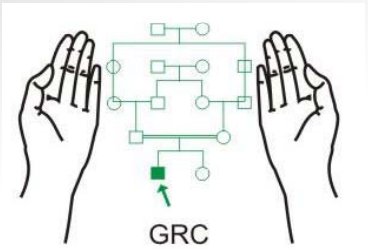
Haemoglobin Research and its Contribution to Evolution of Molecular Medicine

Maj Gen (R) Suhaib Ahmed, HI (M)
MBBS; MCPS; FCPS; PhD (London)

Genetics Resource Centre (GRC)
Rawalpindi

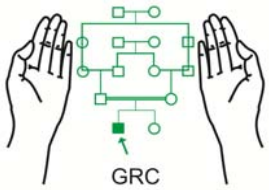


www.grcpk.com

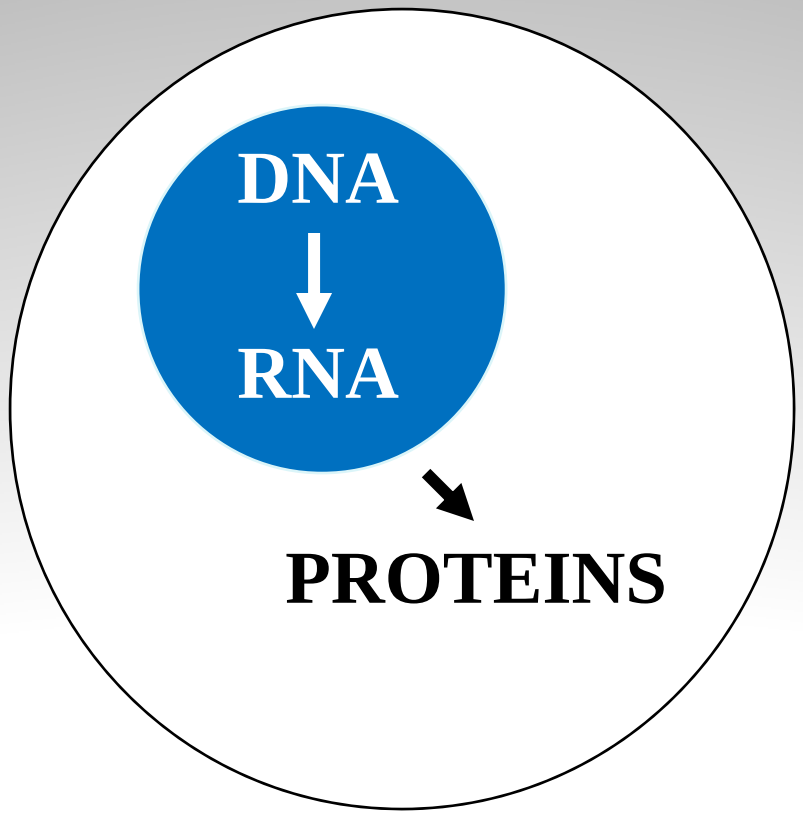
19th Century: Chemistry

20th Century: Physics

21st Century: Biology



The Molecules of Life



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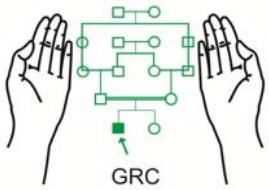
Haemoglobin: History

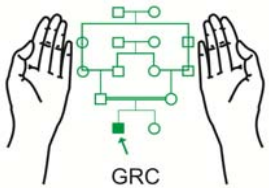
- Kuster: Structure of Haem (1912)
- Adair: Molecular weight of Haemoglobin (1925)
- Svedberg: Haemoglobin: a protein (1927)
- Pauling: Oxygen binds to Haem (1936)
- Pauling: Electrophoretic mobility of Hb-S (1949)
- Ingram: Globin chains of Haemoglobin (1956)



Linus Pauling & Harvey Itano

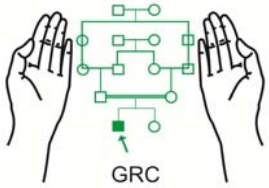
Sickle Cell Anemia: a Molecular Disease

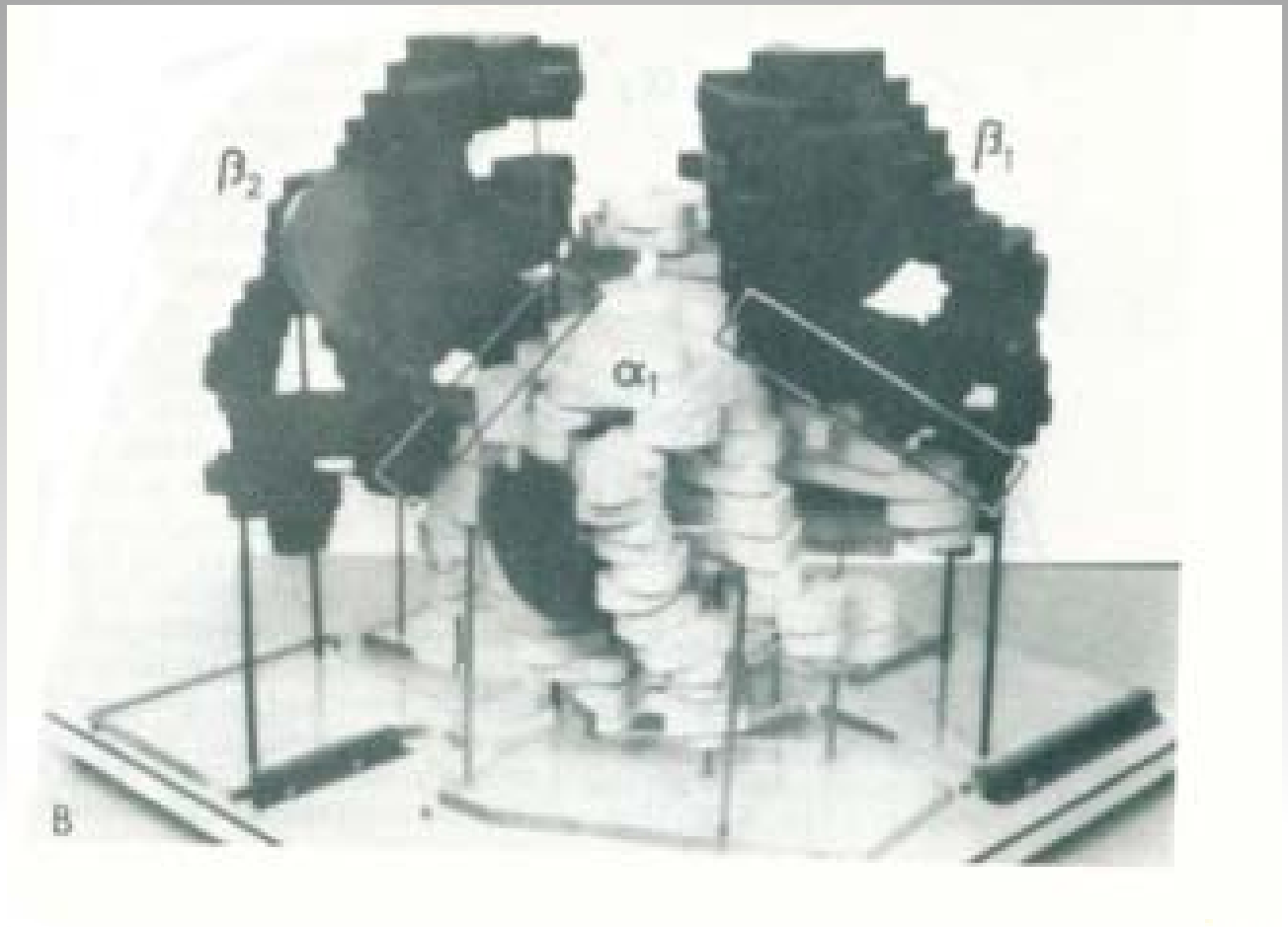
Science November 1949



Max Perutz

Making a discovery is like falling in love and getting to the top of a mountain all in one. When you get to the top after a hard climb, a view of a new landscape opens before you.





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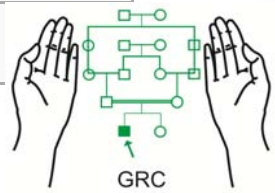
Har Gobind Khorana

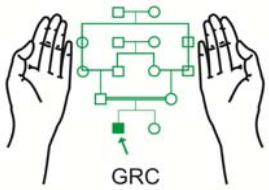
Born	January 9, 1922 (age 89) Raipur (Khanewal), Punjab, (now Pakistan)
Residence	USA
Nationality	American
Fields	Molecular Biology
Alma mater	University of Liverpool (Ph.D.) University of the Punjab (B.S.)(M.S.)
Known for	First to demonstrate the role of Nucleotides in protein synthesis
Notable awards	Nobel Prize in Medicine (1968)



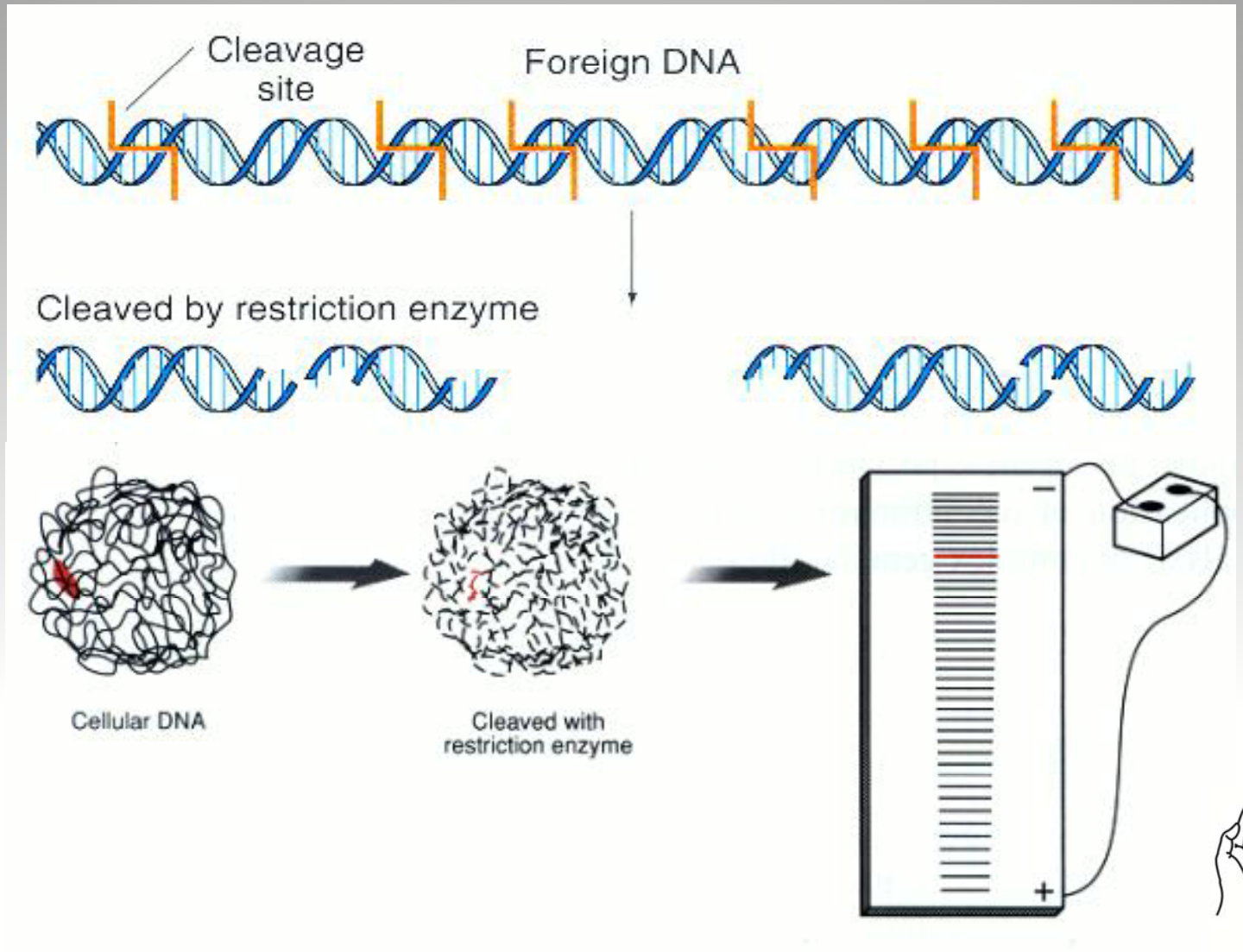
Detection of the Sickle Gene in the Human Fetus — Potential for Intrauterine Diagnosis of Sickle-Cell Anemia

Yuet Wai Kan, M.B., B.S., Andrée M. Dozy, M.T., Blanche P. Alter, M.D., Fredric D. Frigoletto, M.D., and David G. Nathan, M.D.

N Engl J Med 1972; 287:1-5



Southern EM, J Mol Biol 1975; 98: 503



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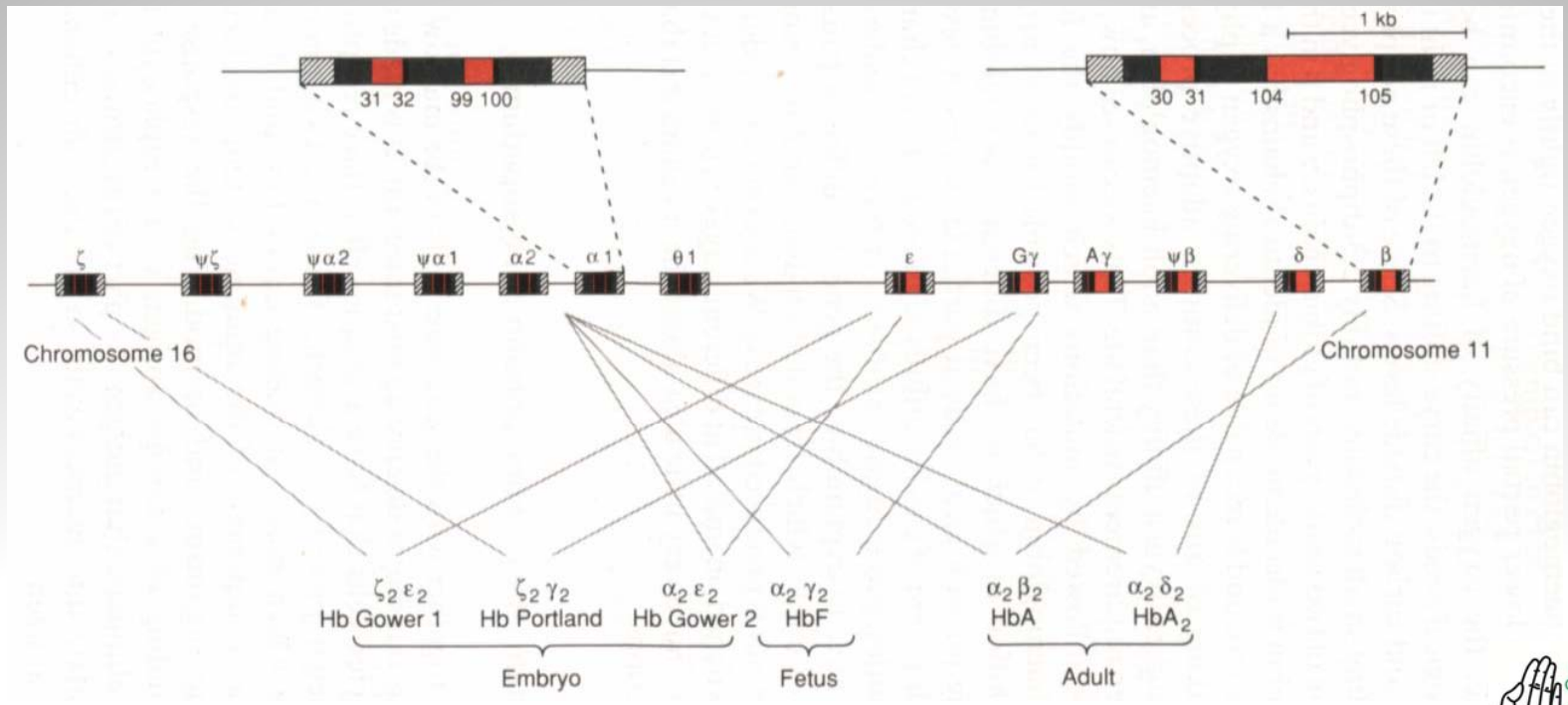
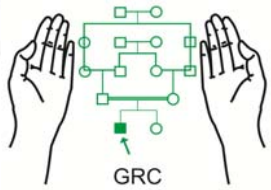
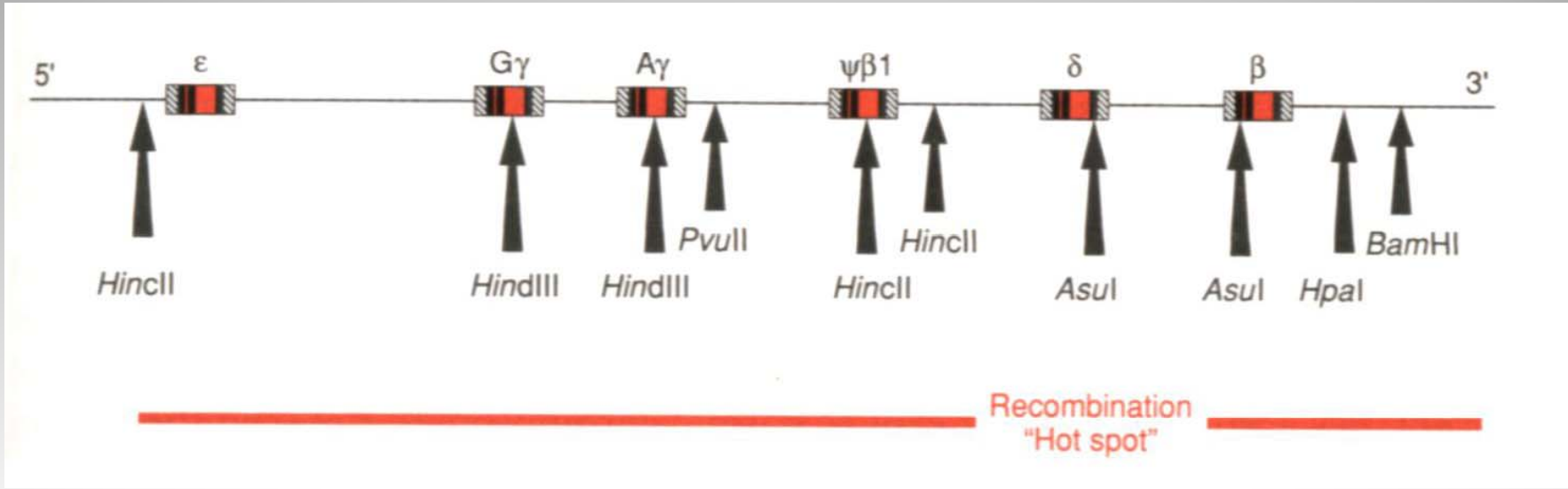
Globin Genes

- Alpha Globin Gene (Deisseroth et al (1977)
- Beta Globin Gene (Deisseroth et al (1978)





Linkage map of Globin Genes



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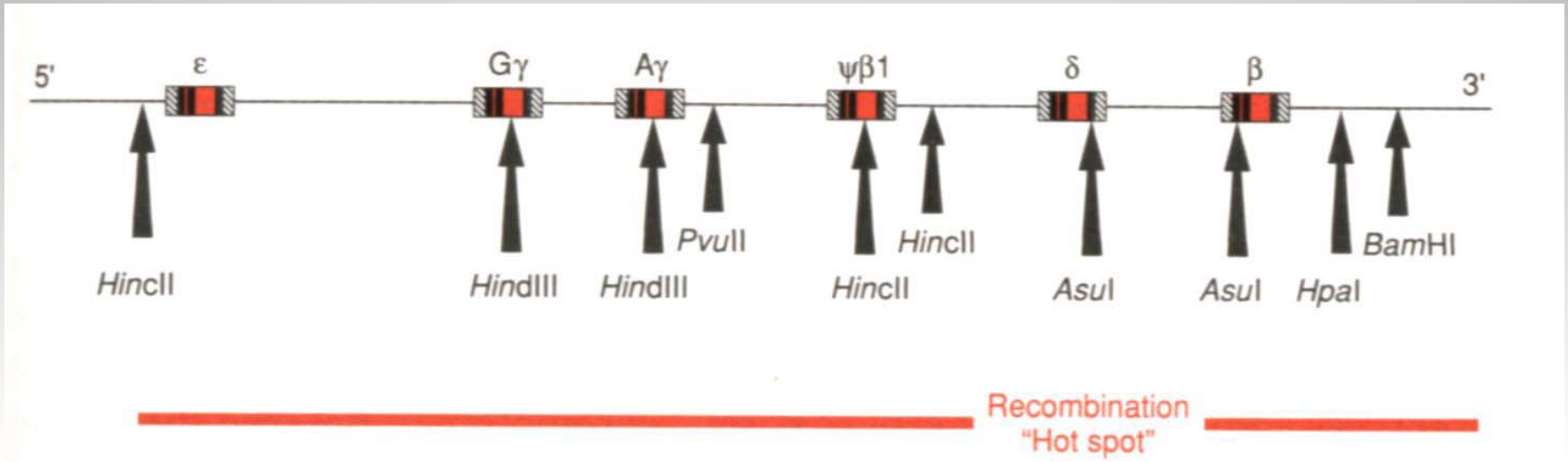
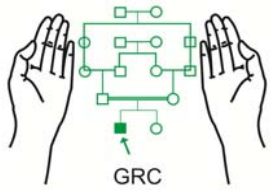
Deletion causing β-Thalassaemia

- Flavel et al, Nucleic Acid Res 1979; 6: 2749
- Orkin et al, Proc Nat Acad Sci USA 1979; 76: 2400





DNA Sequencing

3’-GAGTCGTACGCTACCATGTCATATCAACCATAGTTCCGATCCTAGTACTAAC-5’

5’-CATGCGATGGTACAGTATAG-3’→

3′-GAGTCGTACGCTACCATGTCATATCAACCATAGTTCCGATCCTAGTACTAAC-5′

5′-CATGCGATGGTACAGTATAG-**ddTTP**

5′-CATGCGATGGTACAGTATAGT-**ddTTP**

5′-CATGCGATGGTACAGTATAGTT-**ddGTP**

5′-CATGCGATGGTACAGTATAGTTG-**ddGTP**

5′-CATGCGATGGTACAGTATAGTTGG-**ddTTP**

5′-CATGCGATGGTACAGTATAGTTGGT-**ddATP**

5′-CATGCGATGGTACAGTATAGTTGGTA-**ddTTP**

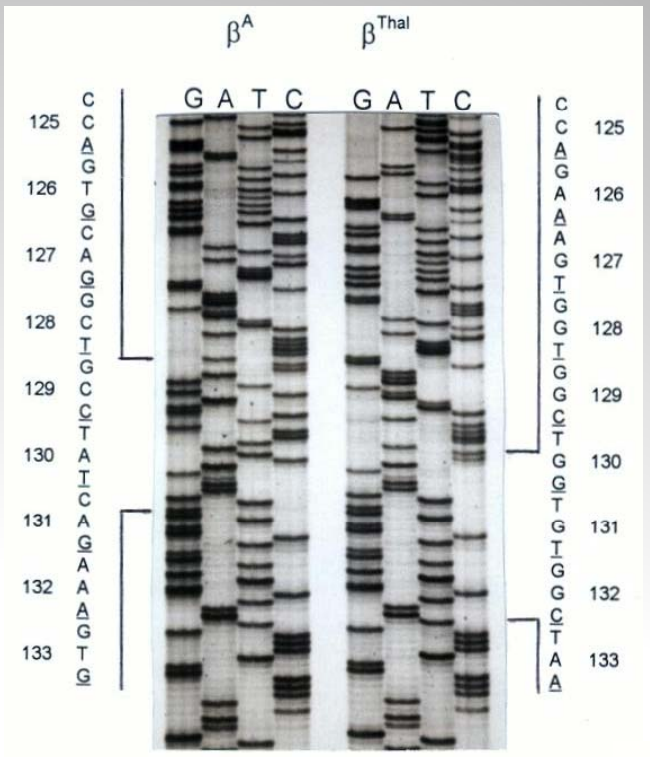
5′-CATGCGATGGTACAGTATAGTTGGTAT-**ddCTP**

5′-CATGCGATGGTACAGTATAGTTGGTATC-**ddATP**

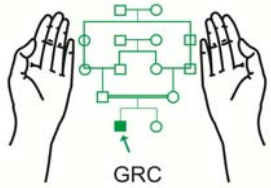
5′-CATGCGATGGTACAGTATAGTTGGTATCA-**ddATP**

5′-CATGCGATGGTACAGTATAGTTGGTATCAA-**ddGTP**

5′-CATGCGATGGTACAGTATAGTTGGTATCAAG-**ddGTP**

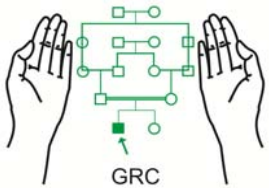


Sanger et al, 1977

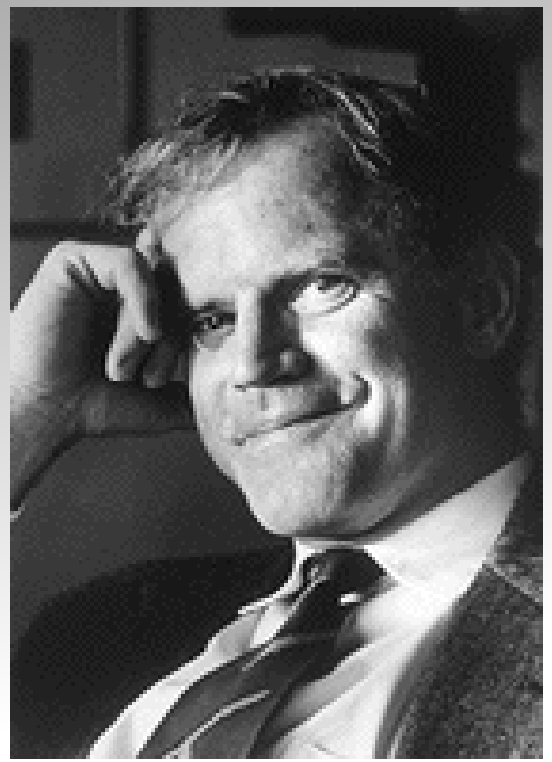


Point mutations causing β-Thalassaemia

- Orkin et al, Proc Nat Acad Sci USA 1980; 77: 3558
- Kazazian et al, EMBO Journal 1984; 3: 593



Kary Mullis



In 1983 Mullis while driving his vehicle late one night with his girlfriend had the idea to use a pair of primers to bracket the desired DNA sequence and to copy it using DNA polymerase, a technique which would allow a small strand of DNA to be copied almost an infinite number of times.

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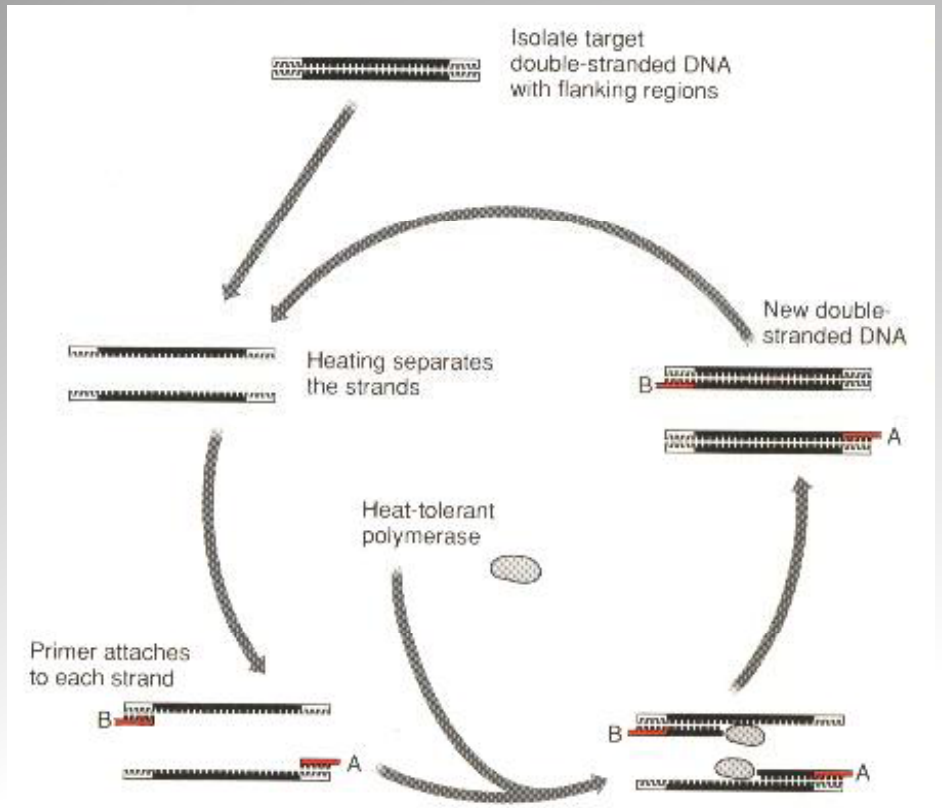
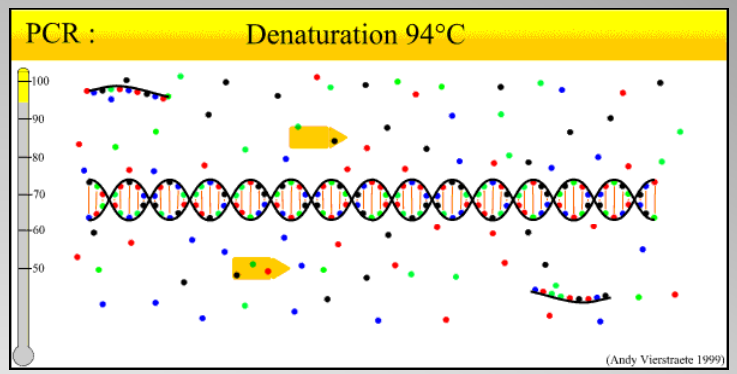
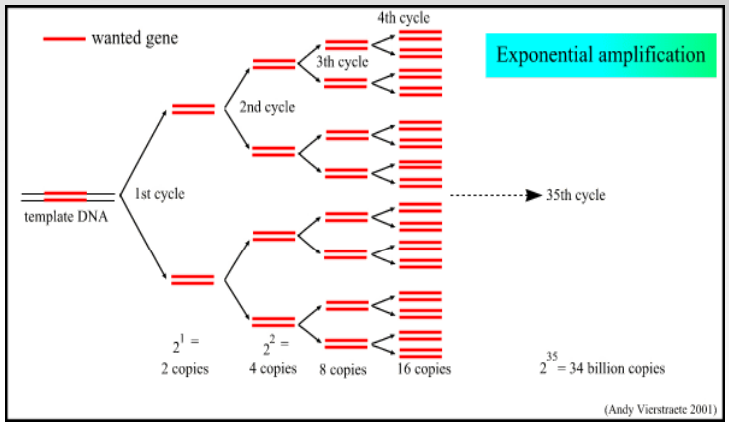
Polymerase Chain Reaction (PCR)







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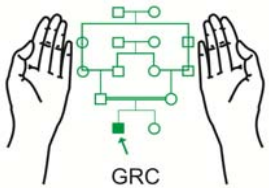
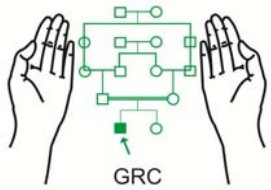
PCR and Thalassaemia

- Mutation analysis
  - Amplification Refractory Mutation System (ARMS)
  - Dot blot hybridization
- PCR-RFLP
- PCR-SSCP
- PCR-DGGE
- Cycle sequencing




IVS – I – 110G→A
CD 39 C→T
IVS – I – 6 T→C
IVS – I – 5 – G→A
IVS – II – 745 C→G
CD 6 – A
IVS – I – 110G→A
CD 39 C→T
IVS – II – 1 G→A
IVS – I – 5 – G→C
CD 8 – AA
CD 44 – C
CD 41/42 – TTCT
CD 17 A→T
IVS – II – 654 C→T
–28 A→G
CD 26 G→A(HbE)
IVS – I – 5 G→C
CD 19 A→G
–30 A→G
–88 C→T
IVS – I – 5 G→C
CD 8/9 + G
IVS – I – 1 G→T
619 bp DEL
(CD 26 G→A(HbE))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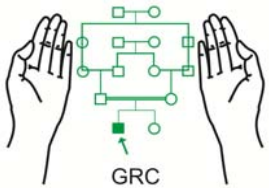
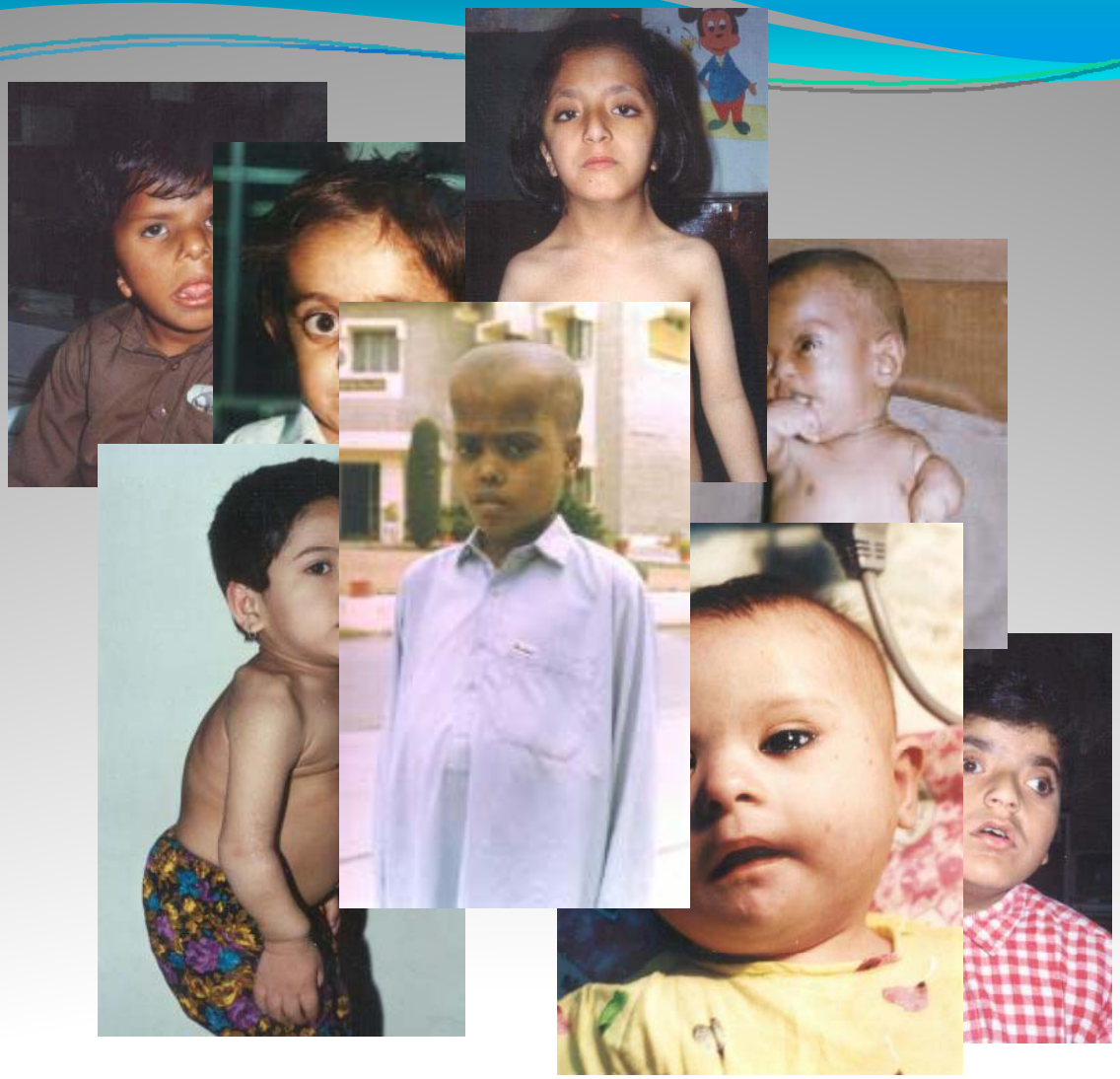
Molecular Medicine: Applications

- Diagnostic
  - Inherited Disorders
  - Cancer
  - Infectious Diseases
  - Human Identification
- Therapeutic
  - Biological Response Modification
  - Repairing the Genes (Anti-sense RNA)
  - Gene Therapy (Replacement)
- Industrial
  - Recombinant DNA Technology





Of the 5000 known Genetic Disorders
2500 can be diagnosed by Genetic Analysis

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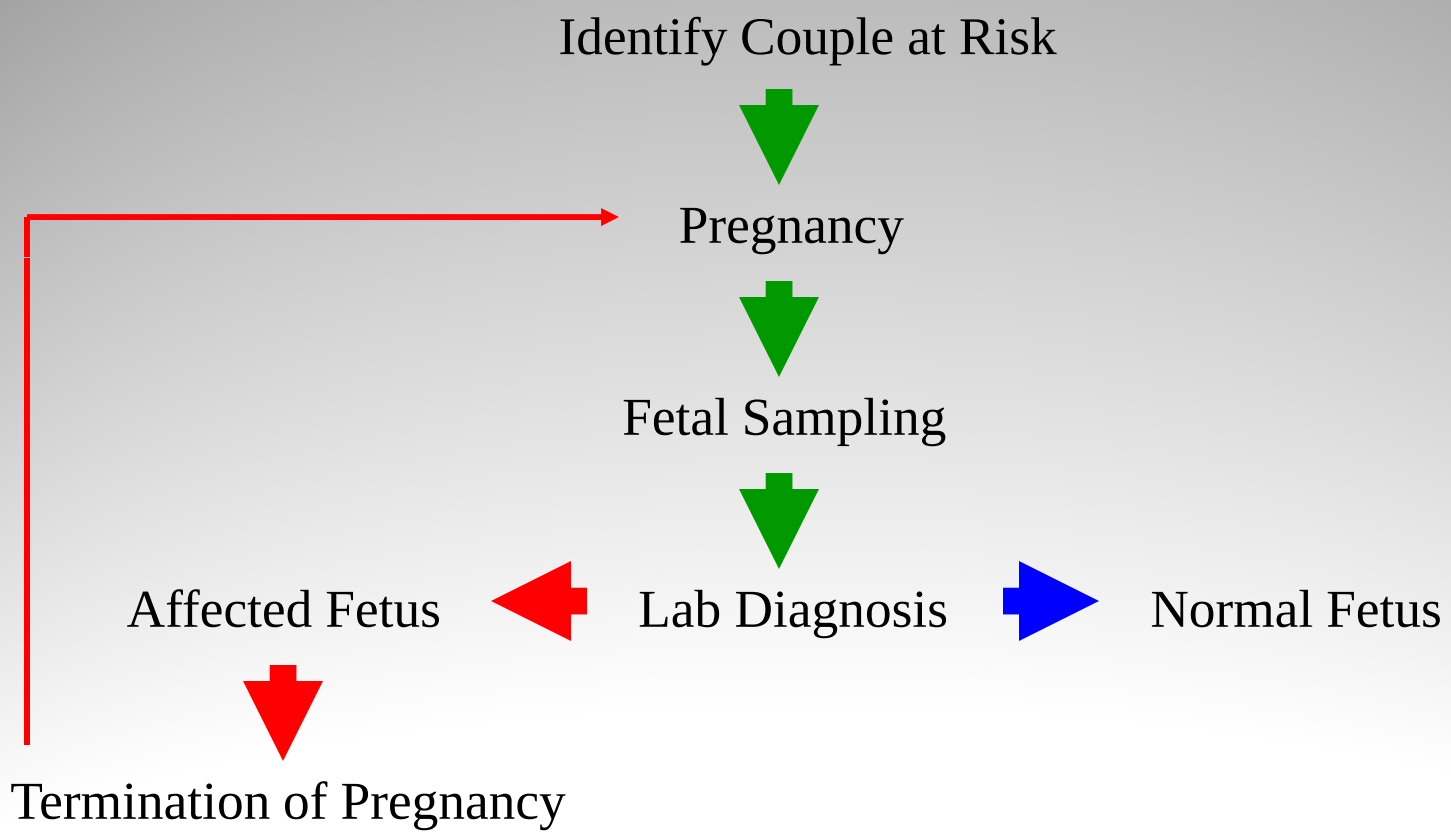
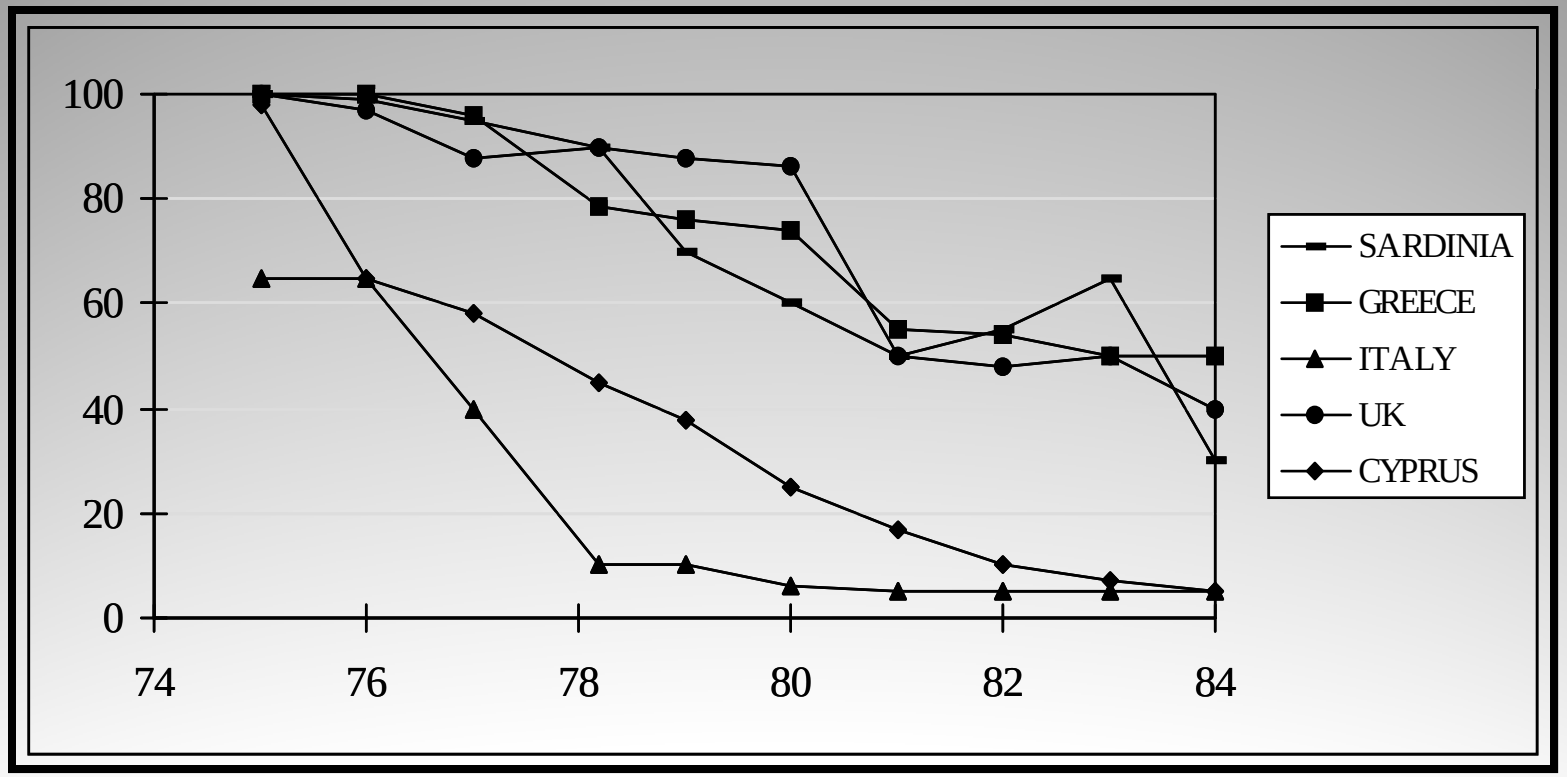
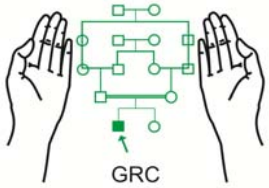
Prenatal Diagnosis




100
80
60
40
20
0
74
76
78
80
82
84
SARDINIA
GREECE
ITALY
UK
CYPRUS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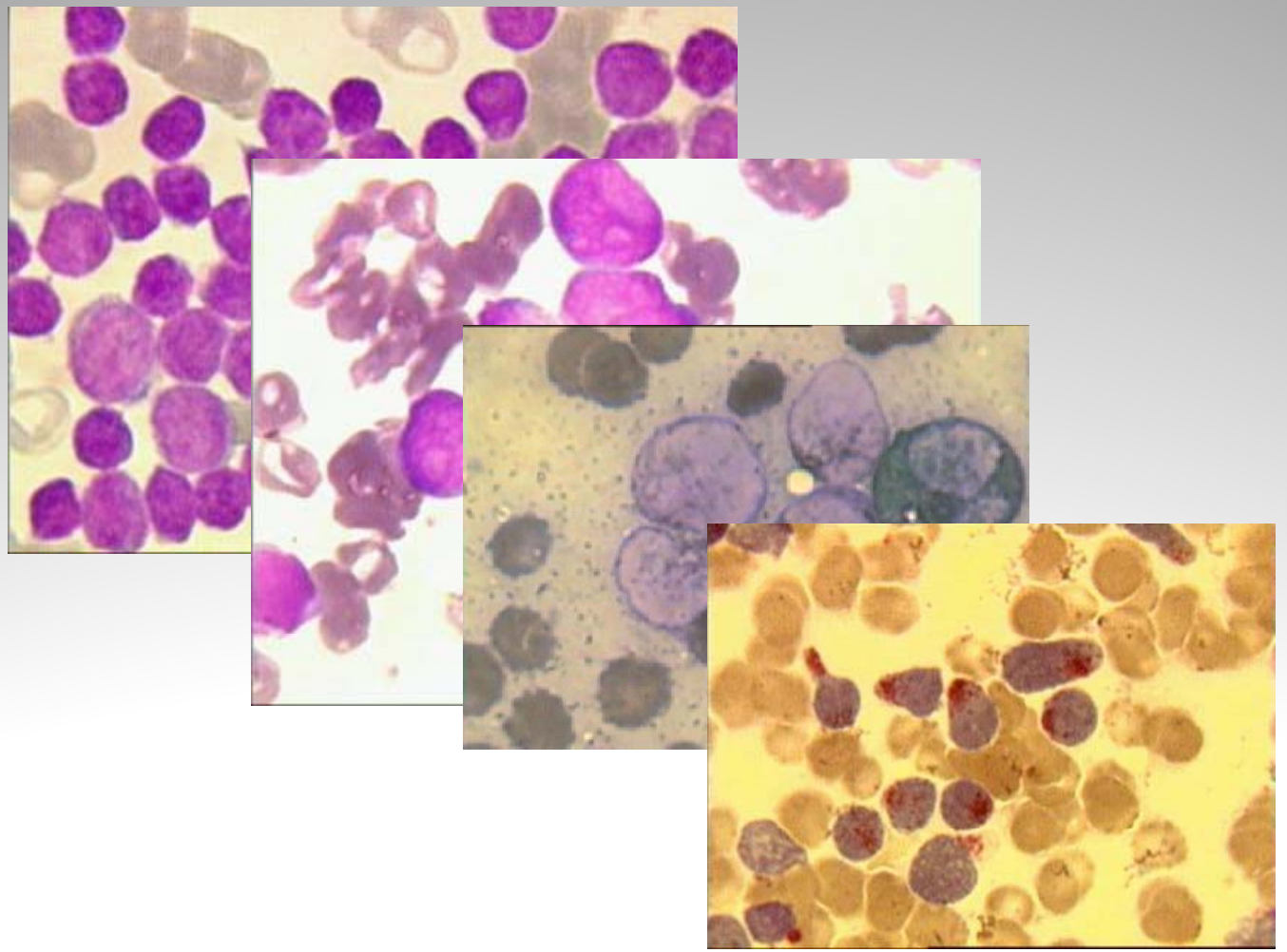
Molecular Diagnosis of Cancer



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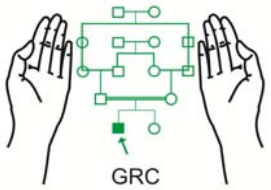
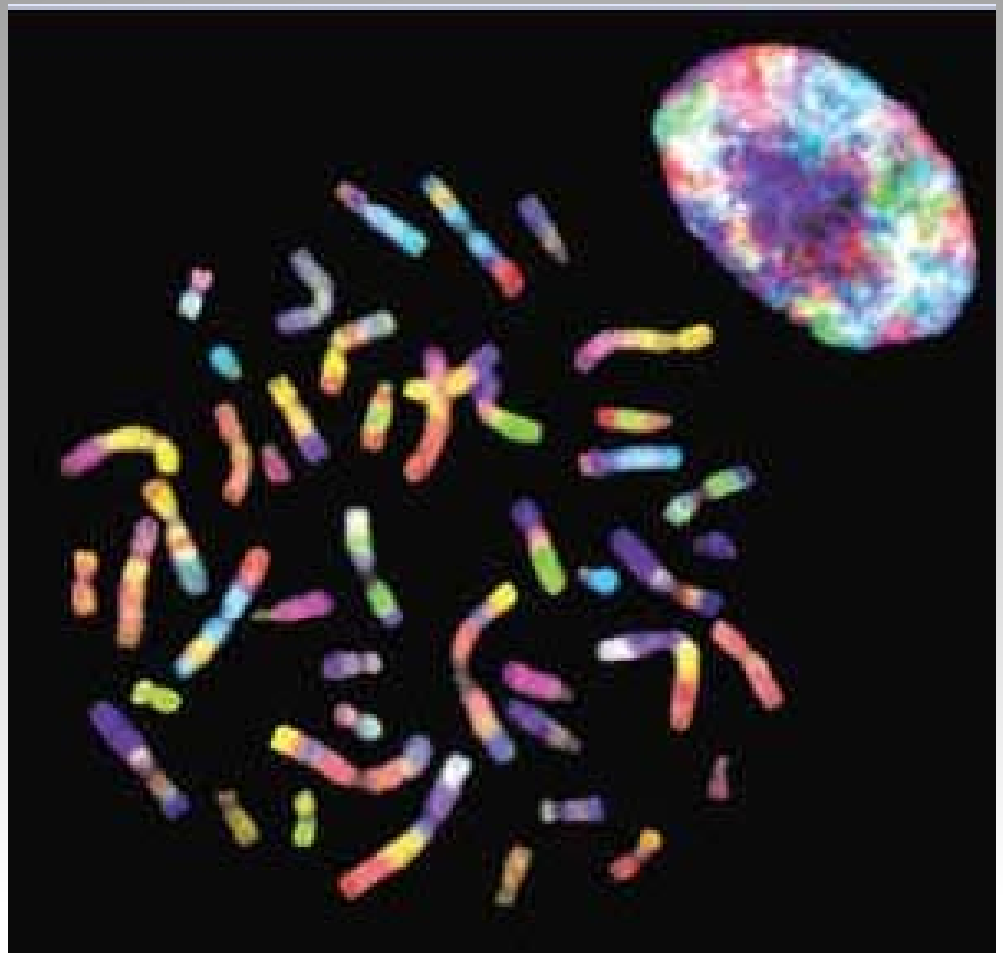
Molecular Markers

- CML bcr-abl
- ALL bcr-abl, Ig-gene, TCR-gene
- AML M2 t(8;21)
- AML M3 t(15;17)
- AML M4Eo inv(16)
- NHL bcl-II, Ig-gene, TCR-gene
- MPD JAK2 mutation





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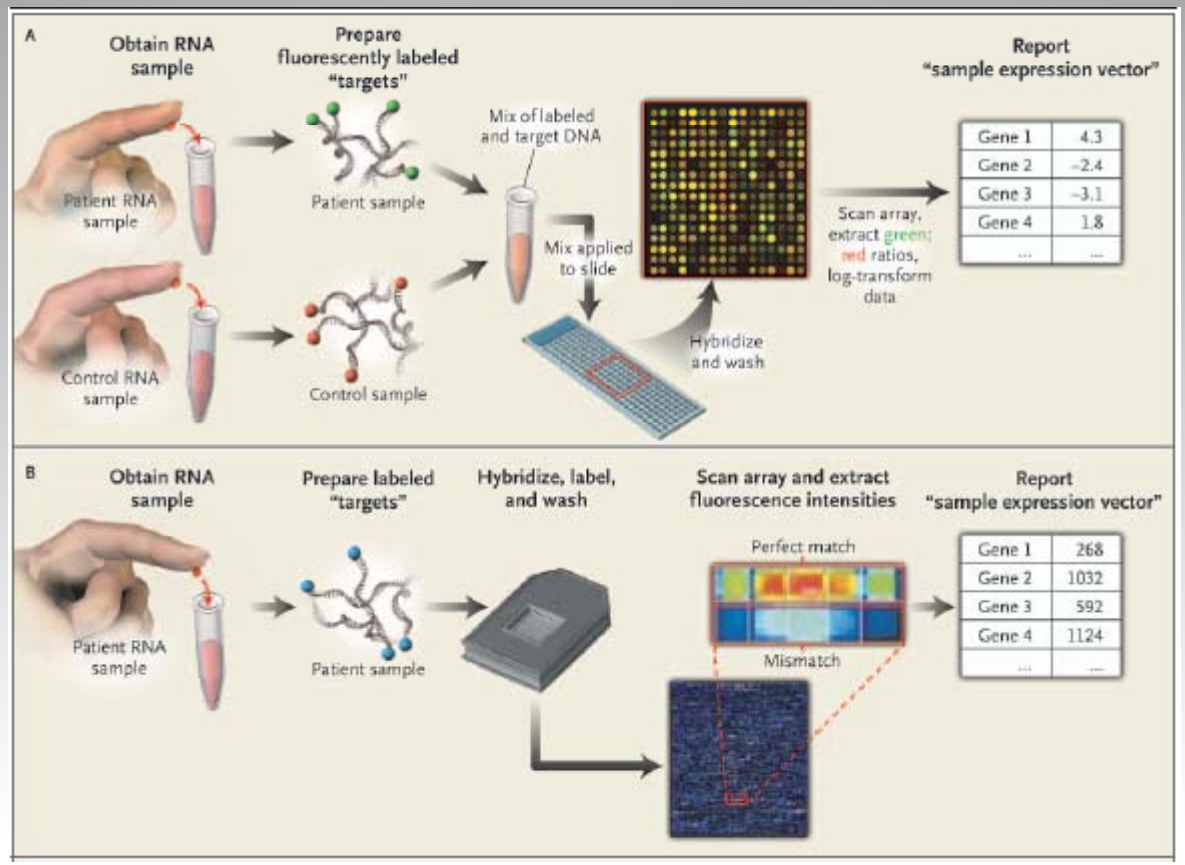
A
Obtain RNA sample
Prepare fluorescently labeled "targets"
Report "sample expression vector"
Patient RNA sample
Patient sample
Mix of labeled and target DNA
Mix applied to slide
Control RNA sample
Control sample
Hybridize and wash
Scan array, extract green: red ratios, log-transform data
Gene 1 4.3
Gene 2 −2.4
Gene 3 −3.1
Gene 4 1.8
B
Obtain RNA sample
Prepare labeled "targets"
Hybridize, label, and wash
Scan array and extract fluorescence intensities
Report "sample expression vector"
Patient RNA sample
Patient sample
Perfect match
Mismatch
Gene 1 268
Gene 2 1032
Gene 3 592
Gene 4 1124

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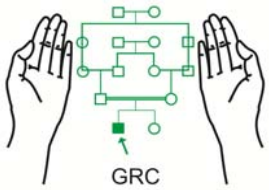
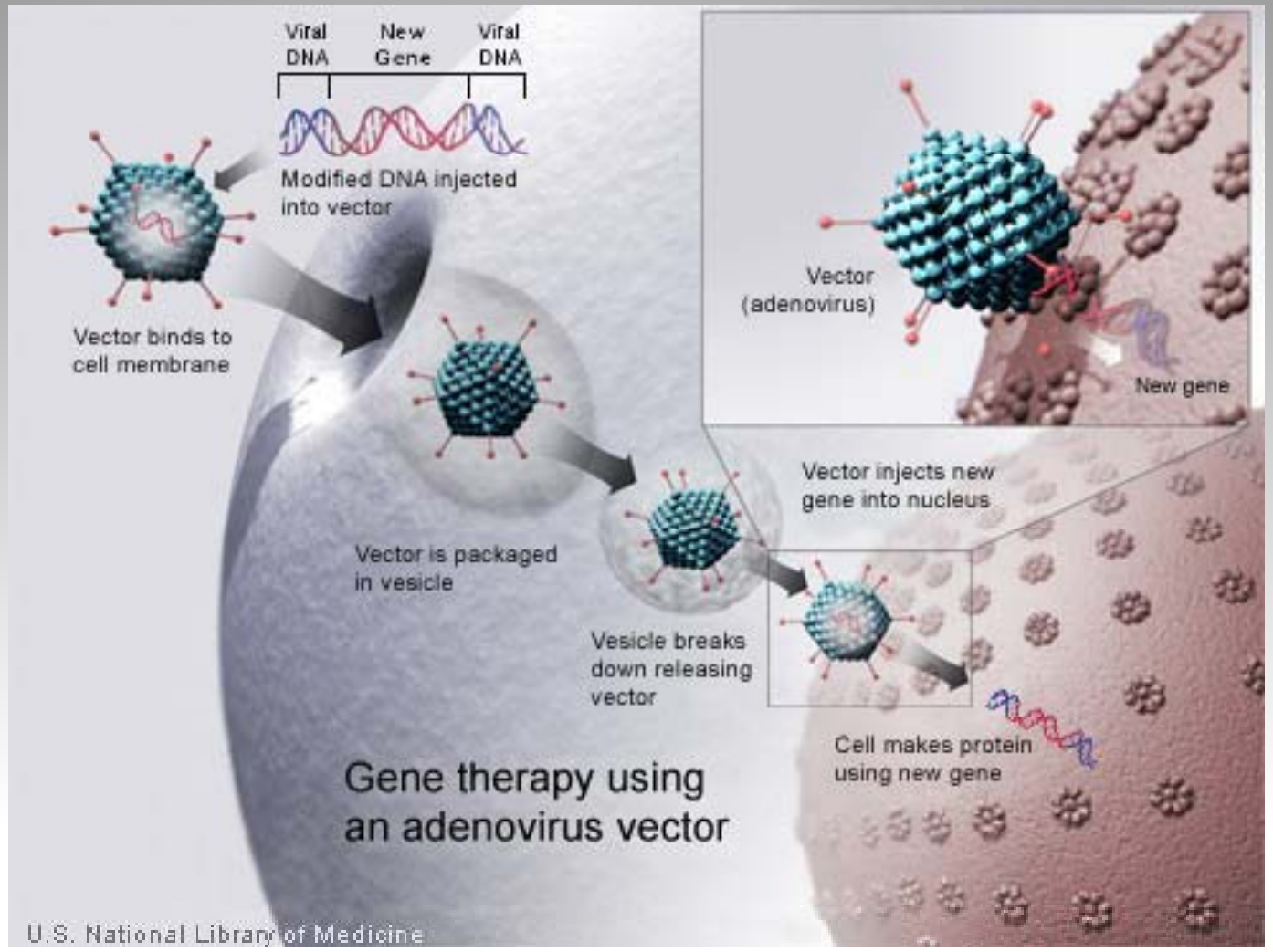
Gene Therapy




Viral DNA
New Gene
Viral DNA
Modified DNA injected into vector
Vector binds to cell membrane
Vector (adenovirus)
New gene
Vector injects new gene into nucleus
Vector is packaged in vesicle
Vesicle breaks down releasing vector
Cell makes protein using new gene
Gene therapy using an adenovirus vector
U.S. National Library of Medicine

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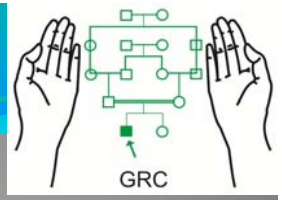
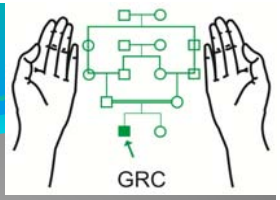


Correction of β-thalassemia major by gene transfer in haematopoietic progenitors of pediatric patients.

Roselli et al, EMBO Molecular Medicine 2010, 2(8): 315–328.

Abstract

We recently showed correction of murine β-thalassemia by gene transfer in HSCs with the **GLOBE lentiviral vector (LV), expressing a transcriptionally regulated human β-globin gene.** Here, we report successful correction of thalassemia major in human cells, by studying a large cohort of pediatric patients of diverse ethnic origin, carriers of different mutations and all candidates to BM transplantation. Extensive characterization of BM-derived $CD34^+$ cells before and following gene transfer shows the achievement of high frequency of transduction, **restoration of haemoglobin A synthesis, rescue from apoptosis and correction of ineffective erythropoiesis**. The procedure does not significantly affect the differentiating potential and the relative proportion of haematopoietic progenitors. Analysis of vector integrations shows preferential targeting of transcriptionally active regions, without bias for cancer-related genes. Overall, these results provide a solid rationale for a future clinical translation.



Gene therapy for Haemophilia: the end of a ‘royal pathology’ in the third millennium?

Haemophilia is an ideal condition for gene therapy because of its monogenetic character and the fact that it requires only a small amount of the expressed protein to achieve palliation. To date, research in the field of gene therapy for haemophilia has largely relied on retroviruses, adenoviruses and adeno-associated viruses as transfer vectors and the major aims will be to achieve stable longlasting in vivo expression of factors VIII or IX (FVIII or FIX) at therapeutic levels. Two clinical trials have been approved by the US Food and Drug Administration (FDA), using miniadenovirus FVIII and the intrahepatic and intramuscular delivery of adeno-associated virus FIX. In the third millennium, haemophilia treatment should encompass more ambitious goals through gene replacement, to result in permanent and safe haemophilia 'eradication', making haemophilia a part of the history of medicine.

Augmentation of Fetal Hemoglobin (HbF) Synthesis in Culture by Human Erythropoietic Precursors in the Marrow and Peripheral Blood: Studies in Sickle Cell Anemia and Nonhemoglobinopathic Adults

By Kinichi Kidoguchi, Makio Ogawa, Jim D. Karam, and Alyce G. Martin

Blood, Vol. 52, No. 6 (December), 1978

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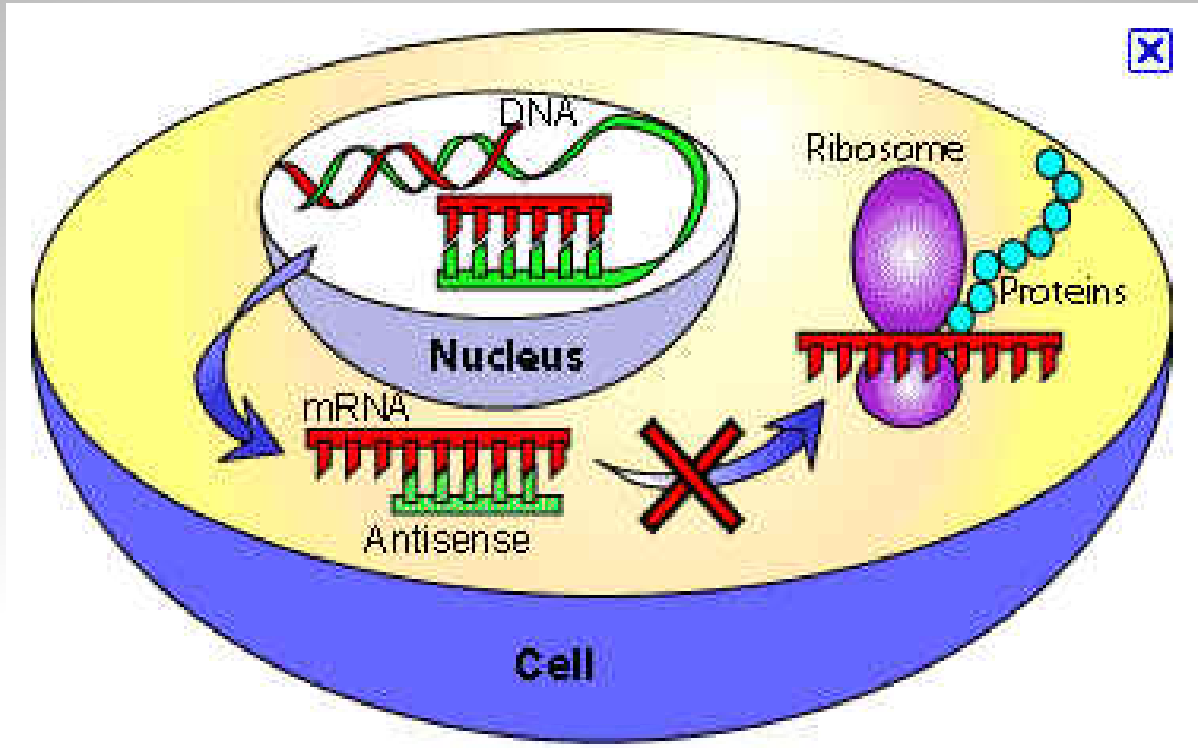
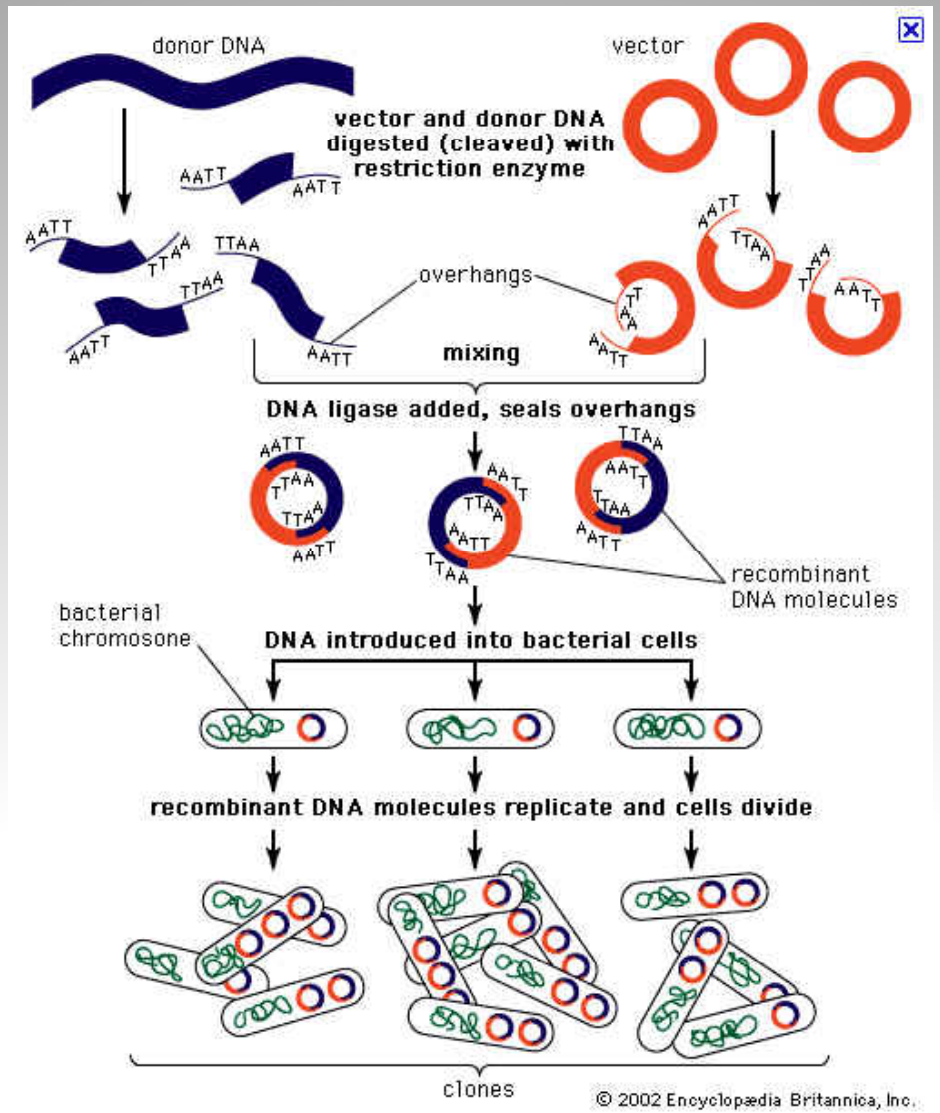
Antisense RNA



Recombinant DNA Technology



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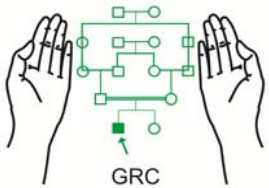
- Haematopoietic growth factors
- Erythropoietin
- Coagulation factors
- Others



The NEW ENGLAND JOURNAL of MEDICINE

EDITORIALS

Individual Genomes on the Horizon

Richard P. Lifton, M.D., Ph.D.

N Engl J Med 2010; 362:1235-1236

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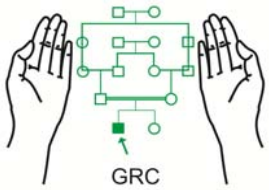
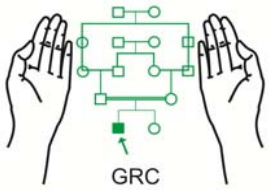
Gene Patenting

Approximately 20% of human genes are patented, and some claims also implicate large numbers of related oligonucleotide sequences or refer to genotype–phenotype associations. Whole-genome testing would thus infringe on a wide variety of claims. To our knowledge, there is no evidence that patent holders would not enforce their rights in this case. There is an urgent need to reform patent policy around genetic diagnostics to ensure that innovation and market competition can work in patients' favor.



Molecular Medicine

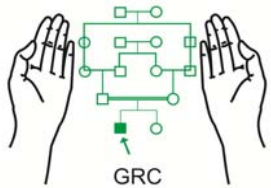
The Pakistani Scenario



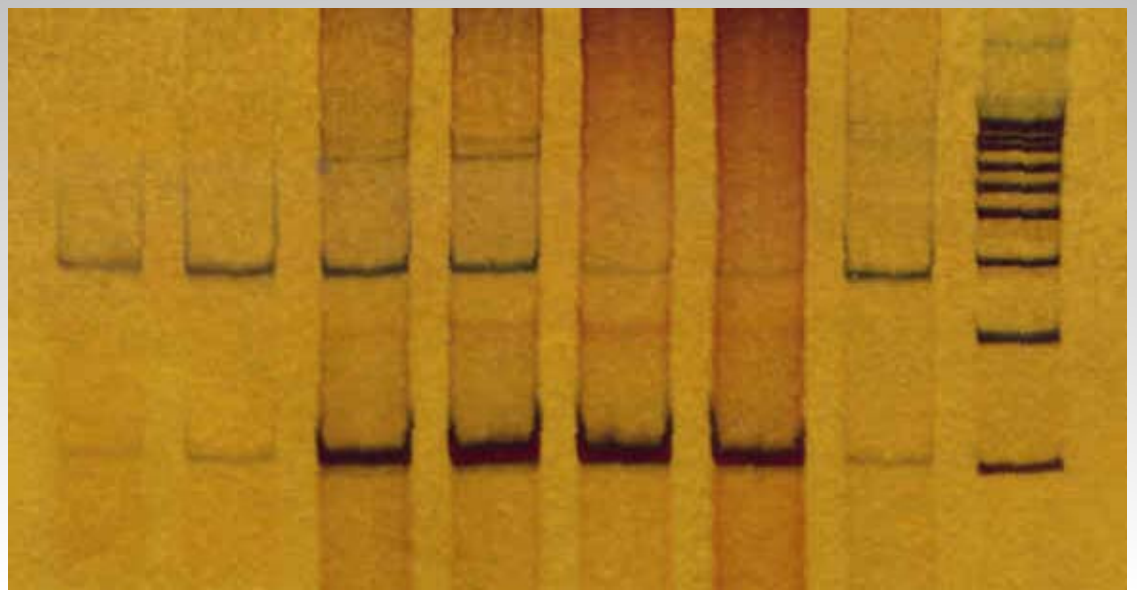
Prenatal Diagnosis of Thalassaemia in Pakistan

- 1st PND of Thalassaemia: May 1994
- Total PNDs at AFIP: 2174
  - Thalassaemia trait: 1106 (50.9%)
  - Normal: 526 (24.2%)
  - Thalassaemia major: 542 (24.9%)
- Misdiagnosis: 6/1632 (0.37%)

(S. Ahmed, Prenatal Diagnosis 2007)



JAK2 Mutation



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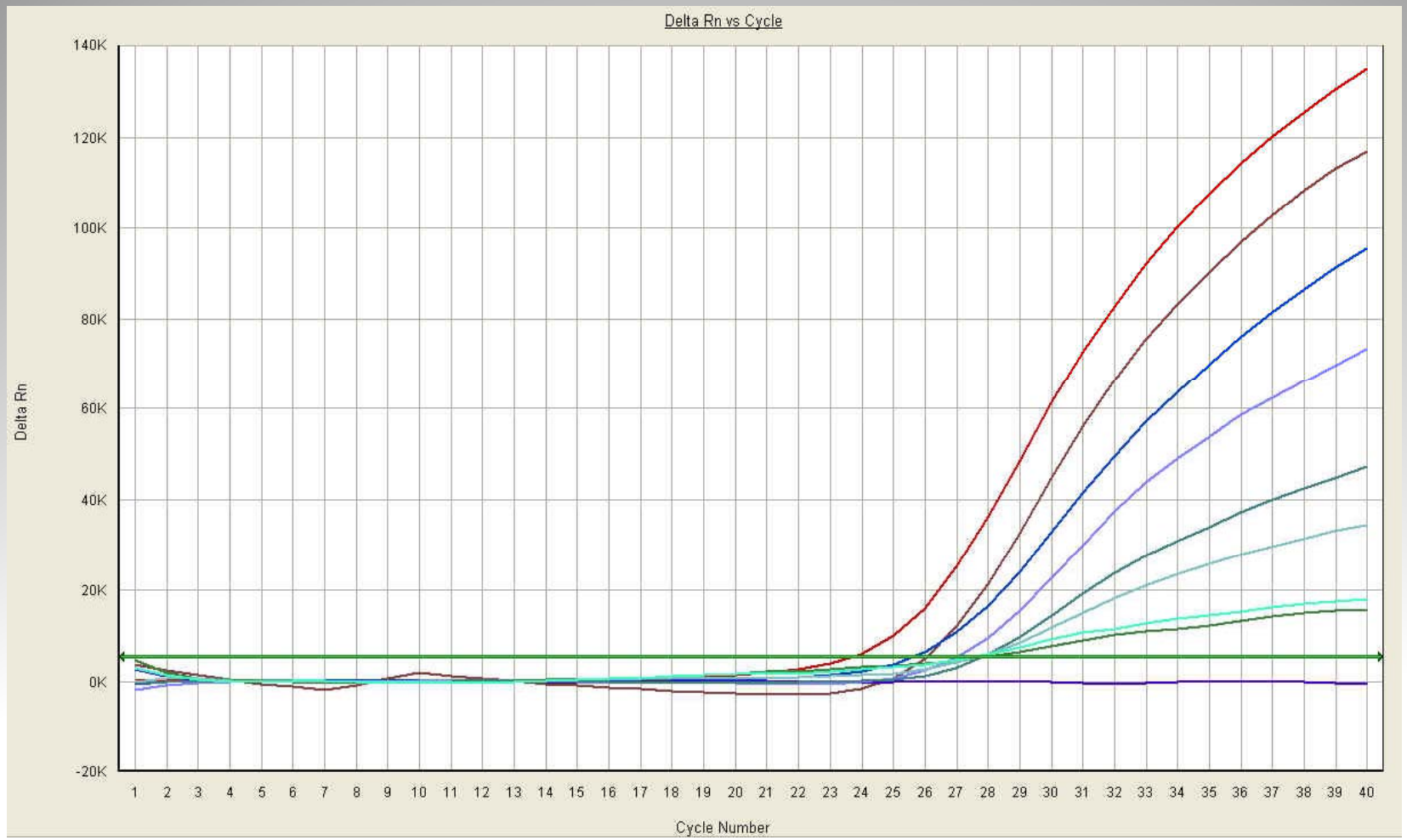
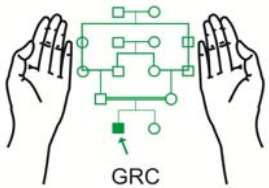
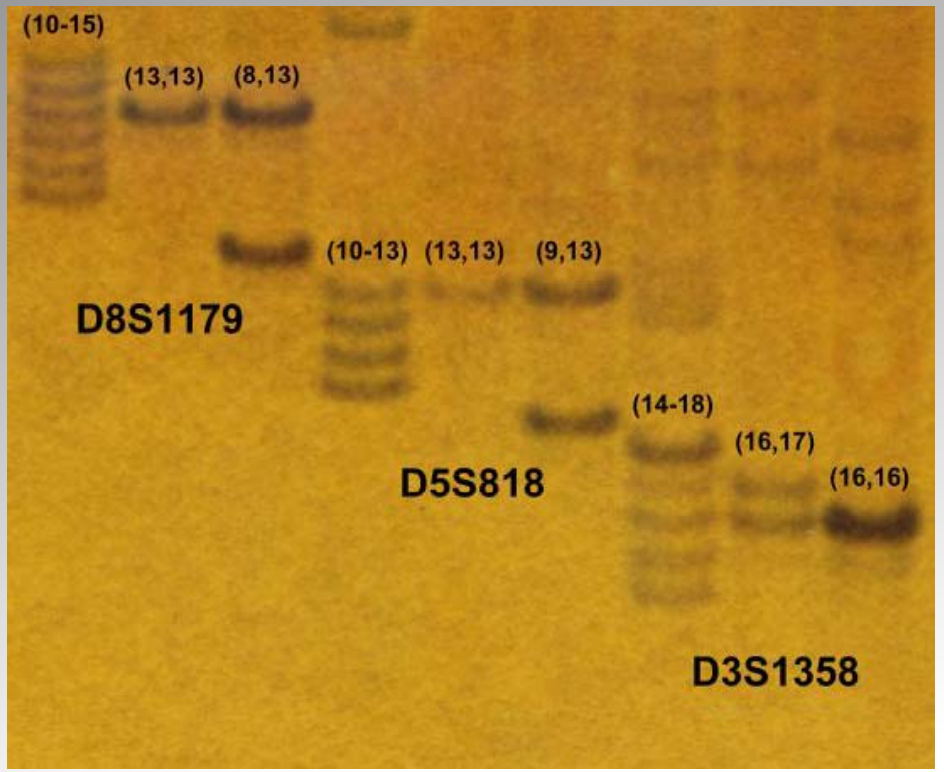
Real Time PCR for *Bcr-abl*



Infectious Diseases

- Viral
  - HCV, HBV, HIV, CMV, HPV, Dengue etc.
- Bacterial
  - Tuberculosis
  - Antibiotic resistance
- Parasitic
  - Leishmania, Malaria
- Others




(10-15)
(13,13)
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(9,13)
D8S1179
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(16,16)
D5S818
D3S1358

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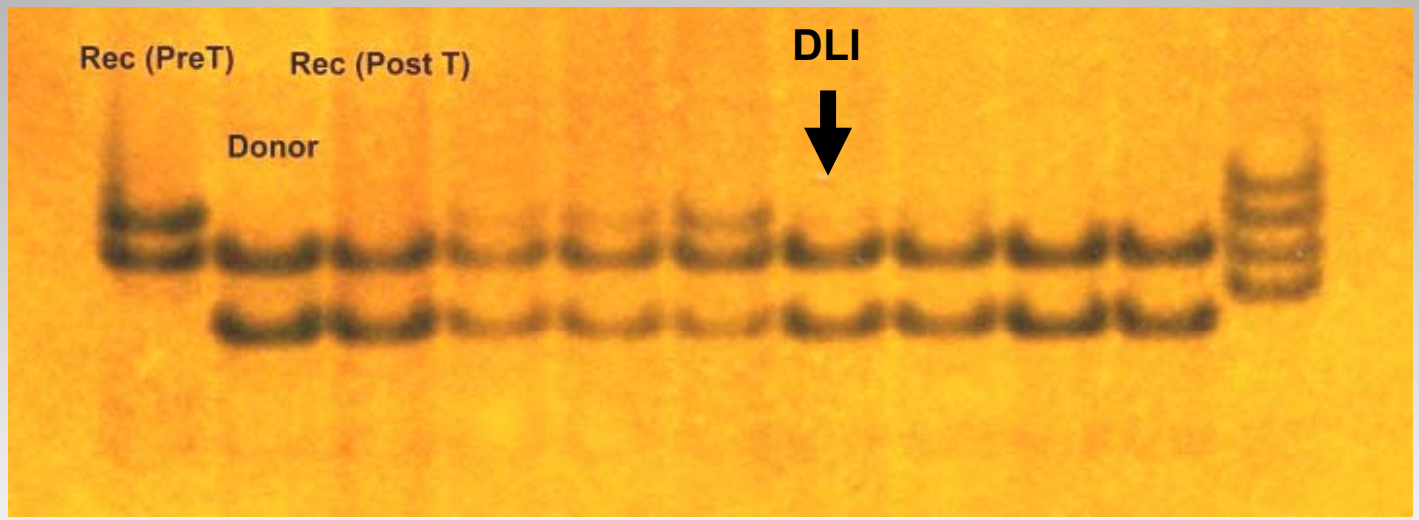
Donor Chimerism



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A Mobile DNA Lab

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Start Tools

Batch Name - test

S. No	Green	Reading	Value	Result	Yellow	Reading	Value	Result
1		275	0	REFRENCE		559	0	REFRENCE
2		273	-2	NEGATIVE		558	-1	NEGATIVE
3		274	-1	NEGATIVE		564	5	NEGATIVE
4		274	-1	NEGATIVE		564	5	NEGATIVE

GRC

How has PCR affected the medical world?

Far less than it has affected the medical journalistic world. The last time I was seriously hospitalized with coronary artery problems was 2004 and there was plenty of testing of blood and imaging work, but information about my DNA was not considered. This is still in the stage of research. It will become more and more a part of medical practice, since individual tolerance and susceptibility to certain drugs, like heparin for instance, is significant and connected to DNA genotype. Personalized medicine is coming. It is still in the research stage.

Transfusions of blood and organs are monitored for histo-compatibility using DNA types, and several genetic disorders as well as infectious diseases are certainly examined at the DNA level.

There is much more to come than has been applied. Practical medicine necessarily moves more slowly than medical research.