Supplemental Experimental Procedures

Preparation of single-stranded sequencing libraries

Adapter 2 was prepared by combining 4.5 ul TE (pH 8), 0.5 ul 1M NaCl, 10 uL 500 uM oligo Adapter2.1, and 10 ul 500 uM oligo Adapter2.2, incubating at 95ºC for 10 seconds, and ramping to 14ºC at a rate of 0.1ºC/s. Purified cfDNA fragments were dephosphorylated by combining 2X CircLigase II buffer (Epicentre), 5 mM MnCl2, and 1U FastAP (Thermo Fisher) with 0.5-10 ng fragments in 20 ul reaction volume and incubating at 37ºC for 30 minutes. Fragments were then denatured by heating to 95ºC for 3 minutes, and were immediately transferred to an ice bath. The reaction was supplemented with biotin-conjugated adapter oligo CL78 (5 pmol), 20% PEG-6000 (w/v), and 200U CircLigase II (Epicentre) for a total volume of 40 ul, and was incubated overnight with rotation at 60ºC, heated to 95ºC for 3 minutes, and placed in an ice bath. For each sample, 20 ul MyOne C1 beads (Life Technologies) were twice washed in bead binding buffer (BBB) (10 mM Tris-HCl [pH 8], 1M NaCl, 1 mM EDTA [pH 8], 0.05% Tween-20, and 0.5% SDS), and resuspended in 250 ul BBB. Adapter-ligated fragments were bound to the beads by rotating for 60 minutes at room temperature. Beads were collected on a magnetic rack and the supernatant was discarded. Beads were washed once with 500 ul wash buffer A (WBA) (10 mM Tris-HCl [pH 8], 1 mM EDTA [pH 8], 0.05% Tween-20, 100 mM NaCl, 0.5% SDS) and once with 500 ul wash buffer B (WBB) (10 mM Tris-HCl [pH 8], 1 mM EDTA [pH 8], 0.05% Tween-20, 100 mM NaCl). Beads were combined with 1X Isothermal Amplification Buffer (NEB), 2.5 uM oligo CL9, 250 uM (each) dNTPs, and 24U Bst 2.0 DNA Polymerase (NEB) in a reaction volume of 50 ul, incubated with gentle shaking by ramping temperature from 15ºC to 37ºC at 1ºC/minute, and held at 37ºC for 10 minutes. After collection on a magnetic rack, beads were washed once with 200 ul WBA, resuspended in 200 ul of stringency wash buffer (SWB) (0.1X SSC, 0.1% SDS), and incubated at 45ºC for 3 minutes. Beads were again collected and washed once with 200 ul WBB. Beads were then combined with 1X CutSmart Buffer (NEB), 0.025% Tween-20, 100 uM (each) dNTPs, and 5U T4 DNA Polymerase (NEB) and incubated with gentle shaking for 30 minutes at room temperature. Beads were washed once with each of WBA, SWB, and WBB as described above. Beads were then mixed with 1X CutSmart Buffer (NEB), 5% PEG-6000, 0.025% Tween-20, 2 uM double-stranded Adapter 2, and 10U T4 DNA Ligase (NEB), and incubated with gentle shaking for 2 hours at room temperature. Beads were washed once with each of WBA, SWB, and WBB as described above, and resuspended in 25 ul TET buffer (10 mM Tris-HCl [pH 8], 1 mM EDTA [pH 8], 0.05% Tween-20). Second strands were eluted from beads by heating to 95ºC, collecting beads on a magnetic rack, and transferring the supernatant to a new tube. Library amplification was monitored by real-time PCR, requiring an average of 4-6 cycles per library.

Simulated reads and nucleotide frequencies

Aligned sequencing data was simulated (SR if shorter than 45 bp, PE 45 bp otherwise) for all major chromosomes of the human reference (GRC37h). Dinucleotide frequencies were determined from real data on both fragment ends and both strand orientations, and for the reference genome on both strands. The insert size distribution of the real data was extracted for the 1-500 bp range. Reads were simulated procedurally: at each step (i.e., at least once at each genomic coordinate, depending on desired coverage), (1) the strand is randomly chosen, (2) the ratio of the dinucleotide frequency in the real data to that in the reference sequence is used to randomly decide whether the initiating dinucleotide is considered, (3) an length is sampled from the insert size distribution, and (4) the frequency ratio of the terminal dinucleotide is used to randomly decide whether the generated alignment is reported. The simulated coverage was matched to that of the original data after PCR duplicate removal.

Analysis of nucleotide composition of 167 bp fragments

Fragments with inferred lengths of exactly 167 bp were filtered within samples to remove duplicates. Dinucleotide frequencies were calculated in a strand-aware manner, using a sliding 2 bp window and reference alleles at each position, beginning 50 bp upstream of one fragment endpoint and ending 50 bp downstream of the other endpoint. Observed dinucleotide frequencies at each position were compared to expected dinucleotide frequencies determined from a set of simulated reads reflecting the same cleavage biases calculated in a library-specific manner.

Analysis of TFBSs and genomic features

We started with clustered FIMO (motif-based) intervals (Grant et al., 2011; Maurano et al., 2012) defining a set of computationally predicted TFBSs. For a subset of clustered TFs (AP-2-2, AP-2, CTCF_Core-2, E2F-2, EBF1, Ebox-CACCTG, Ebox, ESR1, ETS, MAFK, MEF2A-2, MEF2A, MYC-MAX, PAX5-2, RUNX2, RUNX-AML, STAF-2, TCF-LEF, YY1), we retained only predicted TFBSs that overlap with ENCODE ChIP-seq peaks (TfbsClusteredV3 set downloaded from http://hgdownload.cse.ucsc.edu/goldenPath/hg19/encodeDCC/wgEncodeRegTfbsClustered/).

WPS values for CH01 and the corresponding simulation were extracted for each position in a 5 kb window around the start coordinate of each TFBS, and was aggregated within each TF cluster. The mean WPS of the first and last 500 bp (which is predominantly flat and represents a mean offset) of the 5 kb window was subtracted from the original WPS at each position. For L-WPS only, a sliding window mean is calculated using a 200 bp window and subtracted from the original signal. Finally, the corrected WPS profile for the simulation is subtracted from the corrected WPS profile for CH01 to correct for signal that is a product of fragment length and ligation bias. This final profile is plotted and termed the Adjusted WPS. In figures, CTCF binding sites are shifted such that the zero coordinate on the x-axis is the center of its 52 bp binding footprint (Ong and Corces, 2014). Genomic coordinates of transcription start sites, transcription end sites, start codons, and splice donor and acceptor sites were obtained from Ensembl Build version 75. Adjusted WPS surrounding these features was calculated as for TFBSs.

Analysis of CTCF sites

CTCF sites first included clustered FIMO binding site predictions (described above). This set was intersected with ENCODE ChIP-seq peaks (TfbsClusteredV3, described above), and then further intersected with a set of CTCF binding sites experimentally observed to be active across 19 tissues (Wang et al., 2012), to produce three increasingly stringent sets. For each CTCF site, distances between each of 20 flanking nucleosomes (10 upstream and 10 downstream) were calculated. The mean S-WPS and L-WPS at each position relative to the center of the CTCF binding motif were also calculated within bins defined by spacing between -1 and +1 nucleosomes (>160 bp, 161-200 bp, 201-230 bp, 231-270 bp, 271-420 bp, 421-460 bp, and >460 bp).

Analysis of DHS sites

DHS peaks for 349 primary tissue and cell line samples were downloaded from http://www.uwencode.org/proj/Science_Maurano_Humbert_et_al/data/all_fdr0.05_hot.tgz. Samples derived from fetal tissues (233 of 349) were removed from the analysis as they behaved inconsistently within tissue type, possibly because of unequal representation of cell types within each tissue sample. 116 DHS callsets from a variety of cell lineages were retained for analysis. For the midpoint of each DHS peak in a particular set, the nearest upstream and downstream calls in the CH01 callset were identified, and the distance between the centers of those two calls was calculated. The distribution of all such distances was visualized for each DHS peak callset using a smoothed density estimate calculated for distances between 0 and 500 bp.

Fourier transformation and smoothing of trajectories

We use parameters to smooth (3 bp Daniell smoother; moving average giving half weight to the end values) and de-trend the data (i.e. subtract the mean of the series and remove a linear trend). A recursive time series filter implemented in R was used to remove high frequency variation from trajectories. 24 filter frequencies (1/seq(5,100,4)) were used, and the first 24 values of the trajectory were taken as init values. The 24-value shift in the resulting trajectories was corrected by repeating the last 24 values of the trajectory.

Supplemental Tables

Sample name	Library type	Reads	Fragments sequenced	Aligned	Aligned Q30	Coverage	Est. % duplicates	35-80bp	120-180bp
BH01	DSP	2x101	1489569204	97.20%	88.85%	96.32	6.00%	0.65%	57.64%
IH01	DSP	2x101	1572050374	98.58%	90.60%	104.92	21.00%	0.77%	47.83%
IH02	SSP	2x50, 43/42	779794090	93.19%	75.27%	30.08	20.05%	21.83%	44.00%
CH01	--	--	3841413668	96.95%	86.81%	231.32	14.99%	5.00%	50.85%

Table S1: Sequencing statistics for samples BH01, IH01, IH02, and CH01, related to Experimental Procedures. For each sample, sequencing-related statistics, including the total number of fragments sequenced, read lengths, the percentage of such fragments aligning to the reference with and without a mapping quality threshold, mean coverage, duplication rate, and the proportion of sequenced fragments in two length bins, are tabulated. Fragment length is inferred from alignment of paired-end reads. Due to the short read lengths, coverage is calculated by assuming the entire fragment had been read. The estimated number of duplicate fragments is based on fragment endpoints, which may overestimate the true duplication rate in the presence of highly stereotyped cleavage.
SSP, single-stranded library preparation protocol. DSP, double-stranded library preparation protocol.

Oligo Name	Sequence (5'-3')	Notes
CL9	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	HPLC purification
Adapter2.1	CGACGCTCTTCCGATC/ddT/	HPLC purification
Adapter2.2	/5Phos/AGATCGGAAGAGCGTCGTGTAGGGAAAGAG*T*G*T*A	HPLC purification
CL78	/5Phos/AGATCGGAAG/iSpC3/iSpC3/iSpC3/iSpC3/iSpC3/iSpC3/ iSpC3/iSpC3/iSpC3/iSpC3/3BioTEG/	Dual HPLC purification

Table S2: Synthetic oligos used in preparation of single stranded sequencing libraries, related to Experimental Procedures. Sequences adapted from Gansauge et al., 2013. *, phosphorothioate bond. /5Phos/, 5' phosphorylation. /ddT/, dideoxythymidine. /iSpC3/, C3 spacer. /3BioTEG/, 3' biotin-TEG.

Sample ID	Clinical Dx	Stage	cfDNA Yield (ng/ml)	Patient Sex
IC01 †	Kidney cancer (Transitional cell)	IV	242	F
IC02	Ovarian cancer (undefined)	IV	22.5	F
IC03	Skin cancer (Melanoma)	IV	12.0	M
IC04	Breast cancer (Invasive/infiltrating ductal)	IV	12.6	F
IC05	Lung cancer (Adenocarcinoma)	IV	5.4	M
IC06	Lung cancer (Mesothelioma)	IV	11.4	M
IC07 †	Gastric cancer (undefined)	IV	52.2	M
IC08	Uterine cancer (undefined)	IV	15.0	F
IC09	Ovarian cancer (serous tumors)	IV	8.4	F
IC10	Lung cancer (adenocarcinoma)	IV	11.4	F
IC11	Colorectal cancer (undefined)	IV	11.4	M
IC12	Breast cancer (Invasive/infiltrating lobular)	IV	12.0	F
IC13	Prostate cancer (undefined)	IV	12.3	M
IC14	Head and neck cancer (undefined)	IV	27.0	M
IC15 §	Lung cancer (Small cell)	IV	22.5	M
IC16	Bladder cancer (undefined)	IV	14.1	M
IC17 §	Liver cancer (Hepatocellular carcinoma)	IV	39.0	M
IC18	Kidney cancer (Clear cell)	IV	10.5	F
IC19	Testicular cancer (Seminomatous)	IV	9.6	M
IC20 §	Lung cancer (Squamous cell carcinoma)	IV	21.9	M
IC21	Pancreatic cancer (Ductal adenocarcinoma)	IV	35.4	M
IC22	Lung cancer (Adenocarcinoma)	IV	11.4	F
IC23	Liver cancer (Hepatocellular carcinoma)	IV	17.1	M
IC24	Pancreatic cancer (Ductal adenocarcinoma)	IV	37.2	M
IC25	Pancreatic cancer (Ductal adenocarcinoma)	IV	27.9	M
IC26	Prostate cancer (Adenocarcinoma)	IV	24.6	M
IC27	Uterine cancer (undefined)	IV	19.2	F
IC28	Lung cancer (Squamous cell	IV	33.3	M

	carcinoma)			
IC29	Head and neck cancer (undefined)	IV	14.4	M
IC30	Esophageal cancer (undefined)	IV	10.5	M
IC31 †	Ovarian cancer (undefined)	IV	334.8	F
IC32	Lung cancer (Small cell)	IV	9.6	F
IC33	Colorectal cancer (Adenocarcinoma)	IV	13.8	M
IC34	Breast cancer (Invasive/infiltrating lobular)	IV	33.6	F
IC35 §	Breast cancer (Ductal carcinoma *in situ*)	IV	16.2	F
IC36	Liver cancer (undefined)	IV	26.4	M
IC37 §	Colorectal cancer (Adenocarcinoma)	IV	15.9	F
IC38	Bladder cancer (undefined)	IV	6.6	M
IC39	Kidney cancer (undefined)	IV	39.0	M
IC40	Prostate cancer (Adenocarcinoma)	IV	13.8	M
IC41	Testicular cancer (Seminomatous)	IV	16.5	M
IC42	Lung cancer (Adenocarcinoma)	IV	11.4	F
IC43	Skin cancer (Melanoma)	IV	21.9	F
IC44	Esophageal cancer (undefined)	IV	25.8	F
IC45 †	Colorectal cancer (Adenocarcinoma)	IV	3.0	M
IC46 **	Breast cancer (Ductal carcinoma *in situ*)	IV	36.6	F
IC47	Pancreatic cancer (Ductal adenocarcinoma)	IV	19.2	F
IC48 **	Breast cancer (Invasive/infiltrating lobular)	IV	13.8	F

Table S4: Clinical diagnoses and cfDNA yield for cancer panel samples, related to Figure 5. Shown are the clinical and histological diagnoses for 48 patients from whom plasma-borne cfDNA was screened for evidence of high tumor burden, along with total cfDNA yield from 1.0 ml of plasma from each individual and relevant clinical covariates. Of these 48, 44 passed QC and had sufficient material. Of these 44, five were selected for deeper sequencing. cfDNA yield was determined by Qubit Fluorometer 2.0 (Life Technologies).

§: sample was selected for additional sequencing.
******: only 0.5 ml of plasma was available for this sample.
†: sample failed QC and was not used for further analysis.

Sample name	Library type	Reads	Fragments sequenced	Aligned	Aligned Q30	Coverage	Est. % duplicates	35-80bp	120-180bp
IH03	SSP	2x39	53292855	92.66%	72.37%	2.29	15.46%	11.05%	52.34%
IP01 †	DSP	2x101, 2x102	1214536629	97.22%	86.38%	76.11	0.55%	0.08%	62.77%
IP02 †	DSP	2x101, 2x102	855040273	97.16%	87.72%	52.46	0.83%	0.07%	68.10%
IA01	SSP	2x39	53934607	87.42%	68.30%	2.02	22.70%	15.20%	49.77%
IA02	SSP	2x39	42496222	95.42%	76.61%	1.95	4.74%	12.28%	59.00%
IA03	SSP	2x39	51278489	93.12%	71.33%	2.05	25.68%	14.27%	52.57%
IA04	SSP	2x39	50768476	90.30%	70.51%	2.14	7.83%	17.80%	36.76%
IA05	DSP	2x101	194985271	98.80%	90.61%	11.09	12.05%	2.24%	71.67%
IA06	DSP	2x101	171670054	98.90%	90.88%	9.90	5.41%	1.93%	71.26%
IA07	DSP	2x101	208609489	98.67%	90.34%	11.69	11.45%	2.59%	74.84%
IA08	DSP	2x101	193729556	98.81%	90.70%	10.84	11.96%	2.58%	76.24%
IC02	SSP	2x39	57913605	95.07%	75.57%	2.59	5.40%	12.98%	60.00%
IC03	SSP	2x39	63862631	95.78%	75.66%	2.79	8.32%	13.25%	62.20%
IC04	SSP	2x39	55239248	95.47%	76.26%	2.57	8.28%	10.98%	58.48%
IC05	SSP	2x39	39623850	89.80%	69.92%	1.60	9.24%	14.63%	50.33%
IC06	SSP	2x39	59679981	95.57%	74.90%	2.11	3.93%	24.30%	41.46%
IC08	SSP	2x39	46933688	94.38%	74.21%	1.92	5.92%	16.04%	45.25%
IC09	SSP	2x42	59639583	91.22%	71.15%	2.13	6.69%	21.39%	43.50%
IC10	SSP	2x42	53994406	93.73%	73.40%	1.83	2.00%	27.08%	37.62%
IC11	SSP	2x42	59225460	93.25%	72.51%	2.15	5.26%	21.30%	43.33%
IC12	SSP	2x42	57884742	93.52%	74.33%	2.34	2.66%	18.28%	46.58%
IC13	SSP	2x42	71946779	92.94%	72.47%	2.52	2.18%	23.51%	43.97%
IC14	SSP	2x42	61649203	94.54%	73.47%	2.20	3.23%	22.26%	43.37%
IC15	SSP	2x50, 43/42	908512803	95.49%	76.83%	29.77	10.66%	25.42%	38.47%
IC16	SSP	2x42	62739733	92.81%	72.85%	2.47	2.77%	17.71%	48.04%
IC17	SSP	2x50, 2x39	1072374044	96.02%	76.42%	42.08	12.16%	17.08%	50.02%
IC18	SSP	2x39	59976914	87.91%	68.67%	2.24	4.39%	18.85%	44.44%
IC19	SSP	2x39	51447149	89.38%	69.39%	2.02	8.24%	17.30%	46.33%
IC20	SSP	2x50, 2x39	640838540	96.30%	79.11%	23.38	12.43%	25.72%	39.87%
IC21	SSP	2x39	53000679	94.64%	74.57%	1.79	37.39%	29.89%	43.81%
IC22	SSP	2x39	58102606	94.08%	74.08%	2.51	6.24%	13.65%	58.41%
IC23	SSP	2x39	65859970	95.67%	75.67%	2.94	5.34%	11.09%	60.85%
IC24	SSP	43/42	66344431	94.63%	74.46%	2.48	2.00%	22.46%	46.31%
IC25	SSP	43/42	75066833	93.75%	73.66%	2.86	2.24%	21.30%	46.19%
IC26	SSP	43/42	79180860	92.59%	72.32%	2.97	2.93%	22.34%	40.42%
IC27	SSP	43/42	78037377	88.81%	67.04%	2.20	1.50%	31.31%	30.59%
IC28	SSP	43/42	61402081	95.24%	75.74%	2.60	2.46%	18.71%	46.44%
IC29	SSP	2x39	49989522	94.46%	73.36%	1.75	3.03%	25.82%	36.23%
IC30	SSP	2x39	58439504	93.52%	71.19%	1.75	17.35%	29.58%	30.47%
IC32	SSP	43/42	78233981	87.86%	66.80%	2.25	1.79%	30.12%	31.20%
IC33	SSP	43/42	62196185	87.26%	66.71%	1.93	1.93%	27.44%	36.92%
IC34	SSP	43/42	63572169	95.42%	76.74%	2.53	2.35%	19.64%	48.55%

Sample name	Library type	Reads	Fragments sequenced	Aligned	Aligned Q30	Coverage	Est. % duplicates	35-80bp	120-180bp
IC35	SSP	43/42	618554393	86.47%	65.90%	18.22	5.23%	28.18%	35.24%
IC36	SSP	43/42	54402943	94.62%	74.73%	2.21	3.32%	17.02%	52.42%
IC37	SSP	2x50, 43/42	1175553677	93.00%	74.46%	38.22	10.15%	28.47%	35.11%
IC38	SSP	43/42	47981963	89.35%	69.45%	1.78	6.47%	18.59%	43.03%
IC39	SSP	43/42	61968854	95.29%	75.57%	2.62	2.54%	14.42%	57.28%
IC40	SSP	2x39	53228209	93.54%	71.69%	1.81	8.85%	24.88%	34.95%
IC41	SSP	43/42	78081655	87.11%	65.25%	2.26	1.61%	27.94%	35.21%
IC42	SSP	2x39	53017317	93.59%	74.33%	2.02	10.74%	19.04%	44.12%
IC43	SSP	43/42	76395478	88.41%	67.21%	2.40	1.56%	26.68%	37.76%
IC44	SSP	43/42	61354307	95.15%	74.88%	2.45	4.34%	19.10%	46.39%
IC46	SSP	2x39	60123123	94.51%	72.23%	2.13	10.37%	15.46%	50.93%
IC47	SSP	2x39	59438172	95.58%	73.84%	2.07	9.33%	21.67%	43.34%
IC48	SSP	43/42	55704417	91.35%	72.79%	2.01	13.87%	22.56%	38.68%
IC49	DSP	2x101	170489015	99.02%	90.53%	11.19	5.93%	2.41%	59.93%
IC50	DSP	2x101	203828224	98.72%	90.28%	10.82	2.83%	4.81%	66.23%
IC51	DSP	2x101	200454421	98.63%	90.53%	11.77	9.50%	2.58%	67.04%
IC52	DSP	2x101	186975845	98.97%	91.25%	11.37	2.57%	0.83%	68.96%

Table S5: Sequencing statistics for additional samples included in CA01, related to Experimental Procedures. For each sample, sequencing-related statistics, including the total number of fragments sequenced, read lengths, the percentage of such fragments aligning to the reference with and without a mapping quality threshold, mean coverage, duplication rate, and the proportion of sequenced fragments in two length bins, are tabulated. Fragment length is inferred from alignment of paired-end reads. Due to the short read lengths, coverage is calculated by assuming the entire fragment had been read. The estimated number of duplicate fragments is based on fragment endpoints, which may overestimate the true duplication rate in the presence of highly stereotyped cleavage.
SSP, single-stranded library preparation protocol. DSP, double-stranded (conventional) library preparation protocol.
Sample has been previously published (Kitzman JO et al. (2012), Sci Transl Med *4,* 137ra76).