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AutoRELACS: Automated Generation And Analysis Of Ultra-parallel ChIP-seq
2
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11
ABSTRACT

12 Chromatin immunoprecipitation followed by sequencing (ChIP-seq) is a method used to profile
13 protein-DNA interactions genome-wide. RELACS (Restriction Enzyme-based Labeling of
14 Chromatin *in Situ*) is a recently developed ChIP-seq protocol that deploys a chromatin barcoding
15 strategy to enable standardized and high-throughput generation of ChIP-seq data. The manual
16 implementation of RELACS is constrained by human processivity in both data generation and data
17 analysis. To overcome these limitations, we have developed AutoRELACS, an automated
18 implementation of the RELACS protocol using the liquid handler Biomek i7 workstation. We
19 match the unprecedented processivity in data generation allowed by AutoRELACS with the
20 automated computation pipelines offered by snakePipes. In doing so, we build a continuous
21 workflow that streamlines epigenetic profiling, from sample collection to biological interpretation.
22 Here, we show that AutoRELACS successfully automates chromatin barcode integration, and is
23 able to generate high-quality ChIP-seq data comparable with the standards of the manual protocol,
24 also for limited amounts of biological samples.

25

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26 # BACKGROUND

27 Chromatin immunoprecipitation followed by sequencing (ChIP-seq) is a widely used method to
28 study protein-DNA interactions genome-wide (1). Despite the enormous contribution that ChIP-
29 seq has brought to our understanding of epigenetic and transcriptional control, the traditional ChIP-
30 seq protocol (2,3) presents various limitations. For example, it requires substantial amounts of
31 biological input material, which is often a limiting factor in relevant clinical settings, and it is low-
32 throughput, which prevents comprehensive epigenetic profiling. Furthermore, the protocol is
33 poorly standardized across cell types, resulting in a high degree of technical variability that
34 hampers biological interpretation of the data.

35

36 Over the last ten years, much work has been devoted to address these and other shortcomings (4–
37 8). In line with these efforts, we have recently developed RELACS (Restriction Enzyme-based
38 Labeling of Chromatin *in Situ*), a method that employs chromatin barcoding to enable high-
39 throughput generation of ChIP-seq experiments (9). RELACS works reliably with low input
40 material and can be used for quantitative ChIP-seq analysis (9,10). The method is highly
41 standardized, and could potentially be scaled to profile hundreds of samples in parallel for tens of
42 DNA-binding proteins at once. Yet, the current manual implementation is limited by human
43 processivity in both data generation and data analysis.

44

45 To match the ideal potential of this methodology, we have implemented an automated version of
46 the RELACS protocol, named AutoRELACS, using the liquid handler Biomek i7 automated
47 workstation (Beckman Coulter). While other automated ChIP-seq implementations already exist
48 (11,12), they still require a large amount of sample material, and they do not utilize the enormous

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49 multiplexing potential of barcoded chromatin. The scope of these methods is limited to data
50 generation and lack an integrated bioinformatics workflow that streamlines standard
51 computational tasks (e.g. QC, DNA-mapping, peak calling). AutoRELACS, on the other hand,
52 couples the high-throughput generation of ChIP-seq data with the scalable and modular
53 computational pipelines offered by snakePipes (13). From version 1.2.3, snakePipes' DNA-
54 mapping routine can handle RELACS data by performing demultiplexing of fastq files on
55 RELACS adaptors and UMI-based deduplication. Together, AutoRELACS and snakePipes build
56 a continuous workflow that automates ChIP-seq data generation and analysis, allowing for
57 unprecedented processivity.
58
59 In this work, we test the performance of AutoRELACS by assessing 1) the scalability of the
60 chromatin barcode integration step, 2) the quality of the generated data in comparison to the
61 benchmark set by the manual protocol, and 3) the sensitivity of the automated method when
62 working with low (≤ 25.000 cells/sample) and very low (≤ 5.000 cells/sample) cell numbers. We
63 show that AutoRELACS is a scalable method that can generate high quality ChIP-seq data,
64 comparable with the standards of the manual protocol. We finally show that AutoRELACS
65 provides reliable epigenetic profiling also with limited input biological material.
66

67 MAIN

68 AutoRELACS is a scalable method for the generation and analysis of ultra-parallelized 69 ChIP-seq data

70 RELACS (Restriction Enzyme-based Labeling of Chromatin *in Situ*) is a method that enables the
71 high-throughput generation of ChIP-seq experiments (9). To increase the standardization and the

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72 scalability of this approach, we have developed AutoRELACS, an automated implementation of
73 the RELACS protocol using the liquid handler Biomek i7.
74
75 The AutoRELACS workflow is conceptually divided in six parts: four fully automated (A)
76 processes intermitted by two manual (M) steps (Fig 1a). First, cells are manually processed to
77 isolate the nuclei (14) and to digest the chromatin within the nuclear envelope (step 1 - M). Next,
78 using the liquid handler Biomek i7, the chromatin from each sample is barcoded and pooled into
79 a unique masterbatch (step 2 - A). Using focused sonication, nuclei are lysed and the barcoded
80 chromatin is released (step 3 - M). The final three steps of the protocol have been fully automated
81 and require minimal human supervision. These include the chromatin immunoprecipitation (ChIP)
82 reactions and washing steps of beads-bound immunocomplexes (step 4 - A), decrosslinking, DNA
83 purification and PCR amplification (step 5 - A) and, after sequencing, barcode demultiplexing and
84 bioinformatics analysis with snakePipes (step 6 -A) (13).
85
86 The integration of sample-specific RELACS barcodes into the digested chromatin (Fig 1a, step 2)
87 is key to the success of the method. To test the performance of automated and parallelized
88 RELACS barcode integration, 60 custom barcodes were designed, each composed of a 4
89 nucleotide (nt)-long unique molecular identifier (UMI), followed by a 8 nt-long barcode with 50%
90 GC content (note that after combining forward and reverse reads, each fragment is tagged by a 8-
91 nt long UMI). These adaptors were used to label the chromatin of 60 batches of S2 cells
92 (*Drosophila melanogaster*) in duplicates using the Biomek i7 workstation (Fig 1b). Results show
93 that all barcodes are present within the pooled chromatin in both replicates, with a distribution of

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94 barcode representation equal to 1.64% ± 0.22% and 1.64% ± 0.35% for replicate 1 and 2
95 respectively, close to the uniform expectation of 1.667 % (Fig 1b, dashed line).
96
97 In summary, we show that AutoRELACS can be used to uniformly integrate multiple barcodes in
98 a fully automated fashion, allowing for ultra-parallelized processing of a considerable number of
99 samples in one single run.
100
101 **The quality of AutoRELACS ChIP-seq data is comparable with manual RELACS**
102 Next, we test the quality of the ChIP-seq data generated with AutoRELACS and we compare it
103 with the results from the previously published manual RELACS protocol. To this end, we run in
104 parallel a manual and an automated RELACS experiment where we digest and barcode 28 batches
105 of S2 cells and we immunoprecipitate against H3K4me3, H3K27ac and H3K27me3.
106
107 The histone modification profiles generated with manual RELACS and with AutoRELACS are
108 overall similar. The variance present in the first two principal components of the normalized
109 coverage matrix (computed on the merged peaks set) discriminates between the three histone
110 modifications, regardless of the method used (Fig 2a). Comparison of the metaprofiles of the
111 merged scores over peaks shows identical signal for H3K4me3, while H3K27ac and H3K27me3
112 present a slightly lower median coverage in AutoRELACS compared to the manual procedure (Fig
113 2b). Nevertheless, these differences do not impinge on the sensitivity of the assay. Visual
114 inspection of the normalized coverage reveals high similarity between the two RELACS
115 implementations (Fig 2c), while high overlap (80-90%) is observed between the peaks called in
116 the two datasets (Fig S1a).

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117

118 To provide a global overview for all enriched regions, we cluster (k=5) the signal of H3K4me3,
119 H3K27ac and H3K27me3 using the manual and the automated RELACS data on a common
120 merged peaks set (Fig 2d). We do not observe any set of peaks that are specific to manual RELACS
121 or AutoRELACS, which shows no obvious implementation-specific biases.

122

123 Together, we show that AutoRELACS yields high quality ChIP-seq data that are overall
124 comparable with the manual RELACS protocol.

125

126 **AutoRELACS works reliably with low cell numbers**

127 RELACS can generate robust epigenetic profiling with low cell numbers (9). To test the sensitivity
128 limits of AutoRELACS, we barcode 4 batches of HepG2 cells and we aliquote the chromatin into
129 two pools containing 4 x 15,000 and 4 x 75,000 cells respectively. We name the former "Very
130 Low" and the latter "Low" chromatin pool. Next, we divide each chromatin pool into three equal
131 aliquotes for immunoprecipitation against H3K4me3, H3K27ac and H3K27me3, while a small
132 fraction of each pool (~ 1 μl) is set aside as Input control. This setup results in three ChIP reactions
133 with 5000 cells/barcode for the "Very Low" pool and three ChIP reactions with 25,000
134 cells/barcode for the "Low" pool (Fig 3a).

135

136 The normalized genome-wide coverages coming from Low and Very Low experiments are highly
137 correlated within histone modifications groups, which indicates that the profiles generated with
138 different amounts of input material are overall similar (Fig 3b). Although we observe a

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139 deterioration of the signal-to-noise ratio in the Very Low group, the enrichment is preserved and,
140 for narrow euchromatic marks, this is sufficient for robust peak calling (Fig 3c).
141
142 In summary, we show that AutoRELACS can be deployed for automated and parallelized profiling
143 of protein-DNA interactions genome-wide also for limited amounts of biological samples.
144

145 DISCUSSION

146 In this work we present AutoRELACS, an automated implementation of the RELACS protocol (9)
147 that enables the automated high-throughput generation of ChIP-seq experiments. AutoRELACS
148 natively interfaces with the computational pipelines offered by snakePipes (13), thus streamlining
149 the generation and analysis of DNA-binding profiles at unprecedented scale.
150
151 RELACS can parallelize ChIP-seq data generation through *in situ* ligation of sample-specific
152 barcodes into the digested chromatin inside the nuclear envelope. Here, we show that
153 AutoRELACS successfully integrates a high number of barcodes in parallel, ensuring a balanced
154 representation of each adaptor in the final chromatin pool. While we limit our test to 60 barcodes,
155 a single AutoRELACS experiment can support the integration of up to 96 barcodes. The resulting
156 chromatin pool can be split into 96 ChIP reactions, leading to the generation of up to 9,216
157 independent chromatin profiles in only three days. It should be noted that more imbalanced
158 barcode distributions within the final chromatin pool may still lead to a successful profiling, at the
159 cost of increasing the total sequencing depth. It is therefore suggested to perform a preliminary
160 shallow sequencing of the chromatin input to estimate the total sequencing depth needed to ensure
161 a minimum coverage for all samples.

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162

163 We further show that AutoRELACS can generate high quality ChIP-seq data, comparable with the
164 standards of the manual implementation, and that the method can be used for epigenetic profiling
165 of low cell numbers. Together, these features suggest AutoRELACS as a method of choice in
166 various clinical applications, potentially enabling comprehensive screening of epigenetic markers
167 from small amounts of biological material.

168

169 The current AutoRELACS implementation has room for further improvements. To date, the
170 method still requires human intervention in the earliest stages of the protocol. Future developments
171 might integrate the use of focused sonicator platforms into the workflow of the liquid handler
172 workstation, to further reduce user intervention and enable a full walk-away automated solution.

173

174 MATERIALS AND METHODS

175

176 Cell culture

177 S2 cells were cultured in Express Five SFM (Thermo Fisher Scientific) supplemented with
178 glutamax, at 27 °C and were provided by Akhtar's lab (MPI-IE). HepG2 liver hepatocellular
179 carcinoma (ATCC, HB-8065TM) were cultured in Eagle's minimal essential medium (EMEM,
180 Lonza, 06-174) supplemented with 10% fetal bovine serum (Sigma), 2 mM L-glutamine (Lonza),
181 1.8 mM CaCl2, 1 mM sodium pyruvate (Lonza) and penicillin–streptomycin mixture (100
182 units/mL, Lonza), at 37 °C at 5% CO2 in 10 cm plates, up to 70%-80% confluency.

183

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185 **Cell fixation**

186 HepG2 and S2 cells were fixed in 1% methanol-free formaldehyde (Thermo Scientific, 28906) in
187 D-MEM (for HepG2 cells) or Express Five SFM (for S2 cells) for 15 min at room temperature
188 under gentle shaking. Formaldehyde was quenched for 5 min by adding 125 mM glycine final
189 concentration. Cells were rinsed twice with ice-cold PBS, harvested by scraping (HepG2) and
190 pelleted (300 g, 10 min, 4 °C).

191

192 **Detailed AutoRELACS workflow**

193 The AutoRELACS protocol is divided into five main steps (as described in Fig. 1).
194 A separated program file is provided for each automated section and is available for download at
195 https://github.com/FrancescoFerrari88/AutoRELACS/tree/master/AutoRELACS_binaries_Biom
196 ek_i7.

197

198 1) ***Nuclei extraction and chromatin digestion*** (manual protocol): nuclei are extracted from fixed
199 cells, swollen, digested, washed and counted as previously described (9). The resulting digested
200 nuclei are resuspended in 10 mM Tris-HCl pH 8 at the nuclei density of 500,000 nuclei/25 µl
201 (Drosophila S2) and 500,000 nuclei/25 µl (HepG2) for the following nuclei barcoding step.

202

203 2) ***Chromatin barcoding and pooling*** (automated, method file "RELACS barcoding.bmf"): in this
204 step chromatin is barcoded inside the nuclei as previously described (9), but using automation.
205 This method allows the processing for a flexible number of nuclei samples, from 1 to 96.
206 Preparation of reagents: nuclei samples are aliquoted column-wise in a 96-wells PCR plate (25 µl
207 of digested nuclei per well), named "Nuclei Plate". 2 µl of the desired RELACS barcode at 15 µM

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208 are aliquoted in each well of a second 96-wells PCR plate, following the same coordinates of the
209 respective nuclei aliquot (named "Index Plate"). The following reagent mixes are positioned into
210 1.5 ml conical tubes on the Biomek deck in a cold Peltier block: End Repair mix (ER), Ligation
211 mix (LIG) and 3M NaCl, following directions as highlighted in the "guided instrument setup" (a
212 screenshot of the deck is shown in Supplementary Fig. 2a).
213 Steps of the "RELACS barcoding" program: 5 µl of ER mix are added into each occupied well of
214 "Nuclei Plate". The plate is mixed on the orbital shaker present on the deck and incubated into the
215 integrated PCR cycler for 30 min at 20 °C and for 5 min at 65 °C. End-repaired nuclei are
216 transferred from "Nuclei Plate" to the "Index Plate" containing RELACS barcodes. 15.5 µl of LIG
217 mix are added into each occupied well. The "IndexPlate" is shaken and transferred into the
218 integrated PCR cycler for ligation incubation (15 min at 30 °C and for 15 min at 20 °C). The
219 ligation is inactivated adding 5 µl of 3M NaCl into each occupied well of "Index Plate". The plate
220 is shaken and pooling is automatically performed by transferring samples from each occupied well
221 of "Index Plate" to 1.5 ml tubes positioned into the "Final Pool" rack. Wells containing barcoded
222 nuclei can be pooled as specified by the user, by indicating source and destination coordinates of
223 "Index Plate" and "Final Pool" into the .csv file "Nuclei_Pooling_Template.csv".
224
225 3) ***Sonication-assisted nuclei lysis*** (manual protocol): tubes containing nuclei pools are manually
226 collected. Barcoded nuclei are pelletted down (5000 *g* for 10 min). Supernatants are discarded and
227 pellets are resuspended into the desired volume of Shearing buffer supplemented with Protease
228 Inhibitor Cocktail (Roche, 11873580001) and sonicated for 5 minutes in a Covaris E220 sonicator
229 as described (9).
230

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231 4) ***ChIP and elution*** (automated, method file "RELACS ChIP-Elution.bmf"). The method allows
232 for a flexible number of ChIP reactions from 1 to 96 simultaneously. A screenshot of the overall
233 organization of the deck is shown in Supplementary Fig. 2b.
234 All reagents used and the procedure of ChIP largely overlap to the ones described in our former
235 publication (9), with the relevant modifications highlighted here below. Preparation of ChIP plate
236 (named "Sample Plate"): ChIP reactions are carried out in a maximum volume of 150 µl instead
237 of 200 µl used for manual RELACS. 75 µl of chromatin prepared in step 3 are aliquoted column-
238 wise into a 1.2 ml storage plate (Thermo Fisher, AB1127) accordingly to the required number of
239 ChIP. To equilibrate salts and detergents, 73 µl of 1X buffer iC1 (from iDeal ChIP-seq kit for
240 histones, Diagenode C01010173) supplemented with Protease Inhibitor Cocktail (Roche,
241 11873580001) and 2 µl of 5M NaCl are added into each chromatin well. One µg per 100,000 cells
242 of the desired antibody (H3K4me3 C15410003, H3K27ac C15410196, H3K27me3 C15410195,
243 all from Diagenode) is added into each well. Remaining chromatins are set aside at 4 °C to prepare
244 inputs. Please notice that input samples will be manually added later on before the automated
245 decrosslinking step.
246 Preparation of reagents: ChIP Wash buffers 1 to 4 (from iDeal ChIP-seq kit for histones,
247 Diagenode C01010173) are aliquoted into quarter module reservoirs divided by length. ChIP
248 elution buffer (1% SDS, 200 mM NaCl, 10 mM Tris-HCl pH 8, 1 mM EDTA) is also aliquoted
249 into the remaining well of the reservoir as highlighted in the "guided instrument setup". ChIP beads
250 (Dynabeads protein A-conjugated magnetic beads, Invitrogen) are washed twice with 1X buffer
251 iC1 and aliquoted into two 1.5 ml conical tubes before placing them on the deck.
252 Automated protocol: the program involves four main steps (antibody incubation, beads incubation,
253 ChIP washes, elution). Antibody incubation is performed by shaking the "Sample Plate"

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254 containing the ChIP reactions on the orbital shaker, repeating this procedure 12 times: 20 min
255 continuous shaking at 500 rpm, stop for 10 min. In comparison to manual RELACS we carried out
256 ChIP incubation for a total time of 6 hours at room temperature instead of 10 hours at 4 °C as used
257 in manual RELACS. Please notice that we did this modification to overcome technical constraints
258 that would have resulted in loss of samples when mixing by pipetting.
259 Beads incubation: beads placed on the deck are automatically mixed and 15 µl of beads are
260 dispensed into each ChIP reaction. "Sample Plate" is then transferred on the orbital shaker and
261 mixed for a total time of 2 hours at room temperature (5 min continuous shaking at 500 rpm, stop
262 for 5 min, repeated 12 times). In comparison to the procedure used for manual RELACS, beads
263 incubation time for AutoRELACS has been reduced by one hour.
264 ChIP washes: the following procedure is repeated for each of the four wash buffers. "Sample Plate"
265 is transferred onto the magnetic rack and left for 5 minutes to reclaim the beads-bound
266 immunocomplexes to the magnet. Supernatants are aspirated, discarded into the wash station, and
267 150 µl of wash buffer are added into each occupied well. Plate is shaken on the orbital shaker for
268 about 5 minutes to wash the beads (5 seconds pulse shaking at 800 rpm for 60 times).
269 Elution: the last wash supernatants are removed from the beads. 80 µl of ChIP elution buffer is
270 added to the beads and the plate is shaken on the orbital shaker for a total time of about 35 minutes
271 (5 seconds pulse shaking at 800 rpm for 60 times, 4 minutes pause, for four times). "Sample Plate"
272 is placed onto the magnet for 5 minutes and supernatants containing immunoprecipitated material
273 are collected into a fresh 96-well plate (called "ChIP Eluates") and stored overnight into the
274 integrated PCR cycler at 10 °C.
275

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276 5) ***Decrosslink, purification, USER treatment, PCR amplification*** (automated, method file
277 "RELACS Decrosslink-FinalLibrary.bmf"): the plate "ChIP Eluates" is collected from the Biomek
278 and Input samples are manually added column-wise after the ChIP samples (0.1-10% of the
279 original chromatin volume in 80 µl of ChIP Elution buffer). This plate is placed back onto the deck
280 and renamed in the instrument setup as "Sample Plate 2".
281 Reagent preparation: 4 µl of 10 µM Illumina dual index primer cocktails (from IDT) are placed in
282 a 96-well PCR plate column-wise following the desired pattern corresponding to the ChIP samples
283 (plate is named "Index Plate"). The following reagents are required for this section of program, as
284 specified in the instrument setup (Supplementary Fig. 2c): 100% isopropanol, EB (10 mM Tris-
285 HCl pH 8), freshly prepared 85% ethanol (all on the deck at room temperature), proteinase K 20
286 mg/ml (Thermo Fisher, EO0491), glycogen 20 mg/mg (Thermo Fisher, R0561), carboxylated
287 magnetic beads (Invitrogen, 65011), PCR mix (NEBNext Ultra II Q5 Master mix, NEB M0544),
288 USER enzyme (NEB M5505), all placed in 1.5 ml conical tubes in a cold Peltier block. Ampure
289 XP (Beckman Coulter, A63881) are thoroughly mixed and aliquoted column-wise according to
290 the pattern of "Sample Plate 2" in a 96-well storage plate (AB0765, Thermo Fisher), using 100 µl
291 of beads per well.
292 Automated Decrosslink: 2 µl of proteinase K are transferred into each occupied well of "Sample
293 Plate 2" containing ChIP eluates and input samples. The plate is mixed on the orbital shaker and
294 incubated for 2 hours at 65 °C into the integrated PCR cycler.
295 Automated DNA purification: in comparison to manual RELACS, in which decrosslinked DNA
296 is purified using columns (Qiagen minElute PCR purification kit), AutoRELACS uses a custom-
297 made DNA purification by precipitation and sequestration using carboxylated magnetic beads.
298 Decrosslinked samples are transferred from the PCR plate to a larger 96-well storage plate ("ChIP

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299 Purification", 4titude, LB0125). The following reagents are added into each occupied well: 2 µl of
300 glycogen, 10 µl of carboxylated beads (automatically pre-mixed by pipetting before dispensing),
301 and 80 µl of isopropanol. The plate "ChIP Purification" is mixed by shaking and incubated at room
302 temperature for 10 minutes. The beads are reclaimed onto the integrated magnet for 5 minutes and
303 supernatants are discarded. DNA bound to beads is washed twice using 200 µl of 85% ethanol.
304 Beads are dried and DNA is automatically eluted by addition of 28 µl of EB into each occupied
305 well. Plate is placed onto the magnet to discard the beads and to collect purified eluates.
306 USER treatment: 27 µl of purified DNAs are collected into a fresh 96-well PCR plate. 3 µl of
307 USER enzyme is added into each occupied well. Plate is shaken and incubated into the integrated
308 PCR cycler for 15 minutes at 37 °C. Samples are transferred into a 96-well storage plate for
309 purification using Ampure XP (0.9X ratio). After purification, samples are eluted in 22 µl of EB.
310 Automated amplification of final libraries and purification: 21 µl of each purified DNA are
311 transferred to the 96-well PCR plate containing Illumina indexes ("Index Plate"). 25 µl of PCR
312 mix are added into each occupied well and the plate is shaken. The plate is then transferred into
313 the integrated PCR cycler for PCR incubation (hot start 98 °C for 30 sec; PCR cycles: 98 °C for
314 10 sec, 65 °C for 75 sec; final extension 65 °C for 5 min). Notice that before launching the method
315 the user has the possibility of choosing the number of PCR cycles to use (10, 12 or 14). In the
316 experiments presented in this work libraries were amplified using 12 PCR cycles (14 PCR cycles
317 for low input ChIP). Amplified samples are transferred into a 96-well storage plate for double
318 purification using Ampure XP (first at 0.8X ratio second at 1X ratio). Ready libraries are eluted in
319 25 µl of EB and transferred in a clean 96-well PCR plate.
320
321

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322 **Sequencing**

323 Libraries were quality-controlled to check the concentration (Qubit DNA HS, Invitrogen, Q32851)
324 and the fragment size distribution (Fragment Analyzer capillary electrophoresis, NGS 1-6000 bp
325 hs DNA kit). Libraries were pooled and normalized to 1 to 2 nM with 10% PhiX spike-in according
326 to the Illumina guidelines. Libraries were clustered on NovaSeq XP flowcells and sequenced
327 paired-end with a read length of 50 bp on an Illumina NovaSeq 6000 instrument.

328

329 **Bioinformatics analysis**

330 BCL files were converted to fastq format using bcl2fastq2 (v. 2.20.0) and demultiplexed on
331 illumina barcodes. Fastq files were used as input to snakePipes' DNA-mapping and ChIP-seq
332 workflows (v. 1.2.3) (13), using default parameters as listed in
333 https://github.com/FrancescoFerrari88/AutoRELACS/tree/master/snakePipes_defaults. Mapping
334 was performed on the genome build dm6 and hg38 for *D. melanogaster* and *H. sapiens*
335 respectively. Briefly, fastq files were demultiplexed on RELACS adaptor barcodes and reads were
336 mapped to the reference genome using Bowtie2 (v. 2.3) (15). Uniquely mapping read pairs (mapq
337 > 3) were retained and duplicates were filtered on UMI using UMITools (paired mode) (v. 1.0.0)
338 (16). Peaks were called using MACS2 (v. 2.1.2) (17) with default parameters. Merged peak sets
339 were obtained by concatenating, sorting and merging peaks identified in the different experimental
340 conditions included in the analysis, using bedtools sort | merge (v. 2.28) (18).

341 Clustered heatmaps, ChIP-seq metaprofiles and the clustered correlations heatmap were generated
342 using deeptools (v. 3.3.1) (19), using filtered bam files as input. Principal component analysis (Fig
343 2a) was performed using the Python library scikit-learn (v. 0.19.1) on rlog-transformed count

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344 matrix (20). Coverage was obtained using deeptools' multiBamSummary (v. 3.3.1) (19) on the
345 merged peak set. We use pyGenomeTracks (21) to visualize signal tracks on specific genomic loci.
346

347 **Data and code availability**

348 The fully reproducible and documented analysis is available on github at
349 https://github.com/FrancescoFerrari88/AutoRELACS, as Jupyter notebooks and R/python scripts.
350 Raw data and normalized bigWig tracks were deposited to GEO and are available for download
351 using the following accession number: GSE147042.
352

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355 support. This work was funded by the Deutsche Forschungsgemeinschaft - Project ID 192904750
356 - CRC 992 Medical Epigenetics.
357

358 ## SUPPLEMENTARY

359

360 **Biomek i7 requirements and consumables for automation**

361 The following instrument parts are required to perform AutoRELACS on the Biomek i7: Biomek
362 i7 Workstation equipped with left and right pods; 1200 µl 96-multichannel (left pod), Span-8 pipets
363 coupled with 1 ml syringe volume (right pod), gripper tools (one per pod), static Peltier with tube
364 block for conical tubes, shaking Peltier and block for 96-well PCR plates, Orbital shaker, Wash
365 station for multichannel, Wash station for Span-8, Magnet, Peristaltic pump (Masterflex L/S, Cole-
366 Parmer), Automated PCR cycler (Thermo Fisher), seven Tip Loading Stations, twenty-six

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367 Automated Labware Positioners. Deck configuration details are indicated into each respective
368 protocol part (Supplementary Fig. 2). Instrument configuration file is provided in the
369 supplementary material (Biomeki7.bif).
370 The following plastic consumables are used for automation: Hard-Shell 96-well PCR plates
371 (HSP9601, Bio-Rad), 96-Deep well storage microplates (4titude, LB0125), Low profile 1.2 ml
372 square storage plate (AB1127, Thermo Fisher), 0.8 ml 96-well storage plate (AB0765, Thermo
373 Fisher), Auto-sealing plate lids (MSL2022, Bio-Rad), Universal microplate lid (4ti-0290, 4titude),
374 300 ml reservoir (EK-2035, Agilent technologies), Modular reservoir quarter module divided by
375 length (372788, Beckman Coulter), Modular reservoir quarter module (372790, Beckman
376 Coulter), sterile tips with filter (all from Beckman Coulter): 1025 µl (B85955), 190 µl (B85911),
377 50 µl (B85888).
378

379 # FIGURE LEGENDS

380

381 **Figure 1: AutoRELACS workflow ensures comprehensive integration of RELACS barcodes**
382 a) Overview of AutoRELACS protocol. 1-M) Nuclei of formaldehyde-fixed cells are extracted
383 manually using adjusted ultrasound (14). The nuclear envelope is permeabilized, and the
384 chromatin digested *in situ* using a 4-cutter restriction enzyme (RE). 2-A) Digested chromatin from
385 each sample is automatically barcoded. Upon completion, the liquid handler pools all barcoded
386 samples into a unique tube (Biomek i7 program: "RELACS_Barcoding"). 3-M) Pooled samples
387 are collected by the user and nuclei are lysed using focused sonication. 4-A) The barcoded
388 chromatin is aliquoted according to the number of required immunoprecipitation (IP) reactions
389 into corresponding ChIP reaction mixes. The ChIP reactions are carried out overnight in parallel

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390 at room temperature on the Biomek i7 workstation. Upon completion, the ChIP-ped chromatin is
391 sequestrated using beads and automatically washed 4 times at increasing stringency conditions and
392 finally eluted in the elution buffer (Biomek program: "RELACS_ChIP_Elution"). 5-A)
393 Subsequently, the eluted chromatin is decrosslinked and the DNA is purified. DNA is amplified
394 via PCR using primers carrying Illumina dual indexes. Optionally, the liquid handler performs
395 multiple rounds of purification and size selection using Ampure XP beads (Biomek program:
396 "RELACS_Decrosslink_FinalLibraries"). A: Automated; M: Manual.
397 6-A) Libraries are sequenced on Illumina's sequencing devices. Upon completion of the
398 sequencing run, bcl2 files are automatically converted to fastq format and input into the fully
399 automated ChIP-seq workflow available as part of the snakePipes suite (13). SnakePipes' ChIP-
400 seq workflow performs demultiplexing of reads on RELACS custom barcodes, quality controls,
401 mapping and filtering of duplicate reads using unique molecular identifiers (UMI), and further
402 downstream analysis like generation of input-normalized coverage tracks and peak calling.
403 b) Distribution of RELACS barcodes in two independent input chromatin pools. 60 barcodes are
404 integrated into the digested chromatin of two independent batches of S2 cells. Sequencing of the
405 input chromatin pool for replicate 1 (upper panel) and replicate 2 (lower panel), reveals the
406 percentage of input reads for each barcode used (y-axis). The ideal uniform distribution (100/60)
407 is represented as a dotted line. The shaded gray area shows one standard deviation from the mean
408 of the observed distribution.
409
410
411

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412 **Figure 2: AutoRELACS ChIP-seq data are comparable with the standards of the manual**
413 **protocol.**
414 a) Principal component analysis (PCA) of the normalized coverage matrix computed on the
415 merged peak set between H3K4me3, H3K27ac and H3K27me3, as generated by AutoRELACS
416 (Automated) and manual RELACS (Manual). For each mark and protocol implementation, all 28
417 demultiplexed technical replicates are shown. The 10000 most variable loci across all marks are
418 input into the PCA.
419 b) Metaprofile of the median normalized coverage computed over H3K4me3 (upper panel),
420 H3K27ac (central panel) and H3K27me3 (lower panel) peaks. Each panel shows the signal
421 generated with AutoRELACS and manual RELACS from a merge of all 28 technical replicates.
422 c) Data tracks of the merged signal of the 28 technical replicates for H3K4me3 (red), H3K27ac
423 (green), H3K27me3 (grey) and Input (cyan) on the dm6 locus chr2R:7,400,000-7,700,000. For
424 each mark, we show the profile generated by AutoRELACS (Automated) and manual RELACS
425 (Manual) and the merged set of peaks called in the two datasets (Merged Peaks).
426 d) Heatmaps showing the clustered signal (k=5) on a merged set of peaks, as identified in the
427 AutoRELACS (Automated) and in the manual RELACS (Manual) dataset, for H3K4me3 (left
428 panel), H3K27ac (central panel) and H3K27me3 (right panel). The similarity between each pair
429 of tracks indicates that there are no obvious implementation-specific biases.
430
431 **Figure 3: AutoRELACS works with low cell numbers**
432 a) Overview of the experimental design used to test the sensitivity limits of AutoRELACS. Four
433 batches of HepG2 cells are barcoded and pooled into two chromatin masterbatches, the first
434 comprising 4 * 15,000 cells (Very Low input) and the second 4 * 75’000 cells (Low Input). Each

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435 chromatin pool is evenly split into three ChIP reactions (H3K4me3, H3K27ac, H3K27me3), while
436 a small fraction (~ 1µl) is set aside as Input control. For the Very Low pool, about 20,000 cells are
437 used in each ChIP, which corresponds to 5,000 cells/barcode. For the Low pool, about 100,000
438 cells are used in each ChIP, which corresponds to 25,000 cells/barcode.
439 b) Hierarchical clustering of HepG2 ChIP-Seq profiles of H3K4me3, H3K27ac and H3K27me3,
440 generated using Low and Very Low chromatin input, based on the pairwise Pearson Correlation
441 Coefficient (PCC). Each pairwise PCC is computed based on the binned coverage (bin width = 10
442 kb) over the whole genome.
443 c) Metaprofile of the mean enrichment over Input of H3K4me3 (upper panel, red), H3K27ac
444 (central panel, green) and H3K27me3 (lower panel, grey), computed on a consensus set of peaks
445 identified for each mark separately, from the Low and Very Low input chromatin.
446
447 **Supplementary Figure 1: peaks identified in AutoRELACS and RELACS datasets overlap**
448 **to a great extent.**
449 a) Venn diagrams representing the percentage of overlapping peaks and implementation-specific
450 peaks identified in AutoRELACS (Automated) and manual RELACS (Manual) datasets, for
451 H3K4me3 (left panel), H3K27ac (central panel) and H3K27me3 (right panel) profiles of S2 cells.
452
453 **Supplementary Figure 2: Biomek i7 deck configurations for AutoRELACS.**
454 a) Deck configuration for the method “RELACS barcoding”. On the deck are present filtered tips
455 in different volumes (50 µl violet box in position 1, 190 µl green boxes in position 4 and 5), PCR
456 lid for automation (3), magnet (2) and Peltier block containing barcoding reagents at 4 °C (4 °C
457 reagents, 6). Digested nuclei are aliquoted in a 96-well PCR plate (Nuclei plate, 7). RELACS

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458 barcodes are aliquoted in a 96-well PCR plate (Index plate, 10) positioned on top of a cold Peltier.
459 To protect the indexes, a plastic lid is positioned on top of the plate.
460 b) Deck configuration for the method “RELACS ChIP-Elution”. On the deck are present filtered
461 tips in different volumes (190 µl green boxes in position 3, 4 and 5, 1025 µl orange box in position
462 6), PCR lid for automation (2), magnet (1) and Peltier block containing ChIP reagents at 4 °C (4
463 °C reagents, 8). Room temperature ChIP reagents are stored in reservoirs (ChIP-Wash reagents,
464 9). ChIP reactions are aliquoted in a 96-deep well storage plate (Sample plate, 10). Final ChIP
465 eluates are transferred into a 96-well PCR plate (ChIP eluates, 7).
466 c) Deck configuration for the method “RELACS Decrosslink-FinalLibrary”. On the deck are
467 present filtered tips in different volumes (50 µl violet boxes in position 1 8, 9, 190 µl green boxes
468 in position 4, 5, 7, 1025 µl orange boxes in position 6, 10), PCR lid for automation (3), magnet
469 (2) and Peltier block containing the required reagents at 4 °C (4 °C reagents, 18). Room
470 temperature reagents are stored in reservoirs (DNA purification reagents, 23). 85% Ethanol is
471 stored in a lidded reservoir (20). Ampure XP are aliquoted in a 96-deep well storage plate covered
472 with a lid (Ampure XP, 22). ChIP and PCR purification occur in 96-deep well plates (11, 15). 96-
473 well PCR plates in position 12, 16 and 17 are required for several steps of the method and to store
474 the final libraries. ChIP and Input samples, which need to be firstly decrosslinked, are positioned
475 in a 96-well PCR plate (24).
476

477 # REFERENCES

478 1. Furey TS. ChIP-seq and beyond: new and improved methodologies to detect and characterize
479 protein-DNA interactions. Nat Rev Genet. 2012 Dec;13(12):840–52.

480 2. Johnson DS, Mortazavi A, Myers RM, Wold B. Genome-wide mapping of in vivo protein-DNA
481 interactions. Science. 2007 Jun 8;316(5830):1497–502.

482 3. Mikkelsen TS, Ku M, Jaffe DB, Issac B, Lieberman E, Giannoukos G, et al. Genome-wide maps of

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483 chromatin state in pluripotent and lineage-committed cells. Nature. 2007 Aug 2;448(7153):553–60.

484 4. van Galen P, Viny AD, Ram O, Ryan RJH, Cotton MJ, Donohue L, et al. A Multiplexed System for
485 Quantitative Comparisons of Chromatin Landscapes. Mol Cell. 2016 Jan 7;61(1):170–80.

486 5. Schmidl C, Rendeiro AF, Sheffield NC, Bock C. ChIPmentation: fast, robust, low-input ChIP-seq
487 for histones and transcription factors. Nat Methods. 2015 Oct;12(10):963–5.

488 6. Brind'Amour J, Liu S, Hudson M, Chen C, Karimi MM, Lorincz MC. An ultra-low-input native
489 ChIP-seq protocol for genome-wide profiling of rare cell populations. Nat Commun. 2015 Jan
490 21;6:6033.

491 7. Shankaranarayanan P, Mendoza-Parra M-A, van Gool W, Trindade LM, Gronemeyer H. Single-tube
492 linear DNA amplification for genome-wide studies using a few thousand cells. Nat Protoc. 2012 Jan
493 26;7(2):328–38.

494 8. Chabbert CD, Adjalley SH, Klaus B, Fritsch ES, Gupta I, Pelechano V, et al. A high-throughput
495 ChIP-Seq for large-scale chromatin studies. Mol Syst Biol. 2015 Jan 12;11(1):777.

496 9. Arrigoni L, Al-Hasani H, Ramírez F, Panzeri I, Ryan DP, Santacruz D, et al. RELACS nuclei
497 barcoding enables high-throughput ChIP-seq. Commun Biol. 2018 Dec 5;1:214.

498 10. Ferrari F, Arrigoni L, Franz H, Butenko L, Trompouki E, Vogel T, et al. DOT1L Methyltransferase
499 Activity Preserves SOX2-Enhancer Accessibility And Prevents Activation of Repressed Genes In
500 Murine Stem Cells [Internet]. bioRxiv. 2020 [cited 2020 Feb 25]. p. 2020.02.03.931741. Available
501 from: https://www.biorxiv.org/content/10.1101/2020.02.03.931741v1

502 11. Aldridge S, Watt S, Quail MA, Rayner T, Lukk M, Bimson MF, et al. AHT-ChIP-seq: a completely
503 automated robotic protocol for high-throughput chromatin immunoprecipitation. Genome Biol. 2013
504 Nov 7;14(11):R124.

505 12. Gasper WC, Marinov GK, Pauli-Behn F, Scott MT, Newberry K, DeSalvo G, et al. Fully automated
506 high-throughput chromatin immunoprecipitation for ChIP-seq: identifying ChIP-quality p300
507 monoclonal antibodies. Sci Rep. 2014 Jun 12;4:5152.

508 13. Bhardwaj V, Heyne S, Sikora K, Rabbani L, Rauer M, Kilpert F, et al. snakePipes: facilitating
509 flexible, scalable and integrative epigenomic analysis. Bioinformatics. 2019 Nov 1;35(22):4757–9.

510 14. Arrigoni L, Richter AS, Betancourt E, Bruder K, Diehl S, Manke T, et al. Standardizing chromatin
511 research: a simple and universal method for ChIP-seq. Nucleic Acids Res. 2016 Apr 20;44(7):e67.

512 15. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012 Mar
513 4;9(4):357–9.

514 16. Smith T, Heger A, Sudbery I. UMI-tools: modeling sequencing errors in Unique Molecular
515 Identifiers to improve quantification accuracy. Genome Res. 2017 Mar;27(3):491–9.

516 17. Zhang Y, Liu T, Meyer CA, Eeckhoute J, Johnson DS, Bernstein BE, et al. Model-based analysis of
517 ChIP-Seq (MACS). Genome Biol. 2008 Sep 17;9(9):R137.

518 18. Quinlan AR, Hall IM. BEDTools: a flexible suite of utilities for comparing genomic features.
519 Bioinformatics. 2010 Mar 15;26(6):841–2.

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520 19. Ramírez F, Ryan DP, Grüning B, Bhardwaj V, Kilpert F, Richter AS, et al. deepTools2: a next
521 generation web server for deep-sequencing data analysis. Nucleic Acids Res. 2016 Jul
522 8;44(W1):W160–5.

523 20. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq
524 data with DESeq2. Genome Biol. 2014;15(12):550.

525 21. Ramírez F, Bhardwaj V, Arrigoni L, Lam KC, Grüning BA, Villaveces J, et al. High-resolution
526 TADs reveal DNA sequences underlying genome organization in flies. Nat Commun. 2018 Jan
527 15;9(1):189.

Figure 1

a

M 1) Nuclei extraction and chromatin digestion

kHz

A 2) Chromatin barcoding and pooling

M 3) Sonication-assisted nuclei lysis

kHz

A 4) ChIP and elution

A 5) Decrosslink, purification, PCR amplification

A 6) Demultiplexing and analysis

snakePipes

b

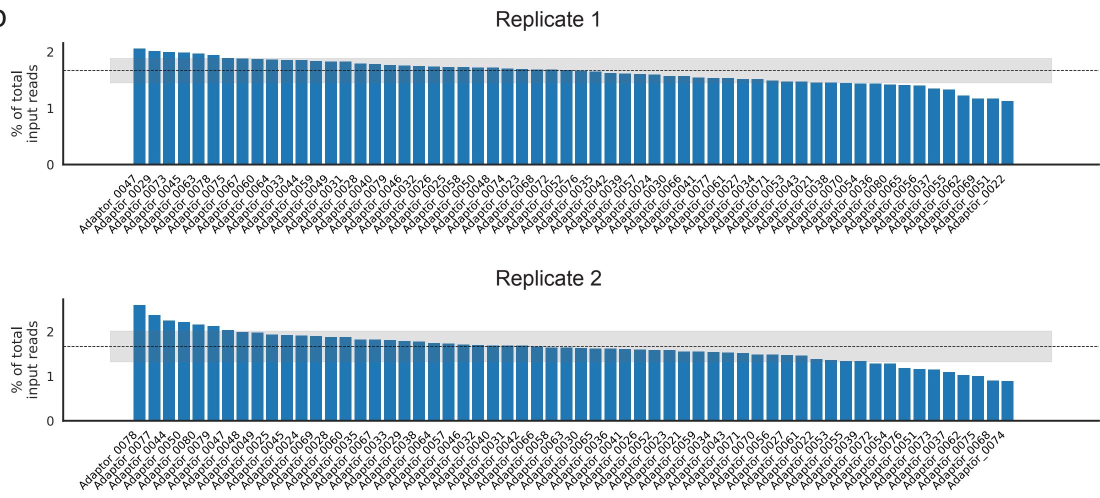


Figure 2

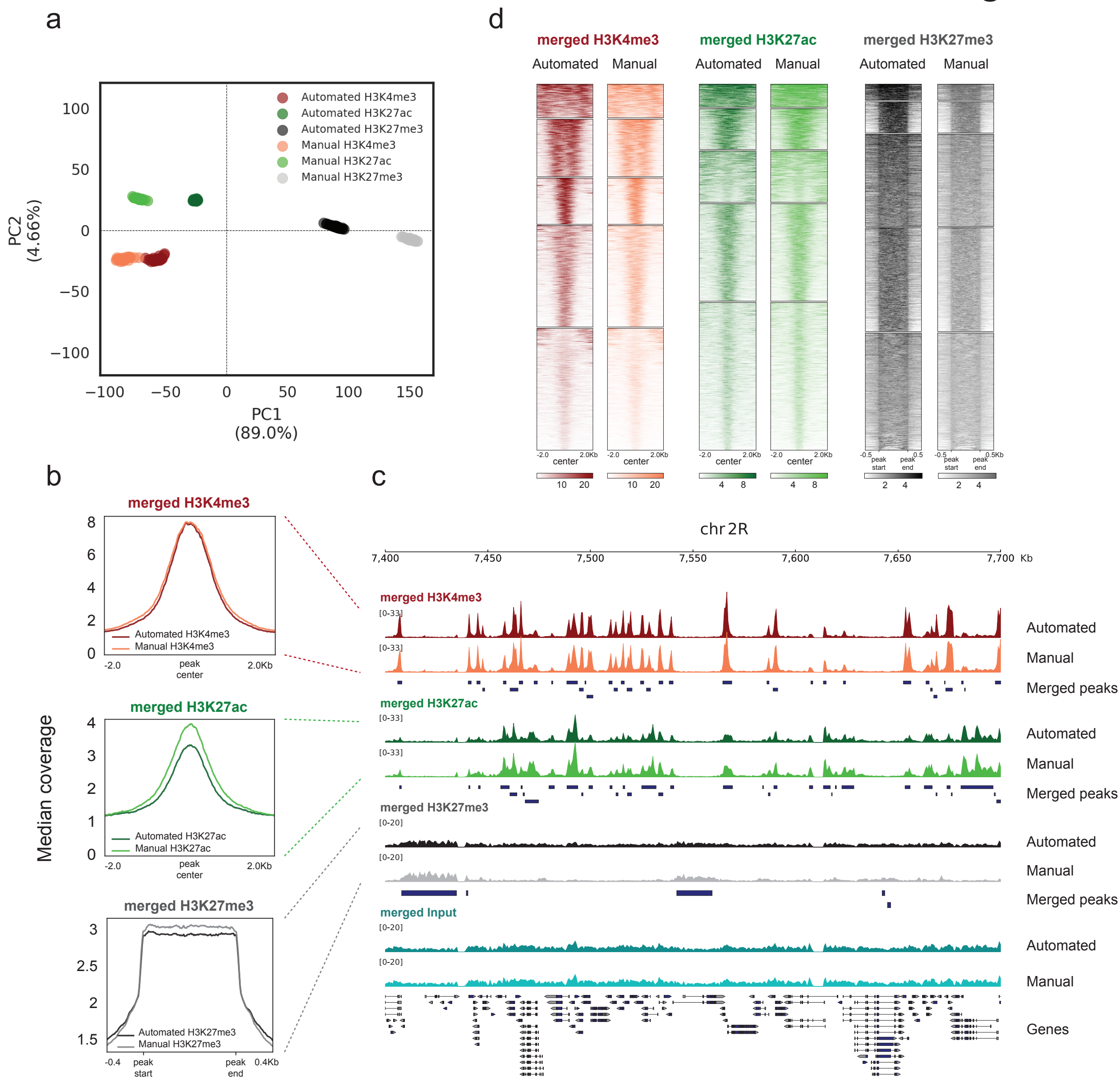


Figure 3

a

4 HepG2 batches

2 chromatin poolings

3 ChIP

Very Low
4 * 15,000 cells

Very Low
20,000 cells/IP
5,000 cells/barcode

Adaptor 0021

Adaptor 0022

Adaptor 0023

Adaptor 0024

15,000 cells

75,000 cells

H3K4me3
H3K27ac
H3K27me3
Input

H3K4me3
H3K27ac
H3K27me3
Input

Low
4 * 75,000 cells

Low
100,000 cells/IP
25,000 cells/barcode

b

H3K4me3 Low 0023
H3K4me3 Low 0022
H3K4me3 Low 0021
H3K4me3 Low 0024
H3K4me3 Very Low 0023
H3K4me3 Very Low 0022
H3K4me3 Very Low 0021
H3K4me3 Very Low 0024
H3K27ac Low 0023
H3K27ac Low 0021
H3K27ac Low 0022
H3K27ac Low 0024
H3K27ac Very Low 0023
H3K27ac Very Low 0022
H3K27ac Very Low 0021
H3K27ac Very Low 0024
H3K27me3 Low 0023
H3K27me3 Low 0024
H3K27me3 Low 0021
H3K27me3 Low 0022
H3K27me3 Very Low 0023
H3K27me3 Very Low 0024
H3K27me3 Very Low 0021
H3K27me3 Very Low 0022

H3K27me3 Very Low 0022
H3K27me3 Very Low 0021
H3K27me3 Very Low 0024
H3K27me3 Very Low 0023
H3K27me3 Low 0022
H3K27me3 Low 0021
H3K27me3 Low 0024
H3K27me3 Low 0023
H3K27ac Very Low 0024
H3K27ac Very Low 0021
H3K27ac Very Low 0022
H3K27ac Very Low 0023
H3K27ac Low 0024
H3K27ac Low 0022
H3K27ac Low 0021
H3K27ac Low 0023
H3K4me3 Very Low 0024
H3K4me3 Very Low 0021
H3K4me3 Very Low 0022
H3K4me3 Very Low 0023
H3K4me3 Low 0024
H3K4me3 Low 0021
H3K4me3 Low 0022
H3K4me3 Low 0023

−1.00 −0.75 −0.50 −0.25 0.00 0.25 0.50 0.75 1.00

Pearson Correlation Coefficient

c

Low cell number (25.000 cells/sample)

Very Low cell number (5.000 cells/sample)

Adaptor 21 22 23 24 21 22 23 24

H3K4me3

3 2 1 0

-2.0 center 2.0Kb

H3K27ac

3 2 1 0

-2.0 center 2.0Kb

Log2(ChIP/Input)

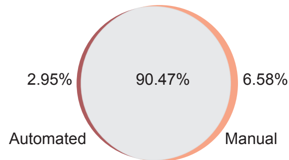
H3K27me3

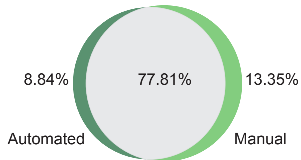
0.2 0.1 0.0 −0.1

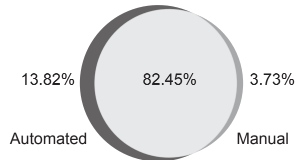
-0.8 peak start peak end 0.8Kb

Supplementary 1

a

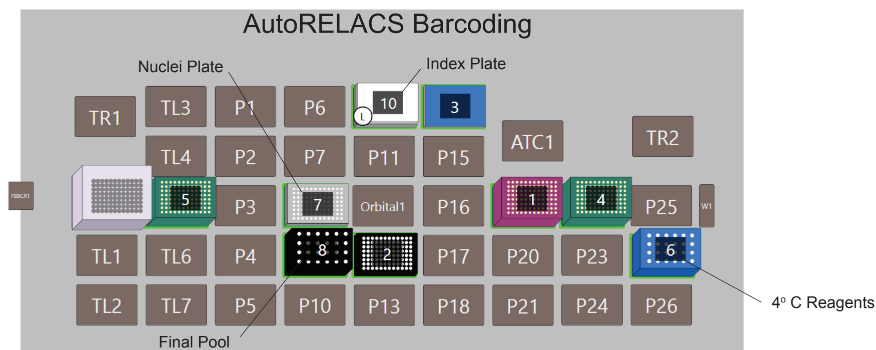






Supplementary 2

a



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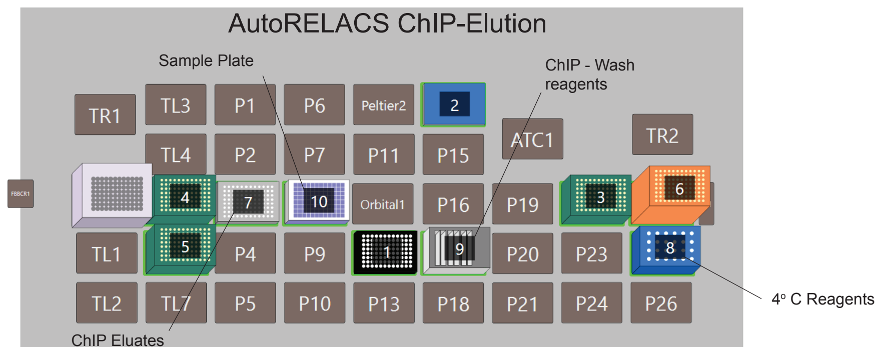

c