HC70A & SAS70A
Winter 2023
Genetic Engineering in Medicine, Agriculture, and Law

Professors Bob Goldberg & John Harada
Bob Goldberg

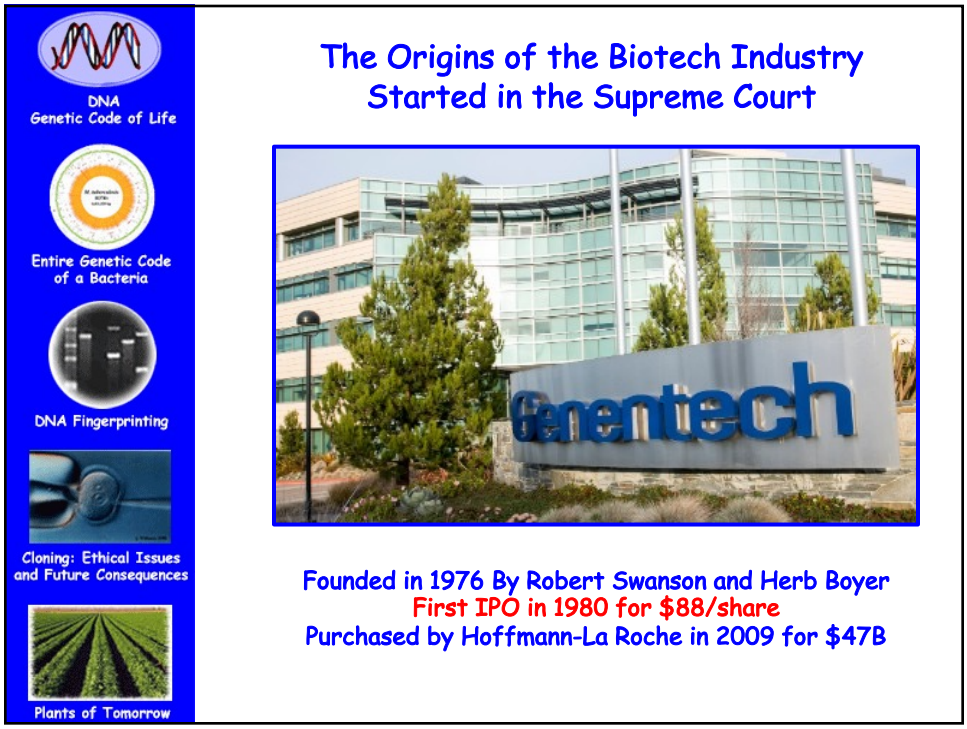
Lecture 5
How Are Genes Cloned & Engineered?
The Insulin and Factor XIII Stories

1

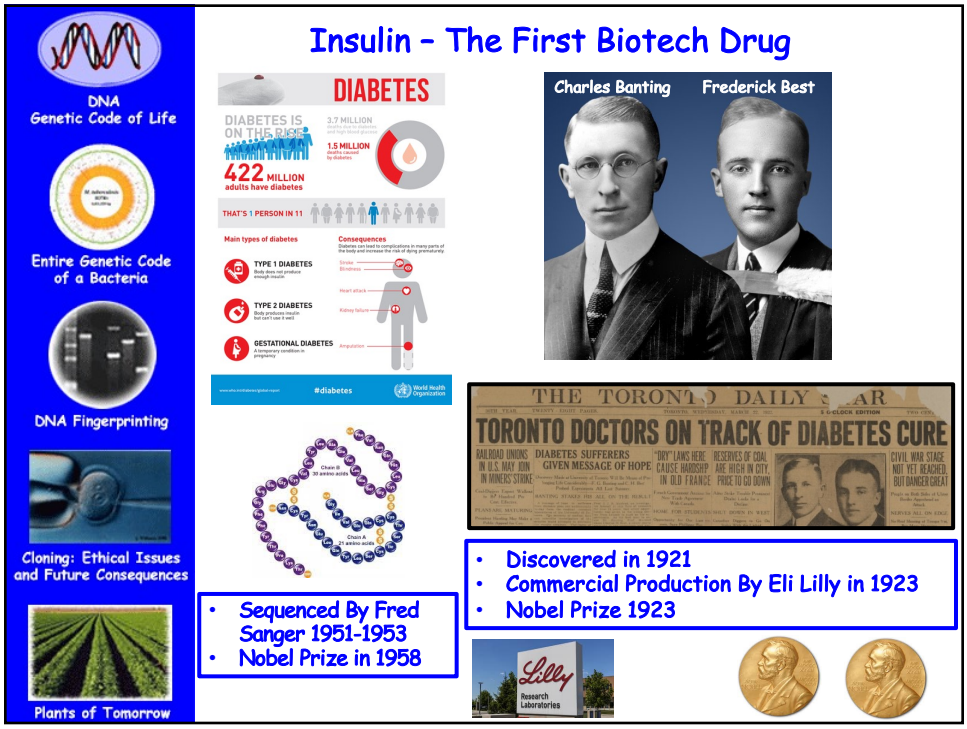
THEMES

1. How Did the Supreme Court Indirectly Give Rise to the Biotechnology Industry?
2. What Strategies Were Developed For Cloning Insulin mRNA and Expressing Insulin in Bacterial Cells? What Strategy "Won" Out?
3. What is Hemophilia and How is it Inherited?
4. How Can a Disease Gene Be Found When It is Not Known Where the Gene is Expressed?
5. What Vectors Can Be Used For Cloning DNA?
6. What is the Advantage of Using a Virus Vector For Constructing Genome Libraries?
7. How To Make a Library of the Human Genome?
8. How Find a Gene With Only a Knowledge of the Protein Sequence?
9. How Use DNA Testing to Detect Factor VIII Disease Alleles?
10. How Isolate a Factor VIII cDNA Clone?
11. Genomic vs. cDNA Libraries
12. How Produce Factor VIII Protein For Use as a Drug

2


DNA
Genetic Code of Life
Entire Genetic Code of a Bacteria
DNA Fingerprinting
Cloning: Ethical Issues and Future Consequences
Plants of Tomorrow
The Origins of the Biotech Industry Started in the Supreme Court
Genentech
Founded in 1976 By Robert Swanson and Herb Boyer
First IPO in 1980 for $88/share
Purchased by Hoffmann-La Roche in 2009 for $47B

3


DNA
Genetic Code of Life
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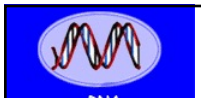
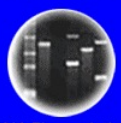
Plants of Tomorrow
Insulin – The First Biotech Drug
DIABETES
422 MILLION adults have diabetes
1.5 MILLION
THAT'S 1 PERSON IN 11
TYPE 1 DIABETES
TYPE 2 DIABETES
GESTATIONAL DIABETES
#diabetes
Charles Banting
Frederick Best
TORONTO DOCTORS ON TRACK OF DIABETES CURE
• Sequenced By Fred Sanger 1951-1953
• Nobel Prize in 1958
• Discovered in 1921
• Commercial Production By Eli Lilly in 1923
• Nobel Prize 1923
Lilly
Research Laboratories

4



DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria



DNA Fingerprinting

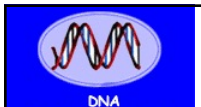
Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

Reasons For Insulin Being the First Biotech Drug

- Diabetes a Major Disease Responsible For Millions of Deaths
- Physiological Basis of the Disease Known
- Site and Mechanism of Insulin Synthesis and Secretion Within the Pancreas Known
- Insulin Was Purified and Amino Acid Sequence Known
- Small Protein Consisting of 51 Amino Acids
- Insulin Protein Structure Understood 110 amino acids Total - A Chain 21 Amino Acids and B Chain 30 Amino Acids)
- Predicted Small Size of mRNA (~390 nts) and Gene
- Insulin Made in Large Quantities in the Pancreas
- Techniques For Cloning mRNA Using Reverse Transcriptase Or Direct DNA Synthesis Known

5



DNA
Genetic Code of Life

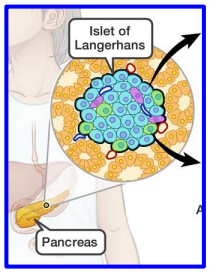
Entire Genetic Code of a Bacteria

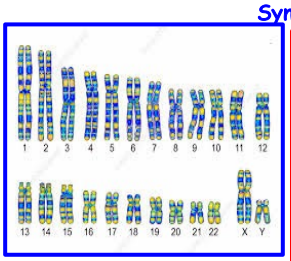
DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

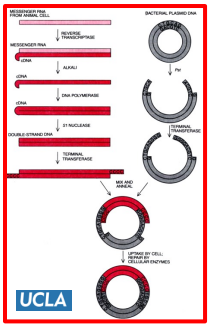
Plants of Tomorrow

Two Strategies For Isolating the Insulin "Gene" And Engineering *E. coli* Cells to Produce Human Insulin



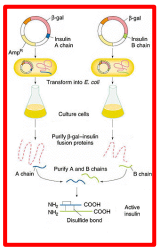


Synthesize & Clone cDNA



Use cDNA/mRNA Sequence

Direct Synthesis and Cloning of A Chain & B Chain cDNAs Separately



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The Winning Strategy Used For Synthesizing Human Insulin in E. coli Cells
β-gal
Insulin A chain
AmpR
β-gal
Insulin B chain
Transform into E. coli
Culture cells
Purify β-gal–insulin fusion proteins
A chain
Purify A and B chains
B chain
NH2
COOH
NH2
COOH
Disulfide bond
Active insulin
Each Chain Made Directly in Separate E. coli Cells
Combined After Synthesis to Make Recombinant Insulin
Note: E. coli cannot process a Pre-Insulin Protein
Genentech
Lilly
Research Laboratories
$30B per Year Market!

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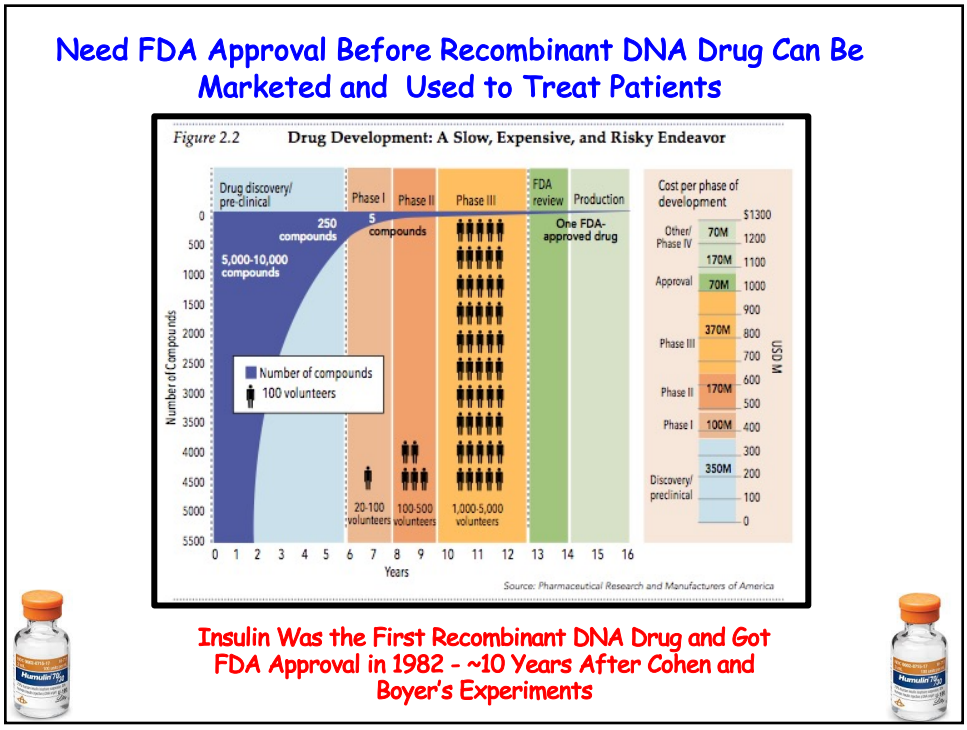
Need FDA Approval Before Recombinant DNA Drug Can Be Marketed and Used to Treat Patients
Figure 2.2 Drug Development: A Slow, Expensive, and Risky Endeavor
Drug discovery/ pre-clinical
Phase I
Phase II
Phase III
FDA review
Production
250 compounds
5 compounds
One FDA-approved drug
5,000-10,000 compounds
Number of compounds
100 volunteers
20-100 volunteers
100-500 volunteers
1,000-5,000 volunteers
Number of Compounds
Years
Source: Pharmaceutical Research and Manufacturers of America
Cost per phase of development
Other/ Phase IV
Approval
Phase III
Phase II
Phase I
Discovery/ preclinical
70M
170M
70M
370M
170M
100M
350M
USD M
Insulin Was the First Recombinant DNA Drug and Got FDA Approval in 1982 - ~10 Years After Cohen and Boyer's Experiments

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The Factor VIII Story is Different and More Complex Than the Insulin Story

The Molecular Genetics of Hemophilia

Hemophiliacs bleed because a defective gene deprives them of a key blood-clotting protein. The protein has now been made artificially by isolating the normal gene and then inserting it into cultured cells

by Richard M. Lawn and Gordon A. Vehar

Sold to Roche For $47B in 2009
Revenue of $26B in 2020

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

First Reference to Hemophilia is in Hebrew Scripture

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.

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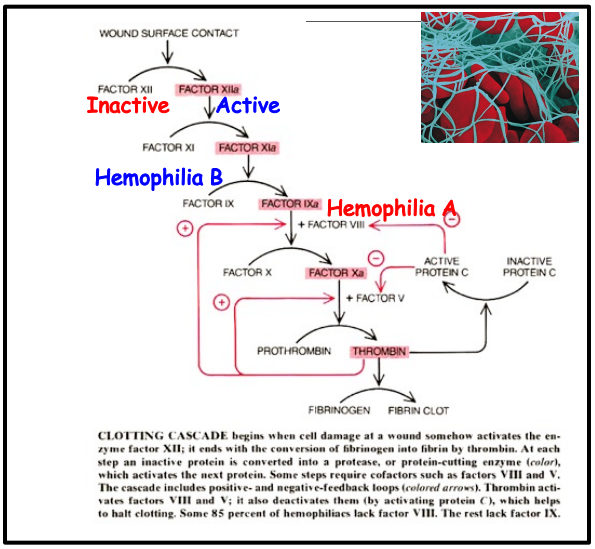
Protein Factors in Blood Lead To Clotting



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.

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 (Loss of Function) ?

↓

No Blood Clot!

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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%

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

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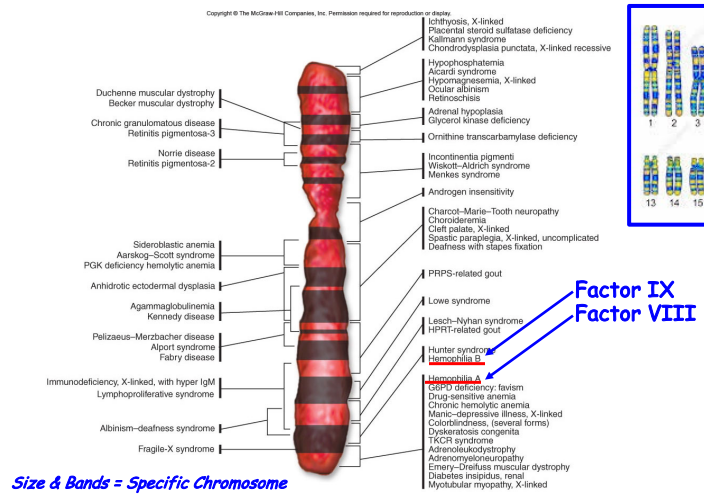
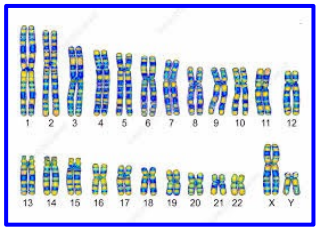
Factor VIII and Factor IX Genes are Closely Linked on the X Chromosome





Nature, March, 2005

The X chromosome has ~1098 Genes and 150,000,000 bp (150 Mb).
168 Mendelian Diseases Explained by 113 X-Linked Genes

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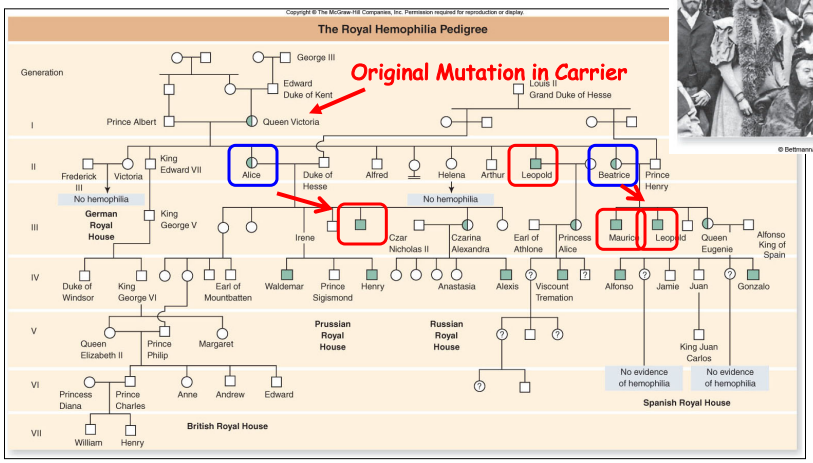
Hemophilia A and B Genes Are Sex Linked & Recessive Traits

© Bettmann/Corbis



Note: 1. Males Obtain Detective Gene From Mothers

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

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What Was Known About Factor VIII
Before Gene Cloned?

- Blood Protein (But Perhaps Synthesized Elsewhere!)
- Not Known Where Site of Synthesis Was
- Could Be Purified In Small Amounts From >20 Liters Of Human Blood + Cow Blood + Pig Blood
- Known Protein Sequence!
- Hemophilia A Could Be Treated By *Blood Transfusions* From Normal Individuals, ∴ Clotting Factor In Blood
- 1980s Aids Epidemic Caused Many Hemophiliacs To Get HIV/AIDs (~50% Of Hemophiliacs Got Aids In 1985)
  - ∴ How To Go From Protein To Gene

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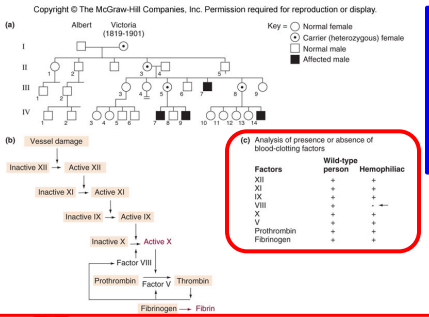
The Problem!!

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

Early 1980's

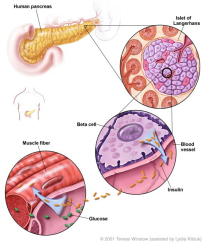


Key Concept

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

Key: Protein Sequence Known

Different Than Insulin Knew Where Protein Made!



How Find Gene & cDNA?

Protein → Gene → mRNA → Drug !

mRNA ⟶ Drug

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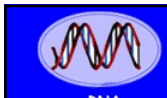
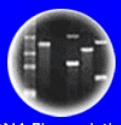
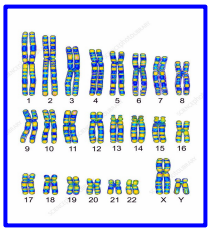


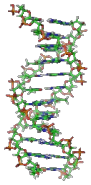


Step One

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

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





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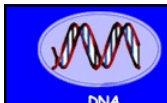
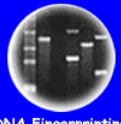
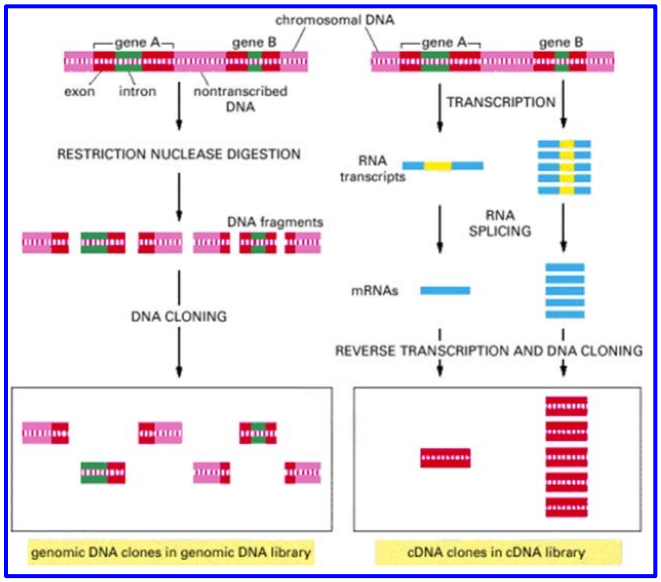




Genomic Libraries vs. cDNA Libraries



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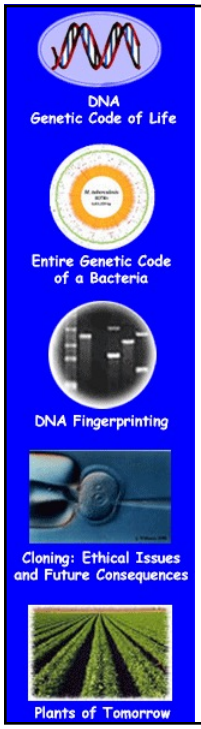

Vectors Used in Genetic Engineering Have Similar Conceptual Properties But are Used in Different Situations

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

Properties of All Vectors

1. Replicate
2. Selectable
3. Can Be Used To Insert Foreign Genes/Restriction Sites
4. Easily Isolated + Transferred Back To Cells

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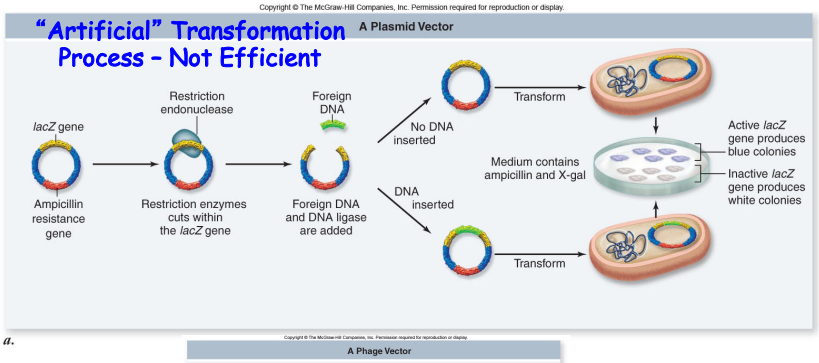
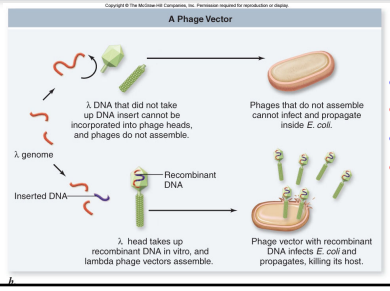
Plasmid vs. Bacteriophage Vectors for Cloning DNA Fragments

"Artificial" Transformation Process – Not Efficient



a.

"Natural" Infection Process



b.

Advantages

- Much More Efficient
- Can Use Less DNA
- Get Lots More Clones
- Need Lots of Clones For Large Genome

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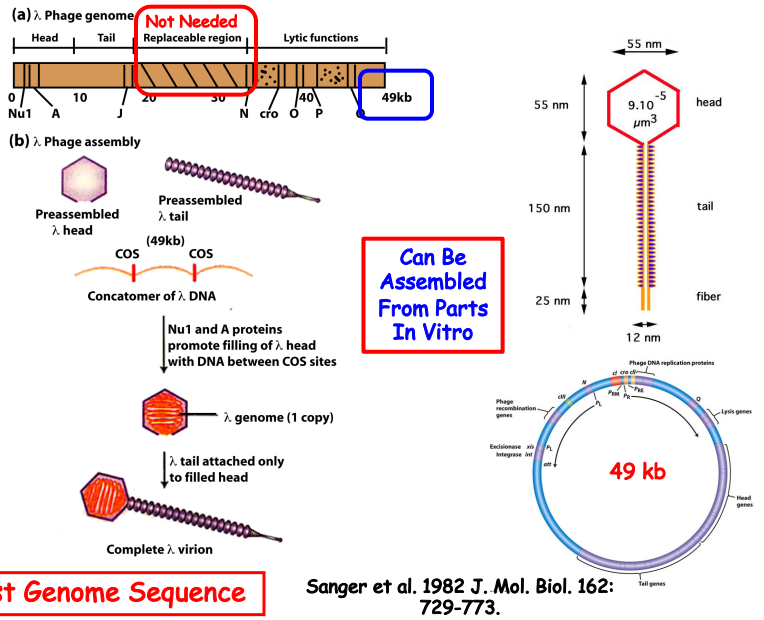
Structure of the λ Phage and Its Genome



Can Be Assembled From Parts In Vitro

First Genome Sequence

Sanger et al. 1982 J. Mol. Biol. 162: 729-773.

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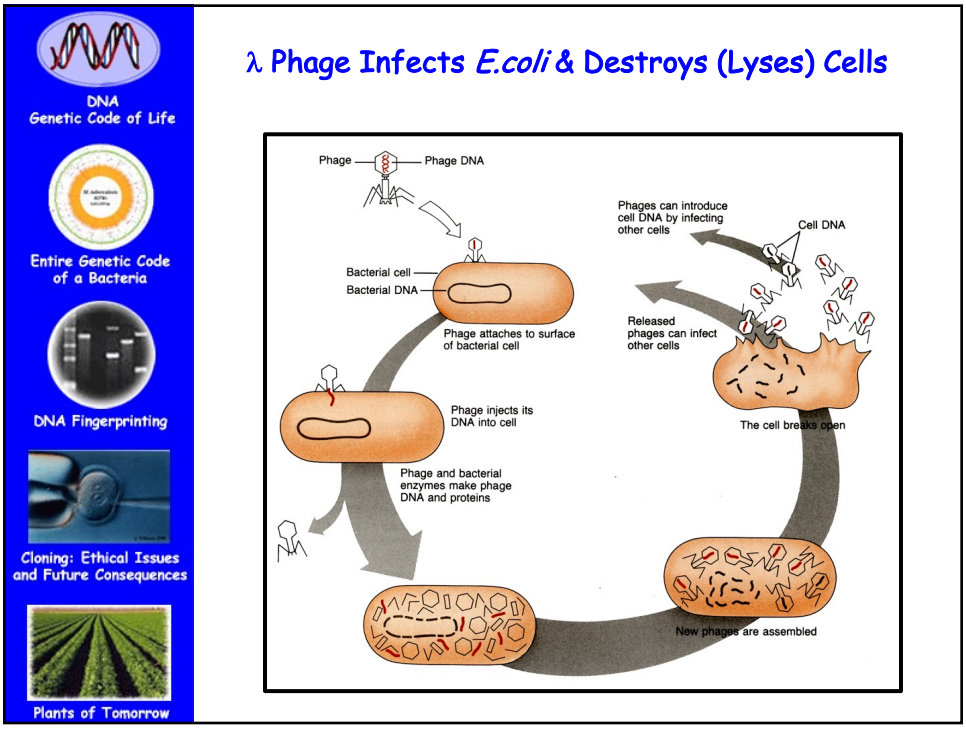
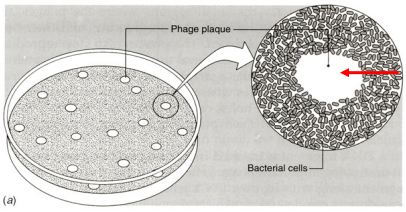


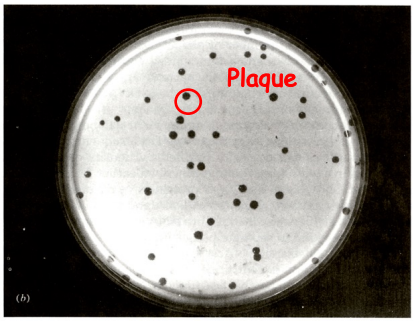
λ Phage Infects *E.coli* & Destroys (Lyses) Cells

22

Lysed Cells Can Be Seen as Clear Plaques on Agar Plates



Clear Plaque
Virus Particles
+ Dead Bacteria Cells



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

2. Selectable Marker is Bacterial Cell Destruction & Plaque Formation

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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!

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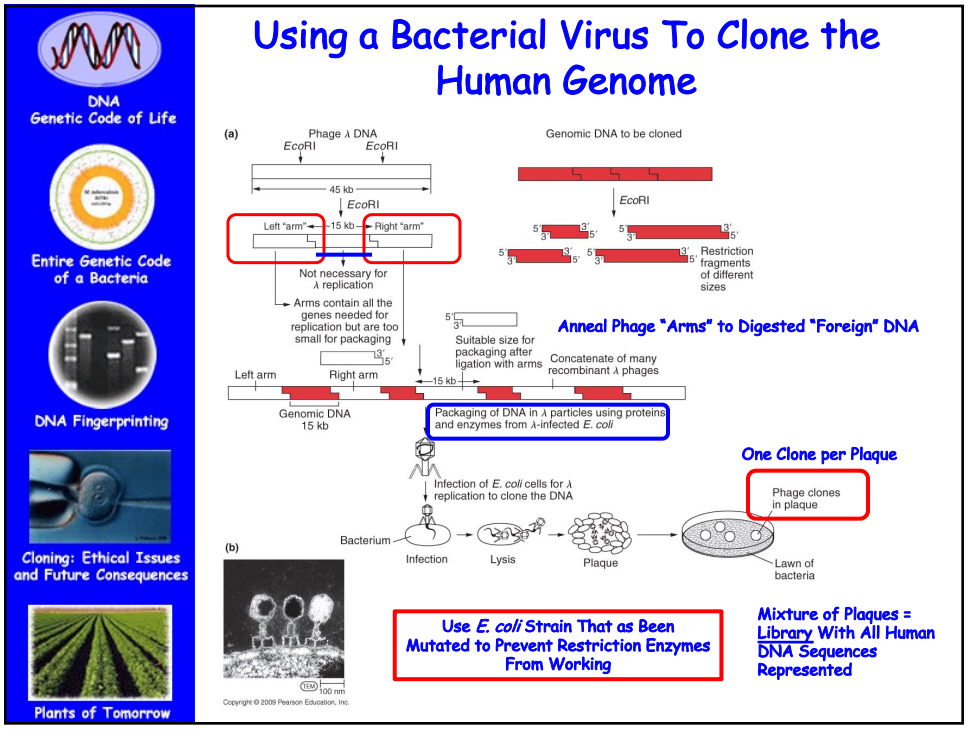
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Genetic Code of Life
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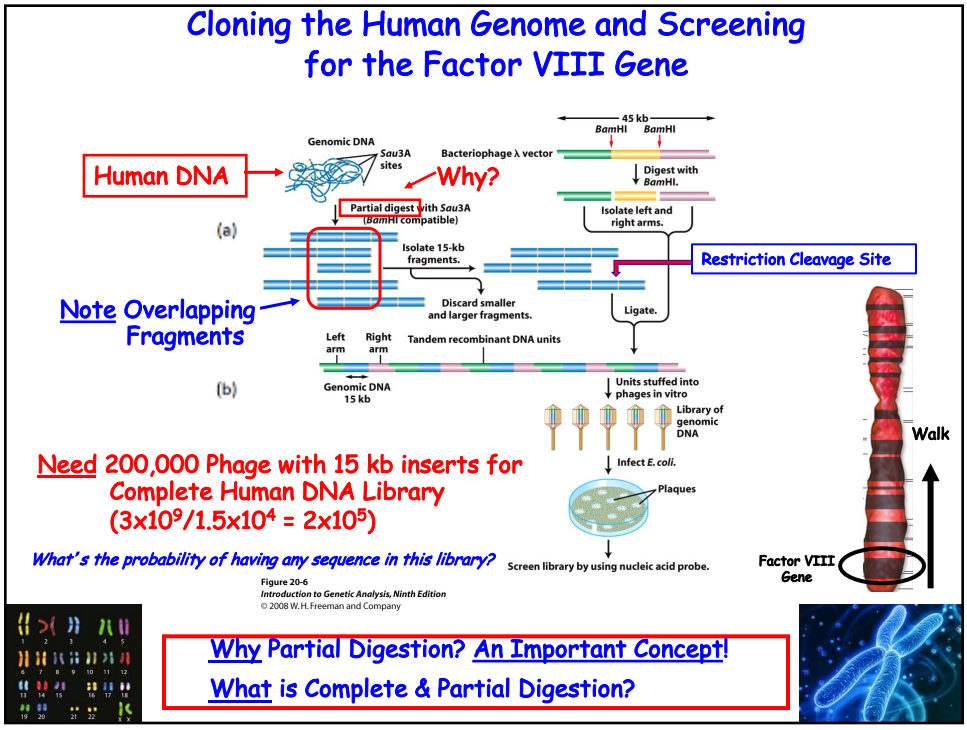
Cloning: Ethical Issues and Future Consequences
Plants of Tomorrow
Using a Bacterial Virus To Clone the Human Genome
(a)
Phage λ DNA
EcoRI
EcoRI
45 kb
EcoRI
Left "arm"
15 kb
Right "arm"
Not necessary for λ replication
Arms contain all the genes needed for replication but are too small for packaging
Genomic DNA to be cloned
EcoRI
Restriction fragments of different sizes
Anneal Phage "Arms" to Digested "Foreign" DNA
Suitable size for packaging after ligation with arms
Concatenate of many recombinant λ phages
Left arm
Right arm
15 kb
Genomic DNA
15 kb
Packaging of DNA in λ particles using proteins and enzymes from λ-infected E. coli
One Clone per Plaque
Infection of E. coli cells for λ replication to clone the DNA
Phage clones in plaque
Bacterium
Infection
Lysis
Plaque
Lawn of bacteria
(b)
TEM
100 nm
Copyright © 2009 Pearson Education, Inc.
Use E. coli Strain That as Been Mutated to Prevent Restriction Enzymes From Working
Mixture of Plaques = Library With All Human DNA Sequences Represented

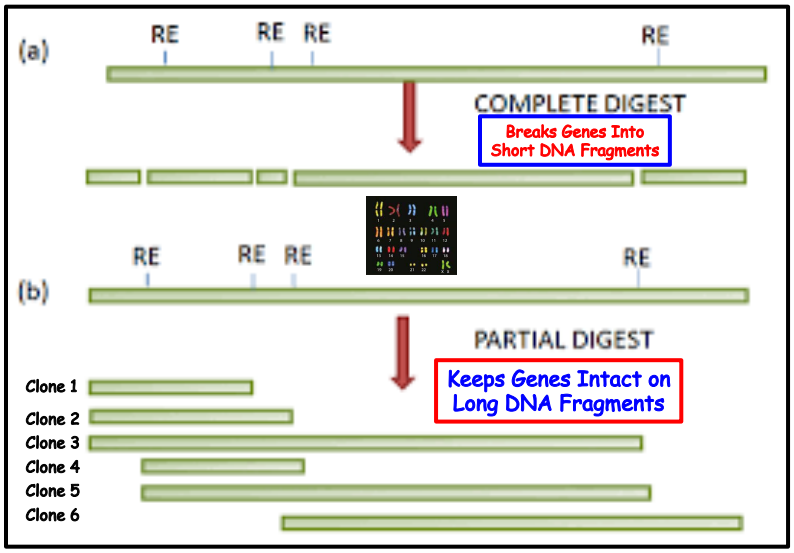
25


Cloning the Human Genome and Screening for the Factor VIII Gene
Human DNA
Genomic DNA
Sau3A sites
Why?
Bacteriophage λ vector
45 kb
BamHI
BamHI
Digest with BamHI.
Isolate left and right arms.
Partial digest with Sau3A (BamHI compatible)
(a)
Isolate 15-kb fragments.
Restriction Cleavage Site
Note Overlapping Fragments
Discard smaller and larger fragments.
Ligate.
Left arm
Right arm
Tandem recombinant DNA units
(b)
Genomic DNA
15 kb
Units stuffed into phages in vitro
Library of genomic DNA
Walk
Need 200,000 Phage with 15 kb inserts for Complete Human DNA Library
(3x10⁹/1.5x10⁴ = 2x10⁵)
Infect E. coli.
Plaques
What's the probability of having any sequence in this library?
Screen library by using nucleic acid probe.
Factor VIII Gene
Figure 20-6
Introduction to Genetic Analysis, Ninth Edition
© 2008 W.H. Freeman and Company
Why Partial Digestion? An Important Concept!
What is Complete & Partial Digestion?

26

Partial Digestion Permits "Walking" From One DNA Region to the Next

Iterative Process of Screening & Rescreening Human Genome Library



27

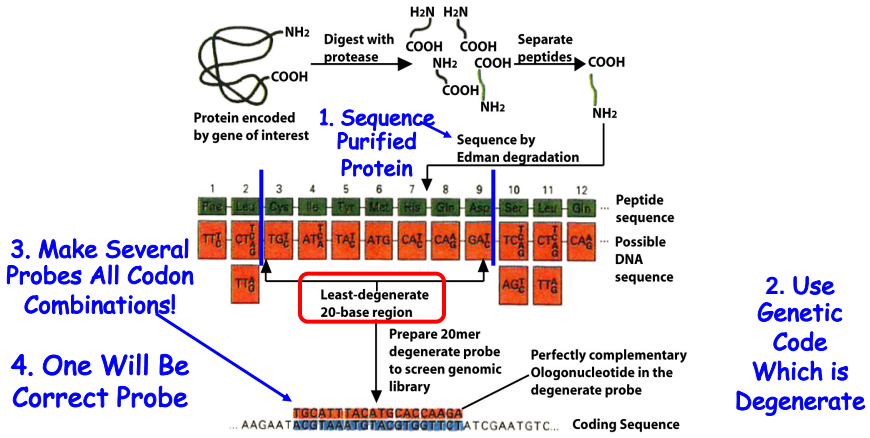
Step Two

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

28

Factor VIII Protein → Gene

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

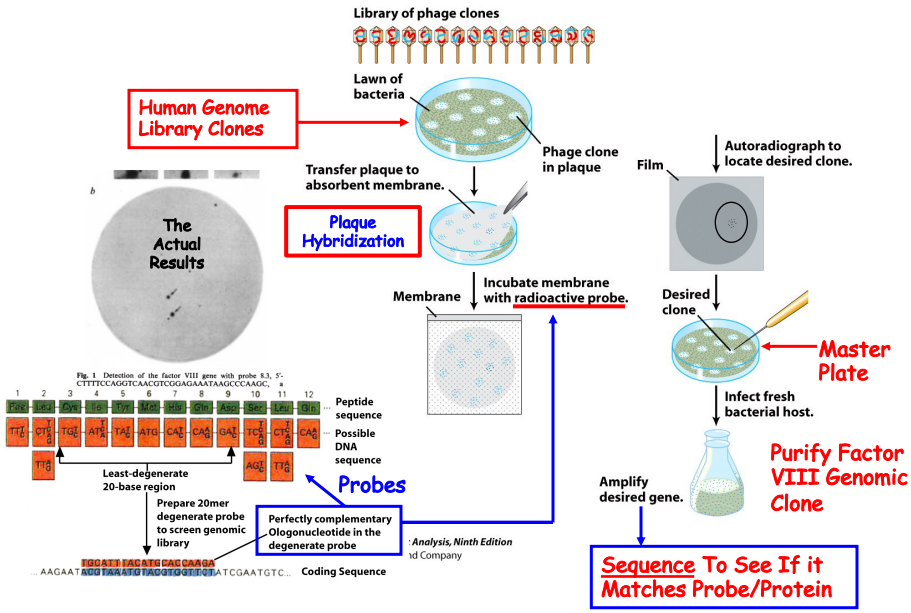


How many Combinations of Synthetic Probes?

2x3x2x1x2x2x2=96

29

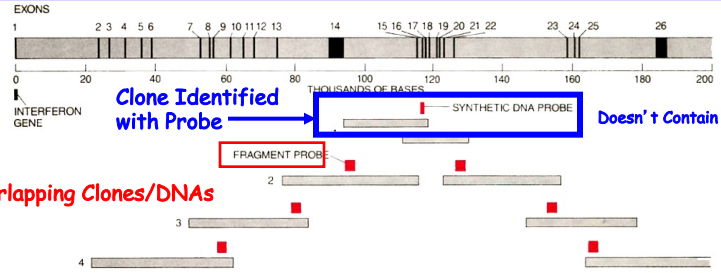
Finding The Factor VIII Gene Or Part of Gene!!



Sequence To See If it Matches Probe/Protein

30

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

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Step Three

Finding the Entire Factor VIII Gene? Walking & Sequencing

Walking Up and Down Genes and Chromosomes

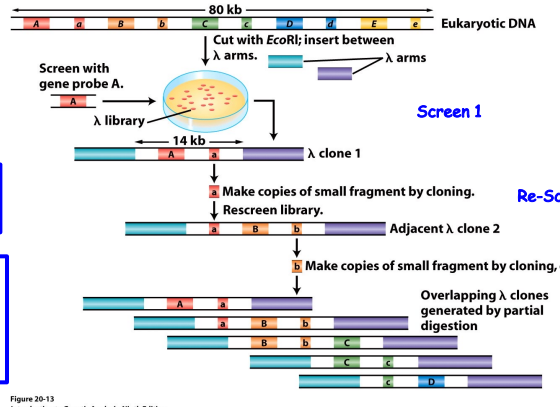

Figure 20-13
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Iterative Library Screening Process

Find Overlapping Clones By Restriction Site Mapping

Re-Screen 3,4,5,etc. Until Entire Gene Found By *Comparing Amin Acid Sequence to DNA Sequence*

Basis of Genome Projects & Whole Genome Sequencing

Key Concepts → How know Find Complete Factor VIII Gene?

→ Compare Protein & DNA Sequences

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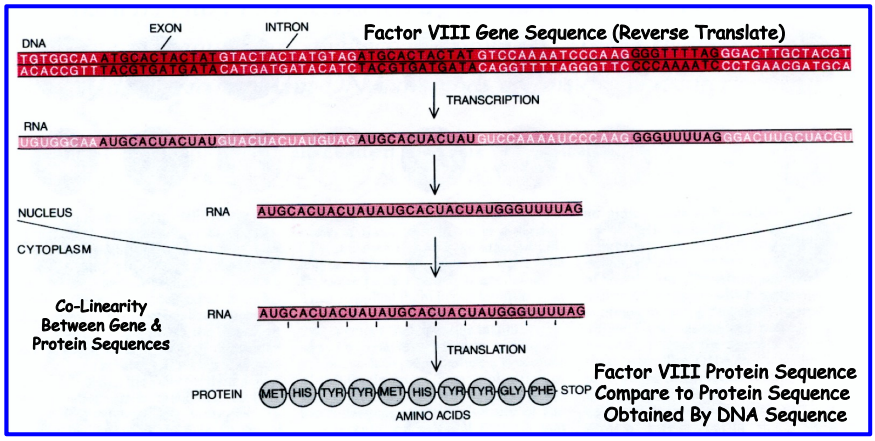
How Find Entire Factor VIII Gene?

1. Sequence Overlapping DNA Fragments



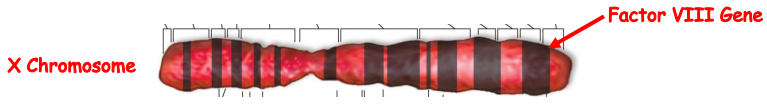
2. Know Factor VIII Protein Sequence & Use Genetic Code

3. Sequence Overlapping Clones Until DNA Sequence Stops Matching Protein Sequence

33

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



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Factor VIII SNP Mutations Occur Throughout the Gene

[*Haemophilia* 11, 481-491 (2005)] *Larger the Gene - Larger Number of Mutations!*

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

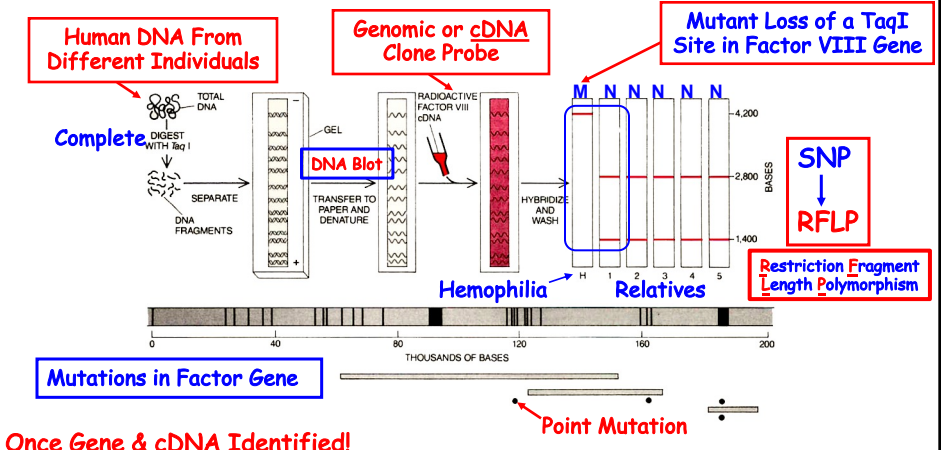
VIII:C (%)	Family history	Consanguinity*	Inversion	Codon†	Mutation	Amino acid change	Exon	Conservation‡
1	Sporadic	NC	Normal	51	TTT → TCT§	Phe → Ser	2	FFFF, identical
1.20	Sporadic	NC	Normal	80	GTT → GAT	Val → Asp	3	VVVV, identical
1	Sporadic	NC	Normal	102	GGT → GTT§	Gly → Val	3	GGGG, identical
2	Sporadic	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
1	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	delCT§	Val → fs (TGA-1517)	14	
1	Sporadic	NC	Normal	1533	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	1833	TGG → TGA§	Trp → Stop	16	WWWW, identical
2.60	Sporadic	C	Normal	1882	ATC → ATA§	Ile → Ile	17	III, identical
1	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

35

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



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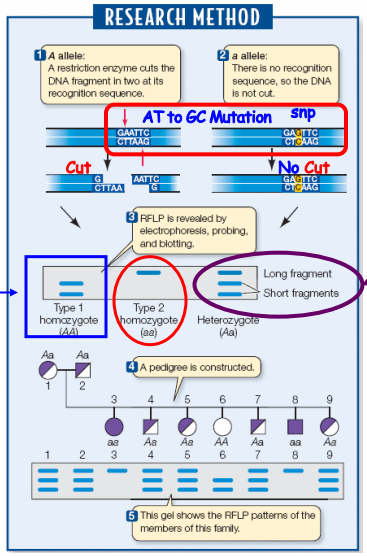
Genetic Diseases Can Also Be Followed in Families Using DNA Methods (e.g., PCR) & Pedigrees – With DNA Markers Linked to the Disease Phenotype



Essential HC70A Concept!

DNA Fingerprints Gene "X"

Mutation in Restriction Enzyme Site = RFLP or Restriction Fragment Length Polymorphism

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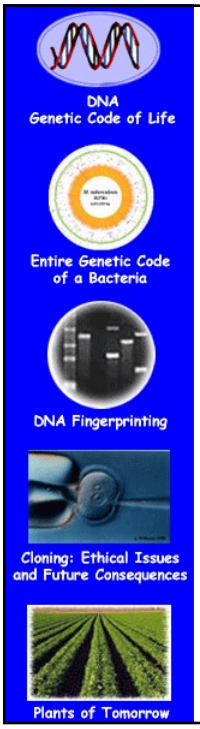
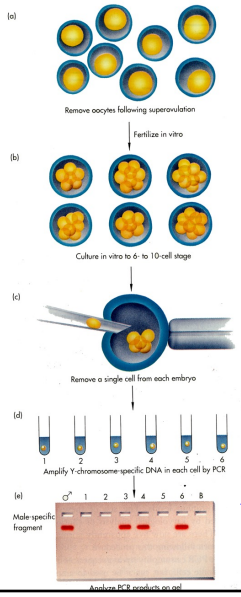
PCR & RFLPs Can Be Used To Analyze Human Genes in A Single Embryo Cell



PGT
Pre-Implantation Genetic Testing

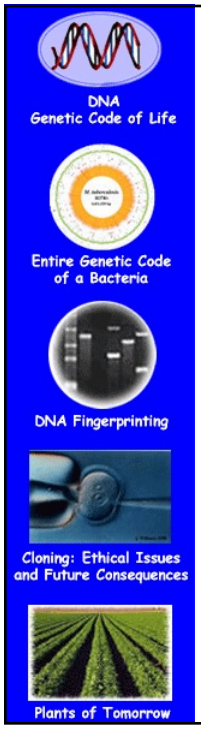
PGT-A
Chromosome #
&
PGT-M
Genes



What is The Implication of This Procedure Considering That The Human Genome Has Been Sequenced?

Sex Determination in 8-cell Embryo!

38



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!

Introns! Switches!

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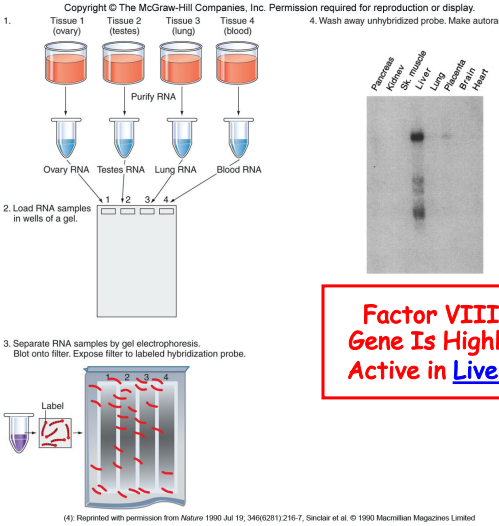
Making the Drug

Need cDNA Not Gene

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



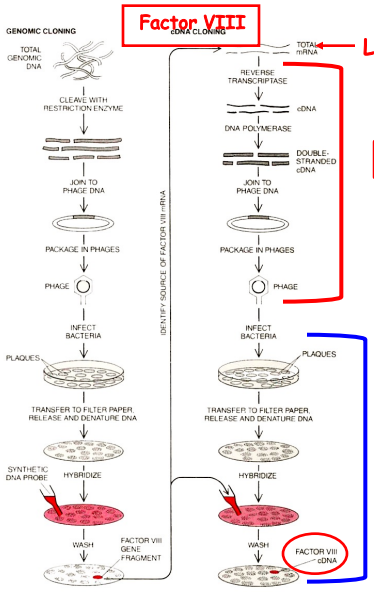
RNA Blot Is Like a DNA Blot Except That RNA is on Gel & Blotted

Factor VIII Gene Is Highly Active in Liver!

Can Also Use PCR (RT-PCR)

40

Using Factor VIII Gene Probe to Identify Factor VIII cDNA clone



Construct Liver cDNA Library

Factor VIII cDNA → Used to Make Drug!
Not Gene!!

Screen Liver cDNA Library

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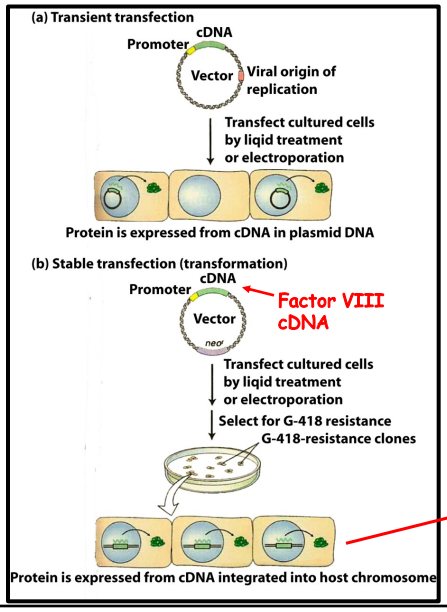
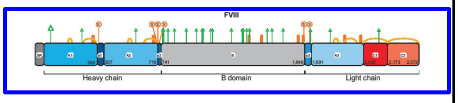
Engineer Factor VIII cDNA to Produce Protein in Host Cell & Synthesize Factor VIII in Mammalian Cells



Why Mammalian Cells?



Purify Factor VIII Protein!

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Recombinant Factor VIII

Bayer Biological Products EU

Bayer HealthCare
Biological Products Division
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- Recombinant Factor VIII
- More Resources
- Haemophilia Centres in Europe

Related Links
- Haemo-QoL Project
- 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.

Replacement Therapy 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)

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Gene Therapy – A Permanent "Cure"

SCIENTISTS WELCOME $3.5-MILLION DRUG — BUT QUESTIONS REMAIN

Haemophilia gene therapy could save lives. But it cannot treat the most common form of the disease.

Factor IX – Hemoglobin B FDA-Approved 2022

Gene Therapy for Hemophilia A

The First Ever In-Human Gene Editing Will Try and Combat Hemophilia

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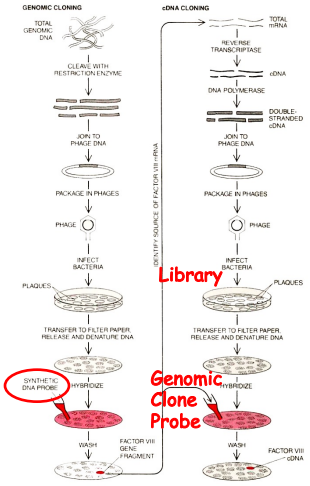
Summary of Steps Required to Clone Factor VIII Gene and cDNA

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!

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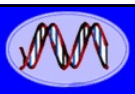
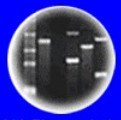


DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

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)

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