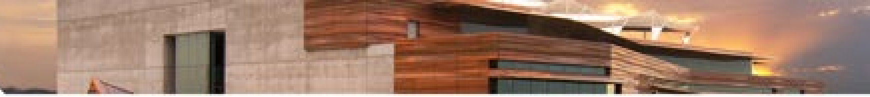


Pharmacogenomic Results From Lab to Phenotype

PGxP4 Symposium 2020

January 17, 2020

A. Min Kang, MD, MPhil

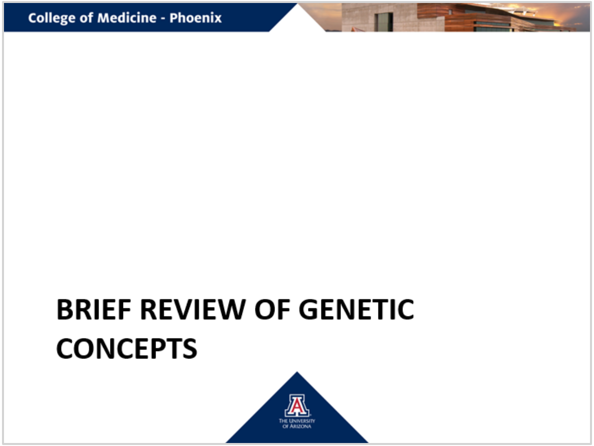
THE UNIVERSITY OF ARIZONA

Disclosures

- None

Goals

- **Discuss laboratory methods for pharmacogenomics**
- **Review limitations in interpretation of results**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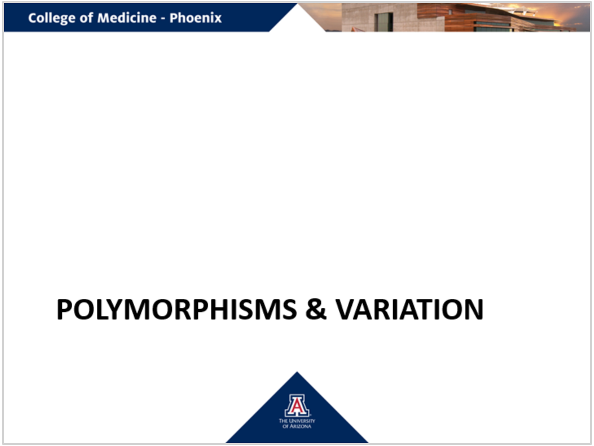
BRIEF REVIEW OF GENETIC CONCEPTS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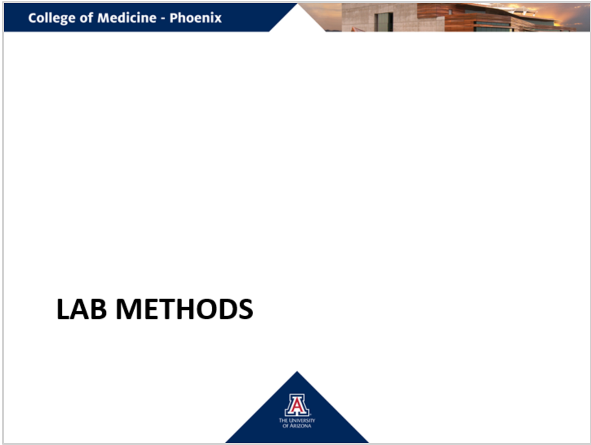
POLYMORPHISMS & VARIATION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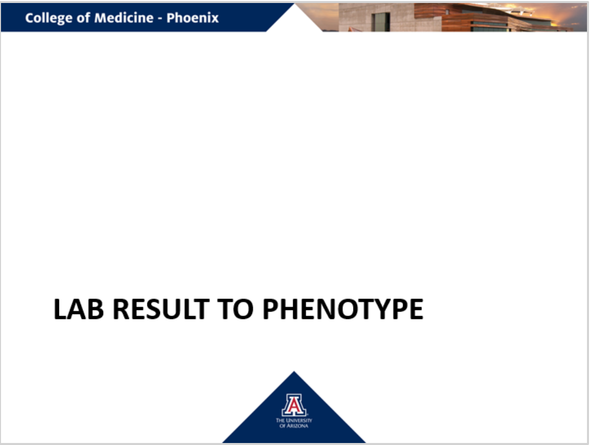
LAB METHODS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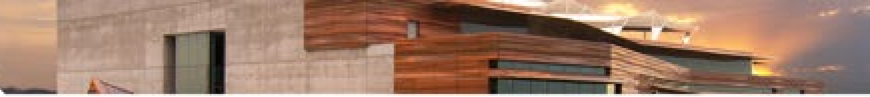
LAB RESULT TO PHENOTYPE



BRIEF REVIEW OF GENETIC CONCEPTS

Chromosomes and DNA

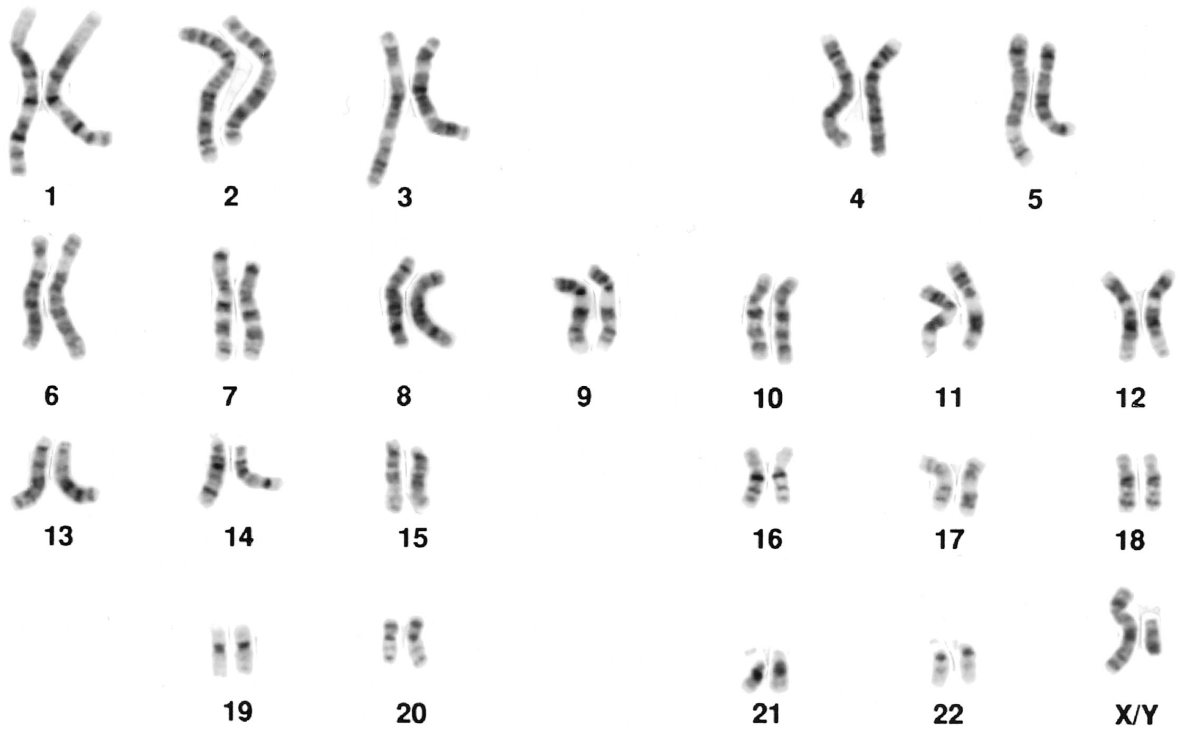
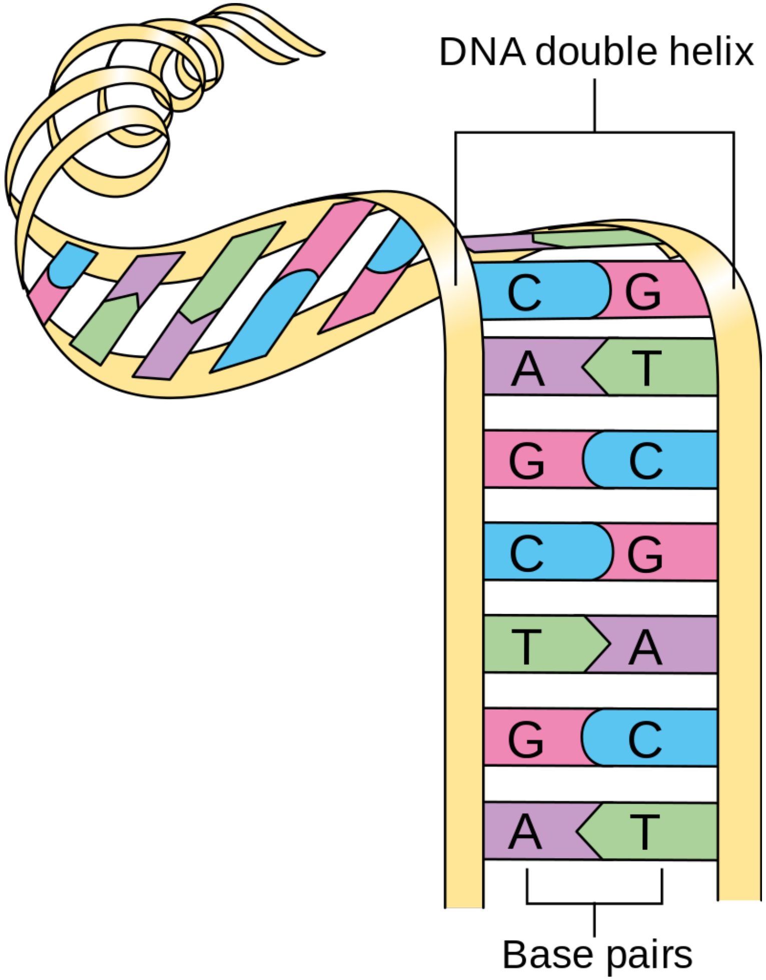
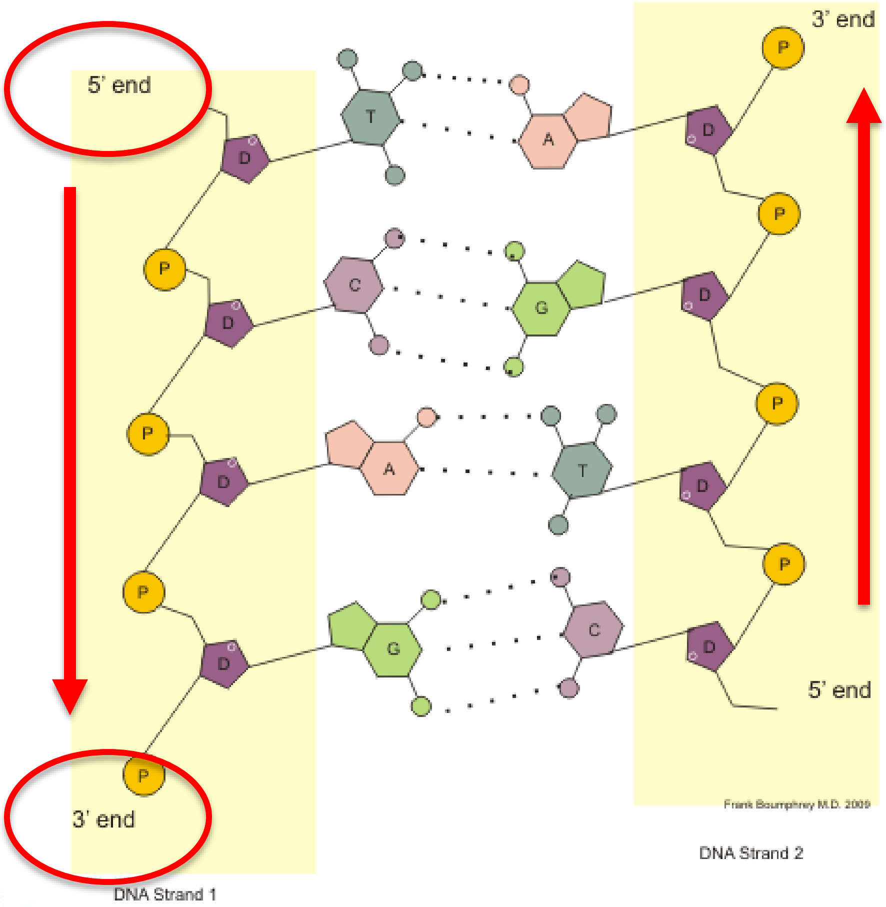


Image Credit: NCI Visuals Online (Public Domain)

Image Credit: Wikimedia Commons

DNA



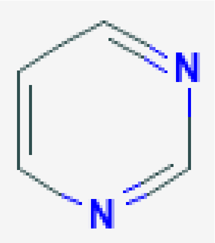


Hydrogen Bonds of DNA Strand

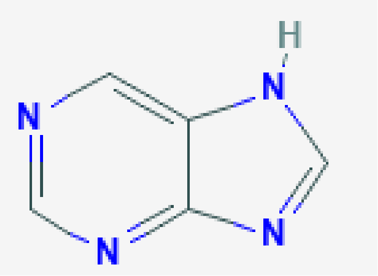
Image Credit: Wikimedia Commons

Nitrogenous Bases

Pyrimidine



Purine



- Thymine / Uracil
- Cytosine

- Adenine
- Guanine

Gene Structure

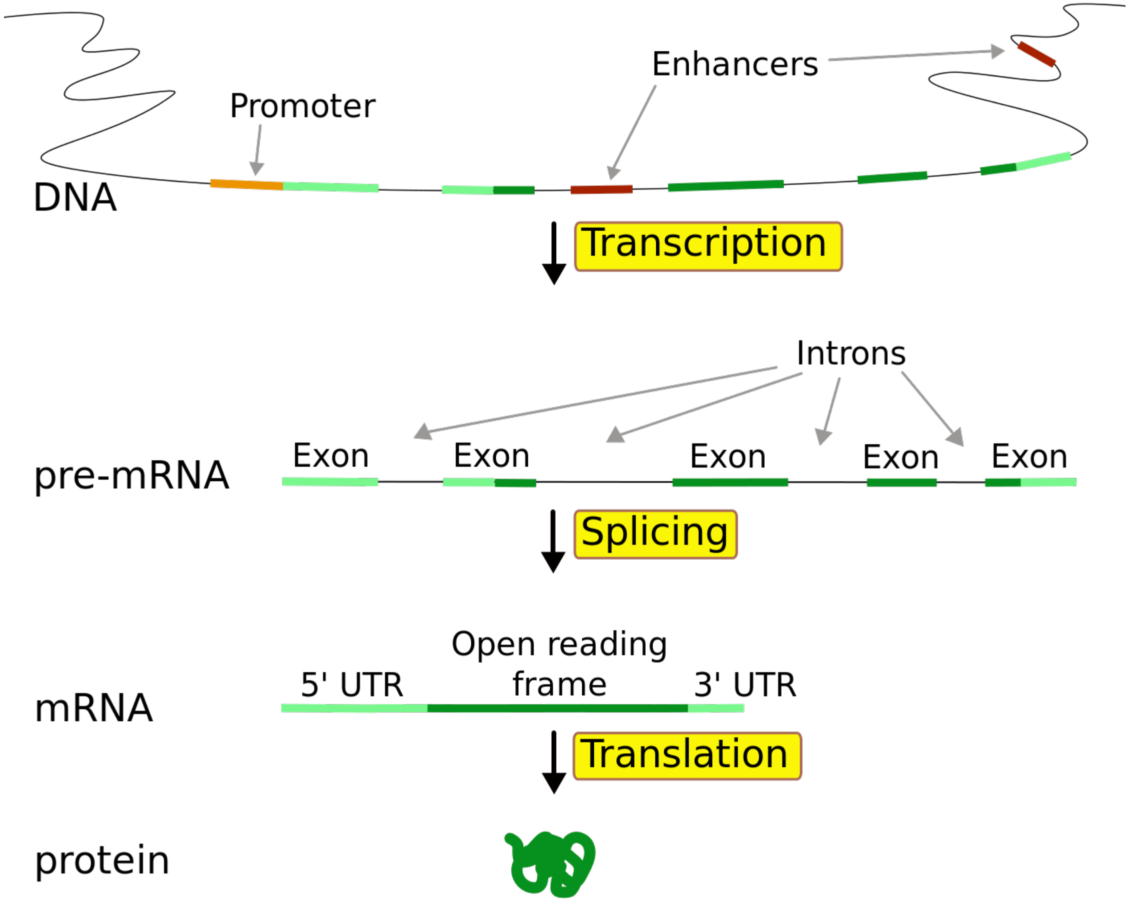


Image Credit: Wikimedia Commons

Alternative Exons

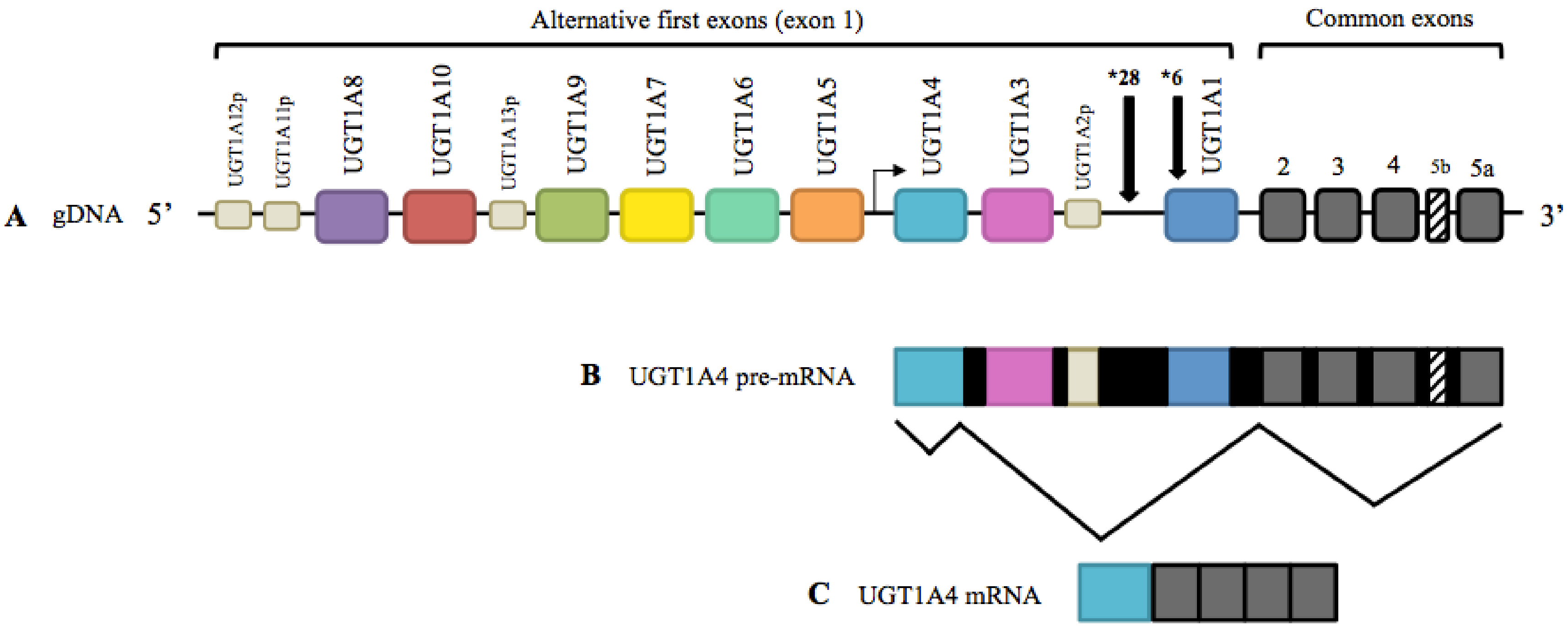
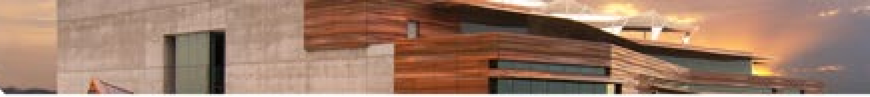


Image Credit: Wikimedia Commons



POLYMORPHISMS & VARIATION

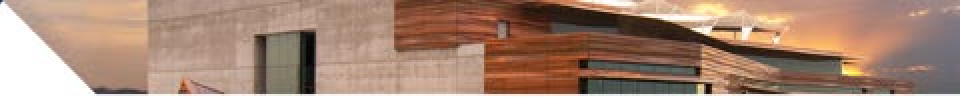
Single Nucleotide Polymorphism (SNP)

Allele 1: 5’-GCACC C GGCCA-3’

↕

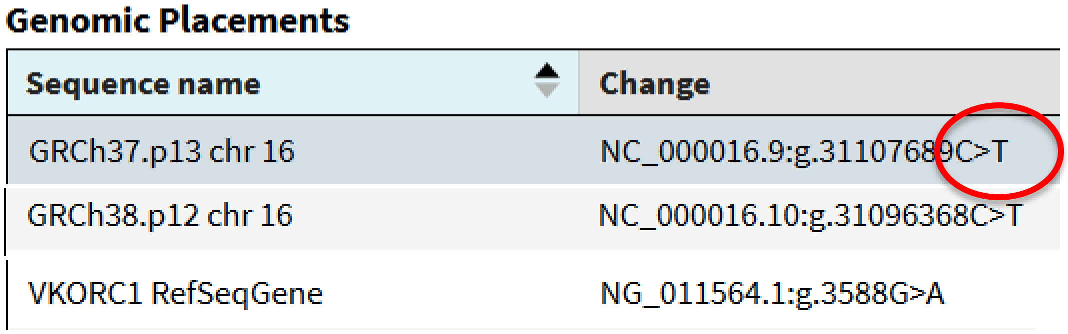
Allele 2: 5’-GCACC T GGCCA-3’

- Reference Sequence
  - GRCh38.p13 chr 16
  - Genome Reference Consortium
    - Human Build 38
    - Patch Release 13



Single Nucleotide Polymorphism (SNP)

- Reference SNP (rs #) - rs9923231



Genomic Placements

Sequence name	Change
GRCh37.p13 chr 16	NC_000016.9:g.31107689C>T
GRCh38.p12 chr 16	NC_000016.10:g.31096368C>T
VKORC1 RefSeqGene	NG_011564.1:g.3588G>A

- VKORC1 -1639G>A

Insertion (Indel)

- rs774671100 – defines CYP2D6*15
  - – CYP2D6 137_138insT (or 137dup)
    - NM_000106.5:c.137dup
    - NP_000097.3:p.Leu47fs

Reference: 5’-CAACC T– GCTGC-3’

Variant: 5’-CAACC TT GCTGC-3’

Deletion (Indel)

- rs9332131 – defines CYP2C9*6
  - – CYP2C9 818delA
    - NM_000771.3:c.818delA
    - NP_000762.2:p.Lys273fs

Reference: 5’-TGGAGA A GGTAA-3’

Variant: 5’-TGGAGA – GGTAA-3’

Dinucleotide Repeats

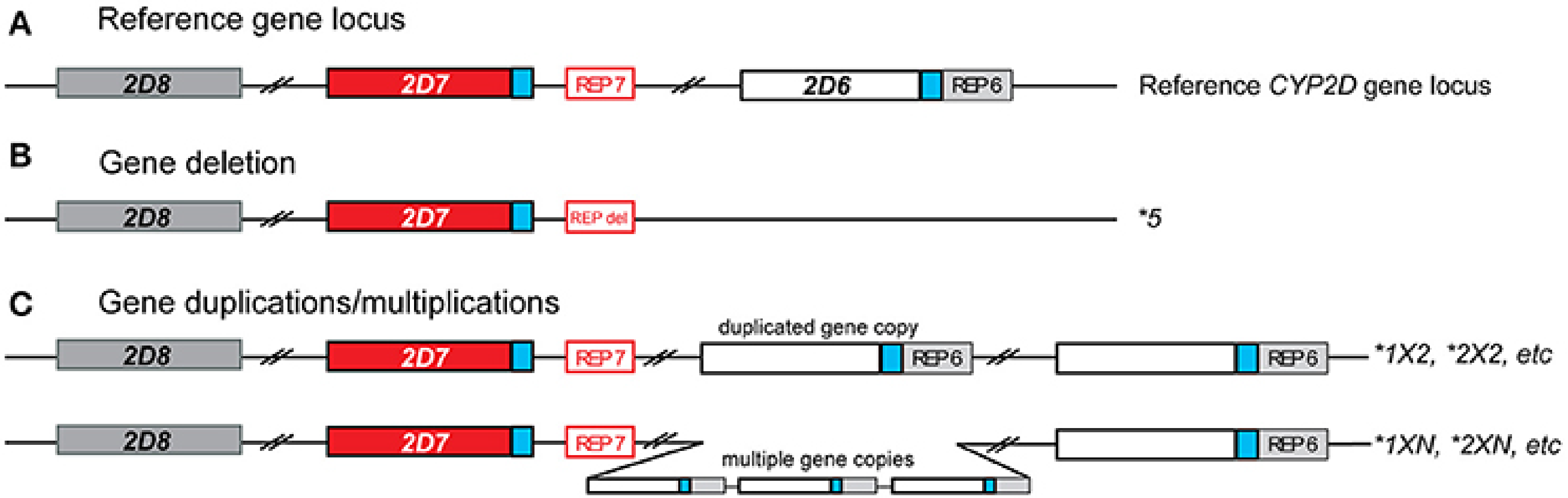
- UGT1A1
  - – Promoter region (TA)n – rs8175347

GRCh38.p12 Chr 2 (NC_000002.12)

A(TATATATATATA)TAA

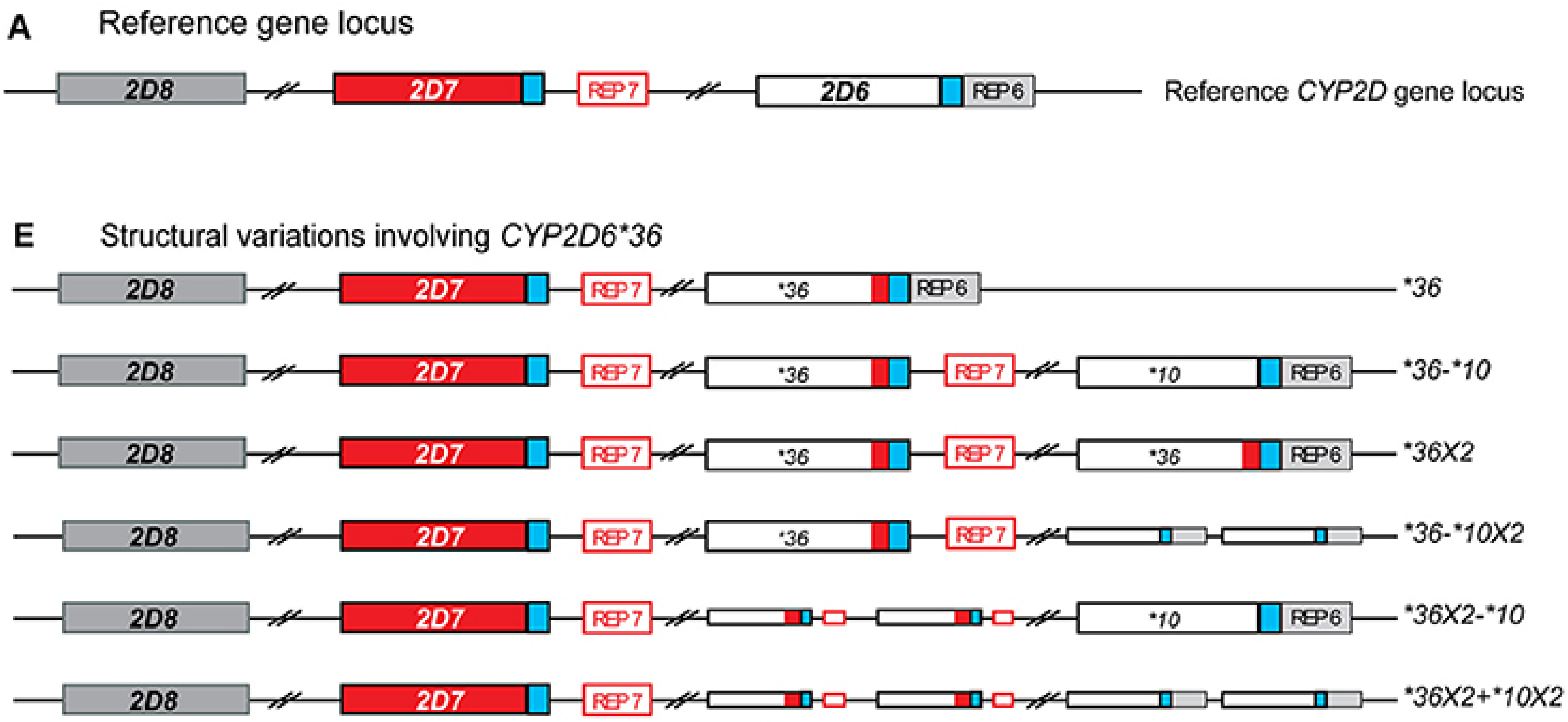
(TA)n	Allele	Effect
5	*36	Increased Activity
6	*1	“Wild Type”
7	*28	Reduced Activity
8	*37	Reduced Activity

Copy Number Variation



Del Tredici et al (2018). DOI: 10.3389/fphar.2018.00305

Structural Variation With Hybrid Genes

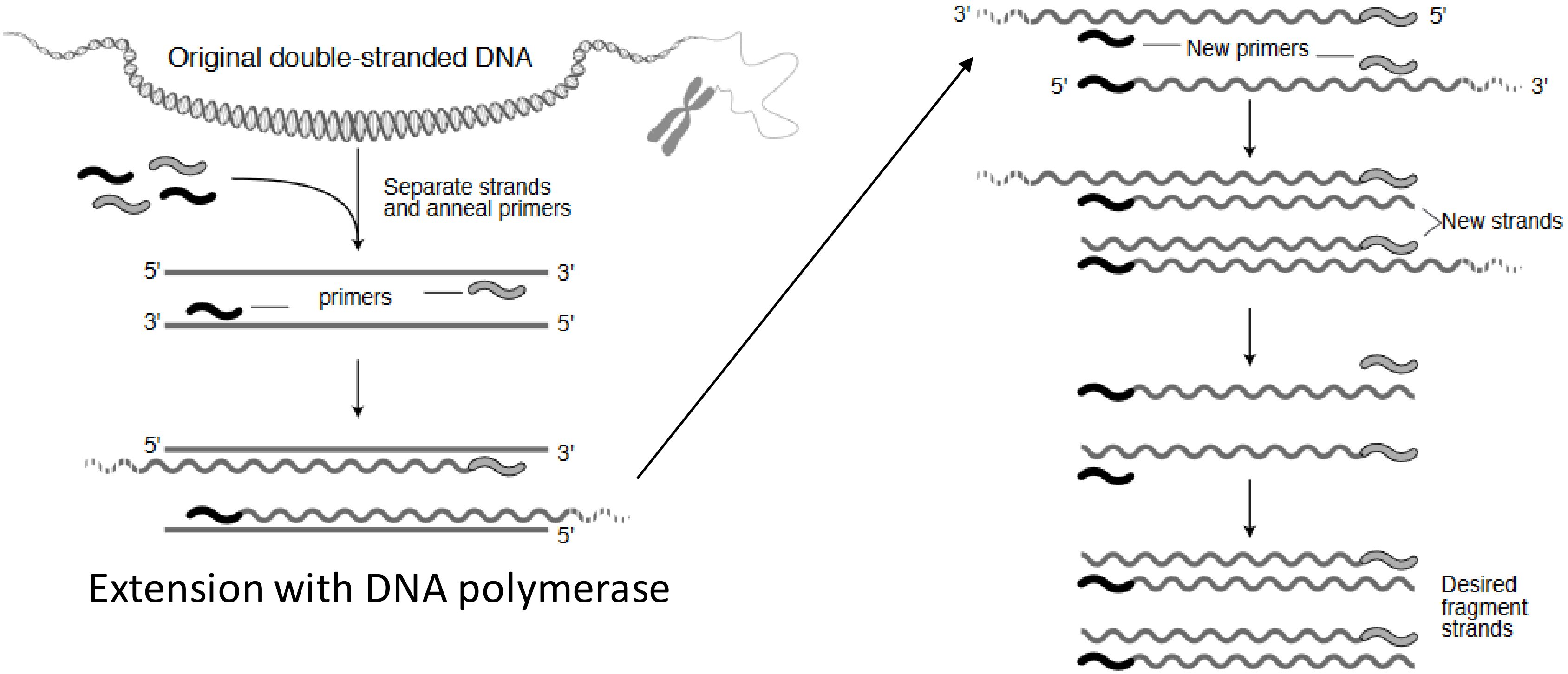


Del Tredici et al (2018). DOI: 10.3389/fphar.2018.00305

LAB METHODS

DNA Sample

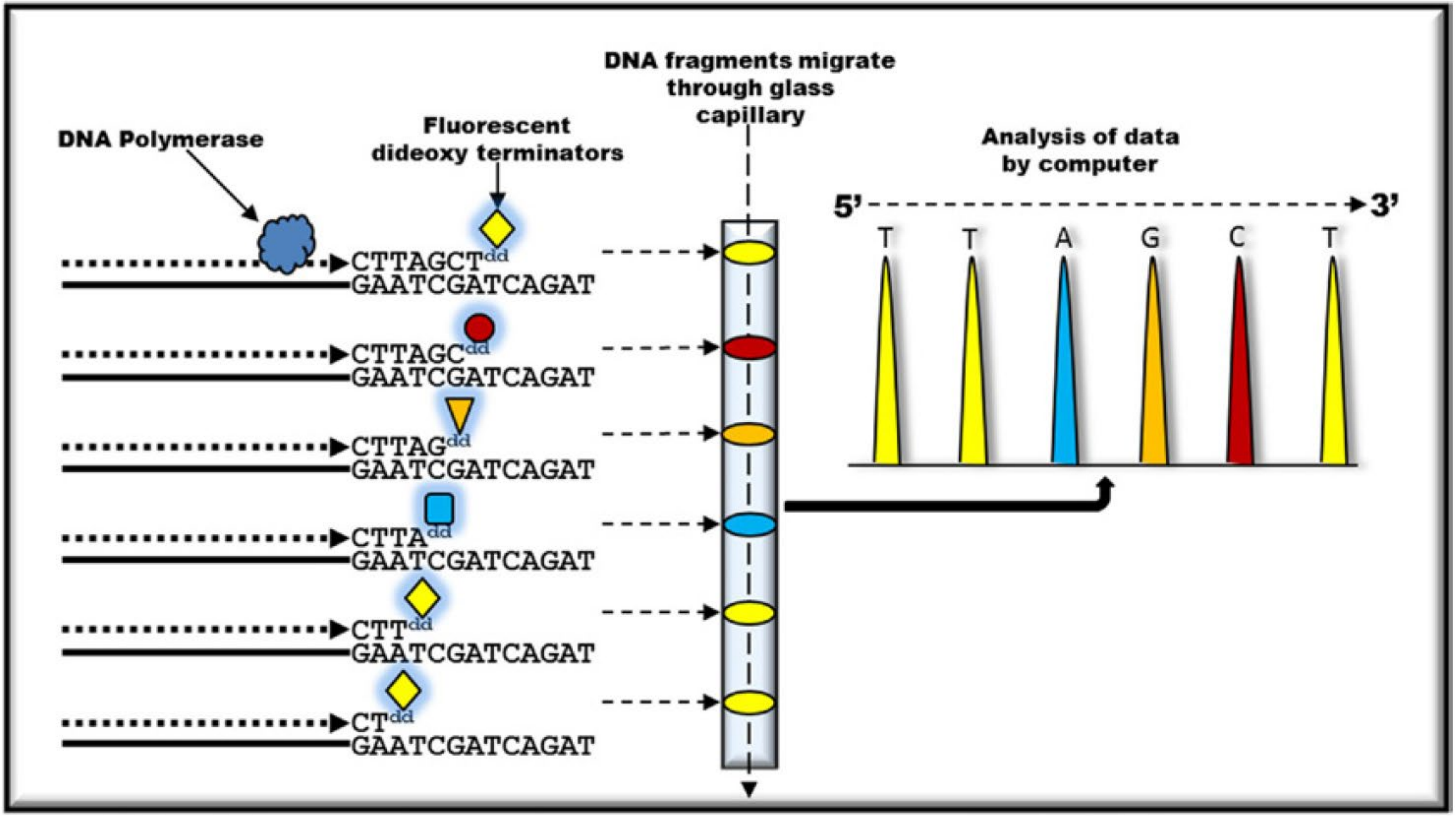
Polymerase Chain Reaction (PCR)



Extension with DNA polymerase

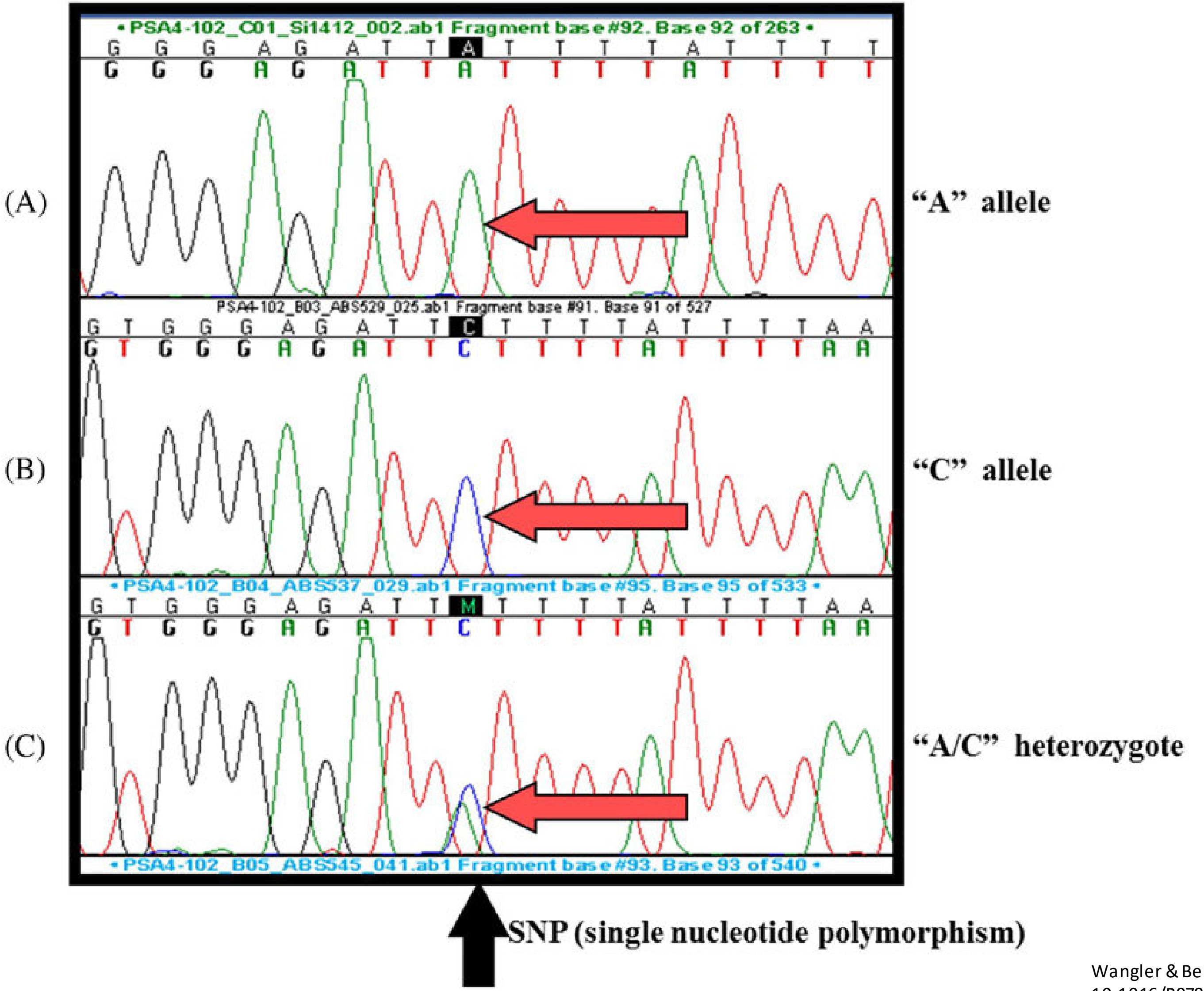
Image Credit: NHGRI

Sanger Sequencing



Wangler & Bellen (2017). DOI: 10.1016/B978-0-12-803077-6.00012-6

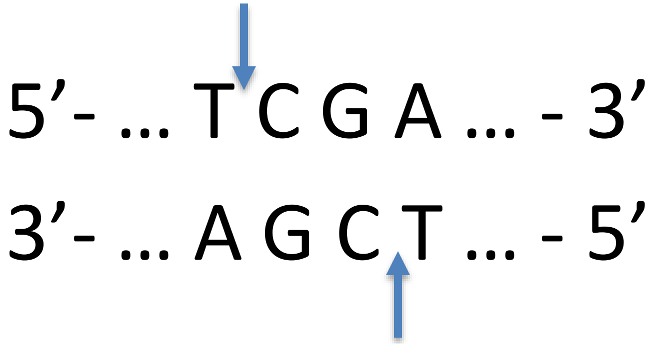
Sequencing



Wangler & Bellen (2017). DOI: 10.1016/B978-0-12-803077-6.00012-6

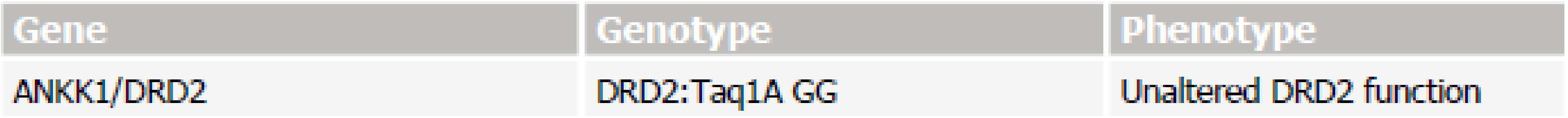
Restriction Fragment Length Polymorphism (RFLP)

- ANKK1/DRD2:TaqIA



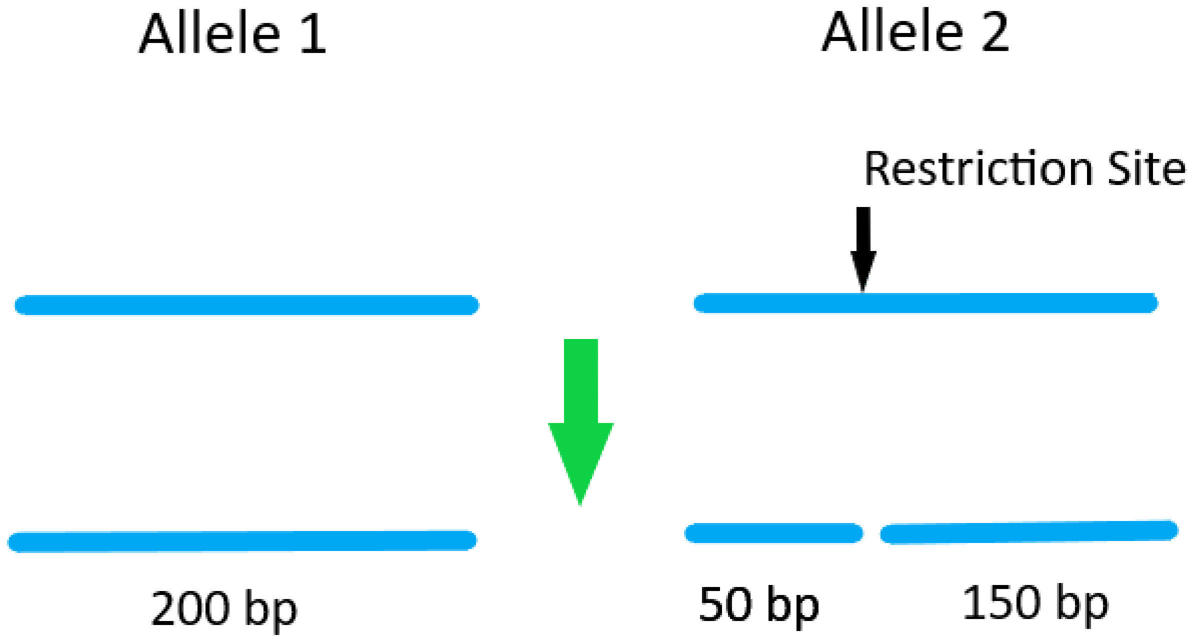
5’- … T C G A … - 3’

3’- … A G C T … - 5’

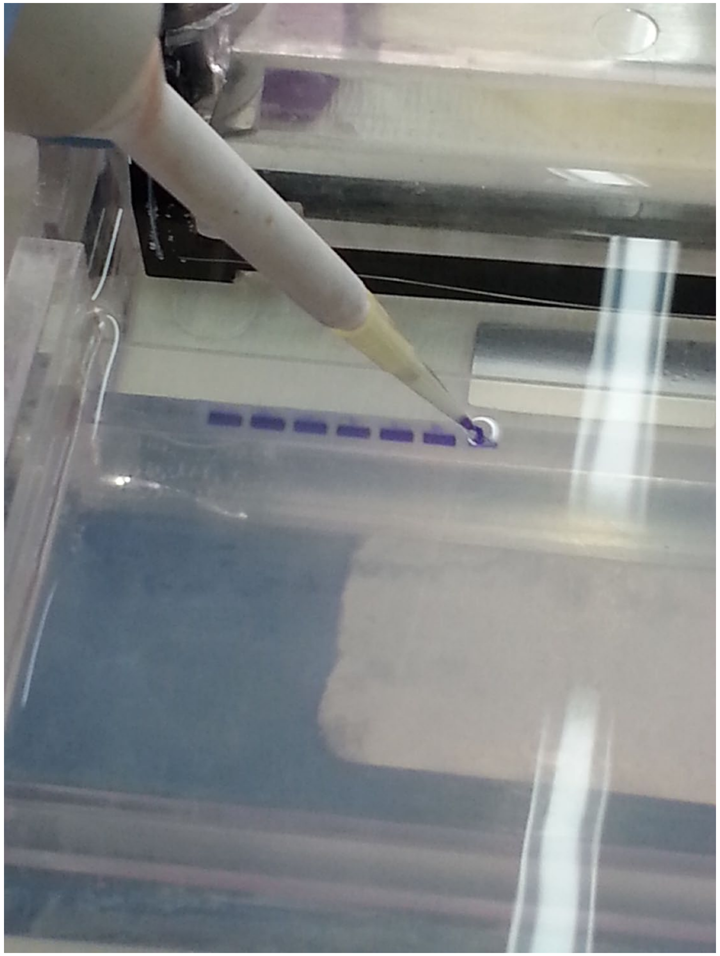


Gene	Genotype	Phenotype
ANKK1/DRD2	DRD2:Taq1A GG	Unaltered DRD2 function

Restriction Fragment Length Polymorphism (RFLP)



Gel Electrophoresis



Allele 1

Allele 2

Size

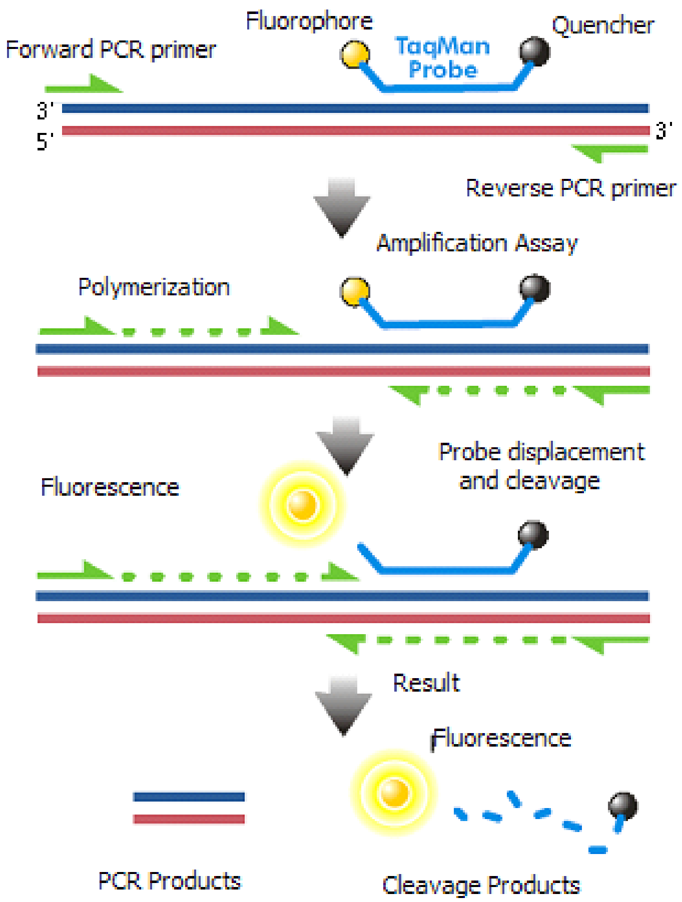
200 bp

150 bp

50 bp

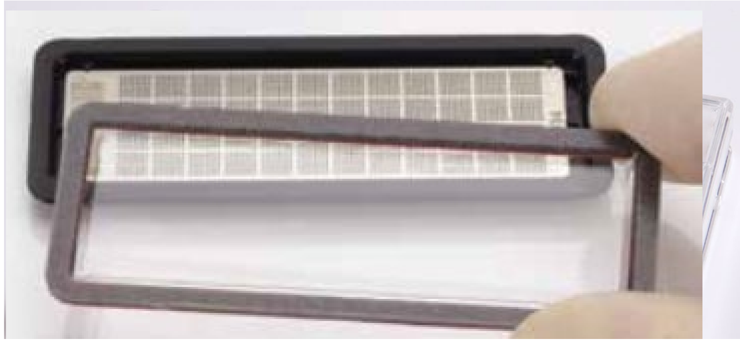
Image Credit: Wikimedia Commons

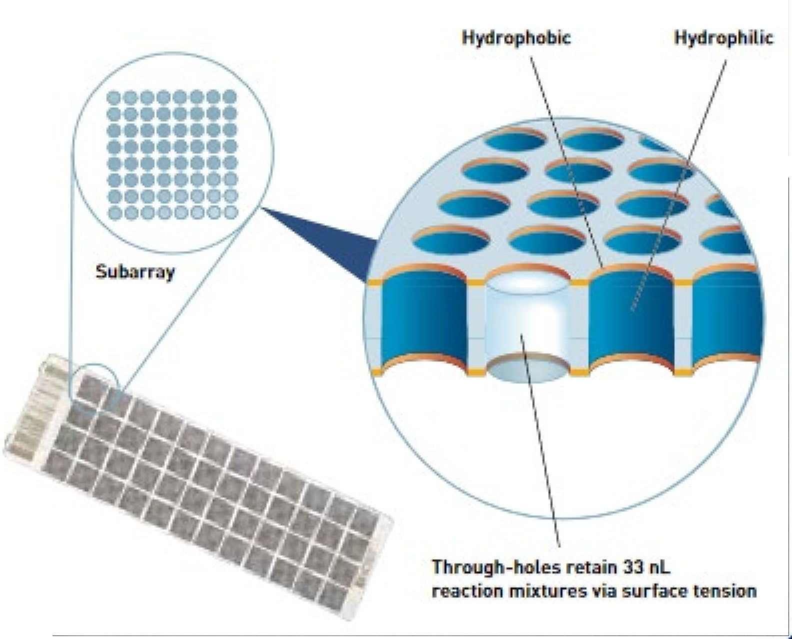
Allele-Specific Probes



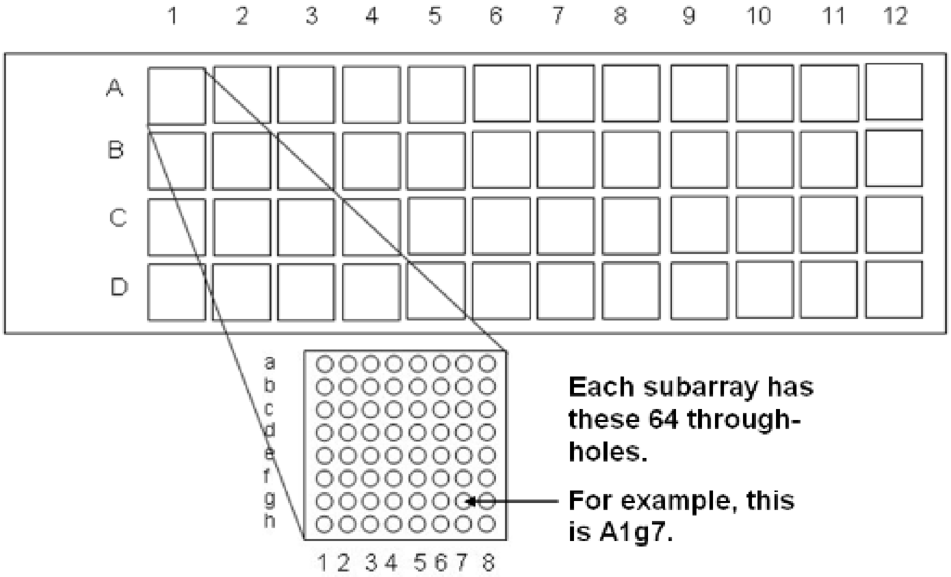
- Fluorescence/Förster Resonance Energy Transfer (FRET)

Allele-Specific Probes





Through-holes retain 33 nL reaction mixtures via surface tension

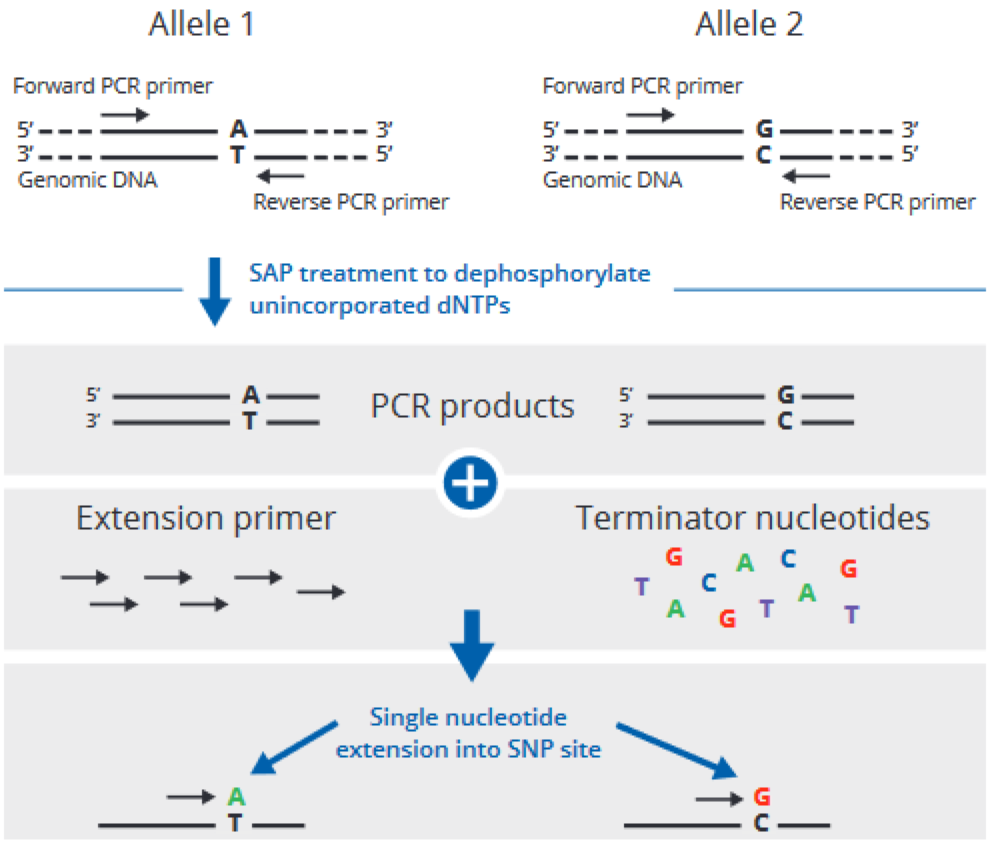


Each subarray has these 64 through-holes.

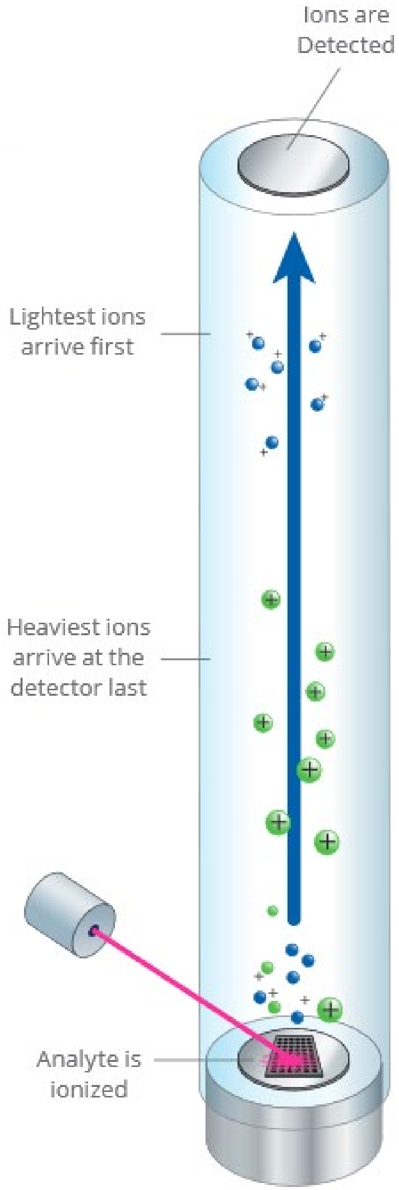
For example, this is A1g7.

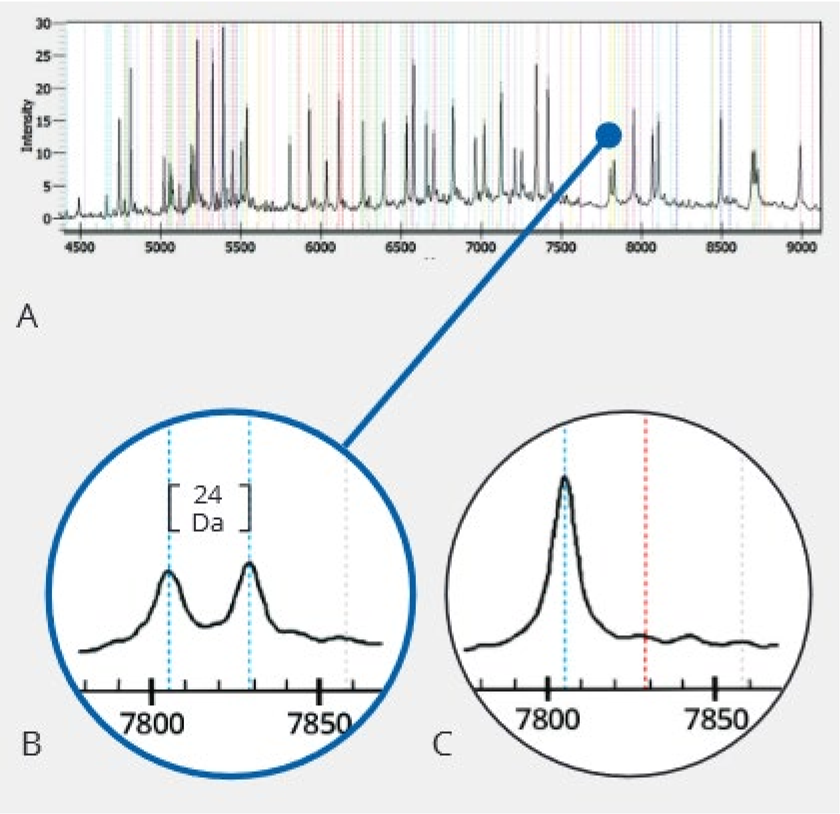
- 4 x 12 grid = 48 subarrays
- 8 x 8 grid in subarray
  = 64 through-holes
- 3,072 reactions

Single Base Extension

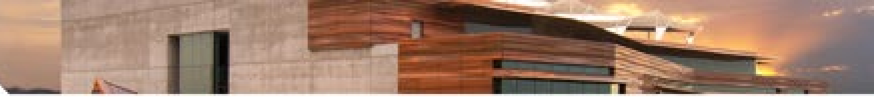


Mass Spectrometry Detection

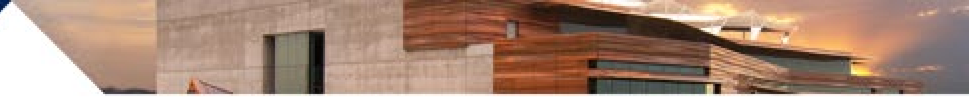




Heterozygous Homozygous



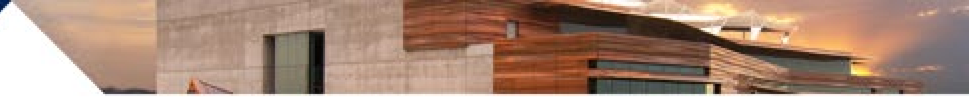
LAB RESULT TO PHENOTYPE



*-Alleles and Haplotypes

- Haplotype = group of polymorphisms on one chromosome

CYP2B6 Allele	SNP 1 rs2279343 A>G	SNP 2 rs3211371 C>T	SNP 3 rs12721655 A>G	SNP 4 rs3745274 G>T
*4	✓			
*5		✓		
*6	✓			✓
*7	✓	✓		✓
*8			✓	
*9				✓
*13	✓		✓	✓



Pitfalls of *-Allele System

Unable to distinguish between *4, *6, and *13 if only SNPs 1 & 2 are tested

CYP2B6 Allele	SNP 1 rs2279343 A>G	SNP 2 rs3211371 C>T	SNP 3 rs12721655 A>G	SNP 4 rs3745274 G>T
*4	✓			
*5		✓		
*6	✓			✓
*7	✓	✓		✓
*8			✓	
*9				✓
*13	✓		✓	✓

Appear as *1

Pitfalls of *-Allele System – Phase

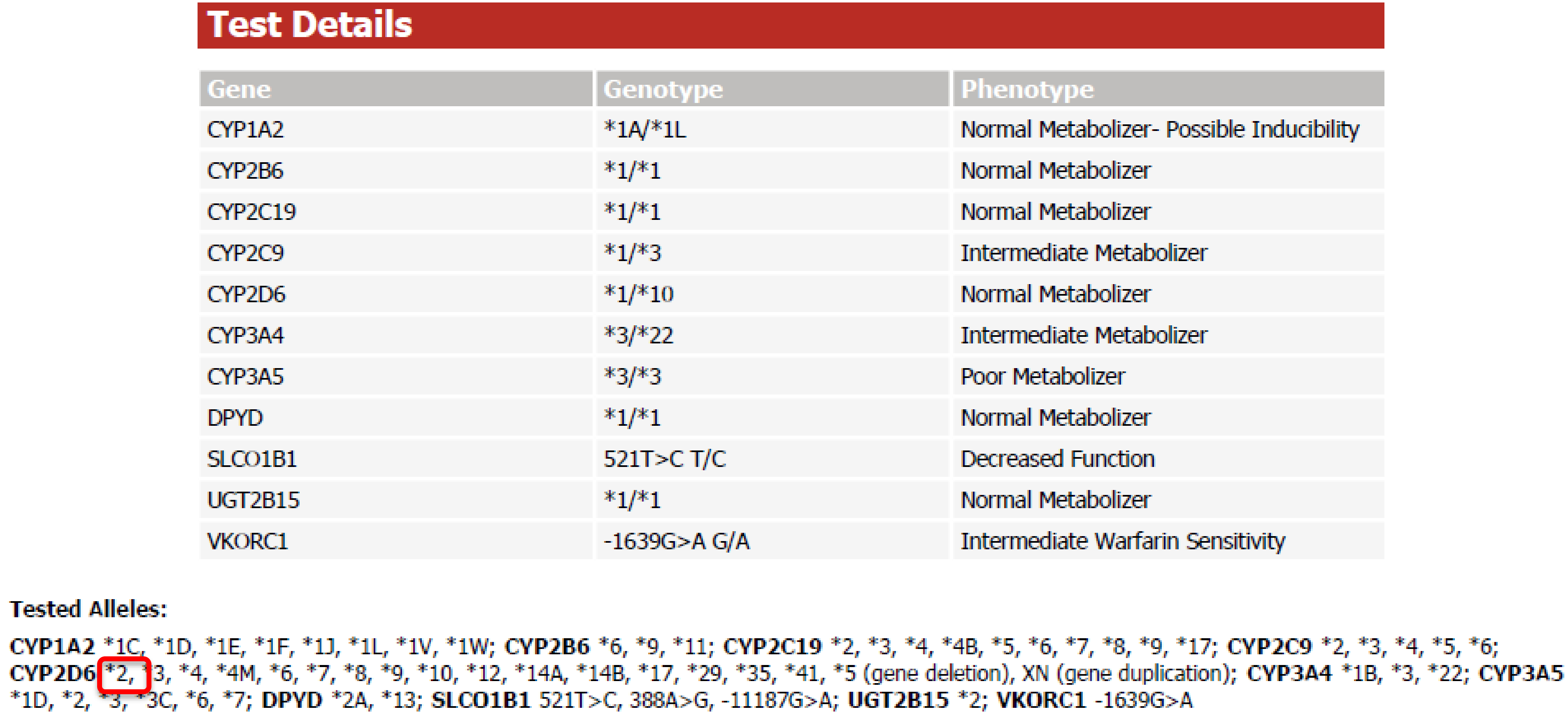
Patient Sample	SNP 1 rs2279343 A>G	SNP 2 rs3211371 C>T	SNP 3 rs12721655 A>G	SNP 4 rs3745274 G>T
1	A/G	C/C	A/A	G/T

Heterozygous

CYP2B6 Allele	rs2279343 A>G	rs3211371 C>T	rs12721655 A>G	rs3745274 G>T
*1	A	C	A	G
*4	G	C	A	G
*6	G	C	A	T
*9	A	C	A	T

2B6 *-Allele Possibilities	SNP 1	SNP 2	SNP 3	SNP 4
*1 *6	A G	C C	A A	G T
*9 *4	A G	C C	A A	T G

Sample Result



Test Details

Gene	Genotype	Phenotype
CYP1A2	*1A/*1L	Normal Metabolizer- Possible Inducibility
CYP2B6	*1/*1	Normal Metabolizer
CYP2C19	*1/*1	Normal Metabolizer
CYP2C9	*1/*3	Intermediate Metabolizer
CYP2D6	*1/*10	Normal Metabolizer
CYP3A4	*3/*22	Intermediate Metabolizer
CYP3A5	*3/*3	Poor Metabolizer
DPYD	*1/*1	Normal Metabolizer
SLCO1B1	521T>C T/C	Decreased Function
UGT2B15	*1/*1	Normal Metabolizer
VKORC1	-1639G>A G/A	Intermediate Warfarin Sensitivity

Tested Alleles:

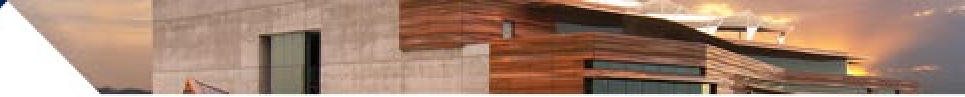
CYP1A2 *1C, *1D, *1E, *1F, *1J, *1L, *1V, *1W; **CYP2B6** *6, *9, *11; **CYP2C19** *2, *3, *4, *4B, *5, *6, *7, *8, *9, *17; **CYP2C9** *2, *3, *4, *5, *6; **CYP2D6** *2, *3, *4, *4M, *6, *7, *8, *9, *10, *12, *14A, *14B, *17, *29, *35, *41, *5 (gene deletion), XN (gene duplication); **CYP3A4** *1B, *3, *22; **CYP3A5** *1D, *2, *3, *3C, *6, *7; **DPYD** *2A, *13; **SLCO1B1** 521T>C, 388A>G, -11187G>A; **UGT2B15** *2; **VKORC1** -1639G>A

CYP2D6*2

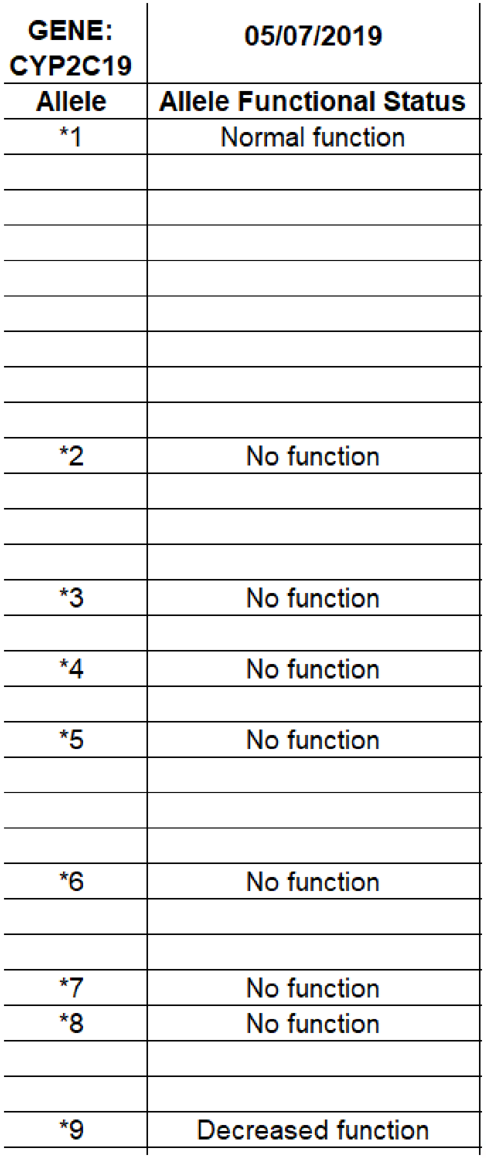
- Defined by 2850C>T (Normal function)
- But many other *-alleles also have 2850C>T

Allele	Function
*8	None
*11	None
*12	None
*14B	Decreased
*17	Decreased
*19	None
*31	None

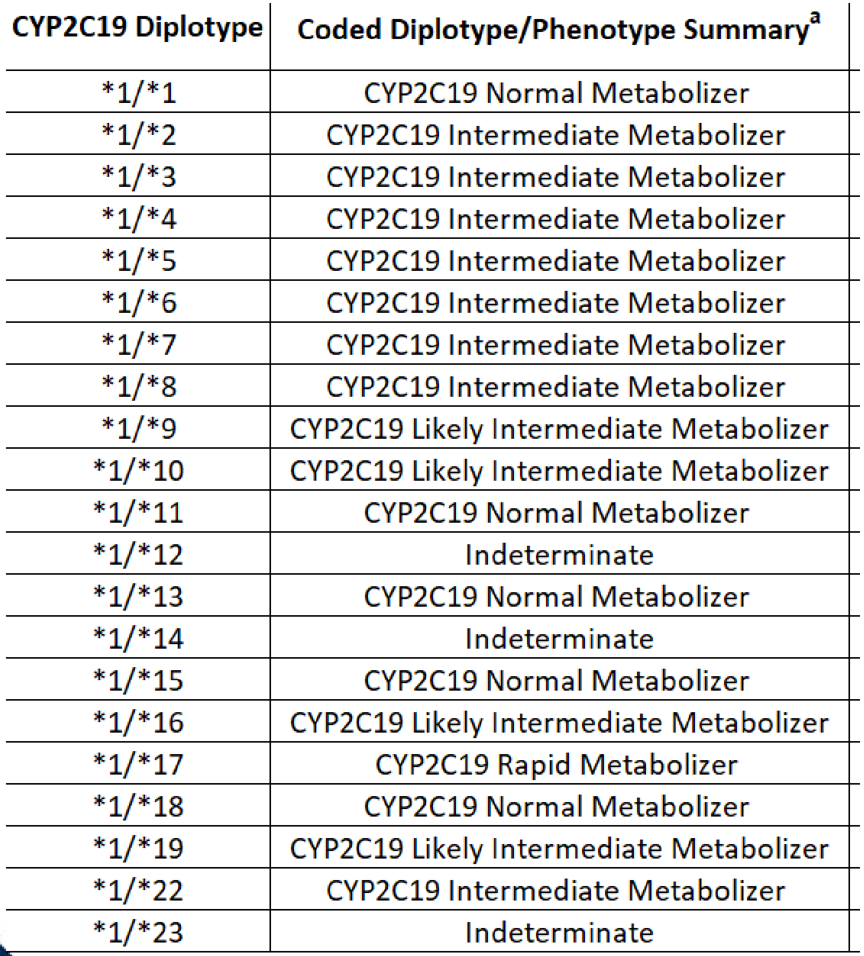
CYP2D6 *2, *3, *4, *4M, *6, *7, *8, *9, *10, *12, *14A, *14B, *17, *29, *35, *41, *5 (gene deletion), XN (gene duplication);



CYP2C19: Diplotype → Phenotype

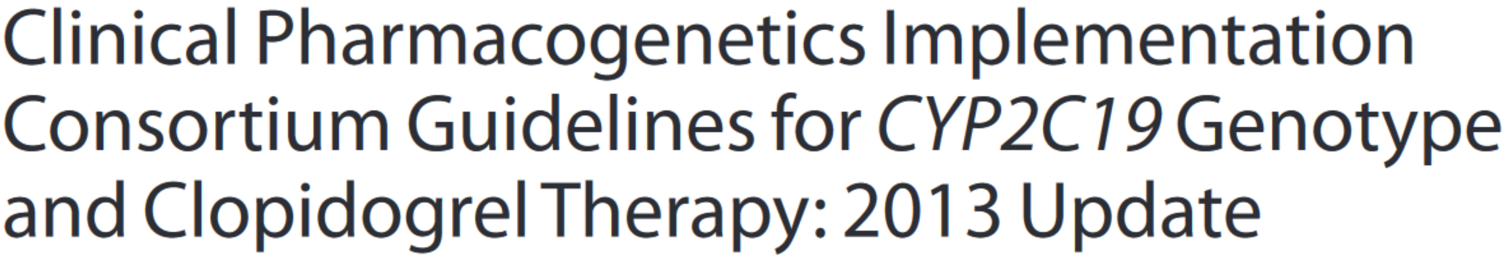


GENE: CYP2C19	05/07/2019
Allele	**Allele Functional Status**
*1	Normal function
*2	No function
*3	No function
*4	No function
*5	No function
*6	No function
*7	No function
*8	No function
*9	Decreased function



CYP2C19 Diplotype	Coded Diplotype/Phenotype Summary[a]
*1/*1	CYP2C19 Normal Metabolizer
*1/*2	CYP2C19 Intermediate Metabolizer
*1/*3	CYP2C19 Intermediate Metabolizer
*1/*4	CYP2C19 Intermediate Metabolizer
*1/*5	CYP2C19 Intermediate Metabolizer
*1/*6	CYP2C19 Intermediate Metabolizer
*1/*7	CYP2C19 Intermediate Metabolizer
*1/*8	CYP2C19 Intermediate Metabolizer
*1/*9	CYP2C19 Likely Intermediate Metabolizer
*1/*10	CYP2C19 Likely Intermediate Metabolizer
*1/*11	CYP2C19 Normal Metabolizer
*1/*12	Indeterminate
*1/*13	CYP2C19 Normal Metabolizer
*1/*14	Indeterminate
*1/*15	CYP2C19 Normal Metabolizer
*1/*16	CYP2C19 Likely Intermediate Metabolizer
*1/*17	CYP2C19 Rapid Metabolizer
*1/*18	CYP2C19 Normal Metabolizer
*1/*19	CYP2C19 Likely Intermediate Metabolizer
*1/*22	CYP2C19 Intermediate Metabolizer
*1/*23	Indeterminate

CYP2C19 Phenotypes



Clinical Pharmacogenetics Implementation Consortium Guidelines for *CYP2C19* Genotype and Clopidogrel Therapy: 2013 Update

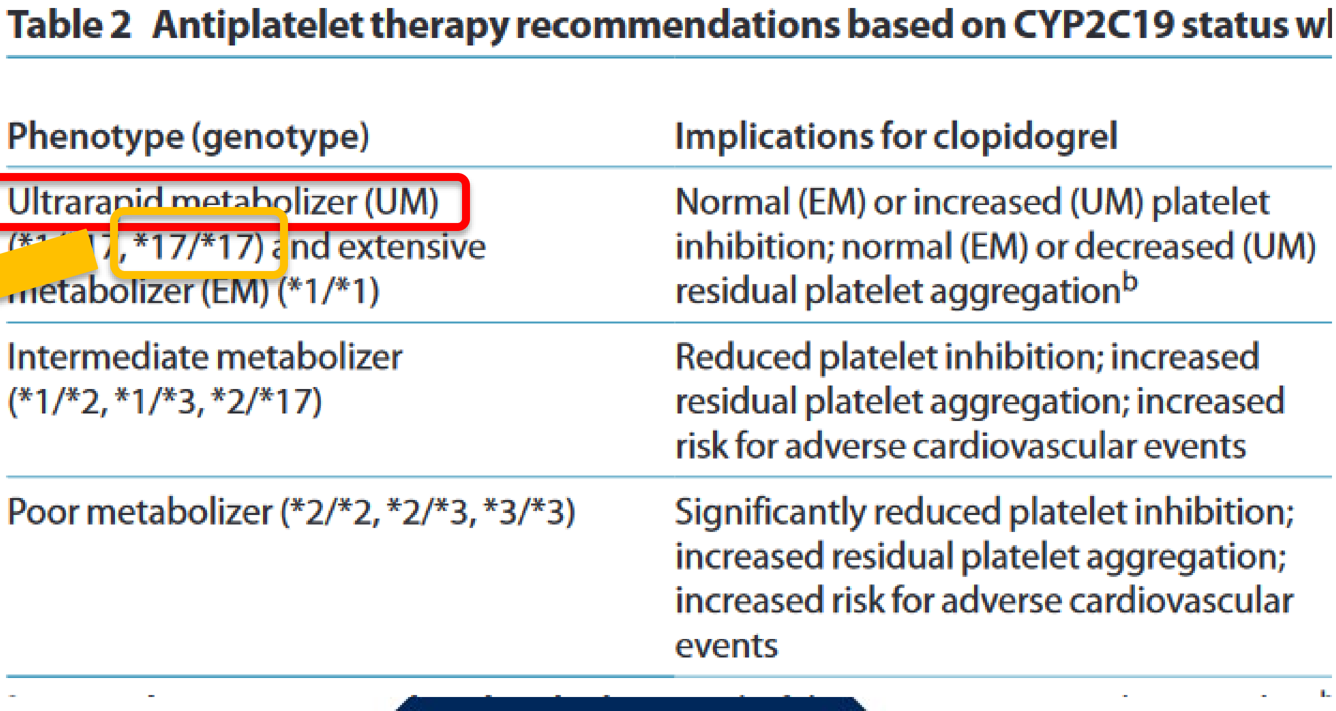


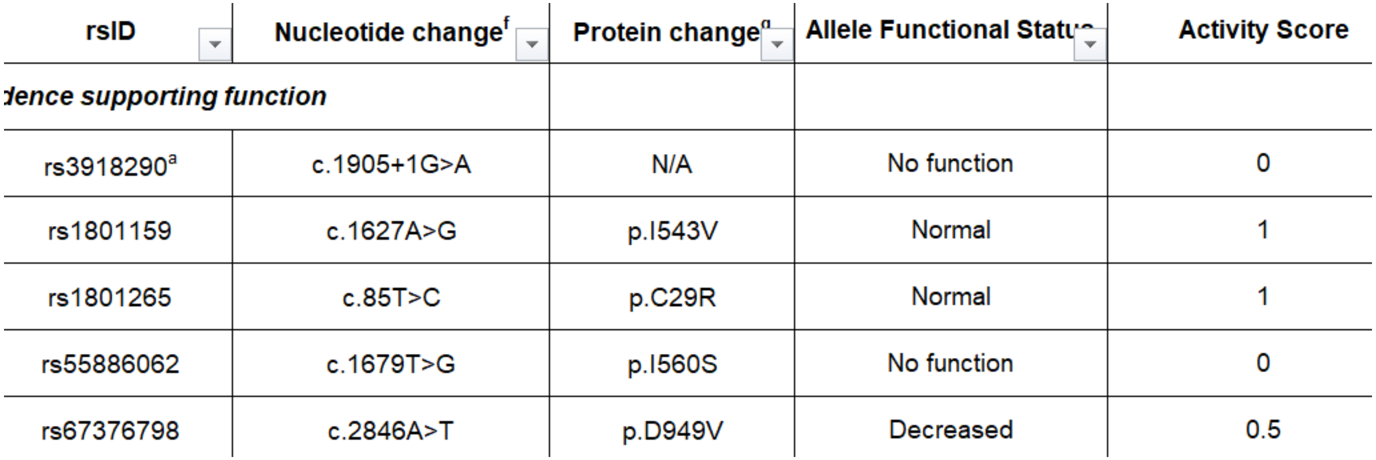
Table 2 Antiplatelet therapy recommendations based on CYP2C19 status w

Phenotype (genotype)	Implications for clopidogrel
Ultrarapid metabolizer (UM) (*1/*17, *17/*17) and extensive metabolizer (EM) (*1/*1)	Normal (EM) or increased (UM) platelet inhibition; normal (EM) or decreased (UM) residual platelet aggregation^{b}
Intermediate metabolizer (*1/*2, *1/*3, *2/*17)	Reduced platelet inhibition; increased residual platelet aggregation; increased risk for adverse cardiovascular events
Poor metabolizer (*2/*2, *2/*3, *3/*3)	Significantly reduced platelet inhibition; increased residual platelet aggregation; increased risk for adverse cardiovascular events

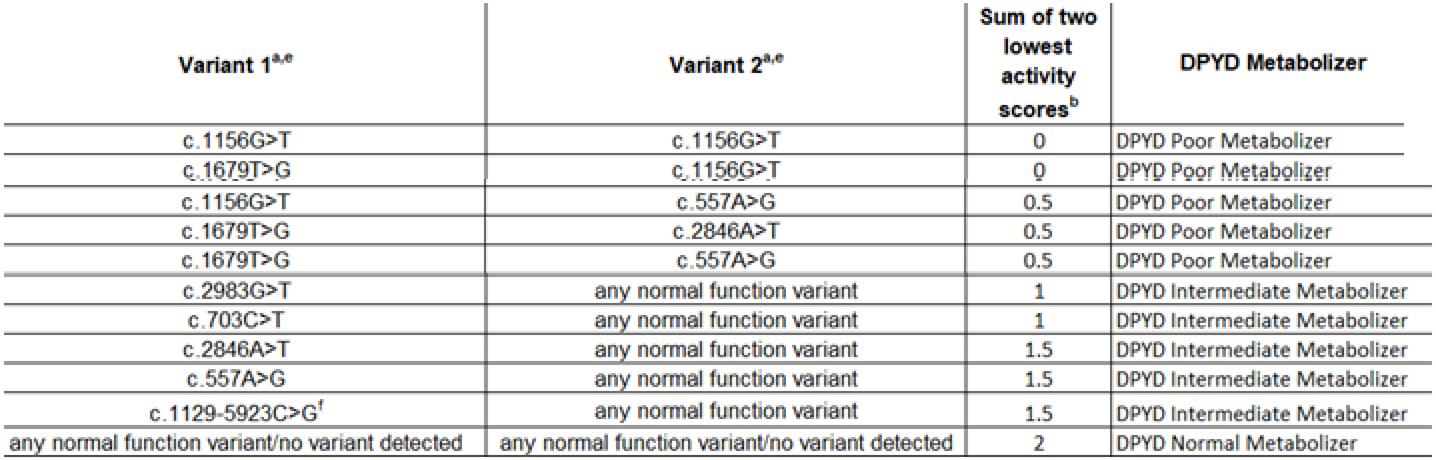
RM

UM

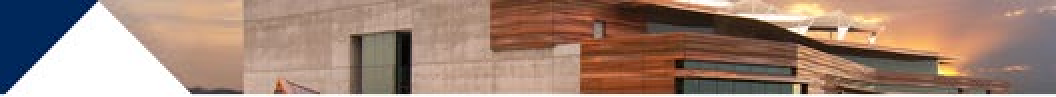
DPYD: Diplotype → Phenotype Activity Scores



rsID	Nucleotide change[f]	Protein change[g]	Allele Functional Statu	Activity Score
dence supporting function				
rs3918290[a]	c.1905+1G>A	N/A	No function	0
rs1801159	c.1627A>G	p.I543V	Normal	1
rs1801265	c.85T>C	p.C29R	Normal	1
rs55886062	c.1679T>G	p.I560S	No function	0
rs67376798	c.2846A>T	p.D949V	Decreased	0.5

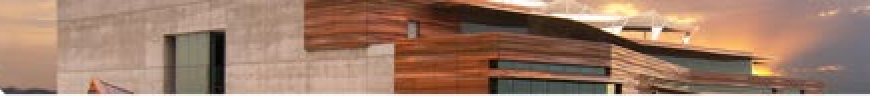


Variant 1[a,e]	Variant 2[a,e]	Sum of two lowest activity scores[b]	DPYD Metabolizer
c.1156G>T	c.1156G>T	0	DPYD Poor Metabolizer
c.1679T>G	c.1156G>T	0	DPYD Poor Metabolizer
c.1156G>T	c.557A>G	0.5	DPYD Poor Metabolizer
c.1679T>G	c.2846A>T	0.5	DPYD Poor Metabolizer
c.1679T>G	c.557A>G	0.5	DPYD Poor Metabolizer
c.2983G>T	any normal function variant	1	DPYD Intermediate Metabolizer
c.703C>T	any normal function variant	1	DPYD Intermediate Metabolizer
c.2846A>T	any normal function variant	1.5	DPYD Intermediate Metabolizer
c.557A>G	any normal function variant	1.5	DPYD Intermediate Metabolizer
c.1129-5923C>G[f]	any normal function variant	1.5	DPYD Intermediate Metabolizer
any normal function variant/no variant detected	any normal function variant/no variant detected	2	DPYD Normal Metabolizer



Other Interpretive Challenges

- Recent blood transfusion
- Transfusion-Associated Microchimerism
- Allogeneic Bone Marrow Transplant (BMT)
- Liver Transplant



QUESTIONS?