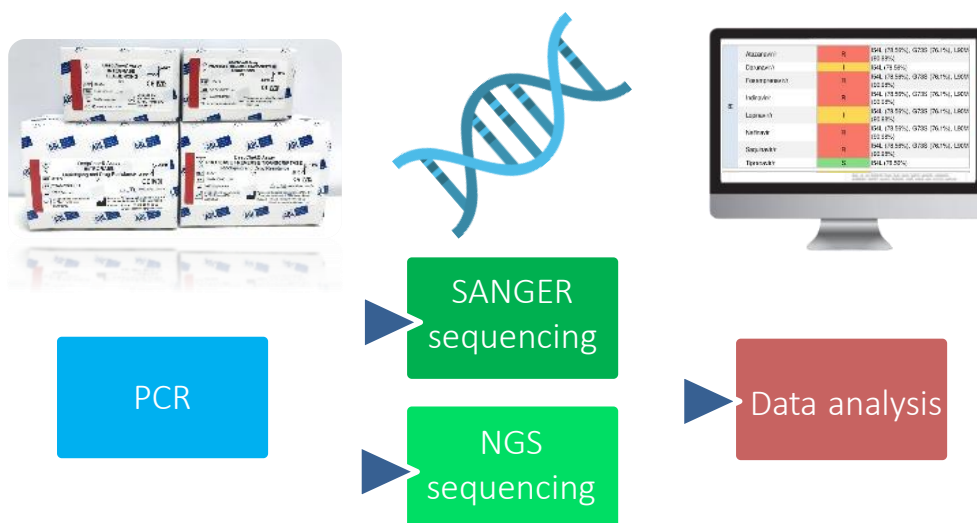


DeepChek[®] Assays for Carrying Out Research About HIV Drug Resistance

An array of Research Use Only (RUO) assays for use on sequencing platforms, next generation sequencing (NGS) and Sanger



Research support | Laboratory efficiency | Cost effectiveness

ROBUST

Pool different RUO applications in the same NGS run

FLEXIBLE

For low to high throughput
1-384 samples in one run

FAST

~1-2 days (SANGER)
~2-4 days (NGS)

OPEN

Compatible with GLP sequencing reagents and instruments (Sanger and NGS)

COMPREHENSIVE

Genotyping, Drug Resistance, Tropism, Reporting, Storage...

SECURED DATA ANALYSIS

Healthcare Cloud Access, Local installation

DeepChek® HIV-1 Assays WORKFLOW OVERVIEW

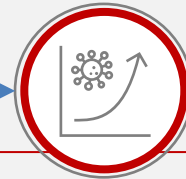


Sample Preparation

Sample collection

RNA or proviral DNA extraction

Lab self-validate



PCR

Whole genome

RT/PR

INT

V3 Loop

DeepChek® HIV assays (RUO)



SANGER sequencing

Purification

Sequencing

DeepChek® SANGER Sequencing Reaction (RUO)



NGS sequencing

Library preparation reagents

Indexes (24/48/96/384)

DeepChek® NGS Library Preparation (RUO)

Sequencing (General Laboratory Products)



SANGER data analysis

Analysis

Reporting

ViroScore® - HIV Software (RUO)



NGS data analysis

Analysis

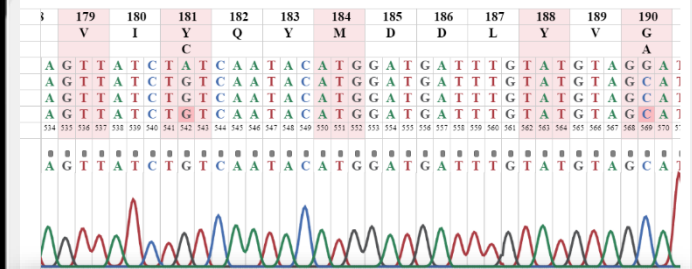
Reporting

DeepChek® - HIV Software (RUO)

SANGER



RUO



Subtype:

Reverse transcriptase B (100% similarity) Protease B (97% similarity) Integrase B (100% similarity) GP41 B (100% similarity) GP120/V3 Loop B (check for 46_BF) (45% similarity)

All Mutations Detected (PKB2 reference Sequence) Resistance mutations in bold based on Stanford v7.0.1 (mutation score # 0)

Reverse transcriptase T27S, T39A, K43E, K49R, **K101E**, I142V, **Y181C**, **G190A**, G196E, **L210W**, L214F, **T215Y**, V245K, P272A, R277K, I293V

Protease V81 L10I, S37N, **I54V**, L63P, H69R, A71V, **G73S**, I84V, **L90M**

Integrase K111R, S119G, T122I, G123S, A124T, T125A, R127K, N232D, R284G

GP41 D78N, I729K, P213S, V311E, R324R/K, N301N/R/S, T303K/T, R304R/K/Q, K305R/K, del306, R306R/Q/K, del307, Q310H, R311I, V318Y, T319A, I320A/T, G321D/E/G, S215N/K/NM/E/D/N/V, K322I, I323E/V/M/K, N325D/N, M326I, Q328K, H330Y

GP120/V3 Loop

Tipism

XI Virus (The 11/25 rule would predict this sequence as an XI-virus.)

Class	Drug	ANRS 25 2015-09	SIZAV 7.0.1 27/02/2014	Z-Score	Interpretation	FPR
NRTI	Zidovudine	R	R	L210W, T215Y 5.137	Resistant	
	Didanosine	S	I	L210W, T215Y 1.713	Intermediate	
NRTI	Stavudine	R	R	L210W, T215Y 4.383	Resistant	
	Lamivudine	S	S	L210W, T215Y 1.467	Sensible	(2)
	Emtricitabine	S	S	L210W, T215Y 1.467	Sensible	(2)
			I	L210W, T215Y 3.849	Resistant	
			I	L210W, T215Y 3.658	Resistant	
NRTI			R	K305E, Y181C, G190A 5.915	Resistant	
			R	K305E, Y181C, G190A 4.136	Resistant	
			R	K305E, Y181C, G190A Not available	Resistant	(3)
			R	K305E, Y181C, G190A Not available	Resistant	(3)
NRTI			R	I54V, G73S, I84V, L90M 11.065	Resistant	
			R	I54V, G73S, I84V, L90M 14.423	Resistant	

SUBTYPING		COVERED POSITIONS	
PROT	B (94.95%)	RT	B (95.83%)
INT	B (95.53%)	PROT	1-99
		RT	1-327
		INT	32-289

(DT, PROT, INT) Subtype B **95.53%** was used as the reference sequence for the alignment (using BWA v0.7.15 alignment tool). Subtyping determination performed using homology testing or a 20% consensus sequence generated from all the reads mapped to the particular region and compared to an updated set of reference sequences.

DEEPCHEK® HIV (Stanford 8.8)			
Generic name	Assessment	Resistance mutations >20.00%	Resistance mutations between >3% and <20.00%
PI	Atazanavir/r	R	I54L (78.56%), G73S (76.1%), L90M (90.98%)
	Darunavir/r	I	I54L (78.56%)
	Fosamprenavir/r	R	I54L (78.56%), G73S (76.1%), L90M (90.98%)
	Indinavir/r	R	I54L (78.56%), G73S (76.1%), L90M (90.98%)
	Lopinavir/r	I	I54L (78.56%), G73S (76.1%), L90M (90.98%)
	Nelfinavir	R	I54L (78.56%), G73S (76.1%), L90M (90.98%)
	Saquinavir/r	R	I54L (78.56%), G73S (76.1%), L90M (90.98%)
	Tipranavir/r	S	I54L (78.56%)
NRTI	Abacavir	I	D67N (96.96%), M184V (99.35%)
	Didanosine	I	D67N (96.96%), M184V (99.35%)
	Emtricitabine	R	M184V (99.35%)
	Lamivudine	R	M184V (99.35%)
	Stavudine	S	D67N (96.96%), M184V (99.35%)
	Tenofovir	S	D67N (96.96%), M184V (99.35%)
Zidovudine	S	D67N (96.96%), M184V (99.35%)	
NNRTI	Doravirine	S	
	Efavirenz	S	
	Etravirine	S	
	Nevirapine	S	
	Rilpivirine	S	
INI	Bictegravir	I	G140S (99.66%), Q148H (99.33%)
	Dolutegravir	I	G140S (99.66%), Q148H (99.33%)
	Elvitegravir	R	G140S (99.66%), Q148H (99.33%)
	Raltegravir	R	G140S (99.66%), Q148H (99.33%)

S Susceptible (S) Potential low-level resistance (PLLR) I Low-level resistance (LLR) Intermediate resistant (IR) R High-level resistance (HLR)

NGS

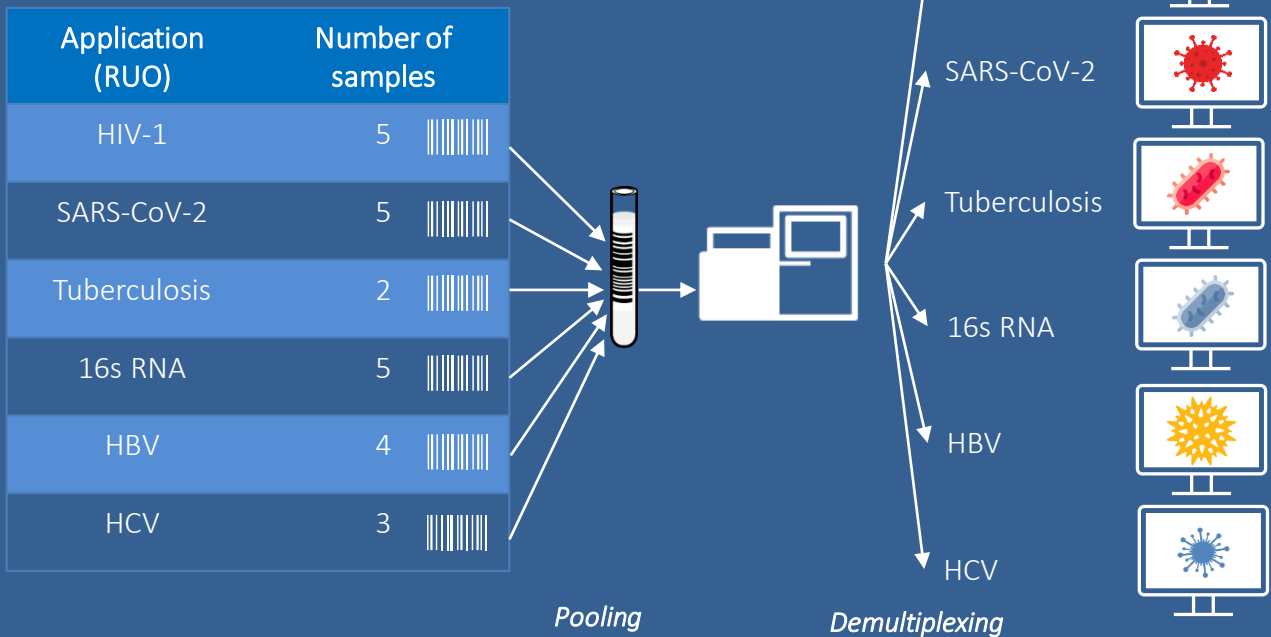


RUO

REDUCE SIGNIFICANTLY NGS RELATED COSTS AND TURNAROUND TIME WHEN USING THE DEEPCHEK® TECHNOLOGY

- The DeepChek® assays & software can be used through NGS on a large variety of research applications simultaneously, providing high efficiency
 - Optimize NGS sequencing costs
 - Optimize turnaround time
 - Bring NGS capacity to any lab settings
- Adapt and customize your NGS panel/run to your research plans

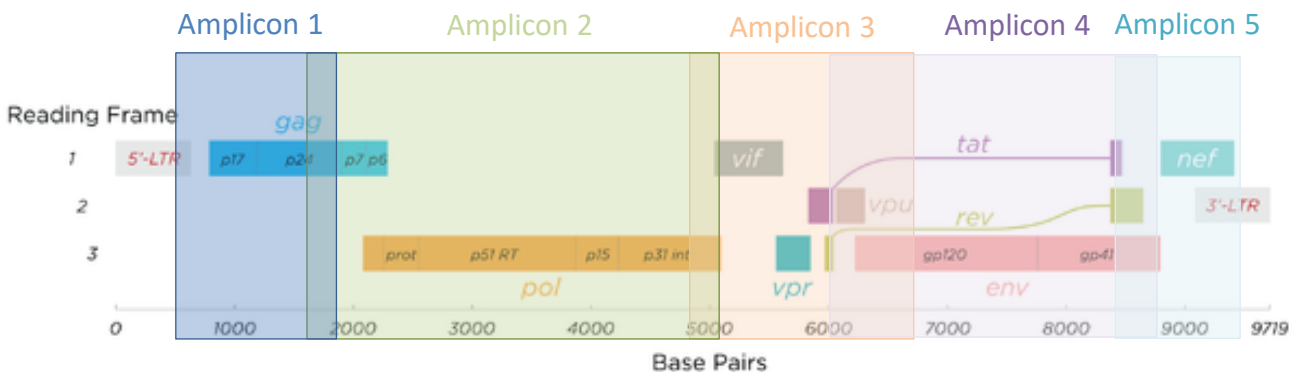
Example of a weekly NGS RUO run on 24 samples



Research Use Only – not for use in diagnostic procedures

DeepChek[®] Assay **Whole Genome** HIV-1 Genotyping (RUO)

- *Access to a comprehensive Next Generation Sequencing (NGS) kit for HIV Whole Genome genotyping.*
- *DeepChek[®] Assay Whole Genome HIV-1 Genotyping has been developed for use with the leading next generation sequencing (NGS) platforms and includes optimized multiplex PCR master mixes with primers to amplify RNA and proviral DNA inputs with manual or automated library preparations.*
- Ability to screen genomic variations in all HIV-1 genome genes targeted by current and under development HIV-1 inhibitors, including **new entry & capsid inhibitors**.
- Number of amplicons: 5x ~700 to 3500 bp obtained from same thermal cycling program in five (5) distinct wells.
- Coverage : ~ 9600 bp.
- Inclusivity: excellent coverage of all representative HIV-1 subtypes from M group strains.
- Cross-reactivity: The assay reacted (i.e., good agarose gel band) for some of the tested microorganisms commonly found in infectious diseases specimens. After NGS and data analysis, no reads mapped with HIV-1.
- Compatible with magnetic-beads RNA/DNA isolation instruments and reagents, and with any PCR thermal cycler with ramp rate of $\geq 1^{\circ}\text{C/s}$, and leading NGS instruments and reagents equivalent to Illumina SBS chemistry.
- Shall be compatible with run of 48 / 96 and 384 samples to reach assay cut-off - minimal median total coverage of 1000 reads for the amplicons and a Phred Quality Score Q30>80% (or equivalent)).
- The sequencing outputs can be analyzed with specific downstream sequencing analysis software (e.g. DeepChek[®] Software) to reduce laboratory staff burden and to alleviate the need for specialized bioinformatics expertise or on the contrary with using generic bioinformatics software and tools for more advanced research teams.



Research Use Only – not for use in diagnostic procedures

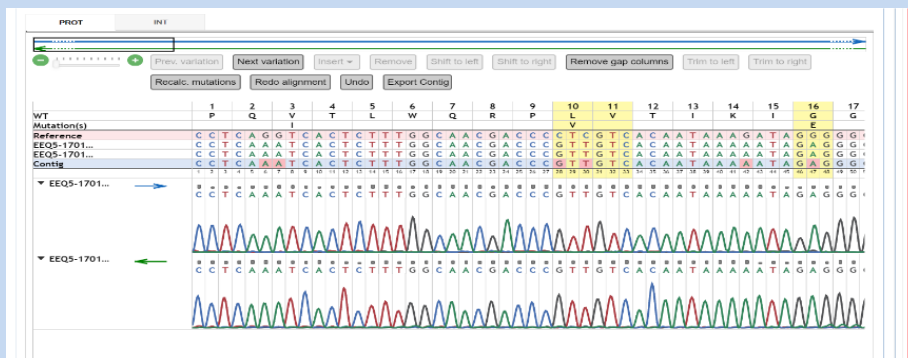
HIV-1 genes associated with antiviral resistance when using the DeepChek® Assay

Whole Genome HIV-1 Genotyping (RUO)

Anti-HIV drug class	HIV-1 gene target	Related drugs	Assay fragment #
Capsid inhibitors	<i>gag</i>	lenacapavir	1
Nucleoside reverse transcriptase inhibitors (NRTIs)	<i>reverse transcriptase</i>	zidovudine, lamivudine, emtricitabine, abacavir, tenofovir disoproxil fumarate, tenofovir alafenamide, islatravir, didanosine and stavudine	2
Non-nucleoside reverse transcriptase inhibitors (NNRTIs)	<i>reverse transcriptase</i>	efavirenz, nevirapine, etravirine, rilpivirine and doravirine	2
Protease inhibitors (PIs)	<i>protease</i>	lopinavir, atazanavir, darunavir, ritonavir, indinavir, saquinavir, nelfinavir, fosamprenavir and tipranavir	2
Integrase inhibitors (IIs)	<i>integrase</i>	raltegravir, elvitegravir, dolutegravir, cabotegravir, bictegravir	2
n.a	<i>vif, vpr, vpu (accessory proteins)</i>	enfuvirtide	3
Fusion inhibitors	<i>gp41</i>	Fostemsavir	4
Post-attachment inhibitors	<i>gp120</i>	n.a	4
n.a	<i>nef (accessory protein)</i>	n.a	5

Research Use Only – not for use in diagnostic procedures

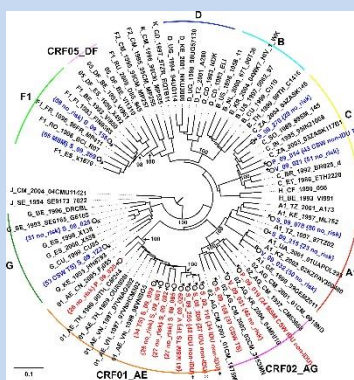
EMBEDDED
CHROMATOGRAM
EDITOR (RUO)



SINGLE or
CUMULATIVE
GENOTYPING (RUO)



SUBTYPING &
DRUG RESISTANCE
ASSESSMENT (RUO)



Drug	ANRS 31 12-2020	STAN 9.0 03-2021
Zidovudine	R	R
Didanosine	Not available	R
Stavudine	Not available	R
Lamivudine	S	S
Emtricitabine	S	S
Abacavir	R	I
Tenofovir	R	I

Algorithm	Sanger based sequencing				
	15.00%	10.00%	5.00%	3.00%	
Abacavir	ANRS	I	I	I	I
	Grade	I	I	I	I
	Rega Institute	S	S	S	S
Didanosine	ANRS	NA	NA	NA	NA
	Grade	I	I	I	I
	Rega Institute	LLR	LLR	LLR	LLR
Emtricitabine	ANRS	R	R	R	R
	Grade	R	R	R	R
	Rega Institute	R	R	R	R
Stanford	HLR	HLR	HLR	HLR	

Public knowledge databases

- HIVDB/STANFORD
- ANRS
- REGA
- GENO2PHENO
- HIV-GRADE



- RIS
- RENAGENO



Research Use Only – not for use in diagnostic procedures



Product References



PCR (RUO)

DeepChek® Assays (RUO)

WHOLE GENOME HIV-1 Genotyping (24 tests)	170B24
PROTEASE / REVERSE TRANSCRIPTASE Genotyping and Drug Resistance (24 or 96 tests)	101B24 96
INTEGRASE Genotyping and Drug Resistance (24 or 96 tests)	102C24 96
V3 LOOP / TROPISM (24 tests)	103A24
GP41/GP120 Genotyping and Drug Resistance (24 tests)	194A24
GAG Genotyping and Drug Resistance (24 tests)	195A24
REVERSE TRANSCRIPTASE SIMPLEX Genotyping and Drug Resistance (24 tests)	197A24
HIV-1 Full PR/RT/INT Drug Resistance (24 tests)	198BA24

NGS SEQUENCING (RUO)

DeepChek® NGS Library Preparation

▪ 24 indexes	116B24+124B24
▪ 48 indexes	116B48+124B48
▪ 96 indexes	116B96+124B96
▪ 384 indexes	116B384+124B384
DeepChek® NGS Clean-up beads	N411-02

SANGER SEQUENCING (RUO)

DeepChek® SANGER Sequencing Reaction (24 rx)	123A24
DeepChek® SANGER Sequencing Reaction (48 rx)	123A48
DeepChek® 96x0.2 mL wells plate	B70501-1
Opti-Seal Optical Sealing Sheet	157300

DATA ANALYSIS (RUO)

DeepChek® - HIV Software (RUO)	S-12-023 (HL HM)
ViroScore® - HIV Software (RUO)	S-09-014
DeepChek Whole Genome HIV-1 (RUO)	S-22-056 (WHL WHM)



AdvancedDx Biological Laboratories USA Inc.



Advanced Biological Laboratories (ABL) S.A.
52-54 avenue du X Septembre, Luxembourg,
Luxembourg



5-7 Perry Way, Unit 15
Newburyport MA 01950
USA