The Beginners Guide to Molecular Biology

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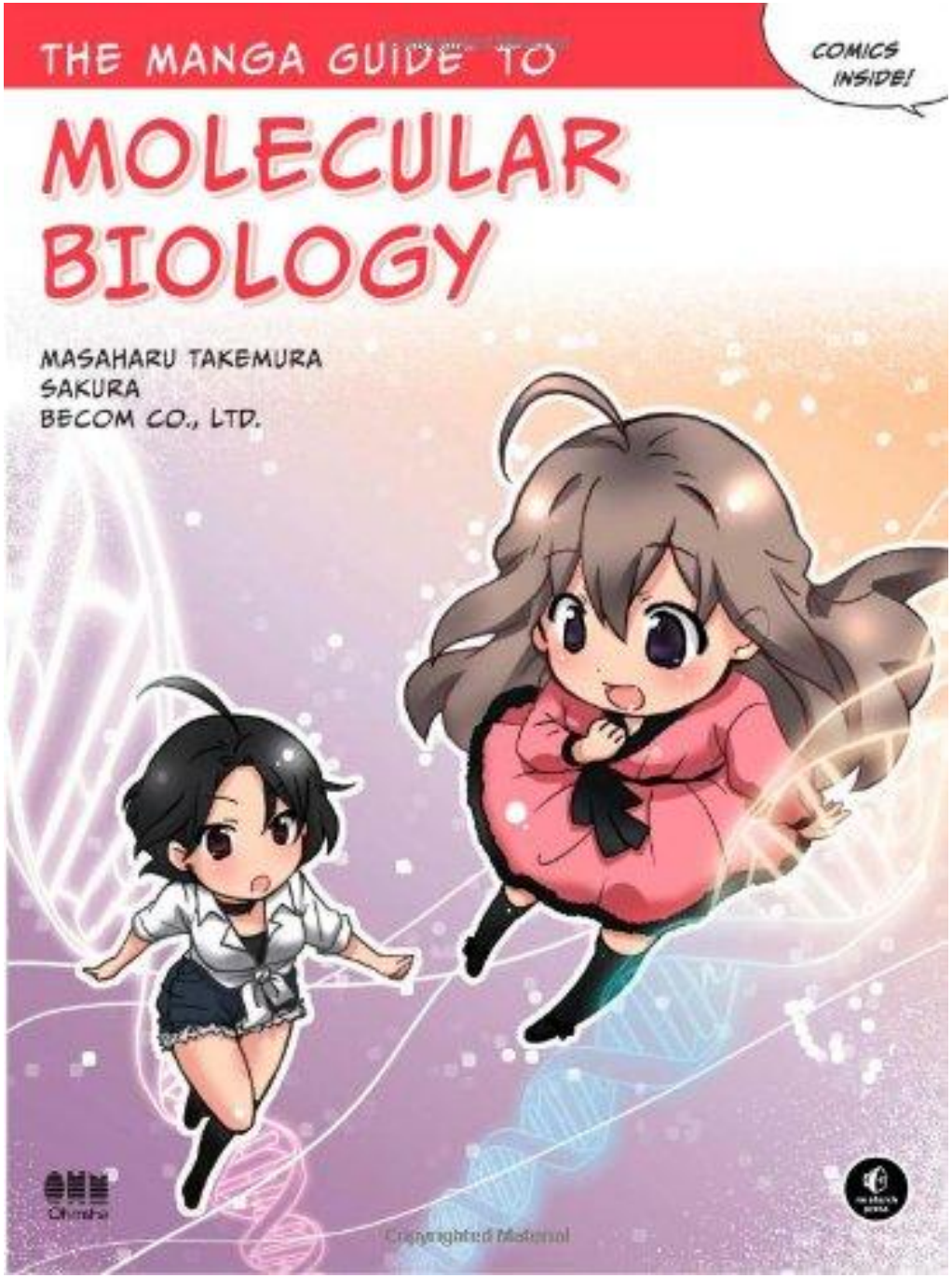
AOTEA PATHOLOGY


THE MANGA GUIDE TO
COMICS INSIDE!
MOLECULAR BIOLOGY
MASAHARU TAKEMURA
SAKURA
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Ohmsha
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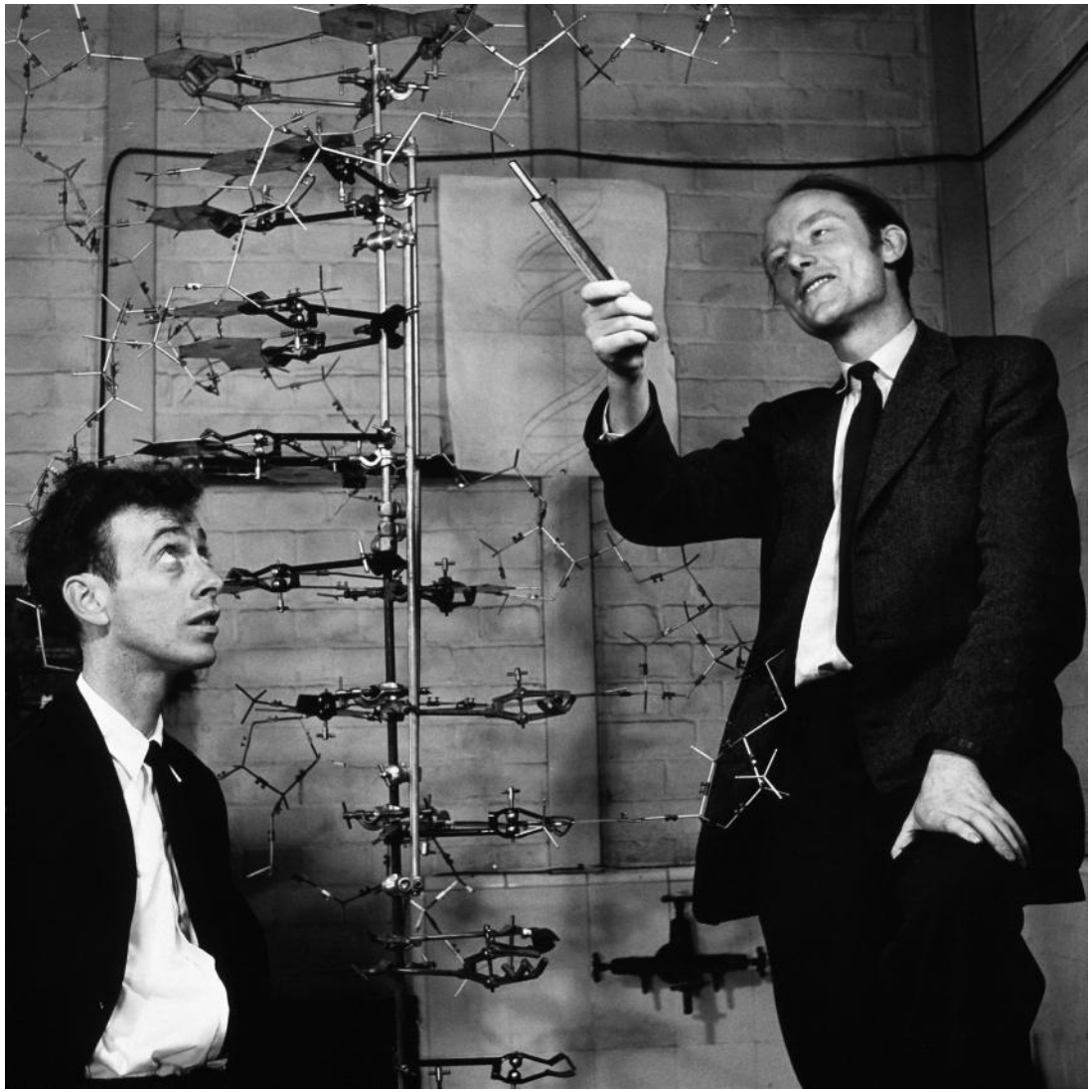
Today you will learn…

- About the structure and properties of DNA and RNA and how to get it out of cells
- About the Polymerase Chain Reaction (PCR)
- How molecular techniques are used in genetics and what they can and cannot tell us
- A bit about epigenetics and the proteome
- Where to go for further information

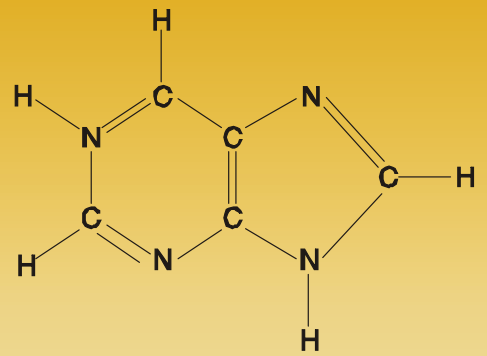
A Structure for Deoxyribose Nucleic Acid

J. D. Watson and F. H. C. Crick (1) April 25, 1953 (2), *Nature* **(3),** 171, 737-738

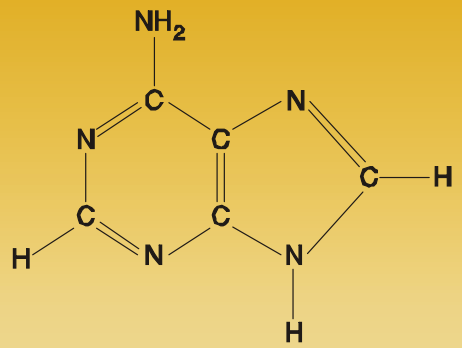
We wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.



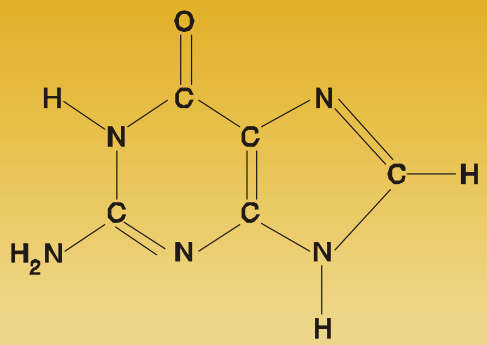
Chemistry of the Nucleobases



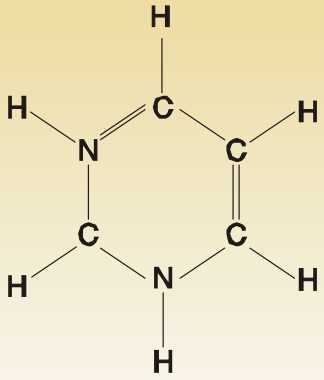
Purine



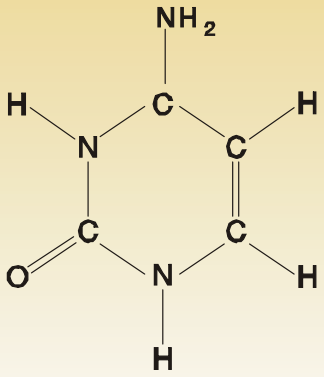
Adenine



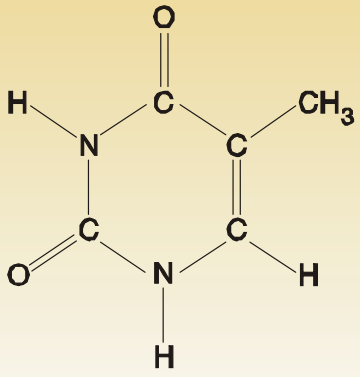
Guanine



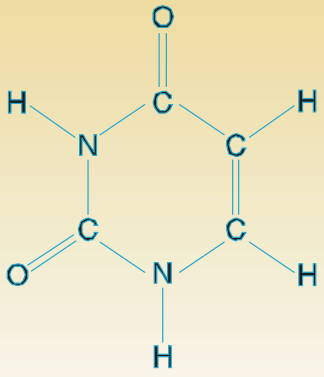
Pyrimidine



Cytosine



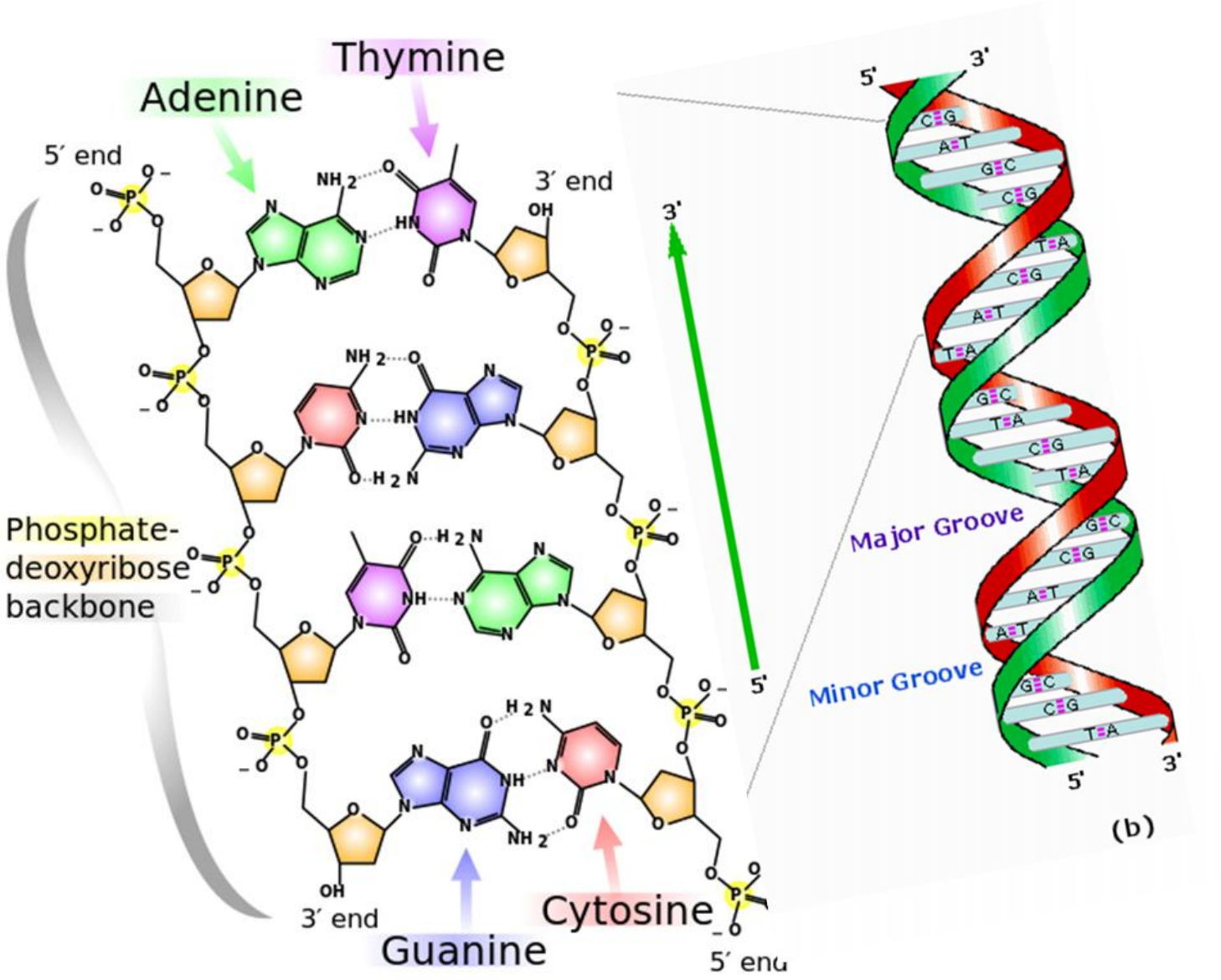
Thymine



Uracil

DNA

Molecular techniques are based on the structure of nucleic acids



RNA

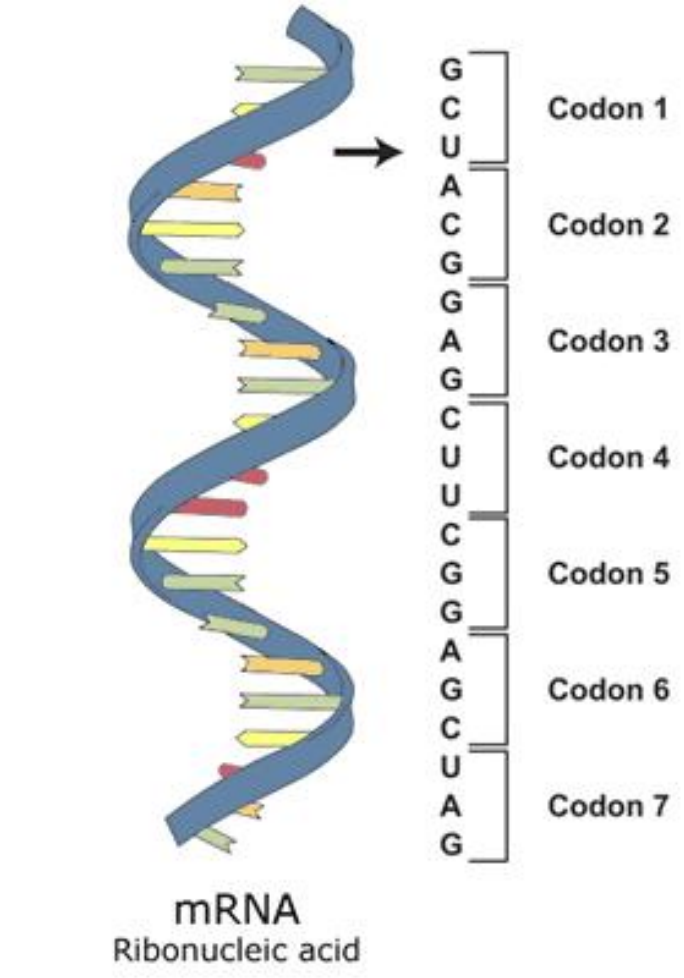
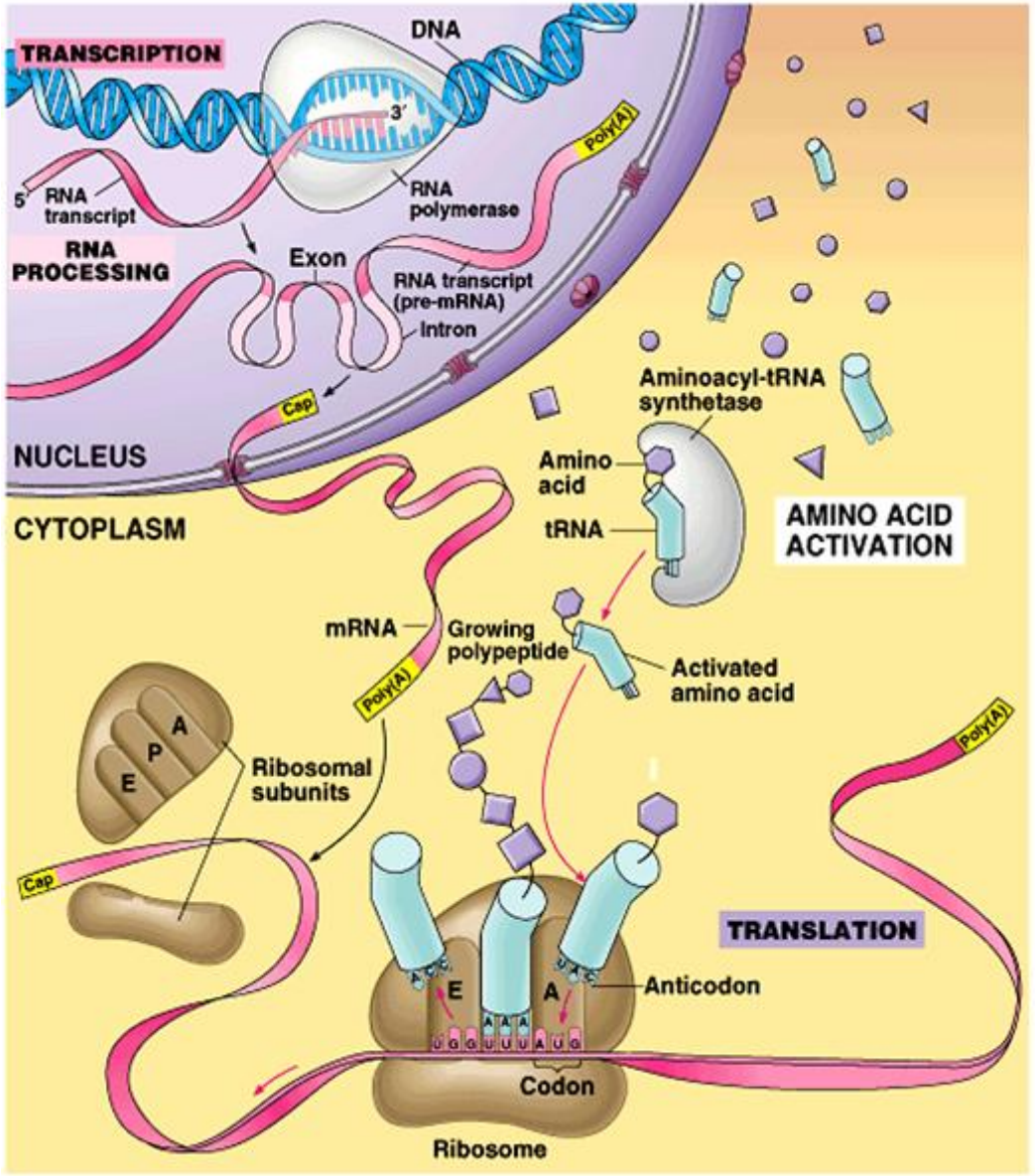
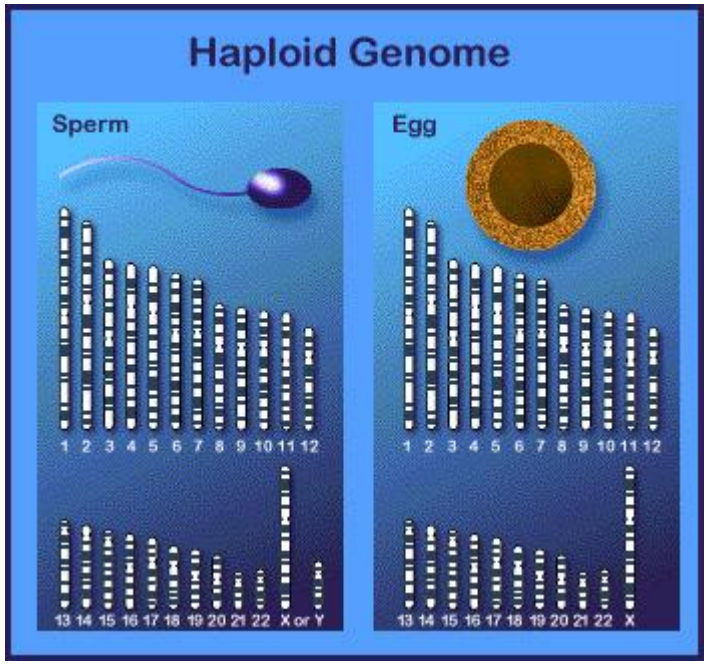
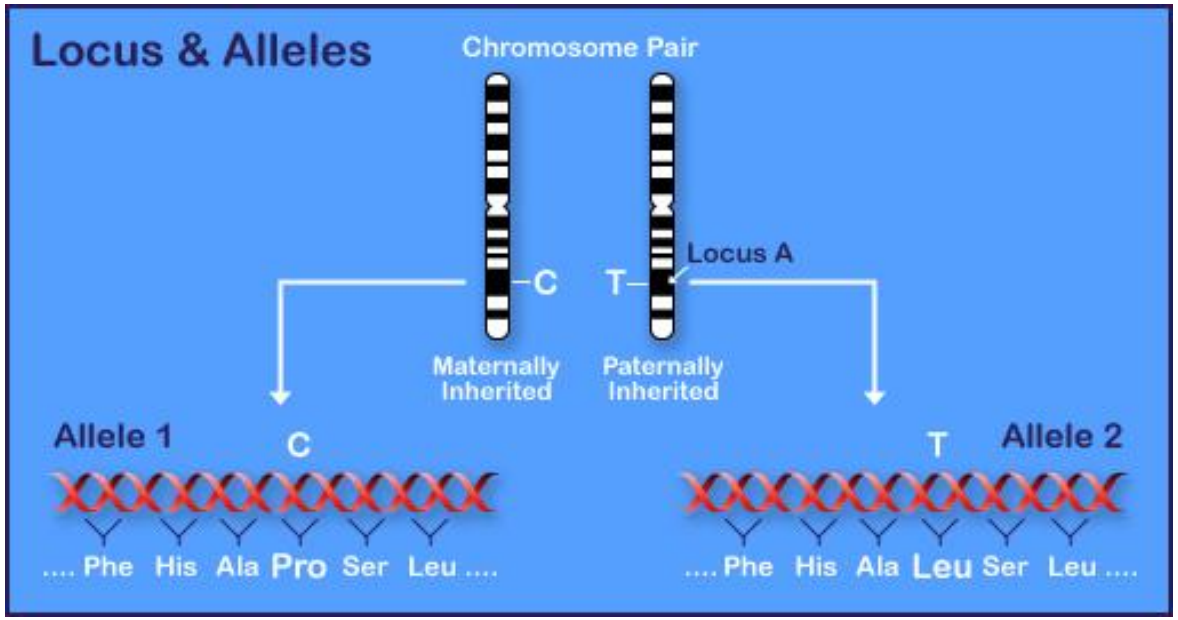


Image adapted from: National Human Genome Research Institute. Talking Glossary of Genetic Terms. Available at: www.genome.gov/Pages/Hyperion/DIR//VIP/Glossary/Illustration/codon.shtml.



This is how it all hangs together



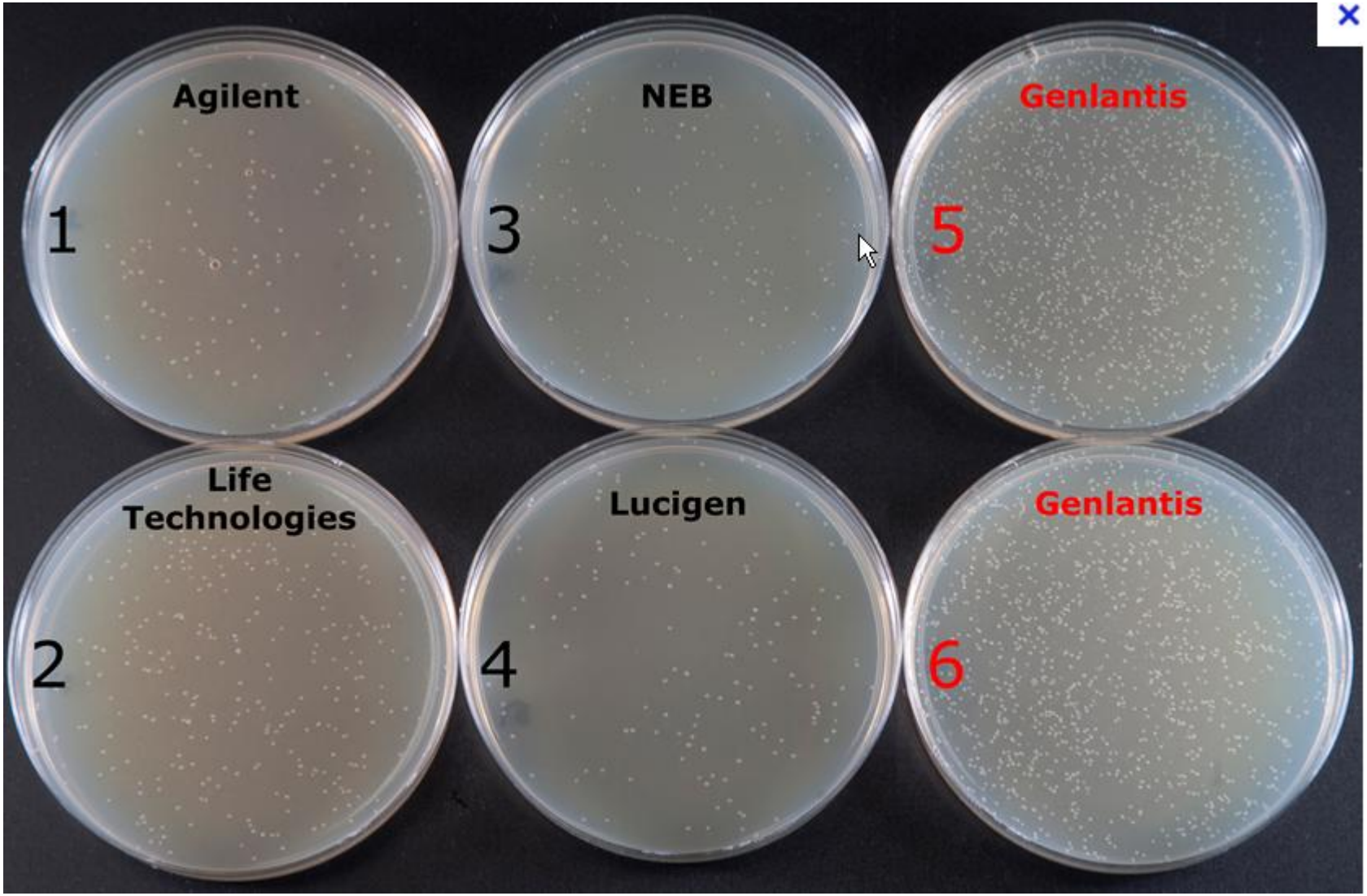


DNA Extraction and Purification

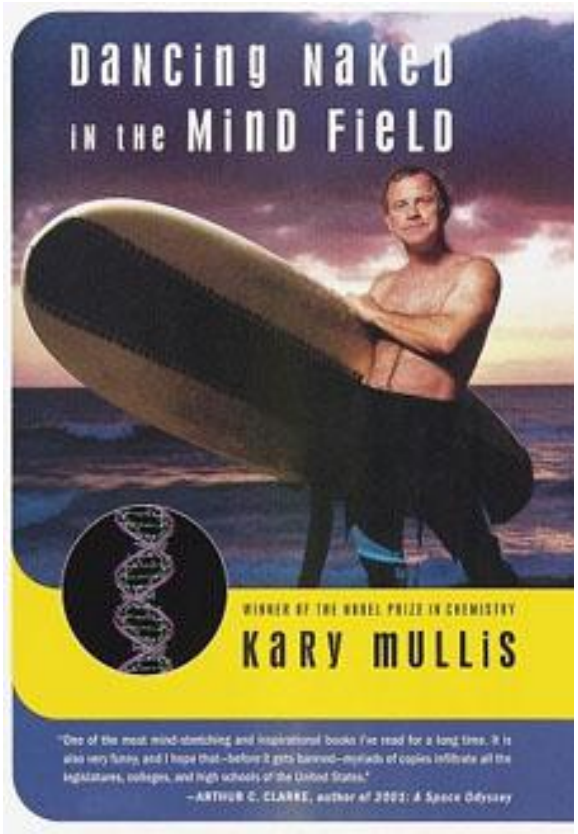
The MagNa Pure System

http://lifescience.roche.com/

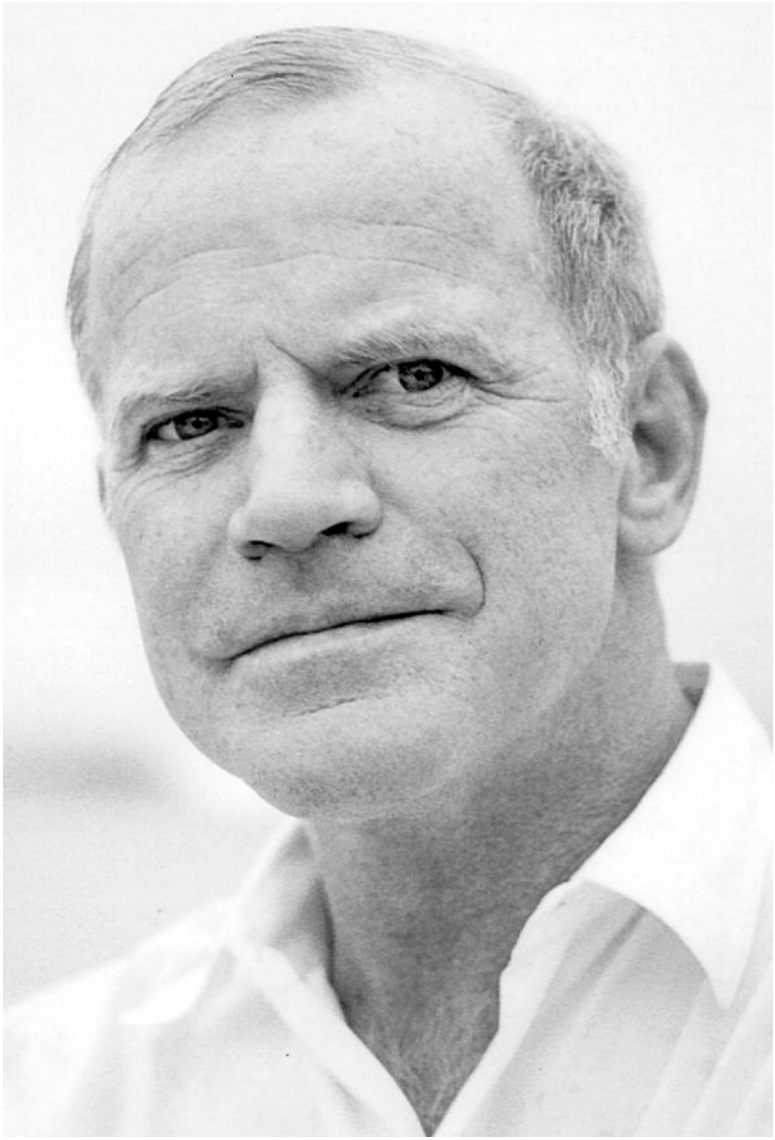
Pre-PCR times: clone libraries




PCR is the Love Child of
Chemistry and Microbiology



http://www.karymullis.com/

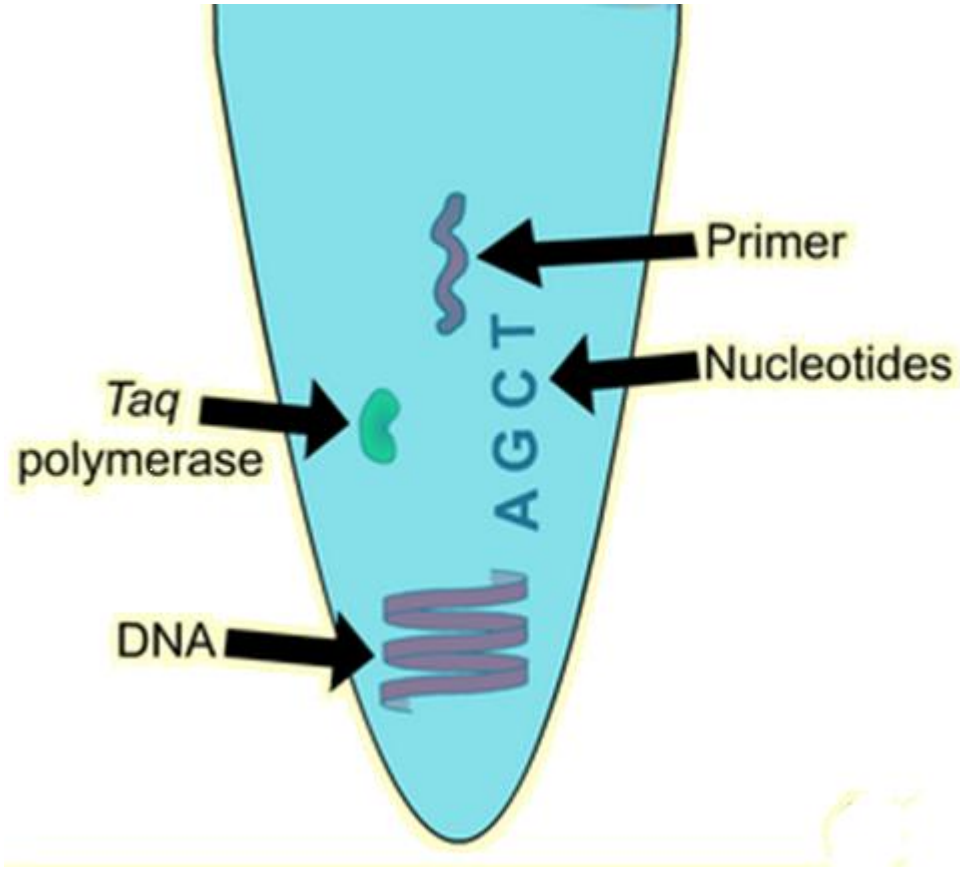


Dr Kary Banks Mullis

Nobel Prize winner for inventing the Polymerase Chain Reaction, 1993

- http://www.dnalc.org/view/15475-The-cycles-of-the-polymerase-chain-reaction-PCR-3D-animation.html

How to do PCR

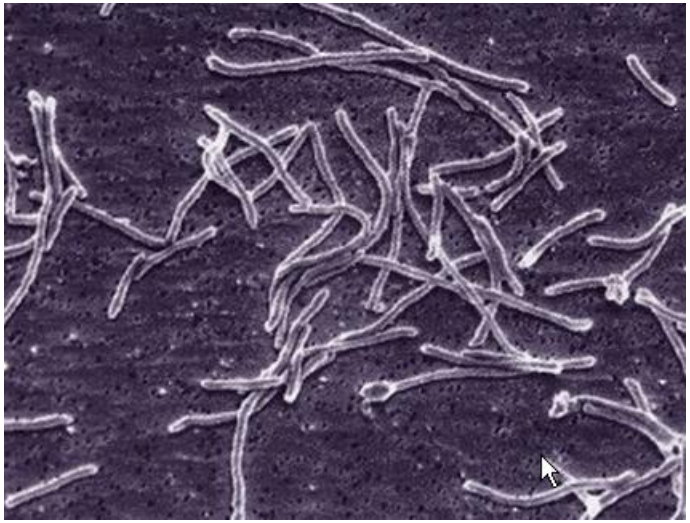


Mix…..

…and cook!

Initially, the DNA was amplified with the Klenow Fragment of DNA polymerase I. However, that enzyme was not stable at high temperatures. Thus, the Klenow in the tube was inactivated during the denaturation step of each PCR cycle, and the experimenter had to add more Klenow before each extension step

Microbiology Fixed Molecular Biology



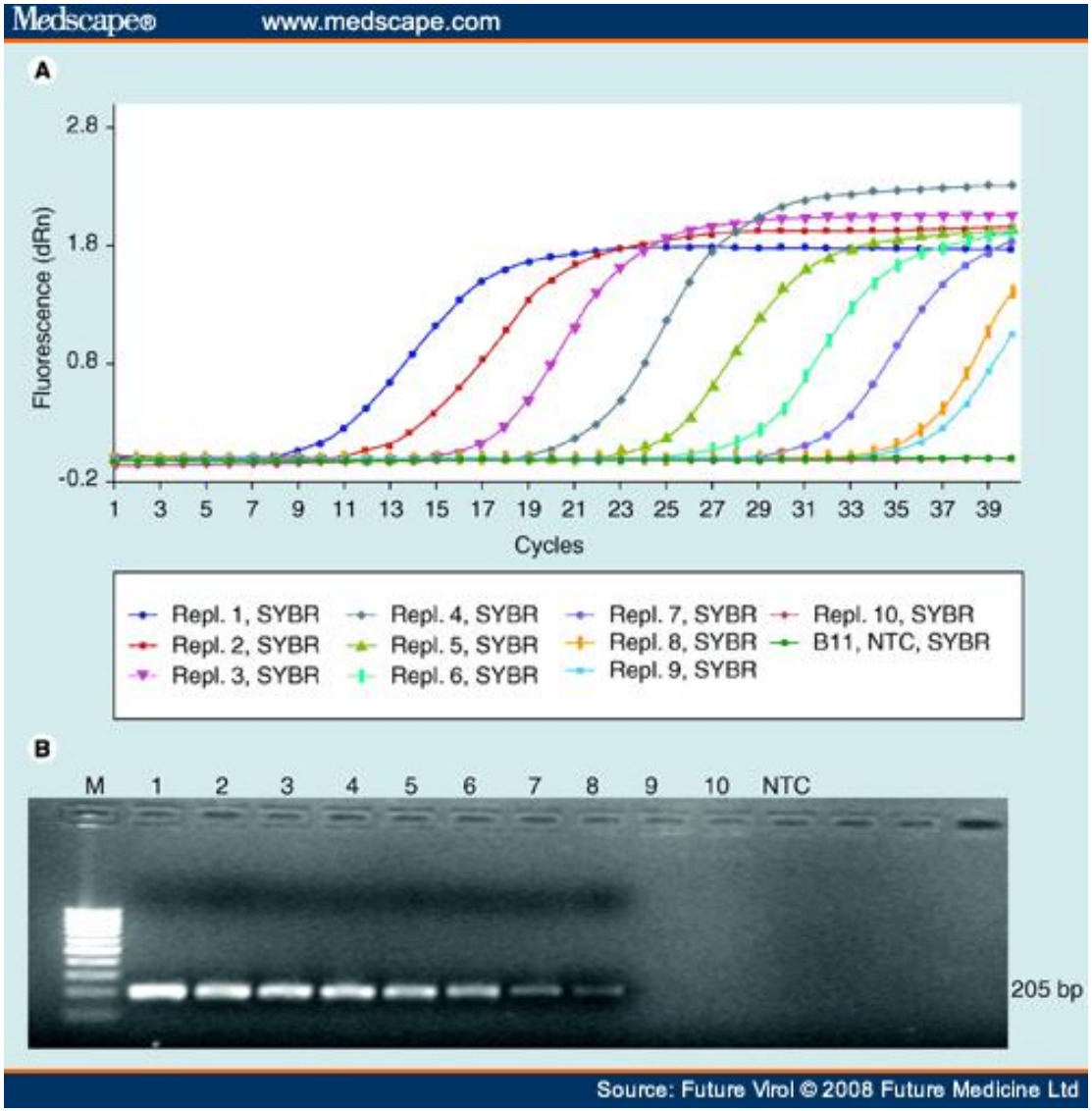
- Thermus aquaticus, first of the Kingdom Archaea
- Lives in boiling water
- Produces Taq Polymerase

The evolution of rapid cycle RTPCR

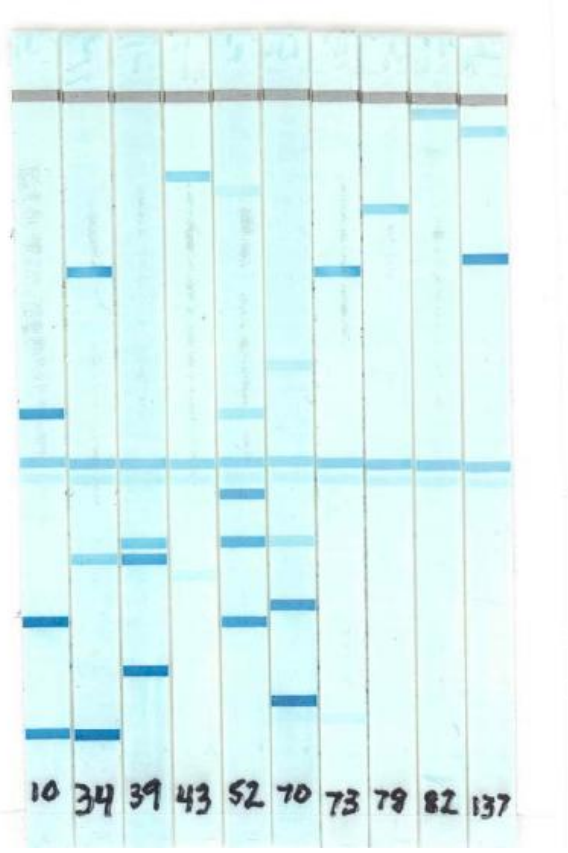
Heating with a Hair Dryer (1995)

Carl Wittwer and Randy Rasmussen proudly showing this loud contraption which (remarkably) worked as a thermal cycler. The light bulb in the thermal cycler was replaced by a hair dryer to allow monitoring by fluorescence. The dryer and vacuum cleaner were connected to the center drum by vacuum hose tubings.

Conventional vs real-time PCR



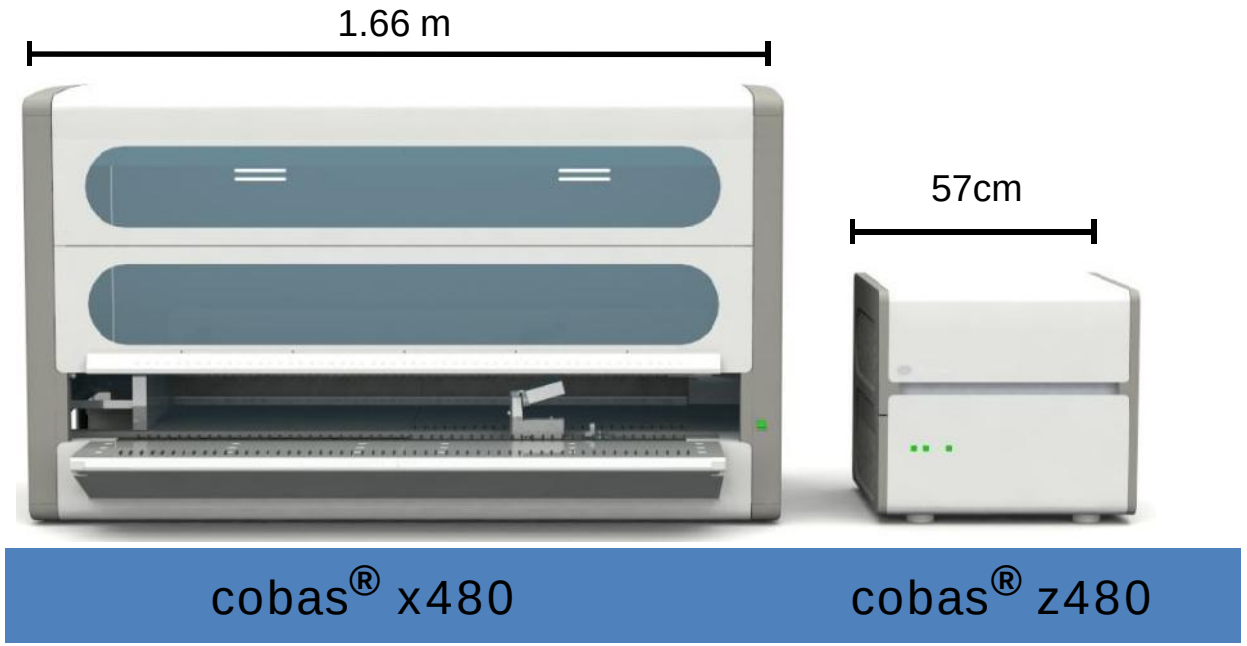
Oligonucleotide probe detection



Roche Linear Array HPV typing Assay

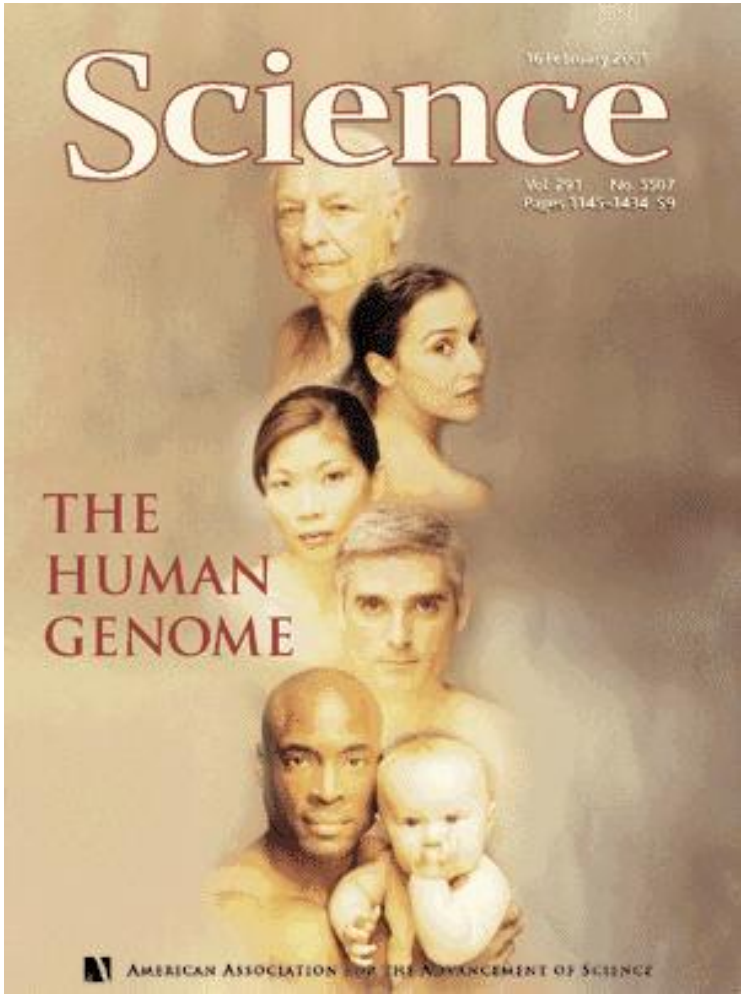
cobas® 4800 System

- cobas® x480 performs fully automated extraction directly from primary vials and automated PCR setup
- cobas® z480 performs amplification and detection



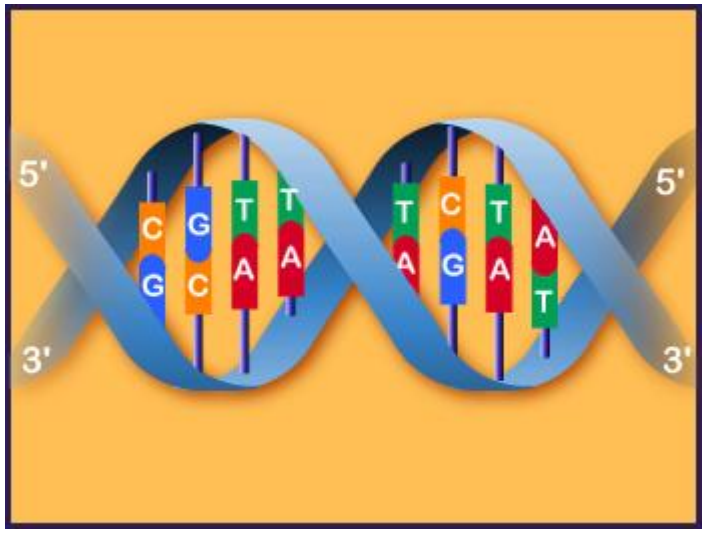
The Human Genome Project 1990-2003

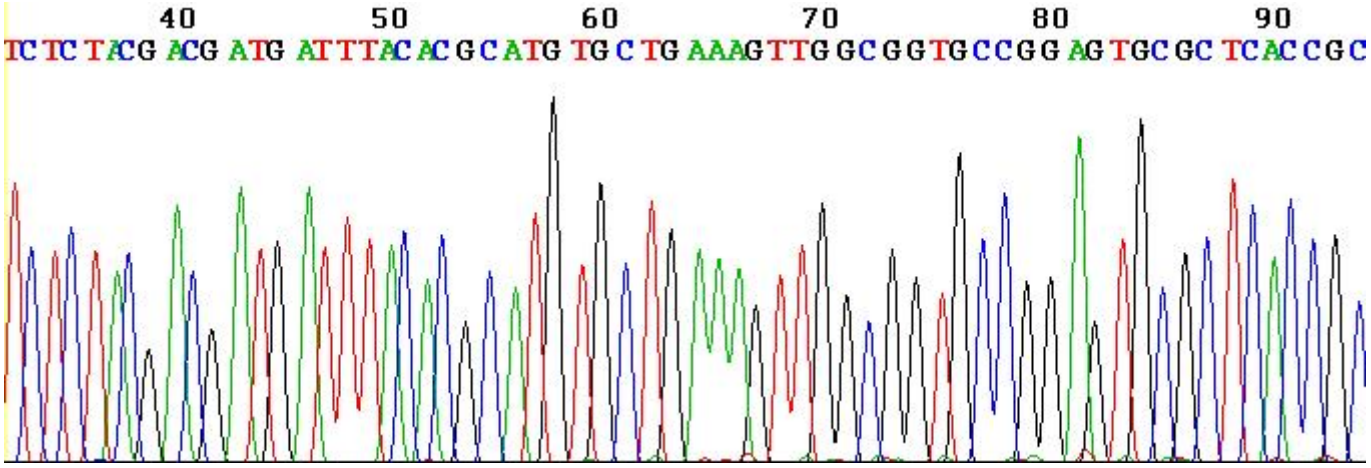
- The HGP was an international 13-year effort to discover all the estimated 20,000-25,000 human genes and determine the complete sequence of the 3 billion DNA bases in the human genome.



Dideoxy sequencing: The Sanger Method

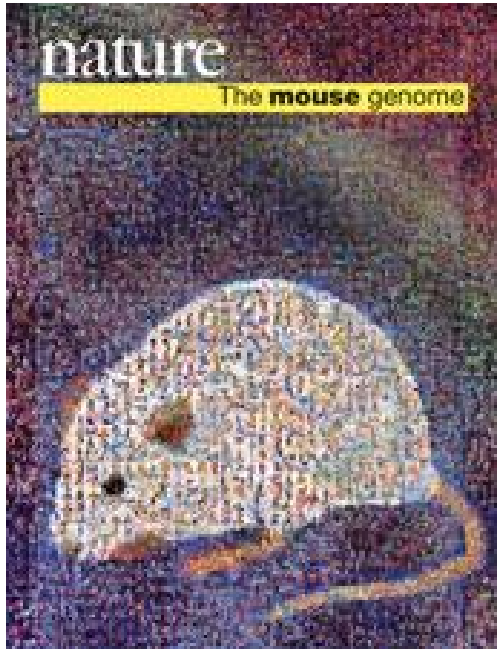
A: Adenine
T: Thymine
C: Cytosine
G: Guanine





Man or Mouse?

- MOUSE: 2.5 billion base pairs and 23,686 genes (EMBL 2007).



- MAN: 3 billion base pairs and 23,000 protein coding genes
- far fewer than expected before its sequencing.
- Most of the genome consists of non-coding RNA genes, regulatory sequences, introns, and "junk" DNA.

What CAN we tell from your genome?

- Diagnose certain diseases eg CF, Huntingtons, HFE
- Predict the likelihood of developing disease or characteristics based on the similarity of your DNA to affected individuals (SNP analysis)
- Predict how you will respond to particular drugs
- Parentage and ancestry

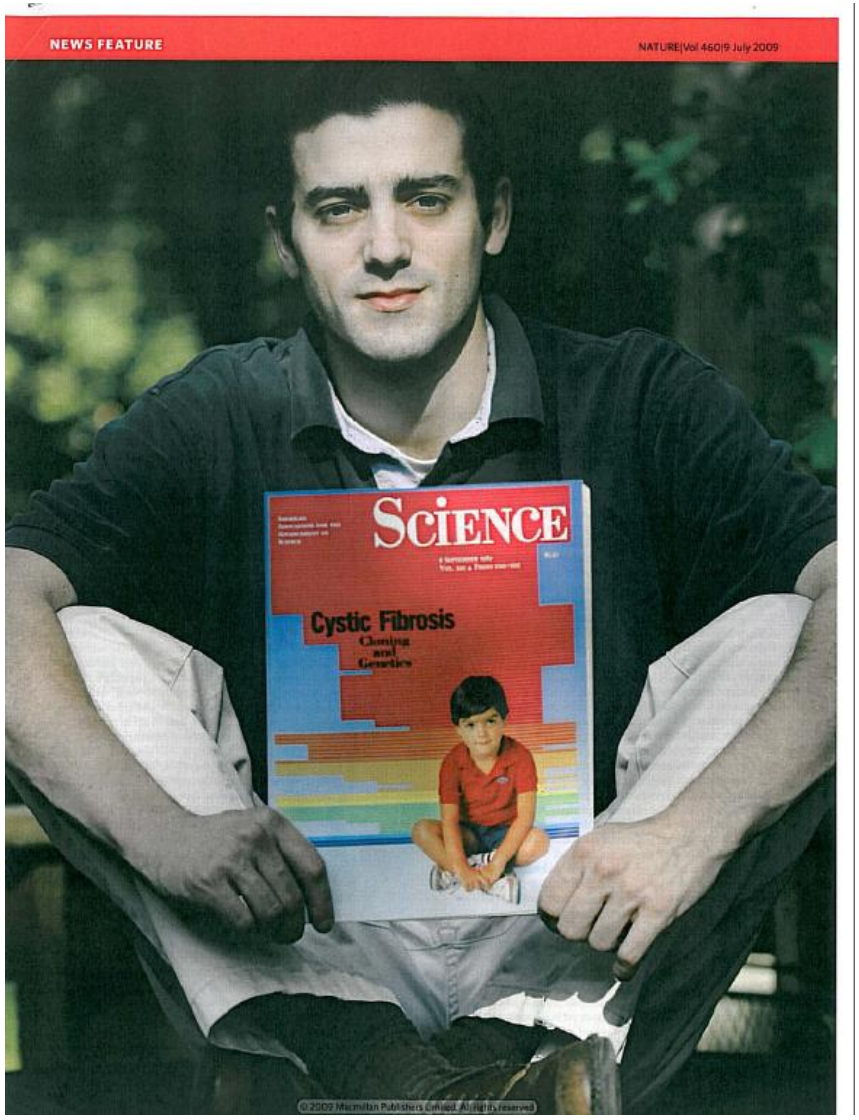
But can we really fix anything and is this the whole story?

Table 2. Examples of Drugs with Genetic Information in Their Labels.*

Drug	Sponsor	Indication	Gene or Genotype	Effect of Genotype	Clinical Directive on Label
Abacavir (Ziagen)	GlaxoSmithKline	HIV-1	*HLA-B*5701*	Hypersensitivity	Black-box warning: "Prior to initiating therapy with abacavir, screening for the *HLA-B*5701* allele is recommended." "Your doctor can determine with a blood test if you have this gene variation."
Azathioprine (Imuran)	Prometheus	Renal allograft transplantation, rheumatoid arthritis	*TPMT*2*, *TPMT*3A*, and *TPMT*3C*	Severe myelotoxicity	"*TPMT* genotyping or phenotyping can help identify patients who are at an increased risk for developing Imuran toxicity." "Phenotyping and genotyping methods are commercially available."
Carbamazepine (Tegretol)	Novartis	Epilepsy, trigeminal neuralgia	*HLA-B*1502*	Stevens–Johnson syndrome or toxic epidermal necrolysis	Black-box warning: "Patients with ancestry in genetically at-risk populations should be screened for the presence of *HLA-B*1502* prior to initiating treatment with Tegretol. Patients testing positive for the allele should not be treated with Tegretol." "For genetically at-risk patients, high-resolution *HLA-B*1502* typing is recommended."
Cetuximab (Erbitux)	Imclone	Metastatic colorectal cancer	*KRAS* mutations	Efficacy	"Retrospective subset analyses of metastatic or advanced colorectal cancer trials have not shown a treatment benefit for Erbitux in patients whose tumors had *KRAS* mutations in codon 12 or 13. Use of Erbitux is not recommended for the treatment of colorectal cancer with these mutations."
Clopidogrel (Plavix)	Bristol-Myers Squibb	Anticoagulation	*CYP2C19*2*3*	Efficacy	"Tests are available to identify a patient's *CYP2C19* genotype; these tests can be used as an aid in determining therapeutic strategy. Consider alternative treatment or treatment strategies in patients identified as CYP2C19 poor metabolizers."
Irinotecan (Camptosar)	Pfizer	Metastatic colorectal cancer	*UGT1A1*28*	Diarrhea, neutropenia	"A reduction in the starting dose by at least one level of Camptosar should be considered for patients known to be homozygous for the *UGT1A1*28* allele." "A laboratory test is available to determine the *UGT1A1* status of patients."
Panitumumab (Vectibix)	Amgen	Metastatic colorectal cancer	*KRAS* mutations	Efficacy	"Retrospective subset analyses of metastatic colorectal cancer trials have not shown a treatment benefit for Vectibix in patients whose tumors had *KRAS* mutations in codon 12 or 13. Use of Vectibix is not recommended for the treatment of colorectal cancer with these mutations."
Trastuzumab (Herceptin)	Genentech	HER2-positive breast cancer	HER2 expression	Efficacy	"Detection of HER2 protein overexpression is necessary for selection of patients appropriate for Herceptin therapy because these are the only patients studied and for whom benefit has been shown." "Several FDA-approved commercial assays are available to aid in the selection of breast cancer and metastatic gastric cancer patients for Herceptin therapy."
Warfarin (Coumadin)	Bristol-Myers Squibb	Venous thrombosis	*CYP2C9*2*3* and *VKORC1* variants	Bleeding complications	Includes the following table: Range of Expected Therapeutic Warfarin Doses Based on *CYP2C9* and *VKORC1* Genotypes.

Adapted from Feero WG, and Guttmacher AE, N Engl J Med 365;11 September 15, 2011

One Gene, Twenty Years



http://www.sciencemag.org/

What CAN'T we tell from your genome?

- Whether you will be killed by a bus or paralysed in a hang gliding accident before your genes get you

Whether you will prefer triangle toast…

Whether you will prefer to sleep outside with a teddy bear…

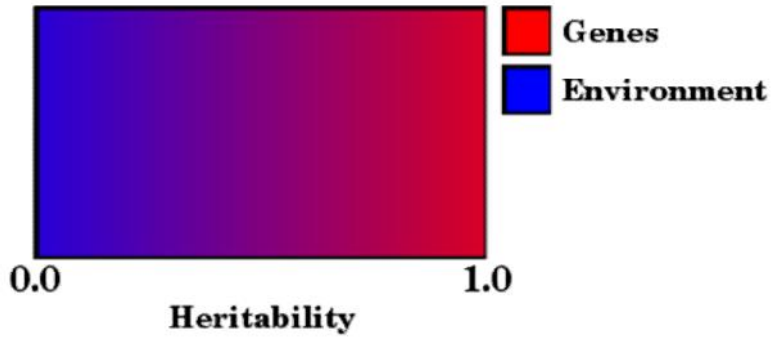
Nor How Happy You Will be!

Finally on this topic

- In the absence of clinical corroboration, gene analysis alone cannot guarantee that you will develop some of the conditions that your genome sequence is associated with.

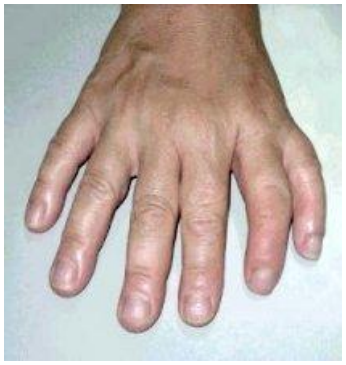
So no need to stress…

Why? Genetics vs Environment



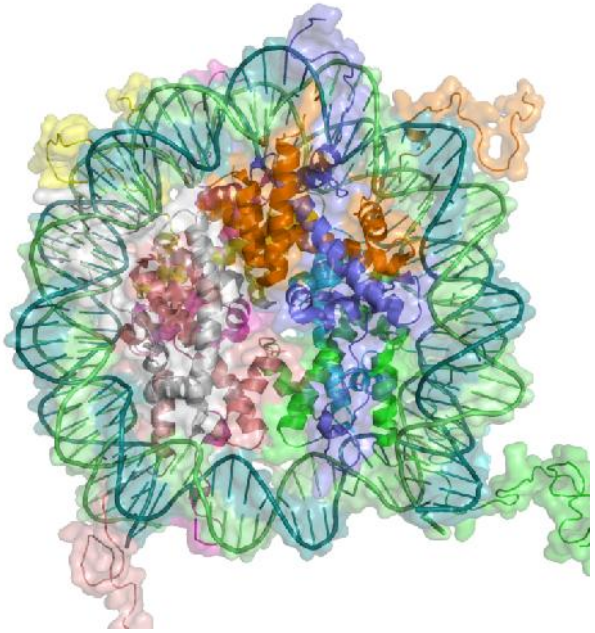
- Coronary artery disease runs in families but does not show mendelian inheritance: smoking, diet, exercise.
- Alzheimer's disease, arthritis, diabetes and cancer are also multifactorial.

Penetrance



- the penetrance of a disease-causing mutation is the proportion of individuals with the mutation who exhibit clinical symptoms.
- For example, if a mutation in the gene responsible for a particular autosomal dominant disorder has 95% penetrance, then 95% of those with the mutation will develop the disease, while 5% will not.

Epigenetics



- =changes in phenotype (appearance) or gene expressior without changes in the underlying DNA sequence
- Basis of cellular differentiation
- Post translational modification of the amino acids that make up histone proteins
- Methylation of gene sequences to reduce transcription

Epigenetics: Imprinting

Prader Willi Syndrome

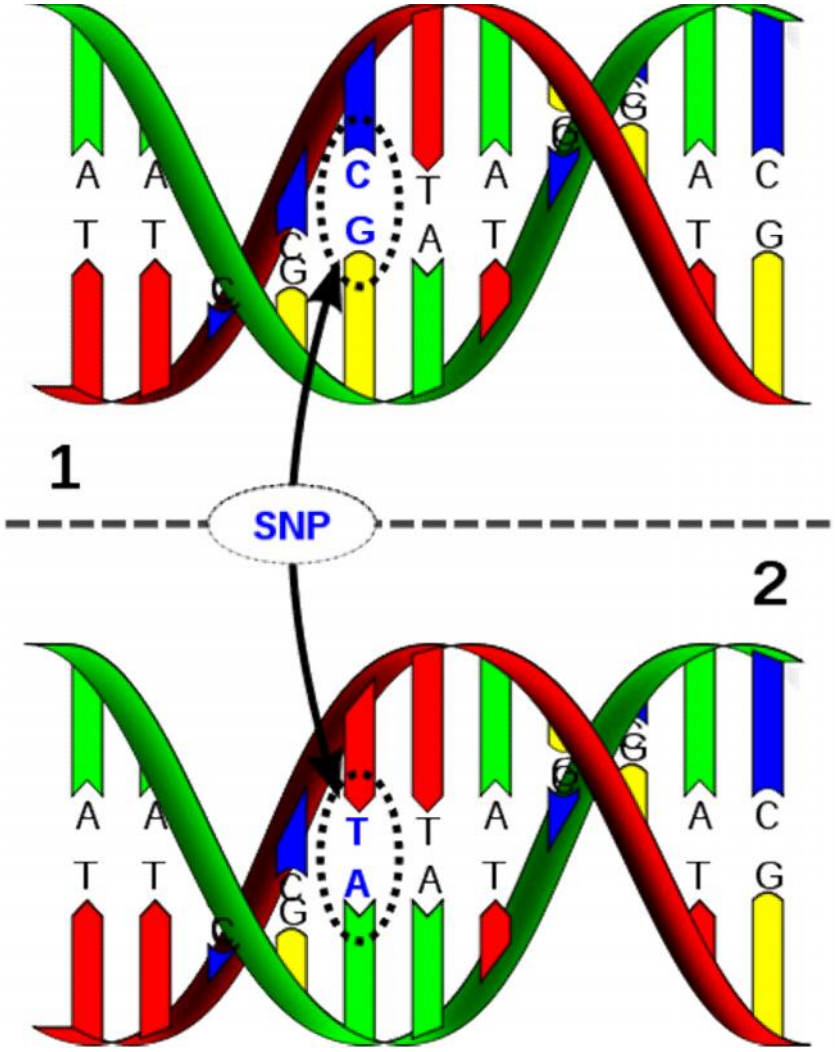
- Hypotonia
- Mental retardation
- Obesity
- Paternal allelle on cs15 deleted (maternal silenced)

Angelmans Syndrome

- Severe mental retardation
- Movement disorder
- Seizures
- Maternal allelle on cs 15 deleted (paternal silenced)

SNP Mutations

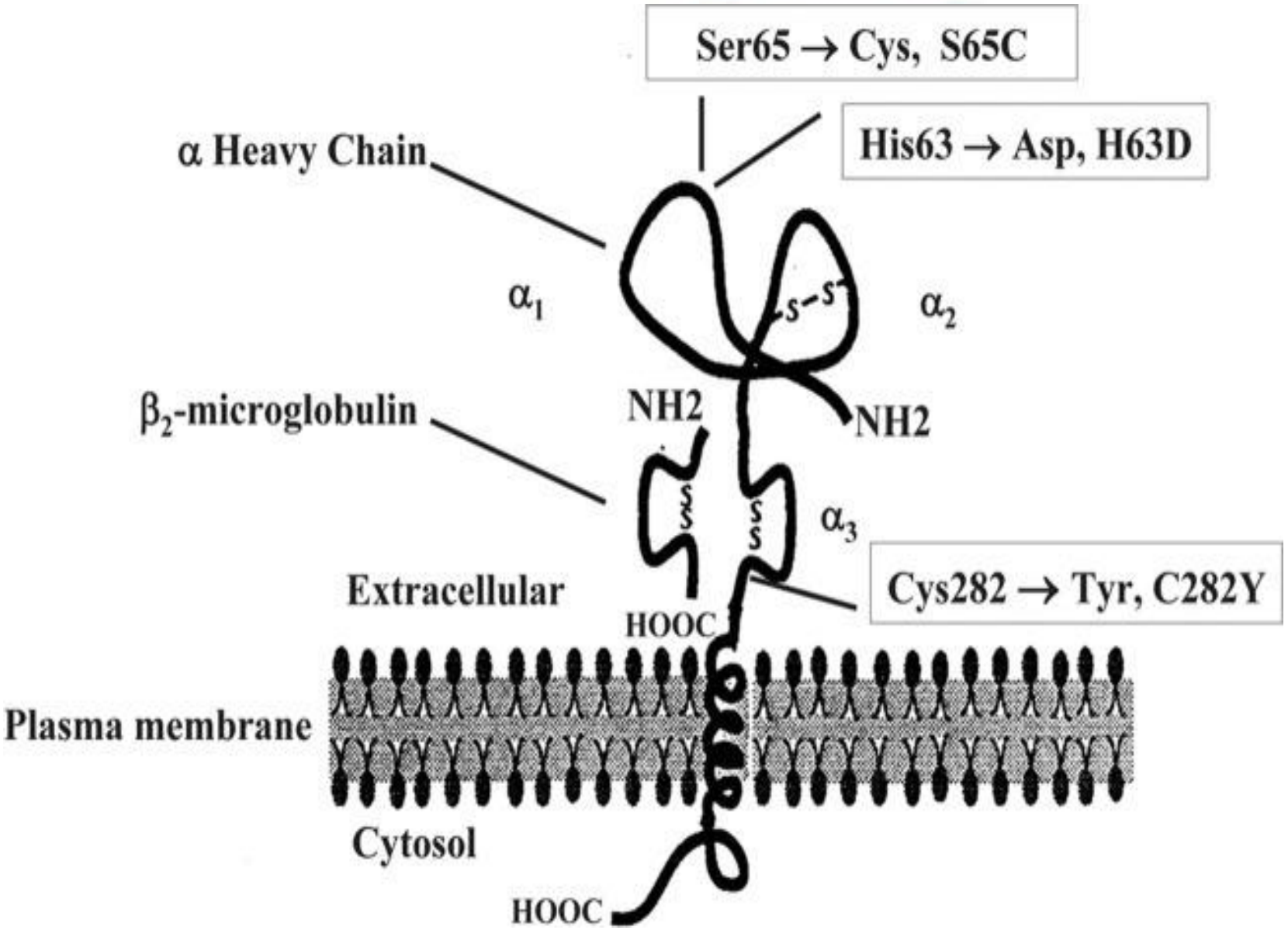
- **Single Nucleotide Polymorphisms** are DNA sequence variations that occur when a single nucleotide (A, T, C, G) differs between individuals or between paired chromosomes in an individual.



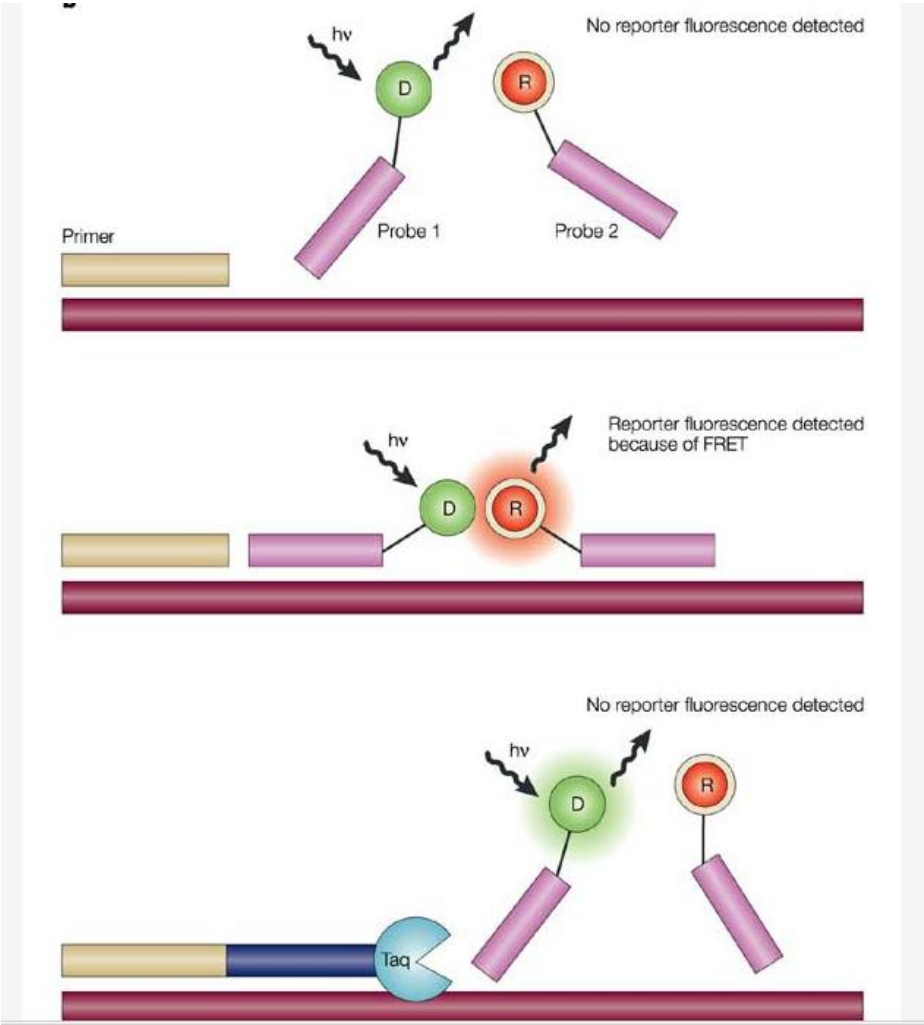
Hereditary Haemochromatosis

- Inherited disease of iron overloading, more common in men.
- Penetrance may be less than 5%
- Caused by SNPs in HFE gene which regulates the production of hepcidin which is a key negative regulator of iron metabolism.

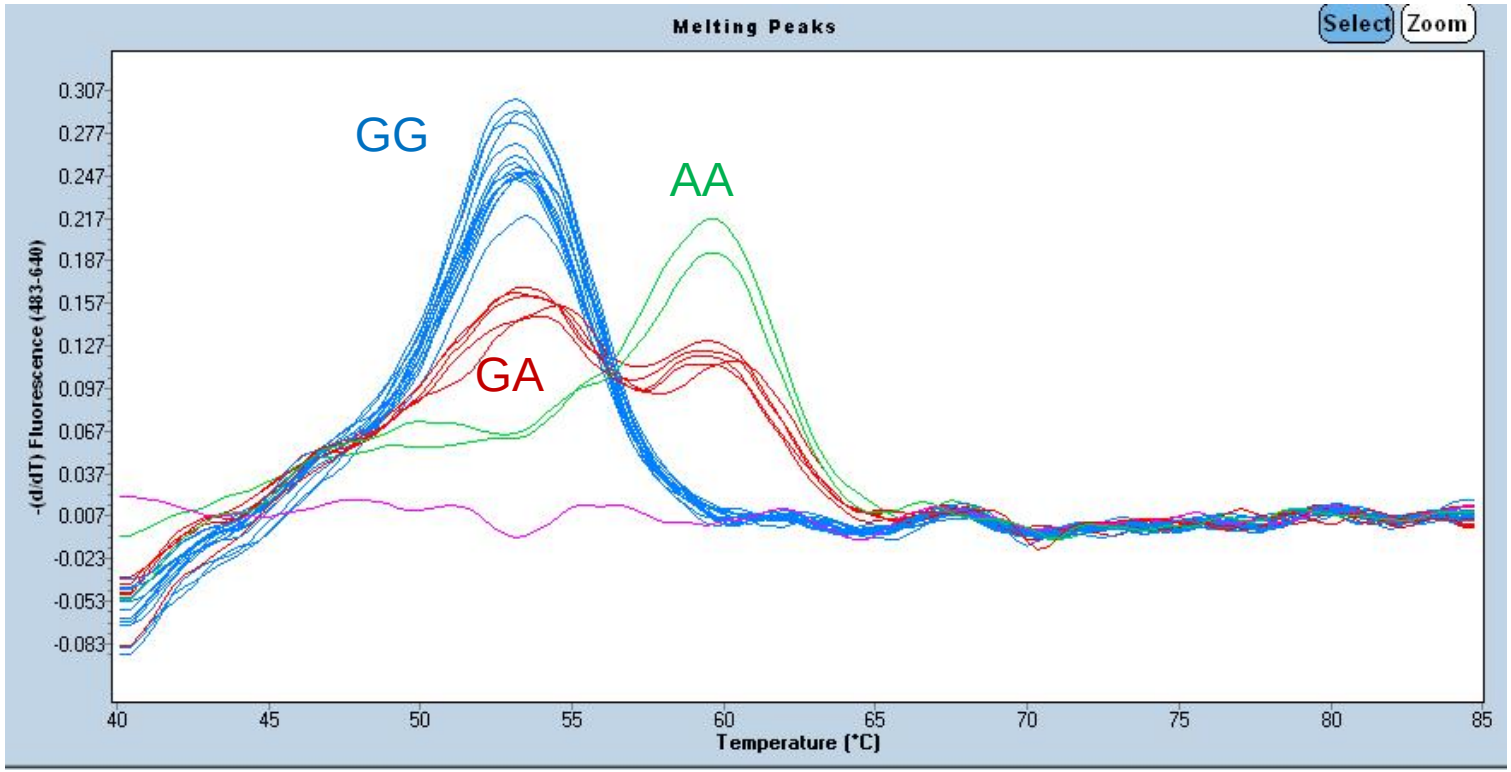
The HFE SNPs


Ser65 → Cys, S65C
His63 → Asp, H63D
α Heavy Chain
α1
α2
S-S
β2-microglobulin
NH2
NH2
α3
Extracellular
Cys282 → Tyr, C282Y
HOOC
Plasma membrane
Cytosol
HOOC

Hybridisation probes



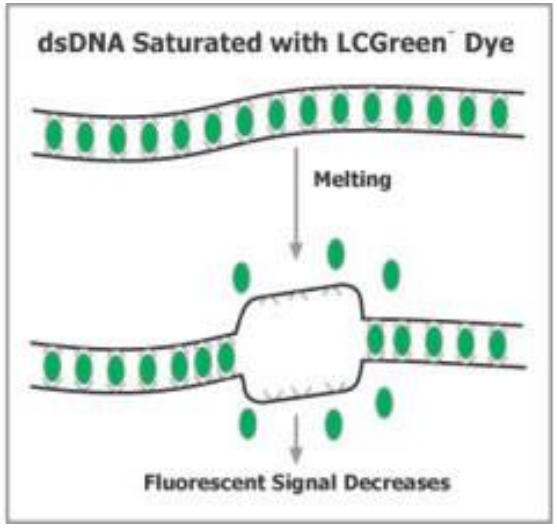
Melting analysis for C282Y SNP

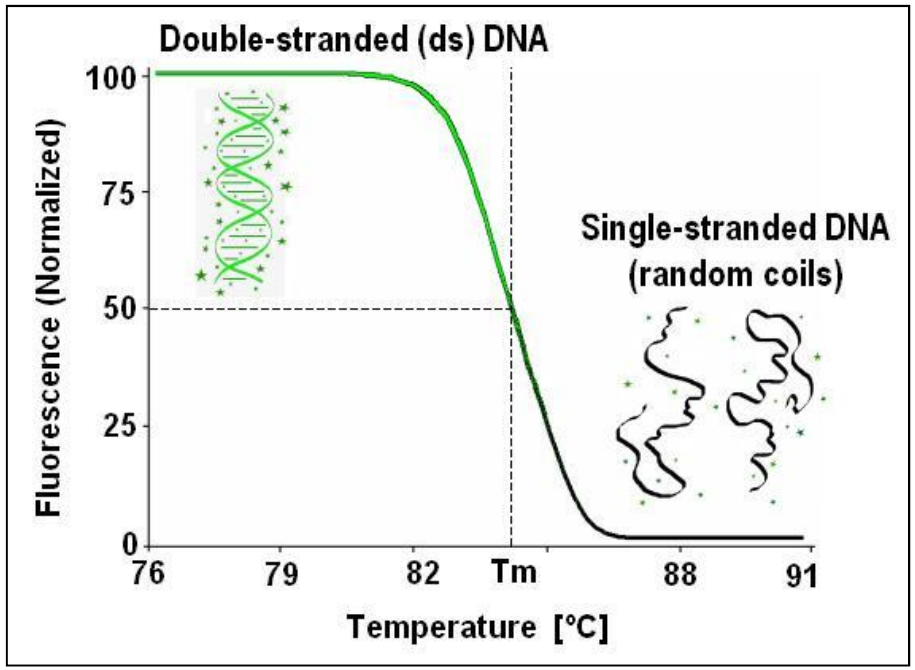


High Resolution Melting

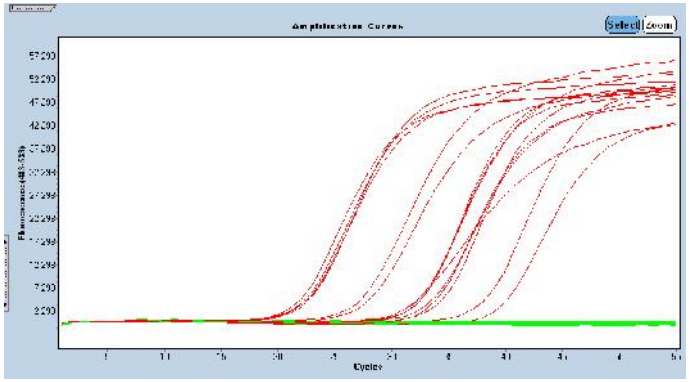
What is High Resolution Melting?

- Melt is monitored in real-time using a fluorescent intercalating dye.
- Very cost effective vs. other genotyping technologies such as sequencing and Taqman SNP typing.



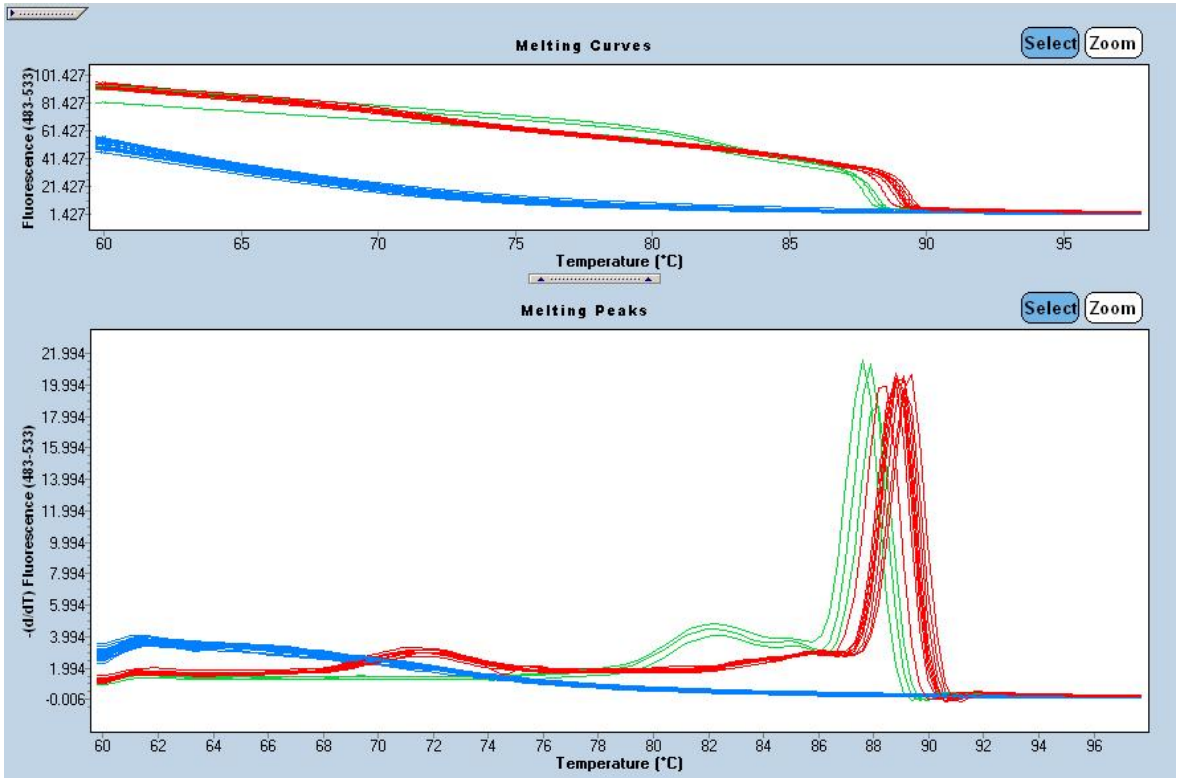


HSV by HRM with Unlabelled Probe

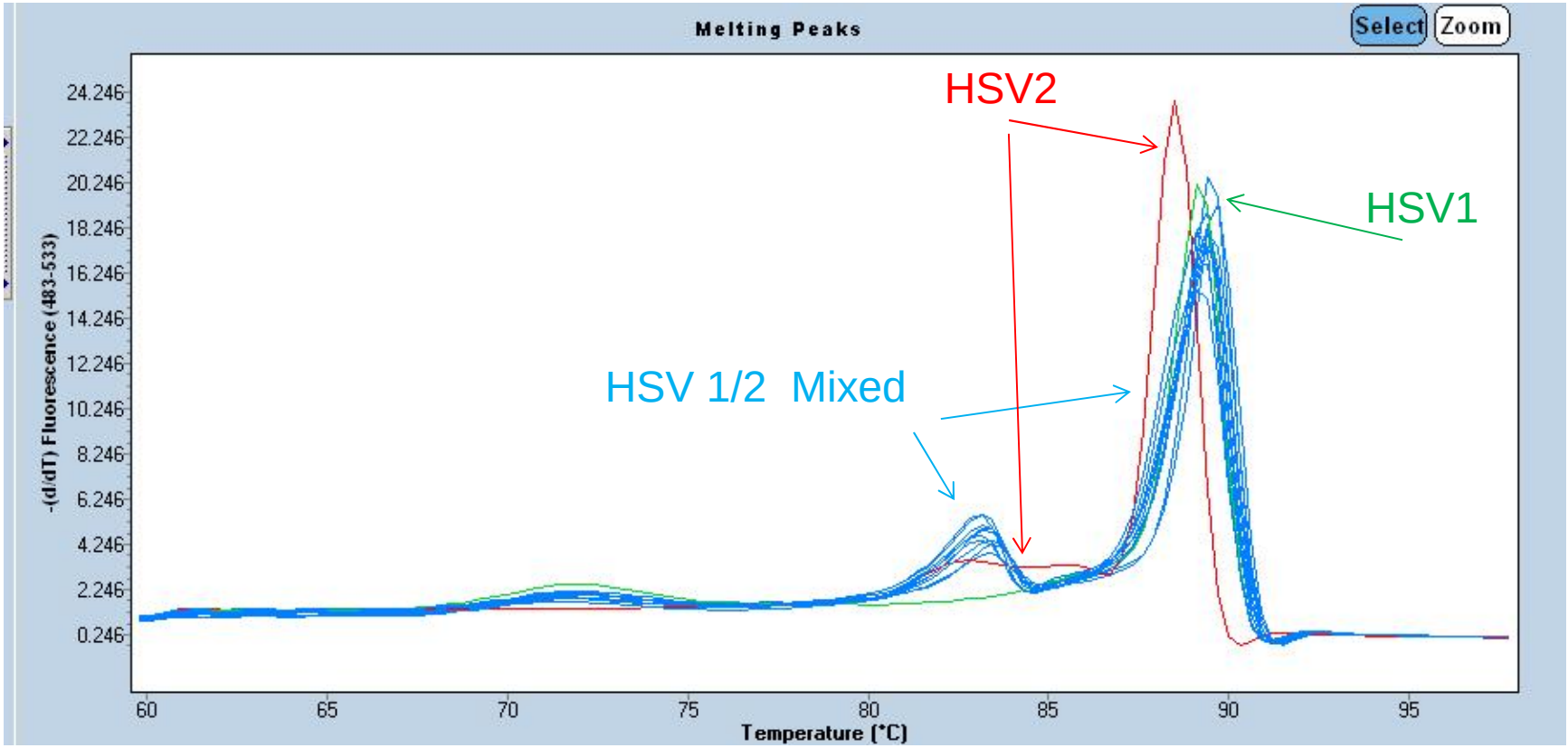


Amplification of glycoprotein D gene…..

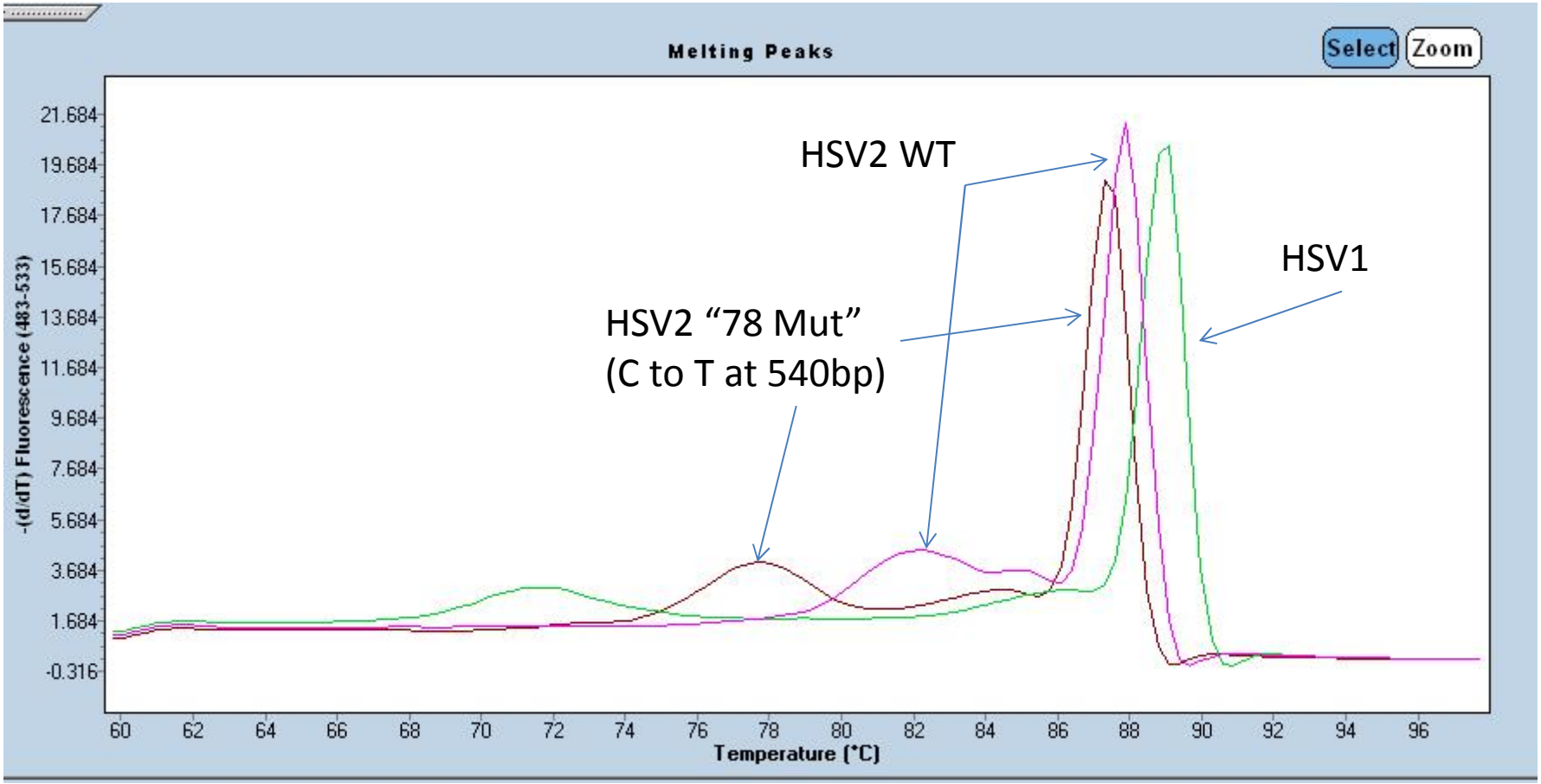
Followed by High Resolution Melting



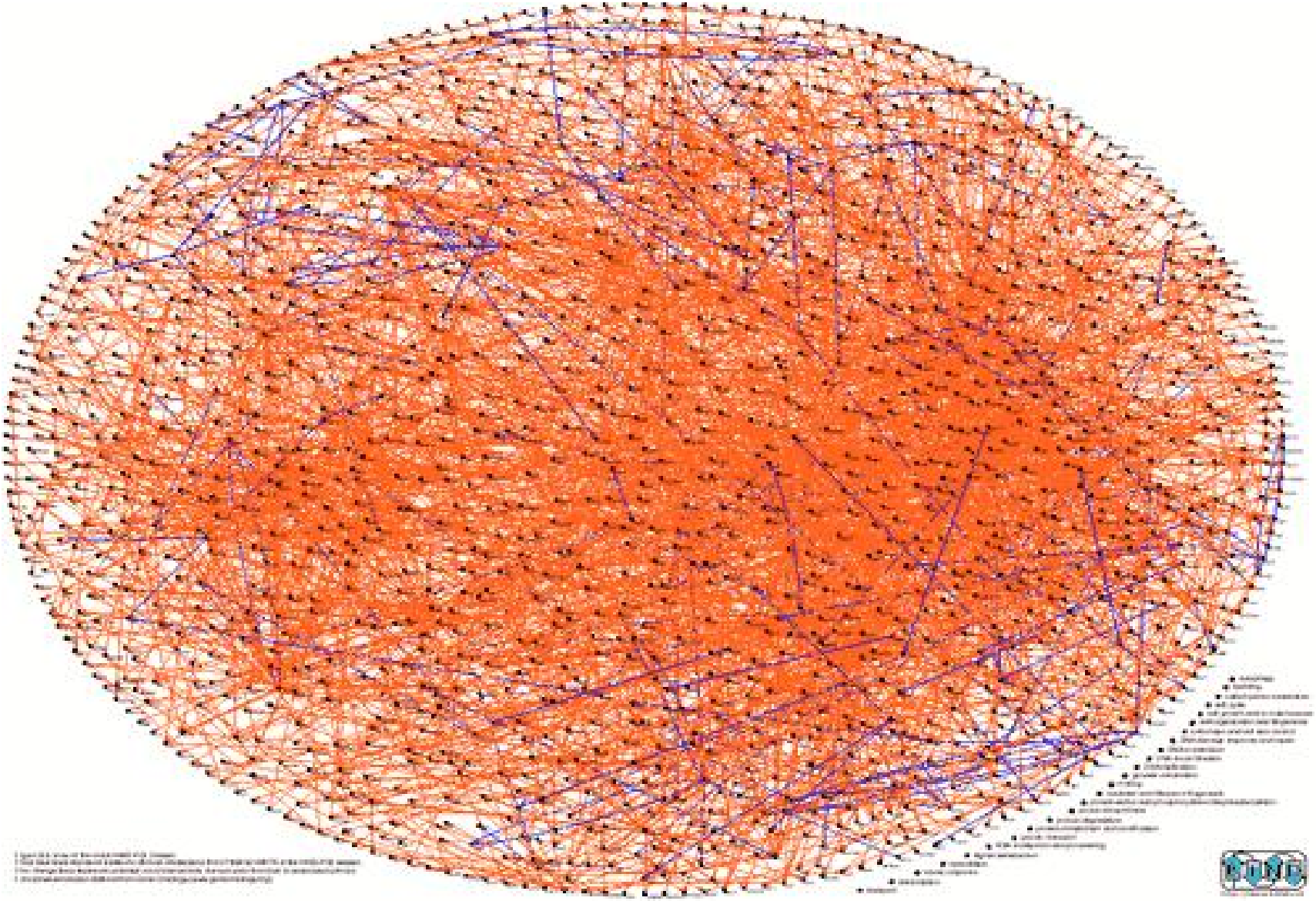
Mixed HSV1/2 Infections



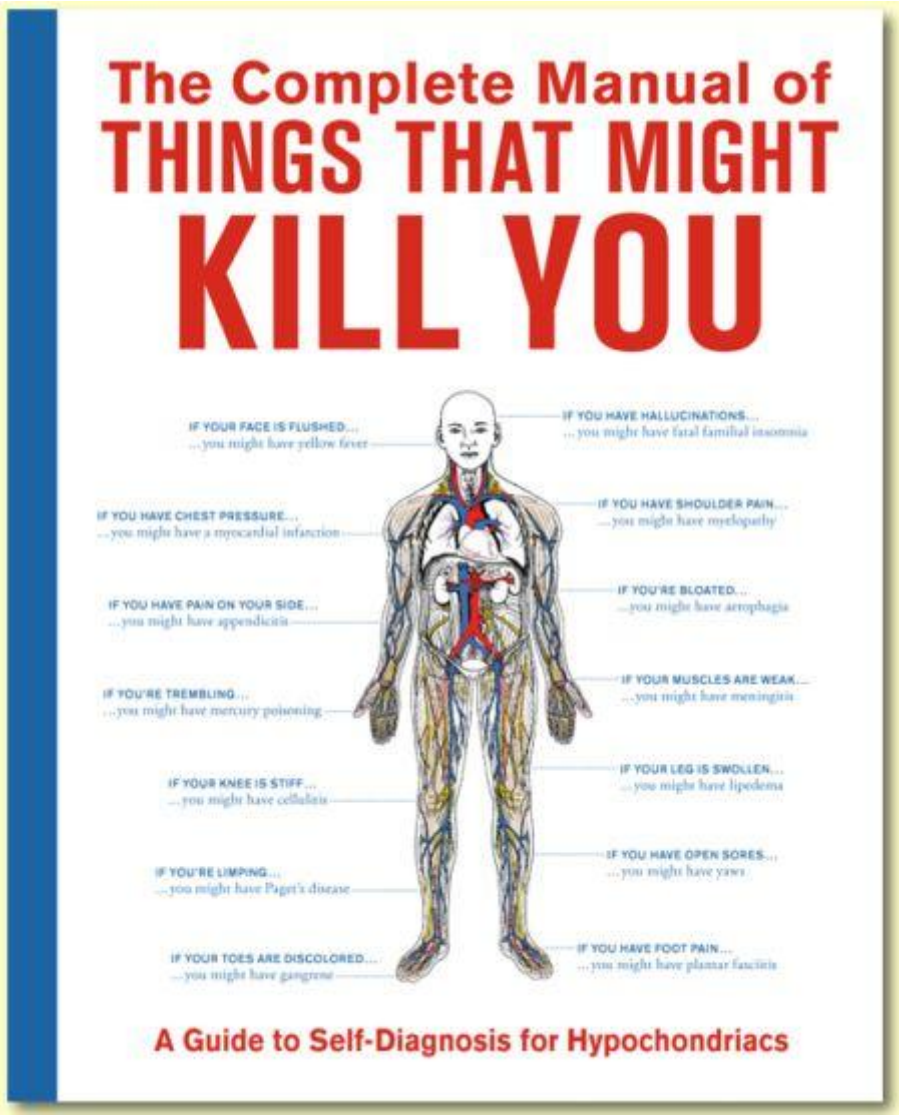
Viral Variants



But what about the Proteome?



The Holy Grail…



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