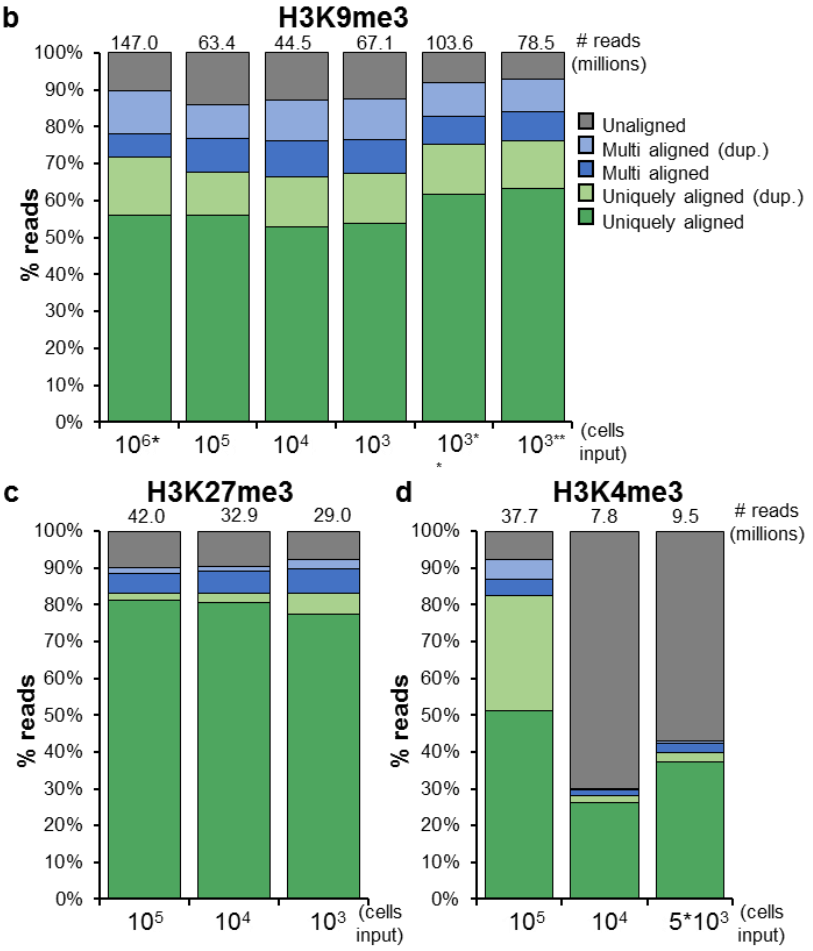
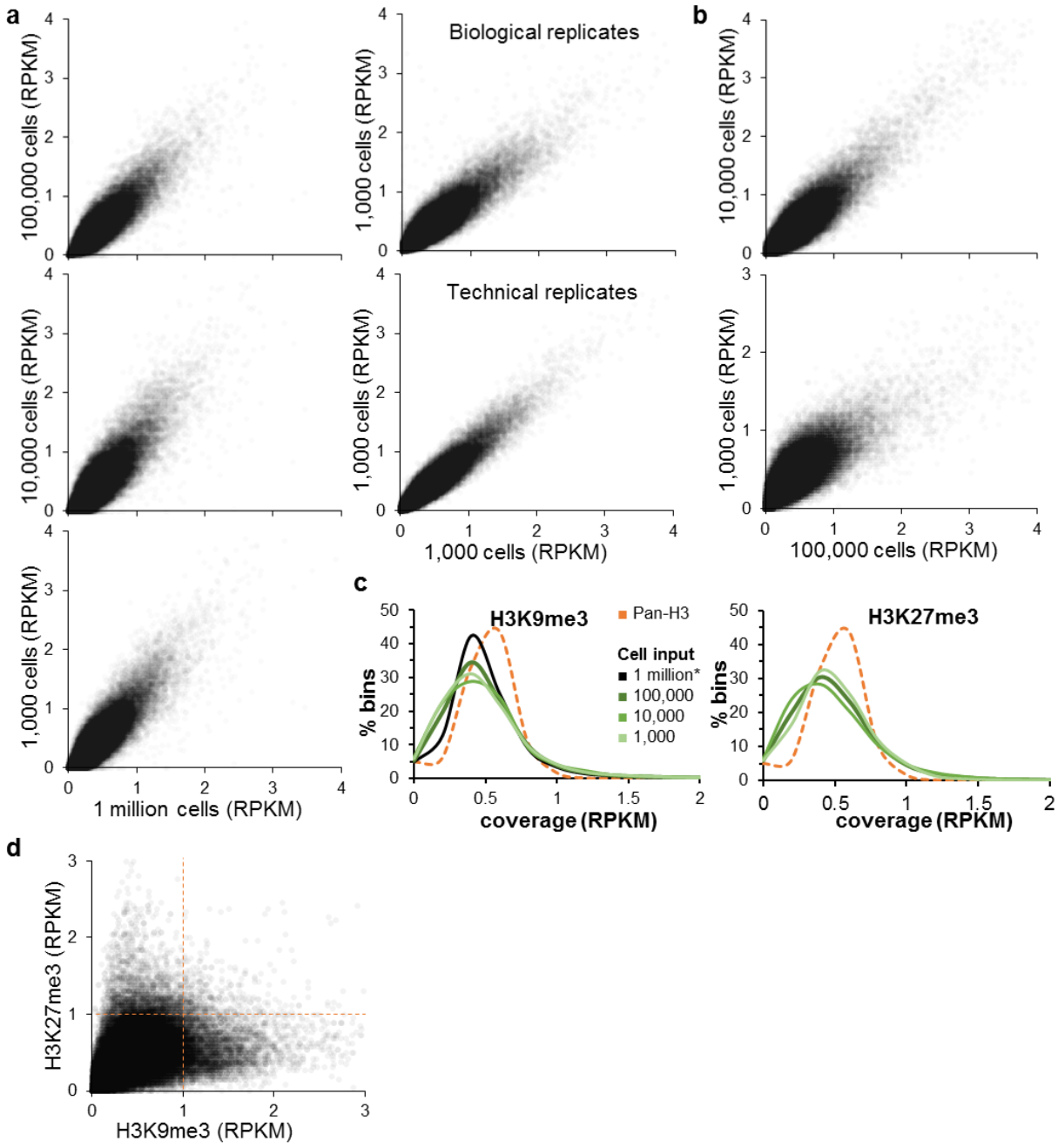
a

# cells	# PCR cycles (library const.)	Total library yield (ng)
H3K9me3		
10^6 (gold standard)	8	n/a *(not all ChIP material in library)*
10^5	8	**57.6**
10^4	10	**18.7**
10^3	10	**9.7**
10^3-2	10	**20.1**
10^3-3	10	**11.2**
H3K27me3		
10^5	8	**22.4**
10^4	10	**18.3**
10^3	10	**4.8**
pan-H3		
10^3-1	10	**20.5**
10^3-2	10	**18.3**
H3K4me3		
10^5	12	**8.4**
10^4	14	**2.2**
$5*10^3$	14	**5.4**



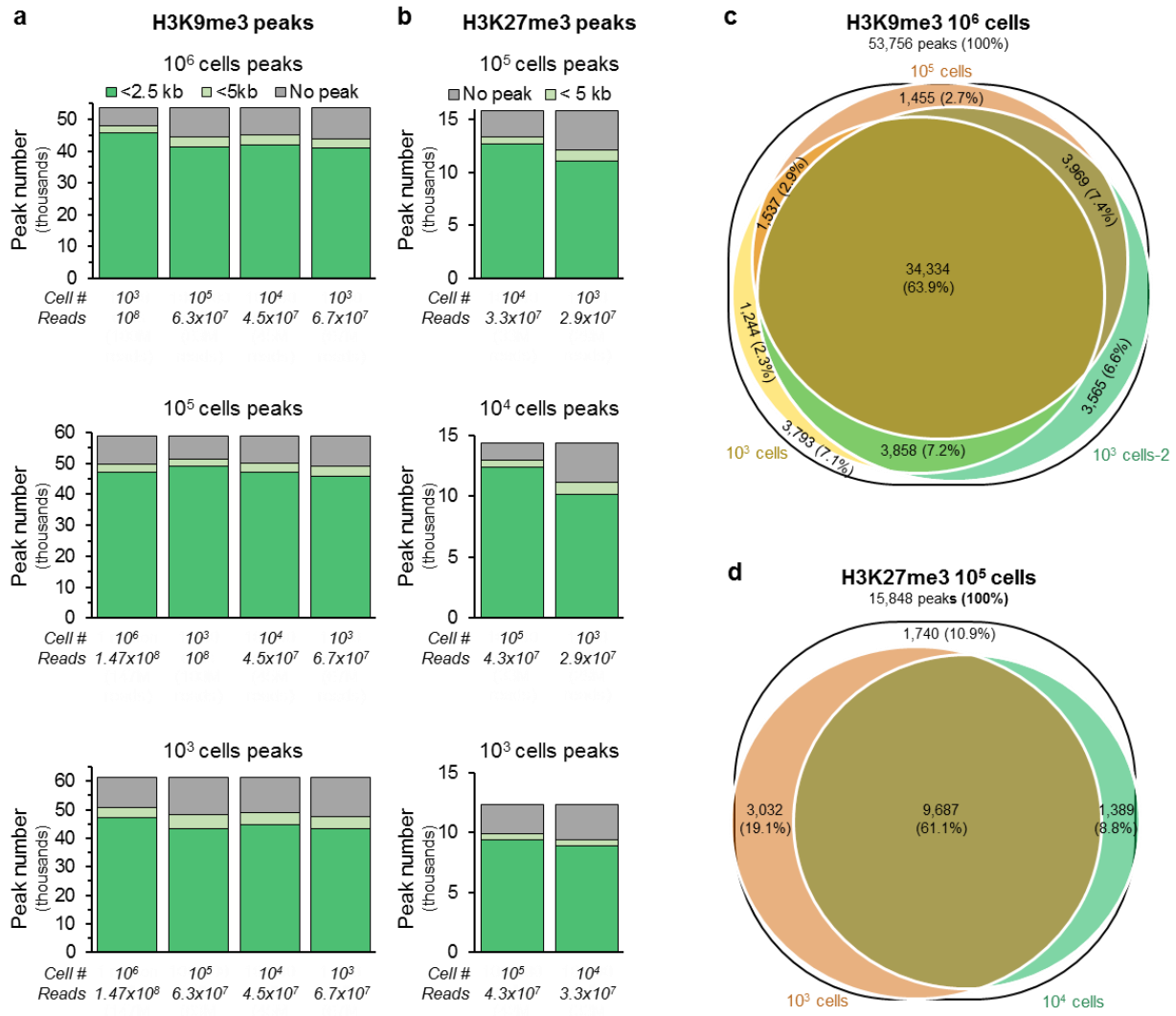
Supplementary Figure 1. Complexity ChIP-seq libraries prepared from 10^3 to 10^6 ESCs.

(a) Preparation of H3, H3K9me3, H3K27me3 and H3K4me3 NChIP-seq libraries from 10^3 to 10^6 cells. Comparison of library complexity in H3K9me3 **(b)** H3K27me3 **(c)** and H3K4me3 **(d)** NChIP-seq libraries prepared with 10^3 to 10^6 cells. The number of reads passing Illumina's chastity filter is presented on top of each stacked bar. Distinct (dark green) and duplicate (light green) uniquely aligned reads. Distinct (dark blue) and duplicate (light blue) multi-aligned reads (MapQ<5). Unaligned reads (gray).

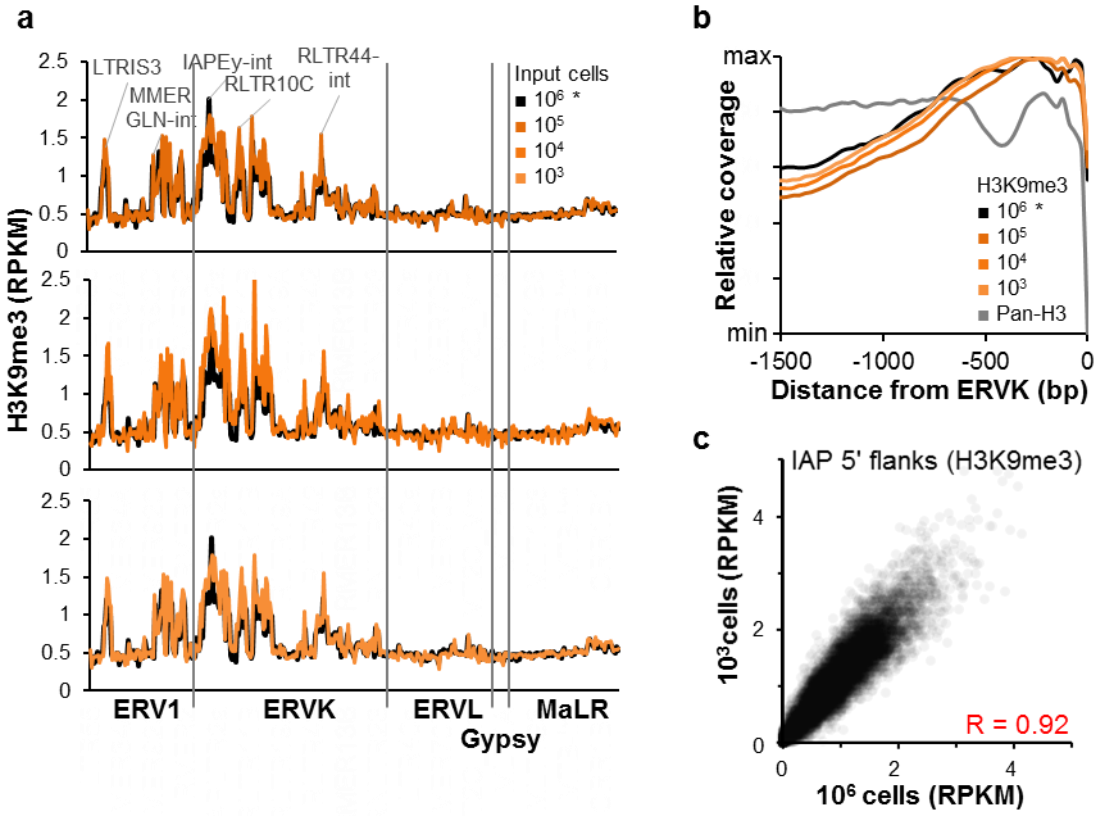


Supplementary Figure 2. Correlation between datasets generated from 10^3 to 10^6 ESCs.

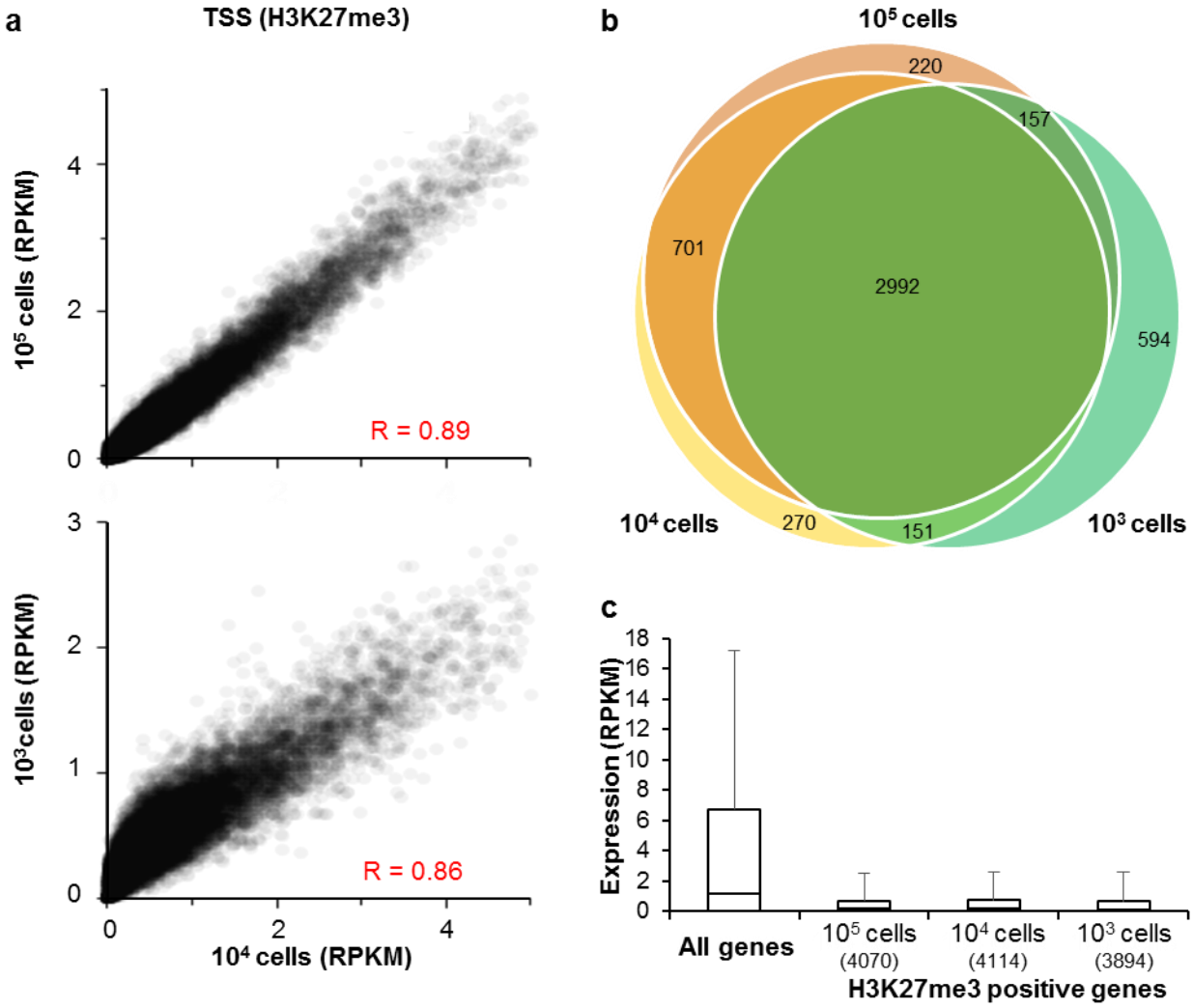
(a) Two-dimensional plots showing the genome-wide relationship (50,000 random 2kb bins) between H3K9me3 datasets generated from 10^3 to 10^6 cells as input material. **(b)** Two-dimensional plot showing the genome-wide relationship (50,000 random 2kb bins) between H3K27me3 datasets generated from 10^3 to 10^5 cells as input material. **(c)** Frequency plot illustrating the distribution of coverage density in genome-wide 2kb bins for H3K9me3 or H3K27me3 libraries. **(d)** Two dimensional plot showing the genome-wide relationship (50,000 random 2kb bins) of H3K9me3 and H3K27me3 datasets generated from 10^3 cells as input material.



Supplementary Figure 3. Sensitivity of H3K9me3 and H3K27me3 ultra-low-input NChIP-seq libraries. Stacked bar graphs showing **(a)** the proportion of H3K9me3 peaks detected in NChIP-seq libraries built from 10^6 (top), 10^5 (middle) and 10^3 (bottom) cells that overlap or are in close proximity (< 2.5 kb, dark green or < 5 kb, light green) to peaks detected in libraries built from 10^3 to 10^6 cells or **(b)** the proportion of H3K27me3 peaks detected in NChIP-seq libraries built from 10^5 (top), 10^4 (middle) and 10^3 (bottom) cells that overlap or are in close proximity (< 2.5 kb, dark green or < 5 kb, light green) to peaks detected in libraries built from 10^3 to 10^6 cells. **(c)** Venn diagram illustrating the proportion of H3K9me3 peaks detected in "gold standard" (10^6 cells) that overlap with peaks detected in ultra-low input libraries built from 10^3-10^5 cells. **(d)** Venn diagram illustrating the proportion of H3K27me3 peaks detected in the library prepared from 10^5 cells that are also detected in ultra-low input libraries prepared from 10^3 to 10^5 cells. Peaks were detected with MACS peak-calling software as described in Methods.

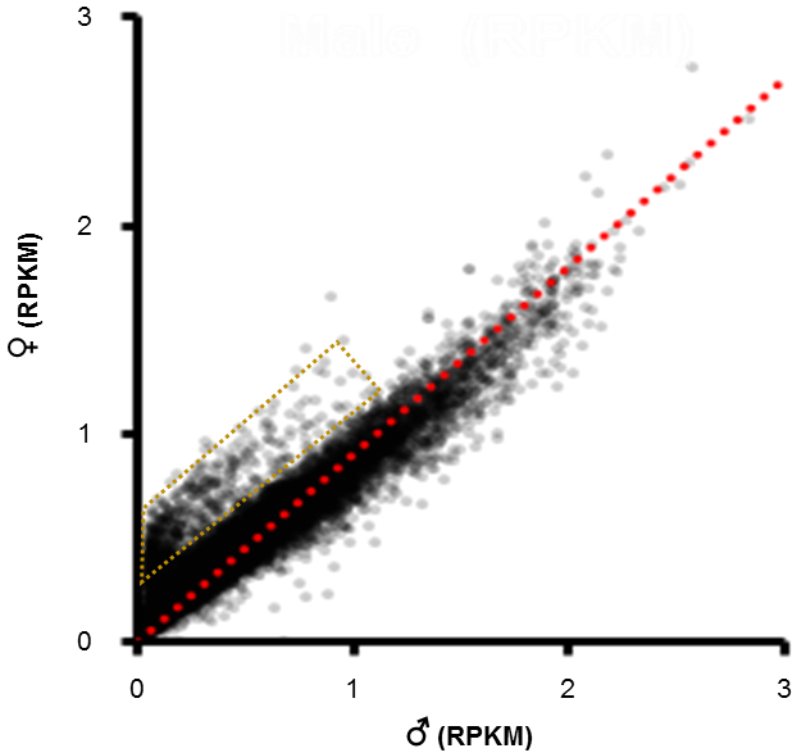


Supplementary figure 4. Correlation between H3K9me3 datasets generated from 10^3 to 10^6 mouse ESCs. (a) Relative H3K9me3 enrichment measured in RPKM at all annotated ERV subfamilies present at >100 copies in the BL6 genome in H3K9me3 NChIP-seq libraries built from 10^3 to 10^6 cells. ERVs classes are sorted alphabetically along the X- axis according to Repeatmasker annotation name within ERV1, ERVK, ERVL, Gypsy and MaLR. **(b)** Relative coverage (presented here as % of maximum coverage) of H3K9me3 libraries (built from 10^3 to 10^6 cells) or pan-H3 library (built from 10^3 cells) in the 5' flank (1.5 kb upstream) of all ERVKs present in the BL6 genome. **(c)** Correlation between H3K9me3 enrichment in the 5' flank (1kb upstream) of individual IAP elements in libraries built from 10^3 and 10^6 cells.

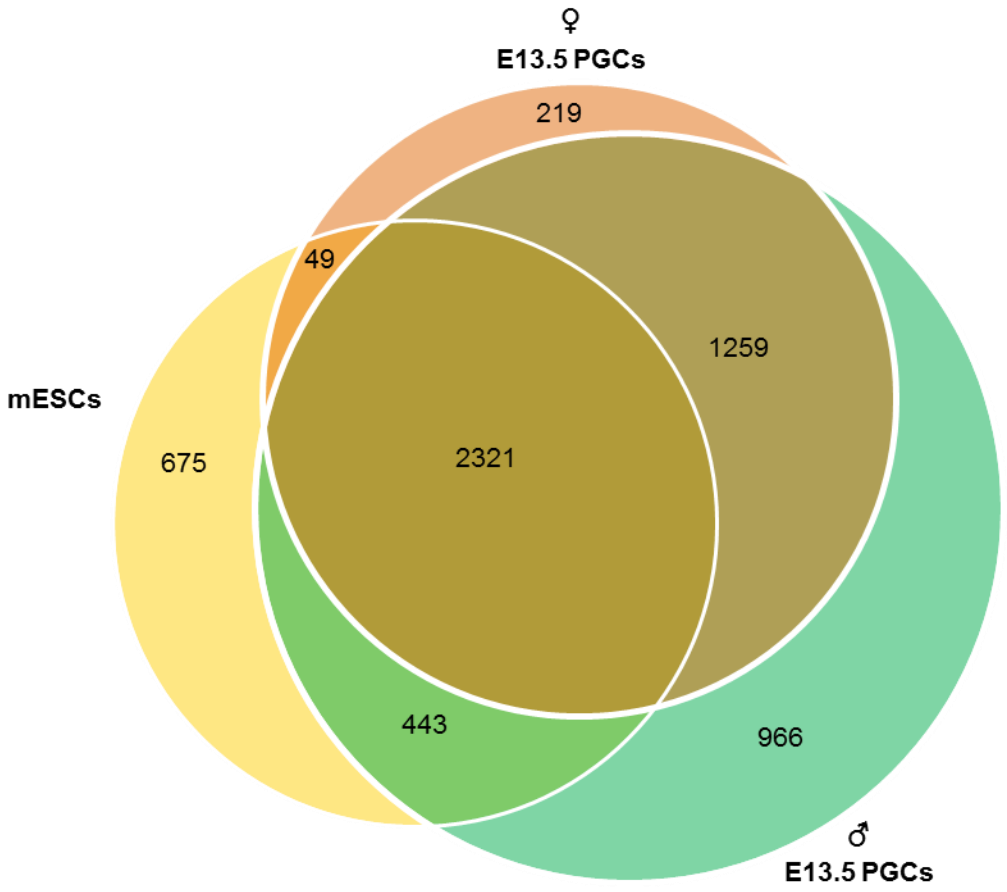


Supplementary figure 5. H3K27me3 enrichment at transcription start sites in mouse ES cells.

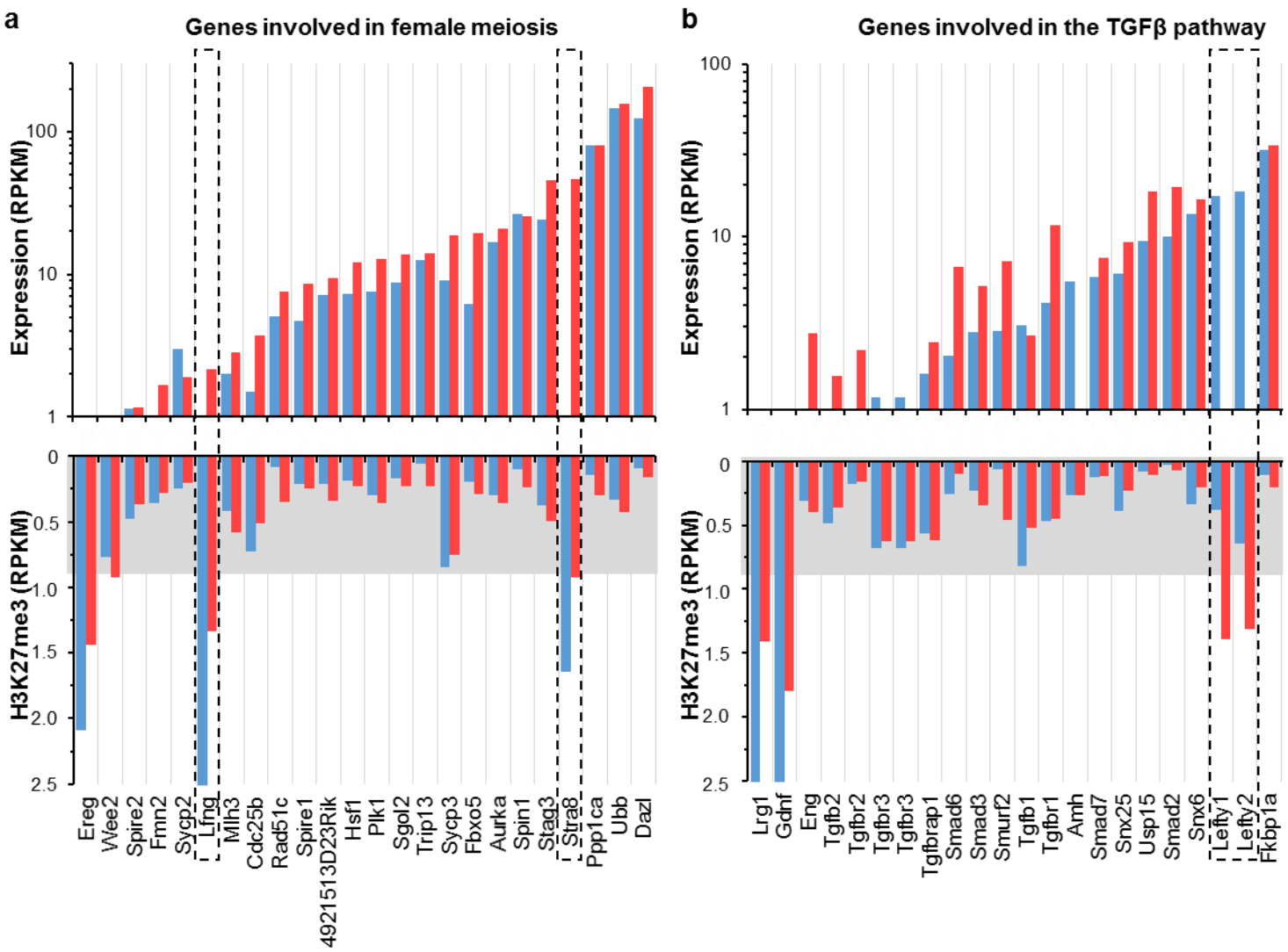
(a) Correlation between H3K27me3 enrichment at annotated TSSs (+/- 1kb) as measured by read coverage in libraries built from 10^3 to 10^5 cells. **(b)** Overlap of the top 15% of H3K27me3 marked genes (TSS +/- 1 kb) as measured by read coverage in libraries built from 10^3 to 10^5 cells. **(c)** Relationship between gene promoter (TSS +/- 1 kb) H3K27me3 signal and gene expression in NChIP-seq libraries prepared from 10^3 to 10^5 cells.



Supplementary figure 6. Correlation of H3K27me3 enrichment at gene promoters in E13.5 PGCs isolated from single male and female embryos. Two-dimensional plot shows H3K27me3 enrichment at gene promoters (TSS +/- 1 kb) measured as read coverage in data generated from 10^3 male versus female PGCs using our low input protocol. Of note, increased X-chromosome reads are observed in the female PGCs (yellow box).



Supplementary figure 7. H3K27me3-marked genes in mouse ES cells and in E13.5 PGCs.
Overlap of the H3K27me3 marked genes (TSS +/- 1 kb) in mouse ES cells, male and female E13.5 PGCs.



Supplementary figure 8. Sex-specific H3K27me3 gene silencing in E13.5 PGCs. Relationship between expression (top) of genes involved in meiosis **(a)** or the TGFβ receptor pathway **(b)** and H3K27me3 enrichment (bottom) in their promoter region (TSS +/- 1kb). Blue: male E13.5 PGCs, red: female E13.5 PGCs.

Supplementary Table 1: Genes expressed specifically in female E13.5 PGCs, marked by H3K27me3 in male E13.5 PGCs

name	ID	Expression M E13.5 PGC (RPKM)	Expression F E13.5 PGC (RPKM)	H3K27me3 M E13.5 PGC (RPKM)	H3K27me3 F E13.5 PGC	M/F H3K27me3 z-score	M/F Gene expression z-score
Lfng	NM_008494	0.74	2.60	2.57	1.34	0.62	-1.02
Repin1	NM_001079901	1.92	4.51	1.55	0.88	0.42	-1.02
Repin1	NM_001079902	1.93	4.52	1.55	0.88	0.42	-1.02
Igsf5	NM_001177887	0.55	2.27	2.21	1.41	0.41	-1.02
Pxmp4	NM_021534	1.32	3.60	0.75	0.32	0.41	-1.03
Repin1	NM_001079903	1.97	4.62	1.55	0.88	0.42	-1.03
Repin1	NM_001079904	1.97	4.62	1.55	0.88	0.42	-1.03
Pacsin3	NM_028733	0.84	2.83	1.51	0.97	0.34	-1.04
Nenf	NM_025424	1.99	4.69	1.52	0.83	0.44	-1.04
Tle3	NM_001083927	2.22	5.05	1.87	1.09	0.45	-1.05
Hapln1	NM_013500	0.82	2.83	1.09	0.62	0.35	-1.05
Tle3	NM_009389	2.24	5.08	1.87	1.09	0.45	-1.05
Repin1	NM_175099	2.04	4.79	1.55	0.88	0.42	-1.05
Repin1	NM_001079905	2.05	4.80	1.55	0.88	0.42	-1.05
Tle3	NM_001083928	2.25	5.10	1.87	1.09	0.45	-1.05
Krt7	NM_033073	1.39	3.80	1.86	0.88	0.59	-1.06
Nceh1	NM_178772	1.52	4.00	0.79	0.48	0.27	-1.06
Slc28a1	NM_001004184	0.59	2.43	1.05	0.66	0.30	-1.06
Ppil6	NM_028430	0.51	2.27	1.04	0.56	0.37	-1.06
Ahnak	NM_009643	0.85	2.90	1.69	1.01	0.41	-1.06
Nynrin	NM_001040072	0.59	2.45	1.22	0.55	0.50	-1.06
Dtx3	NM_030714	1.28	3.64	1.65	1.01	0.39	-1.07
Mme	NM_008604	0.36	2.01	1.31	0.78	0.37	-1.07
Pitpnm1	NM_001136078	0.41	2.15	1.49	0.91	0.37	-1.09
B3gnt5	NM_001159407	0.55	2.43	0.89	0.47	0.36	-1.09
Lrig3	NM_177152	2.04	4.92	1.20	0.77	0.30	-1.09
Ptprr	NM_011217	0.36	2.08	1.47	0.97	0.32	-1.10
B3gnt5	NM_001159408	0.57	2.54	0.89	0.47	0.36	-1.11
Leprel2	NM_013534	0.52	2.44	1.19	0.45	0.57	-1.11
Igsf5	NM_028078	0.66	2.71	2.22	1.41	0.42	-1.12
Src	NM_009271	1.27	3.79	1.54	0.68	0.57	-1.12
Src	NM_001025395	1.28	3.81	1.54	0.68	0.57	-1.12
B3gnt5	NM_054052	0.59	2.60	0.89	0.47	0.36	-1.13
Ppp1r9b	NM_172261	1.90	4.82	0.77	0.38	0.36	-1.13
Mir671	NR_030423	0.70	2.82	2.17	1.04	0.62	-1.13
Neurl1b	NM_001081656	0.31	2.05	1.61	0.81	0.50	-1.13
Cspg5	NM_001166273	0.58	2.62	1.04	0.67	0.28	-1.14
Cspg5	NM_013884	0.59	2.68	1.04	0.67	0.28	-1.15
Slc35f3	NM_175434	0.72	2.94	1.18	0.59	0.44	-1.16

Aldoc	NM_009657	0.78	3.07	1.25	0.74	0.35	-1.17
Pacsin1	NM_011861	0.44	2.42	1.89	1.14	0.43	-1.17
Chst11	NM_021439	1.16	3.78	1.86	1.22	0.36	-1.17
Zfp703	NM_001110508	0.24	2.01	1.51	0.83	0.44	-1.18
Ctdspl	NM_133710	0.40	2.37	0.78	0.50	0.25	-1.18
Pqlc1	NM_025861	1.03	3.57	1.32	0.80	0.35	-1.18
D8Ertd82e	NM_172911	0.35	2.28	1.23	0.45	0.59	-1.19
Necab2	NM_054095	0.59	2.79	1.61	0.99	0.37	-1.19
Negr1	NM_177274	1.95	5.14	1.51	0.78	0.47	-1.20
Slc16a3	NM_001038653	0.86	3.31	1.50	0.94	0.35	-1.20
Pqlc1	NM_001164421	1.06	3.66	1.32	0.80	0.35	-1.20
Pqlc1	NM_001164422	1.06	3.68	1.28	0.77	0.35	-1.20
Zdhhc14	NM_146073	2.37	5.82	1.17	0.75	0.30	-1.20
Zfp703	NM_001101502	0.25	2.10	1.51	0.83	0.44	-1.21
Slc16a3	NM_001038654	0.90	3.45	1.50	0.94	0.35	-1.22
Fam69b	NM_019833	1.60	4.68	1.48	0.93	0.35	-1.23
Lgi2	NM_144945	0.67	3.06	1.13	0.71	0.30	-1.24
Hunk	NM_015755	0.81	3.33	1.88	1.21	0.38	-1.24
Cacna2d2	NM_001174047	0.91	3.56	1.61	1.05	0.34	-1.25
Cacna2d2	NM_020263	0.91	3.56	1.61	1.05	0.34	-1.25
3-Sep	NM_011889	0.39	2.54	2.27	1.24	0.54	-1.26
Cacna2d2	NM_001174048	0.91	3.57	1.61	1.05	0.34	-1.26
Igsf5	NM_001177886	0.90	3.56	2.22	1.41	0.42	-1.26
Cacna2d2	NM_001174050	0.91	3.57	1.61	1.05	0.34	-1.26
Cacna2d2	NM_001174049	0.91	3.58	1.61	1.05	0.34	-1.26
6330403K07Rik	NM_134022	1.22	4.16	1.08	0.55	0.41	-1.27
Edn3	NM_007903	0.20	2.15	1.26	0.65	0.44	-1.27
F2rl1	NM_007974	0.32	2.42	1.05	0.68	0.28	-1.27
Tshz3	NM_172298	0.75	3.34	0.88	0.51	0.31	-1.28
Dmrta2	NM_172296	0.57	2.99	2.77	1.67	0.52	-1.28
Loxl2	NM_033325	1.44	4.62	2.51	1.56	0.46	-1.29
Grik3	NM_001081097	0.35	2.58	2.44	1.60	0.41	-1.30
Lbp	NM_008489	0.32	2.50	0.91	0.53	0.31	-1.30
Wnt4	NM_009523	0.82	3.53	1.38	0.82	0.38	-1.30
F3	NM_010171	0.39	2.68	2.34	1.48	0.43	-1.30
Cpne5	NM_153166	4.37	9.16	0.81	0.48	0.29	-1.30
Gata3	NM_008091	0.11	2.02	0.87	0.50	0.31	-1.31
2610203C20Rik	NR_015483	0.92	3.78	1.08	0.50	0.45	-1.32
Kif21b	NM_001039472	0.57	3.11	0.92	0.49	0.36	-1.32
Cmtm3	NM_024217	1.17	4.26	1.10	0.63	0.36	-1.32
Arhgdig	NM_008113	5.01	10.18	0.92	0.60	0.26	-1.33
Galntl4	NM_173739	0.49	2.95	1.09	0.63	0.34	-1.33
Ccdc8	NM_001101535	1.57	4.97	0.79	0.49	0.26	-1.33
Pde2a	NR_026574	0.40	2.78	1.13	0.57	0.43	-1.33

Pde2a	NM_001143849	0.40	2.79	1.06	0.51	0.44	-1.33
Limk1	NM_010717	3.44	7.95	0.89	0.53	0.30	-1.34
Smarcd3	NM_025891	1.04	4.07	2.74	1.48	0.60	-1.34
Odz4	NM_011858	1.52	4.95	2.44	1.44	0.50	-1.35
Nrcam	NM_176930	0.26	2.52	1.26	0.61	0.47	-1.35
BC051142	NM_001001177	0.06	2.03	0.88	0.50	0.32	-1.36
Lrp3	NM_001024707	0.47	3.02	1.86	1.18	0.38	-1.37
Nrcam	NM_001146031	0.27	2.59	1.26	0.61	0.47	-1.37
Ak4	NM_009647	0.71	3.53	0.96	0.60	0.28	-1.37
Hnf1b	NM_009330	0.64	3.42	2.76	1.71	0.49	-1.38
Gpr153	NM_178406	0.46	3.03	0.90	0.56	0.28	-1.38
Esyt1	NM_011843	0.41	2.94	1.80	1.17	0.35	-1.39
Olfm1	NM_001038612	0.16	2.39	1.30	0.80	0.34	-1.39
Nr2f2	NM_009697	0.64	3.49	1.42	0.93	0.32	-1.40
Obsl1	NM_178884	1.99	5.99	2.38	1.54	0.42	-1.41
Tnfrsf12a	NM_013749	0.75	3.79	0.76	0.42	0.30	-1.42
9530026P05Rik	NR_015530	2.54	6.91	1.88	0.88	0.59	-1.42
Hoxb8	NM_010461	0.23	2.63	2.58	1.49	0.53	-1.42
4931408A02Rik	NM_001199210	0.20	2.58	1.57	0.78	0.51	-1.43
Olfm1	NM_019498	0.26	2.73	1.30	0.80	0.34	-1.43
Hoxc5	NM_175730	0.09	2.32	1.60	0.81	0.50	-1.43
Smpd2	NM_009213	2.90	7.53	0.81	0.47	0.30	-1.43
Col4a2	NM_009932	1.00	4.33	2.66	1.64	0.49	-1.44
Slc25a24	NM_172685	0.76	3.88	0.86	0.38	0.43	-1.45
Tbx2	NM_009324	0.95	4.26	1.94	1.11	0.47	-1.45
Dlk1	NR_033813	1.20	4.77	1.28	0.80	0.33	-1.46
Gjb5	NM_010291	0.11	2.46	0.89	0.55	0.28	-1.46
Sh3pxd2b	NM_177364	0.88	4.18	1.18	0.71	0.34	-1.47
Sepn1	NM_029100	0.31	2.99	1.22	0.74	0.34	-1.47
4931408A02Rik	NM_027627	0.21	2.77	1.57	0.78	0.51	-1.48
Nr2f2	NM_183261	0.77	4.05	1.42	0.93	0.32	-1.49
BC051142	NM_001163855	0.07	2.45	0.88	0.50	0.32	-1.49
Dpysl5	NM_023047	0.93	4.37	1.30	0.75	0.38	-1.50
Tnfrsf12a	NM_001161746	0.85	4.25	0.76	0.42	0.30	-1.51
Limch1	NM_001001980	0.77	4.09	1.48	0.96	0.33	-1.51
Bok	NM_016778	1.76	5.97	1.79	1.15	0.37	-1.51
5-Sep	NM_213614	2.11	6.60	2.25	1.40	0.44	-1.52
Wnt6	NM_009526	1.80	6.09	2.05	1.27	0.42	-1.53
Scarf2	NM_153790	0.88	4.42	2.18	1.09	0.60	-1.54
Chsy3	NM_001081328	0.25	3.08	1.42	0.82	0.39	-1.55
Tbc1d1	NM_019636	6.72	13.82	2.05	1.30	0.41	-1.57
Adcy9	NM_009624	4.81	11.07	0.78	0.46	0.29	-1.57
Dmrtc2	NM_027732	0.19	3.01	1.79	1.06	0.42	-1.58
Efs	NM_010112	0.67	4.13	1.40	0.89	0.33	-1.58

Nbl1	NM_008675	0.44	3.62	1.52	0.84	0.44	-1.58
Irx3	NM_008393	0.08	2.72	2.26	1.49	0.39	-1.58
Hoxb6	NM_008269	0.55	3.92	3.33	2.09	0.52	-1.59
Phlda3	NM_013750	2.06	6.82	1.91	1.17	0.42	-1.60
Gns	NM_029364	4.42	10.67	0.92	0.39	0.46	-1.61
Zbed3	NM_028106	0.81	4.55	0.90	0.54	0.29	-1.61
Pacsin1	NM_178365	1.00	4.96	1.24	0.74	0.35	-1.62
Vill	NM_001164567	0.57	4.09	1.51	0.83	0.44	-1.63
Emp1	NM_010128	0.62	4.21	1.95	1.27	0.37	-1.63
Kcnip3	NM_001111331	0.11	2.99	1.75	1.01	0.44	-1.64
Pde4a	NM_183408	0.74	4.48	1.48	0.81	0.44	-1.64
Agpat3	NM_053014	2.46	7.73	0.89	0.54	0.29	-1.65
Hoxb7	NM_010460	0.31	3.61	3.04	1.95	0.48	-1.67
Zbtb7c	NM_145356	0.64	4.40	0.77	0.44	0.30	-1.67
Ttyh3	NM_175274	2.98	8.79	0.98	0.55	0.34	-1.69
Smo	NM_176996	2.46	7.92	1.30	0.86	0.30	-1.69
Kcnip3	NM_019789	0.14	3.26	1.75	1.01	0.44	-1.70
Ptprf	NM_011213	7.94	16.30	0.83	0.29	0.50	-1.70
Bmp1	NR_033241	0.60	4.44	1.27	0.82	0.31	-1.71
Vill	NM_011700	0.62	4.48	1.51	0.83	0.44	-1.71
Meis2	NM_001159569	1.77	6.76	2.19	1.23	0.51	-1.71
Nkd1	NM_027280	1.59	6.44	1.01	0.58	0.34	-1.71
Meis2	NM_001159570	1.78	6.79	2.19	1.23	0.51	-1.71
Tle6	NM_053254	0.18	3.46	1.68	1.07	0.36	-1.72
Lamb2	NM_008483	0.65	4.60	0.90	0.53	0.31	-1.72
Arhgdib	NM_007486	0.35	4.07	1.15	0.69	0.33	-1.77
Meis2	NM_001159567	1.90	7.27	2.19	1.23	0.51	-1.77
Meis2	NM_001136072	1.91	7.31	2.19	1.23	0.51	-1.78
Gxylt2	NM_198612	0.72	4.97	1.20	0.58	0.46	-1.78
Meis2	NM_001159568	1.94	7.42	2.19	1.23	0.51	-1.79
Palm	NM_023128	3.11	9.47	0.77	0.49	0.25	-1.79
Cdh6	NM_007666	0.84	5.27	0.90	0.52	0.31	-1.79
Gm1673	NM_001033458	5.64	13.48	1.64	0.91	0.45	-1.79
Meis2	NM_010825	1.95	7.46	2.19	1.23	0.51	-1.80
Kcnq3	NM_152923	4.03	11.14	0.93	0.36	0.49	-1.83
Fosl2	NM_008037	1.35	6.48	1.15	0.59	0.42	-1.83
Palm	NM_001161747	3.28	9.97	0.77	0.49	0.25	-1.84
Zfp503	NM_145459	0.53	4.79	2.04	1.08	0.54	-1.85
Scn1b	NM_011322	0.19	3.95	1.29	0.83	0.31	-1.85
Spock2	NM_052994	1.21	6.28	1.34	0.83	0.34	-1.85
Gaa	NM_008064	2.75	9.14	0.97	0.49	0.39	-1.85
Bmp1	NM_009755	0.72	5.26	1.27	0.82	0.31	-1.86
Rtn4rl1	NM_177708	0.49	4.75	1.62	0.98	0.39	-1.86
Tubb4	NM_009451	0.17	3.98	0.84	0.49	0.31	-1.87

Parva	NM_020606	0.90	5.72	1.72	1.03	0.41	-1.87
Grb7	NM_010346	0.60	5.05	1.82	1.12	0.41	-1.87
Gaa	NM_001159324	2.81	9.36	0.96	0.48	0.39	-1.88
Mapre2	NM_001162942	2.65	9.18	0.81	0.48	0.28	-1.90
Fgf9	NM_013518	0.40	4.69	1.82	1.20	0.35	-1.90
P4ha2	NM_011031	1.78	7.73	1.78	0.90	0.53	-1.93
P4ha2	NM_001136076	1.78	7.75	1.78	0.90	0.53	-1.93
Pxdn	NM_181395	4.46	12.39	1.70	1.10	0.35	-1.93
Grina	NM_023168	1.76	7.79	0.87	0.53	0.28	-1.95
Itga3	NM_013565	0.72	5.74	1.66	0.64	0.67	-1.98
Perp	NM_022032	0.38	4.93	1.21	0.69	0.37	-1.98
Runx1	NM_001111023	0.63	5.60	0.96	0.55	0.33	-1.99
Anxa2	NM_007585	9.48	20.47	1.93	1.02	0.52	-2.01
Runx1	NM_009821	0.65	5.75	0.96	0.55	0.33	-2.02
Cldn4	NM_009903	1.80	8.36	1.62	0.96	0.40	-2.06
Pwwp2b	NM_001098636	0.35	5.24	2.32	0.87	0.80	-2.07
Arl8a	NM_026823	1.19	7.28	1.11	0.56	0.43	-2.09
Cd82	NM_007656	0.90	6.65	1.36	0.78	0.39	-2.09
Emx2	NM_010132	0.60	5.97	2.47	1.54	0.46	-2.10
Rbp1	NM_011254	0.33	5.31	0.79	0.50	0.25	-2.10
Lhfp	NM_175386	5.06	14.31	1.85	0.93	0.55	-2.10
Slc22a18	NM_001042760	0.26	5.17	1.23	0.61	0.45	-2.11
Mt2	NM_008630	0.90	6.83	1.94	1.24	0.39	-2.13
Rhbdl3	NM_139228	0.71	6.52	0.94	0.57	0.29	-2.16
Cd82	NM_001136055	0.97	7.18	1.37	0.78	0.39	-2.17
Scd1	NM_009127	0.18	5.27	0.84	0.41	0.38	-2.18
Cnn2	NM_007725	2.50	10.37	0.77	0.41	0.33	-2.19
Rims4	NM_183023	1.01	7.37	1.80	1.08	0.42	-2.20
Runx1	NM_001111021	1.01	7.38	0.96	0.55	0.33	-2.20
Pwwp2b	NM_001033206	0.39	5.93	2.32	0.87	0.80	-2.20
Cd81	NM_133655	10.99	24.21	0.83	0.48	0.30	-2.23
Runx1	NM_001111022	1.04	7.63	0.96	0.55	0.33	-2.24
Mical1	NM_138315	0.81	7.14	1.20	0.49	0.54	-2.24
Fam57a	NM_027773	0.54	6.59	0.89	0.56	0.26	-2.26
Amotl2	NM_019764	1.30	8.46	1.83	0.92	0.54	-2.29
Mical1	NM_001164433	0.87	7.61	1.20	0.49	0.54	-2.32
Cacng4	NM_019431	5.43	16.25	1.44	0.72	0.48	-2.32
Chd3	NM_146019	1.90	10.20	1.10	0.45	0.52	-2.38
H1f0	NM_008197	3.20	13.18	1.20	0.46	0.57	-2.47
Arl4c	NM_177305	1.77	10.41	0.89	0.55	0.28	-2.48
Plk2	NM_152804	0.94	8.74	1.14	0.66	0.36	-2.51
Gsn	NM_001206369	2.36	11.97	1.27	0.75	0.36	-2.54
Rnpep	NM_145417	0.50	7.91	0.84	0.29	0.51	-2.55
Ptprs	NM_011218	8.51	23.26	1.29	0.54	0.55	-2.62

Rnpep	NM_001159624	0.53	8.33	0.84	0.29	0.51	-2.62
Sct	NM_011328	0.07	7.19	1.40	0.72	0.46	-2.64
Cldn11	NM_008770	0.88	9.82	1.66	1.07	0.35	-2.73
Epb4.1	NM_183428	7.22	21.99	2.29	1.36	0.48	-2.73
Aif1l	NM_145144	0.45	9.36	1.10	0.52	0.45	-2.84
Msx2	NM_013601	0.20	9.28	0.98	0.61	0.29	-2.95
Fstl1	NM_008047	3.44	17.13	0.97	0.57	0.31	-3.02
Epb4.1	NM_001128606	9.14	27.42	2.29	1.36	0.48	-3.02
Tead2	NM_011565	1.60	13.44	1.86	0.61	0.79	-3.05
Epb4.1	NM_001128607	9.42	28.70	2.29	1.36	0.48	-3.12
Igfbp5	NM_010518	3.33	17.96	0.91	0.54	0.30	-3.17
Cldn6	NM_018777	1.77	15.70	2.65	1.64	0.48	-3.33
Cdx2	NM_007673	0.04	11.89	2.36	1.29	0.55	-3.43
Krt18	NM_010664	3.54	20.71	1.90	1.07	0.47	-3.49
Gm1564	NM_001127576	1.03	15.28	2.55	1.56	0.48	-3.53
Gpc6	NM_001079844	3.44	20.96	1.40	0.64	0.53	-3.54
Gpc6	NM_011821	3.46	21.05	1.40	0.64	0.53	-3.55
Dsp	NM_023842	0.62	16.10	0.88	0.41	0.41	-3.79
Wfdc2	NM_026323	1.31	18.96	1.09	0.64	0.34	-3.92
Met	NM_008591	0.75	17.97	1.24	0.63	0.44	-3.98
Plagl1	NM_009538	0.78	19.49	1.85	1.04	0.47	-4.15
Bcam	NM_020486	3.23	26.96	1.81	0.94	0.52	-4.32
Ahnak	NM_001039959	14.84	50.50	1.69	1.01	0.41	-4.41
Gata2	NM_008090	0.45	20.94	2.56	0.92	0.87	-4.43
Igfbp4	NM_010517	3.39	29.34	2.15	1.22	0.50	-4.54
Igfbp2	NM_008342	9.11	48.09	1.34	0.82	0.35	-5.15
0610010O12Rik	NM_001081365	8.34	47.33	0.97	0.51	0.37	-5.23
Tsc22d1	NM_001177751	23.47	76.28	2.27	1.17	0.58	-5.29
Vim	NM_011701	4.64	40.87	1.08	0.60	0.36	-5.37
Fscn1	NM_007984	4.38	42.99	1.78	0.82	0.58	-5.61
Id1	NM_010495	2.44	44.54	1.96	0.35	1.05	-6.14
Mdk	NM_010784	7.53	74.99	1.84	0.67	0.73	-7.43
Gnas	NM_201616	13.99	90.25	0.91	0.36	0.49	-7.47
Gnas	NM_001077510	14.33	92.47	0.91	0.36	0.49	-7.56
Stra8	NM_009292	0.78	61.60	1.65	0.92	0.45	-7.70
Mdk	NM_001012335	8.96	88.62	1.84	0.67	0.73	-8.06
Gnas	NR_003258	16.82	108.52	2.80	1.61	0.56	-8.19
Mdk	NM_001012336	9.88	97.99	1.84	0.67	0.73	-8.48
Phlda2	NM_009434	0.70	82.93	2.15	0.78	0.79	-8.99

Supplementary Table 2: Genes expressed specifically in male E13.5 PGCs, marked by H3K27me3 in female E13.5 PGCs

name	ID	Expression M E13.5 PGC (RPKM)	Expression F E13.5 PGC (RPKM)	H3K27me3 M E13.5 PGC (RPKM)	H3K27me3 F E13.5 PGC	M/F H3K27me3 z-score	M/F Gene expression z-score
Gm364	NM_001128625	1.69	0.41	0.64	1.05	-0.31	0.88
Fam3b	NM_020622	2.35	0.77	0.42	0.64	-0.21	0.89
Zcchc12	NM_028325	1.52	0.28	0.64	0.88	-0.20	0.92
Nphs1as	NR_004443	1.87	0.35	0.70	0.95	-0.19	1.01
Ppp2r2b	NM_027531	6.43	3.05	0.28	0.73	-0.44	1.10
Pcp4l1	NM_025557	1.54	0.09	0.75	0.99	-0.18	1.13
Ccr7	NM_007719	1.62	0.08	0.63	1.04	-0.31	1.18
Olig1	NM_016968	4.32	1.41	0.92	1.31	-0.25	1.22
Xkrx	NM_183319	1.91	0.15	0.20	1.11	-0.78	1.22
Nphs1	NM_019459	3.24	0.61	0.59	0.83	-0.20	1.34
Zmym3	NM_001177985	14.99	7.14	0.24	1.05	-0.69	1.67
Usp26	NM_031388	12.41	5.34	0.16	0.72	-0.57	1.68
Tmem63a	NM_144794	5.09	0.72	0.64	1.87	-0.75	1.81
Nodal	NM_013611	5.33	0.79	0.43	0.69	-0.24	1.84
B930007M17Rik	NM_198659	13.53	5.39	0.40	0.68	-0.26	1.87
Otx2	NM_144841	4.09	0.06	0.64	0.91	-0.21	1.98
Cpt1a	NM_013495	13.31	4.81	0.72	1.08	-0.26	2.00
Bcar3	NM_013867	14.30	5.04	0.72	1.25	-0.36	2.11
Pgpep1l	NM_030101	7.03	0.72	0.45	0.68	-0.21	2.27
Gpr56	NM_001198894	11.54	2.73	0.54	0.80	-0.22	2.33
Bcl11a	NM_001159290	16.23	4.68	1.03	1.37	-0.22	2.53
Pitx2	NM_001042502	6.89	0.09	0.99	1.66	-0.40	2.57
Ppp2r2b	NM_028392	21.76	7.12	0.28	0.73	-0.43	2.72
2410004A20Rik	NM_025890	9.10	0.42	0.60	1.05	-0.34	2.81
Hesx1	NM_010420	10.41	0.04	0.41	0.74	-0.30	3.21
Lefty1	NM_010094	18.78	0.05	0.38	1.39	-0.74	4.31
Lefty2	NM_177099	19.30	0.10	0.64	1.31	-0.47	4.36

Supplementary Methods: Step-by-step ULI NChIP procedure

1. CHROMATIN PREPARATION

a. Sort cells by flow cytometry directly in a 1.5 ml sample tube.

	1,000-5,000 cells (µl)	**5,000-20,000 (µl)**	**>20,000 (µl)**
Nuclear isolation buffer (Sigma NUC-101)	10	20	-
PBS	-	-	20-50
	Flash freeze. Keep at -80°C.	Spin down and reduce volume to 10 µl. Flash freeze. Keep at -80°C.	Spin down, remove PBS and flash freeze pellet. Keep at -80°C.

- *Alternatively, the cells can be aliquoted in 1.5 ml sample tubes. Add nuclear isolation buffer as per indicated in the above table. If very few cells are being aliquoted, a small amount of sample buffer can be left behind, as long as it represents less than 1/3 of the nuclear isolation buffer volume. If working with <20,000 cells and the volume of sheath buffer exceeds 1/3rd of the sheath + nuclear isolation buffer volume, add nuclear isolation buffer prior to flash freezing.*

◊ At this stage, samples can be stored at -80°C for a few weeks to a few months.

b. Thaw frozen cell pellet or, for lower cell numbers, cells in nuclear isolation buffer. *If cells frozen as a pellet, re-suspend in appropriate volume of nuclear isolation buffer.*

c. If working with < 10,000 cells (MNase digestion with PEG 6,000), add 10% of the volume of a 1% Triton/1% deoxycholate solution.

d. Pipette samples up and down 15-20 times while swirling and place back on ice.

e. Prepare dilute MNAse stock enzyme in MNAse dilution buffer to 200 U/µl (0.5 µl stock in 4.5 µl dilution buffer). Add MNase enzyme only when you are ready to proceed to digestion. Prepare MNAse digestion buffer as follows* (always prepare just before digestion):

	1,000,000 cells (µl)	**100,000 cells (µl)**	**5,000-10,000 cells (µl)**	**1,000 cells (µl)**
Nuclear isolation buffer (+ sheath)	50	20	~20	~10
1% Triton, 1% DOC sol.	-	-	2	1
MNase Master Mix				
10 X MNase Buffer (NEB)	6	4	10	5
200 mM DTT	0.44	0.37	0.75	0.5
50% PEG 6000 (Sigma 81304)	-	-	10	5
1:10 MNase enzyme (NEB M0247)	0.6	0.4	1	0.5
Ultrapure H_20	2.96	15.23	58.76	29.38
Final MNase Concentration	$2U*µl^{-1}$	$2U*µl^{-1}$	$2U*µl^{-1}$	$2U*µl^{-1}$
Digestion	**7.5 minutes @ 37°C**	**5 minutes @ 37°C**	**7.5 minutes @ 21°C**	**7.5 minutes @ 21°C**
100 mM EDTA	6.6	4.4	11	5.5
1% Triton, 1% DOC sol.	6.6	4.4	8	4
Vortex, 30 seconds				

** MNase is very sensitive to temperature changes, and will lose efficiency with time. Small aliquots of MNase should be prepared and transported to the bench no more than 2-3 times for consistent results. It is recommended to test each new batch of MNase with low numbers of cultured cells prior to use for in vivo samples.*

f. Place samples on rack at room temperature.

g. Add MNAse Master Mix (+MNAse) to each sample. Mix very well (15-20 times) with pipettor.

h. Allow reaction to proceed according to the conditions suggested in the previous table.

i. Stop reaction by adding 10% of the reaction volume of 100 uM EDTA and mix very well with pipette (20-30 times).

j. Add 1% Triton/1% deoxycholate solution as per indicated in the previous table.

k. Rest on ice for >15 minutes.

l. Vortex samples (medium setting) for ~ 30 seconds.

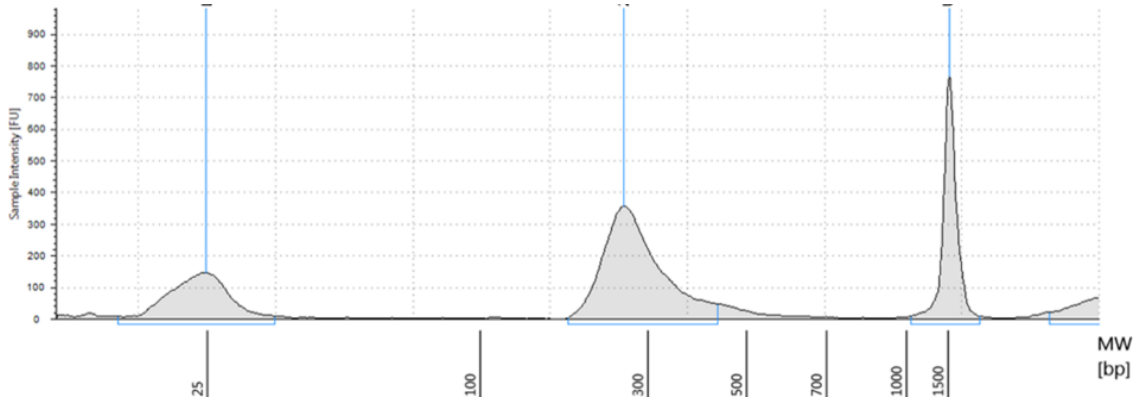
m. Add Complete Immunoprecipitation Buffer to the digested chromatin. Digested chromatin should take <25% of the immunoprecipitation volume. *Depending on the amount of cells available and digestion volume, ChIP is performed in a 100-200 µl final volume.*

n. Rotate chromatin at 4 degrees for 1 hour.

o. Vortex (medium setting) for 30 seconds

p. Take out an input control aliquot (for small samples, inputs tend to be larger than for normal ChIP, to reduce errors due to low cell numbers. For <20,000 cells input per ChIP, the input should be ~ 10% of the sample).

q. This input can be readily extracted if you want to control for fragmentation size. Add 10% volume of 10% SDS, mix well and add EB (Qiagen 19086) to 100 µl. Proceed to DNA extraction as described in section 3.



Example of an Illumina library prepared from 100 cells (10% input of a 1,000 cells chromatin preparation) visualized on and Agilent TapeStation. If using low cell input, any sample will be sub-visible prior to library construction. This is an ideal fragmentation, where the majority of the chromatin has been digested to mononucleosomes (2X forked adapters for Illumina libraries add ~130 bp to the insert size). If the digestion is incomplete, more di- and tri-nucleosomes will be visible in the input. This will lead to bias in coverage and creation of false “peaks” or “domains” in more MNase-accessible regions. These biases can be corrected by sequencing the input in parallel with the ChIP libraries.

2. IMMUNOPRECIPITATION

	1,000-10,000 cells	100,000 cells
A- Antibody-beads complex		
Complete IP buffer	100 µl	100 µl
Protein A:protein G Dynabeads 1:1	5 µl	10 µl
Antibody	0.25 µg	1 µg
Incubate > 3hrs on a rotator at 4°C		
B- Chromatin		
Chromatin lysate	10-50 µl	10-50 µl
Complete IP buffer	to 100-200 µl*	to 100-200 µl*
Protein A:protein G Dynabeads 1:1 (Life Technologies 1006D and 1007D)	5 µl	10 µl
Incubate > 2 hrs on a rotator at 4°C		
Immunoprecipitation		
Take out NChIP buffer from tube A.		
Transfer chromatin lysate from tube B to beads from tube A		
Incubate overnight (8-12 hours) on a rotator at 4°C		

a. Prepare Complete Immunoprecipitation Buffer by adding Protease inhibitor cocktail (Roche #04693132001) to a 1x concentration as per manufacturer's instruction and 0.1 mM PMSF (Sigma P7626). Ice-chill.

b. Pre-wash Protein A/G magnetic beads (Dynabeads, Life Technologies #1006D and 1007D) 3X in Complete Immunoprecipitation Buffer. *Each IP will require 5 µl (<10,000 cells input) or 10 µl (10-100,000 cells input) for pre-clearing and 5-10µl of beads for immunoprecipitation.*

c. Prepare antibody beads complexes (A) in 200 µl PCR strip tubes. Incubate on a rocker @ 4°C for > 3 hours.

d. Pre-clear chromatin (B) prepared in section 1: Add 5 or 10µl of pre-washed magnetic beads per ChIP, depending on input.

e. Rock for > 2 hours at 4°C.

f. After incubation, place Antibody beads complexes (A) on magnetic rack and take out supernatant.

g. Place pre-cleared chromatin (B) on a magnetic rack. Transfer supernatant to antibody-beads complexes.

h. Incubate overnight at 4°C whilst rocking.

i. Transfer the chromatin and beads in 1.5 ml sample tubes. Place on a magnetic rack.

j. Discard unbound chromatin.

k. Wash the beads by re-suspending them in 200 µl Low Salt Wash buffer. Take out the wash buffer and repeat.

l. Wash the beads twice in 200 µl High Salt Wash buffer.

Washes are performed as follows: 200 µl wash buffer is added and the beads are resuspended. ***Last wash: transfer the beads into a new 1.5 ml tube. Take out wash buffer. Close the tubes and pulse-spin the beads. Use a gel loading pipette tip to remove the last few drops of wash buffer.***

m. Re-suspend the beads in 30 µl freshly prepared ChIP elution buffer.

n. Elute DNA for 1-1.5 hours in a 65°C water bath. Vortex tubes regularly.

3. DNA PURIFICATION

a. Transfer eluted chromatin to a pre-spun phase lock tube (Qiagen Maxtract #129046).

b. Wash the beads with 70 µl of EB and transfer to the phase lock tube.

c. Add 100 µl of phenol: chloroform: isoamyl alcohol 25:24:1 to each sample. Vortex vigorously for 20-30 seconds.

d. Spin at max speed (13,000g) for 5 minutes.

e. Take out the upper phase and transfer to a new 1.5 ml tube. Make sure to take all the liquid and to not touch the new tube with the tip of the pipette (which could transfer some of the grease in the following reaction).

f. Add 10 µl of 3M sodium acetate and 1 µl of LPA (linear polyacrylamide, Sigma #56575). Mix well (before adding ethanol).

g. Add 275 µl of cold Ultrapure Ethanol (keep aliquots at -20°C).

h. Mix very well and allow DNA to precipitate for *at least* 30 minutes (can be overnight, or over the weekend) at -20°C.

i. Spin down DNA at max speed (13,000g) for 30 minutes at 4 degrees.

j. Take out supernatant and wash the pellet with 200 µl of freshly prepared 70% ethanol.

k. Allow pellet to dry.

l. Re-suspend in 20 µl (or desired volume) of buffer EB.

At this point, if the DNA is re-suspended in a low volume, the concentration of salts/SDS present will interfere with qPCR or the first step of library construction. We recommend re-purifying the DNA with Agencourt Ampure XP beads (description in custom library construction protocol).

RNAse A treatment is recommended prior to qPCR, depending on amplicon. For 1,000 cells ChIP, we typically take out 10-15% of the eluate and dilute it for qPCR. We can run 2-3 multi-copy amplicons with such an aliquot, but there is typically not enough material for single copy genes.

REAGENTS

Nuclear isolation buffer (complete)

- Sigma EZ nuclei isolation lysis buffer (add protease inhibitor cocktail and PMSF)
    - 0.1% Triton
    - 0.1% deoxycholate

MNAse Dilution buffer (= MNAse storage buffer)

1M Tris, pH7.5	10 µl	0.01M
1M NaCl	50 µl	0.01M
0.5M EDTA	2 µl	0.001M
Glycerol	500 µl	50%
Ultrapure water	438 µl	
Total	*1,000 µl*	

Complete Immunoprecipitation buffer

- 20 mM Tris-HCl pH 8.0
- 2 mM EDTA
- 150 mM NaCl
- 0.1% Triton X-100
- 1x Protease inhibitor cocktail
- 1 mM PMSF

Low Salt Wash buffer

- 20 mM Tris-HCl pH 8.0
- 2 mM EDTA
- 150 mM NaCl
- 1% Triton X-100
- 0.1% SDS

High Salt Wash buffer

- 20 mM Tris-HCl pH 8.0
- 2 mM EDTA
- 500 mM NaCl
-1% Triton X-100
- 0.1% SDS

ChIP Elution buffer

- 100 mM $NaHCO_3$
- 1% SDS

Supplementary Methods: step-by-step library construction procedure

GENERAL RULES:

- Clean all working surfaces with 10% bleach.
- Soak racks and icebox in 10% bleach for about 20 minutes.
- Wipe pipettes and pens with DNA Away (Thermo Scientific #7010).
- Wear a clean lab coat every time a new set of libraries is started.
- Use dedicated filter tip boxes and other consumables.
- Wear clean gloves. Wipe gloves with DNA Away on a regular basis during manipulations.
- Do not reach into bag of eppendorf tubes. Instead, clean a surface of the bench with DNA away and pour a few on it. If you poured too many tubes, do not put them back in the bag. Instead, close the caps and keep them on a clean rack for later.
- Aliquot all reagents
- Use a new (filter) tip for every sample, even when distributing the same reagent.

END REPAIR AND PHOSPHORYLATION

This step will create blunt ends and phosphorylate fragmented DNA (i.e MNAse, sonication). If using enzymatic digestion that creates blunt ends (i.e CviKI-1 or Shearase), this step can be skipped.

1. Thaw ChIP samples (in 20 or 30 µl of EB buffer, Quagen #19086). Keep on ice until End Repair Master Mix is prepared.

2. Thaw End Repair Master Mix components (vortex 10X Phosphorylation Buffer if precipitate present). Keep on ice.

3. Prepare the End Repair Master Mix in a clean 1.5 ml tube as follows:

	100-10,000 cells per ChIP (µl)	**>10,000 cells per ChIP** (µl)
Raw ChIP material in EB	20	30
End Repair Master Mix		
***10X Phosphorylation buffer**	2.5	3.75
10 mM dNTP mix (NEB N0447L)	0.5	1.5
T4 DNA polymerase (NEB M0203L)	0.5	0.75
Klenow DNA polymerase (NEB M0210L)	0.1	0.15
T4 PNK (NEB M0201L)	0.5	0.75
Ultrapure H_2O	0.4	0.6
Total master mix volume	*5*	*7.5*

**10X Phosphorylation buffer is T4 DNA ligase buffer (NEB B0202S)*

4. Mix thoroughly and pulse spin. Keep master mix on ice while distributing to samples.

5. Add 5 (if <10,000 cells per ChIP) or 7.5 µl (if >10,000 cells per ChIP) of End Repair Master Mix to each sample tube. Mix very well (10-15 times) using pipettor.

6. Incubate at room temperature for 30 minutes.

7. Proceed to DNA purification using either phenol:chloroform or Ampure Beads (both procedures described below).

8. Re-suspend samples in 17 or 25.5 µl buffer EB.

PHENOL:CHLOROFORM: ISOAMYL ALCOHOL DNA EXTRACTION

a. Bring final sample volume to 100 µl with buffer EB. Transfer to a phase lock tube (Qiagen MaxTract #129046).

b. In a fume hood, add 100 µl Phenol:Chloroform:isoamyl alcohol 25:24:1 to each sample.

c. Vortex to mix (~ 20 sec.) and spin for 5 minutes at 13,000g (or max speed on most microcentrifuges).

d. In a fume hood, transfer the top (aqueous) layer into a clean labeled 1.5 ml tube. *It is very important to minimize loss by ensuring that all the aqueous layer is transferred and that no liquid remains in the pipette tip. It is recommended not to touch the pipette tip inside the clean tube to avoid transferring grease from the phase lock tube.*

e. At bench, add 1 µl of LPA and 10 µl of 3M sodium acetate to each sample. Mix very well with pipette.

f. Add 275 µl if ice-cold 100% ethanol (aliquots kept at -20°C) to each sample and vortex to mix very well.

g. Allow precipitation to occur at -20°C for *at least* 20 minutes (pause point: overnight OK).

h. Spin in 4 degrees centrifuge at max speed (13,000g) for 30 minutes. *During centrifugation, prepare fresh 70% ethanol (will require about 100 µl per sample).*

i. Aspirate liquid using a P1000 pipette.

j. Wash pellet with 100 µl of 70% ethanol. Mix by aspirating up and down. Discard ethanol.

k. Pulse-spin and use a gel-loading tip to remove the last traces of ethanol. Leave the tubes open to dry.

l. Re-suspend in desired volume of sample buffer.

AMPURE XP BEAD CLEANUP AND SIZE SELECTION

a. Make sure the beads are well re-suspended. Add 1.8x (before adapter ligation) or 0.8x (after adapter ligation) volume of bead slurry per sample. Mix very well with pipette (10-12 times). Once the beads have been added to all samples pulse-vortex 3-4 times.

b. Incubate at room temperature for at least 5 minutes.

c. Place samples on rack for 2 minutes or until the sample is clear of beads.

d. Remove the liquid from the beads.

e. Wash the beads (leave on the magnetic rack) twice with 100 µl of freshly prepared 70% ethanol.

f. Take out the ethanol, pulse-spin and remove the last bit of ethanol using a gel loading tip.

g. Allow the beads to air dry.

h. Elute DNA with appropriate volume of 10 buffer EB.
** Allow beads to rehydrate at room temperature for a few minutes (they are rehydrated when you can re-suspend them by flicking the tube). Pipette up and down 20-30 times (light vortexing will do too)*.

i. Place the tubes back on the magnetic rack and transfer buffer to a new tube, taking care not to transfer any magnetic beads.

A-TAILING

A-tailing and adaptor ligation steps should be performed on the same day (unless precipitating DNA overnight).

20. *Set the heating block or waterbath at 37°C*.

21. Thaw and pulse-spin end-repaired (or digested with blunt cutter) samples (in **17 or 25.5 µl** buffer EB).

22. Thaw A-tailing components, pulse-spin and keep on ice.

23. Prepare A-tailing Master Mix in a clean tube as follows:

	100-10,000 cells per ChIP (µl)	**>10,000 cells per ChIP** (µl)
End-repaired/blunt-ended material in EB	17	25.5
A-tailing Master Mix		
10X NEB buffer 2	2	3
10 mM dATP (NEB N0440S)	0.5	0.75
Klenow (3'-5' exo-) (NEB M0212L)	0.5	0.75
Total master mix volume	*3*	*4.5*

24. Vortex and pulse-spin A-tailing Master Mix and add 3 or 4.5 µl to each sample. Mix thoroughly by pipetting/stirring.

24. Incubate at 37°C for 30 minutes.

25. DNA purification (phenol:chloroform **or** 1.8 X volume Ampure beads purification, as described previously).

26. Samples should be re-suspended in 6.67 or 10 µl of buffer EB. DO NOT freeze samples, keep on ice and proceed to Adaptor Ligation immediately.

ADAPTOR LIGATION

27. Thaw Adaptor Ligation components and keep on ice.

28. Prepare Adaptor Ligation Master Mix in a clean tube as follows:

	100-10,000 cells per ChIP (µl)	**>10,000 cells per ChIP** (µl)
A-tailed material in EB	6.67	10
Adaptor ligation master mix		
2x Quick DNA ligation buffer (NEB)	10	15
1 µM annealed Illumina adapters	0.67	1
Quick DNA ligase (NEB M2200L)	2.67	4
Total master mix volume	*13.34*	*20*

29. Add 13.34 or 20 µl of Adaptor Ligation Master Mix to each sample, mixing very well.

30. Incubate at room temperature for at least 30 minutes. *Can be extended to O/N ligation.*

31. Proceed to Ampure XP beads cleanup. At this stage, use 0.8 X volume beads slurry per volume of sample.

32. Final elution in 11 µl EB.

PCR AMPLIFICATION OF ADAPTOR LIGATED LIBRARIES

33. Thaw PCR components and appropriate indexing primers, pulse spin and keep on ice.

34. Prepare PCR Master Mix as follows (keep on ice until use):

	100-10,000 cells per ChIP (µl)	**>10,000 cells per ChIP** (µl)
Adapter ligated material in EB	10.5	10.5
10 µM Illumina paired-end indexed primer 2.x (different for each sample)	1	1
PCR amplification master mix		
2X Phusion HF Master Mix (NEB M0531L)	12.5	12.5
10 µM Illumina paired-end primer 1.0	1	1
Total master mix volume	*13.5*	*13.5*

35. Distribute 1 µl of the appropriate indexed primers PE 2.x into a PCR plate or 8-tubes strips.

36. Distribute 13.5 µl of PCR Master Mix into sample tubes.

37. Add 10.5 µl of adaptor-ligated samples to PCR tubes and mix very well (10-20 times) with pipette.

38. Pulse-spin tubes or plate

39. Run following PCR program (select appropriate, depending on the material to amplify, suggested in table below):

Hot start (98°C)
98°C 4:00
*10-18 cycles
98°C 0:30
65°C 0:30
72°C 0:30
1 cycle:
72°C 5:00
12°C ∞

Cells in ChIP:	**1,000-10,000 cells**	**100,000 cells**
H3K9me3	10-12	8-10
H3K27me3	10-12	8-10
H3K4me3	14-15	12

40. Clean up PCR reaction in a dedicated post-PCR area using 0.8X volume of Ampure XP beads.

41. Elute in 12-25 µl buffer EB.

These are fully constructed and amplified libraries that can be quantified on an Agilent Bioanalyzer or TapeStation (using Agilent HS DNA chips or screentapes). The libraries can be kept at -20 for several months prior to being pooled and sequenced.

The libraries can be pooled with other libraries that have been amplified using different indexes. We use Life Science's E-Gel system to size select pooled libraries. Note that there is no size selection prior to this last step.

Agilent TapeStation profiles of amplified ULI ChIP-seq libraries: