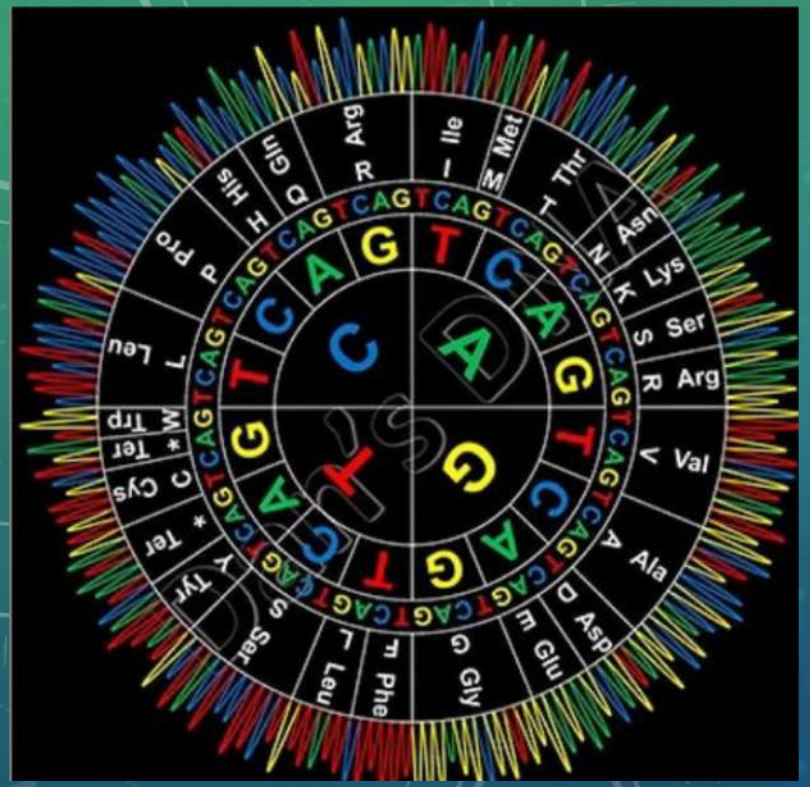
İLERİ ARAŞTIRMA YÖNTEMLERİ 56901007

2020-21 BAHAR

DR. GÜNSELİ ÇUBUKÇUOĞLU DENİZ



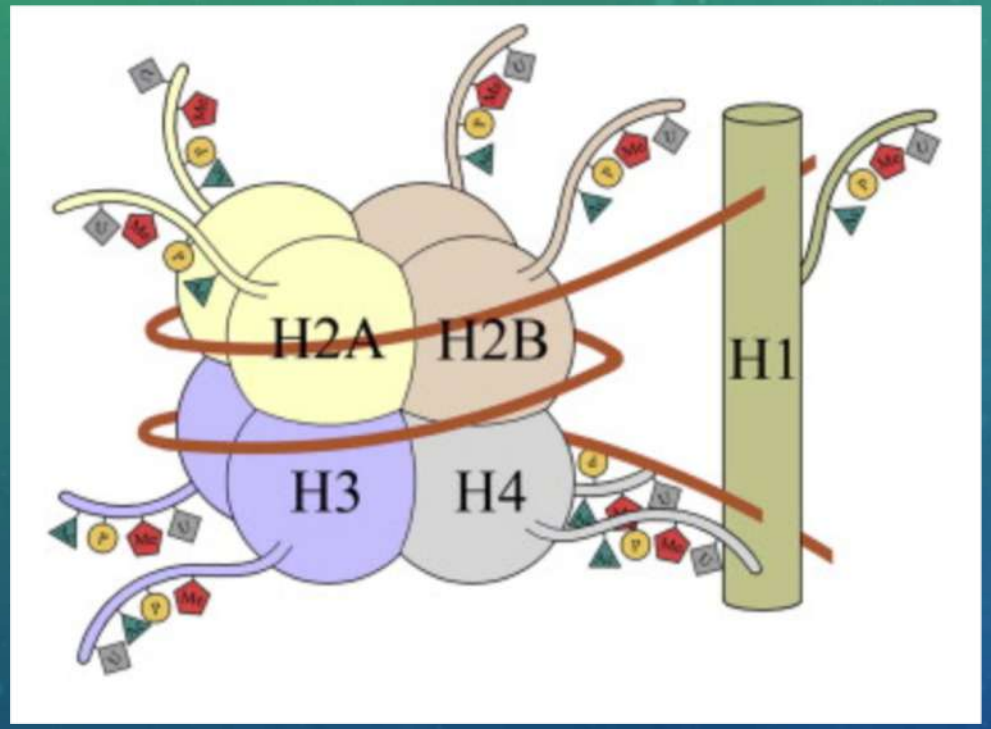
DNA SEKANS (DİZİLEME) YÖNTEMLERİ

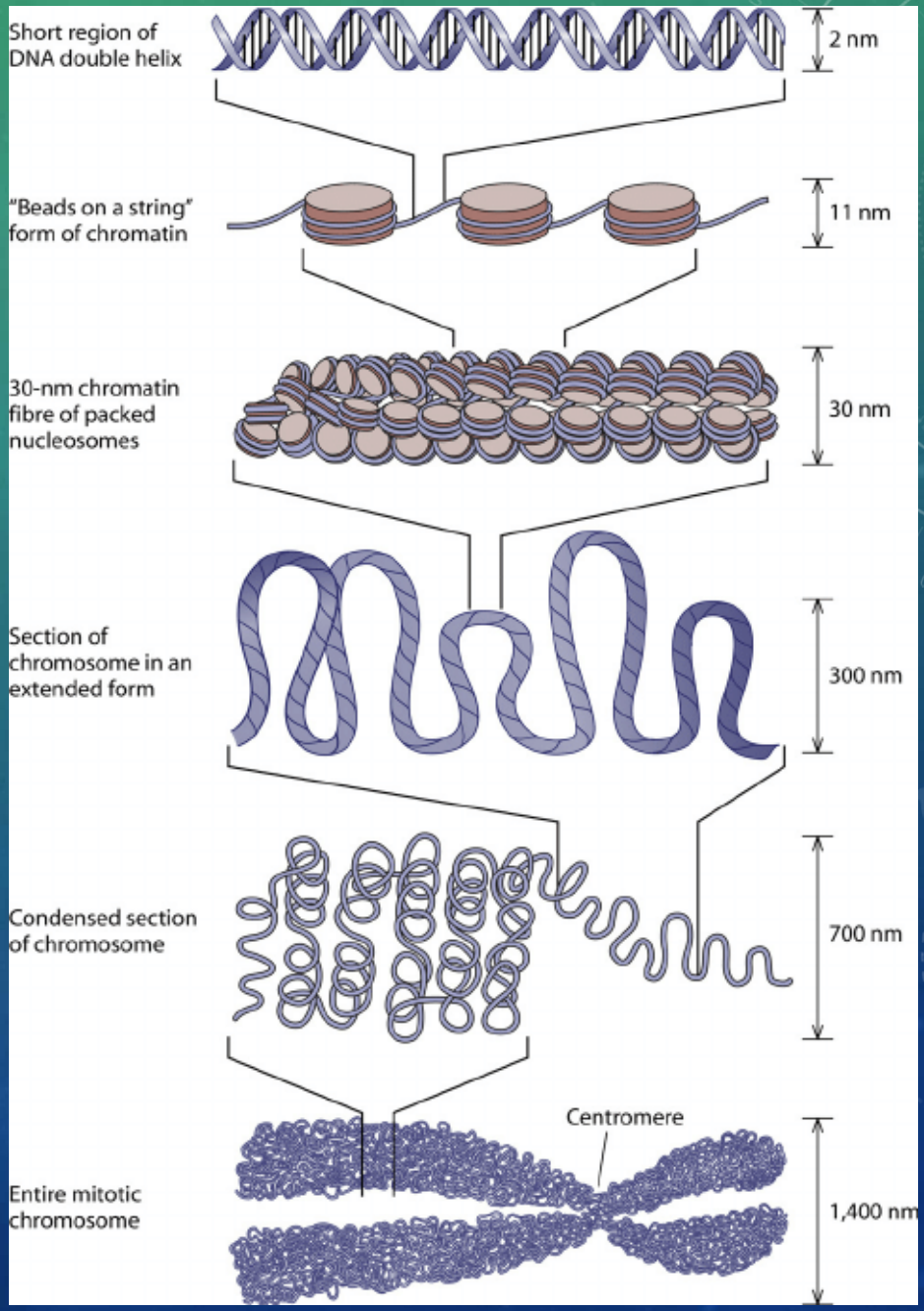
DERS 6

DNA DİZİLEME

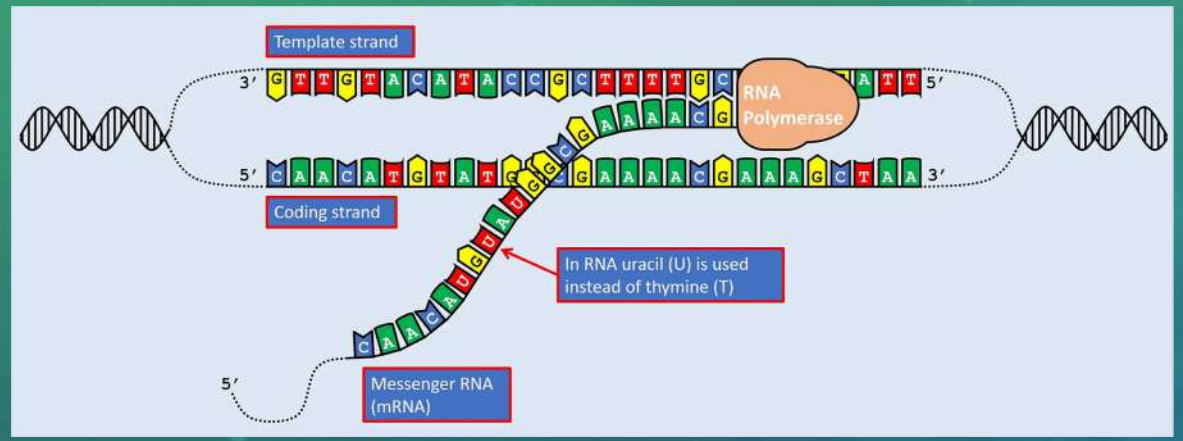
- 1970lerde Walter Gilbert &Allan Maxam ve Fred Sanger ayrı laboratuvarlarda DNA baz dizisinin belirlenmesi üzerine çalışıyorlardı.
- 1977'de her ikisi de kendi dizileme reksiyonlarını yayınladılar.
- 1987'de Leroy Hood&Michael Hunkapiller Applied Biosystems Inc. (ABI) Sanger sekanslamayı otomatize hale getirmeyi başardılar. Radyoaktif moleküller yerine floresan boyaları kullandılar. Data toplama ve analizlerini bilgisayarda yapılabilecek hale getirdiler.
- 1996'da Mostafa Ronaghi, Mathias Uhlen, Pal Nyren pirosekanslama tekniğini geliştirdiler. Pirofosfat sentezinin lüminesansıyla ölçüme dayalı bu otomatize teknik «high-throughput sequencing» olarak değerlendirildi.

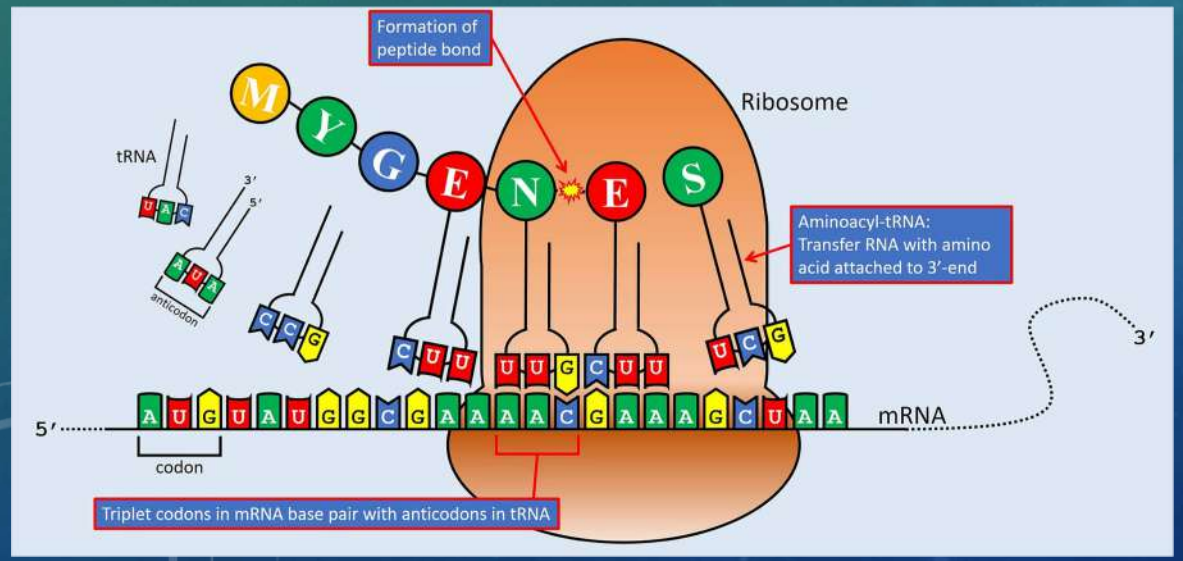
KROMATİN YAPISI





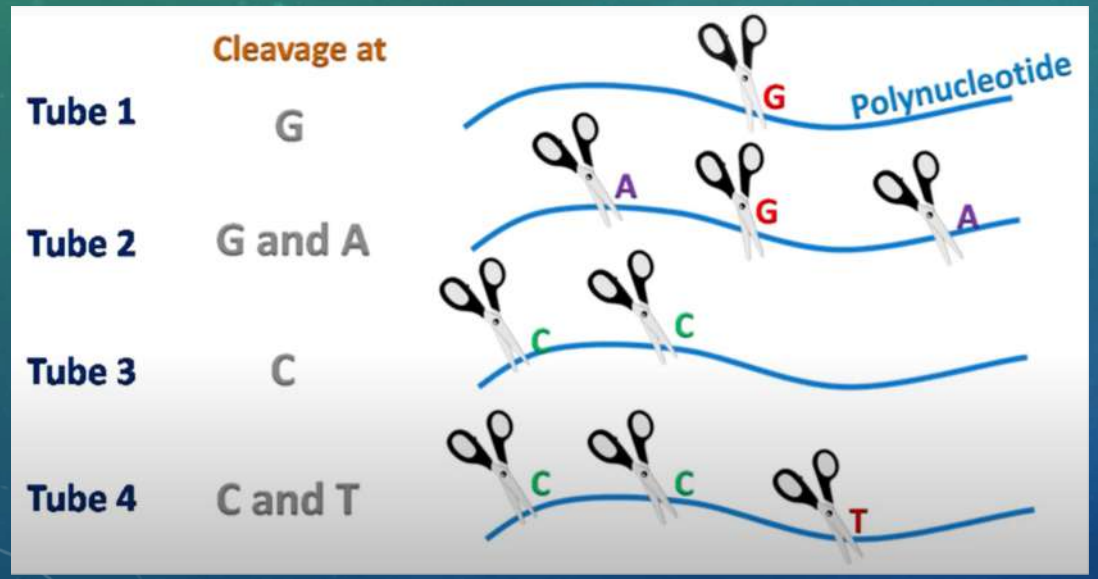
GENETİK KOD





1st base	2nd base								3rd base
	T		C		A		G		
T	TTT	Phe (F)	TCT	Ser (S)	TAT	Tyr (Y)	TGT	Cys (C)	T
	TTC		TCC		TAC		TGC		C
	TTA	Leu (L)	TCA		TAA	Ter (*)	TGA	Ter (*)	A
	TTG		TCG		TAG		TGG	Trp (W)	G
C	CTT		CCT	Pro (P)	CAT	His (H)	CGT	Arg (R)	T
	CTC		CCC		CAC		CGC		C
	CTA		CCA		CAA	Gln (Q)	CGA		A
	CTG		CCG		CAG		CGG		G
A	ATT	Ile (I)	ACT	Thr (T)	AAT	Asn (N)	AGT	Ser (S)	T
	ATC		ACC		AAC		AGC		C
	ATA		ACA		AAA	Lys (K)	AGA	Arg (R)	A
	ATG	Met (M)	ACG		AAG		AGG		G
G	GTT	Val (V)	GCT	Ala (A)	GAT	Asp (D)	GGT	Gly (G)	T
	GTC		GCC		GAC		GGC		C
	GTA		GCA		GAA	Glu (E)	GGA		A
	GTG		GCG		GAG		GGG		G

MAXAM GILBERT METODU



Maxam-Gilbert sequencing - modifications

TABLE 13.2 Chemical Modifications Used in the Maxam-Gilbert Method

Base	Specific modification[a]
G	Methylation of N_7 with dimethyl sulfate at pH 8.0 makes the C_8—C_9 bond specifically susceptible to cleavage by base
A + G	Piperidine formate at pH 2.0 weakens the glycosidic bond of adenine and guanine by protonating nitrogen atoms in the purine rings resulting in depurination
C + T	Hydrazine opens pyrimidine rings, which recyclize in a five-membered form that is susceptible to removal
C	In the presence of 1.5 M NaCl, only cytosine reacts appreciably with hydrazine
A > C	1.2 N NaOH at 90°C results in strong cleavage at A and weaker cleavage at C

[a] Hot (90°C) piperidine (1 M in H_2O) is used to cleave the sugar-phosphate chain of DNA at the sites of chemical modifications.

MAXAM GILBERT METODU

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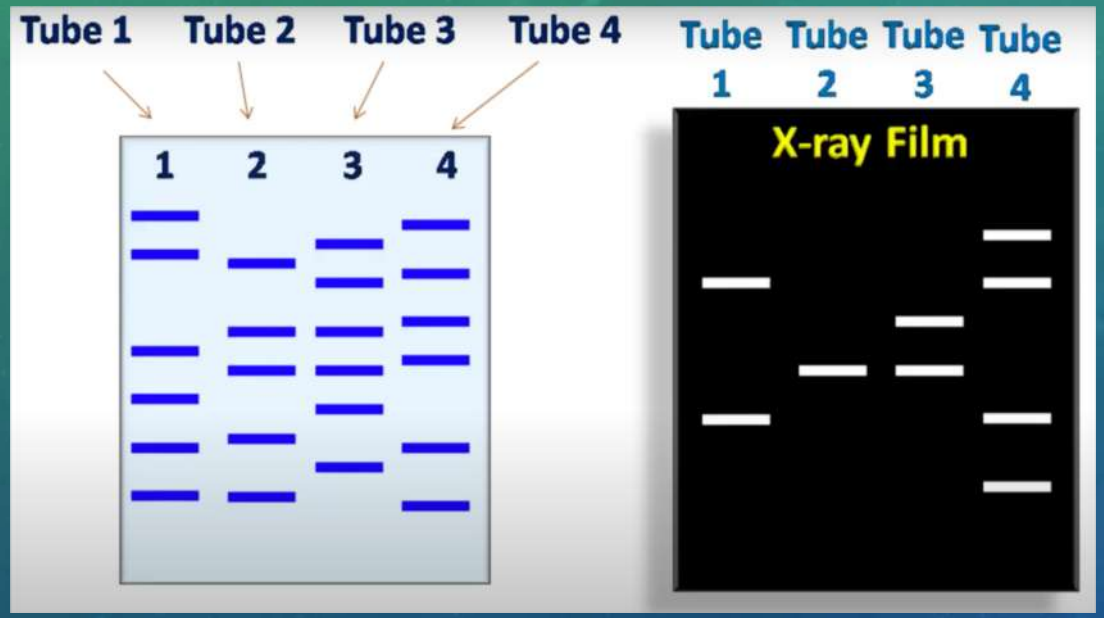
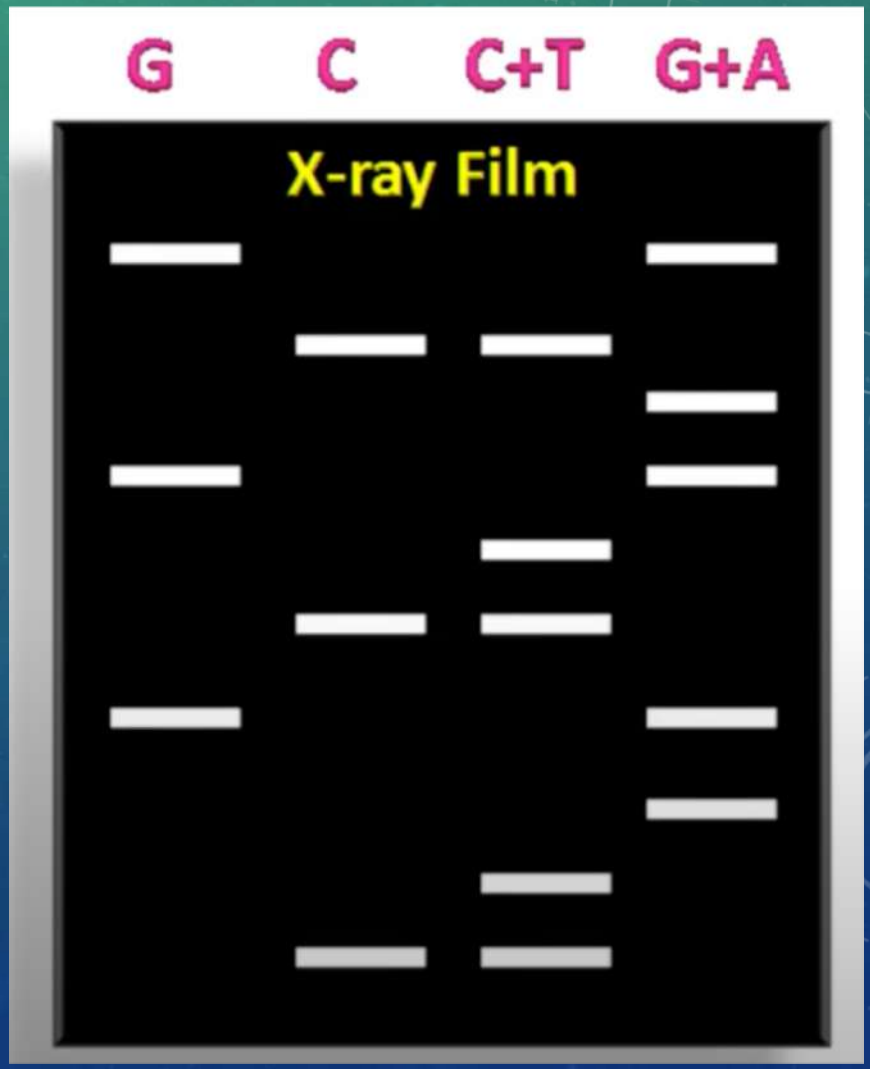
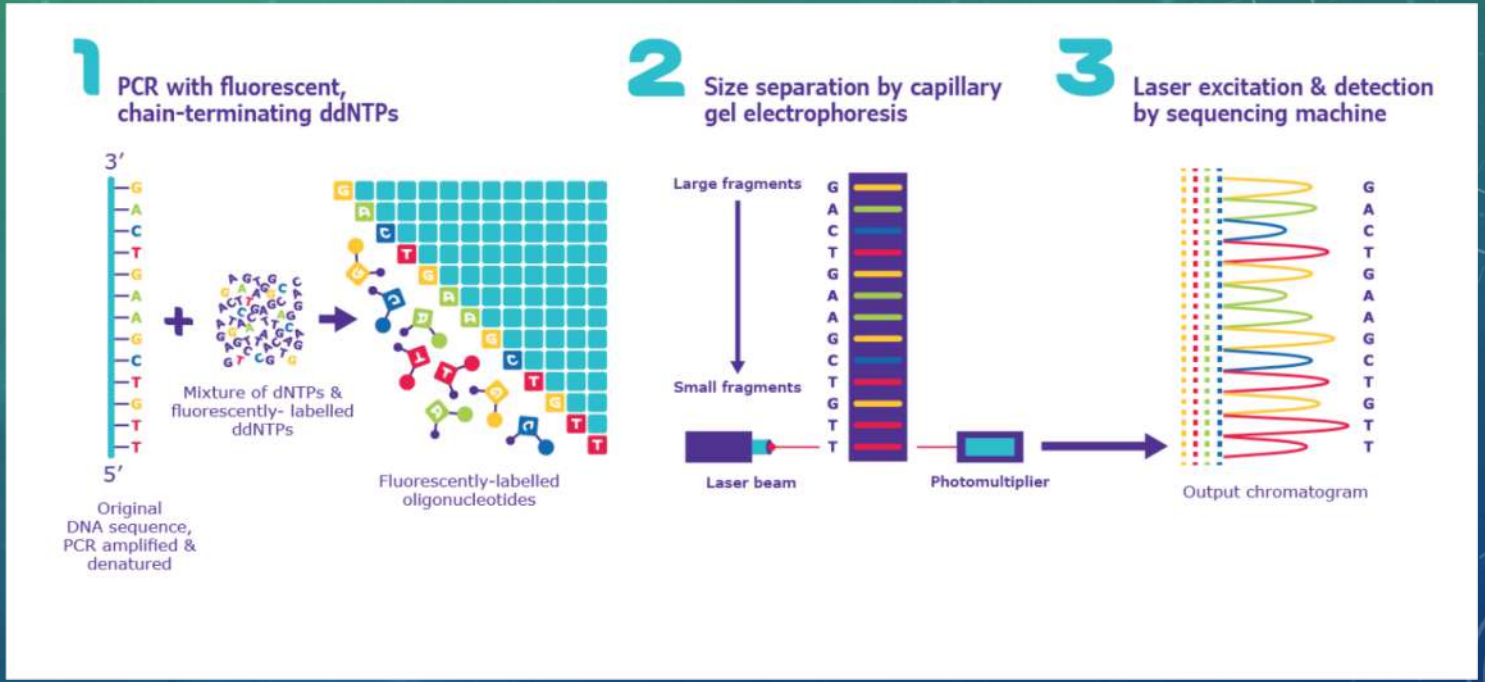


OTORADYOGRAFİ



SANGER SEKANSLAMA



Sanger demonstrated the power of his method by sequencing genomes of ever-increasing size, starting with a simple bacterial virus (5,386 nucleotides) in 1977, then the DNA in the mitochondria of human cells (16,569 nucleotides) in 1981 and, finally, the genome of a complex bacterial virus, bacteriophage lambda (48,502 nucleotides), in 1982.