

Supplementary Material for the article:Newkirk et al., *Human Mutation***Determination of Genomic Copy Number with Quantitative Microsphere Hybridization**

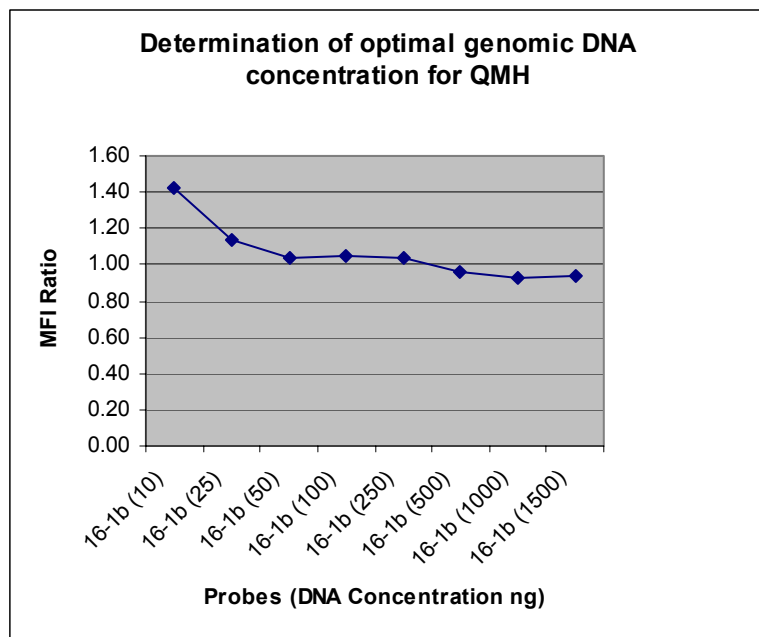
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Supplemental Table 1: Data for QMH optimization reactions using sc probes differing in length

Sample	Cytogenetic findings	Test	Reference	Mean Intensities			Known Genotype
				test	reference	Ratio	
81	t(9;22)(q34;q11.2).ish der(9)(5'ABL1-)	16-1a	HOXB1a	29.06	41.48	0.70	del
138	t(9;22)(q34;q11.2).ish der(9)(5'ABL1+)	16-1a	HOXB1a	105.62	144.17	0.73	wt
46	t(9;22)(q34;q11.2).ish der(9)(5'ABL1-)	16-1a	HOXB1a	89.47	142.28	0.63	del
GM10186	47,XY,+9.ish 9p11q11(D9Z1x3)	16-1a	HOXB1a	62.24	46.64	1.33	dup
81	t(9;22)(q34;q11.2).ish der(9)(5'ABL1-)	16-1a	HOXB1a	235.05	296.97	0.79	del
33	t(9;22)(q34;q11.2).ish der(9)(5'ABL1-)	16-1a	HOXB1a	219.39	276.92	0.79	del
38	t(9;22)(q34;q11.2).ish der(9)(5'ABL1+)	16-1a	HOXB1a	544.61	582.81	0.93	wt
177	t(9;22)(q34;q11.2).ish der(9)(5'ABL1+)	16-1a	HOXB1a	39.33	44.99	0.87	wt
177	ibid	16-1a	HOXB1a	694.58	794.67	0.87	wt
47	t(9;22)(q34;q11.2).ish der(9)(5'ABL1+)	16-2a	HOXB1a	31.24	26.77	1.17	wt
47	ibid	16-2a	HOXB1a	49.55	58.73	0.84	wt
138	t(9;22)(q34;q11.2).ish der(9)(5'ABL1+)	16-2a	HOXB1a	130.74	141.41	0.92	wt
33	t(9;22)(q34;q11.2).ish der(9)(5'ABL1-)	16-2a	HOXB1a	58.76	88.81	0.66	del
33	ibid	16-2a	HOXB1a	69.43	103.09	0.67	del
177	t(9;22)(q34;q11.2).ish der(9)(5'ABL1+)	16-2a	HOXB1a	40.22	44.99	0.89	wt
81	t(9;22)(q34;q11.2).ish der(9)(5'ABL1-)	16-2a	HOXB1a	566.04	1482.05	0.38	del
33	t(9;22)(q34;q11.2).ish der(9)(5'ABL1-)	16-2a	HOXB1a	82.24	285.80	0.29	del
81	t(9;22)(q34;q11.2).ish der(9)(5'ABL-)	16-1c	HOXB1e	140.73	188.25	0.75	del
86	t(9;22)(q34;q11.2).ish der(9)(5'ABL1+)	16-1c	HOXB1e	147.27	105.63	1.39	wt
GM10186	47,XY,+9.ish 9p11q11(D9Z1x3)	16-1c	HOXB1e	64.29	52.45	1.23	dup
81	t(9;22)(q34;q11.2).ish der(9)(5'ABL1-)	16-1c	HOXB1e	178.95	212.91	0.84	del
81	t(9;22)(q34;q11.2).ish der(9)(5'ABL1-)	16-1c	HOXB1e	124.10	136.10	0.91	del
177	t(9;22)(q34;q11.2).ish der(9)(5'ABL1+)	16-1c	HOXB1e	72.68	53.63	1.36	wt
33	t(9;22)(q34;q11.2).ish der(9)(5'ABL1-)	16-1c	HOXB1e	40.15	52.02	0.77	del
GM09286	47,XY,+9.ish 9p11q11(D9Z1x3)	16-1c	HOXB1e	93.92	73.36	1.28	dup
177	t(9;22)(q34;q11.2).ish der(9)(5'ABL1+)	16-2c	HOXB1e	65.59	56.63	1.16	wt
33	t(9;22)(q34;q11.2).ish der(9)(5'ABL1-)	16-2c	HOXB1e	44.49	52.02	0.86	del
124	t(9;22)(q34;q11.2).ish der(9)(5'ABL1+)	16-1d	HOXB1d	462.50	339.80	1.36	wt
81	t(9;22)(q34;q11.2).ish der(9)(5'ABL1-)	16-1d	HOXB1d	1013.43	859.19	1.18	wt
38	t(9;22)(q34;q11.2).ish der(9)(5'ABL1+)	HREP	HOXB1d	120.20	74.37	1.62	wt
CMT1A-1	nuc ish 17p12(PMP22x3)	HREP	HOXB1d	78.78	41.56	1.90	wt
CMT1A-1	nuc ish 17p12(PMP22x3)	PMP22	HOXB1d	151.29	129.85	1.17	dup
CMT1A-2	nuc ish 17p12(PMP22x3)	PMP22	HOXB1d	169.58	144.97	1.17	dup
CMT1A-3	nuc ish 17p12(PMP22x3)	PMP22	HOXB1d	176.35	147.59	1.19	dup

Supplementary Table S2a: Dilution series of genomic target DNA into QMH Reactions to determine the optimal target detection limits

Sample	Concentration (ng)	Test Probe	Geometric Mean of test probe	Geometric mean of reference probe	Ratio
47	10	16-1b (10)	66.47	46.78	1.42
47	25	16-1b (25)	77.80	68.41	1.14
47	50	16-1b (50)	234.44	225.62	1.04
47	100	16-1b (100)	235.19	223.92	1.05
47	250	16-1b (250)	282.48	271.51	1.04
47	500	16-1b (500)	285.20	295.99	0.96
47	1000	16-1b (1000)	279.50	301.56	0.93
47	1500	16-1b (1500)	278.30	298.31	0.93



Supplemental Table 2b: Microsphere-conjugated sc probes detecting homologous PCR product in presence of *Bos taurus* DNA (10ng) to determine lowest detectable target quantity for QMH

PCR product	#genomic equivalents	Geometric Mean
16-1a	3	0.79
16-1a	5	2.04
16-1a	10	2.46
16-1a	20	3.21
16-2a	3	0.00
16-2a	5	3.20
16-2a	10	3.23
16-2a	20	4.62

* Number of genomic equivalents refers to number of copies of each probe found within one human diploid genomic copy

Supplemental Table 3: Flow cytometric detection of chromosome duplication in blind study using 20 normal and 2 CMT1A duplicated samples

Sample	Test Probe	Mean Intensities			Genotype	range within sample runs	variance within replicates
		test probe	HOXB1c	Ratio*			
1A	TEKT3	814.35	805.67	1.01	wt	0.018	0.000106
1B	TEKT3	846.69	853.20	0.99	wt		
1C	TEKT3	726.37	731.00	0.99	wt		
2A	TEKT3	1052.66	1123.97	0.94	wt	0.095	0.002619
2B	TEKT3	1061.07	1150.91	0.92	wt		
2C	TEKT3	1112.92	1094.33	1.02	wt		
3A	TEKT3	1615.23	1529.85	1.06	wt	0.055	0.000232
3B	TEKT3	1616.38	1567.96	1.03	wt		
3C	TEKT3	1631.83	1541.70	1.06	wt		
4A	TEKT3	1874.97	1871.94	1.00	wt	0.042	0.000476
4B	TEKT3	1818.65	1742.10	1.04	wt		
4C	TEKT3	1849.12	1824.29	1.01	wt		
5A	TEKT3	1670.08	1663.20	1.00	wt	0.042	0.000484
5B	TEKT3	1640.09	1618.89	1.01	wt		
5C	TEKT3	1625.97	1674.03	0.97	wt		
6A	TEKT3	2152.80	2117.80	1.02	wt	0.009	0.000036
6B	TEKT3	2332.52	2301.29	1.01	wt		
6C	TEKT3	2168.54	2157.86	1.00	wt		
7A	TEKT3	385.44	406.76	0.95	wt	0.001	0.000037
7B	TEKT3	352.79	375.96	0.94	wt		
7C	TEKT3	361.55	386.24	0.94	wt		
8A	TEKT3	880.67	957.81	0.92	wt	0.033	0.000357
8B	TEKT3	856.13	899.82	0.95	wt		
8C	TEKT3	834.64	875.94	0.95	wt		
9A	TEKT3	698.70	722.54	0.97	wt	0.062	0.001167
9B	TEKT3	701.73	686.48	1.02	wt		
9C	TEKT3	671.94	700.12	0.96	wt		
10A	TEKT3	606.27	615.92	0.98	wt	0.005	0.000007
10B	TEKT3	579.58	591.36	0.98	wt		
10C	TEKT3	610.88	620.37	0.98	wt		
11A	TEKT3	673.18	626.01	1.08	wt	0.027	0.000217
11B	TEKT3	661.83	602.01	1.10	wt		
11C	TEKT3	645.36	601.70	1.07	wt		
12A	TEKT3	1254.05	1243.07	1.01	wt	0.006	0.000011
12B	TEKT3	1220.48	1211.49	1.01	wt		
12C	TEKT3	975.71	973.22	1.00	wt		
13A	TEKT3	1326.44	1298.74	1.02	wt	0.026	0.000187
13B	TEKT3	1125.52	1111.00	1.01	wt		
13C	TEKT3	1095.84	1053.93	1.04	wt		

Supplemental Table 3: Flow cytometric detection of chromosome duplication in blind study using 20 normal and 2 CMT1A duplicated samples (Continued)

14A	TEKT3	1478.91	1434.41	1.03	wt	0.020	0.000094
14B	TEKT3	1385.60	1369.32	1.01	wt		
14C	TEKT3	1343.97	1319.13	1.02	wt		
15A	TEKT3	1402.19	1353.95	1.04	wt	0.020	0.000145
15B	TEKT3	1337.18	1268.01	1.05	wt		
15C	TEKT3	1279.94	1240.01	1.03	wt		
17A	TEKT3	2440.96	2430.98	1.00	wt	0.017	0.000095
17B	TEKT3	2379.22	2371.42	1.00	wt		
17C	TEKT3	2256.53	2211.00	1.02	wt		
18A	TEKT3	2227.55	2171.93	1.03	wt	0.020	0.000109
18B	TEKT3	2024.87	1996.90	1.01	wt		
18C	TEKT3	2068.90	1999.20	1.03	wt		
19A	TEKT3	2269.61	2269.65	1.00	wt	0.030	0.000239
19B	TEKT3	2088.00	2025.51	1.03	wt		
19C	TEKT3	1969.35	1941.78	1.01	wt		
20A	TEKT3	2570.97	2467.60	1.04	wt	0.050	0.000705
20B	TEKT3	2551.68	2430.05	1.05	wt		
20C	TEKT3	2443.81	2442.50	1.00	wt		
22A	TEKT3	1807.33	1745.64	1.04	wt	0.050	0.000579
22B	TEKT3	1654.61	1654.56	1.00	wt		
22C	TEKT3	1617.91	1635.33	0.99	wt		
16A	TEKT3	3313.34	1990.96	1.66	dup	0.034	0.000295
16B	TEKT3	3831.44	2256.39	1.70	dup		
16C	TEKT3	3542.22	2113.40	1.68	dup		
21A	TEKT3	886.15	660.97	1.34	dup	0.060	0.001083
21B	TEKT3	1060.99	760.63	1.39	dup		
21C	TEKT3	916.13	686.02	1.34	dup		

*Ratio of geometric mean fluorescence for test:reference probe

Supplemental Table 4: Data for QMH experiments with samples of known genotype obtained during quality control for each probe-conjugated microsphere level.

(The MFI ratios fall within the 95% confidence intervals determined for all genotypes).

Sample	Probes		Mean Fluorescence Intensity		Ratio	Genotype
	Test	Reference	Test	Reference		
33	16-1b	HOXB1c	235.05	296.97	0.65	del
46	16-1b	HOXB1c	82.77	142.28	0.58	del
46	16-1b	HOXB1c	29.06	41.48	0.60	del
81	16-1b	HOXB1c	89.47	142.28	0.63	del
46	16-2b	HOXB1c	12.87	26.35	0.49	del
81	16-2b	HOXB1c	46.78	88.81	0.53	del
CMT1A-1	TEKT3	HOXB1c	3313.34	1990.96	1.66	dup
CMT1A-2	TEKT3	HOXB1c	3831.44	2256.39	1.70	dup
CMT1A-3	TEKT3	HOXB1c	3542.22	2113.40	1.68	dup
CMT1A-4	TEKT3	HOXB1c	104.09	74.17	1.40	dup
CMT1A-1	TEKT3	HOXB1c	104.09	74.17	1.40	dup
CMT1A-1 ³	TEKT3	HOXB1c	125.56	79.62	1.58	dup
CMT1A-1 ³	TEKT3	HOXB1c	70.65	48.21	1.47	dup
CMT1A-2 ³	TEKT3	HOXB1c	122.33	86.02	1.42	dup
CMT1A-2 ³	TEKT3	HOXB1c	107.62	74.36	1.45	dup
GM09286	16-1b	HOXB1c	65.10	48.72	1.34	dup
81	16-1b	HOXB1c	203.47	202.15	1.01	normal
81	16-1b	HOXB1c	190.66	194.03	0.98	normal
177	16-1b	HOXB1c	182.41	173.86	1.05	normal
177	16-1b	HOXB1c	162.15	161.31	1.01	normal
86	16-1b	HOXB1c	107.40	104.20	1.03	normal
86	16-1b	HOXB1c	124.63	114.80	1.09	normal
124	16-1b	HOXB1c	721.63	678.48	1.06	normal
77	16-1b	HOXB1c	122.30	119.63	1.02	normal
38	16-1b	HOXB1c	97.22	99.60	0.98	normal
GM10186	16-1b	HOXB1c	27.41	26.77	1.02	normal
GM06074	16-1b	HOXB1c	1240.47	1179.37	1.05	normal
CMT1A-1	16-1b	HOXB1c	1121.98	1132.11	0.99	normal
CMT1A-2	16-1b	HOXB1c	41.04	44.74	0.92	normal
CMT1A-3	16-1b	HOXB1c	84.99	82.70	1.03	normal
CMT1A-4	16-1b	HOXB1c	203.47	202.15	1.01	normal
38	16-1b	HOXB1c	182.41	173.86	1.05	normal
77	16-1b	HOXB1c	162.15	161.31	1.01	normal
47	16-2b	HOXB1c	27.41	26.77	1.02	normal
38	16-2b	HOXB1c	721.63	678.48	1.06	normal
77	16-2b	HOXB1c	122.30	119.63	1.02	normal
124	16-2b	HOXB1c	814.35	805.67	1.01	normal
38	PMP22	HOXB1c	846.69	853.20	0.99	normal
77	PMP22	HOXB1c	726.37	731.00	0.99	normal
124	PMP22	HOXB1c	1052.66	1123.97	0.94	normal
177	PMP22	HOXB1c	1061.07	1150.91	0.92	normal

Supplemental Table 4: Data for QMH experiments with samples of known genotype obtained during quality control for each probe-conjugated microsphere level (Continued).

38	TEKT3	HOXB1c	44.31	51.74	0.86	normal
77	TEKT3	HOXB1c	48.72	50.94	0.96	normal
124	TEKT3	HOXB1c	84.99	82.70	1.03	normal
177	TEKT3	HOXB1c	39.22	40.22	0.98	normal
86	TEKT3	HOXB1c	40.22	44.99	0.89	normal
81	TEKT3	HOXB1c	203.47	202.15	1.01	normal
46	TEKT3	HOXB1c	1112.92	1094.33	1.02	normal
33	TEKT3	HOXB1c	1615.23	1529.85	1.01	normal