The Pan-SL-CoV/GD sequences may be from contamination.

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ABSTRACT

Recently, there were much hype about reports of SARS-like coronaviruses being found in samples of Malayan pangolins (Manis Javanica) collected in Guangdong 2019, which appeared to possess nearly identical RBD's to the SARS-CoV-2 coronavirus. Prominent journals cited these discoveries to claim that pangolins may be a possible intermediate host for the zoonotic transmission of SARS-CoV-2 to humans.

Here, we report that all databases used to support such a claim, upon which metagenomic analysis was possible, contained unexpected reads and was potentially contaminated. Here we also report that the presences of these unexpected reads are directly related to the presence of coronavirus reads. Finally, we deduced the actual causative agent of the death of the pangolins sampled in Guangdong 2019 where the claim of coronavirus detections was made.

METHODS

The NCBI Trace tool

The NCBI SRA archive come with it's own tool called Trace, which identifies the origin or reads within the SRA dataset through the recognition of unique K-mers within the nucleotide sequence. Multiple reads of 32 nucleotides is taken from each read to identify the reads toward an origin by comparison with a large database of reference sequences, which produces a classification signal. Then read of 64 nucleotides are taken from each of the read for definitive mapping toward species in the reference database. If any one of the 32nt or 64nt K-mers are found in more than one reference sequence, the reads are instead classified at the lowest phylogenetic classification node where reference sequences containing such a K-mer is found.

The 32nt TRACE generate a "strong signal" classification of sequence origin useful for the deduction of the content of the sample by organism of origin, accessed via the NCBI Krona charting tool,

While the 64nt TRACE generate a definitive classification signal used for the exact tracing of reads to the origin from a specific Species/Taxon, used for the exact classification of reads.

Both the 32nt and 64nt TRACE analysis classify their reads according to the lowest common taxonomical node where K-mers from said read are present in the reference sequence database, a strategy known as "lowest non-ambiguous mapping". Such a strategy avoids the problem with RNA degradation or sequencing errors by excluding potential errors in reads, without introducing potential ambiguous classification by clustering ambiguous reads under the lowest common

classification node such ambiguity is found.

Therefore, if TRACE gives identification to a specific taxonomical node for a sequence read, it could be from any of the taxonomical nodes and species classified under the node, but it could not be from a taxonomical node or species that is not under said node. E.g. if TRACE says hominoidea which was classified under Catarrhini; Simiiformes; Haplorrhini; Primates; Euarchontoglires, Then it can't be from a pangolin since pangolins (Manis Spp.) are classified under Pholidota; Laurasiatheria. The lowest common classification node between Primates and Pangolins is Boreoeutheria—reads from parts of the genomes shared between Primates and Pangolins will only be classified to Boreoeutheria, but not further classified down toward either Laurasiatheria or Euarchontoglires. And definitely will not be classified individually toward Pholidota or Primates, or any child nodes or phylogenetic nodes under them.

Specific BLAST analysis

Whenever a genus or species is provided by analysis, a specific BLAST analysis is performed to confirm the presence of reads toward the exact species by a search of the database in question with representative reference sequences of the specific species in question in look for matches that is either: 100% match, or: contained no 100% matches on BLAST when queried against the Pangolin reference sequences available on GanBank.

RESULTS

The Accession numbers and contents of all Pan-SL-CoV/GD related sequencing experiments are listed under the following table.

Table 1: List of available GD Pangolin sample datasets as provided in the NCBI SRA. By Accession number, size and citation by thesis (if claimed to have SARS-CoV-2 related reads by paper).

Accession number	Size	SARS-CoV-2-like Coronavirus
		Identified and Cited?
SRX6893158	16,491,648	
<u>SRX6893157</u>	9,275,501	Lung12 [3] SRR10168374
<u>SRX6893156</u>	22,220,187	Lung11 [1]
SRX6893155	18,067,615	Lung09 [1] [3] SRR10168376
<u>SRX6893154</u>	16,414,925	Lung08 [1] [3] [4]
		SRR10168377
SRX6893153	19,045,923	Lung07 [1] [3] [4]
		SRR10168378
<u>SRX6893152</u>	13,527,964	
<u>SRX6893151</u>	16,068,654	
<u>SRX6893150</u>	12,967,281	
<u>SRX6893149</u>	12,590,769	
SRX6893148	15,273,939	

SRX6893147	15,975,904	
<u>SRX6893146</u>	19,038,817	
<u>SRX6893145</u>	19,055,973	
<u>SRX6893144</u>	15,350,468	
<u>SRX6893143</u>	11,527,782	
<u>SRX6893142</u>	20,045,443	
<u>SRX6893141</u>	18,903,834	
<u>SRX6893140</u>	19,986,780	
<u>SRX6893139</u>	39,738,679	Lung02 [3] SRR10168392
<u>SRX6893138</u>	22,900,426	
<u>SRX7756769</u>	107,267,359 PRJNA607174**	M1[2]***
<u>SRX7756766</u>	273,651,431 PRJNA607174**	
<u>SRX7756765</u>	196,761,202 PRJNA607174**	
<u>SRX7756764</u>	222,286,763 PRJNA607174**	
<u>SRX7756763</u>	212,161,250 PRJNA607174**	
<u>SRX7756762</u>	232,433,120 PRJNA607174**	M6[2]***
<u>SRX7756761</u>	113,900,941 PRJNA607174**	
<u>SRX7732094</u>	2,633*	"P2S"[3]

*: "Design: This dataset contains coronavirus-like sequence reads, based on BLAST search."

**: All available SRA datasets from PRJNA607174

***:Actual SRA datasets identified from the "Extended Data Table 3" of [2]

Article

Extended Data Table 3 | Identification of SARSr-CoV sequence reads in metagenomes from the lung of pangolins using the SARS-CoV-2 sequence (GenBank accession No. MN908947) as the reference

	bed	No. mapp	Total reads*	Animal species	Sample ID
X7756769 "pangolin 9"	←SR	496	107,267,359	Malayan pangolin	M1
	1	302	38,091,846	Malayan pangolin	M2
		14	79,477,358	Malayan pangolin	мз
lot available		1,100	32,829,850	Malayan pangolin	M4
		56	547,302,862	Malayan pangolin	M5
RX7756762 "pangolin 2"	←SF	10	232,433,120	Malayan pangolin	M6
		12	44,440,374	Malayan pangolin	M8
ot available	No	0	227,801,882	Malayan pangolin	M10
		0	444,573,526	Chinese pangolin	Z1

Fig.1 the "Extended Data Table 3" of [2]. SRA datasets identified in the available database is pointed out by an arrow, while SRA "runs" that failed to be identified in known datasets are outlined in a red square.

Analysis of reads from The Available datasets using NCBI Trace.

Primate-related results n Krona and read size by Kbp N/D Catarrhini 644546	Identification of "Coronaviridae" as by Trace and total read size N/D
oy Kbp I/D	as by Trace and total read size
I/D	total read size
Catarrhini 644546	
	N/D***
Iomo saniens 81948	Pangolin
	coronavirus 2Kbp
Iomininae 3534150	Pangolin
101111111111111111111111111111111111111	coronavirus 5Kbp
Iominoidea 356003	Pangolin
	coronavirus
	154Kbp
Iomo saniens 162180	Pangolin
101110 3001013 102100	coronavirus
	41Kbp
1/D	N/D
170	
I/D	N/D
I/D	N/D
1,0	
imiiformes 313069	N/D
atarrhini 194320	N/D
19 19 20	
Catarrhini 69937	N/D
lominoidea 231755	N/D
	.,_
lomininae 2536765	N/D
	, –
lominoidea 166628	N/D
	, –
I/D	N/D
	,
imiiformes 57084	N/D
	/D miiformes 313069 atarrhini 194320 atarrhini 69937 ominoidea 231755 omininae 2536765 ominoidea 166628 /D

Table 2. The Trace result of Known GD Pangolin datasets when examined using NCBI Trace SRA.

20-Sep-2019			
SRX6893141	Boreoeutheria: 1.41%	N/D	N/D
20-Sep-2019			
SRX6893140	Boreoeutheria: 1.56%	N/D	N/D
20-Sep-2019			
SRX6893139	Homo sapiens: 0.01%	Homo sapiens 491120	Pangolin
20-Sep-2019			coronavirus 2Kbp
SRX6893138	Boreoeutheria: 1.67%	Homininae 2761176	N/D
20-Sep-2019			
<u>SRX7756769</u>	Homo sapiens: 0.03%	Homo sapiens 5457929	Bat SARS-like
18-Feb-2020			coronavirus 2Kbp
			Wuhan seafood
			market
			pneumonia virus
			2Kbp
<u>SRX7756766</u>	Manis javanica: 78.6%	Cercopithecidae 3116	Betacoronavirus
18-Feb-2020			2Kbp**
<u>SRX7756765</u>	Manis javanica: 87.17%	Cercopithecinae 11339	N/D****
18-Feb-2020			
SRX7756764	Manis javanica: 48.39%	Cercopithecidae 22600	N/D
18-Feb-2020			
<u>SRX7756763</u>	Manis javanica: 94.95%	Cercopithecidae 5076	N/D
18-Feb-2020			
<u>SRX7756762</u>	Manis javanica: 95.37%	Catarrhini* 2831	Nidovirales OKbp
18-Feb-2020			
<u>SRX7756761</u>	Manis javanica: 13.63%	Chlorocebus sabaeus	N/D
18-Feb-2020		498506	
SRX7732094	N/A***	N/A	Pangolin
15-Feb-2020			coronavirus***

*: Chlorocebus Sabaeus

**:Not claimed as being SARS-CoV-2 related in the original publication. Likely unrelated.

***Not analyzable. All Non-Coronavirus data filtered out. Leaving only 2,633 reads, all of which can be mapped to the SARS-CoV-2 reference genome.

****8 reads as claimed by [10]

Specific BLAST analysis

In order to determine the authenticity of the Primate-related reads in the datasets, Specific BLAST analysis is carried out for all datasets that possessed claimed or analyzed reads of coronaviridae-related viruses. An 100% full-length match that does not map to non-primates confirms Authenticity of read.

	select all 100 sequences selected					Grap	hics Distance tree of results
	Description			Query Cover	E value	Per. Ident	Accession
	<u>SRX7756762</u>	279	1047	0%	4e-68	100.00%	SRA:SRR11119766.160125840.2
	<u>SRX7756762</u>	279	1366	0%	4e-68	100.00%	SRA:SRR11119766.138036805.1
	<u>SRX7756762</u>	279	967	0%	4e-68	100.00%	SRA:SRR11119766.101239747.1
	<u>SRX7756762</u>	279	1624	0%	4e-68	100.00%	SRA:SRR11119766.46413326.2
Chlo	rocebus sabaeus isolate 1994-021 unplaced genomic sca						
dna							
1339	488						
<u>Dista</u>	nce tree of results MSA viewer 😧						

Fig.2a Specific BLAST analysis on the PRJNA607174 dataset, <u>SRX7756762</u>, that contained claimed SARS-CoV-2 related coronavirus reads. The 100% full-length matches clearly indicate presence of Primate-derived material.

Select all 100 sequences selected		<u>GenB</u>	ank	<u>Grap</u>	<u>hics</u>	Distance	tree of results
Description				Query Cover	E value	Per. Ident	Accession
Macaca mulatta isolate Rh22777_5890-1b major histocompatibility complex genomic sequence		279	279	100%	2e-71	100.00%	KT332833.1
Macaca mulatta isolate Rh22335_5775-3 major histocompatibility complex genomic sequence		279	279	100%	2e-71	100.00%	KT332608.1
Macaca mulatta isolate Rh22335_5725-2 major histocompatibility complex genomic sequence		279	279	100%	2e-71	100.00%	KT332521.1
Macaca mulatta isolate Rh22335_5702-1a major histocompatibility complex genomic sequence		279	279	100%	2e-71	100.00%	KT332463.1
IGATCCTTCAGGAATCGCCATACTCTTTTTCCATAATGGTTGAACTAGTTTACAATCCCAC	230	230	1	00% 2	Pe-56	94.04%	GQ879596.1
	219	219		00% 4			KF661301.1
	219	219					AB553834.1
Mus musculus NOD-derived CD11c +ve dendritic cells cDNA, RIKEN full-length enriched library, clone:F630221F08 productun	204	204	8	16% 1	le-48	94.66%	<u>AK171052.1</u>
Mus musculus bone marrow macrophage cDNA, RIKEN full-length enriched library, clone:G530008A19 product:hypothetical.pj 2	204	204	8	6% 1	le-48	94.66%	<u>AK149653.1</u>
Ralstonia solanacearum genome assembly 9 genomes, chromosome : V	202	202	1	00% 4	le-48	90.73%	LN899823.1
Canis lupus familiaris breed Labrador retriever chromosome 06a	154	3044	g	18%	le-33	86.43%	CP050586.1
Canis lupus familiaris breed Labrador retriever chromosome 04a	154	4569	1	00% 1	le-33	85.23%	CP050572.1
Canis lupus familiaris breed Labrador retriever chromosome 06b	154	3042	g	18% 1	le-33	86.43%	CP050622.1

Fig.2b BLAST result on the returned sequence revealed it as a Primate-derived MHC complex gene that is not found in non-primates, confirming Primate origin.

	select all 100	sequences selected					Grap	hics Distance tree of results
		Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
	SRX7756766		279	279	0%	5e-67	100.00%	SRA:SRR11119762.269072261.2
	SRX7756766		279	279	0%	5e-67	100.00%	SRA:SRR11119762.255768440.2
	SRX7756766		279	279	0%	5e-67	100.00%	SRA:SRR11119762.255768440.1
	SRX7756766		279	279	0%	5e-67	100.00%	SRA:SRR11119762.255318754.2
	SRX7756766		279	279	0%	5e-67	100.00%	SRA:SRR11119762.254520929.1
	SRX7756766		279	6344	0%	5e-67	100.00%	SRA:SRR11119762.251645135.1
	SRX7756766		279	279	0%	5e-67	100.00%	SRA:SRR11119762.234036838.2
	SRX7756766		279	279	0%	5e-67	100.00%	SRA:SRR11119762.211208832.2
	SRX7756766		279	9108	0%	5e-67	100.00%	SRA:SRR11119762.199583624.1
	SRX7756766		279	279	0%	5e-67	100.00%	SRA:SRR11119762.198110623.2
	SRX7756766		279	279	0%	5e-67	100.00%	SRA:SRR11119762.196936636.2
	SRX7756766		279	279	0%	5e-67	100.00%	SRA:SRR11119762.196936636.1
	SRX7756766		279	279	0%	5e-67	100.00%	SRA:SRR11119762.133631622.2
	SRX7756766		279	279	0%	5e-67	100.00%	SRA:SRR11119762.108819247.2
Des	cription	Macaca mulatta isolate AG07107 chromosome 3 genomic sca \dots						
Mol	ecule type	dna						
Que	ery Length	17855752						
Oth	er reports	Distance tree of results MSA viewer						

Fig.3a Specific BLAST analysis of <u>SRX7756766</u> revealed large amount of 100% full-length matches with Macaca Mulatta.

	Macaca mulatta Major Histocompatibility Complex BAC MMU370002, complete sequence	279	279	100%	2e-71	100.00%	AC148706.1
	Macaca mulatta Major Histocompatibility Complex BAC MMU122H23, complete sequence	279	521	100%	2e-71	100.00%	AC148677.1
≤	Macaca mulatta Major Histocompatibility Complex BAC MMU065H09, complete sequence	279	279	100%	2e-71	100.00%	AC148671.1
	Macaca mulatta Major Histocompatibility Complex BAC MMU038L02, complete sequence	279	279	100%	2e-71	100.00%	AC148668.1
≤	Papio anubis clone rp41-22m16, complete sequence	279	554	100%	2e-71	100.00%	AC113268.8
	Papio anubis clone rp41-280n2, complete sequence	279	465	100%	2e-71	100.00%	AC091778.13
≤	Papio anubis clone rp41-5m22, complete sequence	279	279	100%	2e-71	100.00%	AC136143.4
	Papio anubis clone rp41-192i11, complete seguence	279	526	100%	2e-71	100.00%	AC091671.28
≤	Macaca mulatta chromosome 9 CH250-18D2, complete sequence	279	548	100%	2e-71	100.00%	CT573219.3
	Rhesus Macaque CHR4 BAC CH250-23P16 (Children's Hospital Oakland Research Institute Rhesus macaque Adult Male BAC Librar	279	279	100%	2e-71	100.00%	AC169807.2
	Rhesus Macaque CHR4 BAC CH250-476F18 (Children's Hospital Oakland Research Institute Rhesus macaque Adult Male BAC Libra	279	279	100%	2e-71	100.00%	AC171646.5
	Chlorocebus aethiops BAC clone CH252-163P9 from chromosome 5, complete sequence	278	556	99%	7e-71	100.00%	AC239684.4
	MACACA MULATTA BAC clone CH250-192J17 from chromosome unknown, complete sequence	278	552	100%	7e-71	100.00%	AC215693.3
	Macaca mulatta isolate Rh22335_5702-1a major histocompatibility complex genomic sequence	274	274	100%	9e-70	99.34%	KT332463.1
	Macaca mulatta isolate Rh9_6570-3 major histocompatibility complex genomic sequence	274	274	100%	9e-70	99.34%	<u>KT331777.1</u>
≤	Macaca mulatta isolate Rh9_6550-1b major histocompatibility complex genomic sequence	274	274	100%	9e-70	99.34%	KT331733.1
	Macaca mulatta isolate Rh9_6526-2 major histocompatibility complex genomic sequence	274	274	100%	9e-70	99.34%	<u>KT331675.1</u>
✓	Macaca mulatta isolate Rh18665_5547-1b major histocompatibility complex genomic sequence	274	274	100%	9e-70	99.34%	KT329509.1
≤	Macaca mulatta isolate Rh23717 clone 4777 major histocompatibility complex-B genomic sequence	274	274	100%	9e-70	99.34%	KJ913523.1
	Macaca mulatta isolate Rh23108 clone 4769-2 major histocompatibility complex-B genomic sequence	274	274	100%	9e-70	99.34%	<u>KJ913420.1</u>
	Eukaryotic synthetic construct chromosome 18	2	02 41	03 96	% 4e-4	48 91.78%	6 <u>CP034496.</u>
	Eukaryotic synthetic construct chromosome 19	1	96 20	20 96	% 2e-	46 92.09%	CP034522.1
	Eukaryotic synthetic construct chromosome 19	1	96 21	30 96	% 2e-	46 92.09%	6 <u>CP034497.1</u>
	Eukaryotic synthetic construct chromosome 16	1	96 36	54 96	% 2e-	46 91.61%	6 <u>CP034494.1</u>
~	Eukaryotic synthetic construct chromosome 15	1	96 49	94 97	% 2e-	46 92.09%	6 <u>CP034493.1</u>
	Eukaryotic synthetic construct chromosome 14	1	96 77	31 96	% 2e-	46 92.09%	6 <u>CP034492.1</u>
~	Eukaryotic synthetic construct chromosome 13	1	91 79	88 97	% 9e-	45 90.91%	CP034516.1
~	Eukaryotic synthetic construct chromosome Y	1	91 27	81 96	% 9e-	45 91.37%	CP034510.1
	Eukaryotic synthetic construct chromosome 20	1	91 45	17 96	% 9e-	45 91.37%	6 <u>CP034499.1</u>
~	Eukaryotic synthetic construct chromosome 13	1	91 79	88 97	% 9e-	45 90.91%	CP034491.1
~	Eukaryotic synthetic construct chromosome 21	1	85 10	53 96	% 4e-4	43 90.65%	6 <u>CP034500.1</u>
	Eukaryotic synthetic construct chromosome 17	1	85 24	50 96	% 4e-	43 90.65%	6 <u>CP034495.1</u>
~	Eukaryotic synthetic construct chromosome 22	1	83 93	33 96	% 2e-	42 90.58%	6 CP034501.1

Fig.3b BLASTing such matches gives 1005 matches to only Primates, and with no matches outside of Primates. This indicates that <u>SRX7756766</u> also contained significant amount of material derived from primates.

esults for	2:lcl Query_13045 gnl SRA SRR11119762.182596220.2 182596220 (Biologi 🗸		iii uppeui				
rogram	BLASTN ? Citation ~	Type common name,	binomial,	taxid or g	group nam	e	
atabase	nt <u>See details</u> 🛩	+ Add organism					
uery ID	lcl Query_13045	Percent Identity	E valu	e		Query Cov	/erage
escription	gnl SRA SRR11119762.182596220.2 182596220 (Biological)	to		to			to
olecule type	dna						
uery Length	151					Filter	Reset
ther reports	Distance tree of results 😧						
Descriptions	Graphic Summary Alignments Taxonomy						
Sequences	producing significant alignments	Download	× Ма	anage Co	olumns 🗡	Show	1000 🗸 🤇
select all	0 sequences selected				nk <u>Graph</u>		
	Description		Max Score	Total Score	Query E Cover val		Accession
	<u>D: Macaca mulatta LIM domain kinase 2 (LIMK2), transcript variant X3, mRNA</u>		279	279	100% 2e-	71 100.00%	XM_015150059.
0	D: Macaca mulatta LIM domain kinase 2 (LIMK2), transcript variant X3, mRNA D: Macaca mulatta LIM domain kinase 2 (LIMK2), transcript variant X2, mRNA		279 279	279 279	100% 2e- 100% 2e-		
						71 100.00%	 XM_015150059.; XM_015150058.; XM_015150057.;
	D: Macaca mulatta LIM domain kinase 2 (LIMK2), transcript variant X2, mRNA	CaBP-type calcium binding dom	279 279	279	100% 2e-	71 100.00% 71 100.00%	XM_015150058.
PREDICTE PREDICTE Predicte Papio anul	D. Macaca mulatta LIM domain kinase 2 (LIMX2), transcript variant X2, mRNA D. Macaca mulatta LIM domain kinase 2 (LIMX2), transcript variant X1, mRNA	CaBP-type calcium binding dom	279 279	279 279	100% 2e- 100% 2e-	71 100.00% 71 100.00% 71 100.00%	XM_015150058. XM_015150057.
PREDICTE PREDICTE Papio anul PREDICTE Papio anul	D: Macaca mulatta LIM domain kinase 2 (LIMX2). transcript variant X2. mRNA D: Macaca mulatta LIM domain kinase 2 (LIMX2). transcript variant X1. mRNA bis anubis NIPSNAP1 protein (NIPSNAP1) gene., partial cds., and merlin (NP2) and S-100/i	CaBP-type calcium binding dom	279 279 <u>nain</u> 279	279 279 5181	100% 2e- 100% 2e- 100% 2e-	71 100.00% 71 100.00% 71 100.00% 71 100.00%	XM_015150058. XM_015150057. AH012454.2
PREDICTE PREDICTE Papio anul PREDICTE PREDICTE	D. Macaca mulatta LIM domain kinase 2 (LIMX2) transcript variant X2, mRNA D. Macaca mulatta LIM domain kinase 2 (LIMX2), transcript variant X1, mRNA bis anubis NIPSNAP1 protein (NIPSNAP1) gene, partial cds; and merlin (NF2) and S-1007 D. Macaca fascicularis LIM domain kinase 2 (LIMX2), transcript variant X3, mRNA	CaBP4ype calcium binding dom	279 279 aain 279 279	279 279 5181 279	100% 2e- 100% 2e- 100% 2e- 100% 2e-	71 100.00% 71 100.00% 71 100.00% 71 100.00% 71 100.00%	 XM_015150058. XM_015150057. AH012454.2 XM_015457315.
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Fig.3C Presence of Primate-derived mRNA reads in <u>SRX7756766</u> confirms the Primate origin of these reads.

Z :	select all 100 sequences selected					Grap	hics Distance tree of results
	Description	Max Score		Query Cover	E value	Per. Ident	Accession
	<u>SRX7756769</u>	278	278	0%	9e-69	100.00%	SRA:SRR11119759.99831231.2
	<u>SRX7756769</u>	278	278	0%	9e-69	100.00%	SRA:SRR11119759.99831231.1
	<u>SRX7756769</u>	278	4814	1%	9e-69	100.00%	SRA:SRR11119759.88019245.2
	<u>SRX7756769</u>	278	5178	2%	9e-69	100.00%	SRA:SRR11119759.82130976.2
	<u>SRX7756769</u>	278	278	0%	9e-69	100.00%	SRA:SRR11119759.70689253.2
	<u>SRX7756769</u>	278	278	0%	9e-69	100.00%	SRA:SRR11119759.70689253.1
	<u>SRX7756769</u>	278	278	0%	9e-69	100.00%	SRA:SRR11119759.57405658.2
	<u>SRX7756769</u>	278	278	0%	9e-69	100.00%	SRA:SRR11119759.57405658.1
AC	073210.8						
Но	mo sapiens BAC clone RP11-460N20 from 7, complete seq						
nuo	cleic acid						
203	3396						

Fig.4a Similarly, <u>SRX7756769</u> contained large amount of reads that are 100% full-length matches to Human genomic DNA.

select all 0 s	equences selected						
		/lax core	Total Score	Query Cover		Per. Ident	Accession
Homo sapiens	chromosome 22 clone ABC11_000047176300_E22, complete sequence	278	456	100%	6e-71	100.00%	AC279316.1
Homo sapiens	actin related protein 2 pseudogene (LOC284441) on chromosome 19	278	278	100%	6e-71	100.00%	NG_022927.2
Homo sapiens	TBC1 domain containing kinase (TBCK). RefSegGene on chromosome 4	278	2140	100%	6e-71	100.00%	NG_034057.3
<u>Homo sapiens</u>	chromosome 15 clone VMRC59-280106, complete sequence	278	2291	100%	6e-71	100.00%	AC279072.1
Homo sapiens	chromosome 2 clone VMRC59-389K09, complete sequence	278	3905	100%	6e-71	100.00%	AC279037.1
<u>Homo sapiens</u>	chromosome 15 clone VMRC59-359A02_complete sequence	278	3589	100%	6e-71	100.00%	AC278991.1
<u>Homo sapiens</u>	chromosome 16 clone VMRC59-453B14, complete sequence	278	2239	100%	6e-71	100.00%	AC278975.1
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Fig.4b A BLAST analysis on reads sampled from the 100% hit results confirmed that it was found only in humans. Once again confirming human origin.

escription	gnl SRA SRR11119759.706892	253. 1 70689253 (E	Biological)	to		1	to			to	
lolecule type	dna										
uery Length	150								F	Filter	Reset
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Sequences	producing significant alig	nments		Down	load 🗸	Manag	ge Colu	mns	√ Sho	ow 100	0 🗸 🔞
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		Des	cription			Score	Score	Cover	value	Ident	Accession

Fig.4c The sequence have no matches outside of Primates.

🗹 sel	lect all 100	e sequences selected					Graph	ics Distance tree of result
		Description			Query Cover	E value	Per. Ident	Accession
S 5	RX6893156		278	278	0%	2e-69	100.00%	SRA:SRR10168375.5045789.1
🔽 <u>s</u>	RX6893156		278	278	0%	2e-69	100.00%	SRA:SRR10168375.5964.1
Descri	ption	Homo sapiens BAC clone RP11-460N20 from 7, complete seq						
Molecu	ule type	nucleic acid						
Query	Length	203396						
Other	reports	Distance tree of results MSA viewer 🔞						

Fig.5a <u>SRX6893156</u> also returned 100% matched results from the human Genome.

Select all 14 sequences selected	GenBank Graphics Distance tree of results
Description	Max Total Query E Per. Score Score Cover Value Ident
Homo sapiens BAC clone RP11-460N20 from 7, complete sequence	278 278 100% 6e-71 100.00% AC073210.8
Pan troglodytes BAC clone CH251-623C19 from chromosome 7, complete sequence	267 267 100% 1e-67 98.67% AC184799.2
Pan troglodytes BAC clone CH251-2015 from chromosome 7, complete sequence	267 267 100% 1e-67 98.67% AC174000.3
Pan troglodytes BAC clone CH251-565C10 from chromosome 7, complete sequence	267 267 100% 1e-67 98.67% AC148313.3
Description gnl SRA SRR10168375.5045789.1 5045789 (Biological)	
Molecule type dna	
Query Length 150	
Other reports Distance tree of results MSA viewer 😧	
Description gnl SRA SRR10168375.5964.15964 (Biological)	to to to
Molecule type dna	
Query Length 150	Filter Reset
Other reports Distance tree of results ?	
Descriptions Graphic Summary Alignments Taxonomy	
Sequences producing significant alignments	Download ~ Manage Columns ~ Show 1000 ~ (
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Description	Max Total Query E Per. Score Score Cover value Ident
Homo sapiens IncAB572.1 IncRNA gene, complete sequence	278 278 100% 7e-71 100.00% MK280613.1
Pan troglodytes chromosome 2 clone CH251-60P06, complete sequence	278 278 100% 7e-71 100.00% AC279084.1
Pan troglodytes chromosome 2 clone CH251-17022, complete sequence	278 278 100% 7e-71 100.00% AC278930.1
Pan troglodytes chromosome 2 clone CH251-108A24, complete sequence	278 278 100% 7e-71 100.00% AC278921.1

Fig.5b BLAST search on the result returned 100% match only found in humans, confirming origin in human-derived material.

scription	gnl SRA SRR10168375.596	4.1 5964 (Biologica	l)		to			to			to	
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select all	14 sequences selected						Ge	enBank	Grap	hics	<u>Distance t</u>	ree of resu
		Des	cription				Max Score	Total Score	Query Cover	E value	Per. Ident	Accessio
Eukaryotic	synthetic construct chromosome	121					278	7938	100%	7e-71	100.00%	CP03450
Eukaryotic	synthetic construct chromosome	<u>13</u>					267	14570	100%	1e-67	98.67%	CP03451
Eukaryotic	synthetic construct chromosome	<u>16</u>					267	10333	100%	1e-67	98.67%	CP03449
Eukaryotic	synthetic construct chromosome	<u>15</u>					267	9021	100%	1e-67	98.67%	CP03449
Eukaryotic	synthetic construct chromosome	13					267	14570	100%	1e-67	98.67%	<u>CP03449</u>
Eukaryotic	synthetic construct chromosome	18					261	15047	100%	7e-66	98.00%	<u>CP03449</u>
Eukaryotic	synthetic construct chromosome	<u>+ 17</u>					261	6545	100%	7e-66	98.00%	<u>CP03449</u>
Eukaryotic	synthetic construct chromosome	20					219	7949	98%	4e-53	93.79%	<u>CP03449</u>
Eukaryotic	synthetic construct chromosome	<u>19</u>					209	3521	96%	3e-50	93.10%	CP03452
Eukaryotic	synthetic construct chromosome	<u>19</u>					209	3766	96%	3e-50	93.10%	CP03449
Eukaryotic	synthetic construct chromosome	22					207	2291	96%	9e-50	92.47%	<u>CP03450</u>
Eukaryotic	synthetic construct chromosome	<u>14</u>					207	13851	96%	9e-50	92.47%	CP03449
Gossypiur	m hirsutum clone NBRI_GE27093	3 microsatellite seque	nce				189	189	96%	3e-44	90.41%	<u>JX591845</u>

Fig.5c BLAST result of the sequences in question revealed that it is not found outside of Primates.

2 :	select all	00 sequences selected					Graph	nics Distance tree of results
		Description	Max Score	Total Score		E value	Per. Ident	Accession
	SRX689315		278	278	0%	2e-69	100.00%	SRA:SRR10168376.17339580.1
	SRX689315	i	278	278	0%	2e-69	100.00%	SRA:SRR10168376.17013625.2
	SRX689315	i	278	278	0%	2e-69	100.00%	SRA:SRR10168376.17013625.1
	SRX689315	i de la companya de l	278	278	0%	2e-69	100.00%	SRA:SRR10168376.16930714.2
	SRX689315		278	278	0%	2e-69	100.00%	SRA:SRR10168376.16930714.1
	SRX689315	i de la companya de l	278	278	0%	2e-69	100.00%	SRA:SRR10168376.15267479.2
	SRX689315	i	278	278	0%	2e-69	100.00%	SRA:SRR10168376.15267479.1
≤	SRX689315		278	278	0%	2e-69	100.00%	SRA:SRR10168376.13985702.2
	SRX689315	i	278	278	0%	2e-69	100.00%	SRA:SRR10168376.13985702.1
	SRX689315	i	278	278	0%	2e-69	100.00%	SRA:SRR10168376.13353823.2
	SRX689315	i de la constante de	278	278	0%	2e-69	100.00%	SRA:SRR10168376.13353823.1
	SRX689315	i de la companya de l	278	278	0%	2e-69	100.00%	SRA:SRR10168376.11109740.1
	SRX689315	i de la companya de l	278	278	0%	2e-69	100.00%	SRA:SRR10168376.9343845.2
	SRX689315		278	278	0%	2e-69	100.00%	SRA:SRR10168376.9232549.2
escrip	otion	Homo sapiens BAC clone RP11-460N20 from 7, complete seq						
lolecu	ıle type	nucleic acid						
uery	Length	203396						
ther r	reports	Distance tree of results MSA viewer 😵						

Fig.6a Similarly, BLAST research on <u>SRX6893155</u> gives large number of full length 100% matches to the human genome.

✓	select all 57 se	equences selected					GenBan	<u>k</u> <u>G</u>	raphics	<u>Distan</u>	ice tree of results
			Descrip	otion		Max Score		Query Cover	E value	Per. Ident	Accession
	Homo sapiens F(OSMID clone ABC13-48840700E	15 from chromosome	7. complete sequence		278	278	100%	6e-71	100.00%	AC242196.4
	Pan troglodytes 8	BAC clone CH251-340l24 from ch	iromosome 7, complet	<u>te sequence</u>		278	278	100%	6e-71	100.00%	AC185242.2
	Pan troglodytes 8	BAC clone CH251-623C19 from c	hromosome 7, comple	<u>ete sequence</u>		278	278	100%	6e-71	100.00%	AC184799.2
	Pan troglodytes I	BAC clone CH251-114G16 from c	hromosome 7, comple	ete sequence		278	278	100%	6e-71	100.00%	AC183835.2
		BAC clone CH251-2O15 from chr		e sequence		278	278	100%	6e-71		AC174000.3
		AC clone RP11-479O9 from 7, co				278	278	100%	6e-71		AC073107.7
		BAC clone CH251-565C10 from c		ete sequence		278	278	100%	6e-71		AC148313.3
		AC clone RP11-460N20 from 7, c				278	278	100%	6e-71		
	PREDICTED: Ce	bus capucinus imitator small inte	g <u>ral membrane protei</u>	n 11A (SMIM11A), transc	<u>rript variant X6, mRNA</u>	87.9	87.9	49%	1e-13	88.00%	XM_017526193.1
De	scription	gnl SRA SRR10168376.15	267479.2 1526747	9 (Biological)							
M	olecule type	dna									
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Ot	her reports	Distance tree of results	MSA viewer 🔞								
De	cription	gnl SRA SRR10168376.1398	5702.1 13985702 (Biological)	to		to			t	o
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:	Descriptions Sequences pr	Graphic Summary	Alignments	Taxonomy	Download 🗠	Ма	nage Co		s × s		1000 V Ø
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:	Descriptions Sequences pr Select all 8	Graphic Summary	Alignments ignments Descr	iption		Мах	GenBar Total C Score C	t <mark>ik G</mark> Query Cover 1	raphics E value	Distan Per. Ident	ce tree of results
:	Sequences pr Select all 8 Homo sapie	Graphic Summary roducing significant al	Alignments ignments Descr m.7. complete seque	iption		Max Score	GenBar Total C Score C 278 1	n <u>k G</u> Query Cover N 00%	raphics E value 7e-71 1	Distant Per. Ident 00.00%	ce tree of results Accession
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	Descriptions Sequences pr Select all 8 Homo saple Pan troglody Pan troglody	Graphic Summary roducing significant all 37 sequences selected ens BAC clone RP11-460N20 fro ytes BAC clone CH251-623C191 ytes BAC clone CH251-565C101	Alignments ignments Descr m 7. complete seque tom chromosome 7. tom chromosome 7.	tption Ince complete sequence complete sequence		Max Score 278 272	GenBar Total C Score C 278 1 272 1 272 1	uery over 1 00% 3 00% 3	E ralue 7e-71 1 3e-69 9 3e-69 9	Distant Per. Ident 00.00% (99.33% (99.33% (Accession Accor3210.8 AC184799.2
	Cescriptions Cequences pr Sequences pr Select all 8 Homo saple Pan troology Macaca mul Homo saple Homo saple	Graphic Summary roducing significant all 37 sequences selected ens BAC clone RP11-460N20 fro ytes BAC clone CH251-623C191 ytes BAC clone CH251-565C101	Alignments ignments Descr m 7. complete seque tom chromosome 7. tom chromosome 7. in's Hospital Oakland	tption Ince complete sequence complete sequence Research institute Rhe		Max Score 278 272 272	GenBar Total C Score C 278 1 272 1 272 1 272 1 272 1	ak <u>G</u> Query Cover 1 00% 3 00% 3 00% 3	E Palue 7e-71 1 3e-69 9 3e-69 9 2e-52 9	Distant Per. Ident 00.00% 99.33% 99.33% 92.67%	Accession AC073210.8 AC184799.2 AC148313.3
:	Cescriptions Cequences pr Sequences pr Sector all 8 Homo saple Pan troolody Macaca mul Homo saple Homo saple Homo saple	Graphic Summary roducing significant all 37 sequences selected ens BAC clone RP11-460N20 fro vtes BAC clone CH251-623C191 vtes BAC clone CH251-565C101 latta BAC CH250-206B6 (Childre	Alignments ignments Descr m 7. complete seque tom chromosome 7. tom chromosome 7. inis Hospital Oakland (TRIM24), RefSeqGer	iption ince complete sequence complete sequence I Research Institute Rhe ne on chromosome Z		Max Score 278 272 272 272 217	GenBar Total C Score C 278 1 272 1 272 1 272 1 217 1 182 5	Ik G Query V Cover V 00% Cover	E Palue 7e-71 1 3e-69 9 2e-52 9 5e-42 1	Distant Per. Ident 1dent 99.33% 2 99.33% 2 9 92.67% 2 88.59% 3	Accession AC073210.8 AC184799.2 AC148313.3 AC210125.6 NG_023286.1 AC013429.12
	Cescriptions Cequences pr Sequences pr Select all 8 Homo saple Pan troolody Macaca mul Homo saple Homo saple Homo saple Homo saple Homo saple	Graphic Summary roducing significant all 37 sequences selected ens BAC clone RP11-460N20 fro vites BAC clone CH251-623C19 I vites BAC clone CH251-565C10 I latta BAC CH250-206B6 (Childre ens tripartite motif containing 24 ens chromosome 7 clone RP11- ens chromosome 7 clone RP11-	Alignments ignments Descr m7.complete seque tom chromosome 7. inis Hospital Oakland (TRIM24).RefSeqGer 199L18.complete se	iption ince complete sequence complete sequence IResearch Institute Rhe ne on chromosome 7 guence		Max Score 278 272 272 217 182 182 182	GenBar Total C Score C 278 1 272 1 272 1 217 1 182 9 182 9 182 9	hk G cover 1 00% 2 00% 2 00% 2 00% 2 99% 9 99% 9	E F ralue 7e-71 1 3e-69 9 9 3e-69 9 9 2e-52 9 9 5e-42 1 5e-42 5e-42 1 5e-42	Distant Per. Ident Ident 00.00% 4 99.33% 4 99.33% 4 99.33% 4 92.67% 4 88.59% 4 88.59% 4	Accession AC073210.8 AC184799.2 AC148313.3 AC210125.6 NG.023286.1 AC013429.12 AC038265.15
	Cescriptions Cequences pr Sequences pr Select all 8 Homo saple Pan troolody Macaca mul Homo saple Homo saple Homo saple Homo saple Homo saple	Graphic Summary roducing significant all 37 sequences selected ens BAC clone RP11-460N20 fro vtes BAC clone CH251-623C19 f vtes BAC clone CH251-565C10 f latta BAC CH250-206B6 (Childre ens tripartite motif containing 24.1 ens chromosome 7 clone RP11-	Alignments ignments Descr m7.complete seque tom chromosome 7. inis Hospital Oakland (TRIM24).RefSeqGer 199L18.complete se	iption ince complete sequence complete sequence IResearch Institute Rhe ne on chromosome 7 guence		Max Score 278 272 272 272 217 182 182	GenBar Total C Score C 278 1 272 1 272 1 217 1 182 9 182 9 182 9	hk G cover 1 00% 2 00% 2 00% 2 00% 2 99% 9 99% 9	E F ralue 7e-71 1 3e-69 9 9 3e-69 9 9 2e-52 9 9 5e-42 1 5e-42 5e-42 1 5e-42	Distant Per. Ident Ident 00.00% 4 99.33% 4 99.33% 4 99.33% 4 92.67% 4 88.59% 4 88.59% 4	Accession AC073210.8 AC184799.2 AC148313.3 AC210125.6 NG_023286.1 AC013429.12
	Cescriptions Cequences pr Sequences pr Select all 8 Homo saple Pan troolody Macaca mul Homo saple Homo saple Homo saple Homo saple Homo saple	Graphic Summary roducing significant all associated ans BAC clone RP11-460N20 fro wes BAC clone CH251-623C19 I wes BAC clone CH251-623C19 I ista BAC CH250-20686 (Childre ens tripartite motif containing 24 ens tripartite motif containing 24 ens chromosome 7 clone RP11- ens chro	Alignments ignments Descr m7.complete seque tom chromosome 7. inis Hospital Oakland (TRIM24).RefSeqGer 199L18.complete se	iption ince complete sequence complete sequence IResearch Institute Rhe ne on chromosome 7 guence		Max Score 278 272 272 217 182 182 182 182 176	GenBar Total C Score C 278 1 272 1 272 1 217 1 182 9 182 9 182 9	Ik G cover 1 00% 1 00% 1 00% 2 00% 2 00% 2 00% 2 00% 2 00% 2 09% 0 99% 0 99% 2 99% 2	E F ralue 7e-71 1 3e-69 9 9 3e-69 9 9 2e-52 9 9 5e-42 1 5e-42 5e-42 1 5e-42	Distant Per. Ident 100.00% 2 99.33% 2 92.67% 2 88.59% 2 88.59% 2 88.59% 2 88.59% 2 88.59% 2 87.92% 2	Accession AC073210.8 AC184799.2 AC148313.3 AC210125.8 NG_023286.1 AC013429.12 AC008265.15
	Cescriptions Cequences pr Sequences pr Select all s Homo saple Pan troglody Pan troglody Macaca mul Homo saple Homo saple Homo saple Eukaryotic s	Graphic Summary roducing significant all associated ans BAC clone RP11-460N20 fro wes BAC clone CH251-623C19 I wes BAC clone CH251-623C19 I ista BAC CH250-20686 (Childre ens tripartite motif containing 24 ens tripartite motif containing 24 ens chromosome 7 clone RP11- ens chro	Alignments ignments Descr m 7. complete seque tom chromosome 7. tom chromosome 7. t	iption ince complete sequence complete sequence IResearch Institute Rhe ne on chromosome 7 guence		Max Score 278 272 272 217 182 182 182 176	GenBar Total C Score C 278 1 272 1 272 1 217 1 182 9 182 9 353 9 GenBar To	Ik G Ruery V Cover V 000% 7 000% 7 000% 2 010% 2 020% 1 010% 2 010% 2 010% 2 010% 2 010% 2	raphics Falue 7e-71 3e-69 2e-52 3e-69 2e-52 3e-42 3e-42 3e-40 ae-40	Distant Per. Ident 100.00% 2 99.33% 2 92.67% 2 88.59% 2 88.59% 2 88.59% 2 88.59% 2 88.59% 2 87.92% 2	Accession Accession Ac18470.8 Ac184709.2 Ac148313.3 Ac210125.6 NG.023286.1 Ac008265.15 CP034510.1
	Cescriptions Cequences pr Sequences pr Select all s Homo saple Pan troploch Pan troploch Macaca mul Homo saple Homo saple Eukaryotic s Select all 3 seq	Graphic Summary roducing significant all associated ans BAC clone RP11-460N20 fro wes BAC clone CH251-623C19 I wes BAC clone CH251-623C19 I ista BAC CH250-20686 (Childre ens tripartite motif containing 24 ens tripartite motif containing 24 ens chromosome 7 clone RP11- ens chro	Alignments ignments Descr m 7. complete seque tom chromosome 7. tom chromosome 7. t	iption ince complete sequence complete sequence I Research Institute Rhe ne on chromosome 7 quence isquence		Max Score 278 272 272 217 182 182 182 176	GenBar Total C Score C 278 1 272 1 272 1 272 1 272 1 182 9 182 9 353 9 182 9 353 9 182 9 353 9 182 9 183 9 18 18 18 18 18 18 1	Ik G tuery V cover V 000% C	E E raue 38-69 38-69 9 38-69 9 38-69 9 38-69 9 38-69 9 38-64 1 38-64 1 38-64 1 38-64 1 38-64 1 1 1 1 1 1 1 1 1 1 1 1 1	Distant Per. Ident Ident 00.00% 0 99.33% 0 0 99.33% 0 0 88.59% 0 0 88.59% 0 0 88.59% 0 0 97.92% 0 0 Distant 0 0 E Pe Pe	Accession Accession Ac073210.8 Ac184799.2 Ac148313.3 Ac210125.6 NG_023286.1 Ac008265.15 CP034510.1 accession accession
	Cescriptions Cescriptions Cescriptions Cescriptions Cescriptions Cescription	Graphic Summary roducing significant al 37 sequences selected ans BAC clone RP11-460N20 fro Mes BAC clone CH251-623C191 Mes BAC clone CH251-263C101 lata BAC CH250-206B (Childre ens triparitie motif containing 24) ens chromosome 7 clone RP11- synthetic construct chromosome quences selected	Alignments ignments Descr m 7. complete seque tom chromosome 7. tom chromosome 7. t	iption ince complete sequence complete sequence I Research Institute Rhe ne on chromosome 7 quence isquence		Max Score 278 272 272 217 182 182 182 176	GenBar Total C Score C 2778 1 272 1 272 1 272 1 272 1 182 9 353 9 GenBar Tocore Core C I76 3	Ik G tuery V cover V 000% C 000% C	E E 7e-71 1 33e-69 9 36-69 9 36-69 9 36-69 9 36-69 9 36-40 3 36-40 3 age-40 3 age-40 3 age-40 3 age-40 3 age-40 3	Distant Per. Ident 1dent 00.00% 4 99.33% 4 99.33% 4 99.33% 4 99.33% 4 99.33% 4 99.33% 4 99.33% 4 99.33% 4 99.33% 4 99.33% 4 99.33% 4 99.33% 4 99.33% 4 99.33% 4 98.59% 4 88.59% 4 88.59% 4 97.92% 9 Distant 1 E Pe Ide Ide	Accession Accession AC073210.8 AC14813.3 AC210125.6 NG_023286.1 AC008265.15 CP034510.1

Fig.6b The results, when put through BLAST, confirms that the 100% matches are in fact derived from a Hominid origin.

Description Molecule type Query Length Other reports Descriptions	Homo sapiens BAC clone RP11-460N20 from 7, nucleic acid 203396 Distance tree of results MSA viewer Graphic Summary Alignments	complete seq	Percent Identity	E v	alue	to		Query Coverage to	e Reset
Sequences p	roducing significant alignments		Down	load 🗠	Ма	nage Co	olumns	 Show 100 	✓ 0
🗹 select all	100 sequences selected						Graph	nics Distance tre	e of results
	Description			Max Tota Score Scor	Query Cover		Per. Ident	Accessio	on
SRX689315	3			278 278	0%	2e-69	100.00%	SRA:SRR10168378	.1832954.1

Fig.7a <u>SRX6893153</u> have also returned 100% match full-length read on this tiny part of the human genome.

Description	gnl SRA SRR10168378.183	22954 1 1832954 (Bi	ological)					-	-		
Aolecule type	dna	2334.1 1032334 (DI	otoBicati	Percent Identity E	value			Query	/ Covera	ge	
				to	t	ο			to		
Query Length	150										_
ther reports	Distance tree of results M	<u>ISA viewer</u> 😮						F	ilter	Rese	<u>ا</u>
Descriptions	Graphic Summary	Alignments	Taxonomy								
Sequences	producing significant a	lignments		Download 🗡	Manag	e Colu	mns ~	Sho	w 100	00 🗸	0
select all	170 sequences selected				Gen	<u>ıBank</u>	<u>Graph</u>	<u>ics</u>	istance t	ree of res	ults
		De	escription		Max Score		Query Cover	E value	Per. Ident	Access	ion
Homo sapi	ens FOSMID clone ABC18-862111	from chromosome 7, cr	omplete sequence		278	278	100%	6e-71	100.00%	AC24520	<u>5.1</u>
Homo sapi	ens FOSMID clone ABC13-48840	700E15 from chromosor	ne 7, complete sequence		278	278	100%	6e-71	100.00%	AC24219	5.4
Homo sapi	ens BAC clone RP11-460N20 from	n 7. complete sequence			278	278	100%	6e-71	100.00%	AC07321	<u>0.8</u>
Pan trogloo	dytes BAC clone CH251-487D11 fr	rom chromosome 7, con	nplete sequence		272	272	100%	3e-69	99.33%	AC18273	3.3
select all 7	7 sequences selected					Ger	<u>Bank</u>	<u>Grap</u>	hics <u>C</u>	istance (ree of re
		ſ	Description			Max Score	Total Score	Query Cover	E value	Per. Ident	Acces
Eukaryotic s	synthetic construct chromosom	<u>ne 15</u>				211	211	98%	7e-51	92.11%	CP0344
Eukaryotic s	ynthetic construct chromosom	<u>ne 16</u>				206	1214	98%	3e-49	91.45%	CP0344
Eukaryotic s	ynthetic construct chromosom	<u>ne 13</u>				200	200	98%	2e-47	90.79%	CP034
Eukaryotic s	ynthetic construct chromosom	<u>ne 21</u>				200	401	98%	2e-47	90.79%	CP034
Eukaryotic s	synthetic construct chromosom	<u>ie 13</u>				200	200	98%	2e-47	90.79%	CP034
Eukaryotic s	synthetic construct chromosom	<u>ne 18</u>				195	195	96%	7e-46	90.60%	CP034
Eukaryotic s	synthetic construct chromosom	ne 17				195	195	98%	7e-46	90.13%	CP034

Fig.7b similarly, the read is only found in humans—indicating the Homo Sapiens Trace result is

accurate.

escription	Homo sapiens BAC clone RP11-450O3 from 7, complete sequence						
olecule type	nucleic acid						
uery Length	195834						
ther reports	Distance tree of results MSA viewer 😧						
Descriptions	Graphic Summary Alignments						
Sequences	producing significant alignments	Downlo	oad ~	Ма	nage Co	olumns	s ∨ Show 100 ∨
_	producing significant alignments 100 sequences selected	Downlo	oad ~	Ma	nage Co		Show 100 V
_		Мах	Total		E		
_ `	100 sequences selected Description	Мах	Total	Query Cover v a	E alue	<u>Gra</u> Per. Ident	phics Distance tree of resu
select all	100 sequences selected Description	Max Score	Total Score	Query Cover v 0% 1	E alue e-69 10	<u>Grap</u> Per. Ident	phics Distance tree of resu

Fig.8a Reads from the Human PMS1 gene is recovered from <u>SRX6893154</u> with a query sequence only 195834bp in length.

Description	gnl SRA SRR10168377.163	02266.1 16302266 (Biological)						
Molecule type	dna							Filte	r Reset
Query Length	150								
Other reports	Distance tree of results								
Descriptions	Graphic Summary	Alignments	Taxonomy						
Sequences	producing significant a	lignments		Download 🗡	Mar	age Col	umns 🗸	Show	1000 🗸 😨
select all	311 sequences selected					<u>GenBank</u>	Graphic	<u>s Dista</u>	ince tree of results
		Descr	iption			otal Que	ery E ver value	Per. Ident	Accession
Homo sa	piens PMS1 homolog 2, mismato	h repair system comp	onent pseudogene 8	(PMS2P8) on chromosome 7	278	278 100	% 7e-71	100.00%	NG_006447.3
Homo sa	piens PMS1 homolog 2, mismate	h repair system comp	onent pseudogene 1) (PMS2P10) on chromosome 7	278	278 100	% 7e-71	100.00%	NG_023454.4
Homo sa	piens PMS1 homolog 2, mismate	h repair system comp	onent pseudogene 6	(PMS2P6) on chromosome 7	278	278 100	% 7e-71	100.00%	NG_006449.3
Homo sa	piens BAC clone CH17-264B6 fro	<u>m chromosome 7, co</u>	<u>mplete sequence</u>		278	112 100	% 7e-71	100.00%	AC211476.5
Homo sa	piens BAC clone CH17-220H16 f	rom chromosome 7, c	omplete sequence		278	112 100	% 7e-71	100.00%	AC211491.5
Homo sa	piens FOSMID clone ABC10-455	5000F15 from chrome	osome 7, complete s	equence	278	556 100	% 7e-71	100.00%	AC244146.2
 Homo sap Homo sap Homo sap 	viens BAC done RP11-396K3 fro viens BAC done RP11-313P13 f viens chromosome 7 done VMR viens chromosome 7 done VMR viens chromosome 7 done CH1	rom 7, complete sequ C53-89F05, complete C62-404M06, complete	ience e sequence ete sequence		270 271 271 271	2 272 2 272	100% 7 100% 3 100% 3 100% 3	e-69 99 e-69 99	AC005488.2 33% AC278394.1 33% AC278331.1 33% AC270699.1
Homo sapi	iens PMS8 mRNA (yeast misma	ich repair gene PMS1	homologue), partial	cds (C-terminal region)	248	248 1	00% 6e-6	2 96.67	% <u>D38503.1</u>
Homo sapi	iens PMS1 homolog 2. mismatcl	n repair system comp	onent (PMS2), RefSe	gGene (LRG_161) on chromosome 7	244	244 1	00% 7e-6	1 96.00	% <u>NG 008466.1</u>
Canis lupu:	s familiaris breed Labrador retrie	ver chromosome 06a			154	154 8	4% 1e-33	3 88.89%	CP050586.1
Canis lupu:	s familiaris breed Labrador retrie	ver chromosome 06b			154	154 8	4% 1e-33	3 88.89%	CP050622.1
Pipistrellus	pipistrellus genome assembly,	chromosome: 5			139	139 8	6% 3e-29	9 86.15%	LR862361.1
Synthetic co	onstruct Homo sapiens clone ccs	bBroadEn 14772 PM	<u>S2 gene, encodes co</u>	mplete protein	126	126 4	9% 3e-2	5 97.30%	KJ905275.1
	D: Zalophus californianus PMS1	homolog 2, mismatch	repair system comp	onent (PMS2), transcript variant X3, mRNA	124	124 5	4% 9e-2	5 93.90%	XM_027610028.2
	D: Desmodus rotundus PMS1 ho	molog 2, mismatch re	pair system compor	ent (PMS2), transcript variant X2, mRNA	121	121 5	2% 1e-23	3 94.87%	XM_024576931.1
	D: Desmodus rotundus PMS1 ho	molog 2, mismatch re	epair system compor	ent (PMS2), transcript variant X1, mRNA	121	121 5	2% 1e-23	3 94.87%	XM_024576930.1
	D: Myotis lucifugus PMS1 homolo	og 2, mismatch repair	system component (PMS2), transcript variant X4, mRNA	121	121 5	2% 1e-23	3 94.87%	XM_023761457.1
	D: Myotis lucifugus PMS1 homolo	og 2, mismatch repair	system component (PMS2), transcript variant X3, mRNA	121	121 5	2% 1e-23	3 94.87%	XM_023761456.1

Fig.8b This PMS1 read is only found in Humans. This is clearly a contaminant from a hominid origin.

Description	Homo sapiens BAC clone	RP11-611L7 from 7, complete sequence	e	Percent Identity		E va	lue			Query Coverage
Molecule type	nucleic acid			to				to		to
Query Length	173967									
Other reports	Distance tree of results	MSA viewer 😧								Filter Reset
Descriptions	Graphic Summary	Alignments								
Sequences	producing significant	alignments		Dow	nload	· ~	Mar	nage Co	olumns	∽ Show 100 ✔ 💡
🗹 select all	100 sequences selected								Graph	nics Distance tree of results
		Description			Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
SRX689313	<u>19</u>				278	278	0%	3e-69	100.00%	SRA:SRR10168392.39544030.1
SRX689313	<u>19</u>				278	278	0%	3e-69	100.00%	SRA:SRR10168392.28917809.1
SRX689313	<u>19</u>				278	278	0%	3e-69	100.00%	SRA:SRR10168392.14357888.1
SRX689313	19				278	278	0%	3e-69	100.00%	SRA:SRR10168392.2548655.2

Fig.9a similarly, multiple 100% match Full length reads were obtained from <u>SRX6893139</u>. As this query sequence is only 173967 nucleotides in length, the real extent of Human-derived contamination is also extremely severe.

Description	gnl SRA SRR10168392.28917809.1 28917809 (Biological)	Percent Identity	E value		Q	uery Cov	erage
Molecule type	dna	to		to		1	to
Query Length	150						
Other reports	Distance tree of results MSA viewer 😨					Filter	Reset
Descriptions	Graphic Summary Alignments Taxonomy						
Sequences p	producing significant alignments	Download 🗡	Mana	age Columr	is ~	Show	1000 🗸 😨
select all	66 sequences selected		Ge	enBank <u>G</u>	raphics	<u>Distan</u>	ice tree of results
	Description			Total Query Score Cover	E value	Per. Ident	Accession
Momo sapie	ens zinc finger protein 316 (ZNF316), mRNA		278	278 100%	6e-71	100.00%	NM_001278559.2
	D: Homo sapiens zinc finger protein 316 (ZNF316), transcript variant X3, mRNA		278	278 100%	6e-71	100.00%	XM_024446619.1
	D: Homo sapiens zinc finger protein 316 (ZNF316), transcript variant X2, mRNA		278	278 100%	6e-71	100.00%	XM_024446618.1
	D: Homo sapiens zinc finger protein 316 (ZNF316), transcript variant X1, mRNA		278	278 100%	6e-71	100.00%	XM_006715630.4
Homo sapie	ens BAC clone RP11-611L7 from 7, complete sequence		278	278 100%	6e-71	100.00%	AC073343.6
PREDICTE	D: Pongo abelii zinc finger protein 316 (ZNF316), mRNA		272	272 100%	3e-69	99.33%	XM_024250011.1
	Drycteropus afer afer zinc finger protein 316 (ZNF316), mRNA		150	150 100%	2e-32	84.67%	XM_007942750
	<u> Miniopterus natalensis zinc finger protein 853 (ZNF853), mRNA</u>		145	145 100%	7e-31	84.00%	XM_016213621
PREDICTED: C	<u> Dchotona princeps zinc finger protein 316 (ZNF316), mRNA</u>		145	145 98%	7e-31	84.35%	XM_012930995
Pipistrellus pip	<u>sistrellus genome assembly, chromosome: 5</u>		145	145 100%	7e-31	84.11%	LR862361.1

Fig.9b examining these reads revealed that they are only found in humans and apes. This is therefore also clear evidence that there is Human/Hominid-derived contamination in **SRX6893139**.

Description	Homo sapiens chromosor	ne 9, clone hRPK.20	2_H_3, complet	Percent Identity		E val	ue			Query Co	verage	
Molecule type	nucleic acid			to				to			to	
Query Length	187174											
Other reports	Distance tree of results	ISA viewer 🔞								Filter		leset
Descriptions	Graphic Summary	Alignments										
Sequences p	producing significant a	lignments		Down	nload	~	Man	age Co	lumns	Show	100 💊	•
🗹 select all	100 sequences selected								Graphi	<u>cs Dista</u>	nce tree c	of result
		Description			Max Score		Query Cover	E value	Per. Ident		Accession	
SRX689315	7				278	278	0%	8e-70	100.00%	SRA:SRR1	0168374.7	906491

Fig.10a one read is also recovered from <u>SRX6893157</u>, from a query sequence only 187174nt in length.

	PRED	DICTED: Homo sapiens formin binding.protein 1 (FNBP1), transcript variant X13, mRNA	278	278	100%	6e-71	100.00	% <u>XM</u>	005251824	1.2
	PRED	DICTED: Homo sapiens formin binding protein 1 (FNBP1), transcript variant X4, mRNA	278	278	100%	6e-71	100.00	% <u>XM</u>	011518402	<u>11</u>
	PREC	DICTED: Homo sagiens formin binding.protein 1 (FNBP1). transcript variant X3, mRNA	278	278	100%	6e-71	100.00	% <u>XM</u>	011518401	1
	Home	<u>sapiens formin binding protein 1 (FNBP1). RefSeqGene on chromosome 9</u>	278	278	100%	6e-71	100.00	% <u>NG</u>	033946.1	
	Home	sapiens cDNA FLJ13619 fis, clone PLACE1010926, weakly similar to HYPOTHETICAL 72.2 KD PROTEIN C12C2.05C IN CHROMOS(278	278	100%	6e-71	100.00	% <u>AK</u>	<u>023681.1</u>	
	Huma	an DNA sequence from clone RP11-138E2 on chromosome 9q34.11-34.3, complete sequence	278	278	100%	6e-71	100.00	% <u>AL1</u>	136141.13	
	Home	sapiens formin-binding protein 17 (FBP17) mRNA_partial cds	278	278	100%	6e-71	100.00	% <u>AF</u> 2	265550.1	
	Home	sapiens chromosome 9, clone hRPK.202_H_3, complete seguence	278	278	100%	6e-71	100.00	% <u>AC</u>	<u>006241.1</u>	
	Home	sapiens KIAA0554 mRNA for KIAA0554 protein	278	278	100%	6e-71	100.00	% <u>AB</u>	<u>011126.1</u>	
Sec.	PRED	DICTED: Nomascus leucogenys formin binding protein 1 (ENBP1), transcript variant X18, mRNA	272	272	100%	3e-69	99.339	6 <u>XM</u>	030818029	0.1
Image:	PREC	DICTED: Nomascus leucogenys formin binding protein 1 (ENBP1), transcript variant X17, mRNA	272	272	100%	3e-69	99.339	6 <u>XM</u>	030818028	1.1
	PREC	DICTED: Nomascus leucogenys formin binding protein 1 (ENBP1), transcript variant X16, mRNA	272	272	100%	3e-69	99.339	6 <u>XM</u>	030818027	11
Descripti	ion	gnl SRA SRR10168374.7906491.2 7906491 (Biological)								
Molecule	type	dna								
Query Le	ngth	150								
Other rep	ports	Distance tree of results MSA viewer 🔞								
Sciu	irus cai	rolinensis genome assembly, chromosome: 16		17	4 17	74 9	5% 9	e-40	88 81%	LR738606.1
				17						LR738604.1
		rolinensis genome assembly, chromosome: 14								
_		<u>garis genome assembly, chromosome: 15</u>		16						LR738626.1
	DICTE	<u>:D: Loxodonta africana formin binding protein 1 (FNBP1), transcript variant X2, mRNA</u>		13	5 13	35 7	4% 4	e-28	88.50%	XM_010587565.2
	DICTE	D: Loxodonta africana formin binding protein 1 (FNBP1), transcript variant X1, mRNA		13	5 13	35 7	4% 4	e-28	88.50%	XM_023544839.1

Fig.10b this particular sequence is only found in humans—indicating that even the <u>SRX6893157</u> dataset was contaminated by material of human origin.

escription	Chlorocebus aethiops BAC clone CH252-276C1 from chromos	Percent Identity	Ev	alue		Query Coverage
olecule type	nucleic acid	to		to		to
ery Length	160189					
her reports	Distance tree of results MSA viewer 2					Filter Reset
Descriptions	Graphic Summary Alignments					
Sequences	producing significant alignments	Downl	oad ~	Manage	Columns	✓ Show 100 ✔ 6
_	producing significant alignments 100 sequences selected	Downl	oad ~	Manage	Columns <u>Grap</u>	
_		Downl	x Total	Manage Query E Cover value	<u>Grar</u> Per.	
_	100 sequences selected Description	Ma	x Total re Score	Query E	Grag Per. Ident	phics Distance tree of result
Select all	100 sequences selected Description T65	Ma	x Total re Score 9 774	Query E Cover value 0% 4e-69	Grag Per. Ident 100.00%	Chics Distance tree of result
Select all	100 sequences selected Description	Mi Scc 21	x Total Score 9 774 9 375	Query E Cover value 0% 4e-69 0% 4e-69	Grag Per. Ident 100.00%	Accession SRA-SRR11119763.129105044 SRA-SRR11119763.106754018
Select all	100 sequences selected Description	Ma Sco 21 21	x Total re Score 9 774 9 375 9 279	Query Cover E 0% 4e-69 0% 4e-69 0% 4e-69	Grag Per. Ident 100.00% 100.00%	Chics Distance tree of result Accession SRA.SRR11119763.129105044.
 select all SRX775670 SRX775670 SRX775670 	100 sequences selected Description	Mi Scc 23 23 27 27	x Total re Score 9 774 9 375 9 279 9 510	Query Cover E 0% 4e-69 0% 4e-69 0% 4e-69 0% 4e-69 0% 4e-69	Gran Per. Ident 100.00% 100.00% 100.00%	hics Distance tree of result Accession SRA SRR11119763 129105044 SRA SRR11119763 106754018 SRA SRR11119763 75715814 1

Fig.11a The presence of Reads from Somatic Chlorocebus aethiops in <u>SRX7756765</u> confirms the identity of the Cercopithecinae reads there.

scription	gnl SRA SRR11119763.106754018.1106754018 (Biological) to		to				to
lecule type	dna						
ery Length	151					Filter	Reset
her reports	Distance tree of results 🔞						
Descriptions	Graphic Summary Alignments Taxonomy						
Sequences	oroducing significant alignments Download	~	Manage	Colum	ins ~	Show	1000 🗸
select all	1000 sequences selected		GenE	<u>Bank</u>	<u>Graphic</u>	<u>s Dista</u>	nce tree of res
	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Chloroceb	us aethiops BAC clone CH252-276C1 from chromosome 6, complete sequence	279	375	100%	2e-71	100.00%	AC241496.3
_	us aethiops BAC clone CH252-445L10 from chromosome unknown, complete sequence	279	690	100%	2e-71	100.00%	AC238953.2
_	ulatta Y Chr BAC CH250-11J13 (Children's Hospital Oakland Research Institute Rhesus macaque Adult Male BAC I	-	1513		2e-71	100.00%	
_	us aethiops BAC clone CH252-371E9 from chromosome 5, complete sequence	279	1124	100%		100.00%	AC239563.3
_	us aethiops BAC clone CH252-461K13 from chromosome 13, complete sequence	279	629	100%		100.00%	AC239354.3
_	us aethiops BAC clone CH252-138D20 from chromosome 13, complete sequence	279	493	100%			AC239463.3
_	us aethiops BAC clone CH252-417L1 from chromosome 6, complete sequence us aethiops BAC clone CH252-62B18 from chromosome 4, complete sequence	279 279	472 510	100%		100.00%	AC239275.3 AC239442.2
	nthetic construct chromosome 18 Inthetic construct chromosome 16	268 268	1.545e+05 1.008e+05	100% 100%		100.00% 98.68%	CP034496.1 CP034494.1
	nthetic construct chromosome 16						
	nthetic construct chromosome 19	263	47303 1.935e+05	100% 100%		98.01% 99.31%	CP034522.1
	nthetic construct chromosome 13	263 263	1.935e+05		2e-66	99.31%	CP034516.1 CP034510.1
	nthetic construct chromosome Y nthetic construct chromosome 21	263	55262	100%		98.01%	CP034510.1 CP034500.1
	nthetic construct chromosome 20	263	88148			99.31%	01 00 1000.1
Eukarvotic si							CP034499.1
		263	47481		2e-66	99.31% 98.01%	<u>CP034499.1</u> <u>CP034497.1</u>
Eukaryotic s	Inthelic construct chromosome 19 Inthelic construct chromosome 17		47481 74732	100%			
Eukaryotic sy	nthetic construct chromosome 19	263		100% 100%	2e-66	98.01%	CP034497.1
Eukaryotic sy Eukaryotic sy Eukaryotic sy	nthetic construct chromosome 19 nthetic construct chromosome 17	263 263	74732	100% 100%	2e-66 2e-66	98.01% 98.01%	CP034497.1 CP034495.1
Eukaryotic sy Eukaryotic sy Eukaryotic sy Eukaryotic sy	nthelic construct chromosome 19 nthelic construct chromosome 17 nthelic construct chromosome 15	263 263 263	74732 1.384e+05	100% 100% 100% 100%	2e-66 2e-66 2e-66	98.01% 98.01% 98.01%	CP034497.1 CP034495.1 CP034493.1
Eukaryotic su Eukaryotic su Eukaryotic su Eukaryotic su Eukaryotic su	nthelic construct chromosome 19 nthelic construct chromosome 17 nthelic construct chromosome 15 nthelic construct chromosome 14	263 263 263 263	74732 1.384e+05 1.910e+05	100% 100% 100% 100%	2e-66 2e-66 2e-66 2e-66	98.01% 98.01% 98.01% 99.31%	CP034497.1 CP034495.1 CP034493.1 CP034492.1
Eukaryotic sy Eukaryotic sy Eukaryotic sy Eukaryotic sy Eukaryotic sy	nthelic construct chromosome 19 nthelic construct chromosome 17 nthelic construct chromosome 15 nthelic construct chromosome 14 nthelic construct chromosome 13	263 263 263 263 263	74732 1.384e+05 1.910e+05 1.935e+05	100% 100% 100% 100% 100%	2e-66 2e-66 2e-66 2e-66 2e-66	98.01% 98.01% 98.01% 99.31% 99.31%	CP034497.1 CP034495.1 CP034493.1 CP034492.1 CP034491.1
Eukaryotic sy Eukaryotic sy Eukaryotic sy Eukaryotic sy Eukaryotic sy Eukaryotic sy Human gam	nthelic construct chromosome 19 nthelic construct chromosome 17 nthelic construct chromosome 15 nthelic construct chromosome 14 nthelic construct chromosome 13 nthelic construct chromosome 22	263 263 263 263 263 263 257	74732 1.384e+05 1.910e+05 1.935e+05 22814	100% 100% 100% 100% 100% 100%	2e-66 2e-66 2e-66 2e-66 2e-66 9e-65	98.01% 98.01% 98.01% 99.31% 99.31% 97.35%	CP034497.1 CP034495.1 CP034493.1 CP034492.1 CP034491.1 CP034501.1
Eukaryotic si Eukaryotic si Eukaryotic si Eukaryotic si Eukaryotic si Human gam Human gam	nthelic construct chromosome 19 nthelic construct chromosome 17 nthelic construct chromosome 15 nthelic construct chromosome 14 nthelic construct chromosome 13 nthelic construct chromosome 22 maherpesvirus 4 isolate HKNPC60, partial genome	263 263 263 263 263 263 257 248	74732 1.384e+05 1.910e+05 1.935e+05 22814 248	100% 100% 100% 100% 100% 100%	2e-66 2e-66 2e-66 2e-66 9e-65 5e-62	98.01% 98.01% 98.01% 99.31% 99.31% 97.35% 96.03%	CP034497.1 CP034495.1 CP034493.1 CP034492.1 CP034491.1 CP034501.1 MH590571.1
Eukaryotic sy Eukaryotic sy Eukaryotic sy Eukaryotic sy Eukaryotic sy Eukaryotic sy Human gam Human gam Uncultured b	nthetic construct chromosome 19 nthetic construct chromosome 17 nthetic construct chromosome 15 nthetic construct chromosome 14 nthetic construct chromosome 13 nthetic construct chromosome 22 maherpesvirus 4 isolate HKNPC60, partial genome maherpesvirus 4 isolate HKNPC60, partial genome	263 263 263 263 263 263 257 248 248	74732 1.384e+05 1.910e+05 1.935e+05 22814 248 248	100% 100% 100% 100% 100% 100% 100%	2e-66 2e-66 2e-66 2e-66 9e-65 5e-62 5e-62	98.01% 98.01% 99.31% 99.31% 97.35% 96.03% 96.03%	CP034497.1 CP034495.1 CP034493.1 CP034492.1 CP034492.1 CP034491.1 CP034501.1 MH590571.1 MH590409.1

Fig.11b the sequences from the BLAST hits indicate that they were unique to the family Cercopithecinae. Confirming Primate origin.

Analyzing the extent of contamination.

As the Specific BLAST analysis confirmed significant level of Human-derived contamination in all samples positive for SARS-CoV-2 related Coronaviruses, The TRACE result can therefore be

trusted for the analysis on the extent of contamination.

The 32nt Krona Trace system is used for elucidating the ratio of different taxa within a sample. As Specific BLAST analysis confirmed the significant presence of Human and Primate derived Genetic material--The most basal group of primates detected in all Coronavirus-positive samples belong to Catarrhini—or Humans, Apes and Old-World Monkeys. Therefore, Trace classification results that can be classified into sister nodes of Catarrhini should be considered as Contamination by Primate-derived material.

Since Catarrhini is under Simiiformes; Haplorrhini; Primates; Euarchonta; Euarchontoglires and Manis is under Pholidota; Laurasiatheria, If a read is TRACEd down to Catarrhini, it can not be from a Pangolin, and it will have to be from a Primate-derived source—Contamination by material from the lab.

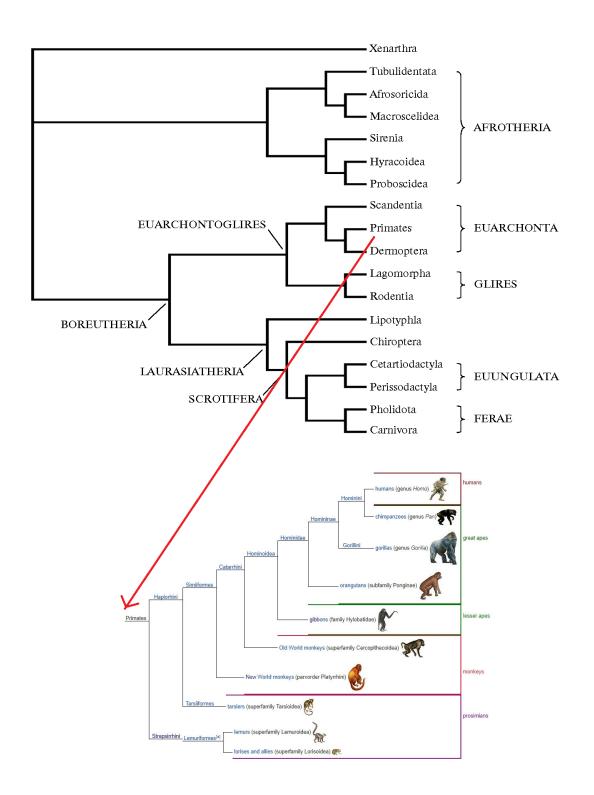


Fig. 12 Family tree of mammals, Including the position and classification of Primates in the lineage of Mammalia.

Table 3a Ratios of Hominid-traced reads to Pangolin-traced reads in the SRA datasets that contained reads of the GD- Pangolin-CoV sequence, and had Hominid reads.

Accession and	Primate	Total traced Kbps	Ratio of	Virus
date	classification and	to Manis Javanica	Primate to	classification
	total traced Kbps	(Pangolin)	Pangolin	and amount of
				reads by Kbps
SRX7756769	Homo sapiens	15401134	0.35	Bat SARS-like
18-Feb-2020	5457929			coronavirus
				2Kbp
				Wuhan seafood
				market
				pneumonia
				virus 2Kbp
<u>SRX6893139</u>	Homo sapiens	5301351	0.0926	Pangolin
20-Sep-2019	491120			coronavirus
				2Кbp
<u>SRX6893157</u>	Catarrhini	1889448	0.34	N/D***
20-Sep-2019	644546			
SRX6893156	Homo sapiens	4765461	0.01719	Pangolin
20-Sep-2019	81948			coronavirus
				2Kbp
SRX6893155	Homininae	525801	6.7214	Pangolin
20-Sep-2019	3534150			coronavirus
				5Kbp
<u>SRX6893154</u>	Hominoidea	2232008	0.159	Pangolin
20-Sep-2019	356003			coronavirus
				154Kbp
<u>SRX6893153</u>	Homo sapiens	3110158	0.05214	Pangolin
20-Sep-2019	162180			coronavirus
				41Kbp

***: No trace result on Coronaviruses, despite claimed reads from [3]

Table 3b Ratios of Primate-traced reads to Coronavirus-traced reads in the SRA datasets that contained reads claimed to be traced to of the GD- Pangolin-CoV sequence, and lacked Hominid reads.

Accession and date	Primate classification	Virus	Ratio of virus
	and reads (in Kbp)	classification and	reads to
		reads	Primate reads
SRX7756766	Cercopithecidae 3116;	Betacoronavirus	0.000642
18-Feb-2020	BLAST to Macaca	2Kbp **	
	Mulatta		
SRX7756762	Catarrhini 2831;	Nidovirales 0Kbp	0.000530
18-Feb-2020	BLAST to Chlorocebus	Claimed	
	sabaeus	10x150bp reads	
SRX7756765	Cercopithecinae 11339	N/D***	N/A
22-Apr-2020	BLAST to Chlorocebus		
	Aethiops		
SRX7732094	N/A*	Pangolin	N/A*
15-Feb-2020		coronavirus	

*: No non-coronavirus reads available in the dataset with a total of 2,633 reads, making analysis impossible.

**: No claimed reads from [2]

***: Claimed 8 reads from [10]

DISCUSSIONS

The extent of contamination in the pangolin sequencing datasets

As the samples were supposed to be pangolin lung tissue, which will neither contact with nor be contaminated by non-pangolin derived mammalian tissues when still inside the animal, any non-pangolin mammalian reads within such a dataset can only be introduced to the sequencing process after the sample itself have been taken and brought into a lab.

As the classification Catarrhini itself is phylogenetically very deep down the Primate line which is itself distinguished from the Pangolin line at a very basal node (Boreoeutheria), and since we have already confirmed that the Primate line in PRJNA573298 traces mostly to humans by using Specific BLAST analysis, (SRX6893157, the only one of the claimed coronavirus read dataset that gives a classification just down to Catarrhini, contained 213 full length 100% matches to the Human Mitochondrial reference genome alone, which is only 16569 bp in length. All other datasets gives definitive TRACE mapping to Homo Sapiens and contained distinct 100% matched reads to even very small parts of the Human genome.), We can deduce the extent of contamination of the PRJNA573298 dataset by Primate-related materials as from a minimum of 1.6% to as high as 87% by sample mass—using the ratio of Primate reads to Pangolin reads on TRACE. Such high level of contamination with Primate-derived material is unacceptable for a sample that was supposed to be Lung tissue. And therefore, the virome data of such samples in PRJNA573298 no longer reflects the original virome of the animal, and an potential "novel" reads from these contaminated samples may have been from in-lab contamination instead.

Deducing the dynamic of contamination in PRJNA607174

Of all 7 PRJNA607174 datasets, only <u>SRX7756769</u> and <u>SRX7756762</u> is claimed by Xiao et. Al to contain SARS-CoV-2-like reads. However, TRACE results revealed low level of contamination by Cercopithecidae (Old World Monkey) reads across all the samples. In particular, the <u>SRX7756762</u> dataset contained definitive mappings to Chlorocebus sabaeus, or African Green Monkey, while <u>SRX7756766</u> which contained 2Kbp unclaimed reads of Betacoronaviruses on TRACE, contained 100% full-length definitive mappings to Macaca Mulatta that may also be mapped to Chlorocebus Aethiops and Homo Sapiens.

<u>SRX7756769</u> genetically resembles other samples in PRJNA573298, in both the kind of contamination and the extent of contamination. It contained an large excess of homo sapiens reads in levels similar to the contaminated samples in PRJNA573298.

From the method section of Lam et.al, we knew that they have performed Virus isolation using VERO E6 cells—Species Chlorocebus Sabaeus on one of the samples that have a positive PCR test for coronaviruses. The low level of contamination by Cercopithecidae-related reads in all the samples in PRJNA607174 except for <u>SRX7756769</u> itself support the possibility that <u>SRX7756769</u> is the first sample to be sequenced, and it happens before the lab begun using VERO E6 cells in the experiment. They then isolated the virus from the contaminated <u>SRX7756769</u> in VERO E6 cells, characterized it but did not sequence it, and this cell culture material then contaminated <u>SRX7756762</u> and possibly <u>SRX7756766</u>, resulting the 10 reads in <u>SRX7756762</u> and the 2Kb Batacoronavirus reads in <u>SRX7756766</u>.

The exact nature of <u>SRX7732094</u> needs to be further scrutinized.

The P2S dataset, SRX7732094, displays very unusual property when compared to other Datasets under the same BioProject. It is the only dataset with all Non-coronavirus reads being filtered out, and contained too little spots for it to be an ILLUMINA NextSeq 550 run. Furthermore, it was the only dataset that did not contain metadata with either an isolation source or a Library prep procedure, other than "This dataset contains coronavirus-like sequence reads, based on BLAST search."

Such a strange designation and the fact of the dataset being heavily filtered, Raises problems on whether such a dataset is an actual BioSample at all. If this sample is really as claimed by Lam et. Al, Why the dataset have to be put through such heavy filtering when the other sequencing runs was clearly not filtered as severely as this dataset? Why there was no BioSample metadata on either Biomaterial provider, Source Tissue or Collector when all other Sequencing runs clearly provided such metadata information?

Unless the complete, unfiltered sequencing reads are made available on **SRX7732094**, and the rest of **PRJNA606875**, this Dataset can not be considered to be a real, reliable sample, and it must be excluded as "evidence" of a SARS-CoV-2-like virus infecting

pangolins in Guangdong, 2019.

Table 4 Sequencing	runs	in	PRJNA696875,	Accession	number,	BioSample,	Content
and designation							

Accession number and date SRX7732094 15-Feb-2020	Size 2,633	Non-Coronavirus reads? No	Source Tissue Provider and Collected by N/A	Virus Designation: GD or GX? GD	Design This dataset contains coronavirus-like
					sequence reads, based on BLAST search.
SRX7732093 15-Feb-2020	470,344	Yes	Intestine Yanling Hu Wuchun Cao	GX	NEBNext Ultra II DNA Library Prep Kit, paired sequencing data has been integrated.
SRX7732092 15-Feb-2020	340,661	Yes	Lung Yanling Hu Wuchun Cao	GX	NEBNext Ultra II DNA Library Prep Kit, paired sequencing data has been integrated.
SRX7732091 15-Feb-2020	416,659	Yes	Intestine Yanling Hu Wuchun Cao	GX	NEBNext Ultra II DNA Library Prep Kit, paired sequencing data has been integrated.
SRX7732090 15-Feb-2020	520,254	Yes	Lung Yanling Hu Wuchun Cao	GX	NEBNext Ultra II DNA Library Prep Kit, paired sequencing data has been

					integrate	d.
SRX7732089	19,607,536	Yes	Blood	GX	lon	Total
15-Feb-2020			Yanling Hu		RNA-Seq	Kit v2
			Wuchun			
			Cao			
SRX7732088	4,550,437	Yes	lung and	GX	lon	Total
15-Feb-2020			intestine		RNA-Seq	Kit v2
			Yanling Hu			
			Wuchun			
			Cao			

By closely examining the P2V dataset, SRX7732088, which claimed to be a culture sample in VERO E6 cells, Chlorocebus Sabaeus, the exact viral load in-culture when compared to Cellular mRNA can be deduced by dividing the total identifiable coronavirus signal to the total identifiable Primate signal within the dataset, 6943Kbp/451932Kbp, which correspond to 0.01536:1 Viral RNA to Cellular RNA.

This places the viral loads on the other datasets with Coronavirus-like reads from GD well within the threshold expected from cell culture contamination of the sequencing samples—including the samples in PRJNA607174.

Potential breach of data availability statement by Xiao et al.[2]

Sequence data that support the findings of this study have been deposited in GISAID with th	e accession numbers EPI_ISL_410721. Raw data of RNAseq are available
from the NCBI SRA under the study accession number PRJNA607174.	

Fig 13. The Data Availability Statement of Xiao et al.

In the Data availability statement, the "Raw data of RNAseq" are clearly stated to be deposited under PRJNA607174. However, only 2 of the "Extended Data Table S3" datasets actually matches the datasets deposited on PRJNA607174. The other 7 datasets were completely unavailable. And the actual deposited datasets on PRJNA607174 does not match what have been claimed by Extended Data Table S3. As the RNA-seq Raw data was stated to be available within PRJNA607174, the failure to publish all the claimed data constitute a breach of the Data Availability statement on the article. Unless such datasets are published and independently examined, All such claimed reads from the strangely unpublished datasets can not be trusted as evidence of a SARS-CoV-2-like virus infecting pangolins in GuangDong, 2019.

Identifying the Etiological agent of the GuangDong 2019 incident.

By using an approach of both SRA TRACE analysis and specific BLAST Analysis, We have uncovered the fact that all samples that does not Contain confirmed Human-derived material, also lacked Claimed reads of a SARS-CoV-2 like virus that can be confirmed using NCBI Trace. All samples with claimed or traced reads of Coronaviruses in general, contained confirmed primate reads with the lowest common phylogenetic node Catarrhini. Samples that does not give a TRACE result on primate-derived material all lacked identifiable or claimed coronavirus reads.

This strongly imply that the Coronavirus-like reads are associated with human/Primate-sourced contamination material.

Most importantly, of all dead pangolins being sampled in the studies, only 9 out of a total of 29

Analyzable samples/datasets contained TRACEd or Claimed Coronavirus reads—despite all dead pangolins displayed similar symptoms in captivity. This imply that the alleged pangolin coronavirus is not the Etiological agent of the death of the pangolins being sampled in the studies. This is further supported by the fact that 4 out of 10 lung samples in PRJNA573298 and 4 out of 7 lung samples in PRJNA607174 lacked any claimed or TRACEd coronavirus reads—despite the same symptoms displayed and similar date of death.

In order to establish the Etiological agent of the dead pangolins in the single GuangDone Accident that leads to the sampling and studies. A full virome TRACE analysis is conducted on the available samples for the determining of the exact etiological agent.

	Mammarenavirus N	airoviridae	Murine respirovirus	Flaviviridae	Nidovirales	Rubulavirus	Nonanavirus	Peribunyavi	Amigovirus	Siphoviridae	Siphoviridae	Pahexavir
SRX6893158				No				No	Yes			No
SRX6893157	Yes Y		No	No	Claimed	No		Yes	No	No	No	No
SRX6893156	No	lo	Yes	Yes	Yes	No	No	No	Yes	No	No	Yes
SRX6893155	No	lo	Yes	No	Yes	No	No	No	No	No	No	No
SRX6893154	No	lo	Yes	No	Yes	No	No	No	No	No		No
SRX6893153	No	lo	Yes	Yes	Yes	No	No	No	Yes	No		No
SRX6893152	Yes Y	es	Yes	Yes	No	No	No	Yes	No	No		No
SRX6893151	Yes Y	es	No	Yes		No		Yes	Yes	No		No
SRX6893150	Yes Y		Yes	No		No		Yes	Yes	No		No
SRX6893149			No	No		No		No	No	No		No
SRX6893148	Yes Y	es	Yes	No	No	No	No	No	Yes	No	No	No
SRX6893147	Yes Y	les	"Respirovirus"	Yes	No	No	Yes	No	Yes	No	No	No
SRX6893146	Yes Y	les	Yes	No	No	No	No	No	No	No	No	No
SRX6893145	Yes Y	les	No	No			No	No	No	No		No
SRX6893144			Yes	Yes		No	No	No	No	No		No
SRX6893143	Yes Y		No	No		No		No	No	No		No
SRX6893142	Yes Y		No	No	No	No		Yes	Yes	No		No
			No	Yes		No	No	No	No	No	No	No
SRX6893140			Yes	No		No		Yes	No	No		No
SRX6893139			Yes	No		No	No	No	No	No		No
SRX6893138	Yes Y			Yes				Yes	Yes	No		No
SRX7756766				Yes		Yes	No	No	No	No		No
SRX7756765				No					No	No		No
			Yes	No		Yes			No	No		No
			Yes	No		Yes		No	No	No		No
SRX7756762	No	lo	Yes	No		Yes		No	No	No		No
SRX7756761	No N		Yes	No	No	Yes		No	No	No		No
SRX7756769	No	lo	Yes	Yes	Yes	No	No	No	No	No	No	No

Extended Data Table S1

Full virome TRACE results of all Analyzable datasets of the GD pangolin incident

A full Virome TRACE result suggest all the dead pangolins were infected by either Mammarenaviruses or Murine Respirovirus, or both. Including both samples that contained Claimed or TRACEd Coronavirus reads and the samples that didn't.

Murine Respirovirus and Mammarenaviruses co-infect 7 out of 29 Available Analyzable datasets, while none of the 29 datasets lacked both—indicating that both viruses were prevalent in the location where the pangolins were captive at The Guangdong Wildlife Rescue Center.

Symptoms of Murine Respirovirus in animals resembles that of SARS-CoV-2 in humans—It forms massive Syncytia in Eukaryotic cells, suppresses the immune system and causes secondary bacterial infections. The virus causes necrosis of Lung tissue in 5 days, with similar inflammation and immunopathological effects in the lung tissues of infected animals [5]—creating the histopathological effect as reported by Xiao et al.

It should be worth pointing out that the only examined lung tissues were examined by Xiao et al. And all Lung tissue samples examined by Xiao et.al contained Reads from the Murine Respirovirus.

Similarly, Mammarenaviruses are also known to cause multi organ, lethal[7] infections, characterized by endothelial pathology and swelling of internal organs. [6] All of which were Symptoms reported in the incident. As these samples were not examined Histopathologically by either the authors of [4] nor by any of the authors of any other article who have used the

datasets/samples, leaving the only mean of elucidating the cause of death being the observed symptoms and the coarse examination of the organs during sampling. Mammarenavirus infection therefore remains the most likely cause of death of the Murine Respirovirus Negative samples in the available datasets.

Is the "GD pangolin CoV" really a virus of the pangolin?

The only examination of the binding affinity of the GD pangolin CoV RBD to different animal receptors was done by Xiao et al [2], which performed molecular dynamic simulation of the RBD docking to the Human ACE2 receptor, The Civet ACE2 receptor and the pangolin ACE2 receptor. If the RBD of GD pangolin CoV in deed evolved in pangolins, we should expect the binding affinity of the RBD toward the pangolin ACE2 receptor to be the highest binding affinity returned from the examination.

However, neither the GD pangolin CoV RBD, nor the RBD of SARS-CoV-2 which is highly similar, produced a higher binding affinity to the pangolin ACE2 receptor than to the human ACE2 receptor, and both binds the Human ACE2 receptor with the highest affinity across all 3 animal species (Human, Civet, Pangolin) examined.

This fact argues strongly against the RBD residues of the GD pangolin CoV being evolved in pangolins, and instead favoring the RBD and the virus being the result of a passage experiment of a possible virus of pangolin origin (The GX/P2V virus was isolated and passaged in VERO E6 cells during it's collection in 2017) in Primate-derived cell lines.

There are only 2 locations of Biological sample storage in Guangdong, the Guangdong Institute of Applied Biological Resources and the China National GeneBank.

As all Credible (Non-filtered and contained analyzable Non-Coronavirus reads) samples were collected in a single incident from the Guangdong Wildlife Rescue Center[1][4][2], which the initial sample collection and storage was carried out by the Guangdong Institute of Applied Biological Resources[4], this experimental culture likely contaminated the GD pangolin samples during their initial collection or Storage, Either by the lab worker doing the initial sampling, or during their storage in the facility.

Epidemiology analysis of SARS-CoV-2 and related viruses argues strongly against the existence of a Coronavirus with the claimed RBD residues and sequence similarity in or near the GuangDong Wildlife Rescue Center at the time and date of the incident and the collection of the samples.

The earliest collection date of the GD pangolin CoV available, MP789, GenBank MT084071.1, is displayed at 29 March 2019.

Since the original location of the animals and samples in question was inside the Guangdong Wildlife Rescue Center which is neither a certified Biosafety Laboratory nor possessed adequate PPE when handling the animals, from the Simulation results by Xiao et al[2] and the observed

high human transmissibility of SARS-CoV-2 which had a very similar RBD, Should the GD pangolin CoV genuinely exists at that date and within the unprotected Guangdong Wildlife Rescue Center, It would almost certainly infect one to multiple On-site workers (Rescue workers which lacked either the Biosafety training or the adequate PPEs required to handle tissues or animals infected with a virus as characterized by the GD pangolin CoV papers) in the Guangdong Wildlife Rescue Center, and caused a SARS-level epidemic in Guangdong 2013 beginning in or around April 2019. However, no such epidemic was recorded, nor there have been any virus that genetically

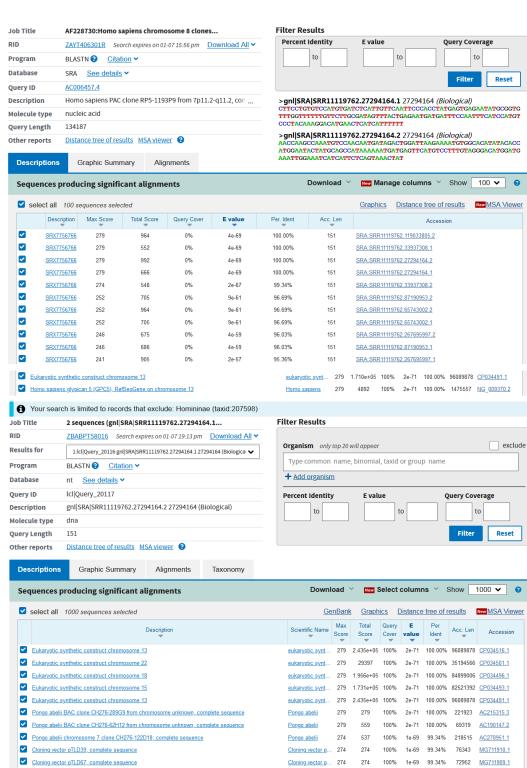
resembled the GD pangolin CoV sequence (which is only 90% similar to SARS-CoV-2) being isolated in humans anywhere in the world even till today.

Nor there is a possibility that the current SARS-CoV-2 pandemic may have stemmed from the 29 March incident with the GD pangolin CoV, since the estimated time of divergence between the current SARS-CoV-2 genome to the GD pangolin CoV Genome was estimated to be at least 100 years ago , ranging from 1851 [1730,1958] to 1877 [1746,1986] [8], for a genome that is only 90% similar to SARS-CoV-2 and possessed significant difference in the sequence and composition of the viral proteins they encodes.

As the Earliest time of discovery and the incident on the GD pangolin CoV is no earlier than the beginning of Year 2019, The time between the incident and the first isolate of SARS-CoV-2 is far too short for GD pangolin CoV incident to be involved in the formation of the current SARS-CoV-2 pandemic, since even the neutral sites on the RBD itself would have taken more than 19.8 years to drift/evolve into what we seen today on the actual SARS-CoV-2 genome. [9]

Homo Sapiens reads are also found in SRX7756766, SRX7756765 and SRX7756762

In addition to Chlorocebus Spp. Indicative of VERO E6 cells, we have also found trace amount of reads uniquely matched to Hominidae within SRX7756766, SRX7756765 and SRX7756762. The presence of such reads may indicate that the Coronavirus-like reads from within such dataset were the result of index-hopping from a more highly contaminated original sample dataset, such as SRX7756769, of which the Homo Sapiens reads within such datasets may have index hopped into SRX7756766, SRX7756765 and SRX7756762, alongside with extremely low level of Coronavirus-related reads. Alternatively, these Homo Sapiens reads may represent the Homo Sapiens host sequence from the original sample which were left in the cell culture medium after inoculation of the VERO E6 cells as indicated by the virus isolation procedure performed by Xiao et al[2]. The presence of low levels of Homo Sapiens sequences within these datasets also confirms the origin of the inoculum into the VERO E6 cells as being samples that had significant amount of Homo Sapiens genetic material within them, which agrees with the hypothesis that the Coronavirus-related reads within these 3 datasets were the result of contamination by the In-lab VERO cell culture used by Xiao et al for virus isolation.



Eukaryotic synthetic construct chromosome 19

Fig 14: In addition to Chlorocebus Aethiops, reads uniquely matched to Hominidae have been found within SRX7756766.

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Fig 15: Reads uniquely matched to Homo Sapiens have been found within SRX7756765.

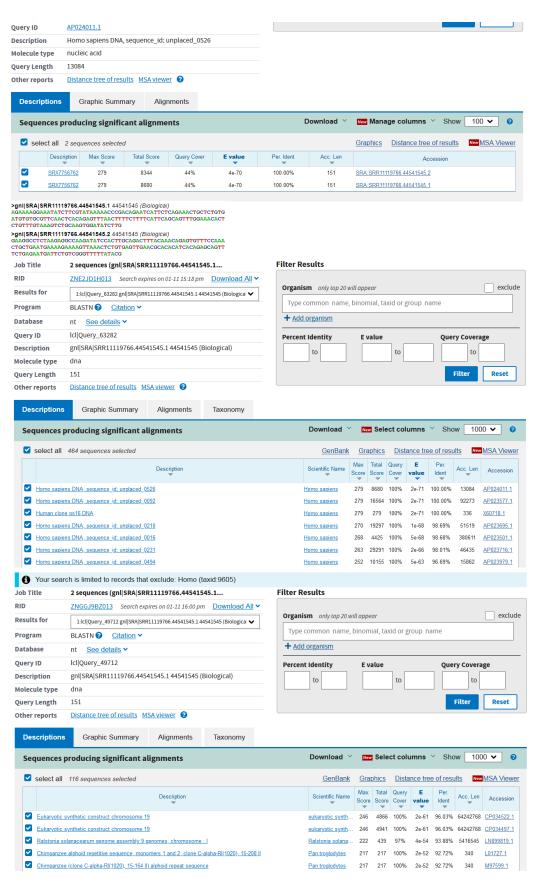


Fig.16: Reads uniquely matched to Homo Sapiens Alpha Satellite DNA in SRX7756762.

The Pan-SL-CoV-GD sequences can not be found in other pangolin sequencing datasets from China.

Recently, we are able to access and perform BLAST analysis on a large dataset of 93 pangolin samples deposited by <u>Hu J *et al.*</u>[12] located under the BioProject PRJNA529540. These samples, alongside with an older sample, SRX1319167, represent a longitudinal survey spanning from between 1990 to 2017 of Both Manis Pentadactyla and Manis Javanica from China. We could not obtain any traces of reads resembling the Pan-SL-CoV-GD sequences from these datasets. Such discovery is in agreement with the conclusion of <u>Hu J *et al.*</u>[12] which failed to find any evidence of SARS-CoV-2-like Coronaviruses within their sequencing study.

Considering that another longitudinal survey of 334 pangolins in Malaysia[13] have also failed to reveal any evidence of Coronaviruses or other potentially zoonotic viruses, the failure to isolate sequences of Coronaviruses from pangolin sequencing datasets are in good agreement that no natural infection of a Coronavirus can happen to a pangolin in the wild. This may be due to their solitary behavior[14] which keep them completely physically isolated from each other for up to 9 months for each year when the population is not in it's mating season which happens from May to July. As this is longer than the time of which a pangolin could stay infected before either clearing the infection or dying in any known incidence of viral infection in captive pangolins[15], any virus species that enters a pangolin population in the wild will either be cleared or kills all of its current hosts when the population is not in or have left its mating season, resulting in the viral population to go extinct. Therefore, the absence of viral infections in pangolin populations is the normal state of such population, and any datasets that claimed viral infection of pangolins must be subjected to the highest level of scrutiny to exclude any potential presence of contamination.

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Fig.17a: No evidence of reads resembling the Pan-SL-CoV/GD sequences could be obtained from

PRJNA529540.

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Fig17b: No evidence of reads resembling the Pan-SL-CoV/GD sequences could be found in SRX1319167.

Potential malpractice associated with Chinese pangolin sequencing data

Recently, we are able to obtain 20 RNA-seq datasets for the transcriptomic sequencing of both Manis Javanica and Manis Pentadactyla skin appendage (skin, scales), deposited by the Guangdong Institute of Applied Biological Resources from a project that is Separate from the Coronavirus-related sequencing project ongoing in the People's Republic of China. Located under the BioProject accession number PRJNA610466.

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Fig 18. No evidence of the Pan-SL-CoV/GD sequences could be found within PRJNA610466.

Although we did not find any evidence of Coronaviruses from these datasets, We noticed that some of the datasets contained several sequences that were associated with vectors associated with the immortalization and engineering of mammalian cells, namely sequences resembling HIV-1, Macaca Mulatta polyomavirus 1 and Human betaherpesvirus 5.

HS14 (SRR11306689)

Metadata Analysis Reads Data access Taxonomy Analysis Unidentified reads: 7.23% Identified reads: 92.77% cellular organisms: 92.77% Eukaryota: 76.86% Opisthokonta: 76.69% Metazoa: 75.87% Boreoeutheria: 74.22% Laurasiatheria: 68.86% -Manis javanica: 64.4% Euarchontoglires: 1.72% Simiiformes: 0.92% Catarrhini: 0.87% Hominoidea: 0.79% Hominidae: 0.7% Homininae: 0.63% -Homo sapiens: 0.3% Fungi: 0.79% Viridiplantae: 0.11% Sar: < 0.01% (188 Kbp) Bacteria: 13.22% Archaea: < 0.01% (132 Kbp)</p> └ Viruses: < 0.01% (222 Kbp) Caudovirales: < 0.01% (100 Kbp) Siphoviridae: < 0.01% (62 Kbp) Tunavirinae: < 0.01% (33 Kbp) Rtpvirus: < 0.01% (29 Kbp) unclassified Rtpvirus: < 0.01% (29 Kbp) Enterobacteria phage vB_EcoS_IME542: < 0.01% (16 Kbp) Escherichia phage vB_Ecos_CEB_EC3a: < 0.01% (12 Kbp) unclassified Tunavirinae: < 0.01% (5 Kbp) Escherichia phage IMM-001: < 0.01% (5 Kbp) Pahexavirus: < 0.01% (15 Kbp) Propionibacterium phage Solid: < 0.01% (9 Kbp) Myoviridae: < 0.01% (23 Kbp)</p> Eneladusvirus: < 0.01% (13 Kbp) Cronobacter phage vB_CsaM_GAP32: < 0.01% (13 Kbp) - Tevenvirinae: < 0.01% (10 Kbp) unclassified Tevenvirinae: < 0.01% (10 Kbp) Riboviria: < 0.01% (74 Kbp) Tymovirales: < 0.01% (35 Kbp) Actinidia seed-borne latent virus: < 0.01% (35 Kbp) Potyviridae: < 0.01% (29 Kbp) Sugarcane mosaic virus: < 0.01% (29 Kbp) Negarnaviricota: < 0.01% (9 Kbp) Mononegavirales: < 0.01% (9 Kbp)</p> Filoviridae: < 0.01% (7 Kbp) Paramyxoviridae: < 0.01% (2 Kbp) Mammalian rubulavirus 5: < 0.01% (2 Kbp) Polyomaviridae: < 0.01% (18 Kbp) Macaca mulatta polyomavirus 1: < 0.01% (18 Kbp) Ortervirales: < 0.01% (16 Kbp) Orthoretrovirinae: < 0.01% (11 Kbp) Gammaretrovirus: < 0.01% (9 Kbp) RD114 retrovirus: < 0.01% (7 Kbp) Lentivirus: < 0.01% (2 Kbp) Human immunodeficiency virus 1: < 0.01% (2 Kbp) Herpesvirales: < 0.01% (6 Kbp) Herpesviridae: < 0.01% (5 Kbp) Gammaherpesvirinae: < 0.01% (3 Kbp) Human gammaherpesvirus 4: < 0.01% (3 Kbp) Betaherpesvirinae: < 0.01% (2 Kbp) Human betaherpesvirus 5: < 0.01% (2 Kbp) Poxviridae: < 0.01% (4 Kbp) Vaccinia virus GLV-1h68: < 0.01% (4 Kbp)

Fig.19: NCBI TRACE analysis result of SRR11306689

We downloaded the dataset with most concentrated occurrence of such sequences, SRR11306689, and performed sequence assembly using MEGAHIT[16]. Contiguous sequences with homology to these cellular engineering-associated sequences were identified using BowTie2[17] and their identities were elucidated through a combination of specific BLAST analysis and through sequence analysis using the Addgene sequence analysis tool.

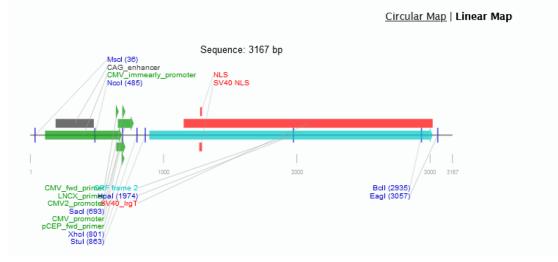


Fig.20: Addgene sequence analysis result of the single contig with detected homology to Macaca Mulatta polyomavirus 1 and Human betaherpesvirus 5.

Sequence analysis of the largest contig with homology to cellular engineering related sequences revealed a Simian Virus 40(SV40) Large T antigen (LTA) placed behind a CMV promoter. Such a sequence is normally used to immortalize cells through the oncogenic properties of the Large T antigen, normally delivered into the cells using an integrative transfection technique, such as lentiviral vectors. This sequence appear to be a partial mRNA transcript.

	Murine retrovirus shuttle vector pZIPneoSV(TAg), complete sequence	<u>syntheti</u> <u>NA</u>	32630	4096	4096	70%	0.0	99.78%	7020	<u>Z93724.1</u>
	Synthetic construct clone pARVA_T-Ag, complete sequence	<u>syntheti</u> <u>NA</u>	<u>32630</u>	3849	5119	89%	0.0	99.30%	5690	MF174873.1
	Mammalian expression vector pSV529HIFNG, complete sequence	<u>Mamma</u> <u>NA</u>	<u>1945111</u>	3502	6652	70%	0.0	99.84%	9455	LT727634.1
	Mammalian expression vector pSV51E6Hf2, complete sequence	<u>Mamma</u> <u>NA</u>	<u>1945103</u>	3502	6652	70%	0.0	99.84%	9574	LT727623.1
	Mammalian expression vector pSV51E6Hf1, complete sequence	<u>MammaNA</u>	<u>1945102</u>	3502	6652	70%	0.0	99.84%	9472	LT727622.1
~	Mammalian expression vector LNXCO3, complete sequence	M	lammalian exp	1463	1463	25%	0.0	99.75%	7484	LT727330.1
	EIAV-based lentiviral vector, complete sequence	E	AV-based lent	1461	1461	24%	0.0	100.00%	7941	<u>GQ872121.1</u>
	Mutant Human betaherpesvirus 5 clone AD169-BAC20, complete genome	H	luman betaher	1448	1448	24%	0.0	100.00%	232314	MN920393.1
	Mutant Human betaherpesvirus 5 clone AD169-BAC2, complete genome	Н	luman betaher	1448	1448	24%	0.0	100.00%	233833	MN900952.1

Fig.21: BLAST result of this contig revealed that this sequence is associated with shuttle vectors (murine retrovirus, lentivirus) carrying the SV40 Large T antigen.

Four contigs within SRR11306689 are found with homology to Human Immunodefiency Virus 1 (HIV-1), which upon specific BLAST analysis reveal themselves to be sequences derived from lentiviral transfer vectors.

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Fig.23: Specific BLAST analysis result of the 4 contigs associated with Human Immunodeficiency Virus 1 (HIV-1).

A deep analysis of these 4 contigs revealed 2 contigs spanning the vector-virus junction for common lentiviral transfer vectors, one contig displaying an Integration junction of the LTR into Manis Pentadactyla DNA and one contig displaying an integration junction of the LTR into Homo Sapiens DNA.

The presence of these fragmented sequences carrying both the payload (SV40 Large T antigen) and the vehicle (Lentiviral transfer vectors) with evidence of delivery into pangolin cells (integration junction of Vector LTR DNA into Manis Pentadactyla genomic DNA), suggesting an ongoing effort of immortalizing pangolin cells and keeping them in culture being conducted in the Guangdong Institute of Applied Biological Resources.

Indeed, the presence of Primary Fibroblast (Skin, Muscle) cells cultures from both Manis Pentadactyla and Manis Javanica and their availability to labs have been recently confirmed by two BioSamples of primary fibroblast cells "collected by Dr. Shujin Luo (Peking University, China)" placed under accession SAMN16895765 and SAMN16895764, with collection date of 20/03/2020 and 08/04/2020 respectively.

Chinese par	ngolin							
Identifiers	BioSample: SAMN1689	5765; Sample name: Sample3814; SRA: SRS7809866	Sunda pang					
Organism	Manis pentadactyla (Ch		Identifiers	BioSample: SAMN1685	5764; Sample name: Sample3826; SRA: SRS7809864			
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	collection date	2020-03-20		collected by	not collected			
	culture collection	not collected		collection date	2020-04-08			
	death date	not collected		culture collection	not collected			
	disease	not collected		death date	not collected			
	disease stage	not collected		disease	not collected			
	genotype	not collected		disease stage	not collected			
	geographic location	not collected		genotype	not collected			
	growth protocol	not collected		geographic location	not collected			
	health state	n/a		growth protocol	not collected			
	isolation source	not collected		health state	n/a			
	latitude and longitude	not collected		isolation source	not collected			
	phenotype	not collected		latitude and longitude	not collected			
	sample type	not collected		phenotype	not collected			
	specimen voucher	not collected		sample type	not collected			
	storage conditions	not collected		specimen voucher	not collected			
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Figure 24: BioSample description of SAMN16895765 and SAMN16895764 as "primary fibroblast cells".

As such a cell line could potentially be used to culture the Pan-SL-CoV/GD virus, should an isolate in VERO E6 [2] exist, these cell lines may potentially be used to contrive "novel" BioSamples and SRAs for this sequence through inoculation and serial passage of the cultured virus in order to eliminate the primate host sequences from the original samples, due to the central role of the RBD of this supposedly "wild" sequence in current publications regarding SARS-CoV-2 origin.

We therefore urge caution when adopting any short read sequencing (SRA) data or viral nucleotide sequences from pangolins with a date of deposition after the collection date for SAMN16895765 and SAMN16895764, especially after the publishing date of PRJNA610466,

24/11/2020, due to the identity of potential "tissue" samples being no longer restricted exclusively to living or dead wild animals once a primary or immortalized cell line of such a species have been established, potentially allowing malpractice when "sequencing" "new" samples from these two species.

The MEGAHIT result and the obtained vector sequences from SRR11306689 have been deposited as

Galaxy266-[Assembly_with_MEGAHIT_on_data_260] SRR11306689.fasta

HIV-1 from SRR11306689.fa

and

SV40 LTA+CMV from SRR11306689.fa

Conclusions

The Extreme lack of transparency and the sheer level of contamination from the original samples, the lack of epidemiological evidence of it's existence at the location of it's collection, and the receptor binding affinity of the Viral RBD itself indicating it as not being evolved nor adapted in pangolins, all strongly argue against the existence of a SARS-CoV-2 like virus infecting pangolins captive in Guangdong at 2019.

Moreover, it suggests that the GD pangolin CoV exists only as a culture in Primate-derived cells within the lab/facility used for the initial collection and/or storage of the samples of the pangolins in question, raising important issues on the serial passage Gain-Of-Function research of viral pathogens.

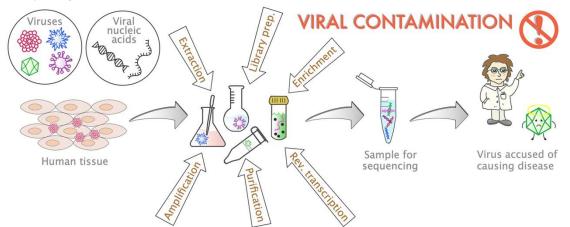


Figure 25. A cartoon diagram of contamination in sequencing experiment leading to false results and false "discoveries".

Note as in 2020/7/23

A recent Dataset, seemingly unrelated to the Xiao et.al Nature dataset, <u>SRX8582289</u>, appeared under <u>PRJNA607174</u>. This dataset seems to be newly sequenced, and it was not referred in [2].

Table S2: TRACE analysis	s result of th	ne <u>SRX8582289</u> d	lataset.			
Accession number and	Primary	Mammalian	Primate-related	results	Identification	of

registration date	Trace results and	in Krona and read size	"Coronaviridae"
	percentage	by Кbp	as by Trace and
			total read size
<u>SRX8582289</u>	Manis javanica: 43.52%	Catarrhini 98913	Pangolin
22-Jun-2020			coronavirus 792

Nevertheless, in-depth analysis revealed significant amount of contamination from the Human genome, with ratio of Virus to cell=0.8%.

escription	Homo sapiens BAC clone	RP11-460N20 from 7, complete sequ	Percent Identity	E va	alue			Query Coverage
olecule type	nucleic acid		to			to		to
uery Length	203396							
ther reports	Distance tree of results	ISA viewer 😮						Filter Reset
Descriptions	Graphic Summary	Alignments						
Sequences	producing significant a	lignments	Downloa	ad ~	Mai	nage C	olumns	✓ Show 100 ✔
🗹 select all	100 sequences selected						<u>Grap</u>	hics Distance tree of result
		Description	Max Scor		Query Cover	E value	Per. Ident	Accession
SRX85822	<u>89</u>		278	278	0%	8e-69	100.00%	SRA:SRR12053850.88444297.
SRX85822	<u>89</u>		278	402	0%	8e-69	100.00%	SRA:SRR12053850.83916175.
SRX85822	<u>89</u>		278	388	0%	8e-69	100.00%	SRA:SRR12053850.83916175
SRX85822	<u>89</u>		278	278	0%	8e-69	100.00%	SRA:SRR12053850.82221130.
SRX85822	<u>89</u>		278	278	0%	8e-69	100.00%	SRA:SRR12053850.71234261
SRX85822	<u>89</u>		278	278	0%	8e-69	100.00%	SRA:SRR12053850.71234261
SRX85822	<u>89</u>		278	5169	2%	8e-69	100.00%	SRA:SRR12053850.51889132
SRX85822	<u>89</u>		278	7268	3%	8e-69	100.00%	SRA:SRR12053850.26027930
SRX85822	<u>89</u>		278	5671	2%	8e-69	100.00%	SRA:SRR12053850.21554419
SRX85822	<u>89</u>		278	278	0%	8e-69	100.00%	SRA:SRR12053850.13271287
SRX85822	<u>89</u>		278	4760	1%	8e-69	100.00%	SRA:SRR12053850.62042.2

Figure S1A: Some BLAST hits out of a human Somatic BAC clone.

escription	gnl SRA SRR12053850.8222	21130.2 82221130 (Biological)		to		to				to
olecule type	dna							L			
uery Length	150									Filte	Reset
her reports	Distance tree of results)									
Descriptions	Graphic Summary	Alignments	Taxonomy								
Sequences pr	roducing significant al	lignments			Download 🗠	М	anage	Colum	ns ~	Show	1000 🗸 🤇
select all 55	9 sequences selected						<u>GenB</u>	ank _	Graphic	<u>s Dista</u>	nce tree of resul
		Descr	iption			Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Homo sapier	ns general transcription factor I	<u>lli pseudogene 14 (GT</u>	F2IP14) on chromosome	7		276	276	99%	3e-70	100.00%	NG_043494.1
Homo sapier	ns FOSMID clone ABC13-4884	0700E15 from chrom	osome 7, complete seque	nce		276	276	99%	3e-70	100.00%	AC242196.4
Homo sapier	ns BAC clone RP11-460N20 fro	om 7, complete seque	ence			276	276	99%	3e-70	100.00%	AC073210.8
Homo sapier	ns general transcription factor I	<u>lli pseudogene 5 (GTF</u>	2IP5) on chromosome 7			270	270	99%	1e-68	99.33%	NG 026590.1
Pan troglody	tes BAC clone CH251-340l24 fi	rom chromosome 7, c	complete sequence			270	270	99%	1e-68	99.33%	AC185242.2
Pan troglody	tes BAC clone CH251-623C19	from chromosome 7,	complete sequence			270	270	99%	1e-68	99.33%	AC184799.2
Pan troglody	tes BAC clone CH251-114G16	from chromosome 7,	complete sequence			270	270	99%	1e-68	99.33%	AC183835.2
Pan troglody	tes BAC clone CH251-2O15 fro	<u>)m chromosome 7, co</u>	mplete sequence			270	270	99%	1e-68	99.33%	AC174000.3
Homo sapier	ns BAC clone RP11-47909 from	<u>m 7, complete sequer</u>	ice			270	270	99%	1e-68	99.33%	AC073107.7
Pan troglody	tes BAC clone CH251-565C10	from chromosome 7,	complete sequence			270	270	99%	1e-68	99.33%	AC148313.3
homo sapier	ns BAC clone CH17-99D2 from	<u>i chromosome 4, com</u>	plete sequence			226	226	99%	3e-55	93.96%	AC278002.1
Homo sapier	ns FOSMID clone ABC27-15411	13 from chromosome	4, complete sequence			226	226	99%	3e-55	93.96%	AC240529.1
Pan troglody	tes BAC clone CH251-4D23 fro	m chromosome 7, co	mplete sequence			226	226	99%	3e-55	93.96%	AC148834.3
Eukaryotic synthe	etic construct chromosome 10	<u>6</u>					193	193	99%	3e-45	90.07% <u>CP034</u>
Eukaryotic synth	etic construct chromosome 19	9					182	182	99%	5e-42	88.74% CP034
Eukarvotic synth	etic construct chromosome 19	9					182	182	99%	5e-42	88.74% CP034

Fig. S1B: BLAST results returned only Homo Sapiens as 100% match. This indicate that the listed Catarrhini reads come from Homo Sapiens.

The significance of this particular dataset is yet unknown.

Note as in 2020/12/26

Two Recent SRAs, <u>SRX9714436</u> and <u>SRX9714921</u>, were recently deposited by the Guangdong Institute of Applied Biological Resources with a listed DOI connection to 10.1371/journal.ppat.1008421 [1]. Both samples have a depositor of LinMao Li, 2020-12-21 the same time as the specified BioProject registration date. Only one of the SRAs contained significant amount of Coronavirus-related reads.

Table S3: TRACE analysis result of SRX9714436 and SRX9714921.

Accession number and	Primary Mammalian	Primate-related results	Identification of
registration date	Trace results and	in Krona and read size	"Coronaviridae"
	percentage	by Кbp	as by Trace and
			total read size
SRX9714436	Manis javanica: 3.14%	Homo sapiens 12332	Pangolin
	Homo sapiens: 0.04%		coronavirus 3
<u>SRX9714921</u>	Homo sapiens: 0.15%	Homo sapiens 9923	N/D

As expected by TRACE results, Reads that are 100% full-length uniquely matched to Homo Sapiens were obtained from **SRX9714436** and **SRX9714921**.

>gnl|SRA|SRR13285085.43 43

CTATACAACAAACCCCCCATGACACGAGTTTACCTATGTAACAAACCTTCA

select all 100 sequences selected					GenBa	ank	<u>Graphics</u>	Distance	e tree of results
Description		Common Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Homo sapiens BAC clone RP11-488C22 from 2, complete sequence	<u>e</u>	<u>human</u>	93.5	93.5	100%	5e-16	100.00%	6 165351	AC019109.9
Pan troglodytes BAC clone CH251-617M1 from chromosome unkno	wn, complete sequence	<u>chimpanzee</u>	87.9	87.9	100%	2e-14	98.00%	188195	AC183921.2
Pan troglodytes BAC clone CH251-564M21 from chromosome unkn	iown, complete sequence	<u>chimpanzee</u>	87.9	87.9	100%	2e-14	98.00%	201789	AC160021.3
Homo sapiens chromosome 5, BAC clone 89K19 (LBNL H179), cor	<u>nplete sequence</u>	<u>human</u>	87.9	87.9	100%	2e-14	98.00%	110312	AC005215.2
Homo sapiens chromosome 5 clone CTD-2332G20, complete sequences	Jence	<u>human</u>	87.9	87.9	94%	2e-14	100.00%	6 146437	AC010489.4
Eukaryotic synthetic construct chromosome Y		eukaryotic syn	84.2	168	90%	3e-13	100.009	64558752	CP034510.1
Pongo abelii BAC clone CH276-222M18 from chromosome 8, comp	<u>plete sequence</u>	Sumatran ora	84.2	84.2	90%	3e-13	100.009	6 196788	AC206339.2
Description	Common Na	ime			otal Q	uery		Per. Acc.	Len Accessio

	Description	Common Name	Score		Cover	value	Ident	Acc. Len	Accession
	Eukaryotic synthetic construct chromosome Y	eukaryotic synthetic construct	84.2	168	90%	3e-13	100.00%	64558752	CP034510.1
≤	Eukaryotic synthetic construct chromosome 15	eukaryotic synthetic construct	80.5	80.5	92%	4e-12	97.83%	82521392	CP034493.1
	Eukaryotic synthetic construct chromosome 14	eukaryotic synthetic construct	76.8	151	94%	5e-11	95.74%	88289540	CP034492.1
	Eukaryotic synthetic construct chromosome 18	eukaryotic synthetic construct	75.0	210	100%	2e-10	95.65%	84899006	CP034496.1
	Eukaryotic synthetic construct chromosome 13	eukaryotic synthetic construct	71.3	71.3	94%	2e-09	93.62%	96089878	CP034516.1
	Eukaryotic synthetic construct chromosome 16	eukaryotic synthetic construct	71.3	71.3	94%	2e-09	93.62%	98200793	CP034494.1
	Eukaryotic synthetic construct chromosome 13	eukaryotic synthetic construct	71.3	71.3	94%	2e-09	93.62%	96089878	CP034491.1
\sim	Eukaryotic synthetic construct chromosome 20	eukaryotic synthetic construct	69.4	138	86%	8e-09	95.35%	68480253	CP034499.1
	Eukaryotic synthetic construct chromosome 19	eukaryotic synthetic construct	62.1	62.1	84%	1e-06	92.86%	64242768	CP034497.1

Fig.S2A: Homo Sapiens 100% full-length Unique matched read obtained from SRX9714436

>gnljSRAjSRR13285570.36 36 ATAGGGAAGTGTGGTACCAAGGAGCAATATTCAATACAGCAACCAGGAAG

Description	Common Name		Score	Query Cover	value	Per. Ident	Acc. Len	Accession
Human DNA sequence from clone XX-DSH1_29E11, complete sequence	<u>human</u>	93.5	93.5	100%	5e-16	100.00%	112322	CU041292.6
Human DNA sequence from clone RP11-535B18 on chromosome 9, complete sequence	<u>human</u>	93.5	93.5	100%	5e-16	100.00%	126815	AL354931.13

Fig.S2B: Homo Sapiens 100% full-length Unique matched read obtained from <u>SRX9714921</u> A provided .fastq file was also found in <u>SRX9714436</u>. Analysis using stand-alone MagicBlast[11] suggests significant presence of Homo Sapiens reads within this fastq file, similar to that of the Run itself. NDX550397_RUO:309:H3FKWBGXH:1:11101:21446:1055 16 AC019109.9 87488 255

	50M * 0 0 TGAAGGTTTGTTACATAGGTAAACTCGTG NH:i:1 AS:i:50 NM:i:0	STCATGGGGG	TTTG	TGT	ATAG	*			
	Description	Common Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
~	Homo sapiens BAC clone RP11-488C22 from 2, complete sequence	human	93.5	93.5	100%	5e-16	100.00%	165351	AC019109.9
/	Pan troglodytes BAC clone CH251-617M1 from chromosome unknown, complete sequence	<u>chimpanzee</u>	87.9	87.9	100%	2e-14	98.00%	188195	AC183921.2
/	Pan troglodytes BAC clone CH251-564M21 from chromosome unknown, complete sequence	<u>chimpanzee</u>	87.9	87.9	100%	2e-14	98.00%	201789	AC160021.3
/	Homo sapiens chromosome 5, BAC clone 89K19 (LBNL H179), complete sequence	human	87.9	87.9	100%	2e-14	98.00%	110312	AC005215.2
/	Homo sapiens chromosome 5 clone CTD-2332G20, complete sequence	human	87.9	87.9	94%	2e-14	100.00%	146437	AC010489.4
/	Eukaryotic synthetic construct chromosome Y	eukaryotic syn	. 84.2	168	90%	3e-13	100.00%	64558752	CP034510.1
ć	Pongo abelii BAC clone CH276-222M18 from chromosome 8, complete sequence	Sumatran ora	84.2	84.2	90%	3e-13	100.00%	196788	AC206339.2
1	Human DNA sequence from clone RP11-987D21 on chromosome X, complete sequence	<u>human</u>	84.2	84.2	96%	3e-13	97.92%	55442	<u>BX119919.5</u>
1	PREDICTED: Callithrix jacchus uncharacterized LOC118154814 (LOC118154814), ncRNA	white-tufted-e	82.4	82.4	94%	1e-12	97.87%	2719	XR 00474507
1	Homo sapiens solute carrier family 26 member 3 (SLC26A3), RefSeqGene (LRG_683) on chrom	ios <u>human</u>	82.4	82.4	100%	1e-12	96.00%	44767	<u>NG_008046.1</u>
1	Eukaryotic synthetic construct chromosome Y eukaryotic synthetic construct	t	4	34.2	168 9	0% 3	e-13 100.	.00% 6455	8752 <u>CP03451</u>
1	Eukaryotic synthetic construct chromosome 15 eukaryotic synthetic construct	t	4	30.5	30.5 9	2% 4	e-12 97.8	83% 8252	1392 <u>CP03449</u>
ł	Eukaryotic synthetic construct chromosome 14 eukaryotic synthetic construct	t	1	76.8	151 9	4% 5	e-11 95.7	74% 8828	9540 <u>CP03449</u>
1	Eukaryotic synthetic construct chromosome 18 eukaryotic synthetic construct	t	1	75.0	210 1	00% 2	e-10 95.6	65% 8489	9006 <u>CP03449</u>
	Eukaryotic synthetic construct chromosome 13 eukaryotic synthetic construct	t	1	71.3	71.3 9	4% 2	e-09 93.6	62% 9608	9878 <u>CP03451</u>
1	Eukaryotic synthetic construct chromosome 16 eukaryotic synthetic construct	t	ī	71.3	71.3 9	4% 2	e-09 93.6	52% 9820	0793 <u>CP03449</u>
)	Eukaryotic synthetic construct chromosome 13 eukaryotic synthetic construct	t	ī	71.3	71.3 9	4% 2	e-09 93.6	62% 9608	9878 <u>CP03449</u>
)	Eukaryotic synthetic construct chromosome 20 eukaryotic synthetic construct	t		59.4	138 8	6% 8	e-09 95.3	35% 6848	0253 <u>CP03449</u>
ł	Eukaryotic synthetic construct chromosome 19 eukaryotic synthetic construct	t		52.1 (52.1 8	4% 1	e-06 92.8	36% 6424	2768 <u>CP03449</u>

Fig.S3: BLAST result of the read NDX550397_RUO:309:H3FKWBGXH:1:11101:21446:1055 TGAAGGTTTGTTACATAGGTAAACTCGTGTCATGGGGGGTTTGTTGTATAG within the provided fastq file. The read is 100% full-length uniquely matched to Homo Sapiens.

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