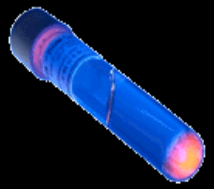
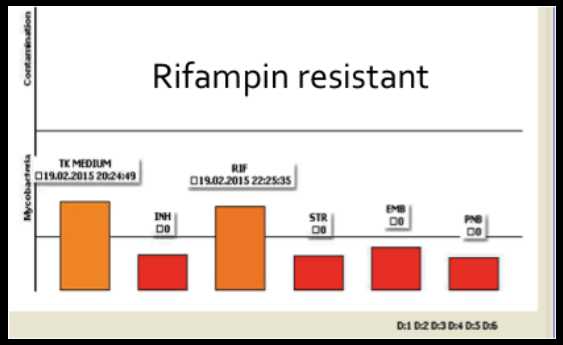
Toplam DNA Dizisi Saptama Yöntemleri ile Mikobakterilerde Hızlı İlaç Direnci Belirlenmesi

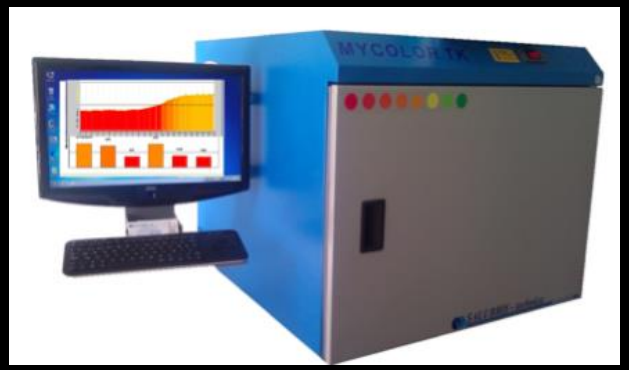
Tanıl Kocagöz

Mikobakterilerde ilaç direnci saptama: Kültür

- Kültür yöntemleri: Evrenseldir. İlaç hedefi, direnç mekanizması bilinmese bile uygulanabilir. Uzun sürede sonuç verir.

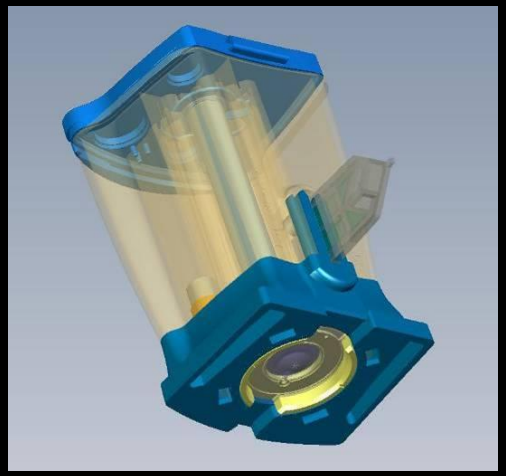


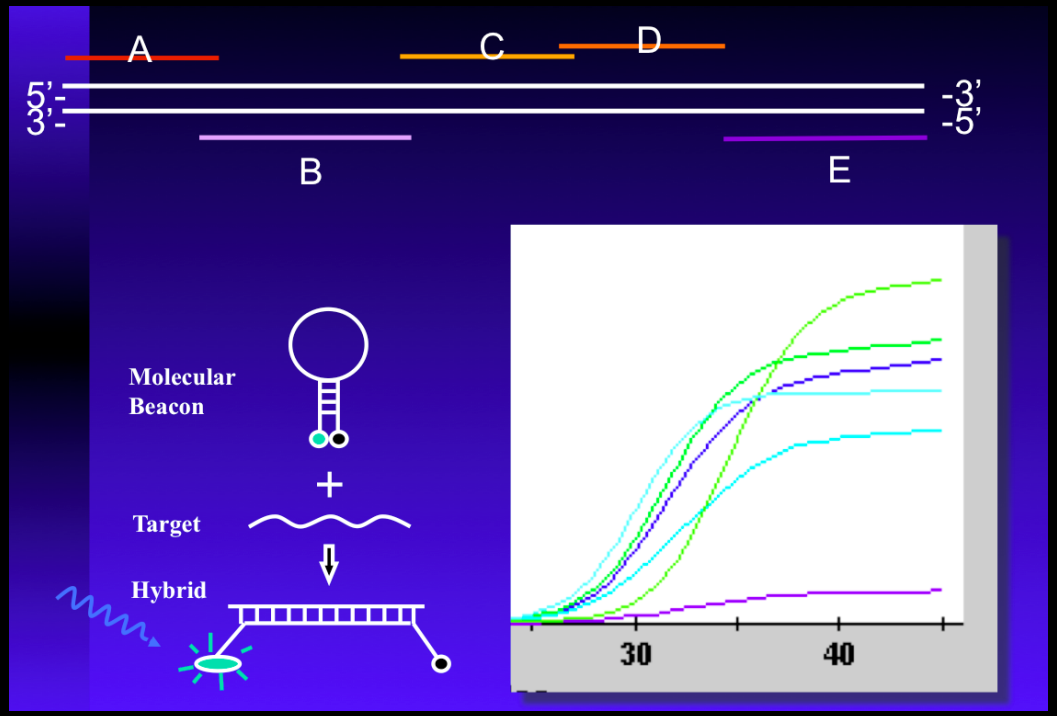




Nükleit asit çoğaltma ve mutasyon saptama yöntemleri

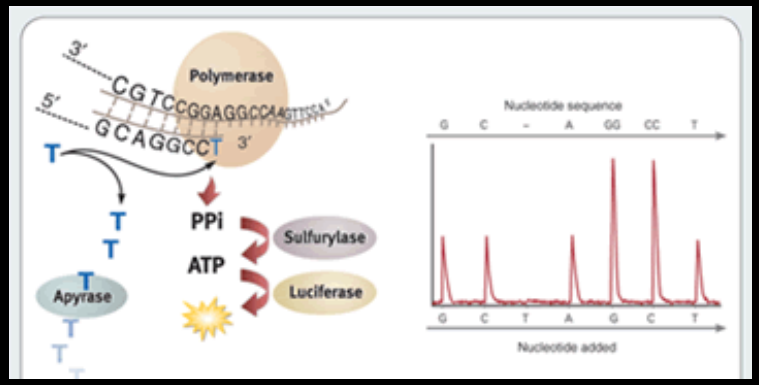
- İlaç direncine yol açtığı bilinen mutasyonların bulunduğu bölgeye bağlanan özgül proplar ile saptama. İlaçlara dirençli tüm suşlar saptanamıyor. (Örneğin Xpert rpoB I491F mutasyonunu saptayamıyor.) Polimorfizimler, duyarlı bakterilerin hatalı olarak dirençli olduğu sonucunu verebiliyor.
- Pahalı yöntemler.

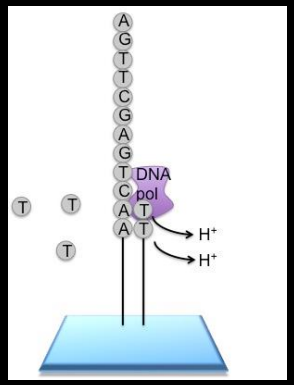


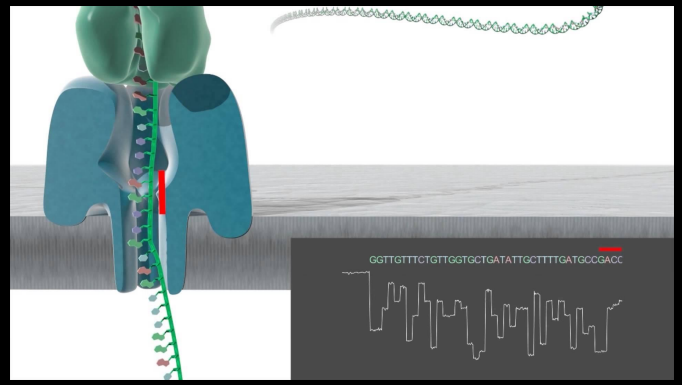


Toplam DNA dizisi saptama yöntemleri

- İlaç direncine yol açan tüm mutasyonları saptayabilir
- İlaç direncine yol açtığı daha önce bilinmeyen mutasyonları ortaya çıkartabilir
- Epidemiyolojik veriler de sağlıyor
- Hala çok pahalı ve zor yöntemler







DNA dizisi saptama yöntemleri

Allan Maxam

Walter Gilbert

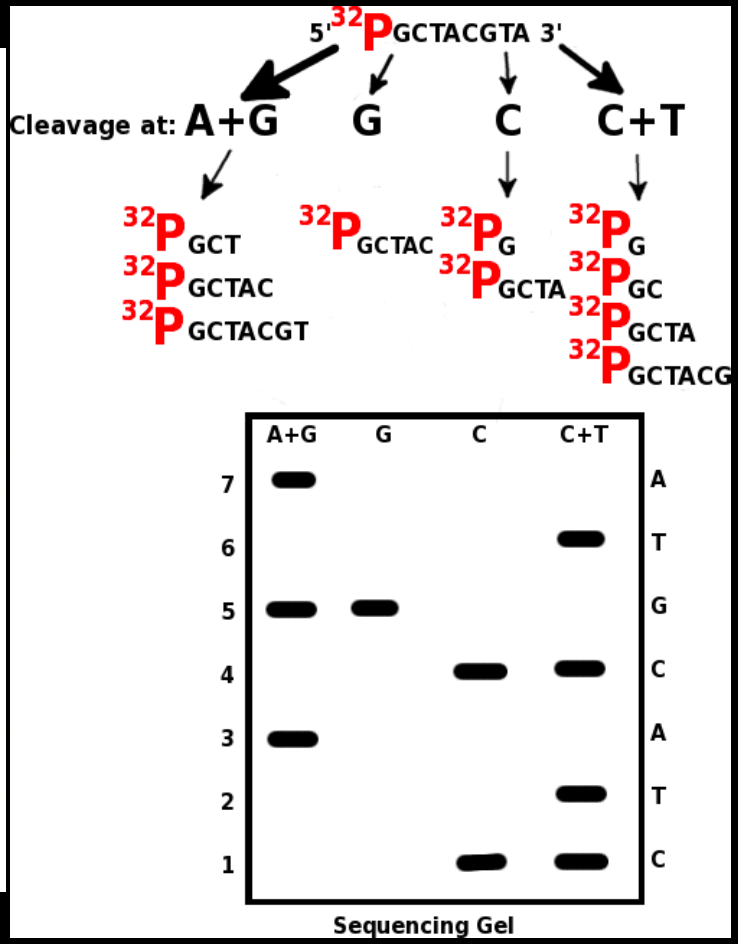
Maxam Gilbert Kimyasal Kırılma Yöntemi

Maxam-Gilbert sequencing - modifications

TABLE 13.2 Chemical Modifications Used in the Maxam-Gilbert Method

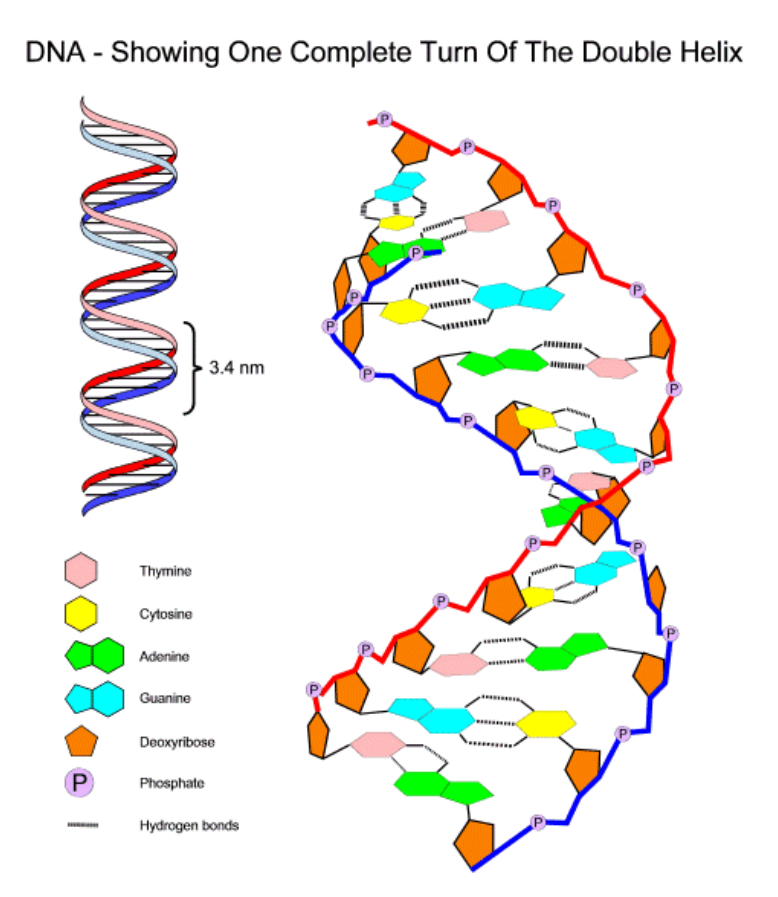
Base	Specific modification[a]
G	Methylation of N_7 with dimethyl sulfate at pH 8.0 makes the C_8—C_9 bond specifically susceptible to cleavage by base
A + G	Piperidine formate at pH 2.0 weakens the glycosidic bond of adenine and guanine by protonating nitrogen atoms in the purine rings resulting in depurination
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

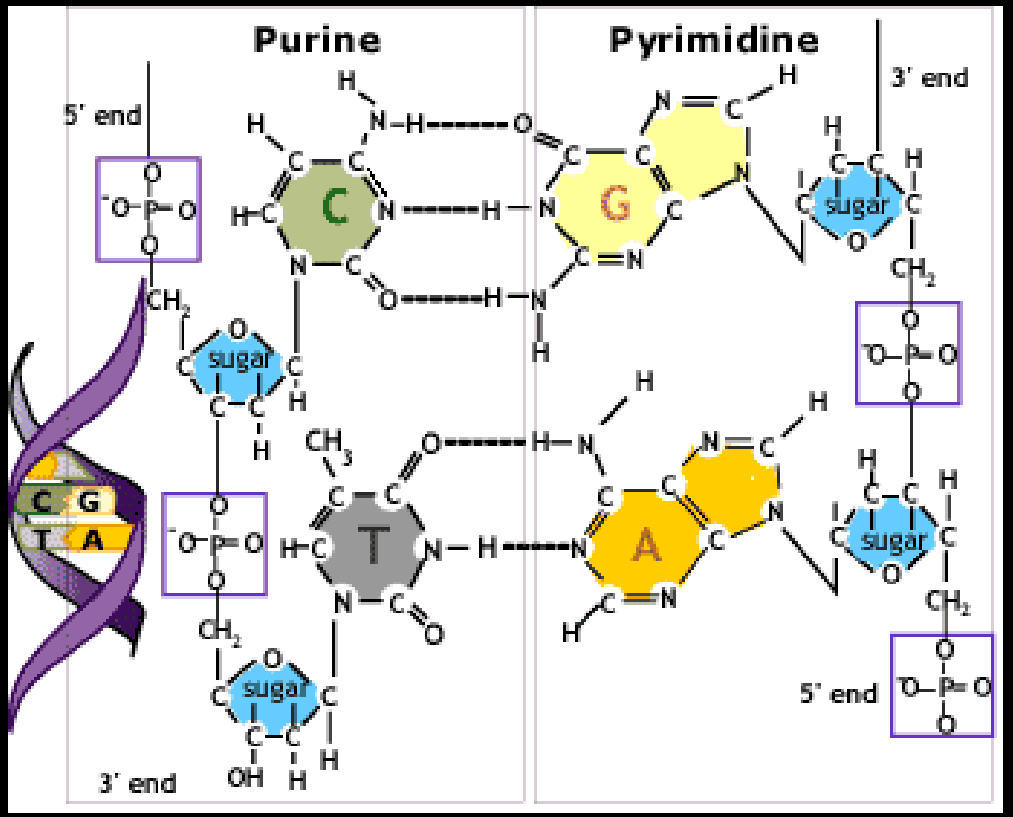
[a] Hot (90°C) piperidine (1 M in H_2O) is used to cleave the sugar-phosphate chain of DNA at the sites of chemical modifications.



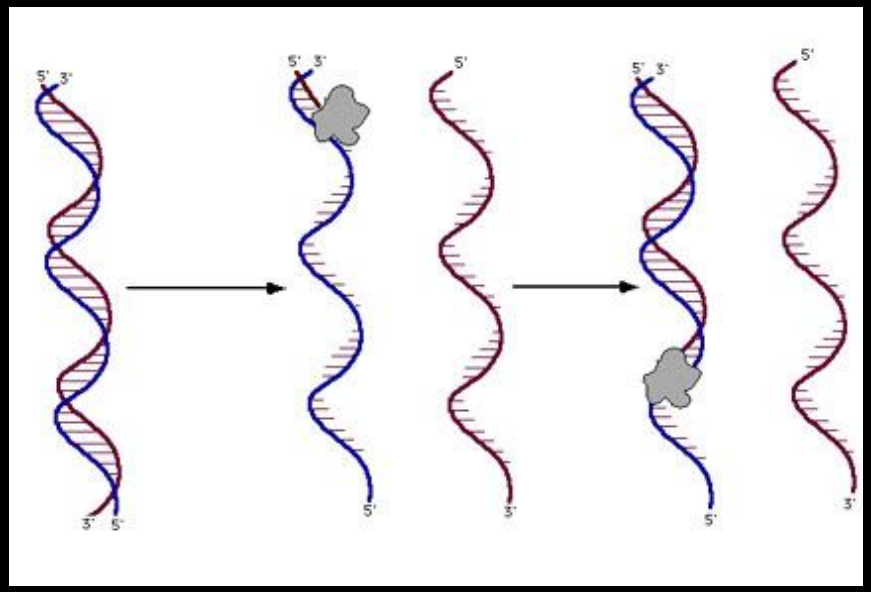
Fred Sanger

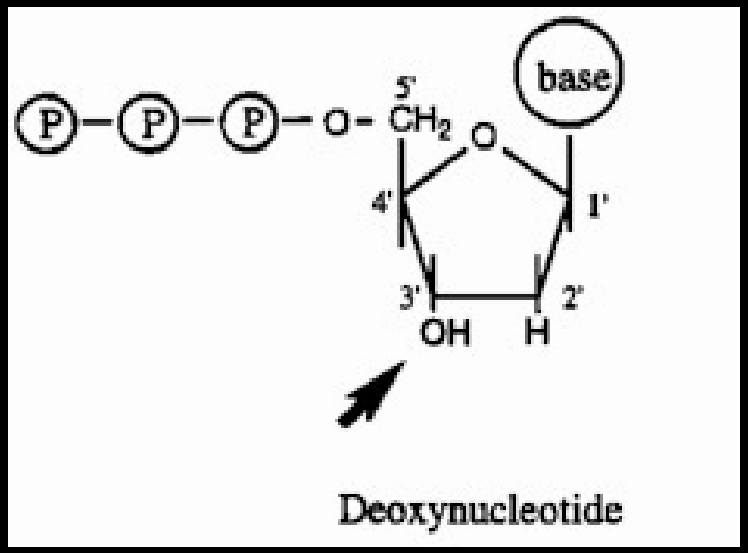
DNA’daki Şeker Fosfat Bağları

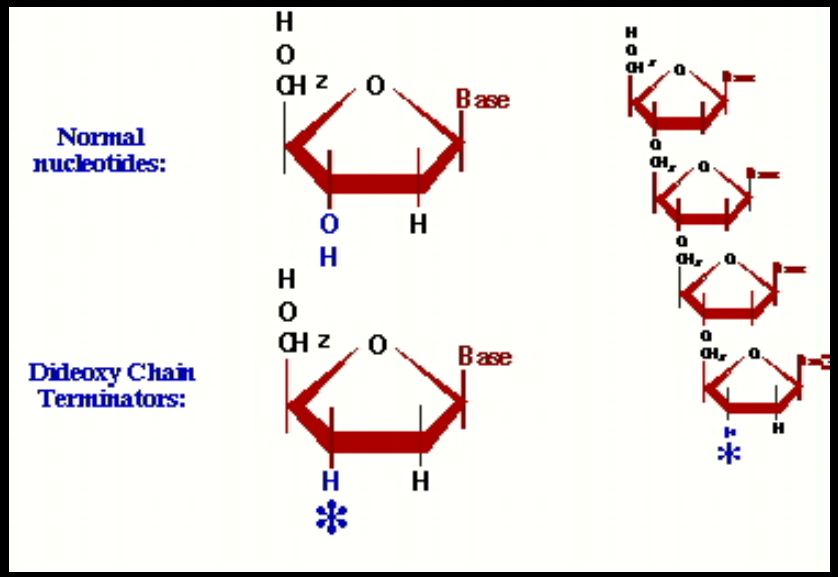


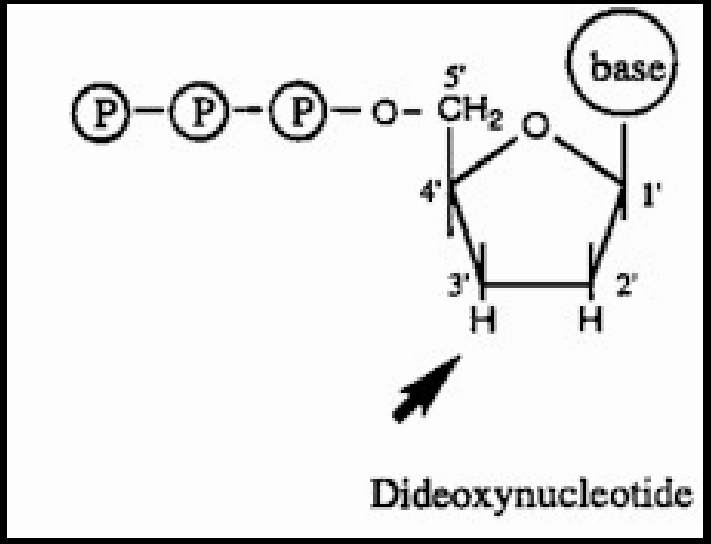


Sanger Zincir Sonlandırma Yöntemi

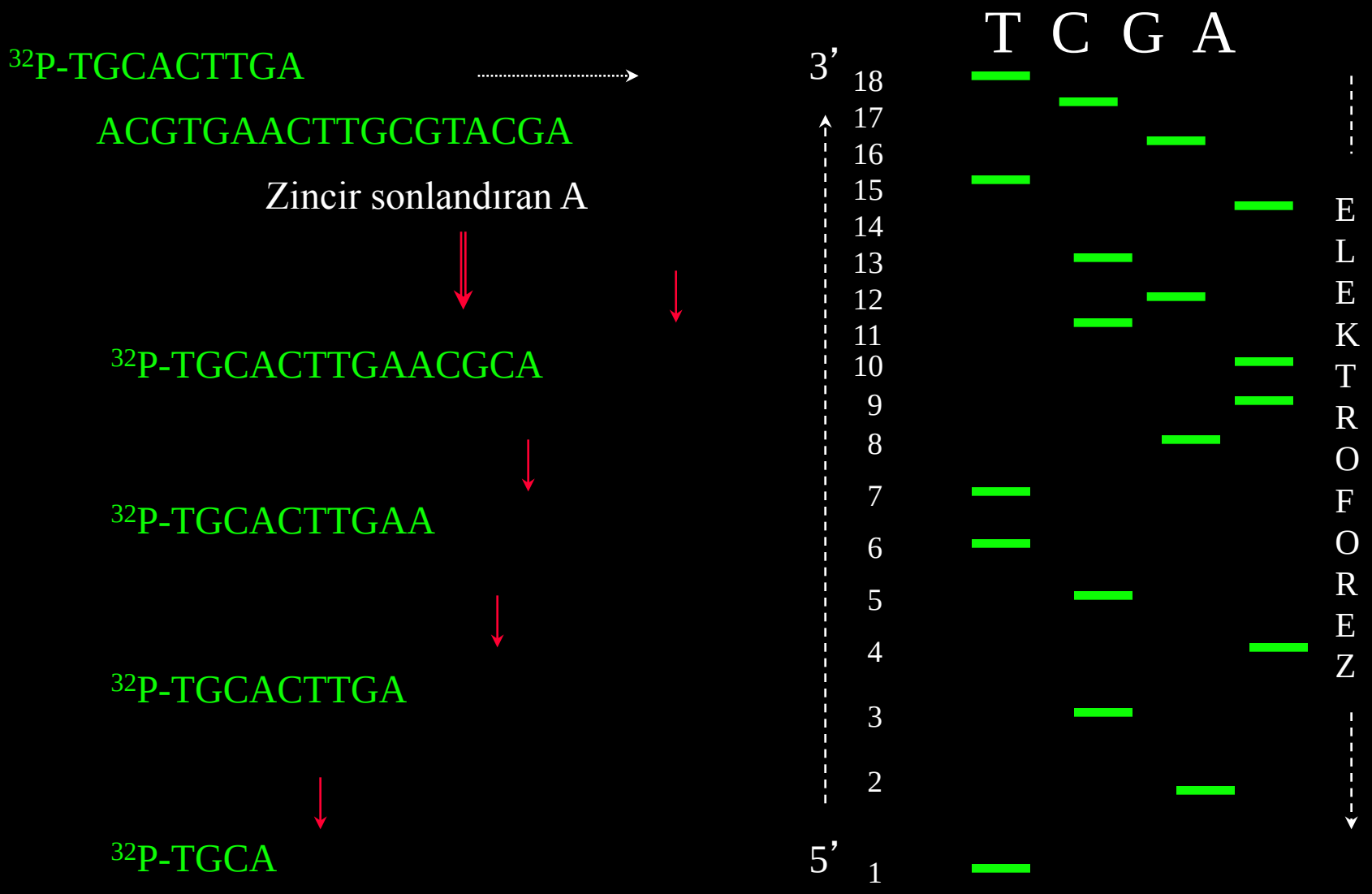




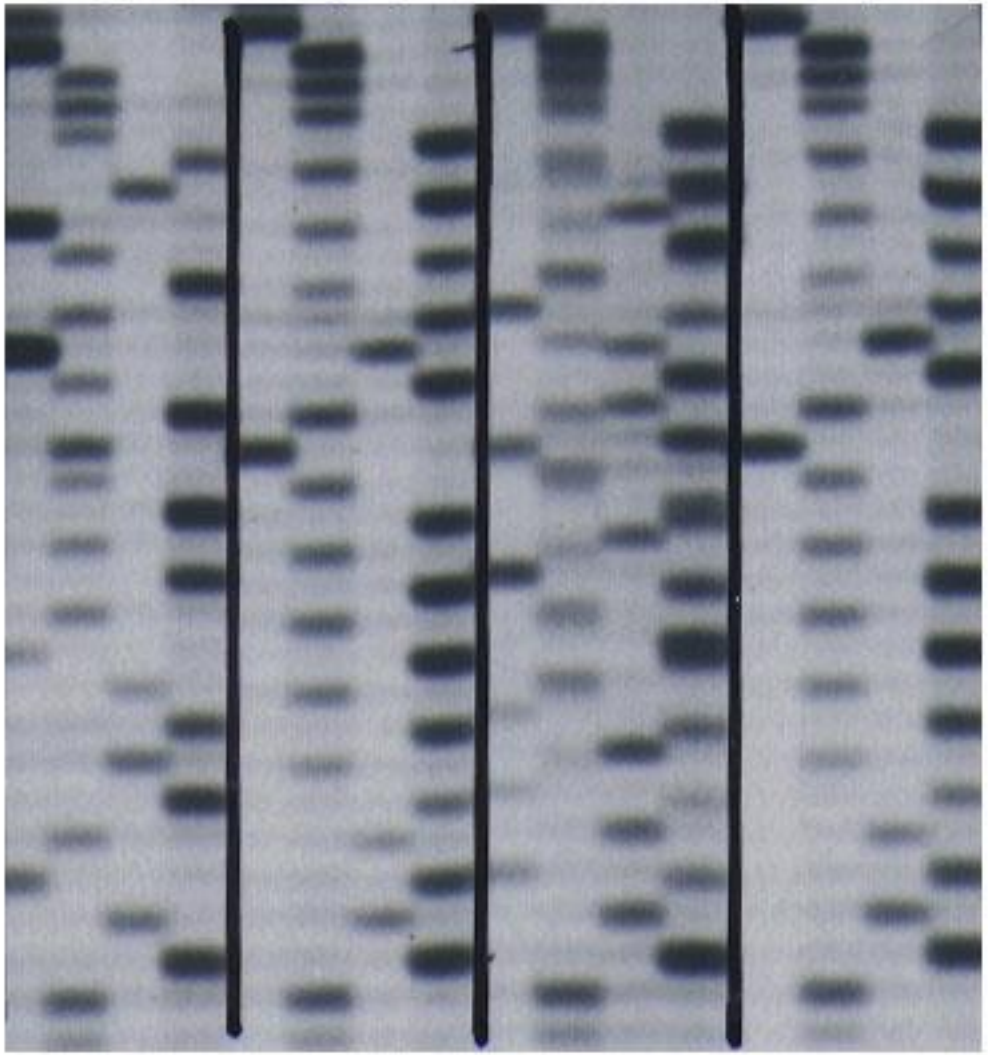




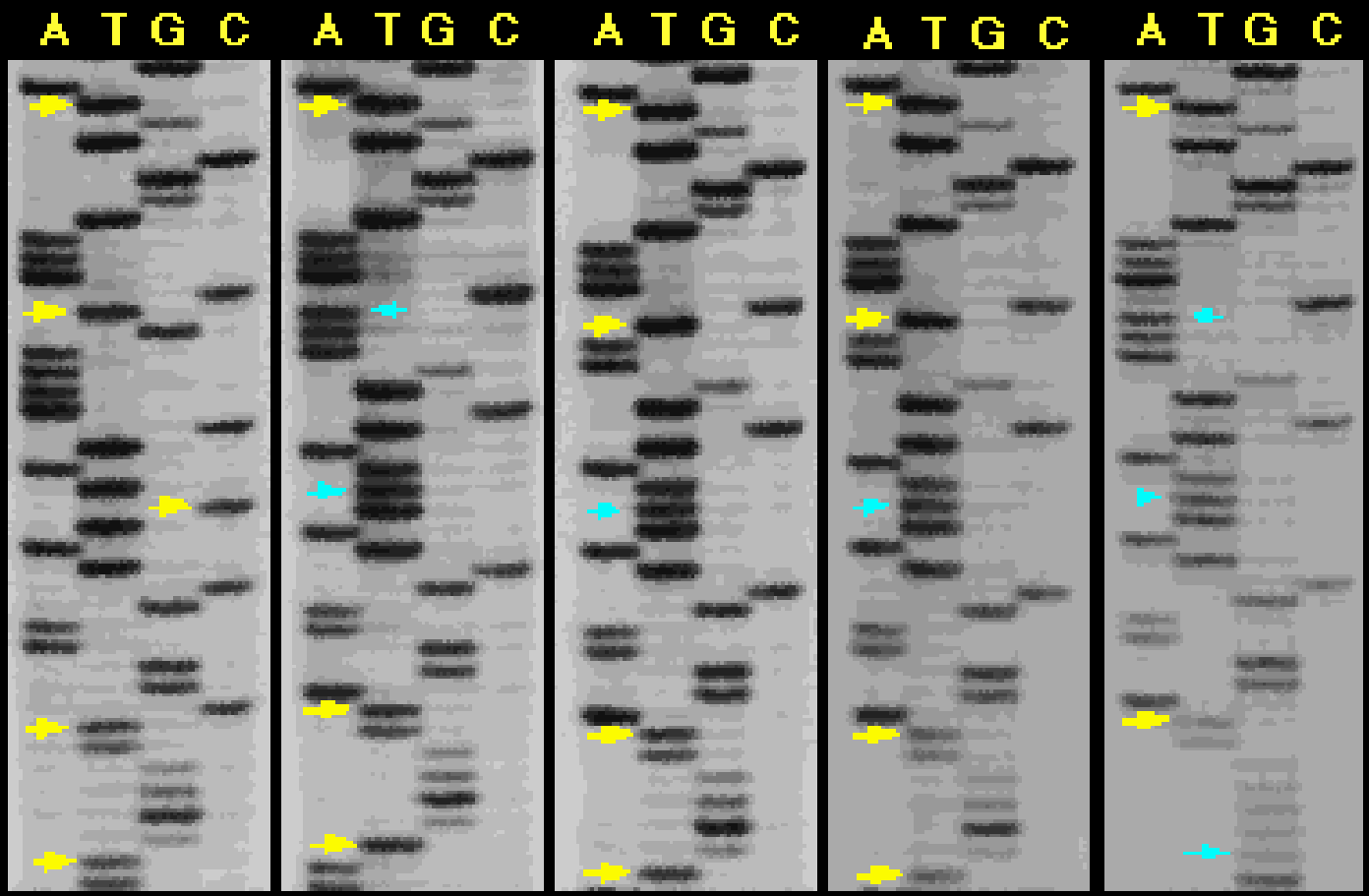
Sanger Zincir Sonlandırma Yöntemi



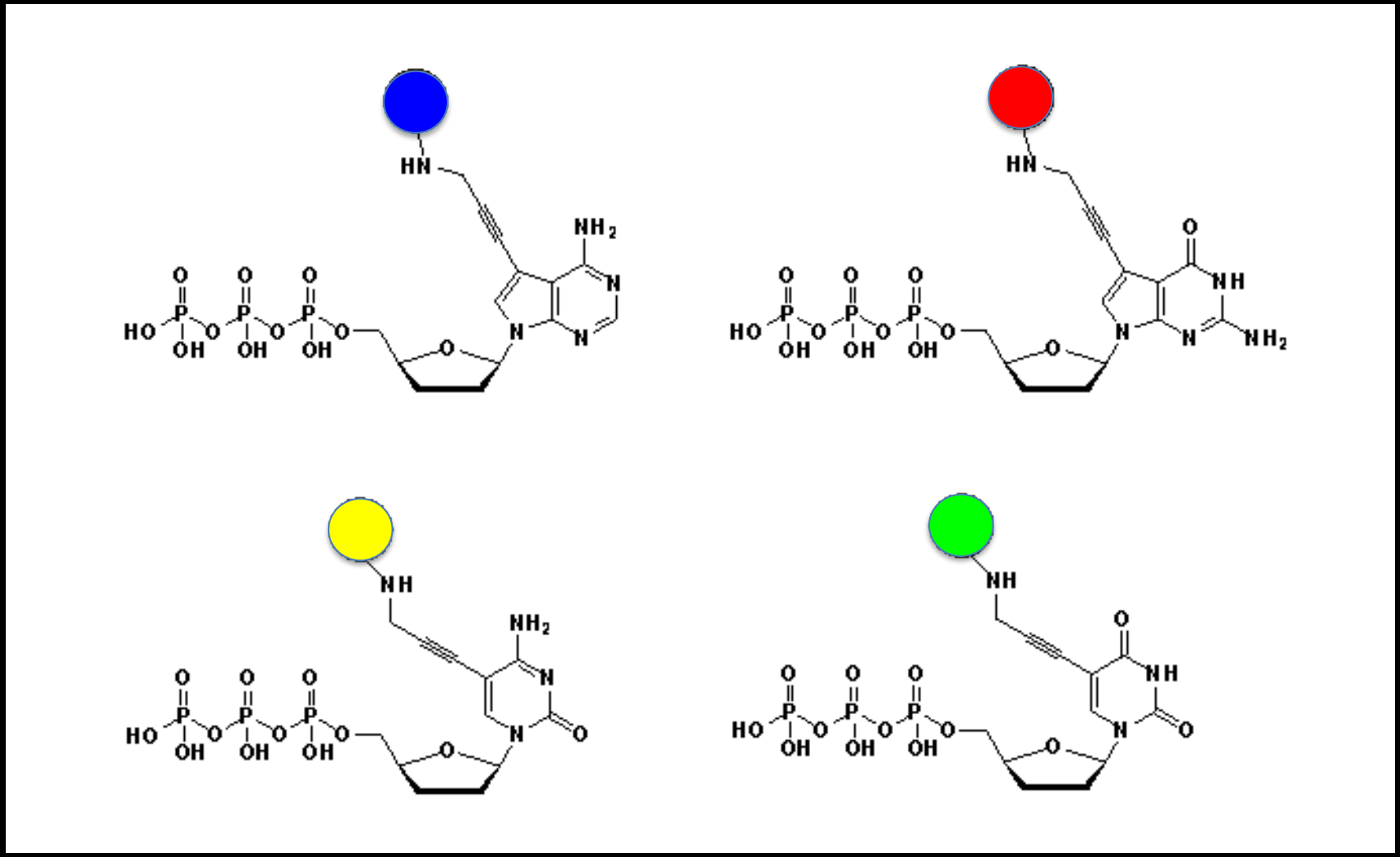

KIRAN
X-Ray Cassette
10x12


1
2
3
4
ctag

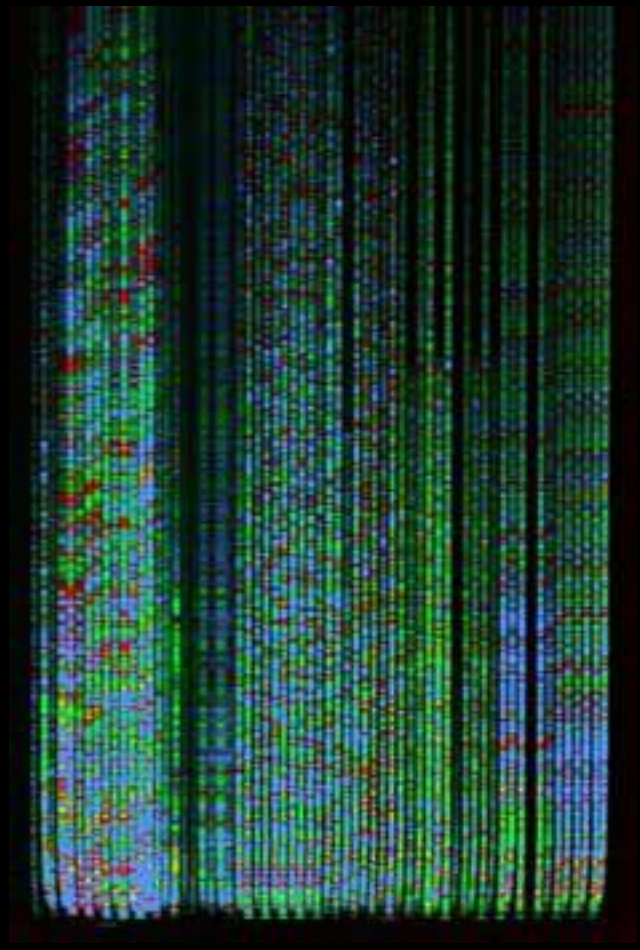
DNA Dizisi Saptama Jelinin Radyogramı

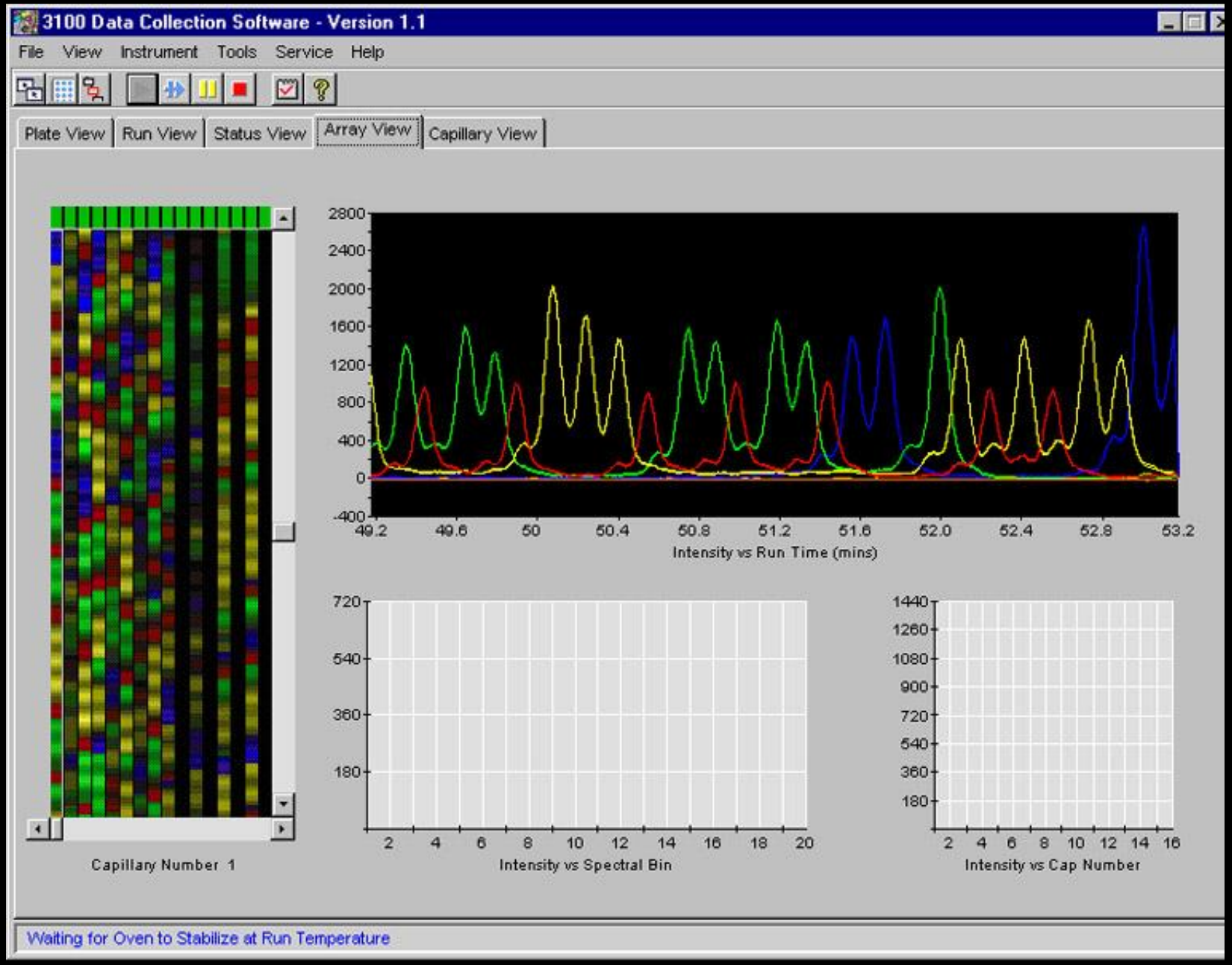


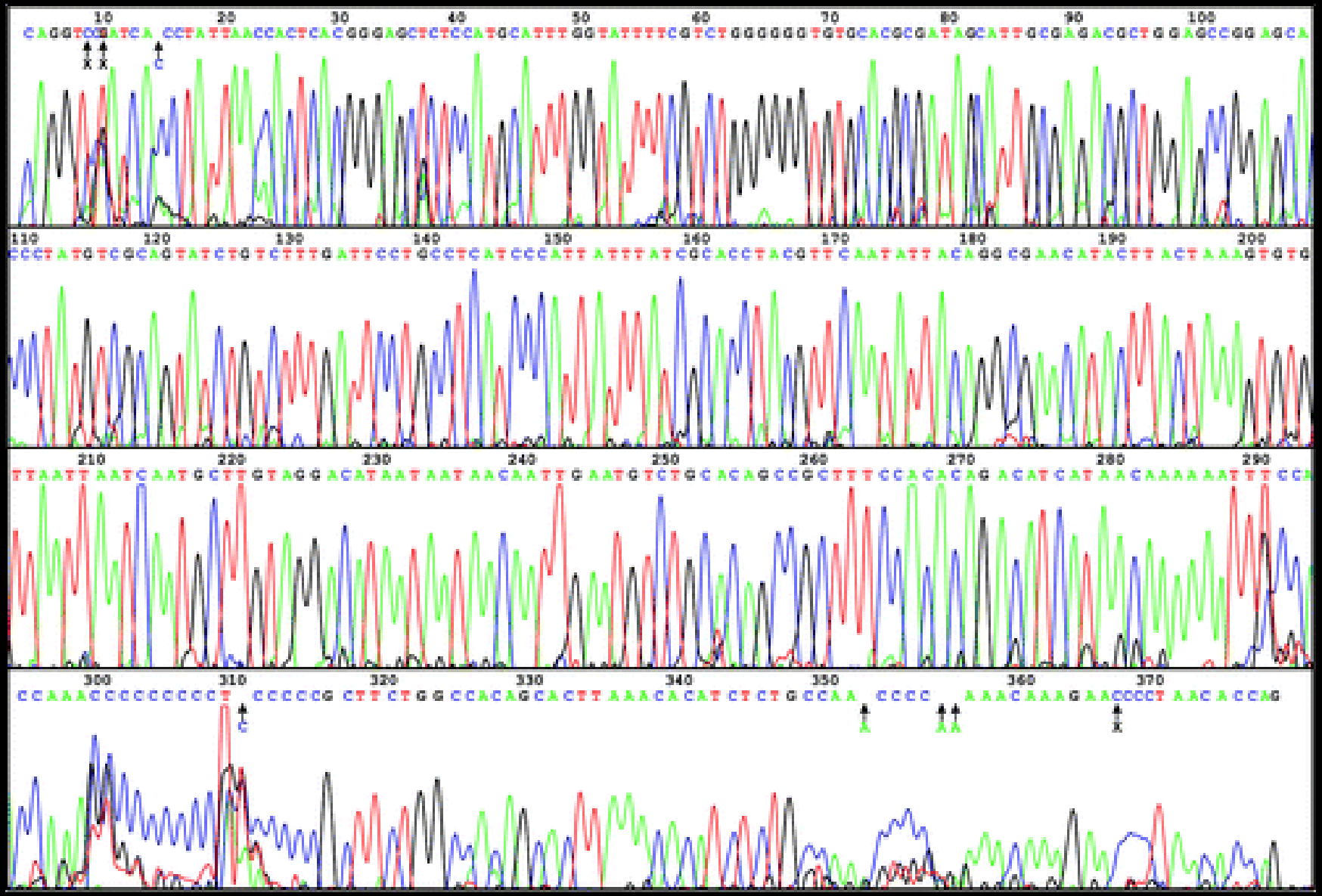
Zincir sonlandıran floresan boya ile işaretli dideoksi-nükleotitler



Kapiler Elektroforez



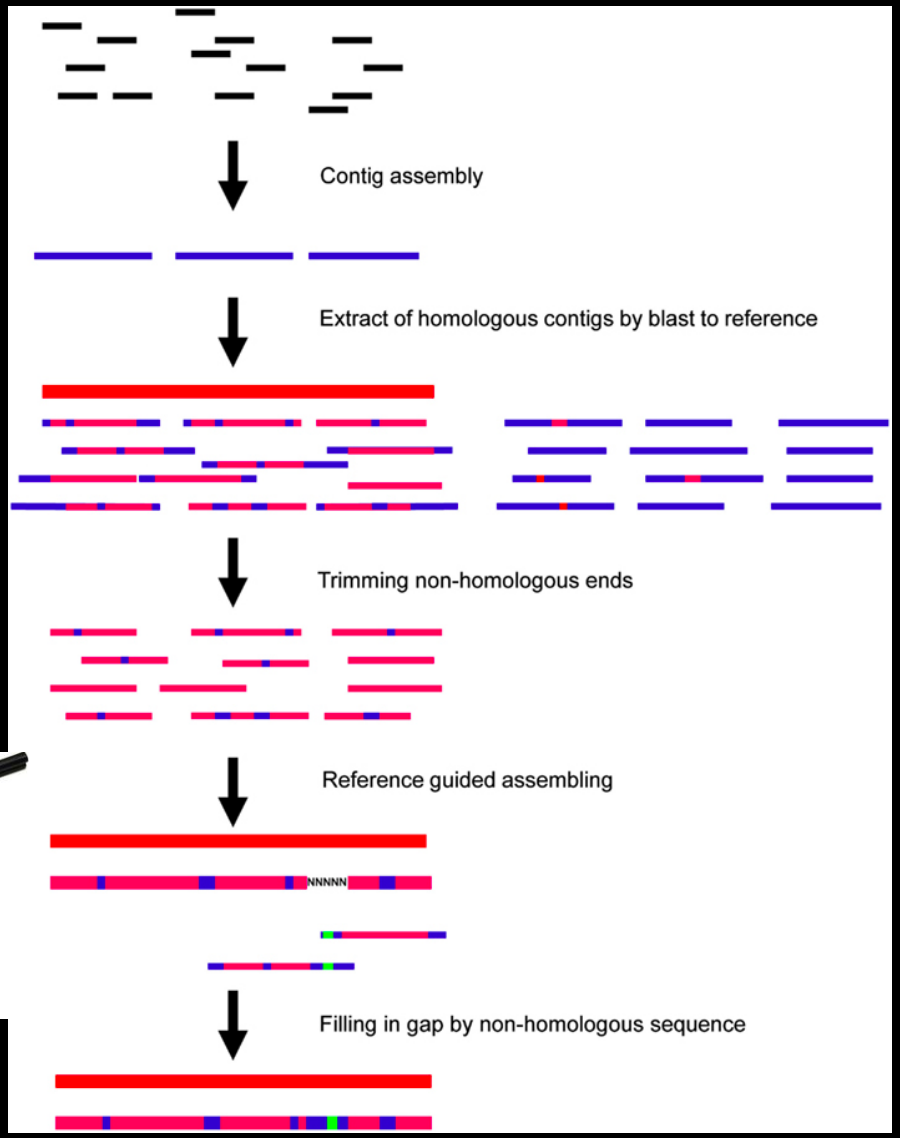

3100 Data Collection Software - Version 1.1
File View Instrument Tools Service Help
Plate View Run View Status View Array View Capillary View
2800 2400 2000 1600 1200 800 400 0 -400
49.2 49.6 50 50.4 50.8 51.2 51.6 52.0 52.4 52.8 53.2
Intensity vs Run Time (mins)
Capillary Number 1
720 540 360 180
2 4 6 8 10 12 14 16 18 20
Intensity vs Spectral Bin
1440 1260 1080 900 720 540 360 180
2 4 6 8 10 12 14 16
Intensity vs Cap Number
Waiting for Oven to Stabilize at Run Temperature



Toplam DNA Dizisi Saptama (Next Generation Sequencing)

"Shot gun sequencing"

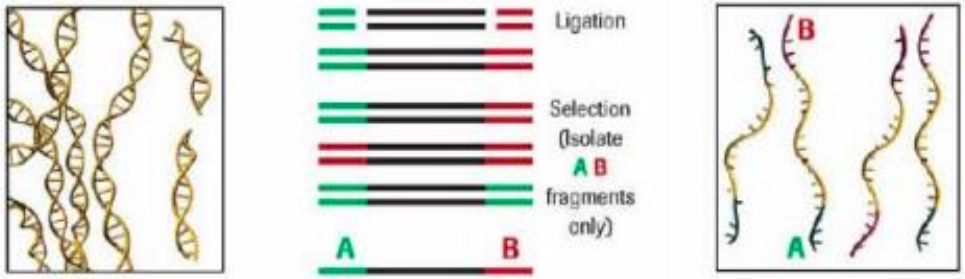
Rastgele atış dizileme



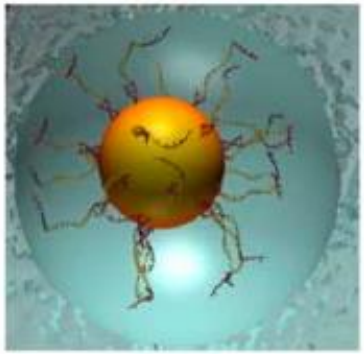
"Delikli demir çıktı Mertlik bozuldu"

Köroğlu

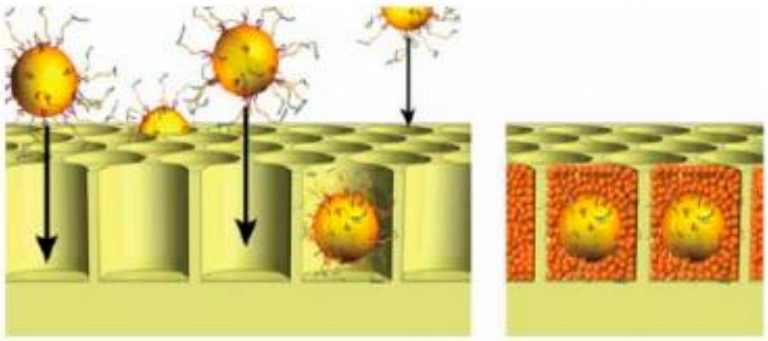
Pirodizileme



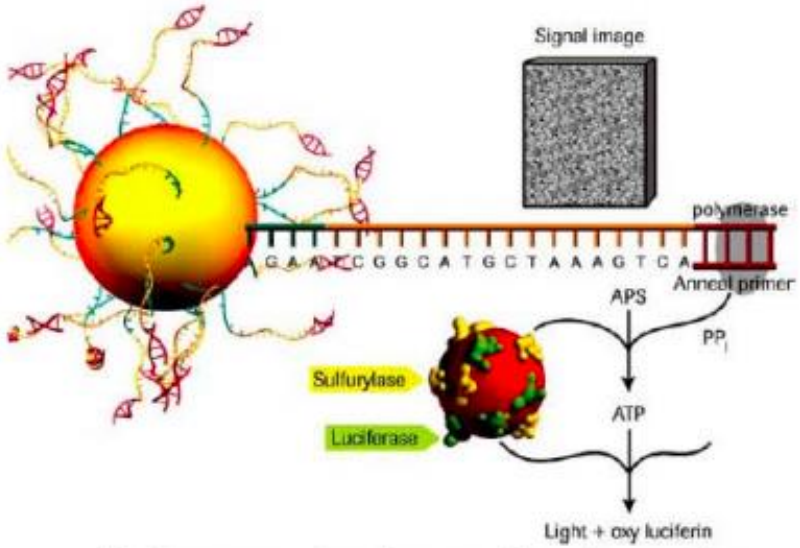
1) Prepare Adapter Ligated ssDNA Library



2) EmPCR: Clonal Amplification on beads

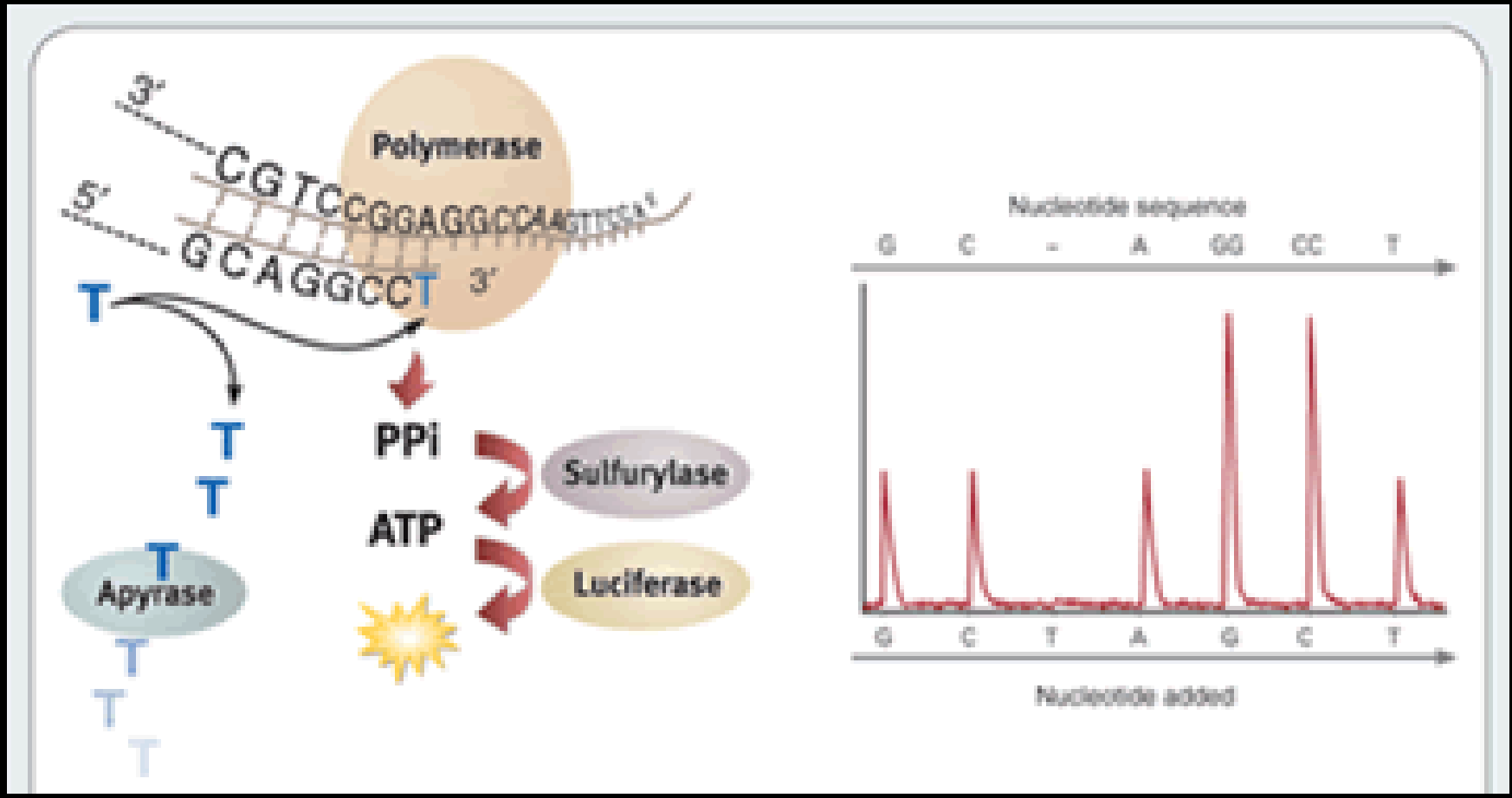


3) Load beads and enzymes in PicoTiter Plate™

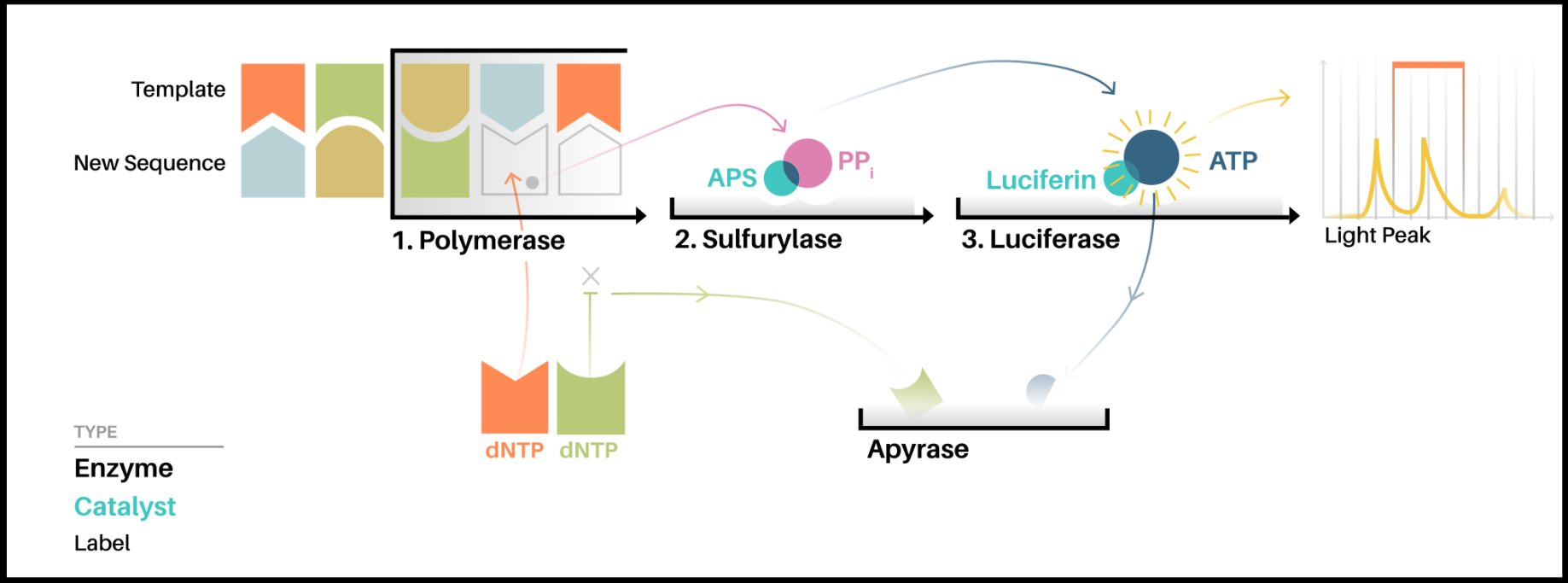


4) Sequencing-by-synthesis on the GS 20 Sequencer

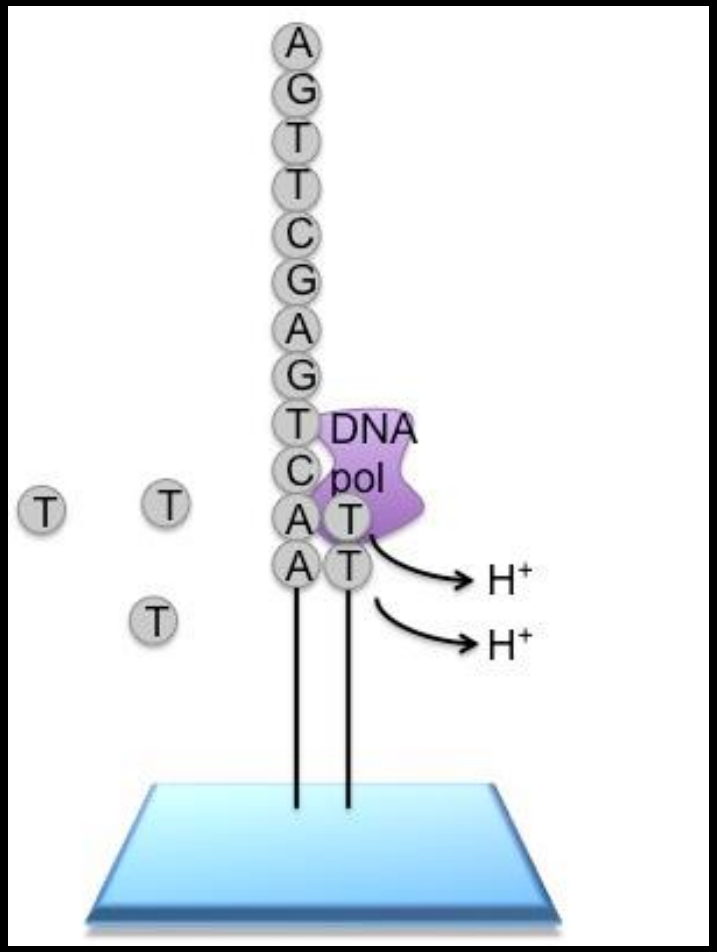
Pirodizileme

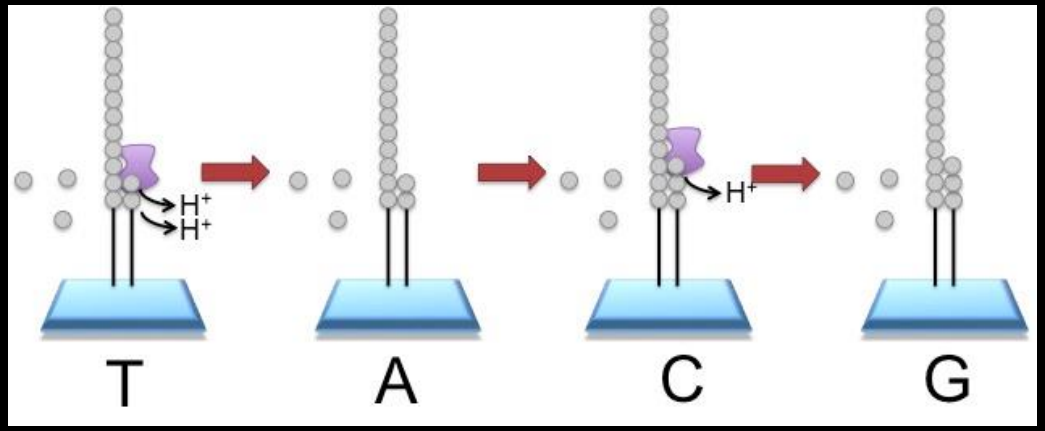


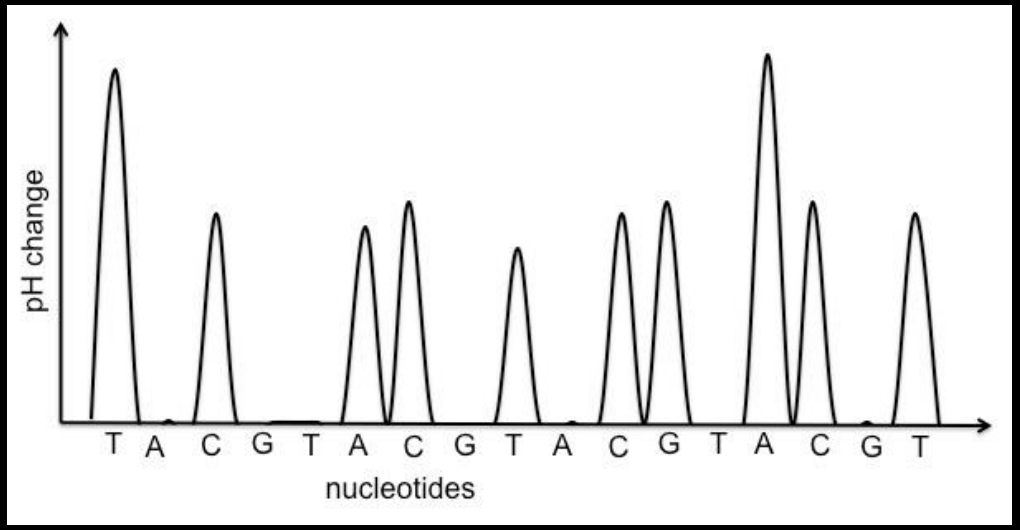
Pirodizileme



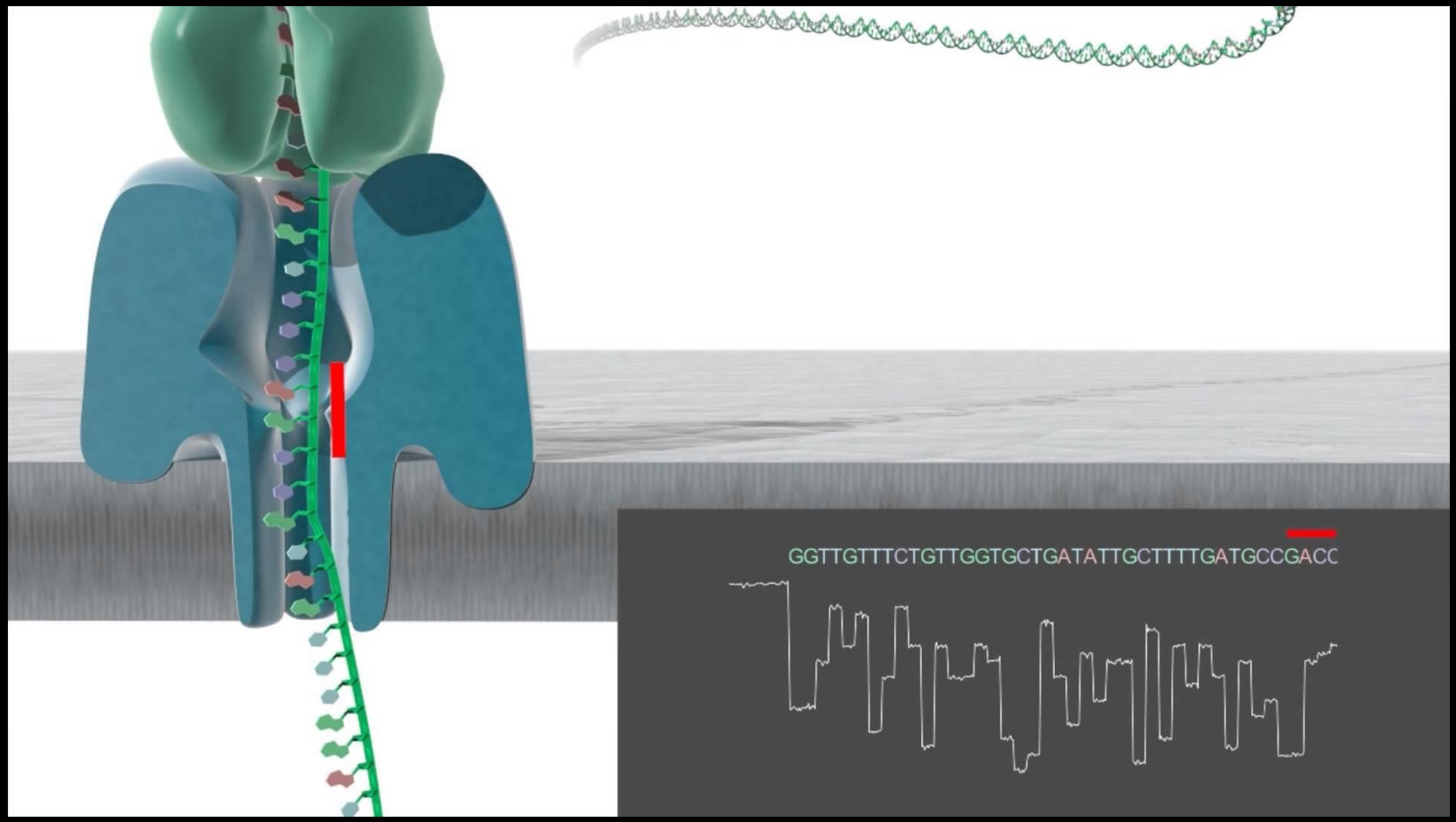
İyona duyarlı dizi incelemesi







Nanopore Dizileme




SpotON
AlinION

JCM Accepted Manuscript Posted Online 8 March 2017
J. Clin. Microbiol. doi:10.1128/JCM.02483-16

Title: Same-day diagnostic and surveillance data for tuberculosis via whole genome sequencing of direct respiratory samples.

Authors

Antonina A. Votintseva[1#▯], Phelim Bradley[2▯], Louise Pankhurst[1▯], Carlos del Ojo Elias[2], Matthew Loose[3], Kayzad Nilgiriwala[4], Anirvan Chatterjee[4], E. Grace Smith[5,6], Nicolas Sanderson[1], Timothy M. Walker[1], Marcus R. Morgan[7], David H. Wyllie[1,6,8], A. Sarah Walker[1,9], Tim E. A. Peto[1,9], Derrick W. Crook[1,6,8+], Zamin Iqbal[2+#]

Toplam DNA Dizi İncelemesi ile Mikobakterilerde İlaç Direnci Saptama

- Tüm DNA'nın tamama yakın okunabilmesi için aynı DNA dizilerinin en az 20 kez okunması gerekiyor. Bu da 2-3 gün sürebiliyor.
- Kültür sonrası yapılan toplam DNA dizilemesi ile ilaç direncinin saptanma süresi 25-30 günü bulmuş (hızlı kültür ile aynı zamanlarda).
- Doğrudan ARB+ örnekten yapılan çalışmalar ile 1-2 günde sonuç elde edilebilmiş. 12 saate indirilmeye çalışılıyor.
- Maliyet 400-1000 USD arasında değişiyor

Kaynağı Kısıtlı Bölgelerde Hızlı Kültür ve İlaç Duyarlılık Testi

DECOMICS

ARB -

ARB +

7-10 gün

TK SLC

TK ANTI TB PNB KIT

TEŞEKKÜRLER