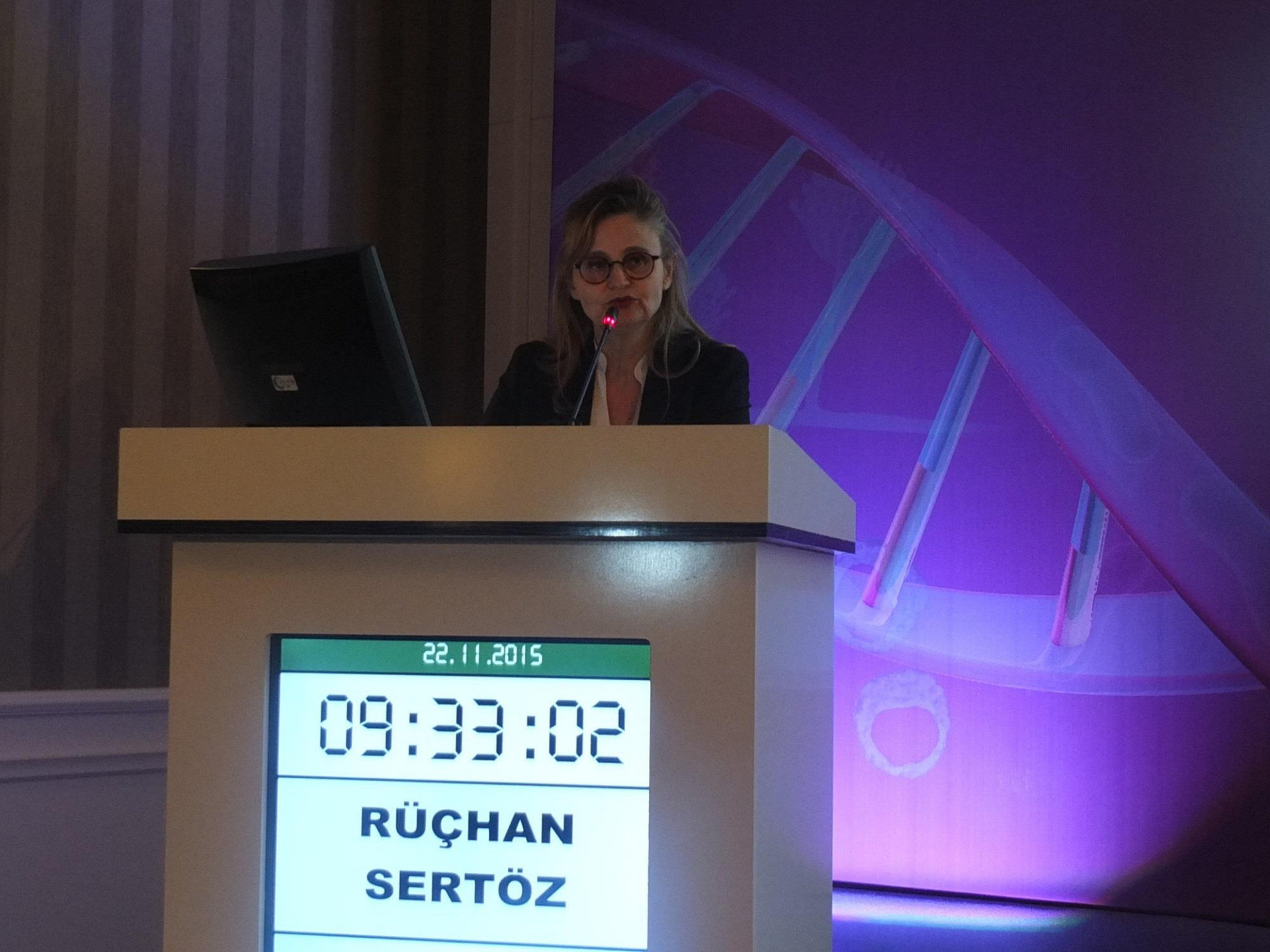
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RÜÇHAN
SERTÖZ

VİRÜS GENOTİPLERİ VE İLAÇ DİRENCİNİN YENİ NESİL DİZİLEME ANALİZİNDEN ÖRNEKLER

RÜÇHAN YAZAN SERTÖZ
EGE ÜNİVERSİTESİ TIP FAKÜLTESİ

Review Journal of Clinical Virology 61 (2014) 9–19

Deep sequencing: Becoming a critical tool in clinical virology

Miguel E. Quiñones-Mateu [a,b,*], Santiago Avila [c,d], Gustavo Reyes-Teran [c,d], Miguel A. Martinez [e]

[a] University Hospital Translational Laboratory, University Hospitals Case Medical Center, Cleveland, OH, USA
[b] Department of Pathology, Case Western Reserve University, Cleveland, OH, USA
[c] Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico
[d] Centro de Investigaciones en Enfermedades Infecciosas, Mexico City, Mexico
[e] Fundació irsicaixa, Universitat Autònoma de Barcelona, Hospital Universitari Germans Trias i Pujol, Badalona, Spain

454

	GS Jr.	GS FLX+
Amplification Method	Emulsion PCR on beads	
Chemistry	Synthesis (pyrosequencing)	
Read length (bp)	400	700
Yield/run (Gb)	0.05	0.9
Primary Error	Indel	
Error rate	~1%	
Run time (hours)	10	20
Virus-related Publications	187	
Advantage(s)	Long reads, maturity	
Disadvantage(s)	Homopolymer misreads, high cost/Mb	

Illumina

	MiSeq	HiSeq
Amplification Method	Bridge PCR in situ	
Chemistry	Synthesis (reversible termination)	
Read length (bp)	250	125
Yield/run (Gb)	8	1,000
Primary Error	Substitution	
Error rate	~0.1%	
Run Time (hours)	39	276
Virus-related Publications	129	
Advantage(s)	Easy work flow, maturity	
Disadvantage(s)	Shortest reads, long run	

Ion Torrent

	PGM	Proton
Amplification Method	Emulsion PCR on beads	
Chemistry	Synthesis (H^+ detection)	
Read length (bp)	400	200
Yield/run (Gb)	2	10
Primary Error	Indel	
Error rate	~1%	
Run time (hours)	7	4
Virus-related Publications	13	
Advantage(s)	Low cost, fast run	
Disadvantage(s)	Homopolymer misreads	

PacBio

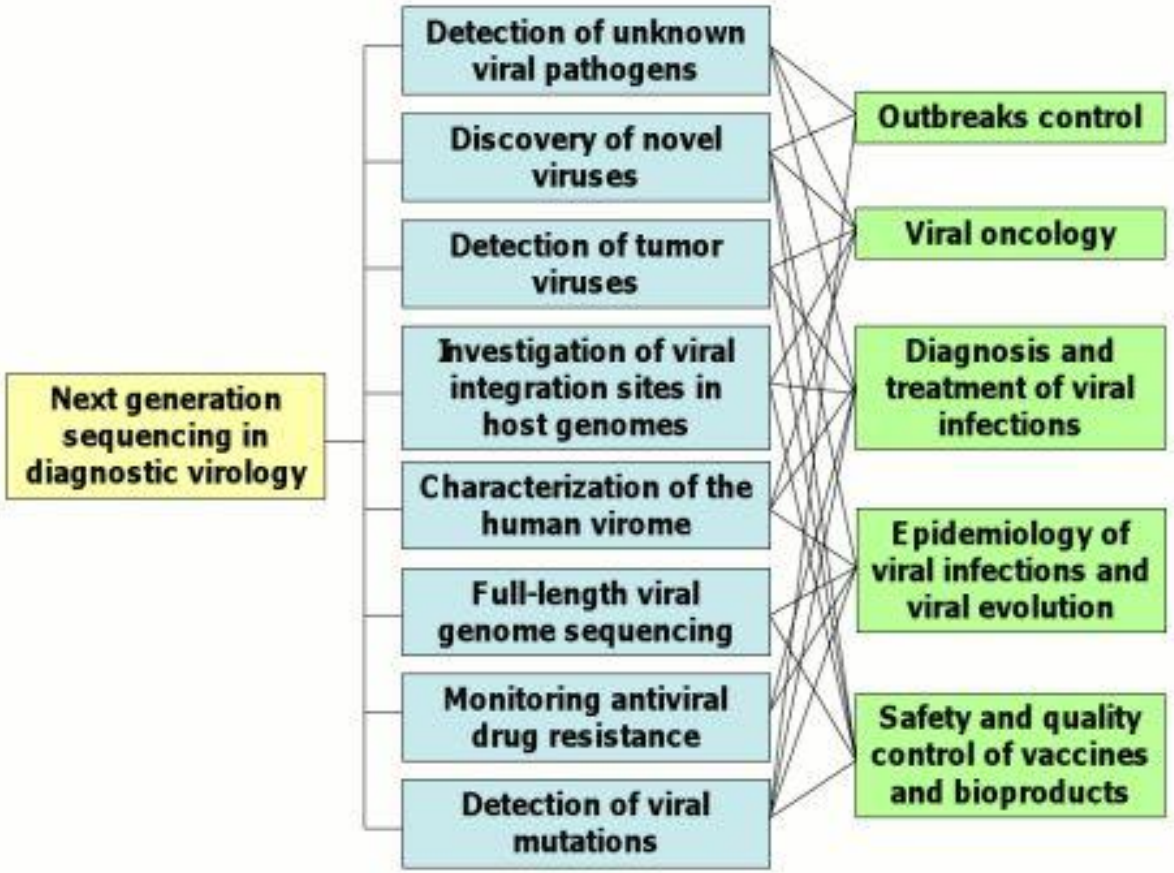
	RS II
Amplification Method	No PCR
Chemistry	Single-molecule real-time sequencing
Read length (bp)	8,500
Yield/run (Gb)	0.15
Primary Error	Indel
Error rate	~13%
Run time (hours)	2
Virus-related Publications	6
Advantage(s)	Longest reads
Disadvantage(s)	High error rate, expensive

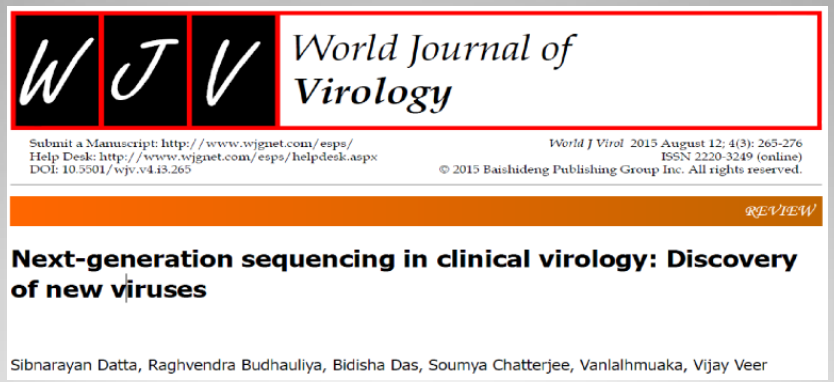
Review *Int. J. Mol. Sci.* **2011,**

Applications of Next-Generation Sequencing Technologies to Diagnostic Virology

Luisa Barzon [1,*], Enrico Lavezzo [1], Valentina Militello [1], Stefano Toppo [2] and Giorgio Palù [1]

- Y… …ması
- Vi… tü…
- İr…
- Ta…
- Vi… …nin ar…
- Ti…
- Ca…
- Ar…





- ***YND temelli metagenomik yaklaşımlar***
  - **Nükleik asit dizilerinden bağımsız**
    - **Nonspesifik amplifikasyon yöntemleri**
      - **Sequence-independent single-primer amplification (SISPA),**
      - **Virus discovery based on cDNA-AFLP (VIDISCA),**
      - **Rolling circle amplification (RCA)**
  - **Virüs yapısı, genomu hakkında ön bilgiye gerek yok**
  - **Çoğaltma, zenginleştirme gerekli**
    - **Fiziksel çoğaltma**
      - **Virüs kapsid pürifikasyonu**
      - **Filtrasyon**
      - **Ultrasantrfüj**

TROPICAL DISEASES

Virus Identification in Unknown Tropical Febrile Illness Cases Using Deep Sequencing

Nathan L. Yozwiak[1☯], Peter Skewes-Cox[2,3,4☯], Mark D. Stenglein[3,4☯], Angel Balmaseda[5], Eva Harris[1], Joseph L. DeRisi[3,4,6*]

1 Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, California, United States of America, **2** Biological and Medical Informatics Program, University of California San Francisco, San Francisco, California, United States of America, **3** Howard Hughes Medical Institute, University of California San Francisco, San Francisco, California, United States of America, **4** Department of Biochemistry and Biophysics, University of California San Francisco, San Francisco, California, United States of America, **5** Departamento de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua, **6** Department of Medicine, University of California San Francisco, San Francisco, California, United States of America

February 2012 | Volume 6 | Issue 2 | e1485

- **Ateş veya bir haftadan kısa süre önce ateş**

- **Baş ağrısı,**
- **Artralji,**
- **Myalji,**
- **Retroorbital ağrı,**
- **Pozitif turnike testi,**
- **Peteşi,**
- **Kanama bulgularından biri**

- ***Dang benzeri hastalıklar***

TROPICAL DISEASES

Virus Identification in Unknown Tropical Febrile Illness Cases Using Deep Sequencing

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- **Çalışma dışında**
  - **Pnömoni tanılılar**
  - **DENV izole edilenler**
  - **DENV RT-PCR pozitifler**
  - **Seroloji pozitifler**

- **123 hasta (6ay-14yaş)**

TROPICAL DISEASES

Virus Identification in Unknown Tropical Febrile Illness Cases Using Deep Sequencing

Nathan L. Yozwiak[1☯], Peter Skewes-Cox[2,3,4☯], Mark D. Stenglein[3,4☯], Angel Balmaseda[5], Eva Harris[1], Joseph L. DeRisi[3,4,6*]

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- **Virochip panviral Mikroarray ve YND (Illumuna) ile metagenomik çalışmalar sonucu**
  - **45/123 pozitif - %37**
    - **13 HHV-6**
  - **Bilinen ve/veya yeni virüslerden gelişmiş olduğu düşünülen**
    - **Herpesviridae,**
    - **Flaviviridae,**
    - **Circoviridae,**
    - **Anelloviridae,**
    - **Asfarviridae,**
    - **Parvoviridae**

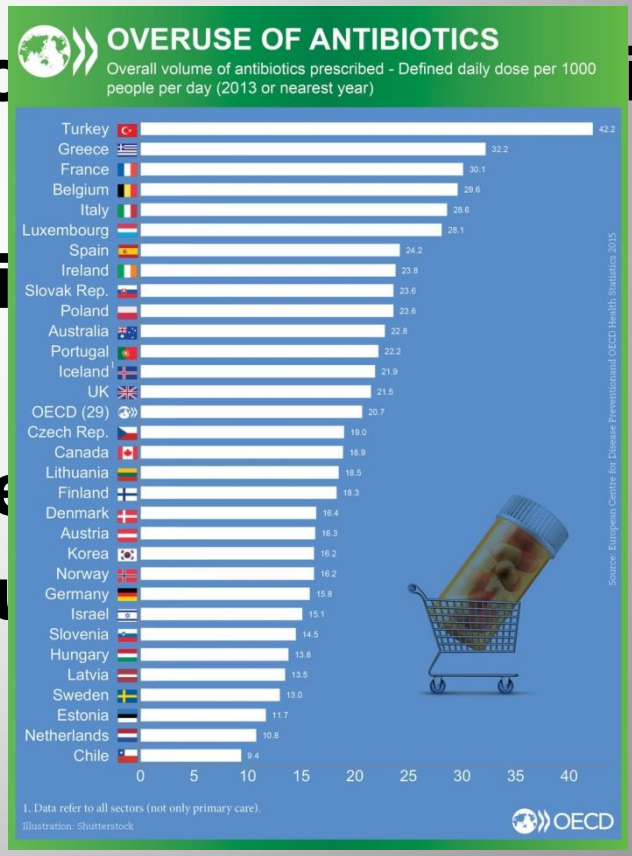
Genome Research 21:1616–1625 © 2011

The human gut virome: Inter-individual variation and dynamic response to diet

Samuel Minot,[1] Rohini Sinha,[1] Jun Chen,[2] Hongzhe Li,[2] Sue A. Keilbaugh,[3] Gary D. Wu,[3] James D. Lewis,[2] and Frederic D. Bushman[1,4]

[1]*Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104, USA;* [2]*Department of Biostatistics and Epidemiology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104, USA;* [3]*Division of Gastroenterology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104, USA*

- **İnsan barsak viromu**
- **Diyet ile insan barsak virom**
- **Aynı diyette bireylerarası**
- **Bakteri florası ile ilişkileri**
- **Fajlar arası rekabet**
- **Fajlar bakteriler birlikte e**
- **AB direnci-dar spektrumlu tedavileri????**



Medicine®

OBSERVATIONAL STUDY

Dynamic of Mixed HCV Infection in Plasma and PBMC of HIV/HCV Patients Under Treatment With Peg-IFN/Ribavirin

Sabrina Bagaglio, PhD, Caterina Uberti-Foppa, MD, Clelia Di Serio, PhD, Filippo Trentini, PhD, Andrea Andolina, MD, Hamid Hasson, MD, Emanuela Messina, MD, Marco Merli, MD, Lucy Porrino, MD, Adriano Lazzarin, MD, and Giulia Morsica, MD

- **Koinfekte bireylerde risk faktörleri ve çoklu karşılaşma nedeniyle mikst infeksiyonlar sık**
- **HIV/HCV koinfekte bireylerde farklı kompartmanlarda HCV mikst genotip dinamiği ve interferon, ribavirin yanıtı ile uyumu**

- **GS FLX (454 Life Sciences, Roche)**

- **19 koinfekte hasta**
  - **10'u yanıtsız veya relaps**
  - **9'u kalıcı viral yanıt**

Medicine
OBSERVATIONAL STUDY

Dynamic of Mixed HCV Infection in Plasma and PBMC of HIV/HCV Patients Under Treatment With Peg-IFN/Ribavirin

Sabrina Bagaglio, PhD, Caterina Uberti-Foppa, MD, Clelia Di Serio, PhD, Filippo Trentini, PhD, Andrea Andolina, MD, Hamid Hasson, MD, Emanuela Messina, MD, Marco Merli, MD, Lucy Porrino, MD, Adriano Lazzarin, MD, and Giulia Morsica, MD

- **Plazmada**
  - **11/19 (%58) mikst genotip**
- **Periferik kan mononükleer hücrelerinde**
  - **HCV pozitif 15 hastanın 6'sı (%40) mikst genotip**

- **Tedavi sırasında 3 yanıtsız hastada iki kompartmanda da baskın genotipler farklılaştı!**
- **Plazma örneklerinde mikst genotipler !!**
  - **Tedavi yanıtını etkileyen en önemli faktör**

- **Farklı tedavi yaklaşımları, genotip test takibi**

Medicine
OBSERVATIONAL STUDY

Dynamic of Mixed HCV Infection in Plasma and PBMC of HIV/HCV Patients Under Treatment With Peg-IFN/Ribavirin

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TABLE 3. Viral Population Analysis by Next-Generation Sequencing During Treatment With P–R and Posttreatment Follow-Up (HCVG–W24PT) in HIV/HCV Coinfected Individuals

	HCVG–BL	HCVG–W12	HCVG–W24	HCVG–W24PT
PT1 Plasma	**1b**-1a-1c-3a	**3a**	**3a**	**1b**-1a-1c-3a
PT1 PBMC	**1b**	**1b**	**1b**-1a	neg
PT2 Plasma	**1a**-1c	**1a**-1c	–	–
PT2 PBMC	**3a**-1b	**1a**	–	–
PT3 Plasma	**4c/d**-1b-1a-3a-2a	**4c/d**-2a-1a	**4c/d**-3a	-
PT3 PBMC	**4c/d**-1b-3a-2a	neg	neg	–
PT4 Plasma	**1a**	**1a**-4c/d	**3a**	**1a**-4c/d-1b-3a
PT4 PBMC	**1c**	**1a**	**1c**	**1a**
PT5 Plasma	**1b**-1a-3a-2a	**1b**-2a	–	–
PT5 PBMC	**1b**	–	–	–
PT6 Plasma	**4c/d**-4a-2a	**4a**-4c/d-3a	**4c/d**-3a	–
PT6 PBMC	**4c/d**-4a-1b	**4a**-4c/d	**3a**-2a	–
PT7 Plasma	**1a**-1b-2a	**1a**-1b	–	–
PT7 PBMC	**1a**	–	–	–
PT8* Plasma	**1b**-3a	neg	neg	**1b**-3a
PT8* PBMC	**1b**	neg	neg	**1b**
PT9* Plasma	**1b**-1a-1c	neg	neg	**1c**-1a-1b
PT9* PBMC	**4c/d**-4a-3a	neg	neg	–
PT10 Plasma	**4c/d**	**4c/d**	**4c/d**	–
PT10 PBMC	**4c/d**	neg	**3a**-4c/d	–

Dominant genotype is indicated in bold. G = genotype, PT = patients. – = specimen not available.
* RE patients.

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Journal of Clinical Virology 58 (2013) 437–442

Next generation sequencing for human papillomavirus genotyping

L. Sara Arroyo[a], Vitaly Smelov[a,b], Davit Bzhalava[a], Carina Eklund[a], Emilie Hultin[a], Joakim Dillner[a,*]

[a] Department of Laboratory Medicine, Karolinska Institutet and Karolinska University Hospital, 141 86 Stockholm, Sweden
[b] Department of Urology and Andrology, Northwestern State Medical University n.a. I.I. Mechnikov, 191015 St. Petersburg, Russia

- **Luminex- MGP primer set**
- **YND-HPV genel primer set PGMY (DSÖ HPV manuel)**
  - **Duyarlılık 100 kopya/mL**
  - **HPV 13/15 10 kopya/mL**
- **36/60 servikal örnekte YND-Luminex uyumlu**

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Table 3b
Cervical samples that showed discrepancies between NGS and Luminex.

Cervical Sample	Genotypes detected by GS Junior	Luminex	Comments[a]
6038	16, 59	16	59 (2)
6047	18, 52, 68b	18	52 (1), 68b (4)
6129	18, 45, 59	18, 18v	45 (2), 59 (1)
6191	11, 33, 39	11, 39	33 (21)
6346	54, 61	61	54 (50)
6959	51, 53	51	53 (3)
6508	42, 58	58	42 (1)
6302	52, 68b, 70	52, 68b	70 (2577)
6251	16, 67	16	67 (2)
6434	53	Neg	53 (2001)
6387	32	Neg	32 NP[b] (2424)
6595	33	Neg	33 (20)
6464	45, 52, 54, 59, 62, 89	45, 52, 70, 89	54 (224), 59 (1), 62 NP[b](58)
6468	114	83, 86	114 NP[b] (749)
6187	18, 89	18, 90	89 (279), 90 PGMYmm[c]
7020	67, 70	59, 70	67 (2)
6060	Neg	91	PGMYmm[c]
6196	Neg	90	PGMYmm[c]
6205	Neg	91	PGMYmm[c]
6217	Neg	91	PGMYmm[c]
6304	Neg	87	PGMYmm[c]
6423	18	18, 68a	68a PGMYmm[c]
6466	Neg	16	
6616	Neg	16	

[a] Genotypes detected by NGS but not detected by Luminex are shown with their number of reads in brackets.
[b] Genotype detected by NGS that were not tested for by any one of the probes included in the Luminex. NP (no probe).
[c] Genotype detected by Luminex but not by NGS, possibly due to a failure in amplification because of mismatch with PGMY primers. PGMYmm (PGMY mismatch).

Cross-clade simultaneous HIV drug resistance genotyping for reverse transcriptase, protease, and integrase inhibitor mutations by Illumina MiSeq

Retrovirology 2014

Dawn M Dudley[1], Adam L Bailey[1], Shruti H Mehta[2], Austin L Hughes[3], Gregory D Kirk[4], Ryan P Westergaard[5] and David H O'Connor[1*]

Antiviral Direnç İzlemi

HIV örneği

- **Mevcut ticari testler**
- **Nonsubtype B de zayıf**
- **RT, proteaz**
- **İntegraz inhibitörleri**
- **Kombine preparatlar ile başlangıç tedavisi!**

YND

- **Multipleks kullanım**
- **Fiyat düşüklüğü**
- **Klonal özellik nedeniyle duyarlılık**
- **MiSeq en az hata eğilimli (%0.1)**

Cross-clade simultaneous HIV drug resistance genotyping for reverse transcriptase, protease, and integrase inhibitor mutations by Illumina MiSeq

Retrovirology 2014

Dawn M Dudley[1], Adam L Bailey[1], Shruti H Mehta[2], Austin L Hughes[3], Gregory D Kirk[4], Ryan P Westergaard[5] and David H O'Connor[1*]

- **Los Alamos referans dizileri ile universal primer çiftleri**
- **Nested PCR**
- **A,B,C,D, CRF 02AG, CRF02AE**
- **799 kopya/mL duyarlılıkta**
- **ALIVE 62 örnek**

Table 1 HIV-1 subtypes tested with the universal primer set

Virus	Subtype	TCID/mL*	P24 ng/mL*	RT-PCR
92UG_029	A	$1.26x10^5$	150	+
93RW_024	A	NA	NA	+
00KE-KER2008	A	$10^{2.60}$	57	+
84US_MNp	B	$1.02x10^4$	634	+
85US_BA-L	B	$5.62x10^6$	39.3	+
91US_1	B	$10^{5.0}$	294	+
94US_33931N	B	$10^{3.10}$	235	+
00TZ_A246	C	$1.45x10^4$	NA	+
02ET_14	C	$10^{4.10}$	12.2	+
94IN_20635-4	C	NA	NA	+
90SE_364	C	$10^{6.0}$	157	+
98US_MSC5016	C	$10^{3.85}$	11.5	+
93UG_065	D	NA	NA	+
98UG_57128	D	$10^{2.39}$	13	+
99UG_A03349M1	D	$2.05x10^4$	290	+
90TH_CM244	CRF01_AE	$4.1x10^4$	88	+
97TH_NP1525	CRF01_AE	$7.11x10^3$	90	+
91DJ_263	CRF02_AG	$2.3x10^4$	NA	+
98US_MSC5007	CRF02_AG	$4x10^4$	127	+

* Provided by the NIH AIDS Research and Reference Reagent Program.

Cross-clade simultaneous HIV drug resistance genotyping for reverse transcriptase, protease, and integrase inhibitor mutations by Illumina MiSeq

Retrovirology 2014

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- **% 54'ünde çeşitli mutasyonlar**
  - **% 59'u %2-20 sıklıkta**
- **Sanger ile kaçırılabilir !**

Table 2 Average sequencing read coverage for all drug resistance mutations identified in the ALIVE cohort

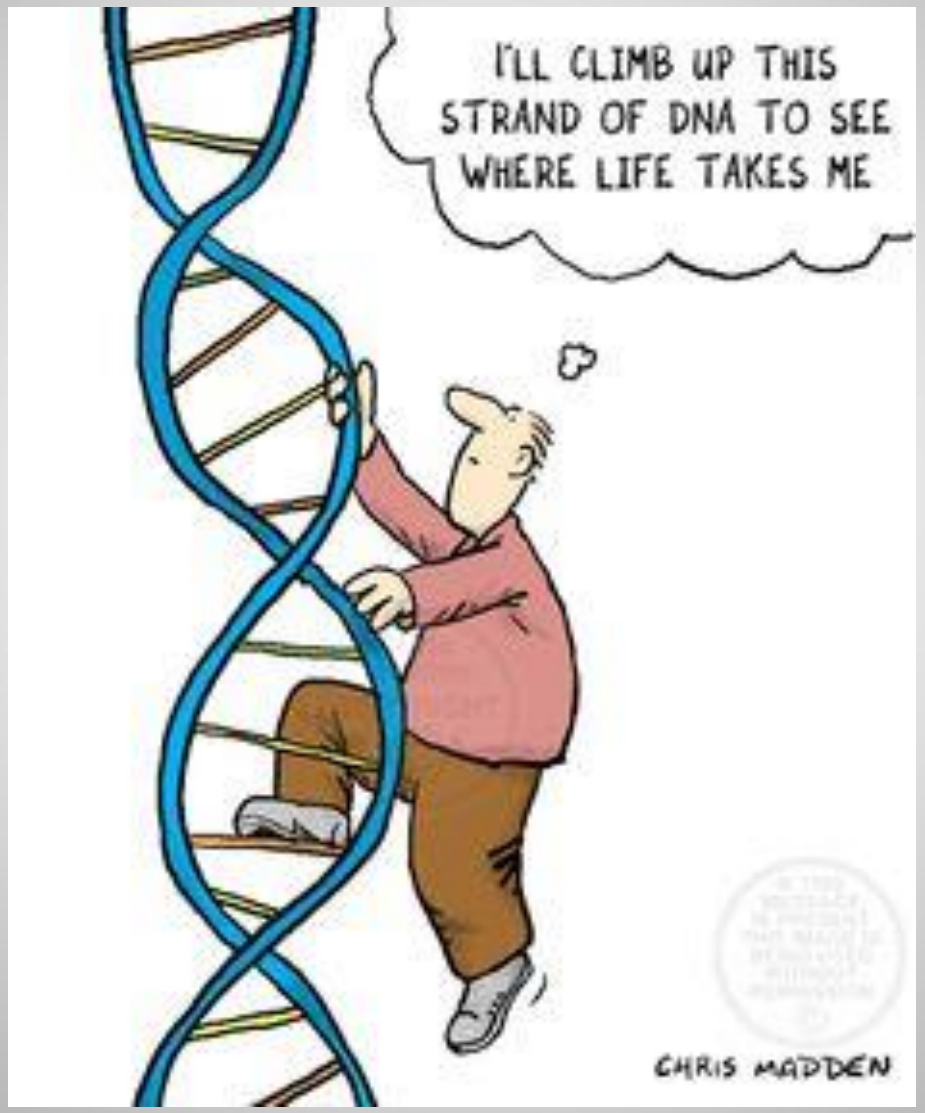
Gene product	Drug resistance mutation*	Ave. sequencing read coverage
PR	L10IFVC	297
	V11I	294
	G16E	1022
	K20RMITV	1917
	L24I	**1039**
	D30N	**2169**
	L33FIV	**3140**
	M36LIV	3639
	M46I/L	**2609**
	I50L/V	**3895**
	F53LY	1213
	I54VTALM	**2122**
	D60E	3065
	I62V	3198
	L63P	3346
	I64LMV	2635
	A71VITL	3800
	G73CSTA	3045
	V82ATFSL	**3300**
	L89MM	4199
	L90M	**4982**
	I93LM	5850
RT	**M41L**	**5014**
	A62V	4941
	K70R	**4324**
	V75I	10460
	V77I	4787
	V90I	4177
	K103N	**6048**
	V106A/M/I	**6492**
	V108I	7424
	E138KAGQR	**7401**
	M184V/I	**5906**
	Y188LHC	**5283**
	G190ASE	**4708**
	P225H	5764
	M230LI	**8668**
IN	**S147G**	**3123**

*Boldface mutations are major drug resistance mutations, non-boldface mutations are accessory mutations.

Yeni Nesil Dizileme

- Hücre kültürü, moleküler testler ve dizi analizinin tamamlayıcısı rolünde

- **İlerlemeler**
- Metagenomik yaklaşımlarla virüs tanımı
- Konak içi ve konaklararası virüs değişkenliği
  - ilaç direnç testleri,
  - genotip,
  - moleküler epidemiyoloji,
  - antiviral ilaç ve aşı geliştirilmesinde
- Konak-virüs ilişkileri
  - RNA sekansı ile transkriptom analizi,
  - epigenomik analiz,
  - protein-nükleik asit ilişkileri
- Konak genomu ve infeksiyon hastalıklarına duyarlılık


I'LL CLIMB UP THIS STRAND OF DNA TO SEE WHERE LIFE TAKES ME
CHRIS MADDEN