Supplemental Table 1. Exons evaluated in the 28 genes sequencing by the bone marrow and ccfDNA assays

Gene	Exon (codons) tested
ABL1 (NM_007313)	1-3 (1-99), 3-4 (92-200), 4-6 (195-317), 6-10 (307-523), 11 (560-576), 11 (567-606), 11 (598-638), 11 (661-700), 11 (691-731), 11 (723-762), 11 (752-790), 11 (781-820), 11 (810-851), 11 (843-882), 11 (873-912), 11 (935-975), 11 (965-1003), 11 (995-1034), 11 (1070-1131)
ASXL1 (NM_015338)	2-4 (20-104), 4-8 (96-253), 8-11 (250-377), 11 (368-407), 11 (398-437), 11-12 (427-589), 12 (581-619), 12 (640-678), 12 (670-709), 12 (701-740), 12 (732-771), 12 (762-801), 12 (792-831), 12 (821-860), 12 (851-889), 12 (880-919), 12 (911-949), 12 (940-979), 12 (970-1009), 12 (999-1038), 12 (1031-1069), 12 (1061-1100), 12 (1091-1129), 12 (1123-1160), 12 (1153-1191), 12 (1182-1221), 12 (1212-1251), 12 (1241-1281), 12 (1271-1311), 12 (1301-1341), 12 (1336-1375), 12 (1369-1542)
BRAF (NM_004333)	2-3 (47-157), 3-17 (147-709), 18 (729-767)
DNMT3A (NM_022552)	3-6 (25-196), 7-8 (214-322), 9-12 (339-486), 12 (477-492), 14-16 (519-641), 16-19 (631-739), 19 (729-768), 19-20 (759-803), 22-23 (827-913)
EGFR (NM_005228)	2-3 (30-96), 3-6 (89-249), 7-9 (273-353), 9-12 (343-444), 12-28 (438-1104), 28 (1096-1133), 28 (1171-1211)
EZH2 (NM_004456)	2-10 (1-404), 10-11 (395-466), 11-15 (460-610), 15-19 (606-728), 19-20 (719-752)
FLT3 (NM_004119)	2-3 (15-112), 3-8 (103-311), 8 (301-340), 8-14 (331-610), 14-15 (606-642), 15-24 (635-994)
GATA1 (NM_002049)	2 (1-16), 2-3 (13-89), 3 (81-120), 3 (111-151), 3 (142-182), 4 (200-248), 5-6 (265-306), 6 (297-336), 6 (377-414)
GATA2 (NM_032638)	2-3 (1-140), 3 (181-220), 3 (212-251), 3 (242-280), 3 (273-291), 4-6 (318-439), 6 (434-474), 6 (466-481)
HRAS (NM_005343)	2-4 (18-129)
IDH1 (NM_005896)	3-4 (1-100), 4 (92-131), 4-6 (121-228), 6-10 (223-415)
IDH2 (NM_002168)	1-2 (1-69), 3-5 (106-220), 5-7 (212-281), 7 (278-317), 7 (308-323), 9-10 (361-417), 10-11 (408-453)
IKZF2 (NM_016260)	2-4 (1-99), 4 (91-129), 4-8 (121-432), 8 (424-463), 8 (454-494), 8 (484-524), 8 (514-527)
JAK2 (NM_004972)	3-7 (11-220), 7-9 (241-364), 9-11 (359-456), 11-17 (451-723), 17-21 (747-949), 21-25 (939-1133)
KIT (NM_000222)	1-3 (1-128), 3 (119-157), 3-5 (152-292), 5 (253-254), 5-6 (292-321), 6-9 (318-460), 9-10 (454-532), 10-12 (523-606), 12-16 (597-765), 17-18 (788-842), 18-21 (834-977)
KRAS (NM_004985)	2-3 (1-87), 3-5 (81-189)
MDM2 (NM_002392)	1-6 (1-134), 7-11 (143-323), 11 (313-352), 11 (342-379), 11 (370-408), 11 (403-498)
MLL (NM_005933)	2-3 (145-182), 3 (178-215), 3 (206-244), 3 (236-274), 3 (265-303), 3 (293-332), 3 (325-362), 3 (353-392), 3 (390-427), 3 (418-456), 3 (476-515), 3 (506-544), 3 (534-573), 3 (564-603), 3 (594-632), 3 (624-662), 3 (653-691), 3 (682-722), 3 (714-753), 3 (743-780), 3 (771-809), 3 (799-837), 3-5 (831-1129), 5-6 (1121-1212), 7-12 (1218-1507), 13-15 (1526-1617), 15-19 (1610-1805), 19-22(1796-1943), 22 (1940-1979), 23 (1985-2024), 24-26 (2032-2114), 26-27 (2111-2186), 27 (2176-2214), 27 (2204-2244), 27 (2236-2274), 27 (2264-2303), 27 (2295-2334), 27 (2324-2362), 27 (2354-2392), 27 (2382-2422), 27 (2413-2451), 27 (2443-2480), 27(2471-2511), 27 (2502-2542), 27 (2533-2570), 27 (2561-2600), 27 (2591-2629), 27 (2619-2660), 27 (2651-2690), 27 (2710-2747),27 (2798-2836), 27 (2827-2864), 27 (2856-2894), 27 (2885-2924), 27 (2914-2953), 27 (2946-2983), 27 (2974-3012), 27 (3003-3041), 27 (3032-3072), 27 (3063-3102), 27 (3092-3131), 27 (3123-3161), 27 (3152-3190), 27-32 (3183-3729), 32 (3720-3758), 32-36 (3755-3891), 36 (3882-3921), 36 (3918-3970)

MPL (NM_005373)	1 (1-17), 1-3 (7-88), 3 (81-122), 4-7 (146-346), 7 (336-375), 7-9 (370-490), 11 (522-551), 12 (559-636)
MYD88 (NM_002468)	1 (1-20), 1 (10-50), 1-3 (41-184), 3 (174-213), 3-5 (211-310)
NOTCH1 (NM_017617)	1-3 (1-128), 4 (197-237), 5 (248-289), 6 (318-357), 6 (350-367), 8 (419-443), 8 (434-475), 8-9 (466-504), 10 (519-557), 11-12(632-672), 13-14 (716-785), 16-17 (823-885), 18 (914-981), 21 (1109-1166), 21 (1157-1170), 23 (1215-1294), 24 (1335-1338), 25(1373-1413), 25 (1449-1491), 25 (1481-1523), 25-26 (1513-1631), 26 (1625-1666), 26 (1657-1673), 27 (1701-1723), 29 (1795-1804), 30 (1825-1843), 30 (1835-1874), 30 (1865-1880), 31 (1895-1935), 31 (1933-1973), 31 (1965-1978), 32 (2012-2028), 34(2061-2085), 34 (2131-2210), 34 (2251-2335), 34 (2326-2364), 34 (2356-2398), 34 (2420-2460), 34 (2451-2489), 34 (2481-2521),34 (2511-2549), 34 (2540-2556)
NPM1 (NM_002520)	1-5 (1-131), 5-11 (123-295)
NRAS (NM_002524)	2 (1-37), 3-5 (77-190)
PTPN11 (NM_002834)	2-3 (5-60), 3-8 (54-299), 9-15 (312-594)
RUNX1 (NM_001754)	3-4 (20-101), 5-6 (118-205), 8-9 (269-435), 9 (428-465), 9 (460-481)
TET2 (NM_001127208)	3 (1-22), 3 (13-52), 3 (44-82), 3 (73-112), 3 (103-140), 3 (132-171), 3 (162-200), 3 (191-229), 3 (220-259), 3 (250-288), 3 (279-318), 3 (308-347), 3 (337-376), 3 (369-408), 3 (399-439), 3 (429-468), 3 (459-498), 3 (488-527), 3 (518-557), 3 (549-586), 3 (577-616), 3 (636-674), 3 (665-704), 3 (694-732), 3 (723-762), 3 (752-790), 3-6 (783-1216), 6-10 (1207-1408), 10 (1399-1438), 10-11(1432-1533), 11 (1523-1563), 11 (1555-1593), 11 (1585-1622), 11 (1612-1651), 11 (1643-1683), 11 (1674-1712), 11 (1703-1740),11 (1736-2003)
TP53 (NM_000546)	4 (41-80), 4 (72-112), 4-6 (107-214), 6 (210-224), 7-10 (234-367)
WT1 (NM_024426)	1 (25-63), 1 (126-204), 1 (197-216), 2 (240-257), 4-10 (291-518)

Supplemental Table 2. 275 genes evaluated in the ccfDNA targeted sequencing panel

ABL1	*BCL2L1*	*CDK12*	*DOT1L*	*FBXW7*	*HIST1H3B*	*KMT2C*	*MYC*	*PIK3CA*	*RHEB*	*STK11*
ACVR1B	*BCL6*	*CDK4*	*EED*	*FGF4*	*HNF1A*	*KMT2D*	*MYCL*	*PIK3R1*	*RHOA*	*SUFU*
AKT1	*BCOR*	*CDK6*	*EGFR*	*FGF6*	*HOXB13*	*KRAS*	*MYCN*	*PIK3R2*	*RIT1*	*SUZ12*
AKT2	*BCORL1*	*CDKN2A*	*EGLN1*	*FGFR1*	*HRAS*	*LRP1B*	*MYD88*	*PIM1*	*RNF43*	*TAL1*
AKT3	*BCR*	*CDKN2B*	*EP300*	*FGFR2*	*HSP90AA1*	*MAP2K1*	*NF1*	*PLCG1*	*ROS1*	*TCF3*
ALK	*BIRC3*	*CDKN2C*	*EPAS1*	*FGFR3*	*ID3*	*MAP2K2*	*NF2*	*PMS1*	*RUNX1*	*TERT*
AMER1	*BLM*	*CEBPA*	*EPHA3*	*FGFR4*	*IDH1*	*MAP2K4*	*NFE2L2*	*PMS2*	*SDHB*	*TET2*
APC	*BRAF*	*CHEK1*	*EPHA5*	*FH*	*IDH2*	*MAP3K1*	*NFKBIA*	*POLD1*	*SETBP1*	*TGFBR2*
AR	*BRCA1*	*CHEK2*	*ERBB2*	*FLCN*	*IGF1R*	*MAP3K14*	*NKX2-1*	*POLE*	*SETD2*	*TNFAIP3*
ARAF	*BRCA2*	*CIC*	*ERBB3*	*FLT3*	*IKZF1*	*MAPK1*	*NOTCH1*	*PPM1D*	*SF3B1*	*TNFRSF14*
ARID1A	*BRIP1*	*CREBBP*	*ERBB4*	*FLT4*	*IKZF3*	*MCL1*	*NOTCH2*	*PPP2R1A*	*SMAD2*	*TP53*
ARID1B	*BTK*	*CRLF2*	*ERG*	*FOXL2*	*IL7R*	*MDM2*	*NOTCH3*	*PRDM1*	*SMAD4*	*TRAF3*
ARID2	*CALR*	*CSF1R*	*ESR1*	*FUBP1*	*INHBA*	*MDM4*	*NPM1*	*PRKAR1A*	*SMARCA4*	*TSC1*
ASXL1	*CARD11*	*CSF3R*	*ETV6*	*GALNT12*	*IRF4*	*MED12*	*NRAS*	*PRKDC*	*SMARCB1*	*TSC2*
ATM	*CBL*	*CTCF*	*EXO1*	*GATA1*	*JAK1*	*MEF2B*	*NSD1*	*PRSS1*	*SMC1A*	*TSHR*
ATR	*CBLB*	*CTNNA1*	*EZH2*	*GATA2*	*JAK2*	*MEN1*	*NTRK1*	*PTCH1*	*SMC3*	*U2AF1*
ATRX	*CBLC*	*CTNNB1*	*FAM175A*	*GATA3*	*JAK3*	*MET*	*NTRK2*	*PTEN*	*SMO*	*U2AF2*
AURKA	*CCND1*	*CUX1*	*FAM46C*	*GEN1*	*KAT6A*	*MITF*	*NTRK3*	*PTPN11*	*SOCS1*	*VHL*
AURKB	*CCND3*	*CXCR4*	*FANCA*	*GNA11*	*KDM5C*	*MLH1*	*PAK3*	*RAC1*	*SOX2*	*WHSC1*
AURKC	*CCNE1*	*CYLD*	*FANCC*	*GNAQ*	*KDM6A*	*MPL*	*PALB2*	*RAD21*	*SOX9*	*WT1*
AXIN1	*CD274*	*DAXX*	*FANCD2*	*GNAS*	*KDR*	*MRE11A*	*PAX5*	*RAD50*	*SPOP*	*XPO1*
AXIN2	*CD79A*	*DDR2*	*FANCE*	*GREM1*	*KEAP1*	*MSH2*	*PBRM1*	*RAD51*	*SRC*	*XRCC2*
B2M	*CD79B*	*DICER1*	*FANCF*	*GRIN2A*	*KIT*	*MSH6*	*PDGFRA*	*RAF1*	*SRSF2*	*XRCC3*
BAP1	*CDC73*	*DNM2*	*FANCG*	*H3F3A*	*KMT2A*	*MTOR*	*PDGFRB*	*RB1*	*STAG2*	*ZNF217*
BCL2	*CDH1*	*DNMT3A*	*FAS*	*HGF*	*KMT2B*	*MUTYH*	*PHF6*	*RET*	*STAT3*	*ZRSR2*

Supplemental Table 3. Mutation VAF in DNA extracted from bone marrow cells and from ccfDNA from one patient tested on 3 consecutive days. Overall similar results were noted between across time and between the two sources.

Gene	HGVSc	HGVSp	Sample type	Day 1	Day 2	Day3
				VAF%	VAF%	VAF%
ASXL1	NM_015338.5:c.2920dupT	p.Tyr974fs	BM Cells	3.11	5.19	5.16
			ccfDNA	7.79	5.84	6.46
U2AF1	NM_006758.2:c.101C>A	p.Ser34Tyr	BM Cells	20.31	17.89	16.67
			ccfDNA	12.5	26.06	17.54

Supplemental Table 4. Mutations and variant allelic frequencies detected in ccfDNA and/or bone marrow in the entire cohort

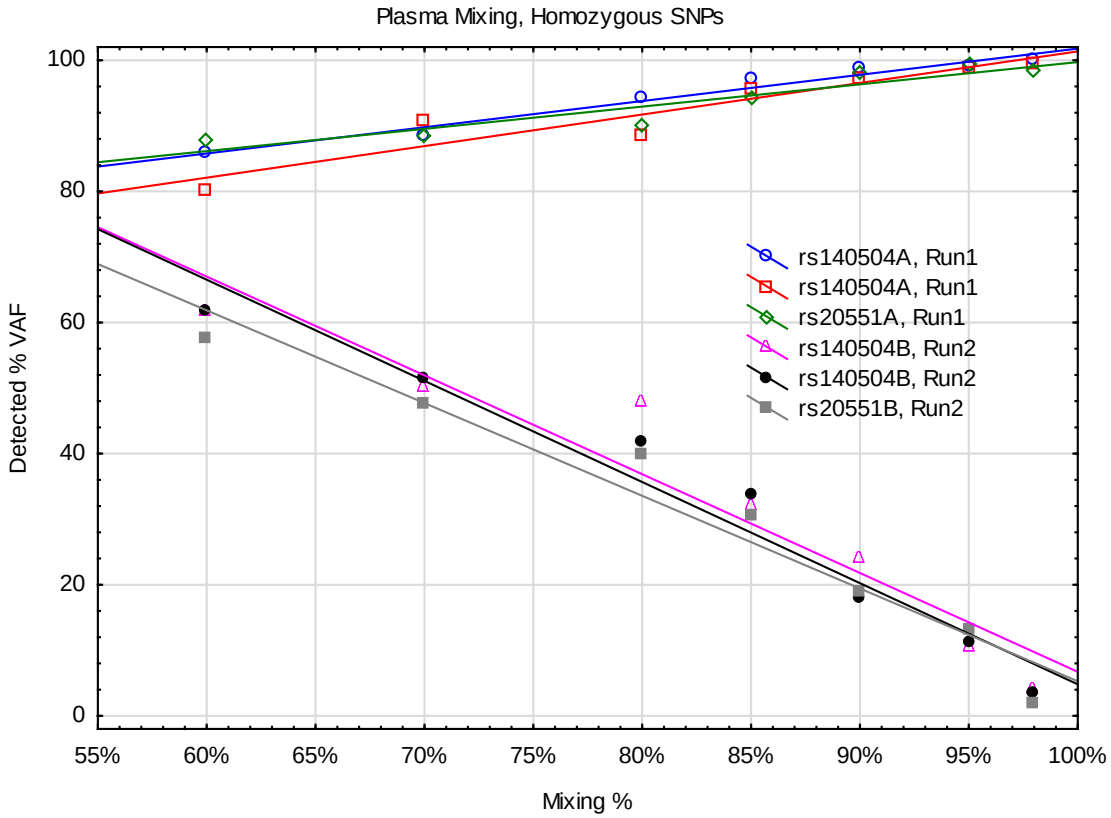
UPN*	ccfDNA mutation	VAF (%)	Bone marrow mutation	VAF (%)
1	*ASXL1* NM_015338.5:c.1900_1922delAGAGAGG CGGCCACCACTGCCAT p.Glu635	25.5	–	–
	RUNX1 NM_001754.4:c.1155C>A p.Tyr385Ter	48.7	*RUNX1* NM_001754.4:c.1155C>A p.Tyr385Ter	58.09
	RUNX1 NM_001754.4:c.319C>T p.Arg107Cys	42.8	*RUNX1* NM_001754.4:c.319C>T p.Arg107Cys	27.2
2	*TET2* NM_001127208.2:c.4138C>T p.His1380Tyr	48.7	*TET2* NM_001127208.2:c.4138C>T p.His1380Tyr	22.15
	TP53 NM_000546.5:c.800G>A p.Arg267Gln	42.9	*TP53* NM_000546.5:c.800G>A p.Arg267Gln	59.19
	TP53 NM_000546.5:c.467G>A p.Arg156His	90.8	*TP53* NM_000546.5:c.467G>A p.Arg156His	54.93
3	*KRAS* NM_033360.2:c.183A>C p.Gln61His	36.7	*KRAS* NM_004985.3:c.183A>C p.Gln61His	30.37
5	*KIT* NM_000222.2:c.1254C>A p.Tyr418Ter	7.7	*KIT* NM_000222.2:c.1254C>A p.Tyr418Ter	5.1
	–	–	*FLT3* NM_004119.2:c.1352C>T p.Ser451Phe	5.87
7	*ASXL1* NM_015338.5:c.1934dupG p.Gly646fs	25.2	*ASXL1* NM_015338.5:c.1934dupG p.Gly646fs	38.0
	RUNX1 NM_001754.4:c.601C>T p.Arg201Ter	43.2	*RUNX1* NM_001754.4:c.601C>T p.Arg201Ter	40.7
	–	–	*NRAS* NM_002524.4:c.38G>A p.Gly13Asp	33.1
	–	–	*NRAS* NM_002524.4:c.35G>T p.Gly12Val	2.35
	–	–	*NRAS* NM_002524.4:c.35G>A p.Gly12Asp	13.19
8	*DNMT3A* NM_175629.2:c.2645G>A p.Arg882His	32.3	*DNMT3A* NM_022552.4:c.2645G>A p.Arg882His	15.55
	IDH1 NM_005896.2:c.394C>T p.Arg132Cys	30.8	*IDH1* NM_005896.2:c.394C>T p.Arg132Cys	18.97
9	–	–	*NRAS* NM_002524.4:c.35G>A p.Gly12Asp	16.0
10	*WT1* NM_024426.4:c.1050C>A p.Cys350Ter	51.3	*WT1* NM_024426.4:c.1050C>A p.Cys350Ter	50.34
12	–	–	*WT1* NM_024426.4:c.1133_1142dupTTGTACGGTC	39.92
14	*DNMT3A* NM_175629.2:c.2644C>T p.Arg882His	47.6	*DNMT3A* NM_022552.4:c.2644C>T p.Arg882His	45.3
	NPM1 NM_002520.6:c.860_863dupTCTG p.Trp288fs	35.1	*NPM1* NM_002520.6:c.860_863dupTCTG p.Trp288fs	48.9
	FLT3 NM_004119.2:c.2503G>T p.Asp835Tyr	13.6	*FLT3* NM_004119.2:c.2503G>T p.Asp835Tyr	11.1
15	*WT1* NM_024426.4:c.1129_1135delACTCTTG p.Thr377fs	33.7	–	–
16	–	–	*NRAS* NM_002524.4:c.35G>C p.Gly12Ala	26.07
17	*NPM1* NM_002520.6:c.860_863dupTCTG p.Trp288fs	29.2	*NPM1* NM_002520.6:c.860_863dupTCTG p.Trp288fs	40.7
18	*IDH1* NM_005896.2:c.603_606delGTCT p.Ser202fs	44.7	*IDH1* NM_005896.2:c.603_606delGTCT p.Ser202fs	49.9

	–	–	*TP53* NM_000546.5:c.830G>A p.Cys277Tyr	4.4
19	NRAS NM_002524.4:c.35G>A p.Gly12Asp	12.67	*NRAS* NM_002524.4:c.35G>A p.Gly12Asp	8.1
	–	–	*KRAS* NM_004985.3:c.35G>A p.Gly12Asp	2.0
	–	–	*FLT3* NM_004119.2:c.2503G>T p.Asp835Tyr	2.8
	–	–	*FLT3* NM_004119.2:c.2505T>A p.Asp835Glu	5.4
20	*IDH2* NM_002168.2:c.419G>A p.Arg140Gln	9.7	*IDH2* NM_002168.2:c.419G>A p.Arg140Gln	45.22
	–	–	*NPM1* NM_002520.6:c.863_864insCCTG p.Trp288fs	36.0
21	–	–	*KIT* NM_000222.2:c.2466T>G p.Asn822Lys	6.6
22	*BRAF* NM_004333.4:c.1799T>A p.Val600Glu	9.83	–	–
	EGFR NM_005228.3:c.2155G>A p.Gly719Ser	2.7	–	–
	EGFR NM_005228.3:c.2235_2249delGGAATTAAGAGAAGC p.Glu746_Ala750del	2.88	–	–
	–	–	*IDH2* NM_002168.2:c.515G>A p.Arg172Lys	22.1
	–	–	*MPL* NM_005373.2:c.1774C>T p.Arg592Ter	6.6

*Patients 4, 6, 11 and 13 did not have any somatic mutations detected at baseline by either assay, among the overlapping 28 genes of interest

UPN: unique patient number, ccfDNA: circulating cell-free DNA, VAF: variant allelic frequency

Supplemental Figure 1. Detected VAF of homozygous SNPs in mixing studies as detected using ccfDNA.



Dilution studies were performed to assess the reliability of the ccfDNA assay at various VAFs. We mixed volume-to-volume blood samples from two normal individuals harboring benign homozygous SNPs. Our goal was to monitor the relative changes in the detected VAF from one dilution to the next. Results from 2 runs of homozygous SNP mixing studies of individual A and individual B are shown. Overall, the results demonstrate linearity and reproducibility of the assay across dilutions.