Diagnostic Assay for the Causative Allele for Jersey Neuropathy with Splayed Forelimbs

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The genetic defect Jersey Neuropathy with Splayed Forelimbs (JNS) was recently identified by the American Jersey Cattle Association ((AJCA), AJCA 2020). Affected calves are born alive but show several symptoms including being unable to stand on splayed forelimbs and neurologic symptoms including spasticity of head and neck and convulsive behavior. While a missense variant in UCLH1 (chromosome 6 60,158,901G->A is the likely causative allele no commercial diagnostic assay currently is offered. Therefore, an animal's JNS status is currently based on a diagnostic SNP haplotype, which is not 100% accurate. To provide a more accurate carrier status information, we developed an in-house diagnostic test for the JNS causative allele. The assay information is provided below to allow diagnostic labs across the globe to quickly implement a causative allele diagnostic test and offer it to their local Jersey producers.

Material and Methods

JNS haplotype status

Individual's JNS haplotype status were obtained via public records reported by the Council on Dairy Cattle breeding ((CDCB) Bowie, Maryland, USA). Individual's JNS haplotype status used for this publication are from April 2021.

Biological Samples

A diagnostic assay validation panel was assembled from 4 haplotype carrier Jersey sires, 12 haplotype free Jersey sires, and 80 haplotype free Holstein sires that we already had extracted DNA from previous internal studies where tissue was collected by staff veterinarians as part of routine health procedures. Animal's JNS haplotype status was obtained via CDCB on December 8, 2021.

After assay validation a set of Jersey animals to test for the JNS diagnostic allele was assembled. Liquid nitrogen semen samples on 33 deceased sires that were JNS haplotype positive were obtained from ABS's stored semen stock. Ear punches were collected from 126 Jersey sires and 42 Jersey females via Allflex tissue sampling unit (TSU) tubes (Merck and Co, Inc. Kenilworth, NJ, USA) as part of routine vet car procedures. Animal's JNS haplotype status on 147 animals was obtained via CDCB on January 6, 2021.

DNA Extraction

Tissue samples were transferred from TSU tubes into Qiagen collection tubes (Cat No./ID: 19560) and 180 µl of ATL Buffer and 30 µl of Proteinase K from the Qiagen DNeasy 96 Blood & Tissue Kit (Cat No./ID: 69582) were added and mixed thoroughly. Samples were then incubated overnight (18 hours) at 56°C. For larger sample cohorts, DNA was purified using manufacturer protocols for Qiagen DNeasy 96 Blood & Tissue Kit (Cat No./ID: 69582) and for smaller sample cohorts purification was performed using Qiagen EZ1 DNA Tissue Kit (Cat No: 953034). Recovered DNA was quantified using the PicoGreen dsDNA High Sensitivity Assay Kit (Cat No: P7589). DNA recovery varied among the two purification techniques with the DNeasy 96 Blood & Tissue kit yielding from 20-70 ng/µl and the EZ1 DNA Tissue Kit yielding from 80-100 ng/µl.

JNS haplotype and causative allele

The JNS causative allele information (chr6 60,158,901G->A) was provided via personal communication from John Cole (United States Department of Agriculture – Agriculture Research Service – Animal Genomics and Improvement Laboratory (USDA-ARS-AIPL) and Cari Wolfe (AJCA) and identified via unpublished work that USDA-ARS-AGIL, AJCA, and the Cooperative Dairy DNA Repository all contributed to (this work is currently submitted for review). Flanking sequence was obtained from the *Bos taurus* genome reference assembly ARS-UCD v1.2 (Rosen, Bickhart et al. 2020)

Taqman JNS Genotyping

A Taqman™ (ThermoFischer Scientific, Waltham, MA, USA) assay was developed according to Malkki and Petersdorf's (Christiansen and Tait 2012) recommendations. Briefly forward and reverse amplification primers were designed with optimal lengths of 20 nucleotides, primer T_m within 1°C of each other, and amplicon length of 75–150 bp. Guanine residue at the 5' end of the probe were avoided, as it would quench the reporter fluorescent even after cleavage. Identical nucleotide in the probe sequence, especially runs of ≥4 consecutive G's were avoided. The targeted SNP site was centered in the central third of the probe, and if not possible then shifted towards the 3' end of the probe but avoiding the last two 3' nucleotides.

Targeted Sequence JNS Genotyping

Primer sets

Three locus-specific primer pairs for the JNS missense allele were designed with an expected product size between 170 - 196 bp. Assay product size and primer sequence information area listed in table 1.

PCR amplification

The missense allele was genotyped using a two-step PCR amplicon sequencing strategy. The first PCR is specific to the missense allele locus and adds Illumina adapters to enable indexing PCRs. For each sample, the three locus-specific PCR reactions had a similar compositions per reaction and differed only in the specific primers added to the reaction well. The per reaction composition was 12.5 µl of Q5 High Fidelity 2X Master Mix, 1.25 µl each of 10 µM Forward and Reverse Primers, and 8 µl of Nuclease-Free Water for a 25 µl total volume. Plates were sealed and vortexed briefly to mix and then spun down

and cycled as follows. Following an initial denaturation for 1 minute at 98°C, 35 cycles of denaturation for 10 seconds at 98°C, annealing for 20 seconds at 60°C, and extension for 30 seconds at 72°C were performed prior to a final extension for 2 minutes at 72°C and an optional indefinite hold at 4°C. Initial PCRs were checked on 3% agarose gels to verify the correct amplicons were generated using ethidium bromide staining and an Alphamager UV transilluminator and imaging software prior to proceeding to the indexing PCR. Verified amplicons were then used as template for the indexing PCR whose composition per reaction is 2 µl DreamTaq Buffer (ThermoFisher Cat. No. EP0704), 2 µl of 2mM dNTP (ThermoFisher Cat. No. N8080261), 5 µl of 2 µM combined F + R custom adaptor primers (IDT for Illumina UD Indexes), 0.1 µl of DreamTaq Polymerase (ThermoFisher Cat. No. EP0704), 8.9 µl Nuclease-Free Water, and 2 µl of locus-specific PCR product for a 20 µl total volume. PCR plates were then briefly vortexed, spun down, and then cycled as follows. Following an initial denaturation for 1 minute at 98°C, 12 cycles of denaturation for 10 seconds at 98°C, annealing for 20 seconds at 60°C, and extension for 30 seconds at 72°C were performed prior to a final extension for 2 minutes at 72°C prior to an optional indefinite hold at 4°C. Indexing PCR amplifications were then verified by electrophoresis on a 3% agarose gel and visualized as before.

Separate final pooling and cleanups were performed with different amplicons to address unusually persistent primer dimer seen in some UCLH1 PCRs. Briefly, combine 5 µl of each Step 2 PCR product from primer pairs UCHL1_A and UCHL1_B into a single tube. Amplicons were purified using a single 0.8X-volume SPRI bead clean with 100 µl of pooled PCR product and 80 µl beads. Primer pair C was pooled separately and double 0.8X SPRI bead cleaned.

Sequencing

Final eluates were quantified on a Qubit 3.0 fluorometer using a dsDNA High Sensitivity Assay Kit according to manufacturer's instructions and then the library concentration was normalized to 1 nM by calculating from the Qubit concentration and the fragment sizes of the amplicons: 319 bp for the 2-amplicon pool and 327 bp for the single amplicon pool. All amplicons were combined into a single pool using 10 µl of UCHL1_A + UCHL1_B and 5 µl of UCHL1_C. Illumina's best practices for denaturation and dilution were followed to have a final sequencing library of 10 pM that was run in paired end 150 bp mode along with a 20% 1.4 pM PhiX spike-in.

Sequencing was performed using paired-end 150bp sequencing reads from Illumina MiniSeq. The resulting demultiplexed and trimmed reads are aligned to *Bos taurus* genome reference assembly ARS-UCD v1.2 using BWA (Li and Durbin 2010) with default parameters. Interrogating the read alignment files for each sample and amplicon, we count the number of reads supporting two alleles G and A at the predesignated site on chromosome 6, 60158901 position using pysam (<https://github.com/pysam-developers/pysam>). The ratio of read counts between two alleles across two amplicons are used to predict the carrier status of the animal/sample. If total read count at the allele site is < 1000X, the amplicon/sample combination is deemed a "dropout". If total read count is ≥1000X, and the ratio of G/A alleles is between 0.1 and 0.9 (including equal to 0.1 and 0.9) for both amplicons, the sample is designated as a "JNS carrier". If both amplicons show total coverage ≥1000X and both ratios are smaller than 0.1 or larger than 0.9, the sample is designated as "JNS free". If one amplicon is labeled as "dropout", the status is called using the same criteria but with results from only one amplicon. If both amplicons are "dropouts" or two amplicons disagree on the ratios, the sample is flagged for retesting with another round of the same amplicon test to determine its status.

Results

Taqman

The development of a diagnostic Taqman assay for the JNS allele failed as it is a very rich GC region (>70% in the SNP design region). There is a poly-G run of which the 5th nucleotide is the JNS allele. For Taqman probes 5' guanine effectively act as a quencher for the fluorophore

Targeted Sequencing

Each diagnostic primer set was run across the validation panel and provided a JNS carrier status that matched the CDCB haplotype status, (results not shown).

When the diagnostic primer sets were run across the full set of 198 animals there were 3 individuals (2 males, 1 female) with JNS sequencing results that differed from their April, 2021 CDCB JNS haplotype status (Table 2) All 3 sample's extracted DNA was assayed again, and the sequencing results did not change. A new tissue sample was collected from the 2 sires via TSU and tested, these biological replicates give the same result as the initial sample.

Conclusion

Due to the genomic structure surrounding the JNS causative allele we were not able to develop a Taqman assay. The three independent targeted sequencing assays work extremely well together or separately to generate a highly accurate JNS test result. We hope that our sharing of the diagnostic assay information and its results will allow other commercial labs to offer the same or a similar diagnostic test for the JNS causative allele soon.

References

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Table 1. Oligonucleotide Primers Used for Genotyping at the UCLH1 Missense Locus

Assay Name	Product Size (bp)	Forward Primer Sequence (5'-3')	Reverse Primer Sequence (5'-3')
F3R3	170	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTGAGCTGTTTGGTGTTTAGAACTT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGGGGGAGAAGCAAAGTGCCC
F3R2	196	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTTGAGCTGTTTGGTGTTTAGAACTT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGATATACTTTCACAATGAAG
F4R1	191	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGTTTCATTTTGAGCTGTTTGGTGT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAATGAAGGGAAGAGGGGGA

Table 2. Comparison of JNS results from sequence bases assay versus haplotype

AnimalID	Sex	Assay read counts: total/ G allele/ A allele			JNS status	
		F3R2	F3R3	F4R1	Diagnostic_JNS	CDCB_haplotype*
2	male	5449/2553/2896	4776/2270/2506	6497/3125/3372	carrier	-
3	male	4793/2179/2614	4155/1934/2221	7174/3314/3860	carrier	-
68	male	5375/2517/2858	7996/3937/4059	5466/2579/2887	carrier	carrier
69	male	5748/2759/2989	7198/3466/3732	4316/2100/2216	carrier	carrier
70	male	5608/2759/2849	5313/2534/2779	3361/1645/1716	carrier	carrier
71	male	6063/2909/3154	7212/3550/3662	4020/1965/2055	carrier	carrier
72	male	5124/2420/2704	8000/3905/4095	4168/2049/2119	carrier	carrier
73	male	4798/2216/2582	4549/2267/2282	2031/985/1046	carrier	carrier
74	male	4577/2236/2341	6020/2878/3142	3673/1782/1891	carrier	carrier
75	male	4441/2100/2341	4891/2528/2363	3664/1788/1876	carrier	carrier
76	male	4538/2242/2296	5308/2613/2695	2917/1319/1598	carrier	carrier
77	male	4458/2128/2330	5953/2875/3078	3074/1404/1670	carrier	carrier
78	male	4330/2097/2233	6353/3064/3289	4740/2353/2387	carrier	carrier
79	male	4319/2037/2282	5363/2728/2635	2192/1055/1137	carrier	carrier
80	male	4198/2043/2155	6752/3357/3395	1695/857/838	carrier	carrier
81	male	3886/1864/2022	5589/2671/2918	3696/1811/1885	carrier	carrier
82	male	4538/2148/2390	6959/3488/3471	4226/2032/2194	carrier	carrier
83	male	4448/2108/2340	5750/2788/2962	4012/1866/2146	carrier	carrier
84	male	5048/2439/2609	6400/3123/3277	5967/2932/3035	carrier	carrier
85	male	5449/2597/2852	7110/3536/3574	3484/1743/1741	carrier	carrier
86	male	5449/2597/2852	7110/3536/3574	3484/1743/1741	carrier	carrier
87	male	6180/2829/3351	7275/3696/3579	3844/1871/1973	carrier	carrier
88	male	6018/2893/3125	7339/3448/3891	3310/1553/1757	carrier	carrier
89	male	4045/1943/2102	6752/3108/3644	4535/2185/2350	carrier	carrier
90	male	4433/2060/2373	6514/3097/3417	4509/2068/2441	carrier	carrier
91	male	558/285/273	3723/1868/1855	2352/1140/1212	carrier	carrier
92	male	4952/2407/2545	6554/3129/3425	5859/2830/3029	carrier	carrier
93	male	4296/2028/2268	5622/2768/2854	3931/1905/2026	carrier	carrier
94	male	406/203/203	2358/1126/1232	2246/1081/1165	carrier	carrier
95	male	6167/2980/3187	7370/3517/3853	4564/2132/2432	carrier	carrier
96	male	2080/1026/1054	2655/1332/1323	1741/828/913	carrier	carrier
97	male	2355/1145/1210	486/216/270	1344/648/696	carrier	carrier
98	male	2371/1132/1239	2619/1251/1368	2296/1100/1196	carrier	carrier
99	male	1791/827/964	816/389/427	1350/686/664	carrier	carrier
100	male	216/97/119	3112/1450/1662	1726/832/894	carrier	carrier
102	male	287/136/151	2230/1008/1222	1137/560/577	carrier	free
159	female	3601/1705/1896	4891/2358/2533	4782/2228/2554	carrier	-
165	male	8007/3721/4286	8001/3701/4300	7996/3753/4243	carrier	-
166	male	8007/3744/4263	8007/3744/4263	8001/3805/4196	carrier	-

187	female	8002/3846/4156	8000/3746/4254	7995/3775/4220	carrier	-
197	female	8008/3705/4303	7998/3629/4369	7996/3678/4318	carrier	free
1	male	2162/2162/0	438/438/0	1806/1804/2	free	-
4	male	2088/2086/2	3286/3284/2	1780/1779/1	free	-
5	male	2088/2086/2	3286/3284/2	1780/1779/1	free	-
6	male	1715/1715/0	4001/4001/0	2181/2180/1	free	-
7	male	2041/2040/1	3077/3077/0	1464/1464/0	free	-
8	male	295/295/0	2726/2726/0	1530/1530/0	free	-
9	male	198/198/0	2377/2377/0	1276/1276/0	free	-
10	male	717/717/0	3499/3499/0	2499/2499/0	free	-
11	male	1920/1920/0	2554/2553/1	1457/1456/1	free	-
12	male	526/526/0	4363/4362/1	2046/2046/0	free	-
13	male	2153/2153/0	512/511/1	1289/1289/0	free	-
14	male	252/252/0	1940/1939/1	1428/1428/0	free	-
15	male	307/307/0	3330/3330/0	1978/1978/0	free	-
16	male	2006/2005/1	4440/4437/3	2053/2053/0	free	-
17	male	625/625/0	2733/2733/0	2266/2266/0	free	-
18	male	2782/2782/0	4042/4042/0	2981/2981/0	free	-
19	male	2327/2327/0	11/11/2000	1796/1795/1	free	-
20	male	2367/2367/0	828/828/0	1491/1491/0	free	-
21	male	2491/2491/0	1540/1540/0	2169/2167/2	free	-
22	male	2706/2706/0	398/398/0	2368/2368/0	free	-
23	male	295/295/0	1019/1019/0	1427/1427/0	free	-
24	male	1723/1722/1	678/678/0	2152/2150/2	free	-
25	male	2288/2287/1	1864/1862/2	1221/1221/0	free	-
26	male	1412/1412/0	409/409/0	1431/1431/0	free	-
27	male	2068/2068/0	528/527/1	1892/1891/1	free	-
28	male	1995/1995/0	821/821/0	1477/1475/2	free	-
29	male	2222/2222/0	3084/3084/0	1545/1545/0	free	-
30	male	291/290/1	2978/2978/0	1558/1558/0	free	-
31	male	2689/2688/1	2225/2224/1	1739/1737/2	free	-
32	male	1225/1224/1	1468/1468/0	1096/1096/0	free	-
33	male	2375/2374/1	3833/3833/0	2768/2768/0	free	-
34	male	2018/2018/0	325/325/0	1219/1218/1	free	-
35	male	2121/2121/0	1296/1296/0	1886/1885/1	free	-
36	male	2330/2329/1	2289/2288/1	2490/2490/0	free	-
37	male	1832/1831/1	2609/2609/0	2404/2403/1	free	-
38	male	366/366/0	2379/2379/0	1250/1249/1	free	-
39	male	2071/2071/0	2360/2359/1	1651/1651/0	free	-
40	male	844/844/0	3901/3901/0	2006/2005/1	free	-
41	male	1550/1550/0	2829/2829/0	1338/1338/0	free	-
42	male	258/258/0	2574/2574/0	1053/1053/0	free	-
43	male	2337/2337/0	2820/2820/0	1918/1918/0	free	-

44	male	243/243/0	3264/3264/0	1085/1085/0	free	-
45	male	1829/1829/0	3563/3563/0	1635/1635/0	free	-
46	male	2405/2405/0	1356/1356/0	1416/1415/1	free	-
47	male	414/414/0	2831/2831/0	1703/1703/0	free	-
48	male	1726/1725/1	410/410/0	1854/1854/0	free	-
49	male	1858/1858/0	3707/3704/3	1662/1662/0	free	-
50	male	2639/2639/0	3318/3318/0	1982/1982/0	free	-
51	male	1794/1794/0	373/373/0	1913/1913/0	free	-
52	male	2351/2350/1	1383/1381/2	1524/1524/0	free	-
53	male	3560/3560/0	5151/5151/0	5247/5246/1	free	-
54	male	3793/3791/2	4251/4249/2	6155/6153/2	free	-
55	male	2934/2934/0	2673/2672/1	4946/4946/0	free	-
56	male	4674/4674/0	3573/3572/1	5781/5781/0	free	-
57	male	3235/3233/2	5226/5224/2	5226/5225/1	free	-
58	male	2020/2020/0	4635/4635/0	5413/5413/0	free	-
59	male	3818/3817/1	4132/4132/0	5367/5367/0	free	-
60	male	3759/3759/0	3458/3457/1	5979/5978/1	free	-
61	male	2516/2516/0	3699/3698/1	4693/4693/0	free	-
62	male	35/35/0	35/35/0	7496/7494/2	free	-
63	male	4013/4011/2	3742/3739/3	5983/5982/1	free	-
64	male	2392/2392/0	4254/4253/1	4549/4548/1	free	-
65	male	2609/2608/1	3039/3039/0	2136/2135/1	free	-
66	male	2051/2051/0	2168/2167/1	2476/2476/0	free	-
67	male	2895/2895/0	1314/1314/0	2641/2641/0	free	-
101	male	2715/2715/0	3206/3206/0	2600/2600/0	free	carrier
103	male	1850/1849/1	3020/3018/2	1848/1848/0	free	free
104	male	326/326/0	3463/3463/0	1915/1915/0	free	free
105	male	276/276/0	2930/2930/0	1413/1413/0	free	free
106	male	1305/1305/0	2184/2183/1	1148/1148/0	free	free
107	male	2376/2376/0	400/400/0	1943/1942/1	free	free
108	male	183/183/0	2899/2898/1	967/967/0	free	free
109	male	1835/1835/0	3235/3232/3	1554/1554/0	free	free
110	male	2198/2198/0	3644/3644/0	1996/1996/0	free	free
111	male	330/330/0	2776/2773/3	1403/1403/0	free	free
112	male	2156/2155/1	2482/2482/0	1528/1528/0	free	free
113	male	244/244/0	1915/1914/1	1164/1164/0	free	free
114	male	628/628/0	3648/3647/1	2104/2104/0	free	free
115	male	736/736/0	4087/4086/1	1947/1947/0	free	free
116	male	2621/2620/1	1424/1424/0	1292/1290/2	free	free
117	male	1711/1711/0	2553/2553/0	1331/1330/1	free	free
118	male	315/315/0	3107/3107/0	1178/1178/0	free	free
119	male	3047/3047/0	2047/2047/0	2076/2075/1	free	free
120	male	2432/2431/1	4328/4328/0	2250/2250/0	free	free

121	male	1922/1922/0	490/489/1	2621/2619/2	free	free
122	male	2678/2678/0	3460/3460/0	2054/2054/0	free	free
123	male	1849/1848/1	2664/2664/0	1450/1448/2	free	free
124	male	1829/1829/0	1274/1274/0	1695/1695/0	free	free
125	male	2090/2089/1	3137/3136/1	1356/1355/1	free	free
126	male	489/489/0	3106/3105/1	1798/1798/0	free	free
127	male	190/190/0	1703/1702/1	957/957/0	free	free
128	male	264/264/0	1904/1904/0	1012/1012/0	free	free
129	male	125/124/1	1439/1439/0	3108/3107/1	free	free
130	male	376/375/1	2889/2887/2	1862/1862/0	free	free
131	male	210/210/0	1849/1849/0	852/852/0	free	free
132	male	152/152/0	2189/2189/0	1231/1231/0	free	free
133	male	2504/2504/0	1223/1223/0	1841/1840/1	free	free
134	male	2831/2830/1	1164/1164/0	1579/1578/1	free	free
135	male	2211/2211/0	633/633/0	2017/2017/0	free	free
136	male	451/451/0	2821/2821/0	1851/1850/1	free	free
137	male	273/273/0	1500/1500/0	931/931/0	free	free
138	male	400/400/0	2068/2068/0	1405/1405/0	free	free
139	male	751/751/0	4072/4072/0	2486/2486/0	free	free
140	male	174/174/0	2446/2446/0	1097/1097/0	free	free
141	male	2398/2395/3	1621/1621/0	2019/2019/0	free	free
142	male	257/257/0	2317/2317/0	1030/1030/0	free	free
143	male	2303/2302/1	1734/1734/0	1666/1666/0	free	free
144	male	246/246/0	1964/1964/0	1308/1308/0	free	free
145	male	5038/5038/0	5671/5671/0	4531/4528/3	free	free
146	male	4778/4776/2	5164/5163/1	3792/3792/0	free	free
147	male	3926/3924/2	4629/4628/1	3682/3682/0	free	free
148	female	3051/3050/1	3711/3710/1	4687/4684/3	free	-
149	female	3133/3131/2	5554/5554/0	1593/1593/0	free	-
150	female	3336/3336/0	5324/5322/2	4672/4670/2	free	-
151	female	5628/5628/0	4728/4727/1	3214/3213/1	free	-
152	female	3829/3826/3	5967/5967/0	5169/5167/2	free	-
153	female	3644/3644/0	6207/6206/1	4944/4944/0	free	-
154	female	6126/6125/1	5354/5353/1	5119/5118/1	free	-
155	female	1914/1912/2	3306/3304/2	4314/4313/1	free	-
156	female	6362/6360/2	6017/6017/0	5200/5199/1	free	-
157	female	4229/4229/0	4846/4846/0	3798/3798/0	free	-
158	female	6560/6559/1	5831/5831/0	5129/5129/0	free	-
160	male	8002/8000/2	8005/8004/1	8005/8002/3	free	-
161	male	8002/8002/0	8002/8002/0	8002/8000/2	free	-
162	male	8006/8006/0	8002/8001/1	7997/7996/1	free	-
163	male	8006/8005/1	8001/8000/1	8000/7998/2	free	-
164	male	7998/7998/0	8001/7999/2	8001/7999/2	free	-

167	male	8009/8008/1	7998/7997/1	8006/8004/2	free	-
168	male	8002/8002/0	8004/8004/0	7999/7996/3	free	-
169	male	8003/8003/0	14/13/1	8000/7998/2	free	-
170	male	8004/8004/0	8002/8001/1	8001/8000/1	free	-
171	male	8008/8008/0	8002/8001/1	8004/8003/1	free	-
172	female	8010/8010/0	8006/8006/0	7996/7992/4	free	-
173	female	8004/8004/0	8001/8000/1	8004/8003/1	free	-
174	female	8008/8005/3	8006/8005/1	8006/8005/1	free	-
175	female	8003/8001/2	6116/6116/0	8003/7999/4	free	-
176	female	8006/8003/3	7998/7997/1	8000/7998/2	free	-
177	female	8007/8005/2	8003/8000/3	8001/8001/0	free	-
178	female	8006/8005/1	8006/8003/3	7999/7998/1	free	-
179	female	8008/8007/1	8008/8008/0	8000/7998/2	free	-
180	female	8007/8007/0	8005/8003/2	7996/7994/2	free	-
181	female	8001/8001/0	7998/7998/0	8001/8000/1	free	-
182	female	8004/8004/0	8000/8000/0	8003/8003/0	free	-
183	female	8001/7997/4	6589/6587/2	7996/7995/1	free	-
184	female	8005/8004/1	8001/8000/1	8002/8001/1	free	-
185	female	7997/7997/0	4896/4895/1	8000/8000/0	free	-
186	female	8010/8008/2	8000/7997/3	8004/8003/1	free	-
188	female	8013/8012/1	8013/8012/1	8009/8007/2	free	-
189	female	8007/8007/0	8001/8001/0	7999/7997/2	free	-
190	female	8007/8006/1	8003/8001/2	7999/7999/0	free	-
191	female	3086/3085/1	6509/6509/0	4337/4337/0	free	-
192	female	8002/8002/0	8000/8000/0	7997/7997/0	free	-
193	female	8007/8006/1	8001/8001/0	7999/7997/2	free	-
194	female	8011/8009/2	8000/7999/1	8003/8001/2	free	-
195	female	8002/8001/1	7998/7998/0	8001/7999/2	free	-
196	female	8004/8004/0	7999/7996/3	8002/8001/1	free	-
198	female	8010/8010/0	8005/8004/1	8004/8003/1	free	-
199	female	8007/8005/2	8002/8001/1	8001/7999/2	free	-
200	female	8001/7998/3	7999/7998/1	7999/7982/17	free	-
201	female	2453/2453/0	5352/5349/3	4562/4562/0	free	-

* CDCB haplotye staus obtained April, 2021