HIV AIDS KONGRESİ
18-21 KASIM 2021
Xanadu Otel ANTALYA
www.hivaidskongresi2021.org

Yeni Nesil Dizileme ile Antiretroviral Direnç Testlerinde İki Farklı Biyoinformatik Değerlendirme Algoritmasının Performanslarının Karşılaştırılması

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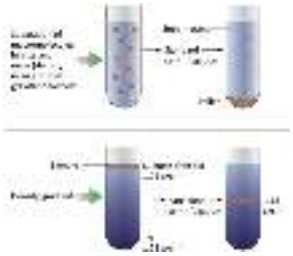
AMAÇ

- Gelişen teknoloji sayesinde genotipik antiretroviral direnç testleri klasik dizileme yerine yeni nesil dizileme (YND) ile daha yaygın bir şekilde yapılmaktadır.

- YND beraberinde biyoinformatik değerlendirme konularını da gündeme getirmiştir. **(Ek maliyet)**

- Bu çalışmada YND verilerinin analizi için iki farklı hizalama algoritmasının kullanıldığı iki yordam ticari valide bir sistem ile karşılaştırılarak performans sınaması yapılmıştır.

Dizi Analizi Protokol ve Analiz



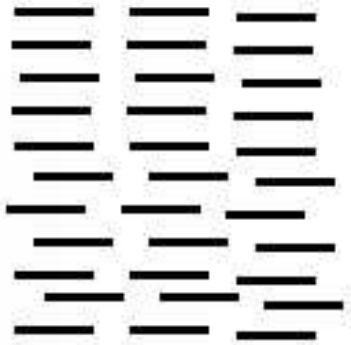
Nükleik Asit İzolasyonu

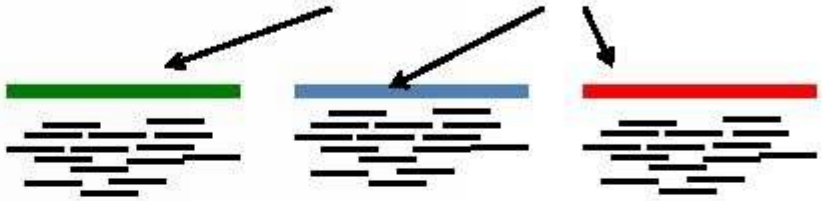
rt PCR ile Hedef DNA Hazırlama

Dizileme

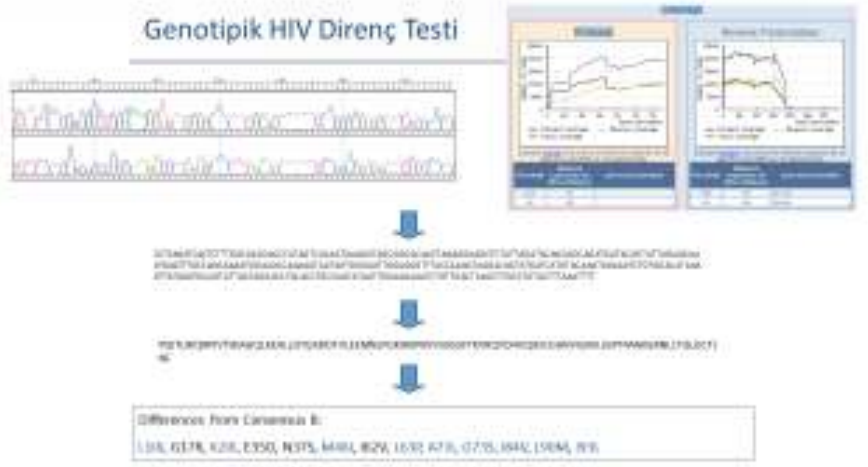
NGS için Kütüphane Oluşturma

Verilerin Okunması ve Ham Data Oluşturma





Okumaların “Contig”ler oluşturacak şekilde birleştirilmesi



Burrows-Wheeler Aligner

Home

Introduction

BWA is a software package for mapping low-divergent sequences against a large reference genome, such as the human genome. It consists of three algorithms: BWA-backtrack, BWA-SW and BWA-MEM. The first algorithm is designed for Illumina sequence reads up to 100bp, while the rest two for longer sequences ranged from 70bp to 1Mbp. BWA-MEM and BWA-SW share similar features such as long-read support and split alignment, but BWA-MEM, which is the latest, is generally recommended for high-quality queries as it is faster and more accurate. BWA-MEM also has better performance than BWA-backtrack for 70-100bp Illumina reads.

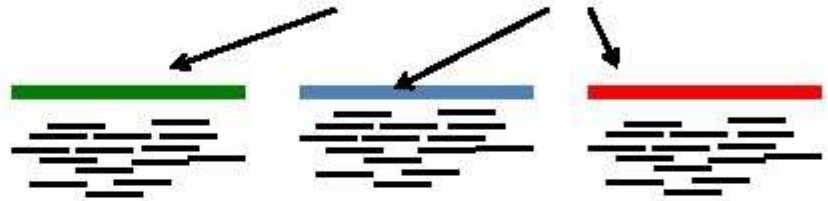
FAQ

BWA:

- SF project page
- SF download page
- Mailing list
- BWA maual page
- Repository

Links:

- SAMtools
- MAQ



Okumaların “Contig”ler oluşturacak şekilde birleştirilmesi

BOW TIE

Bowtie 2

Fast and sensitive read alignment

JOHNS HOPKINS UNIVERSITY

Bowtie 2 is an ultrafast and memory-efficient tool for aligning sequencing reads to long reference sequences. It is particularly good at aligning reads of about 50 up to 100s or 1,000s of characters, and particularly good at aligning to relatively long (e.g. mammalian) genomes. Bowtie 2 indexes the genome with an FM Index to keep its memory footprint small: for the human genome, its memory footprint is typically around 3.2 GB. Bowtie 2 supports gapped, local, and paired-end alignment modes.

OSI certified

SAMtools

SOURCEFORGE github SOCIAL CODING

Home

See http://htslib.org/ for the new 1.x releases of SAMtools, BCFtools, and HTSlib. This website contains information pertaining to the old 0.1.19 samtools release, and so is useful but somewhat out of date. As time permits, this information will be updated for the new samtools/bcftools versions and moved to the new website.

Introduction

SAM (Sequence Alignment/Map) format is a generic format for storing large nucleotide sequence alignments. SAM aims to be a format that

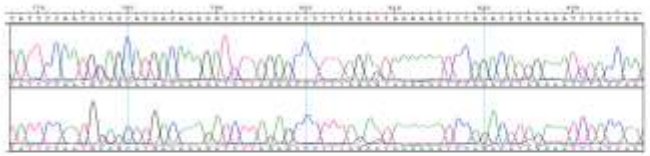
General Information

- SAM Spec v1.4
- SF Project Page
- SF Download Page
- GitHub Project Page
- Mailing Lists
- Related Software
- FAQ

SAMtools in C

- General Introduction

Genotipik HIV Direnç Testi



CCTCAGATCACTCTTTGGCAACGACCCATAGTCACAATAAAGATAGCGGGACAACTAAAGGAAGCTCTATTAGATACAGGAGCAGATGATACAGTATTAGAAGAA
ATGAATTTGCCAGGAAAATGGAAACCAAAAATAATAGTGGGAATTGGAGGGTTTACCAAAGTAAGACAGTATGATCATGTACAAATAGAAATCTGTGGACATAAA
GTTATAGGTGCAGTATTAATAGGACCTACACCTGCCAATATAATTGGAAGAAATCTGTTGACTCAGCTTGGCTGTACTTTAAATTT

PQITLWQRPIVTIKIAGQLKEALLDTGADDTVLEEMNLPGKWKPKIIVGIGGFTKVRQYDHVQIEICGHKVIGAVLIGPTPANIIGRNLLTQLGCTL
NF

Differences from Consensus B:

L10I, G17R, K20I, E35D, N37S, M46I, I62V, L63P, A71I, G73S, I84V, L90M, I93L

iVar

Main Page | Related Pages | Search

Documentation

- Installation
- Manual
- Cookbook

Publication

An amplicon-based sequencing framework for accurately measuring intrahost virus diversity using PrimalSeq and iVar

```
spades.py --pe1-1 lib_pe1_left.fastq --pe1-2 lib_pe1_right.fastq \
--mp1-1 lib_mp1_left.fastq --mp1-2 lib_mp1_right.fastq \
--mp2-1 lib_mp2_left.fastq --mp2-2 lib_mp2_right.fastq \
-o spades_output
```

```
bwa index ref.fa

bwa mem ref.fa reads.fq > aln-se.sam

bwa mem ref.fa read1.fq read2.fq > aln-pe.sam

bwa aln ref.fa short_read.fq > aln_sa.sai

bwa samse ref.fa aln_sa.sai short_read.fq > aln-se.sam

bwa sampe ref.fa aln_sa1.sai aln_sa2.sai read1.fq read2.fq > aln-pe.sam

bwa bwasw ref.fa long_read.fq > aln.sam
```

File Edit Search View Document Project Build Tools Help

Symbols

No symbols found

test.py YMDD_Aligner.pl txttohtml.pl html.pl HIV_Aligner.pl

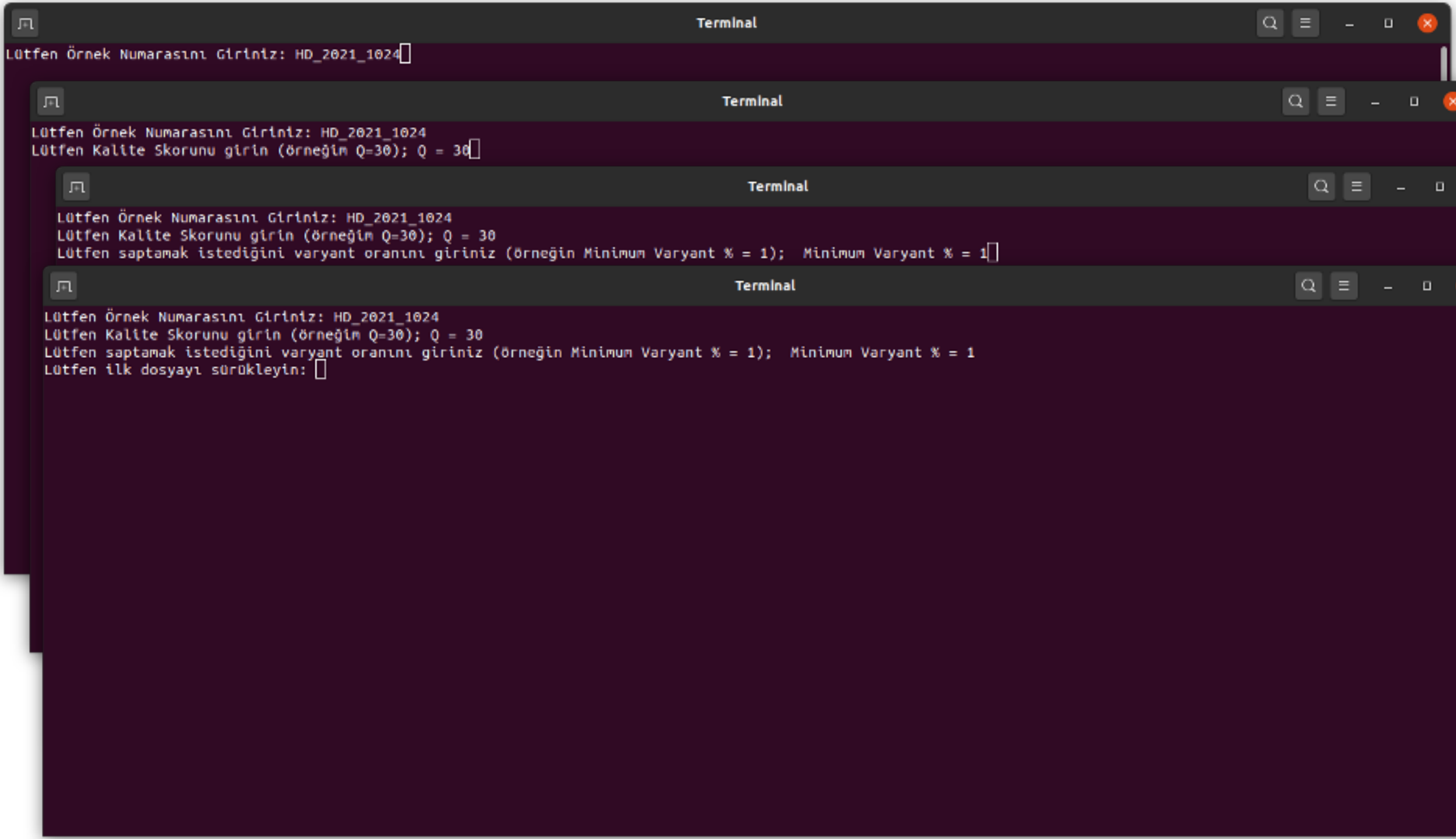
```
1   #Tum Haklari Mert Kuskucuya aittir.
2   #/usr/bin/perl
3
4     use strict;
5     use warnings;
6     use File::Copy;
7     use v5.10;
8
9   # Ilk olarak calisma icin dosya yapisini olustur
18
11  print "Lütfen Örnek Numarasını Giriniz: ";
12  my $sample =  <STDIN>;
13  chomp($sample);
14
15  print "Lütfen Kalite Skorunu girin (örneğin 0-30); 0 = ";
16  my $QS =  <STDIN>;
17  chomp($QS);
18
19  print "Lütfen saptamak istediğini varyant oranini giriniz (örneğin Minimum Varyant= 0.01);  Minimum Varyant = ";
20  my $MV =  1-<STDIN>;
21  chomp($MV);
22
23
24  my $directory0 = "/home/mk/MyBioinf/HCV Genotype/$sample";
25  unless(mkdir $directory0) {
26  die "Girdiginiz örnek numarası ($sample)mevcut, Lütfen kontrol edip tekrar deneyiniz\n";
27  }
28
29  print "Lütfen ilk dosyayi sürükleyin: ";
30  my $Read1 = <STDIN>;
31  chomp($Read1);
32
33  print "Lütfen ikinci dosyayi sürükleyin: ";
34  my $Read2 = <STDIN>;
35  chomp($Read2);
36
37
38
39
40  #BWA 1. Adim : bwa ile index olusturma, Tek sefer yeter
41  my $cmdindexbwa = "bwa index -p /home/mk/MyBioinf/RefandCode/Ref/EU781027.fasta -a is /home/mk/MyBioinf/RefandCode/Ref/EU781027.fasta";
42  system($cmdindexbwa);
43
44
45
46
47  #BWA 2. Adim : bwa mem ile aligment olustur.
48  my $cmdalignbwa = "bwa mem /home/mk/MyBioinf/RefandCode/Ref/EU781027 $Read1 $Read2 > /home/mk/MyBioinf/HCV Genotype/$sample/$sample.sam";
49  system($cmdalignbwa);
```

Status

```
14:44:37: This is Geany 1.36.
14:44:37: File /home/mk/miniconda3/bin/test.py opened (1).
14:44:37: File /home/mk/MyBioinf/RefandCode/Codes/YMDD_Aligner.pl opened (2).
14:44:37: File /home/mk/MyBioinf/RefandCode/Codes/txttohtml.pl opened (3).
14:44:37: File /home/mk/MyBioinf/RefandCode/Codes/html.pl opened (4).
14:45:18: File /home/mk/MyBioinf/RefandCode/Codes/HIV_Aligner.pl opened (5).
```

Compiler

line: 1 / 61 col: 0 sel: 0 INS TAB mode: LF encoding: UTF-8 filetype: Perl scope: unknown


Terminal
Lütfen Örnek Numarasını Giriniz: HD_2021_1024
Terminal
Lütfen Örnek Numarasını Giriniz: HD_2021_1024
Lütfen Kalite Skorunu girin (örneğim Q=30); Q = 30
Terminal
Lütfen Örnek Numarasını Giriniz: HD_2021_1024
Lütfen Kalite Skorunu girin (örneğim Q=30); Q = 30
Lütfen saptamak istediğini varyant oranını giriniz (örneğin Minimum Varyant % = 1); Minimum Varyant % = 1
Terminal
Lütfen Örnek Numarasını Giriniz: HD_2021_1024
Lütfen Kalite Skorunu girin (örneğim Q=30); Q = 30
Lütfen saptamak istediğini varyant oranını giriniz (örneğin Minimum Varyant % = 1); Minimum Varyant % = 1
Lütfen ilk dosyayı sürükleyin:

STANFORD UNIVERSITY
HIV DRUG RESISTANCE DATABASE
A curated public database designed to represent, store, and analyze the divergent forms of data underlying HIV drug resistance.

HIVdb: Genotypic Resistance Interpretation Algorithm

Date: 19-Oct-2014 19:18:25 UTC

Seq ID: Contig_1

Summary Data

Sequence includes PR: codons: 8 - 99

Sequence includes RT: codons: 1 - 235

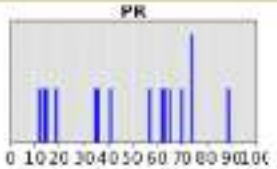
There are no insertions or deletions

Subtype and % similarity to closest reference isolate:

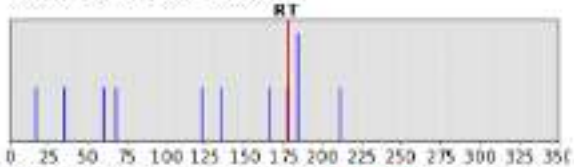
1. *PR: F (90.6%)*
2. *RT: B (93.9%)*

Sequence Quality Assessment

Gene	QA Problem	Codons
PR	Stop Codons, Frame Shifts:	None
PR	Ambiguous Positions:	None
PR	Unusual Residues:	None



Gene	QA Problem	Codons
RT	Stop Codons, Frame Shifts	None
RT	Ambiguous Positions:	None
RT	Unusual Residues:	178



Blue lines indicate differences from consensus B; tall Blue lines indicate sites associated with drug resistance. Red lines indicate QA problems.

Drug Resistance Interpretation: PR

PI Major Resistance Mutations:	None
PI Minor Resistance Mutations:	T74S
Other Mutations:	T12I, K14R, I15V, L19F, E35D, M36I, R41K, R57K, I62V, L63T, E65D, K70R, L89M

Protease Inhibitors	
atazanavir/r (ATV/r)	Susceptible
darunavir/r (DRV/r)	Susceptible
fosamprenavir/r (FPV/r)	Susceptible
indinavir/r (IDV/r)	Susceptible
lopinavir/r (LPV/r)	Susceptible
nelfinavir (NFV)	Low-level resistance
saquinavir/r (SQV/r)	Susceptible
tipranavir/r (TPV/r)	Susceptible

PR Comments

PIMinor
- T74S is a polymorphic mutation weakly selected by most PIs and associated with low-level resistance to NFV.

Other
- L89M is a common polymorphism that is not associated with reduced PI susceptibility. It is the consensus amino acid in most non-B subtypes.

Drug Resistance Interpretation: RT

NRTI Resistance Mutations:	M184V
NNRTI Resistance Mutations:	None
Other Mutations:	D17DG, V35T, V60I, S68G, D123E, I135T, K166R, D177E, I178IT, R211K

Nucleoside RTI		Non-Nucleoside RTI	
lamivudine (3TC)	High-level resistance	efavirenz (EFV)	Susceptible
abacavir (ABC)	Low-level resistance	etravirine (ETR)	Susceptible
zidovudine (AZT)	Susceptible	nevirapine (NVP)	Susceptible
stavudine (D4T)	Susceptible	rilpivirine (RPV)	Susceptible
didanosine (DDI)	Potential low-level resistance		
emtricitabine (FTC)	High-level resistance		
tenofovir (TDF)	Susceptible		

RT Comments

NRTI
- M184V/I cause high-level resistance to 3TC and FTC and low-level resistance to ddI and ABC. However, M184V/I are not contraindications to continued treatment with 3TC or FTC because they increase susceptibility to AZT, TDF and d4T and are associated with clinically significant reductions in HIV-1 replication. In combination with K101E or E138K, M184I synergistically reduces RPV susceptibility.

Bulgular

		PI Majör Direnç Mutasyon Sayısı	PI Aksesuar Direnç Mutasyon Sayısı	NRTI Direnç Mutasyon Sayısı	NNRTI Direnç Mutasyon Sayısı
DeepCheck % 20	Duyarlı 30 Köken	0	0	0	0
Bowtie 2 (%1)		0	1	0	0
Bowtie 2 (%20)		0	0	0	0
BWA (%1)		0	3	1	0
BWA (%20)		0	0	0	0

		PI Majör Direnç Mutasyon Sayısı	PI Aksesuar Direnç Mutasyon Sayısı	NRTI Direnç Mutasyon Sayısı	NNRTI Direnç Mutasyon Sayısı
DeepCheck % 20	Dirençli 30 Köken PI n=2 NRTI n=10 NNRTI n=20	2	0	10	20
Bowtie 2 (%1)		2	0	10	20
Bowtie 2 (%20)		2	0	10	20
BWA (%1)		4	1	10	22
BWA (%20)		2	0	10	20

Direnç mutasyon tipleri uyumlu

Sonuç

- Sınanan algoritmalar sonuçları açısından birbirleriyle uyumlu bulunurken iVar sistemi ile Q=30 kalite skorunda analizin yapılması daha hassas sonuçlar elde edilmesine yol açmıştır.

- Minör varyantların saptanmasında BWA algoritması BT2 algoritmasına göre daha üstün gibi görülmekle birlikte BT2 algoritması bilgisayar kaynaklarını daha etkin kullanmaktadır.

- Mevcut ticari sistemlerin analiz bazlı ücretlendirme yapması nedeniyle geliştirdiğimiz bu yeni nesil dizileme biyoinformatik yazılımının iyi bir alternatif olacağını ve gARDT maliyetlerini azaltabileceğini düşünmekteyiz.