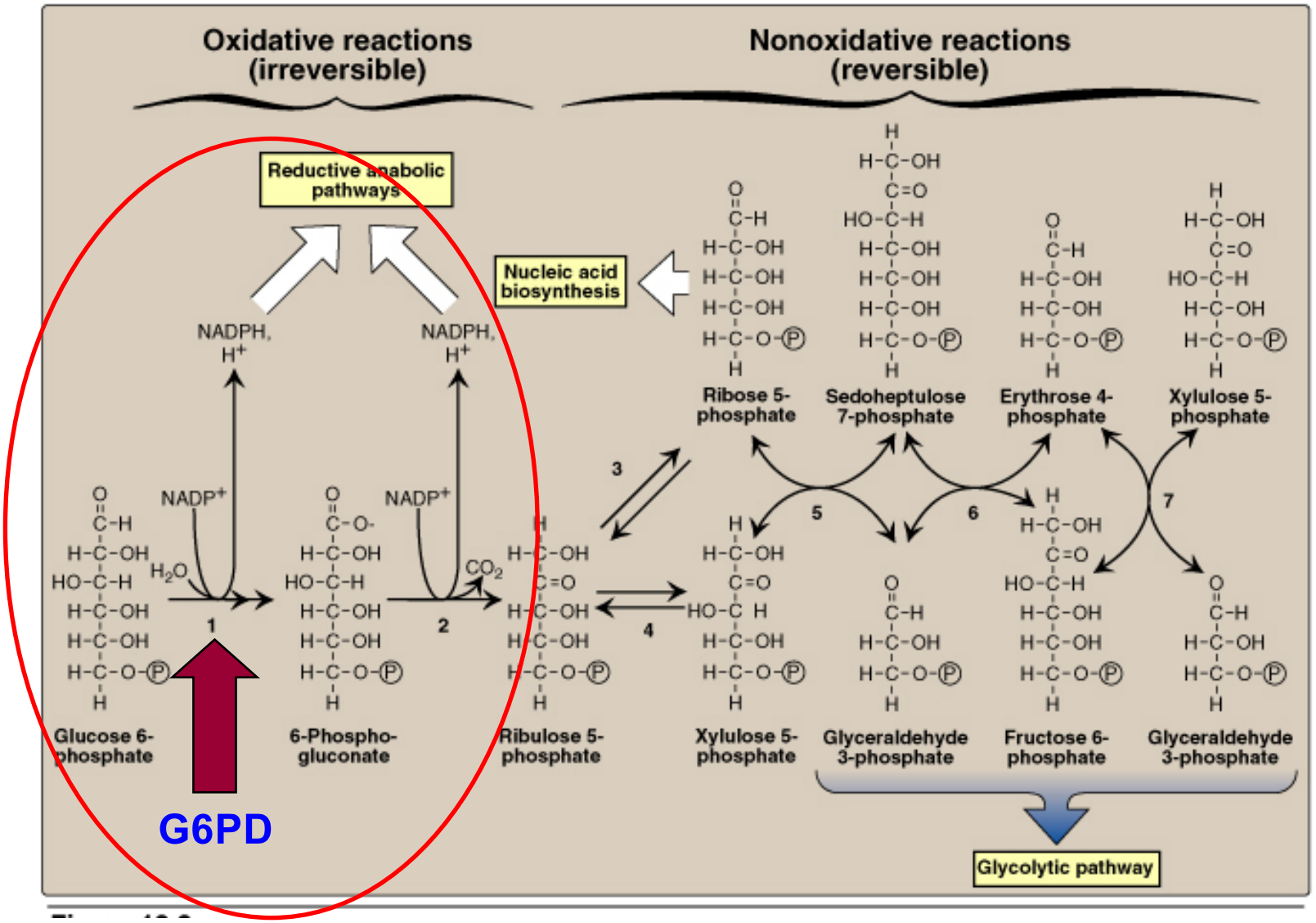
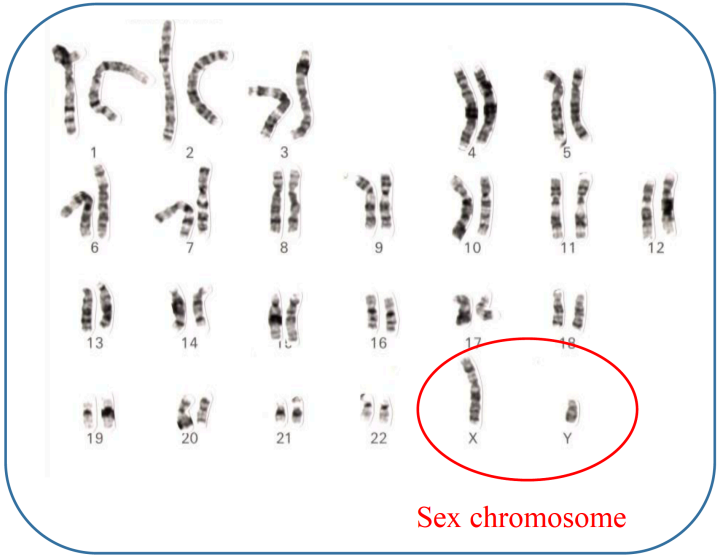
Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency Anemia

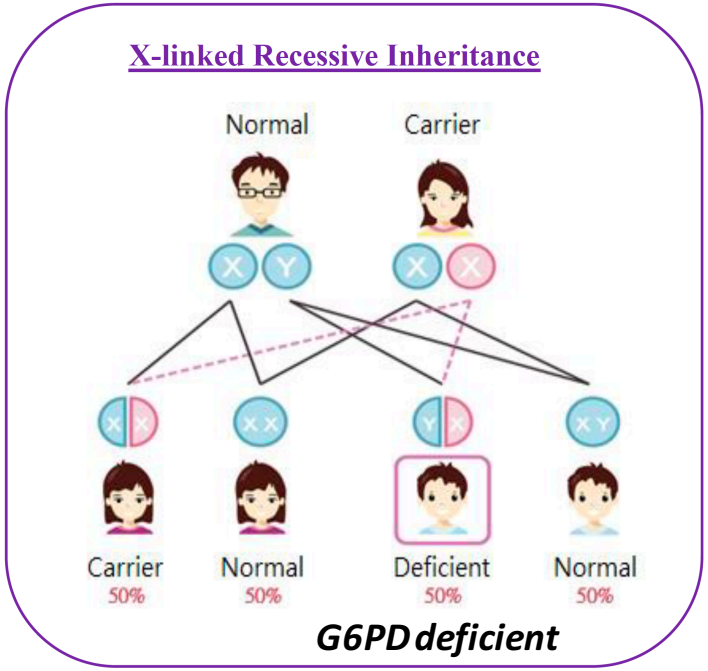
Glucose-6-Phosphate Dehydrogenase produces NADPH



Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency Anemia



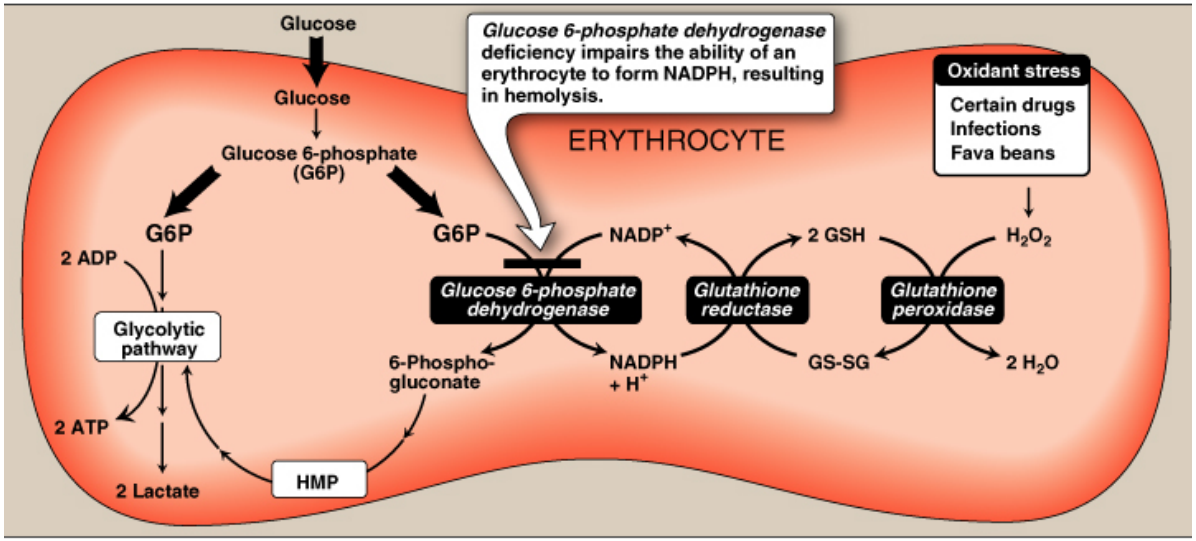
The gene coding for G6PD enzyme is located on the X chromosome.



Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency Anemia is a X-linked recessive disease

Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency Anemia

Glucose-6-Phosphate Dehydrogenase produces NADPH



Uses of NADPH

- **Reductive biosynthesis e.g., fatty acid**
- **biosynthesis**
- **Antioxidant (part of glutathione system)**
- **Oxygen-dependent phagocytosis by WBCs**
- **Synthesis of nitric oxide (NO)**

NADPH: Nicotinamide adenine dinucleotide phosphate

NADPH provides the reducing equivalents for biosynthetic reactions and the oxidation-reduction involved in protecting against the toxicity of reactive oxygen species (ROS),

If mutations in the *G6PD* gene reduce the amount of glucose-6-phosphate dehydrogenase or alter its structure, this enzyme can no longer play its protective role. As a result, reactive oxygen species can accumulate and damage red blood cells. A a consequence, red blood cells to be destroyed faster than the body can replace them = hemolysis. A reduction in the number of red blood cells causes the signs and symptoms of hemolytic anemia.

DETAILLED INFO: https://ghr.nlm.nih.gov/condition/glucose-6-phosphate-dehydrogenase-deficiency

Oxidative stress: imbalance between oxidant production and antioxidant mechanisms

Oxidative damage to:

DNA

Proteins

Lipids (unsaturated fatty acids)

Oxidative stress and diseases:

Inflammatory conditions e.g., Rheumatoid arthritis

Atherosclerosis and coronary heart diseases

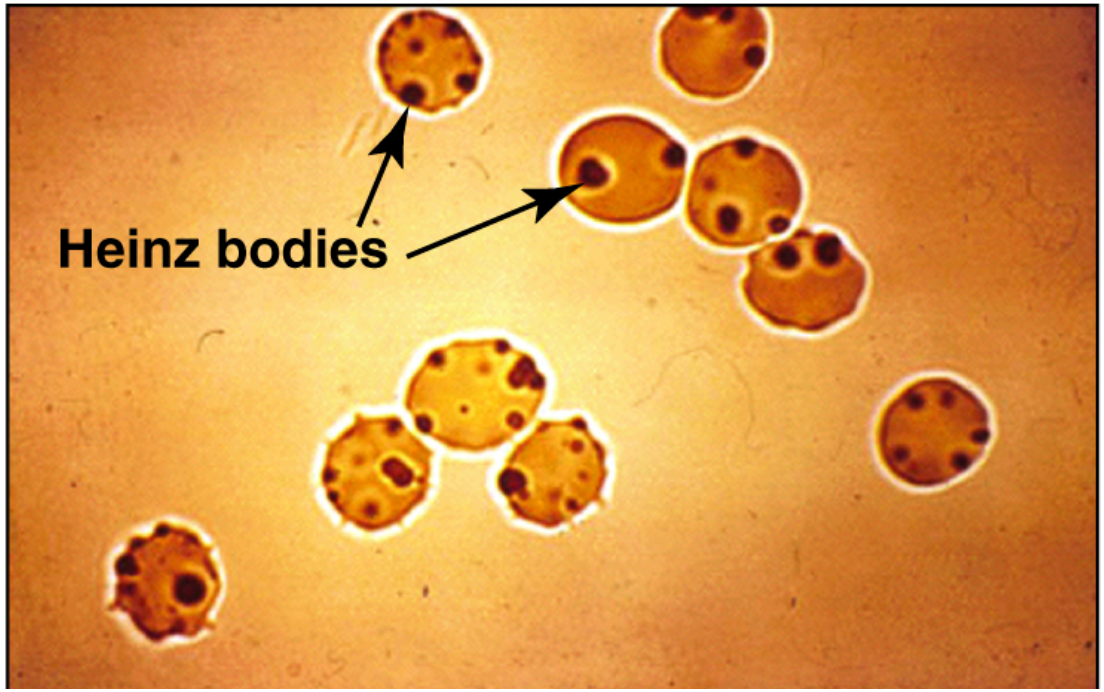
Obesity

Cancers

G6PD deficiency hemolytic anemia

Biochemical basis of G6PD Deficiency Hemolytic Anemia, continued…

Oxidation of sulfhydryl (SH) groups of proteins inside red blood cells (erythrocytes) causes protein denaturation and formation of insoluble masses (Heinz bodies) that attach to red blood cell membranes

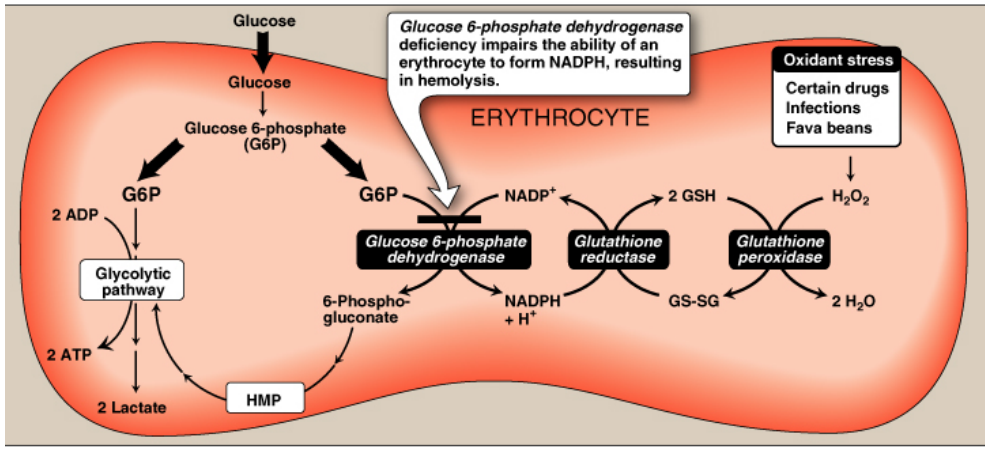


Although G6PD deficiency affects all cells, but it is most severe in red blood cells ……
Why?

Other cells have other sources for NADPH production:

e.g., Malic enzyme that converts malate into pyruvate

Biochemical basis of G6PD Deficiency Hemolytic Anemia, continued…



Carriers of G6PD do not necessarily develop anemia

.. Disease is triggerd by increased increased reactive oxygen species (ROS) levels

G6PD deficient patients will develop hemolytic attack upon:

1.Intake of oxidant drugs (AAA):

- **Antibiotics e.g., sulfa preparation**
- **Antimalarial: e.g., Primaquine**
- **Antipyretics**

2.Exposure to infection

3.Ingestion of fava beans (favism, Mediterranean variant)

<u>Chronic nonspherocytic anemia</u>: Hemolytic attack in absence of precipitating factors. Severe form due to class I mutation

Different Classes of G6PD Deficiency Hemolytic Anemia

- There are 4 different classes:
  - I (Very severe)
  - II (Severe, e.g. Mediterranean)
  - III: (Moderate: G6PD A-)
  - IV: (Normal)
- This classification is based on the residual enzyme activity (Least in class I, and Highest in class IV)

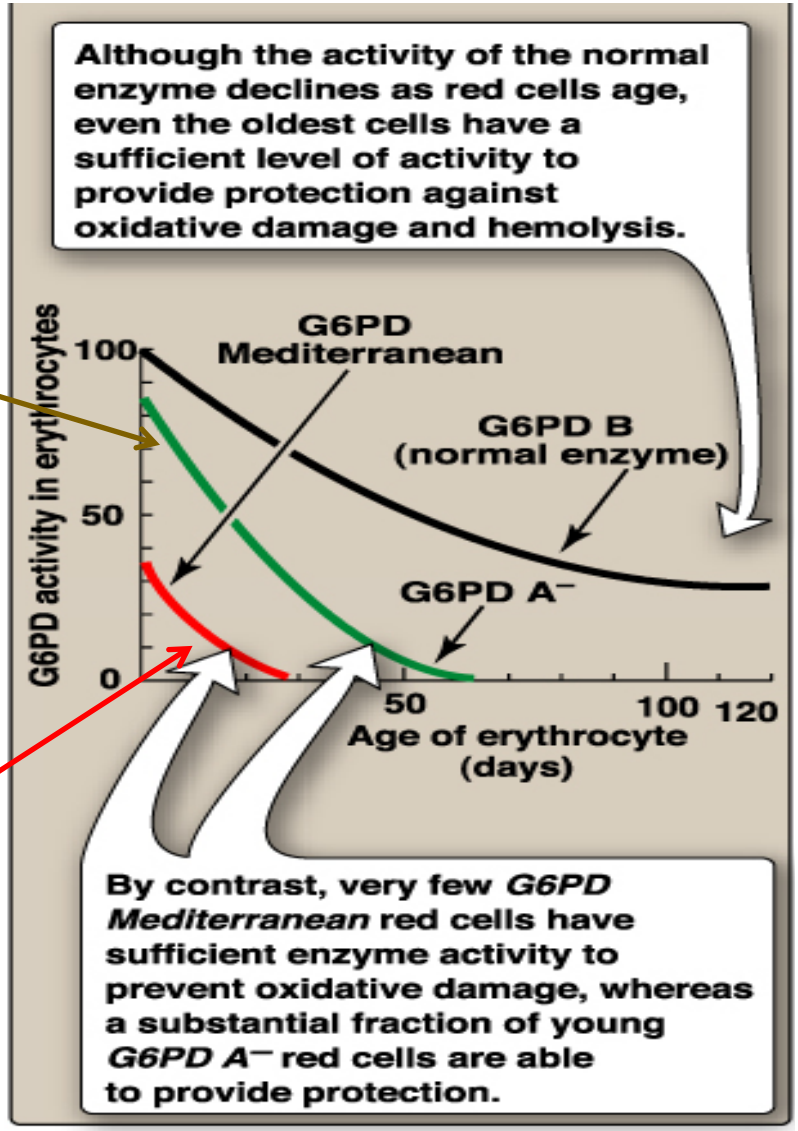
Different Classes of G6PD Deficiency Hemolytic Anemia

G6PD A- (class III):

Moderate, young RBCs contain enzymatic activity.
Unstable enzyme, but kinetically normal

G6PD Mediterranean (II)

Enzyme with decreased stability and activity (severe).
Affect all RBCs
(both young and old)



Diagnosis of
G6PD Deficiency Hemolytic Anemia

Diagnosis of hemolytic anemia

Complete Blood Count (CBC) & reticulocytic count

Screening:

Qualitative assessment of G6PD enzymatic activity
(UV-based test)

Confirmatory test:

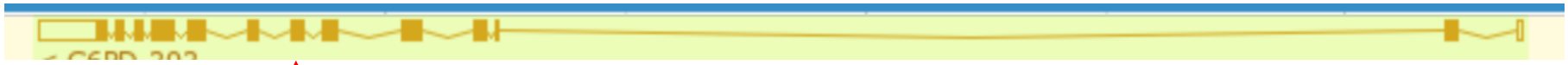
Quantitative measurement of G6PD enzymatic activity

Molecular test:

Detection of G6PD gene mutation

G6PD Mutations linlked with hemolytic anemia

G6PD gene on X chromosome



Exon 6

Descriptive mutations									
Mutation				Gene			Protein		
Designation	Short name	Isoform G6PD-Protein	OMIM-Code	Type	Subtype	Position	Position	Structure change	Function change
G6PD-A(+)	Gd-A(+)	G6PD A	+305900.0001	Polymorphism nucleotide	A→G	376 (Exon 5)	126	Asparagine→Aspartic acid (ASN126ASP)	No enzyme defect (variant)
G6PD-A(-)	Gd-A(-)	G6PD A	+305900.0002	Substitution nucleotide	G→A	376 (Exon 5) and 202	68 and 126	Valine→Methionine (VAL68MET) Asparagine→Aspartic acid (ASN126ASP)	
G6PD-Mediterranean	Gd-Med	G6PD B	+305900.0006	Substitution nucleotide	C→T	563 (Exon 6)	188	Serine→Phenylalanine (SER188PHE)	Class II
G6PD-Canton	Gd-Canton	G6PD B	+305900.0021	Substitution nucleotide	G→T	1376	459	Arginine→Leucine (ARG459LEU)	Class II
G6PD-Chatham	Gd-Chatham	G6PD	+305900.0003	Substitution nucleotide	G→A	1003	335	Alanine→Threonine (ALA335THR)	Class II
G6PD-Cosenza	Gd-Cosenza	G6PD B	+305900.0059	Substitution nucleotide	G→C	1376	459	Arginine→Proline (ARG459PRO)	G6PD-activity <10%, thus high portion of patients.
G6PD-Mahidol	Gd-Mahidol	G6PD	+305900.0005	Substitution nucleotide	G→A	487 (Exon 6)	163	Glycine→Serine (GLY163SER)	Class III
G6PD-Orissa	Gd-Orissa	G6PD	+305900.0047	Substitution nucleotide	C→G	131	44	Alanine→Glycine (ALA44GLY)	NADP-binding place affected. Higher stability than other variants.
G6PD-Asahi	Gd-Asahi	G6PD A-	+305900.0054	Substitution nucleotide (several)	A→G ± G→A	376 (Exon 5) 202	126 68	Asparagine→Aspartic acid (ASN126ASP) Valine→Methionine (VAL68MET)	Class III.

G6PD Mutations linlked with hemolytic anemia

Type of mutations

Table 3. Allele frequency of the most common G6PD mutations in Sardinia and continental Italy.

Geographic area	*Alleles*	*Mediterranean*	*Union*	*Cosenza*	*S. Antioco*	*Partenope°*	*Seattle*	*A–*	*Tokyo°*	*Undefined*
Sardinia	60*	50 (83%)	6 (10%)	1 (1.65%)	2 (3.3%)	–	1 (1.65%)	–	–	–
Southern Italy	57	36 (63%)	2 (3.5%)	1 (1.75%)	–	1 (1.75%)	4 (7%)	4 (7%)	1 (1.75%)	8 (14%)
Northern Italy	45	26 (58%)	2 (4.4%)	–	–	–	5 (11%)	2 (4.4%)	–	10 (22%)
Total	162	112 (69%)	10 (6.2%)	2 (1.2%)	2 (1.2%)	1 (0.6%)	10 (6.2%)	6 (3.7%)	1 (0.6%)	18 (11.1%)

**One female was homozygous; °Not polymorphic.*

Allele frequency: % of total X chromosomes carrying the G6PD deficiency allele

Detection of Mediterranean G6PD mutation by PCR-RFLP

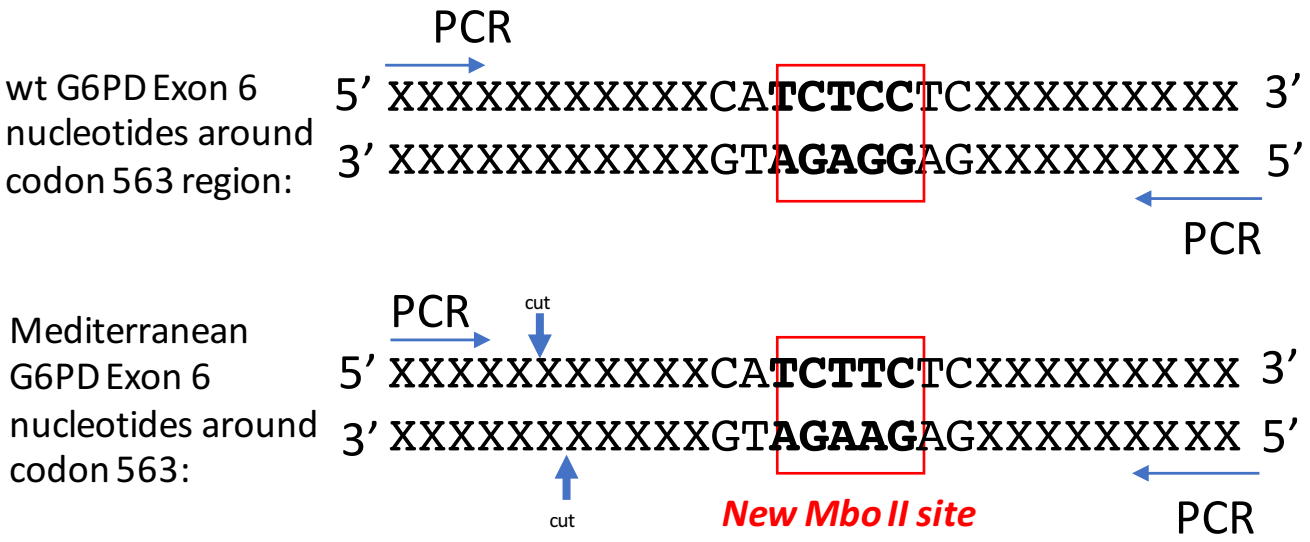
RFLP = Restriction fragment length polymorphism

In molecular biology, restriction fragment length polymorphism (RFLP) is a technique that exploits variations in homologous DNA sequences, known as polymorphisms, in order to distinguish individuals, populations, or species or to pinpoint the locations of genes within a sequence. The term may refer to a polymorphism itself, as detected through the differing locations of restriction enzyme sites, or to a related laboratory technique by which such differences can be illustrated. In RFLP analysis, a DNA sample is digested into fragments by one or more restriction enzymes, and the resulting restriction fragments are then separated by gel electrophoresis according to their size.

PCR-RFLP: 1° step PCR amplicifation of DNA 2° step restiction digest -→ mapping of sequence changes in PCR products derived form different sources of DNA (for example different patients)

G6PD 563C→T variant

PCR oligos amplify region of interest in Exon 6 of the G6PD gene (around aminoacid position 563)

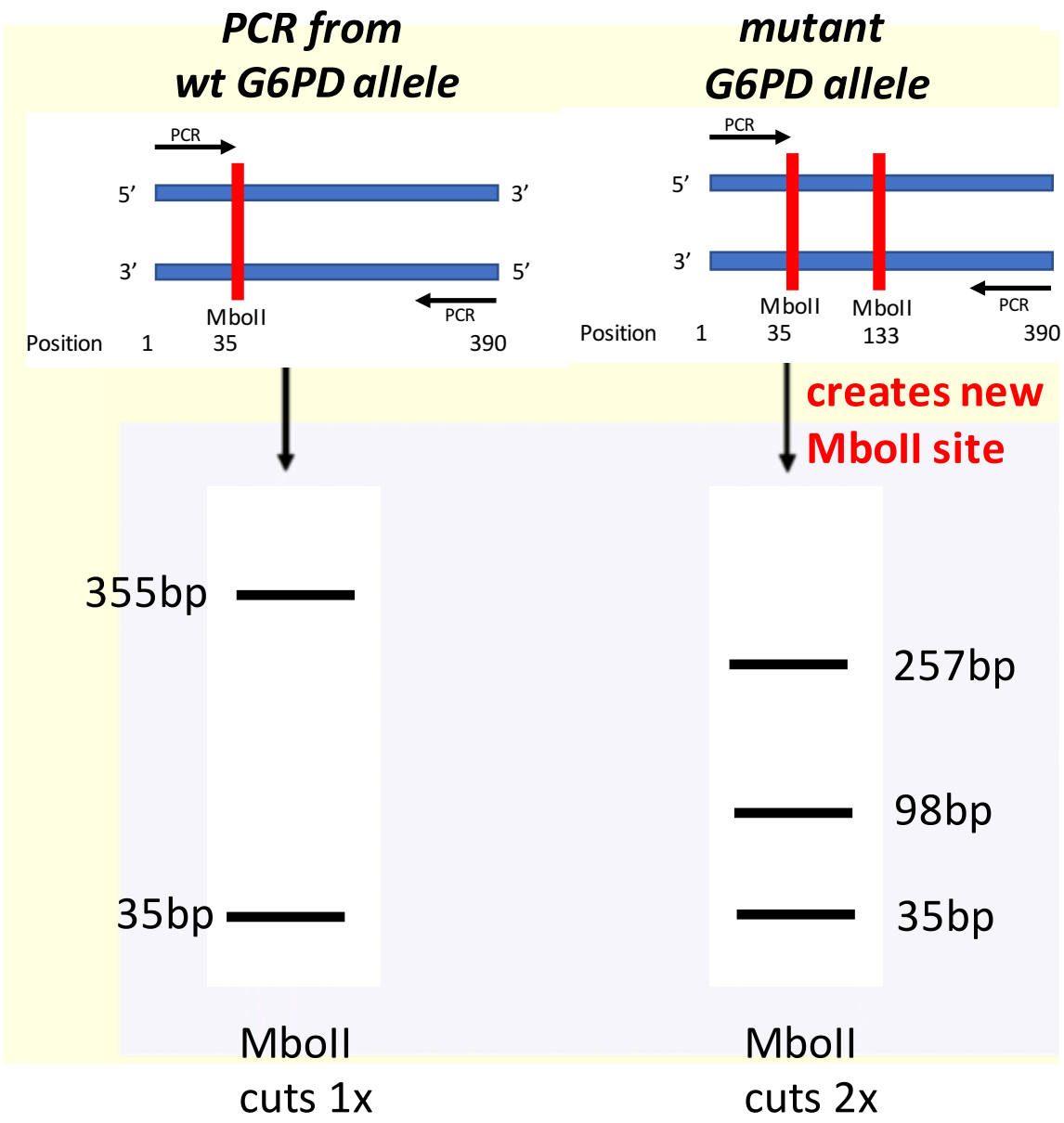


Mbo II Restriction enzyme

MboII is a type II restriction enzymes recognize asymmetric DNA sequences and cleave outside of their recognition sequence

Detection of Mediterranean G6PD mutation

PCR product from Mediterranean, mutant G6PD allele



G6PD 563 C wild-type IN EXON 6
Used primers amplify region that contains 1 MboII site

G6PD 563 C→T variant IN EXON 6
Used primers amplify region that contains 1 **+1** MboII sites

1. G6PD Exon 6 specific primers
2. PCR amplify specific region of students
3. Purify PCR product
4. **Digest purified DNA using MboII**
5. Run agarose gel
6. 563C→T variants results a new MboII site in the PCR fragment
7. Additional band appears in gel

R = sito per enzima di restrizione MboII

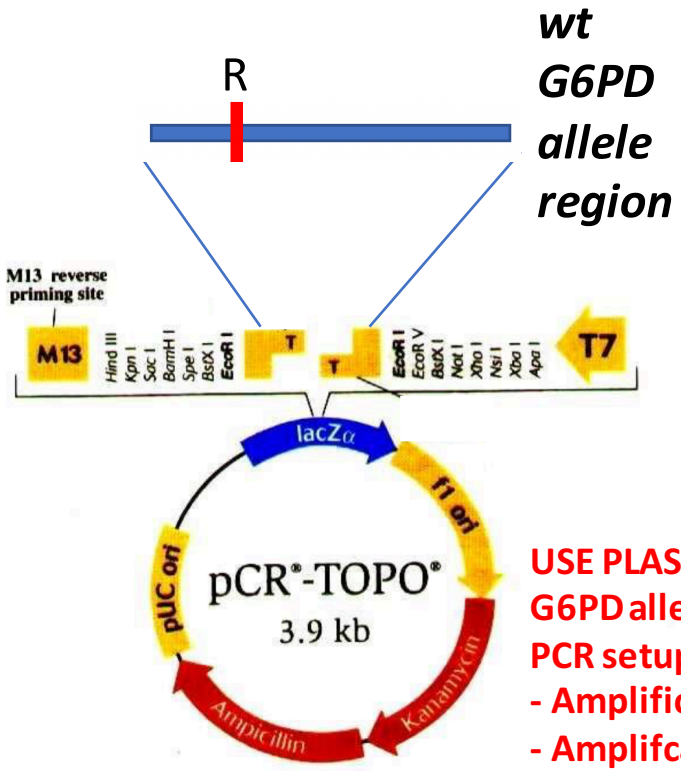
Detection of Mediterranean G6PD mutation

Control for PCR – MUST GIVE AMPLFICATION

DNA amplified from human DNA with wt G6PD (Taq) and cloned via TA-cloning into pCR-TOPOII

→ **Make PCR-RFLP with oligos**
→ **Run gel**
→ **PCR gives band for wt allele**

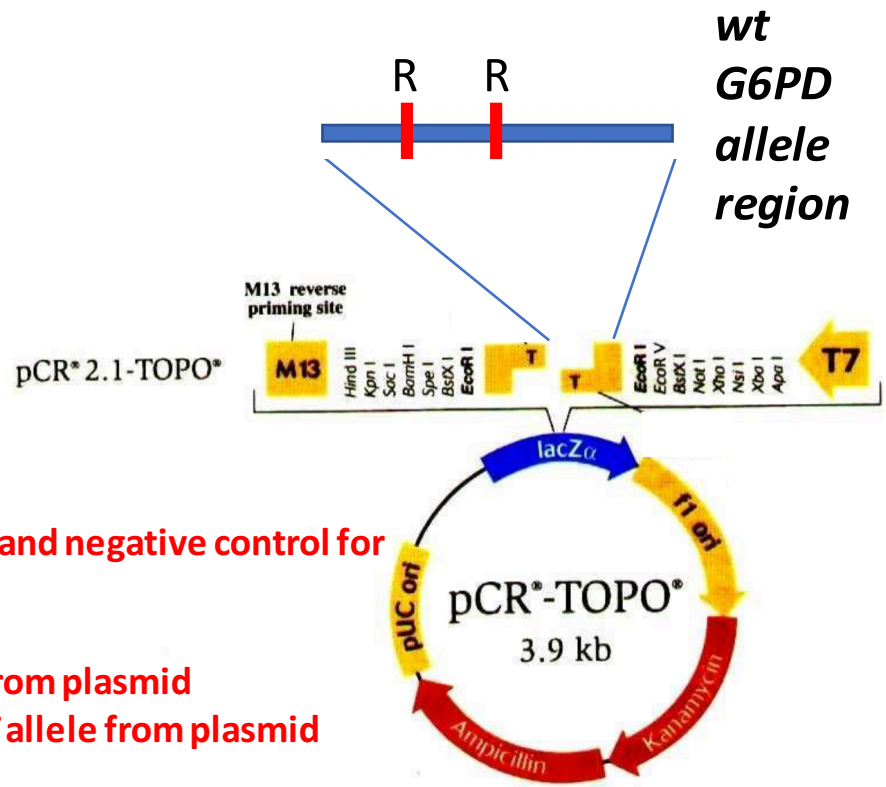
R: MboII



Control for PCR – MUST GIVE AMPLFICATION

DNA amplified from human DNA with wt G6PD (Taq) and cloned via TA-cloning into pCR-TOPOII;
G6PD 563C→T variation inserted (new MboII site)

→ **Make PCR-RFLP with oligos**
→ **Run gel**
→ **PCR gives band for mutant allele**



USE PLASMIDS as positve and negative control for G6PD allele status!!
PCR setup:
- Amplification wt allele from plasmid
- Amplifcation of 563C→T allele from plasmid
- DNA from idividuals