

Supplemental material to: Best molecular tools to investigate coronavirus diversity in mammals: a comparison

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Table S1. Complementarity of primers with *Alfacoronavirus* sequences. The primers that did not bind to the template in *in silico* analyses are underlined (*the mismatches were present within the 3 bp of 3' end of primer). Primer sets where at least one primer had no mismatch with the template sequence are in **bold**.

Species	Subspecies	Isolate	Accession No.	Hu-F	Hu-R	Chu11-F1	Chu11-R1	Chu06-R1	Chu06-R2	Poon-F	Poon-R	DeSouzaLuna-F1+2	DeSouzaLuna-R1+2+3	DeSouzaLuna-F3+4	DeSouzaLuna-R4	Holbrook-F1	Holbrook-R1+2	Holbrook-F2+3	Xiu-F1	Xiu-R	Xiu-F2
<i>Alphacoronavirus 1</i>	TGEV	Purdue	AJ271965	0	0	1	0	1	<u>2*</u>	1	1	3; 0	4; 2; 4	4; 6	3	1	0; 2	2; 0	0	1	2
	Feline CoV	Type I C1Je	DQ848678	0	0	1	1	3	<u>4*</u>	1	3	3; 0	4; 2; 4	4; 6	3	1	1; 3	2; 0	1	2	2
		Type II FIP virus WSU 79-1146	AY994055	0	0	0	0	1	<u>2*</u>	1	1	3; 1	4; 2; 4	4; 6	3	0	0; 2	2; 0	0	1	2
	Porcine respiratory CoV	ISU-1	DQ811787	0	0	1	0	1	<u>2*</u>	1	1	3; 0	4; 2; 4	3; 5	3	1	0; 2	2; 0	0	1	2
	Canine CoV	Type I 23/03	KP849472	0	0	1	1	1	<u>2*</u>	1	1	3; 0	4; 2; 4	4; 6	4	1	0; 2	2; 0	1	2	2
		Type II NTU336/F/2008	GQ477367	0	0	1	0	1	<u>2*</u>	1	1	3; 1	4; 2; 4	4; 6	3	1	0; 2	2; 0	0	0	2
		Type IIa CB/05	KP981644	0	0	1	0	2	<u>3*</u>	1	2	3; 0	3; 2; 3	4; 6	4	1	0; 2	2; 0	0	1	2
		Type IIb 174/06, type IIb	EU856362	0	0	1	0	2	<u>3*</u>	1	2	3; 0	3; 2; 3	4; 6	4	1	0; 2	2; 0	0	1	2
	Bat coronavirus HKU10	183A	NC_018871	0	1	0	0	2	<u>3*</u>	1	2	<u>2*, 3*</u>	3; 2; 3	2; 4	<u>5</u>	1	0; 2	0; 0	0	2	2
	Bat coronavirus CDPHE15	bat/USA/CDPHE15/2006	NC_022103	0	1	0	0	4	<u>5*</u>	2	4	3; 2	7; 5; 3	4; 5	<u>6</u>	1	2; 4	0; 0	0	1	3
	Human coronavirus 229E	KW2E-F151/Hipcf.rub/GHA/2011	KT253269	0	1	0	0	3	<u>3*</u>	0	3	2; 1	4; 1; 4	3; 5	3	1	1; 3	0; 0	0	2	1
		Human coronavirus 229E	NC_002645	1	0	1	0	2	<u>2*</u>	1	2	3; 0	3; 0; 5	4; 6	3	1	1; 3	0; 0	2	0	2
		camel/Riyadh/Ry141/2015	NC_028752	0	0	1	0	1	<u>2*</u>	1	1	3; 1	3; 0; 5	4; 6	2	1	0; 2	0; 0	2	0	2
	Human coronavirus NL63	NL63 Amsterdam I	AY567487	0	1	1	1	2	<u>3*</u>	3	<u>3*</u>	0; 3	3; 1; 5	2; 0	3	1	1; 3	0; 0	0	3	2
Lucheng Rn rat coronavirus		Lucheng-19	NC_032730	0	0	1	0	1	<u>2*</u>	1	1	2; 2	4; 2; 4	3; 3	2	1	0; 2	2; 0	1	0	2
Miniopterus bat coronavirus 1	B	AFCD307	NC_010436	1	1	1	1	3	3	1	3	3; 2	4; 3; 3	4; 6	4	1	1; 3	0; 0	2	1	2
	A	AFCD62	NC_010437	1	0	1	1	2	2	2	2	2; 2	5; 3; 4	3; 3	4	1	2; 4	0; 0	1	0	3
Miniopterus bat coronavirus HKU8		AFCD77/08/05 Mm	NC_010438	0	1	0	1	2	1	2	2	<u>2*, 4*</u>	2; 1; 4	2; 4	3	1	0; 2	2; 0	2	1	1
Mink coronavirus 1		WD1127	NC_023760	0	0	0	1	2	2	3	2	0; 3	5; 3; 4	2; 1	<u>5</u>	2	0; 2	3; 1	0	2	2
Myotis ricketti alphaCoV		Sax-2011	NC_028811	0	1	0	0	<u>6*</u>	<u>7*</u>	0	<u>6*</u>	1; 2	6; 5; 2	1; 3	<u>7</u>	1	2; 4	2; 0	2	1	1
NL-63-related bat coronavirus		BtKYNL63-9b	NC_048216	0	1	0	0	2	<u>4*</u>	2	<u>3*</u>	<u>2*, 3*</u>	3; 2; 3	3; 5	4	1	0; 2	0; 0	2	1	1
Nyctalus velutinus alphaCoV		SC-2013	NC_028833	0	1	0	1	4	<u>5*</u>	1	<u>5*</u>	<u>2*, 3*</u>	4; 3; 2	2; 4	<u>5</u>	1	1; 3	0; 0	0	1	2
Pipistrellus khulii coronavirus		P.kuhlil/Italy/3398-19/2015	NC_046964	0	0	0	1	<u>5</u>	<u>7*</u>	2	<u>6*</u>	<u>2*, 3*</u>	6; 5; 1	2; 4	<u>9</u>	1	3; 5	0; 0	1	1	1
Porcine epidemic diarrhea virus		CV777	NC_003436	0	0	1	1	1	<u>3*</u>	<u>4*</u>	<u>2*</u>	<u>1*, 4*</u>	3; 0; 5	<u>3*, 4*</u>	4	1	0; 2	1; 1	0	1	<u>3*</u>
Rhinolophus ferrumequinum alhaCoV		HuB-2013	NC_028814	0	0	1	1	2	3	3	<u>3*</u>	<u>4*, 2*</u>	5; 3; 4	<u>6; 7</u>	<u>5</u>	2	2; 4	3; 1	0	2	4
Rhinophilus bat coronavirus HKU2		HKU2/HK/46/2006	EF203065	0	1	1	0	2	<u>4*</u>	3	<u>3*</u>	2; 3	3; 2; 3	3; 3	<u>5</u>	1	0; 2	0; 0	1	1	2
Scatophilus bat coronavirus		512/2005	NC_009657	0	1	1	1	2	<u>4*</u>	2	<u>3*</u>	2; 2	4; 1; 5	3; 5	<u>5</u>	1	0; 2	0; 0	1	2	1
Sorex araneus coronavirus		Shrew-CoV/Tibet2014	NC_046955	0	1	0	0	3	<u>4*</u>	3	3	<u>2*, 4*</u>	4; 3; 3	4; 4	4	1	1; 3	0; 0	1	2	2
Suncus murinus coronavirus		Xingguo-101	NC_035191	0	0	2	1	<u>5*</u>	<u>7*</u>	4	<u>6*</u>	2; 1	<u>3*, 5*, 7*</u>	5; 3	<u>8</u>	1	2; 0	2; 0	0	0	3

Table S2. Complementarity of primers with *Betacoronavirus* sequences. The primers that did not bind to the template in *in silico* analyses are underlined (*the mismatches were present within the 3 bp of 3' end of primer). Primer sets where at least one primer had no mismatch with the template sequence are in **bold**.

Species	Subspecies	Isolate	Accession No.	Hu-F	Hu-R	Chu11-F1	Chu11-R1	Chu06-R1	Chu06-R2	Poon-F	Poon-R	DeSouzaLuna-F1+2	DeSouzaLuna-R1+2+3	DeSouzaLuna-F3+4	DeSouzaLuna-R4	Holbrook-F1	Holbrook-R1+2	Holbrook-F2+3	Xiu-F1	Xiu-R	Xiu-F2
<i>Betacoronavirus 1</i>	Human coronavirus OC43	ATCC VR-759	AY585228	0	0	0	0	4	<u>7*</u>	1	4	0; 3	1; 4; 5	0; 2	<u>6</u>	1	2; 0	0; 2*	0	1	0
	Bovine coronavirus	Mebus	BCU00735	0	0	0	0	4	<u>7*</u>	1	4	0; 3	1; 4; 5	0; 2	<u>6</u>	1	2; 0	0; 2*	0	1	0
	Porcine hemagglutinating encephalomyelitis virus	VW572	NC_007732	0	0	1	1	4	<u>7*</u>	2	4	0; 3	1; 4; 5	1; 2	<u>5</u>	1	2; 0	0; 2*	1	2	1
	Equine coronavirus	NC99	NC_010327	0	1	0	0	4	<u>7*</u>	1	4	0; 3	1; 4; 5	0; 2	<u>6</u>	1	2; 0	0; 2*	0	2	0
<i>China Rattus coronavirus HKU24</i>		HKU24-R05005I	NC_026011	0	0	0	1	4	<u>7*</u>	<u>2*</u>	4	1; 3	1; 4; 5	<u>3*, 5*</u>	<u>7</u>	1	3*; 1	2; 0	1	2	1
<i>Eidolon bat coronavirus C704</i>		Kenya/KY24/2006	HQ728482	1	0	0	1	4	<u>7*</u>	0	4	2; 2	2; 3; 7	3; 5	<u>7</u>	1	0; 0	2; 0	1	1	2
<i>Hedgehog coronavirus 1</i>		ErinaceusCoV/2012-174/GER/2012	NC_039207	0	1	0	1	2	<u>4*</u>	2	<u>3*</u>	<u>1*, 4*</u>	4; 1; 5	1; 3	<u>5</u>	1	0; 2*	0; 2	1	1	2
<i>Human coronavirus HKU1</i>		HKU1	NC_006577	0	0	0	1	4	<u>7*</u>	2	4	0; 3	1; 4; 5	1; 1	<u>6</u>	1	0; 2*	2; 0	1	0	1
<i>MERS-related coronavirus</i>	camel/UAE_B21_2015	MF598613	MF598613	0	0	0	0	3	<u>3*</u>	2	3	<u>1*, 4*</u>	3; 2; 4	1; 3	<u>5</u>	4	0; 0	0; 2	1	2	2
	HCoV-EMC/2012	NC_019843	NC_019843	0	0	0	0	3	<u>3*</u>	2	3	<u>1*, 4*</u>	3; 2; 4	1; 3	<u>5</u>	1	0; 2*	0; 2	1	3	2
<i>Murine coronavirus</i>	Rat coronavirus	Rat coronavirus Parker	FJ938068	0	0	0	1	4	<u>7*</u>	0	4	1; 2	1; 4; 5	1; 3	<u>5</u>	1	2; 0	0; 2*	1	1	1
	Murine hepatitis virus	JHM	NC_006852	0	0	0	1	<u>5</u>	<u>8*</u>	1	<u>5</u>	2; 2	2; 5; 6	3; 3	<u>6</u>	1	<u>3*, 4*</u>	0; 2*	1	1	2
<i>Myodes coronavirus 2JL14</i>		RtMruf-CoV-2/JL2014	NC_046954	1	0	1	1	4	<u>7*</u>	3	4	1; 2	1; 4; 5	2; 2	<u>6</u>	1	0; 2*	2; 0	2	2	2
<i>Pipistrellus bat coronavirus HKU5</i>		HKU5-1 LMH03f	NC_009020	0	1	1	0	3	<u>5*</u>	3	<u>4*</u>	<u>2*, 3*</u>	4; 3; 3	4; 4	<u>6</u>	1	0; 2*	1; 3	2	1	2
<i>Rousettus bat coronavirus HKU9</i>		HKU9-1 BF_005I	NC_009021	1	0	0	1	4	<u>7*</u>	1	4	2; 1	2; 3; 6	4; 4	<u>7</u>	1	0; 2*	3; 1	2	2	0
<i>SARS-related CoV</i>	human CoV	Urbani	AY278741	0	1	1	0	2	2	3	<u>3*</u>	0; 3	3; 0; 5	2; 1	4	1	1; 3	0; 0	2	1	2
	palm civet CoV	SZ3/2003	AY304486	0	1	1	0	2	2	3	<u>3*</u>	0; 3	3; 0; 5	2; 1	4	1	1; 3	0; 0	2	1	2
	chinese ferret badger CoV	CFB/SZ/94/03	AY545919	0	0	1	0	2	2	3	<u>3*</u>	0; 3	3; 0; 5	2; 1	4	1	1; 3	0; 0	2	1	2
	SARS-CoV-2	human/ITA/32898/2020	MT483878	0	0	0	1	4	4	2	<u>5*</u>	0; 3	5; 3; 4	1; 1	<u>5</u>	1	3; 5	0; 0	1	0	1
	Rhinopholus bat CoV	Rp3/2004	NC_009693	0	1	1	0	2	2	3	<u>3*</u>	0; 3	3; 0; 5	2; 1	<u>5</u>	1	1; 3	0; 2*	2	1	2
		HKU3-1	NC_009694	0	0	1	1	3	3	3	<u>4*</u>	<u>3*, 3*</u>	4; 1; 4	5; 4	<u>5</u>	1	2; 4	0; 2*	4	0	4
		Rf1/2004	NC_009695	0	0	1	1	2	2	3	<u>3*</u>	<u>2*, 3*</u>	3; 0; 5	4; 3	4	1	1; 3	0; 2*	4	0	4
		Rm1/2005	NC_009696	0	1	1	1	2	2	1	<u>3*</u>	1; 2	3; 0; 5	2; 3	4	1	1; 3	0; 2*	2	1	2
<i>Tylonycteris bat coronavirus HKU4</i>		HKU4-1 BO4f	NC_009019	0	1	0	0	3	<u>4*</u>	2	3	<u>2*, 4*</u>	6; 4; 3	3; 5	4	1	0; 2*	2; 4	1	0	1
<i>Bat Hp-betacoronavirus</i>		Zhejiang2013	NC_025217	1	0	0	0	3	<u>4*</u>	1	3	1; 3	4; 3; 3	1; 3	<u>6</u>	1	0; 2*	1; 3	0	3	1

Table S3. Complementarity of primers with *Gammacoronavirus* and *Deltacoronavirus* sequences. The primers that did not bind to the template in *in silico* analyses are underlined (*the mismatches were present within the 3 bp of 3' end of primer). Primer sets where at least one primer had no mismatch with the template sequence are in **bold**.

Genus	Species	Isolate	Accession No.	Hu-F	Hu-R	Chu11-F1	Chu11-R1	Chu06-R1	Chu06-R2	Poon-F	Poon-R	DeSouzaLuna-F1+2	DeSouzaLuna-R1+2+3	DeSouzaLuna-F3+4	DeSouzaLuna-R4	Holbrook-F1	Holbrook-R1+2	Holbrook-F2+3	Xiu-F1	Xiu-R	Hu-F2
Delta-	<i>Coronavirus HKU15</i>		LC216915	0	0	0	0	<u>5*</u>	<u>9*</u>	2	<u>6*</u>	<u>3*, 5*</u>	<u>5*, 4*, 4*</u>	4; 6	<u>8*</u>	1	0; 2	2*; 0	1	0	1
	<i>Bulbul coronavirus HKU11-934</i>		NC_011547	0	1	1	1	<u>2*</u>	<u>6*</u>	3	<u>3*</u>	<u>3*, 5*</u>	4; 2; 5	<u>5; 5</u>	4	1	0; 2	2*; 0	0	1	2
	<i>Thrush coronavirus HKU12-600</i>		NC_011549	0	0	0	1	<u>5*</u>	<u>8*</u>	2	<u>6*</u>	<u>3*, 4*</u>	4; 3; 3	4; 6	<u>7</u>	1	0; 2	2*; 0	0	1	1
	<i>Munia coronavirus HKU13-3514</i>		NC_011550	0	0	2	0	<u>2*</u>	<u>6*</u>	4	<u>3*</u>	<u>4*, 5*</u>	4; 2; 5	<u>6; 6</u>	4	1	0; 2	1*; 1*	0	1	3
	<i>White-eye coronavirus HKU16</i>		NC_016991	0	0	0	1	<u>4*</u>	<u>7*</u>	2	<u>4*</u>	<u>3*, 5*</u>	<u>5*, 2*, 5*</u>	4; 6	<u>7*</u>	1	0; 2	2*; 1*	0	1	1
	<i>Night-heron coronavirus HKU19</i>		NC_016994	0	1	0	0	<u>3*</u>	<u>5*</u>	3	<u>4*</u>	<u>6*, 7*</u>	2; 1; 4	<u>7; 9</u>	4	0	0; 2	2*; 0	1	1	2
	<i>Wigeon coronavirus HKU20</i>		NC_016995	0	1	0	0	<u>4*</u>	<u>9*</u>	2	<u>5*</u>	3; 5	<u>6*, 4*, 6*</u>	<u>5; 5</u>	<u>8*</u>	1	1; 3	2*; 0	1	0	1
	<i>Common moorhen coronavirus HKU21</i>		NC_016996	0	1	0	1	<u>5*</u>	<u>9*</u>	1	<u>6*</u>	3; 6	<u>7*, 5*, 5*</u>	3; 5	<u>8*</u>	1	1; 3	1*; 1*	0	2	0
	<i>Coronavirus HKU15</i>		NC_039208	0	0	0	0	<u>5*</u>	<u>9*</u>	2	<u>5*</u>	<u>3*, 5*</u>	<u>5*, 4*, 4*</u>	4; 6	<u>8*</u>	1	0; 2	2*; 0	1	0	1
Gamma-	<i>Turkey coronavirus</i>	TCoV-ATCC	EU022526	0	0	0	0	<u>6*</u>	<u>6</u>	2	<u>7*</u>	1; 3	5; 4; 1	1; 3	<u>6</u>	0	2; 4	0; 0	1	1	1
	<i>Avian coronavirus</i>	Baudette	NC_001451	0	1	0	0	<u>6*</u>	<u>7*</u>	1	<u>7*</u>	0; 3	5; 4; 1	0; 2	<u>6</u>	1	2; 4	0; 0	1	2	0
	<i>Beluga whale coronavirus SW1</i>		NC_010646	1	<u>2*</u>	0	0	<u>8*</u>	<u>11*</u>	1	<u>8*</u>	3; 1	<u>7*, 4; 5</u>	3; 5	<u>8</u>	0	3; 1	0; 0	2	2	2
	<i>Goose coronavirus</i>	Cambridge_Bay_2017	NC_046965	0	1	1	1	<u>4*</u>	<u>5*</u>	2	<u>4*</u>	2; 2	4; 3; 2	3; 3	<u>5</u>	1	1; 3	1*; 1*	1	2	3
	<i>Avian coronavirus 9203</i>	IBV Ind-TN92-03	NC_048213	0	0	1	0	<u>6*</u>	<u>6</u>	2	<u>7*</u>	0; 3	5; 4; 1	1; 2	<u>6</u>	1	2; 4	0; 0	1	1	1
	<i>Duck coronavirus 2714</i>	DK/GD/27/2014	NC_048214	0	1	0	0	<u>5*</u>	<u>6*</u>	2	<u>5*</u>	0; 3	5; 4; 3	1; 1	<u>6</u>	1	2; 4	0; 0	0	0	1

Table S4. Field samples and viral strains used to test the inclusivity of the novel assay developed in this study.

Genus	virus	host	matrix tested	Year	Collection strategy	strain
αCoV	Alphacoronavirus 1_Feline Coronavirus	Cat	viral strain (cell culture)			WSU 79-1683 (ATCC VR-989)
	Alphacoronavirus 1_Canine Coronavirus	Dog	viral strain (cell culture)			Jan-71 (ATCC VR-809)
	Alphacoronavirus 1_Ferret Coronavirus	Ferret	feces	2021	Dry	IZSve21RS42-F
	Alphacoronavirus 1_Ferret Coronavirus	Ferret	intestine	2021	Dry	IZSve21RS42-18
	Alphacoronavirus 1_TGE	Pig	viral strain (cell culture)			Virulent Purdue
	Miniopterus coronavirus 1	<i>Miniopterus schreibersii</i>	oral swab	2020	Lysis buffer	IZSve21RS156-83
	PEDV	Pig	feces	2018	Dry	18DIA90174-11
	PEDV	Pig	feces	2018	Dry	18DIA90174-10
	Murine Coronavirus	<i>Mus musculus</i>	feces	2021	Dry	IZSve21RS42-T
	Unclassified pipistrelle αCoV	<i>Pipistrellus kuhlii</i>	feces	2018	Viral transport medium	IZSve19RS495-11
	Unclassified myotis αCoV	<i>Myotis myotis</i>	feces	2016	RNA later	IZSve21RS474-25
	Unclassified myotis αCoV	<i>Myotis myotis</i>	feces	2016	RNA later	IZSve21RS474-36
βCoV	Betacoronavirus 1_PHEV	Pig	feces	2018	Dry	18DIA90174-2
	Betacoronavirus 1_PHEV	Pig	feces	2018	Dry	18DIA90178-5
	Betacoronavirus 1_PHEV	Pig	rectal swab	2021	RNA later	IZSve21RS1031-16
	Betacoronavirus 1_Bovine Coronavirus	cow	viral strain (cell culture)			Mebus
	Betacoronavirus 1_Bovine Coronavirus	antelope	feces	2019	Dry	IZSLER32286-1/2019
	Betacoronavirus 1_Bovine Coronavirus	antelope	feces	2019	Dry	IZSLER32286-2/2019
	Betacoronavirus Erinaceus	<i>Erinaceus europaeum</i>	intestine	2021	Dry	IZSve21RS156-83
	Unclassified Eptesicus βCoV	<i>Eptesicus serotinus</i>	feces	2011	Dry	ITA26_384_2012
	Unclassified Eidolon βCoV	<i>Eidolon helvum</i>	feces	2013	Dry	13RS452-71
	Unclassified Eidolon βCoV	<i>Eidolon helvum</i>	feces	2013	Dry	13RS362-59
	SARS-CoV-2	Dog	oral swab	2020	Dry	20DIA30037-1/2020
	SARS-CoV-2	Human	viral strain (cell culture)			20VIR1935
	SARS-CoV-2	Cat	nasal swab	2020	Dry	VE-IZSve-20DIA30040-2/2020
γCoV	IBV	Chicken	viral strain (egg culture)			IZSve21RS156-64
	duck coronavirus	Duck	feces	2018	Dry	IZSLER138948-2/2018
	duck coronavirus	Duck	feces	2019	Dry	IZSLER288952-1/2019
δCoV	quail coronavirus	<i>Coturnix coturnix</i>	intestine	2019	Dry	IZSLER335273/2019
	quail coronavirus	<i>Coturnix coturnix</i>	intestine	2019	Dry	IZSLER160792/2019