Periodic Paralysis Association
Orlando, 2011

Overview of Periodic Paralysis Genetic Testing and How a Research Study Works

Dr. Michael Segal, Simultconsult, and

Division of Neurophysiology

Frank Lehmann-Horn, Senior Research Professor

Gemeinnützige Hertie-Stiftung

Identification of a mutation in a gene already known

- White blood cells for extraction of genomic DNA

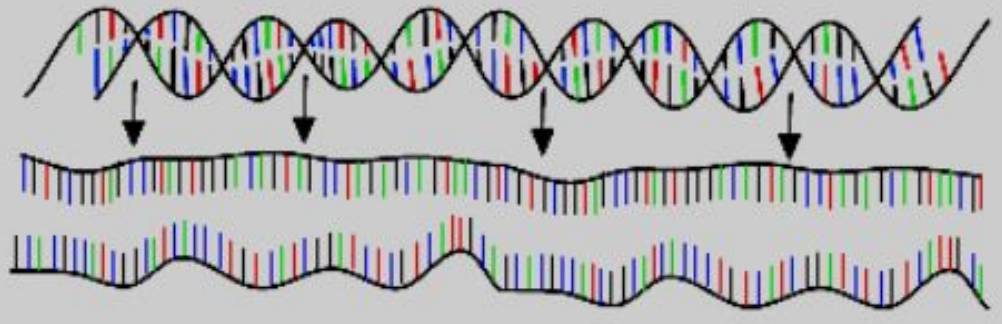
- Amplification of an exon of a certain gene selected by forward and backward primers and the use of the Polymerase Chain Reaction (PCR) to yield products

- Sequencing the PCR products
- Deviations from the normal sequence can be mutations

First PCR step:
Double-stranded DNA has to be separated in two single strands

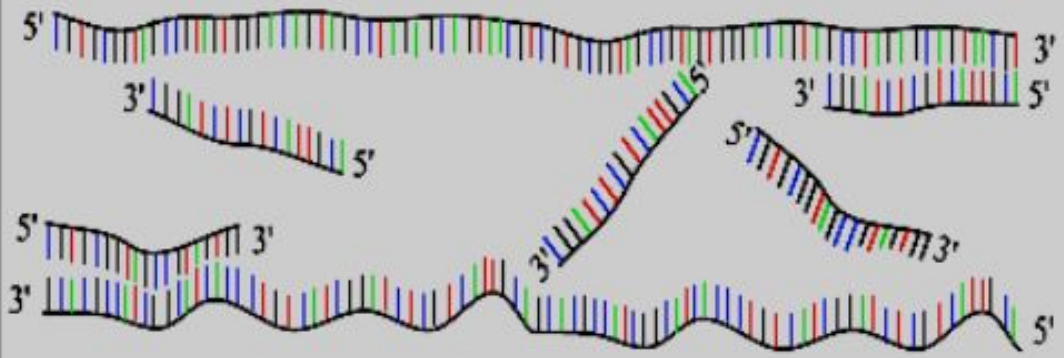
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

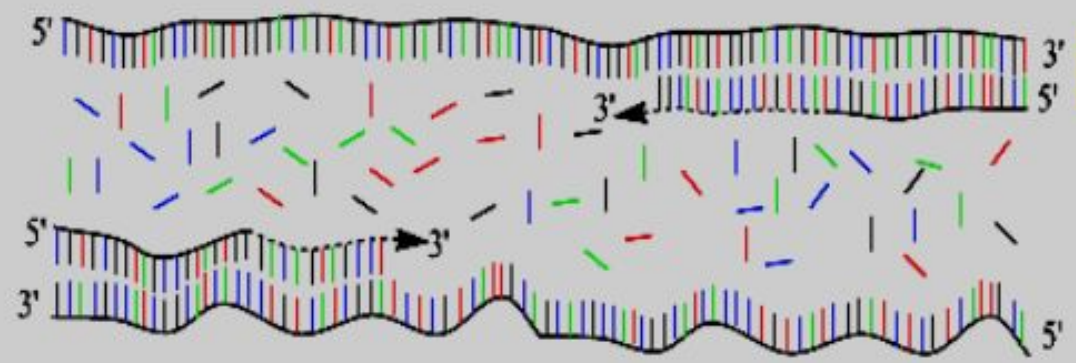
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse primers !!!



Step 3 : extension

2 minutes 72 °C

only dNTP's

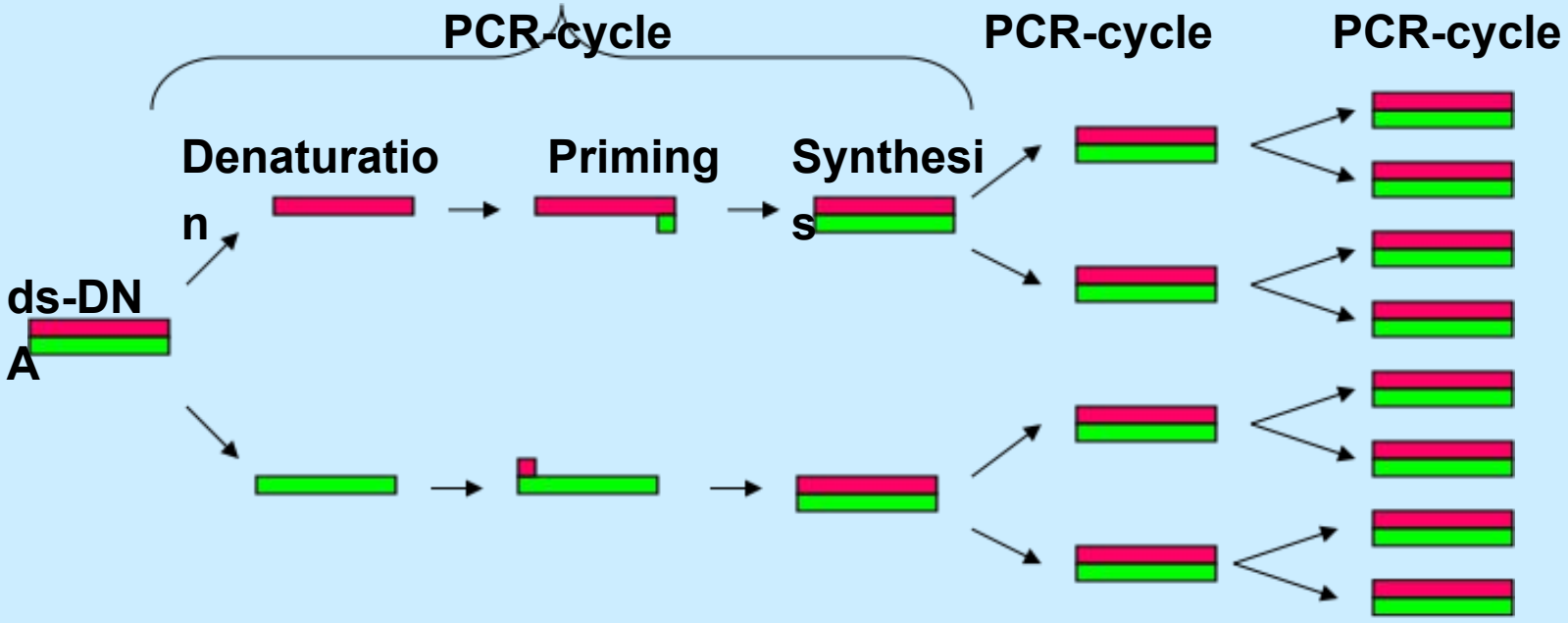
(Andy Vierstraete 1999)

The PCR reaction

... allows us to amplify a certain DNA sequence

Materials:

- 50 ng DNA
- 50 pMol of both primers
- 25 nMol dATP, dCTP, dGTP, dTTP each
- 1,5 units polymerase
- 5 µl 10x-buffer for the polymerase
- Filled up to 50 µl with H_2O

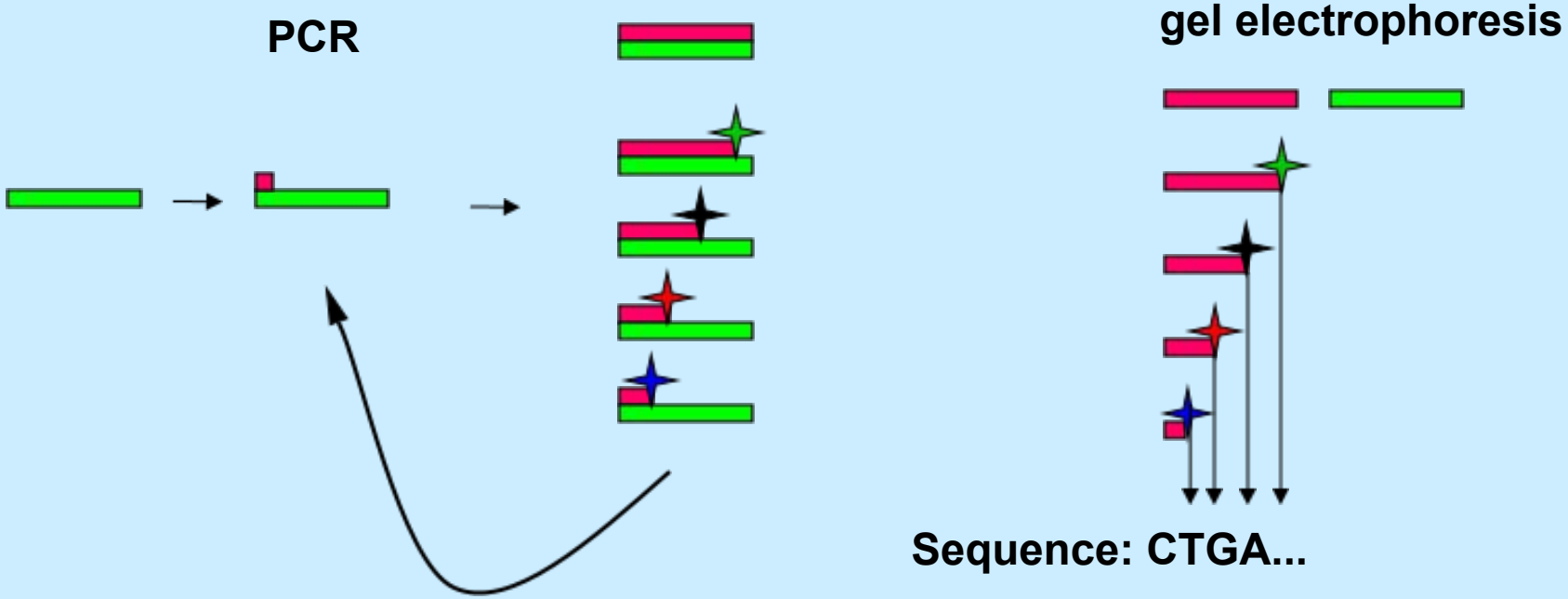


Principle of direct sequencing

The secret is the dNTP mixture!

- around 10 % of dNTP it replaced with ddNTP (>> stops the chain)
- the ddNTP are marked with different colors:

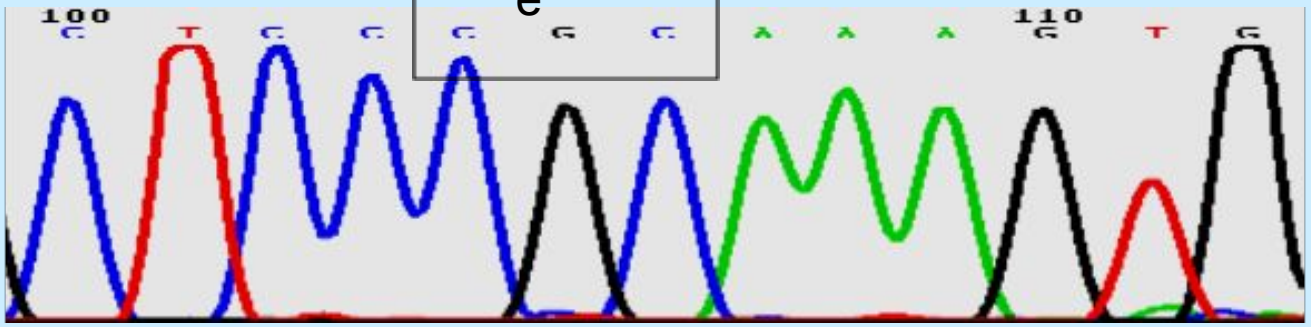
ddATP ddCTP ddGTP ddTTP



Example of sequencing

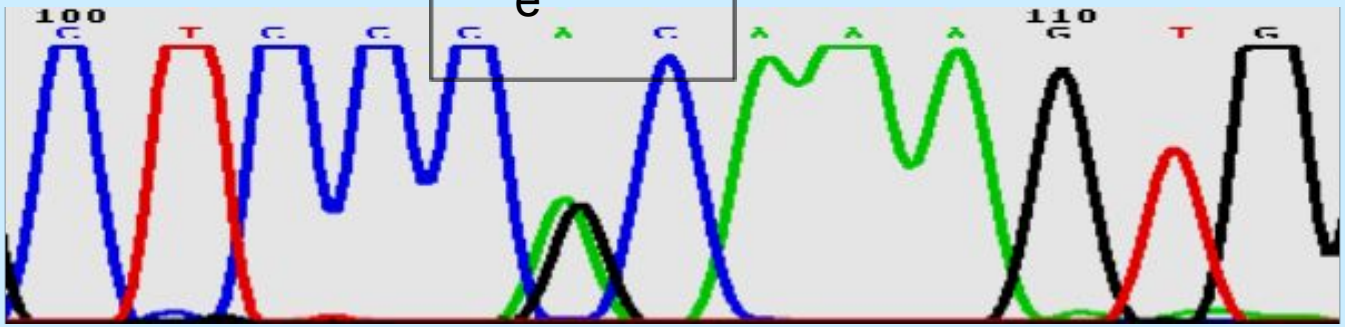
Normal allele

Arginin
e



Heterozygous base exchange G□A

Histidin
e



What evidence suggests a mutation

The genetic alteration leads to an amino acid substitution

The affected amino acid is highly conserved

All affected family members harbor the genetic alteration

All non-affected family members do not harbor the genetic alteration

Functional expression of the mutation shows channel alterations which can explain the clinical phenotype

Absence in large number of controls

Identification of novel PP genes by haplotyping

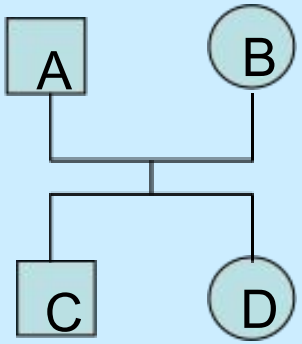
- pedigree requires more than 10 meioses
- the clinical status of the family members must be clear

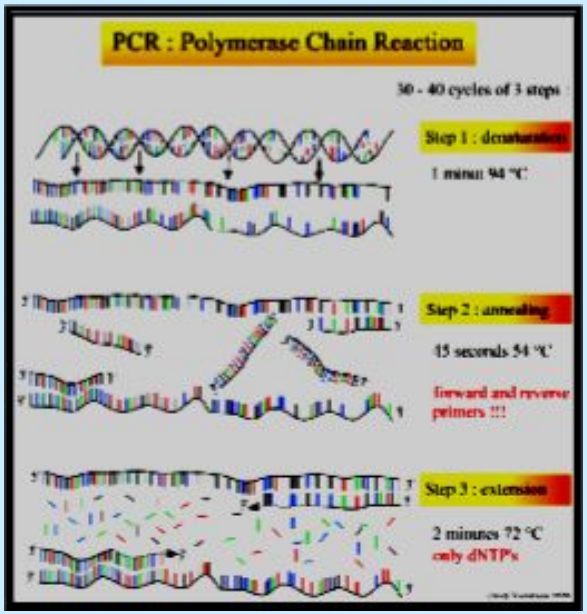
(a single wrong status destroys the search!)

- PCR amplification of DNA sequences characterized by high variability in the population (SNP) & distributed over the entire genome, selected by forward & backward primers
- Sequencing of PCR products and visualization of the pattern (haplotyping)
- Linkage analysis (bioinformatics)

Rationale: if all affected family members and none non-affected member show a certain benign polymorphism, the gene

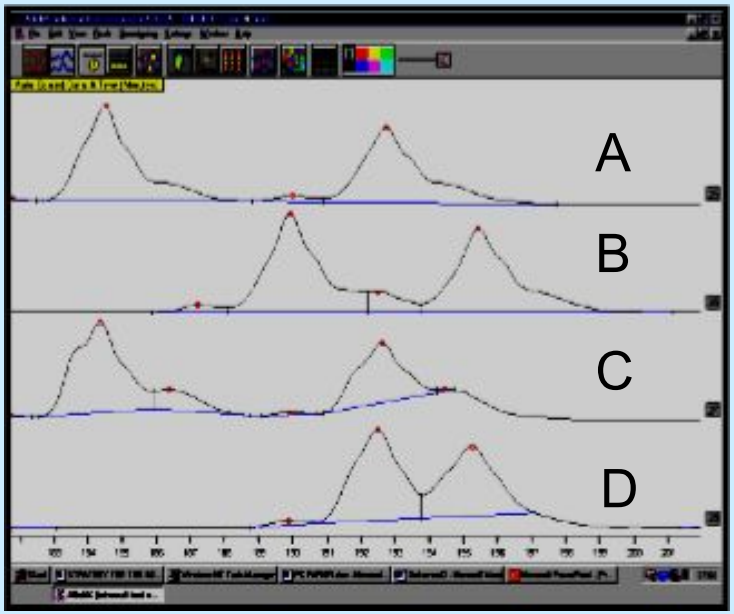
Haplotyping



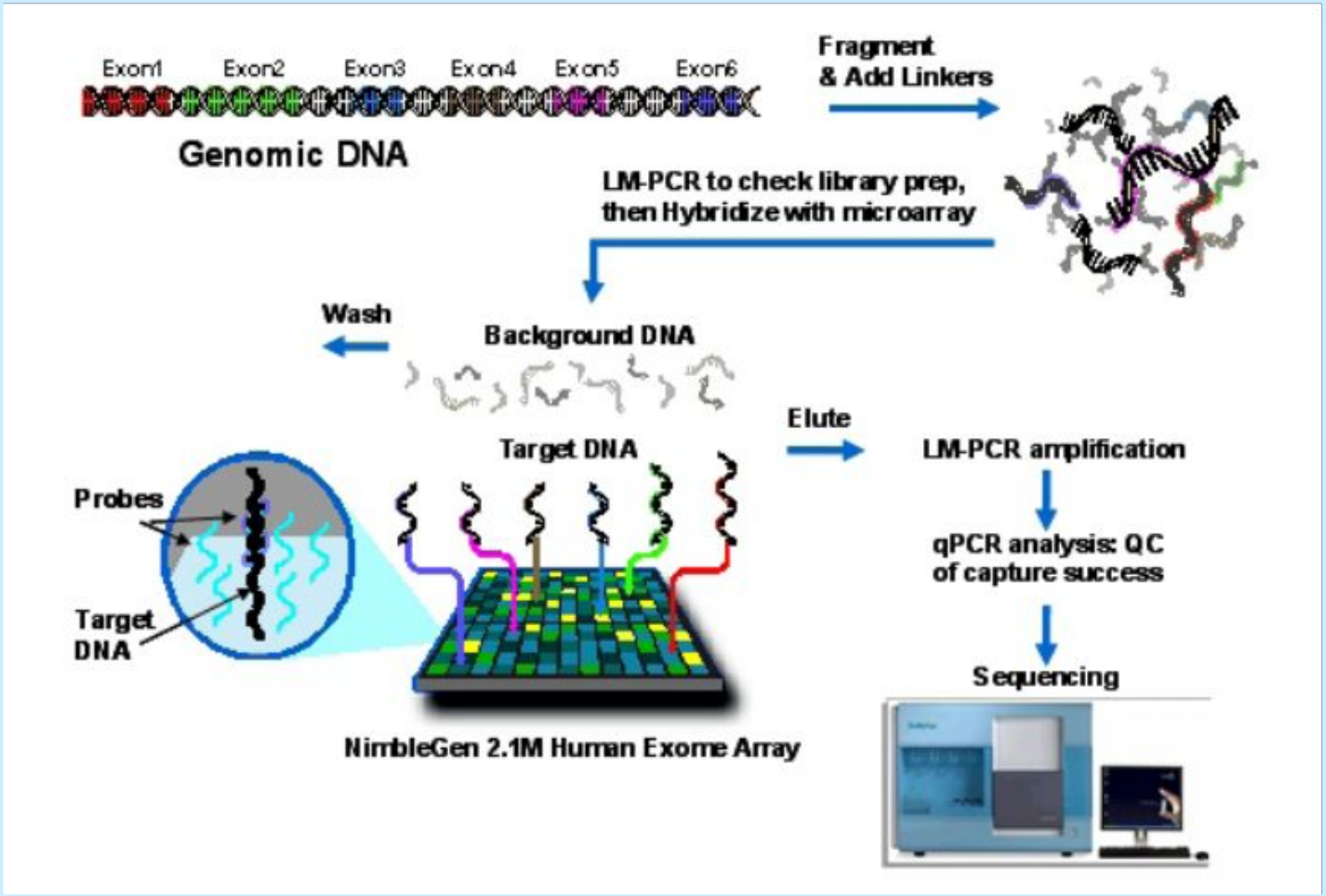


PCR Products

Visualised by denaturating urea-PAGE (ALFexpress™ DNA Sequencer)

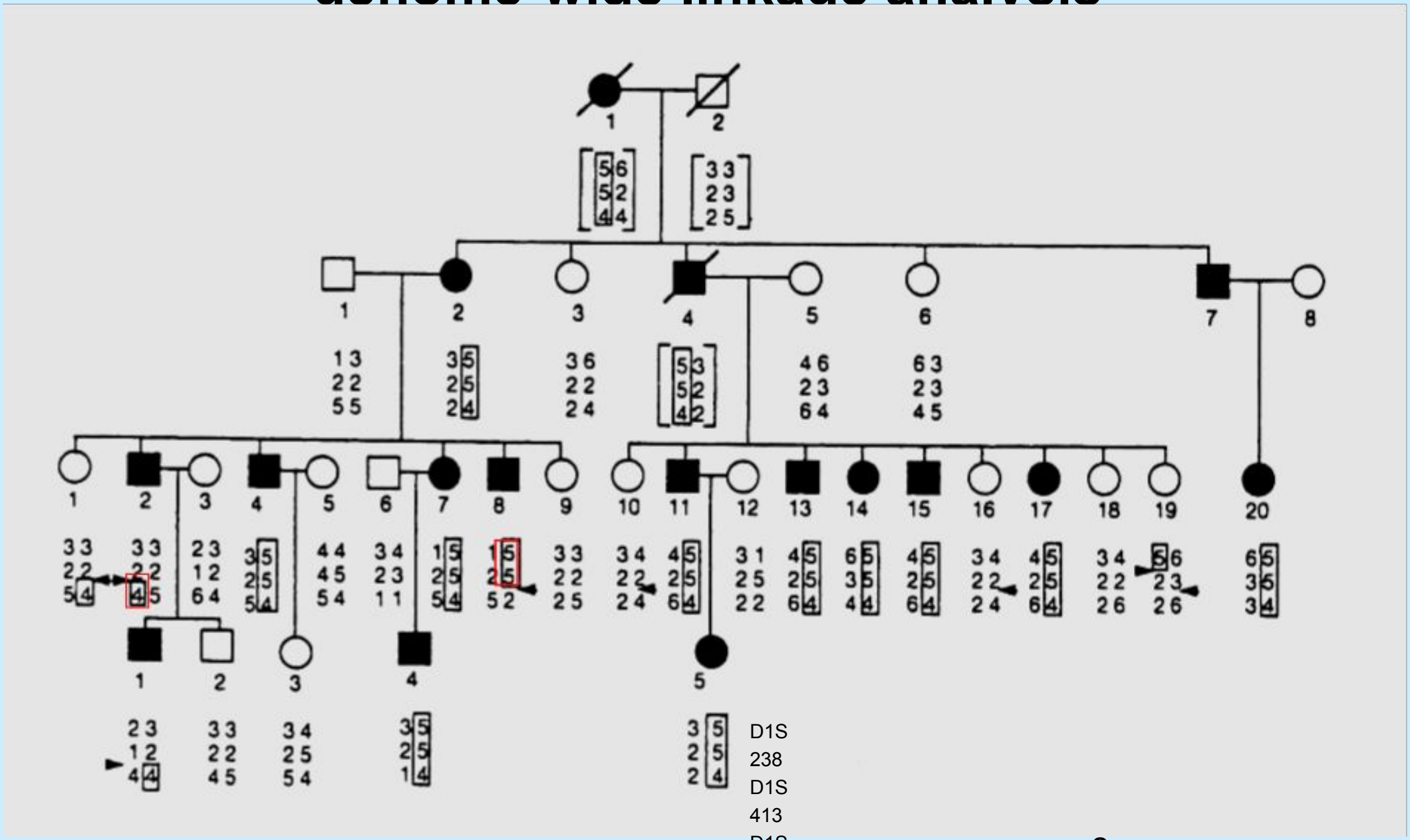


Exome sequencing



For deatails see http://www.genomics.hk/Exome.htm

Hypokalemic periodic paralysis – genome-wide linkage analysis



Chromosomal region of the muscular L-type Ca^{2+} channel

Overview of Periodic Paralysis Genetic Testing and How a Research Study Works

Why medical scientists do things that may seem surprising

Frank Lehmann-Horn MD PhD

Michael Segal MD PhD

Two Types of Studies

- Known mutations: characterizing the disorder
  - Improve diagnosis
    - Narrative educational materials
      - Doctors: traditional articles, textbooks, courses
      - Patients: “Owner’s Manual”
    - Diagnostic software
  - Improve treatment by comparing to similar people
- No known mutation: finding new periodic paralysis genes
  - Look for clusters based on observable differences
    - ADHD / Asperger in “HypoPP+”
    - Diminished effect of lidocaine

When does the scientific method? produce strange results?

- Clinical trials are costly:
  - Non-patentable medicines don't get studied
  - Studying a medication for an orphan drug can raise its price
- Delays in switching to a new understanding: Thomas Kuhn, "The Structure of Scientific Revolutions"

What is being done in Ulm on samples from PPA members?

Mutation screening in the **3 PP genes** in the probands of **69** families (320 people): mutation identified in **14** probands only

Mutation checked in all family members of the 14 probands

Mutation screening in the **Bartter genes** in the probands of **5** families: modifying alterations identified in **2** probands

Mutation screening in the gene areas encoding the **voltage sensors of 13 ion channels** expressed in muscle in the probands of 55 families: no mutation identified

Genome-wide linkage study in **4 large HypoPP-plus families**; reduction of the **35,000** human genes to about **1,000** genes; currently fine mapping in the identified chromosomal areas