

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

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ABSTRACT

Recently, There were much hype about an alleged SARS-like coronavirus being found in samples of Malayan pangolins (*Manis Javanica*) possessing nearly identical RBD to the SARS-CoV-2 coronavirus. Prominent journals cite the alleged discovery to claim that pangolins may be one of 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 in serious risk of contamination. Here we also report that the presence of 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 an 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 under NCBI GenBank. 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	
SRX6893157	9,275,501	Lung12 [3] SRR10168374
SRX6893156	22,220,187	Lung11 [1]
SRX6893155	18,067,615	Lung09 [1] [3] SRR10168376
SRX6893154	16,414,925	Lung08 [1] [3] [4] SRR10168377
SRX6893153	19,045,923	Lung07 [1] [3] [4] SRR10168378
SRX6893152	13,527,964	
SRX6893151	16,068,654	
SRX6893150	12,967,281	
SRX6893149	12,590,769	
SRX6893148	15,273,939	

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

Sample ID	Animal species	Total reads*	No. mapped
M1	Malayan pangolin	107,267,359	496 ←SRX7756769 "pangolin 9"
M2	Malayan pangolin	38,091,846	302
M3	Malayan pangolin	79,477,358	14
M4	Malayan pangolin	32,829,850	1,100
M5	Malayan pangolin	547,302,862	56
M6	Malayan pangolin	232,433,120	10 ←SRX7756762 "pangolin 2"
M8	Malayan pangolin	44,440,374	12
M10	Malayan pangolin	227,801,882	0
Z1	Chinese pangolin	444,573,526	0

Not available

Not available

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.

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

Accession number and registration date	Primary Mammalian Trace results and percentage	Primate-related results in Krona and read size by Kbp	Identification of "Coronaviridae" as by Trace and total read size
SRX6893158 20-Sep-2019	Manis javanica: 14.66%	N/D	N/D
SRX6893157 20-Sep-2019	Boreoeutheria: 1.24%	Catarrhini 644546	N/D***
SRX6893156 20-Sep-2019	Manis javanica: 7.51% Homo sapiens: 0.03%	Homo sapiens 81948	Pangolin coronavirus 2Kbp
SRX6893155 20-Sep-2019	Homo sapiens: 0.37%	Homininae 3534150	Pangolin coronavirus 5Kbp
SRX6893154 20-Sep-2019	Homo sapiens: 0.02%	Hominoidea 356003	Pangolin coronavirus 154Kbp
SRX6893153 20-Sep-2019	Homo sapiens: 0.01%	Homo sapiens 162180	Pangolin coronavirus 41Kbp
SRX6893152 20-Sep-2019	Manis javanica: 2.87% Euarchontoglires: 1.37%	N/D	N/D
SRX6893151 20-Sep-2019	Manis javanica: 7.47%	N/D	N/D
SRX6893150 20-Sep-2019	Boreoeutheria: 1.91%	N/D	N/D
SRX6893149 20-Sep-2019	Manis javanica: 1%	Simiiformes 313069	N/D
SRX6893148 20-Sep-2019	Manis javanica: 0.4%	Catarrhini 194320	N/D
SRX6893147 20-Sep-2019	Manis javanica: 2.71%	Catarrhini 69937	N/D
SRX6893146 20-Sep-2019	Boreoeutheria: 1.72%	Hominoidea 231755	N/D
SRX6893145 20-Sep-2019	Homininae: 0.27% Manis javanica: 1.01%	Homininae 2536765	N/D
SRX6893144 20-Sep-2019	Manis javanica: 0.62%	Hominoidea 166628	N/D
SRX6893143 20-Sep-2019	Manis javanica: 1.63%	N/D	N/D
SRX6893142	Manis javanica: 1.28%	Simiiformes 57084	N/D

20-Sep-2019			
SRX6893141 20-Sep-2019	Boreoeutheria: 1.41%	N/D	N/D
SRX6893140 20-Sep-2019	Boreoeutheria: 1.56%	N/D	N/D
SRX6893139 20-Sep-2019	Homo sapiens: 0.01%	Homo sapiens 491120	Pangolin coronavirus 2Kbp
SRX6893138 20-Sep-2019	Boreoeutheria: 1.67%	Homininae 2761176	N/D
SRX7756769 18-Feb-2020	Homo sapiens: 0.03%	Homo sapiens 5457929	Bat SARS-like coronavirus 2Kbp Wuhan seafood market pneumonia virus 2Kbp
SRX7756766 18-Feb-2020	Manis javanica: 78.6%	Cercopithecidae 3116	Betacoronavirus 2Kbp**
SRX7756765 18-Feb-2020	Manis javanica: 87.17%	Cercopithecinae 11339	N/D****
SRX7756764 18-Feb-2020	Manis javanica: 48.39%	Cercopithecidae 22600	N/D
SRX7756763 18-Feb-2020	Manis javanica: 94.95%	Cercopithecidae 5076	N/D
SRX7756762 18-Feb-2020	Manis javanica: 95.37%	Catarrhini* 2831	Nidovirales 0Kbp
SRX7756761 18-Feb-2020	Manis javanica: 13.63%	Chlorocebus sabaeus 498506	N/D
SRX7732094 15-Feb-2020	N/A***	N/A	Pangolin 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 coronavirus-related viruses. An 100% full-length match that does not map to non-primates confirms Authenticity of read.

select all 100 sequences selected

[Graphics](#) [Distance tree of results](#)

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/> SRX7756762	279	1047	0%	4e-68	100.00%	SRA-SRR11119766.160125840.2
<input checked="" type="checkbox"/> SRX7756762	279	1366	0%	4e-68	100.00%	SRA-SRR11119766.138036805.1
<input checked="" type="checkbox"/> SRX7756762	279	967	0%	4e-68	100.00%	SRA-SRR11119766.101239747.1
<input checked="" type="checkbox"/> SRX7756762	279	1624	0%	4e-68	100.00%	SRA-SRR11119766.46413326.2

Chlorocebus sabaeus isolate 1994-021 unplaced genomic sca ...
dna
1339488
[Distance tree of results](#) [MSA viewer](#) [?](#)

Fig.2a Specific BLAST analysis on the PRINA607174 dataset, [SRX7756762](#), 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

[GenBank](#) [Graphics](#) [Distance tree of results](#)

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/> Macaca mulatta isolate Rh22777_5890-1b major histocompatibility complex genomic sequence	279	279	100%	2e-71	100.00%	KT332833.1
<input checked="" type="checkbox"/> Macaca mulatta isolate Rh22335_5775-3 major histocompatibility complex genomic sequence	279	279	100%	2e-71	100.00%	KT332608.1
<input checked="" type="checkbox"/> Macaca mulatta isolate Rh22335_5725-2 major histocompatibility complex genomic sequence	279	279	100%	2e-71	100.00%	KT332521.1
<input checked="" type="checkbox"/> Macaca mulatta isolate Rh22335_5702-1a major histocompatibility complex genomic sequence	279	279	100%	2e-71	100.00%	KT332463.1

>gn|SRA|SRR11119766.160125840.2 160125840 (Biological)
 TAATCCTTTGGGTATATACCCAGTAATGGGATGGCTGGGTCATATGGTACATCTAGTTCF
 AGATCCTTGAGGAATCGCCATACTGTTTCCATTAATGGTTGAAGTACCAATCCAC
 CAACAGTGTAAAGTGTTCCTATTTCTCCAC

<input checked="" type="checkbox"/> Uncultured organism clone VC1C968TR genomic sequence	230	230	100%	2e-56	94.04%	GQ879596.1
<input checked="" type="checkbox"/> Synthetic construct Pan troglodytes LINE-1L1Pt retrotransposon tagged with EGFP sequence	219	219	100%	4e-53	92.72%	KF661301.1
<input checked="" type="checkbox"/> Human artificial chromosome vector 21HAC4 DNA, isolated from the long arm, clone: YAC/BAC#26-2	219	219	100%	4e-53	92.72%	AB553834.1
<input checked="" type="checkbox"/> Mus musculus NOD-derived CD11c +ve dendritic cells cDNA, RIKEN full-length enriched library, clone F630221F08 product un	204	204	86%	1e-48	94.66%	AK171052.1
<input checked="" type="checkbox"/> Mus musculus bone marrow macrophage cDNA, RIKEN full-length enriched library, clone G530008A19 product hypothetical pr	204	204	86%	1e-48	94.66%	AK149653.1
<input checked="" type="checkbox"/> Ralstonia solanacearum genome assembly 9 genomes_chromosome_V	202	202	100%	4e-48	90.73%	LN899823.1
<input checked="" type="checkbox"/> Canis lupus familiaris breed Labrador retriever chromosome 06a	154	3044	98%	1e-33	86.43%	CP050586.1
<input checked="" type="checkbox"/> Canis lupus familiaris breed Labrador retriever chromosome 04a	154	4569	100%	1e-33	85.23%	CP050572.1
<input checked="" type="checkbox"/> Canis lupus familiaris breed Labrador retriever chromosome 06b	154	3042	98%	1e-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

[Graphics](#) [Distance tree of results](#)

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/> SRX7756766	279	279	0%	5e-67	100.00%	SRA-SRR11119762.269072261.2
<input checked="" type="checkbox"/> SRX7756766	279	279	0%	5e-67	100.00%	SRA-SRR11119762.255768440.2
<input checked="" type="checkbox"/> SRX7756766	279	279	0%	5e-67	100.00%	SRA-SRR11119762.255768440.1
<input checked="" type="checkbox"/> SRX7756766	279	279	0%	5e-67	100.00%	SRA-SRR11119762.255318754.2
<input checked="" type="checkbox"/> SRX7756766	279	279	0%	5e-67	100.00%	SRA-SRR11119762.254520929.1
<input checked="" type="checkbox"/> SRX7756766	279	6344	0%	5e-67	100.00%	SRA-SRR11119762.251645135.1
<input checked="" type="checkbox"/> SRX7756766	279	279	0%	5e-67	100.00%	SRA-SRR11119762.234036838.2
<input checked="" type="checkbox"/> SRX7756766	279	279	0%	5e-67	100.00%	SRA-SRR11119762.211208832.2
<input checked="" type="checkbox"/> SRX7756766	279	9108	0%	5e-67	100.00%	SRA-SRR11119762.199583624.1
<input checked="" type="checkbox"/> SRX7756766	279	279	0%	5e-67	100.00%	SRA-SRR11119762.198110623.2
<input checked="" type="checkbox"/> SRX7756766	279	279	0%	5e-67	100.00%	SRA-SRR11119762.196936636.2
<input checked="" type="checkbox"/> SRX7756766	279	279	0%	5e-67	100.00%	SRA-SRR11119762.196936636.1
<input checked="" type="checkbox"/> SRX7756766	279	279	0%	5e-67	100.00%	SRA-SRR11119762.133631622.2
<input checked="" type="checkbox"/> SRX7756766	279	279	0%	5e-67	100.00%	SRA-SRR11119762.108819247.2

Description: Macaca mulatta isolate AG07107 chromosome 3 genomic sca ...
 Molecule type: dna
 Query Length: 17855752
 Other reports: [Distance tree of results](#) [MSA viewer](#) [?](#)

Fig.3a Specific BLAST analysis of [SRX7756766](#) revealed large amount of 100% full-length matches with Macaca Mulatta.

<input checked="" type="checkbox"/>	Macaca mulatta Major Histocompatibility Complex BAC MMU37002_complete sequence	279	279	100%	2e-71	100.00%	AC148706.1
<input checked="" type="checkbox"/>	Macaca mulatta Major Histocompatibility Complex BAC MMU122H23_complete sequence	279	521	100%	2e-71	100.00%	AC148677.1
<input checked="" type="checkbox"/>	Macaca mulatta Major Histocompatibility Complex BAC MMU065H09_complete sequence	279	279	100%	2e-71	100.00%	AC148671.1
<input checked="" type="checkbox"/>	Macaca mulatta Major Histocompatibility Complex BAC MMU038L02_complete sequence	279	279	100%	2e-71	100.00%	AC148668.1
<input checked="" type="checkbox"/>	Papio anubis clone rp41-22m16_complete sequence	279	554	100%	2e-71	100.00%	AC113268.8
<input checked="" type="checkbox"/>	Papio anubis clone rp41-280n2_complete sequence	279	465	100%	2e-71	100.00%	AC091778.13
<input checked="" type="checkbox"/>	Papio anubis clone rp41-5m22_complete sequence	279	279	100%	2e-71	100.00%	AC136143.4
<input checked="" type="checkbox"/>	Papio anubis clone rp41-192i11_complete sequence	279	526	100%	2e-71	100.00%	AC091671.28
<input checked="" type="checkbox"/>	Macaca mulatta chromosome 9 CH250-18D2_complete sequence	279	548	100%	2e-71	100.00%	CT573219.3
<input checked="" type="checkbox"/>	Rhesus Macaque CHR4 BAC CH250-23P16 (Children's Hospital Oakland Research Institute Rhesus macaque Adult Male BAC Libr	279	279	100%	2e-71	100.00%	AC169807.2
<input checked="" type="checkbox"/>	Rhesus Macaque CHR4 BAC CH250-476F18 (Children's Hospital Oakland Research Institute Rhesus macaque Adult Male BAC Libr	279	279	100%	2e-71	100.00%	AC171646.5
<input checked="" type="checkbox"/>	Chlorocebus aethiops BAC clone CH252-163P9 from chromosome 5_complete sequence	278	556	99%	7e-71	100.00%	AC239684.4
<input checked="" type="checkbox"/>	MACACA MULATTA BAC clone CH250-192J17 from chromosome unknown_complete sequence	278	552	100%	7e-71	100.00%	AC215693.3
<input checked="" type="checkbox"/>	Macaca mulatta isolate Rh22335_5702-1a major histocompatibility complex genomic sequence	274	274	100%	9e-70	99.34%	KT332463.1
<input checked="" type="checkbox"/>	Macaca mulatta isolate Rh9_6570-3 major histocompatibility complex genomic sequence	274	274	100%	9e-70	99.34%	KT331777.1
<input checked="" type="checkbox"/>	Macaca mulatta isolate Rh9_6550-1b major histocompatibility complex genomic sequence	274	274	100%	9e-70	99.34%	KT331733.1
<input checked="" type="checkbox"/>	Macaca mulatta isolate Rh9_6526-2 major histocompatibility complex genomic sequence	274	274	100%	9e-70	99.34%	KT331675.1
<input checked="" type="checkbox"/>	Macaca mulatta isolate Rh18665_5547-1b major histocompatibility complex genomic sequence	274	274	100%	9e-70	99.34%	KT329509.1
<input checked="" type="checkbox"/>	Macaca mulatta isolate Rh23717 clone 4777 major histocompatibility complex-B genomic sequence	274	274	100%	9e-70	99.34%	KJ913523.1
<input checked="" type="checkbox"/>	Macaca mulatta isolate Rh23108 clone 4769-2 major histocompatibility complex-B genomic sequence	274	274	100%	9e-70	99.34%	KJ913420.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 18	202	4103	96%	4e-48	91.78%	CP034496.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 19	196	2020	96%	2e-46	92.09%	CP034522.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 19	196	2130	96%	2e-46	92.09%	CP034497.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 16	196	3654	96%	2e-46	91.61%	CP034494.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 15	196	4994	97%	2e-46	92.09%	CP034493.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 14	196	7731	96%	2e-46	92.09%	CP034492.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 13	191	7988	97%	9e-45	90.91%	CP034516.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome Y	191	2781	96%	9e-45	91.37%	CP034510.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 20	191	4517	96%	9e-45	91.37%	CP034499.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 13	191	7988	97%	9e-45	90.91%	CP034491.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 21	185	1053	96%	4e-43	90.65%	CP034500.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 17	185	2450	96%	4e-43	90.65%	CP034495.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 22	183	933	96%	2e-42	90.58%	CP034501.1

Fig.3b BLASTing such matches gives 1005 matches to only Primates, and with no matches outside of Primates. This indicate that [SRX7756766](#) also contained significant amount of material derived from primates.

Results for

Program **BLASTN** [Citation](#)

Database **nt** [See details](#)

Query ID **lc|Query_13045**

Description **gn|SRA|SRR11119762.182596220.2.182596220 (Biological)**

Molecule type **dna**

Query Length **151**

Other reports [Distance tree of results](#)

Type common name, binomial, taxid or group name

[+ Add organism](#)

Percent Identity to

E value to

Query Coverage to

Filter **Reset**

Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments Download Manage Columns Show 1000

select all 0 sequences selected

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input type="checkbox"/> PREDICTED: Macaca mulatta LIM domain kinase 2 (LIMK2), transcript variant X3 mRNA	279	279	100%	2e-71	100.00%	XM_015150059.2
<input type="checkbox"/> PREDICTED: Macaca mulatta LIM domain kinase 2 (LIMK2), transcript variant X2 mRNA	279	279	100%	2e-71	100.00%	XM_015150058.2
<input type="checkbox"/> PREDICTED: Macaca mulatta LIM domain kinase 2 (LIMK2), transcript variant X1 mRNA	279	279	100%	2e-71	100.00%	XM_015150057.2
<input type="checkbox"/> Papio anubis anubis NIPSNAP1 protein (NIPSNAP1), gene, partial cds, and merlin (NF2) and S-100/CaBP-type calcium binding domain	279	5181	100%	2e-71	100.00%	AH012454.2
<input type="checkbox"/> PREDICTED: Macaca fascicularis LIM domain kinase 2 (LIMK2), transcript variant X3 mRNA	279	279	100%	2e-71	100.00%	XM_015457315.1
<input type="checkbox"/> PREDICTED: Macaca fascicularis LIM domain kinase 2 (LIMK2), transcript variant X2 mRNA	279	279	100%	2e-71	100.00%	XM_005567555.2
<input type="checkbox"/> PREDICTED: Macaca fascicularis LIM domain kinase 2 (LIMK2), transcript variant X1 mRNA	279	279	100%	2e-71	100.00%	XM_005567554.2
<input type="checkbox"/> Chlorocebus aethiops BAC clone CH252-146I12 from chromosome 6_complete sequence	279	3318	100%	2e-71	100.00%	AC241602.2
<input type="checkbox"/> Chlorocebus aethiops BAC clone CH252-138D20 from chromosome 13_complete sequence	279	681	100%	2e-71	100.00%	AC239463.3
<input type="checkbox"/> Macaca mulatta BAC CH250-74N24 (Children's Hospital Oakland Research Institute Rhesus macaque Adult Male BAC Library) complet	279	6774	100%	2e-71	100.00%	AC204493.6
<input type="checkbox"/> Chlorocebus aethiops BAC clone CH252-257N12 from chromosome 6_complete sequence	274	6168	100%	9e-70	99.34%	AC241575.3
<input type="checkbox"/> Chlorocebus aethiops BAC clone CH252-518J9 from chromosome 6_complete sequence	274	4718	100%	9e-70	99.34%	AC241458.2
<input type="checkbox"/> Chlorocebus aethiops BAC clone CH252-124F12 from chromosome 6_complete sequence	274	2252	100%	9e-70	99.34%	AC241509.3
<input type="checkbox"/> Chlorocebus aethiops BAC clone CH252-175F15 from chromosome 6_complete sequence	274	4253	100%	9e-70	99.34%	AC241845.3
<input type="checkbox"/> Chlorocebus aethiops BAC clone CH252-57Q21 from chromosome 6_complete sequence	274	2432	100%	9e-70	99.34%	AC241469.3

Fig.3C Presence of Primate-derived mRNA reads in [SRX7756766](#) confirms the Primate origin of these reads.

select all 100 sequences selected [Graphics](#) [Distance tree of results](#)

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/> SRX7756769	278	278	0%	9e-69	100.00%	SRA-SRR11119759.99831231.2
<input checked="" type="checkbox"/> SRX7756769	278	278	0%	9e-69	100.00%	SRA-SRR11119759.99831231.1
<input checked="" type="checkbox"/> SRX7756769	278	4814	1%	9e-69	100.00%	SRA-SRR11119759.88019245.2
<input checked="" type="checkbox"/> SRX7756769	278	5178	2%	9e-69	100.00%	SRA-SRR11119759.82130976.2
<input checked="" type="checkbox"/> SRX7756769	278	278	0%	9e-69	100.00%	SRA-SRR11119759.70689253.2
<input checked="" type="checkbox"/> SRX7756769	278	278	0%	9e-69	100.00%	SRA-SRR11119759.70689253.1
<input checked="" type="checkbox"/> SRX7756769	278	278	0%	9e-69	100.00%	SRA-SRR11119759.57405658.2
<input checked="" type="checkbox"/> SRX7756769	278	278	0%	9e-69	100.00%	SRA-SRR11119759.57405658.1

[AC073210.8](#)
Homo sapiens BAC clone RP11-460N20 from 7, complete seq ...
nucleic acid
203396

Fig.4a Similarly, [SRX7756769](#) contained large amount of reads that are 100% full-length matches to Human genomic DNA.

select all 0 sequences selected [GenBank](#) [Graphics](#) [Distance tree of results](#)

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input type="checkbox"/> Homo sapiens chromosome 22 clone ABC11_000047178300_E22_complete_sequence	278	456	100%	6e-71	100.00%	AC279316.1
<input type="checkbox"/> Homo sapiens actin related protein 2 pseudogene (LOC284441) on chromosome 19	278	278	100%	6e-71	100.00%	NG_022927.2
<input type="checkbox"/> Homo sapiens TBC1 domain containing kinase (TBCK1)_RefSeqGene on chromosome 4	278	2140	100%	6e-71	100.00%	NG_034057.3
<input type="checkbox"/> Homo sapiens chromosome 15 clone VMRC59-280I06_complete_sequence	278	2291	100%	6e-71	100.00%	AC279072.1
<input type="checkbox"/> Homo sapiens chromosome 2 clone VMRC59-389K09_complete_sequence	278	3905	100%	6e-71	100.00%	AC279037.1
<input type="checkbox"/> Homo sapiens chromosome 15 clone VMRC59-359A02_complete_sequence	278	3589	100%	6e-71	100.00%	AC278991.1
<input type="checkbox"/> Homo sapiens chromosome 16 clone VMRC59-453R14_complete_sequence	278	2239	100%	6e-71	100.00%	AC278975.1

Description gn|SRA|SRR11119759.88019245.2 88019245 (Biological)
Molecule type dna
Query Length 150
Other reports [Distance tree of results](#) [MSA viewer](#)

Query ID Icl|Query_61480
Description gn|SRA|SRR11119759.70689253.1 70689253 (Biological)
Molecule type dna
Query Length 150
Other reports [Distance tree of results](#)

Percent Identity to **E value** to **Query Coverage** to

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Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/> PREDICTED: Pan paniscus endogenous retrovirus group 3 member 1_envelope (ERV3-1)_mRNA	278	278	100%	7e-71	100.00%	XM_034964253.1
<input checked="" type="checkbox"/> Homo sapiens endogenous retrovirus group 3 member 1_envelope (ERV3-1)_transcript variant 1_mRNA	278	278	100%	7e-71	100.00%	NM_001007253.4
<input checked="" type="checkbox"/> PREDICTED: Pan troglodytes endogenous retrovirus group 3 member 1_envelope (ERV3-1)_transcript variant X2_mRNA	278	278	100%	7e-71	100.00%	XM_024357564.1
<input checked="" type="checkbox"/> PREDICTED: Pan troglodytes endogenous retrovirus group 3 member 1_envelope (ERV3-1)_transcript variant X1_mRNA	278	278	100%	7e-71	100.00%	XM_016956774.2
<input checked="" type="checkbox"/> Synthetic construct DNA clone: pFN21AE1261_Homo sapiens ERV3 gene for endogenous retroviral sequence 3_without stop codi	278	278	100%	7e-71	100.00%	AB590999.1
<input checked="" type="checkbox"/> Homo sapiens cDNA FLJ60255 complete cds, highly similar to HERV-R_7q21.2 provirus ancestral Env polyprotein precursor	278	278	100%	7e-71	100.00%	AK295189.1
<input checked="" type="checkbox"/> Pan troglodytes BAC clone CH251-623C19 from chromosome 7_complete sequence	278	278	100%	7e-71	100.00%	AC184799.2
<input checked="" type="checkbox"/> Pan troglodytes BAC clone CH251-565C10 from chromosome 7_complete sequence	278	278	100%	7e-71	100.00%	AC148313.3
<input checked="" type="checkbox"/> Pan troglodytes endogenous retrovirus group 3 member 1_envelope (ERV3-1)_mRNA	278	278	100%	7e-71	100.00%	NM_001135588.1
<input checked="" type="checkbox"/> Homo sapiens BAC clone RP11-460N20 from 7_complete sequence	278	278	100%	7e-71	100.00%	AC073210.8
<input checked="" type="checkbox"/> Homo sapiens cDNA FLJ23884 fis clone LNG13819_highly similar to Human endogenous retrovirus ERV3_pol-env-3LTR region	278	278	100%	7e-71	100.00%	AK074464.1
<input checked="" type="checkbox"/> Human endogenous retrovirus ERV3_pol-env-3LTR region	278	278	100%	7e-71	100.00%	M12140.1
<input checked="" type="checkbox"/> Nomascus leucogenys endogenous retrovirus group 3 member 1_envelope (ERV3-1)_mRNA	272	272	100%	3e-69	99.33%	NM_001308194.1
<input checked="" type="checkbox"/> Gibbon endogenous retrovirus proviral envR gene for R envelope protein	272	272	100%	3e-69	99.33%	AJ862653.1
<input checked="" type="checkbox"/> PREDICTED: Ptilocotobus leproscoteles endogenous retrovirus group 3 member 1_envelope (ERV3-1)_transcript variant X1_mRNA	267	267	100%	2e-67	98.67%	XM_023196289.2
<input checked="" type="checkbox"/> PREDICTED: Papio anubis endogenous retrovirus group 3 member 1_envelope (ERV3-1)_transcript variant X2_mRNA	267	267	100%	2e-67	98.67%	XM_031662056.1
<input checked="" type="checkbox"/> PREDICTED: Papio anubis endogenous retrovirus group 3 member 1_envelope (ERV3-1)_transcript variant X1_mRNA	267	267	100%	2e-67	98.67%	XM_017956880.3
<input checked="" type="checkbox"/> PREDICTED: Rhinopithecus roxellana endogenous retrovirus group 3 member 1_envelope (ERV3-1)_transcript variant X2_mRNA	267	267	100%	2e-67	98.67%	XM_030932630.1
<input checked="" type="checkbox"/> PREDICTED: Rhinopithecus roxellana endogenous retrovirus group 3 member 1_envelope (ERV3-1)_transcript variant X1_mRNA	267	267	100%	2e-67	98.67%	XM_030932629.1

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.

Description
 Molecule type
 Query Length
 Other reports [Distance tree of results](#)

to to to

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select all 1 sequences selected GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/> Synthetic construct DNA clone pFN21AF1261_Homo sapiens ERV3 gene for endogenous retroviral sequence 3 without stop codon in	278	278	100%	7e-71	100.00%	AB590999.1

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

select all 100 sequences selected Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/> SRX6893156	278	278	0%	2e-69	100.00%	SRA-SRR10168375.5045789.1
<input checked="" type="checkbox"/> SRX6893156	278	278	0%	2e-69	100.00%	SRA-SRR10168375.5964.1

Description
 Molecule type
 Query Length
 Other reports [Distance tree of results](#) [MSA viewer](#)

Fig.5a [SRX6893156](#) also returned 100% matched results from the human Genome.

select all 14 sequences selected GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/> Homo sapiens BAC clone RP11-460N20 from 7, complete sequence	278	278	100%	6e-71	100.00%	AC073210.8
<input checked="" type="checkbox"/> Pan troglodytes BAC clone CH251-623C19 from chromosome 7, complete sequence	267	267	100%	1e-67	98.67%	AC184799.2
<input checked="" type="checkbox"/> Pan troglodytes BAC clone CH251-2Q15 from chromosome 7, complete sequence	267	267	100%	1e-67	98.67%	AC174000.3
<input checked="" type="checkbox"/> Pan troglodytes BAC clone CH251-565C10 from chromosome 7, complete sequence	267	267	100%	1e-67	98.67%	AC148313.3

Description
 Molecule type
 Query Length
 Other reports [Distance tree of results](#) [MSA viewer](#)

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Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/> Homo sapiens lncAB572.1 lncRNA gene, complete sequence	278	278	100%	7e-71	100.00%	MK280613.1
<input checked="" type="checkbox"/> Pan troglodytes chromosome 2 clone CH251-60P06, complete sequence	278	278	100%	7e-71	100.00%	AC279084.1
<input checked="" type="checkbox"/> Pan troglodytes chromosome 2 clone CH251-17Q22, complete sequence	278	278	100%	7e-71	100.00%	AC278930.1
<input checked="" type="checkbox"/> Pan troglodytes chromosome 2 clone CH251-108A24, complete sequence	278	278	100%	7e-71	100.00%	AC278921.1
<input checked="" type="checkbox"/> Eukaryotic synthetic construct chromosome 21	278	7938	100%	7e-71	100.00%	CP034500.1

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

Description:
 Molecule type:
 Query Length:
 Other reports: [Distance tree of results](#)

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Sequences producing significant alignments | Download | Manage Columns | Show: 1000

select all 14 sequences selected

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 21	278	7938	100%	7e-71	100.00%	CP034500.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 13	267	14570	100%	1e-67	98.67%	CP034516.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 16	267	10333	100%	1e-67	98.67%	CP034494.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 15	267	9021	100%	1e-67	98.67%	CP034493.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 13	267	14570	100%	1e-67	98.67%	CP034491.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 18	261	15047	100%	7e-66	98.00%	CP034496.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 17	261	6545	100%	7e-66	98.00%	CP034495.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 20	219	7949	98%	4e-53	93.79%	CP034499.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 19	209	3521	96%	3e-50	93.10%	CP034522.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 19	209	3766	96%	3e-50	93.10%	CP034497.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 22	207	2291	96%	9e-50	92.47%	CP034501.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 14	207	13851	96%	9e-50	92.47%	CP034492.1
<input checked="" type="checkbox"/>	Gossypium hirsutum clone NBRI_GE27093 microsatellite sequence	189	189	96%	3e-44	90.41%	JX591845.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome Y	180	2399	96%	2e-41	89.44%	CP034510.1

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

select all 100 sequences selected

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/>	SRX6893155	278	278	0%	2e-69	100.00%	SRA_SRR10168376.17339580.1
<input checked="" type="checkbox"/>	SRX6893155	278	278	0%	2e-69	100.00%	SRA_SRR10168376.17013625.2
<input checked="" type="checkbox"/>	SRX6893155	278	278	0%	2e-69	100.00%	SRA_SRR10168376.17013625.1
<input checked="" type="checkbox"/>	SRX6893155	278	278	0%	2e-69	100.00%	SRA_SRR10168376.16930714.2
<input checked="" type="checkbox"/>	SRX6893155	278	278	0%	2e-69	100.00%	SRA_SRR10168376.16930714.1
<input checked="" type="checkbox"/>	SRX6893155	278	278	0%	2e-69	100.00%	SRA_SRR10168376.15267479.2
<input checked="" type="checkbox"/>	SRX6893155	278	278	0%	2e-69	100.00%	SRA_SRR10168376.15267479.1
<input checked="" type="checkbox"/>	SRX6893155	278	278	0%	2e-69	100.00%	SRA_SRR10168376.13985702.2
<input checked="" type="checkbox"/>	SRX6893155	278	278	0%	2e-69	100.00%	SRA_SRR10168376.13985702.1
<input checked="" type="checkbox"/>	SRX6893155	278	278	0%	2e-69	100.00%	SRA_SRR10168376.13353823.2
<input checked="" type="checkbox"/>	SRX6893155	278	278	0%	2e-69	100.00%	SRA_SRR10168376.13353823.1
<input checked="" type="checkbox"/>	SRX6893155	278	278	0%	2e-69	100.00%	SRA_SRR10168376.11109740.1
<input checked="" type="checkbox"/>	SRX6893155	278	278	0%	2e-69	100.00%	SRA_SRR10168376.9343845.2
<input checked="" type="checkbox"/>	SRX6893155	278	278	0%	2e-69	100.00%	SRA_SRR10168376.9232549.2

Description:
 Molecule type:
 Query Length:
 Other reports: [Distance tree of results](#) | [MSA viewer](#)

Fig.6a Similarly, BLAST research on [SRX6893155](#) gives large number of full length 100% matches to the human genome.

select all 57 sequences selected

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/>	Homo sapiens FOSMID clone ABC13-48840700E15 from chromosome 7, complete sequence	278	278	100%	6e-71	100.00%	AC242196.4
<input checked="" type="checkbox"/>	Pan troglodytes BAC clone CH251-340124 from chromosome 7, complete sequence	278	278	100%	6e-71	100.00%	AC185242.2
<input checked="" type="checkbox"/>	Pan troglodytes BAC clone CH251-623C19 from chromosome 7, complete sequence	278	278	100%	6e-71	100.00%	AC184799.2
<input checked="" type="checkbox"/>	Pan troglodytes BAC clone CH251-114G16 from chromosome 7, complete sequence	278	278	100%	6e-71	100.00%	AC183835.2
<input checked="" type="checkbox"/>	Pan troglodytes BAC clone CH251-2015 from chromosome 7, complete sequence	278	278	100%	6e-71	100.00%	AC174000.3
<input checked="" type="checkbox"/>	Homo sapiens BAC clone RP11-479O9 from 7, complete sequence	278	278	100%	6e-71	100.00%	AC073107.7
<input checked="" type="checkbox"/>	Pan troglodytes BAC clone CH251-565C10 from chromosome 7, complete sequence	278	278	100%	6e-71	100.00%	AC148313.3
<input checked="" type="checkbox"/>	Homo sapiens BAC clone RP11-460N20 from 7, complete sequence	278	278	100%	6e-71	100.00%	AC073210.8
<input checked="" type="checkbox"/>	PREDICTED: Cebus capucinus imitator small integral membrane protein 11A (SMIM11A), transcript variant X6, mRNA	87.9	87.9	49%	1e-13	88.00%	XM_017526193.1

Description [gn|SRA|SRR10168376.15267479.2 15267479 \(Biological\)](#)

Molecule type dna

Query Length 150

Other reports [Distance tree of results](#) [MSA viewer](#) [?](#)

Description [gn|SRA|SRR10168376.13985702.1 13985702 \(Biological\)](#)

Molecule type dna

Query Length 150

Other reports [Distance tree of results](#) [?](#)

to to to

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select all 87 sequences selected

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/>	Homo sapiens BAC clone RP11-460N20 from 7, complete sequence	278	278	100%	7e-71	100.00%	AC073210.8
<input checked="" type="checkbox"/>	Pan troglodytes BAC clone CH251-623C19 from chromosome 7, complete sequence	272	272	100%	3e-69	99.33%	AC184799.2
<input checked="" type="checkbox"/>	Pan troglodytes BAC clone CH251-565C10 from chromosome 7, complete sequence	272	272	100%	3e-69	99.33%	AC148313.3
<input checked="" type="checkbox"/>	Macaca mulatta BAC CH250-206B6 (Children's Hospital Oakland Research Institute Rhesus macaque Adult Male BAC Library) con	217	217	100%	2e-52	92.67%	AC210125.6
<input checked="" type="checkbox"/>	Homo sapiens tripartite motif containing 24 (TRIM24), RefSeqGene on chromosome 7	182	182	99%	6e-42	88.59%	NG_023286.1
<input checked="" type="checkbox"/>	Homo sapiens chromosome 7 clone RP11-199L18, complete sequence	182	182	99%	6e-42	88.59%	AC013429.12
<input checked="" type="checkbox"/>	Homo sapiens chromosome 7 clone RP11-256C24, complete sequence	182	182	99%	6e-42	88.59%	AC008265.15
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome Y	176	353	99%	3e-40	87.92%	CP034510.1

select all 3 sequences selected

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome Y	176	353	99%	3e-40	87.92%	CP034510.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 14	143	143	99%	3e-30	84.00%	CP034492.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 16	111	111	72%	7e-21	85.45%	CP034494.1

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

Description [Homo sapiens BAC clone RP11-460N20 from 7, complete seq ...](#)

Molecule type nucleic acid

Query Length 203396

Other reports [Distance tree of results](#) [MSA viewer](#) [?](#)

Percent Identity to

E value to

Query Coverage to

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select all 100 sequences selected

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/>	SRX6893153	278	278	0%	2e-69	100.00%	SRA-SRR10168378.1832954.1

Fig.7a [SRX6893153](#) have also returned 100% match full-length read on this tiny part of the human genome.

Description
 Molecule type
 Query Length
 Other reports [Distance tree of results](#) [MSA viewer](#)

Percent Identity to
 E value to
 Query Coverage to

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Sequences producing significant alignments Download Manage Columns Show

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	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/>	Homo sapiens FOSMID clone ABC18-862111 from chromosome 7, complete sequence	278	278	100%	6e-71	100.00%	AC245205.1
<input checked="" type="checkbox"/>	Homo sapiens FOSMID clone ABC13-48840700E15 from chromosome 7, complete sequence	278	278	100%	6e-71	100.00%	AC242196.4
<input checked="" type="checkbox"/>	Homo sapiens BAC clone RP11-460N20 from 7, complete sequence	278	278	100%	6e-71	100.00%	AC073210.8
<input checked="" type="checkbox"/>	Pan troglodytes BAC clone CH251-487D11 from chromosome 7, complete sequence	272	272	100%	3e-69	99.33%	AC182733.3

select all *7 sequences selected* [GenBank](#) [Graphics](#) [Distance tree of results](#)

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 15	211	211	98%	7e-51	92.11%	CP034493.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 16	206	1214	98%	3e-49	91.45%	CP034494.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 13	200	200	98%	2e-47	90.79%	CP034516.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 21	200	401	98%	2e-47	90.79%	CP034500.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 13	200	200	98%	2e-47	90.79%	CP034491.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 18	195	195	96%	7e-46	90.60%	CP034496.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 17	195	195	98%	7e-46	90.13%	CP034495.1

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

Description
 Molecule type
 Query Length
 Other reports [Distance tree of results](#) [MSA viewer](#)

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	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/>	SRX6893154	278	556	0%	1e-69	100.00%	SRA-SRR10168377.16302266.1
<input checked="" type="checkbox"/>	SRX6893154	278	556	0%	1e-69	100.00%	SRA-SRR10168377.12014841.2
<input checked="" type="checkbox"/>	SRX6893154	278	556	0%	1e-69	100.00%	SRA-SRR10168377.12014841.1

Fig.8a Reads from the Human PMS1 gene is recovered from [SRX6893154](#) with a query sequence only 195834bp in length.

Description gn|SRA|SRR10168377.16302266.1 16302266 (Biological)
 Molecule type dna
 Query Length 150
 Other reports [Distance tree of results](#) ?

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select all 311 sequences selected

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/> Homo sapiens PMS1 homolog 2_mismatch repair system component pseudogene 8 (PMS2P8) on chromosome 7	278	278	100%	7e-71	100.00%	NG_006447.3
<input checked="" type="checkbox"/> Homo sapiens PMS1 homolog 2_mismatch repair system component pseudogene 10 (PMS2P10) on chromosome 7	278	278	100%	7e-71	100.00%	NG_023454.4
<input checked="" type="checkbox"/> Homo sapiens PMS1 homolog 2_mismatch repair system component pseudogene 6 (PMS2P6) on chromosome 7	278	278	100%	7e-71	100.00%	NG_006449.3
<input checked="" type="checkbox"/> Homo sapiens BAC clone CH17-264B6 from chromosome 7_complete sequence	278	1112	100%	7e-71	100.00%	AC211476.5
<input checked="" type="checkbox"/> Homo sapiens BAC clone CH17-220H16 from chromosome 7_complete sequence	278	1112	100%	7e-71	100.00%	AC211491.5
<input checked="" type="checkbox"/> Homo sapiens FOSMID clone ABC10-4551500F15 from chromosome 7_complete sequence	278	556	100%	7e-71	100.00%	AC244146.2
<input checked="" type="checkbox"/> Homo sapiens BAC clone RP11-450O3 from 7_complete sequence	278	556	100%	7e-71	100.00%	AC105418.5
<input checked="" type="checkbox"/> Homo sapiens BAC clone RP11-396K3 from 7_complete sequence	278	556	100%	7e-71	100.00%	AC006995.5
<input checked="" type="checkbox"/> Homo sapiens BAC clone RP11-313P13 from 7_complete sequence	278	556	100%	7e-71	100.00%	AC005488.2
<input checked="" type="checkbox"/> Homo sapiens chromosome 7 clone VMRC53-89F05_complete sequence	272	272	100%	3e-69	99.33%	AC278394.1
<input checked="" type="checkbox"/> Homo sapiens chromosome 7 clone VMRC62-404M06_complete sequence	272	272	100%	3e-69	99.33%	AC278331.1
<input checked="" type="checkbox"/> Homo sapiens chromosome 7 clone CH17-61K4_complete sequence	272	272	100%	3e-69	99.33%	AC270699.1
<input checked="" type="checkbox"/> Homo sapiens PMS8 mRNA (yeast mismatch repair gene PMS1 homolog) ,partial cds (C-terminal region)	248	248	100%	6e-62	96.67%	D38503.1
<input checked="" type="checkbox"/> Homo sapiens PMS1 homolog 2_mismatch repair system component (PMS2), RefSeqGene (LRG_161) on chromosome 7	244	244	100%	7e-61	96.00%	NG_008466.1
<input checked="" type="checkbox"/> Canis lupus familiaris breed Labrador retriever chromosome 06a	154	154	84%	1e-33	88.89%	CP050586.1
<input checked="" type="checkbox"/> Canis lupus familiaris breed Labrador retriever chromosome 06b	154	154	84%	1e-33	88.89%	CP050622.1
<input checked="" type="checkbox"/> Pipistrellus pipistrellus genome assembly chromosome 5	139	139	86%	3e-29	86.15%	LR862361.1
<input checked="" type="checkbox"/> Synthetic construct Homo sapiens clone ccsbBroadEn_14772 PMS2 gene, encodes complete protein	126	126	49%	3e-25	97.30%	KJ905275.1
<input checked="" type="checkbox"/> PREDICTED: Zalophus californianus PMS1 homolog 2_mismatch repair system component (PMS2), transcript variant X3, mRNA	124	124	54%	9e-25	93.90%	XM_027610028.2
<input checked="" type="checkbox"/> PREDICTED: Desmodus rotundus PMS1 homolog 2_mismatch repair system component (PMS2), transcript variant X2, mRNA	121	121	52%	1e-23	94.87%	XM_024576931.1
<input checked="" type="checkbox"/> PREDICTED: Desmodus rotundus PMS1 homolog 2_mismatch repair system component (PMS2), transcript variant X1, mRNA	121	121	52%	1e-23	94.87%	XM_024576930.1
<input checked="" type="checkbox"/> PREDICTED: Myotis lucifugus PMS1 homolog 2_mismatch repair system component (PMS2), transcript variant X4, mRNA	121	121	52%	1e-23	94.87%	XM_023761457.1
<input checked="" type="checkbox"/> PREDICTED: Myotis lucifugus PMS1 homolog 2_mismatch repair system component (PMS2), transcript variant X3, mRNA	121	121	52%	1e-23	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
 Molecule type nucleic acid
 Query Length 173967
 Other reports [Distance tree of results](#) [MSA viewer](#) ?

Percent Identity E value Query Coverage
 to to to
 Filter Reset

Descriptions Graphic Summary Alignments

Sequences producing significant alignments Download Manage Columns Show 100 ?

select all 100 sequences selected

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/> SRX6893139	278	278	0%	3e-69	100.00%	SRA_SRR10168392.39544030.1
<input checked="" type="checkbox"/> SRX6893139	278	278	0%	3e-69	100.00%	SRA_SRR10168392.28917809.1
<input checked="" type="checkbox"/> SRX6893139	278	278	0%	3e-69	100.00%	SRA_SRR10168392.14357888.1
<input checked="" type="checkbox"/> SRX6893139	278	278	0%	3e-69	100.00%	SRA_SRR10168392.2548655.2

Fig.9a Similarly, multiple 100% match Full length reads were obtained from [SRX6893139](#). 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)
Molecule type dna
Query Length 150
Other reports [Distance tree of results](#) [MSA viewer](#) ?

Percent Identity to E value to Query Coverage to
Filter Reset

Descriptions Graphic Summary Alignments Taxonomy

Sequences producing significant alignments Download Manage Columns Show 1000 ?

select all 66 sequences selected GenBank Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per Ident	Accession
<input checked="" type="checkbox"/> Homo sapiens zinc finger protein 316 (ZNF316) mRNA	278	278	100%	6e-71	100.00%	NM_001278559.2
<input checked="" type="checkbox"/> PREDICTED: Homo sapiens zinc finger protein 316 (ZNF316) transcript variant X3 mRNA	278	278	100%	6e-71	100.00%	XM_024446619.1
<input checked="" type="checkbox"/> PREDICTED: Homo sapiens zinc finger protein 316 (ZNF316) transcript variant X2 mRNA	278	278	100%	6e-71	100.00%	XM_024446618.1
<input checked="" type="checkbox"/> PREDICTED: Homo sapiens zinc finger protein 316 (ZNF316) transcript variant X1 mRNA	278	278	100%	6e-71	100.00%	XM_006715630.4
<input checked="" type="checkbox"/> Homo sapiens BAC clone RP11-611L7 from 7 complete sequence	278	278	100%	6e-71	100.00%	AC073343.6
<input checked="" type="checkbox"/> PREDICTED: Pongo abelii zinc finger protein 316 (ZNF316) mRNA	272	272	100%	3e-69	99.33%	XM_024250011.1
<input checked="" type="checkbox"/> PREDICTED: Ornycteropus afer zinc finger protein 316 (ZNF316) mRNA	150	150	100%	2e-32	84.67%	XM_007942750.1
<input checked="" type="checkbox"/> PREDICTED: Miniopoterus natalensis zinc finger protein 853 (ZNF853) mRNA	145	145	100%	7e-31	84.00%	XM_016213621.1
<input checked="" type="checkbox"/> PREDICTED: Ochotona princeps zinc finger protein 316 (ZNF316) mRNA	145	145	98%	7e-31	84.35%	XM_012930995.1
<input checked="" type="checkbox"/> Pipistrellus pipistrellus genome assembly, chromosome_5	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 are Human/Hominid-derived contamination in [SRX6893139](#).

Description Homo sapiens chromosome 9, clone hRPK.202_H_3, comple ...
Molecule type nucleic acid
Query Length 187174
Other reports [Distance tree of results](#) [MSA viewer](#) ?

Percent Identity to E value to Query Coverage to
Filter Reset

Descriptions Graphic Summary Alignments

Sequences producing significant alignments Download Manage Columns Show 100 ?

select all 100 sequences selected Graphics Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per Ident	Accession
<input checked="" type="checkbox"/> SRX6893157	278	278	0%	8e-70	100.00%	SRA:SRR10168374.7906491.2

Fig.10a One read is also recovered from [SRX6893157](#). From a query sequence only 187174nt in length.

<input checked="" type="checkbox"/> PREDICTED: Homo sapiens formin binding protein 1 (FNBP1) transcript variant X13 mRNA	278	278	100%	6e-71	100.00%	XM_005251824.2
<input checked="" type="checkbox"/> PREDICTED: Homo sapiens formin binding protein 1 (FNBP1) transcript variant X4 mRNA	278	278	100%	6e-71	100.00%	XM_011518402.1
<input checked="" type="checkbox"/> PREDICTED: Homo sapiens formin binding protein 1 (FNBP1) transcript variant X3 mRNA	278	278	100%	6e-71	100.00%	XM_011518401.1
<input checked="" type="checkbox"/> Homo sapiens formin binding protein 1 (FNBP1) RefSeqGene on chromosome_9	278	278	100%	6e-71	100.00%	NG_033946.1
<input checked="" type="checkbox"/> Homo 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%	AK023681.1
<input checked="" type="checkbox"/> Human DNA sequence from clone RP11-138E2 on chromosome 9q34.11-34.3 complete sequence	278	278	100%	6e-71	100.00%	AL136141.13
<input checked="" type="checkbox"/> Homo sapiens formin-binding protein 17 (FBP17) mRNA, partial cds	278	278	100%	6e-71	100.00%	AF265550.1
<input checked="" type="checkbox"/> Homo sapiens chromosome 9, clone hRPK.202_H_3 complete sequence	278	278	100%	6e-71	100.00%	AC006241.1
<input checked="" type="checkbox"/> Homo sapiens KIAA0554 mRNA for KIAA0554 protein	278	278	100%	6e-71	100.00%	AB011126.1
<input checked="" type="checkbox"/> PREDICTED: Nomascus leucogenys formin binding protein 1 (FNBP1) transcript variant X18 mRNA	272	272	100%	3e-69	99.33%	XM_030818029.1
<input checked="" type="checkbox"/> PREDICTED: Nomascus leucogenys formin binding protein 1 (FNBP1) transcript variant X17 mRNA	272	272	100%	3e-69	99.33%	XM_030818028.1
<input checked="" type="checkbox"/> PREDICTED: Nomascus leucogenys formin binding protein 1 (FNBP1) transcript variant X16 mRNA	272	272	100%	3e-69	99.33%	XM_030818027.1

Description gnl|SRA|SRR10168374.7906491.2.7906491 (Biological)
Molecule type dna
Query Length 150
Other reports [Distance tree of results](#) [MSA viewer](#) ?

<input checked="" type="checkbox"/> Sciurus carolinensis genome assembly, chromosome_16	174	174	95%	9e-40	88.81%	LR738606.1
<input checked="" type="checkbox"/> Sciurus carolinensis genome assembly, chromosome_14	174	174	95%	9e-40	88.81%	LR738604.1
<input checked="" type="checkbox"/> Sciurus vulgaris genome assembly, chromosome_15	169	169	95%	4e-38	88.11%	LR738626.1
<input checked="" type="checkbox"/> PREDICTED: Loxodonta africana formin binding protein 1 (FNBP1) transcript variant X2 mRNA	135	135	74%	4e-28	88.50%	XM_010587565.2
<input checked="" type="checkbox"/> PREDICTED: Loxodonta africana formin binding protein 1 (FNBP1) transcript variant X1 mRNA	135	135	74%	4e-28	88.50%	XM_023544839.1

Fig.10b This particular sequence is only found in humans—indicating that even the [SRX6893157](#) dataset was contaminated by material of human origin.

Description Chlorocebus aethiops BAC clone CH252-276C1 from chromo: ...
 Molecule type nucleic acid
 Query Length 160189
 Other reports [Distance tree of results](#) [MSA viewer](#) ?

Percent Identity to E value to Query Coverage to
 Filter Reset

Descriptions Graphic Summary Alignments

Sequences producing significant alignments Download Manage Columns Show 100 ?

select all 100 sequences selected [Graphics](#) [Distance tree of results](#)

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/> SRX7756765	279	774	0%	4e-69	100.00%	SRA_SRR11119763.129105044.1
<input checked="" type="checkbox"/> SRX7756765	279	375	0%	4e-69	100.00%	SRA_SRR11119763.106754018.1
<input checked="" type="checkbox"/> SRX7756765	279	279	0%	4e-69	100.00%	SRA_SRR11119763.75715814.1
<input checked="" type="checkbox"/> SRX7756765	279	510	0%	4e-69	100.00%	SRA_SRR11119763.47541416.1
<input checked="" type="checkbox"/> SRX7756765	279	279	0%	4e-69	100.00%	SRA_SRR11119763.40703161.1
<input checked="" type="checkbox"/> SRX7756765	279	768	0%	4e-69	100.00%	SRA_SRR11119763.38948039.1

Fig.11a The presence of Reads from Somatic Chlorocebus aethiops in [SRX7756765](#) confirms the identity of the Cercopithecinae reads there.

Description gnl|SRA|SRR11119763.106754018.1 106754018 (Biological)
 Molecule type dna
 Query Length 151
 Other reports [Distance tree of results](#) ?

Percent Identity to E value to Query Coverage to
 Filter Reset

Descriptions Graphic Summary Alignments Taxonomy

Sequences producing significant alignments Download Manage Columns Show 1000 ?

select all 1000 sequences selected [GenBank](#) [Graphics](#) [Distance tree of results](#)

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/> Chlorocebus aethiops BAC clone CH252-276C1 from chromosome 6, complete sequence	279	375	100%	2e-71	100.00%	AC241496.3
<input checked="" type="checkbox"/> Chlorocebus aethiops BAC clone CH252-445L10 from chromosome unknown, complete sequence	279	690	100%	2e-71	100.00%	AC238953.2
<input checked="" type="checkbox"/> Macaca mulatta Y Chr BAC CH250-11J13 (Children's Hospital Oakland Research Institute Rhesus macaque Adult Male BAC)	279	1513	100%	2e-71	100.00%	AC240710.4
<input checked="" type="checkbox"/> Chlorocebus aethiops BAC clone CH252-371E9 from chromosome 5, complete sequence	279	1124	100%	2e-71	100.00%	AC239563.3
<input checked="" type="checkbox"/> Chlorocebus aethiops BAC clone CH252-461K13 from chromosome 13, complete sequence	279	629	100%	2e-71	100.00%	AC239354.3
<input checked="" type="checkbox"/> Chlorocebus aethiops BAC clone CH252-138D20 from chromosome 13, complete sequence	279	493	100%	2e-71	100.00%	AC239463.3
<input checked="" type="checkbox"/> Chlorocebus aethiops BAC clone CH252-417L1 from chromosome 6, complete sequence	279	472	100%	2e-71	100.00%	AC239275.3
<input checked="" type="checkbox"/> Chlorocebus aethiops BAC clone CH252-62B18 from chromosome 4, complete sequence	279	510	100%	2e-71	100.00%	AC239442.2
<input checked="" type="checkbox"/> Eukaryotic synthetic construct chromosome 18	268	1.545e+05	100%	4e-68	100.00%	CP034496.1
<input checked="" type="checkbox"/> Eukaryotic synthetic construct chromosome 16	268	1.008e+05	100%	4e-68	98.68%	CP034494.1
<input checked="" type="checkbox"/> Eukaryotic synthetic construct chromosome 19	263	47303	100%	2e-66	98.01%	CP034522.1
<input checked="" type="checkbox"/> Eukaryotic synthetic construct chromosome 13	263	1.935e+05	100%	2e-66	99.31%	CP034516.1
<input checked="" type="checkbox"/> Eukaryotic synthetic construct chromosome Y	263	1.175e+05	100%	2e-66	99.31%	CP034510.1
<input checked="" type="checkbox"/> Eukaryotic synthetic construct chromosome 21	263	55262	100%	2e-66	98.01%	CP034500.1
<input checked="" type="checkbox"/> Eukaryotic synthetic construct chromosome 20	263	88148	100%	2e-66	99.31%	CP034499.1
<input checked="" type="checkbox"/> Eukaryotic synthetic construct chromosome 19	263	47481	100%	2e-66	98.01%	CP034497.1
<input checked="" type="checkbox"/> Eukaryotic synthetic construct chromosome 17	263	74732	100%	2e-66	98.01%	CP034495.1
<input checked="" type="checkbox"/> Eukaryotic synthetic construct chromosome 15	263	1.384e+05	100%	2e-66	98.01%	CP034493.1
<input checked="" type="checkbox"/> Eukaryotic synthetic construct chromosome 14	263	1.910e+05	100%	2e-66	99.31%	CP034492.1
<input checked="" type="checkbox"/> Eukaryotic synthetic construct chromosome 13	263	1.935e+05	100%	2e-66	99.31%	CP034491.1
<input checked="" type="checkbox"/> Eukaryotic synthetic construct chromosome 22	257	22814	100%	9e-65	97.35%	CP034501.1
<input checked="" type="checkbox"/> Human gammaherpesvirus 4 isolate HKNPC60, partial genome	248	248	100%	5e-62	96.03%	MH590571.1
<input checked="" type="checkbox"/> Human gammaherpesvirus 4 isolate HKHD40, partial genome	248	248	100%	5e-62	96.03%	MH590409.1
<input checked="" type="checkbox"/> Uncultured bacterium clone LMOABA39ZH04FM1 genomic sequence	246	246	96%	2e-61	97.24%	EU058958.1
<input checked="" type="checkbox"/> Synthetic construct Pan troglodytes LINE-1L 1Pt retrotransposon tagged with EGFP sequence	241	241	100%	9e-60	95.36%	KF661301.1
<input checked="" type="checkbox"/> Uncultured organism clone VC1C968TR genomic sequence	241	241	96%	9e-60	96.55%	GQ879596.1

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.

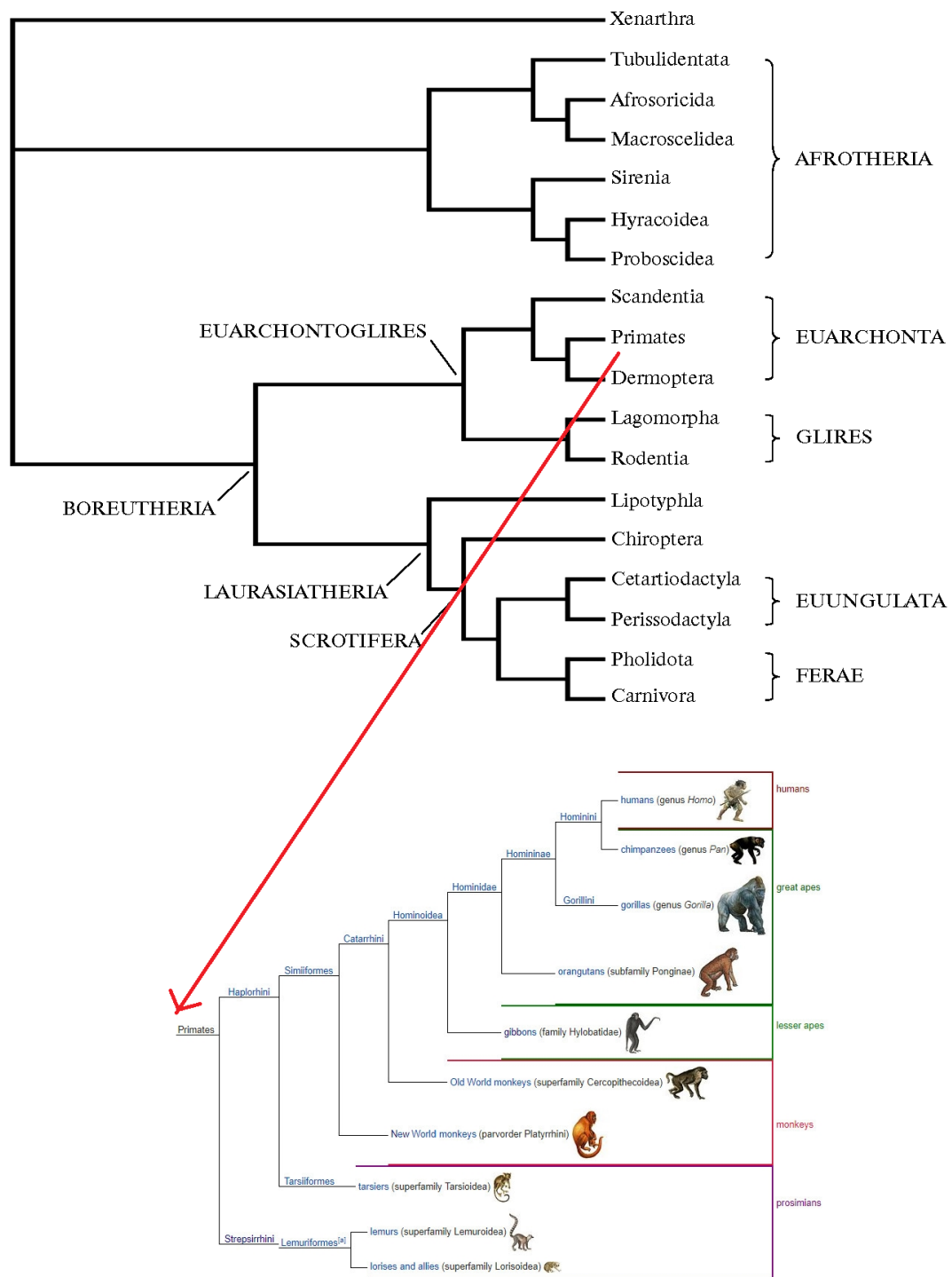


Fig. 12 Family tree of mammals, Including the position and classification of Primates in the lineage of Mammalia.

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 date	Primate classification and total traced Kbps	Total traced Kbps to Manis Javanica (Pangolin)	Ratio of Primate to Pangolin	Virus classification and amount of reads by Kbps
SRX7756769 18-Feb-2020	Homo sapiens 5457929	15401134	0.35	Bat SARS-like coronavirus 2Kbp Wuhan seafood market pneumonia virus 2Kbp
SRX6893139 20-Sep-2019	Homo sapiens 491120	5301351	0.0926	Pangolin coronavirus 2Kbp
SRX6893157 20-Sep-2019	Catarrhini 644546	1889448	0.34	N/D***
SRX6893156 20-Sep-2019	Homo sapiens 81948	4765461	0.01719	Pangolin coronavirus 2Kbp
SRX6893155 20-Sep-2019	Homininae 3534150	525801	6.7214	Pangolin coronavirus 5Kbp
SRX6893154 20-Sep-2019	Hominoidea 356003	2232008	0.159	Pangolin coronavirus 154Kbp
SRX6893153 20-Sep-2019	Homo sapiens 162180	3110158	0.05214	Pangolin 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 and reads (in Kbp)	Virus classification and reads	Ratio of virus reads to Primate reads
SRX7756766 18-Feb-2020	Cercopithecidae 3116; BLAST to Macaca Mulatta	Betacoronavirus 2Kbp **	0.000642
SRX7756762 18-Feb-2020	Catarrhini 2831; BLAST to Chlorocebus sabaeus	Nidovirales 0Kbp Claimed 10x150bp reads	0.000530
SRX7756765 22-Apr-2020	Cercopithecinae 11339 BLAST to Chlorocebus Aethiops	N/D***	N/A
SRX7732094 15-Feb-2020	N/A*	Pangolin coronavirus	N/A*

*: 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 [SRX7756769](#) and [SRX7756762](#) 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 [SRX7756762](#) dataset contained definitive mappings to *Chlorocebus sabaeus*, or African Green Monkey, while [SRX7756766](#) 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*.

[SRX7756769](#) 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 [SRX7756769](#) itself support the possibility that [SRX7756769](#) 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 [SRX7756769](#) in VERO E6 cells, characterized it but did not sequence it, and this cell culture material then contaminated [SRX7756762](#) and possibly [SRX7756766](#), resulting the 10 reads in [SRX7756762](#) and the 2Kb batacoronavirus reads in [SRX7756766](#).

The exact nature of [SRX7732094](#) 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 [PRJNA696875](#), 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	Size	Non-Coronavirus reads?	Source Tissue Provider and Collected by	Virus Designation: GD or GX?	Design
SRX7732094 15-Feb-2020	2,633	No	N/A	GD	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

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

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 the 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.

Extended Data Table S1

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

	Mammarenavirus	Nairoviridae	Murine respirovirus	Flaviviridae	Nidovirales	Rubulavirus	Nonanavirus	Peribunyavi	Amigovirus	Siphoviridae	Siphoviridae	Paheviridae
SRX6893158	Yes	Yes	No	No	No	No	Yes	No	Yes	Yes	No	No
SRX6893157	Yes	Yes	No	No	Claimed	No	No	Yes	No	No	No	No
SRX6893156	No	No	Yes	Yes	Yes	No	No	No	Yes	No	No	Yes
SRX6893155	No	No	Yes	No	Yes	No	No	No	No	No	No	No
SRX6893154	No	No	Yes	No	Yes	No	No	No	No	No	No	No
SRX6893153	No	No	Yes	Yes	Yes	No	No	No	Yes	No	No	No
SRX6893152	Yes	Yes	Yes	Yes	No	No	No	Yes	No	No	No	No
SRX6893151	Yes	Yes	No	Yes	No	No	No	Yes	Yes	No	No	No
SRX6893150	Yes	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No
SRX6893149	Yes	Yes	No	No	No	No	No	No	No	No	Yes	No
SRX6893148	Yes	Yes	Yes	No	No	No	No	No	Yes	No	No	No
SRX6893147	Yes	Yes	"Respirovirus"	Yes	No	No	Yes	No	Yes	No	No	No
SRX6893146	Yes	Yes	Yes	No	No	No	No	No	No	No	No	No
SRX6893145	Yes	Yes	No	No	No	No	No	No	No	No	No	No
SRX6893144	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No	No
SRX6893143	Yes	Yes	No	No	No	No	No	No	No	No	No	No
SRX6893142	Yes	Yes	No	No	No	No	No	Yes	Yes	No	No	No
SRX6893141	Yes	Yes	No	Yes	No	No	No	No	No	No	No	No
SRX6893140	Yes	Yes	Yes	No	No	No	No	Yes	No	No	No	No
SRX6893139	No	No	Yes	No	Yes	No	No	No	No	No	No	No
SRX6893138	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	No	No	No
SRX7756766	No	No	Yes	Yes	Yes	Yes	No	No	No	No	No	No
SRX7756765	No	No	Yes	No	No	Yes	No	No	No	No	No	No
SRX7756764	No	No	Yes	No	No	Yes	No	No	No	No	No	No
SRX7756763	No	No	Yes	No	No	Yes	No	No	No	No	No	No
SRX7756762	No	No	Yes	No	Claimed	Yes	No	No	No	No	No	No
SRX7756761	No	No	Yes	No	No	Yes	No	No	No	No	No	No
SRX7756769	No	No	Yes	Yes	Yes	No	No	No	No	No	No	No

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 Syncytiums 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]

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.

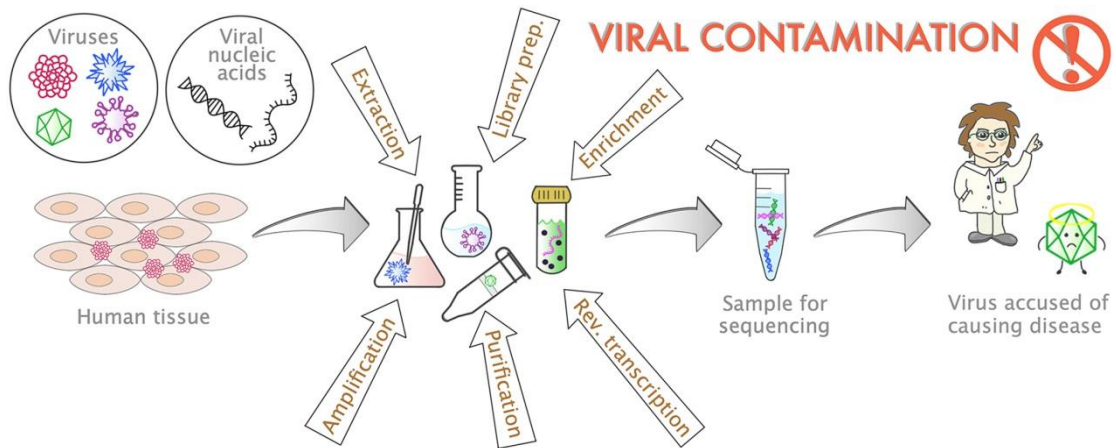


Figure 14. A cartoon diagram of contamination in sequencing experiment leading to false results and false “discoveries”.

Note as in 2020/7/23

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

Accession number and registration date	Primary Mammalian Trace results and percentage	Primate-related results in Krona and read size by Kbp	Identification of “Coronaviridae” as by Trace and total read size
SRX8582289 22-Jun-2020	Manis javanica: 43.52%	Catarrhini 98913	Pangolin coronavirus 792

Table S2: TRACE analysis result of the [SRX8582289](#) dataset.

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

Description: Homo sapiens BAC clone RP11-460N20 from 7, complete seq ...

Molecule type: nucleic acid

Query Length: 203396

Other reports: [Distance tree of results](#) [MSA viewer](#)

Percent Identity: to

E value: to

Query Coverage: to

[Filter](#) [Reset](#)

Descriptions | Graphic Summary | Alignments

Sequences producing significant alignments | Download | Manage Columns | Show: 100

select all | 100 sequences selected

Description	Max Score	Total Score	Query Cover	E value	Per Ident	Accession
<input checked="" type="checkbox"/> SRX8582289	278	278	0%	8e-69	100.00%	SRA_SRR12053850.88444297.1
<input checked="" type="checkbox"/> SRX8582289	278	402	0%	8e-69	100.00%	SRA_SRR12053850.83916175.2
<input checked="" type="checkbox"/> SRX8582289	278	388	0%	8e-69	100.00%	SRA_SRR12053850.83916175.1
<input checked="" type="checkbox"/> SRX8582289	278	278	0%	8e-69	100.00%	SRA_SRR12053850.82221130.1
<input checked="" type="checkbox"/> SRX8582289	278	278	0%	8e-69	100.00%	SRA_SRR12053850.71234261.2
<input checked="" type="checkbox"/> SRX8582289	278	278	0%	8e-69	100.00%	SRA_SRR12053850.71234261.1
<input checked="" type="checkbox"/> SRX8582289	278	5169	2%	8e-69	100.00%	SRA_SRR12053850.51889132.2
<input checked="" type="checkbox"/> SRX8582289	278	7268	3%	8e-69	100.00%	SRA_SRR12053850.26027930.2
<input checked="" type="checkbox"/> SRX8582289	278	5671	2%	8e-69	100.00%	SRA_SRR12053850.21554419.1
<input checked="" type="checkbox"/> SRX8582289	278	278	0%	8e-69	100.00%	SRA_SRR12053850.13271287.2
<input checked="" type="checkbox"/> SRX8582289	278	4760	1%	8e-69	100.00%	SRA_SRR12053850.62042.2
<input checked="" type="checkbox"/> SRX8582289	276	276	0%	3e-68	100.00%	SRA_SRR12053850.82221130.2

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

Description gnl|SRA|SRR12053850.82221130.2.82221130 (Biological)
Molecule type dna
Query Length 150
Other reports [Distance tree of results](#)

to to to
Filter Reset

Descriptions Graphic Summary Alignments Taxonomy

Sequences producing significant alignments Download Manage Columns Show 1000

select all 59 sequences selected

	Description	Max Score	Total Score	Query Cover	E value	Per. ident	Accession
<input checked="" type="checkbox"/>	Homo sapiens general transcription factor III pseudogene 14 (GTF2IP14) on chromosome 7	276	276	99%	3e-70	100.00%	NG_043494.1
<input checked="" type="checkbox"/>	Homo sapiens FOSMID clone ABC13-48840700E15 from chromosome 7, complete sequence	276	276	99%	3e-70	100.00%	AC242196.4
<input checked="" type="checkbox"/>	Homo sapiens BAC clone RP11-460N20 from 7, complete sequence	276	276	99%	3e-70	100.00%	AC073210.8
<input checked="" type="checkbox"/>	Homo sapiens general transcription factor III pseudogene 5 (GTF2IP5) on chromosome 7	270	270	99%	1e-68	99.33%	NG_026590.1
<input checked="" type="checkbox"/>	Pan troglodytes BAC clone CH251-340J24 from chromosome 7, complete sequence	270	270	99%	1e-68	99.33%	AC185242.2
<input checked="" type="checkbox"/>	Pan troglodytes BAC clone CH251-623C19 from chromosome 7, complete sequence	270	270	99%	1e-68	99.33%	AC184799.2
<input checked="" type="checkbox"/>	Pan troglodytes BAC clone CH251-114G16 from chromosome 7, complete sequence	270	270	99%	1e-68	99.33%	AC183835.2
<input checked="" type="checkbox"/>	Pan troglodytes BAC clone CH251-2O15 from chromosome 7, complete sequence	270	270	99%	1e-68	99.33%	AC174000.3
<input checked="" type="checkbox"/>	Homo sapiens BAC clone RP11-479O9 from 7, complete sequence	270	270	99%	1e-68	99.33%	AC073107.7
<input checked="" type="checkbox"/>	Pan troglodytes BAC clone CH251-565C10 from chromosome 7, complete sequence	270	270	99%	1e-68	99.33%	AC148313.3
<input checked="" type="checkbox"/>	homo sapiens BAC clone CH17-99D2 from chromosome 4, complete sequence	226	226	99%	3e-55	93.96%	AC278002.1
<input checked="" type="checkbox"/>	Homo sapiens FOSMID clone ABC27-154I13 from chromosome 4, complete sequence	226	226	99%	3e-55	93.96%	AC240529.1
<input checked="" type="checkbox"/>	Pan troglodytes BAC clone CH251-4D23 from chromosome 7, complete sequence	226	226	99%	3e-55	93.96%	AC148834.3
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 16	193	193	99%	3e-45	90.07%	CP034494.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 19	182	182	99%	5e-42	88.74%	CP034522.1
<input checked="" type="checkbox"/>	Eukaryotic synthetic construct chromosome 19	182	182	99%	5e-42	88.74%	CP034497.1

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.

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