Molecular Methods in Urology and Nephrology

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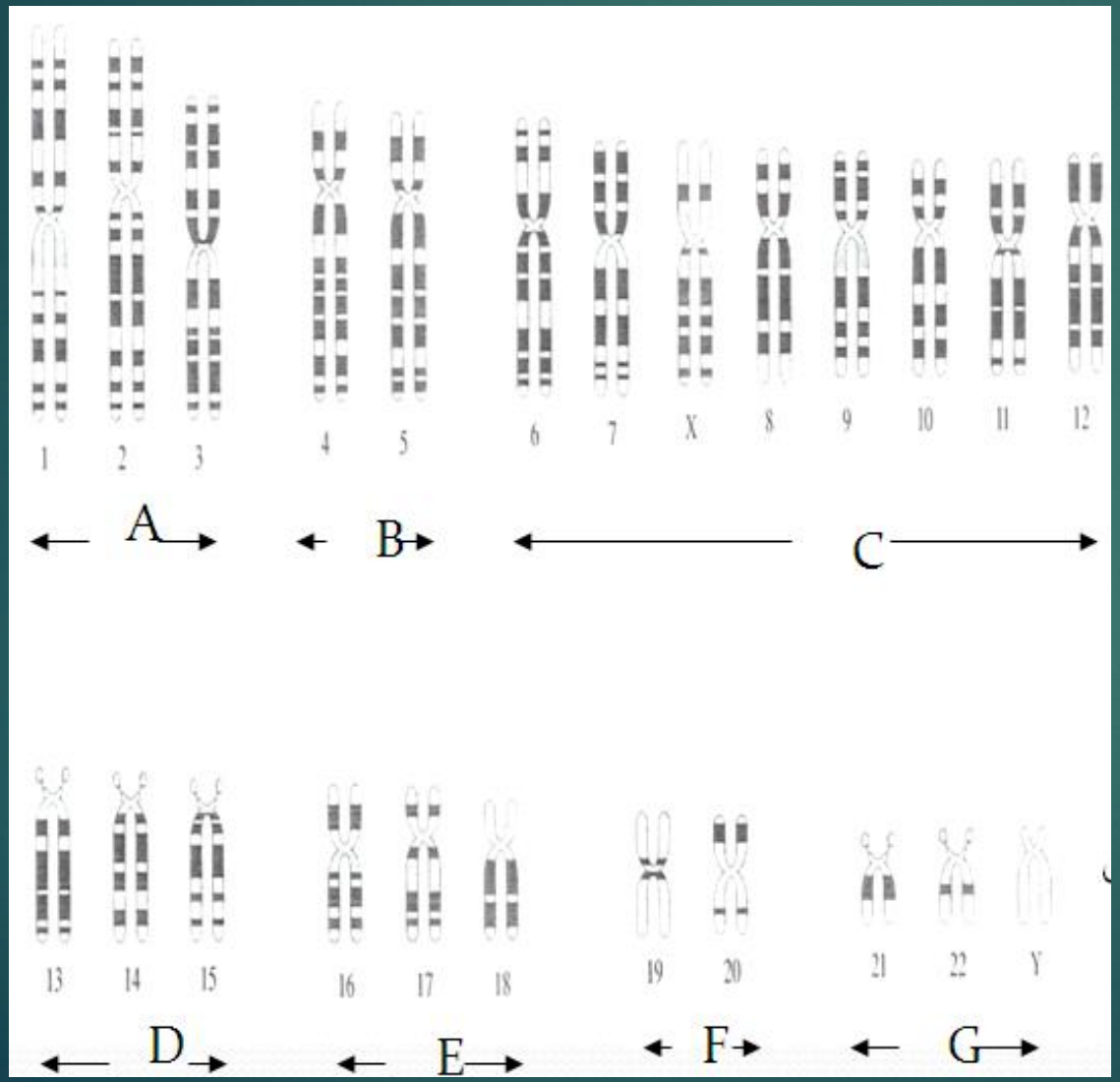
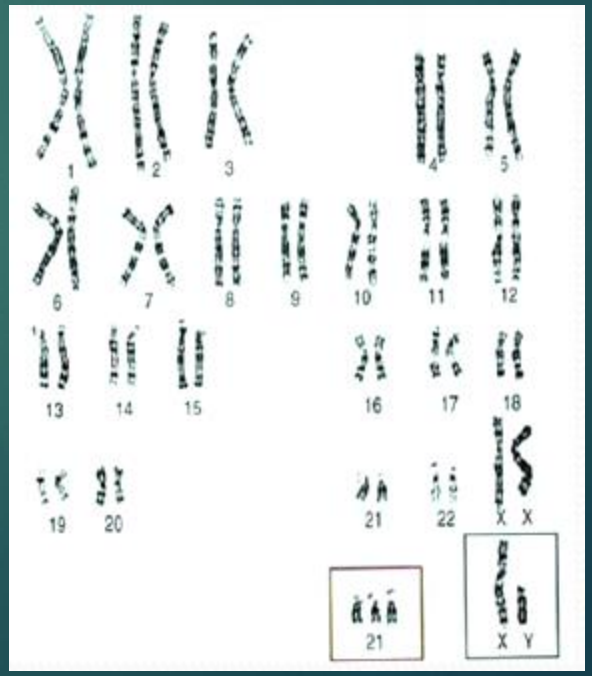
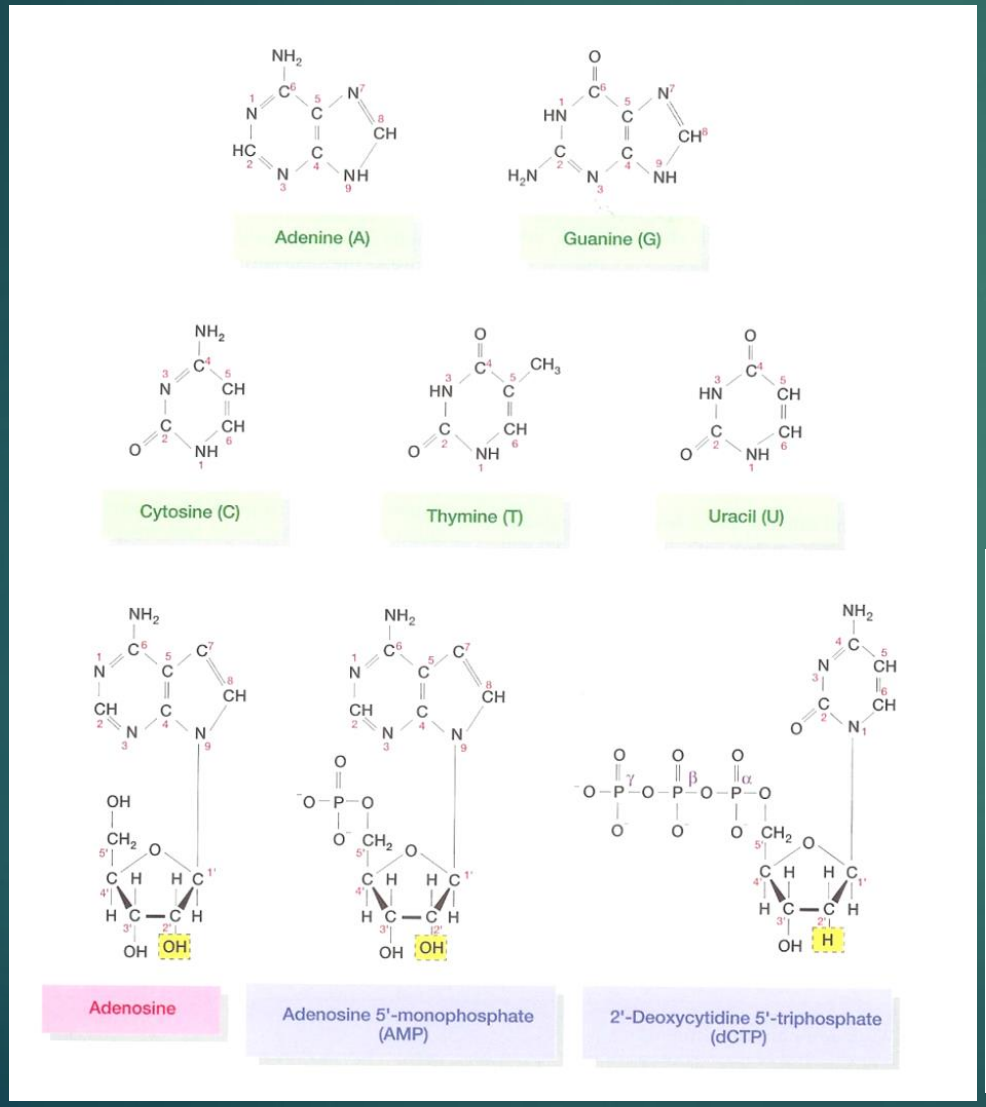
CLASSIFICATION OF CHROMOSOMES





GENE, NUCLEOTIDE AND NUCLEOSIDE



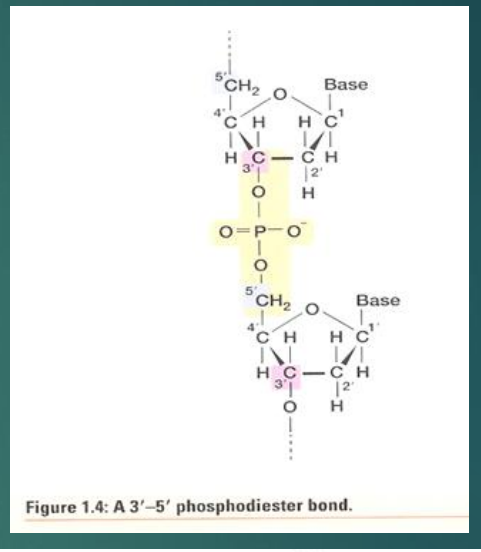
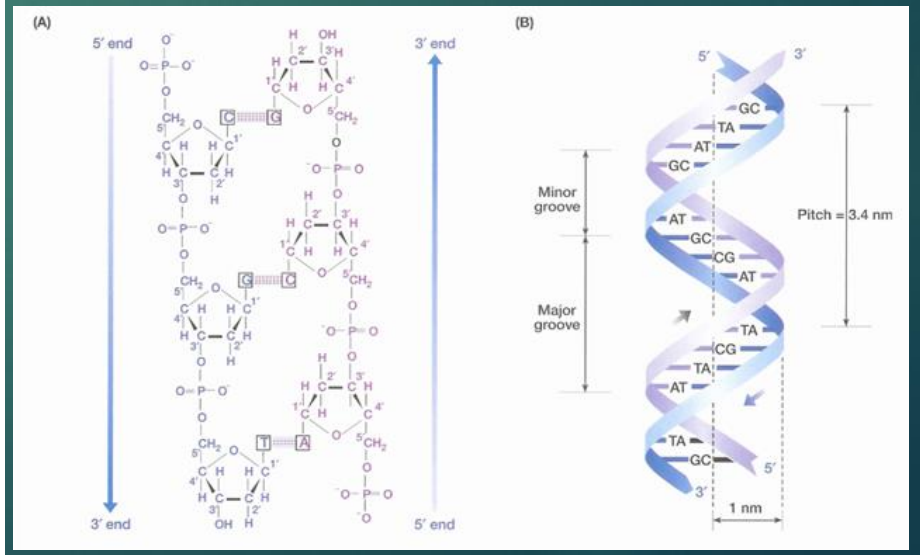
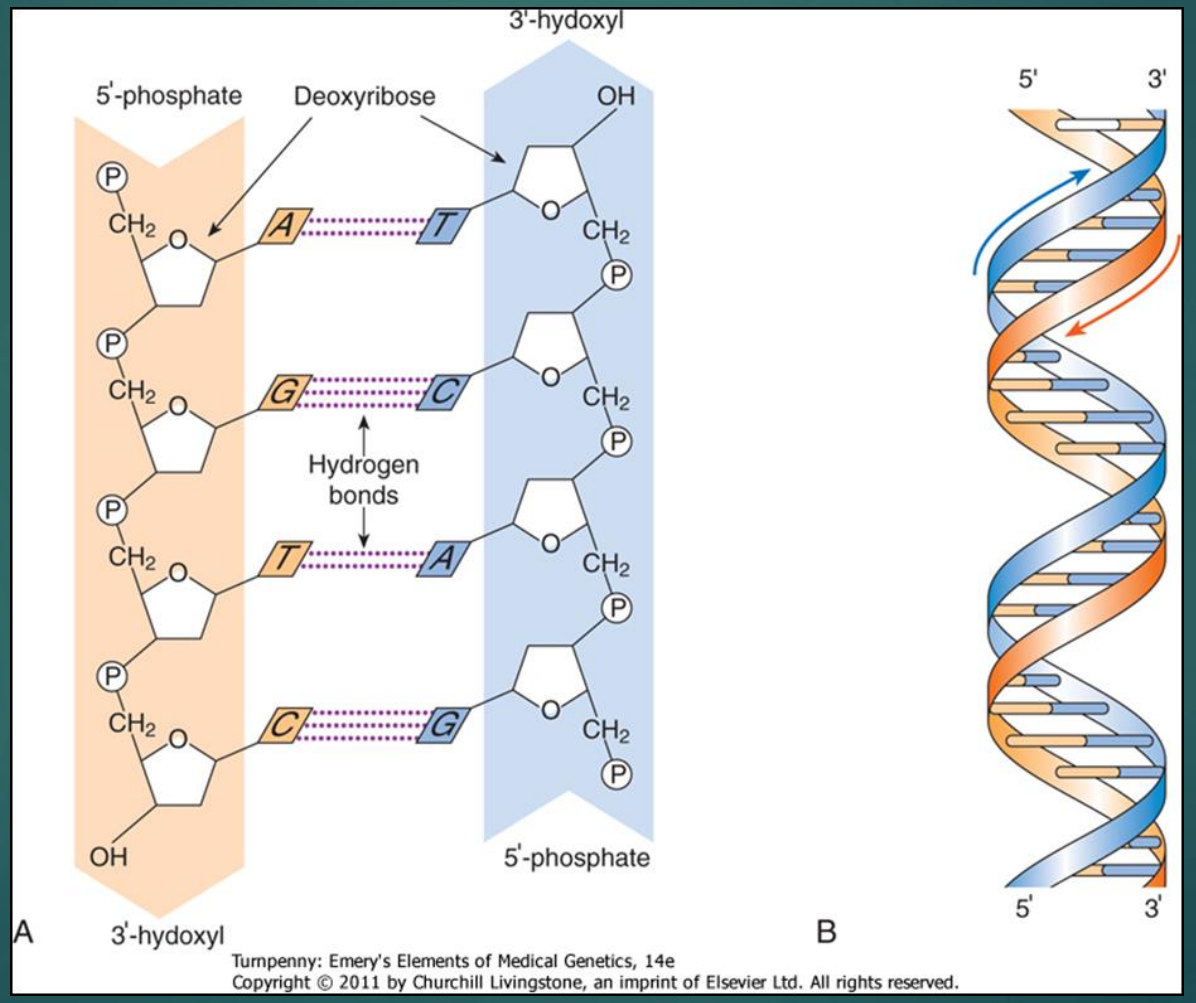


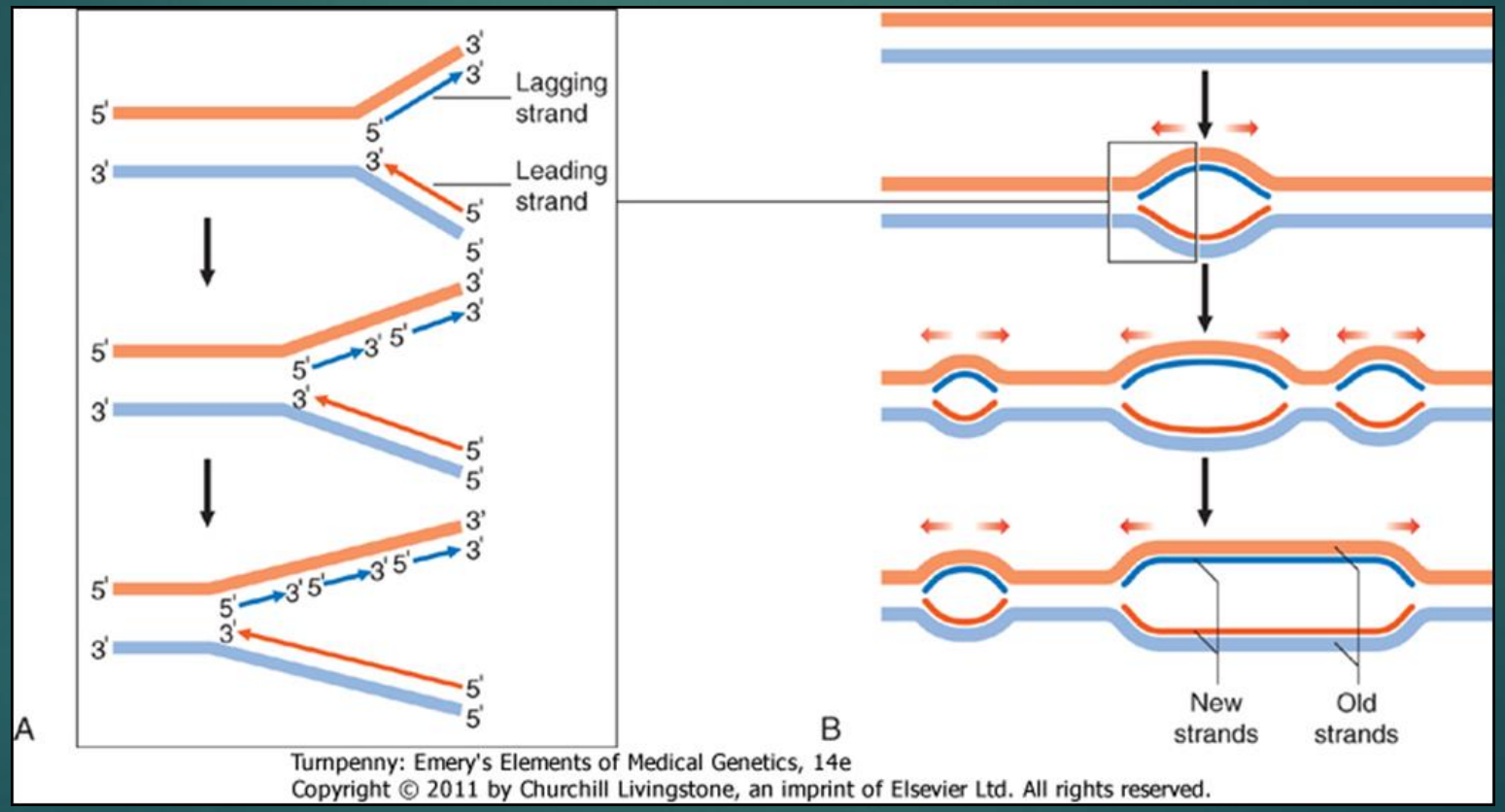
Figure 1.4: A 3′–5′ phosphodiester bond.



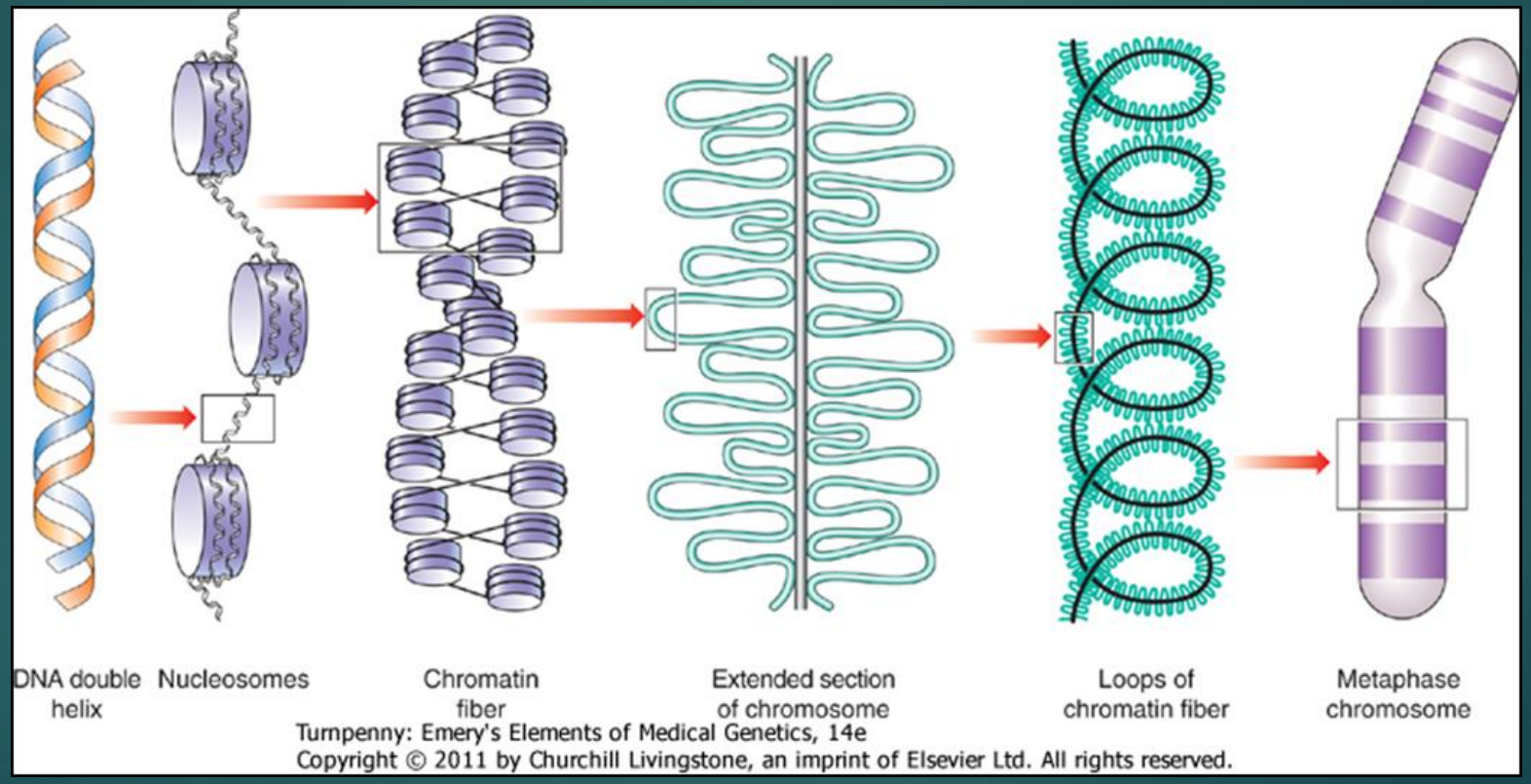
DNA double helix



DNA replication

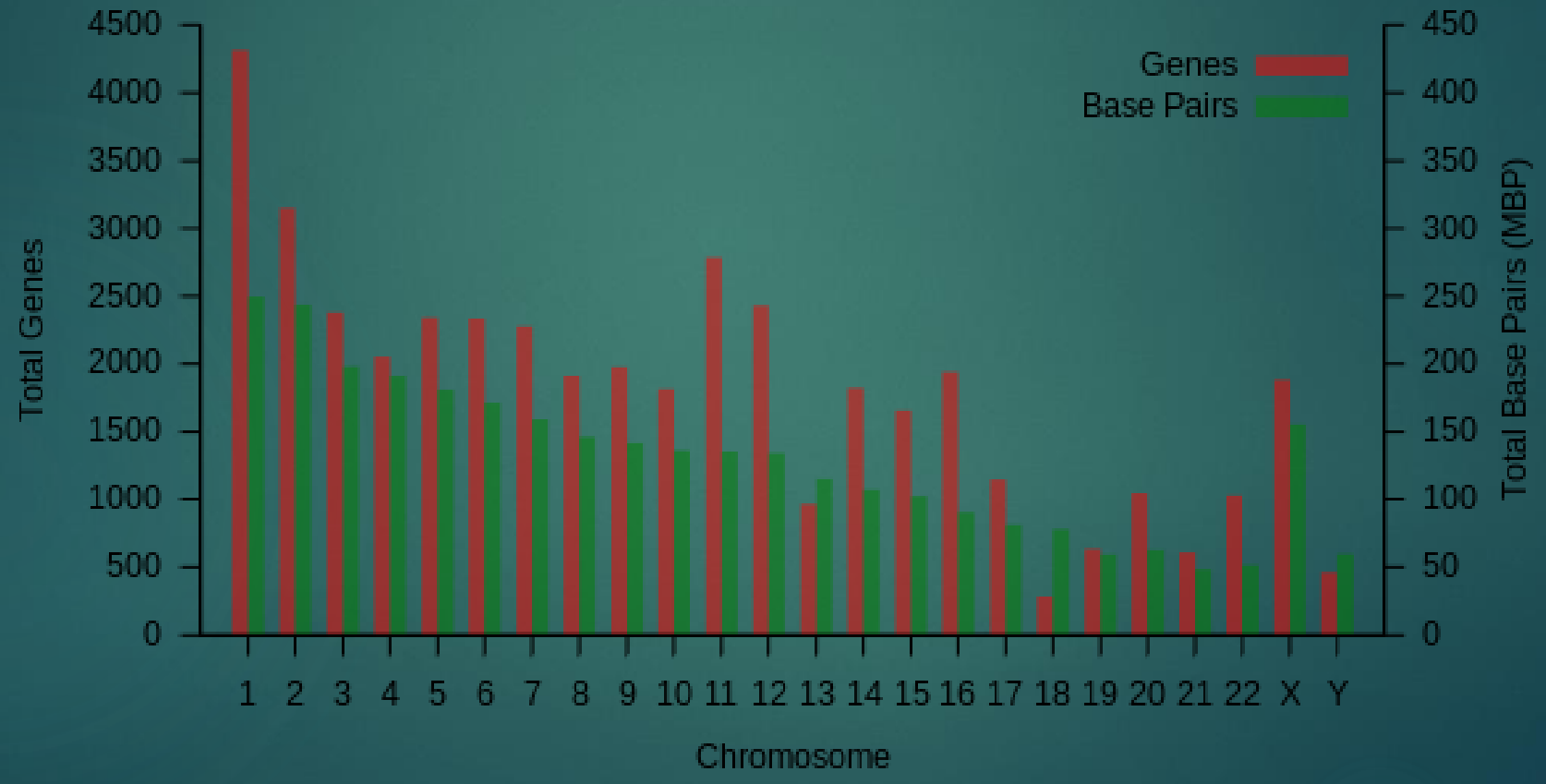


CHROMOSOME STRUCTURE

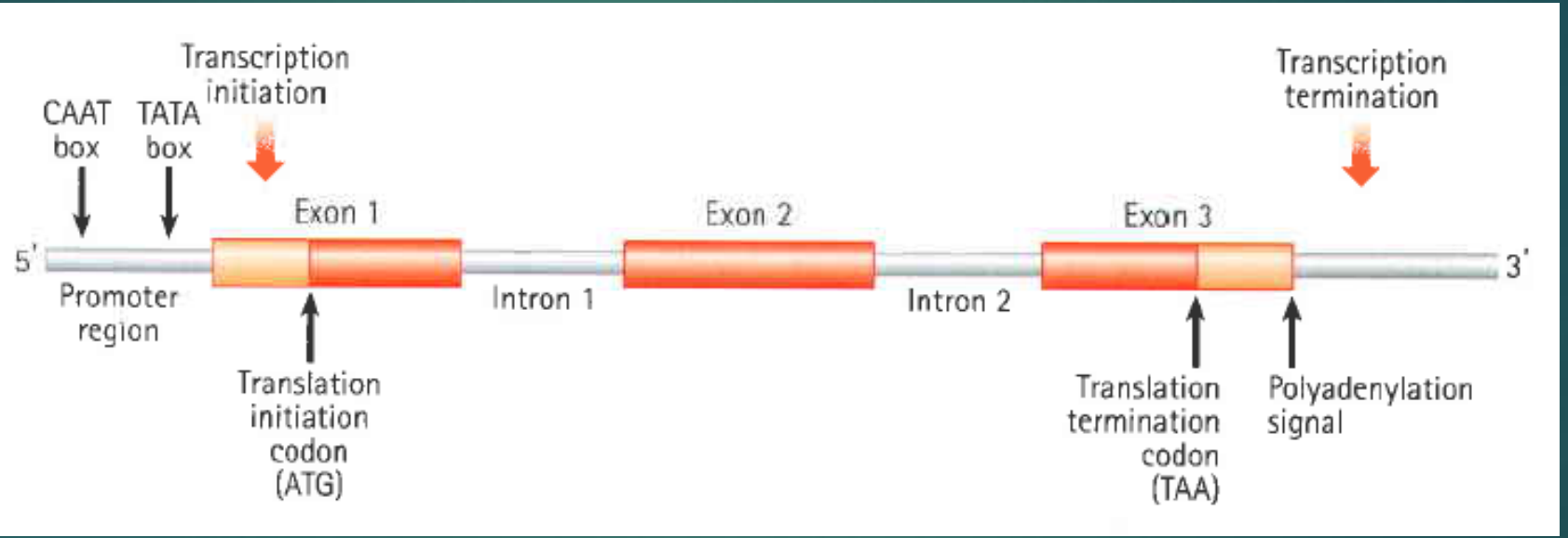


Chromosome	Genes[26]	Total base pairs	% of bases	Sequenced base pairs[27]
1	2000	247,199,719	8.0	224,999,719
2	1300	242,751,149	7.9	237,712,649
3	1000	199,446,827	6.5	194,704,827
4	1000	191,263,063	6.2	187,297,063
5	900	180,837,866	5.9	177,702,766
6	1000	170,896,993	5.5	167,273,993
7	900	158,821,424	5.2	154,952,424
8	700	146,274,826	4.7	142,612,826
9	800	140,442,298	4.6	120,312,298
10	700	135,374,737	4.4	131,624,737
11	1300	134,452,384	4.4	131,130,853
12	1100	132,289,534	4.3	130,303,534
13	300	114,127,980	3.7	95,559,980
14	800	106,360,585	3.5	88,290,585
15	600	100,338,915	3.3	81,341,915
16	800	88,822,254	2.9	78,884,754
17	1200	78,654,742	2.6	77,800,220
18	200	76,117,153	2.5	74,656,155
19	1500	63,806,651	2.1	55,785,651
20	500	62,435,965	2.0	59,505,254
21	200	46,944,323	1.5	34,171,998
22	500	49,528,953	1.6	34,893,953
X (sex chromosome)	800	154,913,754	5.0	151,058,754
Y (sex chromosome)	50	57,741,652	1.9	25,121,652
Total	**21,000**	**3,079,843,747**	**100.0**	**2,857,698,560**

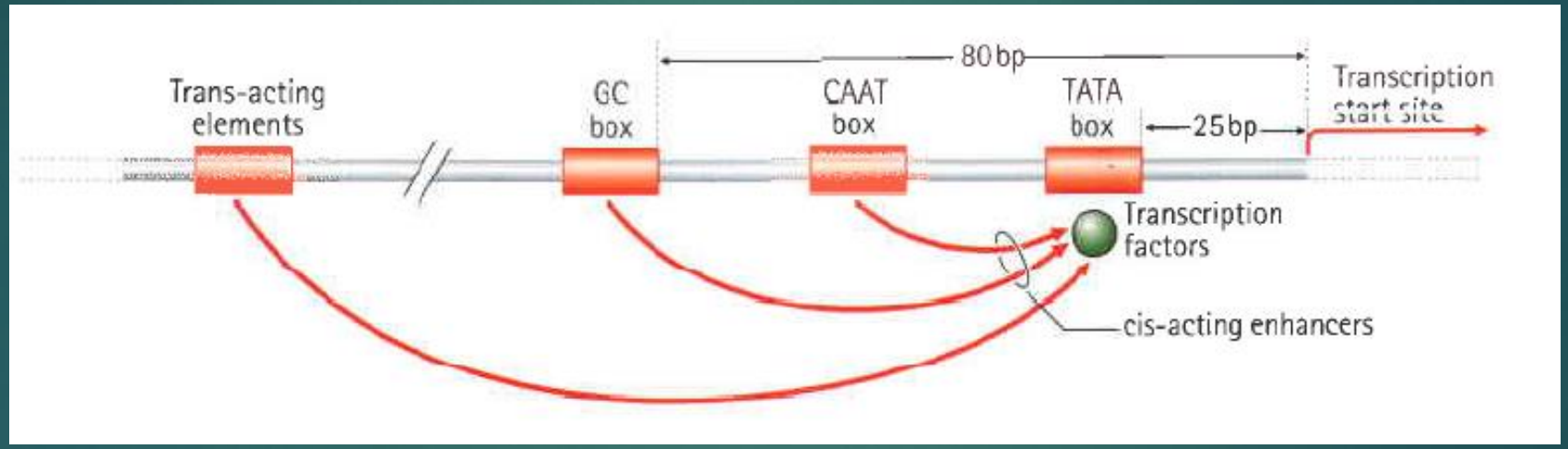
Base pairs of Genes



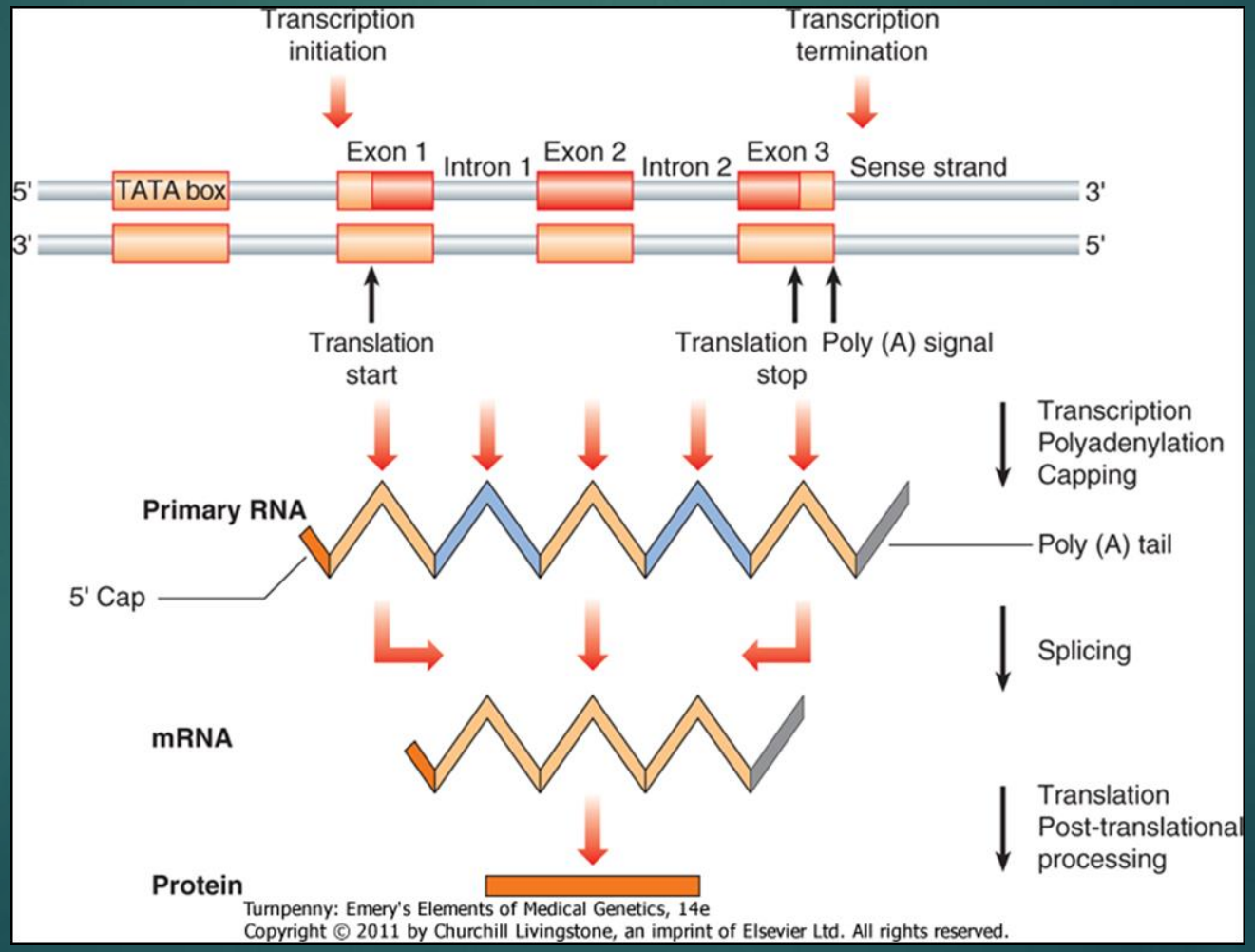
GENE STRUCTURE



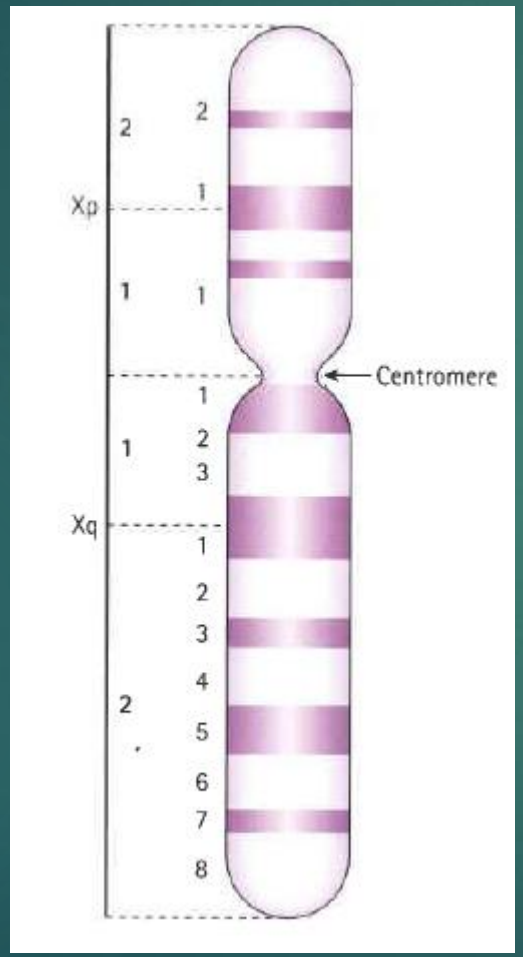
REGULATION OF GENE EXPRESSION

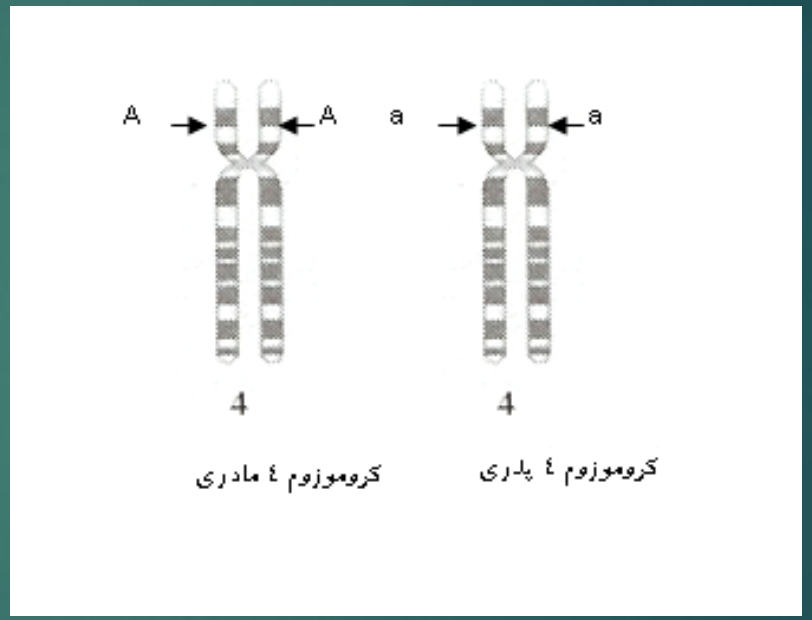


Transcription, post-transcriptional processing, translation, and post-translational processing



ALLELE AND LOCUS





GENETIC DISORDERS

- SINGLE-GENE DISORDER
- CHROMOSOME ABNORMALITY
- MULTFI ACTORIAL DI SORDER

polycystic kidney disease

MIM	Name of Disease	Inheritance	Gene
263100	POLYCYSTIC KIDNEY, CATARACT, AND CONGENITAL BLINDNESS	-	-
600273	- POLYCYSTIC KIDNEY DISEASE, INFANTILE SEVERE, WITH TUBEROUS SCLEROSIS; PKDTS	AD	-
600666	POLYCYSTIC KIDNEY DISEASE 3 WITH OR WITHOUT POLYCYSTIC LIVER DISEASE; PKD3	AD	GANAB
618061	POLYCYSTIC KIDNEY DISEASE 6 WITH OR WITHOUT POLYCYSTIC LIVER DISEASE; PKD6	AD	DNAJB11
617874	POLYCYSTIC LIVER DISEASE 3 WITH OR WITHOUT KIDNEY CYSTS; PCLD3	AD	ALG8
617610	POLYCYSTIC KIDNEY DISEASE 5; PKD5	AR	DZIP1L
617875	POLYCYSTIC LIVER DISEASE 4 WITH OR WITHOUT KIDNEY CYSTS; PCLD4	AD	LRP5
617004	POLYCYSTIC LIVER DISEASE 2 WITH OR WITHOUT KIDNEY CYSTS; PCLD2	AD	SEC63
263210	GILLESSEN-KAESBACH-NISHIMURA SYNDROME; GIKANIS	AR	ALG9
173900	POLYCYSTIC KIDNEY DISEASE 1 WITH OR WITHOUT POLYCYSTIC LIVER DISEASE; PKD1	AD	PKD1
613095	POLYCYSTIC KIDNEY DISEASE 2 WITH OR WITHOUT POLYCYSTIC LIVER DISEASE; PKD2	AD	PKD2
606702	PKHD1 GENE; PKHD1 FIBROCYSTIN; FCYT POLYDUCTIN POLYCYSTIC KIDNEY AND HEPATIC DISEASE 1 GENE	AR	-
263200	POLYCYSTIC KIDNEY DISEASE 4 WITH OR WITHOUT POLYCYSTIC LIVER DISEASE; PKD4	AR	FCYT
174000	MEDULLARY CYSTIC KIDNEY DISEASE 1; MCKD1	AD	MUC1
102500	HAJDU-CHENEY SYNDROME; HJCYS SERPENTINE FIBULA-POLYCYSTIC KIDNEY SYNDROME; SFPKS	AD	NOTCH2
609886	GLOMERULOCYSTIC KIDNEY DISEASE WITH HYPERURICEMIA AND ISOSTHENURIA	-	UMOD

polycystic kidney disease

- PKD is a genetic disorder in which the renal tubules become structurally abnormal, resulting in the development and growth of multiple cysts within the kidney.
- These cysts may begin to develop in utero, in infancy, in childhood, or in adulthood.
- PKD is caused by abnormal genes which produce a specific abnormal protein which has an adverse effect on tubule development.
- PKD is a general term for two types, each having their own pathology and genetic cause:
- autosomal dominant polycystic kidney disease (ADPKD)
- and autosomal recessive polycystic kidney disease (ARPKD).

- Autosomal dominant polycystic kidney disease (ADPKD) is the most common of all the inherited cystic kidney diseases with an incidence of 1:500 live births.

- Studies show that 10% of end-stage kidney disease (ESKD) patients being treated with dialysis in Europe and the U.S. were initially diagnosed and treated for ADPKD.

- There are three genetic mutations in the PKD-1, PKD-2, and PKD3 gene with similar phenotypical presentations.

- Gene PKD1 is located on chromosome 16 and codes for a protein involved in regulation of cell cycle and intracellular calcium transport in epithelial cells, and is responsible for 85% of the cases of ADPKD.

- A group of voltage-linked calcium channels are coded for by PKD2 on chromosome 4.

- PKD3 recently appeared in research papers as a postulated third gene. Fewer than 10% of cases of ADPKD appear in non-ADPKD families.

- Cyst formation begins in utero from any point along the nephron,
- although fewer than 5% of nephrons are thought to be involved.
- As the cysts accumulate fluid, they enlarge, separate entirely from the nephron, compress the neighboring kidney parenchyma, and progressively compromise kidney function.

- Autosomal recessive polycystic kidney disease (ARPKD) (OMIM #263200) is the lesser common of the two types of PKD, with an incidence of 1:20,000 live births.

- And is typically identified in the first few weeks after birth.

- Unfortunately, the kidneys are often underdeveloped resulting in a 30% death rate in newborns with ARPKD.

HYPEROXALURIA

- **Hyperoxaluria** is an excessive urinary excretion of oxalate.
- Individuals with hyperoxaluria often have calcium oxalate kidney stones.
- It is sometimes called **Bird's disease**, after Golding Bird, who first described the condition.
- **Types**
- Primary hyperoxaluria
- Enteric hyperoxaluria
- Idiopathic hyperoxaluria
- Oxalate poisoning

HYPEROXALURIA

259900	HYPEROXALURIA, PRIMARY, TYPE I; HP1 OXALOSIS I GLYCOLIC ACIDURIA ALANINE-GLYOXYLATE AMINOTRANSFERASE DEFICIENCY PEROXISOMAL ALANINE:GLYOXYLATE AMINOTRANSFERASE DEFICIENCY HEPATIC AGT DEFICIENCY SERINE:PYRUVATE AMINOTRANSFERASE DEFICIENCY	AR	AGXT
260000	HYPEROXALURIA, PRIMARY, TYPE II; HP2 OXALOSIS II GLYCERIC ACIDURIA GLYOXYLATE REDUCTASE/HYDROXYPYRUVATE REDUCTASE DEFICIENCY D-GLYCERATE DEHYDROGENASE DEFICIENCY	AR	GRHPR
613616	HYPEROXALURIA, PRIMARY, TYPE III; HP3	-	HOGA1

- Type I (PH1) is associated with AGXT protein, a key enzyme involved in breakdown of oxalate.
- PH1 is also an example of a protein mistargeting disease, wherein AGXT shows a trafficking defect: instead of being trafficked to peroxisomes, it is targeted to mitochondria, where it is metabolically deficient despite being catalytically active.
- Type II is associated with GRHPR.
- It is also a complication of jejunoileal bypass, or in any patient who has lost much of the ileum with an intact colon.
- This is due to excessive absorption of oxalate from the colon.

Cystinuria

- **Cystinuria** is an inherited autosomal recessive disease that is characterized by high concentrations of the amino acid cysteine in the urine, leading to the formation of cystine stones in the kidneys, ureter, and bladder. It is a type of aminoaciduria.
- Cystinuria is caused by mutations in the *SLC3A1* and *SLC7A9* genes.
- These defects prevent proper reabsorption of basic, or positively charged, amino acids: Cysteine, lysine, ornithine, arginine.
- Under normal circumstances, this protein allows certain amino acids, including cysteine, to be reabsorbed into the blood from the filtered fluid that will become urine.

- CYSTINURIA

- *Alternative titles; symbols*
- CSNU
  CYSTINURIA, TYPE I, FORMERLY; CSNU1, FORMERLY
  CYSTINURIA, TYPE II, FORMERLY
  CYSTINURIA, TYPE III, FORMERLY; CSNU3, FORMERLY
  CYSTINURIA, TYPE NON-I, FORMERLY
- Other entities represented in this entry:
- CYSTINURIA, TYPE A, INCLUDED
- CYSTINURIA, TYPE B, INCLUDED
  CYSTINURIA, TYPE A/B, INCLUDED

Phenotype-Gene Relationships

Location	Phenotype	Phenotype MIM number	Inheritance	Phenotype mapping key	Gene/Locus	Gene/Locus MIM number
2p21	Cystinuria	220100	AR, AD	3	SLC3A1	104614
19q13.11	Cystinuria	220100	AR, AD	3	SLC7A9	604144

- Mutations in either of these genes disrupt the ability of this transporter protein to reabsorb these amino acids, allowing them to become concentrated in the urine.

- As the levels of cysteine in the urine increase, it forms cystine crystals, resulting in kidney stones. Cystine crystals form hexagonal-shaped crystals that can be viewed upon microscopic analysis of the urine.

- The other amino acids that are not reabsorbed do not create crystals in urine.

- The overall prevalence of cystinuria is approximately 1 in 7,000 neonates (from 1 in 2,500 neonates in Libyan Jews to 1 in 100,000 among Swedes).

Male infertility

- Genetic factors cause account for 10–15% of male infertility, including chromosomal aberrations and single gene mutations.
- Klinefelter Syndrome
- Y chromosome deletions

Klinefelter Syndrome

- One of the most commonly known causes of infertility is Klinefelter Syndrome, affecting 1 out of 500-1000 newborn males.
- Klinefelter Syndrome is a chromosomal defect that occurs during gamete formation due to a non-disjunction error during cell division.
- Resulting in males having smaller testes, reducing the amount of testosterone and sperm production.

Y chromosome deletions

- Y chromosomal infertility is a direct cause of male infertility due to its affects on sperm production,occurring in 1 out of every 2000 males·
- Usually affected men show no sign of symptoms other than at times can exhibit smaller teste size.
- Men with this condition can exhibit azoospermia (no sperm production), oligospermia (small number of sperm production), or they will produce abnormally shaped sperm.
- These individuals are thereby "Y-linked", although daughters are not affected due to the lack of the Y chromosome.

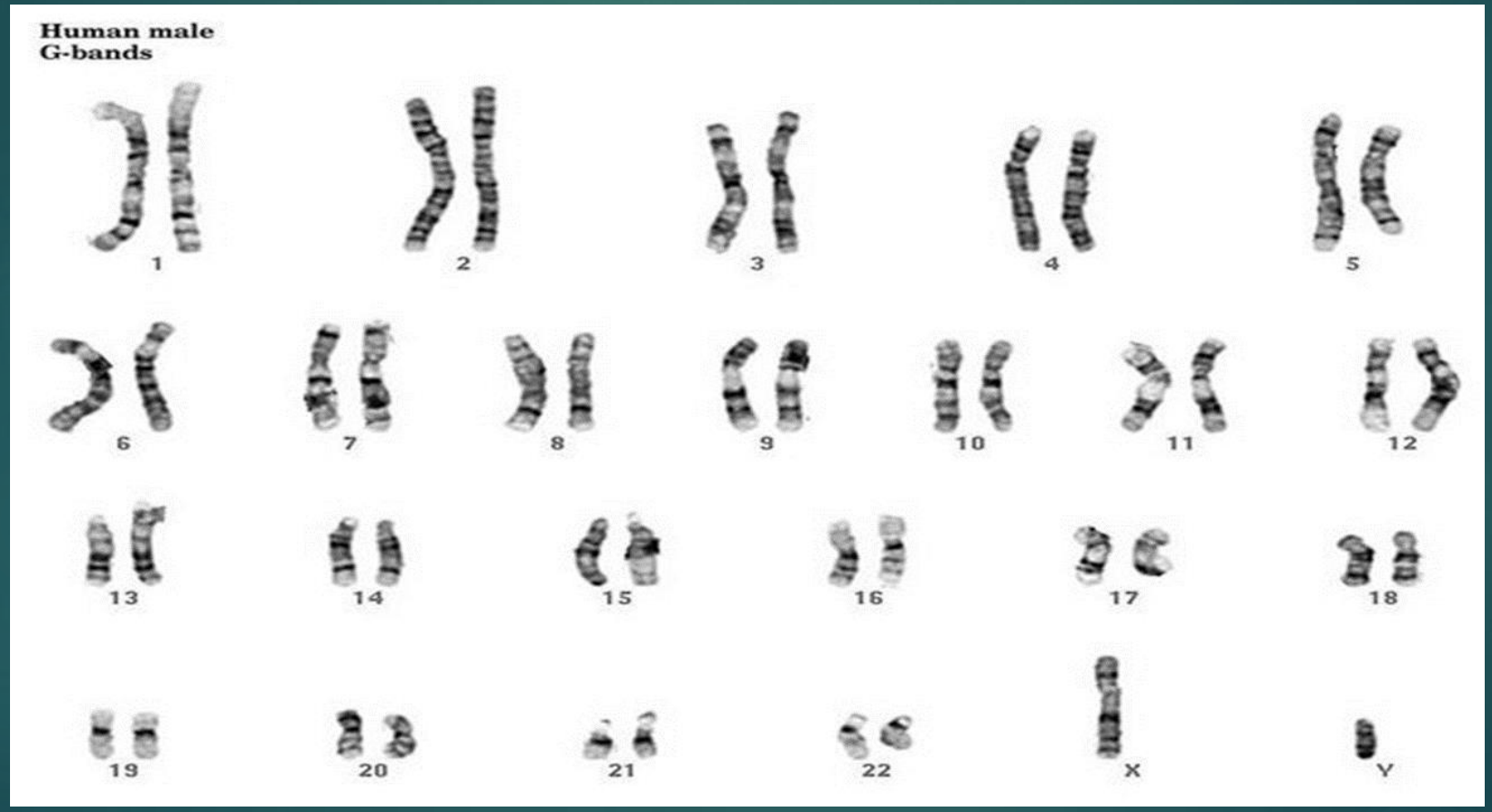
Microdeletions in the Y chromosome

- Microdeletions in the Y chromosome have been found at a much higher rate in infertile men than in fertile controls and the correlation found may still go up as improved genetic testing techniques for the Y chromosome are developed.
- Much study has been focused upon the "azoospermia factor locus" (AZF), at Yq11.
- A specific partial deletion of AZFc called *gr/gr deletion* is significantly associated with male infertility among Caucasians in Europe and the Western Pacific region.
- Additional genes associated with spermatogenesis in men and reduced fertility upon Y chromosome deletions include RBM, DAZ, SPGY, and TSPY.

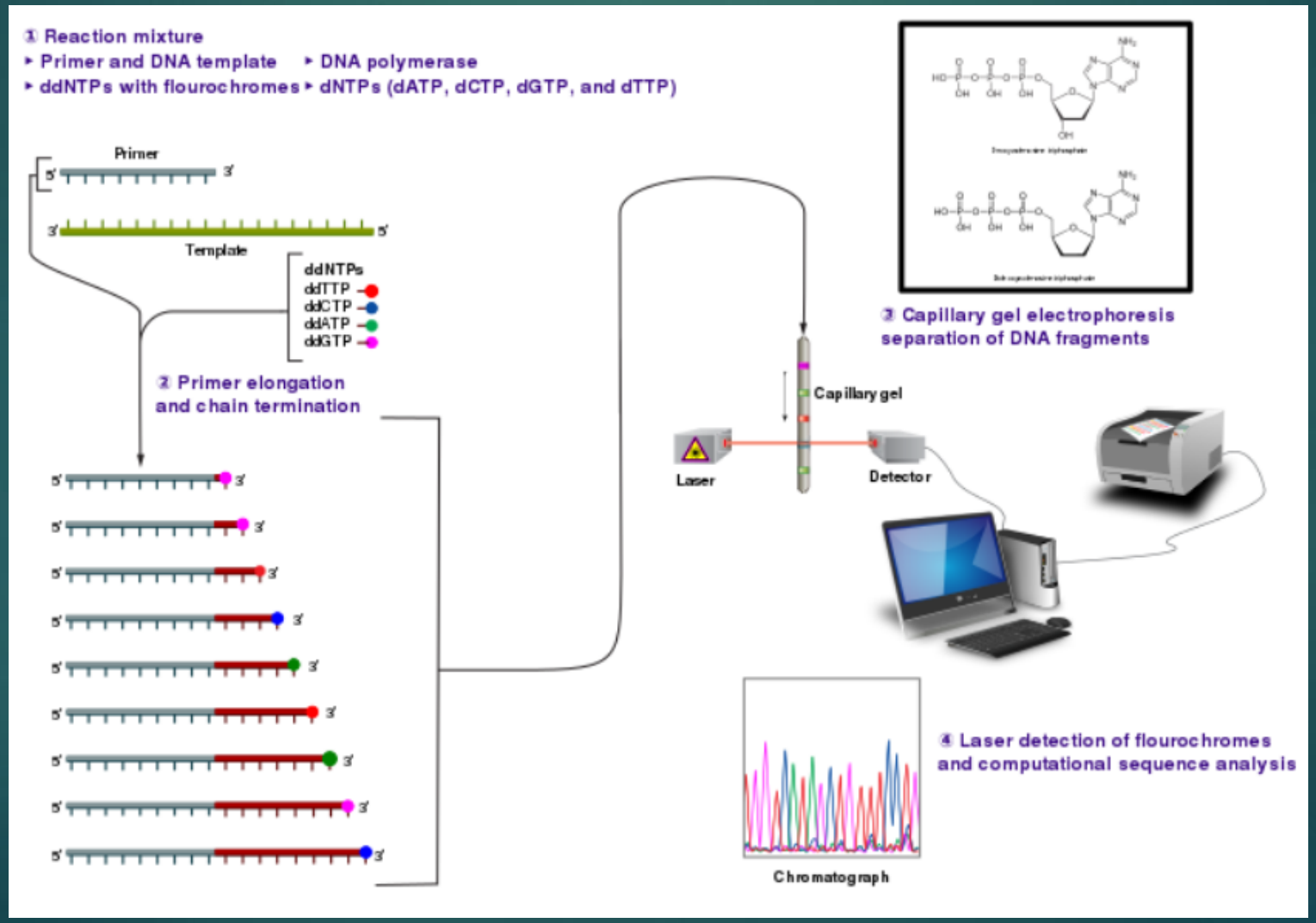
Lab Methods

- Karyotyping
- PCR/Sanger Sequencing
- Deletion/Duplication Analysis
- MLPA
- Next-Gen Sequencing

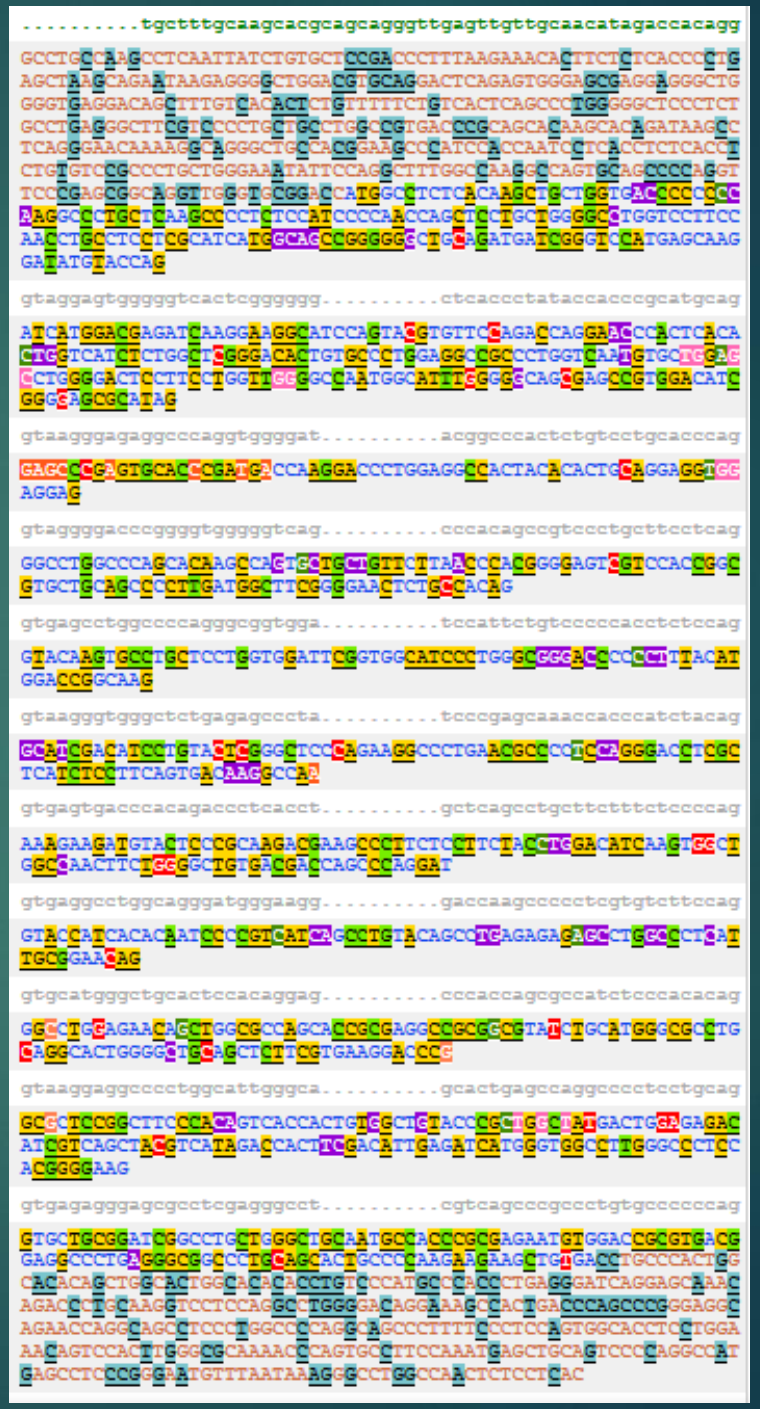
Karyotyping



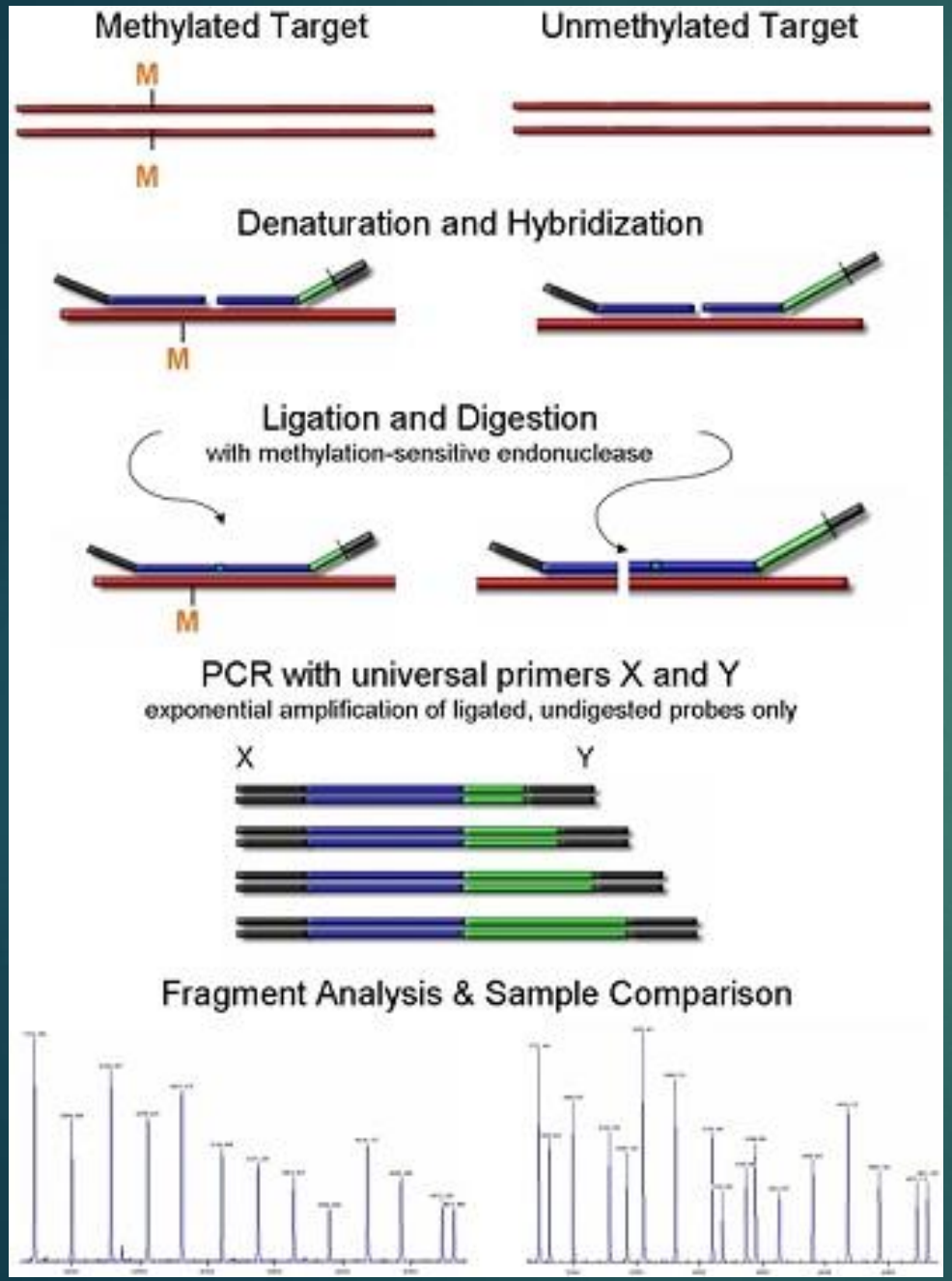
PCR and Sanger sequencing

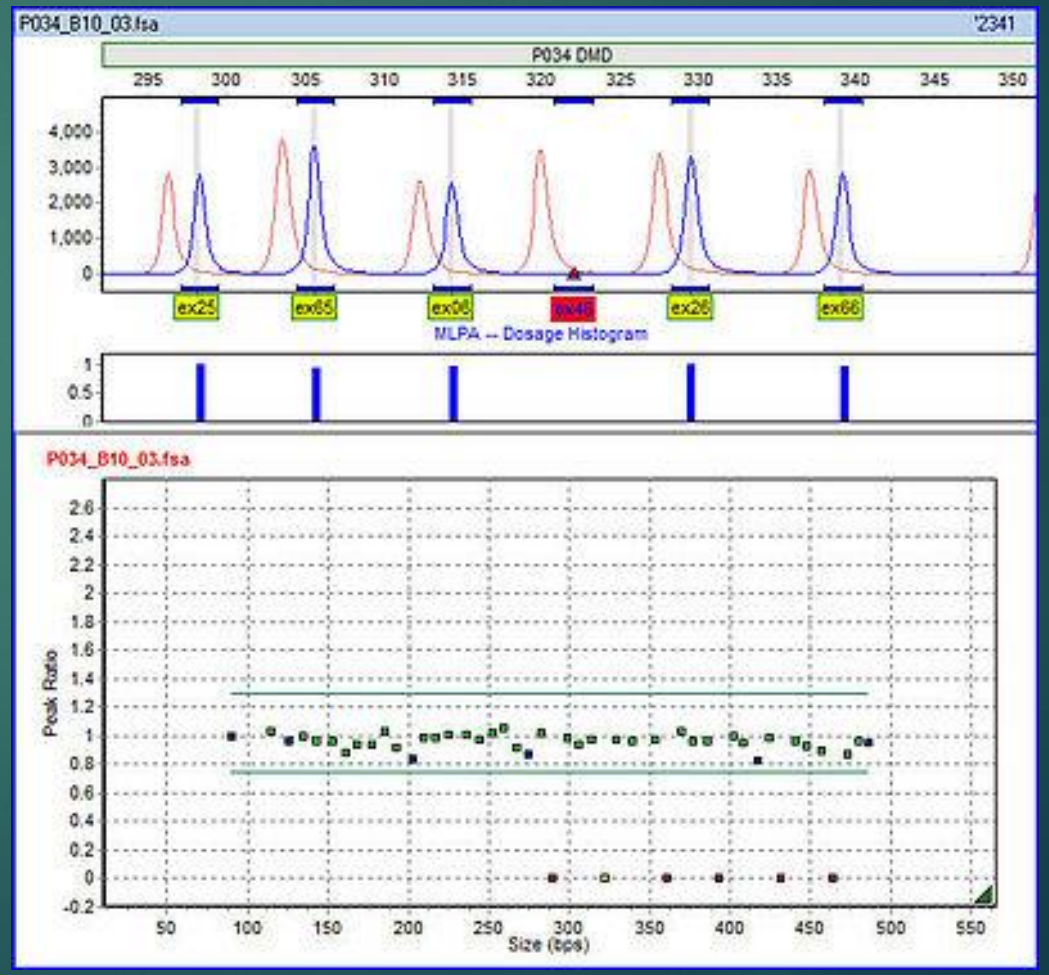


AGXT gene



MLPA


Methylated Target
Unmethylated Target
M
M
Denaturation and Hybridization
M
Ligation and Digestion
with methylation-sensitive endonuclease
M
PCR with universal primers X and Y
exponential amplification of ligated, undigested probes only
X
Y
Fragment Analysis & Sample Comparison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'2341
P034 DMD
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4,000
3,000
2,000
1,000
0
ex25
ex65
ex06
ex46
ex26
ex66
MLPA -- Dosage Histogram
1
0.5
0
P034_B10_03.fsa
Peak Ratio
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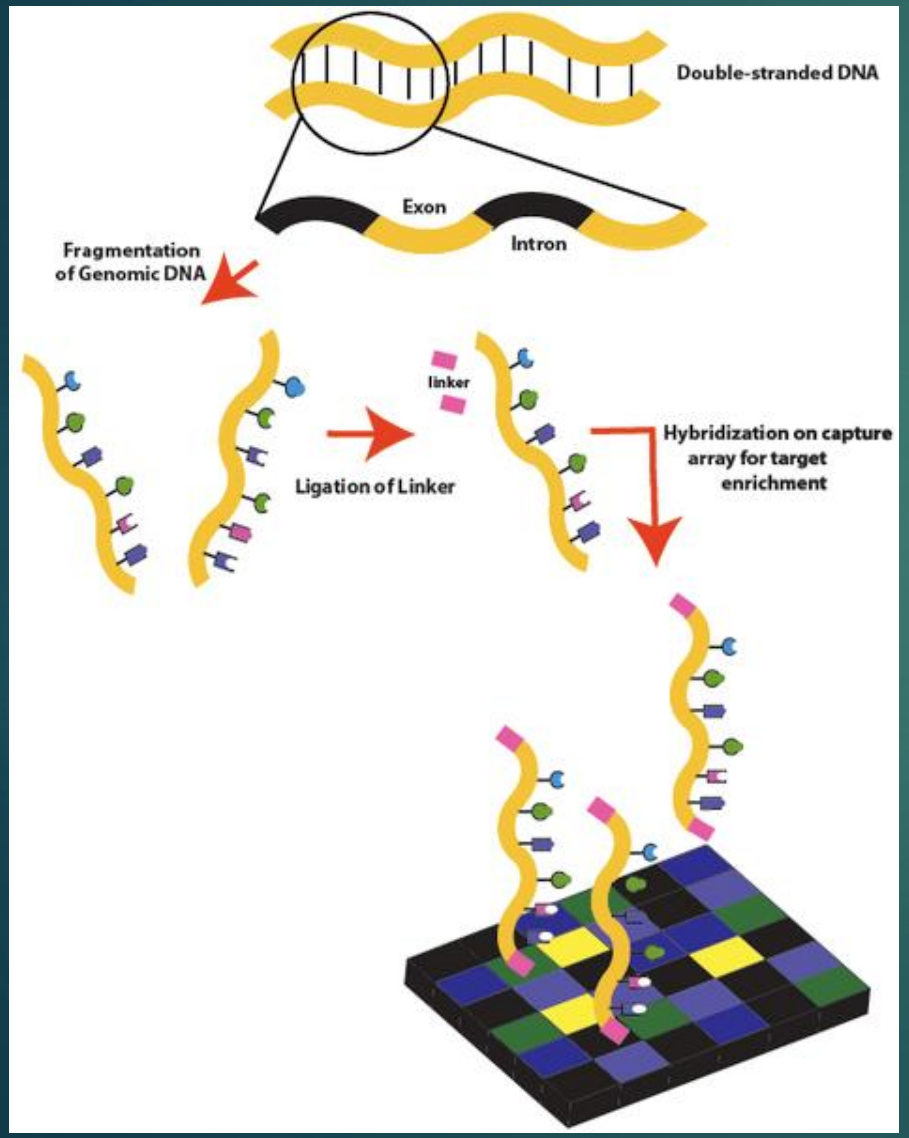
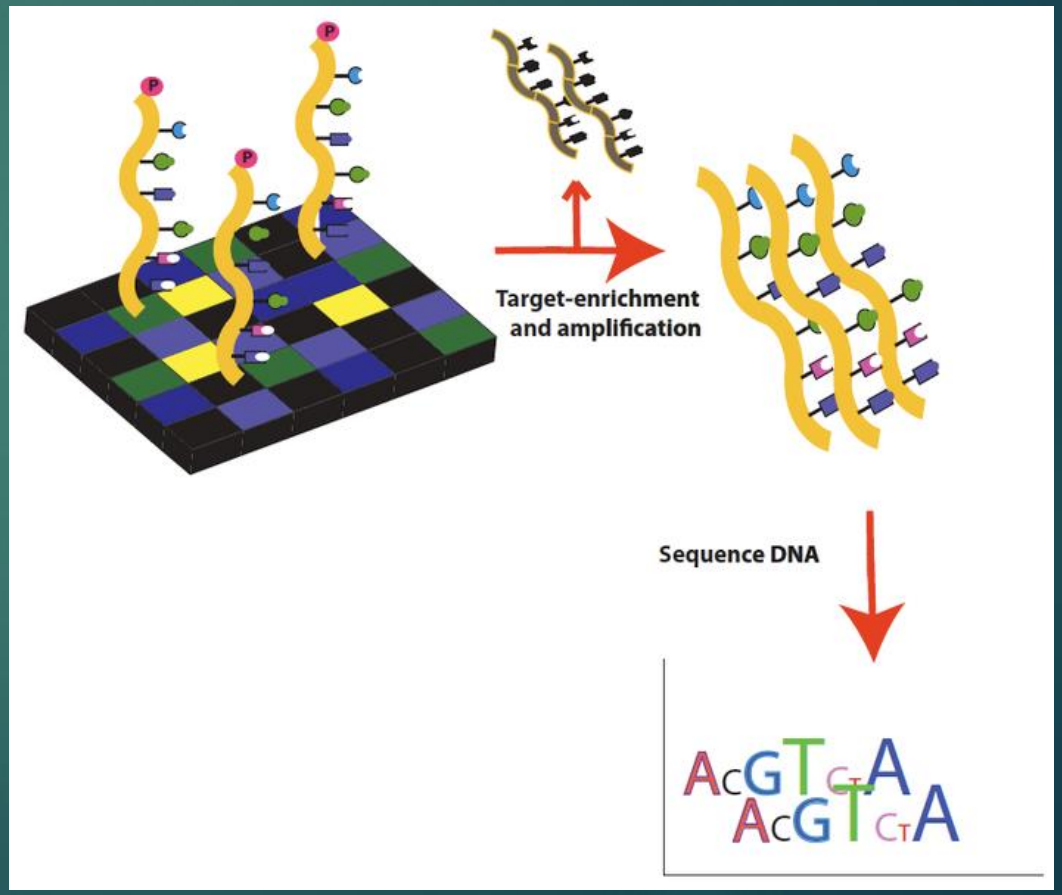
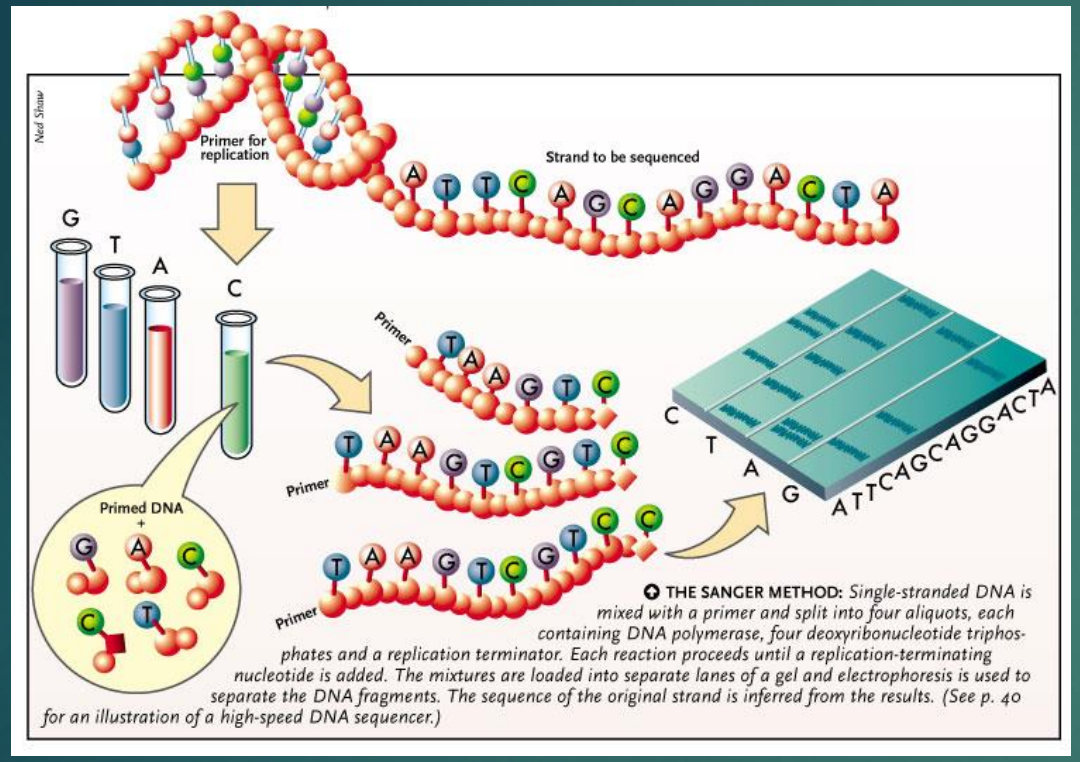
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
50 100 150 200 250 300 350 400 450 500 550
Size (bps)

Whole exome sequencing (NGS)





آزمایش های ngs و پانل



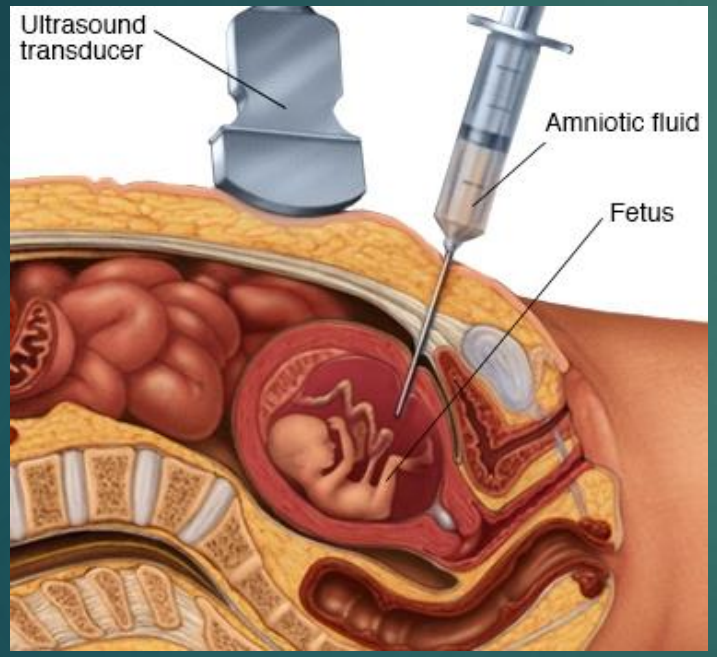
موارد زیر مثال هایی از اختلالاتی هستند که به روش NGSو با بررسی تعدادی ژن یا کل ژنوم مورد بررسی قرار می گیرند.

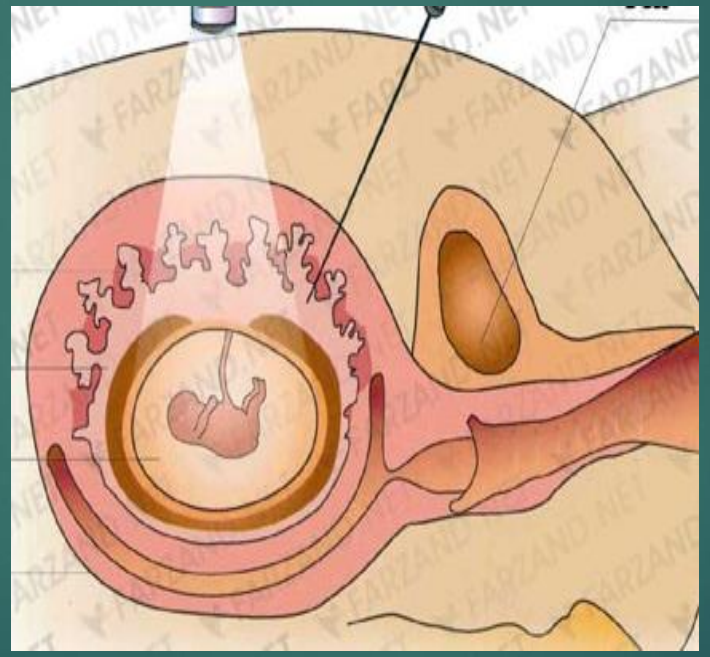
- سرطانها
- ناتوانی ذهنی و تاخیر تکاملی
- ناشنوایی های ارثی
- نابینایی های ارثی
- بیماری های متابولیک ارثی
- بیماری های سیستم اسکلتی و استخوانی
- معلولیت های حرکتی
- بیماری های سیستم اعصاب مرکزی
- بیماری های ژنتیک پوست (ژنودرماتوزها)
- بیماری های ژنتیک قلب (آریتمی ها و...)

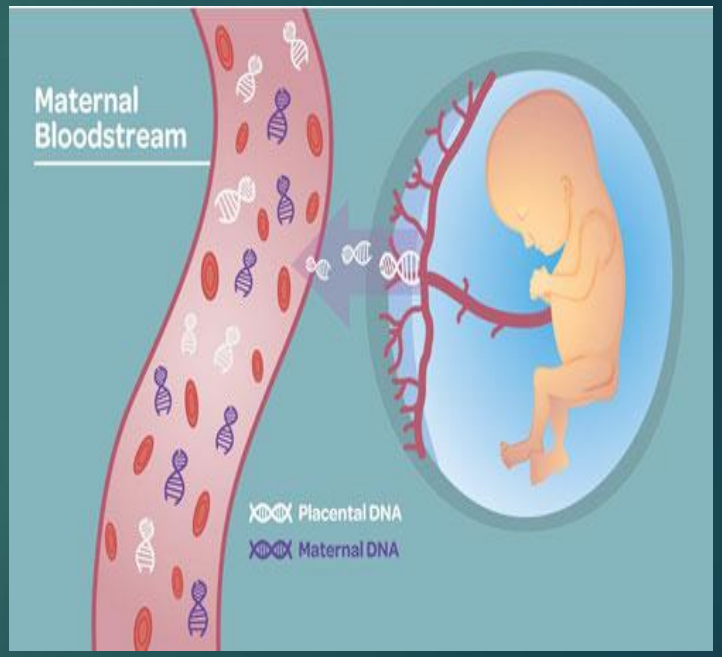
Diagnosis

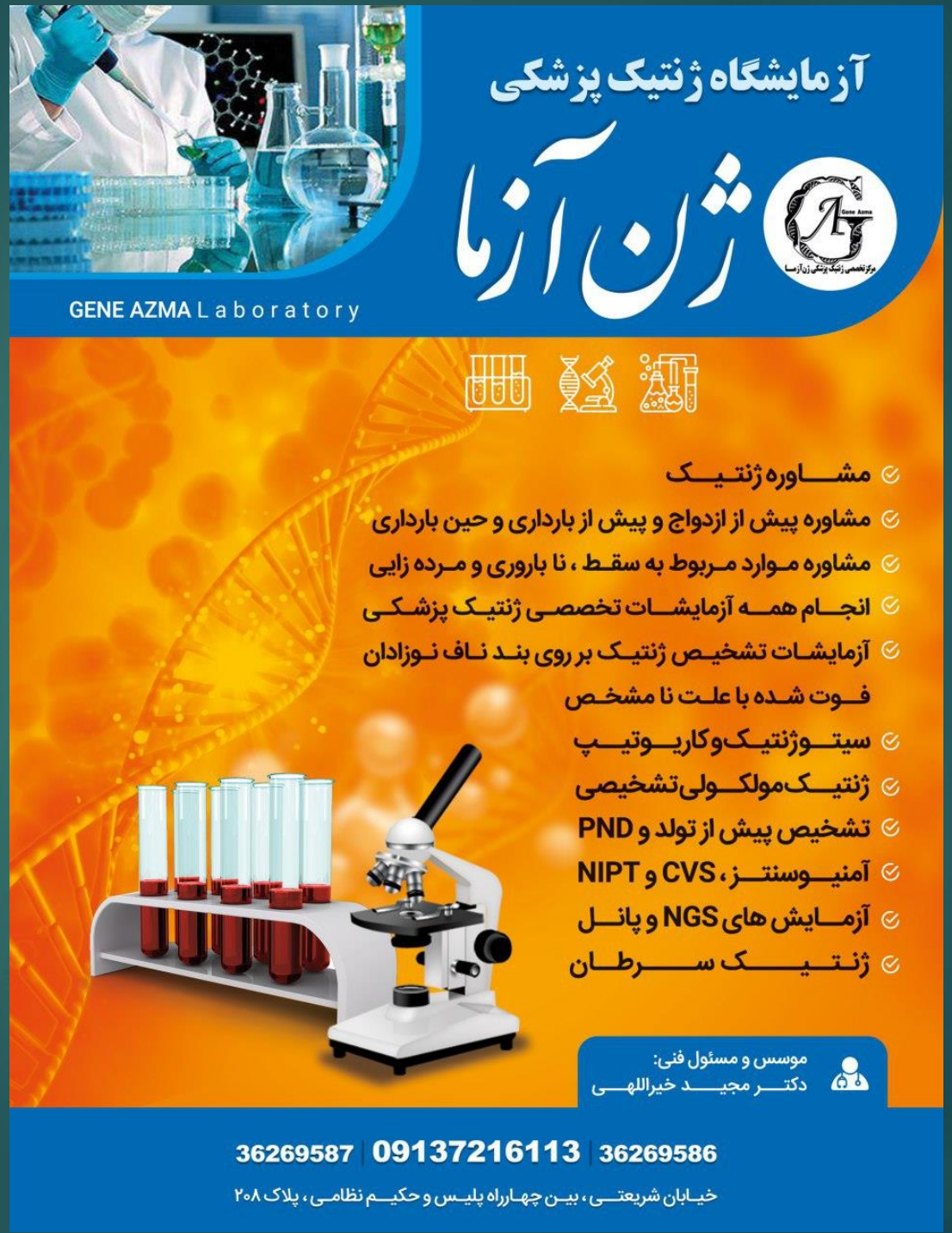
- **Prenatal testing and prenatal expectations**
- Prenatal testing
- in vitro fertilisation, via preimplantation genetic diagnosis
- Chorionic villus sampling
- amniocentesis

آمنیوسنتز، CVS و NIPT








آزمایشگاه ژنتیک پزشکی
ژن آزما
GENE AZMA Laboratory
مشاوره ژنتیک
مشاوره پیش از ازدواج و پیش از بارداری و حین بارداری
مشاوره موارد مربوط به سقط ، نا باروری و مرده زایی
انجام همه آزمایشات تخصصی ژنتیک پزشکی
آزمایشات تشخیص ژنتیک بر روی بند ناف نوزادان
فوت شده با علت نا مشخص
سیتوژنتیک و کاریوتیپ
ژنتیک مولکولی تشخیصی
تشخیص پیش از تولد و PND
آمنیوسنتز ، CVS و NIPT
آزمایش های NGS و پانل
ژنتیک سرطان
موسس و مسئول فنی:
دکتر مجید خیراللهی
36269587 | 09137216113 | 36269586
خیابان شریعتی ، بین چهارراه پلیس و حکیم نظامی ، پلاک ۲۰۸

THANKS