TRICKY CONCEPTS

BIOCHEMISTRY / FMT

BIOCHEMISTRY

A 24-month-old boy is brought to the physician for the evaluation of intellectual and behavioral abnormalities and abnormal movements of his extremities. His mother reports that he often hits his head and limbs against furniture. He is unable to walk without support and speaks in unclear 2-word phrases. Examination shows multiple bruises on the forehead and several healing wounds over the fingers. There is increased muscle tone in all extremities. Laboratory studies show an increased serum uric acid concentration. The serum concentration of which of the following substances is most likely to also be increased in this patient?

Lesch Nyhan

XLR

A) HGPRT
B) Phosphoribosyl pyrophosphate
C) Deoxyadenosine trisphosphate
D) Branched-chain amino acids

A 3-month-old infant presents with poor feeding, failure to thrive, and developmental delay. CBC reveals severe megaloblastic anemia not responding to B12 or folate supplementation. Urine shows markedly elevated orotic acid. Liver function tests are normal. Which enzyme is most likely deficient?

a. Ornithine transcarbamylase
b. UMP synthase
c. Dihydrofolate reductase
d. CPS-I

A neonate becomes lethargic and irritable on day 3 of life. Labs: hyperammonemia, respiratory alkalosis, elevated urinary orotic acid. CBC is normal. No improvement with uridine supplementation. What is the most likely diagnosis?

a. Hereditary orotic aciduria
b. OTC deficiency
c. Folate deficiency
d. B12 deficiency

↑NH_3 → ↑ Hyperventln.

Encephalopathy < hepatic, NH_3 ↑

asterixis

Glutamine

Krebs ↓↓

ATP ↓↓

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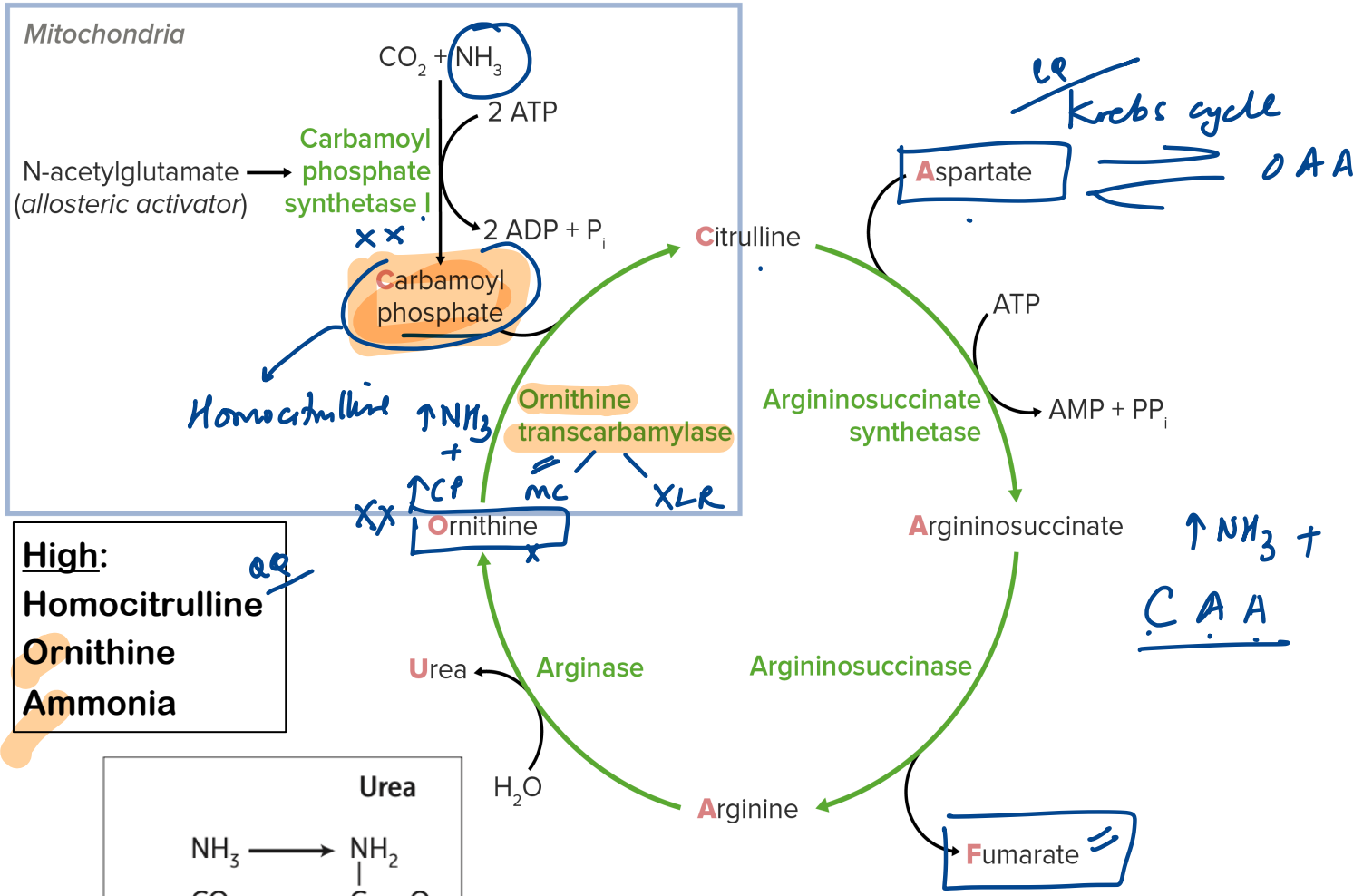
Urea Cycle & Defects



High:
Homocitrulline
Ornithine
Ammonia

Urea

$NH_3 \longrightarrow NH_2$

$CO_2 \longrightarrow C{=}O$ (|)

Aspartate $\longrightarrow NH_2$

CPS-1: Urea cycle- Mitochondria

CO_2 + NH3

CPS-2: Pyrimidine synthesis-Cytoplasm

CO_2 + Glutamine

Orotic Aciduria :

↑NH_3 — OTC def

megaloblastic A

U ← T

+ UMP synthase def

- **Sodium Benzoate** + Glycine → Hippuric acid
- **Phenylacetate, Phenylbutyrate** → Glutamine

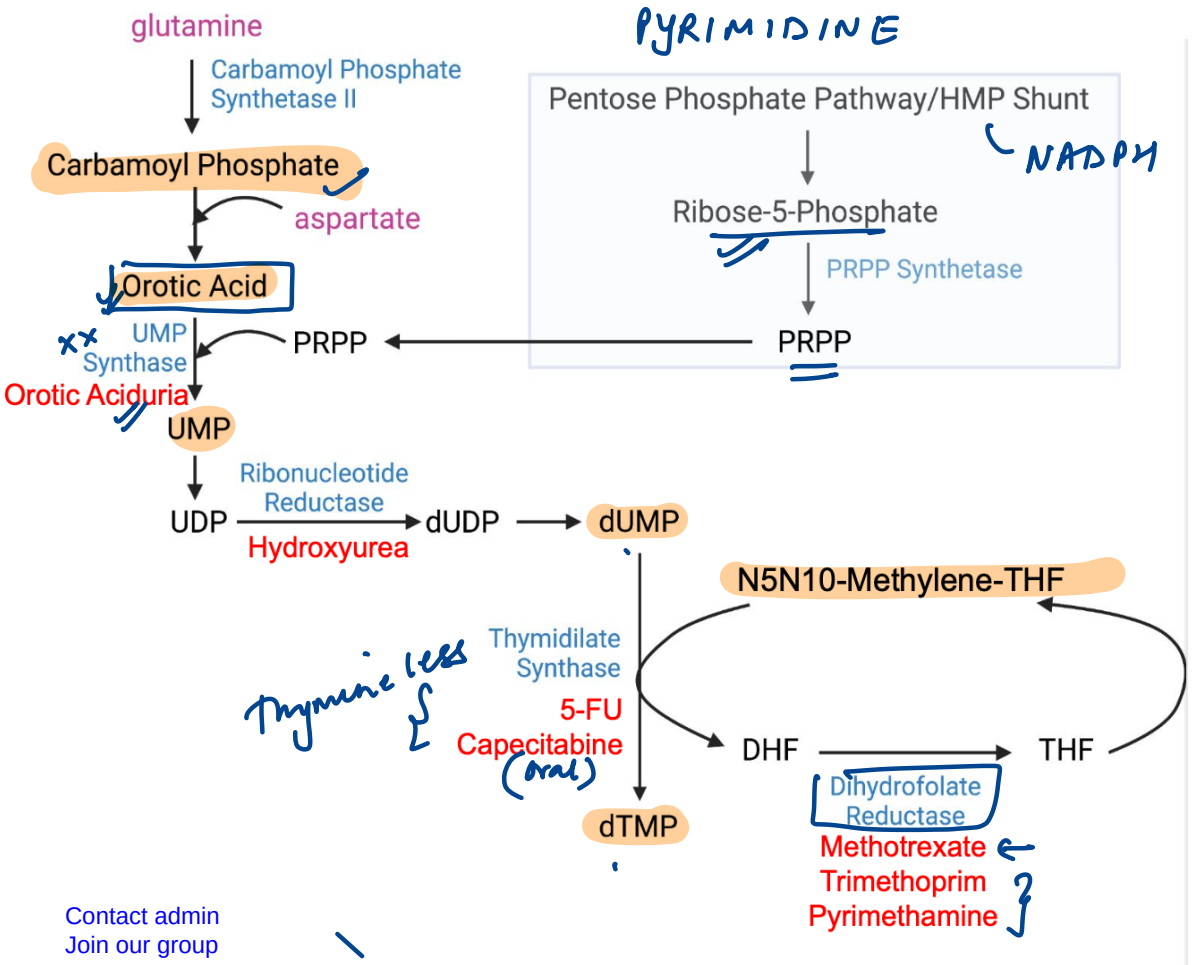
- **Main ammonia transporter in body:** Glutamine
- **Main ammonia transporter in muscle:** Alanine (Cahill)

Nucleic Acids: Purines & Pyrimidines

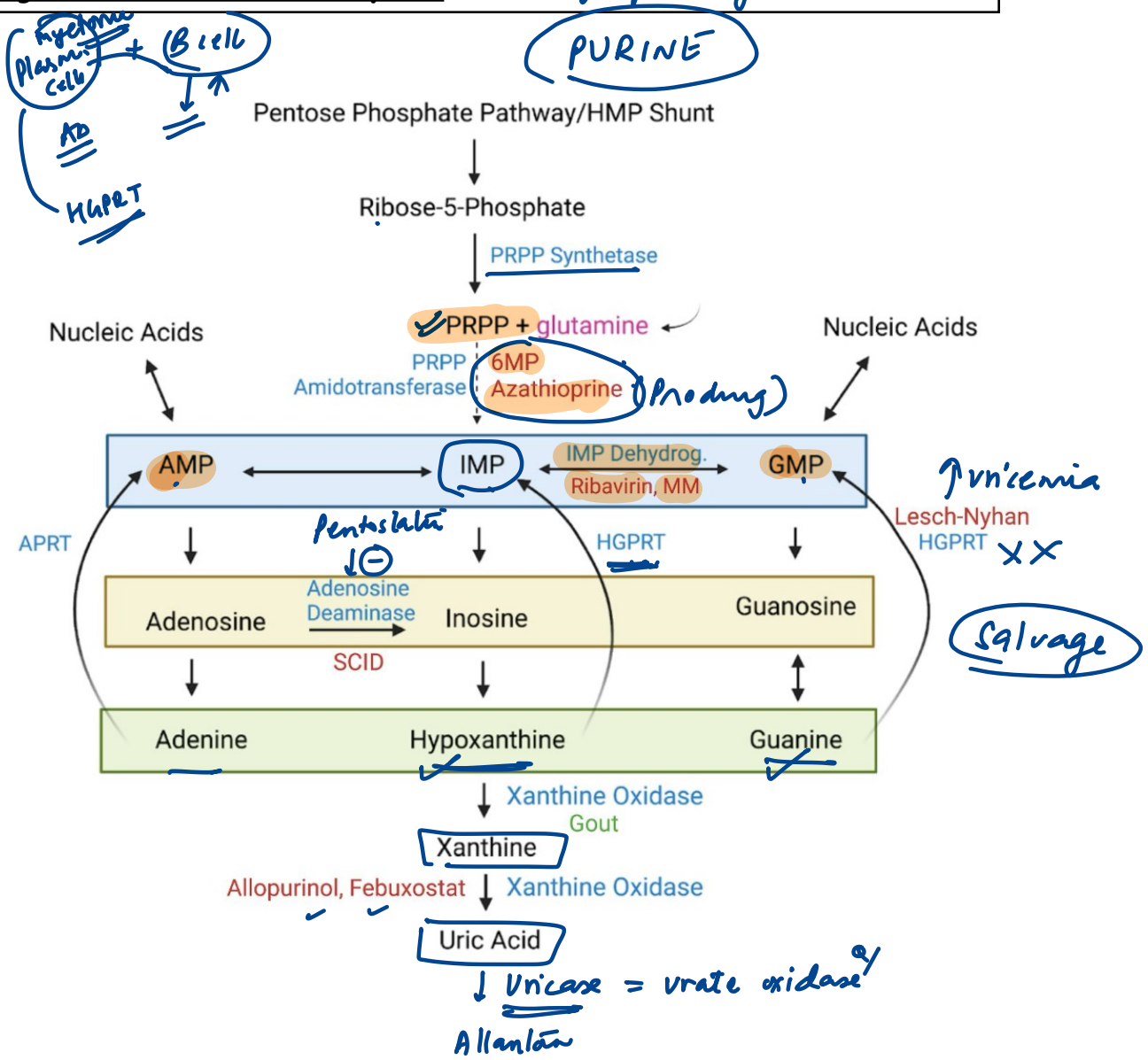
Glycine + Glutamine + Aspartate:

Glutamine + Aspartate:

Deamination:	**Methylation:**
C → U	U → T



Hybridoma technique:



The Klenow fragment retains which of the following enzymatic activities?

1. 5′→3′ polymerase activity ✓
2. 3′→5′ exonuclease activity ✓
3. 5′→3′ exonuclease activity ×
4. Primer removal during replication ×

1, 2

DNA Pol III 5′ → 3′ Pol

proof R · 3′ → 5′ exoN

primer removal 5′→3′ pol I exoN

5′→ 3′

· Polymerase
· DNA replic
· transcrip

× proofreading
× RNA editing

Which of the following statements is incorrect about histones?

a) Histones are synthesized during S phase of the cell cycle ✓
b) H1 histone does not contribute to formation of nucleosome ✓
c) Mitochondrial DNA has histone proteins ××
d) Histones are rich in arginine and lysine

most basic (+ve)

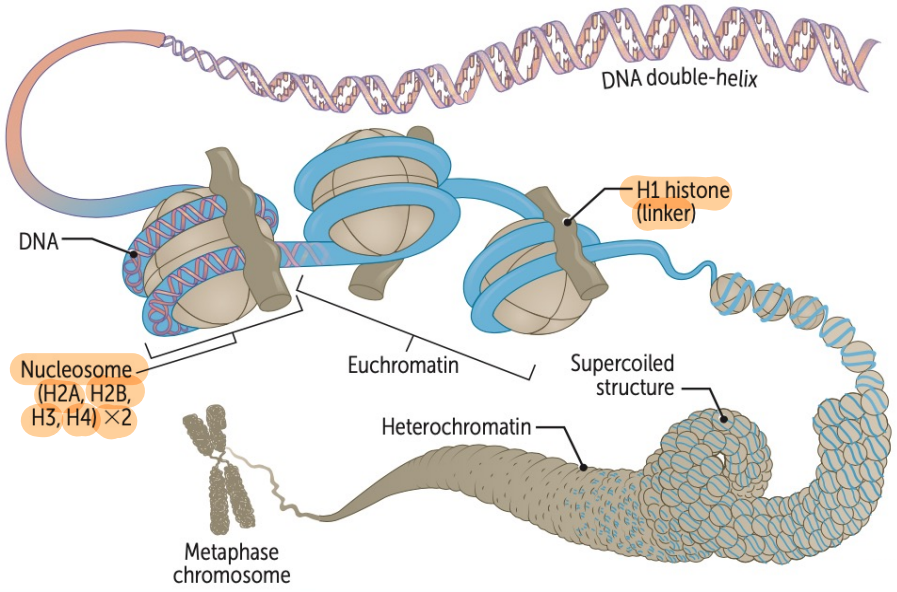
-ve DNA — Phosphodiester bond

ds DNA

Which of the following is not associated with post-transcriptional modification?

A) Methylation ✓ Cap
B) Endonuclease cleavage ✓
C) Adenylation ✓ tail
D) Glycosylation

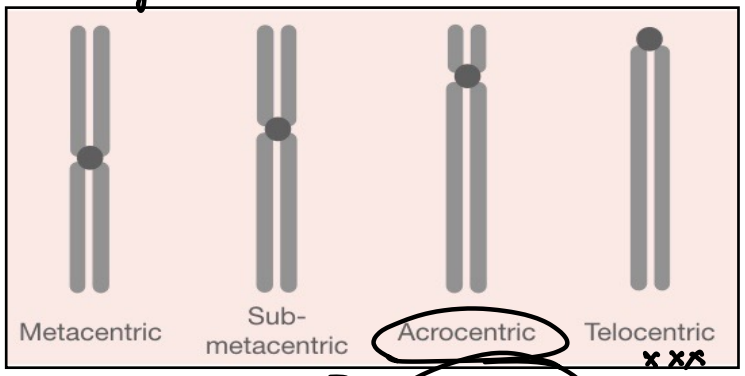
Basics of DNA



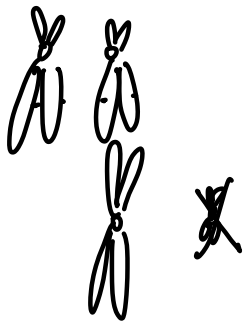
Euchromatin	Heterochromatin
Active / Light/ Loose	Inactive/ Tight/ Dark

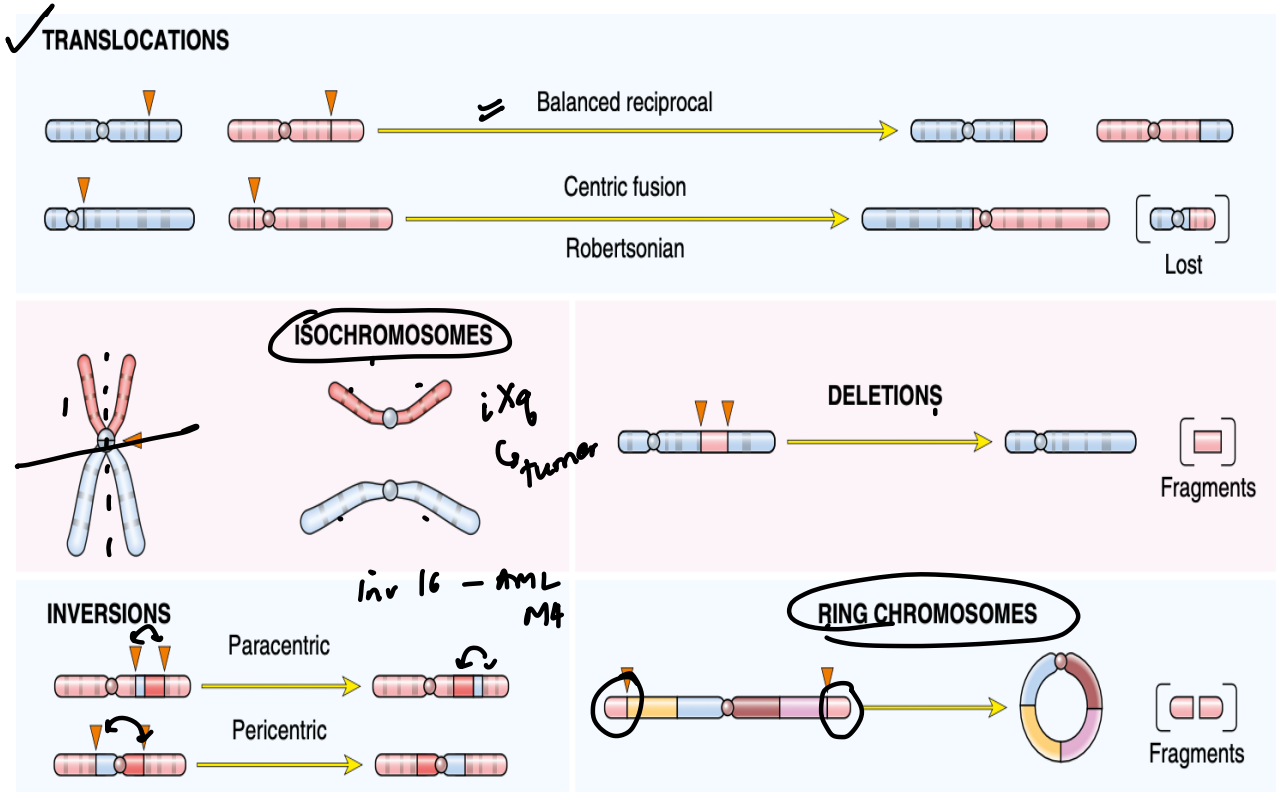
CARBS

arm region band subband

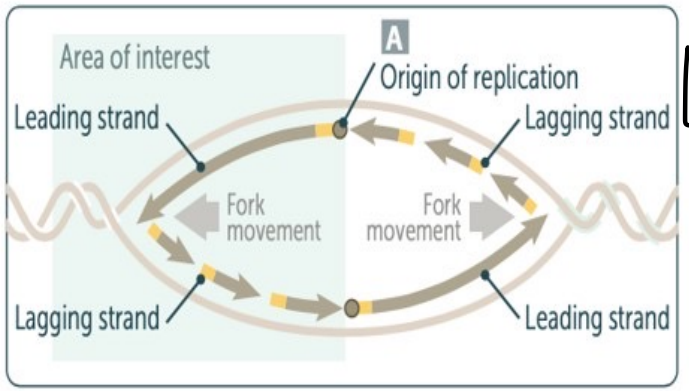


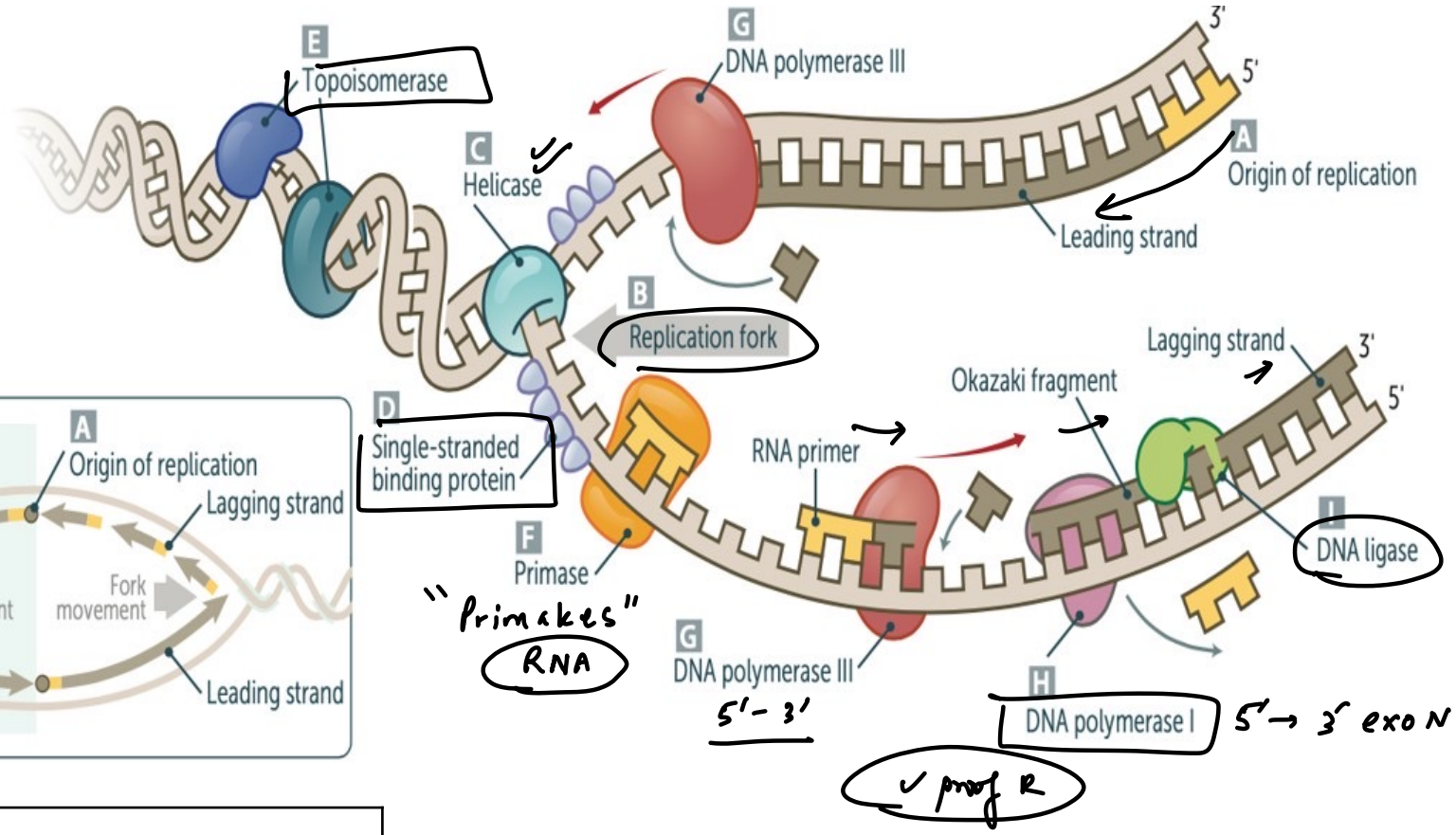
13, 14, 15, 21, 22, Y – Robertsonian

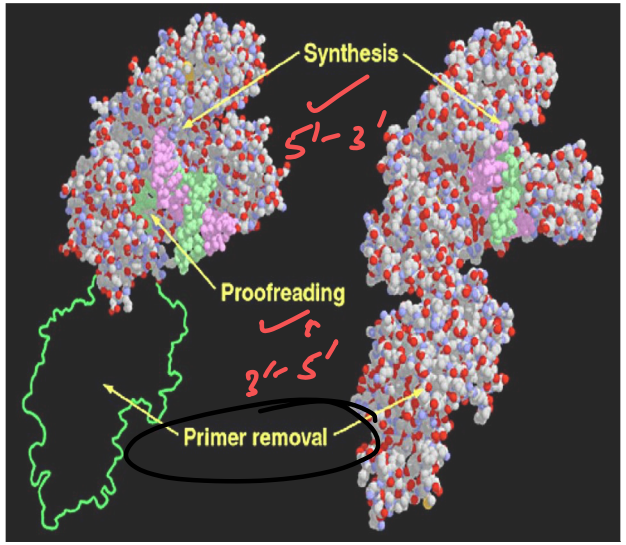




DNA Replication





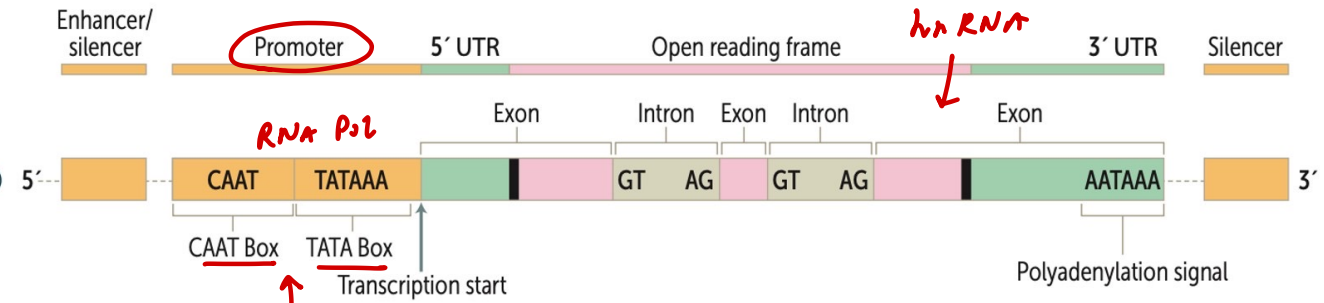


Pol α	Primase activity
Pol β	Repair process
Pol γ	Mitochondrial DNA synthesis
Pol δ	Lagging strand synthesis [Okazaki fragment]
Pol ε	Leading strand

Germ cells: Telomerase — Not Ribozyme
- TTAGGG ✓
- RNA dep DNA polymerase
- Hayflick limit: Somatic cells (30-50)

Ribozyme
- Peptidyl transferase ✓
- snRNA ✓
- Ribonuclease P ✓

Transcription



Eukaryotes

- RNA polymerase I: Makes rRNA
- RNA polymerase II: Makes mRNA, microRNA, snRNA

Amanita phalloides

- RNA polymerase III: Makes 5S rRNA, tRNA
- Prokaryotes: RNA polymerase- *Rifampicin*

Q. The base sequence of the strand of DNA used as the template for transcription has the base sequence GATCTAC. What is the base sequence of RNA product?

A. CUAGAUG

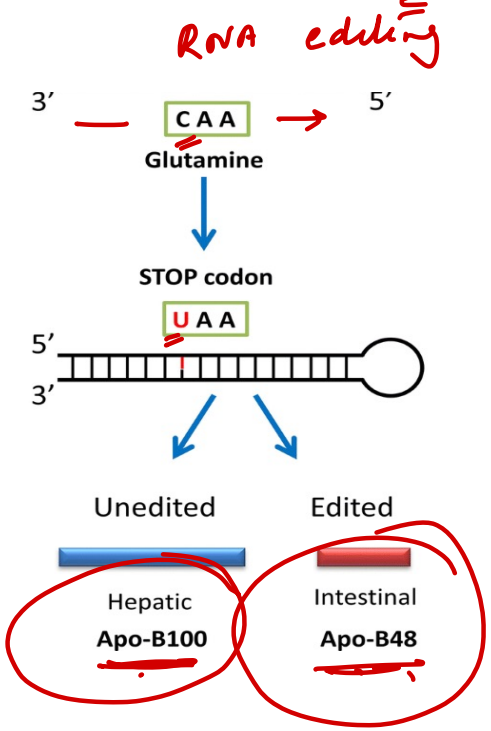
B. GTAGATC

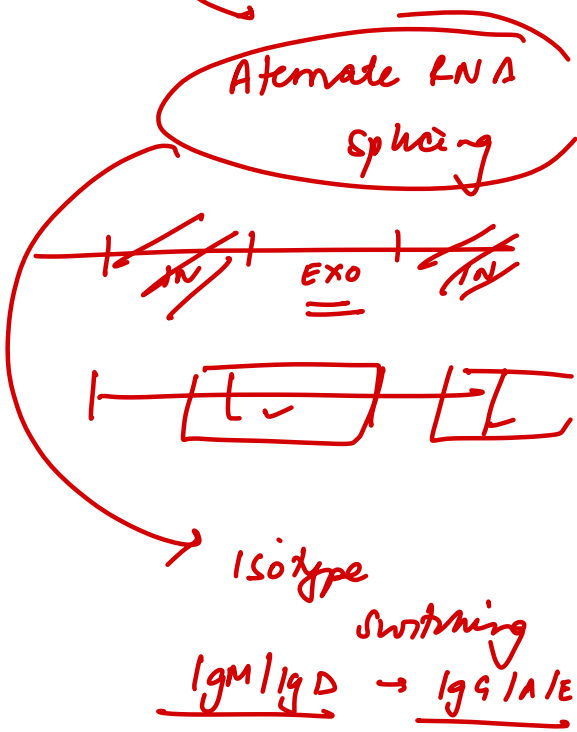
C. GTAGATC

D. GUAGAUC

Post-transcriptional changes

- 7 methyl-Guanosine Cap at 5' end (Not in tRNA/rRNA)
- Polyadenylation (200) at 3' end (Not in histone)
- Splicing out of introns (by snRNA)





A researcher introduces a human gene into a model organism to study its expression pattern. Using Northern blot analysis, he observes that in the brain tissue sample, there are four distinct bands. However, in the heart, kidney, and liver samples, he sees only a single band. Which of the following explanations could account for these findings?

A. RNA methylation – DNA - mutes xx
B. The brain has four different genes encoding this protein
C. Protein folding variations xx
D. Alternative splicing ✓

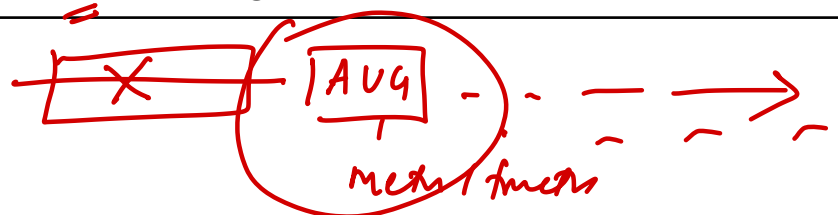
Which of the following would have no effect on the function of the protein product?

A) Glutamine replaced by asparagine (conservative)
B) Glutamine replaced by alanine
C) Glutamine replaced by glutamate
D) Glutamine replaced by arginine

Glu → Val non conservative

A researcher mutates the Shine-Dalgarno sequence in a bacterial mRNA. Which of the following steps in translation will be most directly impaired? (Prokaryotes)

a. Binding of aminoacyl-tRNA to the A-site
b. Alignment of the mRNA with the 30S ribosomal subunit ✓
c. Peptidyl transferase activity of the 50S subunit
d. Binding of EF-G during translocation



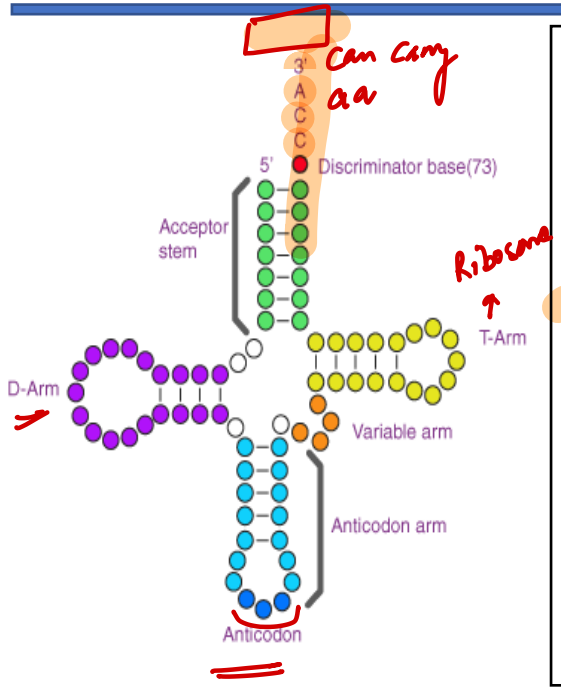
Arrange the following steps of base excision repair in order:

1. Endonuclease excises the sugar backbone
2. Glycosylase removes the altered base
3. DNA-polymerase adds a nucleotide
4. Creation of apurinic/apyrimidinic site

A) 1 → 2 → 3 → 4
B) 2 → 1 → 4 → 3
C) 1 → 2 → 4 → 3
D) 2 → 4 → 1 → 3

2-4-1-3

Translation



Amino-acyl tRNA synthetase-Proofreads and charges AA

Initiation

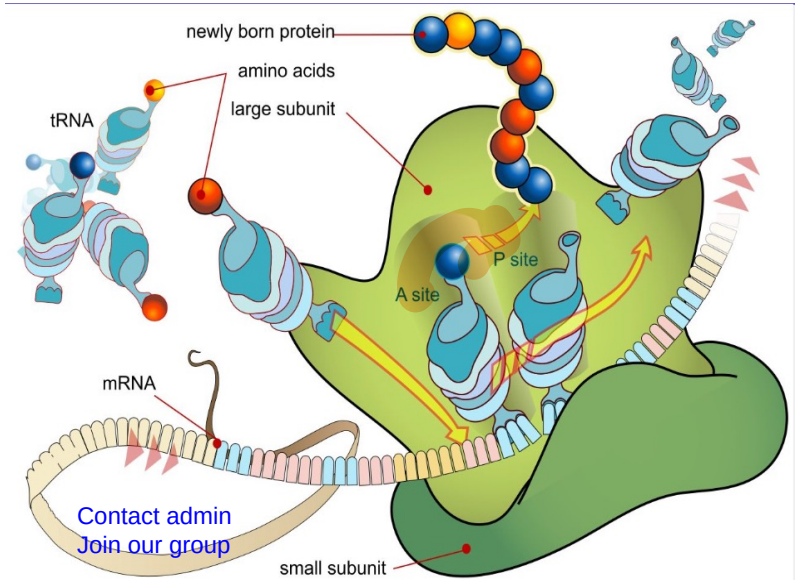
- Initiation factors bind to: **Shine–Dalgarno sequence** (P) / **Kozak sequence** (E)
- AUG start codon codes for **f-Met** (P) / **Met** (E)

Elongation

- **Aminoacyl-tRNA enters the A-site** using 2 **GTP**
- **Peptidyl transferase (rRNA)** catalyzes the **peptide bond formation**
- **Ribosome translocates** along mRNA → polypeptide shifts from **A → P site**
- **Empty tRNA exits** from the **E site**

Termination

- **Stop codons** are recognized by **release factors (RFs)**



Supressor tRNA mutation:

MUTATIONS AND DNA REPAIR

Point mutations: UCA (serine)

Wobble silent → UCU(serine)

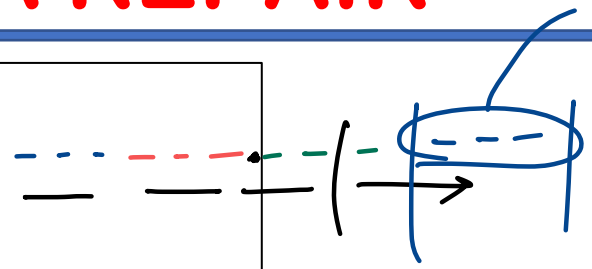
missense → CCA(proline)

nonsense → UAA(stop)

Mis-sense mutation: Sickle cell anemia (Glu → Val)

Frameshift mutations: DMD, Tay-Sachs

Splice site mutations: B-thalassemia, Marfan, Gaucher



not in multiple of 3

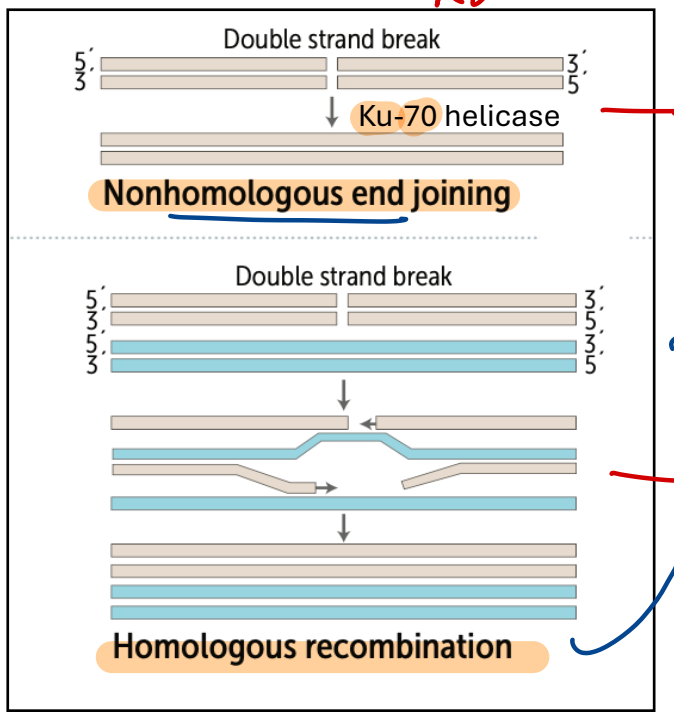
SS DNA

x → XP / Cockayne / trichothiod → Nucleotide excision

→ MUTYH ass. polyposis → Base excision

→ HNPCC / MSI → Mismatch repair

ds DNA → Rt

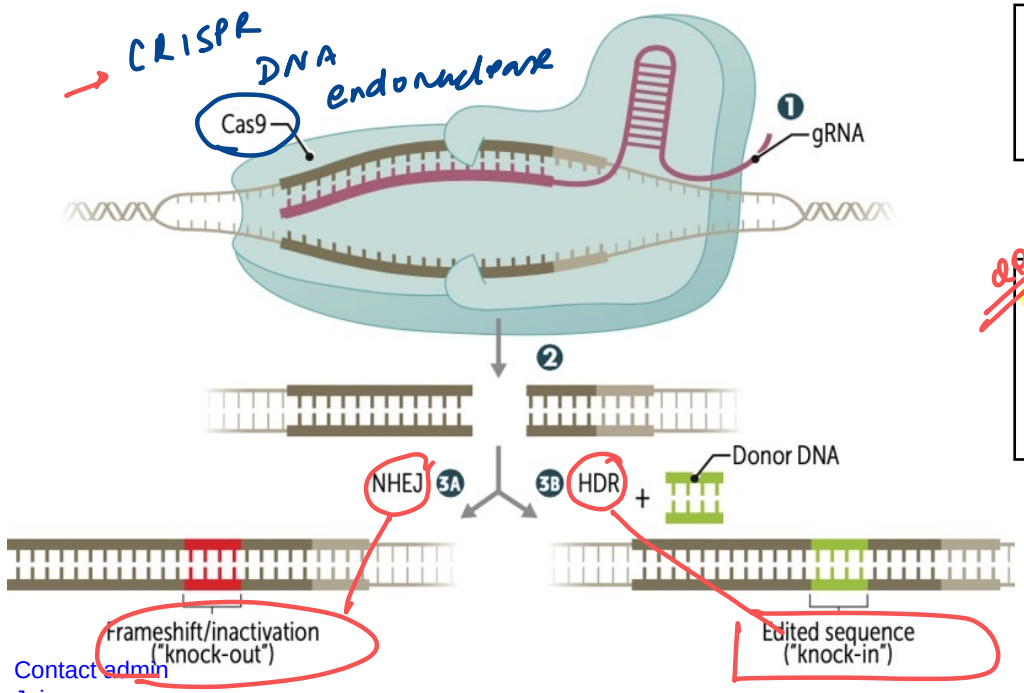


- SCID
- ATM
- Bloom
- Bloom
- BRCA
- Fanconi

GENETIC MODIFICATION

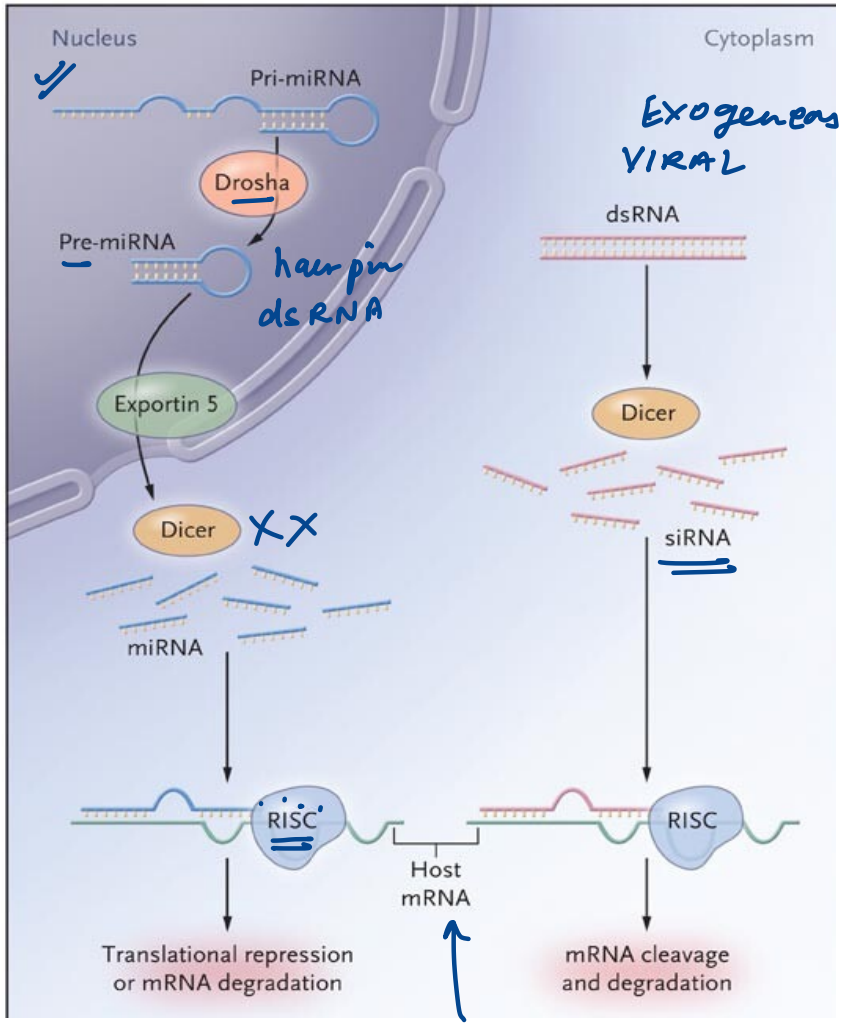
Gene Knock IN
Gene Knock OUT
Gene Knock DOWN = RNA Interference/ PTGS (si/mi RNA)

- 3'UTR
- 20-25 bp
- DICER-1: Pinealoblastoma / WT/ Pleuro-pulmonary blastoma/ Sertoli Leydig/ Multinodular Goitre



Charpentier used the CRISPR technique for gene editing: 2020 Nobel prize

CAR = Chimeric Antigen Receptor
It is an artificial receptor added to patient's T cells



A 12-year-old patient needs to get a molecular diagnosis of sickle cell anemia as three of his maternal cousins are affected. Arrange the steps in the correct sequence.

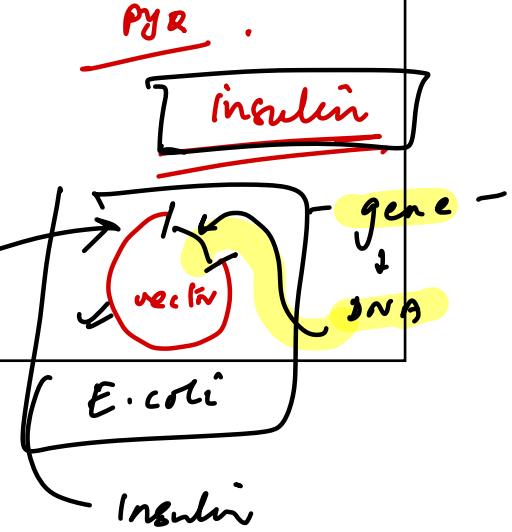
1.RT-PCR
2.Sample collection
3.FISH
4.RFLP
5.Cytogenetics
6.Conventional PCR
7.DNA extraction

2 - 7 - 6 - 4

Enzymes used in recombinant DNA technology are:

Multiple correct

1.Isomerase
2.Phosphatase
3.Terminal transferase
4.CRISPR-Cas9



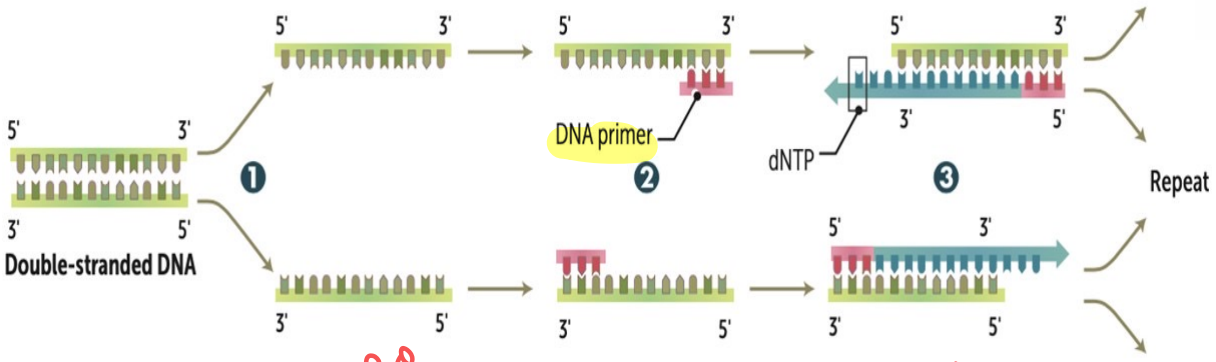
Which of the following is used in diagnosing **aneuploidy**?

Multiple correct

1, 2

1.FISH
2.Cytogenetics
3.Sanger sequencing
4.PCR

PCR



1. Denaturation- 95°C
2. Annealing- 55°C
3. Elongation- 72° C

No of PCR cycles: 2^n

½ cycles (26) = 25

DNA template, DNA primers, Heat stable DNA polymerase (Taq), Deoxynucleotide triphosphates (dNTPs)

flanking sequence

dideoxy NTP

Types of PCR:

- Real-time PCR-Quantitative-Ct value — qPCR, SYBR dye, cycle threshold, viral load, Ph chr
- RT PCR (Tth polymerase) — RNA → DNA — COVID-19 — RT → Real time
- Digital droplet PCR — L level sensitivity / Liquid biopsy — blood — ct DNA, circulating tumor DNA
- Multiplex PCR — meningitis — multiple primer
- Nested PCR — TB — 2 rounds = 2 sets of primers — ↑ specificity

DNA Sequencing:-

- Sanger's — Gold std
- 3.2 billion bp/ 19.5k genes / Only 1.5% exons
- NGS: Whole Genome Sequencing (WGS) → faster / ↑ economical

Chromosome walking

- Gene-mapping to identify unknown DNA sequences that lie adjacent to a known sequence
- "walks" step-by-step along the chromosome

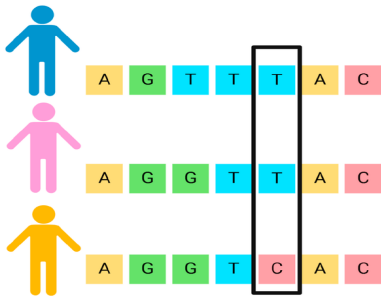
MOLECULAR BIOLOGY TECHNIQUES

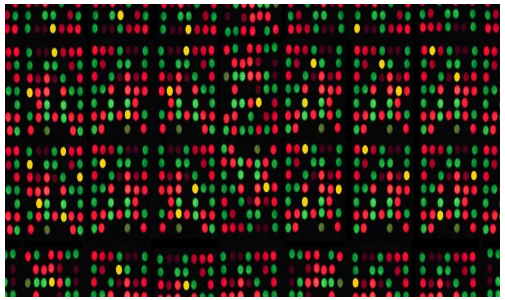
cancers/genetic linkage /disease

DNA Microarray: Identify <u>single nucleotide polymorphisms (SNP)</u>
Small glass slide with short DNA probes attached
Sample DNA binds to complementary microarray DNA probes

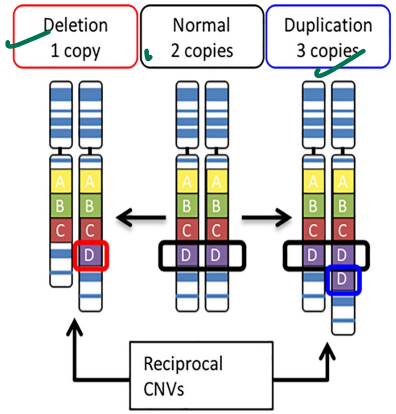
✓ pt mutns ✓ CNV

GWAS:
Microarrays on large population
Assess correlation between SNPs and complex disease



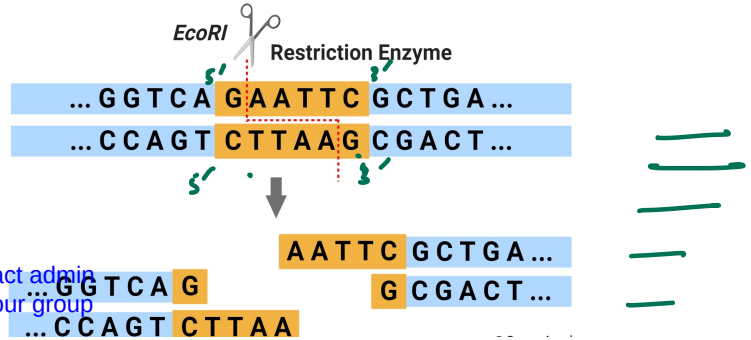


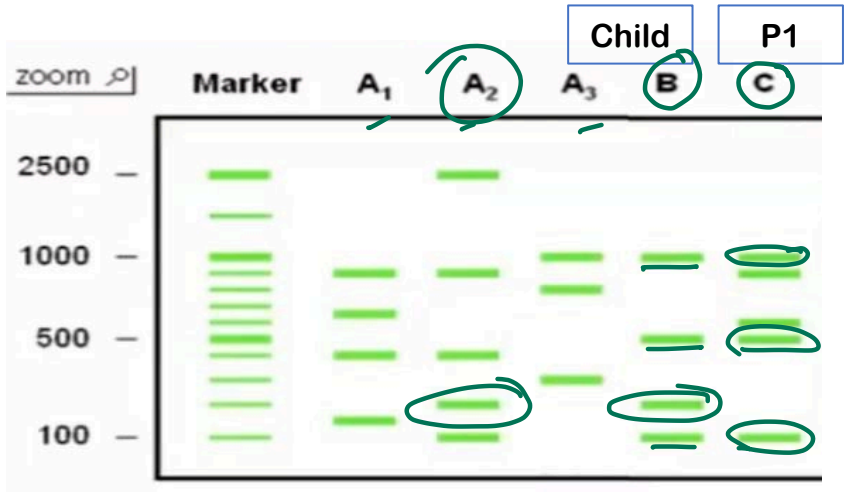
Multiplex Ligation-dependent Probe Amplification (MLPA)
CNV: Changes in the number of copies of a particular gene or genomic region: Deletion/ duplication/ amplification



RFLP: Mutation affecting palindromic sites
Paternal disputes, Crime, Genome mapping

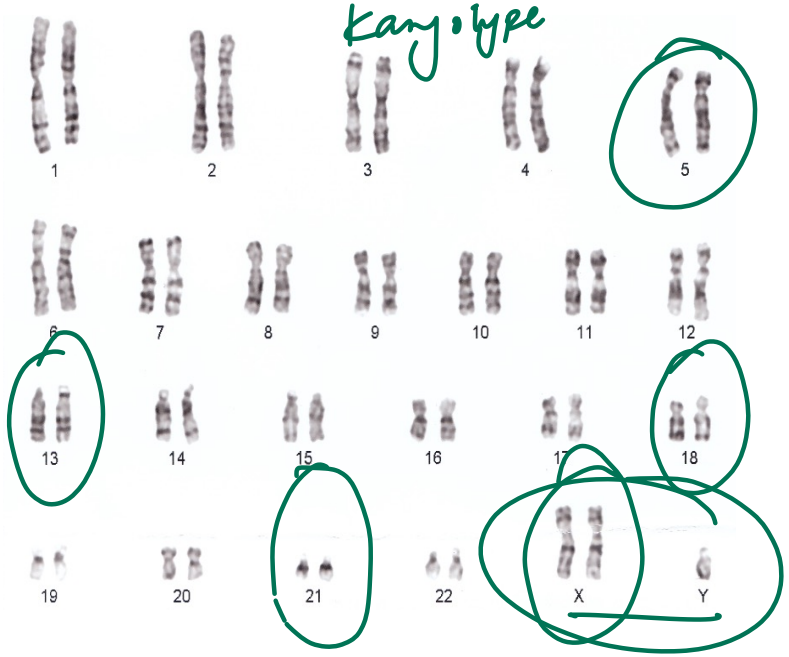
MADAM

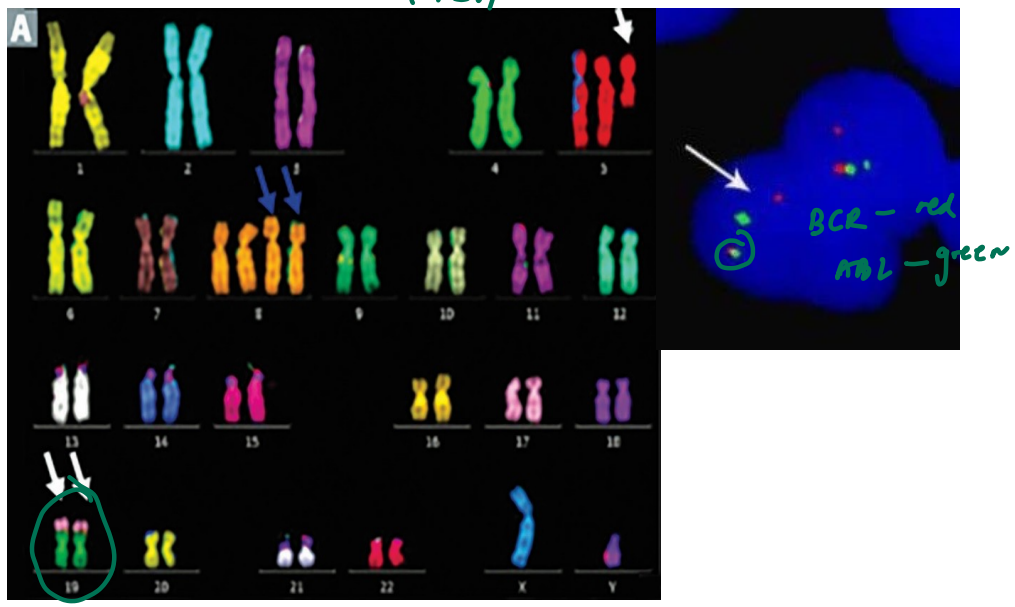




MOLECULAR BIOLOGY TECHNIQUES

Cytogenetics





- Best for ANEUPLOIDY
- Metaphase arrest — Colchicine
- Fixative: Carnoy
- Dicentric staining: C-staining
- G/R/T/C/Q stain — Quinacrine
  - Giemsa
  - Reverse
  - telomere
  - centromere

FISH

QQ

- **M**icrodeletion
- **A**mplification
- **T**ranslocation
- NOT point mutation — Microarray (if known)
  - Sequencing
  - RFLP
  - SSCP

BLOTTING

"SNOW DROP"

SOUTHERN- DNA

NORTHEN- RNA

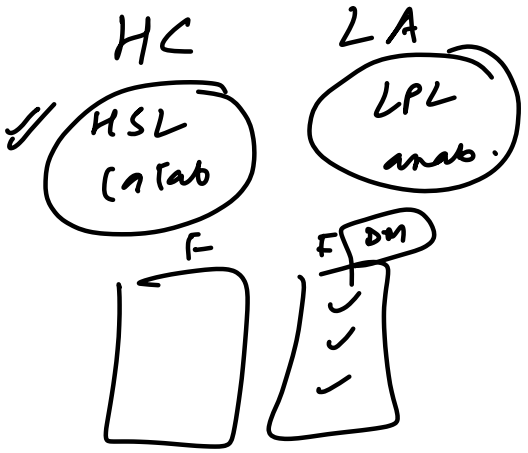
WESTERN- Prtn

SOUTHWESTERN/ DNA

footprinting — DNA binding prtn

A 56-year-old man suffering from uncontrolled diabetes mellitus came for a routine checkup. His lipid profile revealed increased TG (triacylglycerol) and VLDL (very low-density lipoprotein). What is the cause of this abnormal lipid profile?

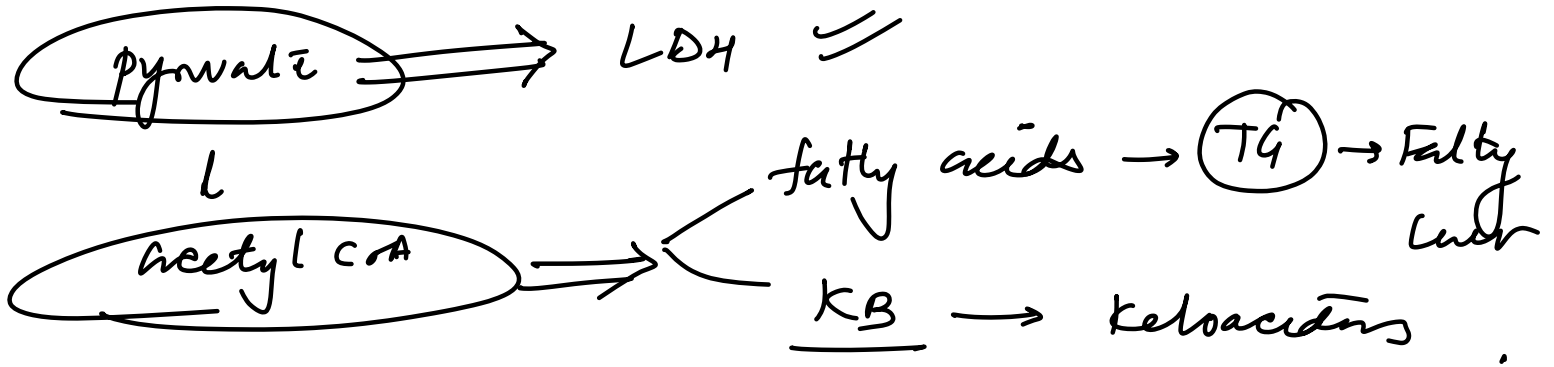
A) Increased activity of lipoprotein lipase and decreased activity of hormone sensitive lipase
B) Increased activity of hormone sensitive lipase and decreased lipoprotein lipase activity
C) Increase in peripheral function of LDL receptors
D) Increased in activity of hepatic lipase



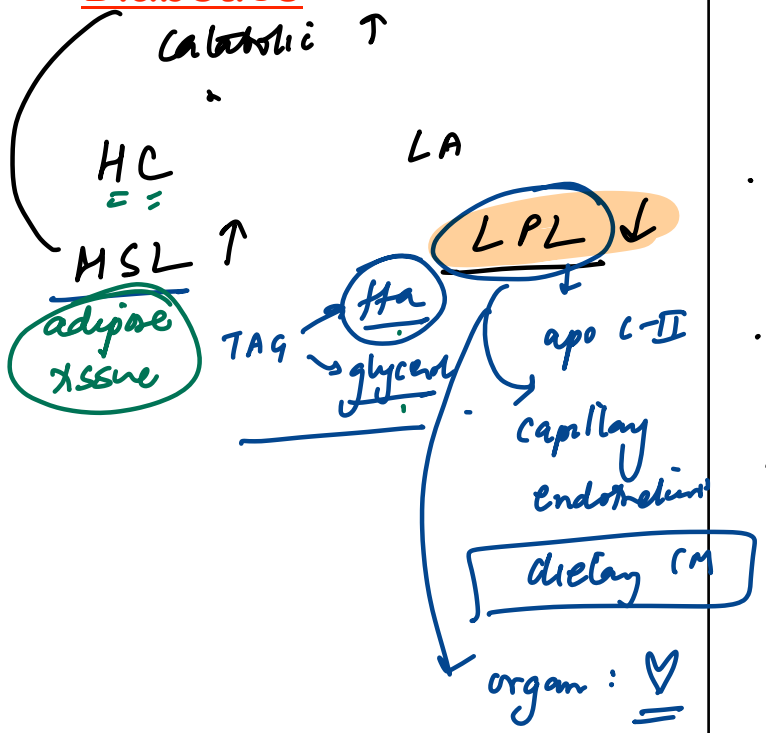
All of the following reactions may be inhibited in an alcoholic except

A) Gluconeogenesis ↓
B) TCA ↓
C) Lactic acid production
D) Fatty acid oxidation

↑NADH



Diabetics



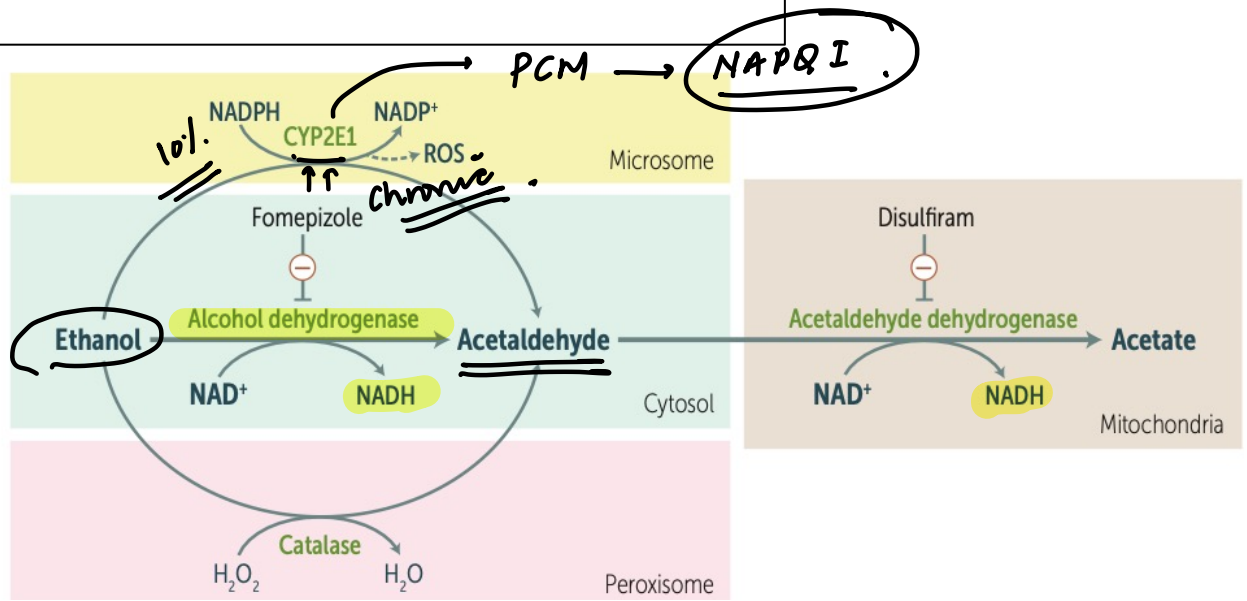
Alcoholics

↑ :

- Lactic acid (LDH)
- KB synthesis
- FA synthesis / TAG

↓ :

- Gluconeogenesis
- Krebs cycle
- FA oxidation



A 5-year-old boy rapidly develops hypoglycemia after moderate activity. Blood examination reveals raised levels of ketone bodies, lactic acid, and triglycerides. Glucagon challenge test was negative. Histopathology of the liver shows deposits of glycogen in an excess amount. What is the diagnosis?

A. Pompe's disease
B. McArdle's disease
C. von Gierke's disease
D. Cori's disease

Match the following disorders with their respective clinical features or lab findings.

Disorder	Clinical Feature or Lab Findings
1. Cori's disease	A. Branched structure in liver biopsy
2. McArdle's disease	B. Accumulation of glycogen in lysosome
3. Pompe's disease	C. Exercise intolerance
4. Von Gierke's disease	D. Hypoglycemia with lactic acidosis

1 - A
3 - B
2 - C
4 - D

Glc ↑ 50% in 10 min

Glucagon Challenge test –ve	Fasting hypoglycemia	Exercise intolerance	Neither
Von Gierke's = G-6-PO4ase absent	0	0	II
Cori – debranching	I	III	IV
Hers	III	V	
	VI	VII (PFK-1)	

Gluconeogenesis ×
Glycogenolysis ↑
18-48 hr
16-18 hr

Glycolysis
Hemolytic anemia
2 anerobic

N exercise / Von Gierke's
↓glc
↑lactate

McArdle's
↓glc
↓lactate

C D
Cori

G-6-P → P → L

Contact admin
Join our group

MPS

MPS: Heparan sulfate + Dermatan sulfate

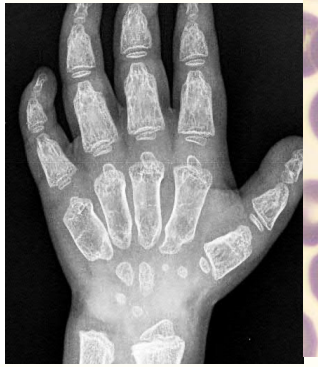
I du wanna Hunt!

No Corneal clouding – Hunter	Iduronate -2-suflatase	XLR
Corneal clouding Hurler	α-L-iduronidase (Partial defect- Sheie)	AR

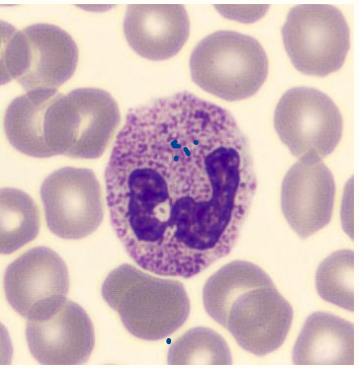
MC MPS: Sanfillipo

Hyaluronidase defect: Natowitz

Coarse facies



Bullet mc



Reiley granule

A 38-year-old man with a strong family history of premature coronary artery disease presents for evaluation. His fasting lipid profile shows:
High LDL
High VLDL
Normal HDL
He has eruptive xanthomas, and mild central obesity and impaired fasting glucose.
Which of the following is the MOST likely Fredrickson classification?
a. Type IIa Hyperlipoproteinemia
b. Type IIb Hyperlipoproteinemia
c. Type IV Hyperlipoproteinemia
d. Type V Hyperlipoproteinemia

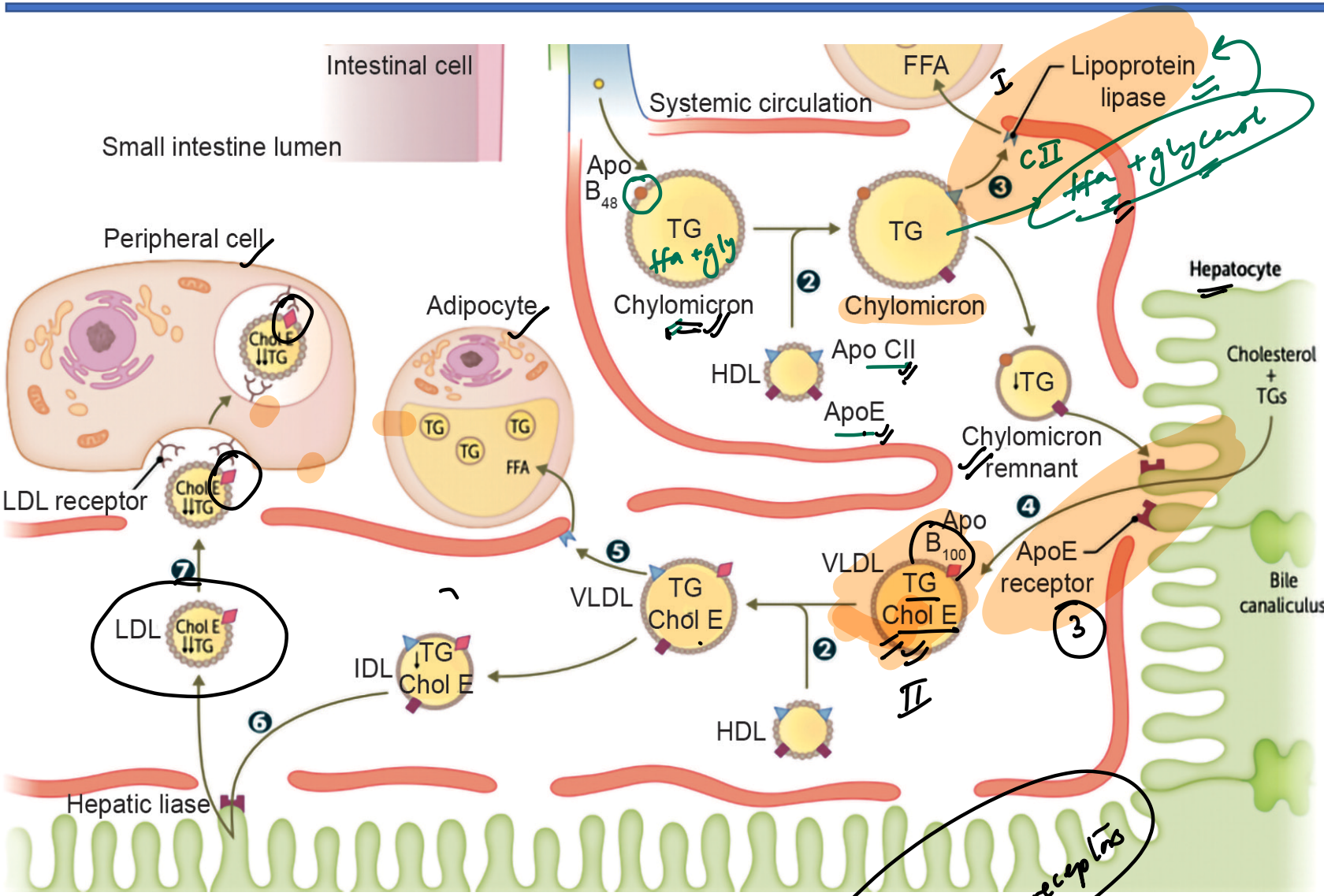
A 6-year-old child presents with recurrent episodes of severe abdominal pain, eruptive xanthomas, and creamy plasma that forms a milky supernatant on standing. Lipid panel shows:
•Triglycerides > 2000 mg/dL
•Massively elevated chylomicrons
•Normal LDL and VLDL
What is the most likely diagnosis?
a. Type IV Hyperlipoproteinemia
b. Type V Hyperlipoproteinemia
c. Type I Hyperlipoproteinemia
d. Type IIb Hyperlipoproteinemia

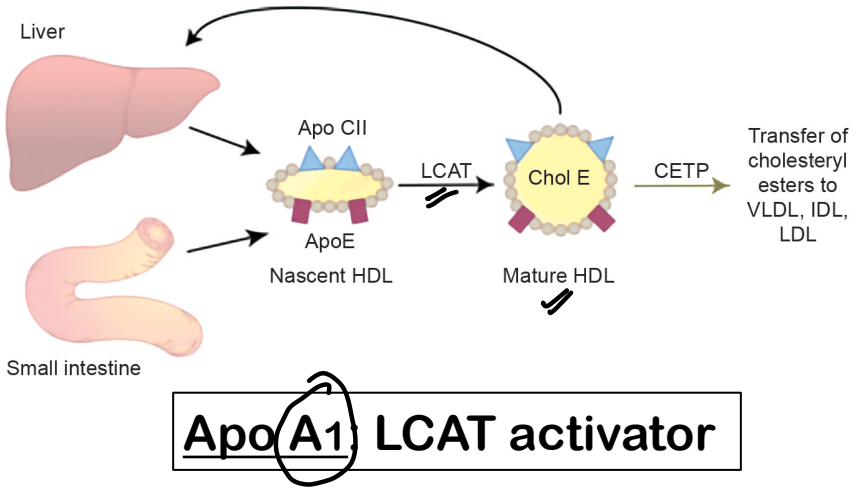
Hyperlipoproteinemias-Frederickson classification

Type	Inherit	Pathogenesis	↑ Blood level	Clinical
I- Hyper-chylomicronemia	AR	Lipoprotein Lipase / ApoC-II deficiency	Chylomicrons, TG, Cholesterol	▪ Pancreatitis ▪ Eruptive/ Pruritic Xanthomas ▪ CREAMY supernatant
II- Familial hyper cholesterolemia	AD	Absent LDL receptors/ ApoB-100	IIa: LDL IIb: LDL + TG	▪ Accelerated atherosclerosis ▪ Tendon Xanthomas ▪ Corneal arcus, Xanthalsema (IIb) ▪ Tubo-eruptive xanthomas (IIb)
III- Dysbeta-lipoproteinemia	AR	Defective ApoE	Chylomicrons, VLDL, LDL	▪ Premature atherosclerosis ▪ Tubero-Eruptive & Palmar Xanthoma ▪ Broad beta band
IV- Hyper-triglyceridemia	AD	↑↑ Hepatic VLDL	VLDL, TG Type 5: VLDL + chylomicrons	▪ Acute pancreatitis ▪ Insulin resistance ▪ Tubero-Eruptive Xanthoma

↑TG → pancreatitis ↑LDL → tendon.

Cholesterol Metabolism





Apo A1: LCAT activator

Apo A1 → LCAT
C-II → LPL
B-48 → chylomicron
B-100 → LDL
Apo E → remnant

Which of the following shows the correct sequence of collagen biosynthesis?

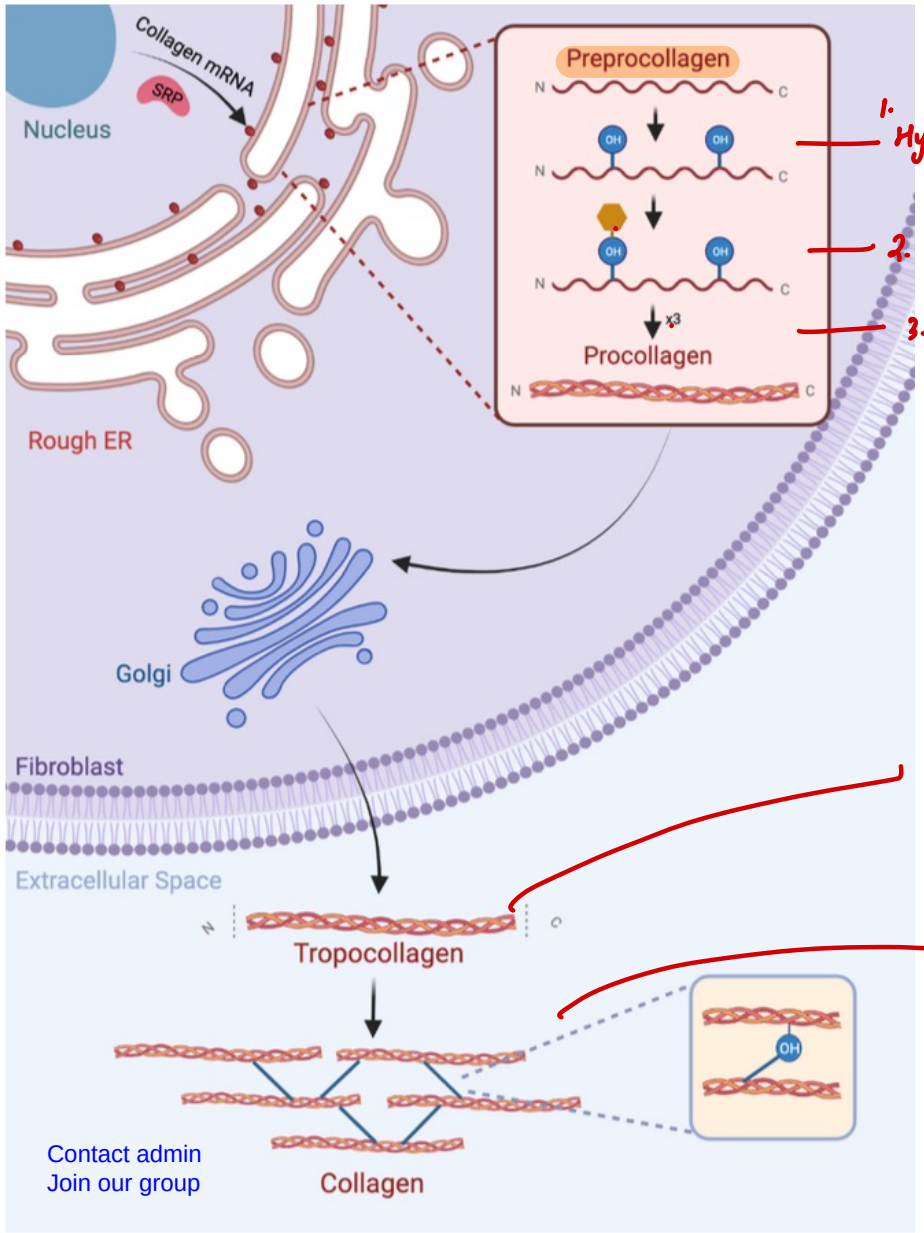
a. Hydroxylation → Glycosylation → Cross-linking → Triple helix

b. Glycosylation → Hydroxylation → Cleavage → Triple helix

c. Hydroxylation → Glycosylation → Triple helix → Cleavage → Cross-linking

d. Triple helix → Hydroxylation → Cleavage → Cross-linking

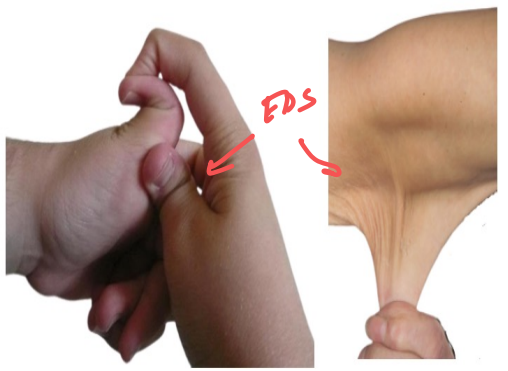
Collagen Synthesis & Disorders



Collagen chain: Gly-X-Y (OH lysine & OH proline)
Disrupter: PROLINE

1. Hydroxylation ← vit C
2. Glycosyl°
3. triple helix ← OI
(disulfide bonds)

4. cleavage of N- C- terminals.
← EDS



5. Crosslinking - lysyl oxidase (Cu)
← Menke's kinky hair

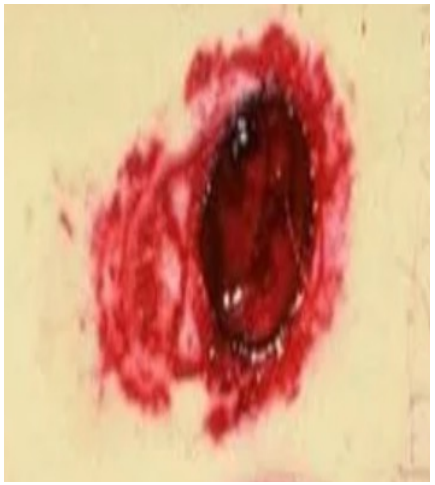
FMT

Identify the image shown below:

A) Contact range rifle
B) Close range rifle
C) Near range Shotgun
D) Intermediate range Rifle

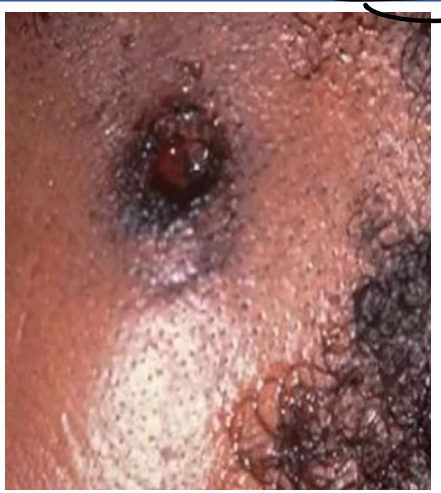
BALLISTIC RANGES

Rifle



Lacerated cherry red

Contact



BB T ✓✓

GC-AC ✓✓

Close

BB ××

T ✓✓ GC-AC ✓✓

Near/Int

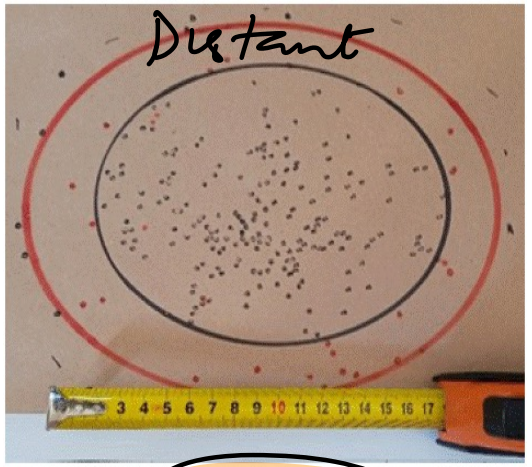
GC-AC ✓✓

Distant

SHOTGUN

Near

Int



Distant

"

"

< 1m

1-2m

2-4m

>4m

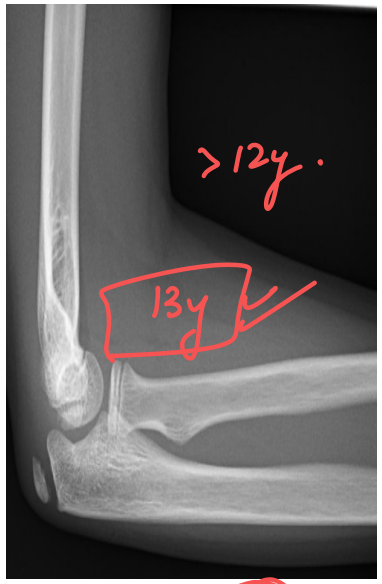
What is the approximate age of a person in whom the medial end of the clavicle is not fused and inferiormost part of body of sternum is fused?

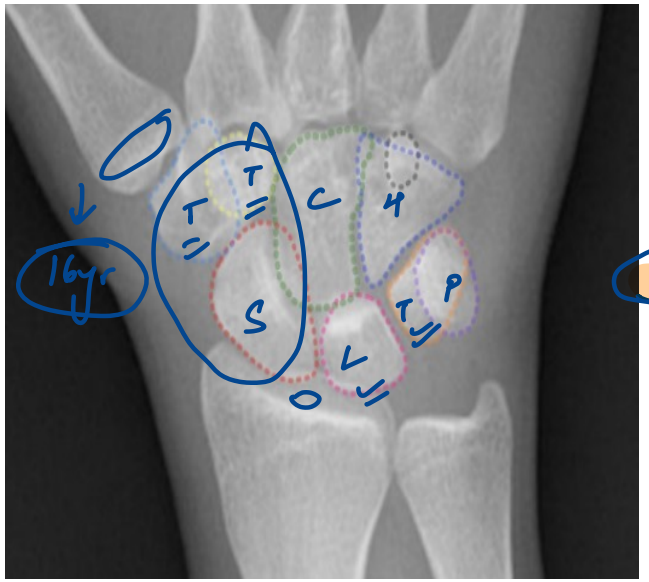
A) 18 yrs
B) 25 yrs
C) 10 yrs
D) 45 yrs

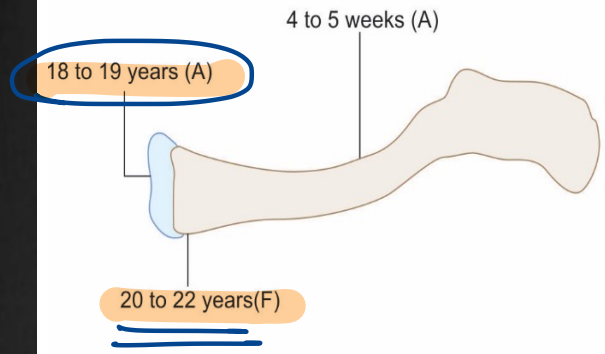
The following ossification centres are present before birth except

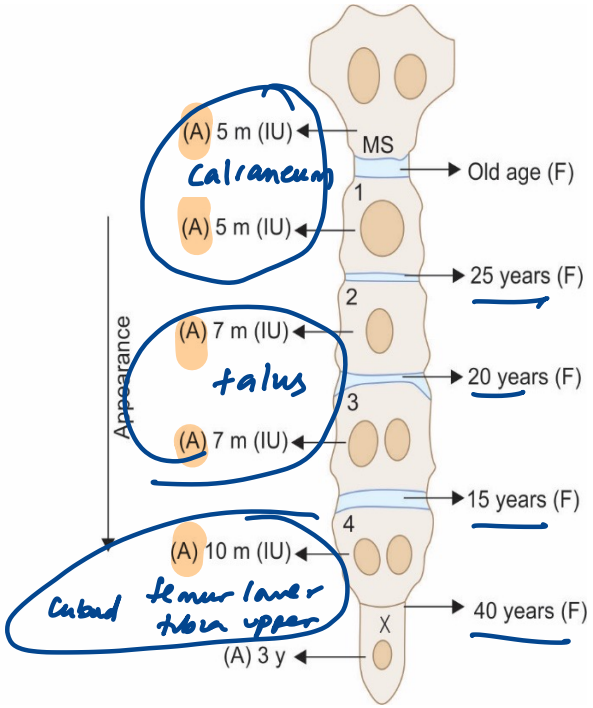
A. Talus
B. Calcaneum
C. Mandible
D. Head of femur

BONE AGE ESTIMATION









CRITOE

2 4 6 8 10 12y

L - 14

M - 15

O - 16

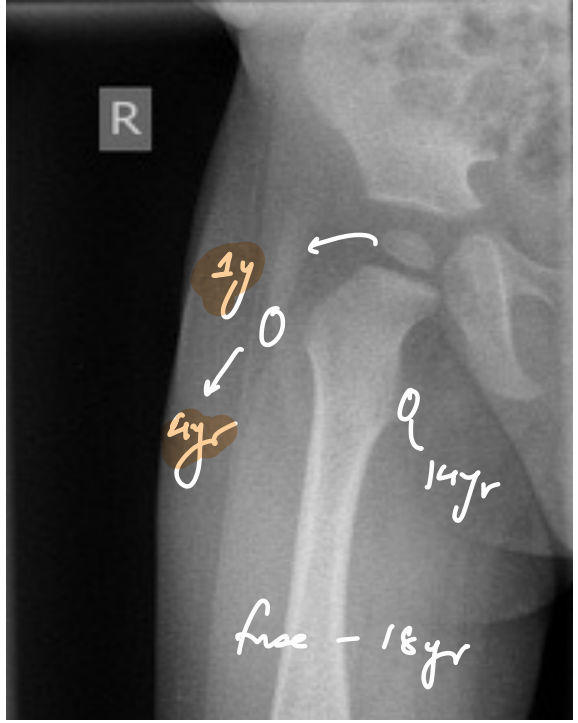
2 mon: C
3 mon: H
3yr: Triquetrum
4yr: L
5-6yr: S-T-T
9-12yr: Pisiform
Radius: 2y
Ulna: 5yr
Fuse: ~18yr

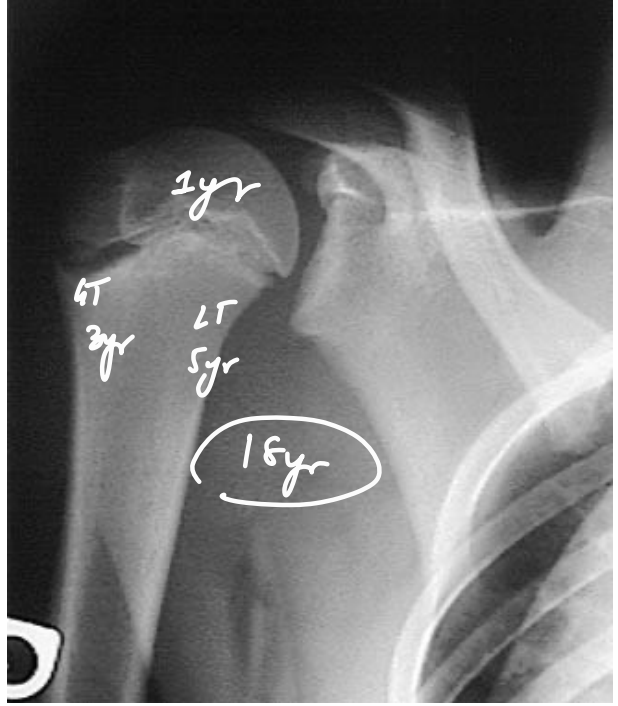
Fontanelle	Time of Fusion
Anterolateral (Sphenoidal) fontanelle	2 – 3 months
Posterior fontanelle	3 – 6 months
Posterolateral (Mastoid) fontanelle	12 months
Anterior fontanelle Metopic suture	1½ – 2 years

Lambdoid suture: 45yrs

12 - 16yr

3yr





DENTAL CHARTING

Haderup

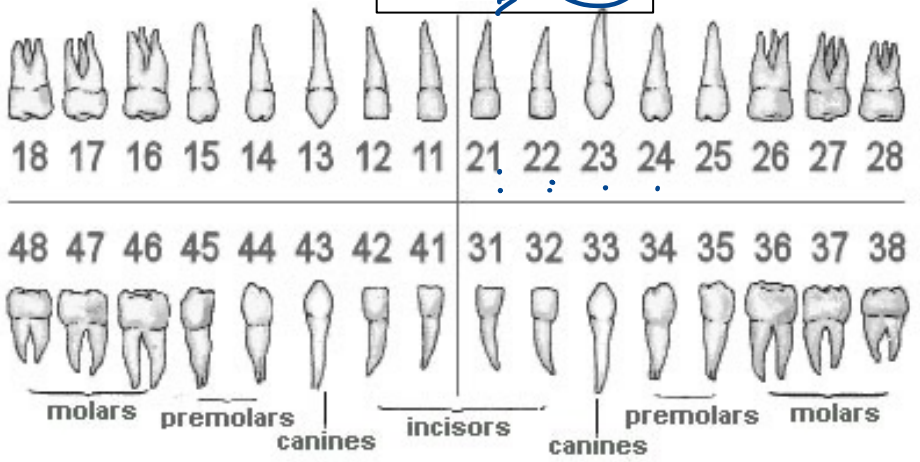
A. Deciduous (Temporary) Teeth

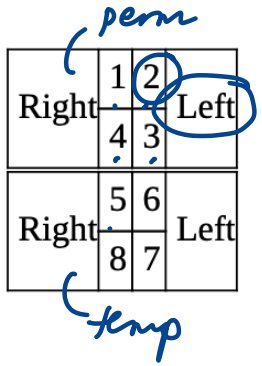
RIGHT	05+ 04+ 03+ 02+ 01+	+01 +02 +03 +04 +05	LEFT
	05- 04- 03- 02- 01-	-01 -02 -03 -04 -05	

B. Permanent Teeth

RIGHT	8+ 7+ 6+ 5+ 4+ 3+ 2+ 1+	+1 +2 +3 +4 +5 +6 +7 +8	LEFT
	8- 7- 6- 5- 4- 3- 2- 1-	-1 -2 -3 -4 -5 -6 -7 -8	

FDI





Palmer

A. Deciduous (Temporary) Teeth

RIGHT	E D C B A	A B C D E	LEFT
	E D C B A	A B C D E	

B. Permanent Teeth

RIGHT	8 7 6 5 4 3 2 1	1 2 3 4 5 6 7 8	LEFT
	8 7 6 5 4 3 2 1	1 2 3 4 5 6 7 8	

- Symbols Represents **Upper Teeth** from **Right and Left Quadrant** respectively. Example: A represents upper left first deciduous incisor.
- Symbols Represents **Lower Teeth** from **Right and Left Quadrant** respectively. Example: 1 represents lower right permanent canine.

Universal

A. Deciduous (Temporary) Teeth

RIGHT	A B C D E F G H I J	LEFT
	T S R Q P O N M L K	

B. Permanent Teeth

RIGHT	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16	LEFT
	32 31 30 29 28 27 26 25 24 23 22 21 20 19 18 17	

A 45-year-old male patient presents with symptoms of increased lacrimation, salivation, sweating, and urinary incontinence. Which of the following is likely to be responsible for the above symptoms?

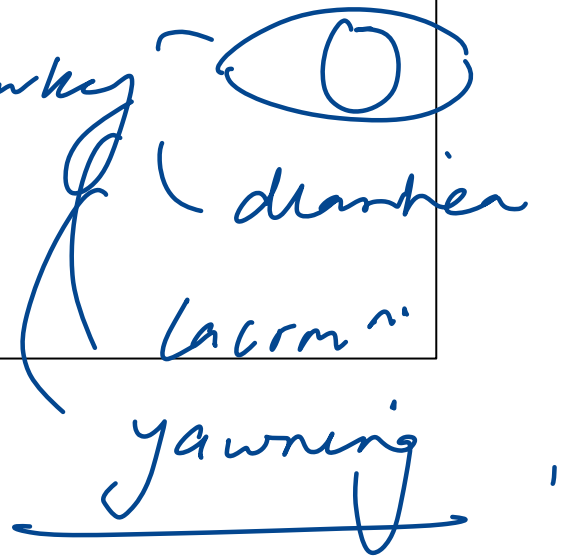
A. Arsenic
B. Strychnine
C. Pesticides
D. Datura

A 28-year-old male arrives in the emergency department after being exposed to an unidentified toxic substance given to him by a friend. His symptoms include dry mouth, urinary retention, and constipation. During the examination, his pulse rate is recorded at 140 beats per minute, and his pupils are observed to be dilated. What is the likely cause?

A) Heroin withdrawal
B) Organophosphate toxicity
C) Cocaine toxicity
D) Belladona toxicity

35-year-old man with a history of bipolar and substance use disorders comes to the emergency department due to depression, auditory hallucinations, and suicidal ideation. His medications include lithium and escitalopram. The patient has a history of 5 psychiatric hospitalizations and 2 past suicide attempts, including overdose on his medications and attempted hanging. The patient has been using "anything I can get my hands on" because his depression is unbearable. He is hospitalized and placed on suicide precautions. His dose of escitalopram is increased to target his depression, and risperidone is added to treat the hallucinations. His lithium level is 1.0 mEq/L. On the second day of hospitalization, the patient reports muscle pains, abdominal cramping, nausea, and diarrhea. His temperature is 37.2 C (99 F), blood pressure is 130/85 mm Hg, and pulse is 84/min. The patient is alert and restless, and his pupils are dilated bilaterally. Bowel sounds are hyperactive and neurologic examination is normal. Which of the following is the most likely explanation for his symptoms?

A. Serotonin syndrome
B. Cocaine withdrawal
C. Lithium toxicity
D. Opioid withdrawal



TOXICOLOGY MASTER-CHART