21. Ulusal Patoloji Kongresi
19 Kasım Cumartesi
Smyrna, 11:30-12:30

Panel: Moleküler patoloji laboratuvarının kurulumu ve testlerin standardizasyonu

Mutasyon tarama teknikleri:
KRAS, EGFR, BRAF mutasyon analiz tekniklerinin avantajları/dezavantajları

Dr. Hüseyin Baloğlu

- I -
Moleküler patoloji laboratuvarının kurulumu ve testlerin standardizasyonu

Klinik laboratuvar testleriyle ilgili kurallar, FDA tarafından ilk kez 1976 yılında başlatıldı.

Laboratuvarda yapılan Klinik testlerin kalite standartlarının sağlanması için 1988 yılında CLIA (Clinical Laboratory Improvement Amendment) oluşturuldu. 2003 yılında son revizyonu yapıldı.

CLIA'nın denetlemesini CMS (Center for Medicare&Medicaid Services) yapıyor. CMS, CLIA dışında, akreditasyon faaliyeti gösteren 3 büyük organizasyonu da tanıyor:

- CAP: College of American Pathologist
- JCAHO: The Joint Commission on Accreditation of Healthcare Organizations
- COLA: The Commission for Office Laboratory Accreditation.

CLIA'da Moleküler Patoloji için özel bir yapılanma yoktur. Bu nedenle Moleküler Patoloji laboratuvarlarının hemen tümünü CAP akredite eder (CAP-Laboratory accreditation. Checklist 12: Molecular Pathology Laboratory)

(CLIA: There are few specific requirements for molecular testing, but the general guidelines and requirements still apply and are considered sufficient).

CLSI: Clinical Laboratory Standarts Institute.

CLIA yaptırımları klinik test yapan tüm laboratuvarların serifikasyonunu gerektirir.

Buna yönelik olarak, CLIA genel laboratuvar sertifikasyonu ve daha spesifik laboratuvarların sertifikasyonu için 'yol gösterici' kurallar ve dökümanlar hazırlar.

LABORATORY CERTIFICATION (LC) CODES
CLIA SPECIALTY AND SUBSPECIALTY INFORMATION

LC Code	Specialty - Subspecialty
010	Histocompatibility
110	Microbiology - Bacteriology
115	Microbiology - Mycobacteriology
120	Microbiology - Mycology
130	Microbiology - Parasitology
140	Microbiology - Virology
210	Diagnostic Immunology - Syphilis Serology
220	Diagnostic Immunology - General Immunology
310	Chemistry - Routine Chemistry
320	Chemistry - Urinalysis
330	Chemistry - Endocrinology
340	Chemistry - Toxicology
400	Hematology
510	Immunohematology - ABO Group & Rh type
520	Immunohematology - Antibody Detection (transfusion)
530	Immunohematology - Antibody Detection (non-transfusion)
540	Immunohematology - Antibody Identification
550	Immunohematology - Compatibility Testing
610	Pathology - Histopathology
620	Pathology - Oral Pathology
630	Pathology - Cytology
800	Radiobioassay
900	Clinical Cytogenetics

CLIA regulations require certification of all laboratories performing clinical testing of any kind and provide both general guidelines and subspecialty-specific standards.

Although **there is a cytogenetic subspecialty** under CLIA, there is **no molecular pathology subspecialty**.

As a consequence, there are few specific requirements for molecular testing, but the general guidelines and requirements still apply and are considered sufficient.

CAP Laboratory Accreditation Program Inspection Checklists and the CLSI documents referenced:

Anatomic Pathology - ANP
Chemistry and Toxicology - CHM
Cytogenetics - CYG
Cytopathology - CYP
Flow Cytometry - FLO
Forensic Drug Testing - FDT
Hematology & Coagulation - HEM
Histocompatibility - HSC
Immunology and Syphilis Serology - IMM
Laboratory General - GEN
Limited Service - LSV
Microbiology - MIC
Molecular Pathology - MOL
Point-of-Care Testing - POC
Reproductive Laboratory - RLM
Team Leader Assessment - TLC
Transfusion Medicine - TRM
Urinalysis and Clinical Microscopy - URN

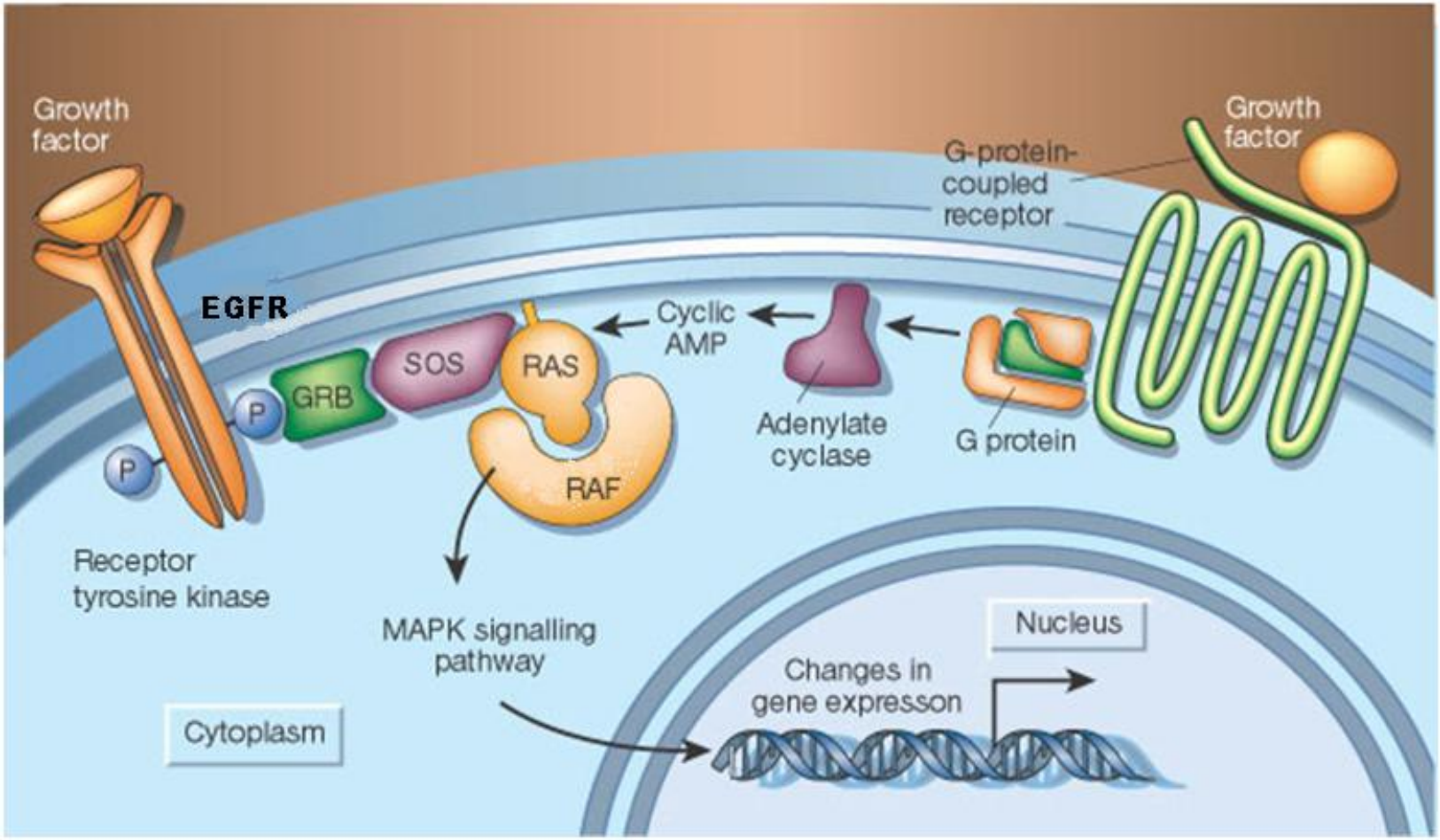
For specific CAP accreditation requirements and information, and to purchase editions of the CAP Laboratory Accreditation Program Inspection Checklists, visit www.cap.org

MOLEKÜLER PATOLOJİ
ÇALIŞMA GRUBU
LABORATUVAR STANDARDİZASYONU VE AKREDİTASYON ÇALIŞMASI
2011

- II -
Mutasyon tarama teknikleri:

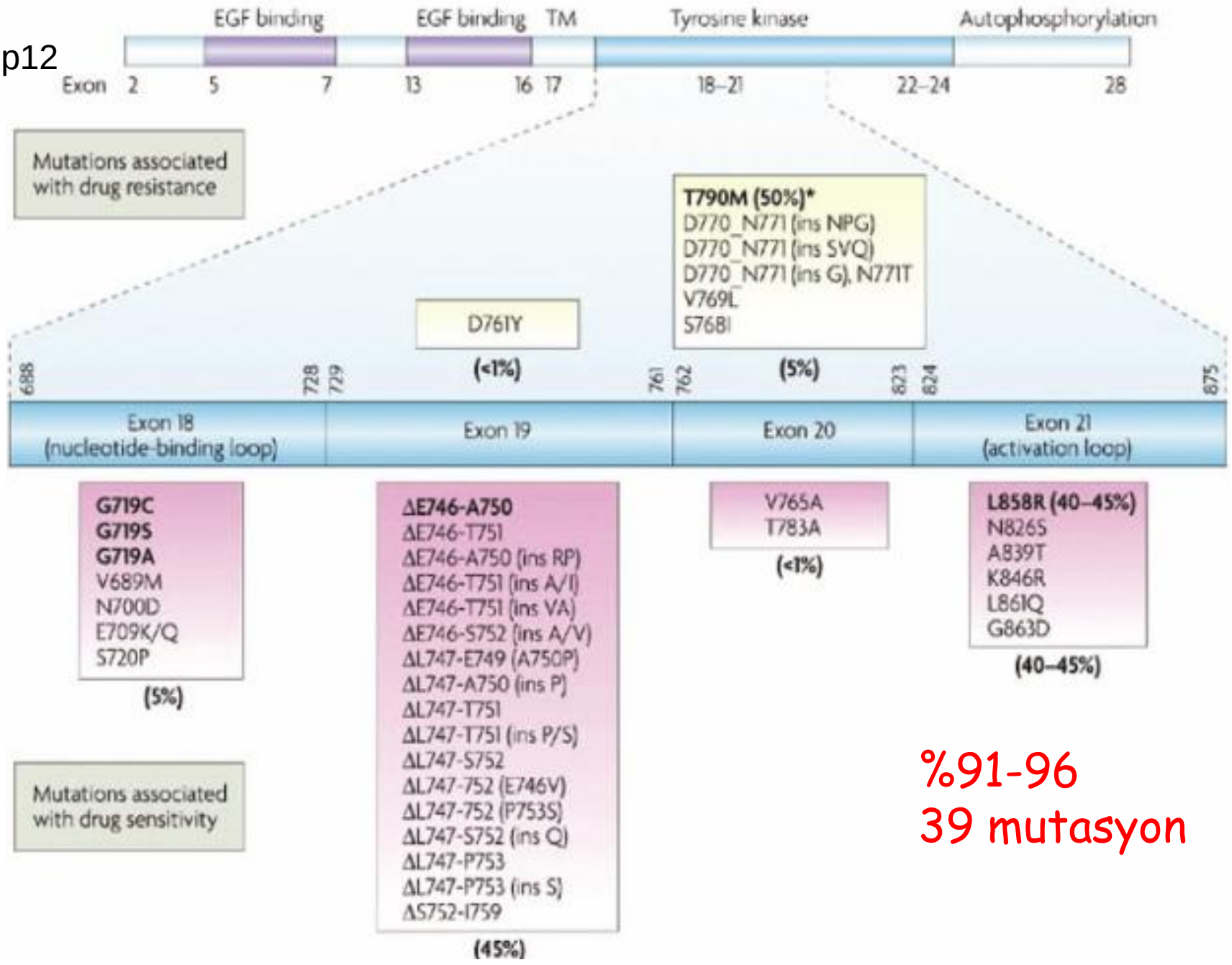
mutasyon analiz tekniklerinin avantajları / dezavantajları

MAPK (mitogen-activated protein kinase) Sinyal Yolağı



http://edrv.endojournals.org/cgi/content/abstract/28/7/742: Endocr Rev.2007; 28: 742-762

7p12



%91-96
39 mutasyon

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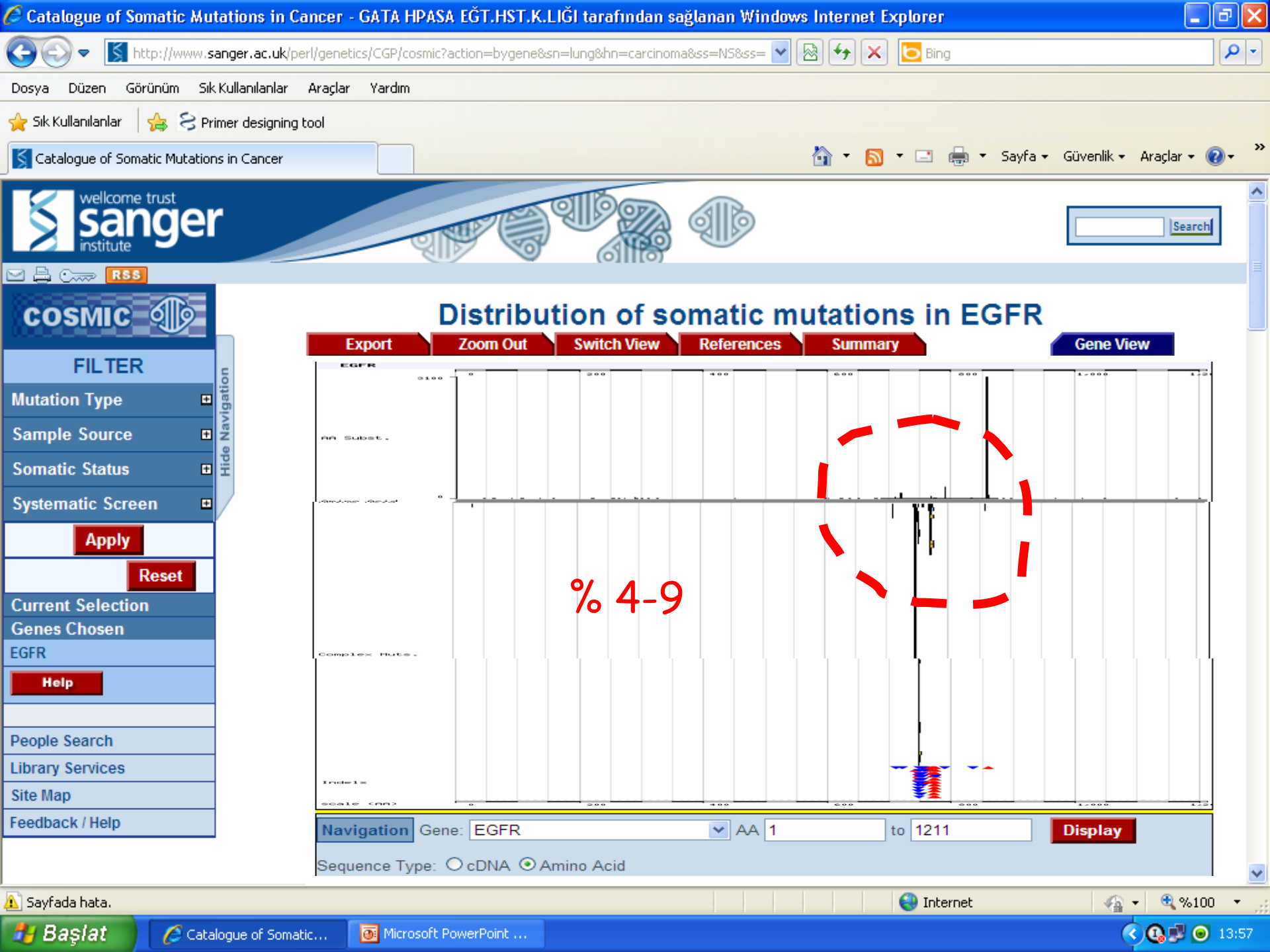

Catalogue of Somatic Mutations in Cancer - GATA HPASA EĞT.HST.K.LİĞI tarafından sağlanan Windows Internet Explorer
http://www.sanger.ac.uk/perl/genetics/CGP/cosmic?action=bygene&sn=lung&hn=carcinoma&ss=NS&ss=
Bing
Dosya Düzen Görünüm Sık Kullanılanlar Araçlar Yardım
Sık Kullanılanlar
Primer designing tool
Catalogue of Somatic Mutations in Cancer
Sayfa Güvenlik Araçlar
wellcome trust sanger institute
Search
RSS
COSMIC
FILTER
Mutation Type
Sample Source
Somatic Status
Systematic Screen
Apply
Reset
Current Selection
Genes Chosen
EGFR
Help
People Search
Library Services
Site Map
Feedback / Help
Hide Navigation
Distribution of somatic mutations in EGFR
Export
Zoom Out
Switch View
References
Summary
Gene View
EGFR
AA Subst.
Complex Muts.
Indels
% 4-9
Navigation
Gene: EGFR
AA 1
to 1211
Display
Sequence Type: cDNA Amino Acid
Sayfada hata.
Internet
%100
Başlat
Catalogue of Somatic...
Microsoft PowerPoint ...
13:57

EGFR: E18, E19, E20, E21

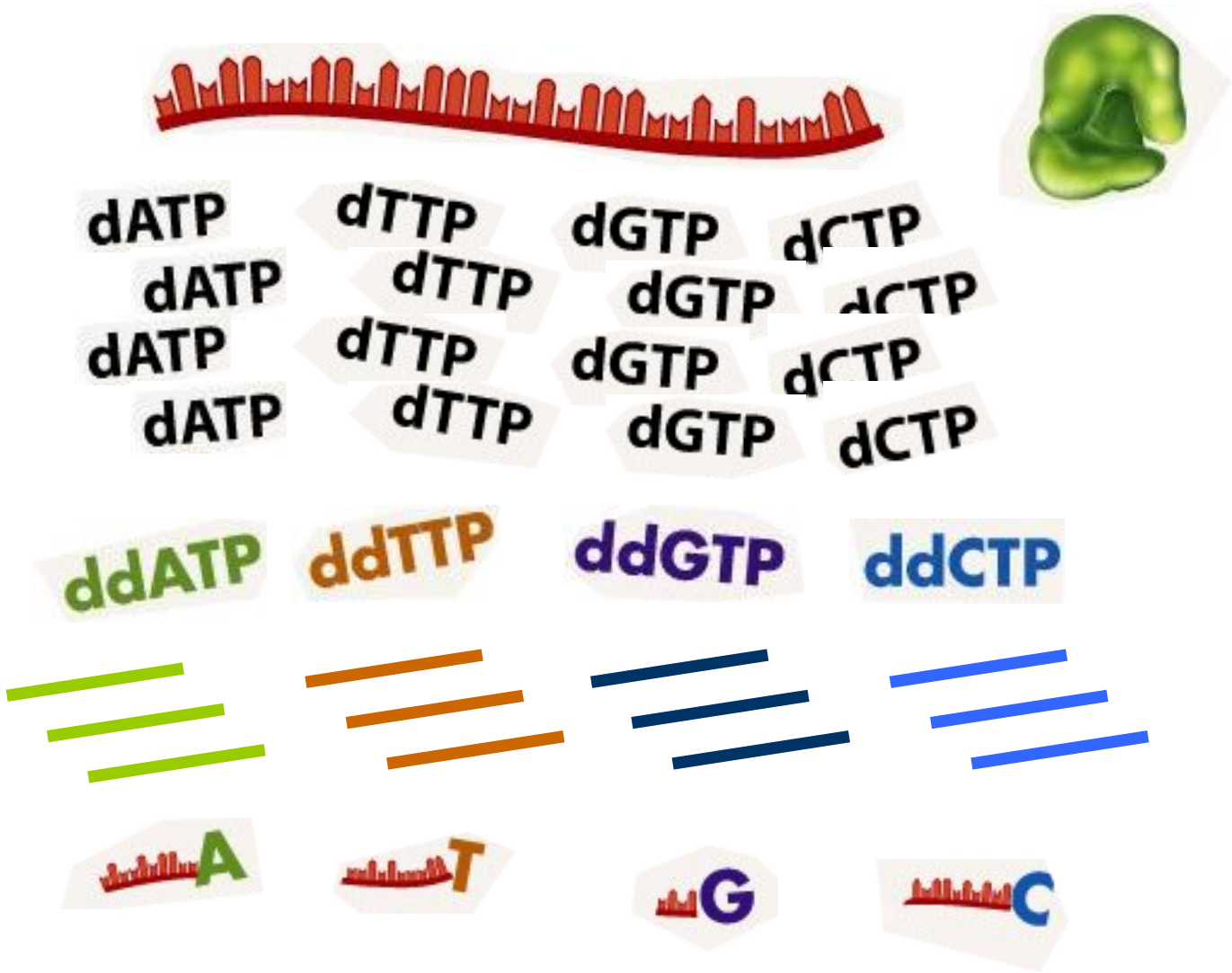
KRAS: E2 (±E3)

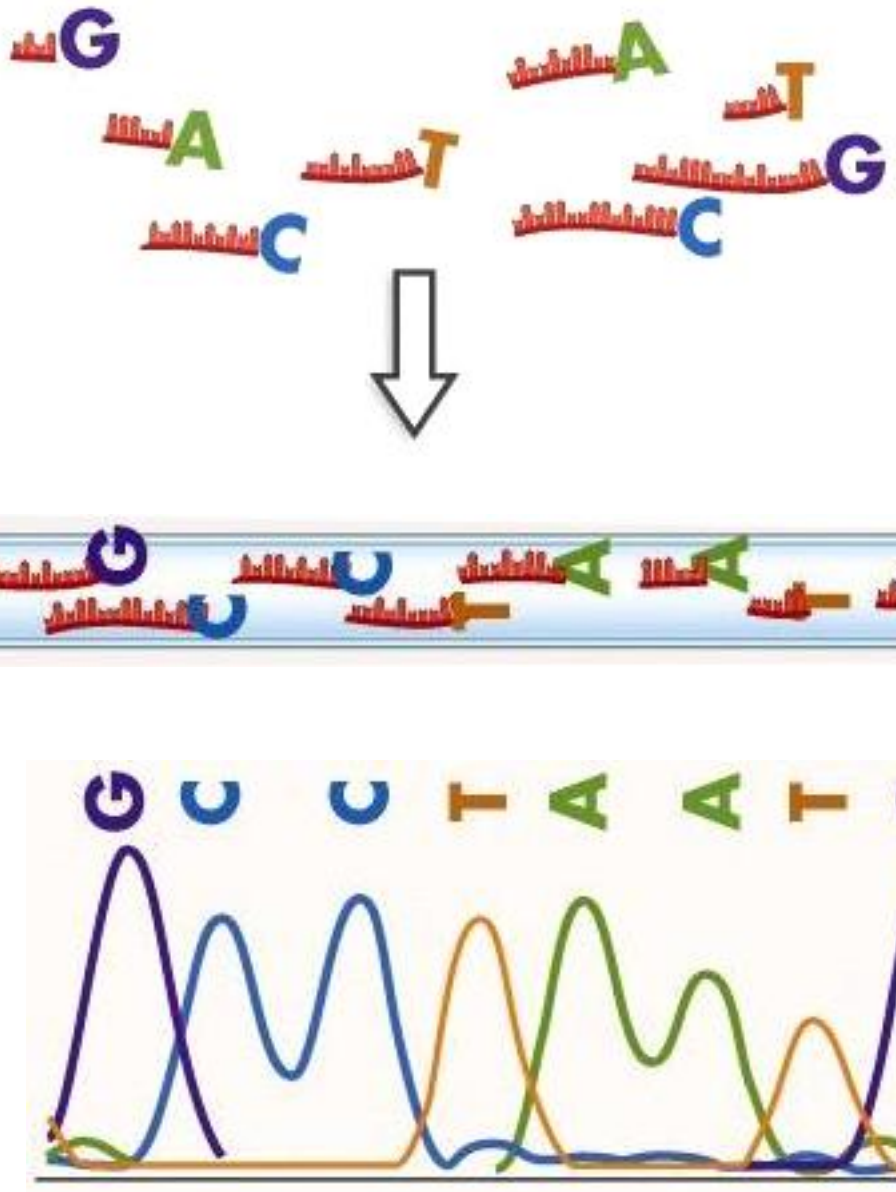
BRAF: E15 (V600E)

Yöntem;

1 – Dideoxysequencing
2 - Pyrosequencing
3 - Ion Torrent
4 - HR-MCA
5 - Sekans (Alel) spesifik PCR
6 - Mikro yonga üzerinde çoklu hibridizasyon

1. Dideoxysequencing



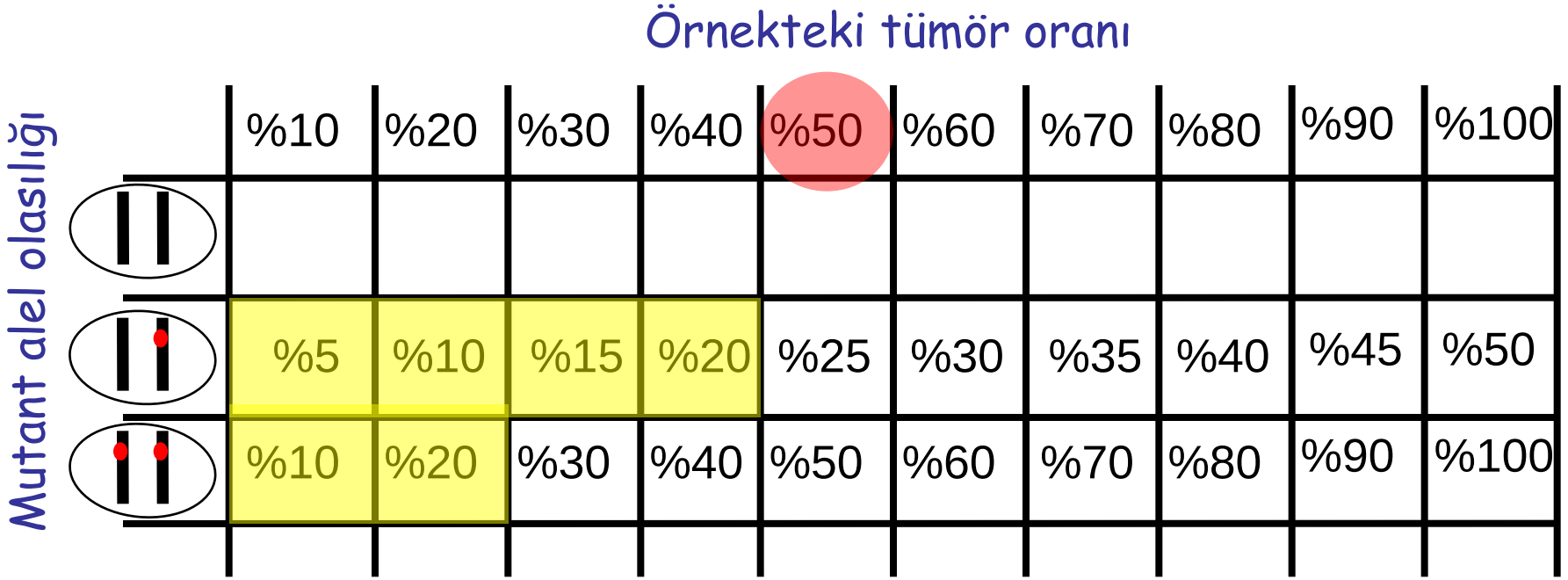

G C C T A A T G

Dideoxysequencing

$$\frac{\text{Mutant alel}}{\text{Wild-type alel}} \geq \frac{15\text{- }20}{100}$$

- Tümör dışı normal doku
- Tümördeki wild-type aleller

MUTASYON PROFİLİ İÇİN OLASILIK MODELLEMESİ

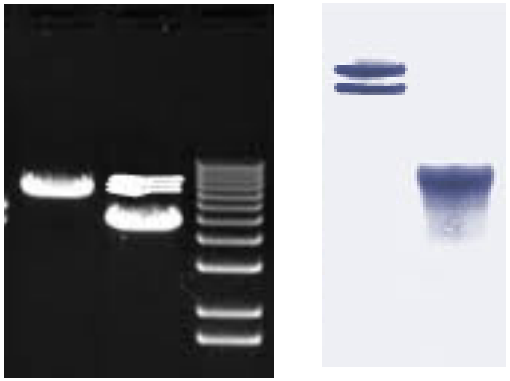


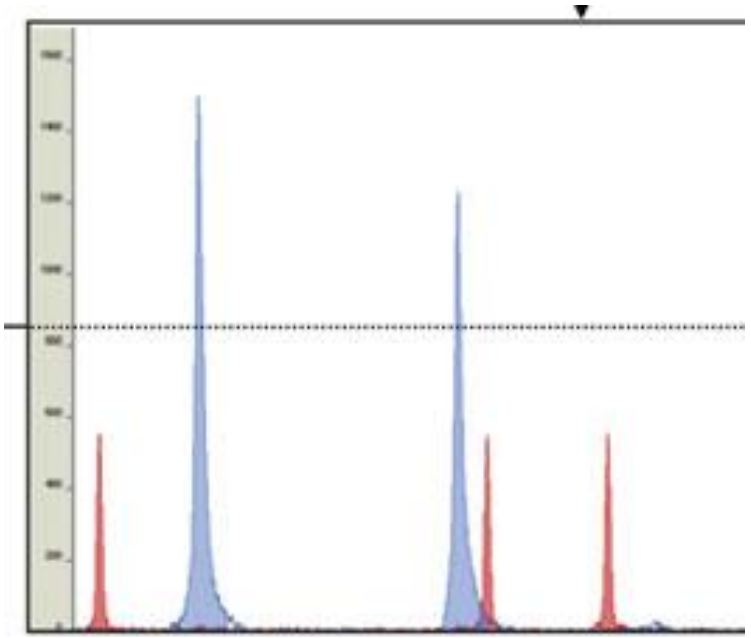
(FLA) Fragment length analysis

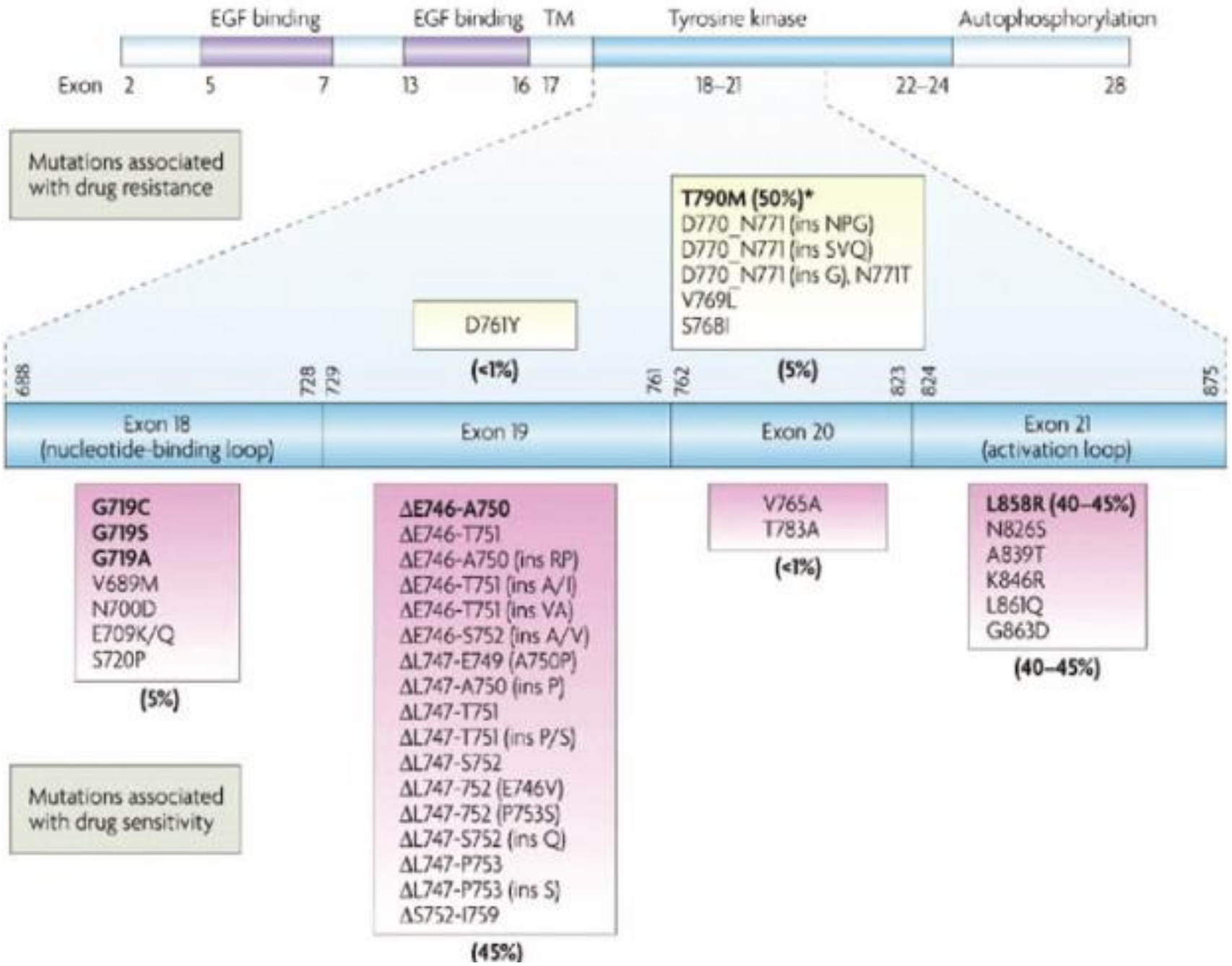
EGFR

Ekzon 19: delsyon

Ekzon 20: insersiyon







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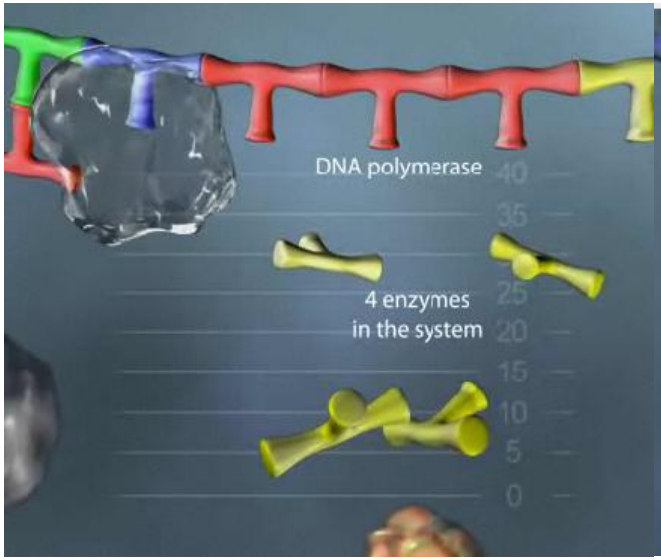
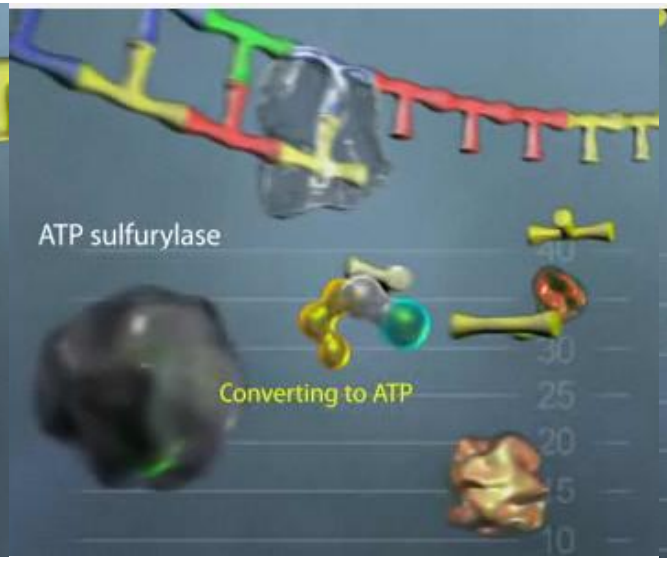
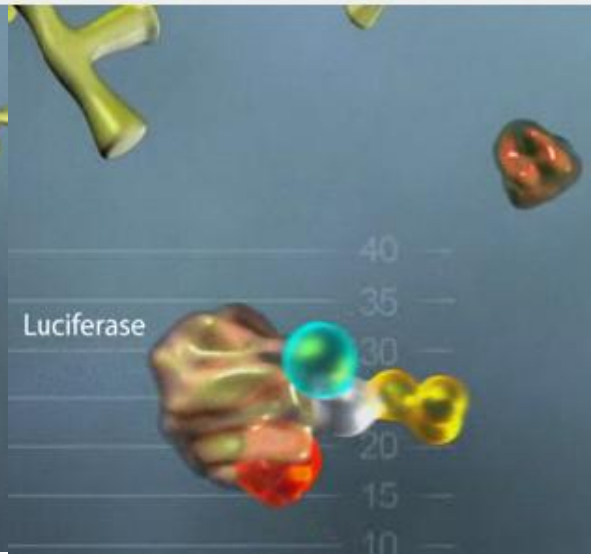
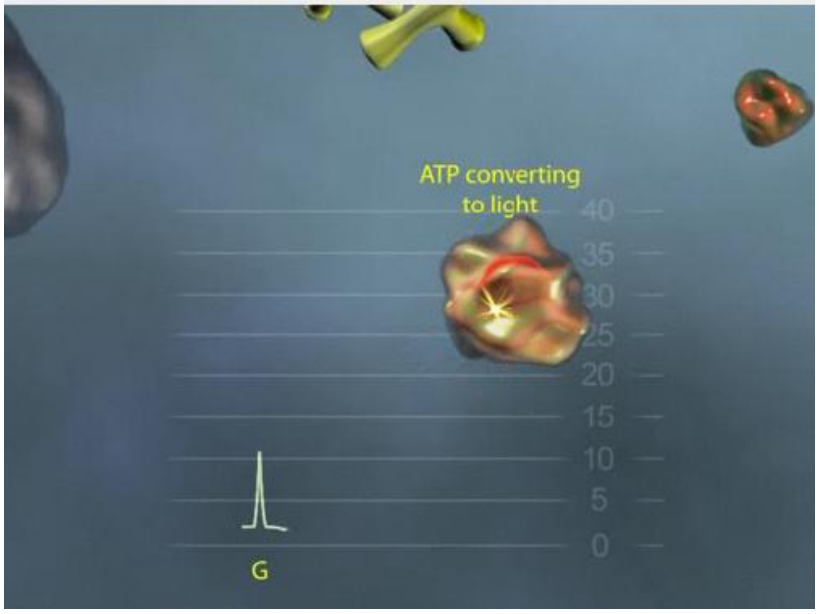
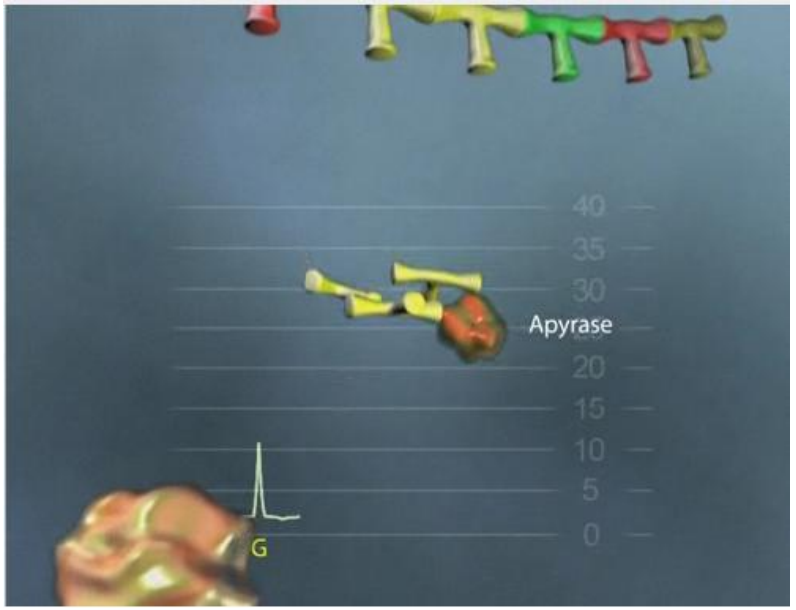
2. Pyrosequencing





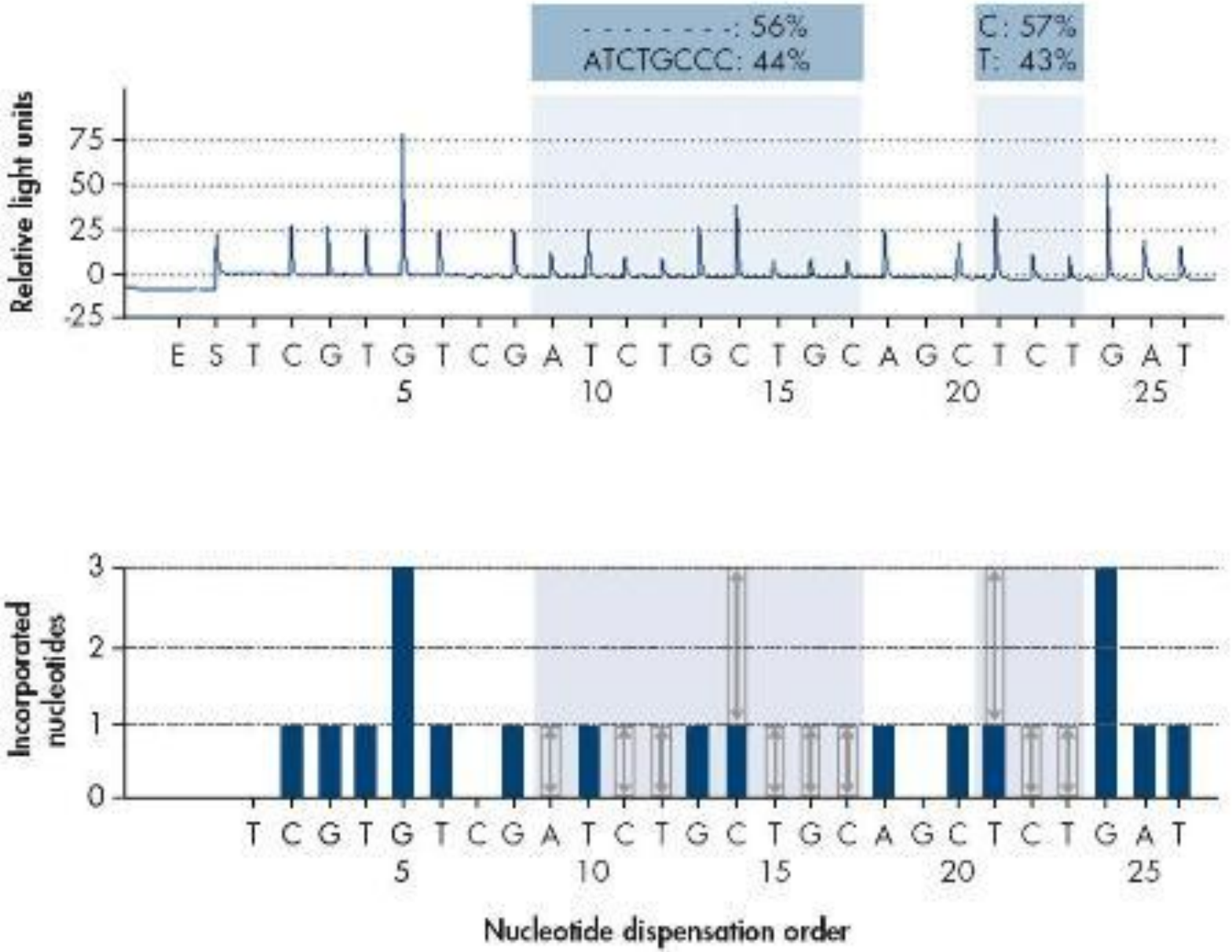






Sequence to analyze:

CGTGGGTG[ATCTGCCC]TGCACTYTGGGATA



Pyrosequencing

$$\frac{\text{Mutant alel}}{\text{Wild-type alel}} \geq \frac{1\text{- }10}{100}$$

- Tümör dışı normal doku
- Tümördeki wild-type aleller

3 - Ion Torrent

nature International weekly journal of science

An integrated semiconductor device enabling non-optical genome sequencing

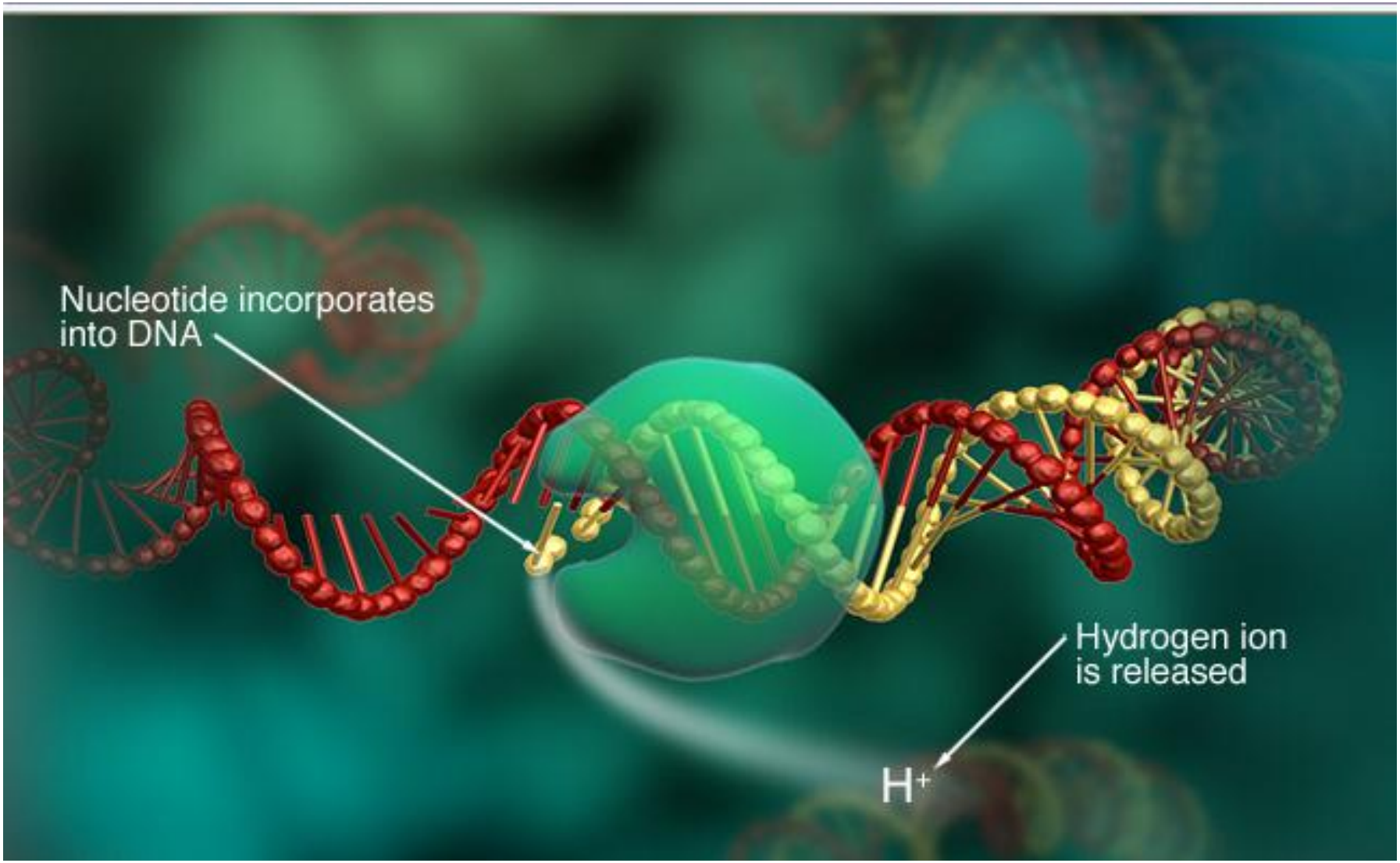
Jonathan M. Rothberg, Wolfgang Hinz, Todd M. Rearick, Jonathan Schultz, William Mileski, Mel Davey, John H. Leamon, Kim Johnson, Mark J. Milgrew, Matthew Edwards, Jeremy Hoon, Jan F. Simons, David Marran, Jason W. Myers, John F. Davidson, Annika Branting, John R. Nobile, Bernard P. Puc, David Light, Travis A. Clark, Martin Huber, Jeffrey T. Branciforte, Isaac B. Stoner, Simon E. Cawley, Michael Lyons + *et al.*

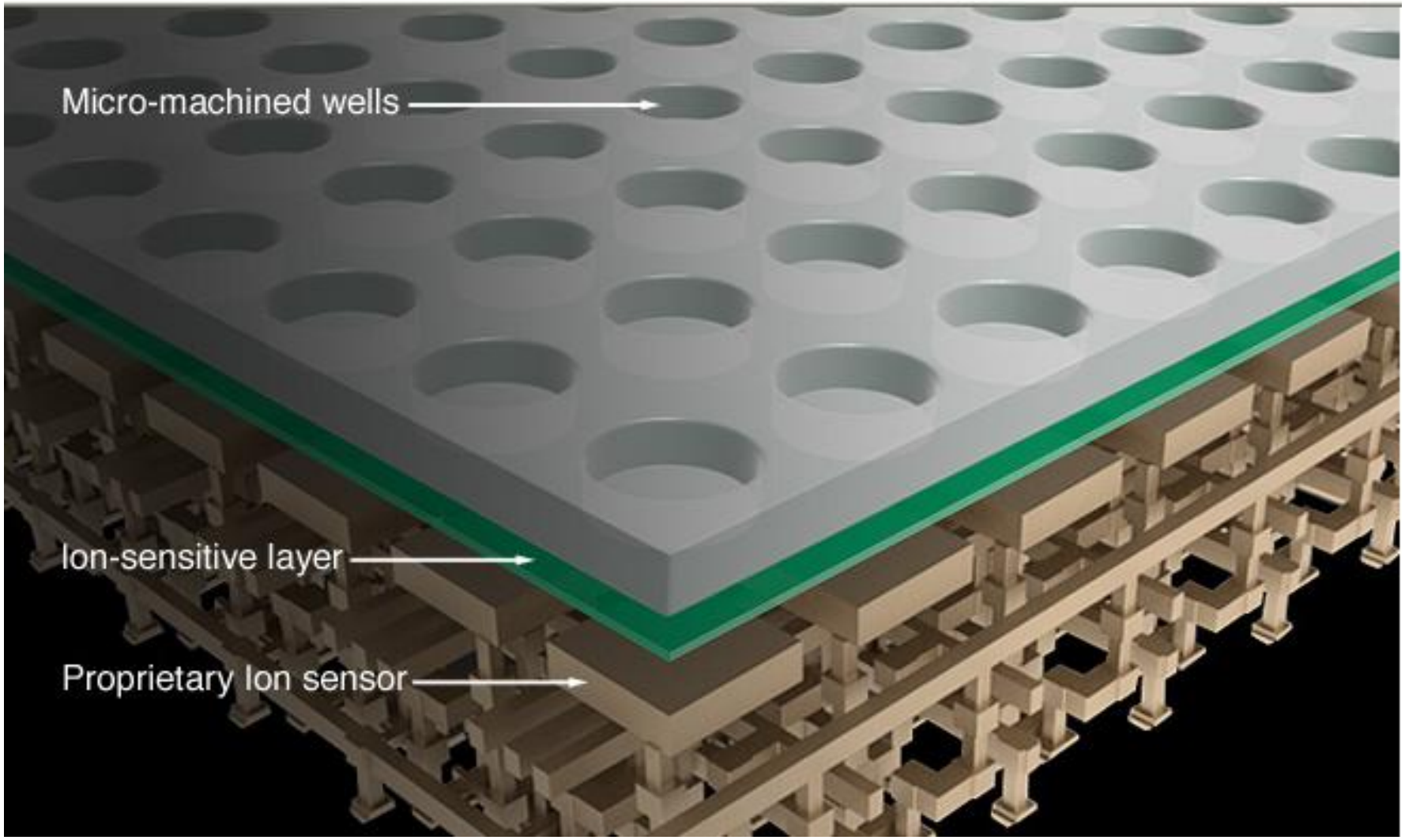
Affiliations | Contributions | Corresponding author

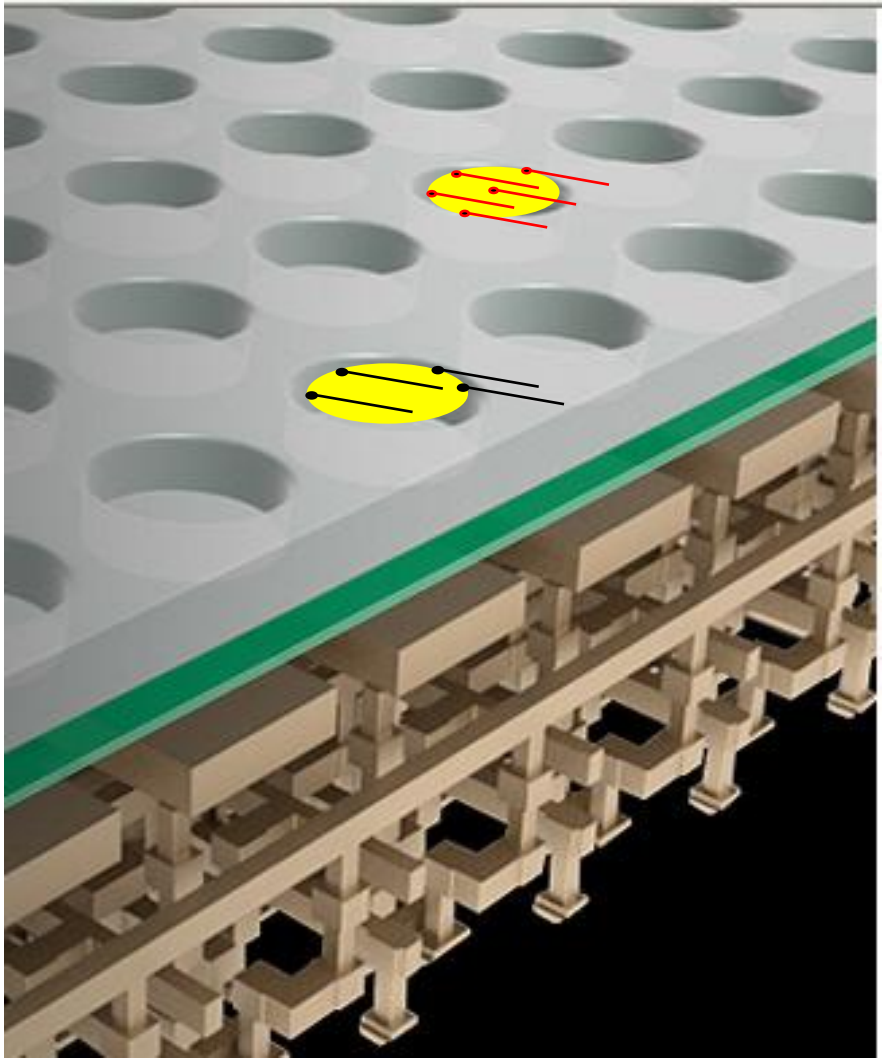
Nature **475**, 348–352 (21 July 2011) | doi:10.1038/nature10242

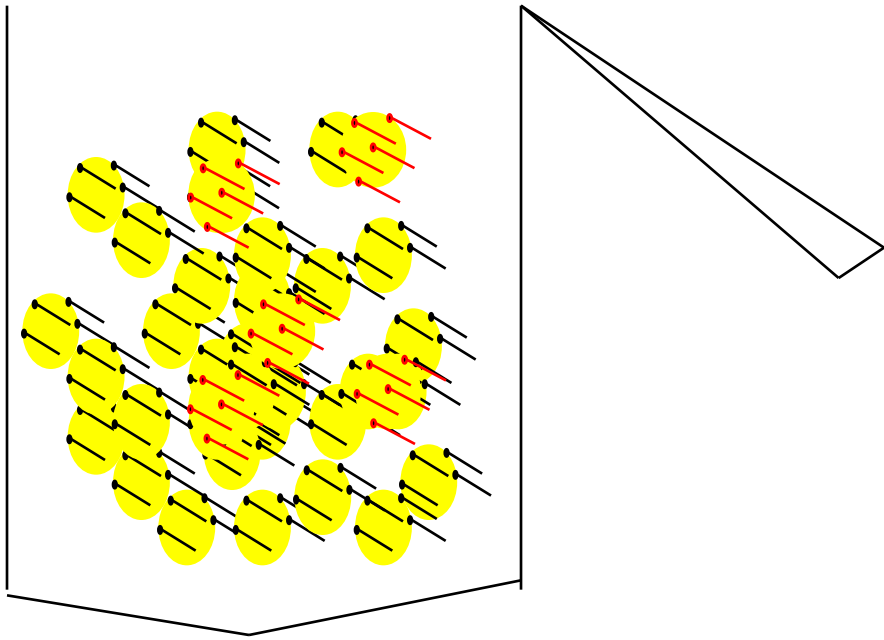
Received 08 March 2011 | Accepted 26 May 2011 | Published online 20 July 2011 | Corrected online 21 July 2011

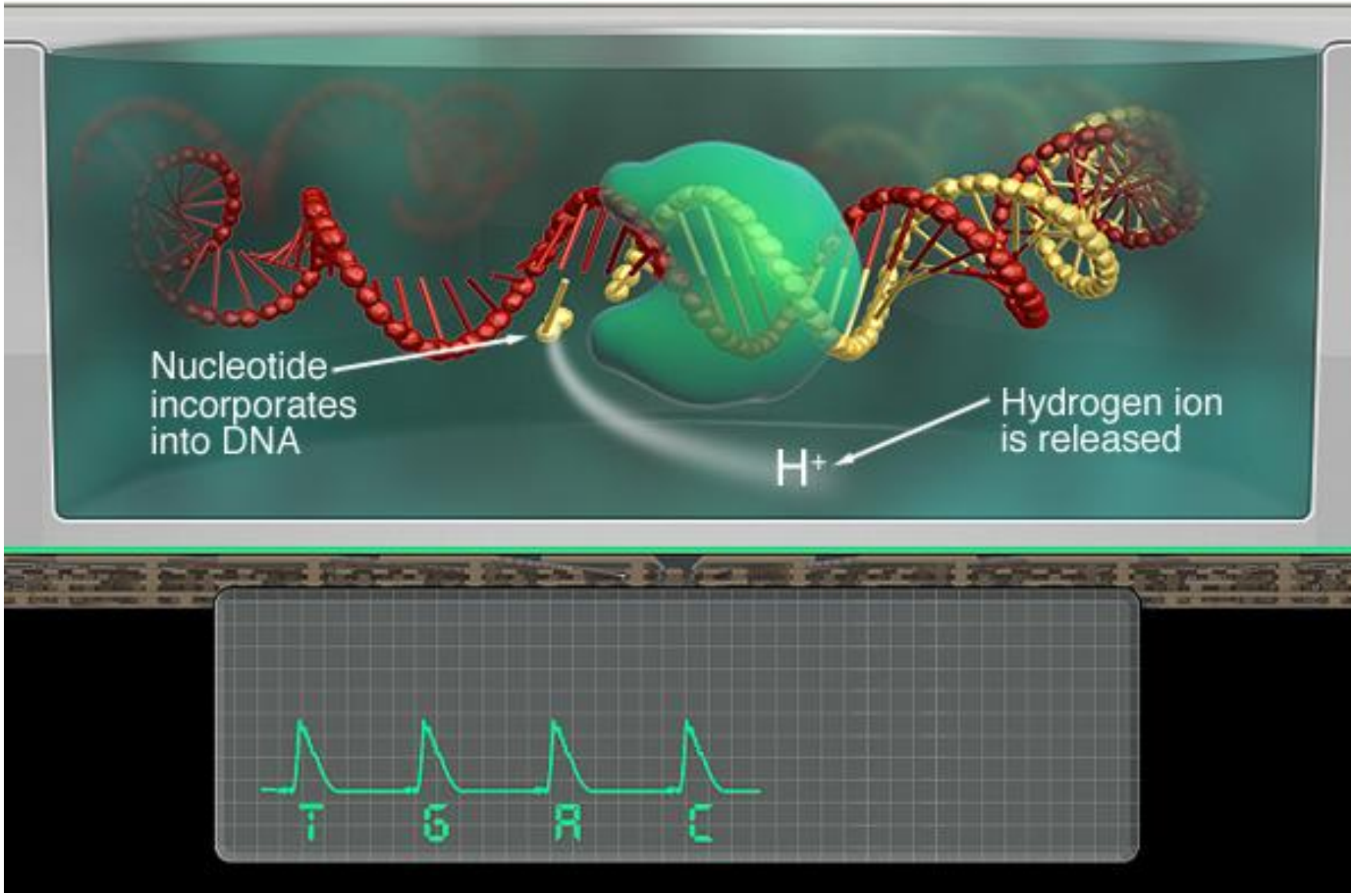
H^+


Nucleotide incorporates into DNA
Hydrogen ion is released
H+

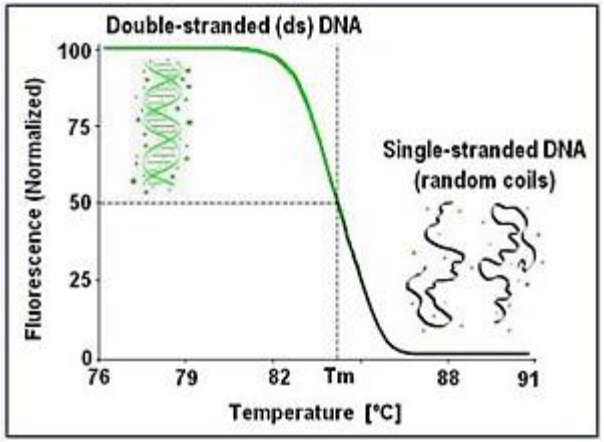

Micro-machined wells
Ion-sensitive layer
Proprietary Ion sensor






Nucleotide incorporates into DNA
Hydrogen ion is released
H+
T
G
A
C

4 - HRMA

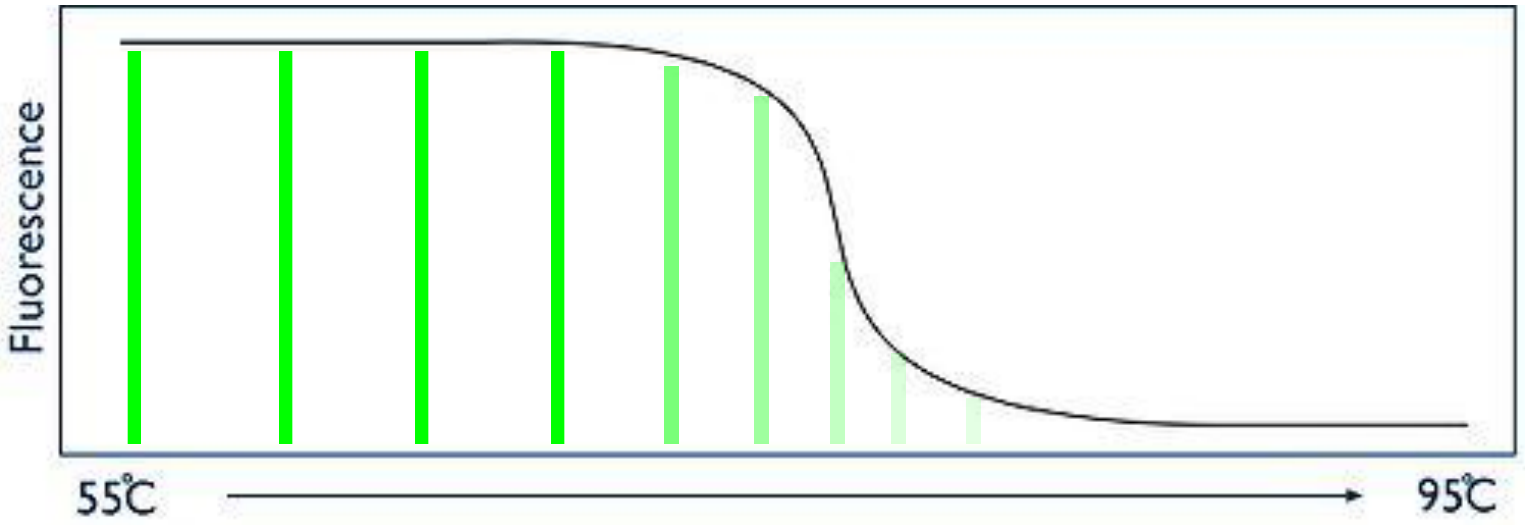


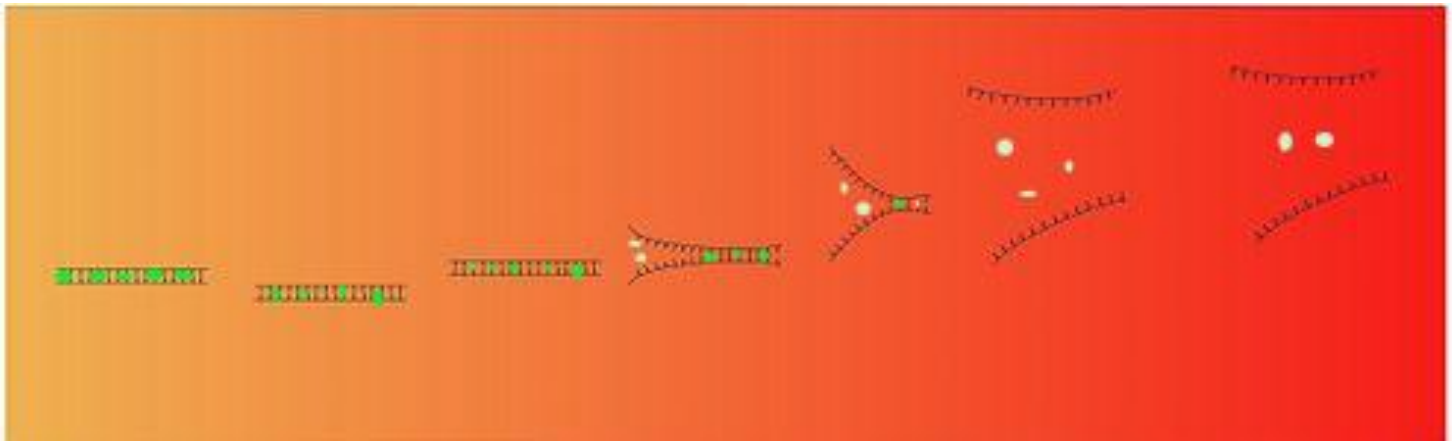
Clinical Chemistry 49:6
853–860 (2003)

High-Resolution Genotyping by Amplicon Melting Analysis Using LCGreen

Carl T. Wittwer,* Gudrun H. Reed, Cameron N. Gundry, Joshua G. Vandersteen, and Robert J. Pryor

HRMA





Detection of Somatic Mutations by High-Resolution DNA Melting (HRM) Analysis in Multiple Cancers

Jesus Gonzalez-Bosquet[1], Jacob Calcei[1], Jun S. Wei[2], Montserrat Garcia-Closas[3], Mark E. Sherman[3], Stephen Hewitt[4], Joseph Vockley[5], Jolanta Lissowska[6], Hannah P. Yang[3], Javed Khan[2], Stephen Chanock[1]*

1 Laboratory of Translational Genomics, Department of Health and Human Services (DHHS), National Cancer Institute (NCI), National Institutes of Health (NIH), Bethesda, Maryland, United States of America, **2** Advanced Technology Center, Pediatric Oncology Branch, Center for Cancer Research, DHHS, NCI, NIH, Bethesda, Maryland, United States of America, **3** Hormonal and Reproductive Epidemiology Branch, Division of Cancer Epidemiology and Genetics, DHHS, NCI, NIH, Bethesda, Maryland, United States of America, **4** Tissue Array Research Program, DHHS, NCI, NIH, Bethesda, Maryland, United States of America, **5** Office of Cancer Genomics, DHHS, NCI, NIH, Bethesda, Maryland, United States of America, **6** Department of Cancer Epidemiology and Prevention, M. Sklodowska-Curie Institute of Oncology and Cancer Center, Warsaw, Poland

BMC Cancer

BioMed Central

Research article

Open Access

High resolution melting analysis for rapid and sensitive *EGFR* and *KRAS* mutation detection in formalin fixed paraffin embedded biopsies

Hongdo Do[1,2], Michael Krypuy[1], Paul L Mitchell[3], Stephen B Fox[1,2] and Alexander Dobrovic*[1,2]

Address: [1]Molecular Pathology Research and Development Laboratory, Department of Pathology, Peter MacCallum Cancer Centre, Locked Bag 1, A'Beckett St, Melbourne, Victoria 8006, Australia, [2]Department of Pathology, University of Melbourne, Parkville, Victoria, 3010, Australia and [3]Ludwig Medical Oncology Department, Austin Hospital, Heidelberg, Victoria, 3084, Australia

Email: Hongdo Do - hongdo.do@petermac.org; Michael Krypuy - mkrypuy@gmail.com; Paul L Mitchell - paul.mitchell@austin.org.au; Stephen B Fox - stephen.fox@petermac.org; Alexander Dobrovic* - alexander.dobrovic@petermac.org

* Corresponding author

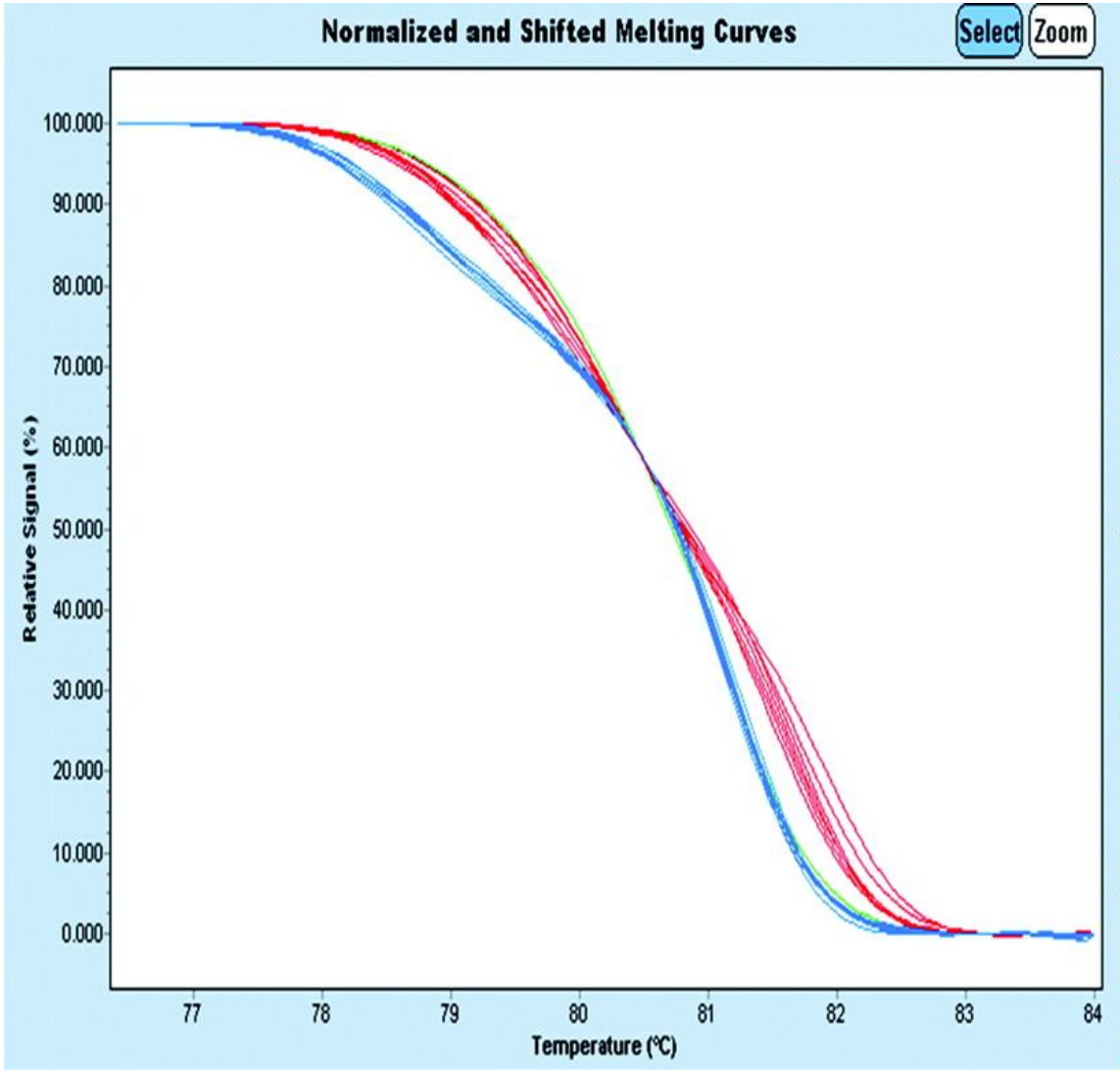
HR-MCA

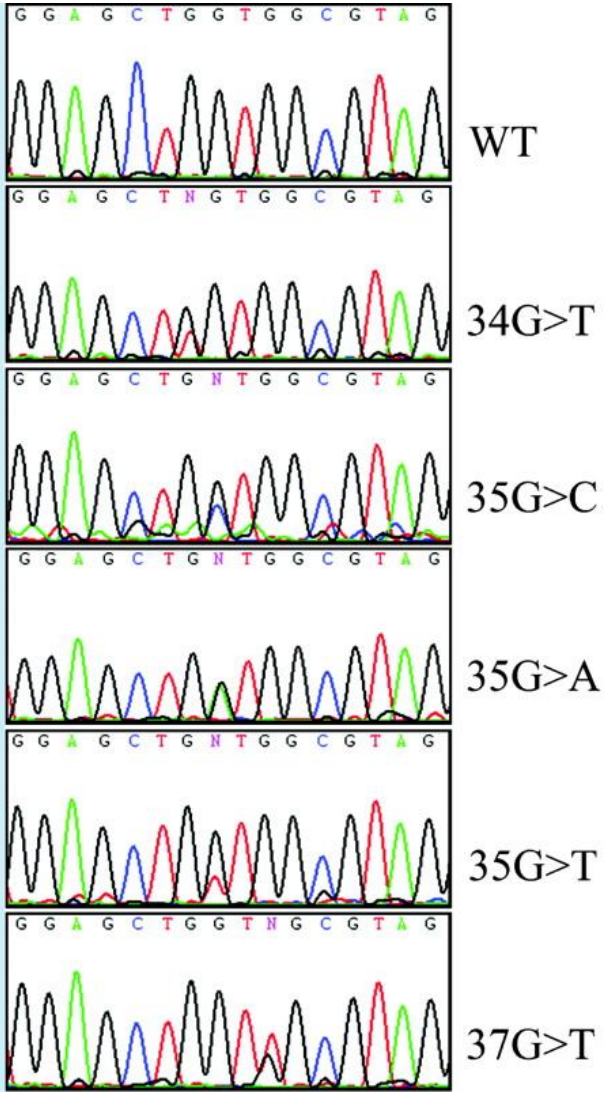
Linda Sabatini, PhD, technical director for molecular pathology at ACL Laboratories in suburban Chicago, has brought up real-time PCR with melt curve analysis to screen for KRAS mutations.

"It is very specific and very reliable,"

"We can quickly report out all wild-type results. When we see an abnormal melt curve, we confirm it with sequencing."

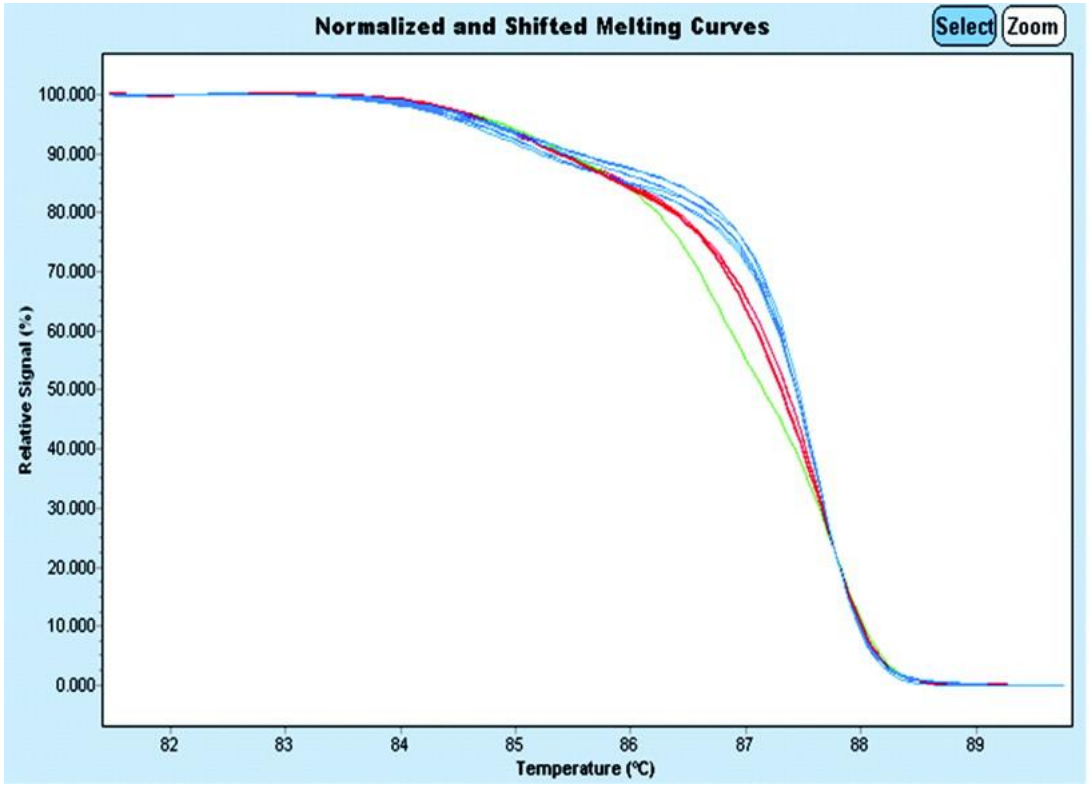
KRAS

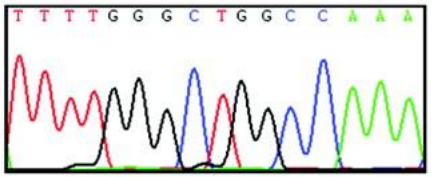




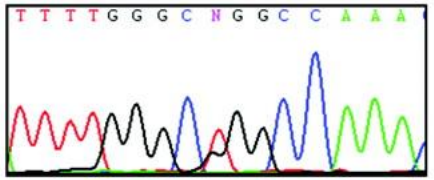
J Clin Pathol 2009;**62:**1096-1102

EGFR



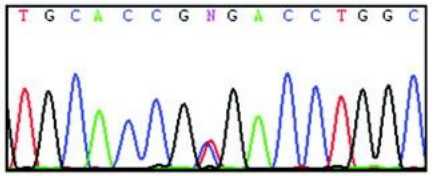


WT



2573T>G

EGFR Ekzon 21, L858R



2508C>T

Exon. 21, R836R

Sonuç:

HRMA iyi bir tarama seçeneği (EGFR, KRAS)

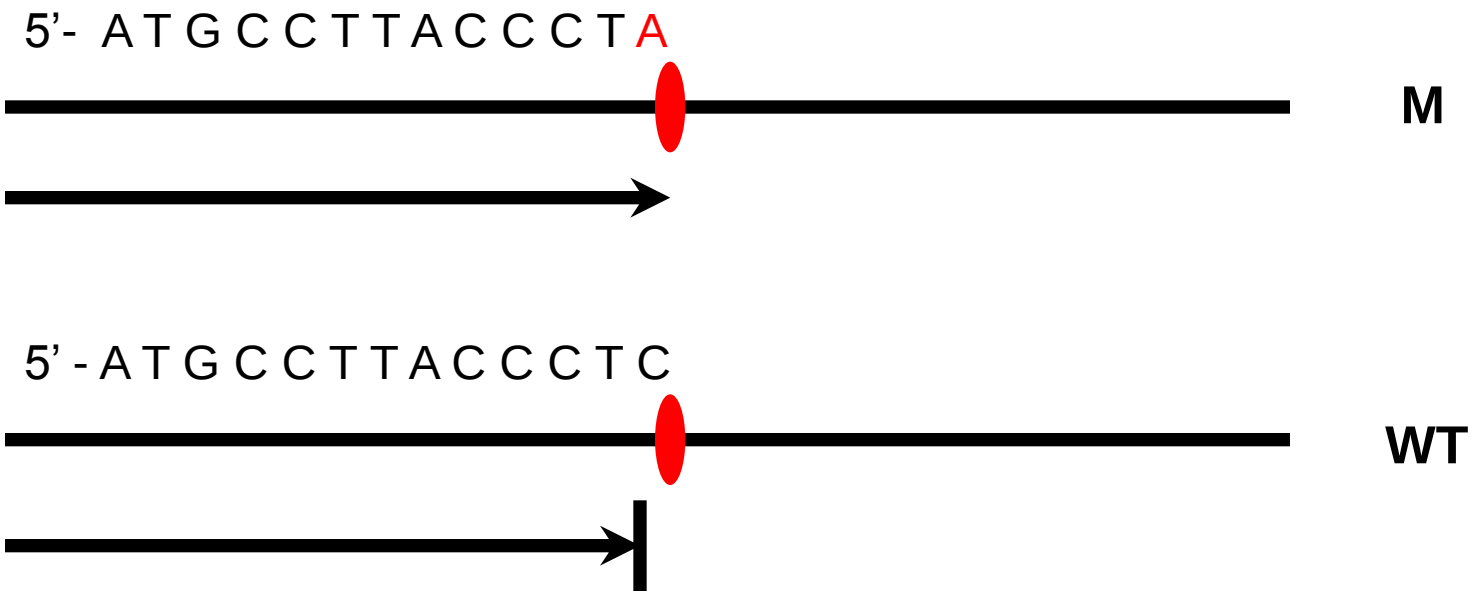
Mutasyon yoksa rapor edilir.

Mutasyon varlığı durumunda ise sekans konfirmasyonu gerekir:

' Her bulunan mutasyon tedavi alakalı olmayabilir'

- varyasyonlar
- sessiz mutasyonlar

5 - Sekans (Alel) spesifik PC



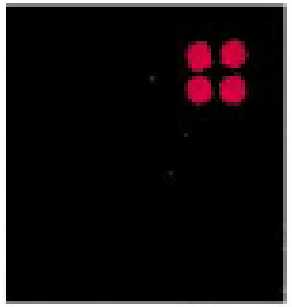
Bilinen mutasyonları hedefler

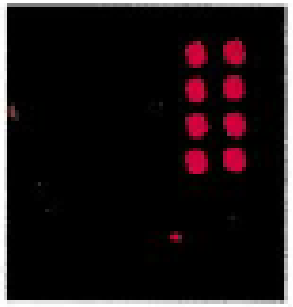
6 – Mikro yonga üzerinde çoklu hibridizasyon teknolojileri

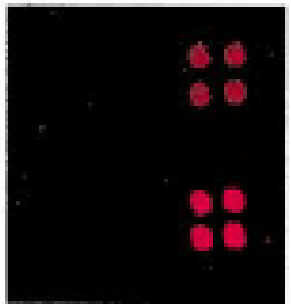
Bilinen mutasyonlar

EGFR
KRAS
BRAF

						16'			23'		TTGA 6			TTAG 8	
		TCTG 1		30'	TCCC 3			TCGT 5							6'
	TGTC 2		36'			TGCG 4						TGAT 7		11'	
					18'		TACA 36			33'					
32'		CTTG 9					CTCA 11	CTGT 13							8'
			CCTA 33					29'				CCAT 15			
CGTT 10		12'					4'					28'			CGAA 16
	34'			25'		CACG 12			CAGC 14		1'			9'	
				GTCT 19	24'				GTGC 22			31'			
GCTT 17		14'											22'		GCAA 23
	20'		GGTA 18	35'							3'		GGAC 24		
		GATG 34			GACC 20		2'	GAGT 21							
						ATCG 28	7'			15'			ATAC 31		
	21'			ACCT 27						ACGG 29	5'			13'	
		AGTG 25			AGCC 35			27'			AGGA 30		19'		
	AATC 26					10'			17'					AAAG 32	







KRAS BRAF EGFR

YÖNTEMLERİN KARŞILAŞTIRILMASI

- İLK YATIRIM MALİYETİ
- TEST MALİYETİ
- DUYARLILIK / ÖZGÜNLÜK
- TEST SÜRESİ
- AVANTAJLARI / DEZAVANTAJLARI

	Sanger Sekans	Pyro Sekans	İon Torrent	Sekans Spesifik PCR	HRMA	MPCR+ Mikro-yonga Hibridizasyon
İlk yatırım maliyeti (Birim)	2-5	2-3	5	3-4	1	3-4
Test maliyeti (Birim)	2	5-6	4-5	5-6	1	5-6
Test süresi (Saat)	8	6	>24	6	4	8
Sensitivite	10-30	≤ 5	≤ 1	1-10	10-20	1-10
Avantaj		Sensitivite	Sensitivite	Sensitivite	Ucuz, Kolay	
Dezavantaj		Poli-N	Ön işlem	Bilinen Mutasyonlar	Sensitivite Limit var	Bilinen Mutasyonlar

LAB KURULUMU ~ STRATEJİ

EGFR, KRAS, BRAF

EGFR; E18, E19, E20, E21

- Sanger sekans
- Pyrosekans
- İon Torrent

  - Mutaston var/yok raporlanır

EGFR; E18, E19, E20, E21

- Sekans (Alel) spesifik PCR
- Mikro-yongo hibridizasyon

→ Mutasyon varsa raporlanır

→ Mutasyon saptanmaz ise mutasyon yok anlamına gelmez.

(Tam sekans okuması: Dideoksi / Pyro / İontorrent)

EGFR: E18, E19, E20, E21

- HRMA

→ Mutasyon yoksa raporlanır
→ Mutasyon var ise tam sekans okuması yapılmalıdır *

(* Dideoksi / Pyro / İontorrent)

KRAS E2 (kodon 12, 13) ; E3 (kodon 61)

- Sangersekans
- Pyrosekans
- İon Torrent
- Sekans (Alel) spesifik PCR
- Mikro-yongo hibridizasyon

  - Mutaston var / yok raporlanır

KRAS E2 (kodon 12, 13) ; E3 (kodon 61)

- HRMA

→ Mutasyon yoksa raporlanır
→ Mutasyon var ise diğer yöntemlerle tanımlanmalıdır.

BRAF E15 (kodon 600)

- Sekans (alel) spesifik PCR
- Sanger sekans
- Pyrosekans
- İon Torrent
- Mikro-yonga

  - Mutaston var/yok raporlanır

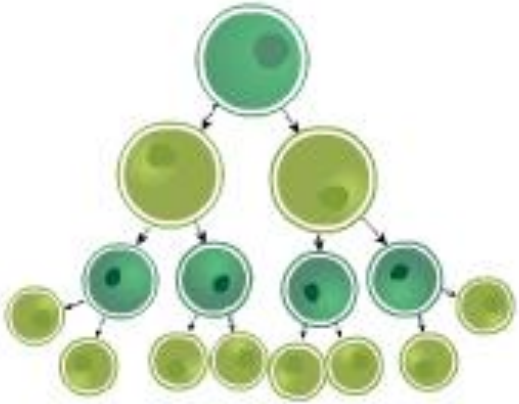
KIT, PDGFRA

- KIT; E9, E11 (Nükslerde E13, E14, E15, E16, E17)
- PDGFRA; E12, E14 (Nükslerde E18)

- Sanger sekans
- Pyrosekans
- İon Torrent

  - Mutaston var/yok raporlanır

YÖNTEMDEN BAĞIMSIZ LİMİTASYONLAR



Heterojenite

Kontaminasyon

Mutasyon analizleri;

1. Hangi hastaya yapılmalı?
2. Biyopsinin teste uygunluğu için kriterler nelerdir?
3. Sitolojik örnekler uygun mu?
4. Hangi hedefler için mutasyon analizleri yapılmalıdır?
5. En uygun DNA ekstraksiyonu nasıl yapılmalıdır?
6. Hangi yöntem en iyisidir?
7. Hangi durumlarda test tekrarı gereklidir?
8. Sonuçlar için makul süre nedir?
9. Sonuç raporu neleri içermelidir?
10. Raporu kim hazırlamalıdır?

- Direct sequencing analysis. *KRAS* mutations can also be identified using a direct sequencing method of exon 1 in the *KRAS* gene. This technique identifies all possible mutations in the exon.
- At this time, there are no FDA-approved tests for *KRAS* testing, but *KRAS* testing can be performed using laboratory-developed tests. Outside the United States, a United Kingdom-based company, DxS, offers a kit (TheraScreen) for its *KRAS* mutations assay. DxS and other vendors are expected to seek US Food and Drug Administration approval for their assays
- Choice of assay defined by which assay the laboratory has validated and routinely uses. Oncologist should consult with laboratory about specific test name to order.

Assay Reporting

KRAS *normal.* No mutation was identified. Report will specify assay type and controls used.

tion was found. Report will specify w
assay was done, and what controls wer

AUTHORS' DISCLOSURES OF
OF INTERE

Although all authors completed the discl
author(s) indicated a financial or other
subject matter under consideration in th
marked with a "U" are those for which n
those relationships marked with a "C" w
description of the disclosure categories, o
ASCO's conflict of interest policy, please
Declaration and the Disclosures of Poten
in Information for Contributors.

Employment or Leadership Position: D
Consultant or Advisory Role: None **Sto**
Honoraria: None **Research Funding:** N
Other Remuneration: None

CCDS
Home
FTP
Process
Statistics
AUG-guidelines

Collaborators
EBI
NCBI
UCSC
WTSI

Contact Us
email CCDS

Genome Displays
E Ensembl
U Genome Browser
N Map Viewer
V VEGA

Related Resources
Gene
HomoloGene
RefSeq
UniGene

Report for CCDS ID CCDS8702.1

CCDS	Status	Species	Chrom.	Gene	NCBI Builds	Links
8702.1	Public	*Homo sapiens*	12	KRAS	35.1 - 37.3	H G G

Sequence IDs included in CCDS 8702.1

Original	Current	Source	Nucleotide ID	Protein ID	Status in CCDS	Seq. Status	Links
✔	✔	EBI,WTSI	ENST00000311936	ENSP00000308495	Accepted	alive	N P N P
✔	✔	EBI,WTSI	OTTHUMT00000412230	OTTHUMP00000245391	Accepted	alive	N P N P
✔	✔	NCBI	NM_004985.3	NP_004976.2	Accepted	alive	N P N P B

Chromosomal Locations for CCDS 8702.1

On '-' strand of Chromosome 12 (NC_000012.11)

Genome Browser links: N U E V

Chromosome	Start	Stop	Links
12	25362729	25362845	N N U E V
12	25378548	25378707	N N U E V
12	25380168	25380346	N N U E V
12	25398208	25398318	N N U E V

CCDS Sequence Data

CCDS Sequence Data

Blue highlighting indicates alternate exons.

Red highlighting indicates amino acids encoded across a splice junction.

Mouse over the nucleotide or protein sequence below and click on the highlighted codon or residue to select the pair.

Nucleotide Sequence (567 nt):

ATGACTGAATATAAACTTGTGGTAGTTGGAGCTGGTGGCGTAGGCAAGAGTGCCTTGACGATACAGCTAA
TTCAGAATCATTTTGTGGACGAATATGATCCAACAATAGAGGATTCCTACAGGAAGCAAGTAGTAATTGA
TGGAGAAACCTGTCTCTTGGATATTCTCGACACAGCAGGTCAAGAGGAGTACAGTGCAATGAGGGACCAG
TACATGAGGACTGGGGAGGGCTTTCTTTGTGTATTTGCCATAAATAATACTAAATCATTTGAAGATATTC
ACCATTATAGAGAACAAATTAAAAGAGTTAAGGACTCTGAAGATGTACCTATGGTCCTAGTAGGAAATAA
ATGTGATTTGCCTTCTAGAACAGTAGACACAAAACAGGCTCAGGACTTAGCAAGAAGTTATGGAATTCCT
TTTATTGAAACATCAGCAAAGACAAGACAGGGTGTTGATGATGCCTTCTATACATTAGTTCGAGAAATTC
GAAAACATAAAGAAAAGATGAGCAAAGATGGTAAAAAGAAGAAAAAGAAGTCAAAGACAAAGTGTGTAAT
TATGTAA

Translation (188 aa):

MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAGQEEYSAMRDQ
YMRTGEGFLCVFAINNTKSFEDIHHYREQIKRVKDSEDVPMVLVGNKCDLPSRTVDTKQAQDLARSYGIP
FIETSAKTRQGVDDAFYTLVREIRKHKEKMSKDGKKKKKKSKTKCVIM

Links Key

Links to:

- H **History report**
- B **BLink report**
- G **Entrez Gene**
- N **Nucleotide report**
- P **Protein report**

Nucleotide Sequence (567 nt):

ATGACTGAATATAAACTTGTGGTAGTTGGAGCTGGTGGCGTAGGCAAGAGTGCCTTGACGATACAGCTAA
TTCAGAATCATTTTGTGGACGAATATGATCCAACAATAGAGGATTCCTACAGGAAGCAAGTAGTAATTGA
TGGAGAAACCTGTCTCTTGGATATTCTCGACACAGCAGGTCAAGAGGAGTACAGTGCAATGAGGGACCAG
TACATGAGGACTGGGGAGGGCTTTCTTTGTGTATTTGCCATAAATAATACTAAATCATTTGAAGATATTC
ACCATTATAGAGAACAAATTAAAAGAGTTAAGGACTCTGAAGATGTACCTATGGTCCTAGTAGGAAATAA
ATGTGATTTGCCTTCTAGAACAGTAGACACAAAACAGGCTCAGGACTTAGCAAGAAGTTATGGAATTCCT
TTTATTGAAACATCAGCAAAGACAAGACAGGGTGTTGATGATGCCTTCTATACATTAGTTCGAGAAATTC
GAAAACATAAAGAAAAGATGAGCAAAGATGGTAAAAAGAAGAAAAAGAAGTCAAAGACAAAGTGTGTAAT
TATGTAA

Translation (188 aa):

MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGETCLLDILDTAGQEEYSAMRDQ
YMRTGEGFLCVFAINNTKSFEDIHHYREQIKRVKDSEDVPMVLVGNKCDLPSRTVDTKQAQDLARSYGIP
FIETSAKTRQGVDDAFYTLVREIRKHKEKMSKDGKKKKKKSKTKCVIM

QIAGEN® *therascreen* KRAS PCR kit enables the detection of seven *KRAS* mutations:

G12A, G12R, G12D, G12C, G12S, G12V, and G13D