

Tüberküloz Tanısında Moleküler Yöntemler

Prof. Dr. Tanıl Kocagöz



ACIBADEM
MEHMET ALİ AYDINLAR
ÜNİVERSİTESİ

STATUS OF THE GLOBAL TB EPIDEMIC AND RESPONSE

GLOBAL TUBERCULOSIS REPORT 2014

QUALITY TUBERCULOSIS (TB) CARE FOR MILLIONS WORLDWIDE HAS DRIVEN DOWN TB DEATHS BUT
TB REMAINS THE SECOND BIGGEST KILLER DISEASE FROM A SINGLE INFECTIOUS AGENT



MILLION

PEOPLE FELL ILL WITH TB
IN 2013 INCLUDING
1.1 MILLION PEOPLE
LIVING WITH HIV



1.5 MILLION

PEOPLE DIED IN 2013
INCLUDING 360 000
PEOPLE WHO WERE
HIV POSITIVE



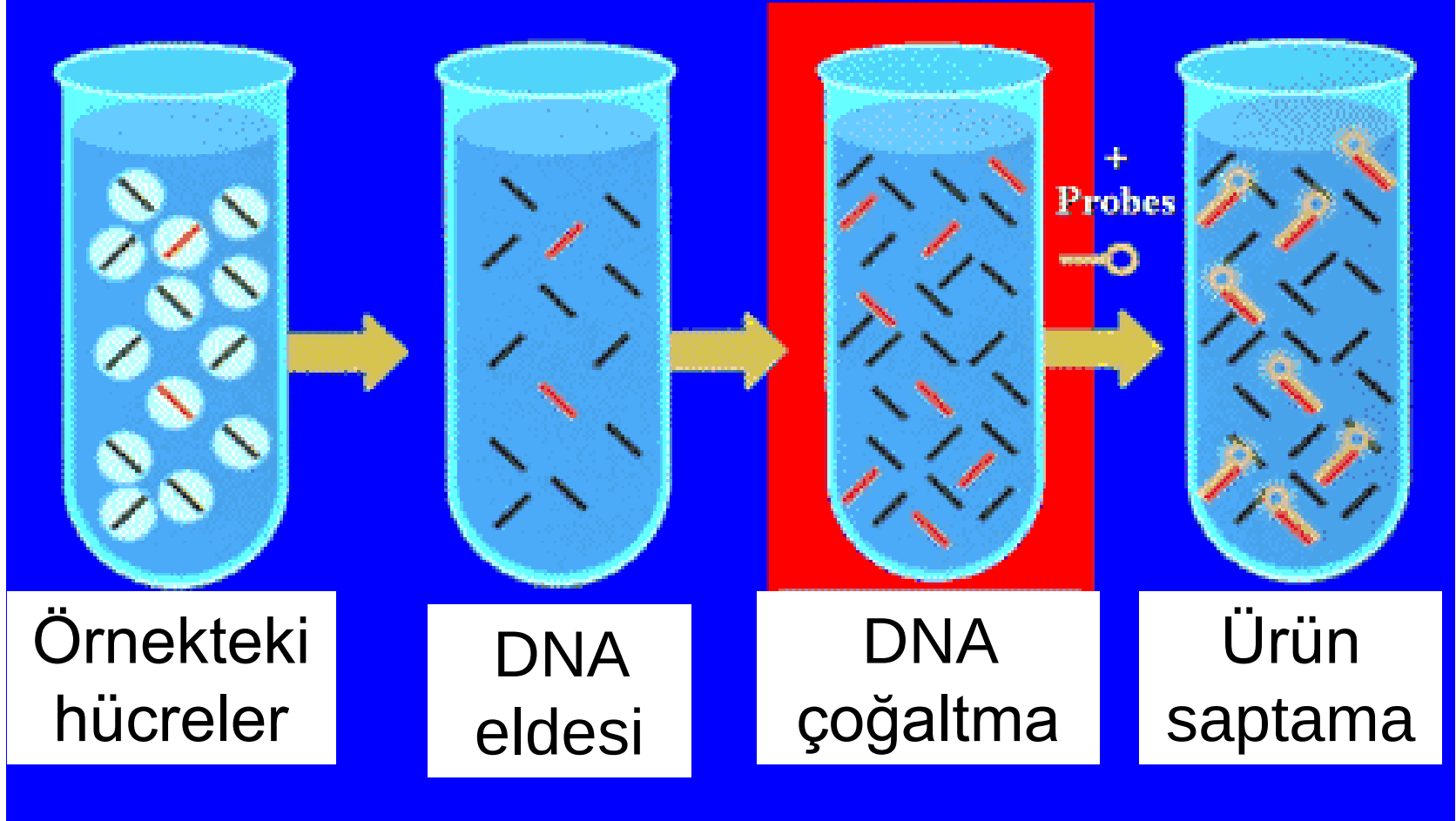
480 000

PEOPLE ESTIMATED TO
HAVE DEVELOPED
MULTIDRUG-RESISTANT TB
(MDR-TB) IN 2013.

Moleküler Tanı

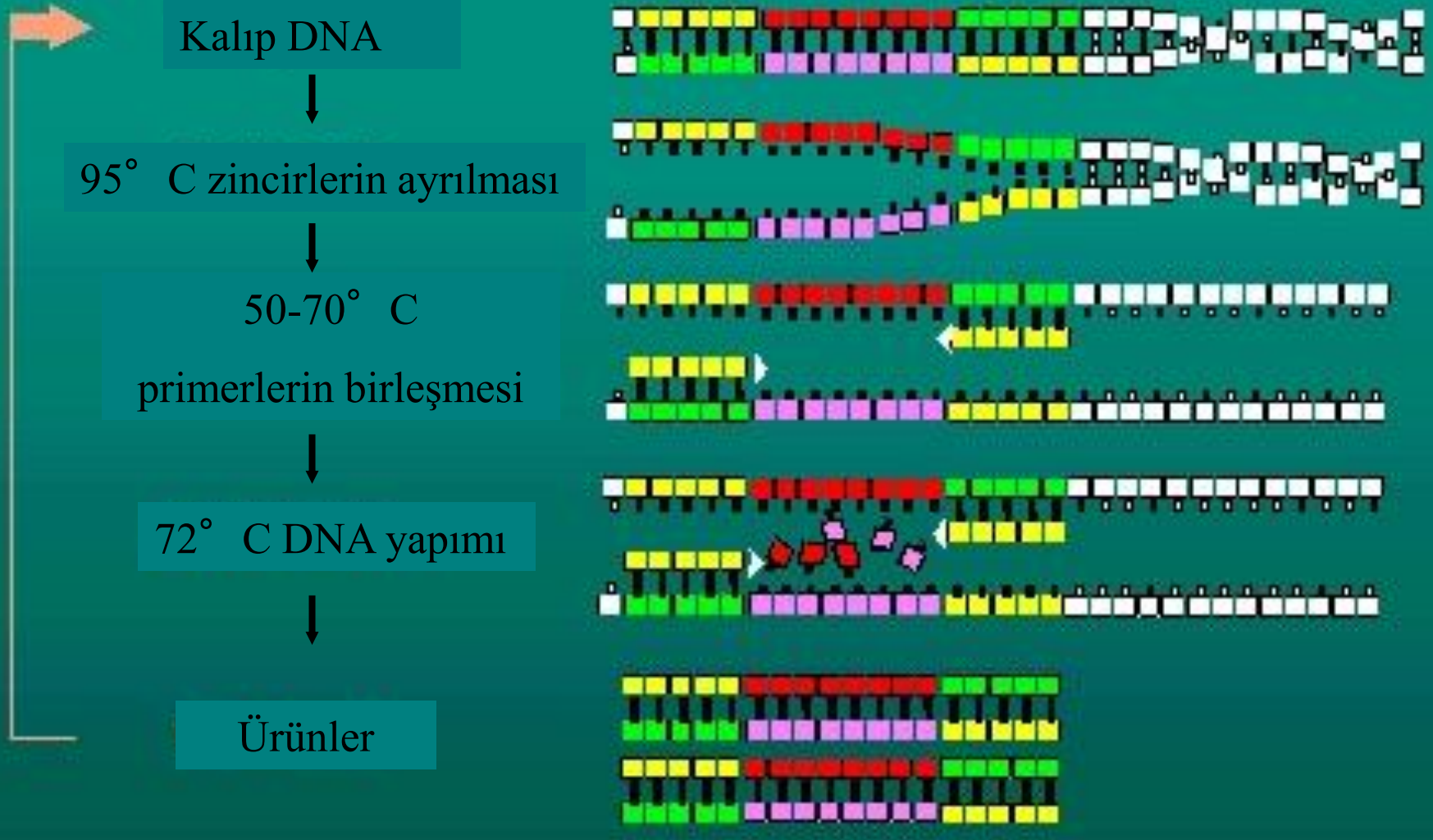
- Duyarlılığı yüksek, yalancı pozitif sonuç verebilir
- İyi standardize edilmezse yüksek oranda yalancı negatif sonuç elde edilebilir
- Canlı ve ölü bakterileri birbirinden ayırt edemez
- Sonuçlar diğer tanı yöntemlerinden elde edilenler ile birlikte değerlendirilmelidir
- Pahalı, karmaşık cihazlar ve özel eğitilmiş çalışanlar gerektirmektedir
- Hızlı tür saptama ve ilaç duyarlılığı belirlemede çok başarılıdır.

N kleik Asit  o altma ile Enfeksiyon Etkenlerinin Saptanması

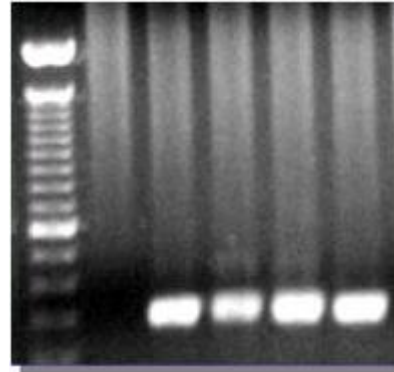
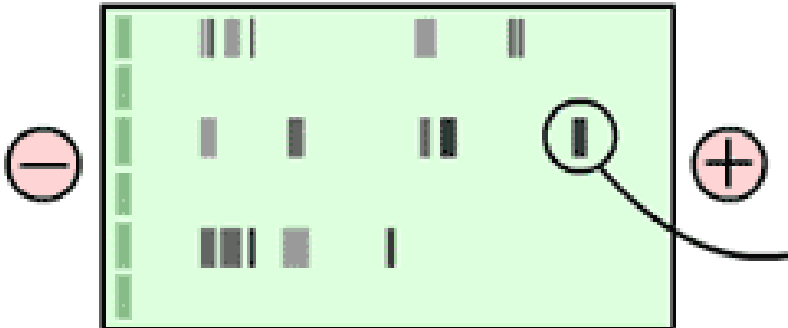
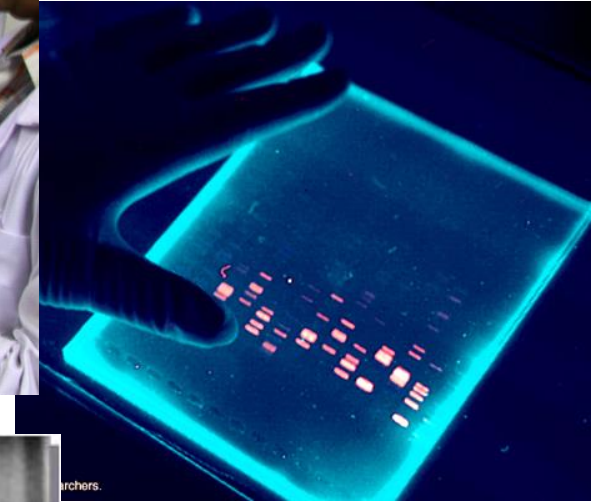
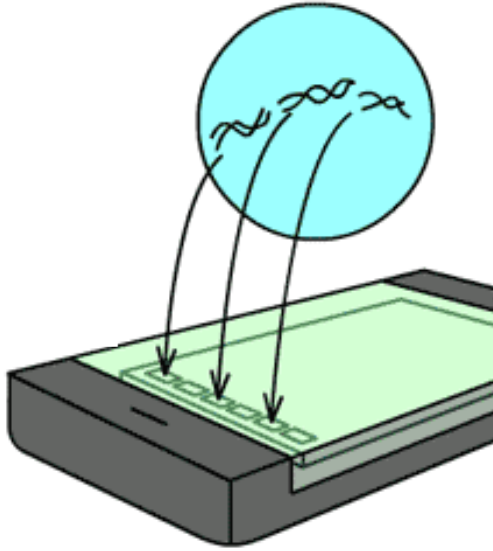


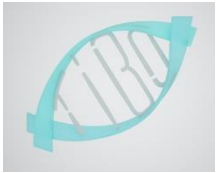
Polimeraz Zincirleme Tepkimesi (PZT)

(Polymerase Chain Reaction –PCR-)



PZT çoğaltma ürünleri elektroforez ile incelenir

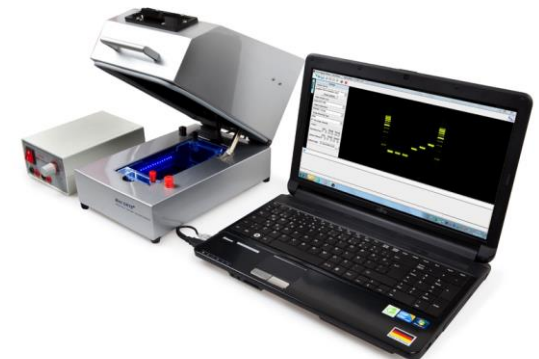
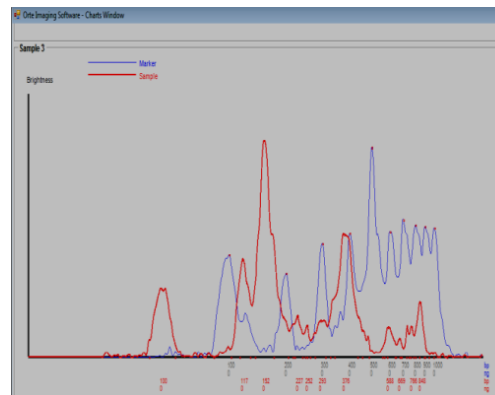
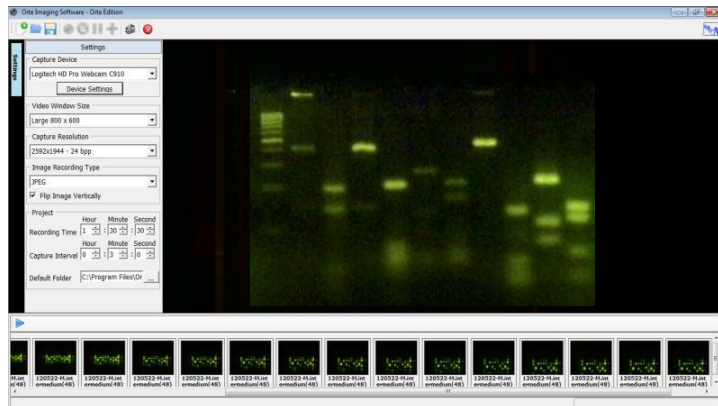
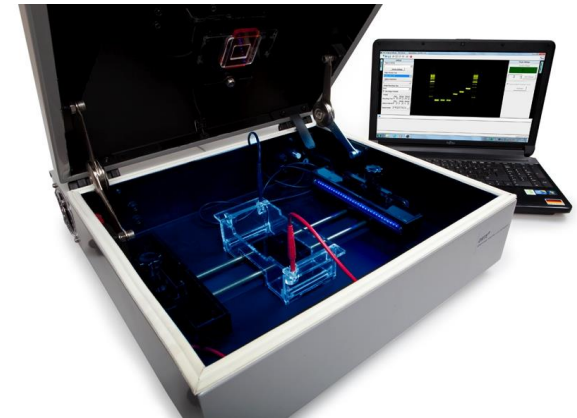
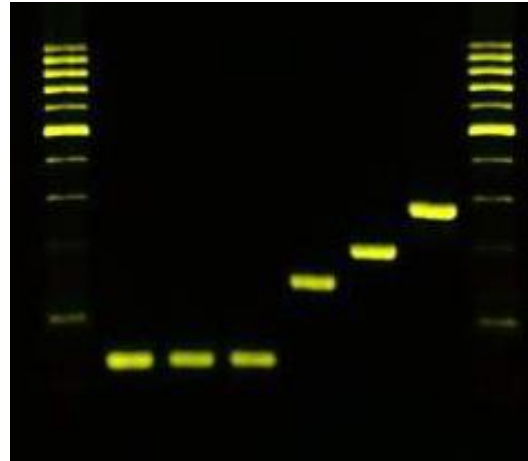
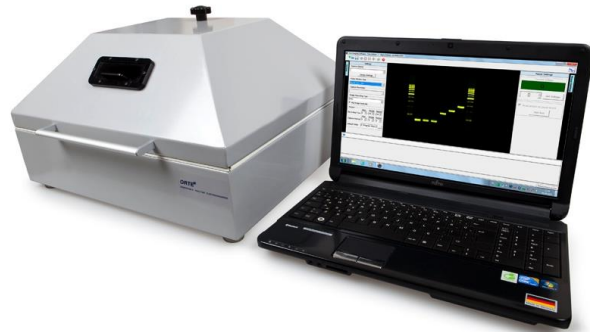




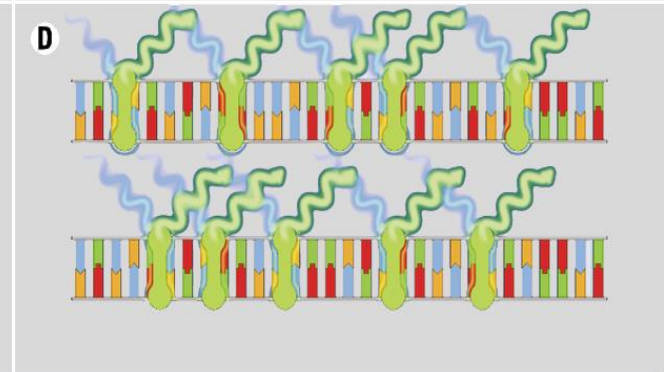
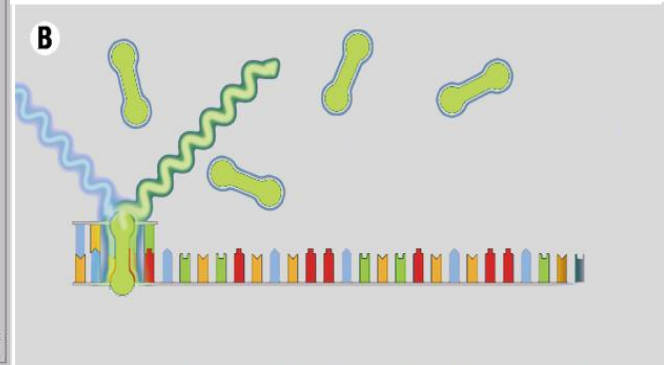
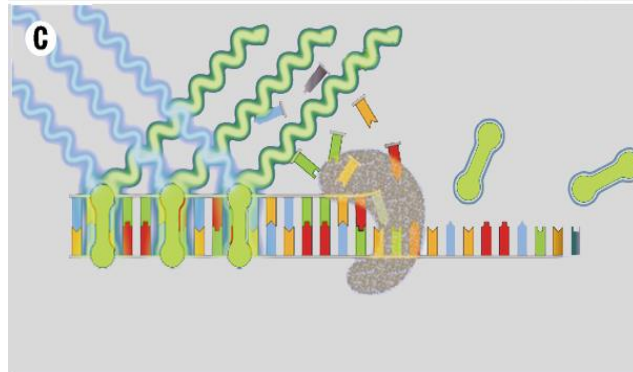
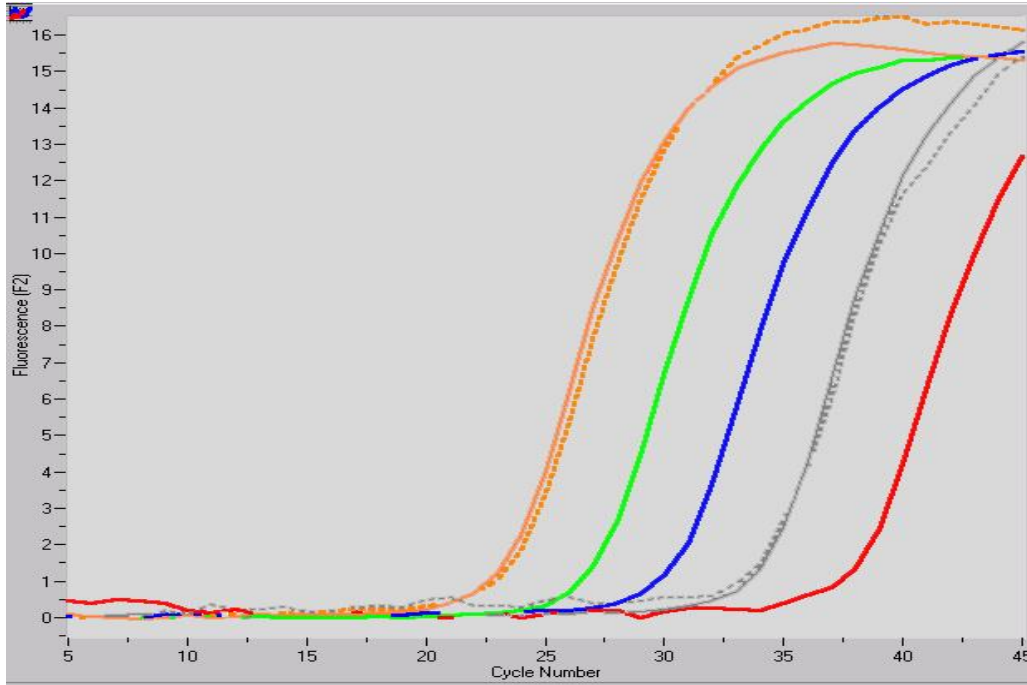
ORTE İzlenebilir Elektroforez

Observable Real Time Electrophoresis

Observable Real Time Electrophoresis



İzlenebilir PZT (Real Time PCR) ile tanı ve ilaç direnci belirleme





DNA saflaştırma



PZT karışımlarının
hazırlanması



İzlenebilir PZT



Uganda'da
bir laboratuvar



Hindistan'da
bir laboratuvar

Kaynakları Sınırlı Ülkelerde Laboratuvar Koşulları

		Environment		Infrastructure		Available equipment						Skills			Communication			Current testing			
Country		Temperature	Humidity	Power	Water	N95	Pipettes	Refrigerator	Incubator	Centrifuge	Water bath	Hood	Pipetting	PCR tests	Computer	Landline	Mobile	Internet	QA Established	ZN	FM
Non-BRICS	Congo																				
	Zimbabwe																				
	Mozambique																				
	Ethiopia																				
	Afghanistan																				
	Myanmar																				
	Uganda																				
	Tanzania																				
	Kenya																				
	Bangladesh																				
	Cambodia																				
	Nigeria																				
	Pakistan																				
	Vietnam																				
	Philippines																				
	Indonesia																				
Thailand																					
BRICS	India																				
	China																				
	South Africa																				
	Brazil																				
	Russia																				
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Detection of *Mycobacterium tuberculosis* in Sputum Samples by Polymerase Chain Reaction Using a Simplified Procedure

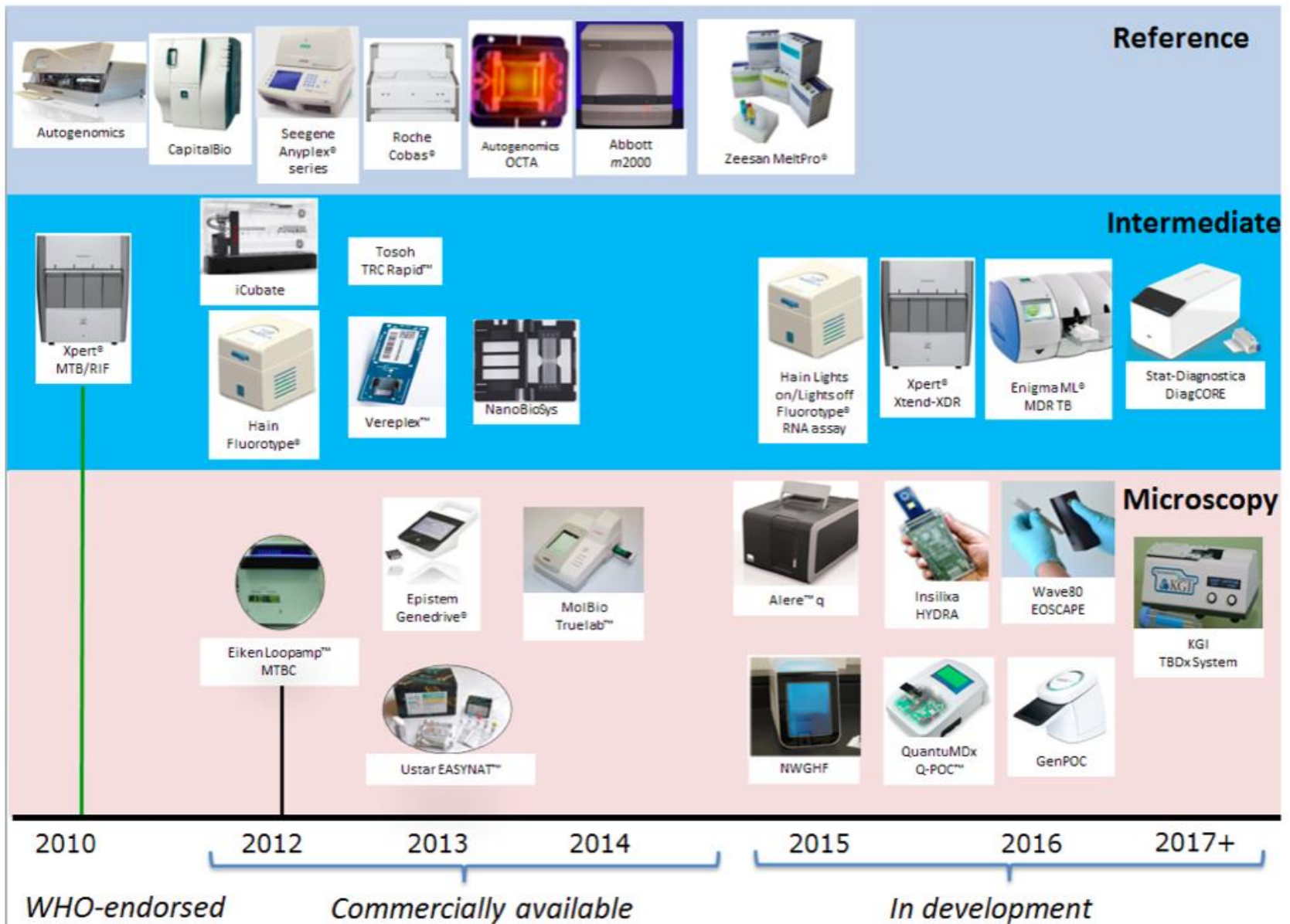
TANIL KOCAGÖZ,^{1*} ENGIN YILMAZ,² ŞEREF ÖZKARA,³ SESİN KOCAGÖZ,¹ MURAT HAYRAN,⁴
MEENA SACHEDEVA,⁵ AND HENRY F. CHAMBERS⁵

Departments of Microbiology,¹ Medical Biology,² and Infectious Diseases,⁴ School of Medicine, Hacettepe University, and Atatürk Chest Diseases and Chest Surgery Center,³ Ankara, Turkey, and The Medical Service, San Francisco General Hospital, and The Department of Medicine, University of California, San Francisco,⁵ San Francisco, California 94110

Received 7 January 1993/Accepted 3 March 1993

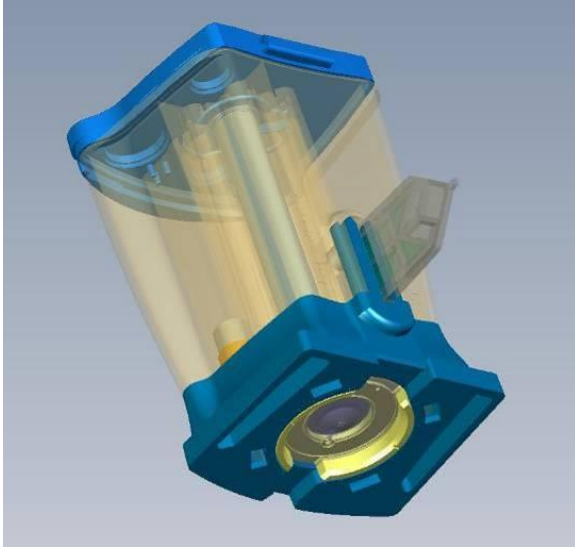
A repetitive sequence of *Mycobacterium tuberculosis* DNA was amplified by polymerase chain reaction (PCR), from sputum samples, for the diagnosis of pulmonary tuberculosis. The method of heating the sample in a boiling water bath to break down the bacterial cell wall and to release the DNA was compared with that of enzymatic lysis of bacteria and then phenol-chloroform extraction of DNA. Heating the sample was the better method with a sensitivity of approximately 10 microorganisms. A total of 78 sputum specimens prepared by heating were examined by PCR, and the results were compared with the results of acid-fast stained smears, cultures, and clinical data. *M. tuberculosis* was detected by PCR in all smear- and culture-positive and smear-negative, culture-positive cases. Additionally, PCR was capable of detecting four of nine cases which were smear and culture negative but clinically suspected of tuberculosis. DNA amplification by PCR is a sensitive and specific method for the diagnosis of tuberculosis, and with this simplified DNA isolation procedure it can be used in routine clinical practice.

Örneği kaynar su üzerinde bekletmek PZT'ne gerekli DNA izolasyonu için yeterli



Nükleik Asit Çoğaltma Testleri

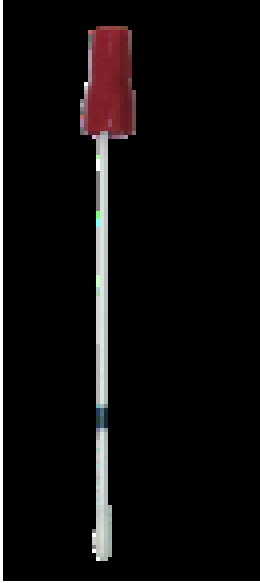
Gene Xpert



M. tuberculosis tanısı ve
Rifampisin direnci belirleme



Test Hazırlama



Balgam örneđi pamuklu çubuk ile parçalama çözeltisine konup çalkalanır. Kartuşa dökölür. Kartuş makineye konup makine çalıştırılır.



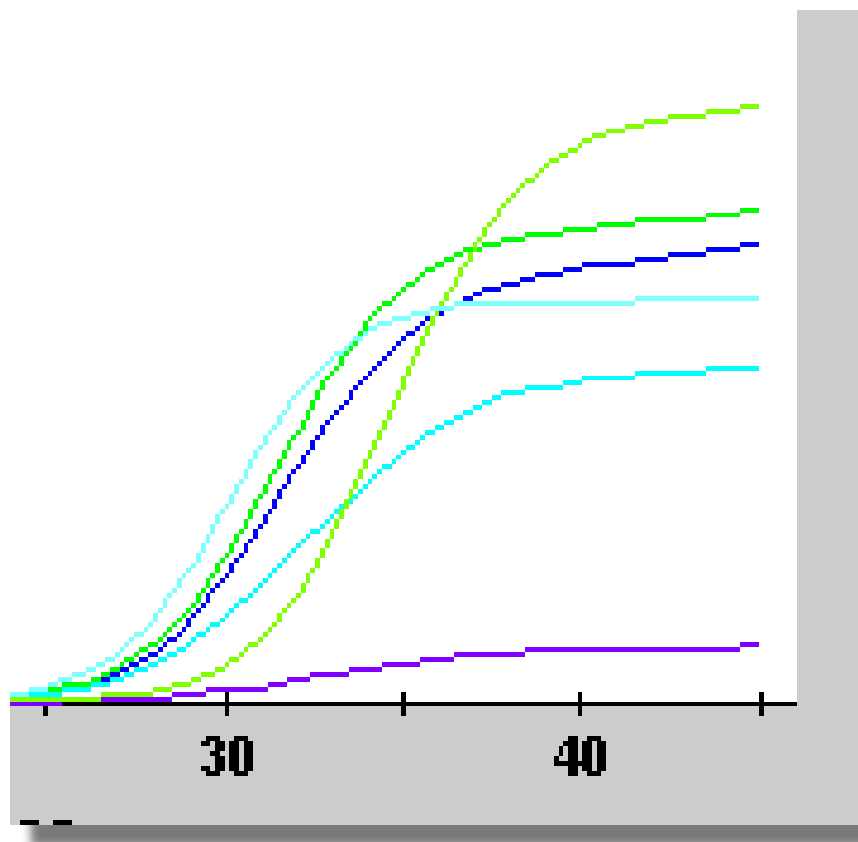
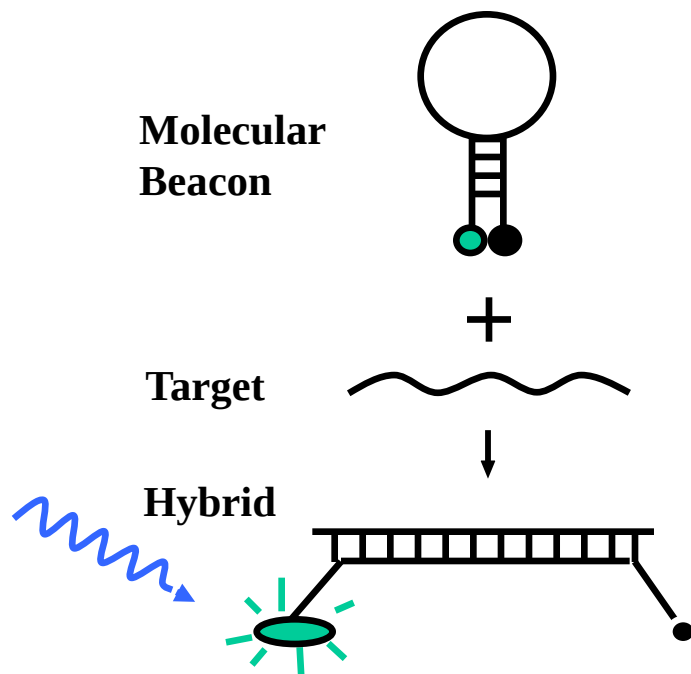
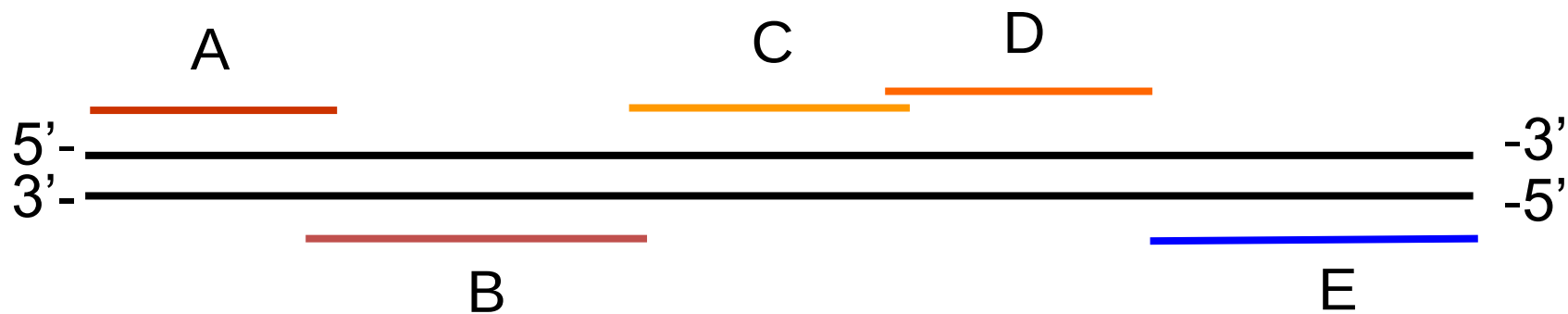
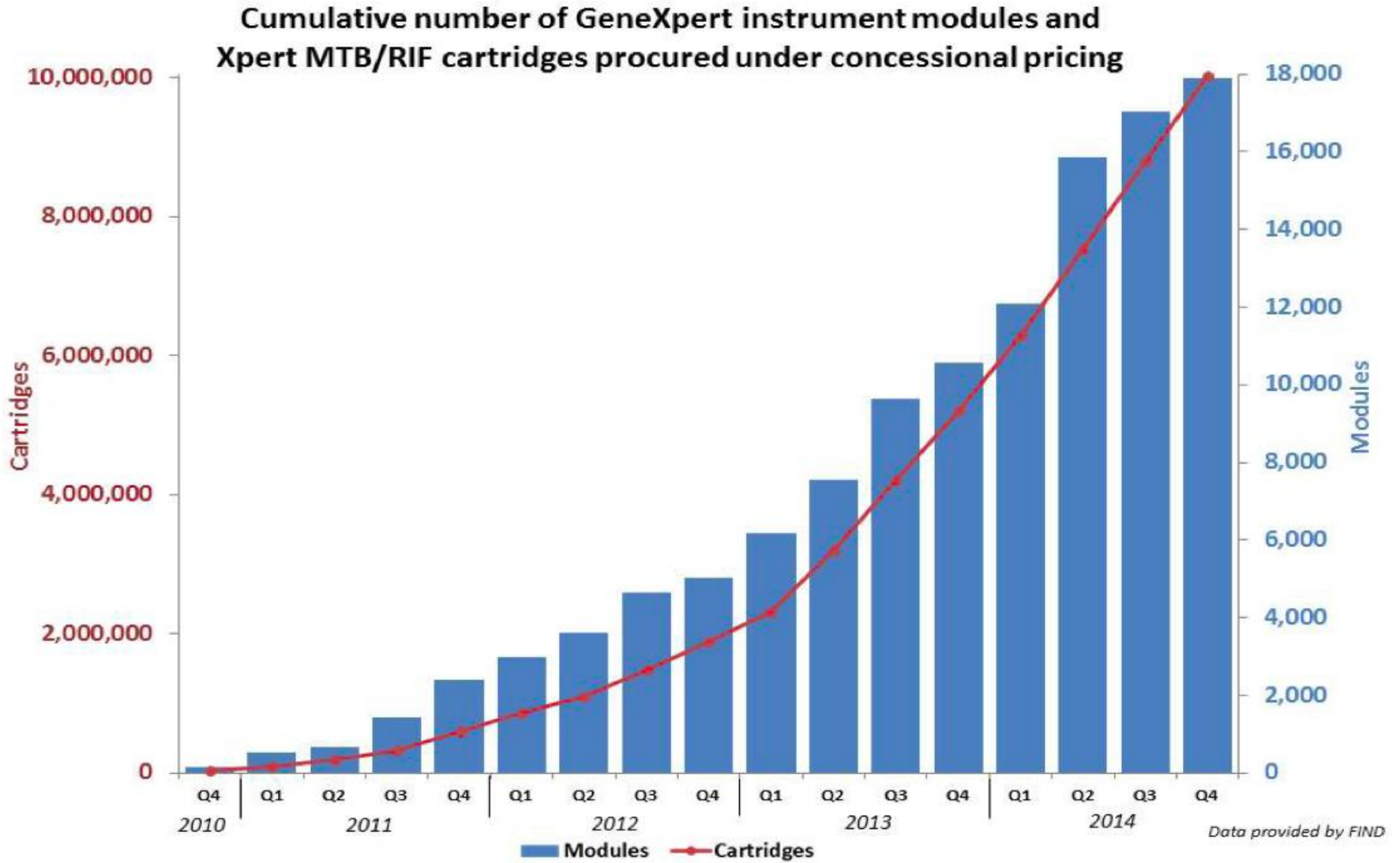


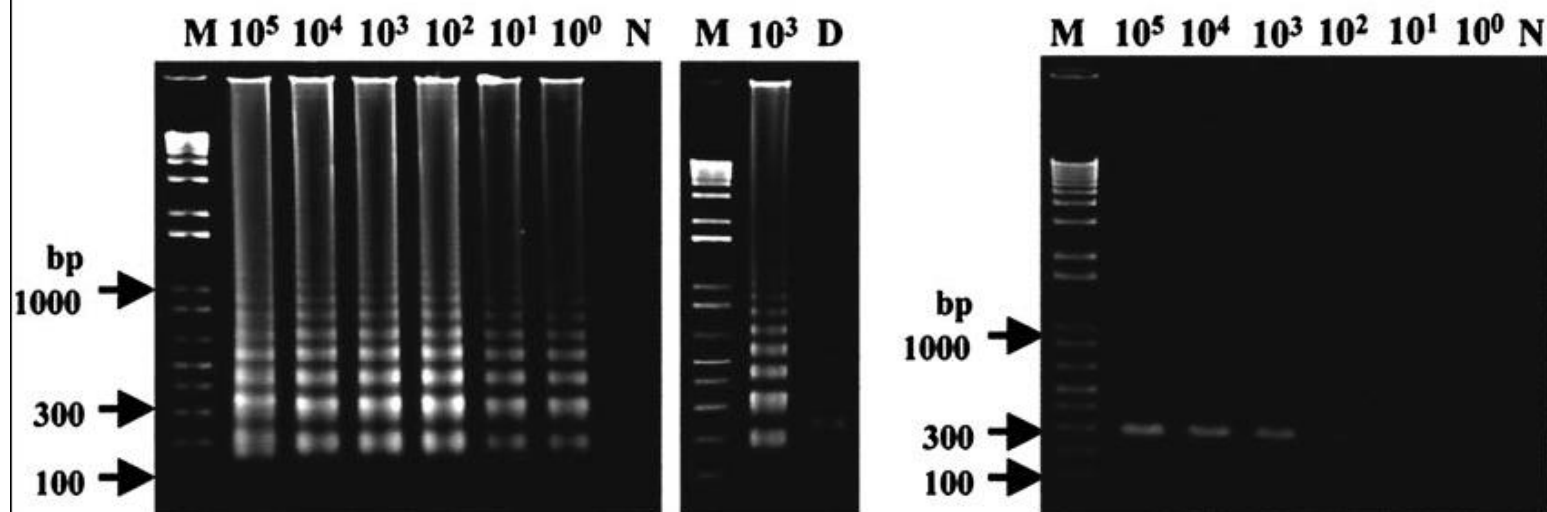
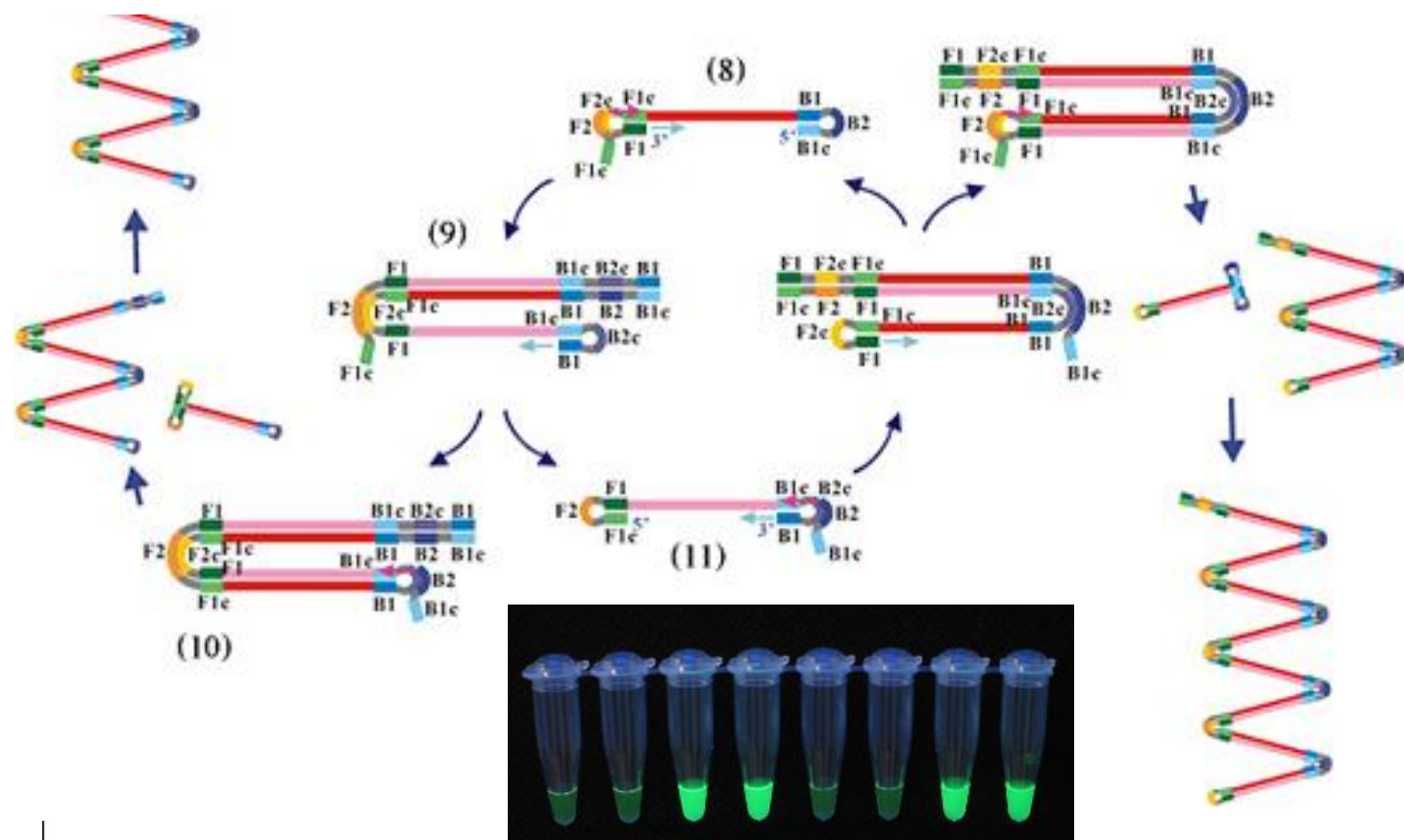
Figure 35. WHO monitoring of Xpert® MTB/RIF scale-up



Çin tüberküloz kontrol programı sadece ARB+ örneklerin Xpert ile çalışılmasına onay vermiş

“Loop Mediated Amplification” (LAMP)

- n 6 değişik bölgeye özgü 4 primer kullanılır.**
- n Özgüllüğü çok yüksek**
- n Sıcaklık döngüsü gerektirmez; izotermal çoğalma**
- n Çoğalma floresans veya tüplerde bulanıklık oluşması ile izlenir**
- n Çoğaltma RNA'dan başlatılabilir**



LAMP (Loop mediated amplification)

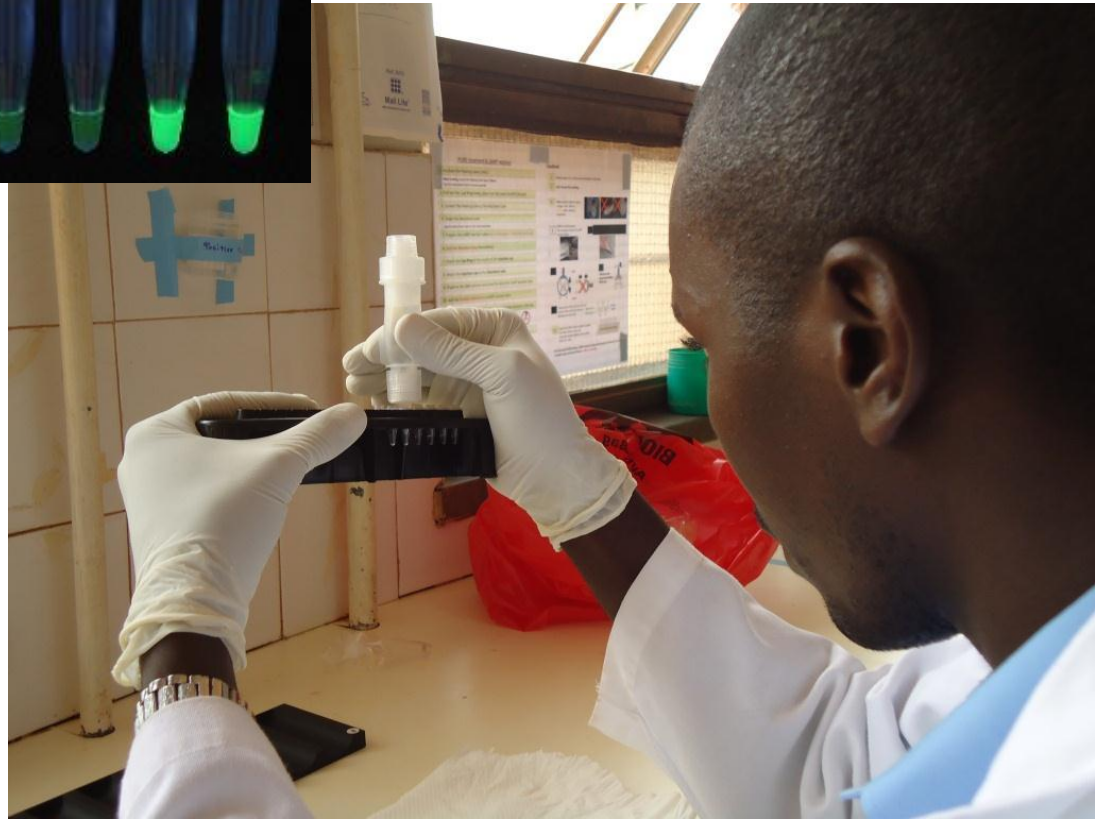
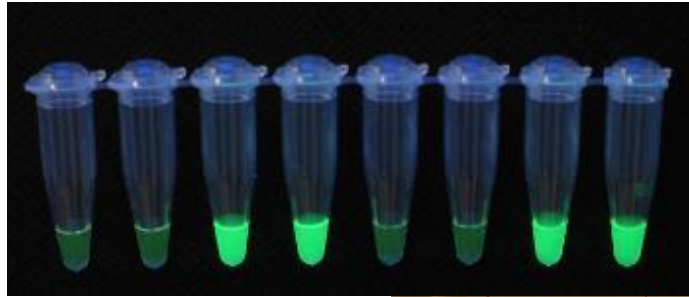
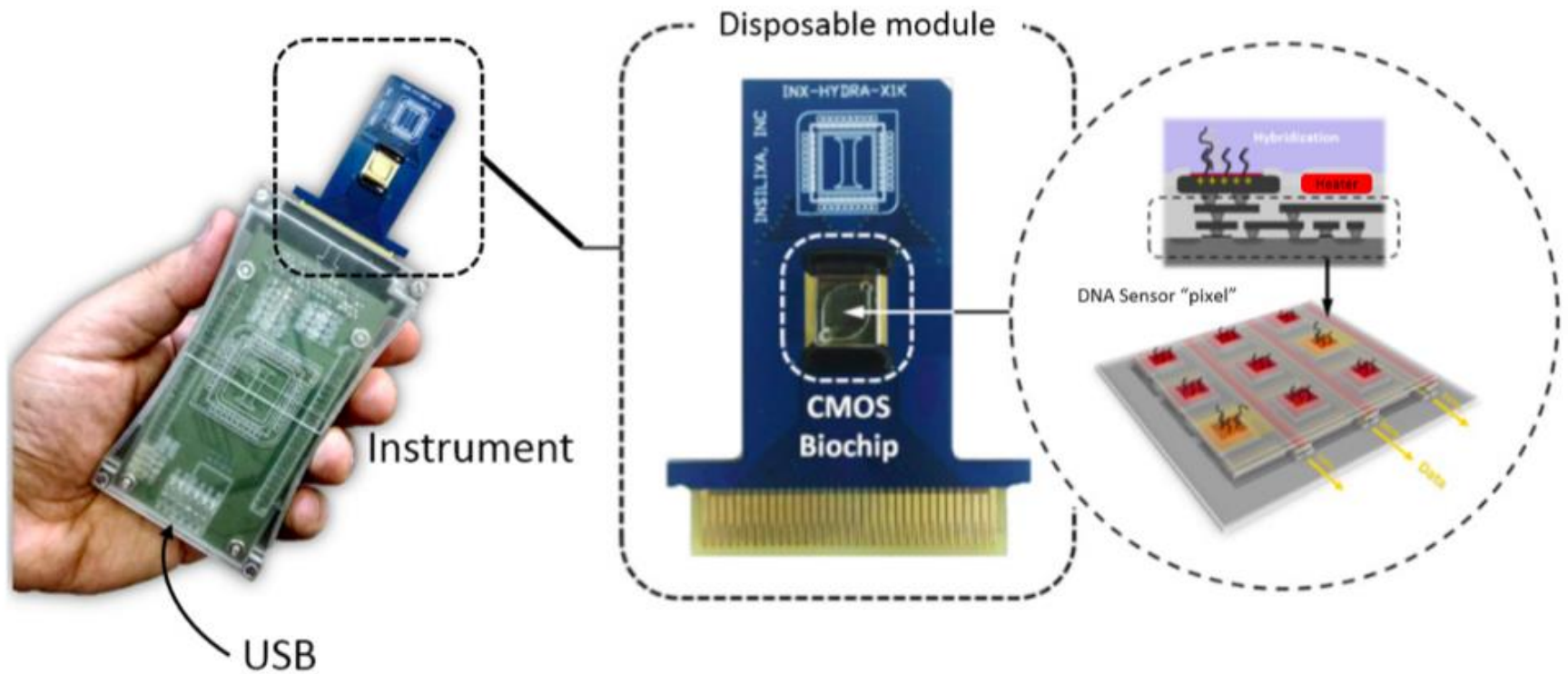


Figure 19. VerePLEX™ Biosystem and VereMTB™ Detection Kit from Veredus Laboratories: A: VereMTB™ Detection chip; B: VerePLEX™ Biosystem and reader



Figure 20. Hydra 1K hand-held platform (left) and chip (centre)



M. tuberculosis saptama ve ilaç direnci

Figure 22. Epistem Genedrive® Mycobacterium iD® assay



M. tuberculosis saptama ve rifampisin direnci

Figure 23. Molbio Diagnostics technology: current products and those in developments for NAAT-based detection of MTBC

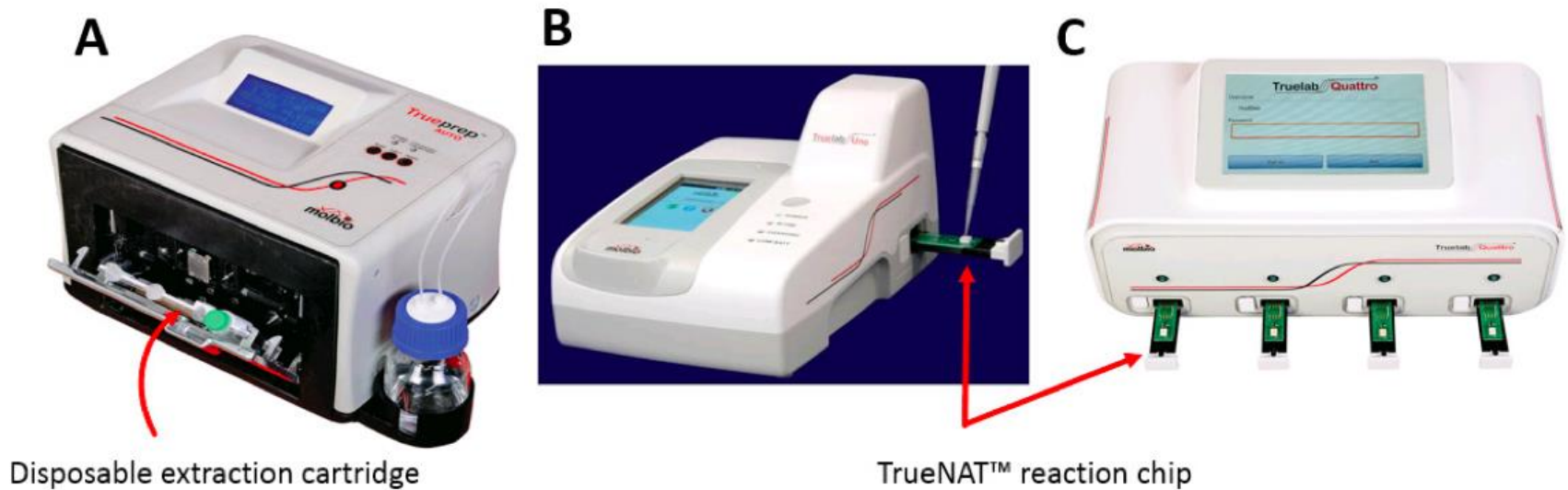


Figure 24. Alere™ q instrument for TB testing, currently in development



Figure 26. GenePOC automated test device

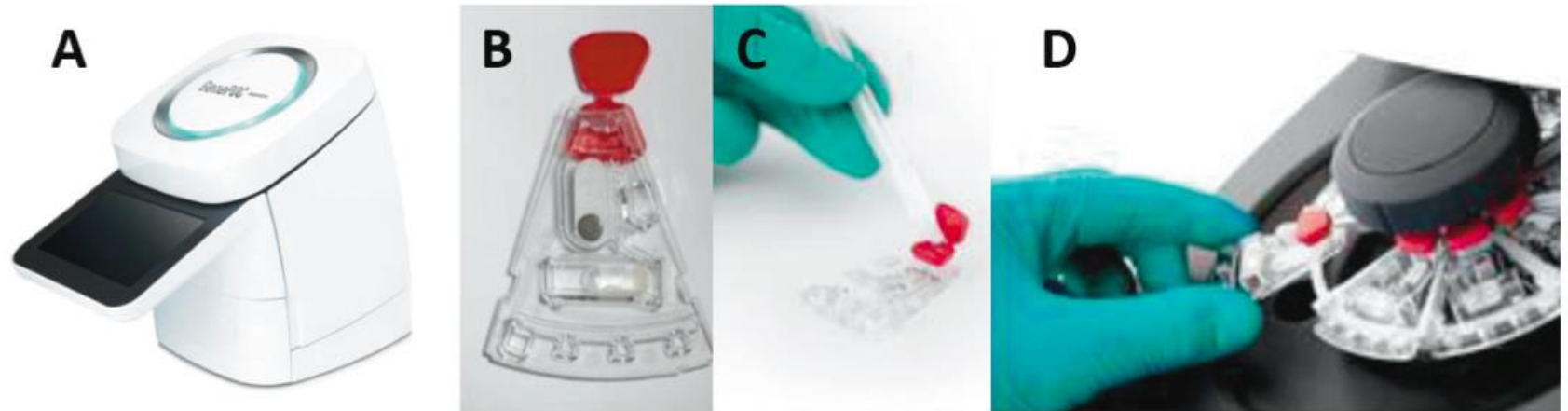


Figure 27. KGI TBDx system: instrument (left) and test cartridge (right)

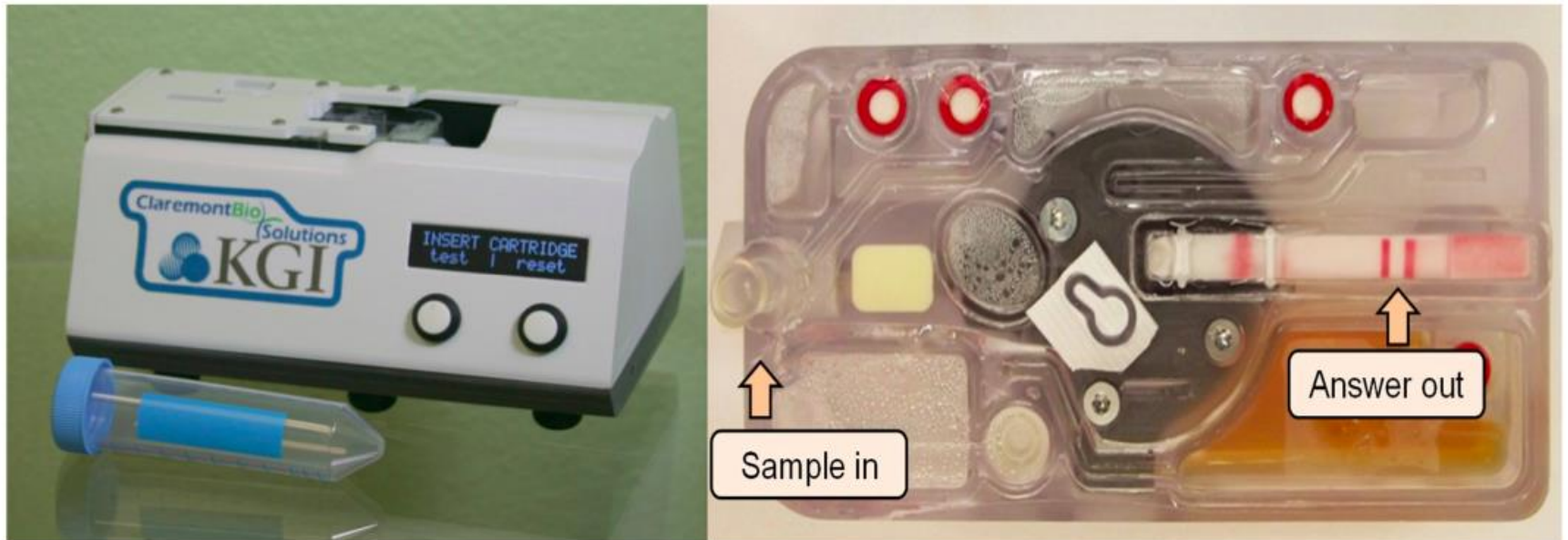


Figure 28. Point of Need (PON) technology in development by Qiagen: a schematic rendering of test cartridge design (left) and an image of a prototype instrument (right)



Figure 29. Rendering of the Q-POC™ platform under development from QuantuMDx

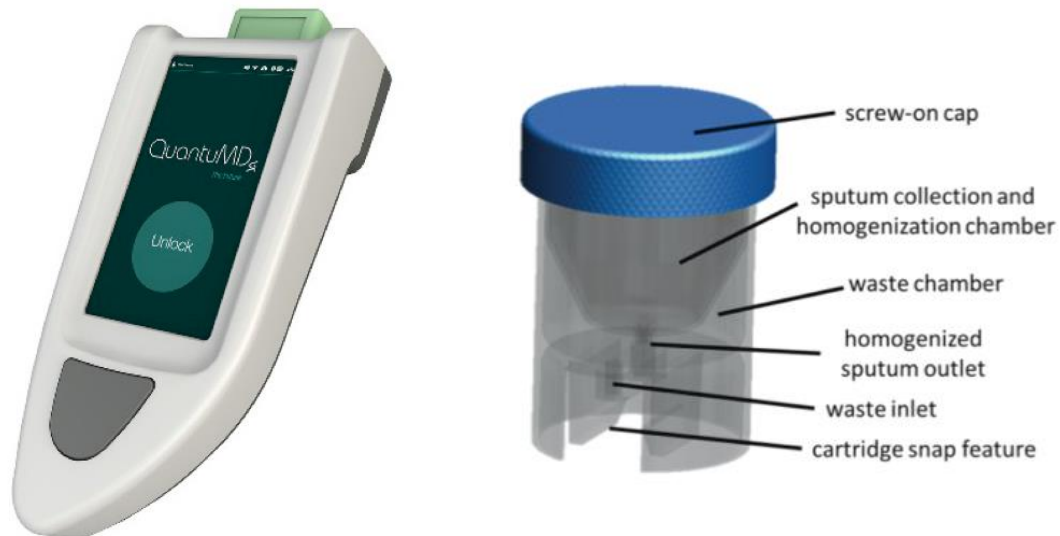
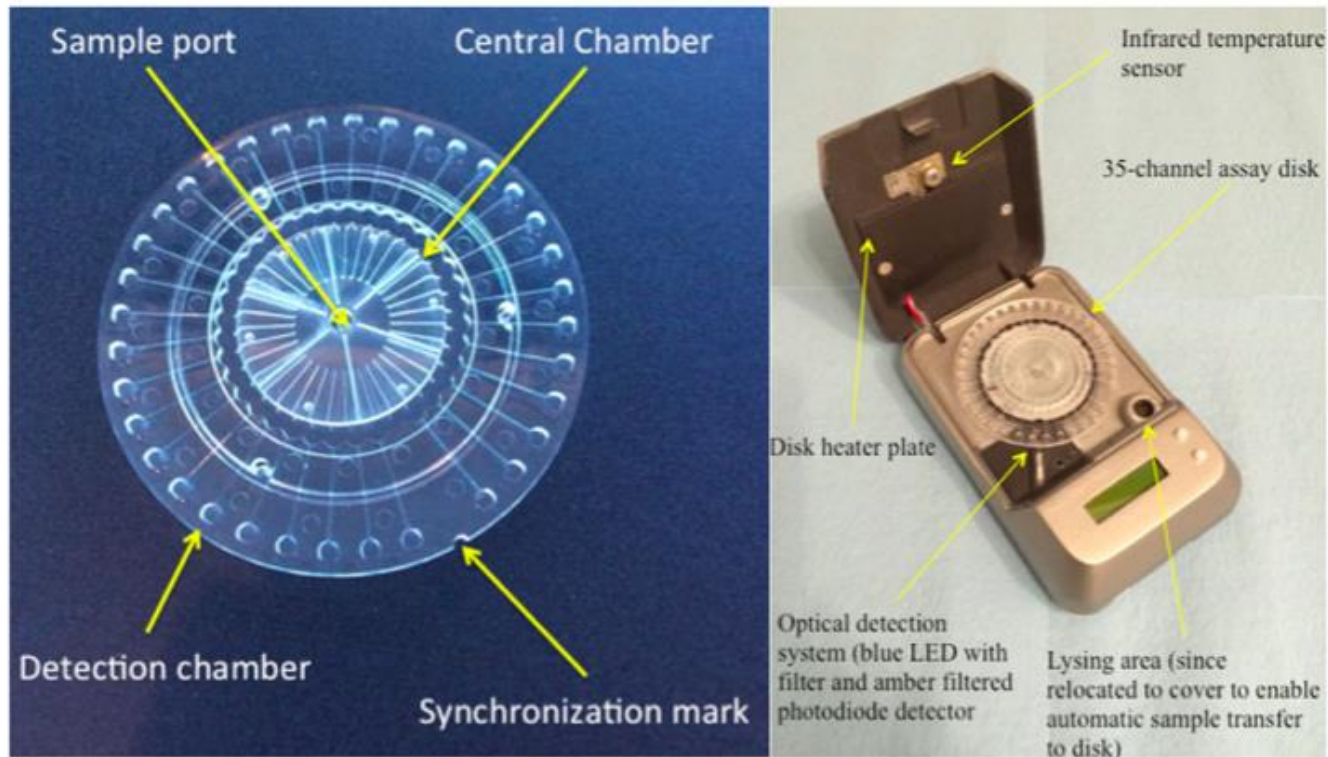
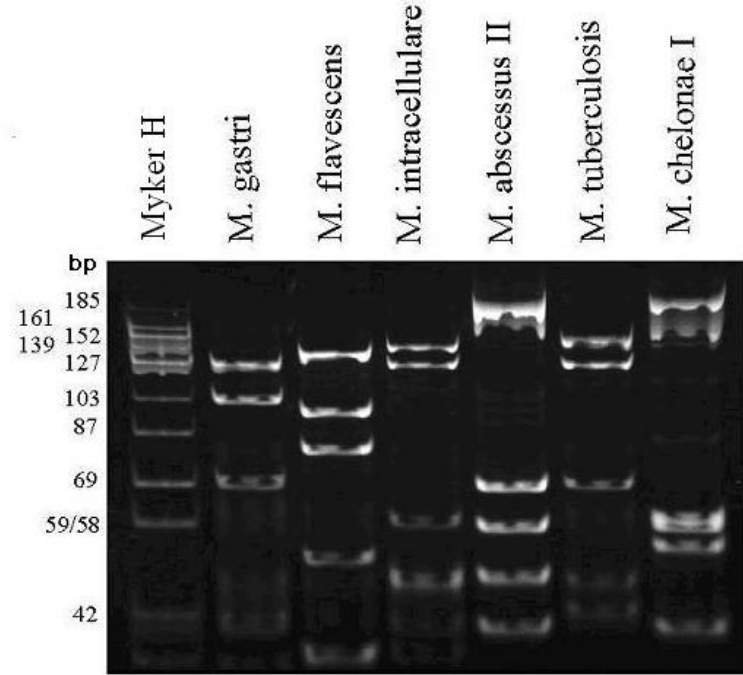
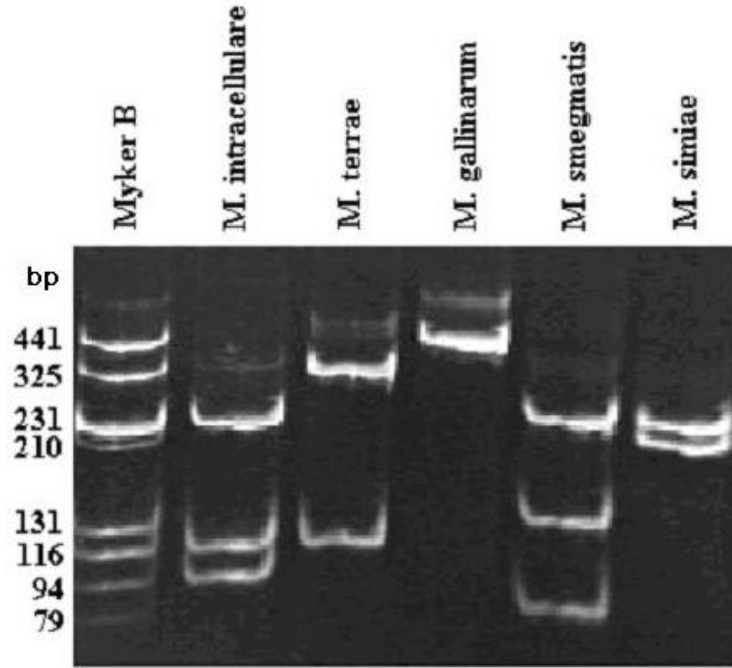


Figure 31. Tangen Biosciences TB diagnostic test disc (left) and platform (right)

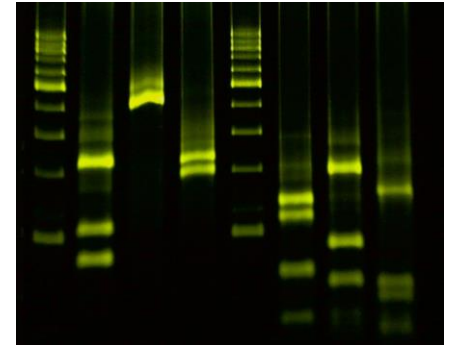


Genotiplendirme

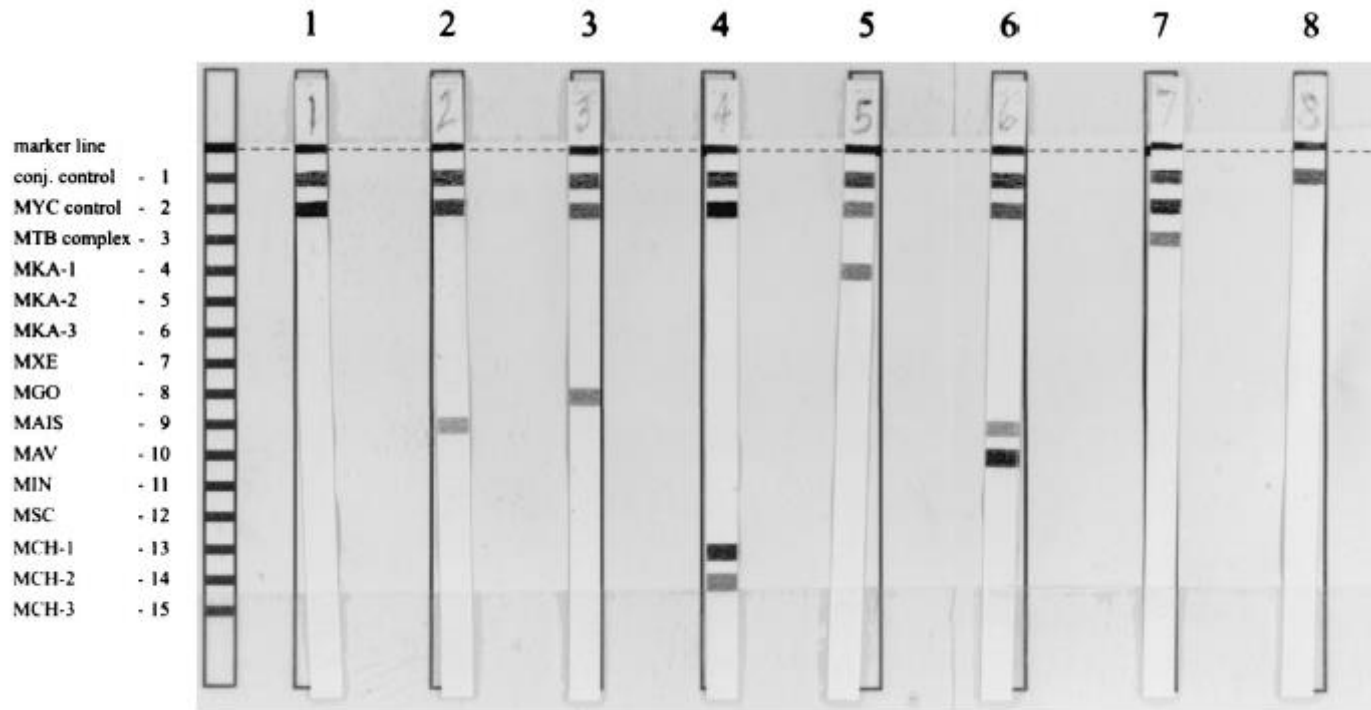
PZT ve Restriksiyon Enzim İncelemesi ile Tür Saptama



- hsp65 geni PZT ile çoğaltılıp BstEII ve HaeIII enzimleri ile kesilir
- Ürünler elektroforez ile ayrıştırılıp incelenir (Myker B ve Myker H. Köksalan & Kocagöz)

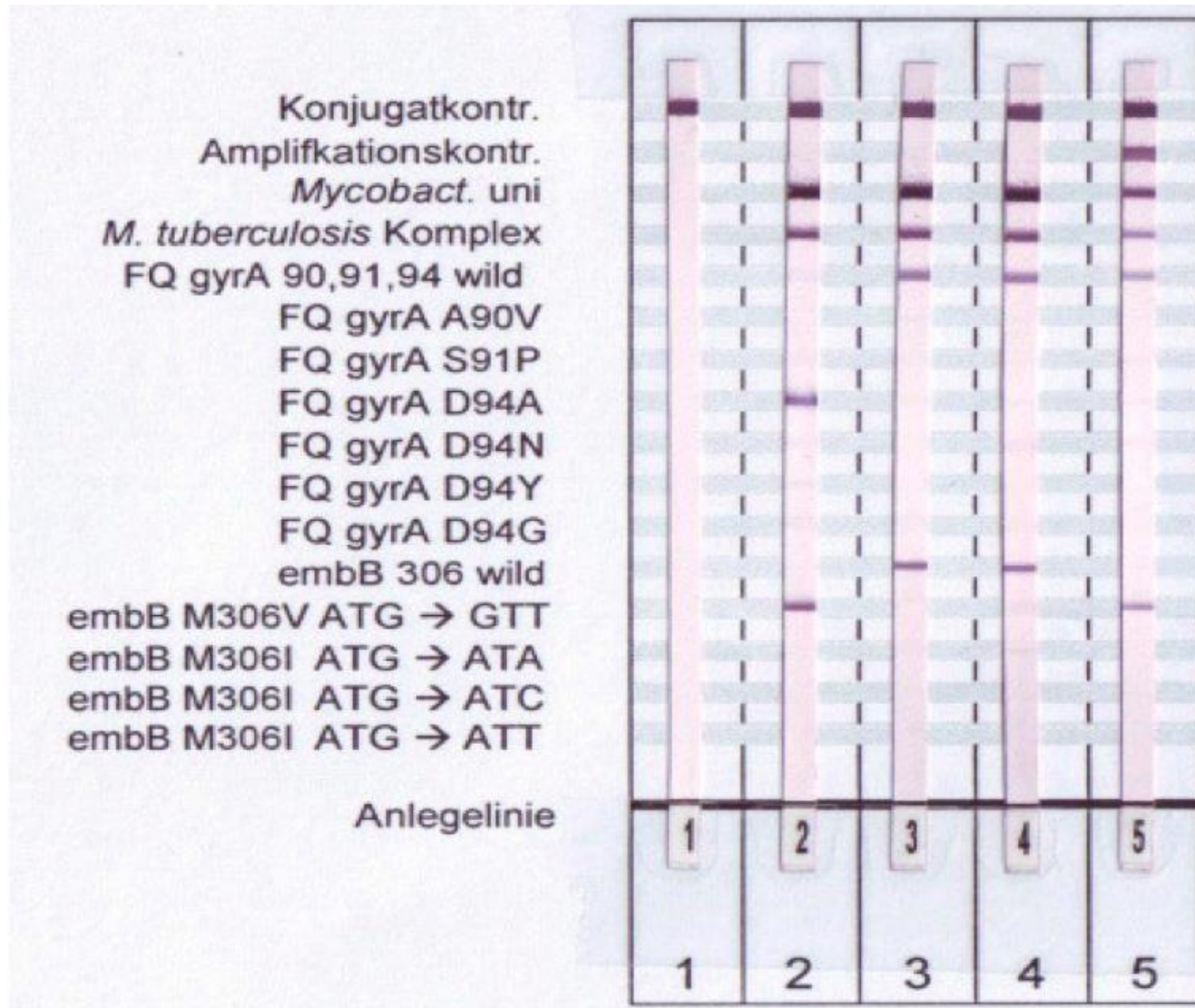


“Line Probe Assay” (LIPA) ile tür saptama



- INNO-LiPA Mycobacteria (Innogenetics, Ghent, Belgium)
- 16S-23S rRNA “spacer region”; Biyotinli primerler ile PZT
- Tersine hibridizasyon; streptavidin alkalen fosfataz ile boyama
- *M. tuberculosis*, *M. kansasii*, *M. xenopi*, *M. gordonae*, *M. avium*, *M. intracellulare*, *M. scrofulaceum*, and *M. chelonae*

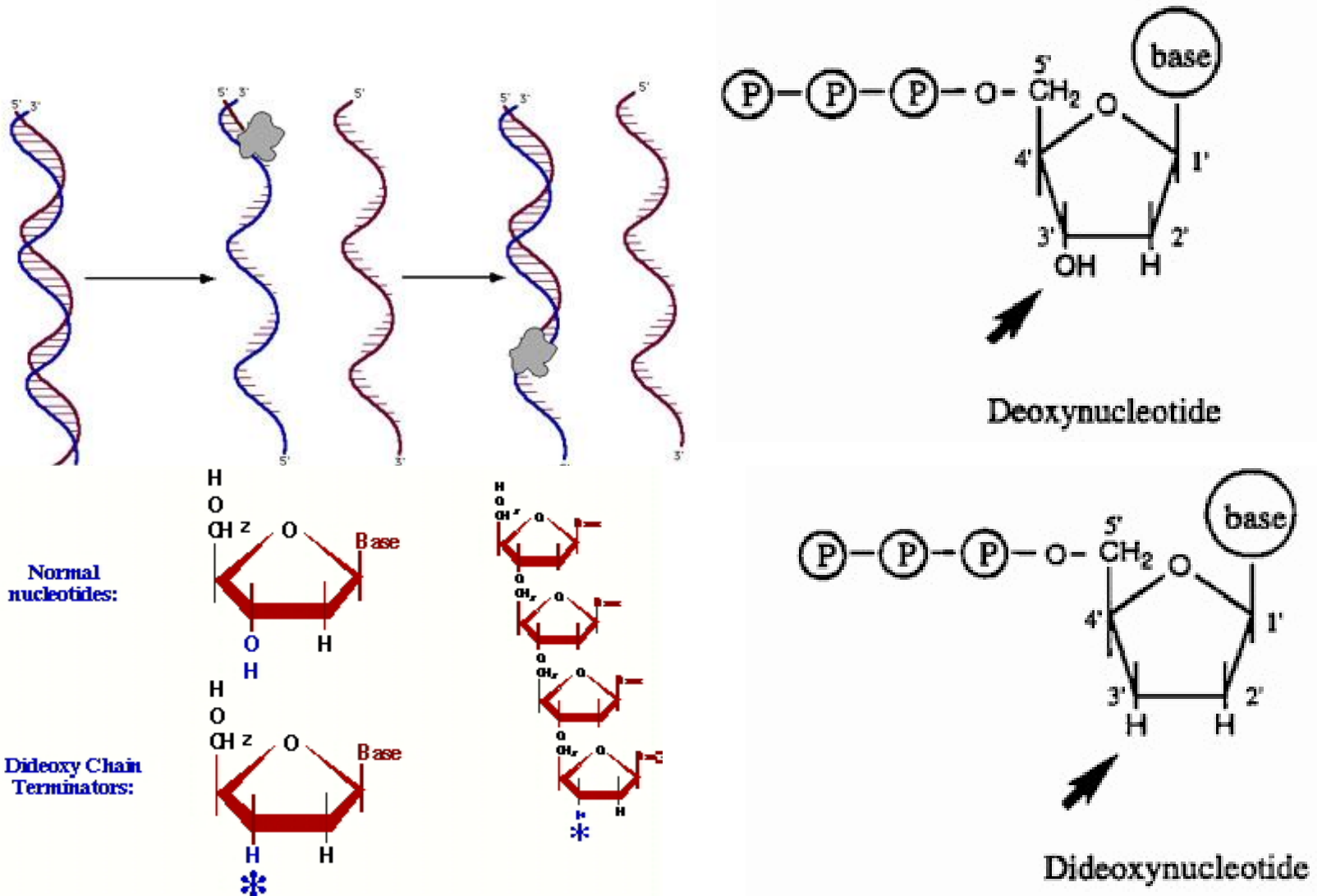
LIPA ile antibiyotik direnci belirleme



Hain Lifesciences Genotype Mtbdn Plus

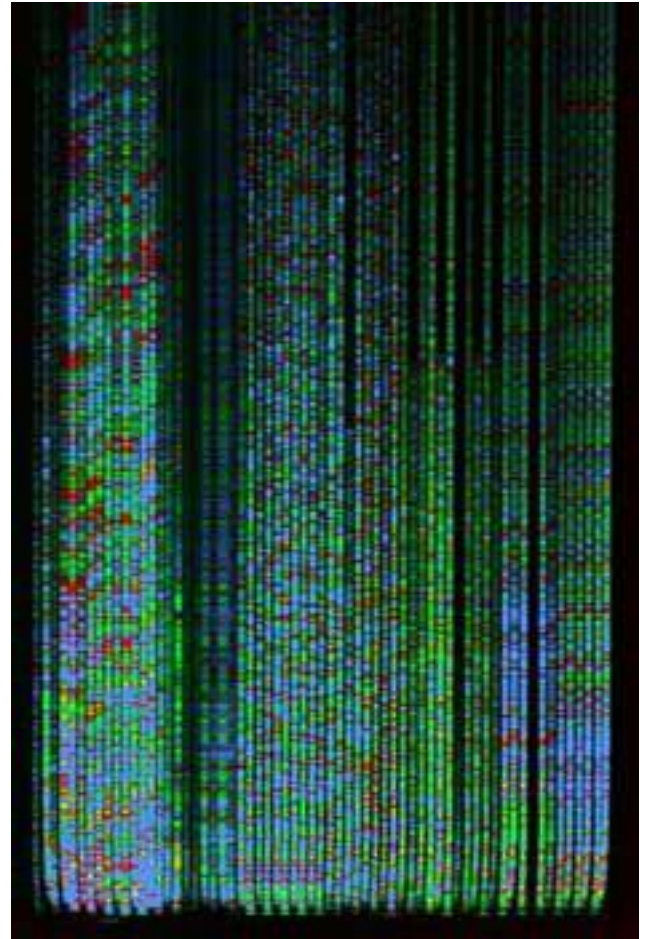
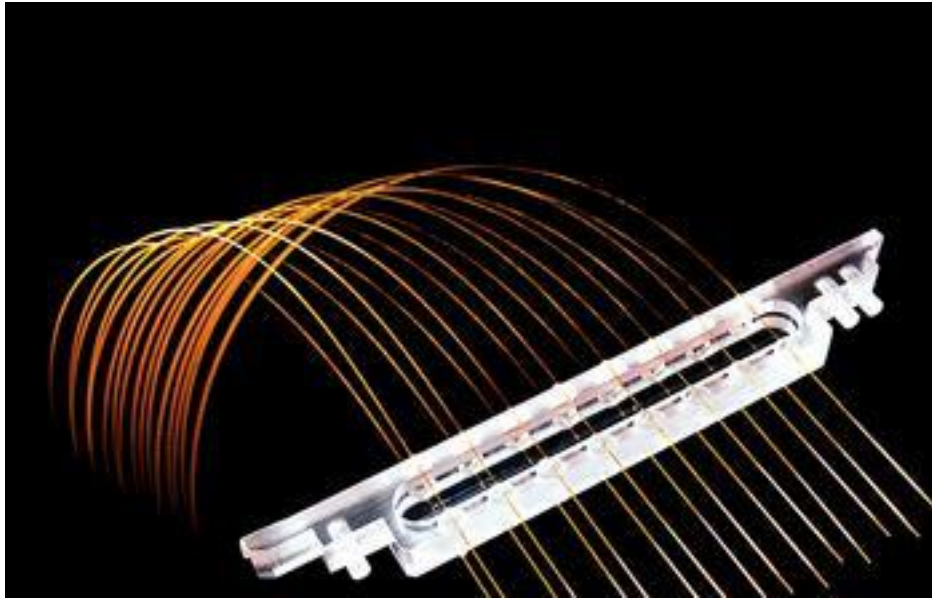
DNA Dizisi Saptama

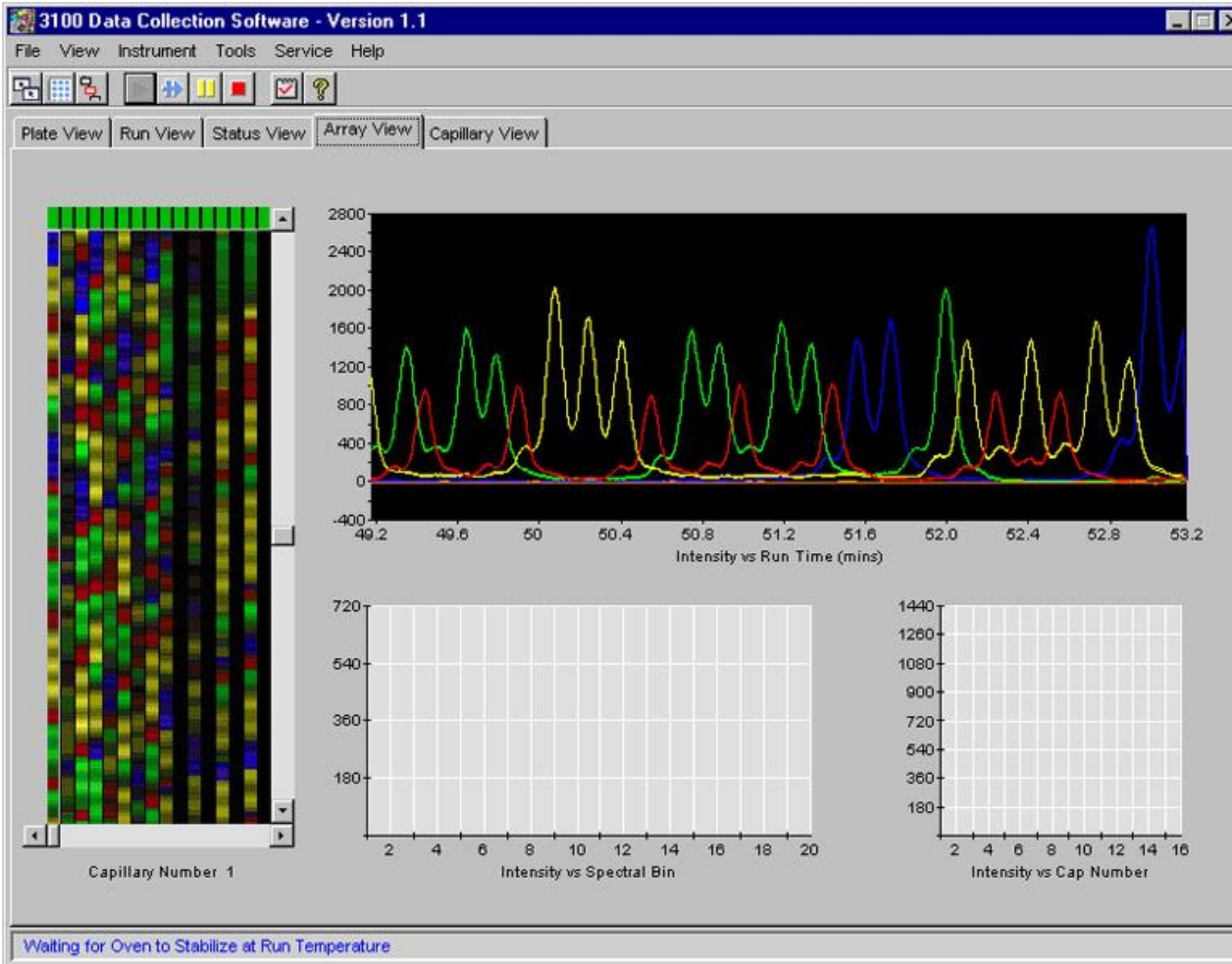
Sanger Zincir Sonlandırma Yöntemi

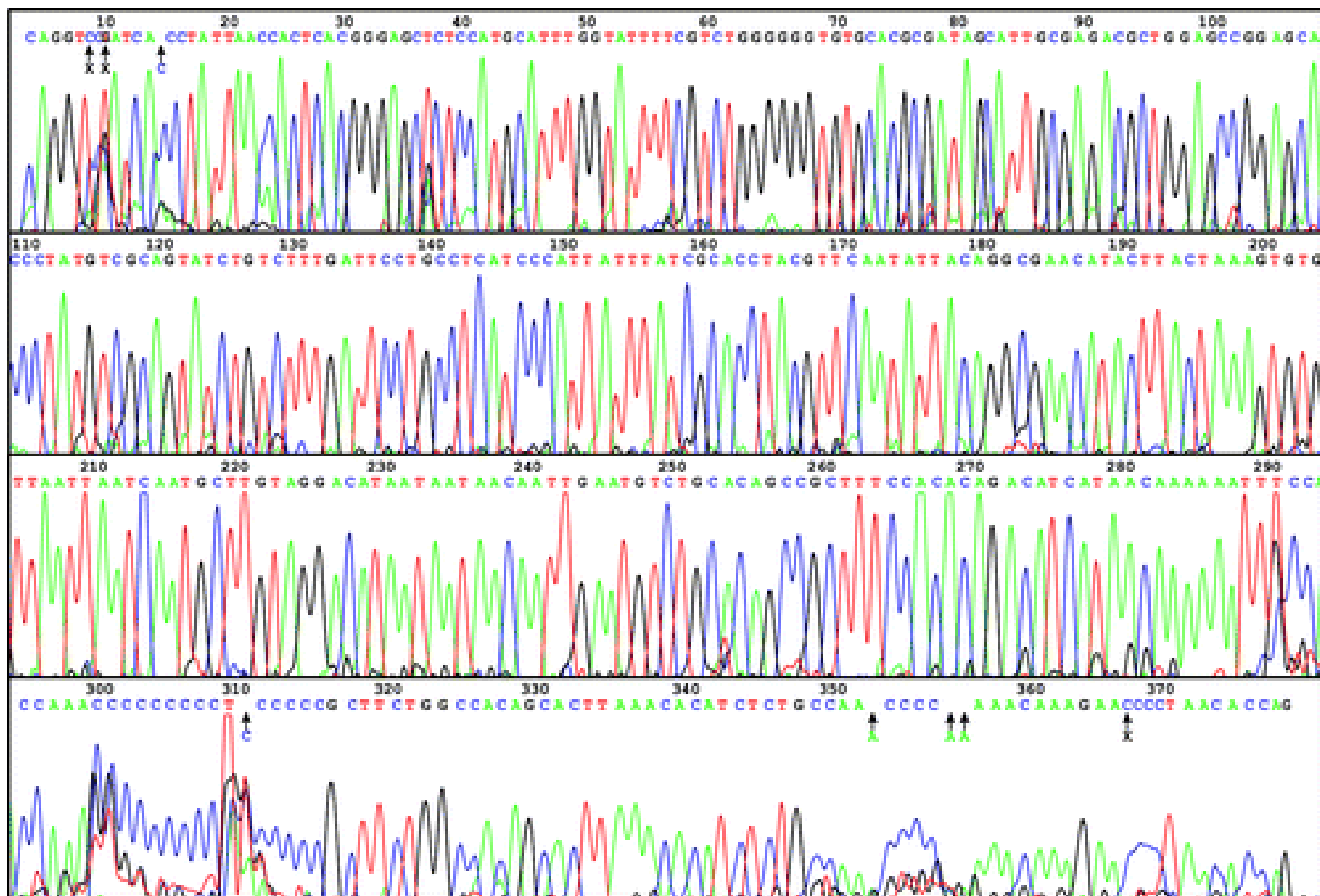


Kapiler Elektrophorez



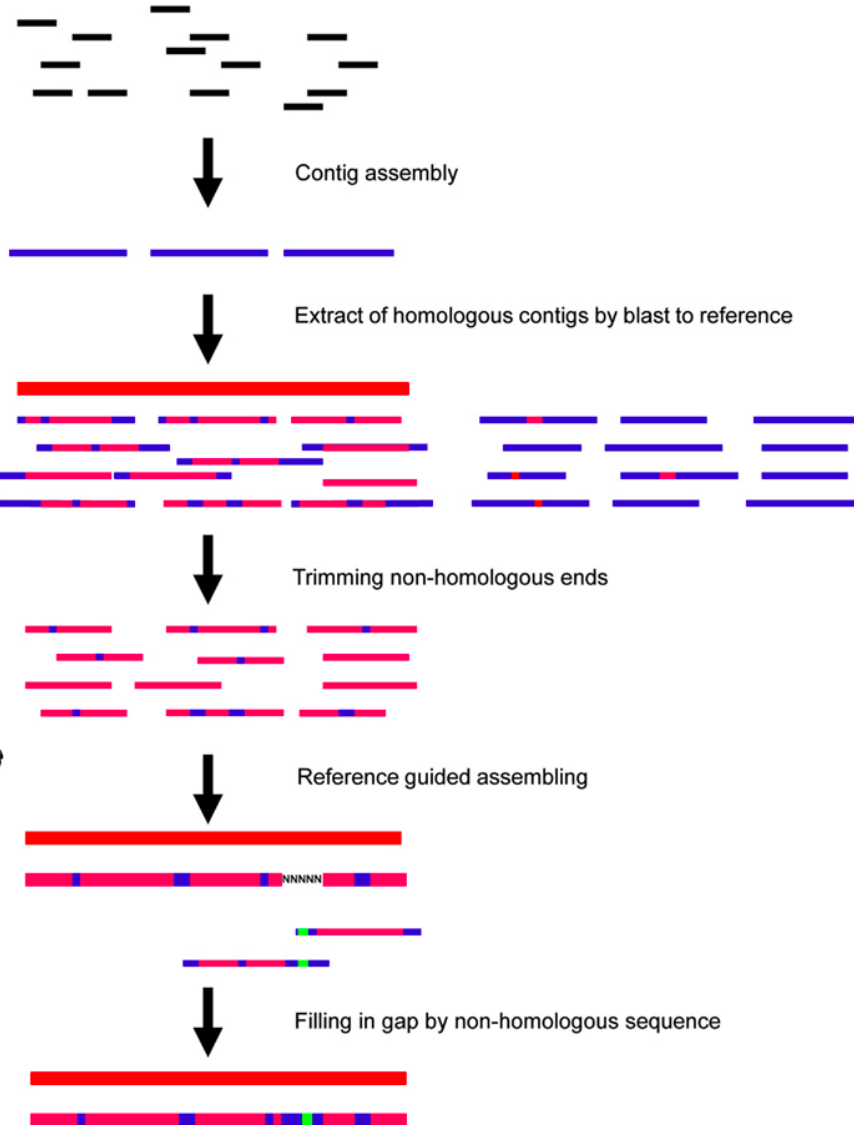






Yeni Nesil Dizileme ile Tüm Genom İncelemesi

Tanı, Epidemiyoloji, İlaç Direnci



“Shot gun sequencing”

Rastgele atış

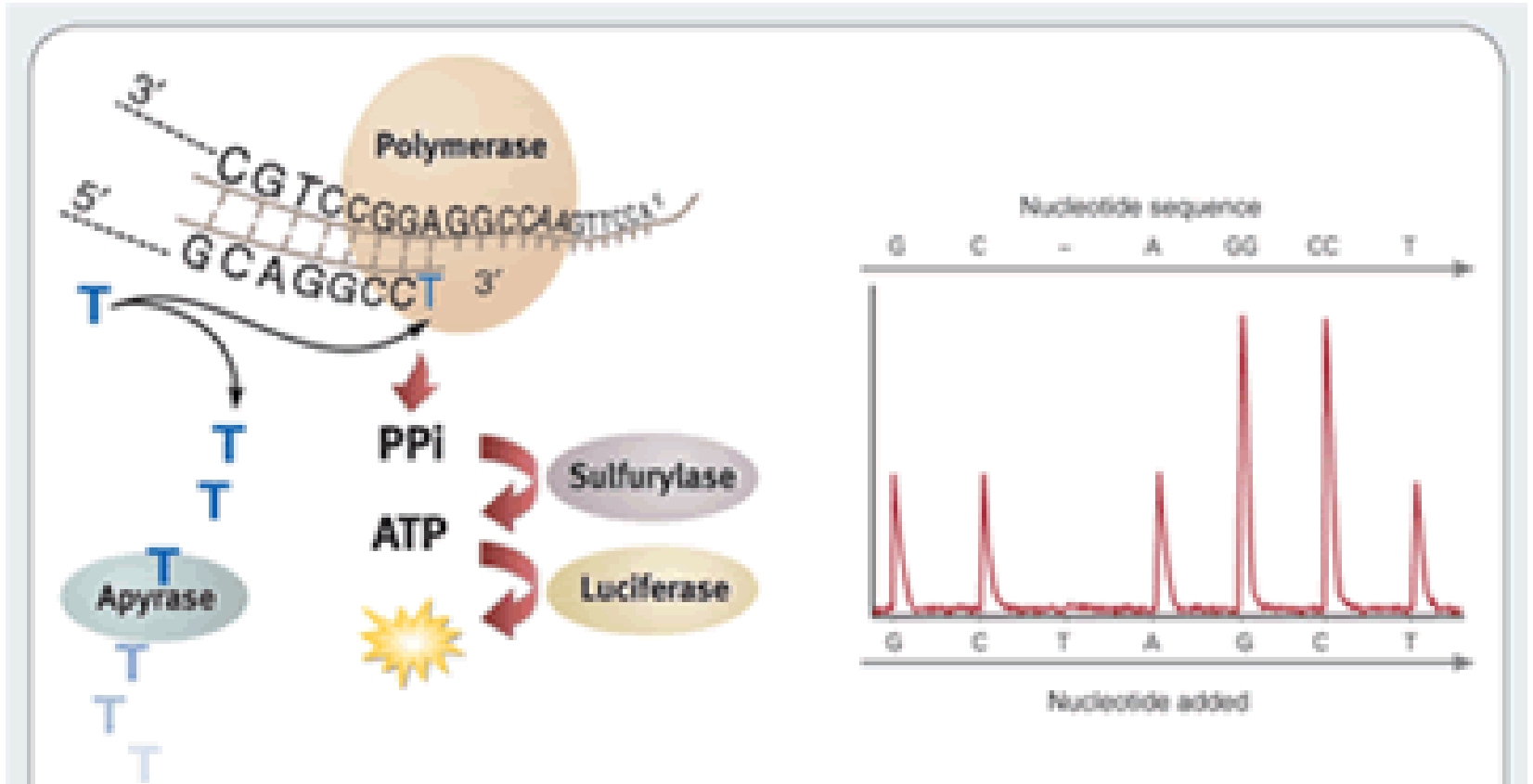


“Delikli demir çıktı
Mertlik bozuldu”

Koroğlu

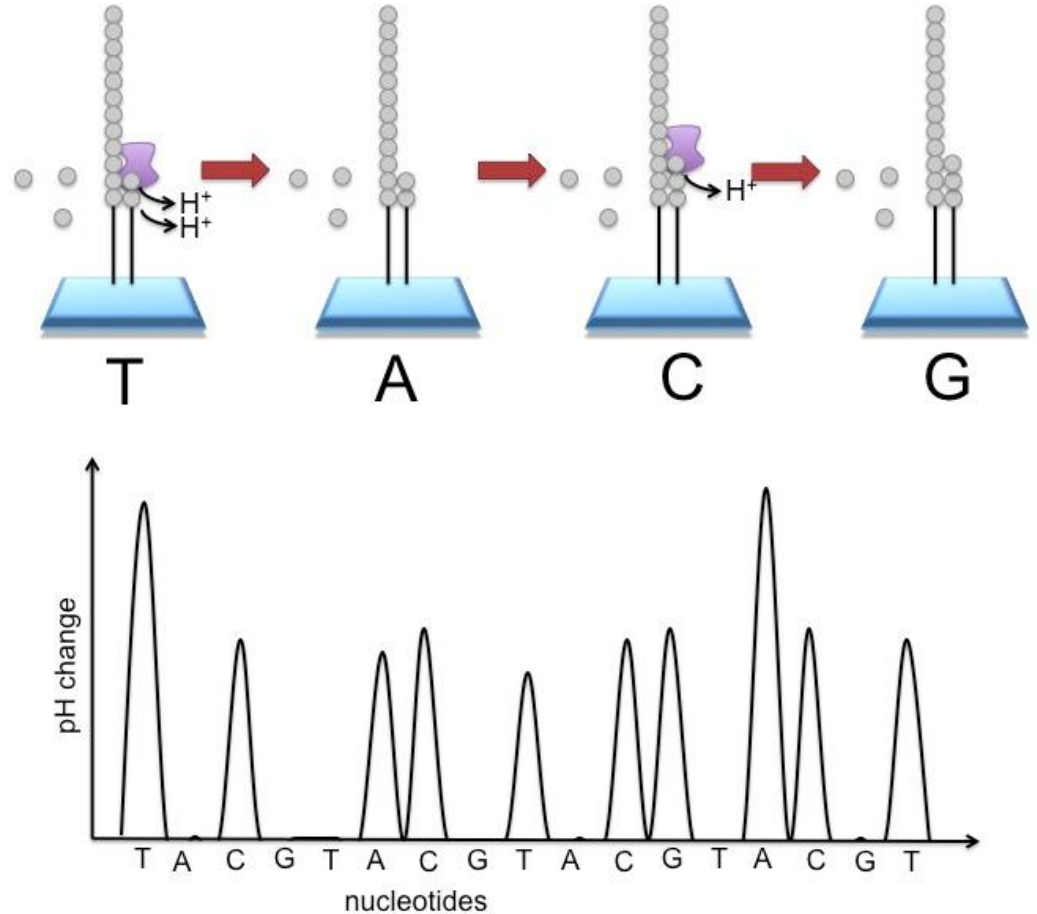
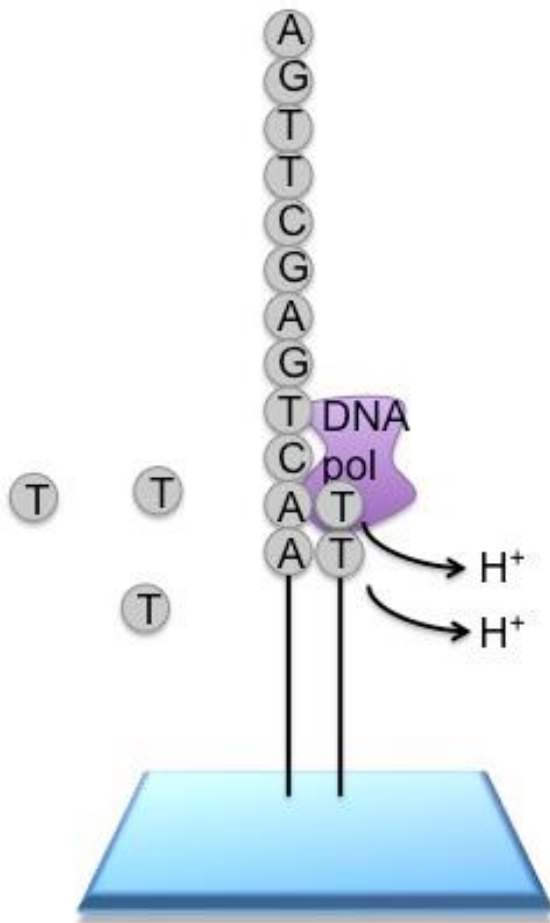
Pirodizileme

Küçük çukurlarda DNA sentezi yapılır. Nükleotitler tek tek sıra ile çukurlara eklenir. Polimeraz nükleotitleri eklerken pirofosfat açığa çıkar. Bu ortama konan sülfirilaz enzimi ile ATP oluşmasını sağlar. Yine ortamdaki Lusiferaz ATP yi kullanarak lusiferini oksitler ve bu sırada ışık oluşur. Işığın oluşması DNA dizisinde o sırada eklenen nükleotitin olduğunu gösterir. Aygıt çukurlardaki ışımları okuyarak DNA dizisini belirler.

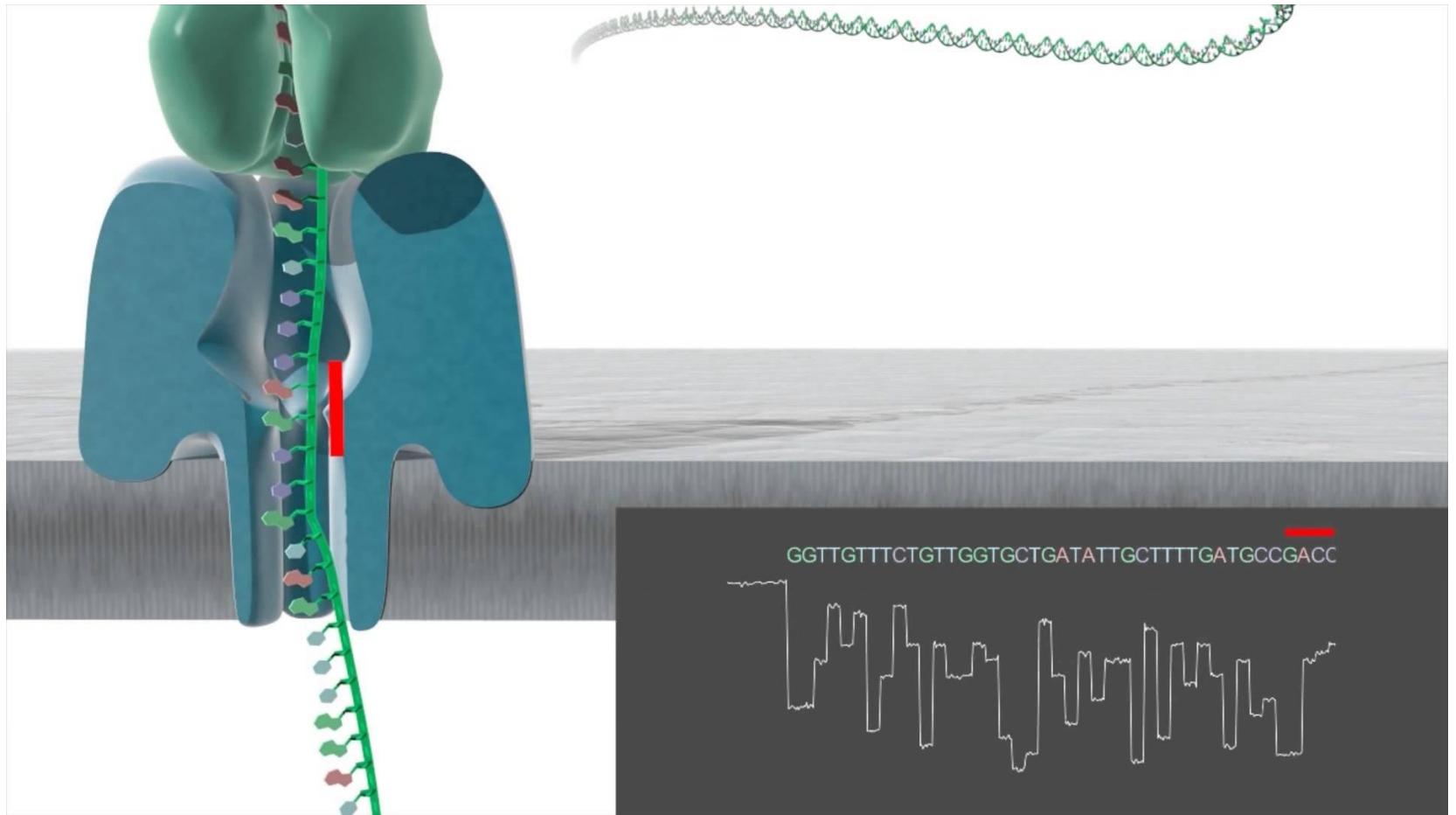


İyon duyarlı dizileme

Aygıtta pH'a duyarlı küçük çukurlar bulunur. Bu yüzeylerde DNA sentezi yapılır. Nükleotitler tek tek sıra ile çukurlara eklenir. Polimeraz nükleotitleri eklerken H^+ açığa çıkararak pH'ı düşürür. Hangi nükleotit ekleniyorsa ona göre DNA dizisi okunur. Her döngüde bağlanmayan nükleotitler çukurlar yıkanarak uzaklaştırılır.



Nanopore Sequencing





2 HAFTADIR ÖKSÜRÜYOR MUSUNUZ?

Tüberküloz olabilir misiniz?



Tüberküloz tedavi edilebilir.

ÖKSÜRÜK VE BALGAM

GECE TERLEMESİ

KİLO KAYBI

**TÜBERKÜLOZUN
DİĞER BELİRTİLERİ**

HALSİZLİK

ATEŞ

İŞTİHAZSIZLIK

- Halk arasında verem diye bilinir.
- Akciğerlerde başlar ve tüm vücuda yayılabilir.
- İnsandan insana hava-yoluyla bulaşır.
- Tüm tedavi masrafları devlet tarafından karşılanır.
- Erken teşhis tüberkülozun yayılmasını engeller.

**BALGAM ÖRNEĞİ VERMENİZ
TANI İÇİN YETERLİ**

**Sizin ve çevrenizdekilerin
sağlığı için...**



**ACIBADEM
ÜNİVERSİTESİ**

**İLETİŞİM: 0534 685 91 32
0539 960 79 57
0505 317 12 12**



**Elaman
tâhden**



Tüberkülozdan arınmış bir dünya için
yaptığınız katkıya teşekkürler...