Supplemental Table 1. Clinical characteristics of the specimens analyzed

Pt ID	BM side analyzed*	ER	PR	Her2	Relapse (months)	Site of Recurrence	Chemo**
3709	L+R	pos	pos	pos	46	chest wall	AC-T
2359	L+R	pos	pos	neg	34	lymph nodes	FEC-T
2827	L+R	neg	neg	neg	15	chest wall, brain, lung	FEC-T
7253	L+R	pos	neg	neg	41	brain	AC
6888	L+R	neg	neg	neg	8	bone, brain, lung	AC-T
7339	L+R	neg	neg	neg	10	brain, lung, breast	AC-T
9191	L+R	neg	neg	neg	24	chest wall, pleural effusion	FEC-T
9789	L+R	pos	neg	neg	17	liver, bone	AC-T
7134	L+R	neg	neg	neg	none		FEC-T
7919	L+R	pos	pos	pos	none		AC-TH
7798	L+R	neg	neg	pos	none		FEC-T
8918	L+R	neg	neg	pos	none		TCH
9193	L+R	neg	neg	neg	none		AC-T
9323	L+R	neg	neg	pos	none		TCH
1756	L+R	pos	pos	pos	none		AC-TH
5077	L+R	neg	neg	neg	none		ET
5808	L+R	neg	neg	pos	none		AC-TH
4262	L+R	pos	pos	neg	none		AC-T
6411	L+R	pos	pos	neg	none		AC-T
6712	L+R	neg	neg	neg	none		FEC-T
2904		neg	neg	neg	15	Liver	ETZ***
9645		neg	neg	neg	12	Lung	ETZ
9290		pos	pos	neg	48	Bone	ETZ
7547		pos	pos	neg	15	Ovary	ETZ
6335		neg	neg	pos	22	Liver, Bone	ETZ

*All bone marrow specimens analyzed were collected prior to treatment

**Abbreviations are: A: Adriamycin, C: Cytoxan, E: Epirubicin, F: 5 Fluorouracil, H: Herceptin, T: Taxane, Z: Zoledronic acid

*** All bone marrow was collected as part of a tissue collection study except those patients who received zoledronic acid. Patients received zoledronic acid with chemotherapy on a clinical trial as described in Ref [37].

Supplemental Table 2. PCR Primer Probes for 38-gene panel

Gene Symbol	`Forward 5'-->3'`	`Reverse 5'-->3'`
AGXT2L1	TCGTCACAATGGGAAAACC	CAAACCAACAGCACAAGATAC
CCND1	CATCTACACCGACAACTCC	TGGCATTTTGGAGAGGAAG
CDH3	AGTTTACCCAGGACACCTTC	CCTTTGGTTCTTGGCTATGG
CLDN4	TGCCAGCAACTACGTGTAAG	TCAGTCTCTGCCCAGTCC
EGFR	CCAAGCCATATGACGGAATCC	GGAACTTTGGGCGACTATCTG
EPCAM	GGACACTGAAATAACCTGCTC	GGATCCAGTTGATAACGCG
ERBB2	AGTGTCAGCCCCAGAATG	TGTAGGAGAGGTCAGGTTTC
ESR1	ACTATATGTGTCCAGCCACC	CCTCTTCGGTCTTTTCGTATC
FGFR4	TGGCTTAAGGATGGACAGG	ACAGCGTTCTCTACCAGG
FOXA1	CTGGATGGTTGTATTGGGC	CCTGAGTTCATGTTGCTGAC
GEMIN2	GGAAGCAAAGTGTGAATATTTCTC	TGTGATTTCCAGTGACTTCTATG
GLI2	GGAAGATCTGGACAGGGATG	GGATGTGCTCGTTGTTGATG
GLI3	ACTCCTTGGTCACGATTCTC	CGGAAGAGTAGGTGAAGCTC
GRB7	TCTGGCCTCTATTACTCCAC	GCTTGACACAGAAACCGAAG
GSC	TCTTTCGGTTTGGTCGGC	TGTCGATGCTGAACATGCTG
HSPB7	CCCTGAGCATGTTTTCCG	TCCACCGCAAACTCATAG
HSPB8	CTCTGAGCCTCTGTTTCTC	CCATGCCAAAGCCATCA
IGFBP4	GGGCAAGATGAAGGTCAAT	GGGATGATGTAGAGGTCCT
KRT17	GGATGCCGAGGATTGGTTC	GTAGCGGTTCTCTGTCTCC
KRT19	GCGAGCTAGAGGTGAAGATC	ACTTGGTTCGGAAGTCATCTG
KRT5	AGAGCTGAGAAACATGCAGG	AGCTCCACCTTGTTCATGTAG
KRT7	AAGCAGGATATGGCACGG	GATATTCACGGCTCCCACTC
KRT8	CAGGAGAAGGAGCAGATCAAG	GTTGTCCATGTTGCTTCGAG
LAMB1	CCCTGGATCACTACCTCTATG	CCAAATAAGCCCCTTCAGG
MAPT	GGGCTGATGGTAAAACGAAG	CCCTGATTTTGGAGGTTCAC
PDGFRB	CCTTCCAGCTACAGATCAATG	TCTGTTCCCCACTGTCAG
PGF	CTGTTCCCTTGCTTCCTG	ACCACTTCCACCTCTGAC
PITX2	GGACCAACCTTACGGAAG	GCTCCCTCTTTCTCCATTTG
PTCH1	TTCCTTCTGAACCCCTGG	TCCACTCCTATGCCAACAG
PTCH2	AGGAGACAAGGCAGAAGG	GAAGAGCACCAAAGAGCAC
S100A3	GGACAAATACAAGCTCTGCC	TCCACAAAGTCCACCTCG
SCGB2A2	CGGATGAAACTCTGAGCAATG	GCAATCCGTAGTTGGTTTCTC
SFRP1	GCTTAAGTGTGACAAGTTCCC	GCCTCAGATTTCAACTCGTTG
SMO	CTGTGAGTGGCATTTGTTTTG	GTGGTTGCTCTTGATGGAG
SNAI1	GAAGCCTAACTACAGCGAGC	CAGAGTCCCAGATGAGCATTG
SNAI2	AACTGCTCCAAAACCTTCTCC	TGTCATTTGGCTTCGGAGTG
TWIST1	CTCAGCTACGCCTTCTCG	ACTGTCCATTTTCTCCTTCTCTG
WNT5A	CCAGTATCAATTCCGACATCG	CTCACCGCGTATGTGAAG

Supplemental Table 3. Genes screened for the nCounter assay panel which are expressed in breast cancer cells and demonstrate absent or low expression in normal bone marrow.

Genes used for 38 gene panel		**Genes tested and excluded**	
AGXT2L1	KRT7	ACADSB	MECOM
CCND1	KRT-8	ACTG2	MGB-1
CDH3	LAMB1	ALDH1A1	MMP11
CLDN4	MAPT	APOD	MYBL-2
EGFR	PDGFRB	BLVRA	MYCN
ERBB2	PGF	BMI1	NID1
ESR1	PITX2	CLEC3B	NID2
FGFR4	PTCH1	EMILIN1	PCOLCE
FOXA1	PTCH2	ETS1	SDC1
GLI2	S100A3	EXO1	SLC22A3
GLI3	SCGB2A2	FZD1	WNT2B
GRB7	SFRP1	GLI-1	ZEB
GSC	GEMIN2	GREM1	
HSPB7	SLUG	IGF1	
HSPB8	SMO	JAG1	
IGFBP4	SNAIL1	LBP	
KRT17	EPCAM	LOXL1	
KRT19	TWIST1	MALAT1	
KRT5	WNT5A	MDM2	

Supplemental Table 4. Realtive Gene expression* in Breast cancer cell lines compared to normal bone marrow

Gene Symbol	MCF-7	MCF-10A	MDAMB231	C1A1
AGXT2L1	1.06	4.63	65.95	0.04
CCND1	0.68	0.07	0.26	0.97
CDH3	10.5	43.69	204.01	0.16
CLDN4	10363.09	3.72	1551.2	1620.81
EGFR	61.57	1472.62	1592.97	1817.2
EPCAM	0.07	84.64	10.83	1.24
ERBB2	0.85	1.26	2.09	1.39
ESR1	0.03	1.99	0.01	0.01
FGFR4	0.56	1.75	0.9	1.59
FOXA1	0.84	902.84	21.28	0.03
GLI2	0.31	0.06	0.19	0.38
GLI3	5.84	5	6.17	4.45
GRB7	91.4	95.5	160.62	12.97
GSC	6.04	1.18	0.03	0.29
HSPB7	0.66	187.73	37.51	1.05
HSPB8	0.12	167.54	0.46	0.06
IGFBP4	3.07	6.19	32.22	1.02
KRT17	109.01	63.48	3528.89	0.32
KRT19	1.74	18539.74	115.76	997.15
KRT5	12.41	555.28	1467.1	7.94
KRT7	45.71	993.41	1185.48	663.79
KRT8	668.02	4.3	45.95	52.06
LAMB1	1963.44	1674.1	0.3	173.55
MAPT	3.38	78.43	4.97	2.69
PDGFRB	0.49	0.25	3.08	0.17
PGF	0.6	3.66	1.96	49.95
PITX2	9.29	0	79.25	0
PTCH1	2.23	0.01	10.27	0.28
PTCH2	4.97	0.24	13.59	0.2
S100A3	33.22	0.55	3.76	14.87
SCGB2A2	18.35	0.38	1.12	0.09
SFRP1	9.29	0	79.25	0
SIP1	13.86	1.07	0.02	0.24
SMO	1.62	0.02	0.97	0.01
SNAI1	0.94	0.06	0.07	0.41
SNAI2	3.56	213.91	168.22	285.53

TWIST1	1.29	725.33	94.95	0.03
WNT5A	721.57	2.31	20.04	0.31

* Samples with expression Above 10 fold relative to normal bone marrow are in red font

Supplemental Table 5. Enhanced nCounter signal detection of DTC-associated transcripts using increased input RNA and Ficoll mononuclear cell enrichment. A 1:1 mixture of MDA-MB231 and ZR75 breast tumor cells were spiked into normal volunteer control BM at a dilution of 1x10^{-4} tumor cells per nucleated BM cells. Cells were either used for direct RNA isolation or first enriched for the mononuclear cell fraction prior to RNA isolation. The indicated quantity of resulting RNA was used for the 9 gene nCounter assay. Numbers indicate total hybridization counts from each sample after subtraction of background signal from normal bone marrow controls and absolute hybridization counts of normal bone marrow controls themselves.

Cells	Input RNA	Ficoll	KRT19	EGFR	SNAI2	TWIST1	SCGB2A2	PITX2	S100A3	KRT17	TACSTD1
MDA-MB231 / ZR75	0.5 ug	N	221	26	12						
MDA-MB231 / ZR75	5 ug	N	2,886	120	126						
MDA-MB231 / ZR75	5 ug	Y	5,276	240	284						
Control BM	0.5 ug	N	23	9	5	9	5	5	5	58	94
Control BM	5 ug	Y	33	35	177	21	0	9	133	489	1520

Supplemental Table 6. Comparison of sample analysis by NS and PCR

	KRT19		KRT17		CCND1		ERBB2		PTCH1	
Pt Number	PCR*	NC**	PCR	NC	PCR	NC	PCR	NC	PCR	NC
7253	0.9	47	2.4	238	3	263	20.6	1063	14.5	1686
7253	1.1	44	2.3	182	2.4	268	28.5	1453	20.3	2026
6888	1.5	36	0.2	15	2.2	372	9.1	578	9.7	1526
6888	5	50	1	18	4.6	394	8.4	775	17.6	1945
7339	1.5	31	0	31	1.7	393	3.7	497	3.1	777
7339	0.6	18	0.3	30	2.3	401	2.6	454	2.6	643
9191	3	66	1.9	95	1.7	293	8.1	1107	8.2	1898
9191	7	31	2.9	66	2.3	290	17.9	1391	14.5	2231
9789	10.4	44	1.5	63	2	285	10.5	1252	5.3	1840
9789	4	36	1.6	66	3.1	312	12.9	1421	7.4	1772

*Numbers are fold over expression compared to normal bone marrow controls. Samples which are 10-fold higher are marked in red

**Numbers are normalized NS counts. Samples which are 2SD higher than the average of control bone marrow are marked in red.

Supplemental Table 7. Normalized Nanostring hybridization counts* for patient Bone marrow specimens

Patient ID	Side	AGXT2L1*	CCND1	CLDN4	EGFR	EPCAM	ERBB2	ESR1	FOXA1	GLI3	IGFBP4	LAMB1	MAPT	PDGFRB	PGF	PTCH1	S100A3	SCGB2A2	SMO	SNAI2	WNT5A
3709	L	-3	562	12	23	1048	662	130	14	24	903	58	29	686	9	1048	44	40	172	57	66
3709	R	-1	727	95	80	1108	577	246	163	95	678	33	33	513	5	1101	51	1	262	40	177
2359	L	0	419	24	21	1971	821	118	24	31	822	14	43	687	12	1356	60	-2	236	21	48
2359	R	-3	354	49	44	1581	986	120	19	43	837	15	50	854	20	1516	56	-2	239	24	58
2827	L	-3	399	17	21	914	592	84	13	30	920	22	26	533	15	1119	65	-5	189	26	41
2827	R	-3	379	29	25	738	680	90	18	30	857	17	33	543	12	1271	55	-5	172	11	44
7253	L	-3	261	28	35	512	1056	98	15	44	920	45	29	825	16	1674	87	-2	187	52	29
7253	R	-2	267	37	41	452	1450	96	27	51	929	55	31	1131	7	2021	67	-3	170	43	37
6888	L	-5	369	24	17	1077	574	172	17	31	677	10	44	785	4	1514	63	-6	239	18	99
6888	R	-3	391	38	50	982	768	216	32	55	1136	64	44	1079	8	1929	62	-3	246	106	106
7339	L	-5	391	26	24	2611	495	126	13	42	745	74	29	547	26	773	399	-1	258	99	32
7339	R	-4	400	9	32	2815	453	118	6	43	821	102	36	635	33	641	376	-4	314	139	19
9191	L	-3	280	38	46	901	1059	202	50	104	955	12	86	684	7	1816	35	-3	180	43	76
9191	R	-7	288	20	15	452	1381	127	22	119	1093	3	102	868	-2	2215	18	-2	124	23	60
9789	L	-3	284	37	46	1229	1249	140	68	83	842	38	58	1504	33	1836	61	-1	291	49	83
9789	R	-6	310	26	41	1364	1411	121	16	54	1010	74	50	2005	26	1760	54	0	251	117	34
7134	L	-1	447	19	19	1465	322	113	8	40	597	28	33	281	13	888	66	-2	252	61	90
7134	R	-4	424	8	15	1196	333	87	0	38	665	58	28	322	11	848	50	-4	263	83	54
7919	L	-1	337	35	57	2600	697	115	11	53	1067	103	23	597	-1	946	110	-2	211	113	33
7919	R	-3	424	42	53	2893	696	110	18	49	1034	122	18	630	6	914	129	-4	219	143	42
7798	L	-2	527	43	43	1911	1154	127	32	44	786	25	27	1459	4	1036	69	-2	235	34	58
7798	R	-3	545	27	40	2124	888	146	15	29	988	61	27	1264	6	804	65	-2	222	78	46
8918	L	-2	432	25	41	1290	697	142	15	82	1547	178	48	1139	75	1251	82	-2	241	185	69
8918	R	-3	455	29	63	1501	710	156	36	104	1894	217	44	1254	61	1261	86	-4	324	278	100
9193	L	-2	402	41	63	2614	569	201	30	92	1834	236	19	1113	35	819	308	-1	318	241	100
9193	R	-2	397	12	21	3154	396	156	12	38	1108	139	9	551	26	509	336	-2	297	129	75
9323	L	0	408	46	71	825	915	147	64	100	1031	83	57	509	7	1352	41	-2	240	121	94
9323	R	-2	464	65	104	875	896	172	106	109	1193	121	49	629	15	1402	49	-2	265	172	132
1756	L	2	319	10	18	955	1413	108	8	63	1202	28	36	546	8	1284	44	-3	145	37	20
1756	R	-3	325	14	14	1367	1080	107	16	67	1117	24	32	461	15	1066	58	-4	186	51	29
5077	L	-2	379	27	38	715	648	114	28	74	1092	87	59	743	10	1439	56	-3	222	98	57
5077	R	-1	424	17	35	901	527	146	14	63	1173	141	65	660	8	1162	50	1	289	139	62
5808	L	-3	264	28	43	654	947	110	18	69	949	81	32	1246	13	1256	51	-2	179	96	45
5808	R	-3	281	23	57	645	824	111	11	51	827	88	26	1088	20	1063	42	-4	211	121	15
4262	L	-2	664	25	51	1256	804	98	28	85	1568	177	41	1320	10	1512	77	-4	242	191	34
4262	R	-5	1116	58	61	1271	949	138	47	94	1160	135	36	1328	14	1583	60	-2	247	139	80
6411	L	-2	260	28	43	1715	729	147	25	38	835	40	43	581	1	1088	43	0	424	72	44
6411	R	-4	191	28	28	1306	761	170	19	31	674	40	43	548	2	1015	33	-4	296	46	28
6712	L	-3	310	24	49	720	1272	106	11	55	1203	95	47	995	22	1864	52	-3	203	127	26
6712	R	-2	233	31	26	550	1469	107	10	55	853	25	57	910	11	2245	37	-2	144	38	26

*Samples 2 standard deviations above the average of 11 normal bone marrows are shown in red boxes