SUPPLEMENTARY INFORMATION

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Supplementary Method I. BrdU IP efficiency measurement.

qPCR to quantify adaptor-ligated DNA fragments (before and after BrdU IP)

This is an example to use Roche FastStart Universal SYBR Green Master (Rox) on ABI 7500Fast. PCR program can be changed depending on the availability of qPCR machine and master mix.

Reagent:
FastStart Universal SYBR Green Master (Roche 04 913 850 001)

primers:

NEBadqPCR_F; ACACTCTTTCCCTACACGACGC
NEBadqPCR_R; GACTGGAGTTCAGACGTGTGC

These anneal to the adaptor region of the DNA fragment.

Standard:
Serial dilution of previously made and quantified (by Qubit) library. 1pg/uL, 0.1pg/uL, 0.01pg/uL, 0.001pg/uL, ,0.0001pg/uL, 0.00001pg/uL.
If you do not have a previously made library, you can make one by amplifying the Illumina PhiX library.

Reaction set-up:

	Volume (uL)	Final concentration
2xmaster mix	7.5	1x
Primer F+R (10uM each)	1.5	1.5uM
H2O	3	
Diluted template*	3	
total	15	

qPCR program:

Step	Cycle	Temperature (˚C)	Time
Pre-incubation	1	95	10 min
Amplification	35	95	15 sec
		60	1 min
Melting Curve	1	95	15 sec
		65	1 min
		95	30 sec
		60	15 sec

* In our experience, pre-IP libraries can be used as 1:10,000 while post-IP libraries can be used as 1:100 to fall within the standard curve.

Supplementary method II. Nuclei preparation by pepsin treatment for nuclei sorting

1. Transfer 2 x 10^6 cells to a 15 mL conical tube.
2. Centrifuge at approximately 200 x g for 5 minutes at room temperature.
3. Decant supernatant carefully.
4. Re-suspend the cell pellet in 2 mL of PBS with 1% FBS (vol/vol). Mix well by tapping the tube.
5. Centrifuge at approximately 200 x g for 5 minutes at room temperature.
6. Decant supernatant carefully.
7. Resuspend cell pellet by tapping (as cells stick to the tip if you pipette up and down) in 4 mL 0.025% pepsin (Sigma, cat. no. P6887) (wt/vol) in 0.01 N HCl and incubate for 1hr at 37°C water bath.
8. Centrifuge at approximately 600 x g for 10 minutes at room temperature.
9. Resuspend nuclei in 4 mL PBS-1% FBS (vol/vol) and centrifuge at 600x g for 10 min at room temperature.
10. Decant supernatant carefully.
11. Resuspend nuclei in 1 mL PBS / 1% FBS / PI / RNase by tapping (this makes 2 x 10^6 cells/mL suspension).
12. Transfer to 5 mL polypropylene round bottom tube.
13. Incubate 20-30 min at RT in the dark. Meanwhile, take an aliquot and check the concentration and shape of your "nuclei" under a microscope. Nuclei in the suspension look grayish under phase contrast while intact cells look shiny.
14. Keep samples on ice in the dark and proceed directly to FACS. Alternatively, add 1/9 vol. DMSO and slowly freeze in -80°C in the dark just like you make a live cell stock. Frozen nuclei at - 80°C are good for at least a few weeks. When ready to sort, thaw the cell suspension in a 37°C water bath. Removing DMSO is not necessary. Once thawed, keep the samples on ice in the dark and proceed to sorting.

Supplementary method III. Merging multiple fastq(.gz) files.
These scripts are useful if you have multiple sequencing files per library.

1. First you need to create one master directory, containing itself one directory per library, and move the fastq or fastq.gz files in the corresponding directory. If you have paired-end data, create separate directories for each paired file.
2. Copy the following script in a script.sh text file, with a text editor, and save it into the master directory.

(A) If you have uncompressed fastq files:

```
for dir in *[^s][^h]; do
   cd $dir;
   cat *.fastq >> ../${dir}.fastq;
   cd ../;
done
```

(B) If you have fastq.gz files:

```
for dir in *[^s][^h]; do
   cd $dir;
   cat *.fastq.gz >> ../${dir}.fastq.gz;
   cd ../;
done
```

3. Open a terminal and go to the master directory:
   $ cd /path/to/the/master/directory
4. Make the script file executable:
   $ chmod 755 script.sh
5. Execute the script:
   $ ./script.sh

The merged files are saved in your master directory.

Supplementary method IV. Generating log ratio coverage files using R with package “travis”

1. Launch R and go to the fastq files directory:
   > setwd("path/to/files/")
2. Load the travis package:
   > library(travis)
3. Set the parameters for the analysis:
   > options(threads=20) # This option specifies the number of threads to use
   > options(chromsizes="path/to/your/genome/index/file.chrom.sizes") # This option specifies the path to access to the chrom.size file of the genome you will you use for the analysis. Many of these files can be directly downloaded from UCSC server at ftp://hgdownload.cse.ucsc.edu/goldenPath/. This file contains two columns: each chromosome name and size in bp, separated by a tabulation.
   CRITICAL STEP This file must be sorted on the first column, following alphabetic order (for example, “chr10” will be before “chr2”).
   (A) If you are analysing single-end data:
      > f=files("*.fastq*") # This command creates a vector containing the names of all your fastq files present in the folder. You can restreint this list by using regular expressions or hand-writing a vector with your files of interest.
   (B) If you are analysing paired-end data, with names of the paired files ending by "R1.fastq" and "R2.fastq", or "R1.fastq.gz" and "R2.fastq.gz":
      > f1=files("*R1.fastq*") # This command creates a vector containing the names of the first files of the pairs.
      > f2=files("*R2.fastq*") # This command creates a vector containing the names of the second files of the pairs.
      CRITICAL STEP Paired-end files must be in the same order in both f1 and f2 vectors.
4. Map the reads on your genome of interest:
   (A) If you are analysing single-end data
      > g=bowtie2(f,"path/to/your/genome",reorder=TRUE)
   (B) If you are analysing paired-end data
      > g=bowtie2(f1,"path/to/your/genome",f2,reorder=TRUE)
   For more informations about the nomenclature to use for the path to the genome, please read bowtie documentation at http://bowtie-bio.sourceforge.net/manual.shtml.
5. Convert the sam files
   > bams<-samtoolsView(g) # This command converts the sam files produced by bowtie2 into bam files.
   > beds<-bamToBed(bams,sortBuffer="5G") # This command converts the bam files into bed files.
   > w<-bedtoolsMakeWindows(windowsize=50000) # This command generates a bed file of 50kb windows coordinates of your genome.
   > bgs<-bedtoolsCoverage(beds,w) # This command calculates the coverage of your mapped reads onto the windows generated above.

6. Calculate the ratio of early versus late reads: files containing early data must contains "_E_", and files containing early data must contains "_L_".

```
> e=bgs[grep("_E_",bgs)] # Select the 50kb coverage files of the early S samples
> l=bgs[grep("_L_",bgs)] # Select the 50kb coverage files of the late S samples
> l2r<-bgOps(e,"log2ratio",l,pattern="_E_",replacement="_T_") # Calculate the log ratio of early to late samples.
```

Supplementary method V. Mapping the reads on two genomes and generating log ratio coverage files using R with package “travis”.

1. Launch R and go to the fastq files directory:
   > setwd("path/to/files/")
2. Load the travis package:
   > library(travis)
3. Set the parameters for the analysis:
   > options(threads=20) # This option specifies the number of CPU threads to use
   > options(chromsizes="path/to/your/genome/index/file.chrom.sizes") # This option specifies the path to access to the chrom.size file of the genome you will you use for the analysis. Many of these files can be directly downloaded from UCSC server at ftp://hgdownload.cse.ucsc.edu/goldenPath/. This file contains two columns: each chromosome name and size in bp, separated by a tabulation.
   CRITICAL STEP This file must be sorted lexicographically by the first column. This can be done with "sort -k1,1 -o file.chrom.sizes file.chrom.sizes" shell command.
   > f=files("*.fastq*") # This command creates a vector containing the names of all your fastq files present in the folder. You can restrain this list by using regular expressions or manually defining a vector with your files of interest.
4. Map the reads on your two genomes of interest. For more informations about the nomenclature to use for the path to the genome, please read bowtie documentation at http://bowtie-bio.sourceforge.net/manual.shtml.
   > g1=bowtie2(f, "path/to/your/genome_1", appendIndexToName=TRUE, reorder=TRUE)
   > g2=bowtie2(f, "path/to/your/genome_2", appendIndexToName=TRUE, reorder=TRUE)
5. Parse reads based on which genome they align better to
   > psams<-samParseGenomes(g1, g2, sortBuffer="5G")
6. Convert the sam files
   > bams<-samtoolsView(psams) # This command converts the sam files produced by bowtie2 into bam files.
   > beds<-bamToBed(bams,sortBuffer="5G") # This command converts the bam files into bed files.
   > w<-bedtoolsMakeWindows(windowsize=50000) # This command generates a bed file of 50kb windows coordinates of your genome.
   > bgs<-bedtoolsCoverage(beds,w) # This command calculates the coverage of your mapped reads onto the windows generated above.
7. Calculate the ratio of early versus late reads: Assumes files containing early data contain "_E_", and files containing late data contains "_L_" in the file names.
   > e=bgs[grep("_E_",bgs)] # Select the 50kb coverage files of the early S samples
   > l=bgs[grep("_L_",bgs)] # Select the 50kb coverage files of the late S samples
   > l2r<-bgOps(e,"log2ratio",l,pattern="_E_",replacement="_T_") # Calculate the log2 ratio of early over late samples.

Supplementary data I. NEB adapter, PCR primer, index info

NEBNext Adaptor for Illumina
#E7337A: 0.24 ml
#E7337AA: 0.96 ml

5 '-/5Phos/GAT CGG AAG AGC ACA CGT CTG AAC TCC AGT C/ideoxyU/A CAC TCT TTC CCT ACA CGA CGC TCT TCC GAT C*T-3 '

NEBNext i501 Primer–NEBNext i508 Primer

NEB #	Product	Index Primer Sequence	Expected Index Primer Sequence Read
#E7603A: 0.060mL	NEBNext i501 Primer	5'-AATGATACGGCGACCACCGAGATCTAC ACTATAGCCTACACTCTTTCCCTACACGA CGCTCTTCCGATC*T-3'	TATAGCCT
#E7604A: 0.060mL	NEBNext i502 Primer	5'-AATGATACGGCGACCACCGAGATCTAC ACATAGAGGCACACTCTTTCCCTACACG ACGCTCTTCCGATC*T-3'	ATAGAGGC
#E7605A: 0.060mL	NEBNext i503 Primer	5'-AATGATACGGCGACCACCGAGATCTAC ACCCTATCCTACACTCTTTCCCTACACGA CGCTCTTCCGATC*T-3'	CCTATCCT
#E7606A: 0.060mL	NEBNext i504 Primer	5'-AATGATACGGCGACCACCGAGATCTAC ACGGCTCTGAACACTCTTTCCCTACACGA CGCTCTTCCGATC*T-3'	GGCTCTGA
#E7607A: 0.060mL	NEBNext i505 Primer	5'-AATGATACGGCGACCACCGAGATCTAC ACAGGCGAAGACACTCTTTCCCTACACG ACGCTCTTCCGATC*T-3'	AGGCGAAG
#E7608A: 0.060mL	NEBNext i506 Primer	5'-AATGATACGGCGACCACCGAGATCTAC ACTAATCTTAACACTCTTTCCCTACACGA CGCTCTTCCGATC*T-3'	TAATCTTA
#E7609A: 0.060mL	NEBNext i507 Primer	5'-AATGATACGGCGACCACCGAGATCTAC ACCAGGACGTACACTCTTTCCCTACACG ACGCTCTTCCGATC*T-3'	CAGGACGT
#E7610A: 0.060mL	NEBNext i508 Primer	5 '-AATGATACGGCGACCACCGAGATCTAC ACGTACTGACACACTCTTTCCCTACACGA CGCTCTTCCGATC*T-3'	GTACTGAC

NEBNext i701 Primer – NEBNext i712 Primer

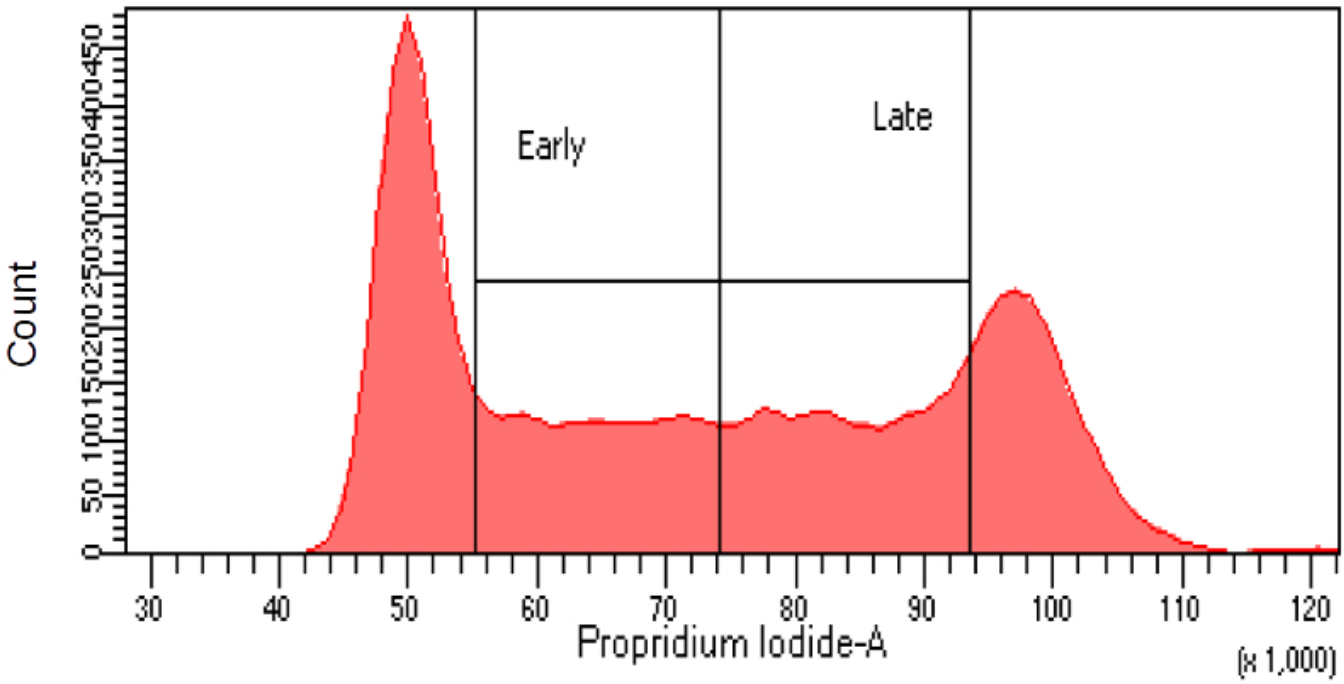
NEB #	Product	Index Primer Sequence	Expected Index Primer Sequence Read
#E7611A:	NEBNex	5'-CAAGCAGAAGACGGCATACGAGATCG	ATTACTCG

0.040ml	t i701 Primer	AGTAATGTGACTGGAGTTCAGACGTGTG CTCTTCCGATC*T-3'	
#E7612A: 0.040ml	NEBNex t i702 Primer	5'-CAAGCAGAAGACGGCATACGAGATTC TCCGGAGTGACTGGAGTTCAGACGTGTG CTCTTCCGATC*T-3'	TCCGGAGA
#E7613A: 0.040ml	NEBNex t i703 Primer	5'-CAAGCAGAAGACGGCATACGAGATAA TGAGCGGTGACTGGAGTTCAGACGTGTG CTCTTCCGATC*T-3'	CGCTCATT
#E7614A: 0.040ml	NEBNex t i704 Primer	5'-CAAGCAGAAGACGGCATACGAGATGG AATCTCGTGACTGGAGTTCAGACGTGTG CTCTTCCGATC*T-3'	GAGATTCC
#E7615A: 0.040ml	NEBNex t i705 Primer	5'-CAAGCAGAAGACGGCATACGAGATTT CTGAATGTGACTGGAGTTCAGACGTGTG CTCTTCCGATC*T-3'	ATTCAGAA
#E7616A: 0.040ml	NEBNex t i706 Primer	5'-CAAGCAGAAGACGGCATACGAGATAC GAATTCGTGACTGGAGTTCAGACGTGTG CTCTTCCGATC*T-3'	GAATTCGT
#E7617A: 0.040ml	NEBNex t i707 Primer	5'-CAAGCAGAAGACGGCATACGAGATAG CTTCAGGTGACTGGAGTTCAGACGTGTG CTCTTCCGATC*T-3'	CTGAAGCT
#E7618A: 0.040ml	NEBNex t i708 Primer	5'-CAAGCAGAAGACGGCATACGAGATGC GCATTAGTGACTGGAGTTCAGACGTGTG CTCTTCCGATC*T-3'	TAATGCGC
#E7619A: 0.040ml	NEBNex t i709 Primer	5'-CAAGCAGAAGACGGCATACGAGATCA TAGCCGGTGACTGGAGTTCAGACGTGTG CTCTTCCGATC*T-3'	CGGCTATG
#E7620A: 0.040ml	NEBNex t i710 Primer	5'-CAAGCAGAAGACGGCATACGAGATTT CGCGGAGTGACTGGAGTTCAGACGTGTG CTCTTCCGATC*T-3'	TCCGCGAA
#E7621A: 0.040ml	NEBNex t i711 Primer	5'-CAAGCAGAAGACGGCATACGAGATGC GCGAGAGTGACTGGAGTTCAGACGTGTG CTCTTCCGATC*T-3'	TCTCGCGC
#E7622A: 0.040ml	NEBNex t i712 Primer	5'-CAAGCAGAAGACGGCATACGAGATCT ATCGCTGTGACTGGAGTTCAGACGTGTG CTCTTCCGATC*T-3'	AGCGATAG

Supplementary data II. TS-Oligo 1 &2 for re-amplification
TS-Oligo 1 (TS-PCROligo1NbGn): AAT GAT ACG GCG ACC ACC GAG A
TS-Oligo 2 (TS-PCROligo2NbGn): CAA GCA GAA GAC GGC ATA CGA G

Supplementary table I.

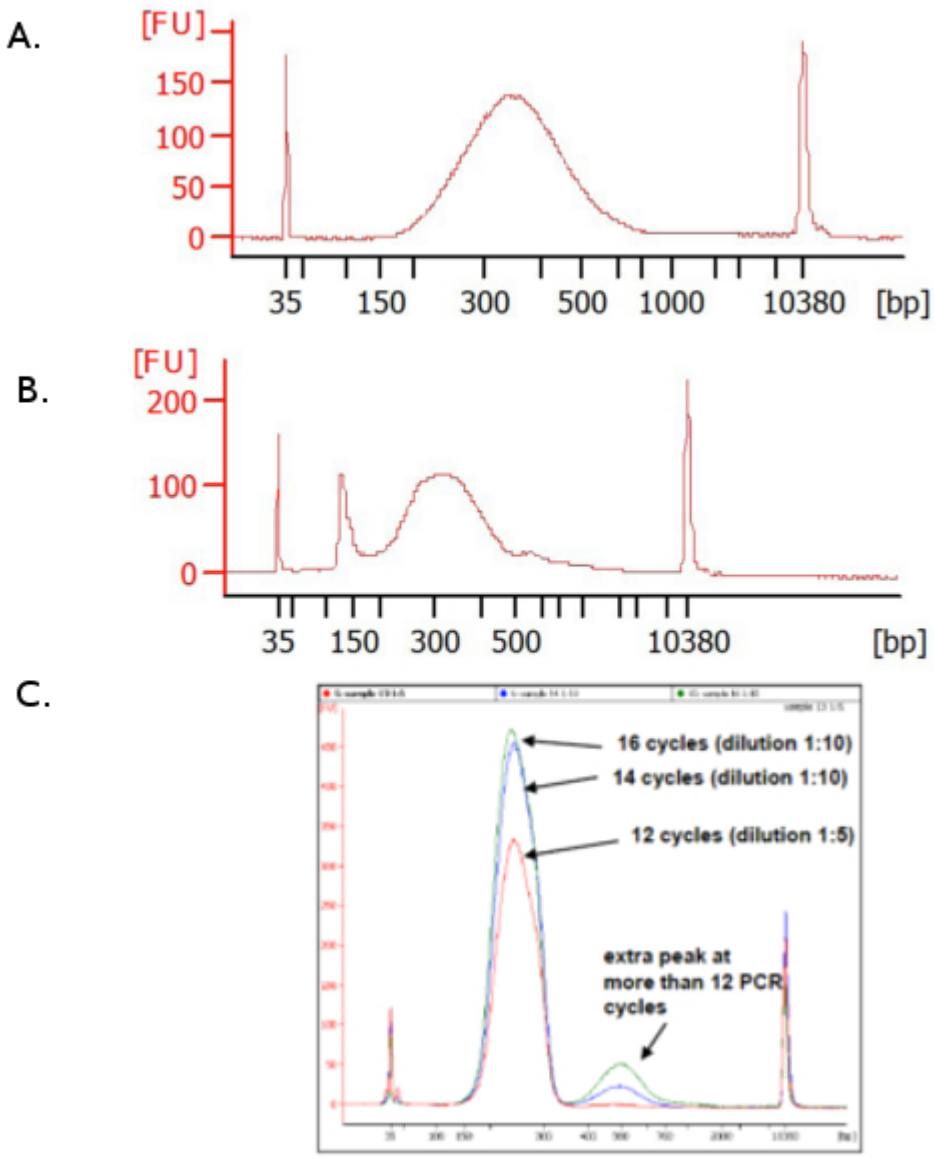
Primer pool name	Locus	PCR product size (bp)	Enrichment	Conc. in primer mix (μM each F and R)	Final conc. MM (in the PCR reaction)
Mouse A	mito	346	E=L	0.625	0.05
	α-globin	439	E	5	0.4
	Pou5f1	194	E	1.25	0.1
Mouse B	β-globin	369	L	1.25	0.1
	Dppa2	199	developmentally regulated	1.25	0.1
Mouse C	Mmp15	360	E	2.5	0.4
	Zfp42	211	E	2.5	0.4
	Akt3	173	L	1.25	0.2
Mouse D	Ptn	230	developmentally regulated	1.25	0.2
	Mash1	182	L	2.5	0.4
Human A	Mito	168	E=L	1.25	0.2
	MMP15	249	E	5	0.4
Human B	α-globin	165	E	2.5	0.4
	β-globin	241	L	1.25	0.2
Human C	BMP1	177	E	2.5	0.4
	hPTGS2	230	L	5	0.4
Human D	hNETO 1	286	L	5	0.4
Human E	hSLITR K6	281	L	2.5	0.4
	hZFP42	233	developmentally regulated	5	0.4
	hDPPA2	168	developmentally regulated	2.5	0.4



Supplementary Figure 1

FACS gating strategy

F121-9 FACS sorting gate.



Supplementary Figure 2

Representative Bioanalyzer results from library quality control.

(A.) Good library (B.) Remaining adaptor dimers around 150 bp (C.) Over-amplification (peak at 2x size)