SUPPLEMENTARY METHODS

Sequenta LymphoSIGHT™ Method

Primer Design: First-stage primers were designed to allow for the amplification of all known alleles of the germline *IgH* and TCR sequences. Each *IgH* V_H segment was amplified by 3 primer sets reducing the chance of somatic mutations interfering with amplification. The numbers of primers and the positions of these primers are shown in Faham and Willis.[1] Similarly, a single primer set was used for each segment of *IgH* DJ_H and TCR amplification. Common sequences, which were complementary to a set of second stage PCR primers, were appended at the 5' ends of V_H primers and a J_H segment primer. The second stage PCR primers contained the common sequence primer and indices for sample multiplexing and cluster formation.

PCR amplification. First stage PCR was carried out using a high-fidelity polymerase (AccuPrime, Life Technologies) for 25 cycles; 1/100 of this amplification reaction was then used as the template for a second PCR reaction, which was carried out for 14 cycles. Multiple samples were pooled and then purified using the QIAquick PCR purification kit (Qiagen). Cluster formation and sequencing reactions were carried out according to the manufacturer protocol (Illumina, La Jolla, CA). Three sequencing reactions were performed: (1) 115 bp from the J_H side through the junctional sequence from J_H to V_H; (2) Sample index for 9 cycles to identify the sample with the sample index; and (3) 95 bp from the V_H- to-J_H direction providing ample sequence to map the V_H segment accurately.

Clonotype determination. Algorithmic methods were utilized for clonotype determination. Low quality reads, defined as those that do not map to a J and a V segment and do not demonstrate

high-quality sequence data in the clone-defining region, were excluded. Sequence data were analyzed to determine the clonotype sequences including mapping to germline V and J consensus sequences. First, the forward sequence read was used to map the J segment. After J segment identification, V segments were mapped using the reverse sequence read. The V_H primer was mapped and the bases under this primer were excluded from further analysis of the reverse read. Thereafter, the next ~70 bases of the reverse read were mapped to the known V_H segments. Read pairs that did not map to V segments were excluded. The next step in mapping involved identifying the frame that related the forward and reverse reads and this allowed a continuous sequence from J to V to be constructed.

To generate a clonotype, identification of at least two identical sequences was required. We developed an algorithm to determine whether similar sequencing reads are the result of biological differences in the initial sample or technical artifact (i.e., sequencing or PCR error). The algorithm takes into account the number of sequencing reads and the degree of sequence variation between the clonotypes in question. For example, assume that 2 clonotypes are found in the same sample, which have a 2 base difference from each other. If 100 reads are obtained for clone 1 and 70 reads for clone 2, these clonotypes are not likely to be the result of a technical artifact. Alternatively, assume that 2 clonotypes are found in the same sample, which differ by 1 base. If 300 reads are obtained from clone 1 and 2 reads from clone 2, these clonotypes are likely to be the result of sequencing or PCR error.

REFERENCES CITED

1. Faham M, Willis T. Monitoring health and disease status using clonotype profiles. U.S. Patent Application No. 2011/0207134A1, August 25, 2011.

SUPPLEMENTARY TABLES

Supplementary Table 1. Technical performance data: relative standard deviation for dilution replicates.

Frequency	Average RSD Replicates (%)
1.00E-06	49
3.00E-06	20
1.00E-05	16
3.00E-05	6.9
1.00E-04	4.1
3.00E-04	7.6
1.00E-03	5.5

Supplementary Table 2. Technical performance data: r^2 and slope for dilution replicates.[a]

Clone	r^2	Slope
1	0.981	1.14
2	0.985	0.878
3	0.994	0.907
4	0.992	1.08
5	0.992	1.08
6	0.977	0.977
7	0.989	1.08
8	0.991	0.925
9	0.996	0.935
10	0.993	0.968
11	0.98	1
12	0.993	0.939
13	0.989	1.12

[a]Lines were fit for the relationship between expected and observed level (after conversion to log) of the diluted clonotypes. Shown are the r^2 and the slope for each of the clones.

Supplementary Table 3. MRD results for sequencing, flow cytometry and ASO-PCR methods in 106 ALL samples.

			% MRD		
Patient ID	**Time of Collection**	**Input Cells**	**Sequencing**	**Flow Cytometry**	**Standard PCR**
1	day 19	396291	0.0028	0.0110	0.0130
2	wk 7	181863	UD	UD	UD
3	day 26	227287	0.3473	1.7530	1.1000
4	day 46	245156	UD	UD	UD
5	wk 7	375583	0.0186	0.0450	0.0360
6	day 46	229625	UD	UD	UD
7	day 19	377086	0.0342	0.0630	0.2300
8	wk 7	229458	UD	UD	UD
9	day 46	248496	0.00004	UD	UD
10	day 46	272210	0.0841	0.1330	0.2200
11	day 46	190345	1.9667	1.8700	0.0160
12	day 46	133669	0.0027	UD	0.0017
14	day 19	141266	0.0362	0.0120	0.0110
15	day 19	127499	1.8536	0.6580	1.1000
16	wk 7	914645	UD	UD	UD
17	day 46	171789	0.0041	0.0080	0.0029
18	day 46	927850	UD	UD	UD
19	wk 7	737057	UD	UD	UD
20	day 19	117546	0.3635	0.2350	0.0760
21	day 46	138268	0.0134	0.0030	0.0022
22	day 19	86143	0.2799	0.0450	0.0690
23	day 46	938554	UD	UD	UD

25	day 46	142310	0.0106	UD	0.0005
26	wk 120	893286	UD	UD	UD
27	day 46	126252	0.0569	0.0100	0.0150
28	wk 17	836207	UD	UD	UD
29	day 19	161142	0.9607	UD	1.2000
30	day 19	120307	0.0032	UD	0.0076
31	day 46	1109253	0.0014	UD	UD
32	wk 120	726829	UD	UD	UD
33	wk 17	902662	UD	UD	UD
34	day 19	1043573	UD	UD	UD
35	day 46	1763995	UD	UD	UD
36	wk 7	1070242	UD	UD	0.0020
37	wk 146	1405104	UD	UD	UD
38	wk 120	210397	UD	UD	UD
39	day 46	280097	0.0362	0.0200	0.0360
40	wk 120	564621	UD	UD	UD
41	day 46	626380	0.0005	UD	0.0051
42	wk 120	1125658	UD	UD	UD
43	day 46	1227371	UD	UD	UD
44	wk 48	1556654	UD	UD	UD
45	wk 48	1398000	UD	UD	UD
46	wk 17	206141	UD	UD	UD
47	day 19	144025	0.0320	0.0860	0.0240
48	day 46	273636	UD	UD	UD
49	wk 17	1357339	UD	UD	UD
50	day 46	248321	0.5495	0.4290	0.1600
51	wk 17	201563	UD	UD	UD

52	wk 7	222551	0.0988	0.0570	0.0670
53	wk 48	654390	UD	UD	UD
54	wk 120	565318	UD	UD	UD
55	wk 120	990287	UD	UD	UD
56	day 19	191148	0.6396	0.4780	0.3500
57	day 46	197881	0.0832	0.0700	0.0370
58	day 19	171326	0.0102	UD	0.0120
59	wk 48	1252982	UD	UD	UD
60	wk 7	619176	UD	UD	UD
61	wk 48	893485	UD	UD	UD
62	day 46	132038	0.3035	0.0560	0.0940
63	wk 7	830152	UD	UD	UD
64	wk 48	848615	UD	UD	UD
66	wk 48	1206717	UD	UD	UD
67	day 46	118115	0.0339	0.0070	0.0040
68	day 46	146424	0.0241	0.0270	0.0180
69	day 46	113497	0.0385	0.0300	0.0310
70	wk 48	791378	UD	UD	UD
71	wk 120	838569	UD	UD	UD
72	wk 7	1211859	UD	UD	UD
73	day 46	983289	0.0004	UD	UD
74	wk 146	869338	UD	UD	UD
75	wk 48	1358960	UD	UD	UD
76	day 19	153787	0.0312	0.0340	0.0100
77	wk 120	826209	UD	UD	UD
78	wk 17	778707	UD	UD	UD
79	day 46	716734	UD	UD	UD

80	wk 120	982602	UD	UD	UD
81	wk 7	1291093	UD	UD	UD
82	wk 7	714626	UD	UD	UD
83	wk 146	806382	UD	UD	UD
84	wk 7	779402	UD	UD	UD
85	wk 17	772165	UD	UD	UD
86	wk 17	624304	UD	No assay	UD
87	wk 7	841964	UD	UD	UD
88	day 26	115013	32.2783	30.1400	17.0000
89	wk 7	1092445	UD	UD	UD
90	day 26	125822	0.5258	0.5330	0.1600
91	wk 17	736134	UD	UD	UD
92	wk 7	878092	UD	UD	UD
93	wk 120	746149	UD	UD	UD
94	day 19	122267	20.1995	18.6000	14.0000
95	wk 120	665950	UD	UD	UD
96	wk 48	624069	UD	UD	UD
97	wk 17	150808	UD	UD	UD
98	day 46	162217	0.0097	UD	0.0038
99	wk 17	754359	UD	UD	UD
101	day 19	134193	0.0377	0.0250	0.0190
102	wk 48	706041	UD	UD	UD
103	wk 17	864971	UD	UD	UD
104	wk 7	1005772	UD	UD	UD
105	wk 48	936670	UD	UD	UD
106	wk 7	695583	UD	UD	UD
107	wk 120	721544	UD	UD	UD

108	wk 120	773343	UD	UD	UD
109	wk 7	1157994	UD	UD	UD
110	wk 7	1250016	UD	UD	UD

UD = Undetectable

Supplementary Table 4. MRD results for *IgH* and second receptor using sequencing method in 9 ALL samples.

Patient ID	**Time of collection**	**Input cells**	**% MRD (*IgH*)**	**Receptor 2**	**% MRD (Receptor 2)**
22	day 19	86143	0.2799	TRD@	0.0109
26	wk 120	893286	UD	TRD@	UD
29	day 19	161142	0.9607	TRG@	0.3221
66	wk 48	1206717	UD	TRG@	UD
71	wk 120	838569	UD	TRD@	UD
81	wk 7	1291093	UD	TRG@	UD
91	wk 17	736134	UD	TRG@	UD
99	wk 17	754359	UD	TRG@	UD
107	wk 120	721544	UD	TRD@	UD

UD = Undetectable