Supplemental Figure S1. Schematic of long PCR and sequencing for determination of the complete HTLV-1 proviral genome.

As described in the Materials and Methods and Supplemental Table S6, four long DNA fragments in the full HTLV-1 genome were amplified using primers indicated by orange arrows. Using the sequencing primers indicated by blue arrows (also listed in Supplemental Table S7), nucleotide sequences were read from both strands. Sequence tags in the sense orientation are indicated as black lines, and those in antisense orientations are indicated by red lines. The size of the line is set as ~600 bases in this figure. Nucleotide numbering is based on the ATK-1 prototypic sequence (Genbank: J02029).

Supplemental Figure S2. Correlation between the CLEIA cutoff index (COI) and HTLV-1 qPCR results.

CLEIA COIs at the initial screening test of PV+ and PV- blood donors were plotted. PV+: WB indeterminate samples that were provirus positive in HTLV-1 qPCR. PV-: WB indeterminate samples that were provirus negative in HTLV-1 qPCR.

Supplemental Table S1. Interpretations of results of the Problot HTLV-1 WB test kit used in this study.

Env	Gag			Interpretation
gp46	p19	p24	p53	
+	At least a band is +			Positive
-	-	-	-	Negative
-	At least a band is +/- or +			Indeterminate
+/-	Any patterns			
+	Any bands are - or +/-			

Supplemental Table S2. HTLV-1 antibody titers, band patterns of WB results, and PVL of WB indeterminate.

ID	Age	Gender	CLEIA (Cutoff index)			WB (Probrot HTLV-1)					PVL[*1]
						p19	p24	p53	gp46	Result	
K-1001	51	F	1.6	1.5	1.5	±	-	-	-	Ind[*2]	UD[*3]
K-1002	52	M	2.4	2.4	2.5	-	-	-	±	Ind	0.0143
K-1003	51	M	1.8	1.6	1.6	+	+	-	-	Ind	0.0010
K-1004	57	M	5.2	5.0	4.9	+	+	-	±	Ind	0.0058
K-1005	41	M	1.8	1.7	1.7	-	+	-	-	Ind	UD
K-1006	46	M	10.0	10.0	10.0	+	+	+	±	Ind	0.0341
K-1007	33	M	6.3	6.6	6.4	+	+	+	±	Ind	0.0292
K-1008	42	M	1.0	1.0	1.0	+	+	+	-	Ind	UD
K-1009	38	M	1.1	1.1	1.1	+	+	+	-	Ind	UD
K-1010	58	M	1.6	1.6	1.7	+	+	-	±	Ind	UD
K-1011	34	F	10.0	10.0	10.0	+	+	+	-	Ind	0.0415
K-1012	42	M	1.0	1.1	1.0	+	+	+	-	Ind	UD
K-1013	55	M	5.9	6.0	6.1	+	+	+	±	Ind	0.0063
K-1014	60	M	1.6	1.7	1.6	±	-	-	-	Ind	0.0011
K-1015	47	M	1.5	1.6	1.6	+	-	-	±	Ind	0.2444
K-1016	57	F	2.8	2.8	2.9	+	±	-	±	Ind	0.0015
K-1017	26	M	1.8	1.8	1.9	-	+	-	-	Ind	UD
K-1018	58	F	5.7	5.3	5.2	±	-	-	-	Ind	0.0596
K-1019	26	M	1.2	1.3	1.3	+	+	±	-	Ind	0.0843
K-1020	65	M	1.5	1.6	1.5	-	+	-	-	Ind	UD
K-1021	52	F	1.7	1.7	1.6	+	-	-	-	Ind	UD
K-1022	20	M	1.1	1.1	1.1	±	-	-	-	Ind	0.0627
K-1023	32	M	1.0	1.0	1.0	+	+	+	-	Ind	UD

K-1024	65	M	1.2	1.1	1.1	+	+	+	-	Ind	UD
K-1025	42	M	1.2	1.3	1.3	+	+	+	-	Ind	UD
K-1026	52	F	2.0	1.9	1.9	-	±	-	-	Ind	UD
K-1027	57	M	1.0	1.0	1.1	+	-	-	-	Ind	UD
K-1028	51	M	1.2	1.2	1.1	+	-	-	-	Ind	UD
K-1029	48	F	2.7	2.6	2.7	+	+	+	-	Ind	0.0094
K-1030	39	F	1.1	1.0	1.0	+	+	+	-	Ind	UD
K-1031	38	F	3.3	3.4	3.3	+	-	-	-	Ind	0.0005
K-1032	30	M	1.0	1.0	1.0	+	+	+	-	Ind	UD
K-1033	45	F	8.0	8.1	8.3	+	+	+	-	Ind	0.0052
K-1034	51	M	1.6	1.6	1.5	+	+	-	-	Ind	0.0023
K-1035	34	M	1.2	1.2	1.2	+	+	+	-	Ind	UD
K-1036	65	F	2.6	2.5	2.4	-	±	-	-	Ind	UD
K-1037	58	M	2.9	2.9	2.9	+	+	+	-	Ind	UD
K-1038	25	M	2.1	2.1	2.1	+	+	+	-	Ind	UD
K-1039	59	M	4.7	5.2	5.0	+	+	-	-	Ind	0.0009
T-1001	32	F	1.0	0.9	1.0	+	+	+	-	Ind	UD
T-1002	21	F	1.0	1.0	1.0	+	+	+	-	Ind	UD
T-1003	50	M	1.1	1.1	1.0	+	+	+	-	Ind	UD
T-1004	58	M	1.6	1.5	1.4	+	+	+	-	Ind	UD
T-1005	43	M	1.0	1.0	1.0	+	+	+	-	Ind	UD
T-1006	39	F	1.6	1.7	1.6	+	+	+	-	Ind	UD
T-1007	53	M	8.3	8.5	8.4	+	+	+	±	Ind	0.0006
T-1008	58	M	1.5	1.5	1.5	+	+	+	-	Ind	UD
T-1009	58	M	1.4	1.4	1.4	+	±	±	-	Ind	UD
T-1010	39	F	4.2	3.9	3.9	+	-	-	-	Ind	UD
T-1011	21	M	1.1	1.1	1.1	+	±	-	-	Ind	UD
T-1012	20	M	2.5	2.3	2.4	+	+	+	±	Ind	0.0192
T-1013	39	F	1.6	2.4	2.3	+	+	+	-	Ind	UD
T-1014	51	M	1.8	1.1	1.1	+	+	+	-	Ind	UD
T-1015	40	M	1.9	1.4	1.1	+	+	+	-	Ind	UD
T-1016	58	M	1.2	1.4	1.4	+	±	-	-	Ind	UD
T-1017	24	F	2.3	2.3	2.2	+	+	+	-	Ind	UD
T-1018	56	M	1.0	1.1	1.1	+	+	+	-	Ind	UD
T-1019	29	M	1.1	1.1	1.0	+	+	+	-	Ind	UD
T-1020	58	M	1.3	1.2	1.2	+	±	±	-	Ind	UD
T-1021	39	F	2.3	2.2	2.2	+	+	+	-	Ind	UD
T-1022	37	M	1.4	1.4	1.4	+	+	+	-	Ind	UD
T-1023	48	F	1.6	1.5	1.5	+	+	+	-	Ind	UD
T-1024	32	F	1.4	1.5	1.5	+	+	+	-	Ind	UD
T-1025	57	M	7.1	7.3	7.3	+	-	+	-	Ind	0.0025
T-1026	58	M	1.2	1.1	1.2	+	±	±	-	Ind	UD
T-1027	16	F	1.0	1.1	1.1	-	±	-	-	Ind	UD

T-1028	45	M	1.5	1.6	1.6	+	+	+	-	Ind	UD
T-1029	50	M	2.6	2.3	2.4	+	+	+	-	Ind	UD
T-1030	50	M	1.9	1.9	1.9	+	+	±	-	Ind	UD
T-1031	30	F	1.2	1.2	1.2	+	+	+	-	Ind	UD
T-1032	25	M	1.7	1.6	1.7	+	+	+	-	Ind	UD
T-1033	39	M	3.9	4.0	4.1	±	-	-	-	Ind	UD
T-1034	58	M	1.1	1.2	1.2	+	+	+	-	Ind	UD
T-1035	21	F	1.7	1.8	1.8	+	+	+	-	Ind	UD
T-1036	42	M	1.0	1.0	1.0	?	?	?	?	Ind[*4]	UD
T-1037	38	M	1.0	1.1	1.0	+	+	+	-	Ind	UD
T-1038	28	M	2.1	2.1	2.2	+	-	-	-	Ind	UD
T-1039	21	M	1.3	1.1	1.1	+	+	+	-	Ind	UD
T-1040	39	F	2.4	2.2	2.3	+	-	-	-	Ind	UD
T-1041	27	F	1.4	1.4	1.2	+	-	-	-	Ind	UD
T-1042	28	F	1.1	1.1	1.1	+	+	+	-	Ind	UD
T-1043	58	M	1.1	1.2	1.2	+	-	-	-	Ind	UD
T-1044	37	M	1.4	1.3	1.3	+	+	+	-	Ind	UD
T-1045	52	M	1.1	1.0	1.0	+	+	+	-	Ind	UD
T-1046	38	M	1.6	1.6	1.6	+	+	+	-	Ind	UD
T-1047	54	F	1.0	1.0	1.0	+	-	-	-	Ind	UD
T-1048	59	M	6.5	6.1	6.1	+	+	+	-	Ind	0.0131
T-1049	43	M	1.2	1.1	1.1	+	+	+	-	Ind	UD
T-1050	37	M	1.3	1.1	1.1	+	+	+	-	Ind	UD
T-1051	58	M	1.2	1.1	1.2	+	+	+	-	Ind	UD
T-1052	55	F	6.8	6.3	6.3	+	±	-	-	Ind	UD
T-1053	32	M	1.1	1.1	1.1	?	?	?	?	Ind	UD
T-1054	21	F	1.6	1.7	1.7	+	+	+	-	Ind	UD
T-1055	32	M	1.1	1.1	1.1	+	-	-	-	Ind	UD
T-1056	18	F	4.2	4.0	4.0	±	+	+	±	Ind	0.0324
T-1057	36	M	1.3	1.3	1.4	+	+	+	-	Ind	UD
T-1058	19	F	2.0	2.0	2.0	+	+	+	-	Ind	UD
T-1059	30	M	1.2	1.1	1.2	?	?	?	?	Ind	UD
T-1060	45	M	1.1	1.1	1.0	+	+	+	-	Ind	UD
T-1061	39	M	1.0	1.0	1.0	?	?	?	?	Ind	UD

*1: PVL indicates proviral loads (copies/100 cells).

*2: Ind indicates indeterminate.

*3: UD indicates undetermined by qPCR.

*4: ? of all WB bands indicates indeterminate because of excessive background signals.

Supplemental Table S3. Concentration of CFSE-stained TL-Om1 cells analyzed by flow cytometry in the low copy HTLV-1 panel.

Sample name	Total cells analyzed	CFSE (+) cells	TL-Om1 concentration
0.05%	372,576	195	0.052%
0.02%	608,950	137	0.023%
0.005%	1,777,902	97	0.0055%
0.002%	1,768,789	37	0.0021%

Supplemental Table S4. Concentrations of CFSE-stained TL-Om1 cells diluted with PBMCs which were analyzed by flow cytometry in the extremely low copy HTLV-1 panel.

Sample Name	Total cells analyzed	CFSE (+) cells	TL-Om1 concentration
0.02%	1,977,510	312	0.0158%
0.005%	1,964,932	86	0.0044%
0.002%	6,897,479	116	0.0017%
0.0005%	7,845,021	46	0.0006%
0.0002%	7,852,649	12	0.00015%

Table S5. List of primer and probe sequences, IC genes, and extraction methods of the laboratories[*1].

Method	Sequence of HTLV-1 primer and probe (5’ to 3’ orientation)		IC gene	DNA extraction method	Reference
1	Forward:	CAAACCGTCAAGCACAGCTT	ACTB	Silica membrane based column	1
	Reverse:	TCTCCAAACACGTAGACTGGGT			
	Probe:	TTCCCAGGGTTTGGACAGAGTCTTCT			
2	Forward:	GAAGACTGTTTGCCCACCACC	RAG1	Silica membrane based column	2
	Reverse:	TGAGGGTTGAGTGGAACGGA			
	Probe:	CACCCGTCACGCTAACAGCCTGGCAA			
3	Forward:	TGGACAGAGTCTTCTTTTCGGATA	CD81	Silica membrane based column	3
	Reverse:	CACCAGTCGCCTTGTACACAGT			
	Probe:	CCAGTCTACGTGTTTGG			
4*	Forward:	CGGATACCCAGTCTACGTGTT	RPPH1	Silica membrane based column	4
	Reverse:	CAGTAGGGCGTGACGATGTA			
	Probe:	CTGTGTACAAGGCGACTGGTGCC			
5	Forward:		ALB	Silica membrane based column	5
	Reverse:	Same as method 4			
	Probe:				
6	Forward:	CCCACTTCCCAGGGTTTGGA	HBB	Silica membrane based column	6
	Reverse:	GGCCAGTAGGGCGTGA			
	Probe:	CCAGTCTACGTGTTTGGAGACTGTGTACA			
7	Forward:	ACAAAGTTAACCATGCTTATTATCAGC	ACTB	SDS-PK digestion Phenol-Chloroform	7
	Reverse:	ACACGTAGACTGGGTATCCGAA			
	Probe:	TTCCCAGGGTTTGGACAGAGTCTTCT			

*1: This table is also listed as Supplemental data in reference S8.
*2: Method number 4 was used by two laboratories.

Supplemental Table S6. Nested genomic long-PCR primers for HTLV-1 full length genome sequence

		Name	Sequence	Name	Sequence	Size (bps)	Nucleotide region equivalent to ATK-1
Fragment 1	1st PCR	209F	CATTTCCTCCCCATGTTTGTCAAG	071R	CGGTGACTGTGGTGGAGTAAGAT	3556	241-3796
	2nd PCR	008F	CCCTGCTTGCTCAACTCTACGT	066R	GGGCATGTAGCCAGACAAGTG	3016	585-3600
Fragment 2	1st PCR	053F	GGAGGCAGGCCATATCGAA	122R	AATGGAGATTAATATTGAGGTGTGAAAC	3000	2663-5662
	2nd PCR	056F	TAGTCCCACCCTGTTCGAAATG	109R	TGGTGTTGGTGGTCTTTTTCTTT	2209	2996-5203
Fragment 3	1st PCR	092F	CTCCAATATCCTGCGCTCTTG	173R	CCGAACATAGTCCCCCAGAGA	3033	4400-7432
	2nd PCR	101F*	TGCCTATATTTCCCAAGACTTCCT	153R	AGGGATGACTCAGGGTTTATAAGAGA	1975	4691-6665
Fragment 4	1st PCR	122F	CCTAGGGTGCCAATCATGGA	008R	GGCAGTCAGTCGTGAATGAAAG	3418	5544-8961
	2nd PCR	137F	CCCCACCTGACGTTACCATT	005R*	TCAGTCGTGAATGAAAGGGAAAG	2962	5995-8956

* these primers were reported previously in reference S9

ATK-1 (J02029) is the prototype sequence of HTLV-1

Supplemental Table S7. Sequence primer sets corresponding to nested genomic long-PCR fragments

Primers for fragment 1		Primers for fragment 2		Primers for fragment 3		Primers for fragment 4	
15R	AGGCTGGCTAGGAGGGAGTAGTT	**056F**	TAGTCCCACCCTGTTCGAAATG	**107F**	AGCCGATCCCAGAGACACATT	**153F**	TTGTCGCGCTACTCCTTCTTG
18F[*1]	CCAAGTCCTTCCAGTCATGCA	**066F**	TGCCGCAGTAGACTAGTCCAAAC	**107R**	GCCTCCTGTGGTCCTTTCC	**153R**	AGGGATGACTCAGGGTTTATAAGAGA
18R[*]	CGCTTGGGAGACTTCTTGCT	**071F**	CTGCCAAACCATACATCATAACATC	**109F**	ATCCCGGTAAGCGCTAGTTCT	**158F**	GCACACAATTATTGCAACCACAT
25F	CCGAAACTCGAGGTATTACAGGTT	**071R**	CGGTGACTGTGGTGGAGTAAGAT	**109R**	TGGTGTTGGTGGTCTTTTTCTTT	**158R**	TGGAGTCCTTGGAGGCTGAA
25R	GTTGCTGGTATTCTCGCCTTAATC	**084F**[*1]	CCTGCCCCGCTTACTATCG	**117F**	CTACAGCCCCCCTGCCCTAA	**167F**	TCCTCCTTGTCCTTTAACTCTTCCT
34F	GGAGATATGTTGCGGGCTTGT	**084R**[*1]	GGCATCTGTGAGAGCGTTGA	**122F**	CCTAGGGTGCCAATCATGGA	**173F**	TCCCAGGGTTTGGACAGAGT
34R	CGGAAGCACGGCTGATTT	**092F**	CTCCAATATCCTGCGCTCTTG	**122R**	AATGGAGATTAATATTGAGGTGTGAAAC	**173R**	CCGAACATAGTCCCCCAGAGA
41F[*1]	ACAGTCCTTCCGATAGCCTTGTT	**092R**	TGCCAGATGTGGTTAGGGAGTAG	**131mF**	GAGCCCTCTATACCATGGAAAT	**180F**[*1]	GGCCTTCCTCACCAATGTTC
41R[*]	GGGAGGTGAGCTTAAAGTGATCTT	**101F**[*1]	TGCCTATATTTCCCAAGACTTCCT	**137F**	CCCCACCTGACGTTACCATT	**187F**	CAAGGCCTACCACCCCTCAT
48F	CCGCCTGTAATCTTGCCAATA	**101R**[*1]	TTCTACAAGTCCGGAGCTGGTT	**137R**	GGGAACAGGTGACAAGGAAAAG	**187R**	GGGATGTTGGTGTATTCTTCAAATAG
48R	GGAGGCGTTCTGGTTTAAAGG					**202R**	TTAGTCTGGGCCCTGACCT
53F	GGAGGCAGGCCATATCGAA					**209F**	CATTTCCTCCCCATGTTTGTCAAG
56R	GCCAGGAGAATGTCATCCATGT					**209mR**	TCCACGCTTTTATAGACTCCTG
66R	GGGCATGTAGCCAGACAAGTG					**pX2-AS**[*2]	CAGTAGGGCGTGACGATGTA
568F	CTCAACTCTACGTCTTTGTTTCG					**627R**	GTGGAACTTTCGATCTGTARCG

* These primers were reported previously in reference S8. ** the primer was reported previously in reference S4.

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