Leiden University
Medical Center

Farmacogenetica – analysetechnieken

PUOZ labdag 22-11-2016

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Associate Professor of Pharmacogenetics
Sectiehoofd Laboratorium
Klinische Farmacie & Toxicologie
Leids Universitair Medisch Centrum

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Is Your Medicine Right for Your Metabolism?

More genetic tests aim to help predict how people might respond to many common medications

By MELINDA BECK

Updated March 14, 2016 3:05 p.m. ET

People can respond to drugs very differently. A medication that brings relief for some patients might show no benefit at all in others, or even cause harmful side effects.

deVolkskrant

'Geef elke Nederlander een medisch dna-paspoort'

Dna-paspoort kan ernstige bijwerkingen voorkomen

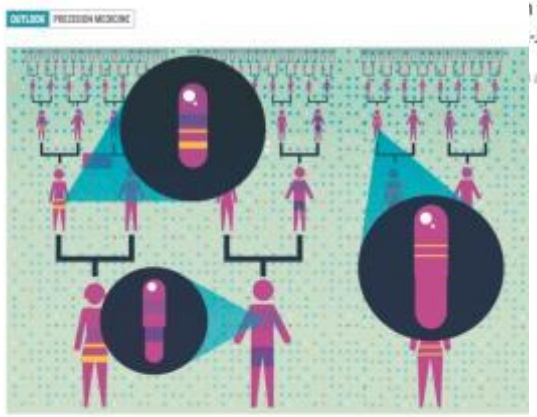
Geef elke Nederlander een medisch dna-paspoort. Dat kan veel ernstige bijwerkingen tijdens behandelingen en zelfs sterfgevallen voorkomen. Dat
farmacogenetica Ron van Schaik, in het Erasmus MC in
-Jan Guchelaar in het Leidse LUMC.

Gezondheid van Nederland

'DNA-paspoort voor alle Nederlanders'

Gepubliceerd: 30 april 09:04
Update: 30 april 21:28

Alle Nederlanders moeten een DNA-paspoort krijgen. Daarin staan de genen die bepalen of iemand afwijkend op een medicijn of behandeling reageert. Verschillende artsen en hoogleraren van het Erasmus MC en het LUMC pleiten daarvoor.



OUTLOOK PRECISION MEDICINE

The right drug for you

Personalized prescribing is gaining momentum, but is there enough evidence for it to become standard clinical practice?

BY LIAM DEEN

Medical News & Perspectives

Getting Pharmacogenomics Into the Clinic

Jennifer Abbasi

Personalised = trendy

‘Most drugs don’t work’

Effective (%)…..

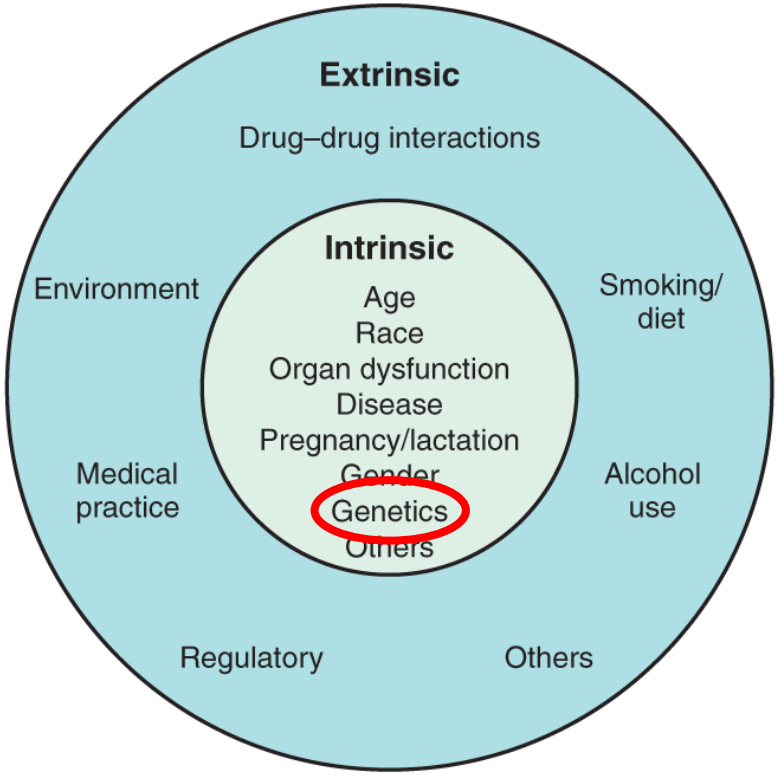
- Alzheimer 30
- Depression (SSRI) 62
- Asthma 60
- Diabetes mellitus 57
- Migraine (acute) 52
- Migraine (profyl.) 50
- Cradiac dysrhythmia 60
- Tumors 25
- Schizophrenia 60
- Reumatoid arthritis 50
- Reumat. art. (Cox-2) 80
- Hepatitis C 47

Spear, Trends Mol Med 2001;7(5):201

How is this possible?

- Non-adherence
- Wrong drug-use
- Interacting co-medication
- Organ dysfunction
- Different stage of the disease
- Environmental factors
  - food, alcohol

….



Heritable (genetic) differences in drug sensitivity

Farmacogenetica

DNA Test

100% dosis

Geneesmiddel A

50% dosis

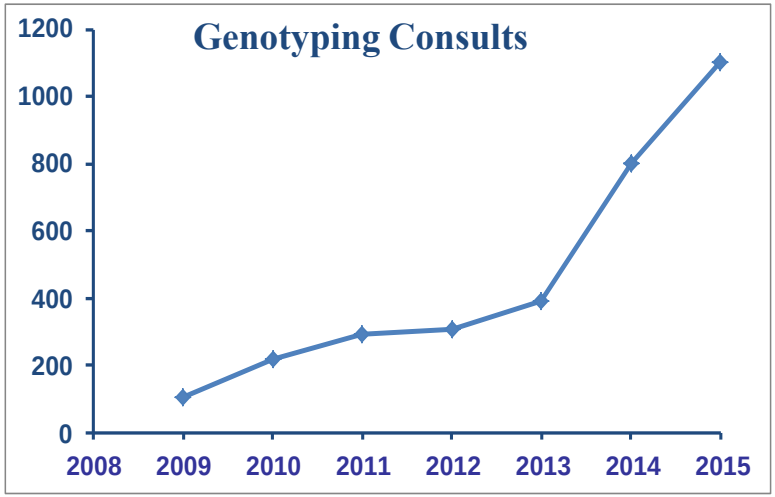
Geneesmiddel A

Geneesmiddel B

Pharmacogenetics @ LUMC

Prospective test:

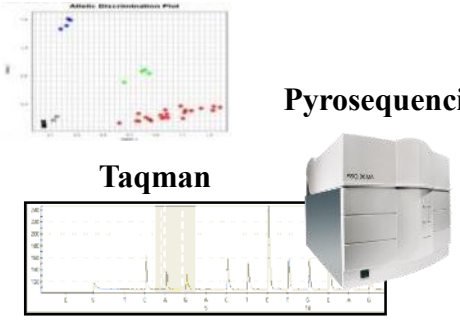
- Oncology: patients receiving CAP or 5-FU tested for *DPYD* (rs3918290, rs55886062, rs67376798, rs56038477)
- Nephrology: kidney transplant patients tested for *CYP3A5* (rs776746, rs10264272).
- Psychiatry: patients with a therapy resistant depression, referred to LUMC for ECT, tested for *CYP2D6,* and *CYP2C19*.
- PGx consultation service for outpatients



On request: VKORC1, CYP2C9, SLCO1B1, UGT1A1, THYMS, ABCB1, NAT1, NAT2, CYP3A4, CYP2B6 other….

Workflow example: Individualization of fluoropyrimidine therapy

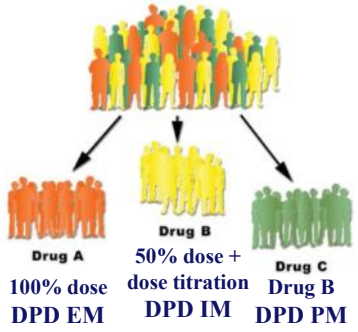
Rx fluoropyrimidine



Genotyping with 2 independent techniques

Technical approval

Interpretation and Consult: Hospital Pharmacist



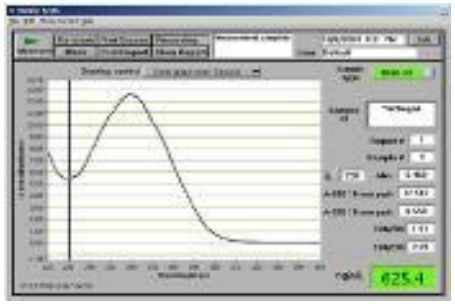
Individualized Dose Recommendation

Van monster naar DNA

Monstername → **DNA isolatie** → **“QC”** → **DNA voor analyse**

2 ml speeksel

4 ml EDTA bloed

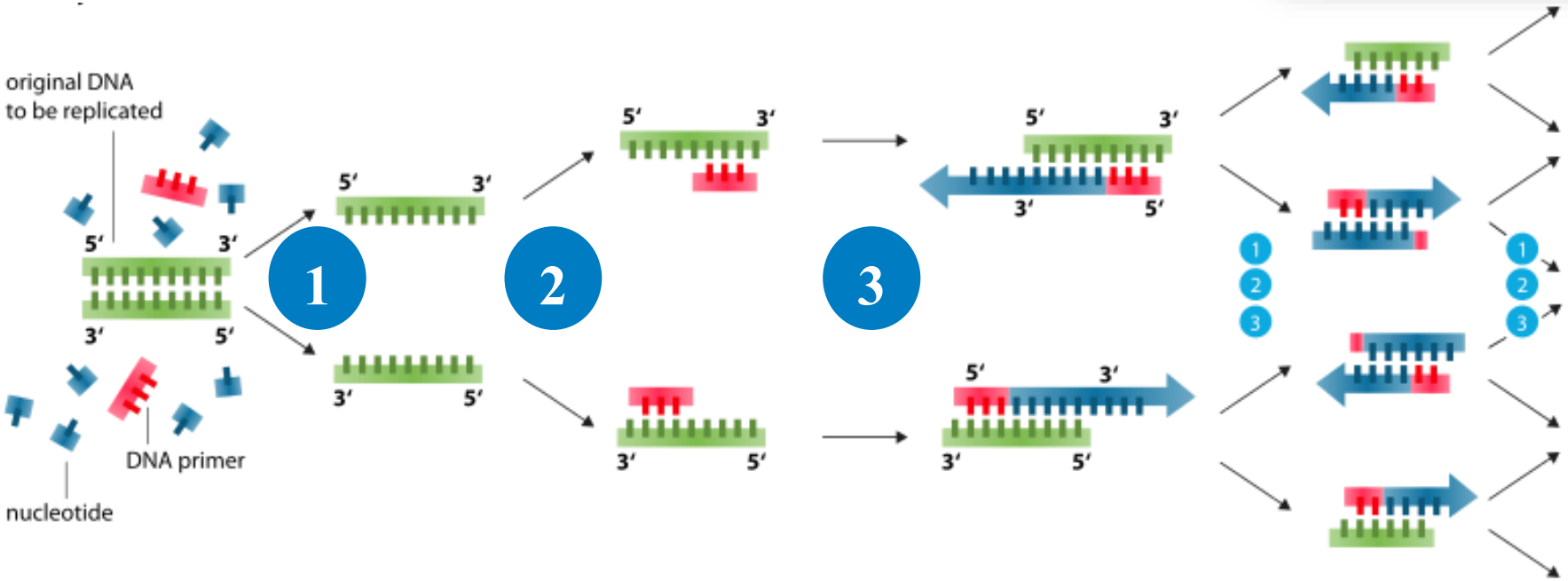


260/280 ratio → 1,5 - 2

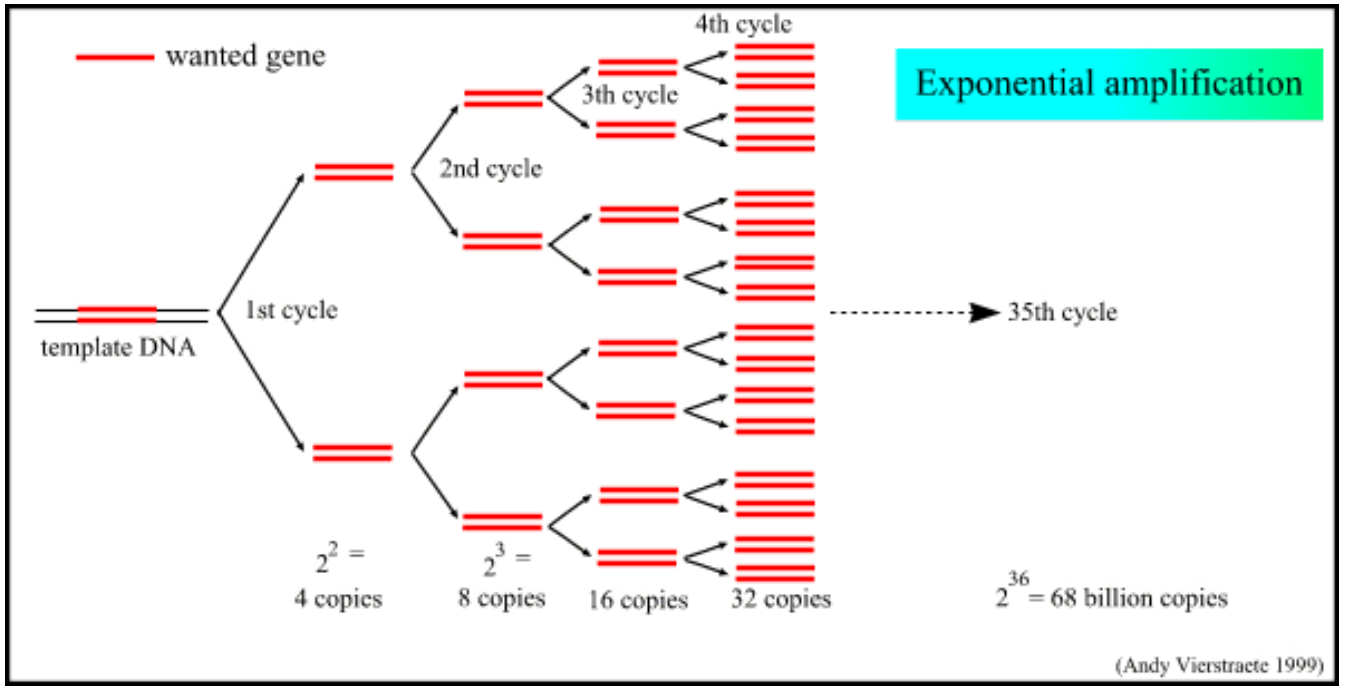
Polymerase Chain Reaction

Invented in 1983 by Kary Mullis, Nobelprice in 1993

1. Denaturation (90 - 96°C)
2. Annealing of primers (45 - 65°C)
3. Elongation by thermostable polymerase (72°C)



Polymerase Chain Reaction



1983

1993

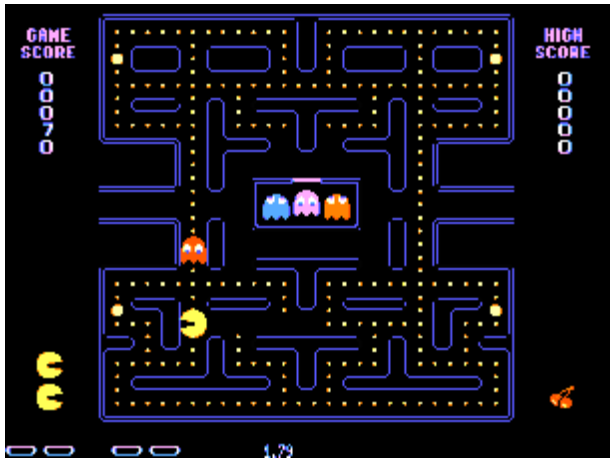
2003

Beschikbare methoden

- PCR gel electroforese
- Real-time PCR genotyping assays
- Pyrosequencing
- Genotyping SNP Array
- High Resolution Melting
- Sanger sequencing
- Next Generation Sequencing

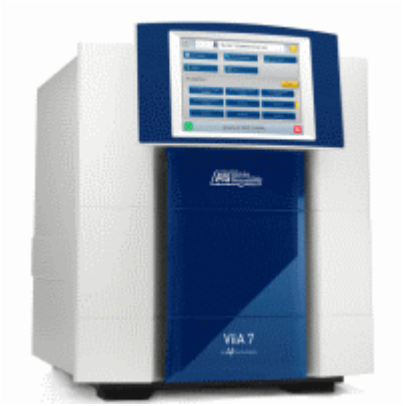
Techniek 1 Real-time PCR: TaqMan

PacMan



- Real-time PCR
- Addition of labeled probes
- Easy to perform; pre-designed assays
- Fluorescence

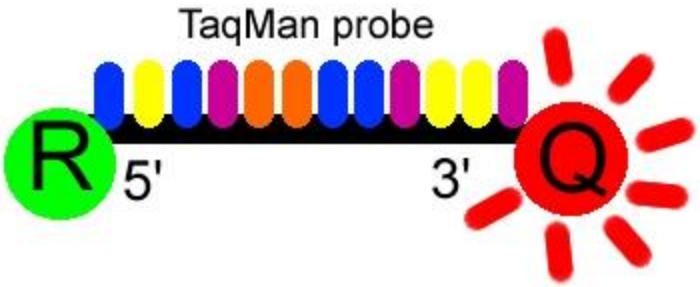
LightCycler® 480 System



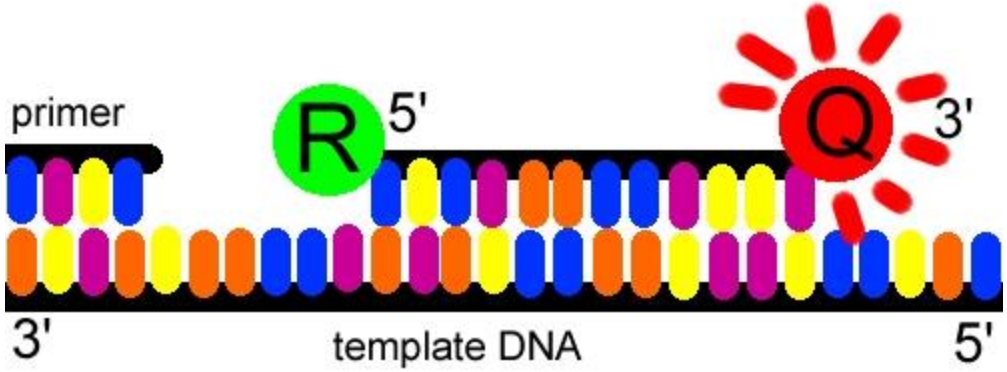
ViiA™ 7 Real-Time PCR System

QuantStudio 12K Flex RT PCR System

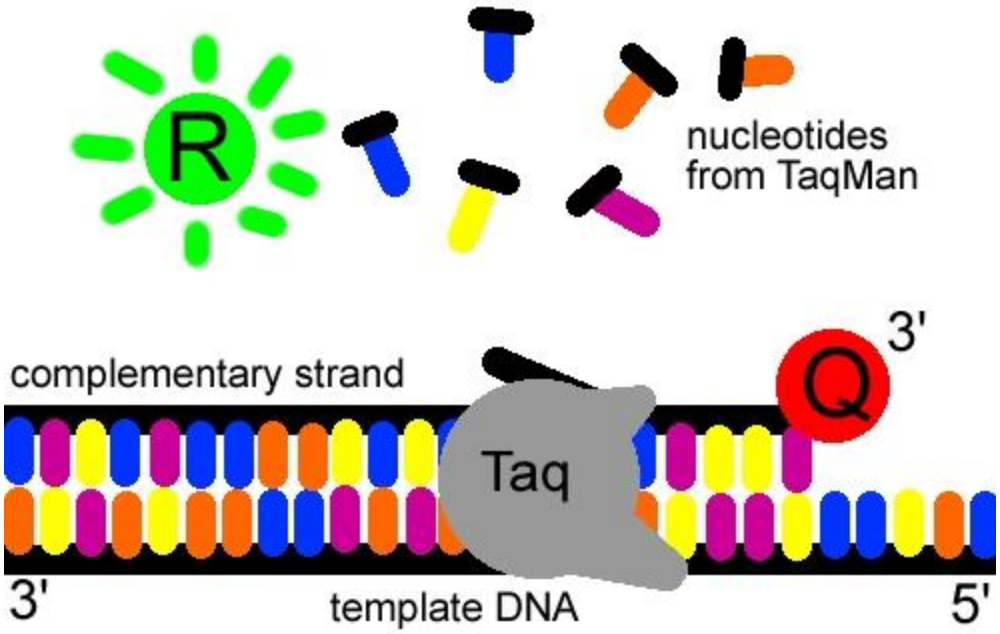
Principle: probe based SNP detection



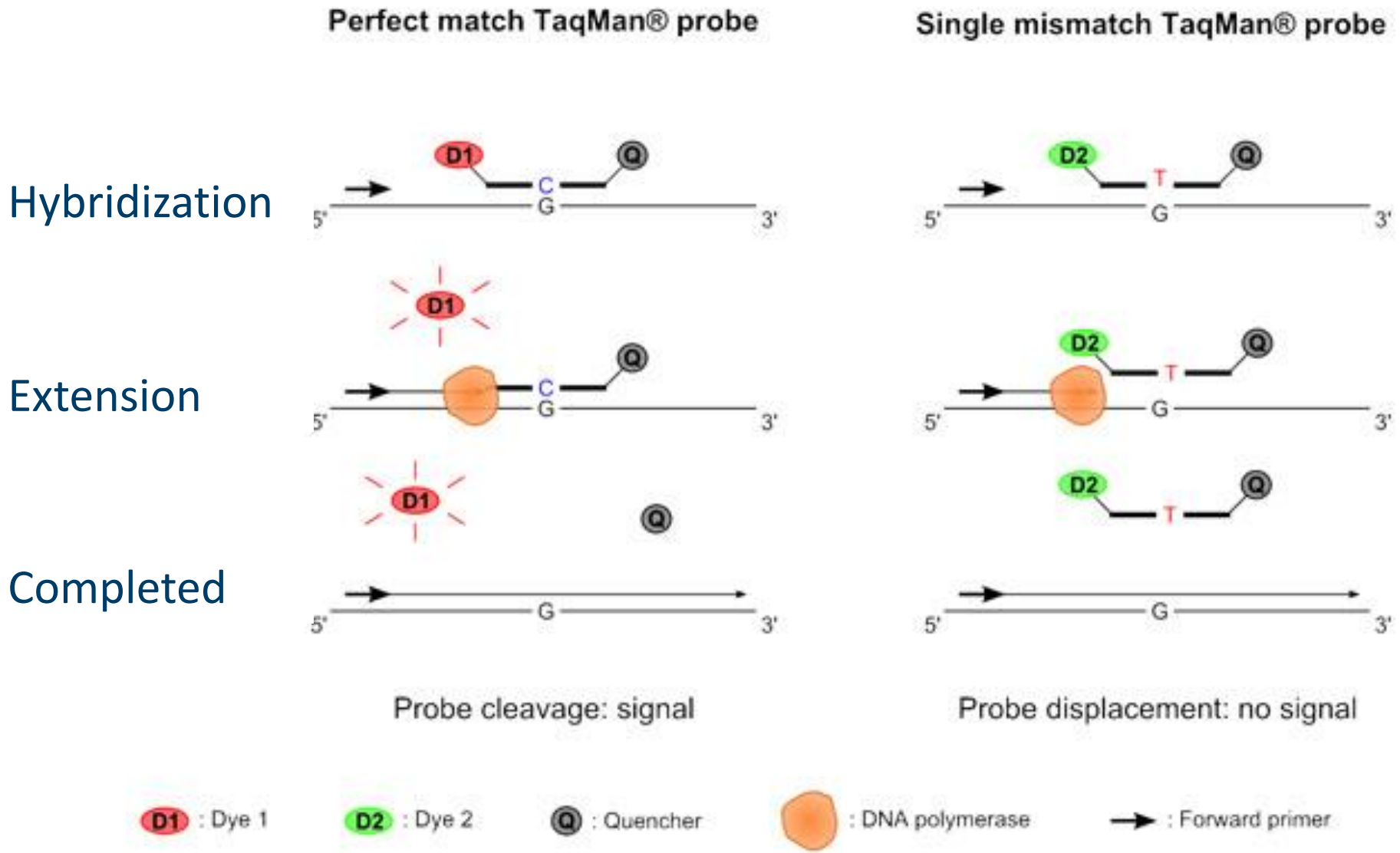
Annealing



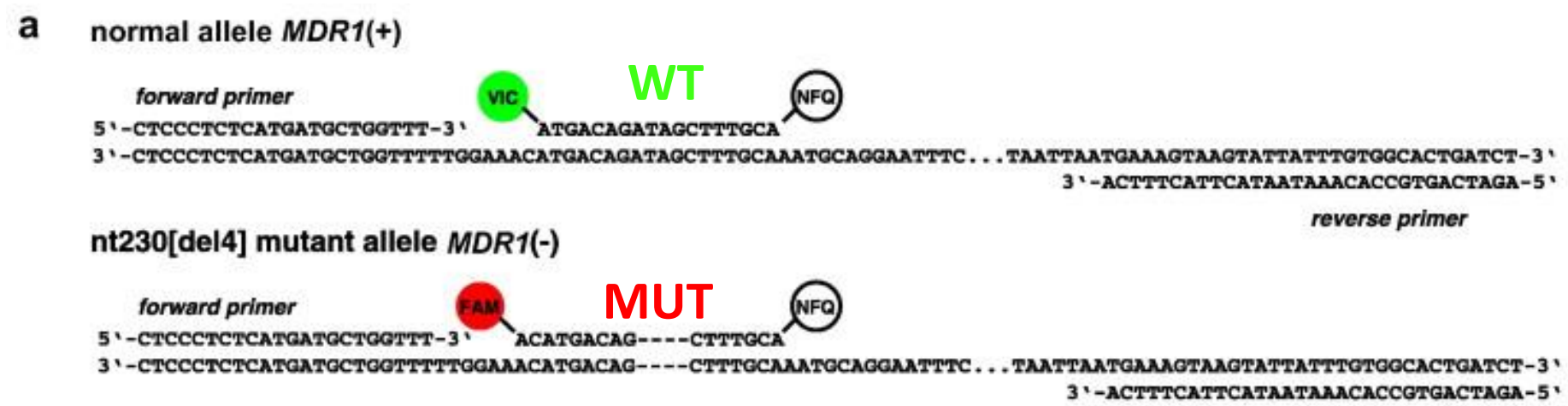
Cleavage


R
nucleotides
from TaqMan
complementary strand
3'
Q
Taq
3'
template DNA
5'

SNP detectie; verschillende probes



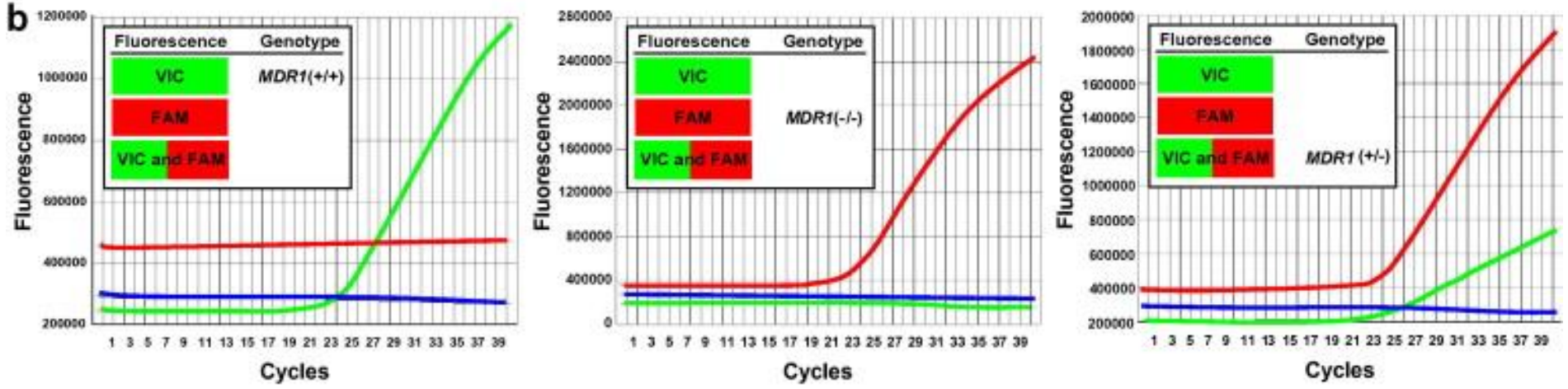
Hoe ziet dat er in de praktijk uit?



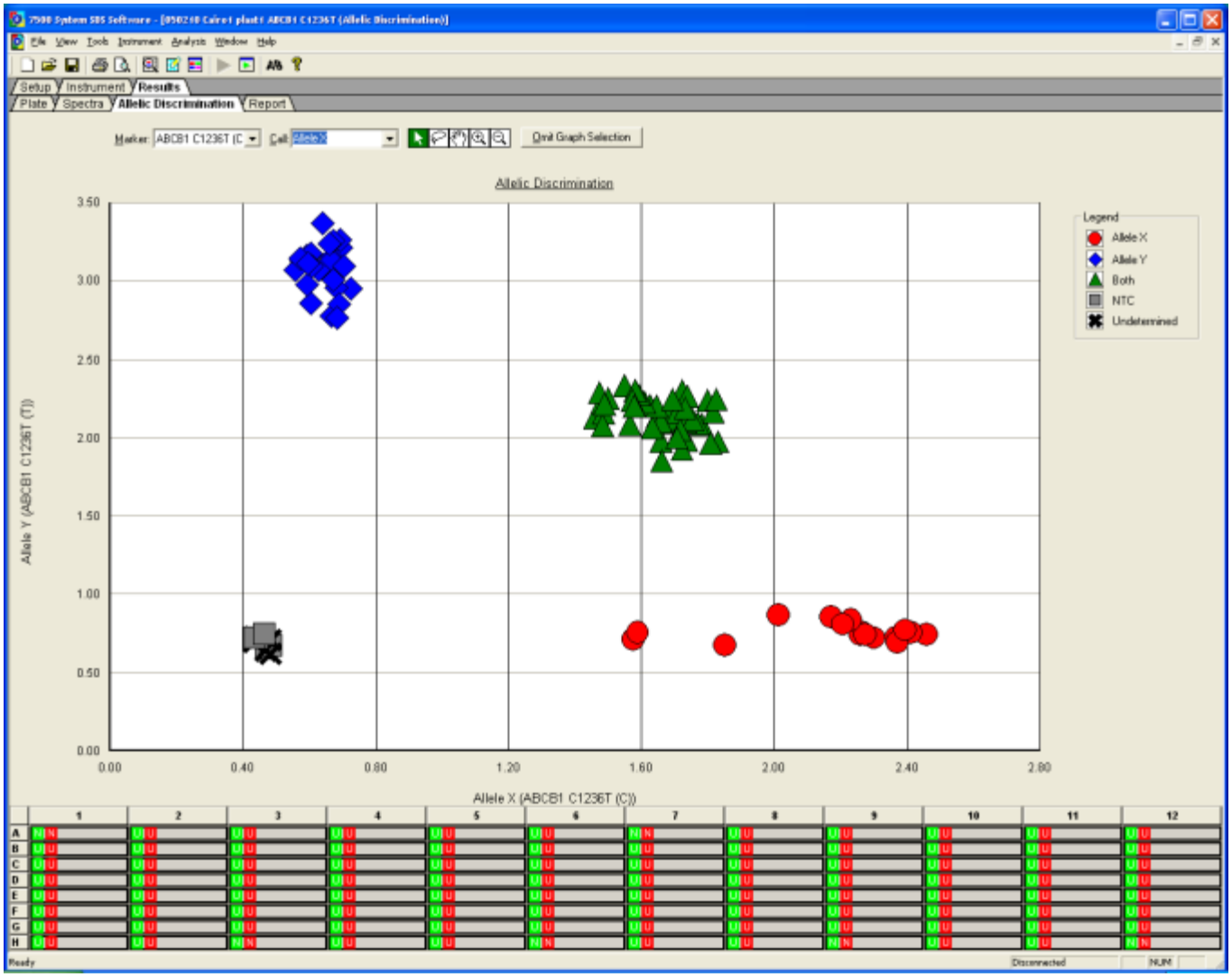
WT/WT

MUT/MUT

WT/MUT



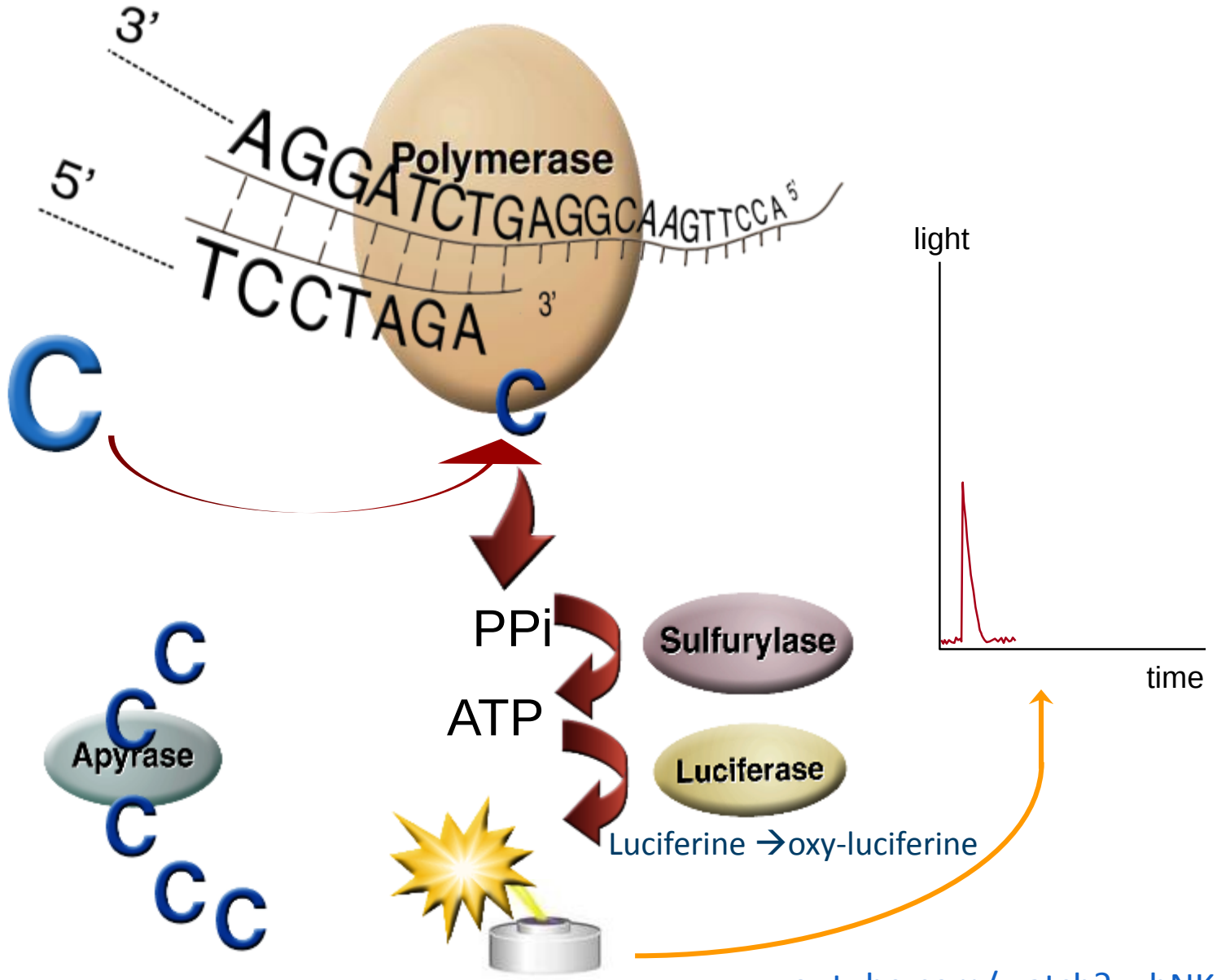
Een typisch resultaat



Techniek 2: Pyrosequencing

- “Sequencing by synthesis” principle
- Ontwikkeld in 1996
- 4 enzymen
- Chemiluminescentie,
- Reads van ~300-500 nucleotiden

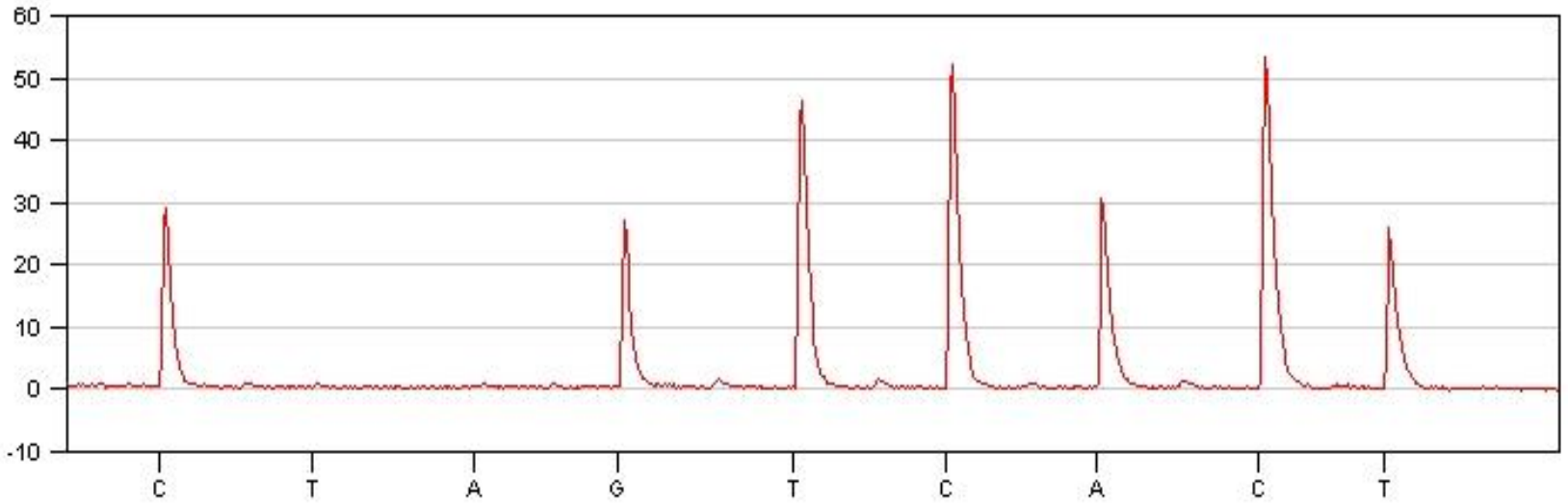
Principle



www.youtube.com/watch?v=bNKEhOGvcaI

- Each peak represents the incorporation of 1 nucleotide.
- 1 nucleotide is added at a time.

3’------GCAAGGTGGA------5’



Quality Control– plasmide controles

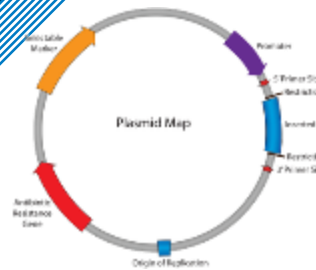
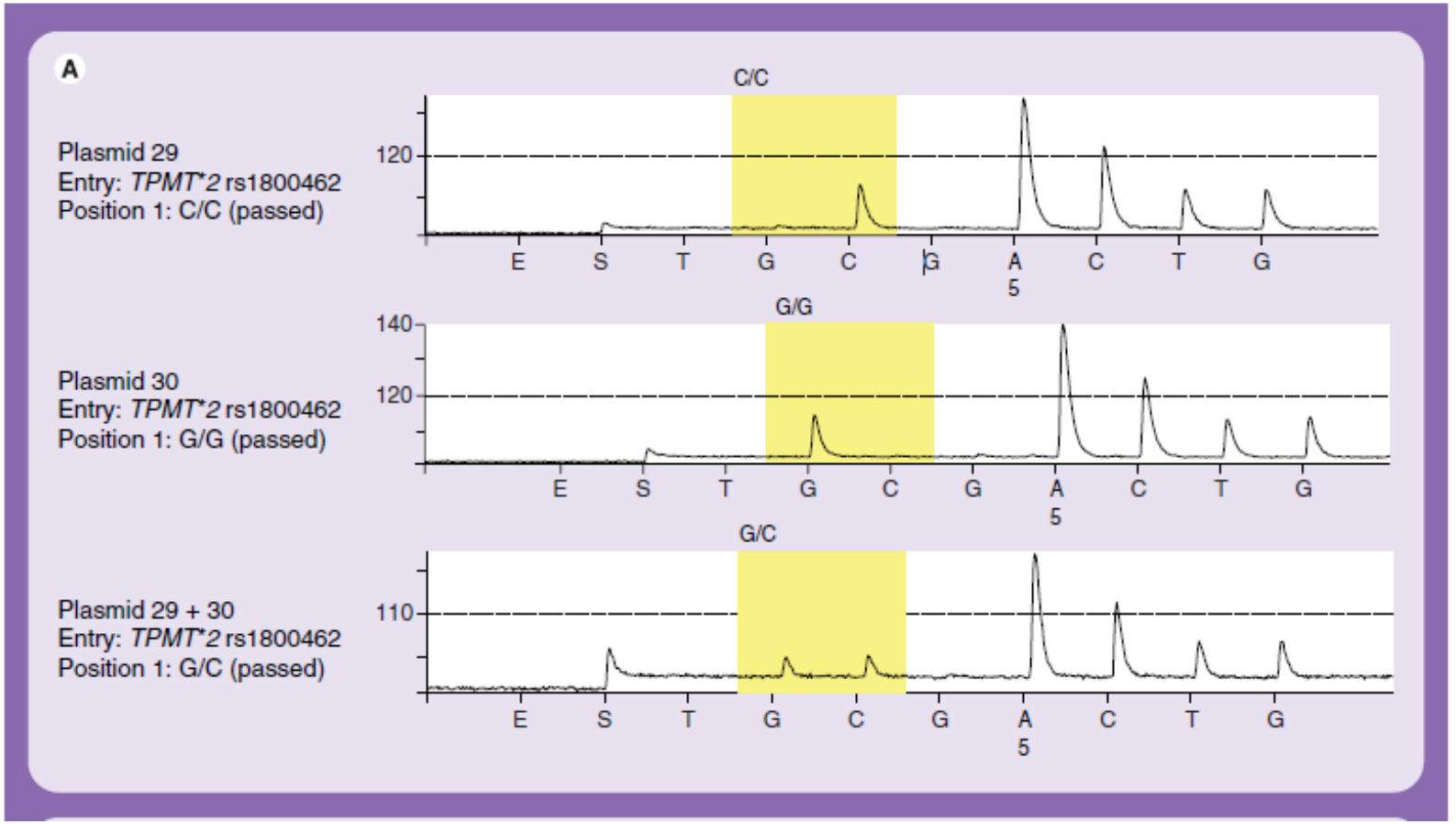
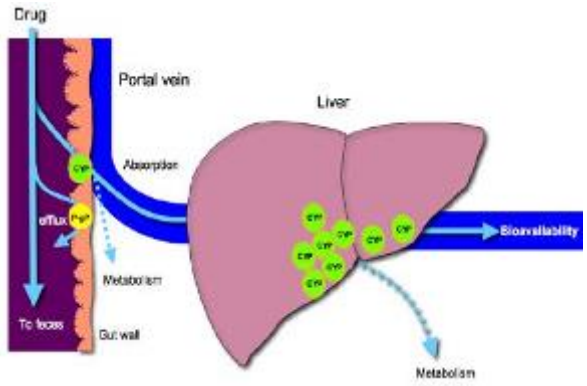


Figure 1. Example of validation report of *TPMT*2*.



Example 1:tacrolimus



Tacrolimus → (**CYP3A4**, **CYP3A5**) → **Metabolites**

***1/*3, *1/*1**

Expressers

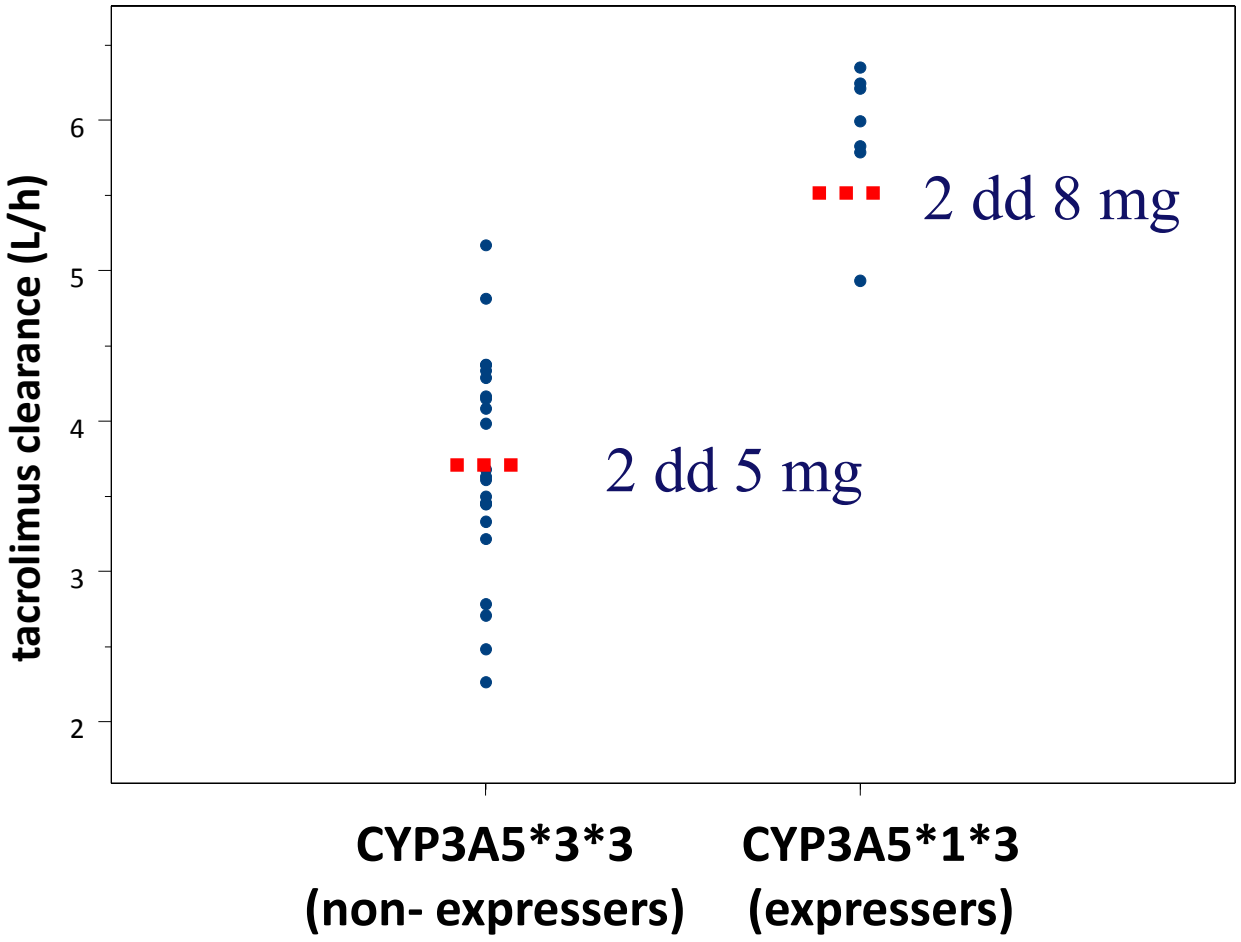
60-80% afro american pop

***3/*3**

Non-expressers

80-90% caucasian pop

Adult kidney transplant patients



Case: Mind the Mix

20 yr old female, referred for PGx test prior to kidney transplantation

PGx tests : *CYP3A5*3* (rs776746)
*CYP3A5*6* (rs10264272)

Conflicting genotype results…….

nature publishing group

PERSPECTIVES

LETTERS TO THE EDITOR

Pharmacogenetics in Transplant Patients: Mind the Mix

MH ten Brink[1], T van der Straaten[1], H Bouwsma[2], R Baak-Pablo[1], HJ Guchelaar[1] and JJ Swen[1]

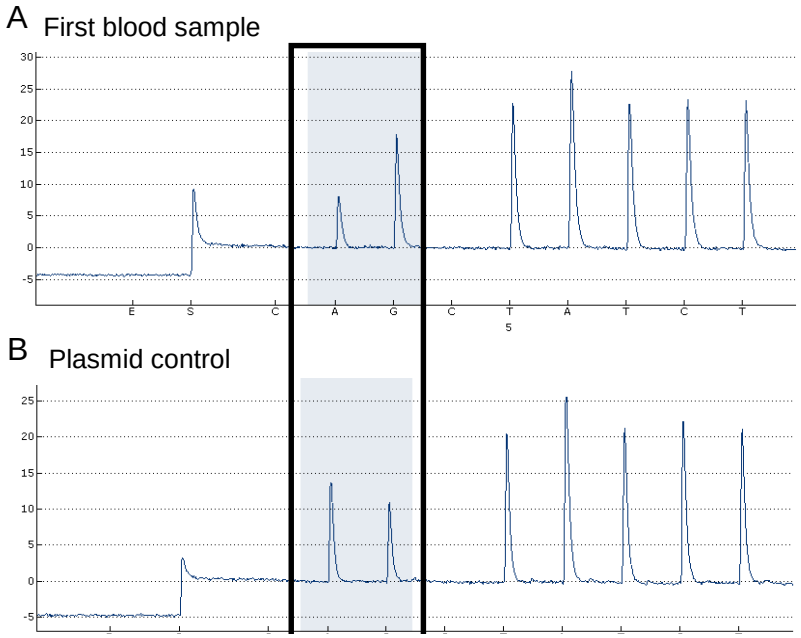
To the Editor: Several consortia have published guidelines to aid clinicians with the interpretation of pharmacogenetic test results,[1,2] and an increasing number of medical centers are implementing prospective genotyping.[3] Among these, there are many highly specialized care centers with complex patient populations. These patients may present unexpected challenges, as demonstrated by this case.

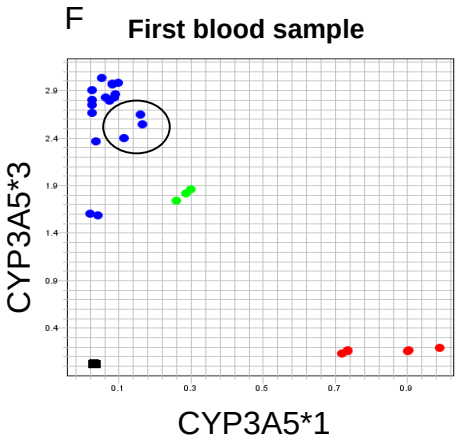
A 20-year-old women was admitted for a living–related kidney transplant. A standard quadruple immunosuppressive regimen was prescribed (basiliximab induction, tacrolimus, mycophenolate, and prednisolone). Tacrolimus is metabolized by cytochrome P450 (CYP)3A4 and

Clin Pharmacol Ther. 2013 Oct;94(4):443-4

First blood sample: conflicting results

PSQ





TaqMan

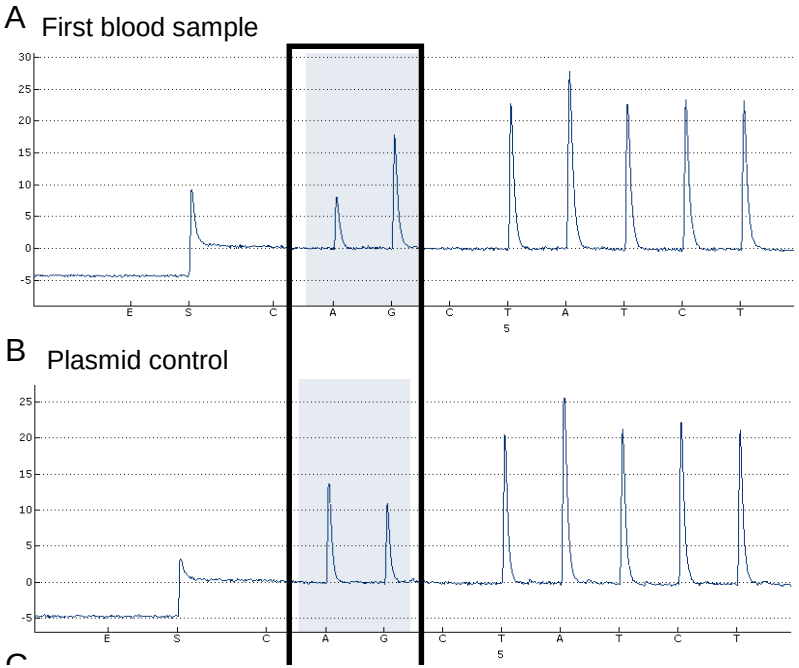
CY3A5*1/*3

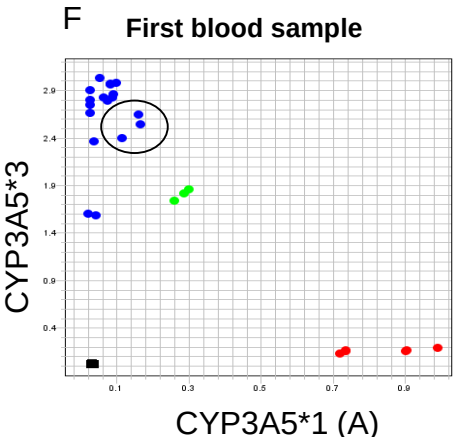
→ 1.5x normal dose

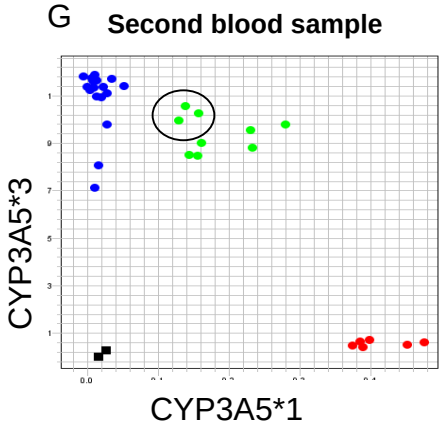
CYP3A5*3/*3

→ normal dose

Second blood sample







CYP3A5*1/*3

→ 1.5x normal dose

CY3A5*1/*3

→ 1.5x normal dose

Consulted attending nephrologist:

- Hematopoietic stem cell transplantation
- Mixed hematopoietic chimerism
- 28% autologous, 72% donor

Saliva results of patient and donor

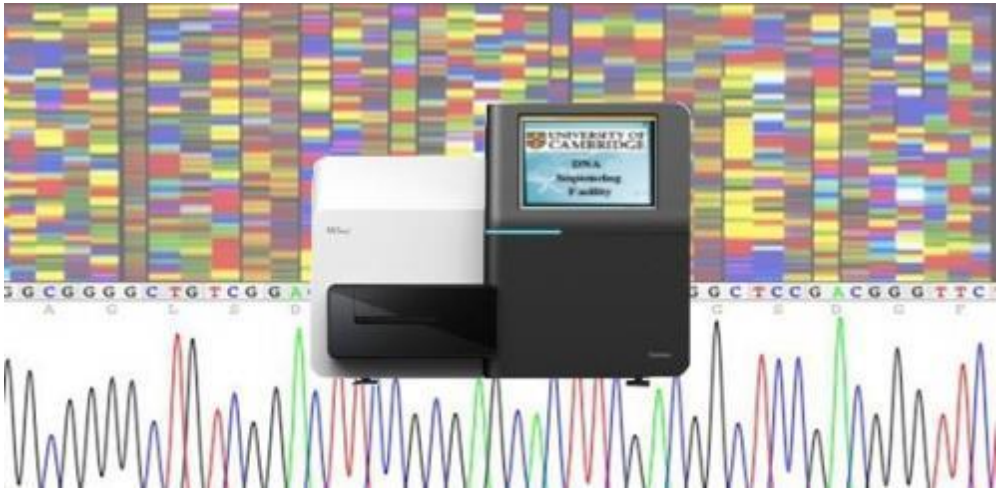
- Patient: *CYP3A5*1/*3*
- Donor: *CYP3A5*3/*3*

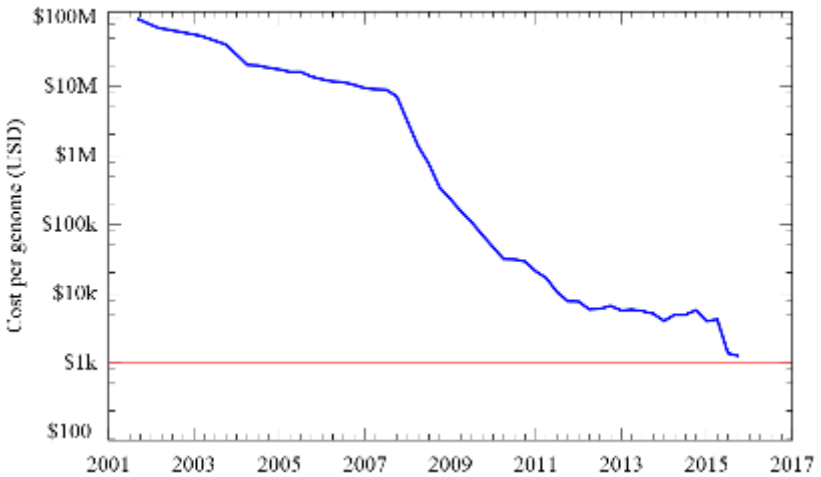
Patient tacrolimus trough level: 5.5 µg/L,

AUC: 110 µg/hr*l → 2dd 8 mg of tacrolimus

WAAR GAAT HET VELD NAAR TOE?

Cost to sequence a human genome





For $999, Veritas Genetics Will Put Your Genome on a Smartphone App

Getting your entire genome decoded is now more affordable than ever. Will consumers buy it?

by Antonio Regalado March 4, 2016

MIT Technology Review

Price of sequencing your genome falls to $999

By Gary Robbins | 2:17 p.m. March 4, 2016

VeritasGenetics

Announced at Future of Genomic Medicine Conference
4 March 2016

U-T San Diego

Common Medical Test/Scan/Med	Cost+
Head CT scan	$1,200
Abdominal CT scan	$1,420
MRI scan	$2,611
Echocardiogram	$1,300
10 "Most Important" Lab Tests*	$ 319
Crestor 1 year supply from Costco	$3,222
Xarelto 1 year supply from Costco	$4,444
3 Pharmacogenetic tests Quest^	$ 975
Whole Genome Sequence	**$ 999**

* A "package" significantly discounted via DirectLabs.com
\+ vary widely, these are average US charges from centers w/ data available on the web
^ 3 SNPs which account for 0.00000000001% of the genome

Complimentary pharmacogenetic information

- WES, WGS data in clinical genetics

- WGS data in oncology

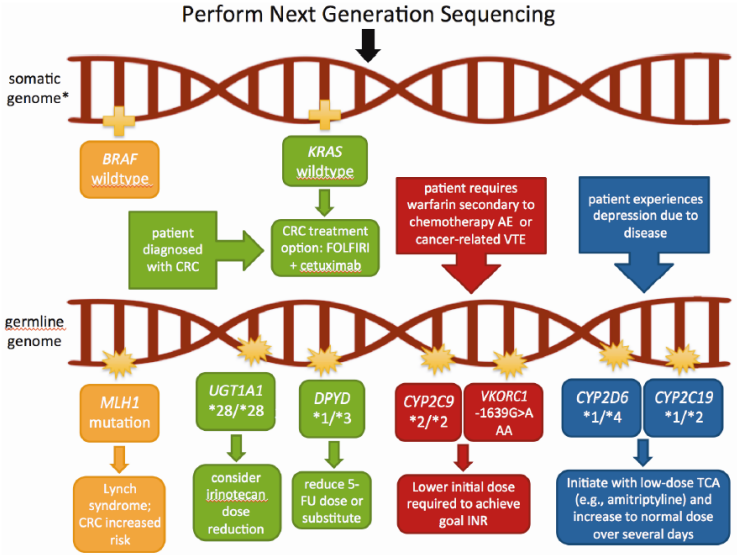
CPCT
Center for Personalized Cancer Treatment

Genomics england

THE PRECISION MEDICINE INITIATIVE

Tumor Genome

Germline Genome



Dutch PGx Working Group (DPWG) guidelines (N=84)

CYP2D6

- Amitriptyline
- Aripiprazol
- Atomoxetine
- Carvedilol
- Citalopram
- Clomipramine
- Clozapine
- Codeine
- Doxepine
- Flecainide
- Flupentixol
- Haloperidol
- Imipramine
- Metoprolol
- Mirtazapine
- Nortriptyline
- Olanzapine
- Oxycodeine
- Paroxetine
- Propafenon
- Risperidone
- Tamoxifen
- Tramadol
- Venlafaxine
- Zuclopenthixol

DPYD

- Capecitabine / 5-FU

CYP2C9

- Acenocoumarol
- Phenprocoumon
- Phenytoin
- Glibenclamide
- Glicazide
- Glimepride
- Tolbutamide

UGT1A1

- Irinotecan

VKORC1

- Acenocoumarol
- Phenprocoumon

CYP3A5

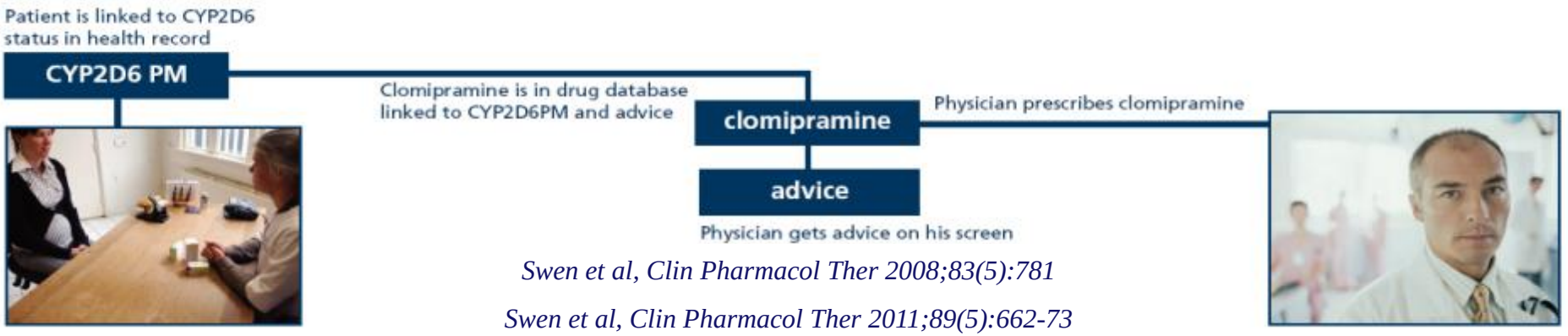
- Tacrolimus

CYP2C19

- Citalopram
- Clopidogrel
- Imipramine
- Lansoprazol
- Moclobemide
- (es)Omeprazol
- Pantoprazol
- Rabeprazol
- Sertraline
- Voriconazol

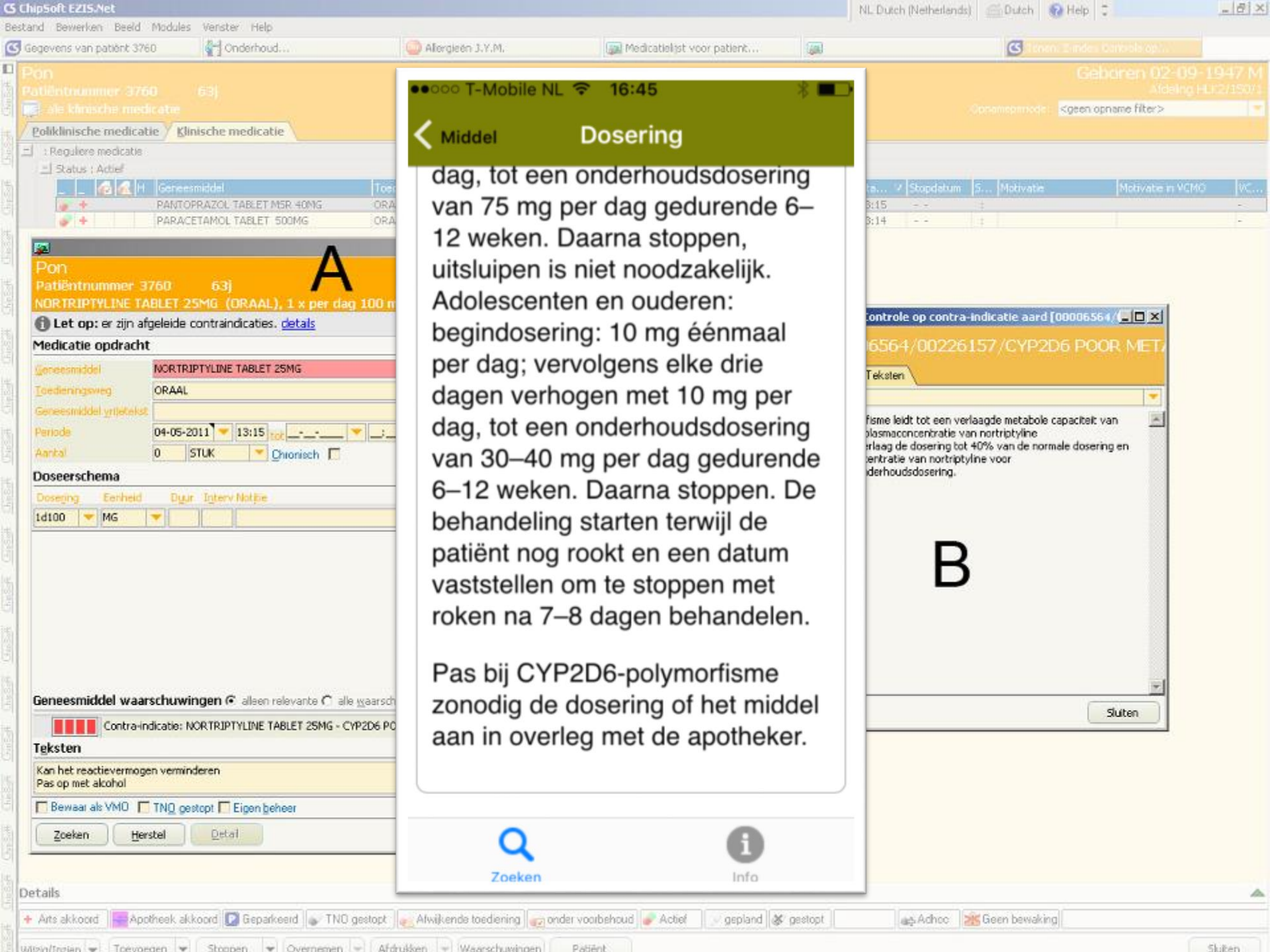
TPMT

- Azathioprine
- Mercaptopurine
- Thioguanine



Swen et al, Clin Pharmacol Ther 2008;83(5):781

Swen et al, Clin Pharmacol Ther 2011;89(5):662-73


T-Mobile NL 16:45
Middel
Dosering
dag, tot een onderhoudsdosering van 75 mg per dag gedurende 6–12 weken. Daarna stoppen, uitsluipen is niet noodzakelijk. Adolescenten en ouderen: begindosering: 10 mg éénmaal per dag; vervolgens elke drie dagen verhogen met 10 mg per dag, tot een onderhoudsdosering van 30–40 mg per dag gedurende 6–12 weken. Daarna stoppen. De behandeling starten terwijl de patiënt nog rookt en een datum vaststellen om te stoppen met roken na 7–8 dagen behandelen.
Pas bij CYP2D6-polymorfisme zonodig de dosering of het middel aan in overleg met de apotheker.
Zoeken
Info
A
Pon
Patiëntnummer 3760 63j
NORTRIPTYLINE TABLET 25MG (ORAAL), 1 x per dag 100 m
Let op: er zijn afgeleide contraindicaties. details
Medicatie opdracht
Geneesmiddel NORTRIPTYLINE TABLET 25MG
Toedieningsweg ORAAL
Periode 04-05-2011 13:15
Aantal 0 STUK Chronisch
Doseerschema
Dosering 1d100 Eenheid MG
Geneesmiddel waarschuwingen
Contra-indicatie: NORTRIPTYLINE TABLET 25MG - CYP2D6 PO
Teksten
Kan het reactievermogen verminderen
Pas op met alcohol
Bewaar als VMO TNO gestopt Eigen beheer
Zoeken Herstel Detail
B
Controle op contra-indicatie aard
6564/00226157/CYP2D6 POOR MET
Teksten
fisme leidt tot een verlaagde metabole capaciteit van
plasmaconcentratie van nortriptyline
rlaag de dosering tot 40% van de normale dosering en
entratie van nortriptyline voor
derhoudsdosering.
Sluiten
Details
Arts akkoord
Apotheek akkoord
Geparkeerd
TNO gestopt
Afwijkende toediening
onder voorbehoud
Actief
gepland
gestopt
Adhoc
Geen bewaking

Enquête arts en apotheker

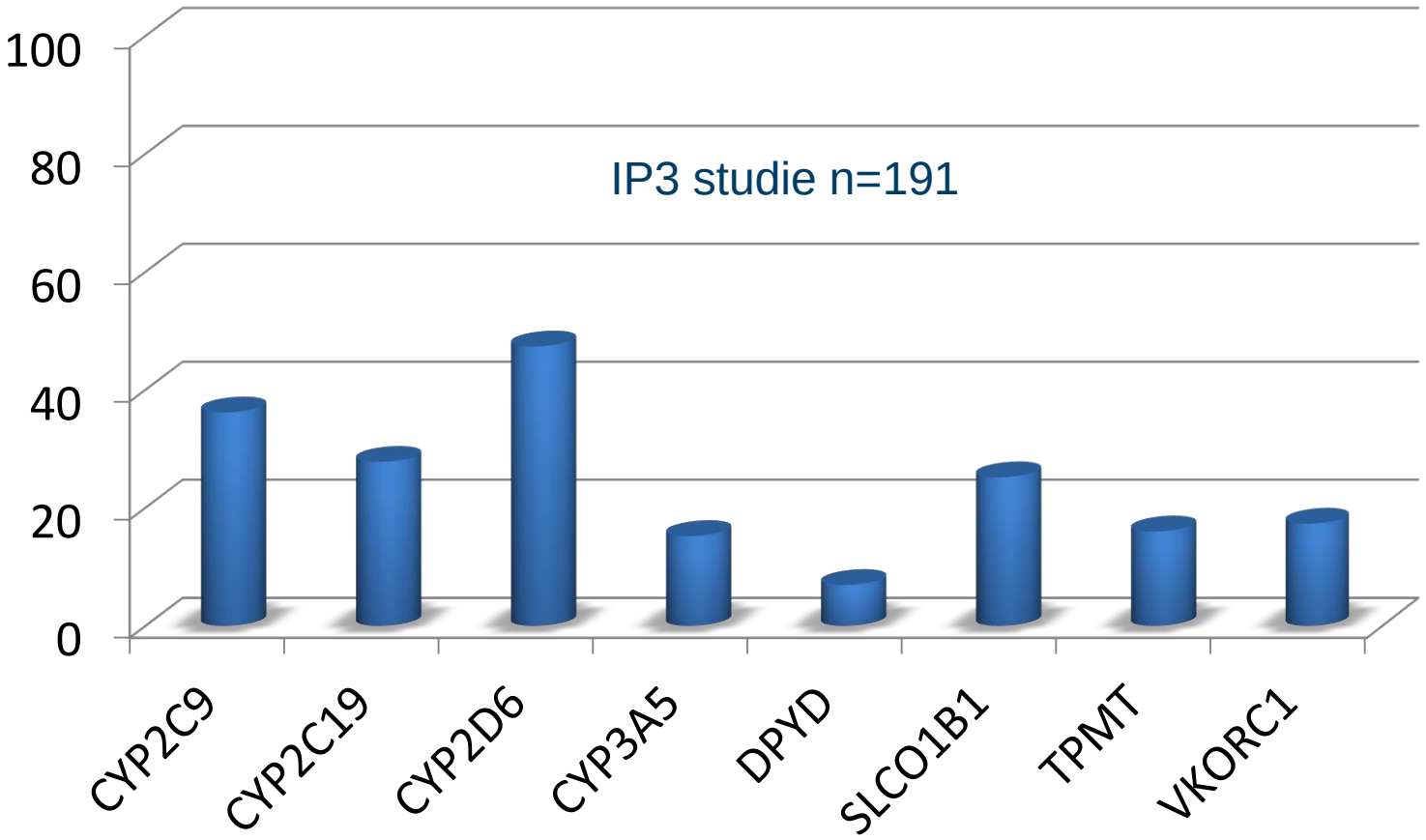
Heeft u in de afgelopen 6 maanden een farmacogenetische test aangevraagd of aanbevolen?

Implementing PGx in Primary Care Project (IP^3)

- 200 patients
  - Primary care, vicinity Leiden
  - Apply PGx in own practice
- Focus on primary care drug with recommendation

  CYP2D6: TCAs, atomoxetine, venlafaxine
  - *CYP2C9*: acenocoumarol, fenprocoumon
  - *CYP2C19*: (es)citalopram, imipramine,
  - *SLCO1B1*: simvastatine, atorvastatine
- Genotyping
  - Panel of 8 genes: *CYP2C9, CYP2C19, CYP2D6, CYP3A5, DPYD, SLCO1B1, TPMT, VKORC1*

Hoe vaak komt farmacogenetische variatie voor?



→ ~95% van de patiënten heeft ten minste 1 ***actionable*** genotype, 10% heeft er ≥4

U-PGx | Ubiquitous Pharmacogenomics

- €15 million, HORIZON2020, 10 EU countries
- Implement pre-emptive PGx testing in a real world clinical setting across 7 EU sites
- Evaluate **patient outcome** and **cost effectiveness** using solid **scientific methodology**
- Start 1-1-2016, 5 yr, n=8,000
- Consortium members:

WWW.UPGX.EU

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- M Pirmohamed, R Turner, UOL
- J Stingl, FDMD
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- VHM Deneer STZHM
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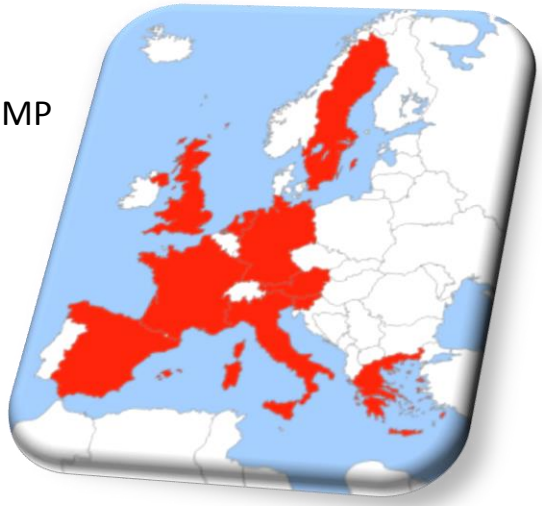
- CL Davila Fajardo, SAS
- G Patrinos, UPAT
- V Dolžan, ULMF
- A Cambon-Thomsen, UPS
- G Toffoli, E Cecchin, CROA



Boodschap:

- Geneesmiddelrespons is erfelijk.
- Real-time PCR meest gebruikte techniek.
- Technologie en bewijs ontwikkelen zich razendsnel; het aantal mensen met een bekend genotype neemt toe.
- Farmacogenetica is:
  - niet ‘iets zeldzaams’
  - niet ‘iets academisch’, ‘voor de toekomst’ etc.
- Arts en apotheker kunnen komende week nog een patiënt tegenkomen waarvoor een farmacogenetische test nuttig zou zijn.

"Here's my sequence..."

New Yorker, 2000

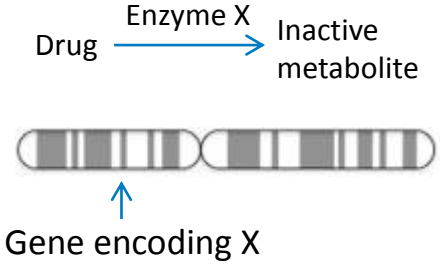
Dank voor uw aandacht!

Email: J.J.Swen@lumc.nl

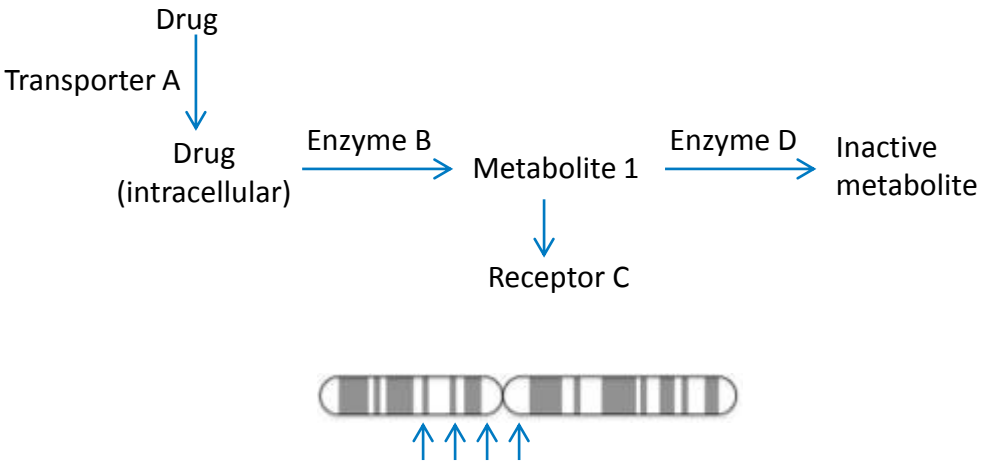
Wat betekent dit voor mij?

Genotype	Fenotype	Geneesmiddel	Actie
CYP2D6*2/*5	IM	Codeine Metoprolol Nortriptyline	Alternatief 50% dosis 60% dosis
CYP2C9*1/*1	EM	Acenocoumarol	Normale dosis
CYP2C19*1/*2	IM	Citalopram Clopidogrel	Max 20 mg Alternatief
SLCO1B1TT	Norm act	Simvastatine	Normale dosis
TPMT*1/*1	EM	Azathioprine	Normale dosis

Ontwikkelingen in onderzoek



Candidate gene

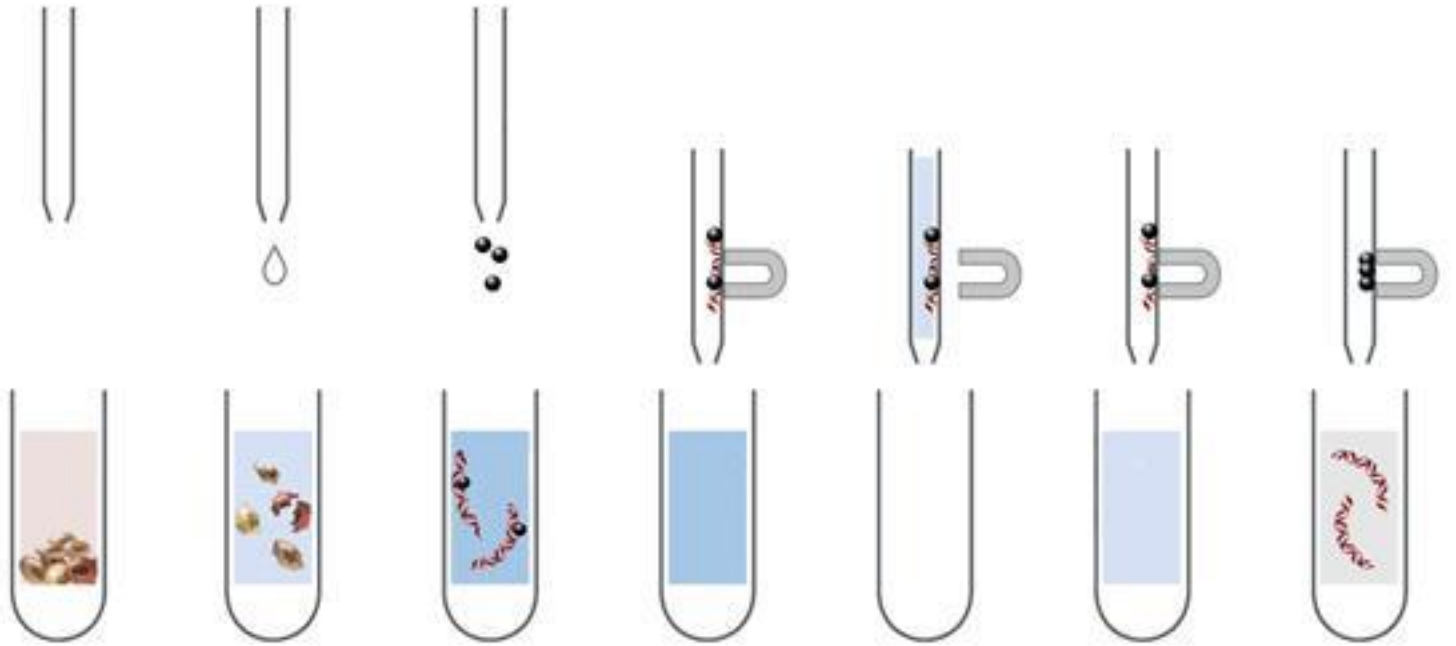


Pathway approach

Hypothesis free approaches

GWAS

WES
WGS


Sample material
Cell disruption and protein digestion by the addition of Lysis Buffer and Proteinase K
NA binding to the surface of Magnetic Glass Particles. (DNA is removed by DNase digestion)
Magnetic separation of the nucleic acid-bead complex
Removal of cellular debris by extensive washing steps
Magnetic separation of the nucleic acid-bead complex
Nucleic acid elution at high temperatures during the removal of the Magnetic Glass Particles