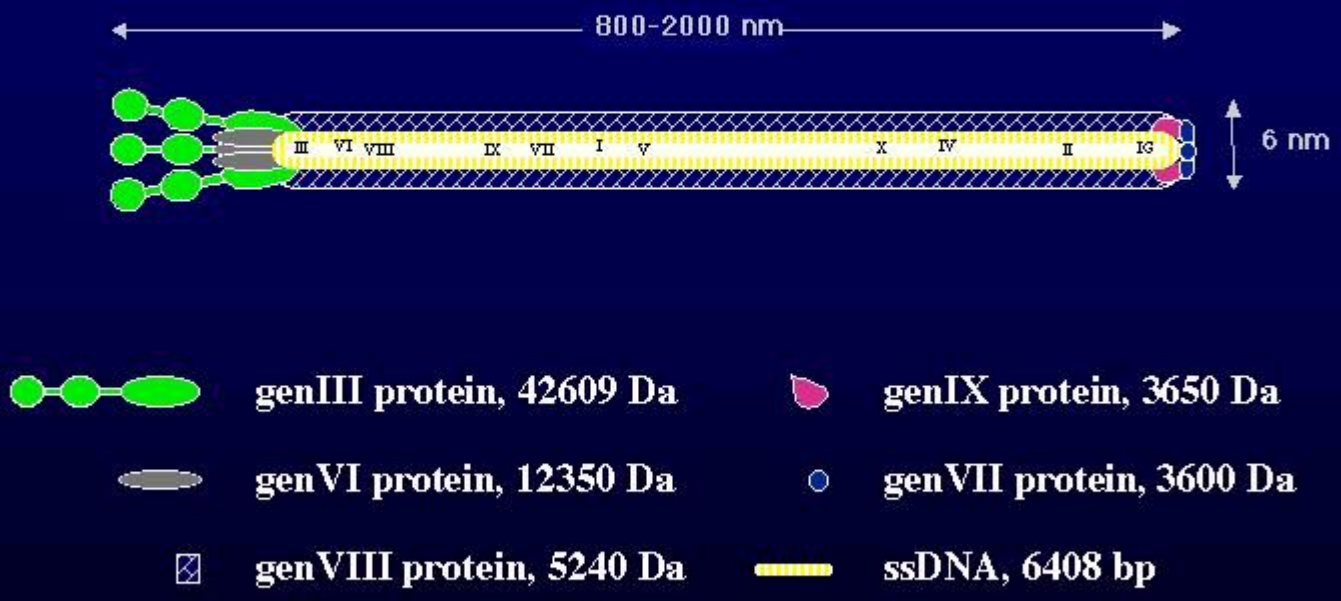
Display peptide or protein on surface of bacterial virus
(in principle can use other viruses but phage viruses easiest to prepare etc.)
Some proteins on viral coats can accommodate peptides or proteins and
will present them on the surface.

The phage genome (or alternatively phagemid) contains the sequence for the protein or peptide so isolation of the phage with desired phenotype will also provide the genotype.

Most popular is filamentous phage f1 or M13.
pIII on the end or pVIII along the length of the rod-like virion
for pVIII ~10% can be loaded with alternate peptide
Advantage of phage display: easy to screen over 10^9 sequences

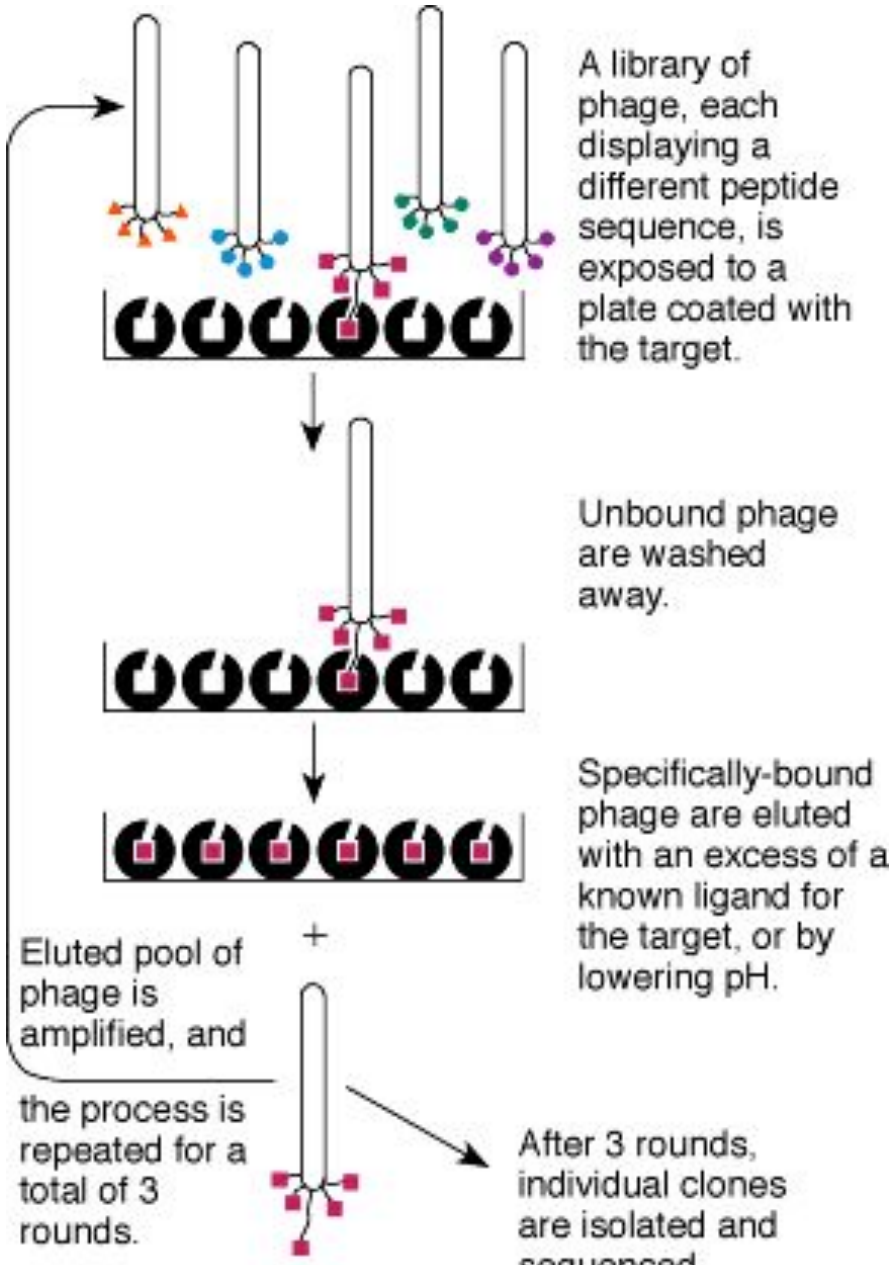
Can either clone library directly into phage genome
or use a phagemid (plasmid that contains f1 ori) with replication
deficient helper phage

Filamentous phage architecture



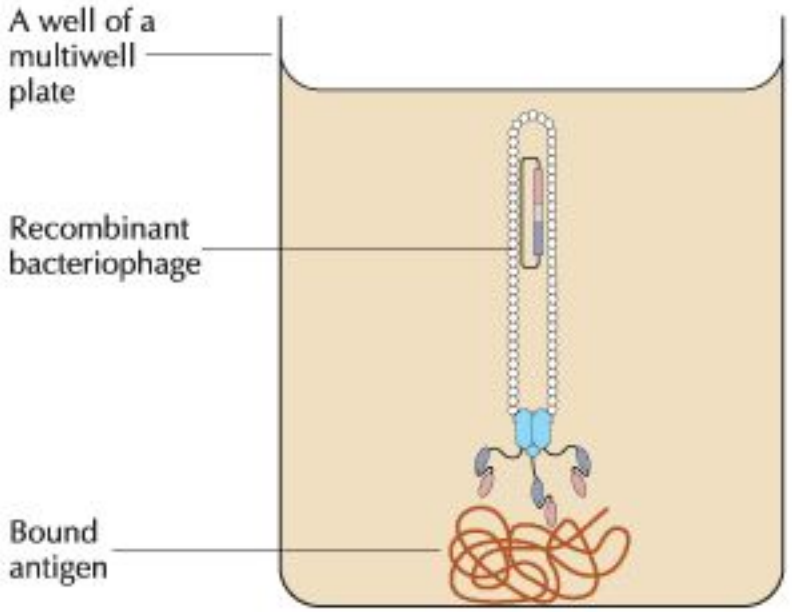
5 copies of pIII and pVI 2800 copies pVIII - all can accommodate peptides

http://www.biochem.unizh.ch/plueckthun/teaching/Teaching_slide_shows/filamentous_phages/index.htm



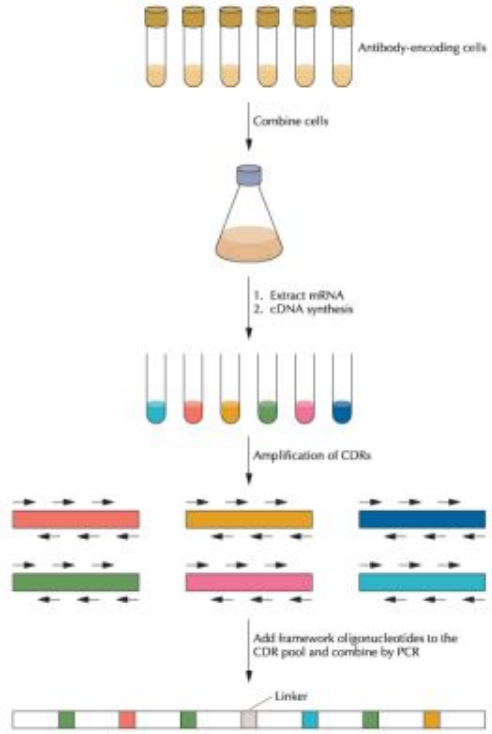
www.neb.com/nebecomm/products/productE8100.asp

Combinatorial library in M13



- Assay expressed mAb by
- Immunological screening
- ELISA-like system
- Multiwell plate coated with target Ag
- Bind, wash
- Score with chromogenic substrate cleaved by Ab-enzyme complex

Shuffling CDR sequences



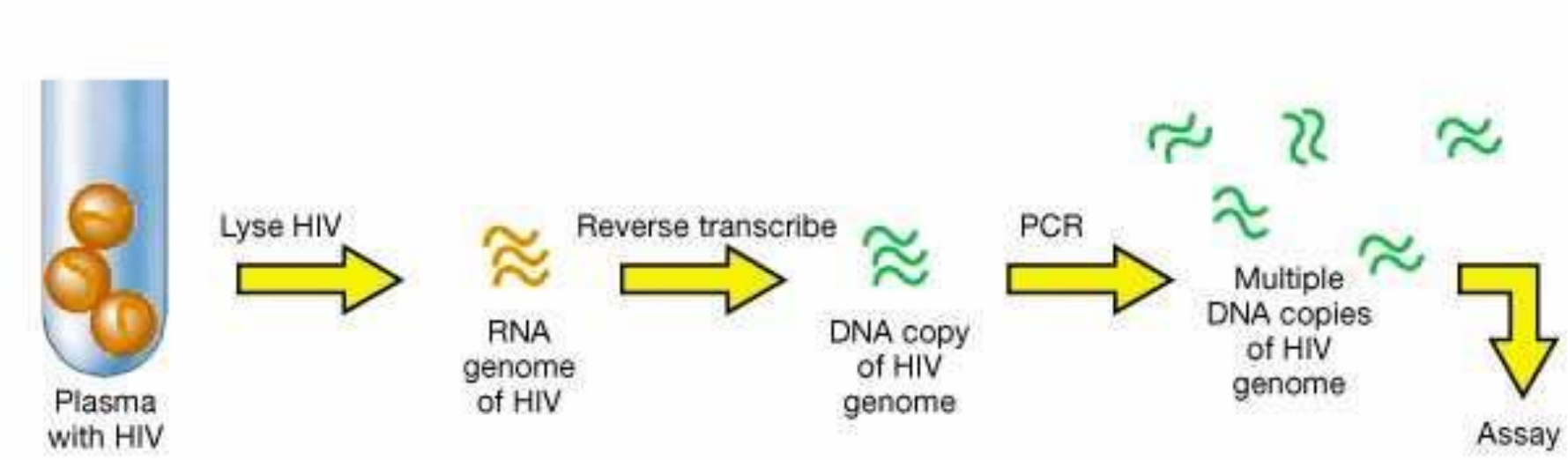
- Very large libraries yield wider range of Abs
- B cells from several non-immunized individuals collected and pooled
- mRNA isolated; cDNA synthesized
- PCR amplify all six CDR regions separately
- Pool with oligos encoding the framework regions and linker
- Overlap extension PCR gives variable L and H domains
- At 2×10^9 different single-chain Ab

Diagnostics

Using PCR to Detect for HIV

- **RT-PCR** (reverse transcriptase PCR).
- HIV has a ssRNA genome.
- Lyse plasma cells from the potentially infected person to release HIV RNA genome.
- The RNA is precipitated using isopropanol.
- Reverse transciptase is used to make a **cDNA** copy of the RNA of the virus.
- This cDNA is used as a template to make **dsDNA**.

RT-PCR Diagnosis of HIV



Using PCR to Detect for HIV

- Specific primers are used to amplify a 156 bp portion of the HIV ***gag* gene**.
- Using standards the amount of PCR product can be used to determine the **viral load**.
- PCR can also be used as a prognostic tool to determine viral load.
- This method can also be used to determine the effectiveness antiviral therapy.

Gene polymorphism

Polymorphic refers to the existence of two or more forms of the same gene, or genetic marker

Each form must be too common in a population to be merely attributable to a new mutation

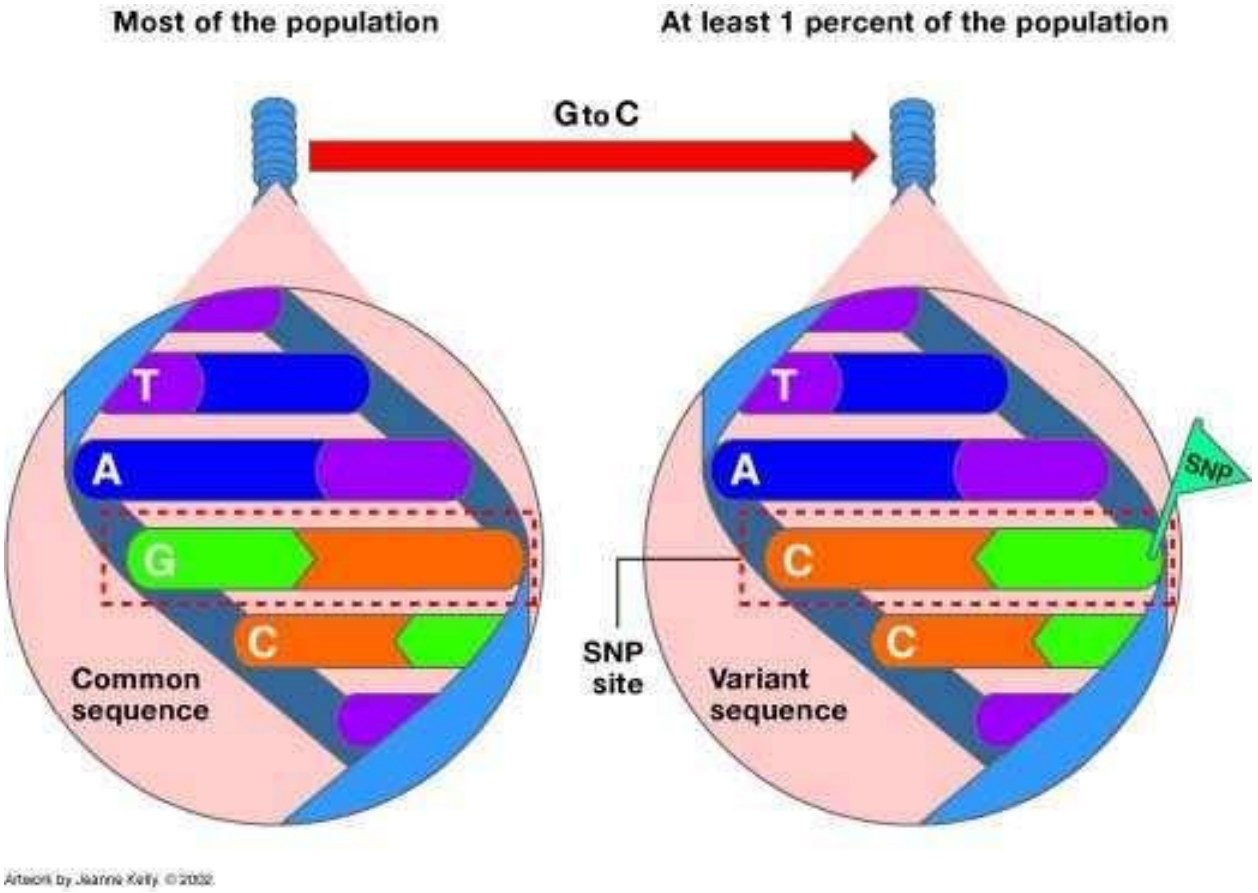
One type of polymorphism, Single Nucleotide Polymorphisms (SNPs), can be used as a diagnostic tool

SNPs are the 3.2 million single nucleotide changes that differ between genomes

Most SNPs occur outside of genes, but some occur in gene promoters & a few occur in genes themselves

For SNPs to be useful, a person's DNA must be examined for the presence of specific SNPs

SNPs



http://press2.nci.nih.gov/sciencebehind/snps_cancer/snps_cancer/snps_cancer13.htm

How do we identify SNPs?

DNA microarrays (DNA chips) make it possible to examine person for the presence of specific SNPs quickly and affordably

A single microarray can now be used to screen 100,000 SNPs found in a patient's genome in a matter of hours

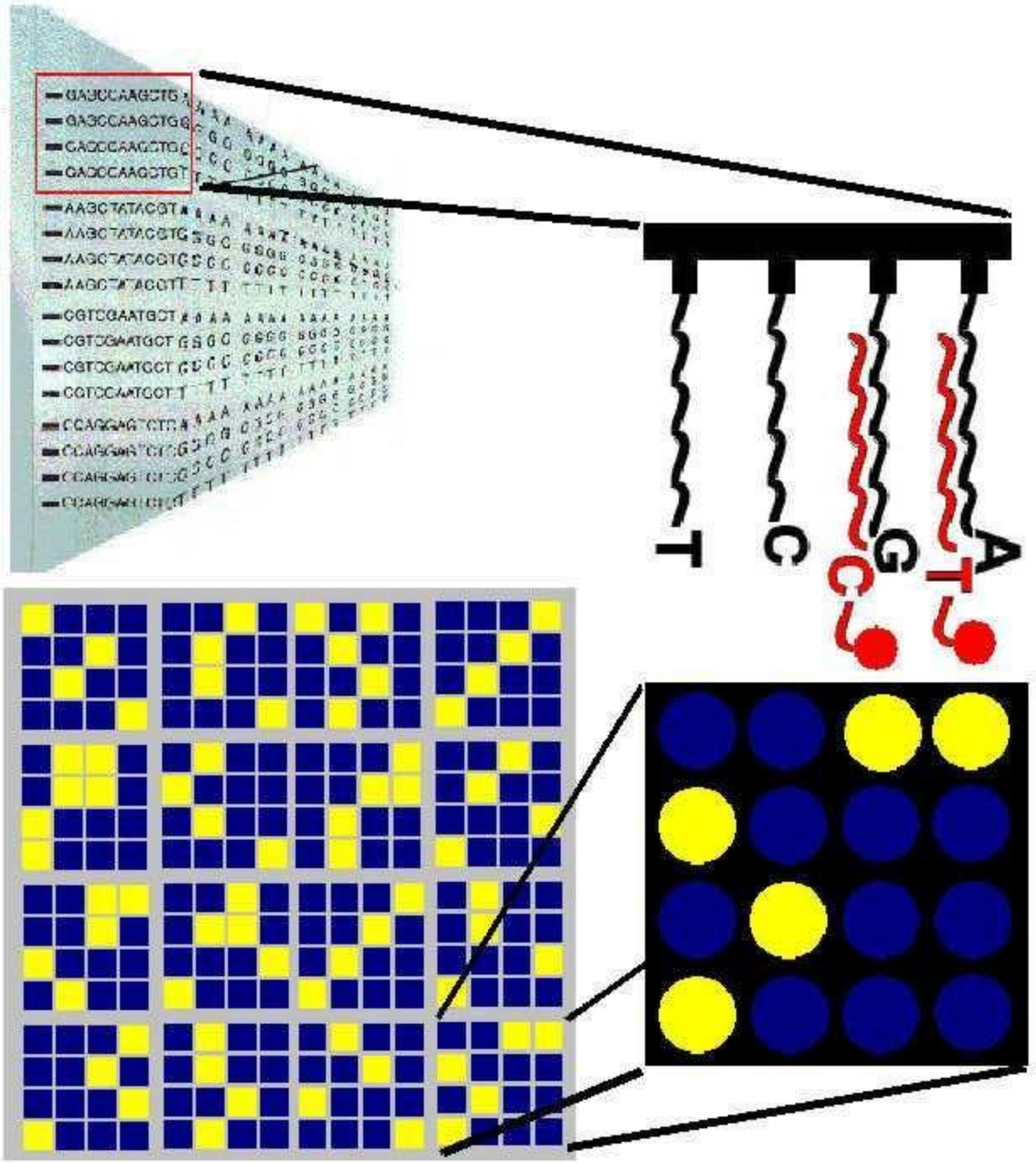
How Are Microarrays Made?

Short fragments of DNA (oligonucleotides) corresponding to each version of all known SNPs are spotted onto a glass slide in a known order

A patients DNA is fragmented and each fragment is linked to a fluorescent dye

This pool of fragments is allowed to hybridize to its corresponding oligonucleotide on the chip

The pattern of fluorescence determines which SNPs are found in the patient


T
C
G
A

Whole Human Genome Microarray by Agilent Technologies

1” x 3” glass slide with 44,000 genes dotted

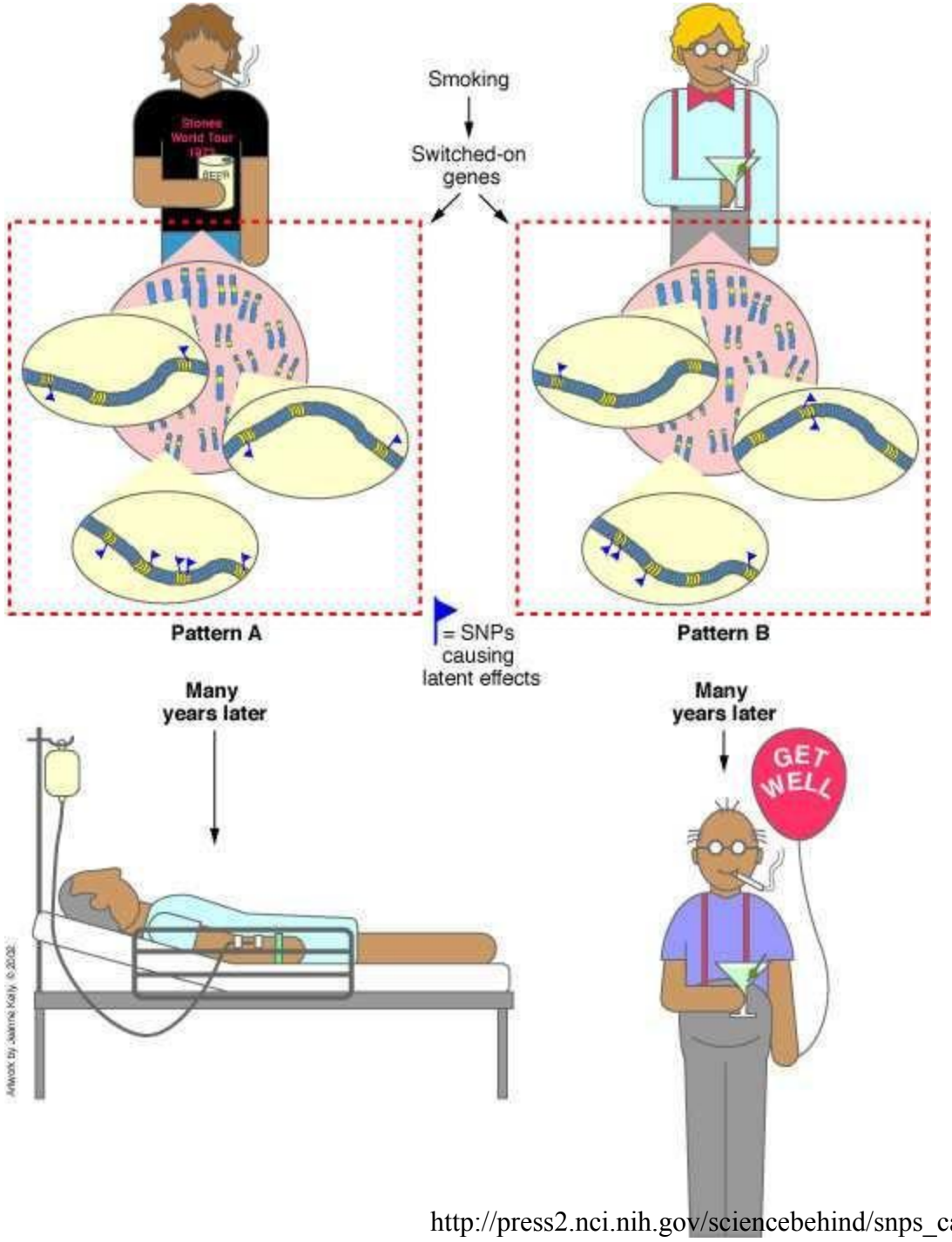
What Can SNPs Be Used to Predict?

A person's susceptibility to disease is linked to which alleles they carry as well as how those alleles interact with the environment

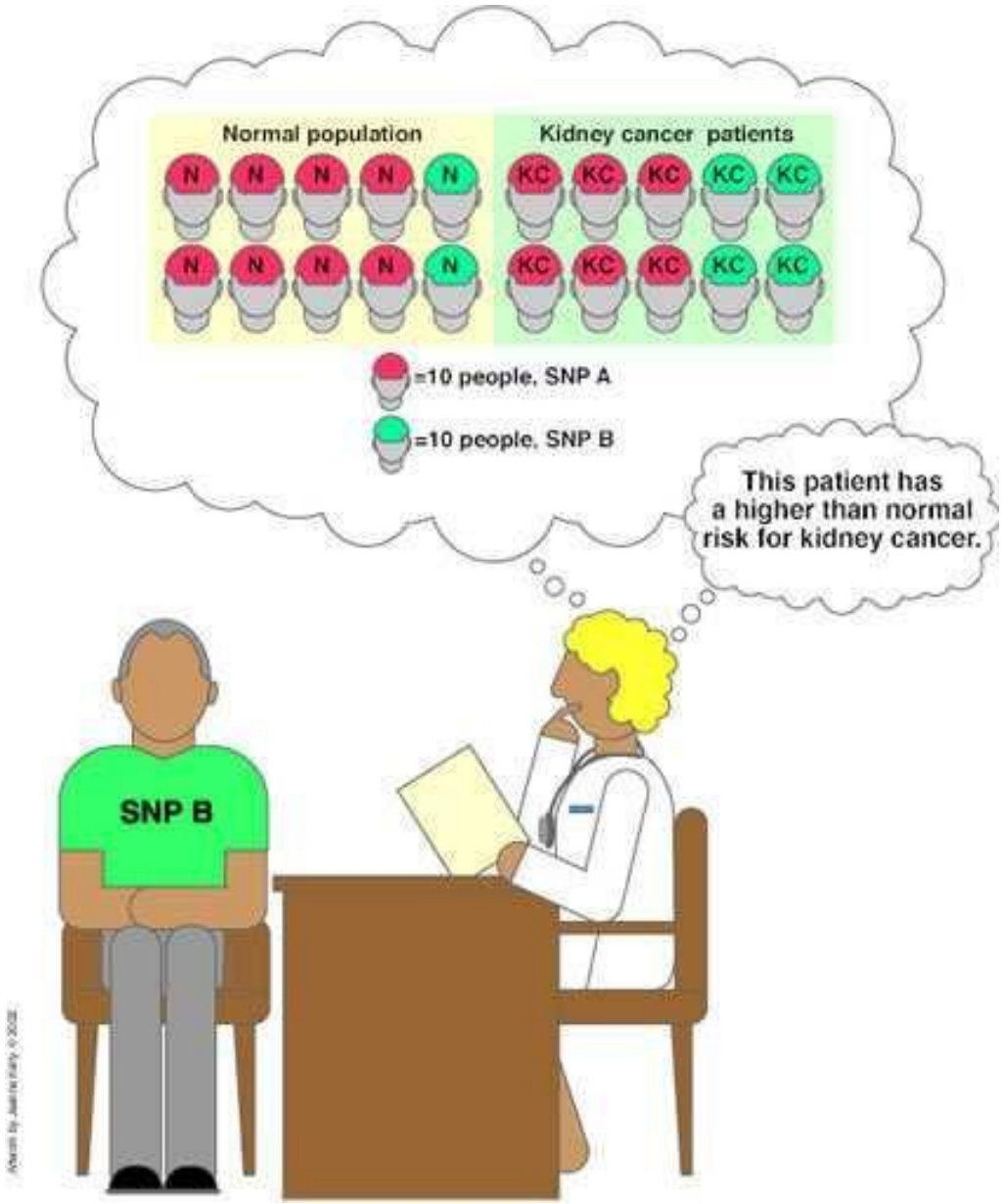
SNPs can be used to build a profile of a person's susceptibility to various diseases

Example:

Craig Venter (Celera genomics) has an increased risk of heart attack based on a SNP in the promoter of the MMP-3 gene



http://press2.nci.nih.gov/sciencebehind/snps_cancer/snps_cancer/snps_cancer25.htm



http://press2.nci.nih.gov/sciencebehind/snps_cancer/snps_cancer/snps_cancer38.htm

Drug Dosing/Reaction

Average patient

There is no simple way to determine how particular patient will respond to a medication

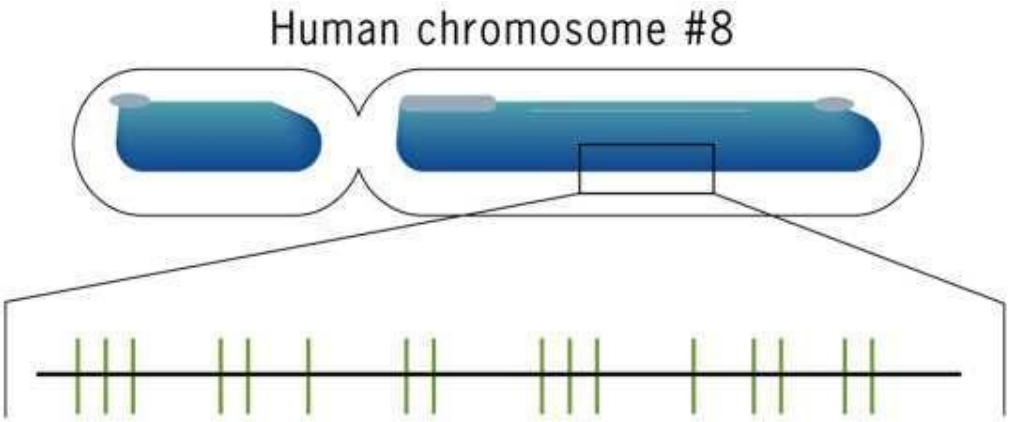
Adverse Drug Reactions (ADRs) one of the important causes of hospitalization and death in the United States

Medical drugs are developed using a ”average” patient

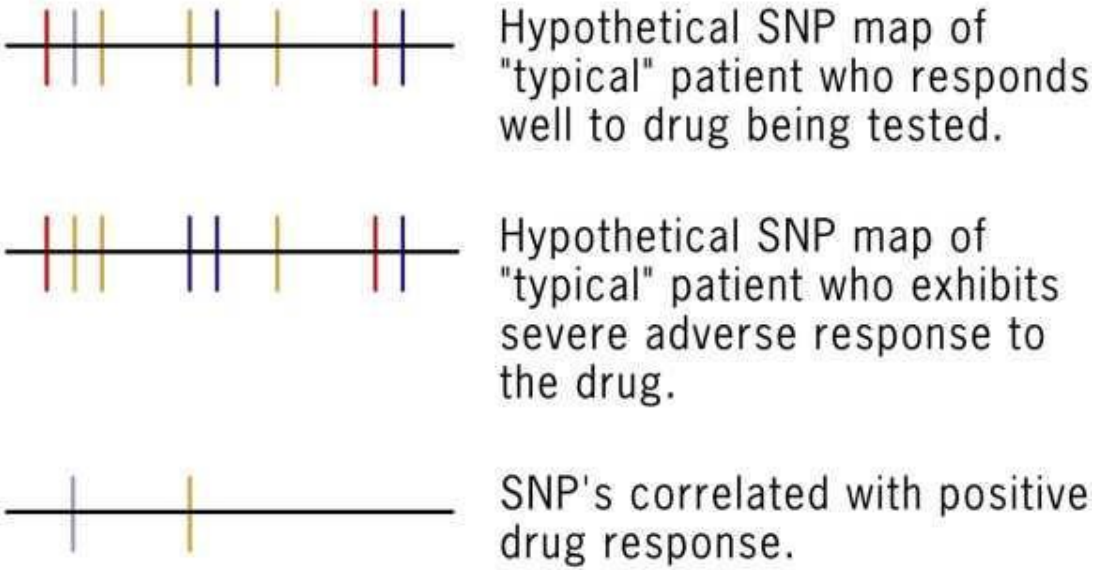
Pharmacogenomics examines the DNA variations that is correlated to drug response

Can be used to predict if a patient will have a good response to a drug, a bad response to a drug, or no response at all

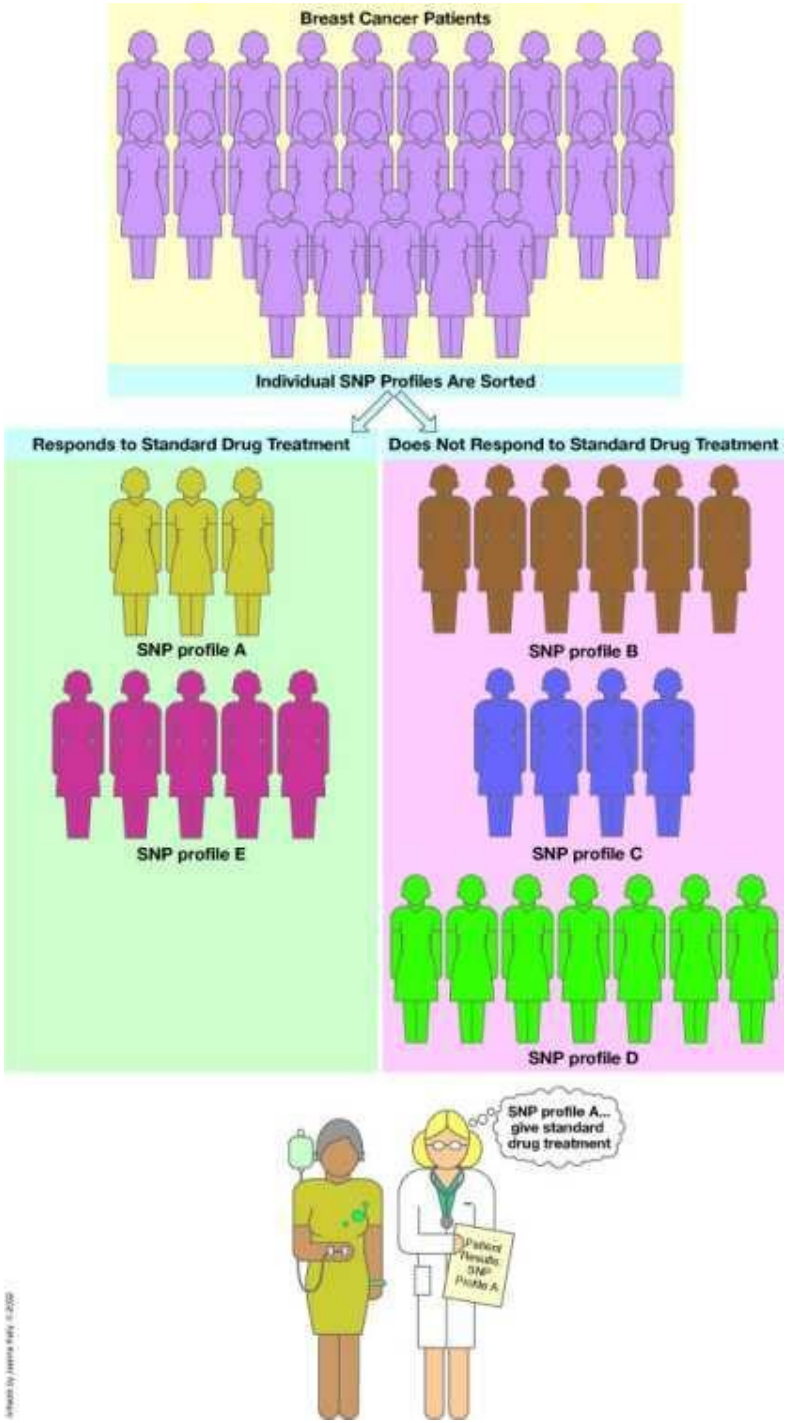
[1]http://www.fda.gov/CDER/drug/drugReactions/default.htm


Human chromosome #8

(a)


Hypothetical SNP map of "typical" patient who responds well to drug being tested.
Hypothetical SNP map of "typical" patient who exhibits severe adverse response to the drug.
SNP's correlated with positive drug response.
Key: =A =G =C =T

(b)



Testing for Variation

Cytochrome p450 (CYP450) involved in drug metabolism
Four major types; CYP3A, CYP2C9, CYP2D6 & CYP2C19

Variations in at least 3 genes regulate drug metabolism

By looking at the alleles a person has of these genes it is possible to predict how a patient will react to a drug

Dosing can be regulated so that a patient gets the maximum benefit without possible toxic side effects

CYP Genes & Their Metabolites

CYP3A	CYP2D6
Antihistamines	Codeine
Statins	Beta-Blockers
Ca^{+} Channel Blockers	Tricyclic Antidepressants
Benzodiazepines	Tamoxifen
HIV protease inhibitors	

CYP2C9	CYP2C19 (Missing in 30% of Asians)
NSAIDs	Proton pump inhibitors
Anti-epileptics	Valium
Warfarin	

Herceptin targets HER2 & is effective in stopping breast cancer growth

Only 25 to 30% of breast cancers overexpress HER2

Erbitux effective in colorectal cancers by stopping signaling through EGFR

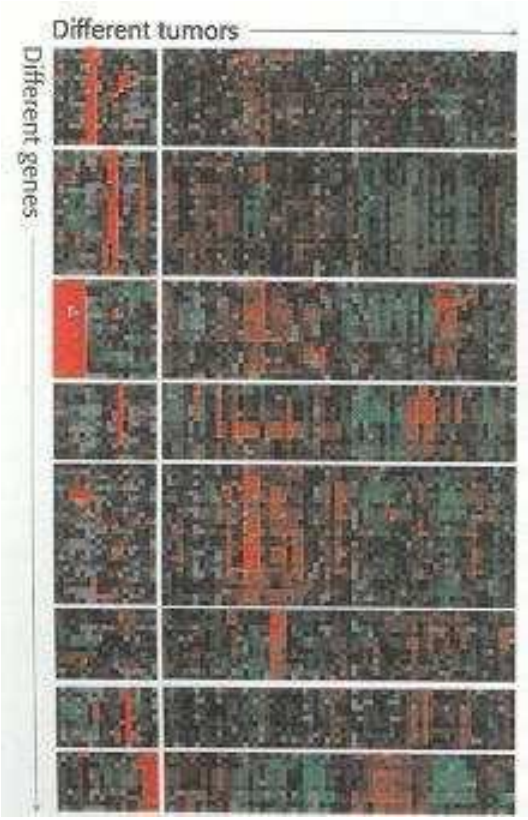
Not all colorectal cancers overepress EGFR

Diagnostic tests are used to detect which tumors will benefit from treatment allowing better use of treatment time & money

Tumors Are Not Identical So Why Should Every Patient be Treated the Same?

Red =gene induction

Green = gene repression

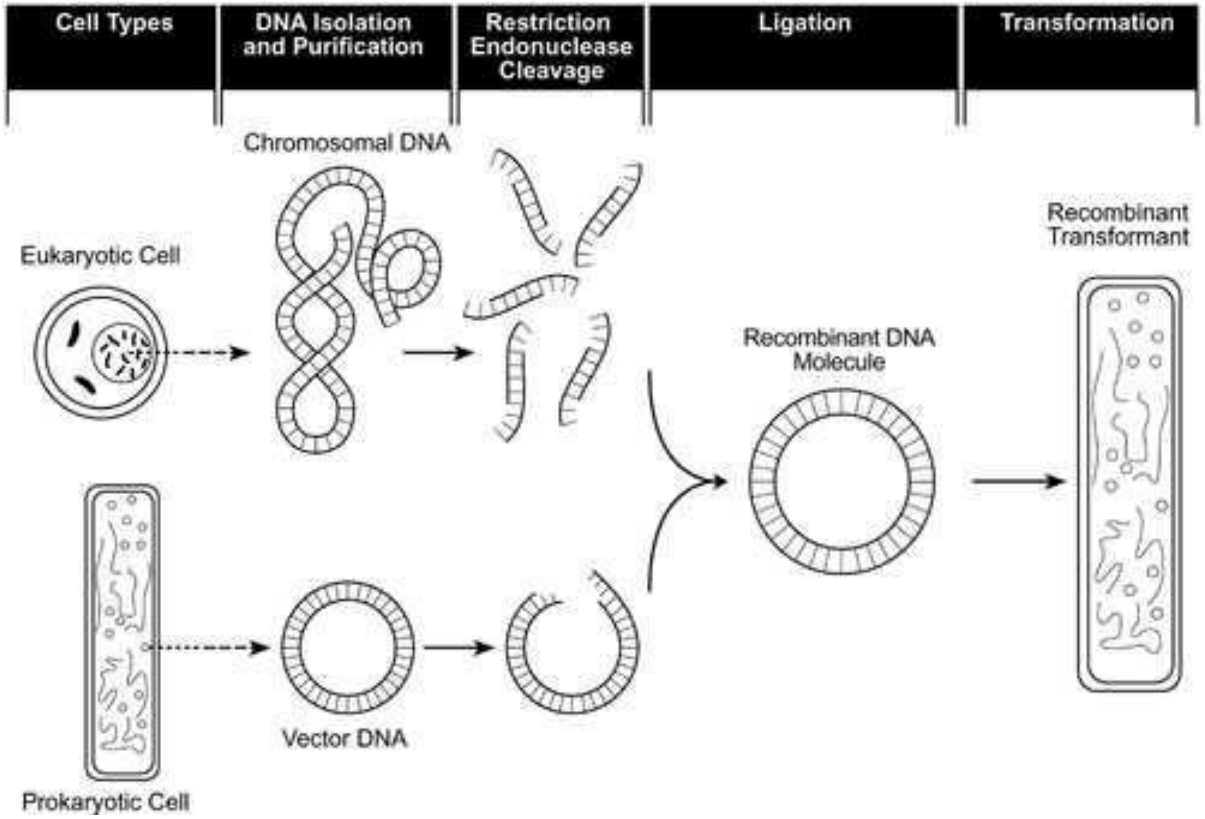


1733 Genes

84 breast tumor samples

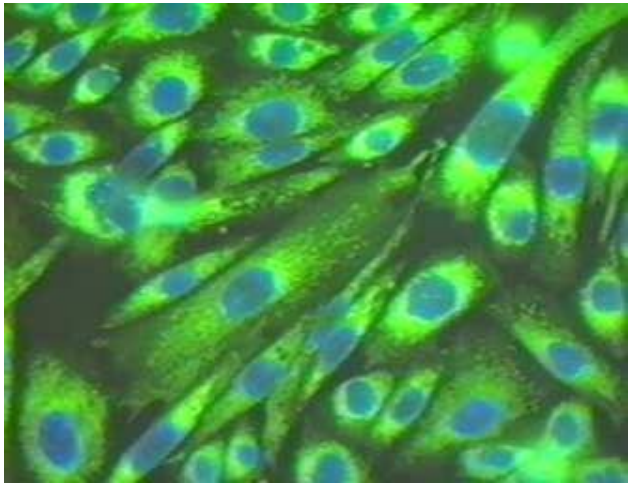
http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=stryer.figgrp.832

Recombinant DNA Drugs



http://history.nih.gov/exhibits/genetics/sect3.htm

Chinese Hamster Ovary Cells



Most popular cells for producing proteins that are not able to be produced in *E. coli*

These are proteins that are difficult to fold, glycosylated, or even toxic to the bacteria

http://dept.kent.edu/projects/cell/IMAGES3.HTM

Mammalian Cell Bioreactor

http://www.nbsc.com/ferm_eq/bf6000.asp

Protein Drugs Made in CHO Cells

Avonex (Interferon Beta-1a) Multiple Sclerosis - Biogen

Herceptin (Trastuzumab) Breast Cancer - Genentech

Humira (Adalimumab) Rheumatoid Arthritis - Abbott Labs

Remicade (Infliximab) Crohn's Disease - Centocor

Embrel (Etanercept) Rheumatoid Arthritis - Amgen

GENE THERAPY

Gene Therapy

Cells are removed from a patient and modified either by having a working copy of a defective gene inserted or a therapeutic gene added

Once the cells are expressing the new gene correctly, they are inserted back into the patient (*ex vivo*)

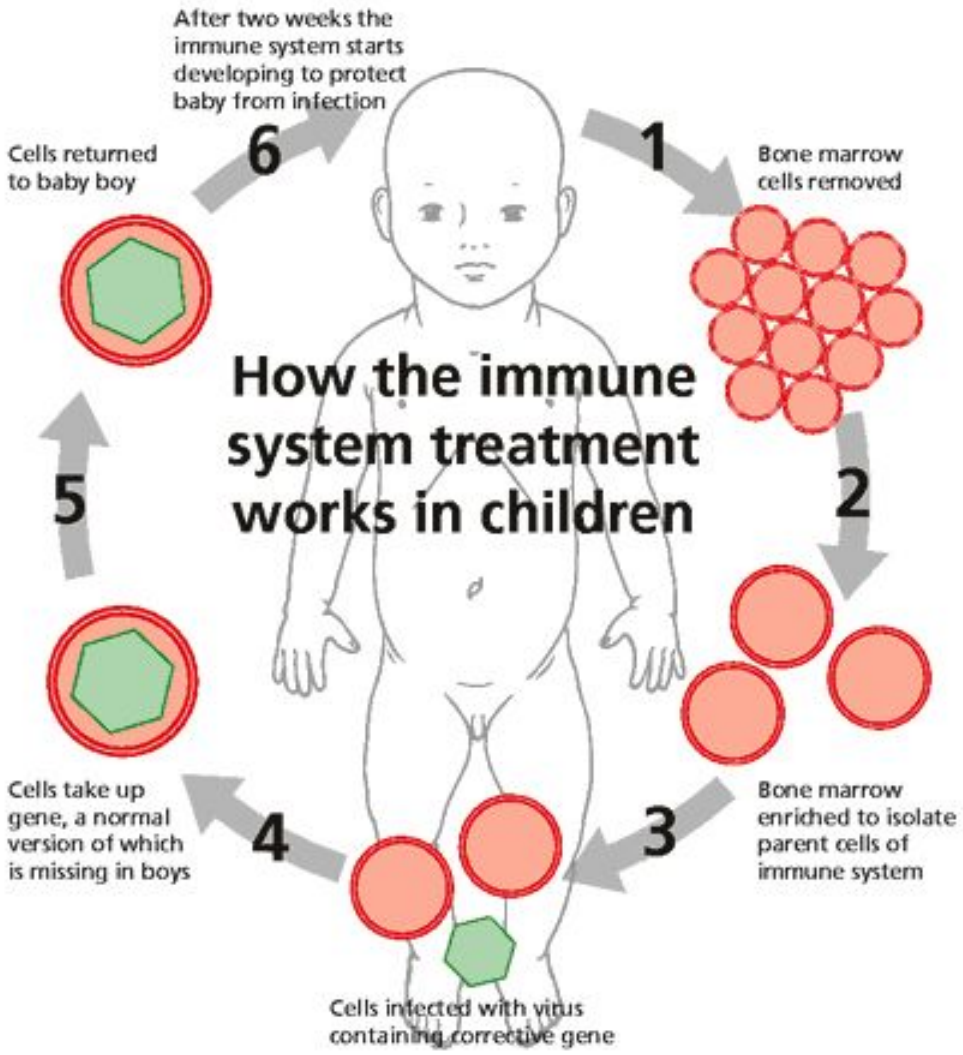
The gene is usually delivered using a defective virus

Sometimes the virus is delivered directly into the patient (*in vivo*)

Jak je to delano,

priklady, doplnit

Gene Therapy



http://www.jeansforgenes.com/images/2070_illustration.gif

Gene-Therapy and SCIDs

Severe Combined Immune Defiency (SCID): no T cells

Two types: ADA-SCID & SCID-X1

>20 SCID patients have been successfully treated

The FDA has not approved any human gene therapy

Current State of Gene Therapy

Little progress has been made since the first gene therapy clinical trials begun in 1990

In 1999, gene therapy suffered a major setback with the death of 18-year-old Jesse Gelsinger

Part of a gene therapy trial for ornithine transcarboxylase deficiency (OTCD)

Died from multiple organ failures 4 days post-treatment

Death was caused by a severe immune response

In 2003, the FDA placed a temporary halt on all gene therapy trials using retroviral vectors in blood stem cells

FDA took this action after it learned that two childern treated in a French gene therapy trial had developed a leukemia-like condition

These children in August 2002 had been successfully treated by gene therapy (SCID-X1)

Stem Cells

Stem cells are unspecialized cells that renew themselves for long periods through cell division.

Under certain physiologic or experimental conditions, they can be induced to become cells with special functions such as the beating cells of the heart muscle or the insulin-producing cells of the pancreas

These cells could then be used to repair or replace damaged organs or tissues

Three Types of Stem Cells

- Embryonic
- Adult/Somatic
- Induced Pluripotent

Human Embryonic Stem Cells

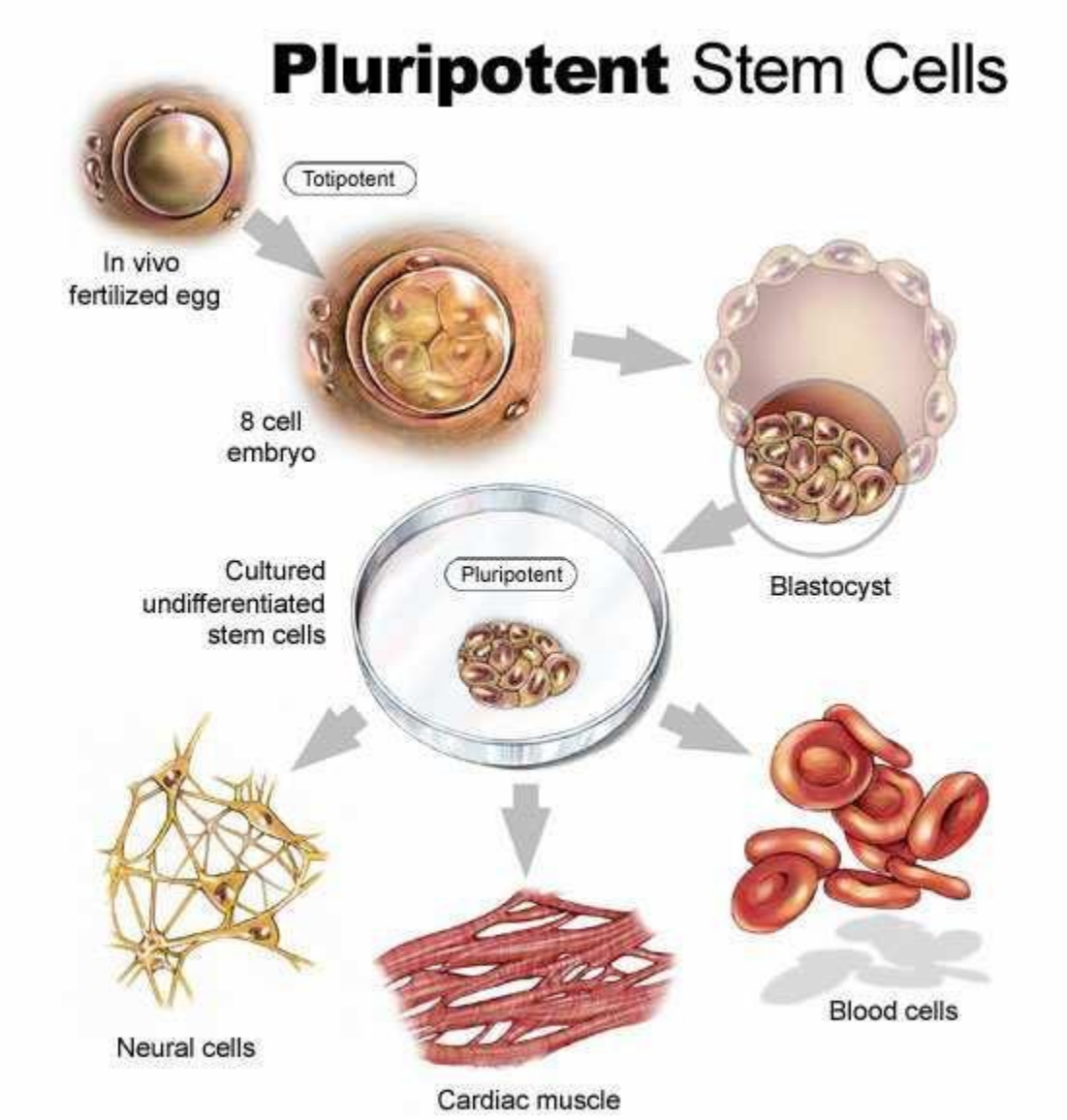
In 1998, human embryonic stem cells (hES) were isolated and grown in the laboratory

hES cells are derived from the ICM of human blastocysts

These cells are pluripotent just like mouse ES cells

The embryos used in these studies were created for infertility purposes through *in vitro* fertilization

They were donated for research with the informed consent of the donor



http://www.csa.com/discoveryguides/stemcell/images/pluri.jpg

Therapeutic Cloning

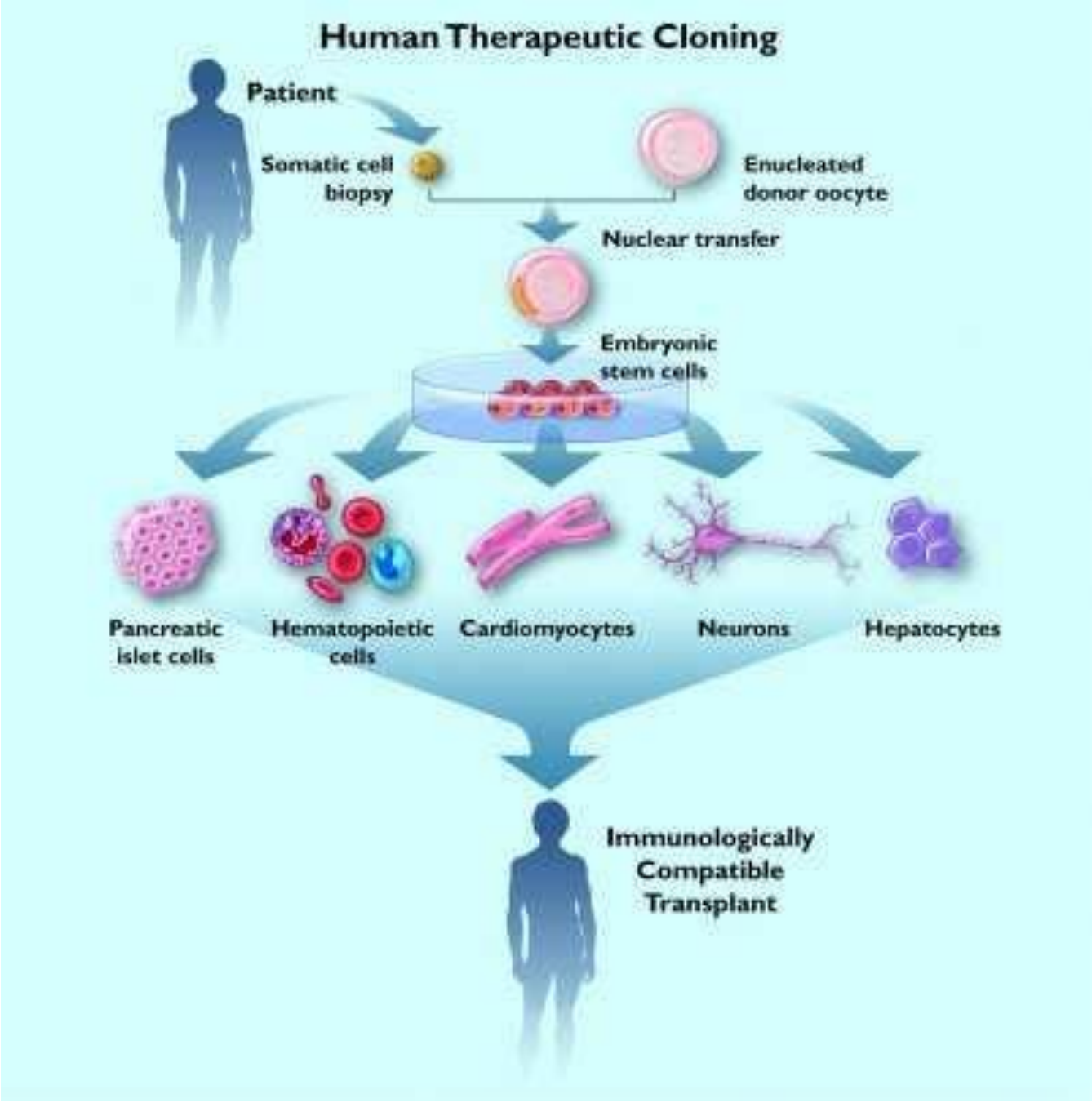
Isolation of cloned cells/tissue for curing disease or injury

The nucleus from an adult cell is placed in an enucleated egg

Instead of implanting the egg and letting it grow into a fetus, it is cultured until the blastocyst stage where ES cells are removed and cultured

These ES cells are coaxed down a specific developmental pathway such that they differentiate into a specific tissue

This allows for the creation of cells identical to the donor thus preventing rejection



http://www.advancedcell.com/testimony-12-4-2001.html

Adult Stem Cells

An adult or somatic stem cell is an undifferentiated cell found among differentiated cells in a tissue or organ

It can renew itself, and can differentiate to yield the major specialized cell types of the tissue or organ

The primary roles of adult stem cells are to maintain and repair the tissue in which they are found

These cells are more restricted as to what cell types they can become & are thus said to be multipotent

1960s, two stem cell populations identified in bone marrow

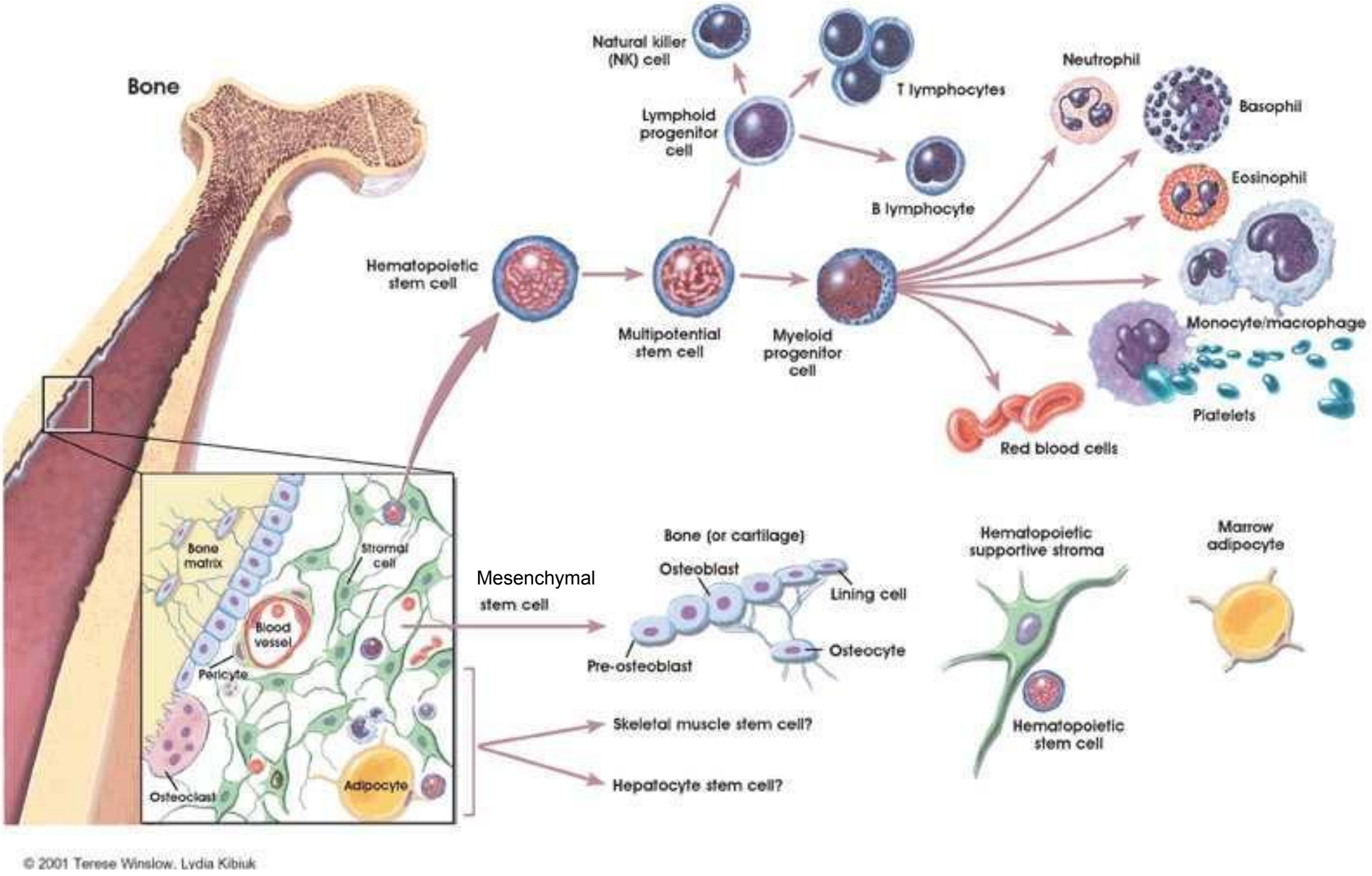
One population, called hematopoietic stem cells, forms all the types of blood cells in the body

The second, called mesenchymal stem cells generate bone, cartilage, fat, & connective tissue

Hematopoietic stem cells have also been isolated from umbilical cord blood

Mesenchymal stem cells have now been isolated from amniotic fluid, umbilical cord blood, and adipose tissue

Bone Marrow Stem Cells

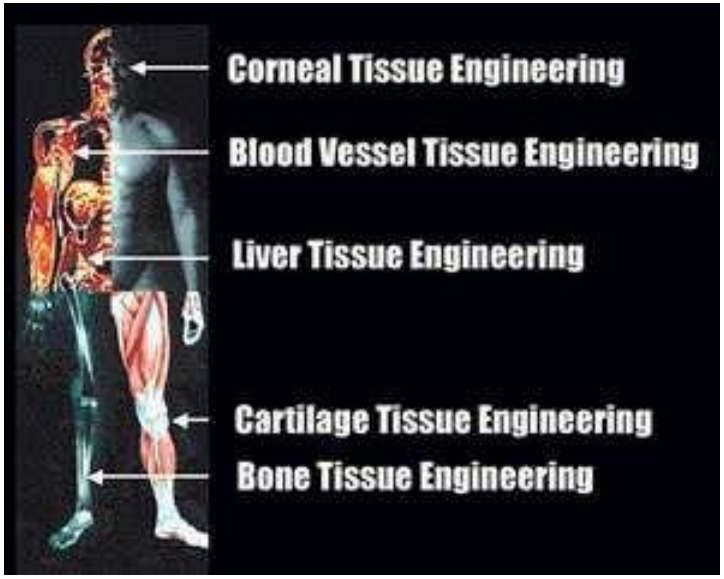


http://www4.od.nih.gov/stemcell/figure2cropbig.gif

Tissue Engineering

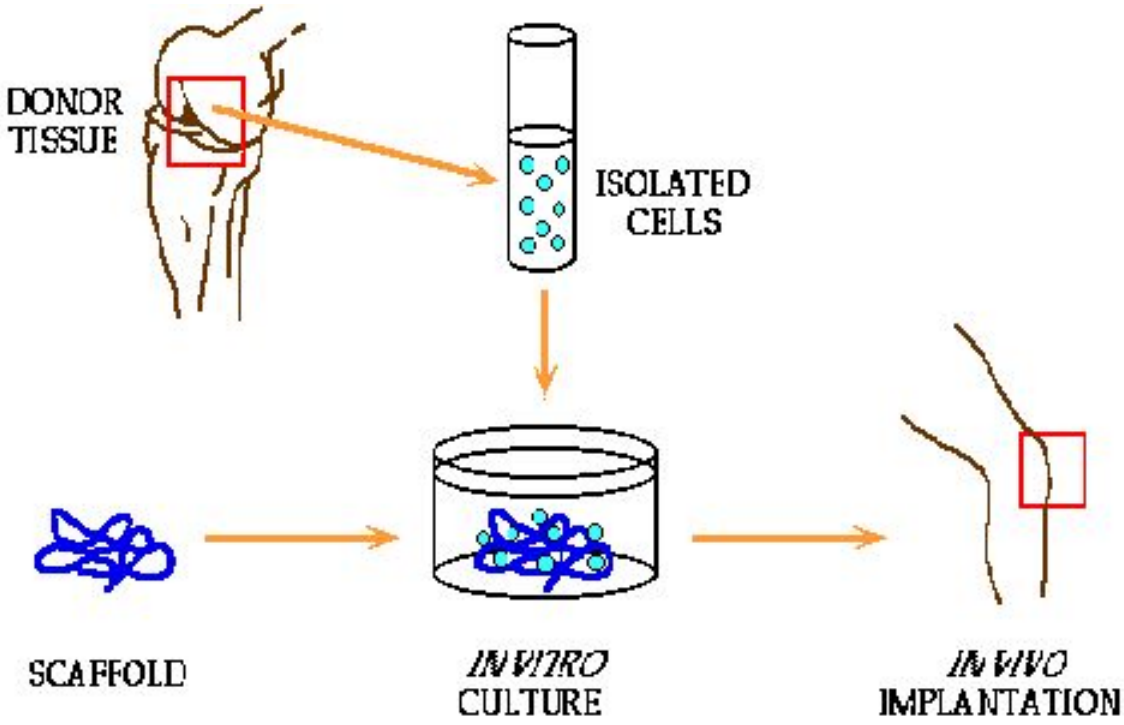
Tissue engineering or regenerative medicine is a multidisciplinary field combining biology, medicine, and engineering & involving the restoration, maintenance, or enhancement tissue & organ function

Often involves the growth of new tissue or organs within a 3D matrix to mimic natural organ growth



http://www2.mahidol.ac.th/spectrum/pic3_vol10_no3.gif

Generation of Replacement Knee Cartilage

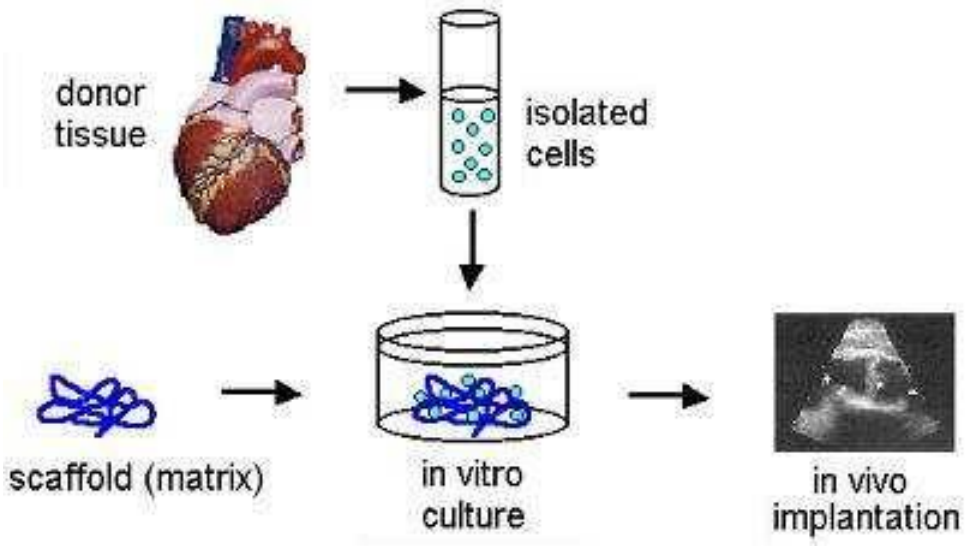


http://txtell.lib.utexas.edu/stories/media/t0003-2.html

Valvular heart disease is a major cause of mortality

Currently available substitutes for failing heart valves have serious limitations

An alternative is to tissue engineer heart valves



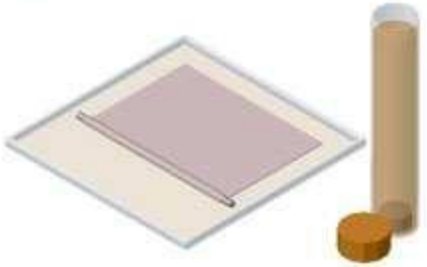
http://navier.ugent.be/public/biomed/research/kris/res_kris.php

Engineering a Blood Vessel

A California company can create blood vessels in the lab using the patient's own cells.

HARVEST A small piece of skin the size of a postage stamp is removed from the back of the patient's hand. Skin cells from the biopsy are isolated and placed in a culture dish.

GROWTH Over several weeks, the skin cells grow into a thin sheet.



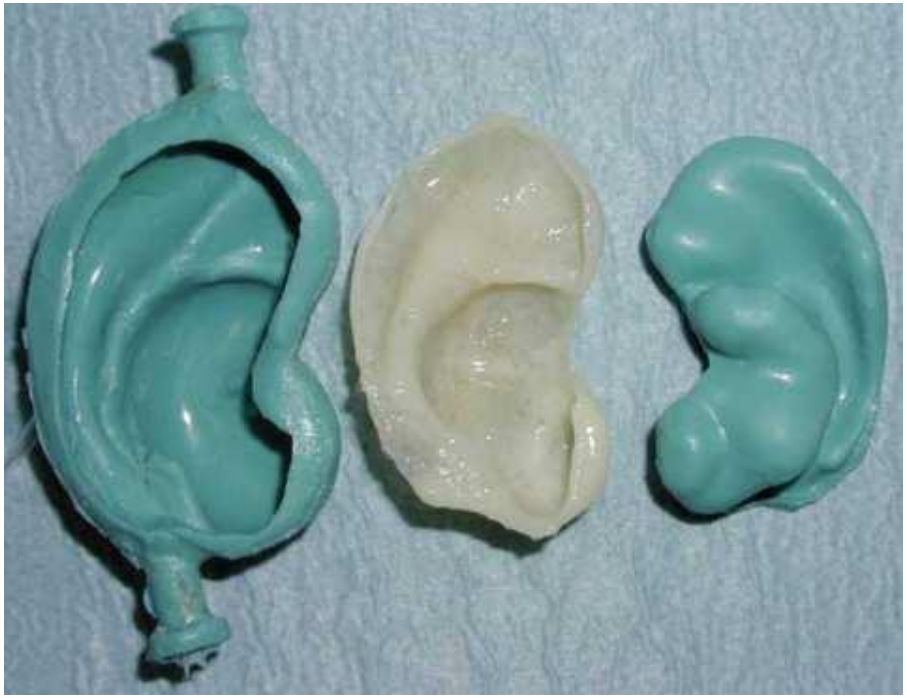
CONSTRUCTION The sheet is rolled onto a metal cylinder and placed in a serum. After eight weeks the cylinder is removed, and the tube of cells is seeded with cells from the lining of the patient's veins.

FINISH After several weeks the vein cells have lined the tube, and the vessel is ready for implanting.

Source: Cytograft

THE NEW YORK TIMES

Cartilage scaffold of a human ear implanted under the skin of a mouse



http://www.swr.de/swr2/biotopien/expeditionen/tissue/tissue3.html
http://www.medizin.fu-berlin.de/hno/arbeitsgruppen/haischgruppe.htm