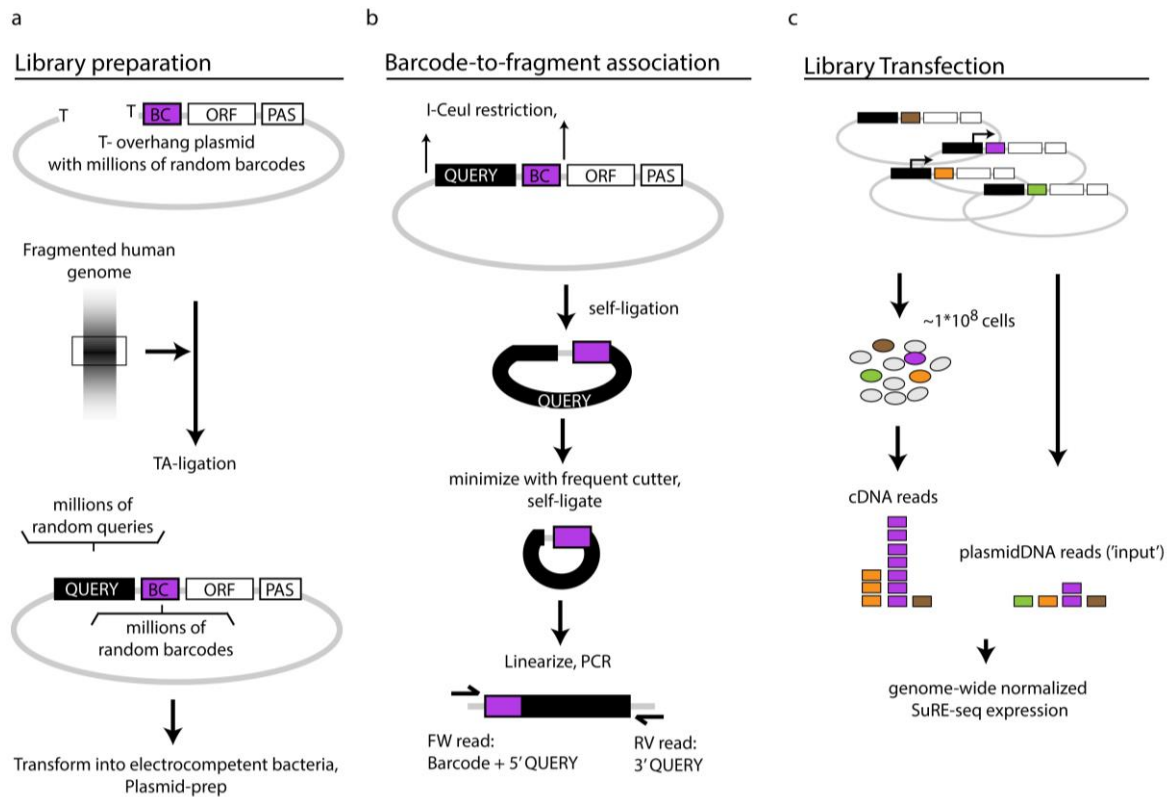




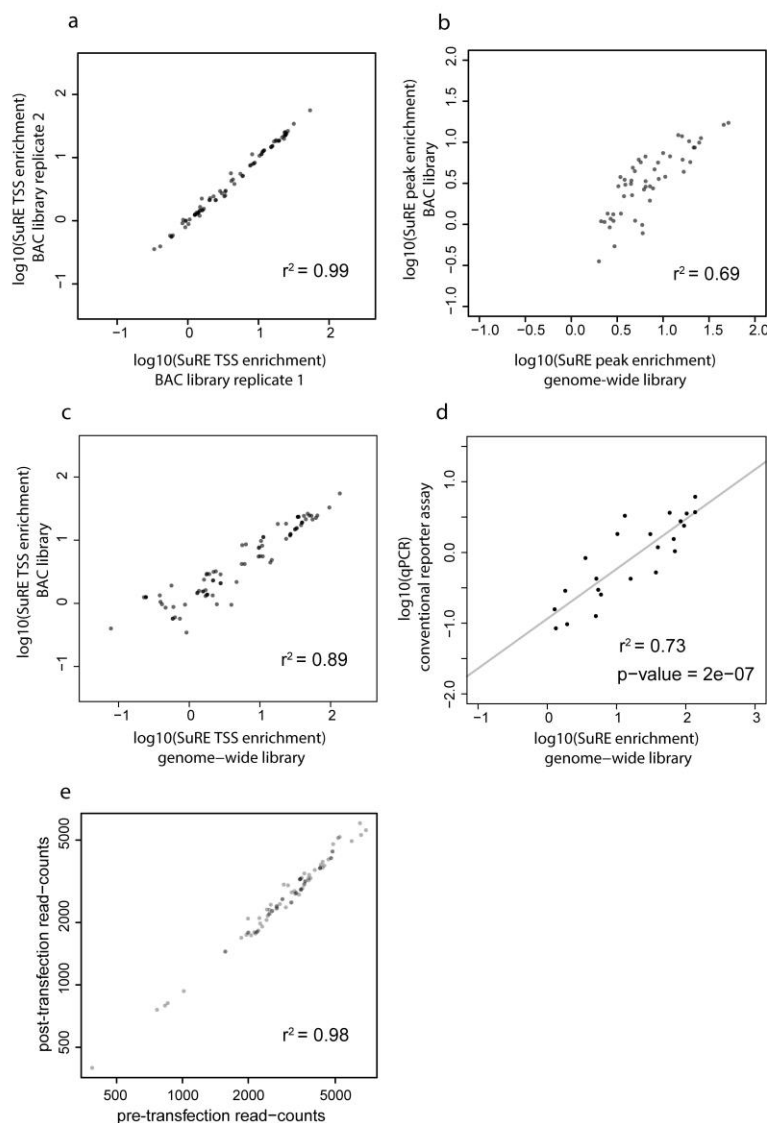
Supplementary Figure 1

Detailed schematic representation of SuRE methodology.

See Methods for detailed description. **a.** Size-selected and A-tailed random fragments ('queries') of the human genome are inserted in bulk into barcoded T-overhang plasmids by ligation. BC, barcode; ORF, open reading frame; PAS, polyadenylation signal. **b.** The library is digested by endonuclease I-CeuI so that the barcode with the query sequence is released. This is then self-ligated and again digested with a frequent cutter restriction enzyme to reduce the insert size. After another self-ligation the circle is linearized, PCR amplified and subjected to high-throughput sequencing. **c.** Per biological replicate ~ 100 million cells are transfected. Those plasmids that contain promoter activity in the direction of the barcode will transcribe the barcode into RNA. Cells are harvested after 24 hours, RNA is extracted, polyA purified, reverse transcribed, PCR amplified and subjected to high-throughput sequencing. By normalization to estimated barcode frequencies in the SuRE plasmid library a genome-wide SuRE expression profile is generated.

maximum of 1.

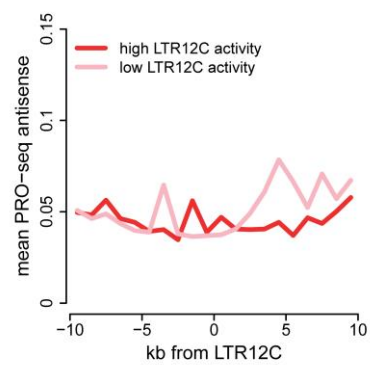
1. Encode Project Consortium. An integrated encyclopedia of DNA elements in the human genome. *Nature* 489, 57-74 (2012).
2. FANTOM Consortium. A promoter-level mammalian expression atlas. *Nature* 507, 462-470 (2014).
3. Zhang, Y. et al. Model-based analysis of ChIP-Seq (MACS). *Genome Biol* 9, R137 (2008).



Supplementary Figure 3

Focused BAC library.

a. Correlation between biological replicates for the focused SuRE library. Data is shown for all TSSs within in the BAC library. **b.** Correlation between SuRE enrichment obtained with the genome-wide library (x-axis) and the focused library (y-axis) for all peaks overlapping the BAC library. **c.** Same as (b) but for all TSSs in the BAC library. **d.** Correlation between SuRE enrichment obtained with the genome-wide library (x-axis) and a conventional reporter assay (y-axis) for 23 promoters. Grey line shows linear fit. **e.** Correlation between pre-transfection read-counts and post-transfection read-counts for all TSSs in the BAC library.

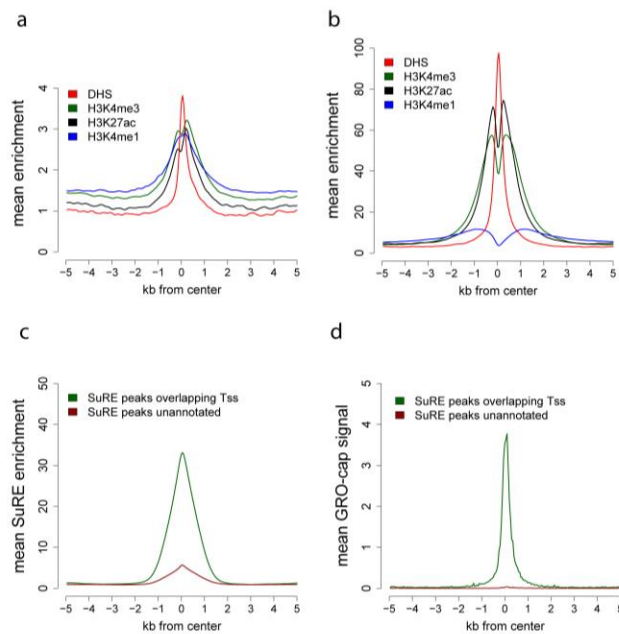


Supplementary Figure 4

Run-on transcription around LTR12C elements, antisense.

Average PRO-seq run-on transcription activity⁴ around LTR12C elements as in Fig. 5e, but in antisense orientation.

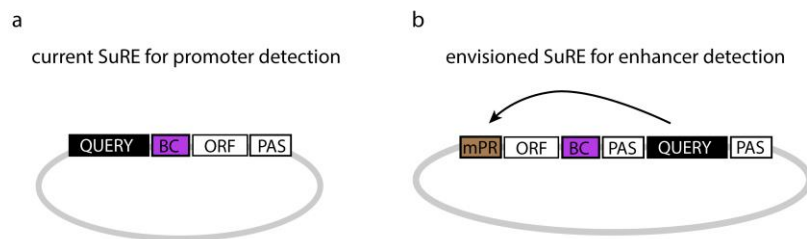
4. Core, L.J. et al. Analysis of nascent RNA identifies a unified architecture of initiation regions at mammalian promoters and enhancers. *Nat Genet* 46, 1311-1320 (2014).



Supplementary Figure 5

Chromatin marks associated to unannotated SuRE peaks.

- a.** Mean enrichment for 4 chromatin marks centered on the summit of unannotated SuRE peaks, i.e. peaks that did not overlap ENCODE annotated promoters or enhancers ('Tss' or 'Enh' chromatin state) or repetitive elements of the ERV1 or ERVL-MaLR family.
- b.** Same as (a) but for SuRE peaks that overlapped encode annotated promoters. **c.** Mean SuRE enrichment for all peaks overlapping ENCODE annotated promoters (green) and unannotated SuRE peaks. **d.** Same as (c) but for mean GRO-cap signal.



Supplementary Figure 6

Envisioned SuRE methodology for enhancer detection.

a. Current SuRE reporter construct for promoter detection. **b.** Envisioned reporter construct for enhancer detection. Query: genomic fragment, BC: barcode, ORF: open reading frame, PAS: polyadenylation signal, mPR: minimal promoter.

Supplementary Table 1. hg19 coordinates and references for Bacterial Artificial Chromosomes

chr	start	end	BAC reference
chr16	146,255	277,801	CTD-2100B21
chr4	74,192,088	74,388,711	CTD-2610J6
chr5	139,961,667	140,166,117	CTD-3252A18
chr1	109,571,381	109,687,521	CTD- 3156P24
chr6	26,115,655	26,242,415	CTD-2153L18
chr1	155,087,497	155,235,687	CTD-3075C4
chr2	11,628,199	11,749,760	CTD-2079O10
chr17	46,595,129	46,735,928	CTD-2508F13
chr2	176,924,832	177,067,161	CTD-2521G12

Supplementary Table 2. Oligo nucleotide sequences

name	description	synthesis type	sequence
256JvA	Forward primer for usage on barcoding template	standard	TGTGATGGTTGGCCAACCTTGAATTCCGGAAGGGATCTGGTTAACCTTGG AACC
254JvA	barcoding template	ultramer	AAGGGATCTGGTTAACCTTGAACCTTGGCCAACGTACGACTGGAGATCG GAAGAGCACACGTCTGAACTCCAGTCACTAGGGATAACAGGGTAATACACT CTTCCCTACACGACGCTCTCCGATCT
264JvA	Reverse primer for usage on barcoding template (containing barcode)	standard	TTGGTTCCTAGG(N)(N)(N)(N)(N)(N)(N)(N)(N)(N)(N)(N)(N)(N)(N)(N))AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT
247JvA	First strand primer of reverse transcription	standard	CCTCTCCGCCGCCACCAGCTCGAACTCCAC
211JvA	Reverse primer for cDNA or plasmidDNA; containing S2, index and P7	standard	CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTG TGCTCTTCCGATCTGGTGATGCGGCACTCGATCTTCATGGC
117JvA	Reverse primer for iPCR; containing S2 and P7	standard	CAAGCAGAAGACGGCATACGAGATACATCGGTGACTGGAGTTCAGACGTG TGCTCTTCCGATC
AR151	Forward primer for cDNA, plasmidDNA or iPCR; containing S1 and P5	standard	AATGATACGGCGACCACCGAGATCTACACTCTTCCCTACACGACGCTCTT CCGATCT
383JvA	TBP FW	standard	ACTTCGTGCCCCGAAACGC
384JvA	TBP FW	standard	ATCCTCATGATTACCGCAGCAAAC
419JvA	Alpha globin 1 FW	standard	CTCGGTGGCCATGCTTCTT
420JvA	Alpha globin 1 RV	standard	GCCGCCCACTCAGACTTTAT

421JvA	Alpha globin 2 FW	standard	TCAAGCTCCTAAGCCACTGC
422JvA	Alpha globin 2 RV	standard	CAGGAGGAACGGCTACCGAG
431JvA	Theta1 globin FW	standard	CCTGAGCCACGTTATCTCGG
432JvA	Theta1 globin RV	standard	GGCTTTACTCAAACACGGGG
433JvA	Zeta globin FW	standard	CTGAGCAGGCCCAACTCC
434JvA	Zeta globin RV	standard	GATCTTGGCCCACATGGACA
446JvA	BetaActin FW	standard	ACAGAGCCTCGCCTTTGCC
447JvA	BetaActin RV	standard	GAGGATGCCTCTCTTGCTCTG
AR68FW	GFP FW	standard	AGGACAGCGTGATCTTCACC
AR68RV	GFP RV	standard	CTTGAAGTGCATGTGGCTGT
444JvA	YFP FW	standard	GATCTGCACCACCGCAAG
445JvA	YFP RV	standard	GCTGCTTCATGTGGTCGGG
PAIP2_FW	confirmation set supplementary figure 3d	standard	TGTCTGAGTGCGGATGTTTGC

PAIP2_RV	confirmation set supplementary figure 3d	standard	GGCAGCCAACGAATCCTGTC
PARK7_FW	confirmation set supplementary figure 3d	standard	TGTGCGCAGCACTGCTCTAGT
PARK7_RV	confirmation set supplementary figure 3d	standard	GCCCGTTGGGTACCACTCAC
TIMM10B_FW	confirmation set supplementary figure 3d	standard	GGTCTCCCCTCCTCCGTCTC
TIMM10B_RV	confirmation set supplementary figure 3d	standard	CGTCTGGCCTCGAAACGACT
HIST1H2BD_FW	confirmation set supplementary figure 3d	standard	CGAAGGGCTACATTCAAGTGC
HIST1H2BD_RV	confirmation set supplementary figure 3d	standard	TCTTTGGGGCAGGAGCAGAC
PKLR_FW	confirmation set supplementary figure 3d	standard	TTTCCCTGGGGGTAGGAGTC
PKLR_RV	confirmation set supplementary figure 3d	standard	ACCGAAGCTGCAGGGATGAT
ALG3_FW	confirmation set supplementary figure 3d	standard	CCCCCAACGCTCAAACCTCTG
ALG3_RV	confirmation set supplementary figure 3d	standard	TGGCAGTACAGCCGGAGGAT
CNOT4_FW	confirmation set supplementary figure 3d	standard	AATGCGCAAGGACAGGGAAA
CNOT4_RV	confirmation set supplementary figure 3d	standard	GCTTCAGCGAGTCCGACCTT

GLMN_FW	confirmation set supplementary figure 3d	standard	TCTGGGGGAAGAGGGGAGTC
GLMN_RV	confirmation set supplementary figure 3d	standard	TCCACTTACCGGCCAGAACC
ZNF669_FW	confirmation set supplementary figure 3d	standard	CATCCCCAACCTTGGCAAAA
ZNF669_RV	confirmation set supplementary figure 3d	standard	CTCCGGCGAAGGAGAGACAA
C9orf156_FW	confirmation set supplementary figure 3d	standard	TTTCCCACCACCCAGGGATA
C9orf156_RV	confirmation set supplementary figure 3d	standard	CGCATGGCTACTGGTTGCTG
POLR2J_FW	confirmation set supplementary figure 3d	standard	TTGTCCCTCCCGGCTAACAA
POLR2J_RV	confirmation set supplementary figure 3d	standard	GCCCTCGAAGAGCAAGAACG
RPL37_FW	confirmation set supplementary figure 3d	standard	AAAGTCAGCGTCGGCCAAAA
RPL37_RV	confirmation set supplementary figure 3d	standard	CCCCAAGCACAGCAAACAGA
TOMM7_FW	confirmation set supplementary figure 3d	standard	TGTGCAGCCAGGGTTGAGAA
TOMM7_RV	confirmation set supplementary figure 3d	standard	CGGGAATCCGAAAGGGAAAG
NHSL1_FW	confirmation set supplementary figure 3d	standard	TGCTTTGGAACACACAATGCTG

NHSL1_RV	confirmation set supplementary figure 3d	standard	TTCCCCCGGTCTCATATCCTT
IL1R1_FW	confirmation set supplementary figure 3d	standard	CGCCCCTGGTGTGTCAGGTA
IL1R1_RV	confirmation set supplementary figure 3d	standard	TGGGTGTCACCTCCCATTITT
PRKAG2_FW	confirmation set supplementary figure 3d	standard	CTTGCTGGGAGGTGGGATTG
PRKAG2_RV	confirmation set supplementary figure 3d	standard	GGCAGCAGGTTCCAGATGTGT
GANC_FW	confirmation set supplementary figure 3d	standard	GCCTGGCCTGAGTCTTTTCTG
GANC_RV	confirmation set supplementary figure 3d	standard	TCAGGCCCAAAGTAGCGTTTC
DMKN_FW	confirmation set supplementary figure 3d	standard	AGAATGGGGGCAGGACTGTG
DMKN_RV	confirmation set supplementary figure 3d	standard	TCCCCCTCTTTAGCCTGTTGG
OSBPL6_FW	confirmation set supplementary figure 3d	standard	AGCGCTGGAGCCGTTCTG
OSBPL6_RV	confirmation set supplementary figure 3d	standard	CCCAAGCAATCCCTTTGCAG
TLR4_FW	confirmation set supplementary figure 3d	standard	TGGTGGGCCCTAATCCAACA
TLR4_RV	confirmation set supplementary figure 3d	standard	GCGAGGCAGACATCATCCTG

SLC13A4_FW	confirmation set supplementary figure 3d	standard	CTTTGCCAGGGAGGCAGCTA
SLC13A4_RV	confirmation set supplementary figure 3d	standard	GGGCCTGCAAAGCAGAAAAG
CEP85L_FW	confirmation set supplementary figure 3d	standard	CCACCCCAAATCCCACTGAA
CEP85L_RV	confirmation set supplementary figure 3d	standard	TGCTCCACAATTGGAGAAACAA
MGST3_FW	confirmation set supplementary figure 3d	standard	GCCAGCTCTCGGCAAACTAA
MGST3_RV	confirmation set supplementary figure 3d	standard	CCTTCGAACAGCTGGAGCAGA