Development of chromatin immunoprecipitation for the analysis of histone modifications in red macroalga *Neopyropia yezoensis* (Rhodophyta)

Abstract

Epigenetic regulation by histone modification can activate or repress transcription through changes in chromatin dynamics and regulates development and the response to environmental signals in both animals and plants. Chromatin immunoprecipitation (ChIP) is an indispensable tool to identify histones with specific post-translational modifications. The lack of a ChIP technique for macroalgae has hindered understanding of the role of histone modification in the expression of genes in this organism. In this study, a ChIP method with several modifications, based on existing protocols for plant cells, has been developed for the red macroalga, *Neopyropia yezoensis,* that consists of a heterogeneous alternation of macroscopic leaf-like gametophytes and microscopic filamentous sporophytes. ChIP method coupled with qPCR enables the identification of a histone mark in generation-specific genes from *N. yezoensis*. The results indicate that acetylation of histone H3 at lysine 9 in the 5' flanking and coding regions from generation-specific genes was maintained at relatively high levels, even in generation-repressed gene expression. The use of this ChIP method will

contribute significantly to identify epigenetic regulatory mechanisms through histone modifications that control a variety of biological processes in red macroalgae.

Key words: chromatin immunoprecipitation (ChIP); epigenetics; histone modifications; red algae; life cycle

Introduction

Histones are subject to an assortment of dynamic and reversible post-translational modifications (e.g., methylation, acetylation, phosphorylation, ubiquitination, etc.) that serve as a "histone code" to active or repress gene transcription (Jenuwein and Allis, 2001). For example, histone acetylation marks (especially H3 and H4 acetylation) are associated with gene activation to increase DNA access, which results from the neutralization of the basic charge in histones and the weak interaction of histones with DNA (cis effects; Allis and Jenuwein, 2016; Onufriev and Schiessel, 2019). Furthermore, the presence or absence of methylation on Lys or Arg in histones alters their association with reader proteins, which results in modifications of chromatin structure and either transcriptional repression or activation (Teperino et al., 2010). Thus, unraveling the role of the "histone code" will provide insight into many physiological processes affected by alterations in gene expression. In contrast to animals and plant, however, knowledge on the roles of post-

translational modifications of histone molecules in macroalgae remains limited, because of a lack of tools for histone modifications studies, except for the model brown alga *Ectocarpus* (Bourdareau et al. 2021).

Chromatin immunoprecipitations (ChIP) is a powerful tool to investigate interactions between DNA-binding proteins and genomic DNA. It provides important information about the role of DNA-binding proteins and putative target genes (Nelson et al., 2006). ChIP is also used to analyze the abundance and distribution of histone carrying specific covalent modifications under diverse conditions. Two methods for ChIP have been developed: X-ChIP (cross-linked chromatin followed by immunoprecipitation) and N-ChIP (native chromatin immunoprecipitation). In X-ChIP, chromatin is cross-linked using formaldehyde and then sheered by sonication or fragmented with enzymes. This is applicable to non-histone proteins that bind weakly (or indirectly) to DNA (Orlando, 2000). In N-ChIP, chromatin is isolated without cross-linking and micrococcal nuclease (MNase) is used to digest the linker DNA between the nucleosomes to maintain a native chromatin state (O'neill and Turner, 2003; Huang et al., 2020). Although N-ChIP requires stable interactions between DNA and proteins, such as histones, it has advantages with respect to antibody specificity for analyzing histones modifications (O'neill and Turner, 2003). Thus, it is important to develop N-ChIP methods in macroalgae to clarify their roles of histone modifications.

The red alga, *Pyropia/Neopyropia* (formerly *Porphyra*), belongs to the

67 Bangiales and is an important marine crop that is harvested to produce nori.
68 The life cycle of Bangiales generally consists of a heterogeneous alternation
69 of macroscopic leaf-like gametophytes and microscopic filamentous
70 sporophytes. The gametophytes, which are harvested to produce food for nori
71 or laver, form non-motile male (spermatia) and female (carpogonia) gametes
72 on the thallus during sexual reproduction. Fertilization occurs when the
73 female gametes are still retained on the gametophytes and successive cell
74 divisions produce clones of the zygotes that are referred as carpospores.
75 These develop into sporophytes, known as a *Conchocelis* phase, which was
76 previously considered to be another species, *Conchocelis rosea* (Drew 1949;
77 Kurogi 1953; Iwasaki 1961). The unravelling of the complete
78 *Pyropia/Neopyropia* life cycle with an alteration of two heteromorphic
79 generations contributes to the establishment of the mariculture system.
80 *Pyropia/Neopyropia* gametophytes usually grow in winter and
81 produce male and female gametes at the beginning of spring. This results in
82 the sporophytes that germinate from the zygotes growing in the summer
83 during exposure to high temperatures. Consistent with this observation,
84 previous studies showed differential expression of genes associated with heat
85 stress tolerance between gametophytes and sporophytes (Luo et al., 2014; Uji
86 et al., 2019). In addition to heat stress, generation phase-preferential genes
87 associated with stress response and development have been characterized in
88 *Pyropia/Neopyropia* (Uji et al., 2012; 2013; Inoue et al., 2015; Matsuda et al.,

89 2015; Uji et al., 2022).

90 To achieve the different expression pattern during generation,
91 modifications of chromosome structure appear to be necessary for the
92 transitions between the gametophyte and sporophyte stages of its life cycle.
93 Indeed, the differential expression of subtypes of histones during the
94 generation of Bangiales has been reported previously as well as the
95 identification of putative genes encoding histone-modifying enzymes, such as
96 histone acetyltransferase, deacetylases, histone-lysine N-methyltransferase,
97 histone-arginine N-methyltransferase, and lysine-specific histone
98 demethylase (Chan et al., 2012). However, there is scant evidence indicating
99 a role for chromatin remodeling in the generation transition of red
100 macroalgae.

101 In addition to its economic importance, fossil evidence (Butterfield,
102 2000) and molecular phylogenetic analysis (Sutherland et al. 2011) suggest
103 that Bangiales is an important group for elucidating the primitive
104 mechanisms that regulate gene expression. In this study, N-ChIP method with
105 several modifications based on existing protocols for plant cells (Saleh et al.
106 2008; Huang et al. 2018) has been developed in *N. yezoensis*. This new ChIP
107 method is suitable for the examination of the acetylation of histone H3 at
108 lysine 9 (H3K9ac) in differential expression genes between the gametophyte
109 and sporophyte stages of *N. yezoensis*. The method will increase our
110 understanding of the role of chromatin remodeling for regulating not only life

111 cycles in the ancient lineage but also many physiological processes involved
112 in improve productivity and quality of nori.
113
114 **Materials and Methods**
115 *Algal materials*
116 The leafy gametophytes and filamentous sporophytes of *N. yezoensis* strain
117 TU-1 (Kuwano et al., 1996) were cultured in a medium consisting of sterile
118 vitamin-free Provasoli's enriched seawater (PES; Provasoli, 1968) under a 10
119 h light/14 h dark photoperiod with cool-white, fluorescent lamps at a light
120 intensity of 40 μmol photons m^{-2} s^{-1}.
121
122 *Chromatin extraction and shearing*
123 For chromatin extraction, 0.1 g (FW) of gametophytes or sporophytes were
124 ground in liquid nitrogen with a pestle and mortar. The homogenates were
125 mixed with 50 mL of nuclei isolation buffer [0.25 M sucrose, 15 mM 0.1 M
126 PIPES (pH 6.8), 5 mM $MgCl_2$, 60 mM KCl, 15 mM NaCl, 1 mM $CaCl_2$,
127 0.9% (w/v) Triton X-100] and kept on ice for 10 min, followed by filtering
128 through a nylon mesh (10 μm) to remove cell debris. The filtered
129 homogenates were centrifuged at 6,500 ×g for 20 min at 4°C and the
130 supernatant was discarded for the isolation of nuclei. The nuclei pellets were
131 repeatedly washed with 1 mL of nuclei isolation buffer and then with 1 mL of
132 TE buffer, and centrifuged at 6,500 ×g for 20 min at 4°C. The supernatant

133 was discarded and the pellet was resuspended in 500 μL of micrococcal
134 nuclease buffer [50 mM Tris-HCl (pH 7.9), 5 mM $CaCl_2$] by gently pipetting
135 up and down. The suspension was mixed with 0.5 μL of micrococcal
136 nuclease (MNase, New England Biolabs), 5 μL of 10 mg/ml BSA, and 5 μL
137 of Protease Inhibitor Cocktail Set V (EDTA-free; FUJIFILM Wako Pure
138 Chemical Corporation, Osaka, Japan) and sonicated (40 kHz, 5min, 4°C) by
139 MCS-2 (AS ONE, Osaka, Japan), followed by incubation at 37°C for 15 min.
140 After stopping the reaction with the addition of 2.5 μL of 0.5 M EDTA, the
141 sample was centrifuged at 20,000 ×g for 10 min and the supernatant was
142 transferred to a new 1.5 ml microcentrifuge tube as soluble chromatin. A 5
143 μL aliquot of each sample was set aside for verifying the efficiency of
144 chromatin shearing.
145
146 *Chromatin immunoprecipitation*
147 Before immunoprecipitation, Protein G Mag Sepharose beads (Cytiva,
148 Tokyo, Japan) were rinsed with TE buffer three times, suspended in 1 mL
149 blocking buffer (50 μL of 10 mg/mL sheared salmon sperm DNA
150 (Biodynamics Laboratory Inc, Tokyo, Japan), 50 μL of 10 mg/mL BSA (New
151 England Biolabs), and 900 μL of TE buffer) and gently rotated at 4°C
152 overnight to block the beads. Blocked beads were rinsed with TE buffer and
153 then resuspended with TE buffer equivalent to the amount of beads added.
154 For preclearing, 500 μL of chromatin solution was transferred to a 1.5 mL

155 tube containing 30 μL of blocked beads, 7 μL of Protease Inhibitor Cocktail
156 Set V (EDTA free), and 700 μL of TE buffer and rotated with a tube rotator
157 at 4°C for 2h. After removing the beads using a magnetic stand, 400 μL of
158 pre-cleared chromatin was split into three 1.5 mL tubes (antibody of interest,
159 negative and positive controls) containing 400 μL of TE buffer. Pre-cleared
160 chromatin (10 μL) was kept at 4°C to serve as an 'input' DNA control. For
161 immunoprecipitation, 2 μL (2 μg) of monoclonal antibody (anti-H3K9ac) and
162 2 μL (2 μg) of monoclonal H3 antibody (positive control) were added to each
163 of the three tubes containing pre-cleared chromatin. The other tube without
164 any antibody served as a negative control. All tubes were incubated with
165 gentle rotation overnight at 4 °C. After incubation, 25 μL of newly blocked
166 beads were added to each tube, which were incubated with gentle rotation at
167 4°C for 2 h. After removing the supernatant, the beads were washed three
168 times sequentially with low salt wash buffer [75 mM NaCl, 1 mM EDTA, 10
169 mM Tris-HCl (pH 8.1), 0.1% SDS, 1% Triton X-100]. For eluting the DNA,
170 200 or 190 μL of ChIP elution buffer (1% SDS, 100 mM $NaHCO_3$)
171 containing 1.0 μL of RNase A (100 mg/ml) (NIPPON GENE, Tokyo, Japan).
172 was added to each tube containing beads or chromatin solution for input,
173 respectively, and incubated at 68°C for 2 h. The antibodies were purchased
174 from Monoclonal Antibody Research Institute, Inc. (Nagano, Japan).
175
176 *ChIP-qPCR analysis*

177 For ChIP-qPCR experiments, the eluted DNA was purified using a QIAquick
178 PCR Purification Kit (Qiagen, Hilden, Germany) following the
179 manufacturer's instructions. For each reaction, 1.0 μl of DNA was used as a
180 template in a 20 μL reaction volume containing KOD SYBR® qPCR Mix
181 (TOYOBO, Osaka, Japan). ChIP-qPCR was performed based on the
182 manufacturer's instructions using a LightCycler® 480 System (Roche
183 Diagnostics, Basel, Switzerland) under the following conditions: 2 min at
184 98°C followed by 45 cycles of 10 s at 98°C, 10 s at 55°C, and 30 s at 68°C.
185 ChIP-qPCR data were analyzed to determine the pull-down efficiency
186 relative to the input. Ct values were used for performing the calculation,
187 which consisted of evaluating the fold-difference between the experimental
188 sample and normalized input as follows: ΔCt [normalized ChIP] = (Ct [ChIP]
189 - (Ct [Input] - Log_2 (Input Dilution Factor))). The percentage (Input %) value
190 for each sample was calculated as follows: % Input = $2^{(-\Delta Ct\ [normalized}$
191 $^{ChIP])}$*100. The "Input %" value represents the enrichment of a histone
192 modification in a specific region. qPCR was performed in triplicate. Table S1
193 lists the primers that were used for these analyses.
194
195 *Transcriptional analysis*
196 Total RNA from gametophytes and sporophytes (Fresh weight: 0.05–0.1 g)
197 was separately extracted using the RNeasy Plant Mini Kit (Qiagen, Hilden,
198 Germany) in liquid nitrogen with a mortar and pestle, following the

199 manufacturer's instructions. The extracted RNA was purified using a
200 TURBO DNA-free kit (Invitrogen/Life Technologies, Carlsbad, CA) to
201 obtain DNA-free RNA. First strand cDNA was synthesized from 0.5 μg total
202 RNA using the PrimeScript II First Strand cDNA Synthesis Kit (TaKaRa
203 Bio, Shiga, Japan). The cDNA was diluted 10-fold for qRT-PCR analysis.
204 For each reaction, 1.0 μl of the diluted cDNA was used as a template in a 20
205 μL reaction volume containing KOD SYBR® qPCR Mix. Quantitative RT-
206 PCR was performed as per the manufacturer's instructions using a
207 LightCycler® 480 System under the following conditions: 2 min at 98°C
208 followed by 40 cycles of 10 s at 98°C, 10 s at 55°C and 30 s at 68°C. The
209 mRNA levels were calculated using the $2^{-\triangle\triangle Ct}$ method and normalized to the
210 expression of the 18S ribosomal RNA (*18SrRNA*) gene (Uji et al., 2016).
211 Relative expression levels were calculated as the ratio of the observed mRNA
212 levels present in the gametophytes. The PCR reactions were performed in
213 triplicate. Table S2 lists the primers that were used for these analyses.
214

215 **Results and discussion**

216 *Nuclei isolation from N. yezoensis*

217 To establish the ChIP procedure (Fig. 1), a ChIP-qPCR experiment targeting
218 *18SrRNA* in *N. yezoensis* gametophytes was performed using antibodies to
219 histone H3. Chromatin extracts from homogenates prepared from the
220 gametophytes were filtered through a 10 μm mesh to remove large cell

221 debris. It was also an important step to wash three times with 1 mL of nuclei
222 isolation buffer and TE buffer to remove large amounts of polysaccharides
223 and photosynthetic pigments (e.g., phycobiliproteins), respectively (Fig. 2).
224 The pull-down efficiency of *Ny18SrRNA* against H3 antibody (positive
225 control) and no antibody (mock) using chromatin extracts without washing
226 showed 0.39% and 0.20% relative to the input DNA in the gametophytes,
227 respectively. In contrast, the pull-down efficiency of *Ny18SrRNA* against H3
228 antibody and no antibody using chromatin extracts washed three times with
229 buffer showed 1.78% and 0.06%, respectively. Thus, repeated washes were
230 recommended using buffer before chromatin fragmentation.
231
232 *Chromatin fragmentation*
233 A suitable size distribution of DNA fragments is crucial for ChIP. Thus, the
234 size distribution of genomic DNA was checked by gel electrophoresis after
235 15 min of MNase digestion. Large amounts of chromatin fragments of
236 suitable size (100-250 bp) for further immunoprecipitation were produced by
237 MNase treatment with sonication, which promotes nuclease digestion of
238 samples containing polysaccharides (Fig. 3).
239
240 *Chromatin immunoprecipitation and validation*
241 Next, the quality of the fragmented chromatin and the pull-down efficiency
242 of immunoprecipitation were checked to determine the enrichment of H3.

243 The pull-down efficiency of *Ny18SrRNA* against H3 antibody and no
244 antibody without blocking beads was 4.67% and 1.52% relative to the input
245 DNA in the gametophytes, respectively (Fig. 4). In contrast, the pull-down
246 efficiency of *Ny18SrRNA* against H3 antibody and no antibody using
247 blocking beads showed 1.31% and 0.06% relative to the input DNA in the
248 gametophytes, respectively. The fold positive control relative to mock
249 treatment with and without blocking beads was 3.07- and 21.83-fold,
250 respectively. Finally, in this protocol, we obtained a quantity of DNA that was
251 sufficient to conduct 50 qPCR assays.
252 To test the quality of chromatin and the efficiency of
253 immunoprecipitation, the protocol was applied to study histone modification
254 patterns of the generation-preferential genes in *N. yezoensis*. Based on
255 previous studies (Uji et al., 2012; Inoue et al., 2015; Matsuda et al., 2015),
256 two highly upregulated genes for each generation, gametophyte and
257 sporophyte (Table 1) were selected. As illustrated in Figure 5, the transcripts
258 of *NyBPO* and *NyKPA1* were in high abundance in sporophytes compared
259 with gametophytes. In contrast, the expression of *NyAly* and *NyKPA2* was
260 decreased at sporophyte stages, whereas they were overexpressed in
261 gametophytes. As shown in Fig. 6, ChIP-qPCR exhibited higher levels of
262 H3K9ac enrichment for both the 5'flanking and coding regions in the
263 generation with increased gene expression, with the exception of *NyBPO*.
264 Histone acetylation and methylation are important chromatin modifications

265 that regulate gene expression during development and the response to
266 environmental stress in animals and plants (Loidl 2004; Vastenhouw et al.
267 2012; Ali et al., 2022). In particularly, H3K9ac is closely associated with
268 genes exhibiting high expression levels (Zhou et al., 2010; Kurita et al.
269 2017). In the present study, the strong correlation between the enrichment
270 levels of H3K9ac with transcription was not observed, suggesting that other
271 histone marks, such as histone methylation, highly regulate the
272 transcriptional dynamics of the *N. yezoensis* life cycle. A previous study
273 showed that the induction of *NyKPA1* transcripts occurred rapidly in the
274 gametophytes exposed to cold stress (Uji et al. 2012). Thus, the enrichment
275 of H3K9ac, even in generations with suppressed expression, may be
276 necessary for the synthesis of mRNA in response to environmental stress. In
277 a microalga *Nannochloropsis*, H3K9ac was predominantly enriched in the 5'
278 flanking regions and the distribution was relatively sparse in the exon regions
279 (Wei and Xu 2018). In contrast, a decrease in acetylation in exon regions was
280 not observed in *N. yezoensis* genes in the present study.
281 A previous study showed variations in the daily expression of genes
282 encoding MSI1-like WD40 repeat (MSIL) proteins and SET-domain proteins
283 in the gametophytes of *N. yezoensis* (Kominami et al., 2022). MSIL proteins,
284 which are conserved histone-binding proteins in eukaryotes, function
285 together with histone deacetylases and methyltransferases in mammals and
286 plants (Hennig et al. 2005). SET-domain proteins methylate lysine residues in

287 histone tails and play a fundamental role in the epigenetic regulation of gene
288 activation and silencing in all eukaryotes (Rea et al. 2000). Thus, our ChIP
289 method can elucidate the role of histone post-translational modifications
290 associated with diurnal rhythm regulation in *N. yezoensis*.
291 In addition to histone modifications, epigenetic studies have revealed
292 that DNA methylation and non-coding RNAs can alter the configuration of
293 chromatin, which results in various chromatin states that epigenetically
294 regulate transcriptional outputs (Ahmad et al. 2010; Skvortsova et al. 2018).
295 To date, DNA methylation patterns during heat stress (Yu et al. 2018) and
296 noncoding small RNAs in generations as well as under osmotic stress (He et
297 al. 2012; Cao et al., 2019) has been identified in Bangiales. However,
298 knowledge of epigenetic regulation of gene expression in red algae remains
299 scant. Future studies clarifying the link among epigenetic regulators will
300 provide insight into the role of primitive gene expression systems.
301
302 Conclusion
303 A ChIP method based on ChIP-qPCR, which can determine the histone
304 modification status at different genomic regions has been developed in *N.*
305 *yezoensis*. This method may be used for the analysis of the genome-wide
306 distribution of specific histone modifications combined with high-throughput
307 sequencing, such as ChIP-seq analyses. Thus, the method described here is an
308 essential tool for elucidating the role of the “histone code” for many

physiological processes involved in improve productivity and quality of nori.

Acknowledgments

We wish to thank Drs. Katsutoshi Arai, Takafumi Fujimoto, and Toshiya Nishimura (Hokkaido University, Japan) for kindly providing the LightCycler 480 system.

Conflict of interest

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Funding

This work was supported by the Grant-in-Aid for Young Scientists [grant number19K15907 and 22K05779 to TU] from the Japan Society for the Promotion of Science.

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Figure legends

Fig. 1. Outline of the ChIP-qPCR protocol in *Neopyropia yezoensis*.

500 **Fig. 2.** Optimization of Chromatin extraction.

501 After filtering, chromatin extracts were not washed with any buffer (a),
502 washed three times with only nuclei isolation buffer (b), or with both nuclei
503 isolation buffer and TE buffer (c). The chromatin solution was treated with
504 sonication and MNase. ChIP-qPCR experiment targeting the *Ny18SrRNA*
505 gene was performed with an antibody specific to histone H3. Relative
506 amounts of the PCR products were calculated and normalized with respect to
507 the input chromatin. The results are presented as % Input (IP). Mock
508 indicates the signals from the no antibody controls. The data are presented as
509 means ± standard deviations (n = 3).

510

511 **Fig. 3.** Optimization of Chromatin shearing conditions.

512 After chromatin extraction, chromatin extracts were repeatedly washed three
513 times with nuclei isolation buffer and then with TE buffer. For shearing, the
514 chromatin solution pretreated without or with sonication was incubated with
515 MNase at 37°C for 15 min. M: DNA marker.

516

517 **Fig. 4.** Optimization of Chromatin immunoprecipitation.

518 After chromatin extraction, chromatin extracts were repeatedly washed three
519 times with nuclei isolation buffer and then with TE buffer. Then, the
520 chromatin solution was treated with sonication and MNase. The ChIP-qPCR
521 experiment targeted the *Ny18SrRNA* gene with an antibody specific to

522 histone H3, without blocking beads (a) or with blocking beads (b).

523

524 **Fig. 5.** Relative expression levels of genera genes from *Neopyropia yezoensis*

525 in gametophytes and sporophytes. RNA samples were prepared from

526 gametophytes (GA) and sporophytes (SP), and expression levels were

527 determined using the *Ny18SrRNA* gene for normalization. Relative

528 expression levels were calculated as the ratio of the observed mRNA level

529 present in the gametophytes. The data are presented as means ± standard

530 deviations (n = 3).

531

532 **Fig.6.** ChIP-qPCR analysis of generation-preferential genes in *Neopyropia*

533 *yezoensis*.

534 (a) Location of amplicons used in the ChIP-qPCR analysis. The boxed

535 regions indicate part of the coding sequence. The amplified sequences are

536 indicated by bars.

537 (b) The pull-down efficiencies of H3K9ac by ChIP-qPCR analysis

538 Chromatin was isolated from gametophytes (GA) and sporophytes (SP) and

539 ChIP-qPCR was targeted to *NyBPO*, *NyKPA1*, *NyAly* and *NyKPA2* using an

540 antibody specific to histone H3K9ac. Chromatin extracts were repeatedly

541 washed three times with nuclei isolation buffer and then with TE buffer.

542 Then, the chromatin solution was treated with sonication and MNase. The

543 relative amounts of PCR products were calculated and normalized with

544 respect to the input chromatin. The results are represented as % Input (IP).

545 Mock indicates the signals from the no antibody controls. The data are

546 presented as means ± standard deviations (n = 3).

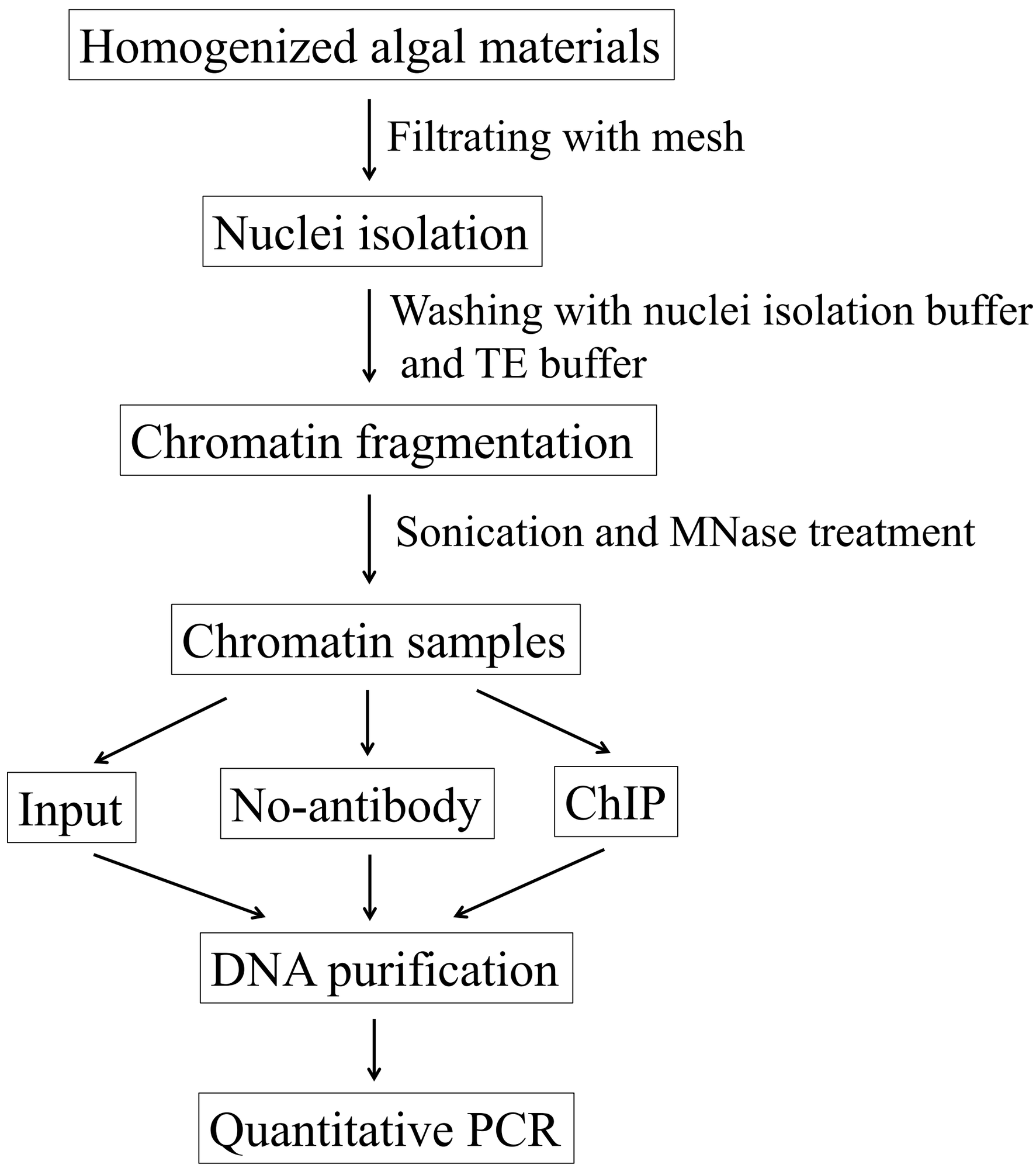
Homogenized algal materials
Filtrating with mesh
Nuclei isolation
Washing with nuclei isolation buffer
and TE buffer
Chromatin fragmentation
Sonication and MNase treatment
Chromatin samples
Input
No-antibody
ChIP
DNA purification
Quantitative PCR

Fig.1

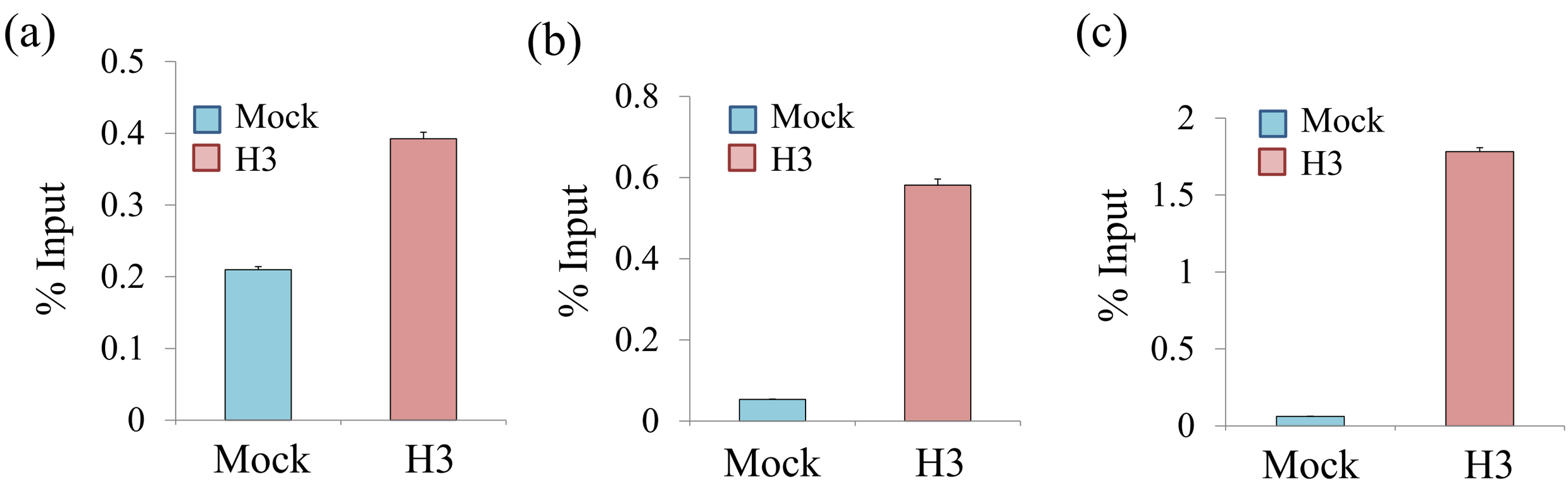
(a)
0.5
0.4
0.3
0.2
0.1
0
% Input
Mock
H3
Mock
H3
(b)
0.8
0.6
0.4
0.2
0
% Input
Mock
H3
Mock
H3
(c)
2
1.5
1
0.5
0
% Input
Mock
H3
Mock
H3

Fig.2

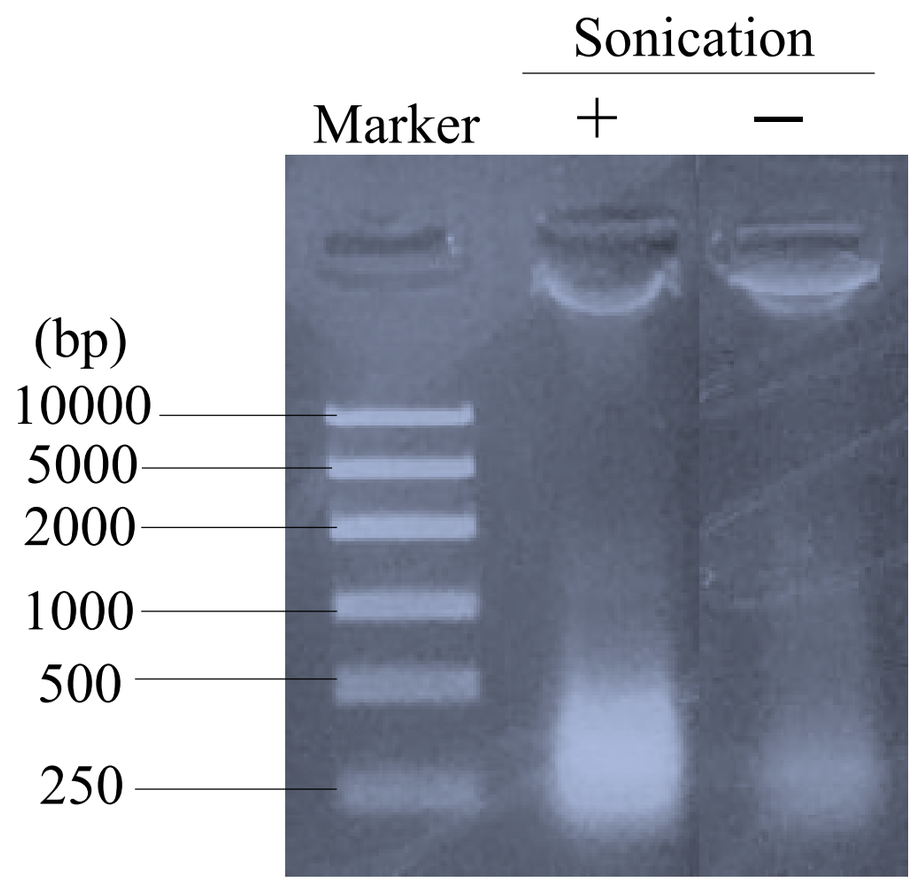

Fig.3

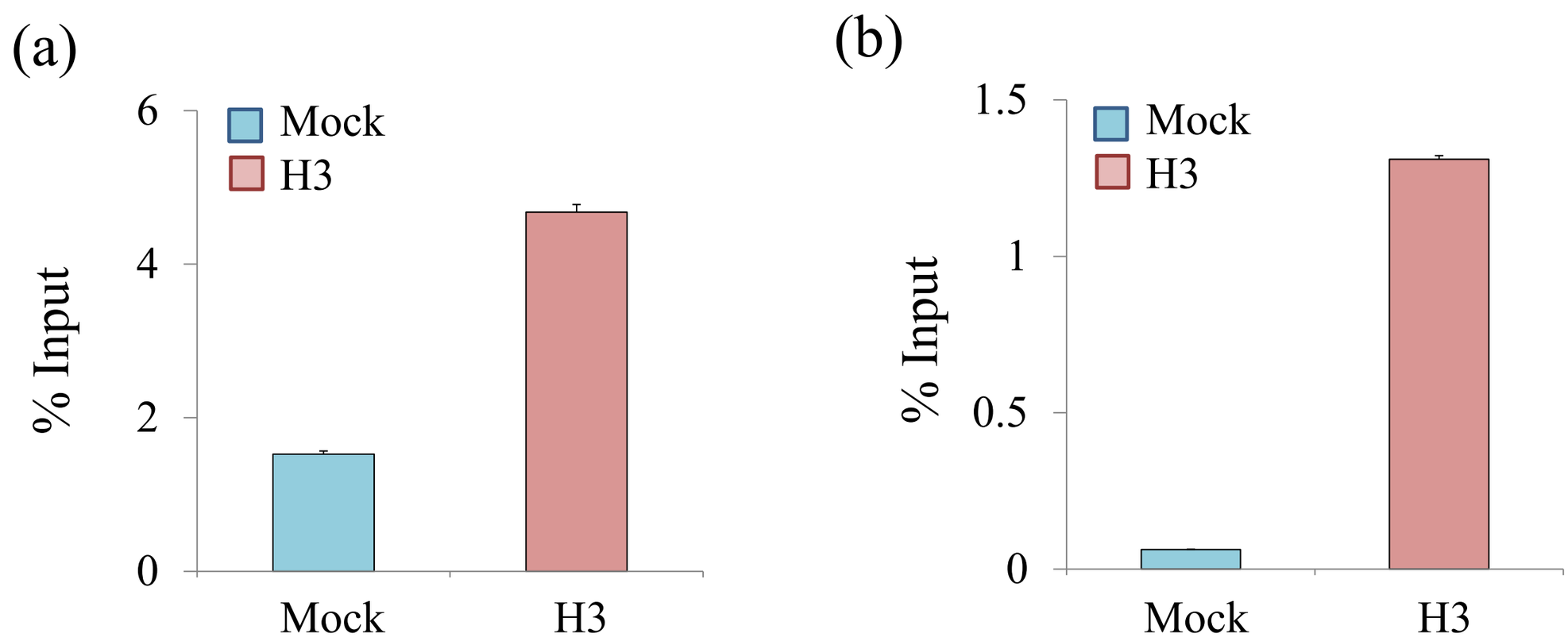

Fig.4

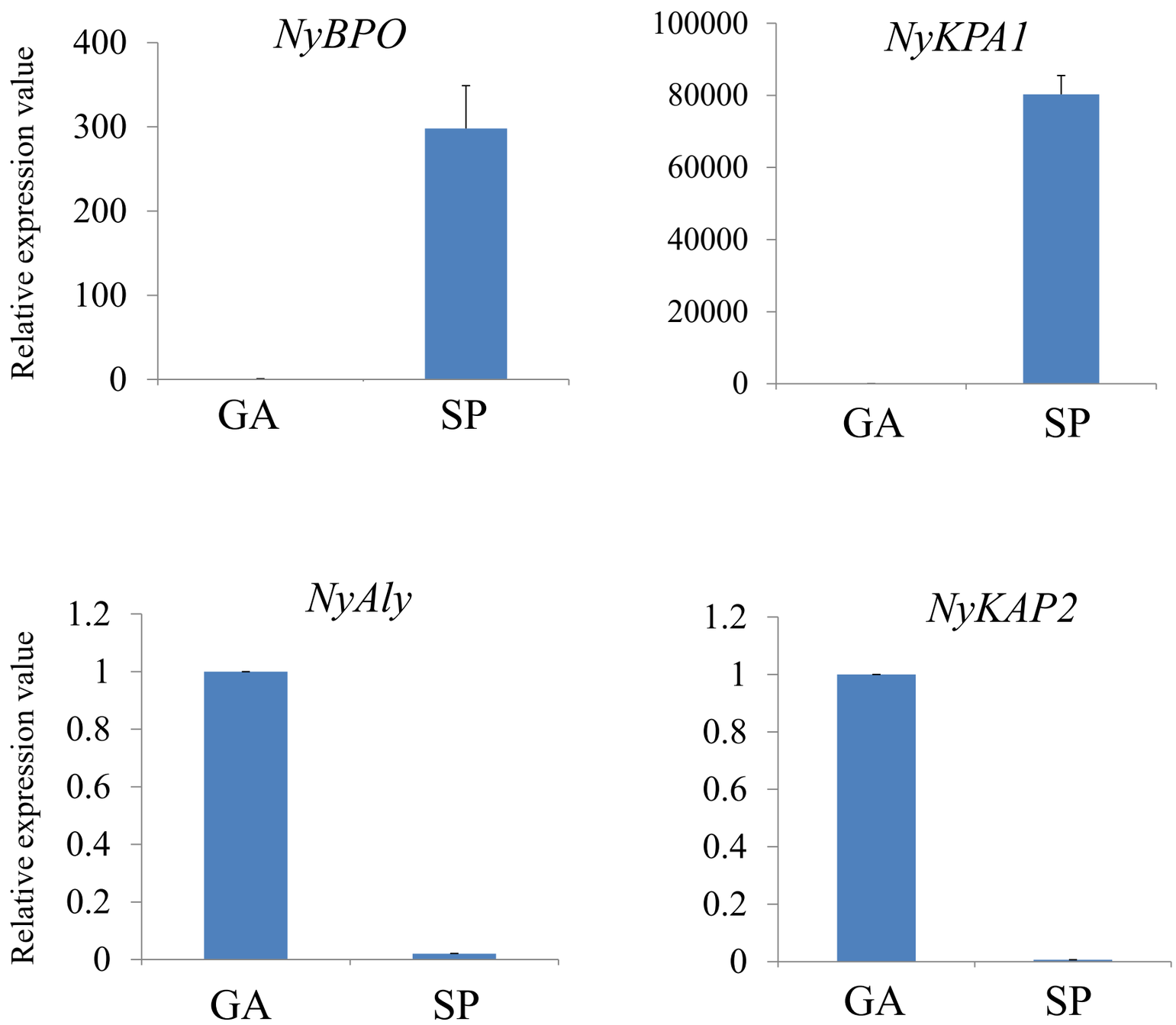
NyBPO
Relative expression value
400
300
200
100
0
GA
SP
NyKPA1
100000
80000
60000
40000
20000
0
GA
SP
NyAly
Relative expression value
1.2
1
0.8
0.6
0.4
0.2
0
GA
SP
NyKAP2
1.2
1
0.8
0.6
0.4
0.2
0
GA
SP

Fig.5

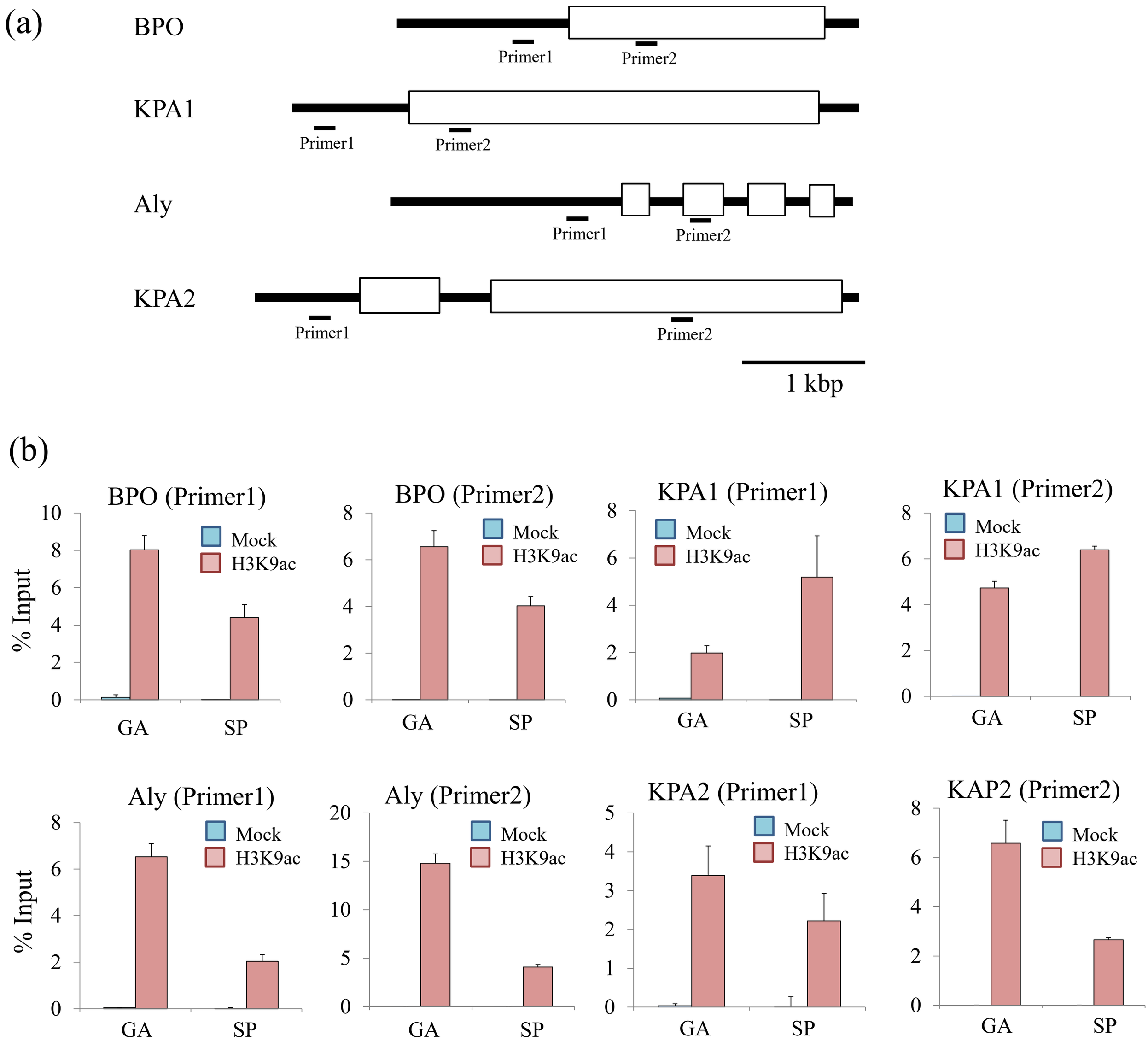
(a)
BPO
Primer1
Primer2
KPA1
Primer1
Primer2
Aly
Primer1
Primer2
KPA2
Primer1
Primer2
1 kbp
(b)
BPO (Primer1)
BPO (Primer2)
KPA1 (Primer1)
KPA1 (Primer2)
Aly (Primer1)
Aly (Primer2)
KPA2 (Primer1)
KAP2 (Primer2)
% Input
Mock
H3K9ac
GA
SP

Fig.6