Bildercollage © Gorodenkoff/shutterstock.com; U.PSD/shutterstock.com; PublicDomainPictures/pixabay.com

Comparison of Non-Targeted and Broad-Spectrum Targeted NGS for Adventitious Virus Detection

Dr. Gibran Horemheb Rubio Quintanares
Viral Security Assessor

Bundesinstitut für Impfstoffe und biomedizinische Arzneimittel
Federal Institute for Vaccines and Biomedicines

Das Paul-Ehrlich-Institut ist ein Bundesinstitut im Geschäftsbereich des Bundesministeriums für Gesundheit.

The Paul-Ehrlich-Institut is an Agency of the German Federal Ministry of Health.

Paul-Ehrlich-Institut

Disclaimer

The views expressed in this presentation are the personal views of the presenter(s). They shall not be understood or cited as opinions of the Paul-Ehrlich-Institut. The presenter has not received any funding or grants from companies or from associations representing companies.

INTRODUCTION

Problem

Blood Derivate Products

Table 1. Effect of manufacturing scale on risk of exposure. Adapted from Lynch et al. 1996 with permission.

Manufacturing Scale (Number of Donors)	Number of Independent Infusions		
	1	10	100
Prevalence of agent = 5×10^{-5}			
60,000	11%	70%	100%
25,000	2%	18%	86%
1000	0.2%	2%	18%
Prevalence of agent = 5×10^{-4}			
60,000	70%	100%	100%
25,000	39%	99%	100%
1000	2%	18%	86%
Prevalence of agent = 5×10^{-3}			
60,000	100%	100%	100%
25,000	99%	100%	100%
1000	18%	86%	100%

Vaccines and biomedicines

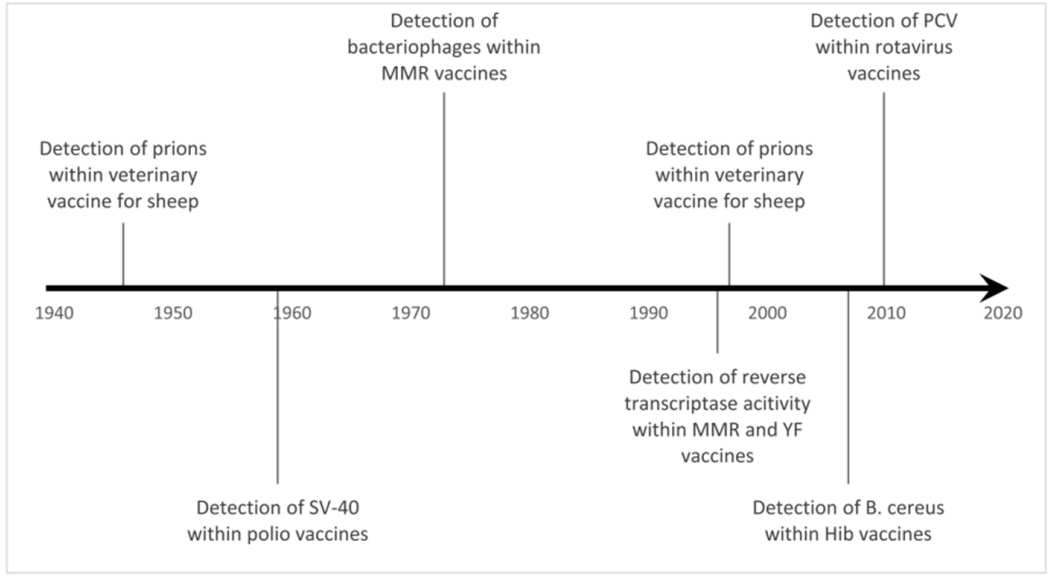


Fig. 1. A selected timeline of adventitious agent contamination
Each of the discussed events of adventitious agent contamination are listed chronologically, with the date in reference to the year the contamination was detected.

Unbiased sequence does not exist!

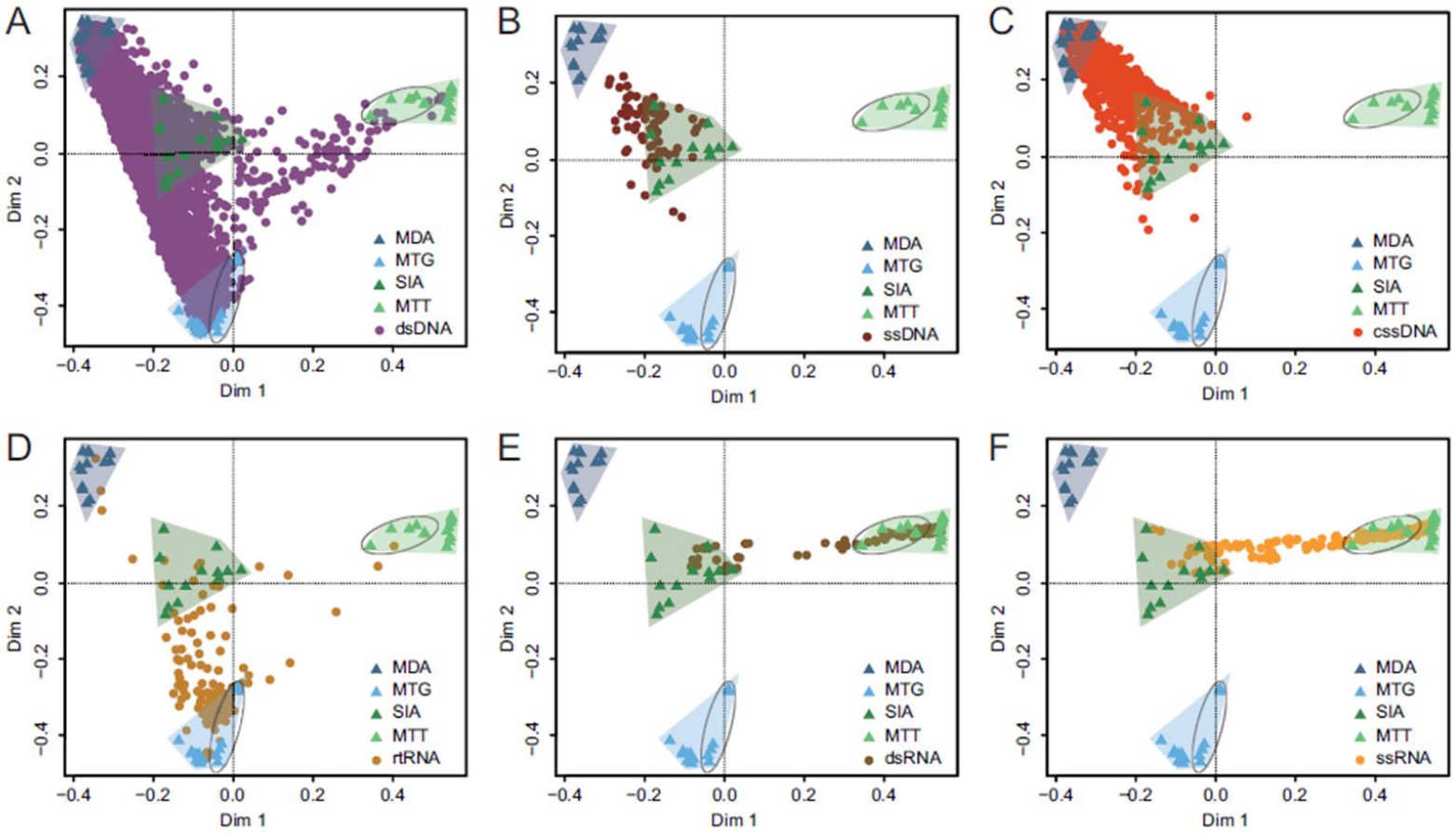


FIG 3 Principal coordinates analysis (PCoA) analyses revealed that libraries (triangles) aggregated into four clusters corresponding to MDA, MTG, SIA, and MTT viromic techniques that show different preferences to vOTUs (filled circles) of double-stranded DNA (dsDNA) (A), single-stranded DNA (ssDNA) (B), circular single-stranded DNA (cssDNA) (C), reverse transcribing RNA (rtRNA) (D), double-stranded RNA (dsRNA) (E), single-stranded RNA (and ssRNA) (F). The circled libraries are rebuilt MTG and MTT ones that detach from the initial ones due to batch effects.

Sun Y, Qu Y, Yan X, Yan G, Chen J, Wang G, Zhao Z, Liu Y, Tu C, He B. Comprehensive Evaluation of RNA and DNA Viromic Methods Based on Species Richness and Abundance Analyses Using Marmot Rectal Samples. mSystems. 2022 Aug 30;7(4):e0043022. doi: 10.1128/msystems.00430-22. Epub 2022 Jul 14. PMID: 35862817; PMCID: PMC9426427.

Broad capture system (VirCapSeq-VERT)

TABLE 1

Virus Taxa Selected for VirCapSeq-VERT Probe Design

Name	tax_id	ParentName	parent tax_id
Adenoviridae	10508	dsDNA viruses, no RNA stage	35237
Alloherpesviridae	548682	Herpesvirales	548681
Alphacoronavirus	693996	Coronavirinae	693995
Alphaherpesvirinae	10293	Herpesviridae	10292
Alphanodavirus	143920	Nodaviridae	12283
Alphapapillomavirus	333750	Papillomaviridae	151340
Alphapermutotetravirus	1283211	Permutotetraviridae	1283210
Alpharetrovirus	153057	Orthoretrovirinae	327045
Alphatorquevirus	687331	Anelloviridae	687329
Alphavirus	11019	Togaviridae	11018
Amdoparvovirus	310911	Parvovirinae	40119
Anelloviridae	687329	ssDNA viruses	29258
Aphthovirus	12109	Picornaviridae	12058
Aquabirnavirus	39750	Birnaviridae	10993
Aquamavirus	1330065	Picornaviridae	12058
Aquaparamyxovirus	1232658	Paramyxovirinae	11159
Aquareovirus	10979	Spinareovirinae	689831
Arenaviridae	11617	ssRNA negative-strand viruses	35301
Arenavirus	11618	Arenaviridae	11617
Arteriviridae	76803	Nidovirales	76804
Arterivirus	11046	Arteriviridae	76803
Asfarviridae	137992	dsDNA viruses, no RNA stage	35237
Asfivirus	39743	Asfarviridae	137992
Astroviridae	39733	ssRNA positive-strand viruses, no DNA stage	35278
Atadenovirus	100953	Adenoviridae	10508
Aurivirus	1513230	Malacoherpesviridae	548685
Avastrovirus	249589	Astroviridae	39733
Aveparvovirus	1511864	Parvovirinae	40119
Aviadenovirus	10552	Adenoviridae	10508
Avibirnavirus	39751	Birnaviridae	10993
Avihepadnavirus	10437	Hepadnaviridae	10404
Avihepatovirus	691955	Picornaviridae	12058
Avipoxvirus	10260	Chordopoxvirinae	10241
Avisivirus	1511771	Picornaviridae	12058
Avulavirus	260963	Paramyxovirinae	11159
Bafinivirus	694018	Torovirinae	694017
Batrachovirus	692605	Alloherpesviridae	548682
Betacoronavirus	694002	Coronavirinae	693995
Betaherpesvirinae	10357	Herpesviridae	10292
Betanodavirus	143919	Nodaviridae	12283
Betapapillomavirus	333922	Papillomaviridae	151340
Betaretrovirus	140052	Orthoretrovirinae	327045
Betatorquevirus	687332	Anelloviridae	687329
Birnaviridae	10993	dsRNA viruses	35325
Blosnavirus	564643	Birnaviridae	10993
Bocaparvovirus	1507401	Parvovirinae	40119
Bornaviridae	178830	Mononegavirales	11157
Bornavirus	186458	Bornaviridae	178830
Bracorhabdovirus	490109	unclassified Rhabdoviridae	35303
Bunyaviridae	11571	ssRNA negative-strand viruses	35301
Caliciviridae	11974	ssRNA positive-strand viruses, no DNA stage	35278

Name	tax_id	ParentName	parent tax_id
Capripoxvirus	10265	Chordopoxvirinae	10241
Cardiovirus	12103	Picornaviridae	12058
Cervidpoxvirus	573055	Chordopoxvirinae	10241
Chipapillomavirus	934800	Papillomaviridae	151340
Chloridovirus	10491	Iridoviridae	10486
Chordopoxvirinae	10241	Poxviridae	10240
Circoviridae	39724	ssDNA viruses	29258
Circovirus	39725	Circoviridae	39724
Coltivirus	10911	Spinareovirinae	689831
Copiparvovirus	1511888	Parvovirinae	40119
Coronaviridae	11118	Nidovirales	76804
Coronavirinae	693995	Coronaviridae	11118
Cosavirus	586418	Picornaviridae	12058
Crocodylidpoxvirus	1285599	Chordopoxvirinae	10241
Cuevavirus	1513236	Filoviridae	11266
Cyprinivirus	692606	Alloherpesviridae	548682
Cytomegalovirus	10358	Betaherpesvirinae	10357
Cytorhabdovirus	11305	Rhabdoviridae	11270
Deltacoronavirus	1159901	Coronavirinae	693995
Deltapapillomavirus	325454	Papillomaviridae	151340
Deltaretrovirus	153136	Orthoretrovirinae	327045
Deltatorquevirus	687334	Anelloviridae	687329
Deltavirus	39759	Viruses	10239
Dengue virus group	11052	Flavivirus	11051
Densovirinae	40120	Parvoviridae	10780
Dependoparvovirus	10803	Parvovirinae	40119
Dicipivirus	1330067	Picornaviridae	12058
Dinornavirus	674976	Alvernaviridae	866787
Dyodeltapapillomavirus	936056	Papillomaviridae	151340
Dyoepsilonpapillomavirus	935646	Papillomaviridae	151340
Dyoetapapillomavirus	935641	Papillomaviridae	151340
Dyoiotapapillomavirus	934804	Papillomaviridae	151340
Dyokappapapillomavirus	1513238	Papillomaviridae	151340
Dyolambdapapillomavirus	1513239	Papillomaviridae	151340
Dyomupapillomavirus	1513240	Papillomaviridae	151340
Dyorupapillomavirus	1513241	Papillomaviridae	151340
Dyoomikronpapillomavirus	1513242	Papillomaviridae	151340
Dyopipapillomavirus	1513243	Papillomaviridae	151340
Dyorhopapillomavirus	1513244	Papillomaviridae	151340
Dyosigmapapillomavirus	1513245	Papillomaviridae	151340
Dyothetapapillomavirus	1052159	Papillomaviridae	151340
Dyoxipapillomavirus	1513246	Papillomaviridae	151340
Dyozetapapillomavirus	934803	Papillomaviridae	151340
Ebolavirus	186536	Filoviridae	11266
Enterovirus	12059	Picornaviridae	12058
Entomopoxvirinae	10284	Poxviridae	10240
Ephemerovirus	32613	Rhabdoviridae	11270
Epsilonretrovirus	153137	Orthoretrovirinae	327045
Epsilontorquevirus	687335	Anelloviridae	687329
Equine lentivirus group	11654	*Lentivirus*	11646
Erbovirus	194961	Picornaviridae	12058
Erythroparvovirus	40121	Parvovirinae	40119
Etapapillomavirus	325458	Papillomaviridae	151340
Etatorquevirus	687337	Anelloviridae	687329
Ferlavirus	1283308	Paramyxovirinae	11159
Filoviridae	11266	Mononegavirales	11157
Flaviviridae	11050	ssRNA positive-strand viruses, no DNA stage	35278
Flavivirus	11051	Flaviviridae	11050
Gallivirus	1511775	Picornaviridae	12058
Gammacoronavirus	694013	Coronavirinae	693995
Gammaherpesvirinae	10374	Herpesviridae	10292
Gammapapillomavirus	325455	Papillomaviridae	151340
Gammaretrovirus	153135	Orthoretrovirinae	327045
Gammatorquevirus	687333	Anelloviridae	687329
Gyrovirus	227307	Circoviridae	39724
Hantavirus	11598	Bunyaviridae	11571
Henipavirus	260964	Paramyxovirinae	11159
Hepacivirus	11102	Flaviviridae	11050
Hepadnaviridae	10404	Retro-transcribing viruses	35268
Hepatovirus	12091	Picornaviridae	12058

Name	tax_id	ParentName	parent tax_id
Hepeviridae	291484	ssRNA positive-strand viruses, no DNA stage	35278
Hepevirus	186677	Hepeviridae	291484
Herpesvirales	548681	dsDNA viruses, no RNA stage	35237
Herpesviridae	10292	Herpesvirales	548681
Humnivirus	1431456	Picornaviridae	12058
Ichtadenovirus	691957	Adenoviridae	10508
Ictalurivirus	172653	Alloherpesviridae	548682
Iltovirus	180255	Alphaherpesvirinae	10293
Influenzavirus D	1511083	unclassified Orthomyxoviridae	35324
Intracisternal A-particles	11749	unclassified Retroviridae	35276
Iotatorquevirus	687339	Anelloviridae	687329
Iridoviridae	10486	dsDNA viruses, no RNA stage	35237
Iridovirus	10487	Iridoviridae	10486
Isavirus	324913	Orthomyxoviridae	11308
Japanese encephalitis virus group	11071	*Flavivirus*	11051
Kappapapillomavirus	325457	Papillomaviridae	151340
Kappatorquevirus	1218487	Anelloviridae	687329
Kobuvirus	194960	Picornaviridae	12058
Kokobera virus group	303179	*Flavivirus*	11051
Lagovirus	95339	Caliciviridae	11974
Lambdapapillomavirus	325462	Papillomaviridae	151340
Lambdatorquevirus	1218489	Anelloviridae	687329
Lentivirus	11646	Orthoretrovirinae	327045
Leporipoxvirus	10270	Chordopoxvirinae	10241
Lymphocryptovirus	10375	Gammaherpesvirinae	10374
Lymphocystivirus	10494	Iridoviridae	10486
Lyssavirus	11286	Rhabdoviridae	11270
Macavirus	548687	Gammaherpesvirinae	10374
Malacoherpesviridae	548685	Herpesvirales	548681
Mamastrovirus	249588	Astroviridae	39733
Marburgvirus	186537	Filoviridae	11266
Mardivirus	180252	Alphaherpesvirinae	10293
Mastadenovirus	10509	Adenoviridae	10508
Megalocytivirus	308906	Iridoviridae	10486
Megrivirus	1330069	Picornaviridae	12058
Metapneumovirus	162387	Pneumovirinae	11244
Mischivirus	1511778	Picornaviridae	12058
Modoc virus group	29260	*Flavivirus*	11051
Molluscipoxvirus	10278	Chordopoxvirinae	10241
Mononegavirales	11157	ssRNA negative-strand viruses	35301
Morbillivirus	11229	Paramyxovirinae	11159
Mosavirus	1481451	Picornaviridae	12058
mosquito-borne viruses	59562	*Flavivirus*	11051
Mupapillomavirus	334202	Papillomaviridae	151340
Muromegalovirus	10365	Betaherpesvirinae	10357
Nairovirus	11592	Bunyaviridae	11571
Nebovirus	696855	Caliciviridae	11974
Negevirus	1307798	unclassified ssRNA positive-strand viruses	38173
Nidovirales	76804	ssRNA positive-strand viruses, no DNA stage	35278
Nodaviridae	12283	ssRNA positive-strand viruses, no DNA stage	35278
Norovirus	142786	Caliciviridae	11974
Novirhabdovirus	186778	Rhabdoviridae	11270
Ntaya virus group	29261	*Flavivirus*	11051
Nucleorhabdovirus	11306	Rhabdoviridae	11270
Nupapillomavirus	475861	Papillomaviridae	151340
Nyamiviridae	1513294	Mononegavirales	11157
Nyavirus	1513295	Nyamiviridae	1513294
Omegapapillomavirus	936061	Papillomaviridae	151340
Orbivirus	10892	Sedoreoviridae	689832
Orthobunyavirus	11572	Bunyaviridae	11571
Orthohepadnavirus	10405	Hepadnaviridae	10404
Orthomyxoviridae	11308	ssRNA negative-strand viruses	35301
Orthopoxvirus	10242	Chordopoxvirinae	10241
Orthoreovirus	10882	Spinareovirinae	689831
Orthoretrovirinae	327045	Retroviridae	11632
Oscivirus	1511780	Picornaviridae	12058
Ostreavirus	548686	Malacoherpesviridae	548685

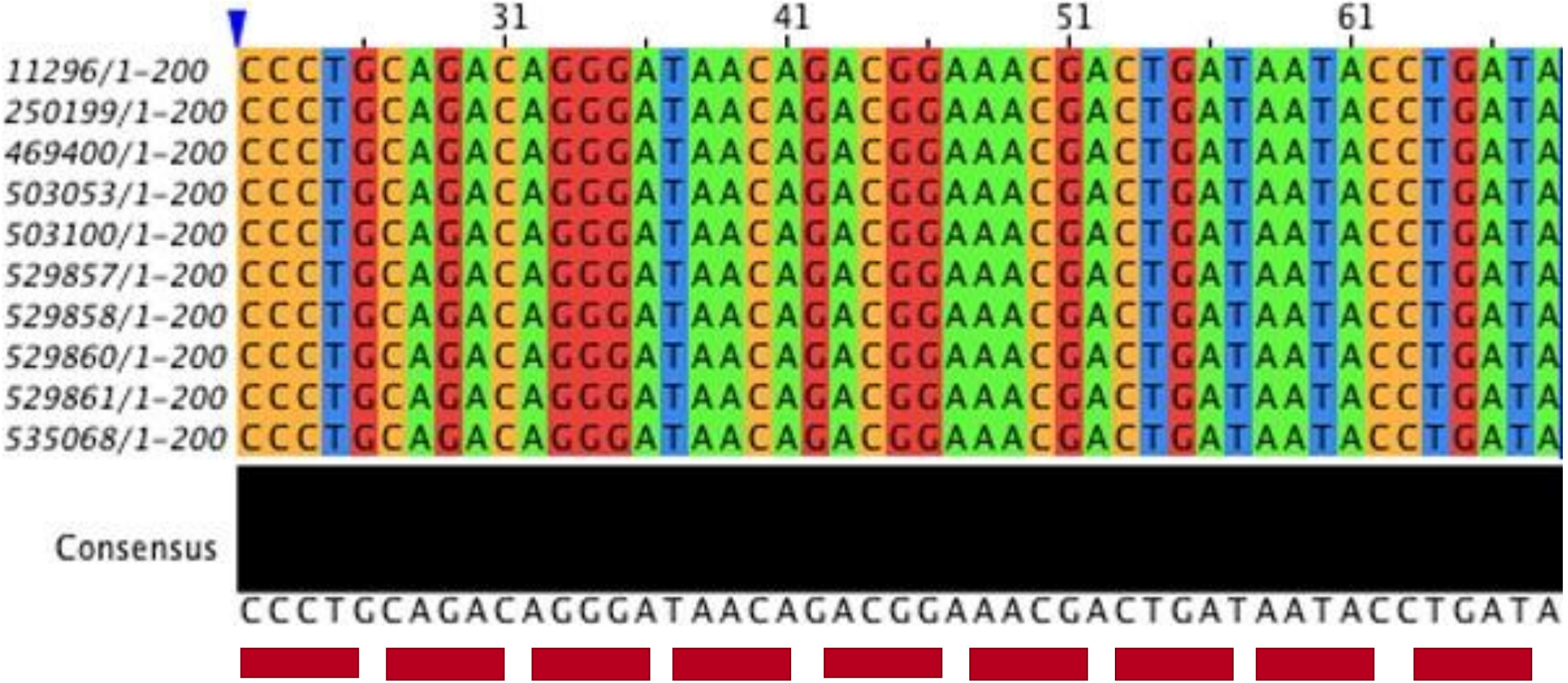
Name	tax_id	ParentName	parent tax_id
Papillomaviridae	151340	dsDNA viruses, no RNA stage	35237
Paramyxoviridae	11158	Mononegavirales	11157
Paramyxovirinae	11159	Paramyxoviridae	11158
Parapoxvirus	10257	Chordopoxvirinae	10241
Parechovirus	138954	Picornaviridae	12058
Parvoviridae	10780	ssDNA viruses	29258
Parvovirinae	40119	Parvoviridae	10780
Pasivirus	1511782	Picornaviridae	12058
Passerivirus	1511802	Picornaviridae	12058
Pegivirus	1307799	Flaviviridae	11050
Percavirus	548688	Gammaherpesvirinae	10374
Perhabdovirus	1298653	Rhabdoviridae	11270
Pestivirus	11095	Flaviviridae	11050
Phipapillomavirus	934802	Papillomaviridae	151340
Phlebovirus	11584	Bunyaviridae	11571
Picobirnaviridae	585893	dsRNA viruses	35325
Picobimavirus	104394	Picobirnaviridae	585893
Picornavirales	464095	ssRNA positive-strand viruses, no DNA stage	35278
Picornaviridae	12058	Picornavirales	464095
Pipapillomavirus	334211	Papillomaviridae	151340
Pneumovirinae	11244	Paramyxoviridae	11158
Pneumovirus	11245	Pneumovirinae	11244
Polyomaviridae	151341	dsDNA viruses, no RNA stage	35237
Polyomavirus	10624	Polyomaviridae	151341
Poxviridae	10240	dsDNA viruses, no RNA stage	35237
Proboscivirus	548689	Betaherpesvirinae	10357
Protoparvovirus	1506574	Parvovirinae	40119
Psipapillomavirus	935650	Papillomaviridae	151340
Quadrivirus	1299297	Quadriviridae	1299296
Quaranjavirus	1299308	Orthomyxoviridae	11308
Ranavirus	10492	Iridoviridae	10486
Recovirus	873551	Caliciviridae	11974
Reoviridae	10880	dsRNA viruses	35325
Respirovirus	186938	Paramyxovirinae	11159
Retroviridae	11632	Retro-transcribing viruses	35268
Rhabdoviridae	11270	Mononegavirales	11157
Rhadinovirus	10379	Gammaherpesvirinae	10374
Rhopapillomavirus	936057	Papillomaviridae	151340
Rio Bravo virus group	29262	*Flavivirus*	11051
Rosavirus	1511804	Picornaviridae	12058
Roseolovirus	40272	Betaherpesvirinae	10357
Rotavirus	10912	Sedoreoviridae	689832
Rubivirus	11040	Togaviridae	11018
Rubulavirus	39744	Paramyxovirinae	11159
Salivirus	688449	Picornaviridae	12058
Salmonivirus	692607	Alloherpesviridae	548682
Sapelovirus	686982	Picornaviridae	12058
Sapovirus	95341	Caliciviridae	11974
Scutavirus	1232637	Alphaherpesvirinae	10293
Seaborne tick-borne virus group	29264	*Flavivirus*	11051
Seadornavirus	208294	Sedoreovirinae	689832
Sedoreovirinae	689832	Reoviridae	10880
Senecavirus	586425	Picornaviridae	12058
Siadenovirus	129876	Adenoviridae	10508
Sigmapapillomavirus	935635	Papillomaviridae	151340
Sigmavirus	1308858	Rhabdoviridae	11270
Simplexvirus	10294	Alphaherpesvirinae	10293
Spinareovirinae	689831	Reoviridae	10880
Sprivivirus	1513299	Rhabdoviridae	11270
Spumaretrovirinae	327046	Retroviridae	11632
Spumavirus	11640	Spumaretrovirinae	327046
Suipoxvirus	10275	Chordopoxvirinae	10241
Taupapillomavirus	934799	Papillomaviridae	151340
Teschovirus	118139	Picornaviridae	12058
Tetraparvovirus	1511911	Parvovirinae	40119
Thetapapillomavirus	334213	Papillomaviridae	151340
Thetatorquevirus	687338	Anelloviridae	687329
Thogotovirus	35323	Orthomyxoviridae	11308
Tibrovirus	1299306	Rhabdoviridae	11270

Virus Taxa Selected for VirCapSeq-VERT Probe Design

Name	tax_id	ParentName	parent tax_id
tick-borne encephalitis virus group	29263	*Flavivirus*	11051
Togaviridae	11018	ssRNA positive-strand viruses, no DNA stage	35278
Torovirinae	694017	Coronaviridae	11118
Torovirus	11155	Torovirinae	694017
Tremovirus	689759	Picornaviridae	12058
Tupavirus	1513300	Rhabdoviridae	11270
Upsilonpapillomavirus	936058	Papillomaviridae	151340
Varicellovirus	10319	Alphaherpesvirinae	10293
Vesiculovirus	11271	Rhabdoviridae	11270
Vesivirus	95337	Caliciviridae	11974
Yatapoxvirus	10282	Chordopoxvirinae	10241
Yellow fever virus group	40005	*Flavivirus*	11051
Zetapapillomavirus	333918	Papillomaviridae	151340
Zetatorquevirus	687336	Anelloviridae	687329

- >207 Taxa
- Coding sequences
- Homology grouping >85-96%
- 50-120nt Probe lenght (70nt-79nt)
- 700.000 Hibridization Probes

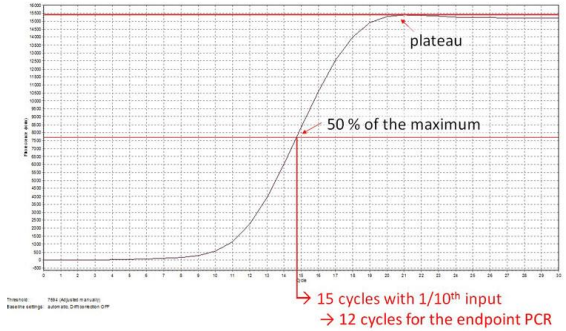
Broad Capture System (VirCapSeq-VERT)



LABORATORY WORKFLOW

Non – Target NGS

Paul-Ehrlich-Institut



Pretreatment

Depletion

Determine the optimum # cycles

Pre-amplification

Barcoding PCR

1. NA Extraction

2. Library Prep

cDNA synthesis

RNA

cDNA

2nd strand synthesis

DNA

2nd strand synthesis

Amplification

Double stranded DNA/cDNA

Fragmentation

Amplified DNA/cDNA

End repair dA tailing

Fragmented NA

Ligation

End repaired A-tailed NA

Amplification + Barcoding

Library

Quality control Normalization

Barcoded Amplified Library

Sequencing

Pooled libraries

Raw reads

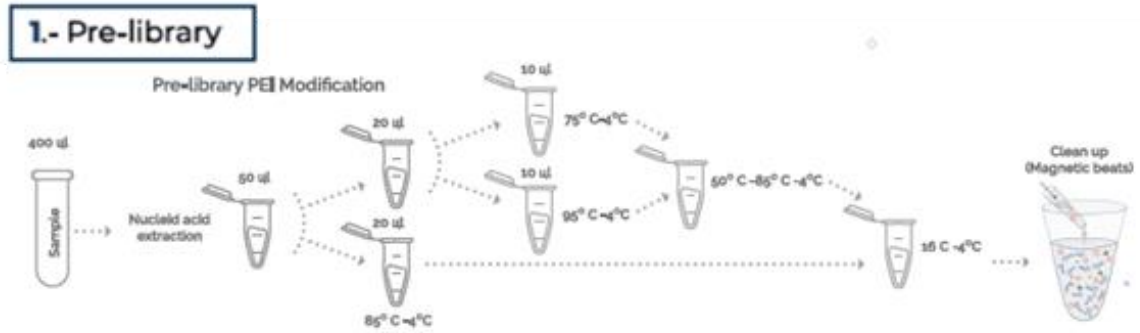
Minimum input: 0.5 ng

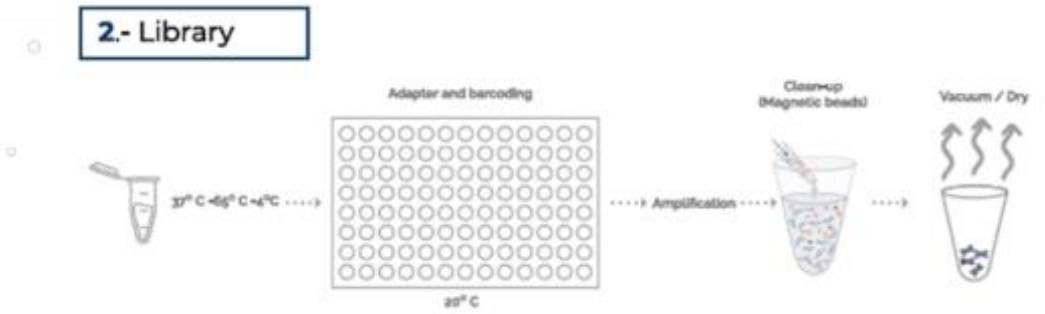
NEBNext® Ultra™ II DNA Library Prep Kit

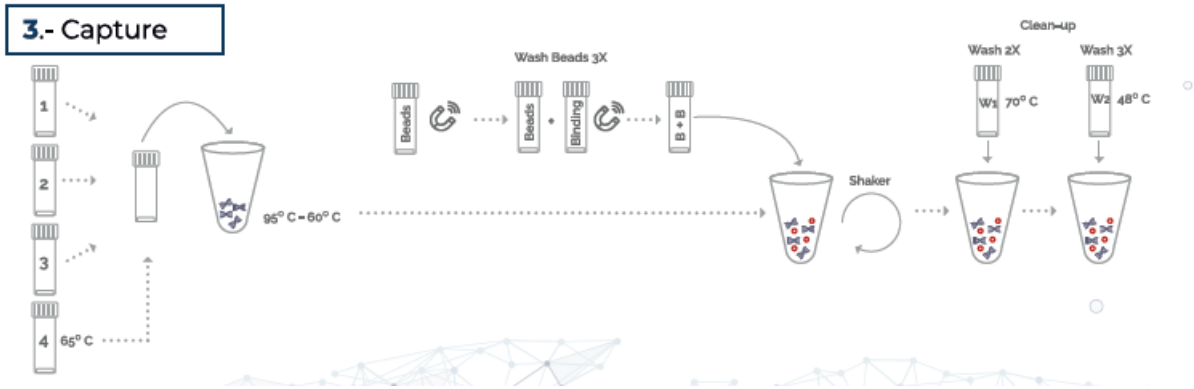
QC and Pooling

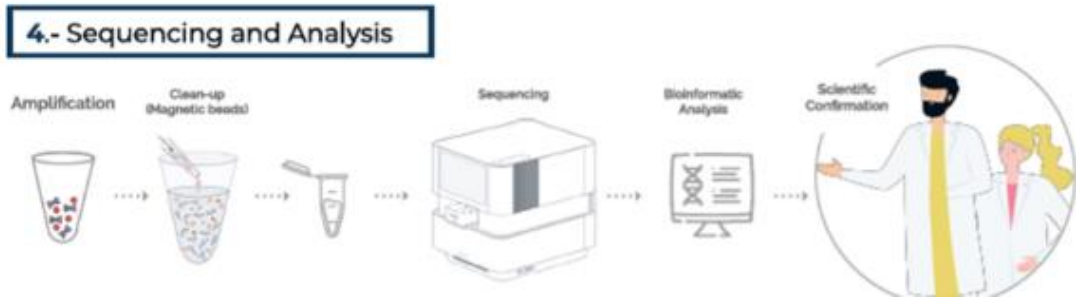
No QC

Target NGS


1.- Pre-library
Pre-library PEI Modification
400 ul
Sample
Nucleid acid extraction
50 ul
20 ul
20 ul
10 ul
10 ul
75° C-4°C
95° C-4°C
85° C-4°C
50° C-85° C-4°C
16 C-4°C
Clean up (Magnetic beats)


2.- Library
37° C-65° C-4°C
Adaptar and barcoding
20° C
Amplification
Clean-up (Magnetic beads)
Vacuum / Dry


3.- Capture
1
2
3
4
65° C
95° C - 60° C
Wash Beads 3X
Beads
Beads
Binding
B + B
Shaker
Clean-up
Wash 2X
Wash 3X
W1 70° C
W2 48° C


4.- Sequencing and Analysis
Amplification
Clean-up (Magnetic beads)
Sequencing
Bioinformatic Analysis
Scientific Confirmation

Multiplexing

2-Plex **12-Plex** **24-Plex**

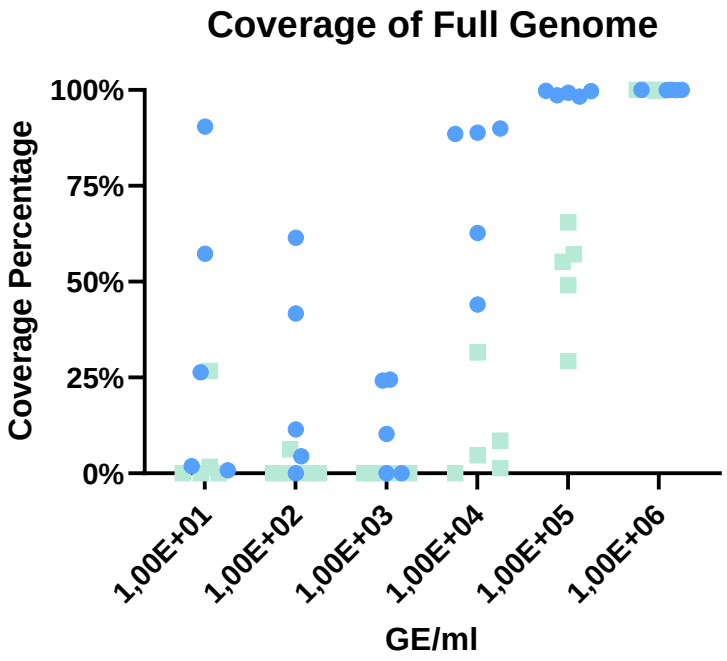
	Virus length	Total HIT length			% Coverage			# Reads aligned		
Human beta-herpesvirus 6B	162.114	16.699	14.922	11.420	10,30%	9,20%	7,04%	3.254	864	282
Human pegivirus	9.367	9.210	9.173	9.122	98,32%	97,76%	97,38%	1.900.941	454.492	139.177
SEN virus	3.087	2.795	2.530	2.177	90,54%	81,96%	70,52%	3.185	681	181
Human endogenous retrovirus K	9.234	1.625	628	409	17,60%	6,80%	4,43%	53	11	5
Adeno-associated virus	4686	1385	1393	1194	29,56%	29,73%	25,48%	1185	315	81

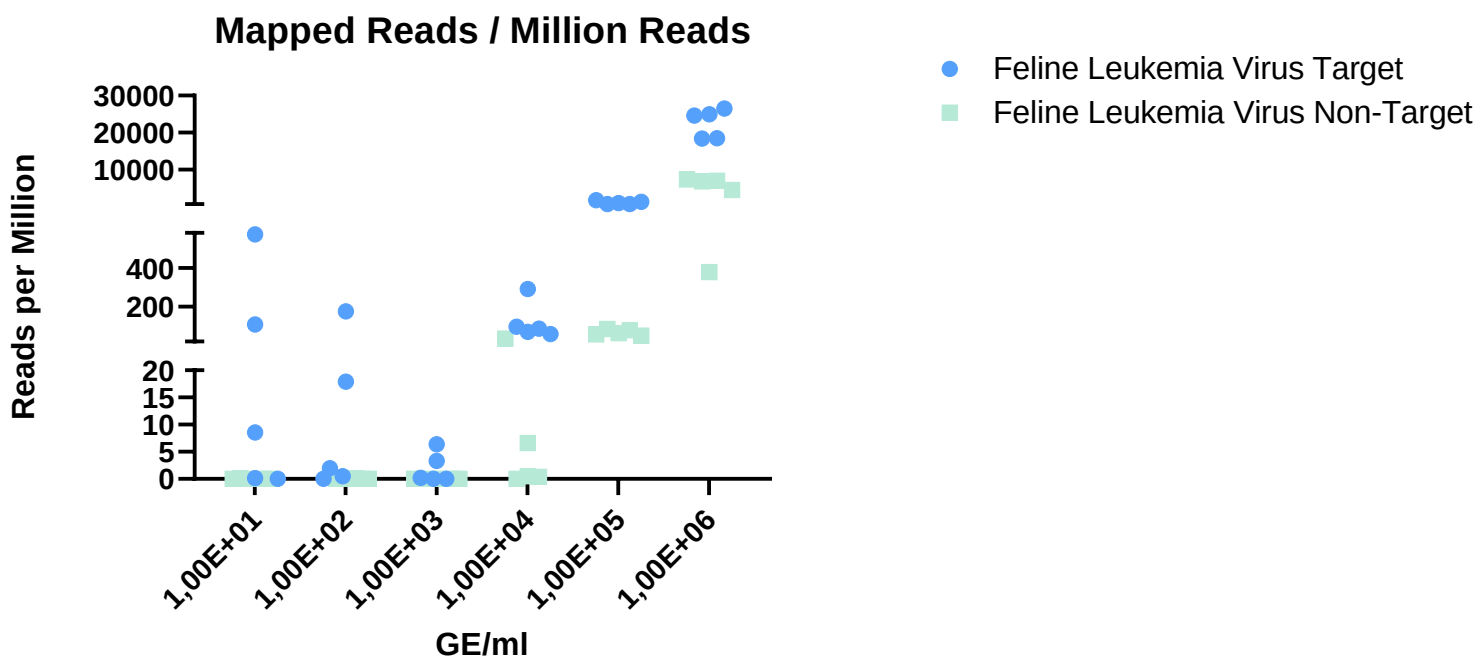
COMPARISON

WHO Panel for LoD comparison

Paul-Ehrlich-Institut

Feline Leukemia Virus												
	10^1 GE/ml		10^2 GE/ml		10^3 GE/ml		10^4 GE/ml		10^5 GE/ml		10^6 GE/ml	
	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target
Replicate 1												
Replicate 2												
Replicate 3												
Replicate 4												
Replicate 5												

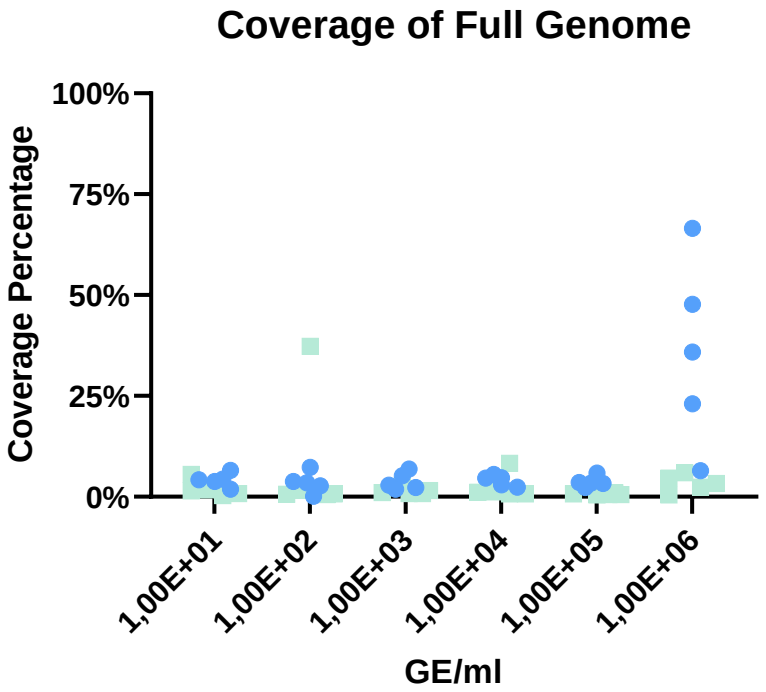


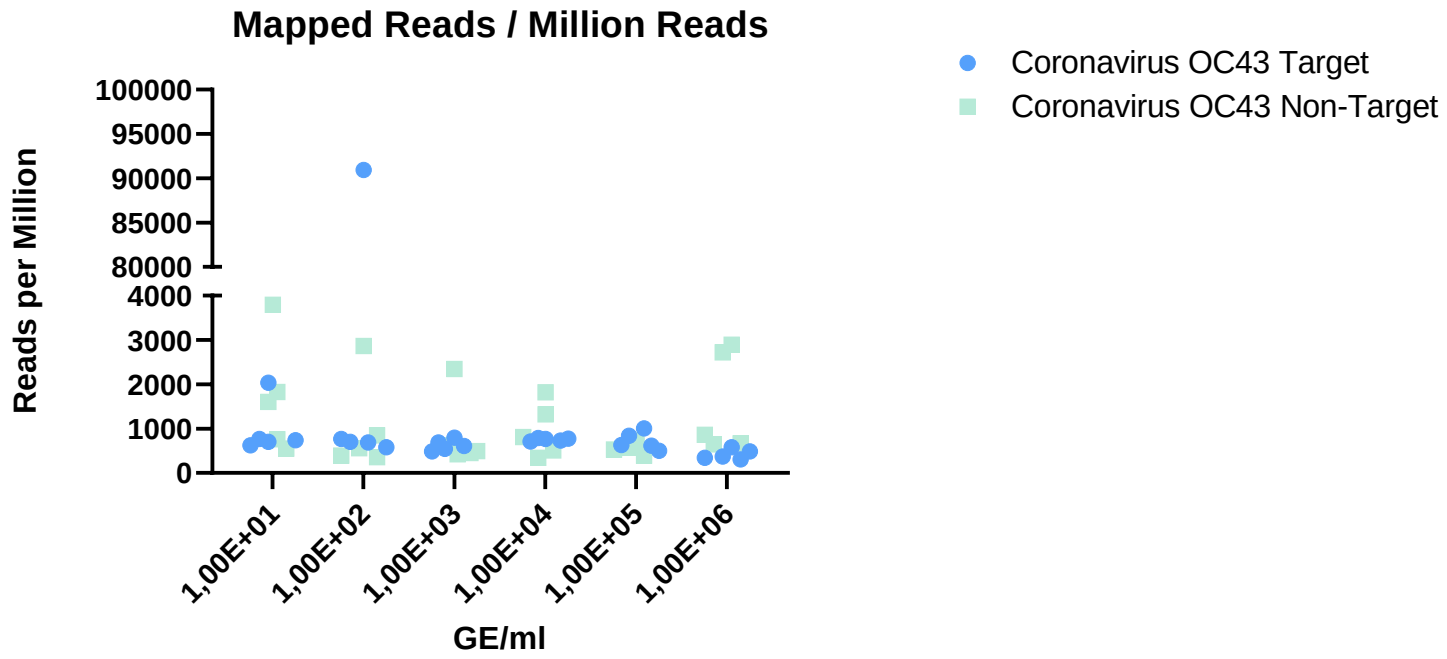


WHO Panel for LoD comparison

Paul-Ehrlich-Institut

Human Coronavirus OC43												
	10^1 GE/ml		10^2 GE/ml		10^3 GE/ml		10^4 GE/ml		10^5 GE/ml		10^6 GE/ml	
	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target
Replicate 1												
Replicate 2												
Replicate 3												
Replicate 4												
Replicate 5												

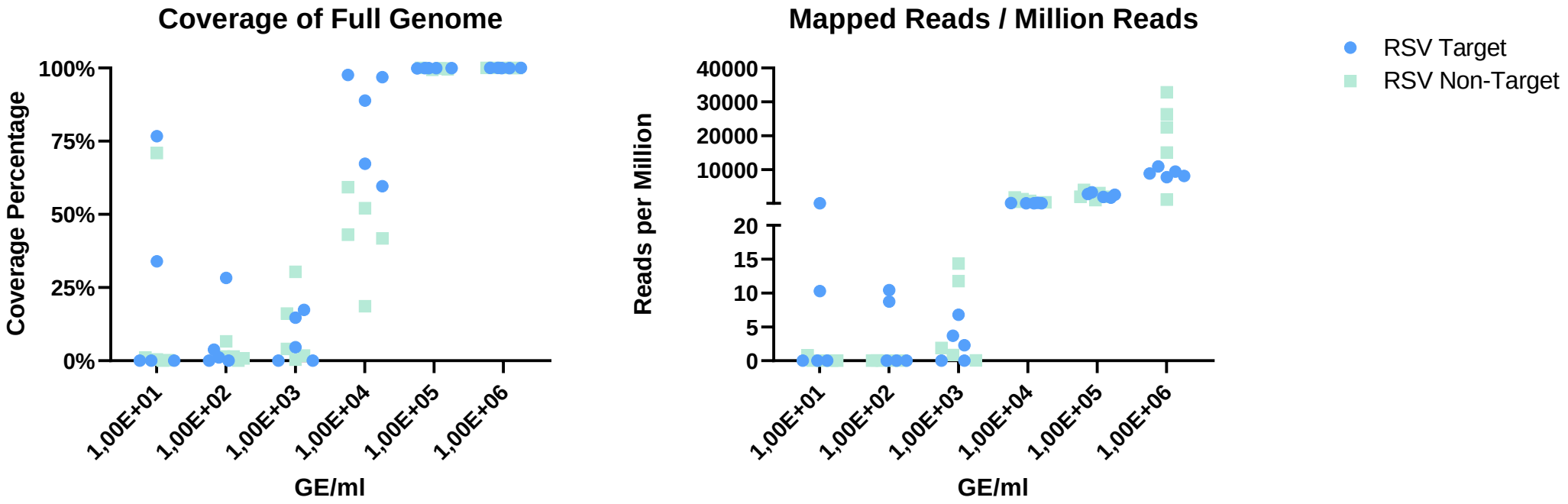




WHO Panel for LoD comparison

Paul-Ehrlich-Institut

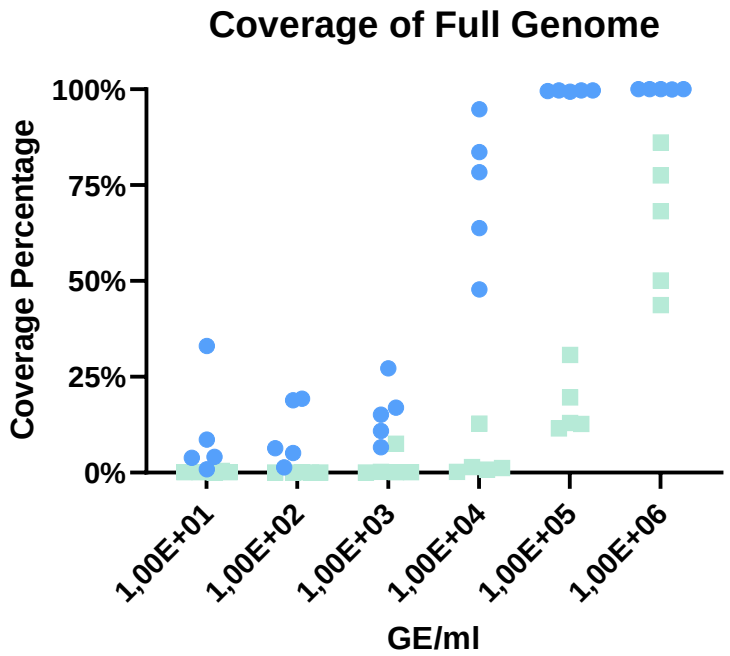
	RSV											
	10^1 GE/ml		10^2 GE/ml		10^3 GE/ml		10^4 GE/ml		10^5 GE/ml		10^6 GE/ml	
	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target
Replicate 1												
Replicate 2												
Replicate 3												
Replicate 4												
Replicate 5												

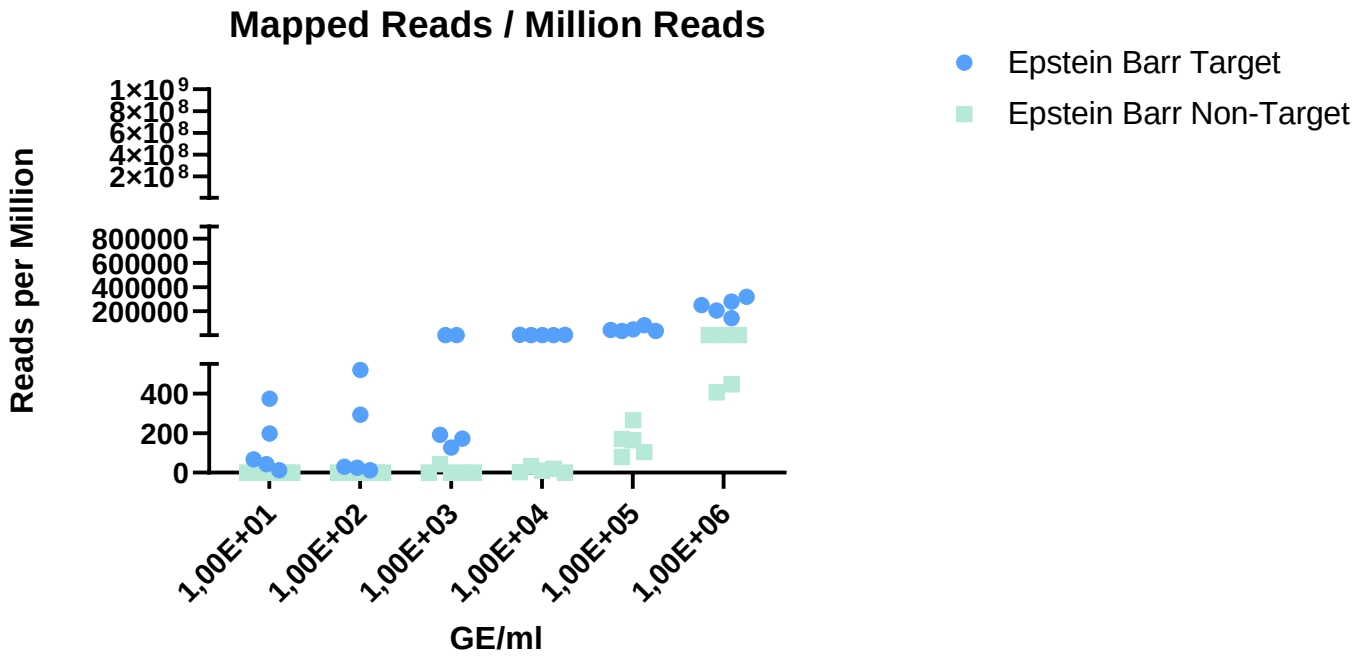


WHO Panel for LoD comparison

Paul-Ehrlich-Institut

Epstein Barr Virus												
	10^1 GE/ml		10^2 GE/ml		10^3 GE/ml		10^4 GE/ml		10^5 GE/ml		10^6 GE/ml	
	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target
Replicate 1												
Replicate 2												
Replicate 3												
Replicate 4												
Replicate 5												

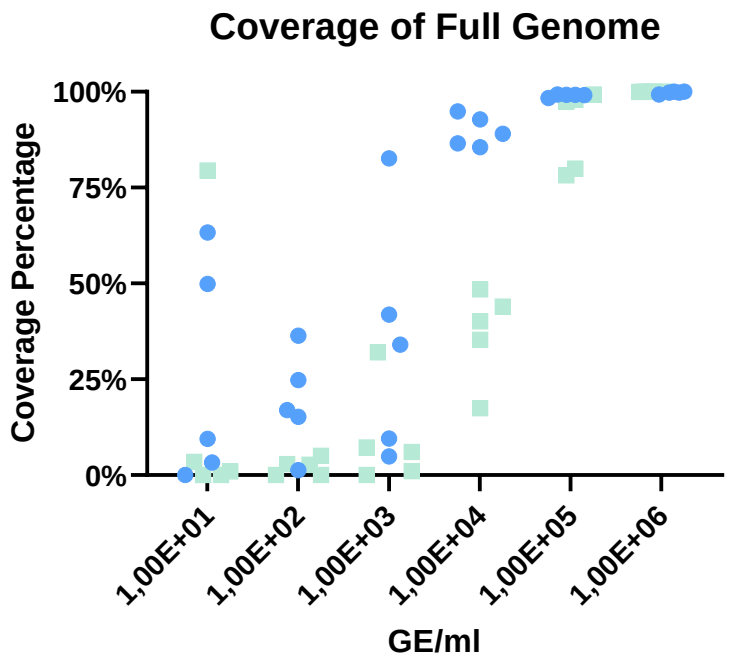


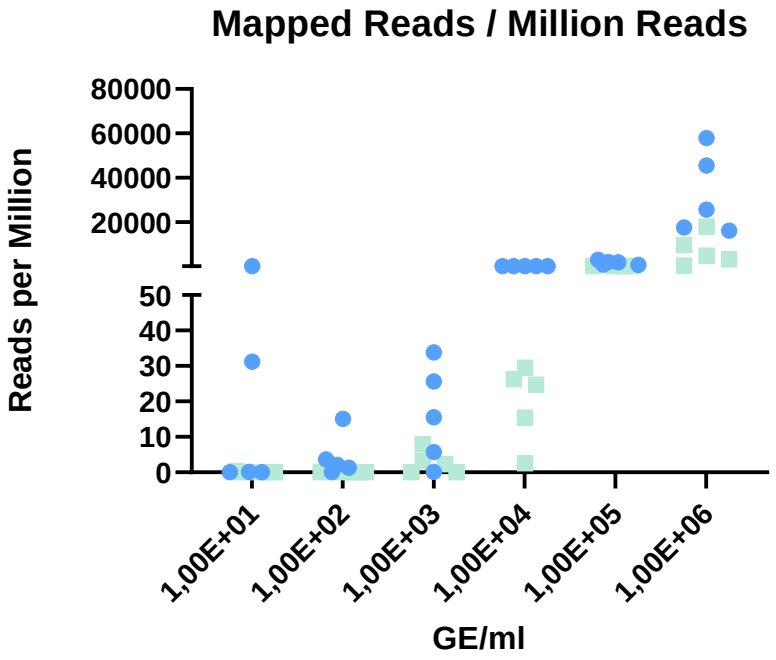


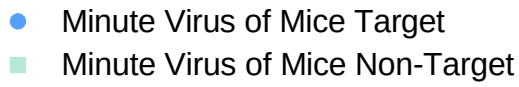
WHO Panel for LoD comparison

Paul-Ehrlich-Institut

Minute Virus of Mice												
	10^1 GE/ml		10^2 GE/ml		10^3 GE/ml		10^4 GE/ml		10^5 GE/ml		10^6 GE/ml	
	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target
Replicate 1												
Replicate 2												
Replicate 3												
Replicate 4												
Replicate 5												



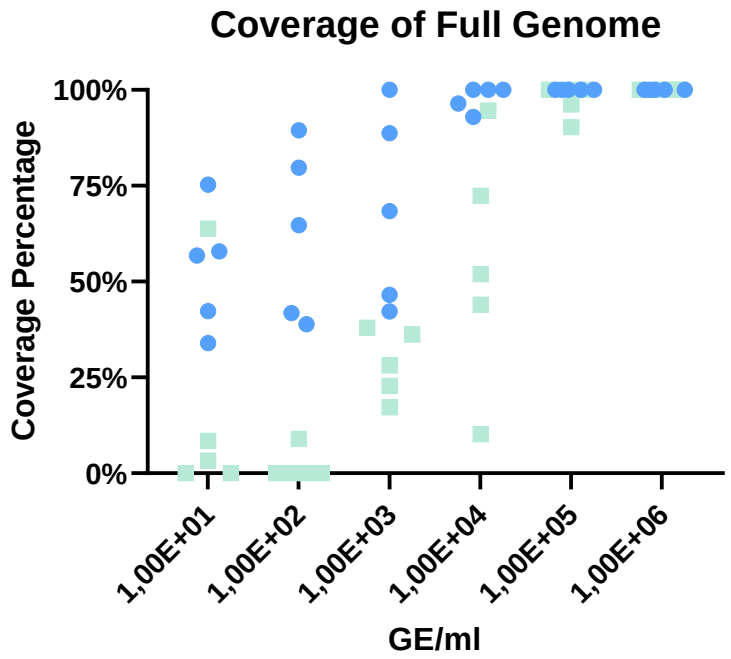


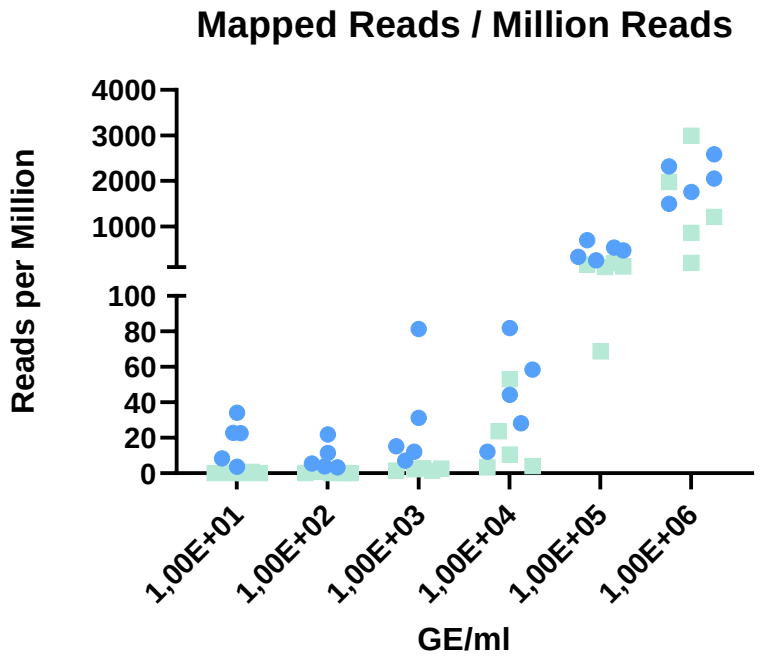


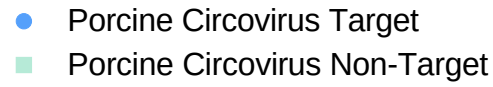
WHO Panel for LoD comparison

Paul-Ehrlich-Institut

Porcine Circovirus												
	10^1 GE/ml		10^2 GE/ml		10^3 GE/ml		10^4 GE/ml		10^5 GE/ml		10^6 GE/ml	
	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target
Replicate 1												
Replicate 2												
Replicate 3												
Replicate 4												
Replicate 5												



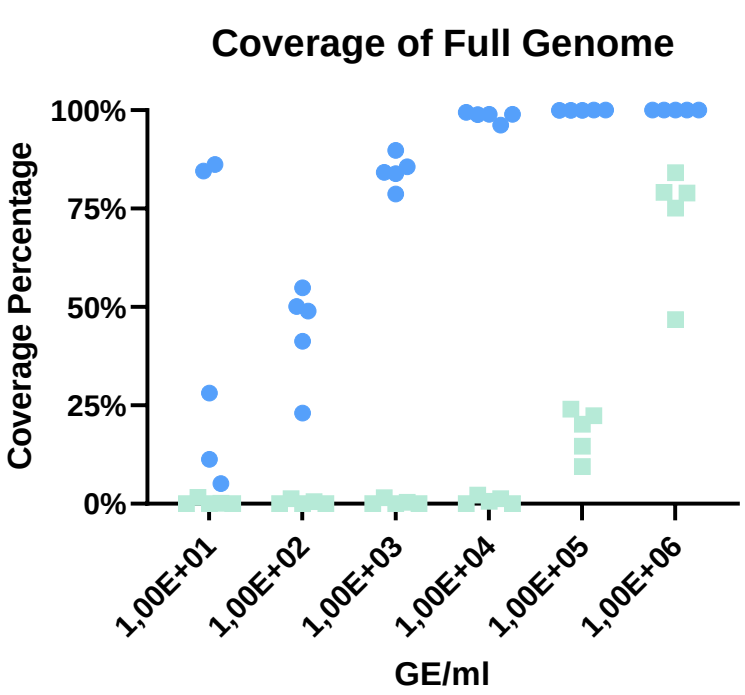


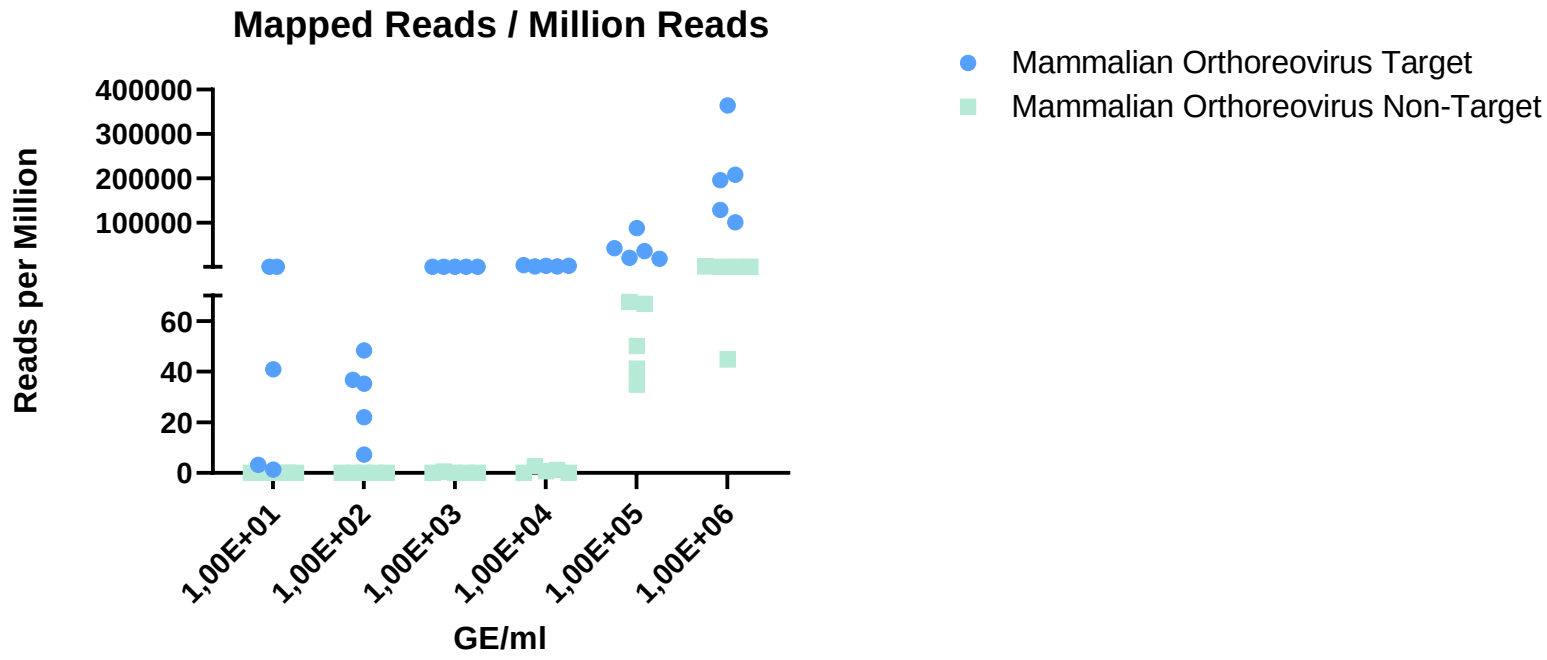


WHO Panel for LoD comparison

Paul-Ehrlich-Institut

Mammalian Orthoreovirus												
	10^1 GE/ml		10^2 GE/ml		10^3 GE/ml		10^4 GE/ml		10^5 GE/ml		10^6 GE/ml	
	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target	Target	Non-Target
Replicate 1												
Replicate 2												
Replicate 3												
Replicate 4												
Replicate 5												





Big In-house Panel for breadth of detection comparison

Paul-Ehrlich-Institut

21-Virus Panel									
#	Virus	Family	Genus	Nucleic Acid	Segmented	Baltimore	Enveloped	Target	Non-Target
1	Hepatitis C genotype 1	Flaviviridae	Hepacivirus	ssRNA+	No	IV	Yes		
2	Usutu virus	Flaviviridae	Orthoflavivirus	ssRNA+	No	IV	Yes		
3	Zika virus; PF13/251013-18	Flaviviridae	Orthoflavivirus	ssRNA+	No	IV	Yes		
4	West Nile Virus	Flaviviridae	Orthoflavivirus	ssRNA+	No	IV	Yes		
5	Bovine viral diarrhea virus	Flaviviridae	Pestivirus	ssRNA +	No	IV	Yes		
6	Hepatitis B virus	Hepadnaviridae	Orthohepadnavirus	dsDNA-RT	No	VIII	Yes		
7	Hepatitis E virus	Hepeviridae	Orthohepevirus	ssRNA+	No	IV	No		
8	Herpes simplex virus type 1	Herpesviridae	Simplexvirus	dsDNA	No	I	Yes		
9	Suid herpesvirus 1 strain Kaplan	Herpesviridae	Varicellovirus	dsDNA	No	I	Yes		
10	Influenza A virus A/PR/8/34 (H1N1)	Orthomyxoviridae	Orthomyxovirus	ssRNA -	Yes	V	Yes		
11	Bovine parvovirus 1	Parvovirinae	Bocaparvovirus	ssDNA-	No	II	No		
12	Porcine parvovirus	Parvovirinae	Bocaparvovirus	ssDNA-	No	II	No		
13	Schmallenberg virus	Peribunyaviridae	Orthobunyavirus	ssRNA-	Yes	V	Yes		
14	Murine encephalomyelitis virus	Picornaviridae	Cardiovirus	ssRNA +	No	IV	No		
15	Bovine enterovirus 2	Picornaviridae	Enterovirus	ssRNA +	No	IV	No		
16	Human poliovirus strain Sabin 1	Picornaviridae	Enterovirus	ssRNA +	No	IV	No		
17	Hepatitis A virus	Picornaviridae	Hepatovirus	ssRNA +	No	IV	No		
18	Simian virus 40	Polyomaviridae	Polyomavirus	cdsDNA	No	I	No		
19	Vesicular stomatitis virus	Rhabdovirus	Vesiculovirus	ssRNA-	No	V	Yes		
20	Semliki forest virus	Togaviridae	Alphavirus	ssRNA+	No	IV	Yes		
21	Chikungunya	Togaviridae	Alphavirus	ssRNA+	No	IV	Yes		
22	Parainfluenza 5 virus	Paramyxoviridae	Orthorubulavirus	ssRNA-	No	V	Yes		
23	Human Pegivirus	Flaviviridae	Pegivirus	ssRNA+	No	IV	Yes		

Worflow Summary and Cost Comparison

Paul-Ehrlich-Institut

Process steps	VirCapSeq-VERT	Standard Metagenomic
Nuclease sample pre-treatment	No	Yes
Elution	55ul	
Pre-amplification	No	
Denature temperature	Multiple	One
Retrotranscription	2 hours	1 hour
Second strand synthesis	Nick translation based	
Library preparation Kit	Library Preparation EF 2.0 with Enzymatic Fragmentation and Twist Universal Adapter System	NEBNext Ultra II FS DNA Library Prep Kit for Illumina
PCR amplification	11 Cycles	Variable
Hybridization/capture	Yes	No
second PCR amplification	Yes	No
Sequencing equipment	Illumina Nextseq 2000	
Sequencing kit	P3	P3
Sequence depth/sample	17 million reads	100 million reads

	VirCapSeq-VERT	Standard-Metagnomik
Extraktion	5	5
Wirts-DNA Reduktion	0	20
cDNA-Synthese	15	10
2-Strand DNA-Synthese	13	13
Library Präparation	76	64
Hybridizierung (VirCapSeq-VERT)	100	0
Sequenzierung (FLowCell P3)	100 / 76	600 / 457
Analyse	50	80
Total pro Probe	**359** / 335	**792** / 649

433(54%) – 314(48%)

4.427(92%)

REAL SAMPLE TEST

Test (Challenge) our implemented system with real blood (serum) samples

Mexican samples

Batch 1: Oct 2023-February 2024

- Mexico City
- Puebla
- Cancun
- Durango

Batch 2: March 2024 – October 2024 (375 – 17

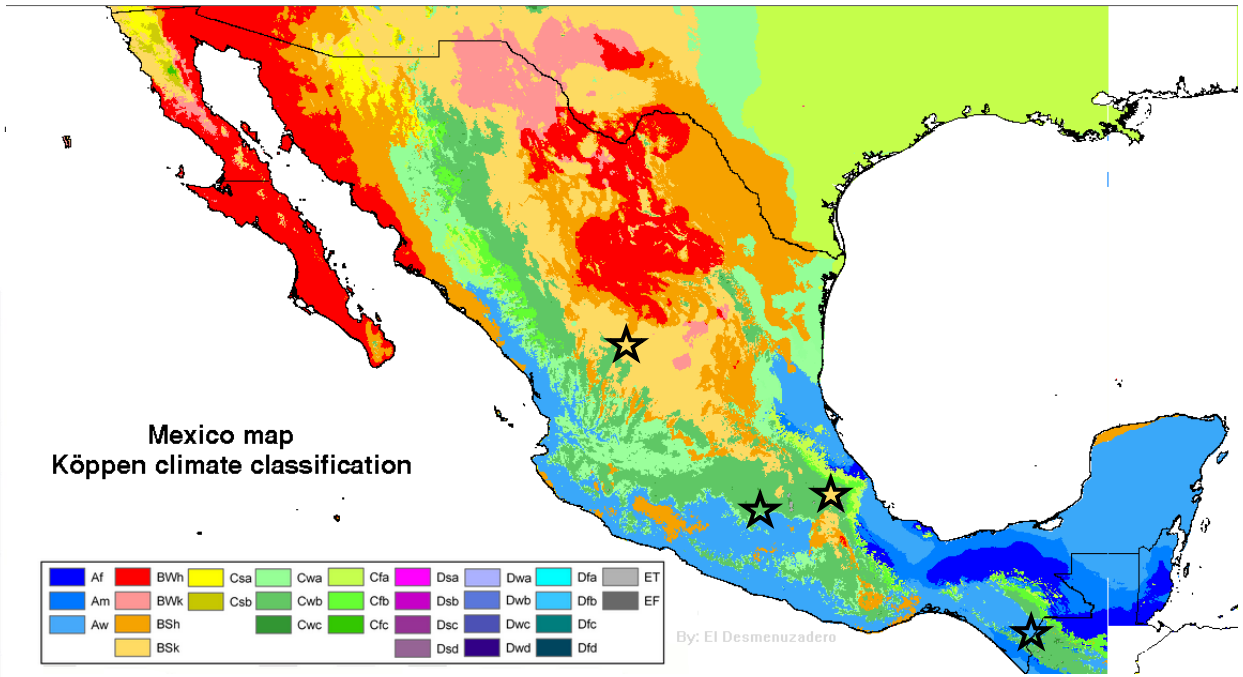
- Mexico City
- Puebla
- Cancun
- Durango
- Chiapas
- Baja California

895 Pools = 89.500 Donors

	Pools (100)		Metapools (1.000)		
	Acepted Donors	Rejected Donors	Acepted Donors	Rejected Donors	Total
Mexico City	240	158	25	16	439
Durango	3	0	2	0	5
Cancun	19	3	3	1	26
Puebla	5	0	1	0	6
Total	267	161	31	17	476

Köppen climate classification scheme symbols description table[8][7][10]

1st	2nd	3rd
A (Tropical)	f (Rainforest) m (Monsoon) w (Savanna, dry winter) s (Savanna, dry summer)	
B (Dry)	W (Arid desert) S (Semi-arid steppe)	h (Hot) k (Cold)
C (Temperate)	w (Dry winter) f (No dry season) s (Dry summer)	a (Hot summer) b (Warm summer) c (Cold summer)
D (Continental)	w (Dry winter) f (No dry season) s (Dry summer)	a (Hot summer) b (Warm summer) c (Cold summer) d (Very cold winter)
E (Polar)	T (Tundra) F (Ice cap)	



Sample: 2NR_Chiapas_S9

The following table lists the detected virus candidates. Taxa selected by the pipeline *PASSED* reclassification as well as filtering.

TaxID	Taxon name	Read number	Reclassification	Filtering
3052610	*Pegivirus platyrrhini*	1,670,224	PASSED	PASSED
3052608	*Pegivirus hominis*	1,044,266	PASSED	PASSED
2793982	*Chimpanzee GB virus C*	4,318	PASSED	PASSED
3052464	*Orthoflavivirus denguei*	4,085	PASSED	PASSED
2993523	*nabpantry virus 9*	1,169	PASSED	PASSED
2055263	*Anelloviridae sp.*	406	PASSED	PASSED
3050298	*Roseolovirus humanbeta7*	145	PASSED	PASSED
3050299	*Lymphocryptovirus humangamma4*	123	PASSED	PASSED
68887	*Torque teno virus*	38	PASSED	PASSED
67082	*BeAn 58058 virus*	14	PASSED	PASSED
3047840	Okavirus branchiae	156	PASSED	FAILED
3048415	Alphatorquevirus homin13	64	PASSED	FAILED
2809145	Alphatorquevirus sp.	33	PASSED	FAILED
432261	Torque teno midi virus	11	PASSED	FAILED
3048459	Orthoflavivirus zikaense	8	PASSED	FAILED
3050295	Cytomegalovirus humanbeta5	6	PASSED	FAILED
3052606	Pegivirus columbiaense	3	PASSED	FAILED
2993529	ticpantry virus 5	4,063	FAILED	FAILED
3052631	Phasivirus phasiense	1,871	FAILED	FAILED
136966	SEN virus	207	FAILED	FAILED
3048428	Alphatorquevirus homin3	91	FAILED	FAILED
3048419	Alphatorquevirus homin18	70	FAILED	FAILED

dengue virus type 1 (NC_001477.1, TaxID 11053, 1-level-up TaxID 12637, 2-levels-up TaxID 3052464) (10.7Kbp)

```
>  90.00% |.: : ::. . ::..: ::::::::: : :  :: :    | Number of reads: 5937
>  80.00% |:: :.::: ::::::: ::::::::: : :  ::.: :   |     (40 filtered)
>  70.00% |:: ::::: ::::::::::::::::: :.:. :::: :   | Covered bases:   9.2Kbp
>  60.00% |:::::::: ::::::::::::::::::::.:::: :     | Percent covered: 85.39%
>  50.00% |:::::::: ::::::::::::::::::::::::: :     | Mean coverage:   70.8x
>  40.00% |:::::::: ::::::::::::::::::::::::: :     | Mean baseQ:      39.2
>  30.00% |:::::::: ::::::::::::::::::::::::: :     | Mean mapQ:       59.7
>  20.00% |:::::::: ::::::::::::::::::::::::: ::
>  10.00% |:::::::::::::::::::::::::::::::::::.::   | Histo bin width: 268bp
>   0.00% |:::::::::::::::::::::::::::::::::::::   | Histo max bin:   100%
          1        2.7K      5.4K      8.0K      10.7K
```

Copy consensus sequence

Human hepegivirus (NC_038436.1, TaxID 1704090, 1-level-up TaxID 3052606, 2-levels-up TaxID 1307799) (9.5Kbp)

```
>  12.10% |                    :                   | Number of reads: 14
>  10.76% |                    :                   |
>   9.41% |                    :                   | Covered bases:   32bp
>   8.07% |                    :                   | Percent covered: 0.3355%
>   6.72% |                    :                   | Mean coverage:   0.0295x
>   5.38% |                    :                   | Mean baseQ:      36.7
>   4.03% |                    :                   | Mean mapQ:       1.5
>   2.69% |                    :                   |
>   1.34% |                    :                   | Histo bin width: 238bp
>   0.00% |                    :                   | Histo max bin:   13.445%
          1        2.4K      4.8K      7.1K       9.5K
```

Copy consensus sequence

GB virus C (NC_001710.1, TaxID 54290, 1-level-up TaxID 3052608, 2-levels-up TaxID 1307799) (9.4Kbp)

```
>  90.00% |.:::::::::::::::::::::::::::::::::::::: | Number of reads: 1138997
>  80.00% |:::::::::::::::::::::::::::::::::::::::|     (13107 filtered)
>  70.00% |:::::::::::::::::::::::::::::::::::::::| Covered bases:   9.3Kbp
>  60.00% |:::::::::::::::::::::::::::::::::::::::| Percent covered: 99.32%
>  50.00% |:::::::::::::::::::::::::::::::::::::::| Mean coverage:   1.42e+04x
>  40.00% |:::::::::::::::::::::::::::::::::::::::| Mean baseQ:      39.1
>  30.00% |:::::::::::::::::::::::::::::::::::::::| Mean mapQ:       48.8
>  20.00% |:::::::::::::::::::::::::::::::::::::::
>  10.00% |:::::::::::::::::::::::::::::::::::::::| Histo bin width: 234bp
>   0.00% |:::::::::::::::::::::::::::::::::::::::| Histo max bin:   100%
          1        2.3K      4.7K      7.0K       9.4K
```

Copy consensus sequence

Query ID	lcl|Query_1134533
Description	2NR_Chiapas_S9 NC_001477.1 dengue virus type 1 taxid 1 ...
Molecule type	dna
Query Length	10259
Other reports	Distance tree of results MSA viewer

Percent Identity [ ] to [ ] E value [ ] to [ ] Query Coverage [ ] to [ ] Filter Reset

Descriptions | **Graphic Summary** | Alignments | Taxonomy

hover to see the title *click to show alignments*

Alignment Scores < 40 | 40 - 50 | 50 - 80 | 80 - 200 | >= 200

100 sequences selected

Distribution of the top 1192 Blast Hits on 100 subject sequences

Description 1NR_G4_H15Chiapas_S1 NC_001477.1 dengue virus type ...

Molecule type

Query Length

Other reports

1NR_G4_H15Chiapas_S1 NC_001477.1 dengue virus type 1
taxid 11053

Percent Identity to

E value to

Query Coverage to

Filter Reset

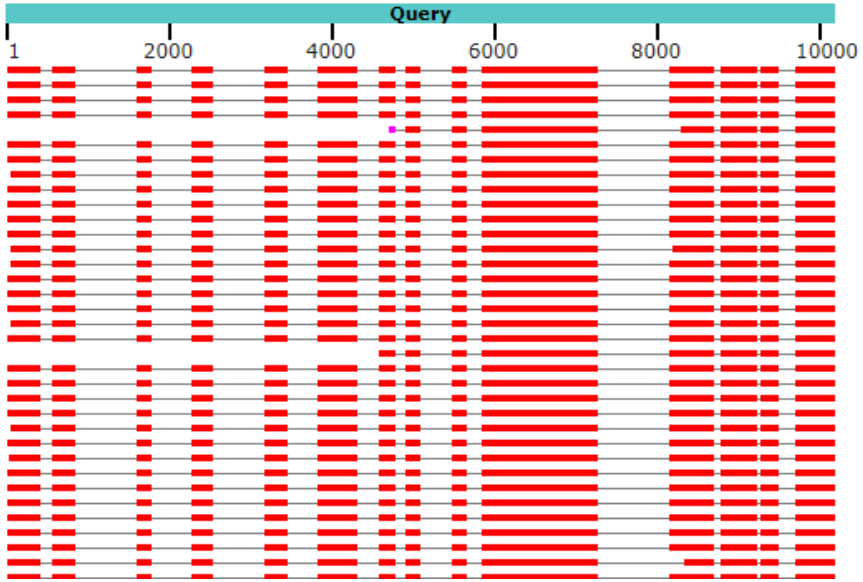
Descriptions | **Graphic Summary** | Alignments | Taxonomy

hover to see the title *click to show alignments*

Alignment Scores < 40 | 40 - 50 | 50 - 80 | 80 - 200 | >= 200

100 sequences selected

Distribution of the top 1366 Blast Hits on 100 subject sequences



Molecule type

Query Length

Other reports

28NR_Cancun_S5 KY315532.2 Human betaherpesvirus 6 taxid 10368

to to to

Filter Reset

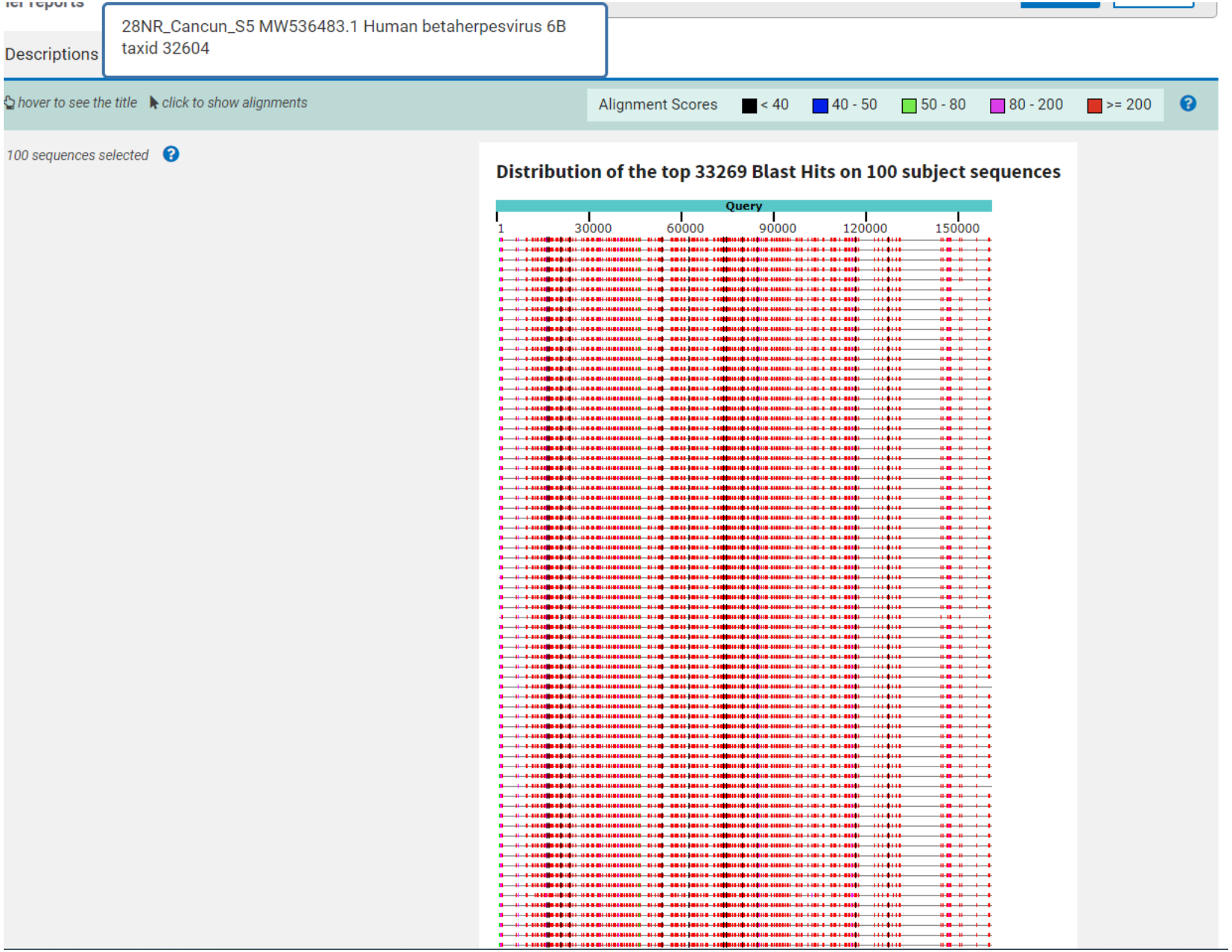
Descriptions | **Graphic Summary** | Alignments | Taxonomy

hover to see the title *click to show alignments*

Alignment Scores < 40 | 40 - 50 | 50 - 80 | 80 - 200 | >= 200

100 sequences selected

Distribution of the top 37131 Blast Hits on 100 subject sequences


28NR_Cancun_S5 MW536483.1 Human betaherpesvirus 6B
taxid 32604
Descriptions
hover to see the title
click to show alignments
Alignment Scores
< 40
40 - 50
50 - 80
80 - 200
>= 200
100 sequences selected
Distribution of the top 33269 Blast Hits on 100 subject sequences
Query
1
30000
60000
90000
120000
150000

Description 28NR_Cancun_S5 MF994817.1 Human betaherpesvirus 6A ...

Molecule type

Query Length

Other reports

28NR_Cancun_S5 MF994817.1 Human betaherpesvirus 6A
taxid 32603

Percent Identity to

E value to

Query Coverage to

Filter Reset

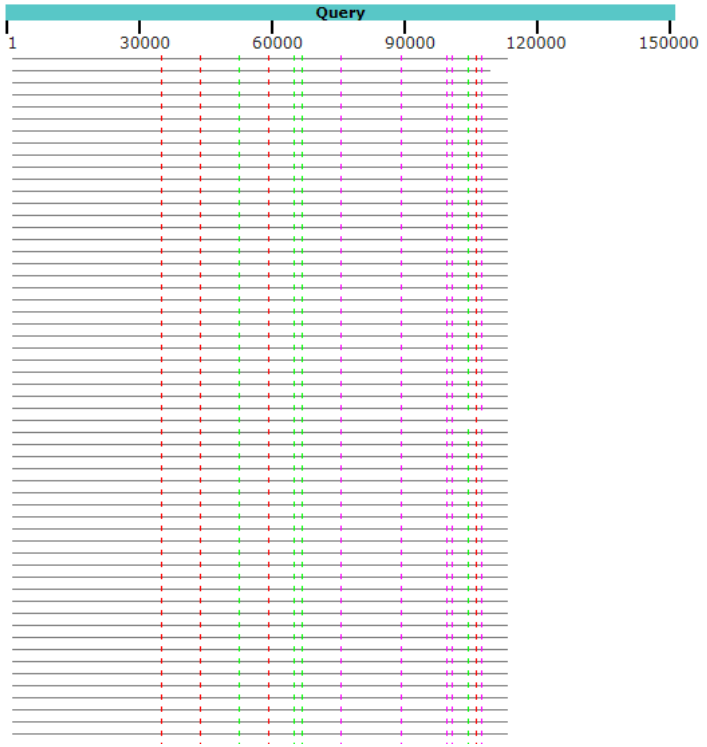
Descriptions | **Graphic Summary** | Alignments | Taxonomy

hover to see the title *click to show alignments*

Alignment Scores < 40 | 40 - 50 | 50 - 80 | 80 - 200 | >= 200

100 sequences selected

Distribution of the top 4090 Blast Hits on 100 subject sequences



Partial Summary

Pools	Origin	Pegivirus	TTV	HHV-6b	HHV-6a	HPV	EBV	HEV	ADV-11	Parvo B19	HHV-8	DNV-1	DNV-2	DNV-3
34	CDMX	31	33	7	1	1	2	2	1	1	1		1	
2	Puebla	2	2	1										1
3	Chiapas	3	3							1		2		
1	Cancun	1	1	1										
40	**Pools**	**37 (92,5%)**	**39 (97,5%)**	**9 (22,5%)**	**1 (2,5%)**	**1 (2,5%)**	**2(5%)**	**2(5%)**	**1 (2,5%)**	**2(5%)**	**1 (2,5%)**	**2(5%)**	**1 (2,5%)**	**1 (2,5%)**
400	**Donors**	**9,25%**	**9,75%**	**2,25%**	**0,25%**	**0,25%**	**0,50%**	**0,50%**	**0,25%**	**0,50%**	**0,25%**	**0,50%**	**0,25%**	**0,25%**

Acknowledgments

Project Leaders
Johannes Blümel
Renate König

Non Target NGS
Csaba Miskey
Dora Spekhardt
Pauline Santos

Target NGS
Janice Brückman

Bionformatics
Markus Braun
Martin Machyna

VirCapSeq-VERT
Thomas Bries
Kenneth Wickiser
Vishal Kappoor
Alexandra Petrosov

THANK YOU

FOR YOUR ATTENTION