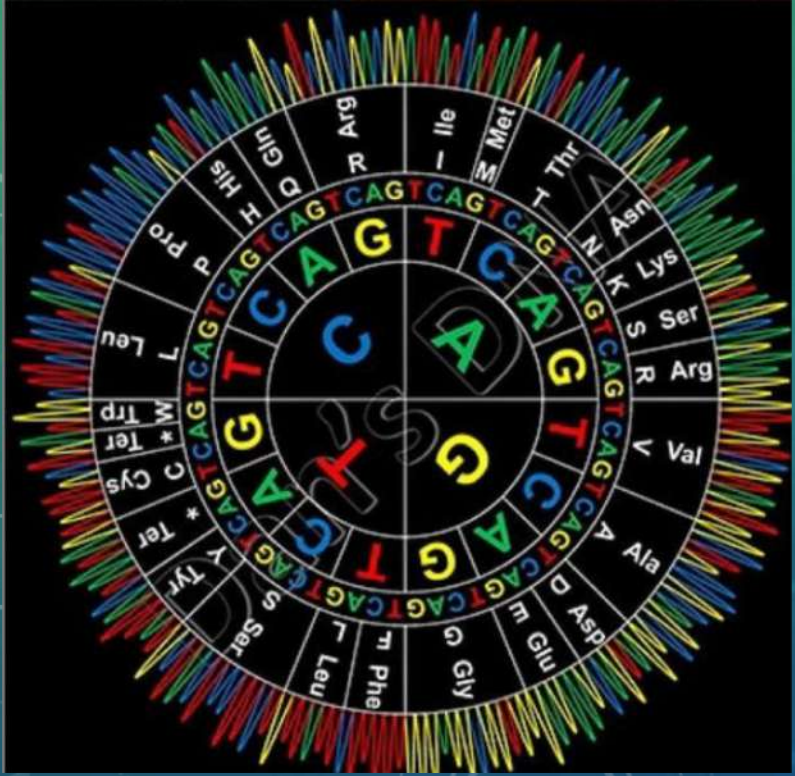


İLERİ ARAŞTIRMA YÖNTEMLERİ 56901007

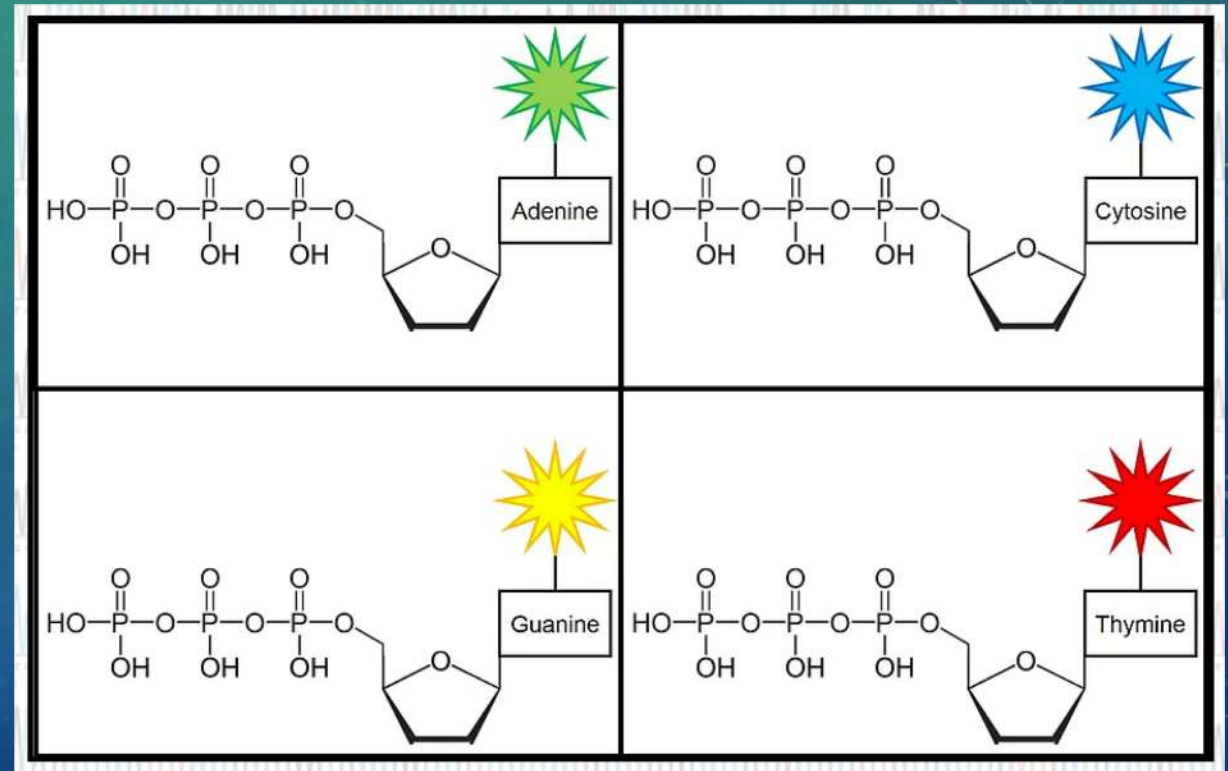
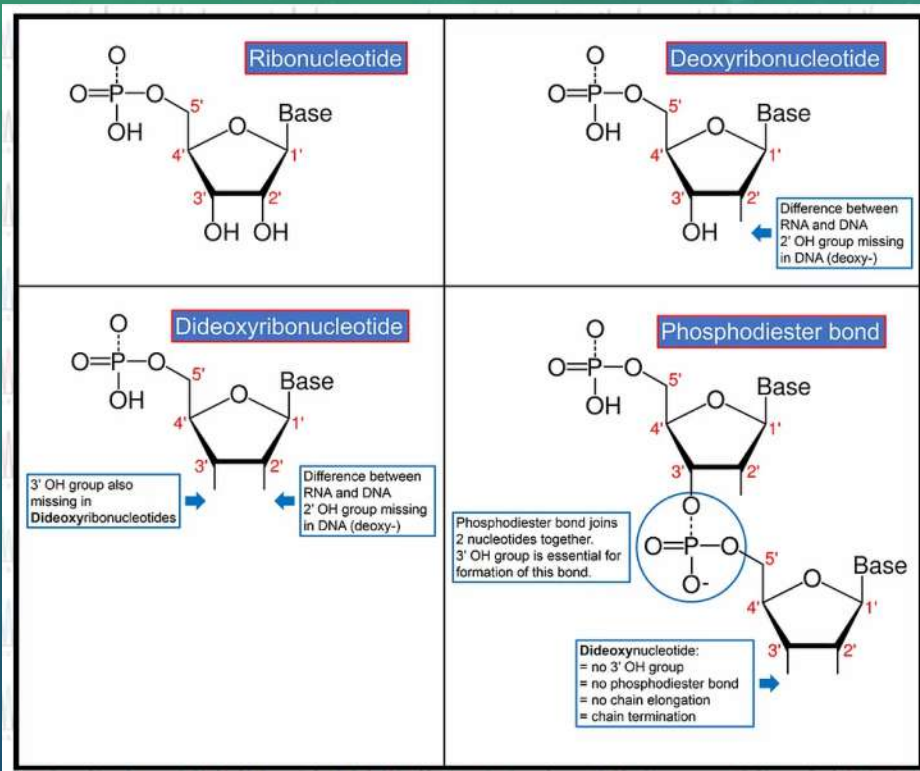
2020-21 BAHAR

DR. GÜNSELİ ÇUBUKÇUOĞLU DENİZ

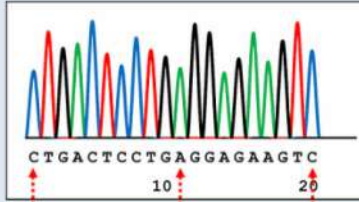


DNA SEKANS (DİZİLEME) YÖNTEMLERİ-2

SANGER SEKANS LAMA



3 Primer: short sequence (~20 bases)
 same sequence as forward strand
 - binds to reverse strand through
 complementary base pairing



8 DNA sequence chromatogram:
 read-out of fluorescence
 after capillary electrophoresis.
 Tells us the sequence of the DNA

Primer

5'-GACACCATGGTGCATC
 5'-GACACCATGGTGCATCT
 5'-GACACCATGGTGCATCTG
 5'-GACACCATGGTGCATCTGA
 5'-GACACCATGGTGCATCTGAC
 5'-GACACCATGGTGCATCTGACT
 5'-GACACCATGGTGCATCTGACTC
 5'-GACACCATGGTGCATCTGACTCC
 5'-GACACCATGGTGCATCTGACTCCT
 5'-GACACCATGGTGCATCTGACTCCTGA
 5'-GACACCATGGTGCATCTGACTCCTGAG
 5'-GACACCATGGTGCATCTGACTCCTGAGGA
 5'-GACACCATGGTGCATCTGACTCCTGAGGA
 5'-GACACCATGGTGCATCTGACTCCTGAGGAGA
 5'-GACACCATGGTGCATCTGACTCCTGAGGAGAA
 5'-GACACCATGGTGCATCTGACTCCTGAGGAGAAG
 5'-GACACCATGGTGCATCTGACTCCTGAGGAGAAGTC

|||||
 3'-CTGTGGTACCACGTAGACTGAGGACTCCTCTTCAG-5'

6 Small DNA fragments:
 run quickly through capillary tube

4 DNA polymerase makes new copies
 of forward strand by adding
 bases to 3'-end of primer.
 Occasionally "dideoxy" bases are
 added that terminate the DNA chain

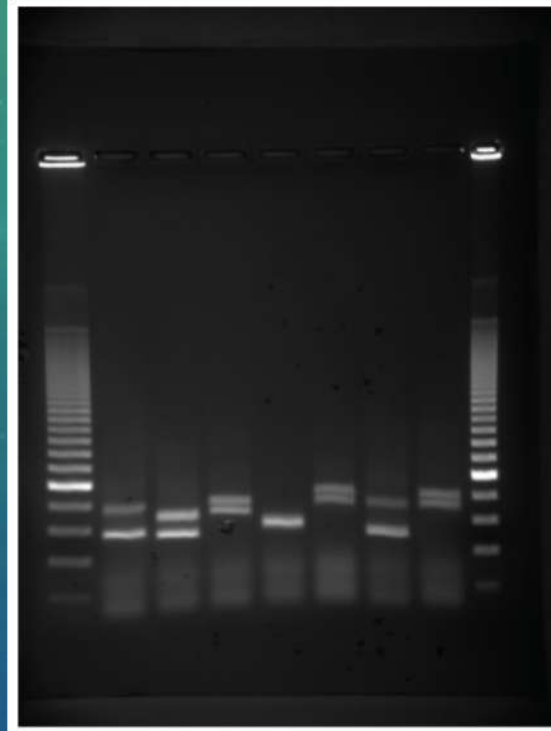
5 DNA fragments extended from primer.
 Many different sizes terminated
 by a fluorescent "dideoxy" base

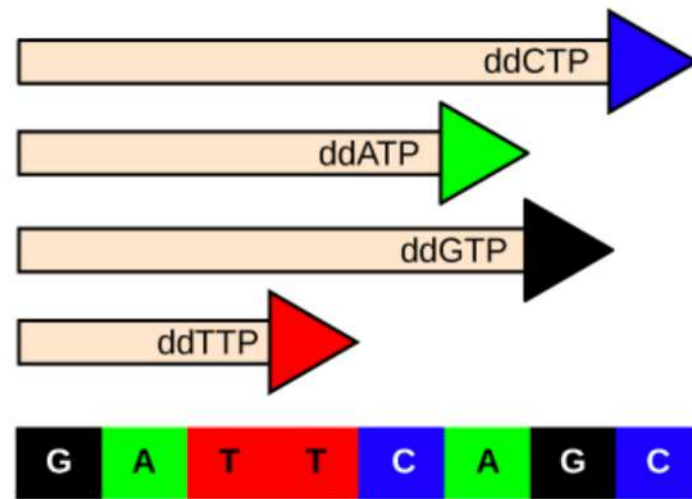
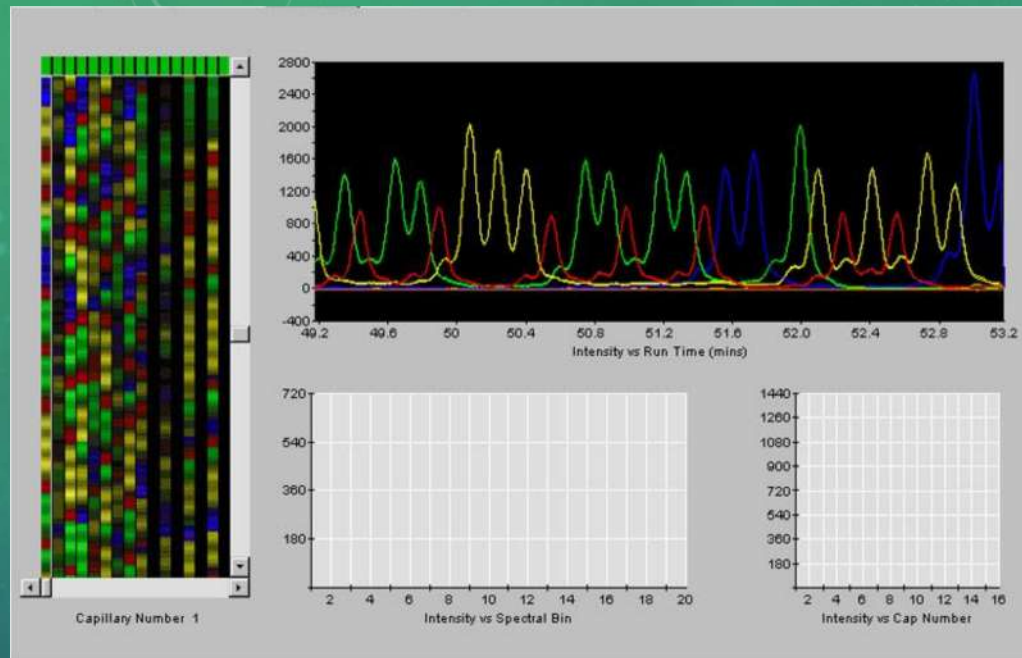
7 Longer DNA fragments:
 can be up to 900 bases long:
 run slowly through capillary tube

2 Reverse strand acts as template
 to make new copies of forward strand

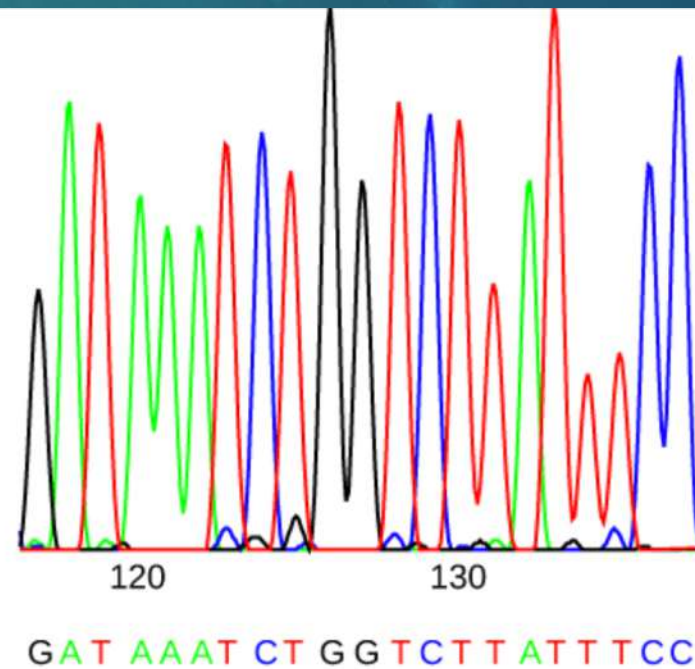
5'-GACACCATGGTGCATCTGACTCCTGAGGAGAAGTC-3' -forward strand
 |||||
 3'-CTGTGGTACCACGTAGACTGAGGACTCCTCTTCAG-5' -reverse strand

1 Double stranded DNA molecule to be sequenced.
 Contains 2 anti-parallel strands held together
 by complimentary base pairs: A=T and C≡G

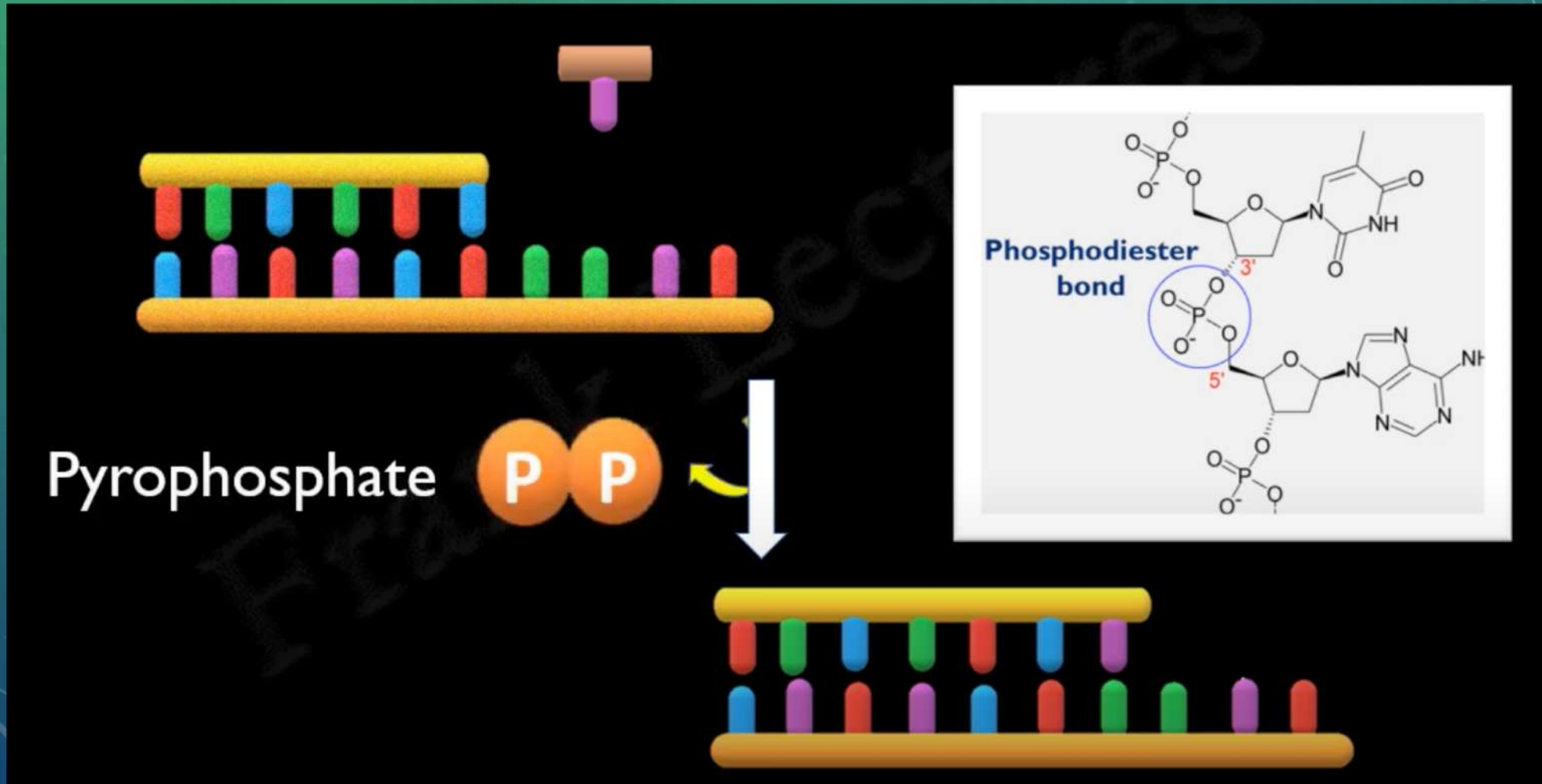




Dye-labeled dideoxynucleotides are used to generate DNA fragments of different lengths



PIROSEKANSLAMA



PIROSEKANSLAMA

