Supplementary information

Profiling the genetic determinants of chromatin accessibility with scalable single-cell CRISPR screens

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Supplementary materials

Scalable pooled CRISPR screens with single-cell chromatin accessibility profiling

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а	Pooled CR virus pr	RISPR library roduction	Pooled viral infection	Nuclei isolation	Tagmentatio	n	Reverse transcrip	tion
			Pla	asma horane visis C C C C C C C C C C C C C C C C C C	lei	posome SC1 Transfer 2,000 nuclei well-by-well	Pool all nuclei	go I
	U6 inner P7 prin BC3	gRNA 4 2nd PCR P5 prime	Amplify gRNA	ATAC P5 primer BC ATAC P BC ATAC Transfer well-by-well gRNA U6 outer primer Ist PCR RT oligo BC	7 primer Multiplex amplifi 23 ATAC fragments a 000000000000000000000000000000000000	Ration of Place Place 15-20 nuclei per well		•
b	gD	NA	AT	AC P5 primer		C1		
	tag PC (Se	gmented DNA CR1 amplicon equencing library)		↓ ↓	BC3 ATAC	P7 primer		
С	CF inte gei	ROP-Seq construct egrated into nomic DNA	-EF-1a	Puro WPRE	hU6 gRNA	Reverse transcriptase		
	m⊢ cD	NA		U6 outer primer	BC1 BC1 BC2 1st	^r oligo gRNA scaffold PCR primer		
	PC PC (Se	R 1 amplicon R 2 amplicon equencing library)	U6 inner i	2nd PCR	P5 primer			
d	ATAC PCR P5 primer	SCIATAC prin Index 2: 8 cyc P5 AATGATACGGCGACCA		2 PCR anneal on T5 oligo	Read 1: 45	cycles		
	ATAC PCR P7 primer	P7 CAAGCAGAAGACGGC	BC3 ATACGAGAT NNNNNN (Tagmentation oligo (PCR anneal on 17 oligo STCTCGTGGGGCTCGG Spacer on T7 STCTCGTGGGGCTCGG CTGTCCCTGTCC	Read 2: 4	5 cycles		
е		gRNA prime	r design and se	equencing strategy	Index 1: 4	3 cycles		
2nd F P5 pr	PCR P5 imer AATGAT	TACGGCGACCACCGAGAT	Read 1: 162 cy	Cles Diversity 2nn nucleotides on 1 GACGCTCTTCCGATCT NNNNNN CG 1st PCR primer CG	I PCR anneal st PCR primer ccgtccagctga ccgtccagctga NNNNNNN RT ollag	1st PCR anneal on RT oligo CCGGGAGCTGCATG CCGGGAGCTGCATG	T BC1 gRNA	RT oligo AAGTTGATAA
U6 P inner	7 · primer	P7 CAAGCAGAAGACGGCAT	BC3 21 ACGAGAT NNNNNNN TT	nd PCR anneal on U6 GTGGAAAGGACGAAACAC	U6 outer primer T	Ist PCR anneal on GCATATACGATCACA	U6 AGGCTGTTAG	

Supplementary Figure 1. CRISPR-sciATAC library preparation and sequencing. (a) Workflow for CRISPR-sciATAC. BC, barcode. Cell barcodes consist of a unique combination of

BC1, BC2, and BC3. (b) CRISPR-sciATAC schematic for ATAC-seq library preparation. (c) CRISPR-sciATAC schematic for guide RNA (gRNA) library preparation. (d) CRISPR-sciATAC primer design and sequencing strategy for ATAC fragments. (e) CRISPR-sciATAC primer design and library sequencing strategy for gRNA amplicons. Staggered P5 oligos were introduced in the library preparation to introduce sequence diversity. BC 1, 2, and 3 are matched for ATAC-seq and gRNA libraries, e.g. the ATAC-seq BC 1 in well A1 in the 96-well plate where tagmentation is performed is the same as the gRNA BC 1 in well A1 in the 96-well plate where reverse transcription is performed.