### Supplemental Figure 1

Overview of main ChIP-seq procedures pioneered for low-input and/or automated ChIP-seq. In green the features that are advantageous, in red the disadvantageous features, in orange strategies that are compatible with single cell readout. Current automated low-input ChIP-seq workflows require custom-built platforms and can handle only a low number of parallel samples, as also indicated in the red boxes.

**Authors/PMID** | **Year** | **Technology** | **Automated Chip** | **Microfluidic/miniaturized** | **Readout** | **#Fp parallel samples** | **Ease of implementation** | **Minimum R cells started** | **Native proteins proﬁled** | **Handling time (cells to DNA)** | **Cell readout** | **Pooled indexed chromatin** | **Fragmentation**
--- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | ---
Murphy et al. 2942781 | 2018 | LifeChip (pre) | Yes | Valve microfluidic | NGS | 4 | Custom POMSchip, custom operation script | 1000000 | H3K4me1, H3K27ac | 1 day | No | No | Sonication
Cao et al. 26214128 | 2015 | MOW-ChIP-seq | Yes | Valve microfluidic | NGS | 1 | Custom POMSchip, custom operation script | 10000 to 100 | H3K4me1, H3K27ac | Not described | No | No | Sonication
Shen et al. 25178839 | 2015 | - | Yes | Valve microfluidic | NGS | 4 | Custom POMSchip, custom operation script | 10000 | H3K4me1 | No | No | No | Sonication/MNase
Bergert et al. 25549003 | 2014 | - | Yes | No liquid handler robot | NGS | Not Specified | Commercial platform (BPSstar) | 100000 to 10000 (H3K4me3) | H3K4me3, H3K27ac, H3K27me3, H3K36me3 | 1 day | No | No | Sonication
Gaspar et al. 24918486 | 2014 | R-ChIP | Yes | No liquid handler robot | NGS | 96 | Commercial platform (Nanoseq) | 1000000 (Flow cytometry) | H3K4me1, CIBPA, H4A21, p300, H3K4A | 5 days | (400 samples) | No | No | Sonication
Alridge et al. 24200198 | 2013 | Anti-ChIP-seq | Yes | No liquid handler robot | NGS | 96 | Commercial platform (Agilent Bravo) | 1000000 | H3K27me3 | 5 days | Yes | No | Sonication

### Supplemental Figures

“A plug and play microfluidic platform for standardized sensitive low-input Chromatin Immunoprecipitation”, Dirks et al., Genome Research 2021
Supplemental Figure 2. Overview of the conventional ChIP-seq workflow and the part of the ChIP-seq workflow that we automated on the microfluidic platform.
Supplemental Figure 3.

Details of the microfluidic IFC. (A) Pipette map on the newly developed microfluidic plates for ChIP. All control valves as present in the Integrated Fluidic Circuitries (Fig. 1C) can be individually pressurized by the use of 0.05% Tween 20 solution that is loaded within the wells C1-C4 and within the accumulators. The PDMS circuitry chip is present in the center. (B) Volumes of the main parts of a single reactor unit within the microfluidic IFC.

| Carrier inlet | Buffer                  |
|---------------|-------------------------|
| C1, C2, C3, C4| 25µL 0.05% Tween-20    |
| Accumulators  | 200µL 0.05% Tween-20    |
| Beads inlet   | 15µL ProA/G beads       |
| 1             | 20µL Frit beads         |
| 2             | 25µL Equilibration buffer|
| 3             | 25µL Equilibration buffer|
| 4             | 200µL High Salt wash buffer|
| 5             | 5µL DNA Extraction buffer|
| 6             | 200 µL DNA Elution buffer|
| Sample inlet  | 1-8µL chromatin + antibody|
| Harvest outlet| ~ 3µL output            |
Supplemental Figure 4. Overview and reproducibility of the antibody binding columns as generated within the microfluidic chip for ChIP. (A) Phase-contrast image of microfluidic bead columns that are packed to various sizes. These are not the exact but representative columns for the experiments as shown in Fig. 2F and Supplemental Fig. S4C. (B) Reproducibility of bead packing column across the 24 parallel reactors of the Integrated Fluidic Circuit. (C) Reproducibility of ChIP-qPCR across the 24 parallel reactors of a single microfluidic plate (A-P), and between 2 microfluidic plates run on different days (run 1 and 2), as shown for H3K4me3 per individual ChIP.
**Supplemental Figure 5.** Overview of the automatic microfluidic protocol that we developed. (A) Hands-on and machine time of the newly developed protocol. (B) Overview of the running time of the individual steps that are performed during the ChIP on the plates. (C) Overview of the adaptable script associated with running of the automated microfluidic for ChIP. Pink numbers refer to panel (B).

### A

**Carrier inlet**

| Details | Hands-on time |
|---------|---------------|
| Acquire chromatin, fresh or frozen, crosslinked sonicated or native digested. | -- |
| Combine chromatin with ChIP buffer with antibody of choice | 10 minutes |
| Pre-incubate chromatin at 4°C (30 minutes) | -- |
| Pipette beads, reagents and chromatin into carrier | 10 minutes |
| Run automated ChIP protocol in instrument (4.5 hours) | -- |
| Harvesting | 10 minutes |
| **Total** | **30 minutes** |

### B

| Step | Details | Time |
|------|---------|------|
| 1) Load chip | Load microfluidic chip into the controller, pressurize the valves and inlets, priming of valves and reagents to avoid air bubbles in the system. | 15 min. |
| 2) Pack column | Primes branch structure and reactors, primes column bypass, packs 5 cycles of ~200 pL frit beads into the reactor, packs ProAG beads into reactor for 10 minutes (~5 nL volume). | 30 min. |
| 3) Wash | Removes bead buffer from ChIP column. | 10 min. |
| 4) IP | Loads chromatin and antibody across the bead column at the specified pressure for the specified amount of time. | 35 min. |
| 5, 6) Wash (x2) | Equilibrates the ChIP to physiological salt condition, then performs a high salt wash to remove non-specific binding proteins and DNA segments. | 20 min. |
| 7) Air purge | Removes the high salt wash buffer and any remaining non-specific DNA carrying proteins. | 10 min. |
| 8) Extraction | Loads a Proteinase K buffer on to the column and increases the temperature to elute specific DNA from the column. | 90 min. |
| 9) Harvest | Pushes the specific DNA out of the PDMS circuitry into the carrier for pipetting off-chip. | 60 min. |

### C

```python
def info():
    script.require("LIBRARY");
    script.name="Miniaturized ChIP-seq";
    script.version="x.x.x";

def main():
    ChIP_Names()
    NGS_Names()
    AP=30
    load_chip(True,65,10)
    off("B2")
    ChIPColumn(AP,9,13,5,10)
    AP=35
    DilutionWash(AP,EQUILIBRATION,5,4)
    DirectIP(AP,30,4,11)
    set_temp(4)
    DilutionWash(AP,AIR PURGE,20,12)
    DilutionWash(AP,EQUILIBRATION,15,12)
    DilutionWash(AP,HIGH SALT WASH,15,12)
    DilutionWash(AP,AIR PURGE,20,12)
    set_temp(25)
    AP=40
    Extraction(AP,EXTRACTION,60,65,12,beads=False')
    AP=30
    set_temp(40)
    HarvestChIP(AP,11,300)
    LockChip(AP)
```

**Settings, optional tweaks**

- **Load library containing valve operation schematics**
- **Anvil pressure**
- **5x0.05nL frit bead pack at 9 psi, 13 psi ProAG packing for 10 min**
- **Washing cycles, temperature**
- **Chromatin loading for 30 minutes, 4 °C, 11 psi**
- **Air purge, 20 cycles of 5nL, 12 psi**
- **150mM NaCl wash, 15 cycles of 5nL, 12 psi**
- **450mM NaCl wash, 15 cycles of 5nL, 12 psi**
- **Air purge, 20 cycles of 5nL, 12 psi**
- **Proteinase K, 60 min. column incubation, 65 °C, 12 psi**
- **11 psi, 300 harvest cycles of 10nL volume (~3uL)**
- **Output chip**
Supplemental Figure 6. PnP-ChIP-seq using small quantities of bulk-sonicated crosslinked chromatin. (A) Genome browser view of a 4Mb locus for PnP-ChIP-seq of H3K4me3, H3K4me1, H3K27ac and H3K36me3. (B) Average profile of H3K4me3 over all H3K4me3 peaks of profiles generated by PnP-ChIP-seq using small quantities of bulk-sonicated crosslinked chromatin. The start and end of the peaks are indicated with 5'end and 3'end, respectively.
**Supplemental Figure 7.** Use of low-volume sonication on low numbers of mESCs for PnP-ChIP-seq. (A) Genome browser view of a gene-rich locus for PnP-ChIP-seq of H3K4me3 using a series of mESC input quantities for sonication. (B) Average profile of H3K4me3 over all H3K4me3 peaks of profiles generated by PnP-ChIP-seq using a series of mESC input quantities for sonication. The start and end of the peaks are indicated with 5'end and 3'end, respectively.
Supplemental Figure 8. PnP-ChIP-seq using small cell quantities by the use of MNase shearing on 15,000 mESCs. (A) Genome browser view of a 3Mb locus for PnP-ChIP-seq of H3K4me3, H3K4me1, H3K27ac and H3K36me3. (B) Average profile of H3K4me3 over all H3K4me3 peaks of profiles generated by PnP-ChIP-seq using small cell quantities by the use of MNase shearing on 15,000 mESCs. The start and end of the peaks are indicated with 5'end and 3'end, respectively. (C) A comparison of H3K4me3 over all H3K4me3 peaks of profiles generated by PnP-ChIP-seq using small cell quantities by the use of sonication or MNase shearing. The start and end of the peaks are indicated with 5'end and 3'end, respectively. (D) Overlap between de novo H3K4me3 peak calls of replicate PnP-ChIP-seq using small cell quantities by the use of MNase shearing on 15,000 mESCs.
Supplemental Figure 9. Comparison between MNase-based PnP-ChIP-seq and alternative low-cell input ChIP-seq methods developed by (Brind’Amour et al., 2015) or an automated microfluidic platform developed by Shen et al. (2015) (A) Intersections between de novo peak calls of H3K4me3 PnP-ChIP-seq and H3K4me3 profiles generated using alternative low-cell input ChIP-seq. (B) Average profile of H3K4me3 over all H3K4me3 peaks of profiles generated by PnP-ChIP-seq using small cell quantities by the use of MNase shearing on 15,000 mESCs (in blue called “ChIP merged”) or using H3K4me3 profiles generated by alternative low-cell input ChIP-seq methods performed on mESCs (in other colors). The start and end of the peaks are indicated with 5’end and 3’end, respectively. (C) Cross-correlation between H3K4me3 PnP-ChIP-seq (labeled “Dirks”) and H3K4me3 profiles generated using alternative low-cell input methods for ChIP-seq.
Supplemental Figure 10. PnP-ChIP-seq using small cell quantities using MNase shearing on 15,000 mESCs for H3K4me1, H3K27ac and H3K36me3. (A) Cross-correlations of PnP-ChIP-seq using tag counts of merged peak set for H3K4me1, H3K27ac or H3K36me3 (B) Overlap between de novo peak calls of PnP-ChIP-seq and bulk ChIP-seq. (C) Overlap between de novo peak calls of PnP-ChIP-seq of replicate experiments using 3000 mESC chromatin equivalent as input.
Supplemental Figure 11. PnP-ChIP-seq of H3K27me3 and H3K9me3. (A) Exemplary genome browser views for PnP-ChIP-seq of H3K27me3 and H3K9me3. (B) Average profile of H3K27me3 over all H3K27me3 peaks in mESCs (van Mierlo et al., 2019) of profiles generated by PnP-ChIP-seq as compared to bulk ChIP-seq profiles and an alternative low-cell input ChIP-seq method called STAR ChIP-seq (Zhang et al., 2016). The start and end of the peaks are indicated with 5’end and 3’end, respectively. (C) Genome browser views for PnP-ChIP-seq of H3K9me3. The bottom example shows a genomic region which is highly enriched for major satellite repeats. (D) Presence of PnP-ChIP-seq sequence tags of various hPTMs in two types of repeats, confirming the presence of H3K9me3 over major satellites.
**Supplemental Figure 12.** PnP-ChIP-seq allows to detect significant differences in the closely-related cell types 2i and serum mESCs. Scatterplot of DESeq2-normalised tag counts (replicate means) of H3K4me3, H3K27ac and H3K4me1 PnP-ChIP-seq on 2i and serum mESCs. Significant enrichment (FDR-adjusted p-value < 0.05) depicted in red. We detect in total 25,617 H3K4me3 peaks (3,459 peaks significantly higher in either 2i ESCs or serum ESCs), 8,329 H3K27ac peaks (161 peaks significantly higher in either 2i ESCs or serum ESCs), and 5,752 H3K4me1 peaks (41 peaks significantly higher in either 2i mESCs or serum mESCs). Please find more details in Supplemental Table S1.
Supplemental Figure 13. PnP-ChIP-seq allows the detection of significant differences in H3K4me3 between the closely-related cell types 2i and serum ESCs (serum is abbreviated as FCS (foetal calf serum)). (A) Genome browser views for H3K4me3 PnP-ChIP-seq of 2i and serum mESCs for 3 genes that are known to be higher expressed in 2i mESCs. (B) Genome browser views for H3K4me3 PnP-ChIP-seq of 2i and serum mESCs for 3 genes that are known to be higher expressed in serum mESCs. r = replicate; H3K4me3peaks_all = merge track of all H3K4me3 peaks detected in 2i mESCs and serum mESCs. H3K4me3peaks_sig = merge track of all H3K4me3 peaks that are significantly increased in either 2i mESCs or serum mESCs. Significant differences in gene expression between 2i and serum ESCs from Marks et al., 2012 (PMID: 22541430).
Supplemental Figure 14. PnP-ChIP-seq allows the detection of significant differences in H3K27ac between the closely-related cell types 2i and serum ESCs (serum is abbreviated as FCS (foetal calf serum)). (A) Genome browser views for PnP-ChIP-seq of loci showing a significant increase in H3K27ac in 2i mESCs as compared to serum mESCs (boxed) (B) Genome browser views for PnP-ChIP-seq of loci showing a significant increase in H3K27ac in serum mESCs as compared to 2i mESCs (boxed). r = replicate; H3*peaks_all = merge track of all peaks of a hPTM detected in 2i mESCs and serum mESCs. H3*peaks_sig = merge track of all peaks of a hPTM that are significantly increased in either 2i mESCs or serum mESCs.
Supplemental Figure 15. PnP-ChIP-seq allows the detection of significant differences in H3K4me1 between the closely-related cell types 2i and serum ESCs (serum is abbreviated as FCS (foetal calf serum)). (A) Genome browser views for PnP-ChIP-seq of loci showing a significant increase in H3K4me1 in 2i mESCs as compared to serum mESCs (boxed). (B) Genome browser views for PnP-ChIP-seq of loci showing a significant increase in H3K4me1 in serum mESCs as compared to 2i mESCs (boxed). r = replicate; H3*peaks_all = merge track of all peaks of a hPTM detected in 2i mESCs and serum mESCs. H3*peaks_sig = merge track of all peaks of a hPTM that are significantly increased in either 2i mESCs or serum mESCs.
Supplemental Figure 16. Gating of embryonic stem cell subpopulations on fluorescent markers Venus (driven by the *Hhex* promoter), tdTomato (driven by the MERVL promoter) and emGFP (driven by the *Zscan4c* promoter).
**Supplemental Figure 17.** Epigenome analysis of 2C-like cells as compared to wildtype mESCs. (A) Scatterplot of DESeq2-normalised ChIP-seq tag counts of H3K27ac, H3K4me1 (both from Zhang et al., 2019) and H3K27me3 (Hayashi et al., 2016) of 2C-like cells as compared to wildtype mESCs. DESeq2 analysis detected no significant differences (FDR-adjusted p-value < 0.05) in H3K27ac, H3K4me1 or H3K27me3 between 2C-like cells and wildtype mESCs. The axes of the plots are in log₂.

(B) Boxplots of DESeq2-normalised tag counts of H3K4me3 PnP-ChIP-seq profiles of Hhex-, MERVL- and Zscan4c-positive (“pos”) and negative (“neg”) mESC cell populations of all genes, and genes that have been reported to be higher or lower in 2C-like cells as compared to wildtype mESCs (Fu et al., 2019). Significant differences between boxplots calculated using the Wilcoxon rank-sum test are indicated.