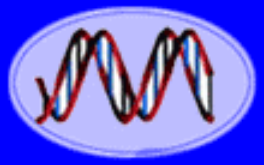
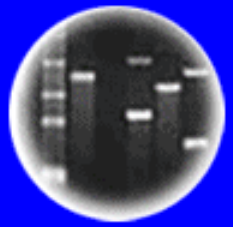


DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

HC70A & SAS70A Winter 2013
Genetic Engineering in Medicine, Agriculture, and Law

Professors Bob Goldberg, John Harada, & Channapatna Prakash

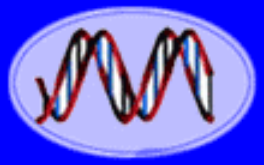
Lecture 5

The Nuts & Bolts of Genetic Engineering:The Factor VIII Story - From Gene To Drug

UCLA

TUSKEGEE UNIVERSITY

UCDAVIS
UNIVERSITY OF CALIFORNIA



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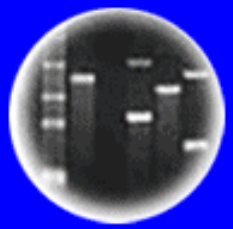


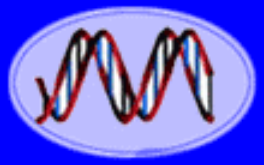
DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

THEMES

1. What is Current Status of Recombinant DNA Drugs?
2. How Do We Go From Disease to Gene to Drug? Hemophilia – A Case Study
3. How Is Hemophilia Inherited?
4. What is the Pedigree Pattern of a Sex-Linked Gene?
5. How Find a Disease Gene When It is Not Known Where the Gene is Expressed?
6. What Vectors Can Be Used For Cloning DNA?
7. What Are the Advantage of Using a Virus Vector For Constructing Genome Libraries?
8. How Make a Library of the Human Genome?
9. How Find a Gene With Only a Knowledge of the Protein Sequence?
10. What is Chromosome Walking & What Role Did it Play in Cloning the Factor VIII Gene?
11. How Use DNA Testing to Detect Factor VIII Disease Alleles?
12. How Isolate a Factor VIII cDNA Clone?
13. How Produce Factor VIII Protein For Use as a Drug?
14. Transgenic Protein Patent & Regulatory Concerns?
15. Diagnostic Disease DNA Testing Legal Concerns?
16. How About Gene Therapy?



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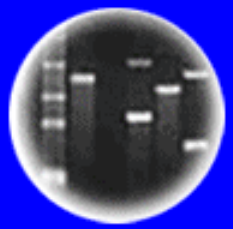
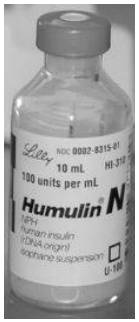


DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

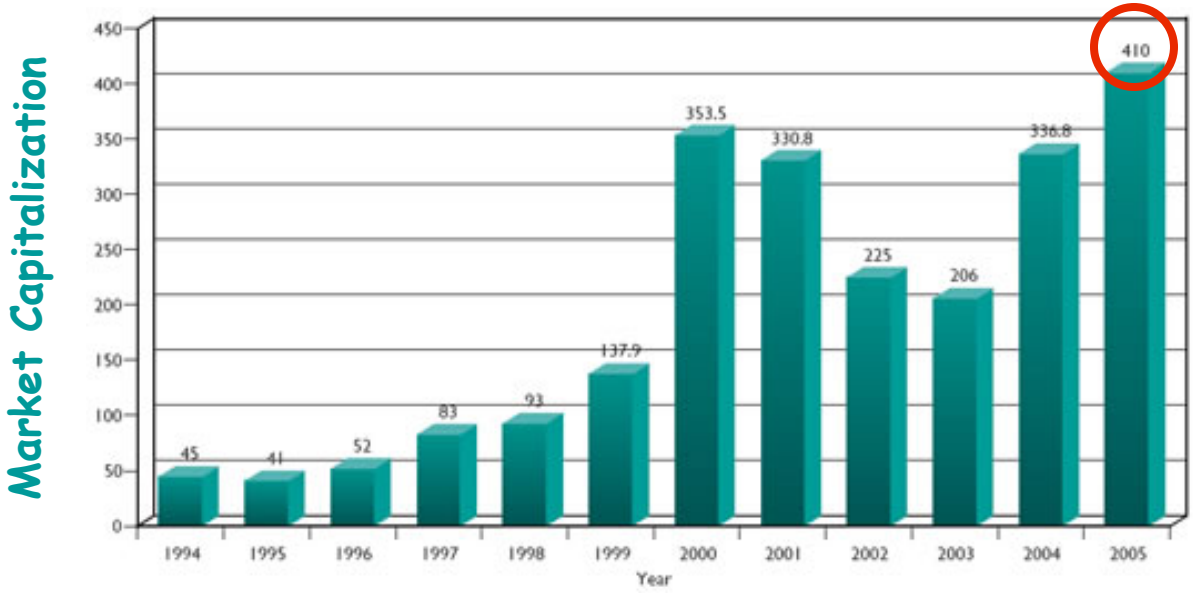
Plants of Tomorrow

One of the Most Important Applications of Genetic Engineering Technology Has Been To Manufacture Drugs to Treat Human and Animal Diseases

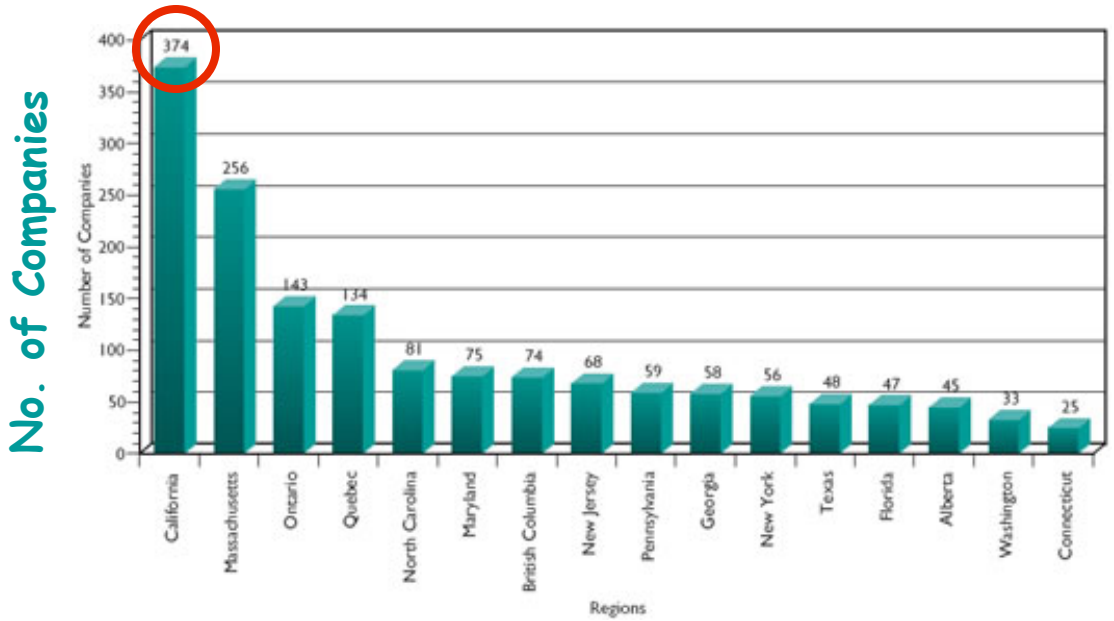


Created a Multibillion Dollar Biotechnology Industry, Was Responsible For the Acceptance of Recombinant DNA Technology in the 1970s,& Lead to Pioneering Decisions in Patent Law

Biotech in the United States is a Huge Success and a Big Business



350 Billion Dollars In 2011



Note:

There Was No Biotech Industry Before 1976

With No Gene Patent Protection There Would Be no Biotech Industry!!

Life Is Patentable

SCIENCE MAY PATENT NEW FORMS OF LIFE, JUSTICES RULE, 5 TO 4

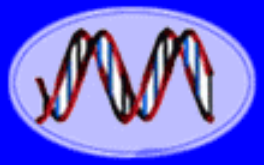
Diamond vs. Chakrabarty

6/17/1980

1980
The Supreme Court rules that Ananda Chakrabarty's bacterium is not a "product of nature" and so can be patented; other living things "made by man" are declared patentable as well

Ananda Chakrabarty

Decision Assists Industry in Bioengineering in a Variety of Projects



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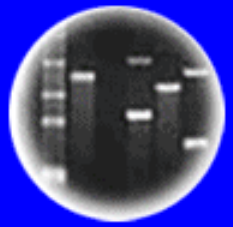


DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

Should You Be Able To Patent Genes & Have Intellectual Property Rights?

a. Yes
b. No

Examples of Recombinant DNA Drugs

TABLE 1.2 EXAMPLES OF PROTEINS MANUFACTURED FROM CLONED GENES

Product	Application
Blood factor VIII (clotting factor)	Treat hemophilia
Epidermal growth factor	Stimulate antibody production in patients with immune system disorders
Growth hormone	Correct pituitary deficiencies and short stature in humans; other forms are used in cows to increase milk production
Insulin	Treat diabetes
Interferons	Treat cancer and viral infections
Interleukins	Treat cancer and stimulate antibody production
Monoclonal antibodies	Diagnose and treat a variety of diseases including arthritis and cancer
Tissue plasminogen activator	Treat heart attacks and stroke

TABLE 1.1 TOP 10 BIOTECHNOLOGY DRUGS (WITH SALES OVER $1 BILLION) 2012

Drug	Developer	Function (Treatment of Human Disease Conditions)
Enbrel	Amgen & Wyeth	Rheumatoid arthritis
Remicade	Johnson & Johnson	Rheumatoid arthritis
Rituxan	Roche	Non-Hodgkin's lymphoma
Avastin	Roche	Colon cancer
Herceptin	Roche	Breast cancer
Humira	Abbott Labs	Rheumatoid arthritis
Levenox	sanofi-aventis	Blood clots
Lantus	sanofi-aventis	Diabetes
Aranesp	Amgen	Anemia

These Include: Hormones, Blood Factors, Anticoagulents, Growth Factors, Interferons, Vaccines, Monoclonal Antibodies, Bone Morphogenic Proteins, & Many Others

Examples of Recombinant DNA Drugs-1

I. Amgen: (Nasdaq: AMGN)

	Protein Product: what human protein is formulated as the drug	Indication: why drug is prescribed and what it does in the body	Other notes (Alternate forms of this drug, competing products on the market, "Dark Side" etc)
1:Epogen /Procrit	**rErythropoetin** [FDA approval 1989] Epo binds the Epo receptor (Epo R) on bone marrow erythroid progenitors, inducing proliferation, maturation, and differentiation of red blood cells (MOA)	**For patients with anemia due to Dialysis/ Chronic Kidney Disease / Renal Failure / Chemo / HIV** Stimulates production of RBCs. Truly revolutionized treatment of anemia! **Epo:** For patients on dialysis with anemia. **Procrit:** For non-dialysis use only = Cancer, Chronic Kidney Disease, HIV (anemia due to AZT treatment); some blood transfusions	Sales of ~$2.4 B annually Increases the risk of thromboembolic complications (Stroke) GOOD OR BAD FOR CANCER? Epo has pleiotropic effects Epo Doping
2. Aranesp	**rErythropoetin "+"** [FDA approval September 2001] (Darbepoetin)	**For patients with anemia due to Dialysis/ Chronic Kidney Disease / Renal Failure / Chemo** 2 additional **N-linked carbohydrate chains** creates a longer lasting effect (3X greater that Epo), requires fewer injections - 1 shot every 3 weeks, rather than once/week	Glycoengineering increased half life from 8.5 to 25 hours (see below) Sales of $2.8 B annually increases the risk of stroke Muehlegg, Lazutina stripped of gold medals February 24, 2002
3: Neulasta	**rGranulocyte colony-stimulating factor (G-CSF)** [FDA approvals 1991 for Neupogen, Feb 2002 for Neulasta]	**For Neutropenia:** low WBC count febrile neutropenia (low WBC count with fever/ infection) due to chemo, BMT, AML. **G-CSFs** are glycoprotein cytokine hormones that stimulate proliferation and growth of granulocytes, particularly neutrophils (WBCs) but also eosinophils, and basophils	**Tagline:** "Are you ready to start Chemotherapy?" ... "Be Ready" **Neulasta:** Addition of a polyethylene glycol (PEG) molecule extends the half-life = only a **single dose per chemotherapy cycle** vs daily injections with Neupogen. Sales of $1.4 B annually
4. Infergen:	**rInterferon alpha** [FDA approval 1997] A synthetic 'consensus' interferon based on the most common amino acid sequences in 12 natural interferons	**For patients with Chronic, non-responding, or relapsing hepatitis C viral (HCV) infection,** 4 M Americans have Hepatitis C! -	**MOA is unknown:** "No one knows exactly how interferons work" but Interferons are immunity-boosting proteins in WBCs with "antiviral, antiproliferative, and 'immunomodulatory' activities". Sales of ~$10

II. Biogen (Nasdaq: BGEN)

rDNA products on the Market

	Protein Product: what human protein is formulated as the drug	Indication: why drug is prescribed and what it does in the body	Other notes (Alternate forms of this drug, competing products on the market, etc)
1. Avonex	**rInterferon beta-1a** [FDA Approval May 1996]	**Treatment of relapsing forms of MS.** Slows the progression of MS by regulating the body's immune response against myelin. Given as an IM injection (ouch!) once per week.	MOA: "Calms" or down-regulates the immune system* Made in CHO cells. Sales of ~1 B annually
2. Intron A	**rInterferon alpha-2b** [FDA Approval 1986	**Over 20 indications,** * Malignant melanoma * Non-Hodgkin's lymphoma * Hairy cell leukemia * Kaposi's sarcoma * Chronic hepatitis B (HBV) * Chronic hepatitis C (HCV) * Condylomata acuminata	**Interferons** - see MOA Above. Sales exceeding $440 million in each of the last three years."
3. Engerix-B/ Recombivax	**rHepatitis B vaccine** [FDA approval 8/99, 1/2000 for teens]	**Infection with HBV** Prevention of 1° liver cancer New HepB Law (2005) for all Indiana 9th and 12th graders	"The global HBV market exceeds $1 billion dollars annually. It will grow as more countries adopt WHO recommendations for the vaccination of newborns, teenagers, healthcare workers and other at-risk populations. "
4. Amevive	A recombinant fusion protein between **IgG1 and the "leukocyte function-associated antigen-3"** (LFA-3). [FDA approval 1/31/03]	**Moderate-to-severe chronic plaque psoriasis** Suppresses overactive T lymphocytes found in autoimmune diseases (more on this when we get to mABs)	Injected by physician (15 mg IM, into the muscle) once a week for a total of 12 doses. Because Amevive reduces T-cell counts (important for fighting off infections, etc), T-cell levels are monitored closely after MOA

Examples of Recombinant DNA Drugs-2

III. Chiron (a Novartis partnership) recently acquired by Novartis, agreements with Bayer...

	Protein Product: what human protein is formulated as the drug	Indication: why drug is prescribed and what it does in the body	Other notes (Alternate forms of this drug, competing products on the market, etc)
1. Betaseron	**rInterferon beta-1b** [FDA approval July 93]	**Multiple Sclerosis:** Significantly delays the progression of secondary MS, including relapsing-remitting MS.	SubQ every other day. Sales of ~$118 M annually
2. Proleukin	**Interleukin-2 - IL-2** [FDA approval 1992, 1998]	**Cancer:** Metastatic renal cell carcinoma, and metastatic melanoma. IL-2 activates lymphokine-activated killer (LAK) cells, NK cells that normally destroy tumor cells.	MOA: IL-2 activates the immune system in several ways, but the major one is to stimulate T cell and natural killer (NK) cell proliferation, increasing and activating these immune system cells to find and destroy cancer cells. (See figure) Sales of ~$92 M annually; MOA
3. Regranex	**rPlatelet-derived growth factor. PDGF** [FDA approval 12/97]	**Treatment of diabetic foot ulcers.** PDGF is a cytokine (growth factor) that stimulates skin cell and blood vessel production	PDGF is involved in developing protective tissue and skin after a wound or ulcer (a process called granulation; MOA) Sales of $48 M annually

IV. Genentech (NYSE: DNA)

rDNA products on the Market:

>

	Protein Product: what human protein is formulated as the drug	Indication: why drug is prescribed and what it does in the body	Other notes (Alternate forms of this drug, competing products on the market, etc)
1. Activase 1a.CathFlo Activase:	**rTPA, tissue-plasminogen activator.** [FDA approval 4/95; CathFlo 9/00]	**Thrombolytic:** Approved for treatment of **AMI, cardiac ischemia, acute massive pulmonary embolism, and management of stroke.**	**CathFlo Activase:** can be directly injected into an occluded central line catheter. Converts plasminogen to plasmin, which activates fibrin, breaking down blood clot.
2. TNKase	**rTPA, tissue-plasminogen activator "+"** TNKase can be administered over five seconds in a single dose, **fastest administration of any thrombolytic**	**Thrombolytic: TNKase is bioengineered** with 3 amino acid substitutions from natural t-PA: **T, N and K** (Thr, Asp, Lys). =Increased Fibrin Specificity =Long Plasma Half-Life =Greater Resistance to Plasminogen Activator Inhibitor-1	Investigate the Molecule "An advanced lytic by design" 1,000 variants of rt-PA were evaluated, using site-directed mutagenesis
3. Pulmozyme	**rDeoxyribonuclease I** (rhDNase) [FDA approval 1994]	**For management of cystic fibrosis in children 3 months - 5 years old.** Inhalation Solution - Pulmozyme hydrolyzes the DNA in sputum and airways of CF patients.	**MOA:** In CF patients, lungs and airways become clogged with mucous containing high concentrations of extracellular DNA released by degenerating leukocytes (neutrophils).
4: Neutropin	**rHuman growth hormone** (Somatropin; synthetic growth hormone) [FDA approval 11/93] What is a depot form?	**Treatment of GHD in children and in adults** Identical to pituitary-derived hGH. Also indicated for growth failure associated with chronic renal insufficiency (CRI) prior to kidney transplantation, and short stature associated with Turner syndrome (45, Xo)	**Neutropin replaced Protropin** [FDA approval October 1985; Genetech's first product]; Protropin stopped production in 2002 **4a: Neutropin AQ:** liquid formulation; **4b: Neutropin pen:** same stuff, single use throw-away 'pen' injection

Examples of Recombinant DNA Drugs-3

V. Eli Lilly: (NYSE: LLY)
rDNA products on the Market: (rDNA origin; there)

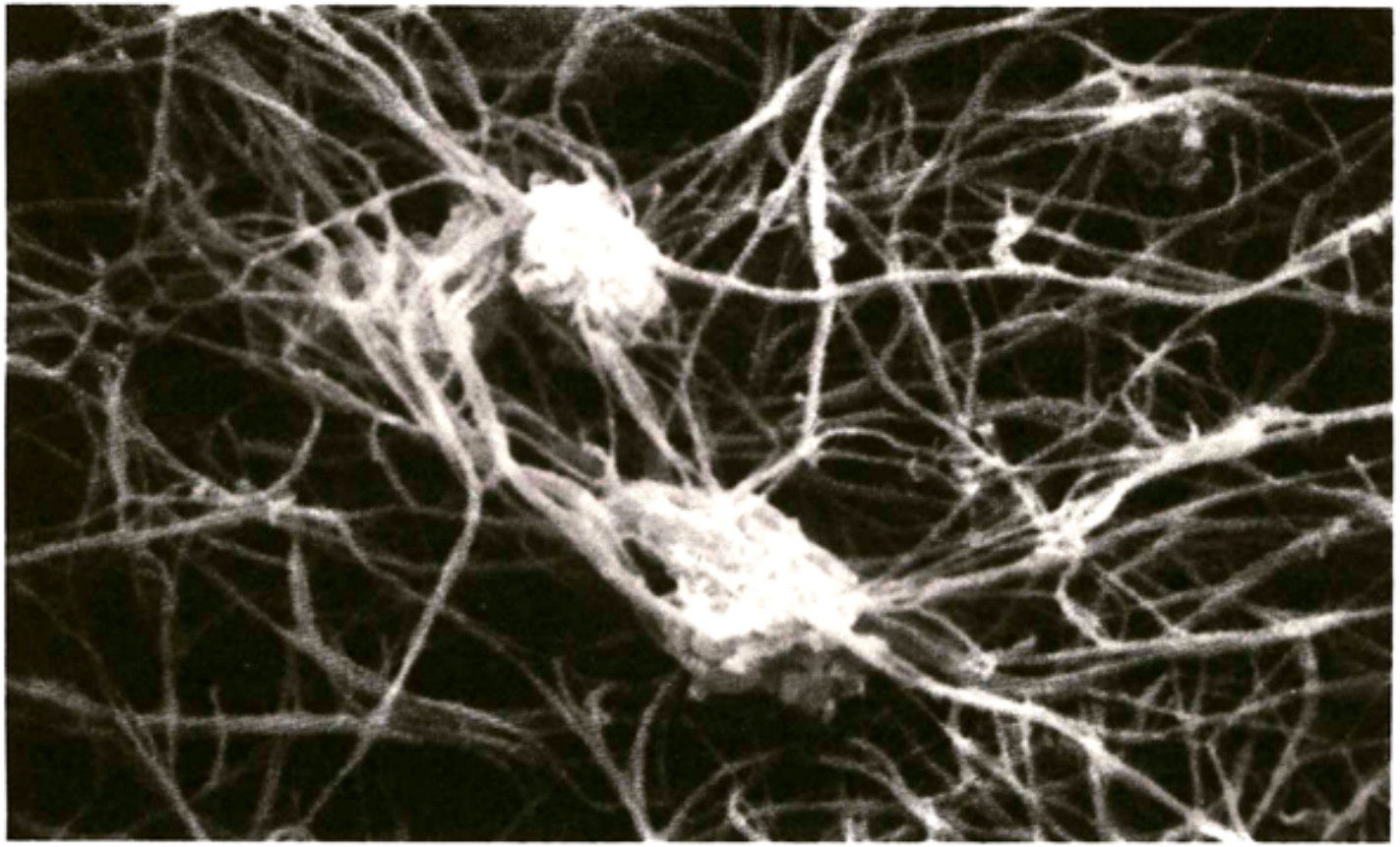
	Protein Product: what human protein is formulated as the drug	Indication: why drug is prescribed and what it does in the body	Other notes (Alternate forms of this drug, competing products on the market, etc)
1. Humulin Chart comparing Time Activity Profiles (go here)	**rInsulin** [FDA approval 1982]	**Diabetes: Used by over 3.5 million people in the U.S. every day** Rapid-acting 'mealtime' form	Worldwide revenues of $2.5 B annually
1a: Humulin pens 1b: Humalog: 1c: Humalog Mix	**rInsulin**	1a: Single use injectable 1b. Rapid acting insulin 1c.Rapid acting + long acting 'basal' insulin	Chart comparing Time Activity Profiles July 6, 2005 Lilly discontinues little-used insulins: Pork Insulin, and Humulin U and L
2. Humatrope	**rHuman growth hormone** (hGH) (Somatropin) [FDA approval 8/96]	**For Somatropin Deficiency Syndrome (SDS)** in adults and GHD in children due to hypopituitarism, a pituitary tumor or other pituitary disorder, or Turner Syndrome	**Annual revenues of ~$330 million.** July 25, 2003: FDA Approves Humatrope for Short Stature: (only the shortest 2-3% of children qualify).
3. Xigris	**rActivated Protein C.** [FDA approval 11/01] Activated Protein C acts inside the blood vessel as an **anticoagulant** / antithrombotic - reduces blood clots; controls inflammation. Activated by thrombin.	**Treatment of severe sepsis**, a fast-moving, dramatic, and often fatal acute response to infection that claimed 215,000 lives each year (pre-Xigris) Costs associated with treatment of sepsis are $17B annually in the US. Increases the odds of survival by over 38% (efficacy somewhat disputed)	FDA approved Xigris in a split vote by a 20-member advisory panel; its efficacy is somewhat disputed. Standard treatments for sepsis (antibiotics, blood pressure drugs) usually cost less than $50 per day, while Xigris costs **$6,800 per treatment**. Worldwide sales of ~$100M annually.
4. Forteo	**rParathyroid hormone**, N-terminal 34 amino acids (of 84) [FDA Approval Nov 26, 2002]	**Treatment of osteoporosis** in women and men - Anabolic Therapy: stimulates new bone formation, osteoblasts, bone mineral density (BMD) and bone strength.	About 10 million people in the US suffer from osteoporosis. **Black box warning:** Forteo may promote bone cancer (osteosarcoma) by stimulation of osteoblasts. Worldwide sales of ~$400M annually.

And one new one from Merck:

	Protein Product: what protein is formulated as the drug	Indication: why drug is prescribed and what it does in the body	Other notes (Alternate forms of this drug, competing products on the market, etc)
1. Gardasil:	**rQuadrivalent HPV (human papilloma virus) types 6, 11, 16, 18** [FDA Approval 6/8/06] L1 capsid protein of all 4 viruses made individually in yeast cells and combined into one vaccine.	**First Cancer Vaccine:** Approved for the immunization of children aged 9 to 15 years and of adult females aged 16 to 26 years for the prevention of cervical cancer, high-grade cervical dysplasia (CIN 2/3), and warts caused by HPV s types 6, 11, 16 and 18	Currently 3,700 U.S. and 233,000 worldwide cervical-cancer deaths. Virtually 100% effective in protecting against the HPV-16 and HPV-18 strains. **Downside:** The wholesale price for Gardasil will be $120 per dose; $360 for all three doses.

From Gene To Drug

The Molecular Genetics of Hemophilia (Potentially Lethal Disease)



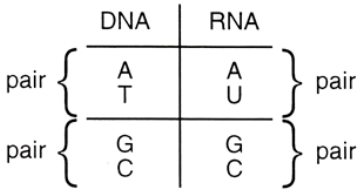
FIBRIN STRANDS stabilize a blood clot at the site of a wound by trapping the platelets that form the bulk of the clot. The electron micrograph, which was made by Jon C. Lewis of Wake Forest University, shows a clot formed in a suspension of platelets and fibrin.

A clot in the bloodstream is the result of a complex cascade of enzymatic reactions culminating in the conversion of fibrinogen, a soluble protein, into insoluble fibrin strands. In hemophiliacs a crucial protein in the blood-clotting cascade is either missing or defective.

A Case Study of Cloning Genes and mRNAs

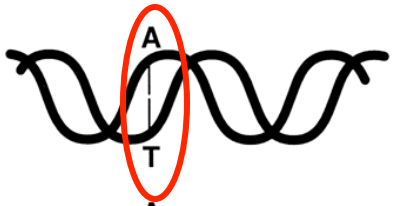
Reference: Lawn & Vehar, Sci. Amer., January, 1986

DNA Replication is Precise
But Mistakes or Mutations Can Occur – A Review!



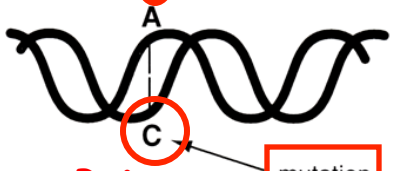
BASE PAIR RULES

Gene A



Rare Base Mismatch

Replication①



MUTATION DURING REPLICATION

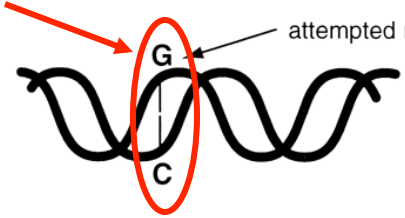
mutation

C mispairs with A

New Base Pair

See Mutation As Change in Phenotype

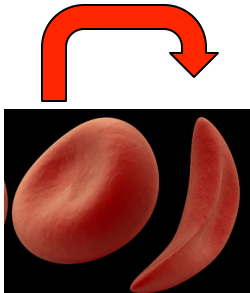
Replication②
Gene A'
Allelic Variant

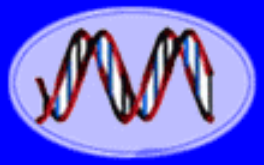


RESULTING DEFECT

Change DNA Sequence From A = T to G ≡ C

∴ Change Protein Amino Acid Sequence ⇨ Alter Function!





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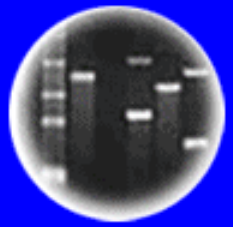
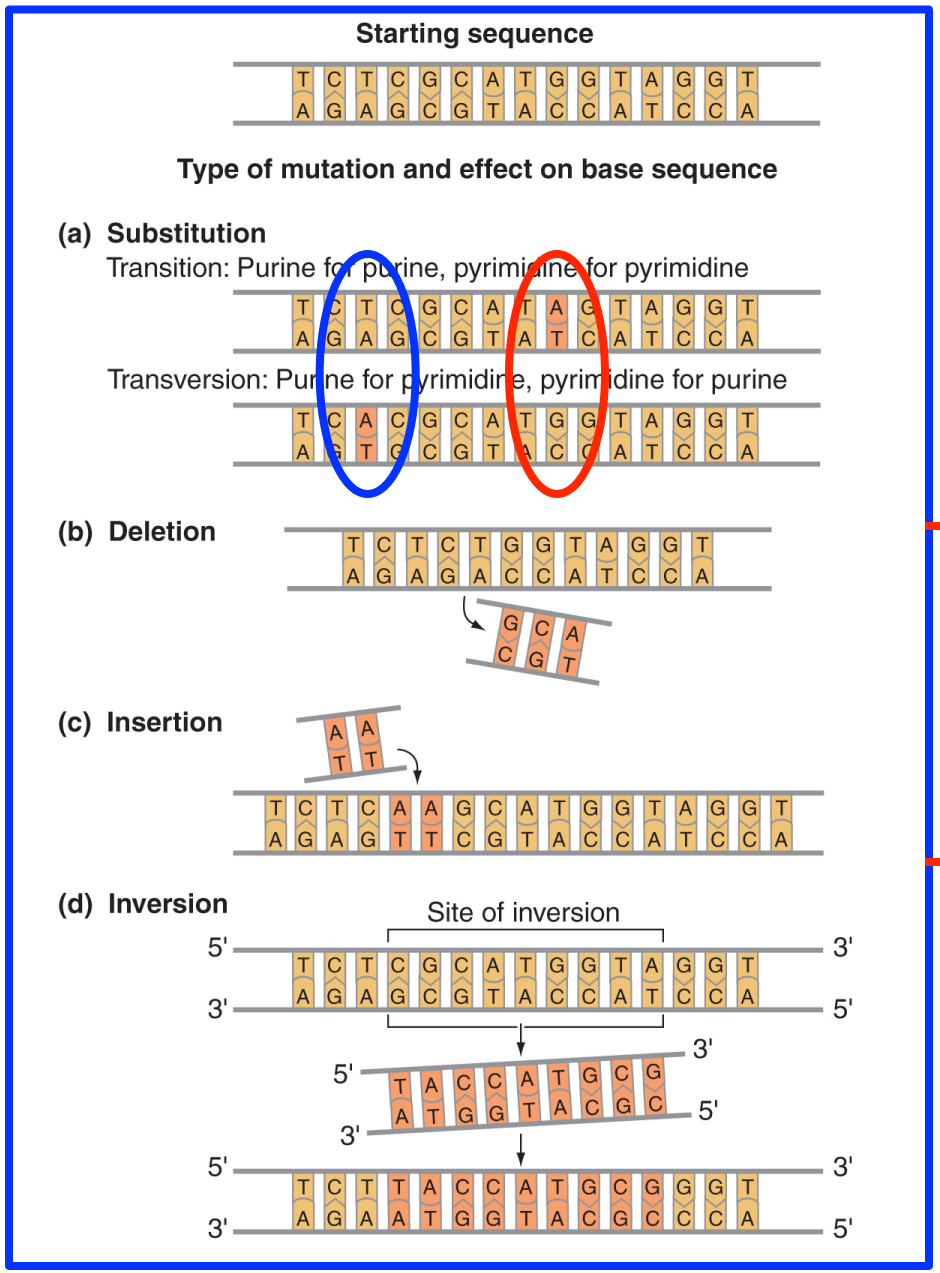


DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

Different Events Cause Gene Mutations

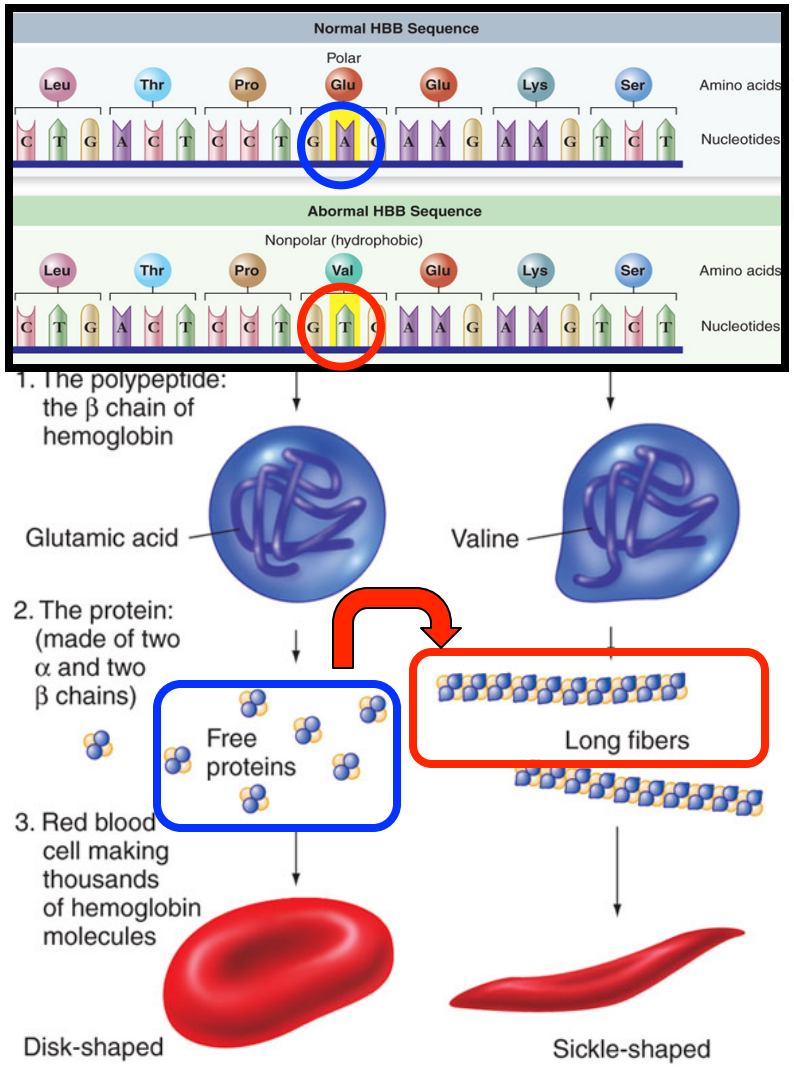


snps

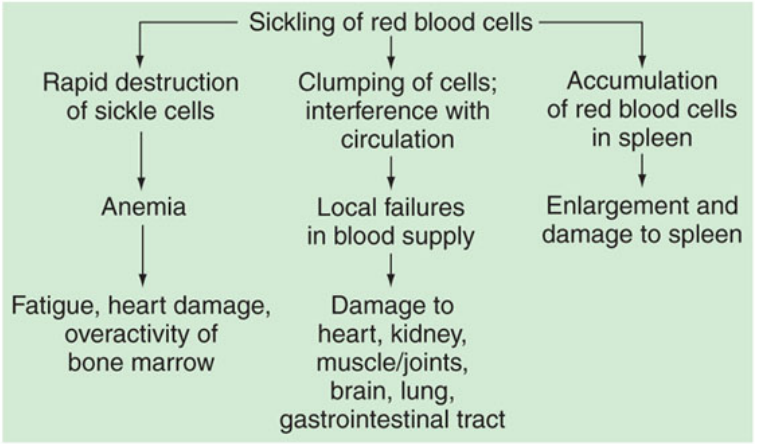
indels

Human Genetic Disorders Occur As A Result of Mutations: *Change Code-Alter Protein*

Chromosome 11



(b) Sickle-cell anemia is pleiotrophic



(c) β-chain substitutions/variants

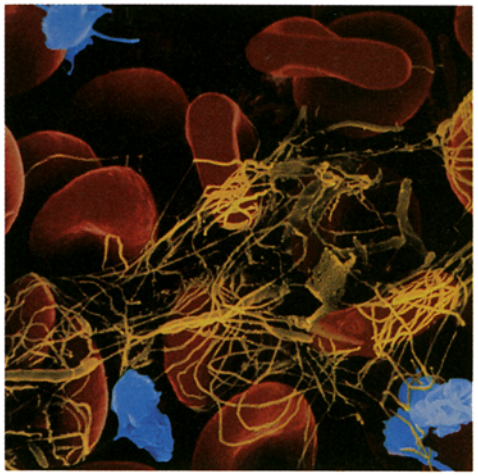
	Amino-acid position									
	1	2	3 ··· 6		7 ··· 26 ··· 63 ··· 67				125 ··· 146	
Normal (HbA)	Val	His	Leu	Glu	Glu	Glu	His	Val	Glu	His
HbS	Val	His	Leu	Val	Glu	Glu	His	Val	Glu	His
HbC	Val	His	Leu	Lys	Glu	Glu	His	Val	Glu	His
HbG San Jose	Val	His	Leu	Glu	Gly	Glu	His	Val	Glu	His
HbE	Val	His	Leu	Glu	Glu	Lys	His	Val	Glu	His
HbM Saskatoon	Val	His	Leu	Glu	Glu	Glu	Tyr	Val	Glu	His
Hb Zurich	Val	His	Leu	Glu	Glu	Glu	Arg	Val	Glu	His
HbM Milwaukee 1	Val	His	Leu	Glu	Glu	Glu	His	Glu	Glu	His
HbDβ Punjab	Val	His	Leu	Glu	Glu	Glu	His	Val	Gln	His

Note Change in Protein Structure Leading to Sickle-Cell Anemia Phenotype!

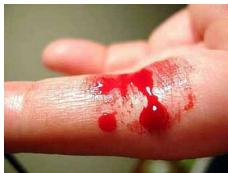
Hemophilia Has Been Known As An Inherited Disease For >2500 Years!

Old Testament-Circumcisions

Royal Family-Europe



a = activated form



First Reference to Hemophilia is in the Old Testament

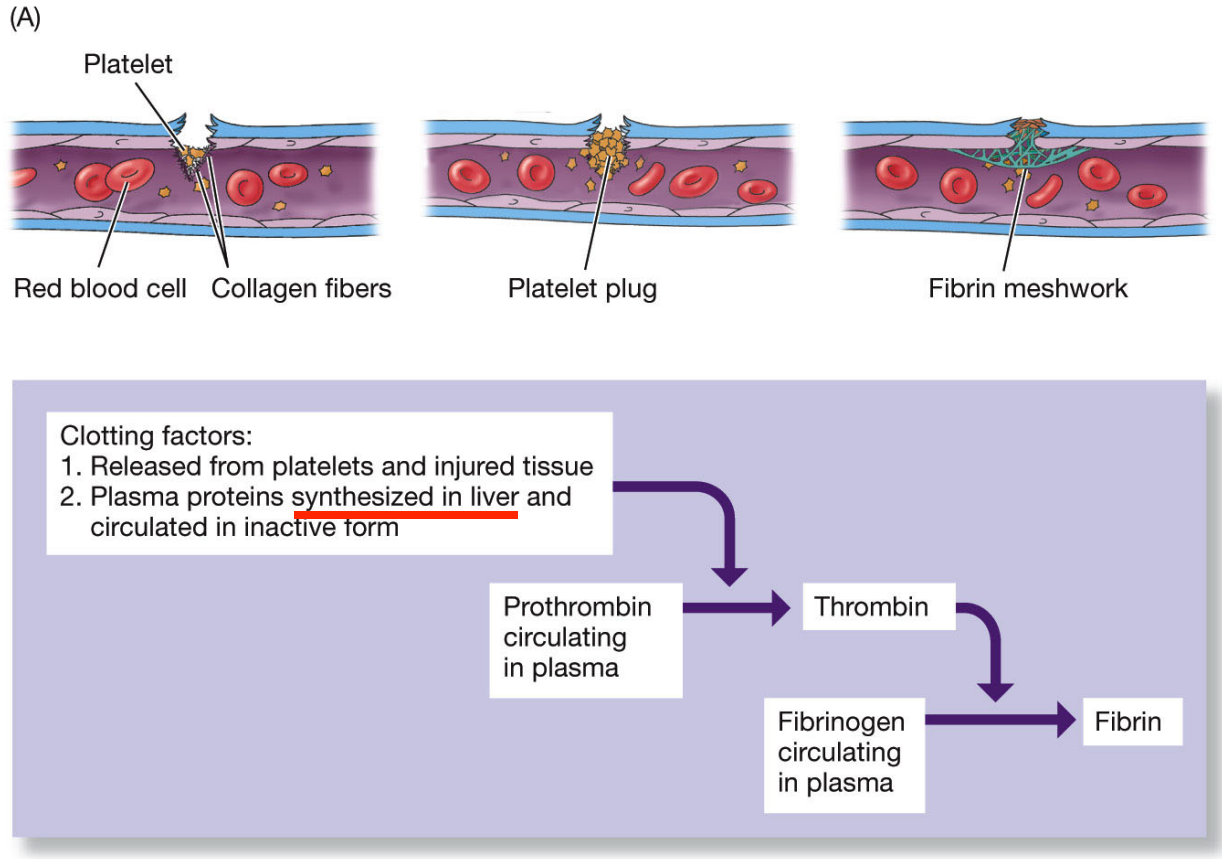
Genesis 17:10-14

'This is My covenant that you shall keep between Me and you and your descendants after you: every male among you shall be circumcised. You shall circumcise the flesh of the foreskin.....At the age of eight days every male among you shall be circumcised throughout your generations.....an uncircumcised male...that soul shall be cut off from its people, he has invalidated My covenant.'

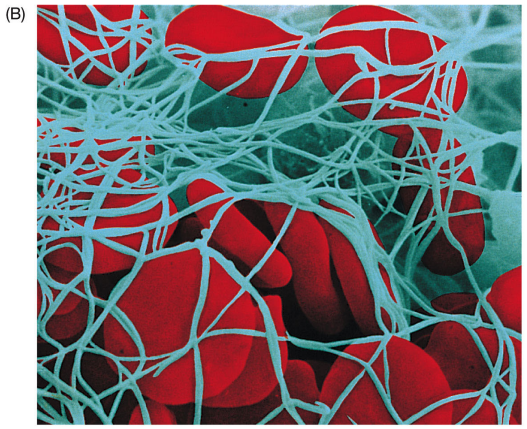
The Talmud also makes reference to families in whom children have died as a result of circumcision (Babylonian Talmud, Chapter *Yevamoth* p64b) [6]. Should a mother lose two children or should two sisters lose a child each after circumcision, subsequent children of the woman, the two sisters or of any other sisters of the same family should not be circumcised until they are older, or possibly not at all. This is thought to be the earliest reference to haemophilia; it was recognized in the Talmud that this condition was transmitted by the mother.

Abraham was circumcised at 93 and gave birth to Isaac at 99. His wife - Sarah - was 90!

A Cascade Of Events After Wounding Leads to A Fibrin Clot



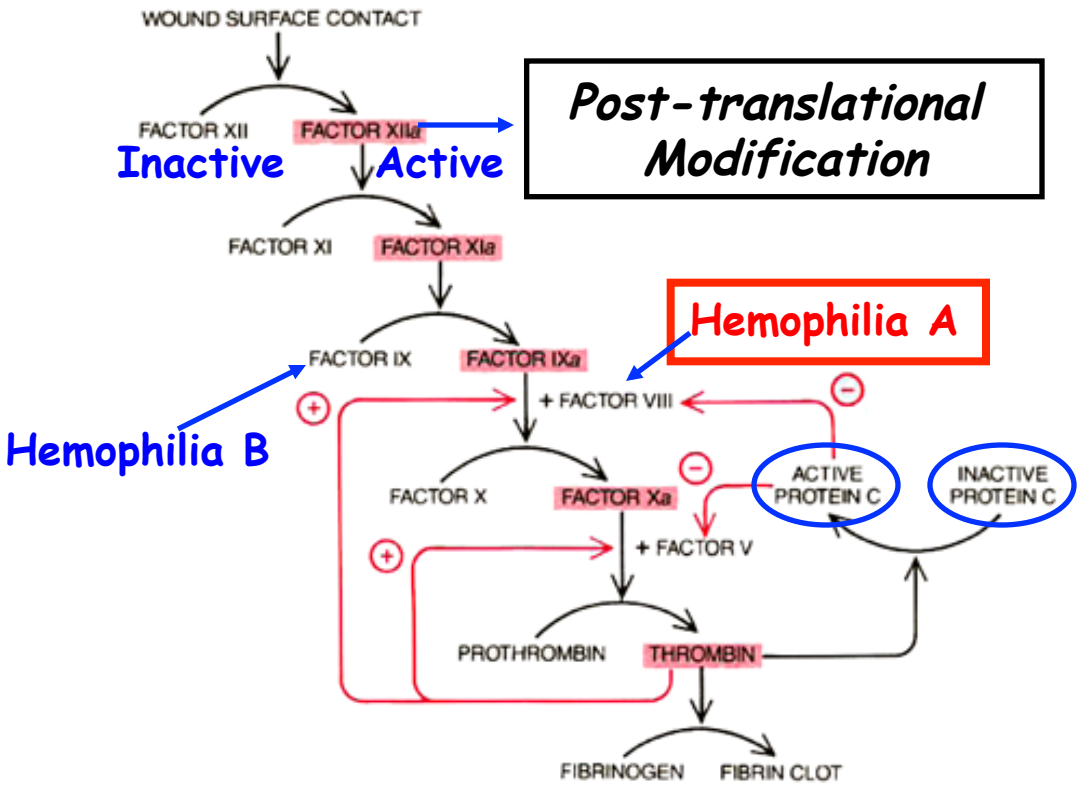
LIFE 8e, Figure 49.10 (Part 1)



LIFE 8e, Figure 49.10 (Part 2)

Clotting Factors Such As Factor VIII Play A Critical Role in This Process

How Does Blood Clot After Wounding?



CLOTTING CASCADE begins when cell damage at a wound somehow activates the enzyme factor XII; it ends with the conversion of fibrinogen into fibrin by thrombin. At each step an inactive protein is converted into a protease, or protein-cutting enzyme (*color*), which activates the next protein. Some steps require cofactors such as factors VIII and V. The cascade includes positive- and negative-feedback loops (*colored arrows*). Thrombin activates factors VIII and V; it also deactivates them (by activating protein *C*), which helps to halt clotting. Some 85 percent of hemophiliacs lack factor VIII. The rest lack factor IX.

Post-translational Modification

Hemophilia A

Hemophilia B

Eight Proteins/Genes Required:

1. Factor VII
2. Factor XI
3. Factor IX
4. Factor VIII
5. Factor X
6. Protein C
7. Prothrombin
8. Fibrinogen

What Happens If Any Of These Proteins Or Genes Are Mutated?

↓

No Blood Clot!

Aryn® 2009

Anti-Thrombin?? → **Anti-Thrombin Deficiency (At-III) genetic disease**

Cascade

Hemophiliacs Have Mutations in Factor VIII, Factor IX, or Factor XI Genes

TABLE 13.2 Some Important Genetic Disorders

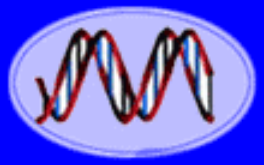
Disorder	Symptom	Defect	Dominant/ Recessive	Frequency Among Human Births
Cystic fibrosis	Mucus clogs lungs, liver, and pancreas	Failure of chloride ion transport mechanism	Recessive	1/2500 (Caucasians)
Sickle cell anemia	Blood circulation is poor	Abnormal hemoglobin molecules	Recessive	1/600 (African Americans)
Tay–Sachs disease	Central nervous system deteriorates in infancy	Defective enzyme (hexosaminidase A)	Recessive	1/3500 (Ashkenazi Jews)
Phenylketonuria	Brain fails to develop in infancy	Defective enzyme (phenylalanine hydroxylase)	Recessive	1/12,000
Hemophilia	Blood fails to clot	Defective blood-clotting factor VIII	X-linked recessive	1/10,000 (Caucasian males)
Huntington disease	Brain tissue gradually deteriorates in middle age	Production of an inhibitor of brain cell metabolism	Dominant	1/24,000
Muscular dystrophy (Duchenne)	Muscles waste away	Degradation of myelin coating of nerves stimulating muscles	X-linked recessive	1/3700 (males)
Hypercholesterolemia	Excessive cholesterol levels in blood lead to heart disease	Abnormal form of cholesterol cell surface receptor	Dominant	1/500

18,000 People in US Have Hemophilia & 400 Babies/Year Are Born With Disorder
Prior to 1960s – Average Life Span Was 11 Years

Hemophilia A	Defective Factor VIII Gene	1/10,000 males	80%
Hemophilia B	Defective Factor IX Gene	1/30,000 males	20%
Hemophilia C	Defective Factor XI Gene	Autosomal	<1%

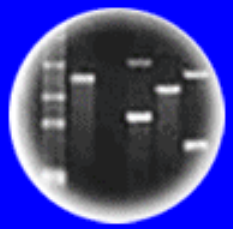
Hypothesis For High Frequency in Males?

Both Factor VIII & IX Genes
on X-Chromosome (♀→♂'s)



DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria

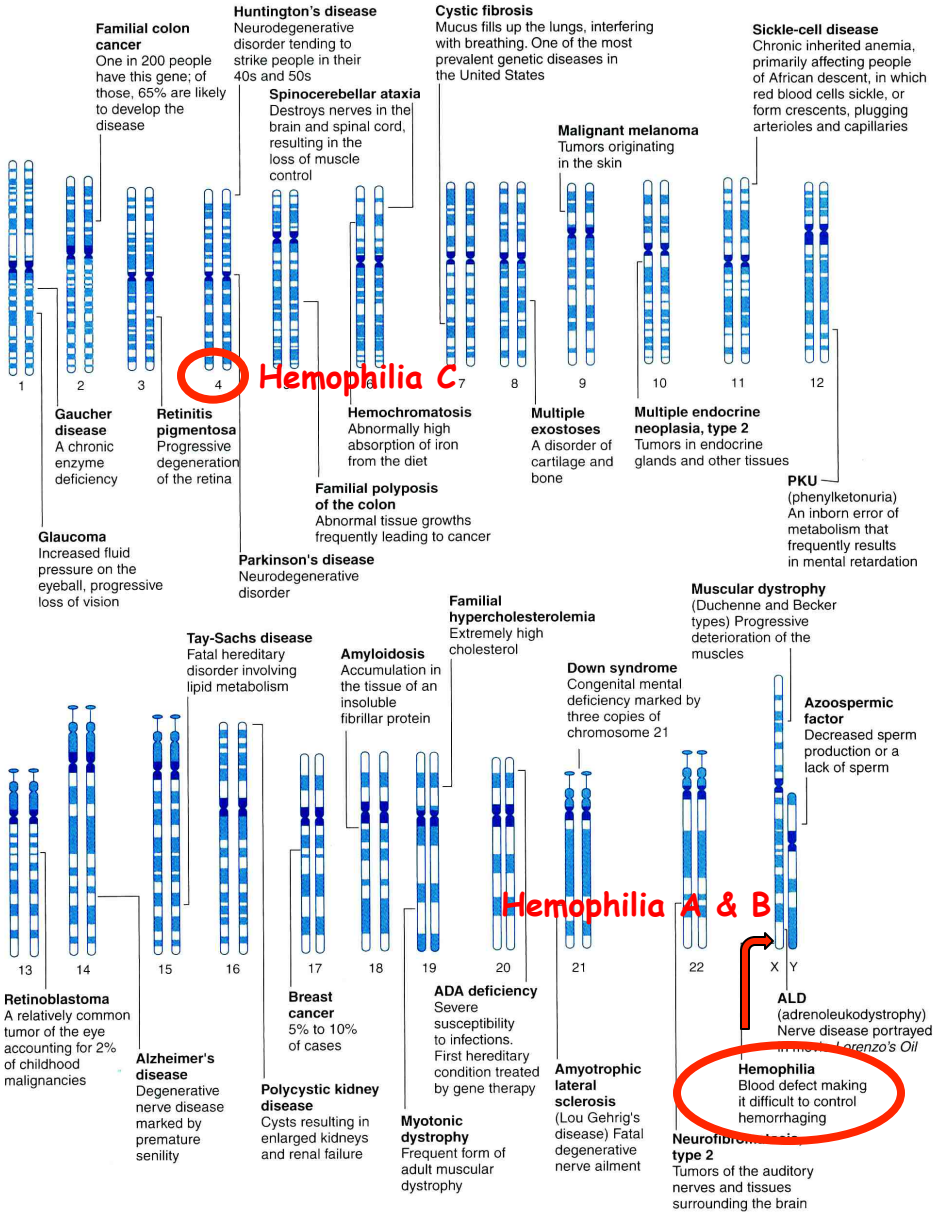


DNA Fingerprinting

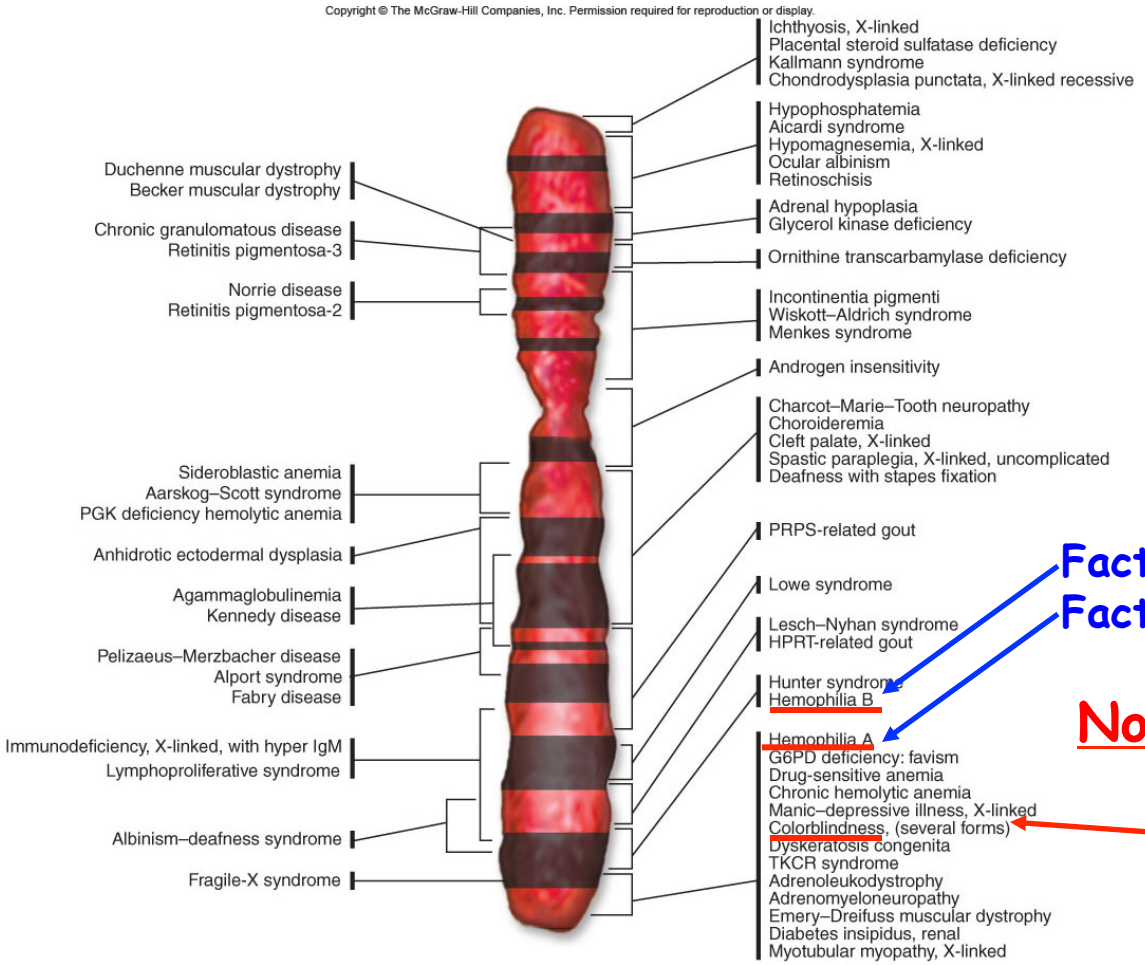
Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

Human Disease Genes Have Been Mapped To Specific Chromosomal Locations

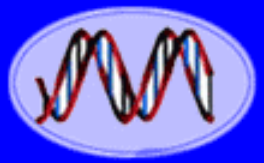


Factor VIII and Factor IX Genes are Closely Linked on the X Chromosome



Note: Factor VIII *gene* is closely *linked* to Colorblindness Gene

The X chromosome has ~1500 Genes (2008) and 150,000,000 bp (150 Mb)



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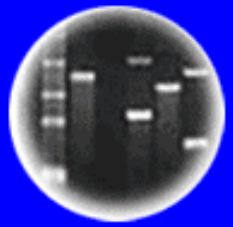


DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

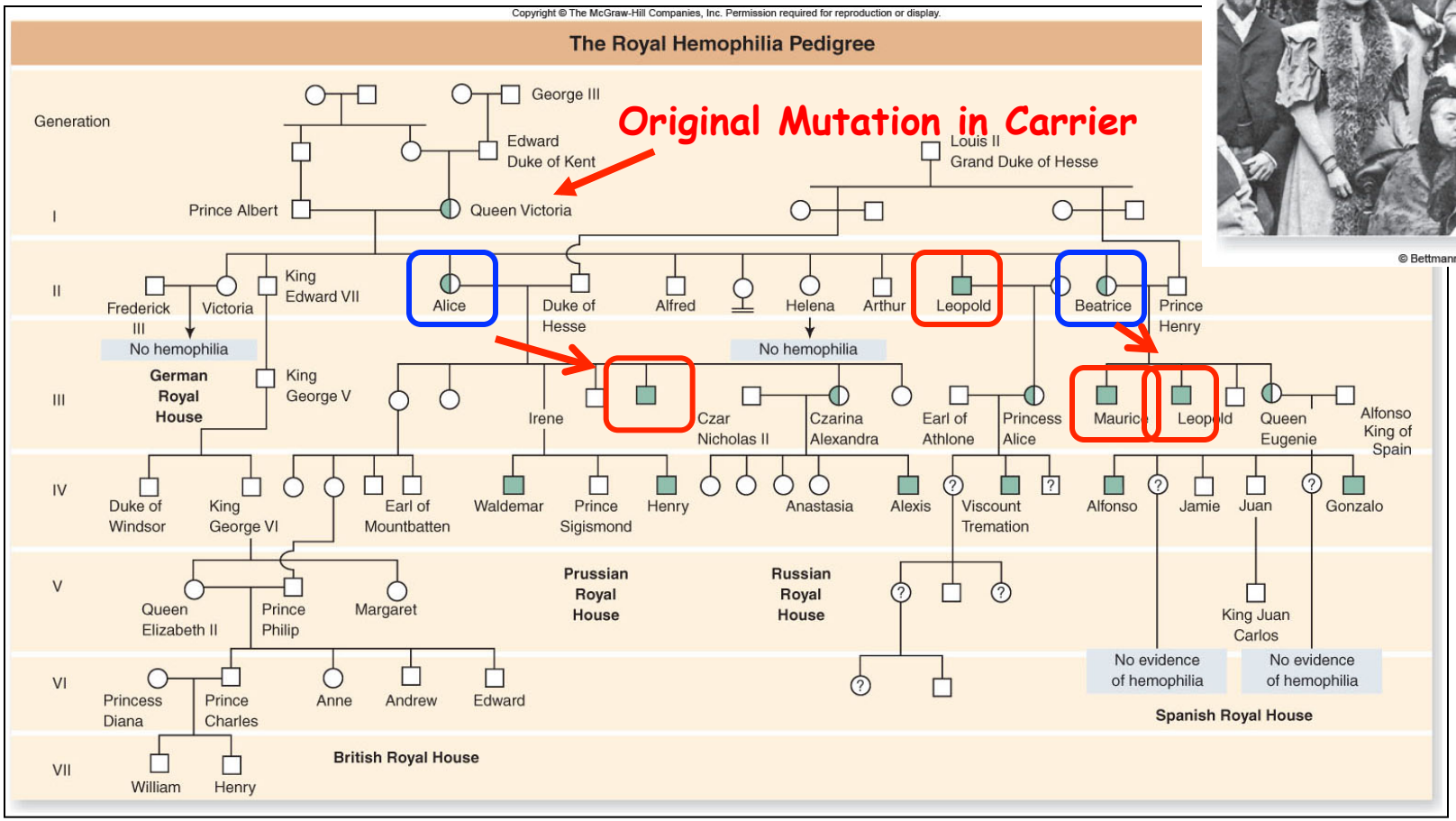
Plants of Tomorrow

Pedigrees Can Be Used To Determine If a Trait is Dominant or Recessive

Each Type of Inheritance Predicts Specific Results in Each Generation

Hemophilia A and B Genes (Traits) Are Sex Linked

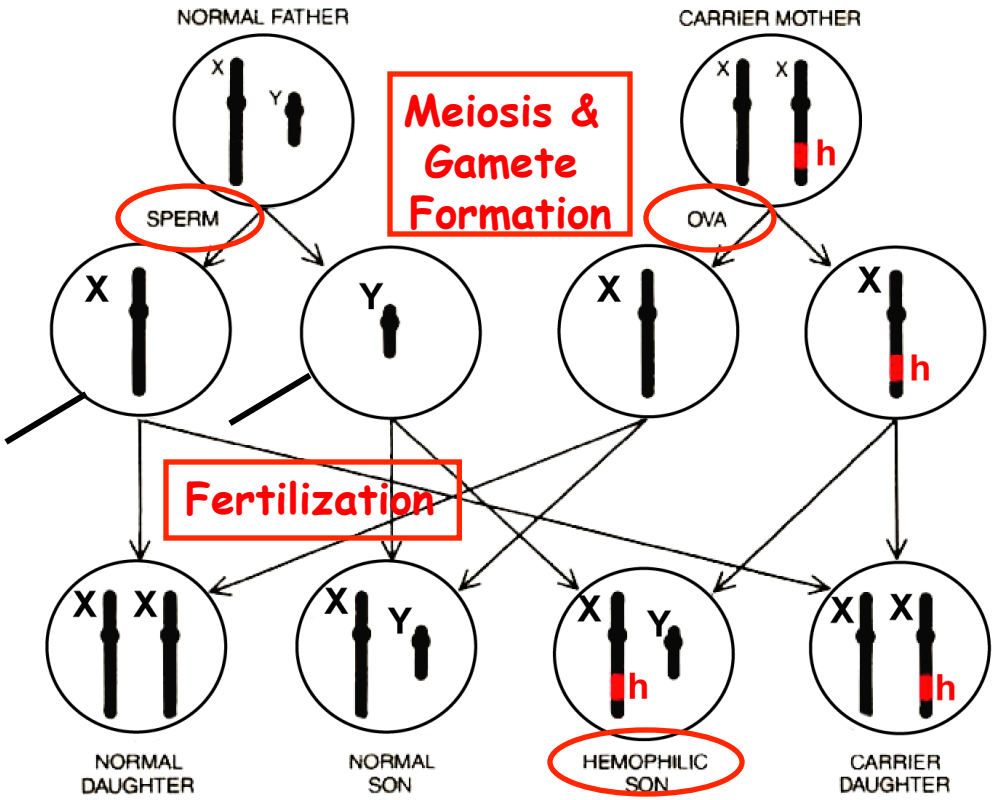
© Bettmann/Corbis



Note: 1. Males Obtain Detective Gene From Mothers

2. 50% of Sons Of A Maternal Carrier Have The Defective Gene

Hemophilia A and B Inheritance



SEX-LINKED INHERITANCE of hemophilia results from the location of the factor VIII gene on the X chromosome. A male carrying a mutant factor VIII gene lacks normal factor VIII and is hemophilic. A female carrier is protected by the normal gene on her second X chromosome, but half of her daughters will be carriers and half of her sons will be hemophilic. In the case of a hemophilic father (not shown), his sons will not be hemophilic, because they receive his Y (not his X) chromosome, but his daughters will be carriers.

Parents 2n

Meiosis

Gametes n

Fertilization

Children 2n

Sex-Linked Inheritance

♀ Carriers → 1/2 Sons + No Daughters!

Only One X-Chromosome is ♂

Hemophilia A and B Sex-Linked Inheritance

Carrier Female

Healthy Male

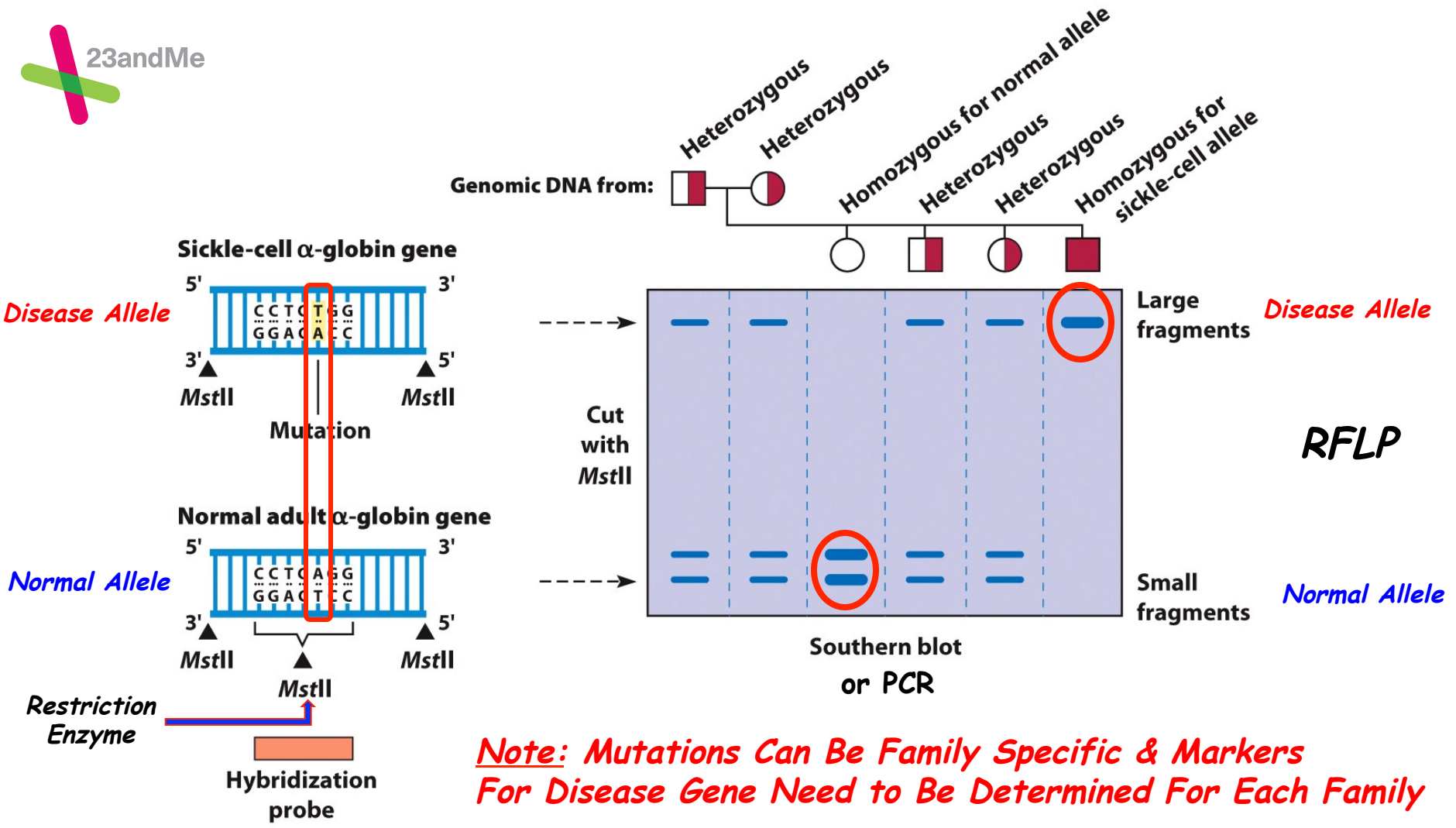
Egg / Sperm	X	X
X	XX ♀ *Carrier*	XX ♀ *Healthy*
Y	XY ♂ Hemophiliac	XY ♂ *Healthy*

Sex-Linked Inheritance

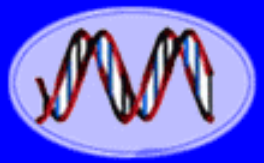
♀ Carriers → 1/2 Sons Afflicted + No Daughters!

Only One X-Chromosome is in ♂

DNA Testing Can Be Used To Detect The Presence of Disease Gene Alleles: *This is Now Done Using PCR*



<u>Note:</u> Mutations Can Be Family Specific & Markers For Disease Gene Need to Be Determined For Each Family



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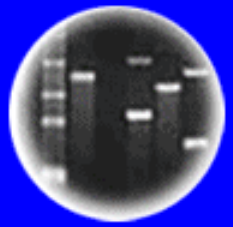


DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

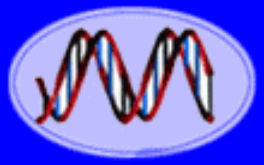
Should You Be Able To Patent a Diagnostic DNA Test For a Human Disease Gene?

a. Yes
b. No

DNA Tests Can Now Be Used To Detects Hundreds of Genetic Disease Alleles

TABLE 11.1 GENETIC DISEASE TESTING

Genetic Disease Condition	Genetic Basis for Disease and Symptoms
Cancers (brain tumors; urinary bladder, prostate, ovarian, breast, brain, lung, and colorectal cancers)	A variety of different mutant genes can serve as markers for genetic testing.
Cystic fibrosis	Large number of mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene on chromosome 7. Causes lung infections and problems with pancreatic, digestive, and pulmonary functions.
Duchenne muscular dystrophy	Defective gene (dystrophin) on the X chromosome causes muscle weakness and muscle degeneration.
Familial hypercholesterolemia	Mutant gene on chromosome 19 causes extremely high levels of blood cholesterol.
Hemophilia	Defective gene on the X chromosome makes it difficult for blood to clot when there is bleeding.
Huntington disease	Mutation in gene on chromosome 4 causes neurodegenerative disease in adults.
Phenylketonuria (PKU)	Mutation in gene required for converting the amino acid phenylalanine into the amino acid tyrosine. Causes severe neurological damage, including mental retardation.
Severe combined immunodeficiency (SCID)	Immune system disorder caused by mutation of the adenosine deaminase gene.
Sickle cell disease	Mutation in ß-globin gene on chromosome 11 affects hemoglobin structure and shape of red blood cells, which disrupts oxygen transport in blood and causes joint pain.
Tay-Sachs disease	Rare mutation of a gene on chromosome 5 causes certain types of lipids to accumulate in the brain. Causes paralysis, blindness, retardation, and respiratory infections.



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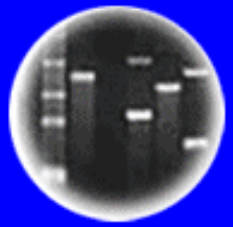


DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

Genetic Screening Issues

- Why Screen For Genes?
- When is a Test Accurate Enough?
- Mandatory or Voluntary Screening?
- Who Should Be Tested?
- Employer & Insurance Company Testing?
- Protection From Genotype Discrimination?
- Testing for Genetic Diseases With No Cures?
- How Ensure Privacy & Confidentiality?
- Obligations to Inform Others (Spouse/Sibling) of Genetic Disorder Knowledge?
- Genetic Databases??
- Patents on Tests?

What Was Known About Factor VIII *Before Gene Cloned?*

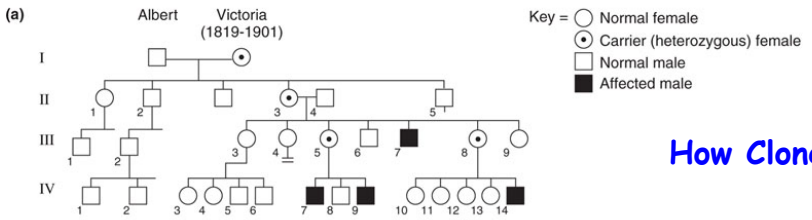
1. Blood Protein (But Perhaps Synthesized Elsewhere!)
2. Could be purified in small amounts from >20 Liters of human blood + cow blood + pig blood
3. Short Stretch of Protein Sequenced = Known Protein Sequence!
4. Hemophilia A could be treated by *blood transfusions* from normal individuals, ∴ clotting factor in blood.

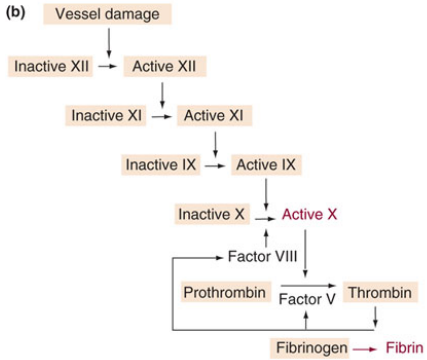
∴ How to go From Protein to Gene

The Problem

For Factor VIII- *Not Known Where Gene is Expressed* ∴ Must Use Genome Library

Early 1980's





(c) Analysis of presence or absence of blood-clotting factors

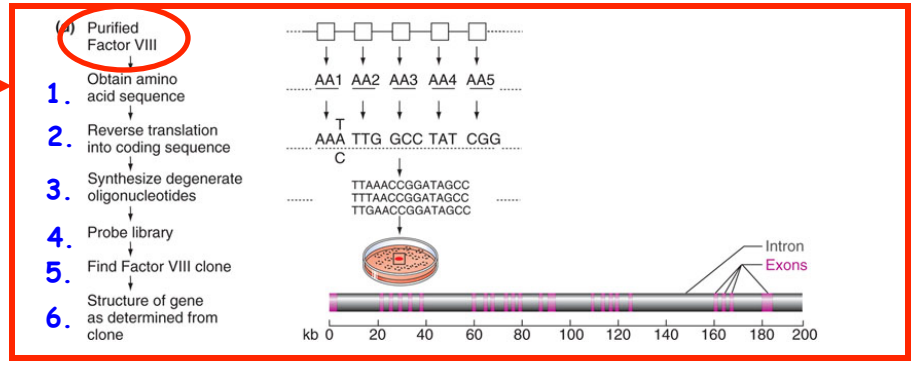
Factors	Wild-type person	Hemophiliac
XII	+	+
XI	+	+
IX	+	+
VIII	+	-
X	+	+
V	+	+
Prothrombin	+	+
Fibrinogen	+	+

Key Concept

⬇

How Clone A Gene When You Don't Know Where it is Expressed !

Key:
Protein Sequence Known



How Find Gene & cDNA?

Protein → Gene → mRNA → Drug !

Knowledge of the Protein Sequence and the Genetic Code Makes it Possible to Identify a Gene

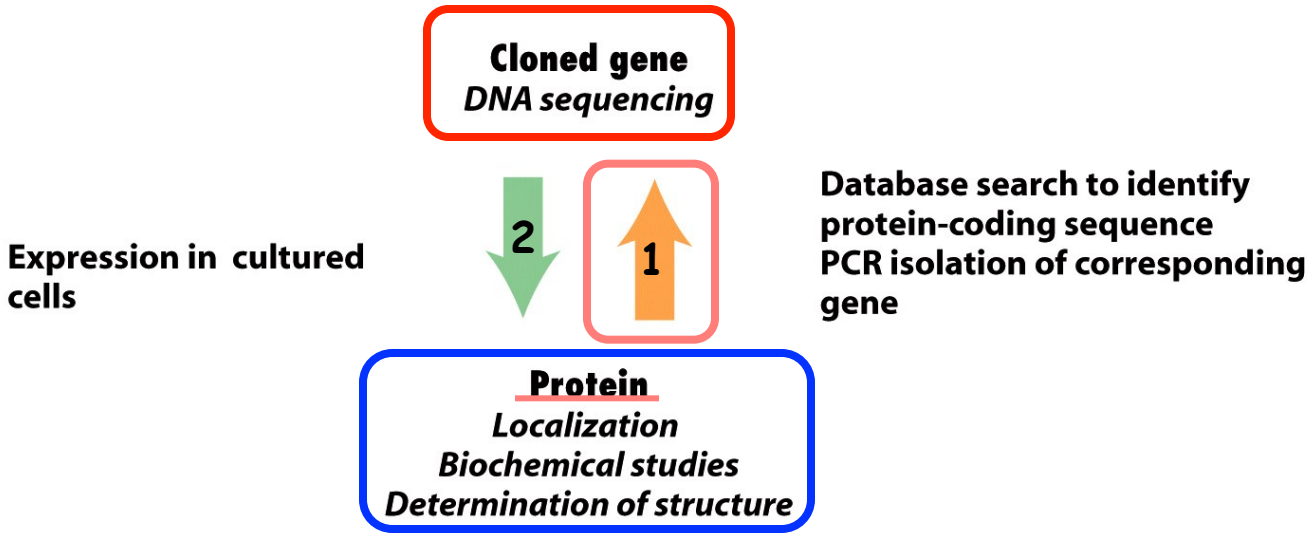


Figure 5-1
Molecular Cell Biology, Sixth Edition

∴ 1. Protein → Gene → Drug

Factor VIII Strategy (1985)

or

Genomics

2. Gene → Protein Using Sequencing and Genetic Code

GenBank

2013

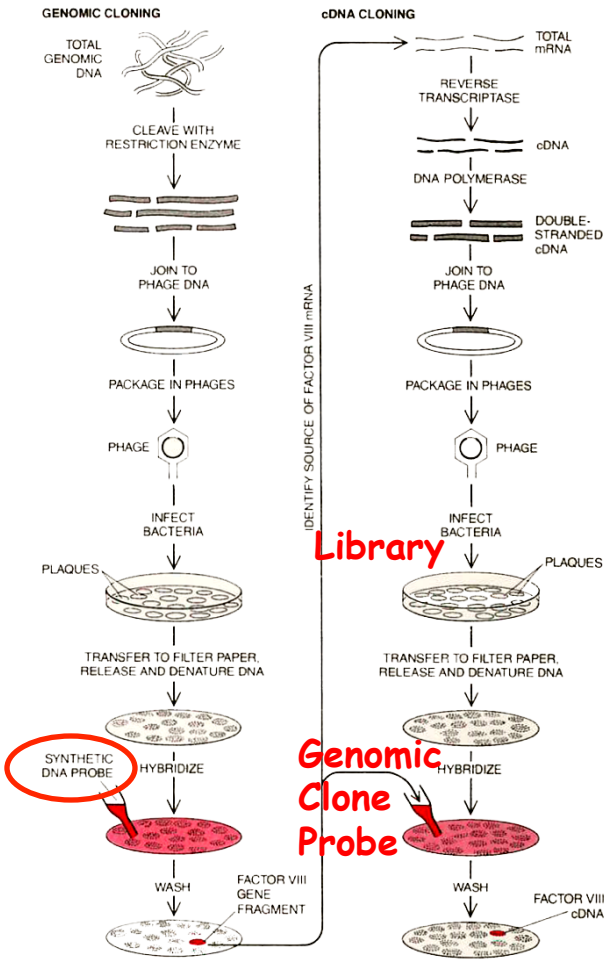
3. Just Sequence Everything + Identify Protein-GenBank Huge

Steps Required to Clone Factor VIII Gene and cDNA

Gene

1. Make Genome Library Because Factor VIII Gene in Genome!
2. Purify Protein from Blood- that's where it works (wasn't known where made)
3. Reverse Translate using the genetic code a portion of the protein sequence
4. Synthesize a DNA probe complementary to Factor VIII gene corresponding to protein sequence
5. Screen Genome Library

Entire Gene on The Clone?



cDNA

1. Use Gene probe to screen cDNA library for Factor VIII cDNA clone
2. How know what mRNA to use to make cDNA library?
3. Use gene probe to probe RNA blots containing mRNA from all major organs (liver, kidney, blood, etc.)
4. Find Factor VIII mRNA in liver- male, liver- secrete into blood

Why Need cDNA?

Story continued

Want cDNA to Manufacture Factor VIII as a Drug to Treat Hemophilia A!

Step One

How to Construct a Human Genome Library to Find the Factor VIII Gene?

If It is Not Known Where Gene is Active Can "Look" to Genome Instead of mRNA to Find + Clone Gene!

Vectors Used in Genetic Engineering Have Similar Conceptual Properties But are Used in Different Situations

Table 3.2 A COMPARISON OF DNA VECTORS AND THEIR APPLICATIONS

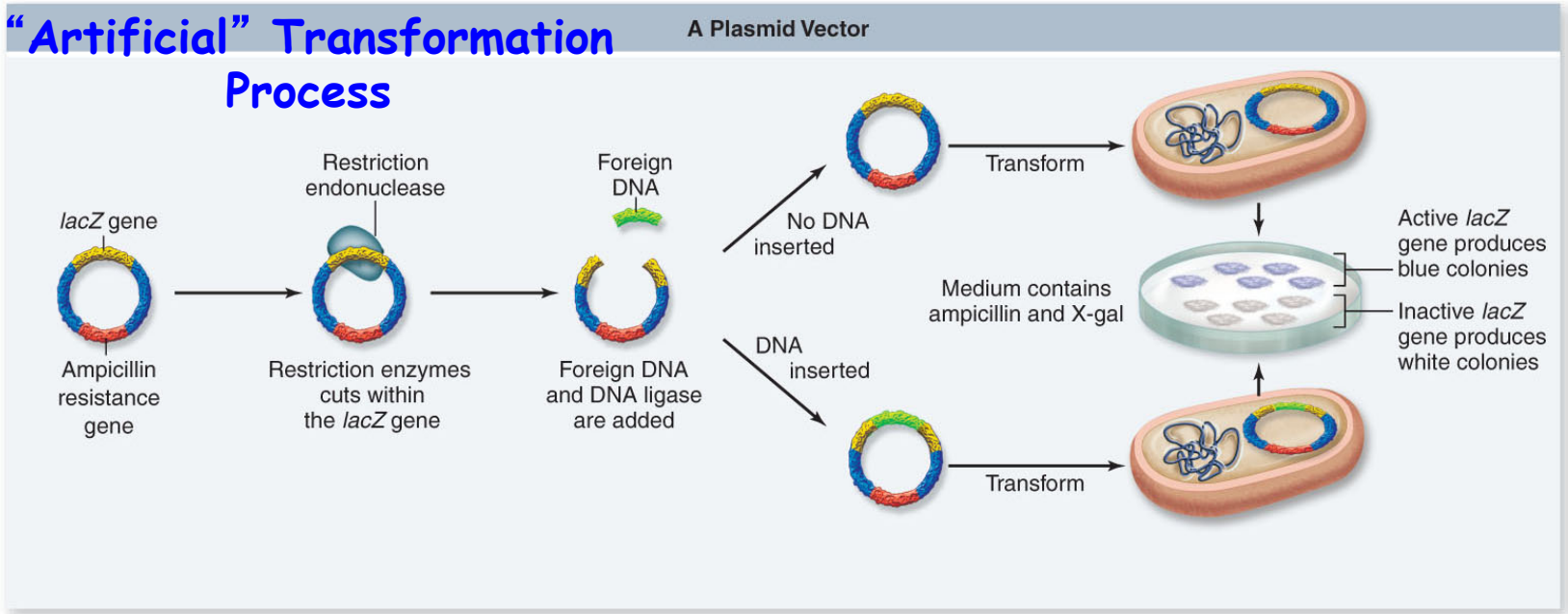
Vector Type	Maximum Insert Size (kb)	Applications	Limitations
Bacterial plasmid vectors (circular)	~6–12	DNA cloning, protein expression, subcloning, direct sequencing of insert	Restricted insert size; limited expression of proteins; copy number problems; replication restricted to bacteria
	DNA	DNA	
Bacteriophage vectors (linear)	~25	cDNA, genomic and expression libraries	Packaging limits DNA insert size; host replication problems
Cosmid (circular)	~35	cDNA and genomic libraries, cloning large DNA fragments	Phage packaging restrictions; not ideal for protein expression; cannot be replicated in mammalian cells
Bacterial artificial chromosome (BAC, circular)	~300	Genomic libraries, cloning large DNA fragments	Replication restricted to bacteria; cannot be used for protein expression
Yeast artificial chromosome (YAC, circular)	200–2,000	Genomic libraries, cloning large DNA fragments	Must be grown in yeast; cannot be used in bacteria
Ti vector (circular)	Varies depending on type of Ti vector used	Gene transfer in plants	Limited to use in plant cells only; number of restriction sites randomly distributed; large size of vector not easily manipulated

Plasmids vs. Bacteriophage Vectors

1. Replicate
2. Selectable
3. Can be used to insert foreign genes/restriction sites
4. Easily isolated + transferred back to cells

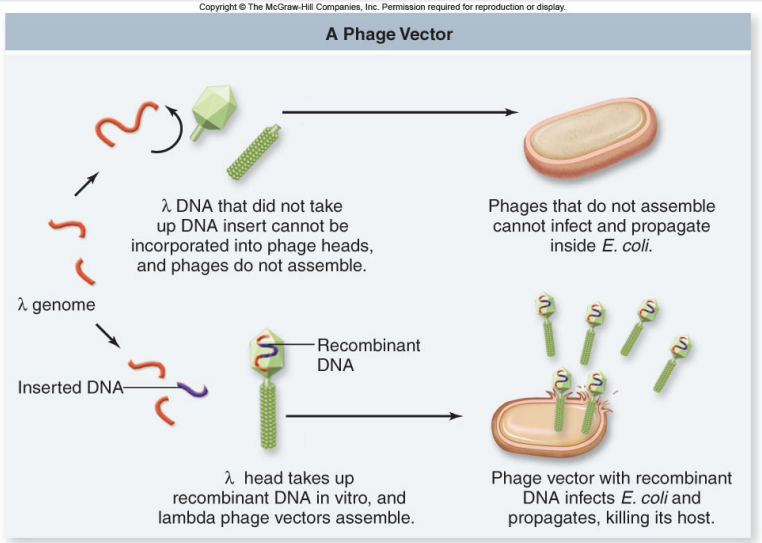
Plasmid vs. Bacteriophage Vectors for Cloning DNA Fragments

"Artificial" Transformation Process



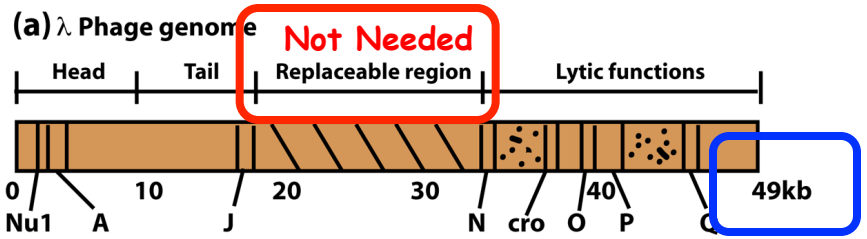
a.

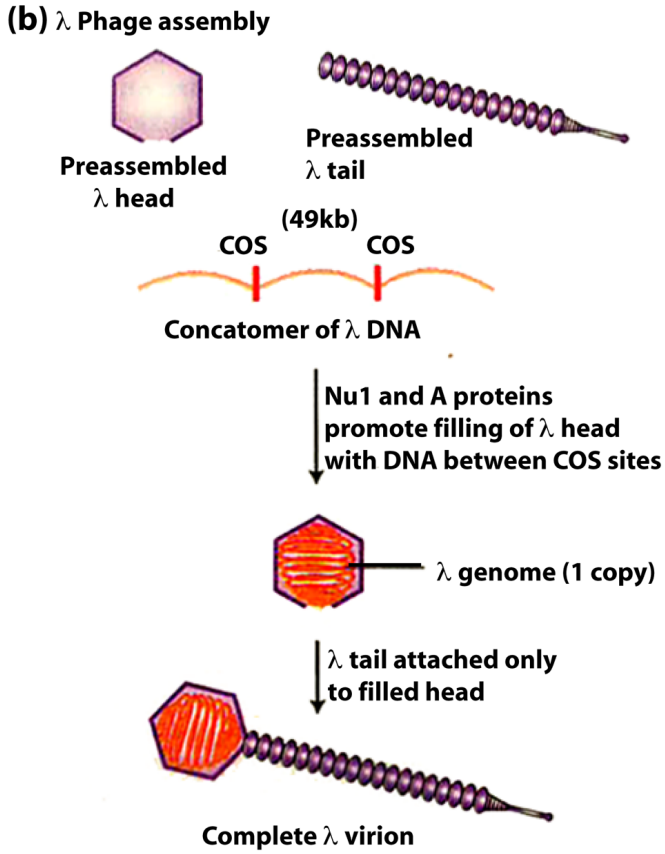
Natural Infection Process

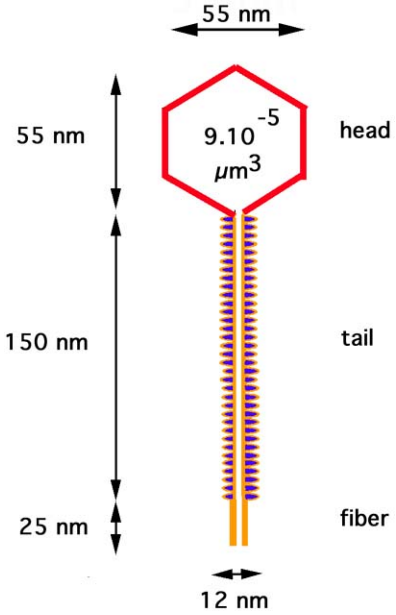


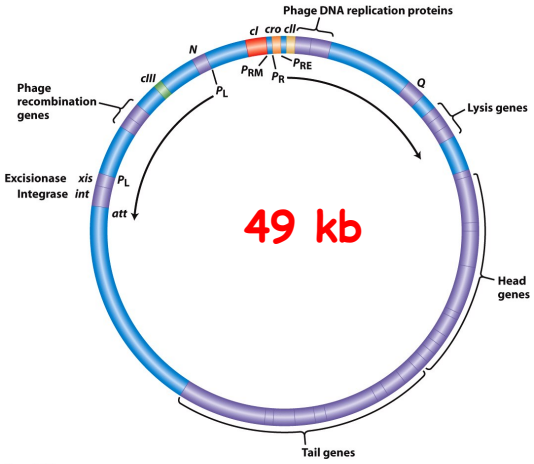
b.

Structure of the λ Phage and Its Genome





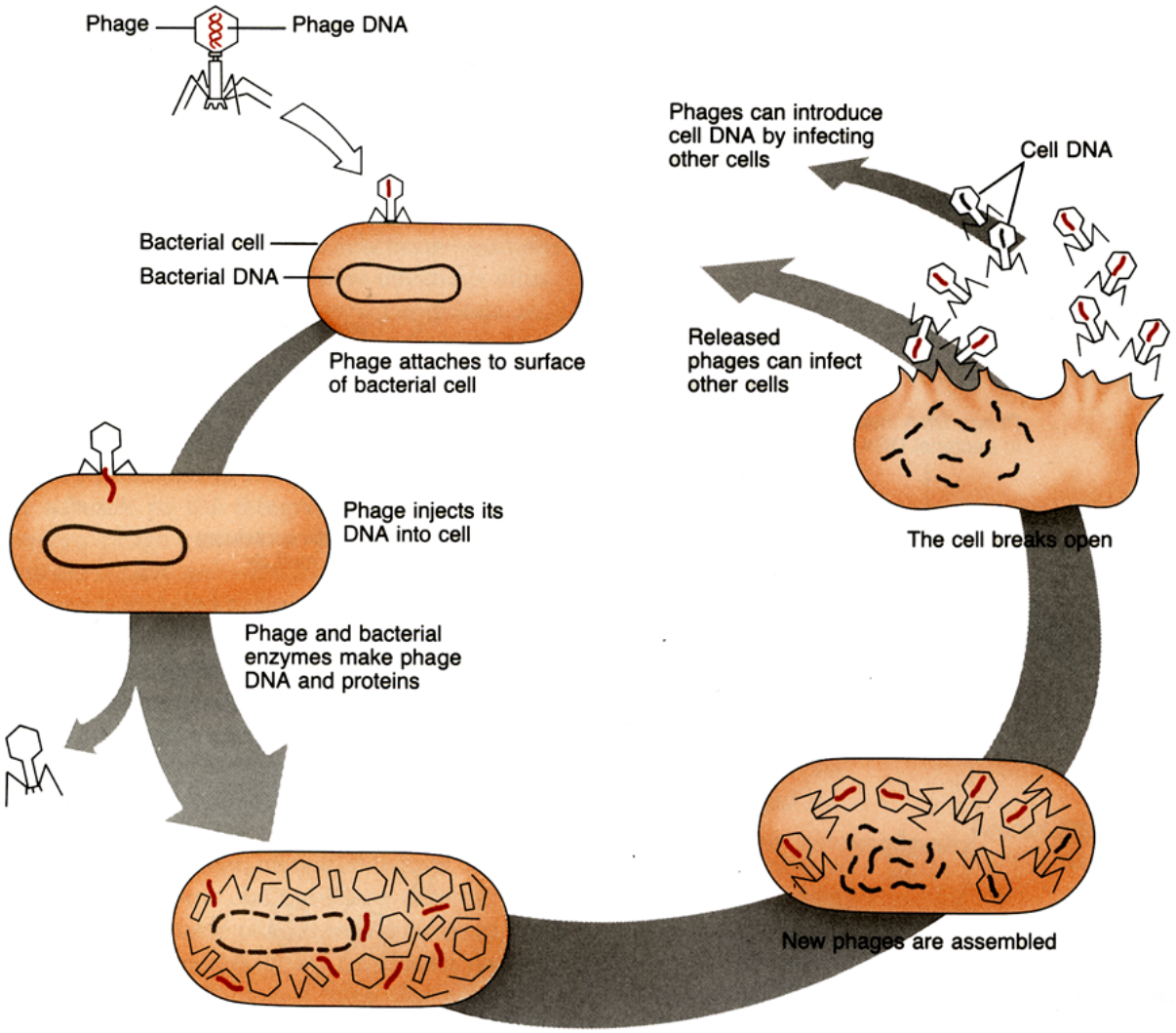




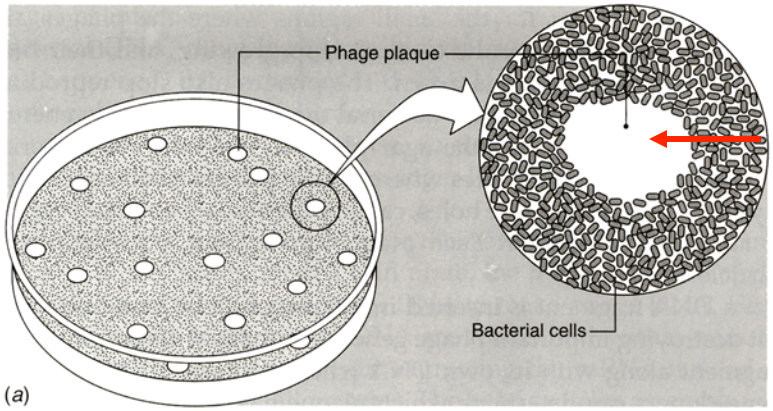
One of First Genome Sequences

How can the Genome "fit" into the Head?

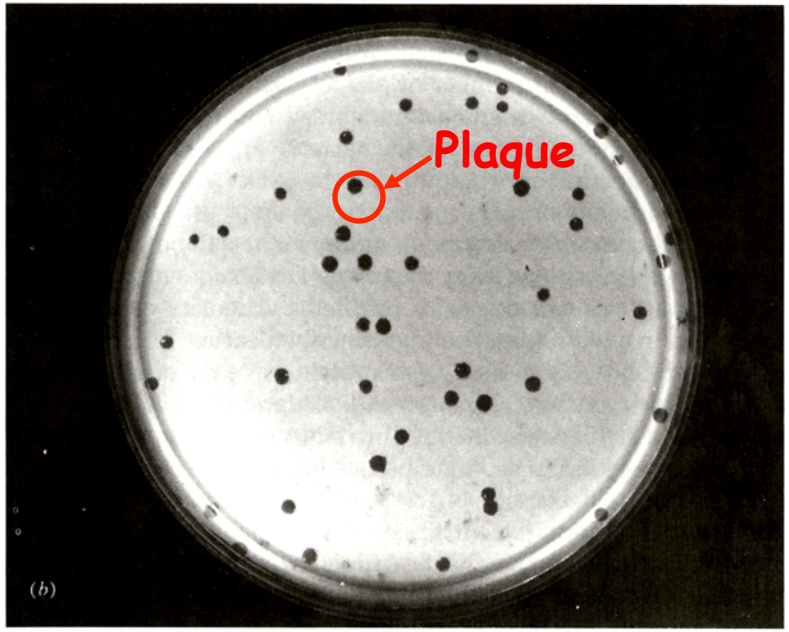
λ Phage Infects E.coli & Destroys (Lyses) cells



Lysed Cells Can Be Seen as Clear Plaques on Agar Plates



Plaque



1. Each Plaque is a Virus Clone Representing One Viral Infection!

2. Selectable Marker is Bacterial Cell Destruction & Plaque Formation

Advantages of λ Virus as a Vector for Cloning DNA

1. Long DNA Segments can be Cloned (~20kb) Need fewer clones for whole Genome!

2. Can clone DNA Segments in Viral Genome & Self-Assemble with viral proteins into virus in a test tube!

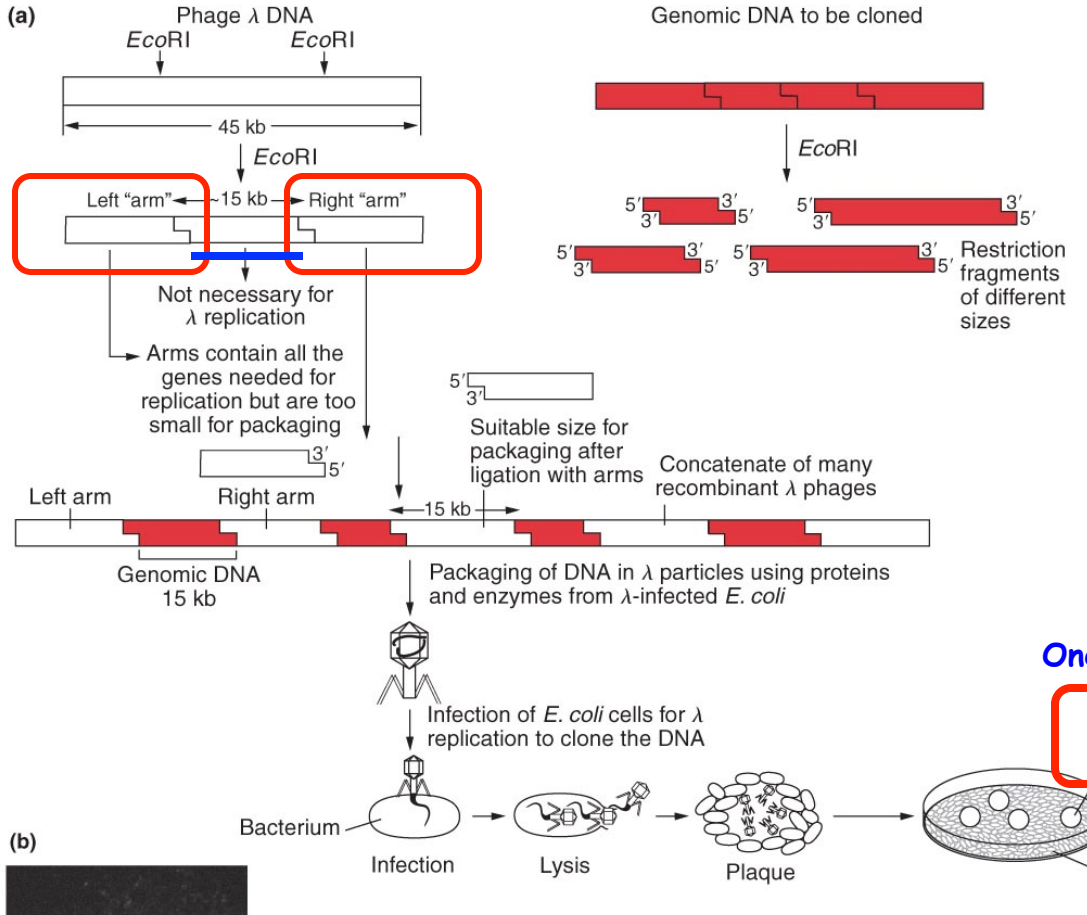
   ∴ Make Recombinant Viruses in the Lab!

3. Use "Natural" Infection process to Generate Large Number of Clones for a Eukaryotic Genome Library.

   Much higher efficiency for getting recombinant DNA →bacterial cells compared with DNA transformation.

   ∴ set more clones per amount of recombinant DNA!

Using a Bacterial Virus To Clone the Human Genome

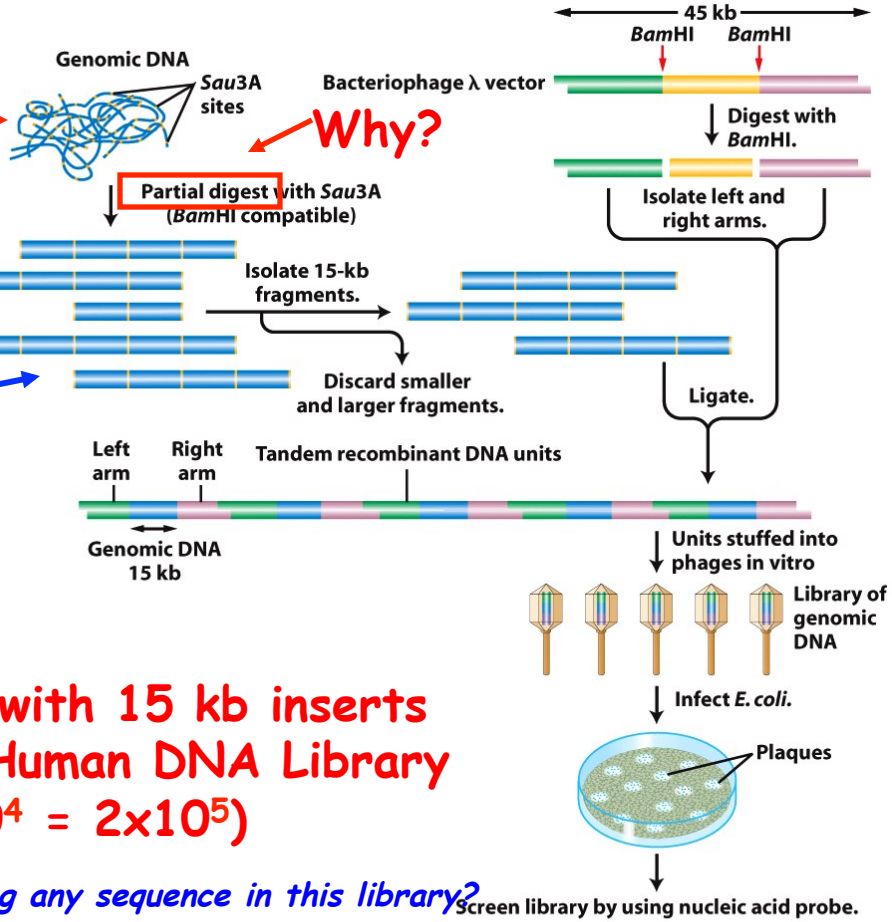


One Clone per Plaque

Mixture of Plaques = Library With All Human DNA Sequences Represented

Cloning the Human Genome and Screening for the Factor VIII Gene

Human DNA



Note Overlapping Fragments

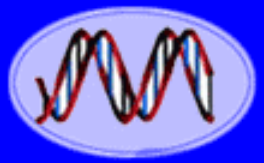
Need 200,00 Phage with 15 kb inserts for Complete Human DNA Library ($3x10^9/1.5x10^4 = 2x10^5$)

What's the probability of having any sequence in this library?

Figure 20-6
Introduction to Genetic Analysis, Ninth Edition

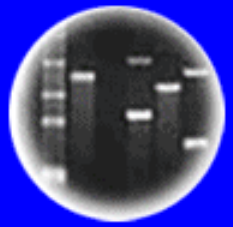
Why Partial Digestion? An Important Concept!

What is Complete & Partial Digestion?



DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

An EcoRI Restriction Enzyme Site is Found Only Once in the Human Genome:

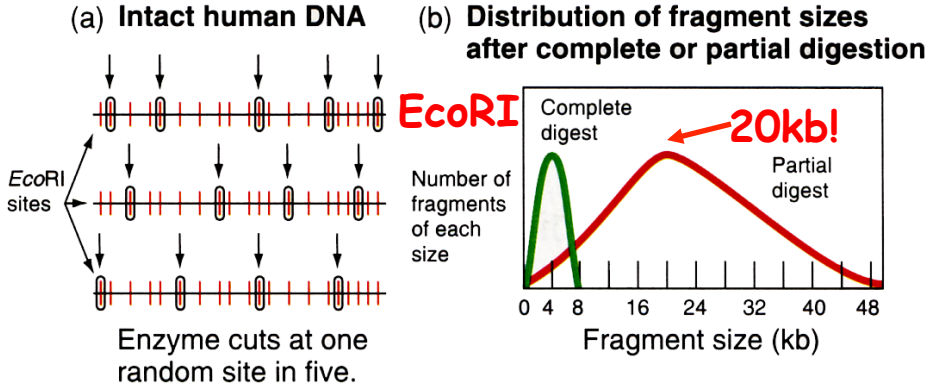
a. Yes
b. No

What is the Purpose of Partial Digestion of Human DNA?

Sau 3A= 4bp= $^{5'}GATC^{3'}$ ∴ 1 site every 280bp if digest to completion = $1x10^7$ DNA fragments

Eco RI= 6bp= $^{5'}GAATTC^{3'}$ ∴ 1 site every 3100 bp if digest to completion (cleaves every site) = 972,000 DNA fragments

1. Complete Digestion Produces fragments that are too small to clone in λ virus (need 20Kb)
2. Complete Digestion would create huge genome libraries with large # clones to screen
3. Complete Digestion would break up genes of different DNA fragments- particularly if human genes big- ∴ would have one gene on many different clones- parts separated !
4. Complete Digestion provides no way to find neighbors of clones in genome- what's next to gene in chromosome!

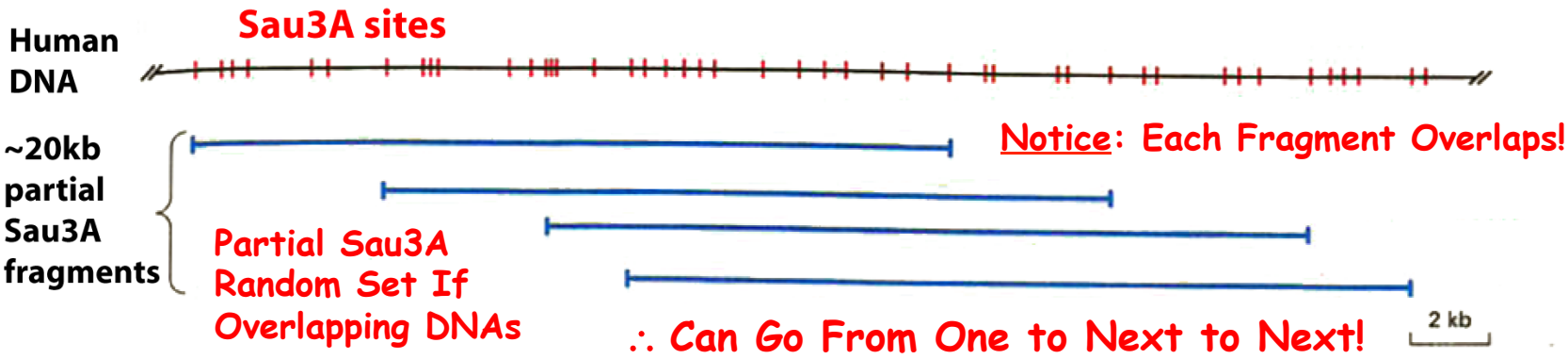


Principle of Genome Sequencing Too!!

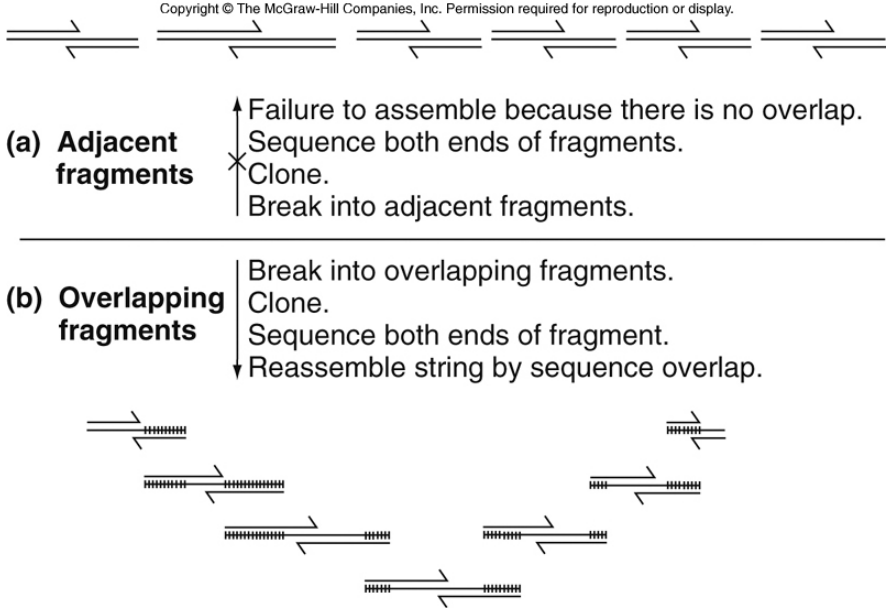
Partial Digestion Produces A series of Large, Overlapping DNA Fragments/ Clones

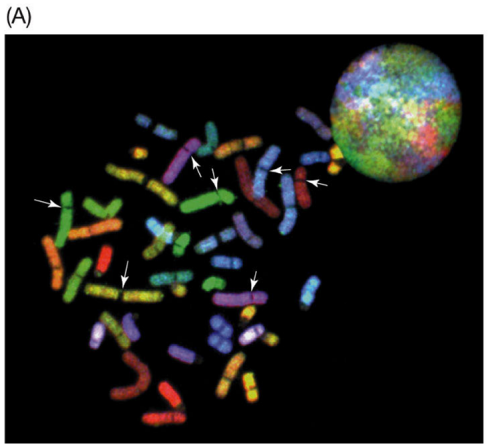
Can connect one clone with another!! Build up clones of each chromosome!!

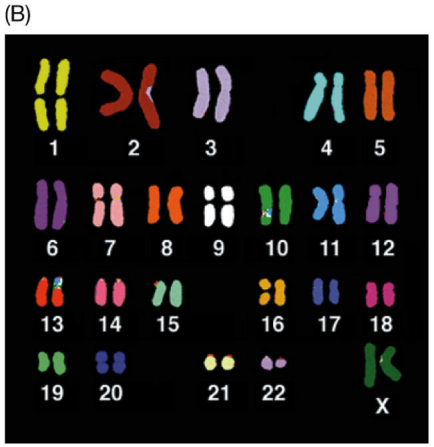
Constructing a Human Genome Library by Partial Digestion Creates a Set of Overlapping DNA Fragments/ Clones



∴ An overlapping set for each of the 24 chromosomes would allow clones to be ordered from beginning to end by restriction mapping because each chromosome contains one DNA molecule !

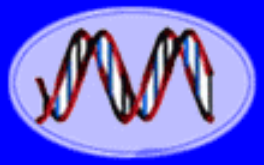






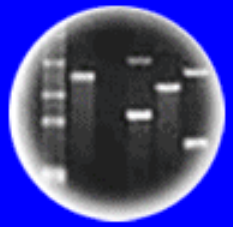
Step Two

How Find the Factor VIII Gene in a Human Genome Library?



DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

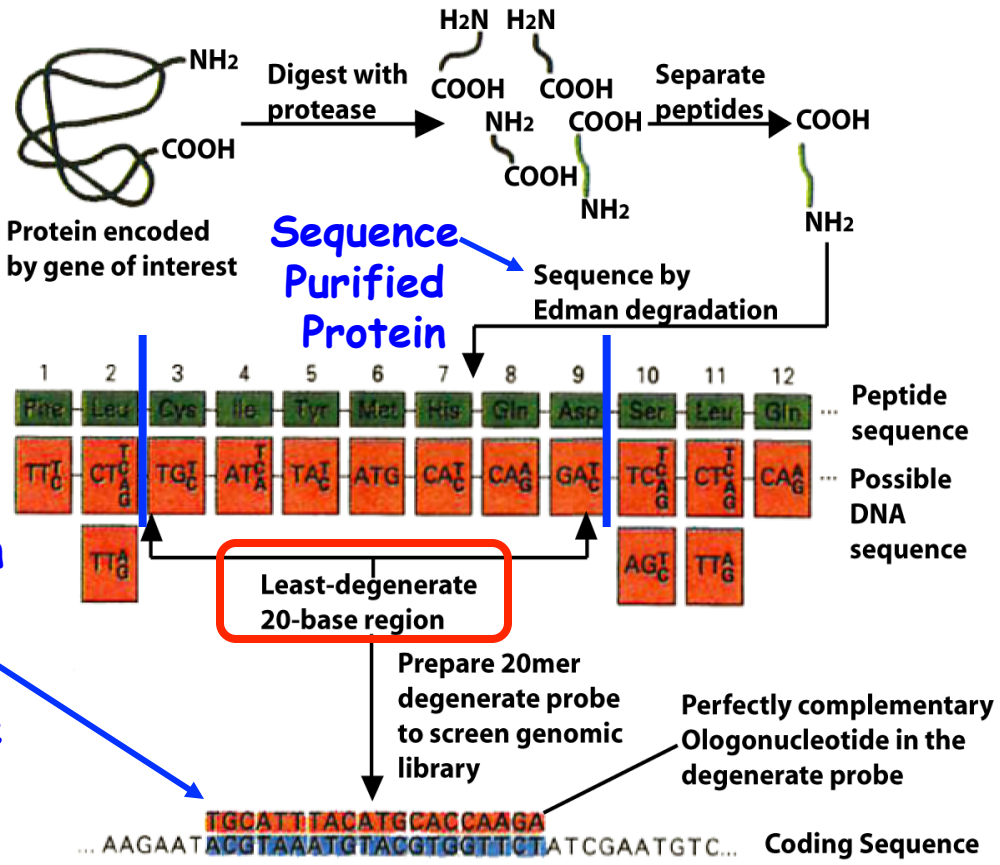
Plants of Tomorrow

A Specific Gene Can Be Identified in a Genome Library if the Amino Acid Sequence of its Protein is Known Because of the :

a. Double Helical Structure of DNA
b. Antisense Strand DNA Sequence
c. Genetic Code
d. Mutant Gene Phenotype

Factor VIII Protein → Gene

Using the Factor VIII Protein Sequence and Genetic Code as a Guide to Synthesize a Factor VIII Probe



1. Use Genetic Code

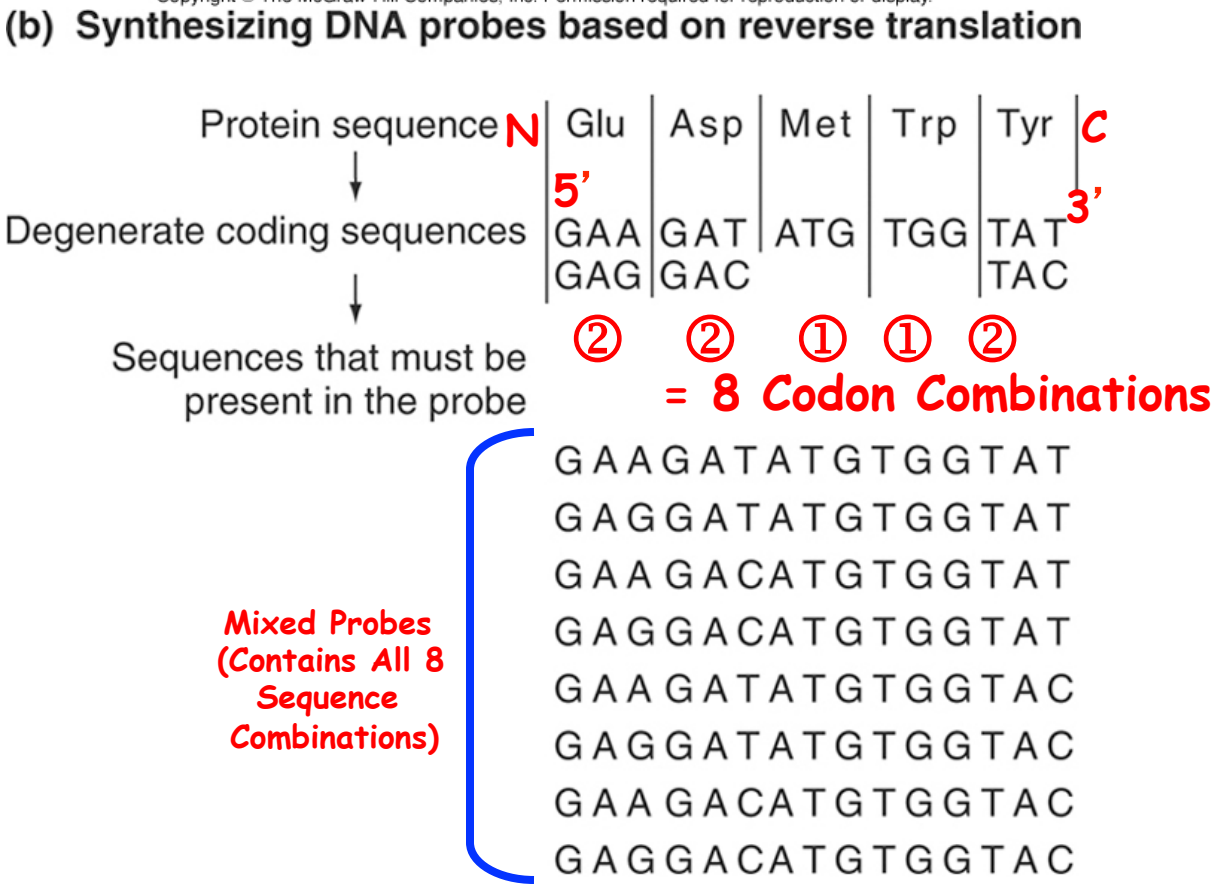
2. Make Several Probes All Codon Combinations!

3. One Will Be Correct Probe

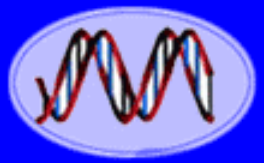
How many Combinations of Synthetic Probes?

2x3x2x1x2x2x2=96

Using the Genetic Code to go From Protein Sequence to Gene Sequence



1. Need Amino Acid Sequence of Part of the Protein
2. Need DNA Sequences Representing all Codon Combinations
3. Synthesize DNA Sequence Probes!



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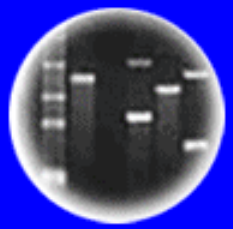


DNA Fingerprinting

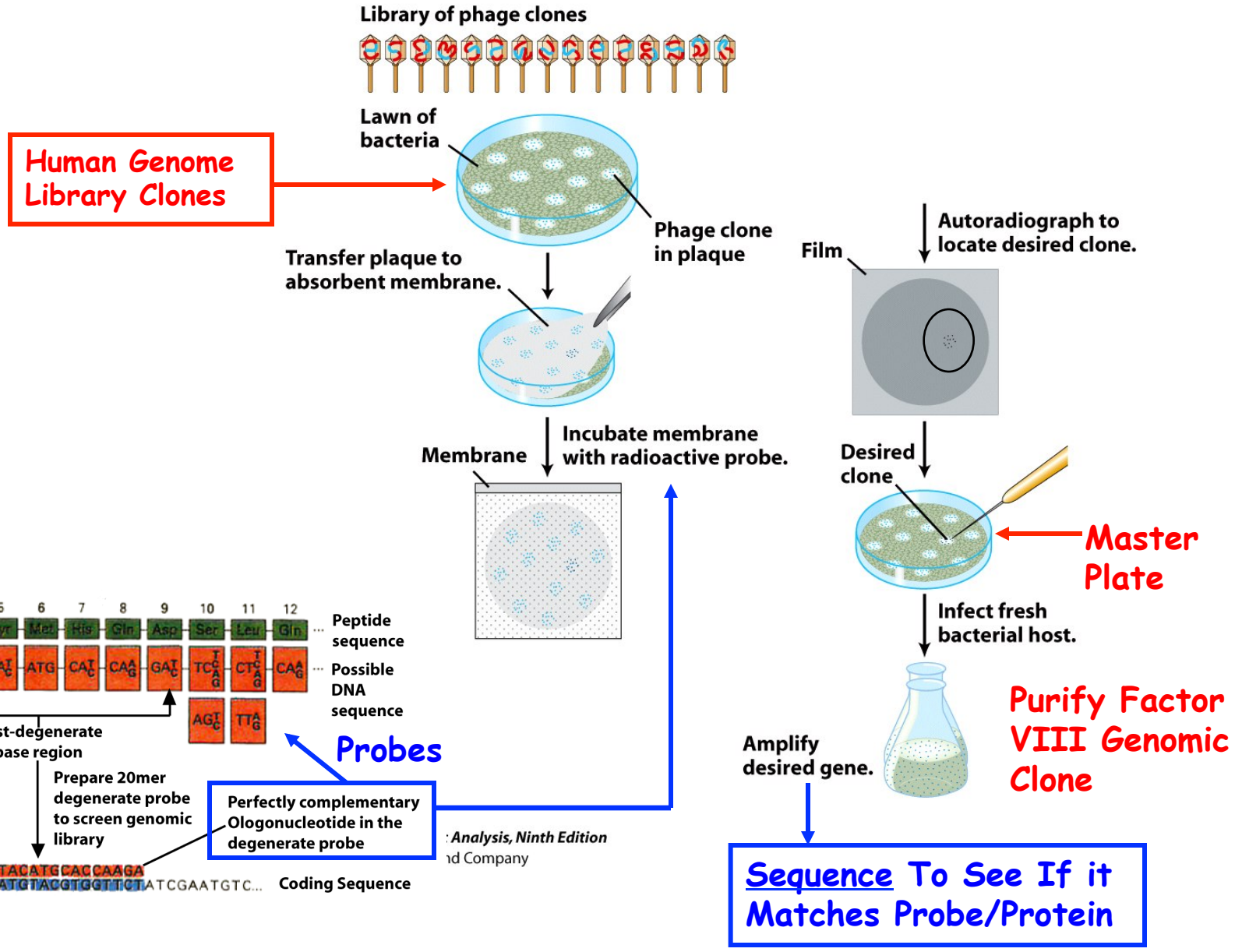
Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

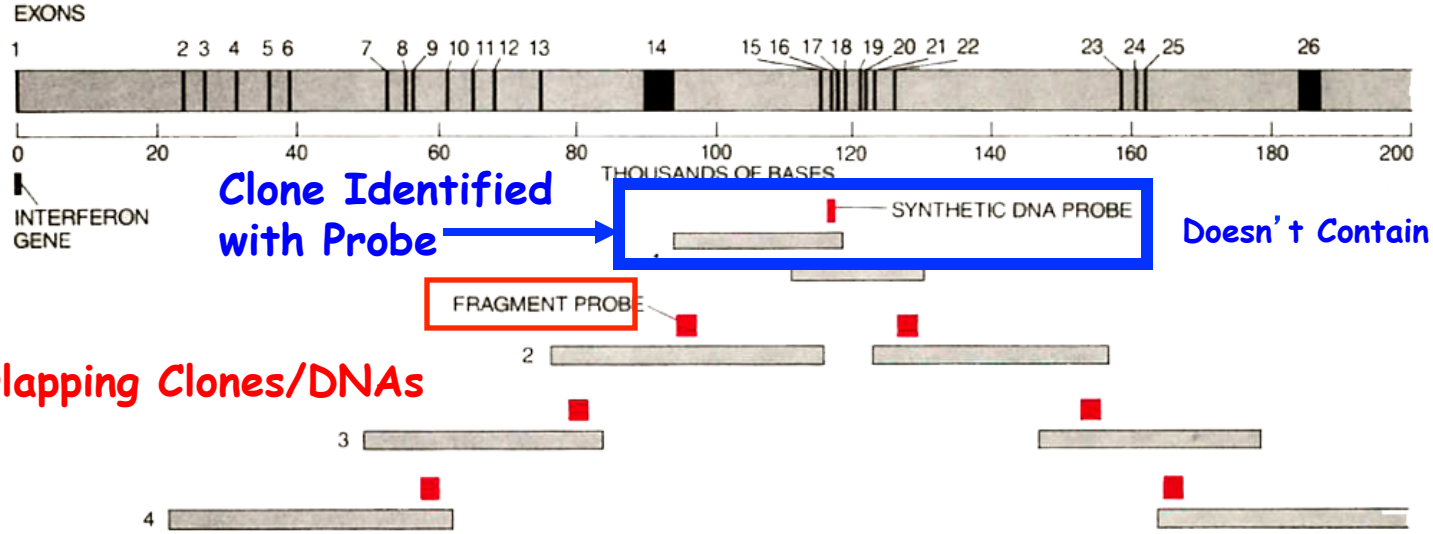
Probes Can Identify Genes in a Genome Library Because They Are: ?

a. Synthetic
b. Complementary to Specific DNA Sequences
c. Contain the Correct Amino Acid Sequence
d. Are Non-Radioactive

Finding The Factor VIII Gene Or Part of Gene!!



The Result-The Factor VIII Gene is Huge- 186,000 bp- The Probe Identified a Clone Containing Only One Part of Gene !!! Why?



How Find Clones with Rest of Gene? Key Question !

Remember - the library contains overlapping DNA clones ∴ can use one part of first clone to re-screen library & "walk" to other gene regions- using restriction maps & sequencing (compare with protein sequence) as guides!

Sequence ----------> GenBank

Step Three

Finding the Entire Factor VIII Gene? Walking & Sequencing

Walking up and down Genes and Chromosomes

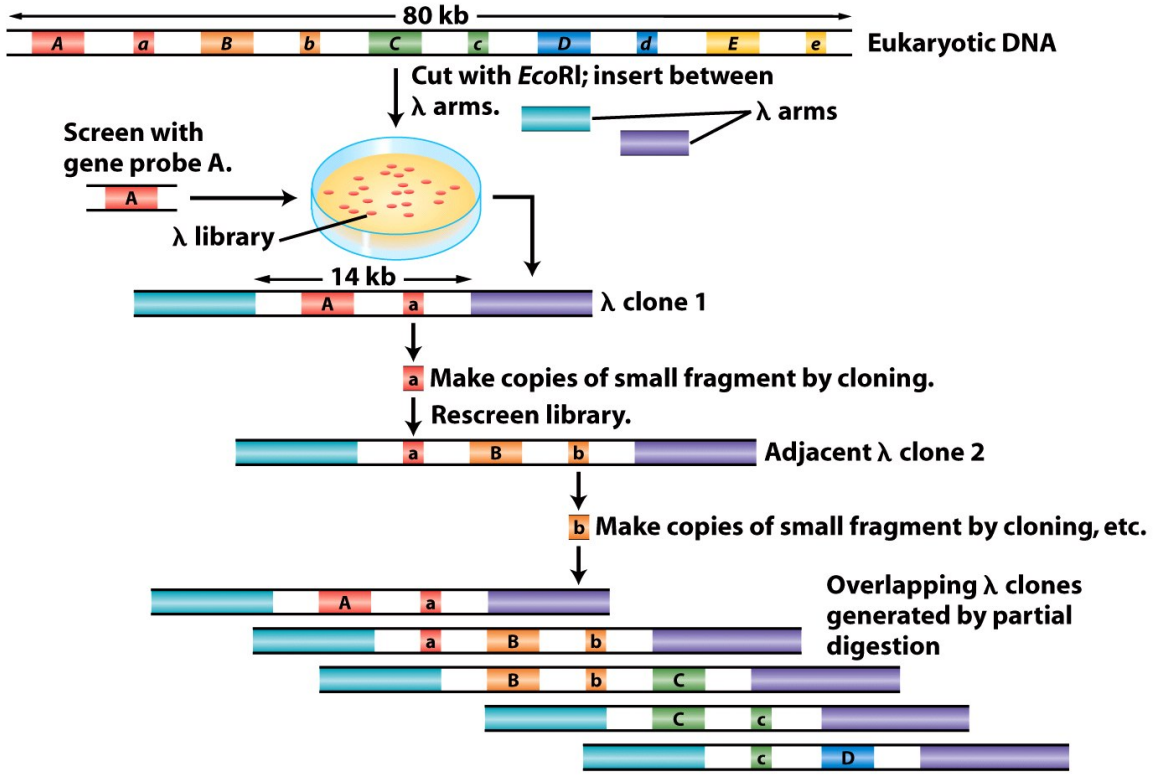


Figure 20-13
Introduction to Genetic Analysis, Ninth Edition

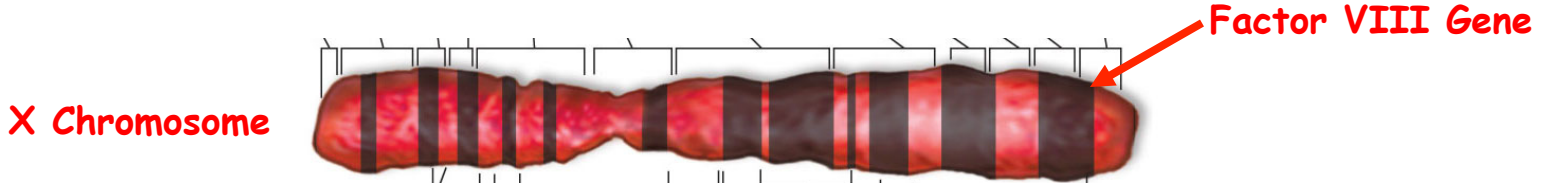
Basis of Genome Projects & Whole Genome Sequencing

Key Concepts → **How know Find Complete Factor VIII Gene?**

→ **Compare Protein & DNA Sequences**

The Factor VIII Gene Was Found To Be Very Large

- 186,000 Nucleotides in Length (Won't Fit in One Phage Clone)
- 25 Introns
- 9,000 Nucleotide Coding Sequence (cDNA)
- 2,351 Amino Acids in Protein

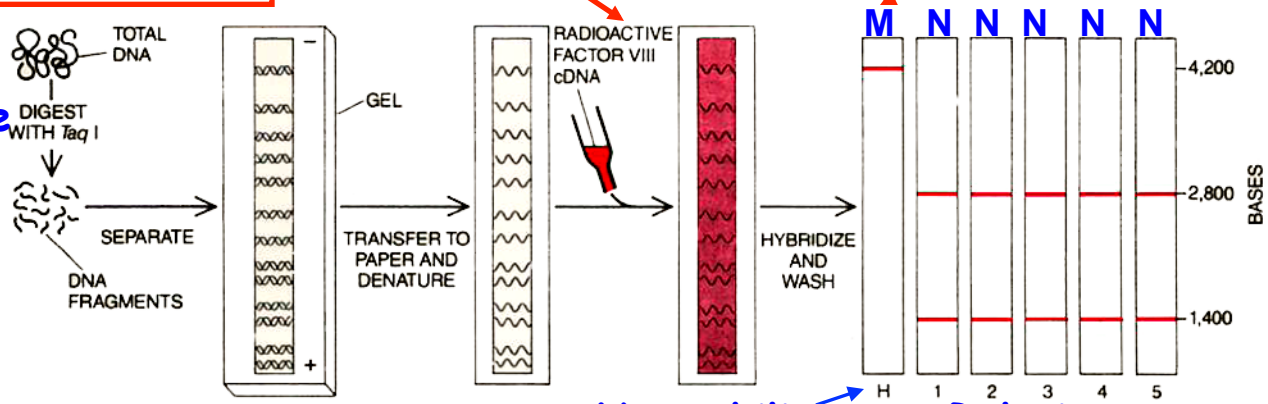


Factor VIII Gene Probes/ Sequence Can Be Used to Characterize Mutant Genes & Do DNA Testing for Carriers

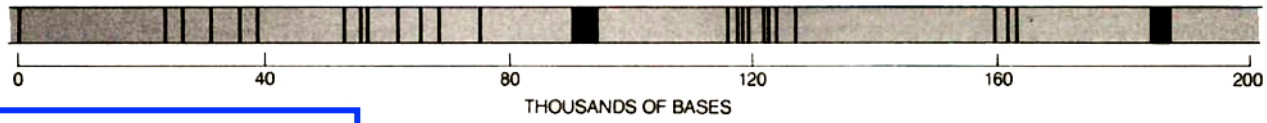
Human DNA From Different Individuals

Genomic or cDNA Clone Probe

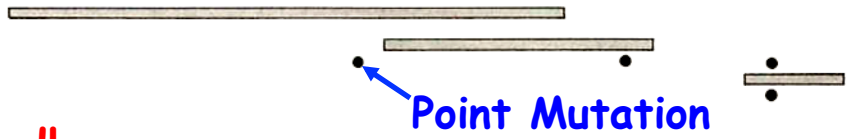
Loss of a TaqI site in Factor VIII Gene



RFLP



Mutations in Factor Gene



Once Gene & cDNA Identified!

Use DNA Gel Blots (or PCR) & Factor VIII Probes to Investigate Presence of Mutant Alleles in Families (carriers)

Mutations Arise Independently in Families

Factor VIII Mutations Occur Throughout the Gene

[*Haemophilia* 11, 481-491 (2005)]

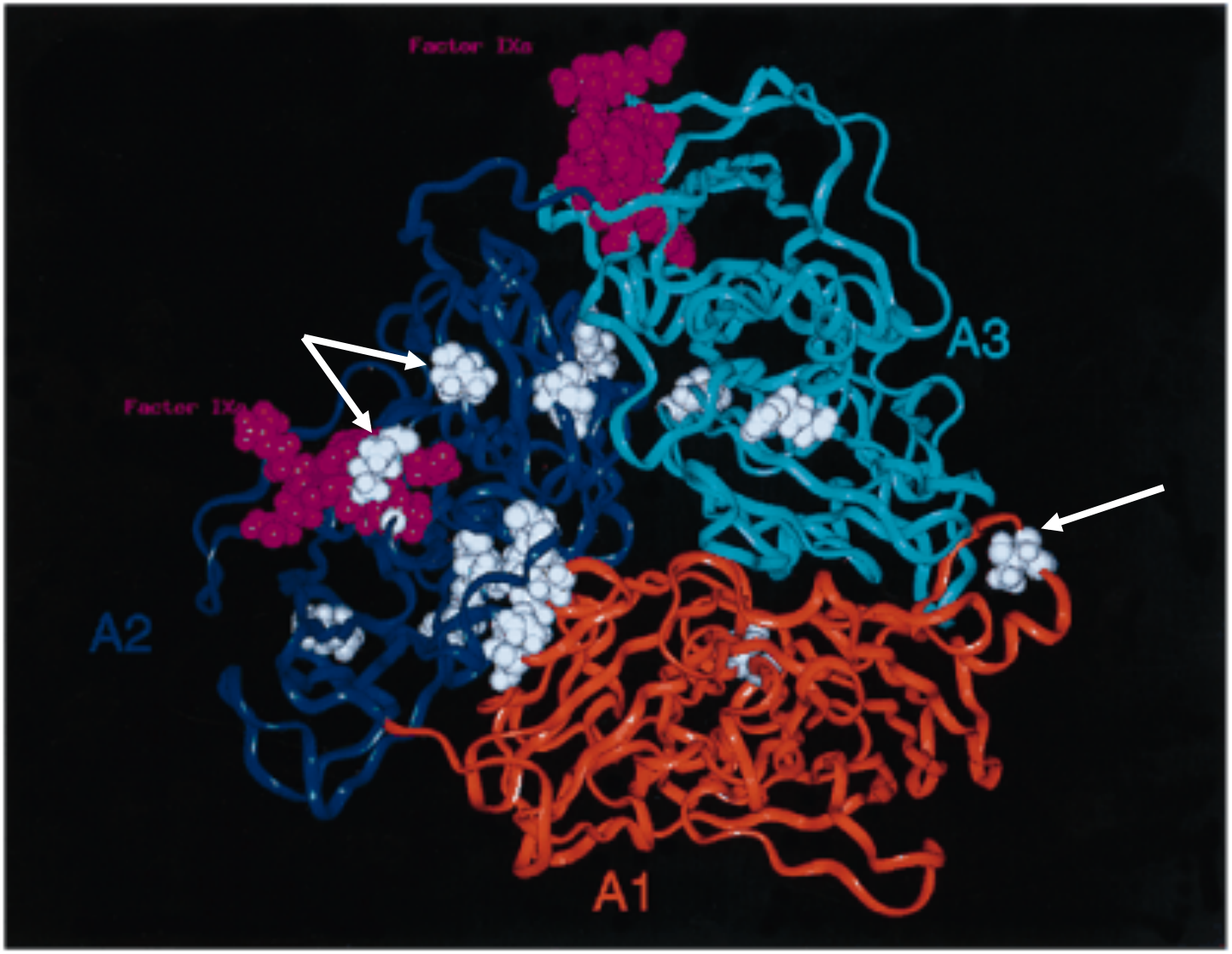
Factor VIII gene mutations in haemophilia A patients without intron 22 inversion.

VIII:C (%)	Family history	Consanguinity*	Inversion	Codon†	Mutation	Amino acid change	Exon	Conservation‡
1	Sporadic	NC	Normal	51	TTT → TCT§	Phe → Ser	2	FFFF, identical
1.20	Sporadic	NC	Normal	80	GTT → GAT	Val → Asp	3	VVVV, identical
1	Sporadic	NC	Normal	102	GGT → GTT§	Gly → Val	3	GGGG, identical
2	Sporadic	NC	Normal	104	TCC → CCC§	Ser → Pro	3	SSSS, identical
6	Sporadic	NC	Normal	143	GAG → AAG§	Glu → Lys	4	EEEE, identical
1	Sporadic	NC	Normal	233	delCA§	Thr → fs (TGA-264)	6	
2.70	Inherited	NC	Normal	321	GAA → AAA	Glu → Lys	8	EEEE, identical
0	Sporadic	NC	Normal	372	CGC → CAC	Arg → His	8	RRRR, identical
3	Inherited	NC	Normal	527	CGG → TGG	Arg → Trp	11	RRRR, identical
1	Sporadic	NC	Normal	528	TGC → TAC§	Cys → Tyr	11	CCCC, identical
1	Inherited	NC	Normal	592	CAA → TAA	Gln → Stop	12	QQQQ, identical
1	Inherited	NC	Normal	864	delGACA insCAATTAAATGAGAA§	Gly → fs [TAA-867]	14	
1	Sporadic	NC	Normal	948	insA§	Lys → fs (TGA-984)	14	
1	Sporadic	NC	Intron 1	1107	AGG → TGG§	Arg → Trp	14	RGKK, dissimilar
1	Sporadic	NC	Normal	1107	AGG → TGG§	Arg → Trp	14	RGKK, dissimilar
1	Inherited	NC	Normal	1191–1194	delA	Ile → fs (TAG-1198)	14	
1.40	Sporadic	NC	Normal	1191–1194	insA	Ile → fs (TAA-1220)	14	
1	Sporadic	C	Normal	1227	delC§	Leu → fs (TGA-1231)	14	
2.10	Sporadic	NC	Normal	1241	GAC → GAG	Asp → Glu	14	DGGE, similar
1	Sporadic	NC	Normal	1392	1392del1418§	Pro → fs (TAG-1446)	14	
1	Inerited	C	Normal	1392	1392del1418§	Pro → fs (TAG-1446)	14	
1	Sporadic	NC	Normal	1441	insA§		14	
1	Inerited	C	Normal	1441	insA§			
1	Inherited	NC	Normal	1502	CAG → TAG§	Gln → Stop	14	QREQ, dissimilar
1	Inherited	NC	Normal	1504	delGT§	Val → fs (TGA-1517)	14	
1	Sporadic	NC	Normal	1535	TGG → TGA	Trp → Stop	14	WLWM, dissimilar
hibitor 96 BU								
1	Sporadic	NC	Normal	1571	TAT → TAA§	Tyr → Stop	14	Y-YY, dissimilar
1	Sporadic	NC	Normal	1581	AAA → TAA§	Lys → Stop	14	KEKK, dissimilar
0.20	Sporadic	NC	Normal	1696	CGA → GGA	Arg → Gly	14	RRRR, identical
1.80	Sporadic	NC	Normal	1729	delA§	Gln → fs (TAA-1752)	15	
1	Inherited	NC	Normal	1751	GAA → AAA§	Glu → Lys	15	EEEE, identical
1	Sporadic	NC	Normal	1775	TTC → TCC§	Phe → Pro	16	FFFF, identical
1	Sporadic	NC	Normal	1835	TGG → TGA§	Trp → Stop	16	WWWW, identical
7.60	Sporadic	C	Normal	1882	ATC → ATA§	Ile → Ile	17	IIII, identical
3	Inherited	C	Normal	1966	CGA → CAA	Arg → Glu	18	RRRR, identical
1	Sporadic	NC	Normal	1966	CGA → TGA	Arg → Stop	18	RRRR, identical

FVIII GENE MUTATIONS IN INDIAN PATIE

Need To Screen Across the Gene for Markers -- Family Specific

Factor VIII Protein Structure & Positions Where Mutations Disrupt Protein Function and Lead to Hemophilia

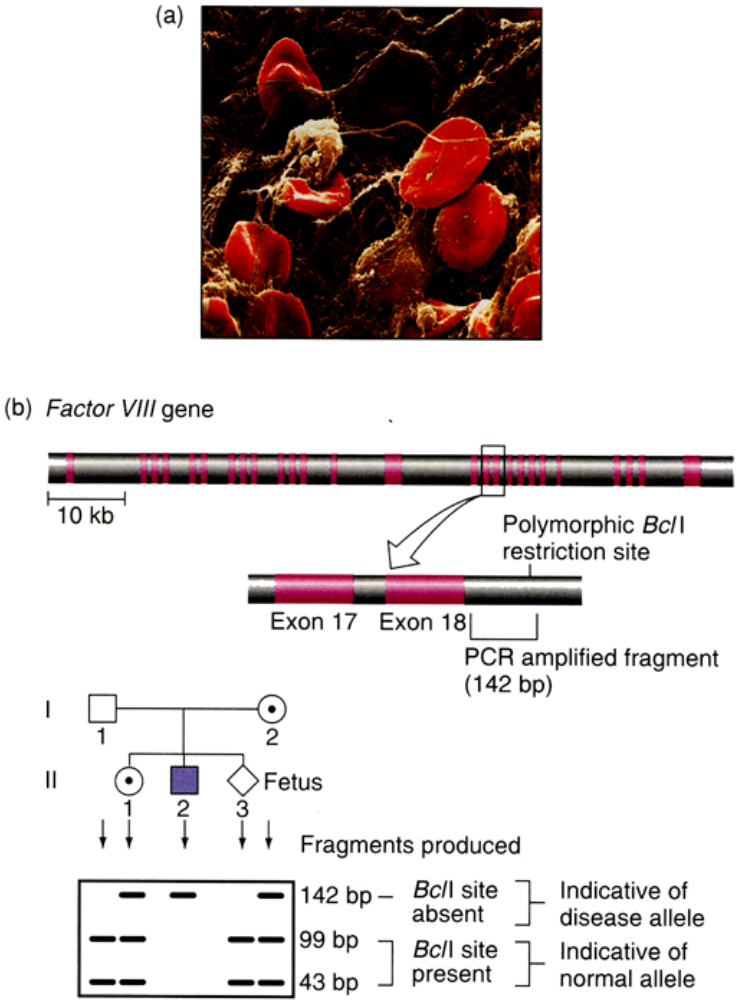


Using PCR and RFLPs (Markers) to Detect the Hemophilia A Disease Allele/Gene

1. Use PCR to amplify a specific Factor VIII gene region
2. Use restriction enzyme (BcL I) to distinguish between normal allele (1 site) & disease allele (no site)

[=] = Normal allele

[–] = Disease allele



The 21st Century Approach!

1. Sequence the Entire Gene & Find Mutation
2. Then Synthesize Primers to Test Family Members Using PCR

Only Can Do This With a Knowledge of DNA Sequence of Wild-type (Normal) and Disease Genes (Can Vary family to Family)

RESEARCH ARTICLE **New Non-Invasive DNA Tests Are Available Based on PCR**

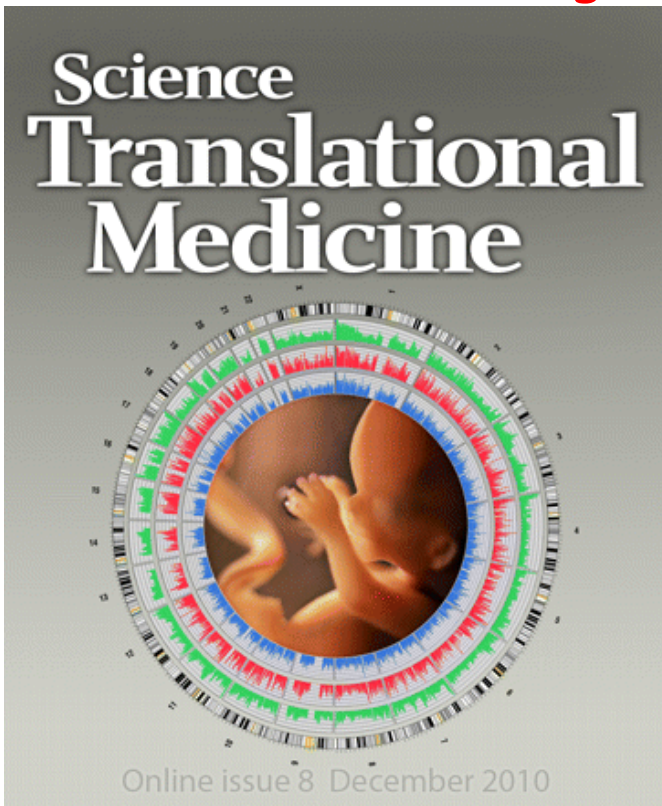
PRENATAL DIAGNOSIS

Maternal Plasma DNA Sequencing Reveals the Genome-Wide Genetic and Mutational Profile of the Fetus

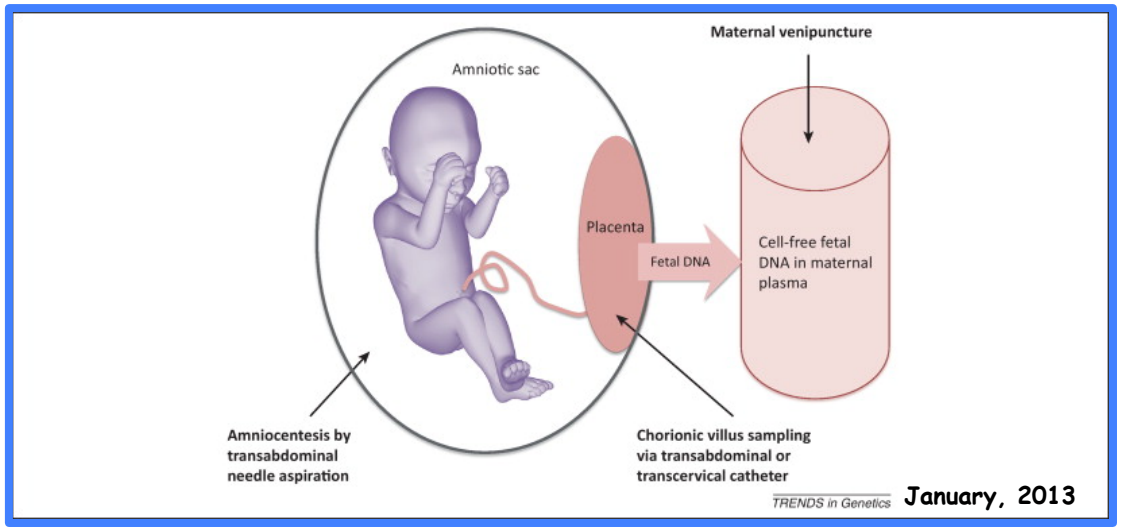
Science Translational Medicine, December 8, 2010 (61,1-12)

Sequencing DNA From the Blood of a Pregnant Woman Allows the Complete Genome Of the Fetus to Be Decoded!

A New Era in DNA Testing!!

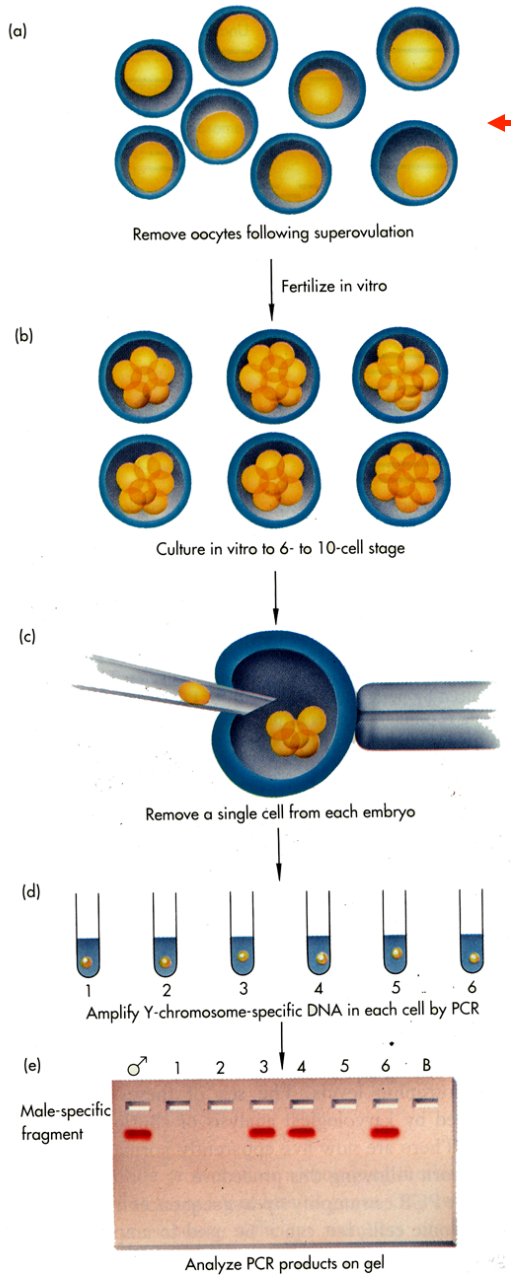


~10% of DNA in Maternal Plasma is From the Fetus



January, 2013

Using PGD to Detect Hemophilia A Disease Alleles



Mother is a Carrier X^HX^h

1. Test for Male Embryos
2. Test for Presence of Hemophilia A Disease Alleles!

X^hY

Step Four

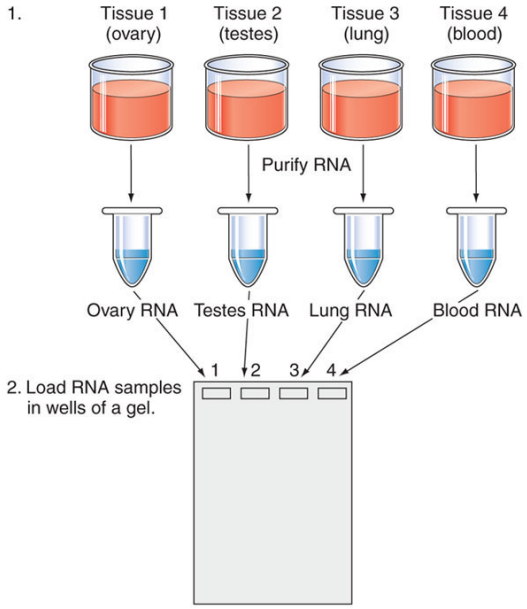
How Find Factor VIII mRNA to Generate a cDNA for Protein Production in Host Cells?

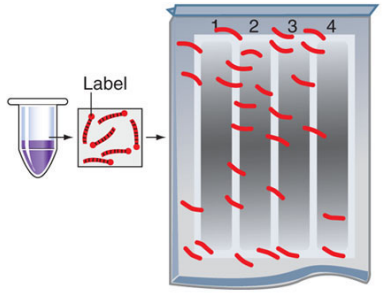
Recall: Eukaryotic Genes Provide Obstacles for Efficient Protein Production in Genetically Engineered Cells! Reasons???

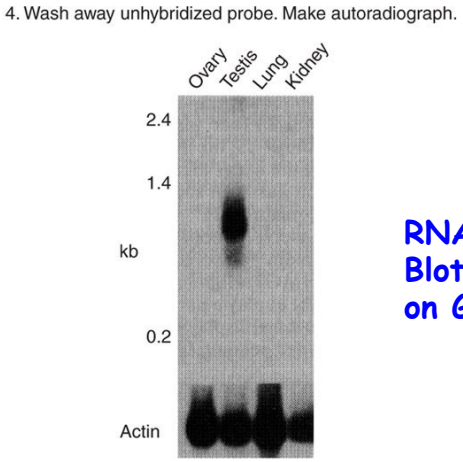
Making the Drug

Need cDNA Not Gene

Factor VIII Gene Can Be Used to Find Out Where It is Active Using RNA Blots





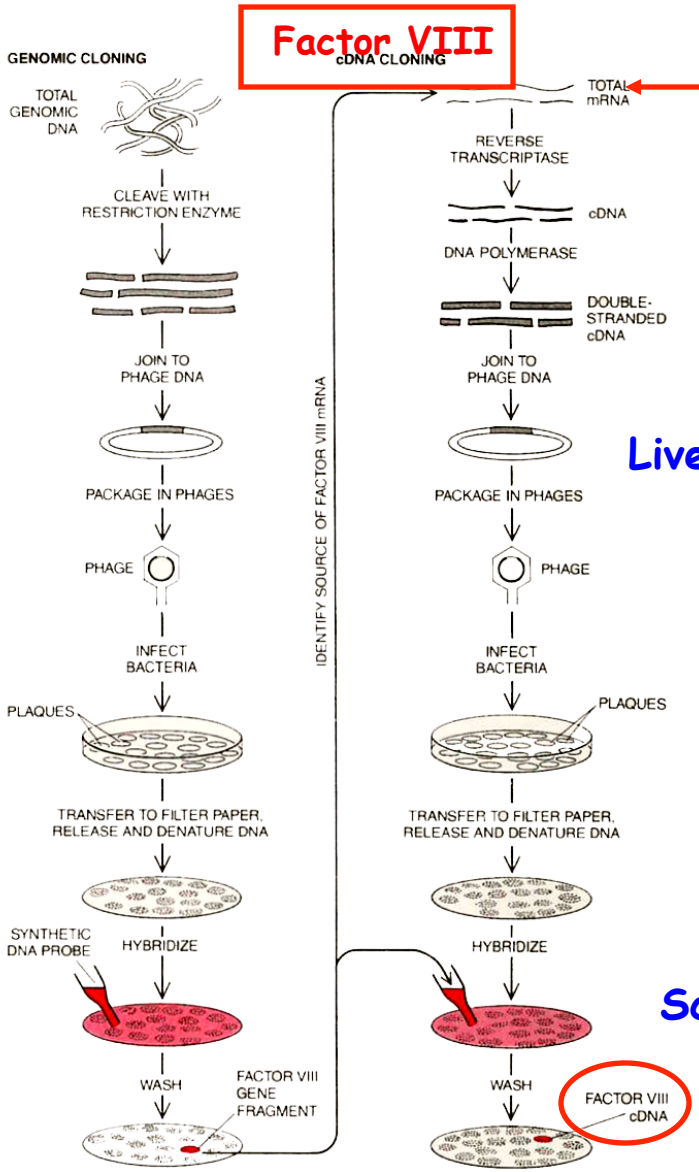


RNA Blot Is Like a DNA Blot Except That RNA is on Gel & Blotted

Factor VIII Gene Is Highly Active in Liver!

Could Also Use PCR (RT-PCR)

Using Factor VIII Gene Probe to Identify Factor VIII cDNA clone



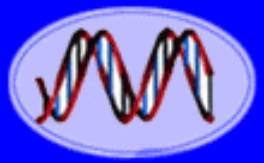
Liver mRNA

Liver cDNA Library

Factor VIII cDNA →

Used to Make Drug !

Screen Liver cDNA Library



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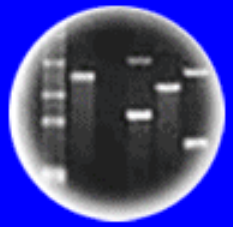


DNA Fingerprinting

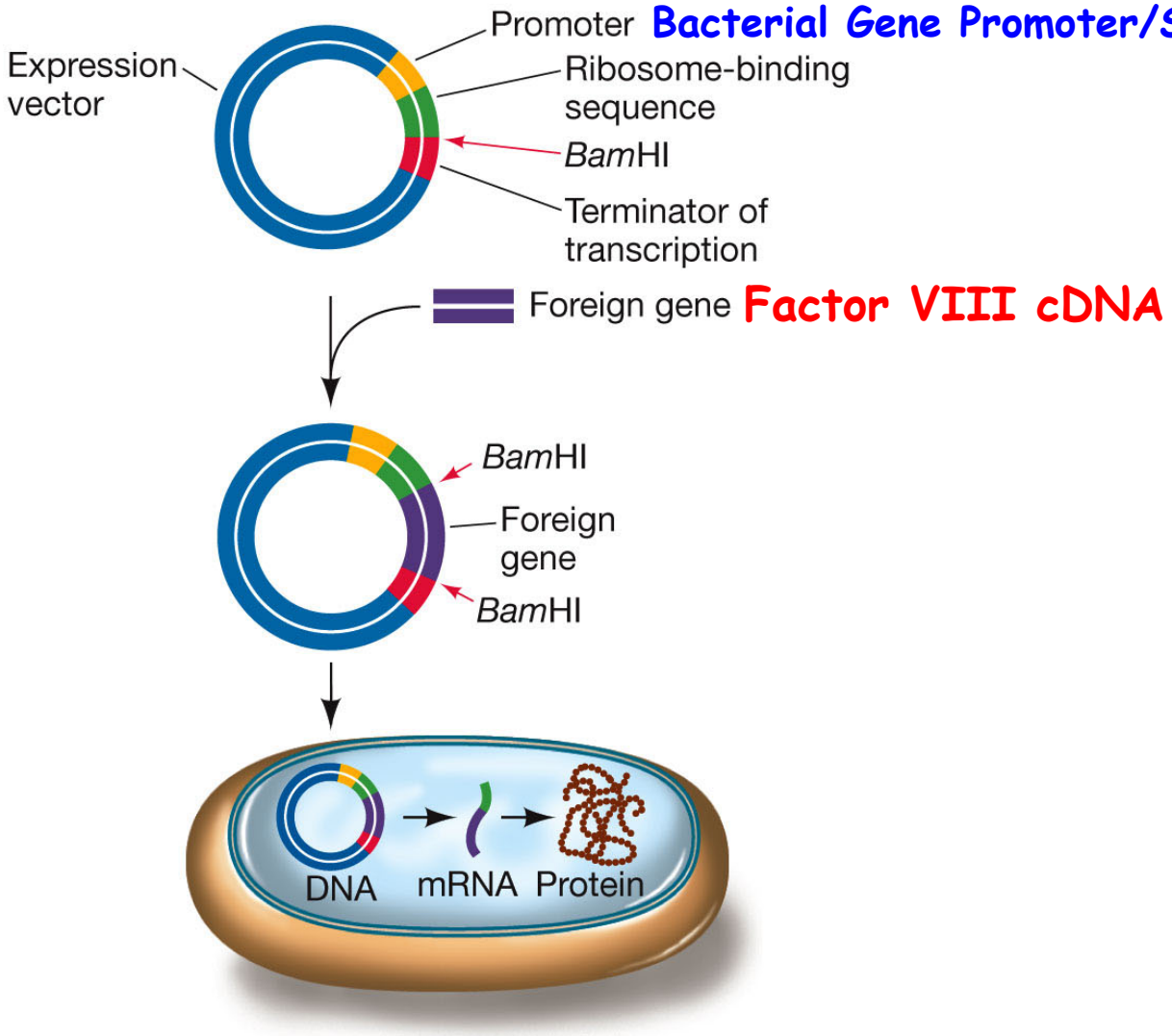
Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

The sequence of a cDNA clone is the same as:

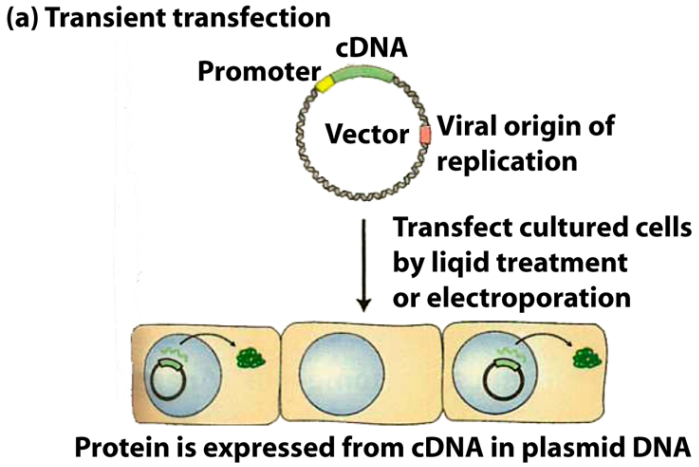
a. The Sense Strand of the Corresponding Gene
b. The mRNA Template
c. The Antisense (Template Strand) of the
d. Corresponding Gene
e. The Sense and Antisense Strands of the Corresponding Gene Minus Introns

Use Expression Vector to Allow cDNA to Produce Protein in Host Cell

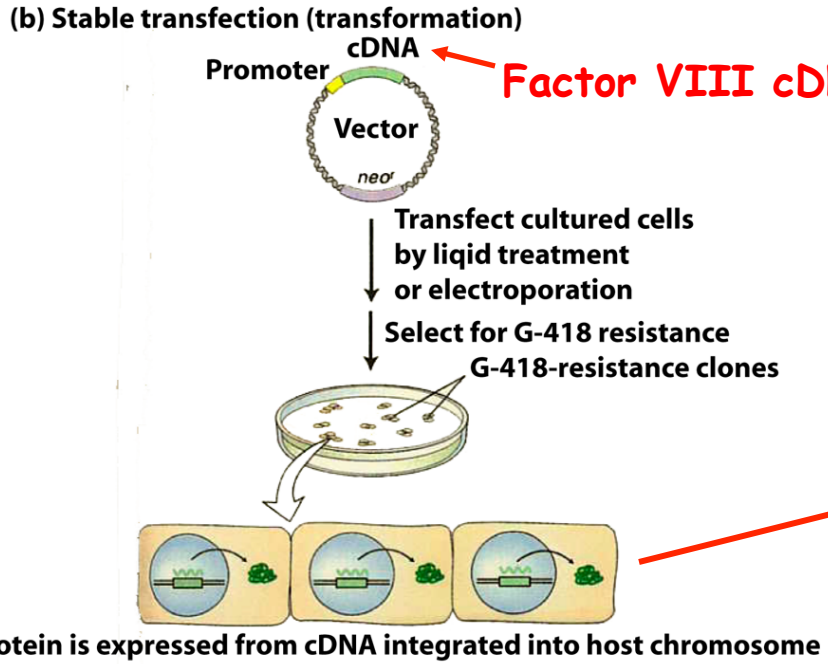


LIFE 8e, Figure 16.16

A Factor VIII Drug/"Cure"
Making Factor VIII in Mammalian Cells

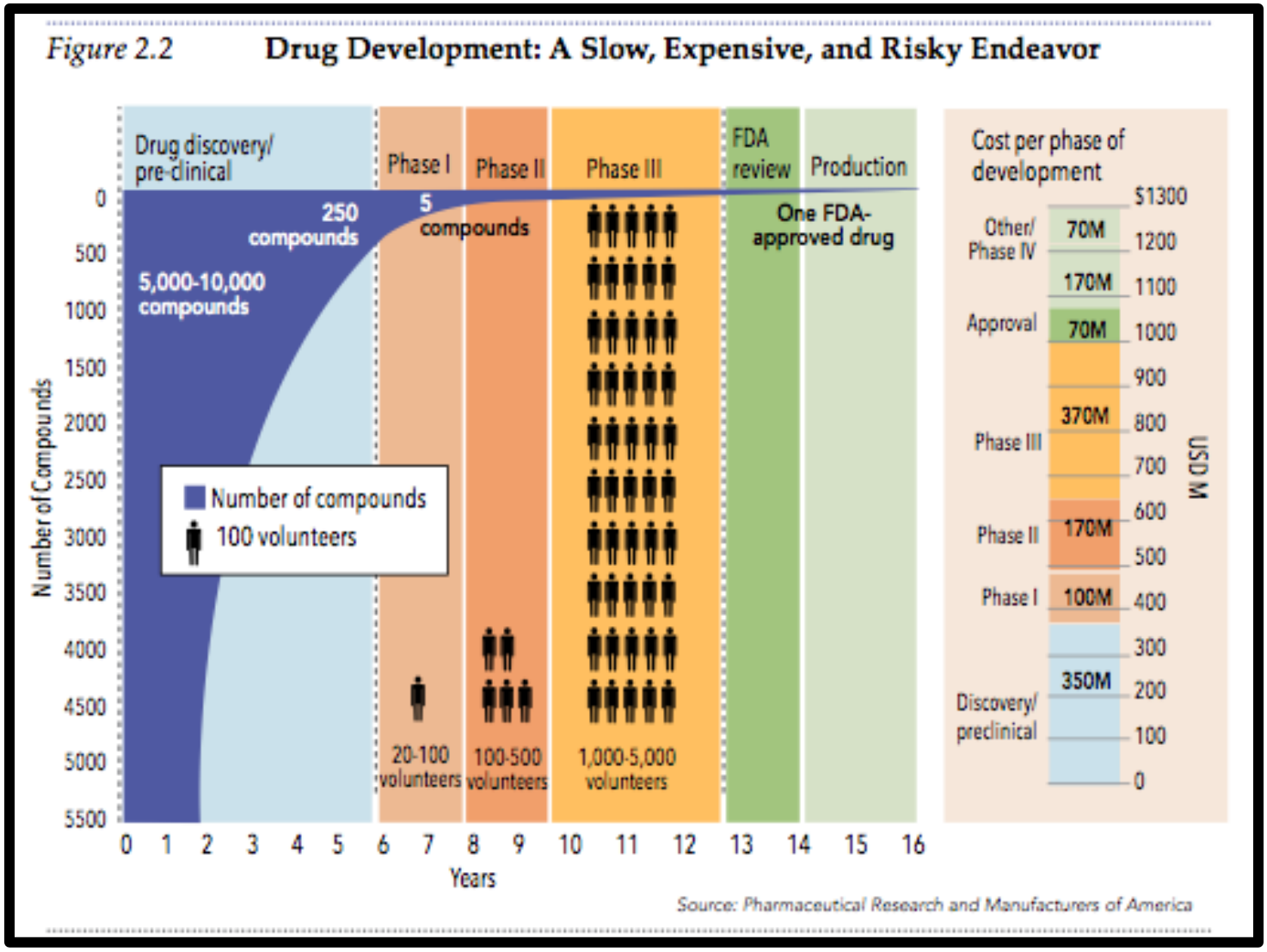


[Why Mammalian Cells?]



Purify Factor VIII Protein!

Need FDA Approval Before Recombinant DNA Drug Can Be Marketed and Used to Treat Patients



A Long and Expensive Process!

Recombinant Factor VIII

Bayer Biological Products EU

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- More Resources
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- Hemophilia Research Awards

Recombinant factor VIII

Recombinant factor VIII (rFVIII) is the antihaemophilic factor A, obtained using recombinant DNA technology. With this technology, pure protein is synthesized in the laboratory instead of being extracted from blood. In the following pages, it will be explained in detail how the knowledge and analysis of DNA, using the new instruments of molecular genetics, have represented both the beginning and follow-up stages in the development of recombinant FVIII.

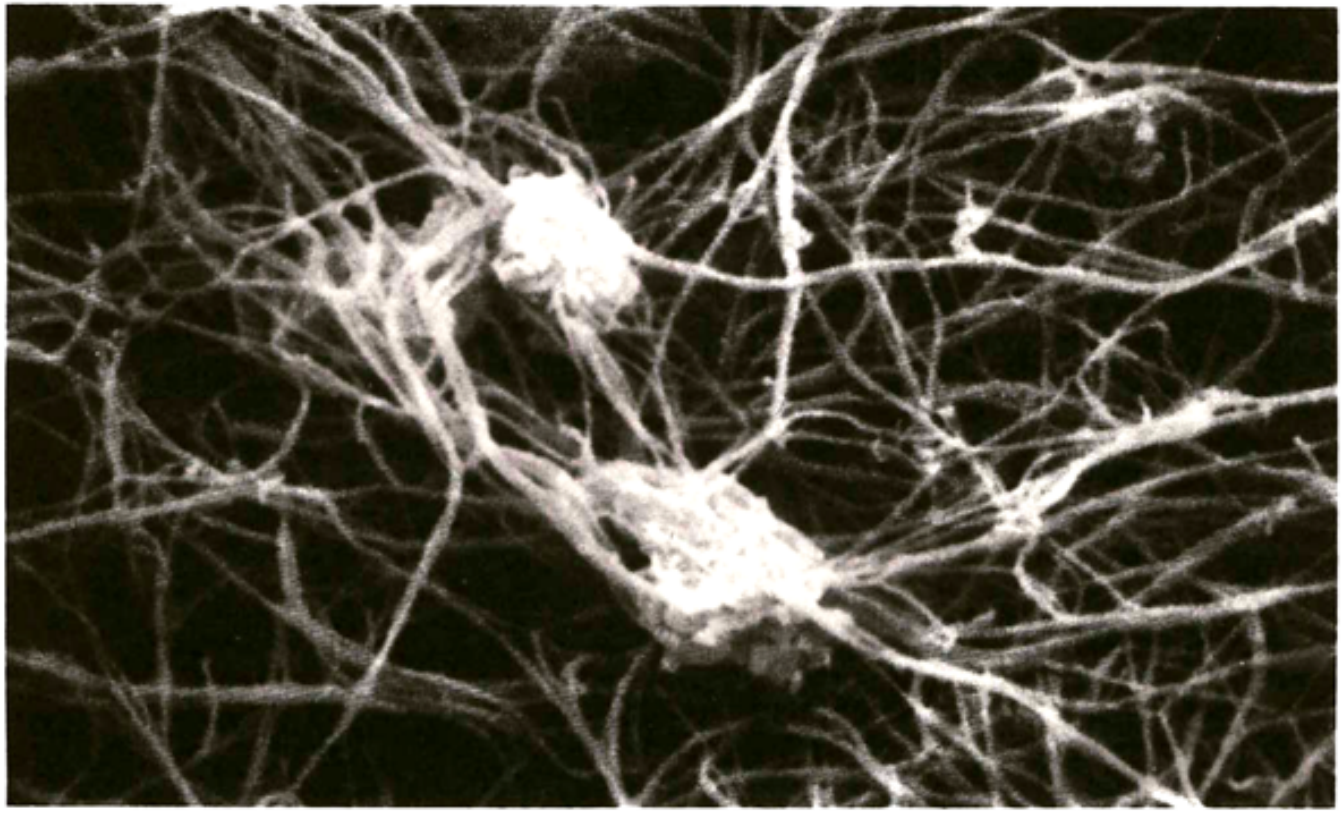
Prophylactic Treatment Costs $300,000/ Year!
Most Hemophiliacs Use "On Demand" or As Needed

Factor VIII gene cloned in 1983

Factor VIII (recombinant) approved as drug in 1993!
Ten years from gene → drug! (Off Patent in 2011)

Using Factor VIII to Treat Hemophilia

Formation of a Blood Clot



FIBRIN STRANDS stabilize a blood clot at the site of a wound by trapping the platelets that form the bulk of the clot. The electron micrograph, which was made by Jon C. Lewis of Wake Forest University, shows a clot formed in a suspension of platelets and fibrin.

A clot in the bloodstream is the result of a complex cascade of enzymatic reactions culminating in the conversion of fibrinogen, a soluble protein, into insoluble fibrin strands. In hemophiliacs a crucial protein in the blood-clotting cascade is either missing or defective.

A Triumph of Genetic Engineering

The Future: Gene Therapy – A Permanent "Cure"

December 10, 2011

Treatment for Blood Disease Is Gene Therapy Landmark

By NICHOLAS WADE

TIME Partners with CNN

Gene Therapy Shows Promise for Treating Hemophilia

By ALICE PARK Monday, December 12, 2011

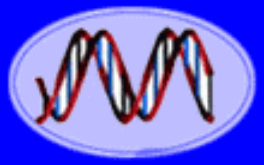
The NEW ENGLAND JOURNAL of MEDICINE

December 12, 2011

ORIGINAL ARTICLE

Factor IX

Adenovirus-Associated Virus Vector–Mediated Gene Transfer in Hemophilia B



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Entire Genetic Code
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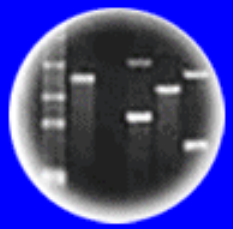


DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

There is A Patent on YOUR Factor VIII Gene (in fact many)!

United States Patent **5,618,788**
Capon , et al. **April 8, 1997**

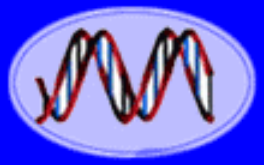
Preparation of functional human factor VIII and pharmaceutical treatment therewith

Abstract

Functional human factor VIII produced recombinantly is used in the treatment of human beings diagnosed to be deficient in factor VIII coagulant activity. Also provided are DNA solates and expression vehicles encoding functional human factor VIII, as well as transformed host cells and processes for producing human factor VIII by use of recombinant DNA echnology.

Inventors: **Capon; Daniel J.** (San Mateo, CA), **Lawn; Richard M.** (San Francisco, CA), **Vehar; Gordon A.** (San Carlos, CA), **Wood; William I.** (San Mateo, CA)
Assignee: **Genentech, Inc.** (South San Francisco, CA)
Appl. No.: **07/570,096**
Filed: **August 20, 1990**

All Have Expired



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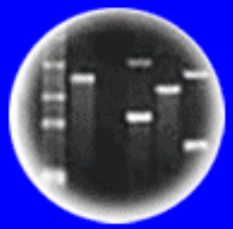

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Publication Number	Application Date	Title	Abstract	Assignee/Applicant Name	Number of Fwrd Ref.
EP150735A2	11/01/1985	Method and composition for preparation of factor VIIIC	Methods and compositions are provided for recombinant DNA production of Factor VIIIC and truncated derivatives thereof. Based on amino acid sequences, probes are developed for isolating messenger RNA and/or chromosomal DNA encoding for Factor VIIIC. The Factor VIIIC gene in its entirety or encoding for a fragment thereof is then used for expression of Factor VIIIC in a host.	CHIRON CORPORATION | NOR DISK GENTOFTE A/S	0
EP157556A2	22/03/1985	Recombinant factor VIII-C	Human Factor VIII-C antihemophilic factor in essentially pure form is provided. Among the processes for its production is that of expressing DNA encoding human Factor VIII-C in a self-replicating recombinant host system. A composition for preparing human Factor VIII-C from a hererogeneous mRNA mixture containing mRNA for said protein, which composition comprises: a) means for	MELOY LABORATORIES, INC.	0
EP160457A1	18/04/1985	Human factor VIII, Compositions containing it, methods and materials for use in it production	The full DNA coding sequence of human factor VIII is identified herein. Also disclosed is the recombinant means useful to isolate and express this coding sequence in the preparation of functional human factor VIII polypeptide and functional derivatives thereof.	GENENTECH, INC.	0
EP169562A1	25/07/1985	Recominant factor VIII-R	Human Factor VIII-R essentially free of other proteins of human origin is disclosed. Characteristically, the Factor VIII-R protein is glycosylated. The Factor VIII-R is produced by recombinant DNA techniques in host cells or other self-replicating systems and is provided in essentially pure form. Also provided are methods and compositions for preparing the above-described Factor VIII-R as well as therapeutic compositions and uses for the Factor VIII-R protein in the treatment of coagulation disorders in humans and animals. The invention further provides replicable expression	MELOY LABORATORIES, INC.	0
US4757006	28/10/1983	Human factor VIII:C gene and recombinant methods for production	The protein having factor VIII:C procoagulant activity has been produced by culturing a cell transformed with a recombinant expression vector encoding the gene for that activity.	Genetics Institute, Inc.	97

Publication Number	Application Date	Title	Abstract	Assignee/Applicant Name	Number of Fwrd Ref.
US4965199	07/08/1987	Preparation of functional human factor VIII in mammalian cells using methotrexate based selection	A method for producing factor VIII in recombinant mammalian host cells utilizing an expression vector containing a selectable marker DNA and an amplifiable marker DNA. The initial selection is based upon the selectable marker and subsequent amplification of factor VIII DNA and amplifiable marker DNA is conducted in cells not deficient in the amplifiable marker.	Genentech, Inc.	70
US5004804	12/01/1984	Method and composition for preparation of factor VIIIC	Methods and compositions are provided for recombinant DNA production of Factor VIIIC and truncated derivatives therfeof. Based on amino acid sequences, probes are developed for isolating messenger RNA and/or chromosomal DNA encoding for Factor VIIIC. The Factor VIIIC gene in its entirety or encoding for a fragment thereof is then used for expression of Factor VIIIC in a host.	Nordisk Gentofte	14
US5045455	14/06/1990	Factor VIII:C cDNA cloning and expression	Methods and compositions are provided for recombinant DNA production of Factor VIIIC and truncated derivatives thereof. Based on amino acid sequences, probes are developed for isolating messenger RNA, cDNA and/or chromosomal DNA encoding for Factor VIIIC. The Factor VIIIC gene in its entirety, a fragment	Chiron Corporation	13
US5171844	10/06/1988	Proteins with factor VIII activity: process for their preparation using genetically-engineered cells and pharmaceutical compositions containing them	Novel polypeptides having Factor VIII activity are provided as well as compositions and methods for their preparation. The polypeptides comprise derivatives and fragments of Factor VIII and have sequences substantially similar to portions of naturally occuring Factor VIII. The polypeptides find use in treatment of Hemophilia A.	Gist-Brocades N.W.	21
US5198349	23/05/1991	Method for producing factor VIII:C and analogs	An improved method for producing Factor VIII:c is disclosed. The method involves culturing mammalian cells which contain DNA encoding Factor VIII:c and which are capable of expressing Factor VIII:c. In accordance with this invention the cells are cultured in a	Genetics Institute, Inc.	29

Court upholds patenting of genes in Myriad case

Fri, Jul 29 2011

By Julie Steenhuysen

CHICAGO (Reuters) - A federal appeals court affirmed the right of Myriad Genetics to patent two genes linked to breast cancer, overturning a lower court ruling that threatened a key element of the biotech business.

July 29, 2011

Ruling Upholds Gene Patent in Cancer Test

By ANDREW POLLACK

The court ruled that DNA isolated from the body was eligible for patents because it was "markedly different" in its chemical structure from DNA that exists inside the chromosomes in the body. As a result, the isolated DNA is not simply a product of nature, which would not be eligible for a patent.

March 29, 2010

Judge Invalidates Human Gene Patent

By JOHN SCHWARTZ and ANDREW POLLACK

A federal judge on Monday struck down patents on two genes linked to breast and ovarian cancer. The decision, if upheld, could throw into doubt the patents covering thousands of human genes and reshape the law of intellectual property

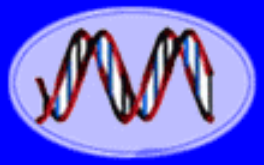
United States District Court Judge Robert W. Sweet issued the 152-page decision, which invalidated seven patents related to the genes BRCA1 and BRCA2, whose mutations have been associated with cancer.

Oct 30, 2010

US Government Argues in Court that Isolated Genes are Unpatentable

AMP v. Myriad (Fed. Cir. 2010)

In March, 2010, District Court Judge Robert Sweet held Myriad's gene patent claims invalid for failing to satisfy the subject matter eligibility requirements of 35 U.S.C. 101. The ruling was directed toward claims that cover particular isolated DNA molecules (genes) and processes of detecting and screening for those genes, but was written broadly enough to essentially invalidate all patents covering genes that were isolated from an organism.



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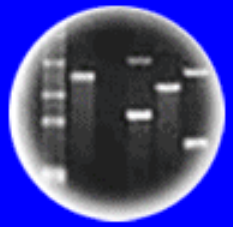

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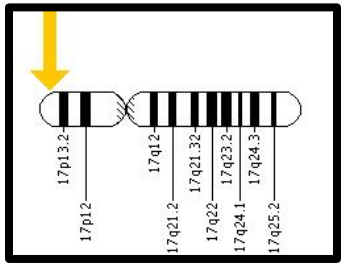
A DNA Testing Ethical Issue-The Greenberg Case

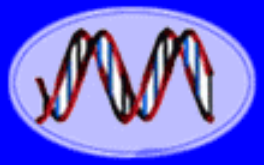
Canavan's Disease – *Defect In N-acetyl-L-aspartic Acid (NAA) Metabolism Which Causes Myelin Breakdown In Brain.*
1/10,000 Ashkenazi Jews



GREENBERG vs. MIAMI CHILDREN'S HOSPITAL RESEARCH INSTITUTE, INC.,

Parents Suing Over Patenting Of Genetic Test

They Say The Researchers They Assisted Are Trying To Profit From A Test For A Rare Disease.



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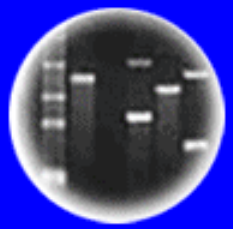

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The Factor VIII Story -- A Summary

1. Purify Small Amounts of Factor VIII
2. Obtain Partial or Complete Amino Acid Sequence
3. Use the Genetic Code to Synthesize Degenerate DNA Probes
4. Isolate Factor VIII DNA Clones Complementary to Probe in Genome Library
5. Determine if Factor VIII Clones Contain the Complete Gene By Sequencing and Comparing With Protein Sequence
6. If Not, "Walk" to Obtain Overlapping DNA Clones That Collectively Contain the Factor VIII Gene
7. Sequence Clones To Determine Where the Factor VIII Gene Starts and Stops
8. Use Factor VIII Genome Probe to Find Out What Body Organ/Tissue Expresses the Factor VIII Gene
9. Make a cDNA Library From the Target Organ/Tissue and Isolate a Factor VIII cDNA Clone
10. Sequence the Factor VIII cDNA Clone and Compare With Factor VIII Gene Sequence to Map its Anatomy (I.e., introns, exons, swtiches) and Ensure That it Contains the Complete Protein Coding Sequence
11. Use Factor VIII cDNA and/or Genome Fragments as a Probe to Find RFLP Markers For Disease Alleles -- Or Sequence Disease Alleles to Find Relevant RFLP Markers By Comparison With Wild-Type Sequence
12. Insert Factor VIII cDNA Into an Expression Vector and Synthesize Factor VIII Protein in Host Cells (e.g., Mammalian Cells)