| Description | DCC Biosample | Barcodes | Raw reads for both replicates (M) | Uniquely mapped reads | Reads with valid barcodes | Estimated PCR duplication rate | Mitochondrial reads | Final read count (M) | Reads for each replicate (M) |
|---|---|---|---|---|---|---|---|---|---|
| E115_Rep1 | ENCBS745VOM | Set_1; p5 only 7 bp called | 683 | 82% | 46% | 49% | 4% | 116 | 54 |
| E115_Rep2 | ENCBS720JOH | Set_2; p5 only 7 bp called | | | | | | | 62 |
| E125_Rep1 | ENCBS974GPH | Set_1 | 491 | 85% | 71% | 56% | 5% | 113 | 68 |
| E125_Rep2 | ENCBS617DPG | Set_2 | | | | | | | 46 |
| E135_Rep1 | ENCBS273RVL | Set_1 | 486 | 84% | 46% | 36% | 2% | 109 | 57 |
| E135_Rep2 | ENCBS793ZRY | Set_2 for p5, p7 and i7; i5: S502-S511;X528-X535 | | | | | | | 52 |
| E145_Rep1 | ENCBS002AAA | Set_1 | 436 | 87% | 58% | 39% | 4% | 118 | 61 |
| E145_Rep2 | ENCBS014AAA | Set_2 | | | | | | | 57 |
| E155_Rep1 | ENCBS811RMD | Set_1 | 476 | 84% | 93% | 53% | 2% | 160 | 72 |
| E155_Rep2 | ENCBS841GER | Set_2 | | | | | | | 88 |
| E165_Rep1 | ENCBS156DHU | Set_1 | 545 | 88% | 34% | 39% | 4% | 86 | 43 |
| E165_Rep2 | ENCBS512LKA | Set_2 | | | | | | | 43 |
| P0_Rep1 | ENCBS038AAA | Set_1 | 361 | 85% | 83% | 52% | 2% | 108 | 51 |
| P0_Rep2 | ENCBS554AAA | Set_2 | | | | | | | 57 |
| P56_Rep1 | | Set_1 | 680 | 89% | 65% | 73% | 4% | 90 | 47 |
| P56_Rep2 | | Set_2 | | | | | | | 44 |
| hu_GM12878_mu_E155_mix | | p5:1-8, p7:1-12 i5: S502-S511; i7: S701-S715 | 27 | 87% | 89% | 38% | 2% | 13 | |

**Barcode sets (for sequences see Supplementary Table 1)**

| Set_2 | |
|---|---|
| p5 | 1-8 |
| p7 | 1-12 |
| i5 | X520-X535 |
| i7 | N731- X754 |

| Set_1 | |
|---|---|
| p5 | 1-8 |
| p7 | 1-12 |
| i5 | S502-S511,X512-X519 |
| i7 | N701-N729,N730 |

**Supplementary Table 1: Sequencing statistics for single nuclei ATAC-seq libraries.** General overview of sequencing for single nuclei ATAC-seq libraries including PCR duplication rates and fraction of mitochondrial reads. Please note that paired end reads were treated as separate reads. Sequencing libraries for replicate 1 and 2 were sequenced together and single cell datasets were assigned based on replicate specific barcode combinations (Set_1 or Set_2). One exception was E13.5 where replicate 1 and 2 were sequenced on separate lanes. Please note that for E11.5 7 out of 8 bp were detected for the p5 barcode. M: million.

| Sample | Cells pass QC (#) | Reads per cell (median) | Promoter coverage (median) | Fraction of reads in peaks (median) |
|---|---|---|---|---|
| E11.5_Rep1 | 528 | 16,023 | 10.6% | 17.5% |
| E11.5_Rep2 | 685 | 16,499 | 11.1% | 17.5% |
| E12.5_Rep1 | 781 | 18,397 | 11.2% | 15.7% |
| E12.5_Rep2 | 303 | 17,945 | 10.3% | 14.9% |
| E13.5_Rep1 | 651 | 16,891 | 11.8% | 18.4% |
| E13.5_Rep2 | 646 | 14,669 | 11.1% | 18.7% |
| E14.5_Rep1 | 976 | 14,440 | 11.3% | 19.8% |
| E14.5_Rep2 | 905 | 14,489 | 11.5% | 19.2% |
| E15.5_Rep1 | 1,591 | 16,789 | 12.2% | 20.4% |
| E15.5_Rep2 | 2,235 | 17,840 | 12.9% | 20.5% |
| E16.5_Rep1 | 317 | 12,758 | 11.0% | 23.2% |
| E16.5_Rep2 | 738 | 10,460 | 11.4% | 29.3% |
| P0_Rep1 | 1,044 | 9,275 | 13.3% | 36.0% |
| P0_Rep2 | 1,333 | 8,703 | 12.6% | 37.4% |
| P56_Rep1 | 1,569 | 12,509 | 11.6% | 27.1% |
| P56_Rep2 | 1,465 | 12,689 | 11.5% | 26.4% |
| Average | 985 | 14,399 | 11.6% | 22.6% |
| Total | 15,767 | | | |

**Supplementary Table 2: Overview of single nuclei ATAC-seq data after filtering out low quality cells.** Overview of nuclei that pass quality control and general properties of data sets including promoter coverage and fraction of reads in peaks.

| Parameter | this study | Cusanovich et al. 2015[12] | Buenrostro et al. 2015[13] |
|---|---|---|---|
| Single cell strategy | Combinatorial indexing | Combinatorial indexing | Microfluidics |
| Sample type | Frozen tissues | Cell lines | Cell lines |
| Total cells/nuclei (per experiment) | 15,676 (303-2,235) | 15,814 (533-1,459) | 1886 (n.r.) |
| Reads per cell/nucleus (median) | 9,375-18,397 | 1,390-3,094 | 73,000 (average) |
| Fraction of reads in peaks | 15.7%-37.4% | 41-59 % | > 15% |
| Promoter coverage per cell/nucleus | 11.60% | n.r. | 9.40% |

**Supplementary Table 3: Comparison of single nuclei ATAC-seq with previously published initial single cell ATAC-seq studies.** Table illustrating several characteristics of single nuclei/cell ATAC-seq libraries. n.r. not reported