OXFORD

BIOLOGY Methods & Protocols

Biology Methods and Protocols, 2025, **10(1)**, bpaf007

https://doi.org/10.1093/biomethods/bpaf007

Advance Access Publication Date: 24 January 2025

Methods Article

An improved cell nuclear isolation method

Pengfei Li[1,2], Jingyao Zhang[3], Xiaojuan Liu[1,4], Zhijuan Wu[5], Y. James Kang[1], Wenjing Zhang[6,*]

[1]Regenerative Medicine Research Center, Sichuan University West China Hospital, Chengdu 610041, China
[2]Key Laboratory of Molecular Pathology, Inner Mongolia Medical University, Huhhot 010059, China
[3]Core Facilities of West China Hospital, Sichuan University, Chengdu 610041, China
[4]Institute of Medical Biology, Chinese Academy of Medical Sciences and Peking Union Medical College, Kunming 650118, China
[5]Key Laboratory of Qinghai-Tibetan Plateau Animal Genetic Resource Reservation and Utilization, Sichuan Province and Ministry of Education, Southwest Minzu University, Chengdu 610041, China
[6]Department of Pathology and Laboratory Medicine, College of Medicine, University of Tennessee Health Science Center, Memphis, TN 38163, United States

*Corresponding author. Department of Pathology and Laboratory Medicine, College of Medicine, University of Tennessee Health Science Center, 3 N. Dunlap Street, Van Vleet Building C329, Memphis, TN 38163, United States. Tel: +1 9015030686; E-mail: wzhang67@uthsc.edu.

Abstract

Nuclear isolation is crucial for studying gene expression and regulatory mechanisms in eukaryotic cells. This study aimed to improve nuclear isolation and compare the yield, purity, and efficiency of several methods. Human umbilical vein endothelial cells were used to evaluate four different techniques: sucrose centrifugation, a simplified method, homogenization, and the NE-PER kit. For sucrose centrifugation, cells were scraped in Tween buffer, washed with sucrose buffer, and homogenized in a Dounce homogenizer. The pellet was washed with glycerol buffer to isolate the nuclei. In the simplified method, cells were scraped in scraping buffer, washed with sucrose buffer, and the pellet was washed with glycerol buffer to isolate the nuclei. For homogenization, cells were washed with phosphate buffered saline, followed by two washes in extract buffer and lysed with 10 strokes in a Kontes Dounce homogenizer. The NE-PER kit was used according to the manufacturer's protocol. Nuclei isolated by each method were tested by immunoblotting, co-immunoprecipitation (Co-IP), and chromatin immunoprecipitation (Ch-IP) assays. The simplified method produced nuclei with fewer organelles and less cytoplasm than those isolated by homogenization or the NE-PER kit. It was similarly effective as sucrose centrifugation but faster. Co-IP and Ch-IP assays confirmed that the simplified method enriched target proteins and DNA fragments. Overall, the simplified method provides a highly pure nuclear sample optimal for downstream applications requiring purified nuclei.

Keywords: cell nuclear isolation; co-immunoprecipitation; chromatin immunoprecipitation

Introduction

The genetic material of eukaryotes is mainly concentrated in the nucleus. Nuclear extraction technology is becoming increasingly important to study gene expression and regulatory mechanisms in eukaryotes. Many transcription factors, such as hypoxia-inducible factor 1 (HIF-1), P65, and STAT [1], are present in the cytoplasm and translocate into the nucleus when the cell obtains a signal. HIF-1, a transcription factor that regulates cellular responses to hypoxia, is composed of HIF-1α and HIF-1β. Under hypoxic conditions, HIF-1α accumulates in the cytosol and is translocated into the nucleus [2]. Then, the two subunits combine to form HIF-1, which binds hypoxia-responsive element (HRE) sites in target genes. This leads to the transactivation of target gene expression [3–5]. In the NF-κB signaling pathway, IκBs are phosphorylated and degraded through ubiquitination, and the free active form of the p50/p65 complex is transported to the nucleus [6–8]. To study the interactions between molecules in the nucleus, nuclear isolation methods are widely used in several experiments, including co-immunoprecipitation (Co-IP) assay and chromatin immunoprecipitation (Ch-IP) assay [9]. In Co-IP assays of proteins in the nucleus, nuclear samples are used, as they contain a larger percentage of nuclear proteins than whole-cell samples. Additionally, nuclear samples are not affected by the cytoplasmic proteins. In Ch-IP assays, nuclear samples are used, as they contain lower levels of inactivated proteins than the whole-cell samples. And we can enrich more target DNA fragments with nuclear samples. Therefore, nuclear samples have a better performance in Co-IP for nuclear proteins such as transcriptional factors and Ch-IP assays.

In previous research, cell nuclei were isolated by centrifugation with a sucrose buffer or lysed by a homogenizer. The sucrose centrifugation method used in this study is a part of the PICh protocol [10]. We can obtain a pure nuclear sample with this method, but this method is time-consuming [11]. Some proteins may be degraded over the long isolation process. The homogenization method takes a very short time, but the nuclear samples isolated by this method usually contain cytoplasm. In the homogenization method, different cell lines require different times of homogenization, and the homogenates should be checked with a microscope [12]. Cell nucleus extraction kits are user-friendly but costly. However, no studies have compared different methods of nuclear isolation. In this study, we improved the sucrose centrifugation method and named it the Simplified Method. We then compared the performance of nuclei isolated by different methods in Co-IP and Ch-IP assays.

Received: 12 December 2024; **Revised:** 17 January 2025; **Editorial decision:** 20 January 2025; **Accepted:** 22 January 2025

Materials and methods

Cell culture and treatment

Human umbilical vein endothelial cells (HUVECs) were purchased from American Type Culture Collection (ATCC). Cells were cultured with DMEM (Gibco) containing 10% FBS (Gibco) and 1% penicillin/streptomycin. To induce HIF-1α nuclear localization, cells were incubated with 100 μM $CoCl_2$ for 22 h as we described before [13–15].

Sucrose centrifugation method

Cells in a 150 mm dish were washed twice with phosphate buffered saline (PBS) and collected with a scraper. The cell mass was resuspended in 3 mL of Tween buffer (PBS, 0.05% Tween-20) and centrifuged at 1500 rpm for 5 min. Cell pellets were resuspended in PBS with 1 mM PMSF and centrifuged (3200×*g* for 10 min) for four times. After removal of the PBS-PMSF supernatant, the cell pellets were either stored at −80°C or processed further.

The cell pellets were washed with a sucrose solution (0.3 M sucrose, 10 mM HEPES-NaOH pH 7.9, 1% Triton-X100, 2 mM MgOAc, 1 mM PMSF) and centrifuged at 3200×*g* for 10 min. Then, the cell pellets were resuspended in 2 ml of a sucrose solution, transferred to a precooled homogenizer kept on ice and homogenized 50 times using a tight pestle. The cell pellets were transferred to a new tube, and homogenizer was washed two times with 1 ml of a sucrose solution to reduce sample loss. The mixture was centrifuged at 3200×*g* and 4°C for 10 min. The pellets were resuspended in glycerol buffer (25% glycerol, 10 mM HEPES-NaOH pH 7.9, 0.1 mM EDTA, 5 mM MgOAc) and centrifuged at 3200×*g*, for 10 min at 4°C. The precipitate contained the nuclei.

The simplified method

The Simplified Method uses scrapping buffer and does not require a homogenization step.

Cells from a 150 mm culture dish were collected when they reached 80% confluency. They were washed twice with PBS and then harvested using a scraper. The cell mass was resuspended in 2 mL of PBS per 150-mm dish and centrifuged at 1500 rpm for 5 min. After removal of the supernatant, the cell pellets were either stored at −80°C or processed further.

The cell pellets were resuspended in 1 ml of scrapping buffer (PBS, 0.1% Triton-X100) and let stand on ice for 10 min. The mixture was centrifuged at 3200×*g* and 4°C for 10 min. The cell mass was washed in PBS with 1 mM PMSF and centrifuged (4°C, 3200×g for 10 min) two times. Then, the pellets were resuspended in 1.2 ml of a sucrose solution (0.3 M sucrose, 10 mM HEPES-NaOH pH 7.9, 1% Triton-X100, 2 mM MgOAc, 1 mM PMSF) and centrifuged (4°C, 3200×g for 10 min) two times. The pellets were resuspended in 1 mL of a glycerol solution and centrifuged at 3200×*g* and 4°C for 10 min. The precipitate contained the nuclei.

Differences between sucrose centrifugation method and the simplified method

The main differences between the two methods are scrapping buffer and homogenization step. In cell pellets collection step, The Simplified Method used scrapping buffer (PBS, 0.1% Triton-X100) instead of Tween buffer (PBS, 0.05% Tween-20). 0.1% Triton-X100 is used to perforate cell membrane and have a better performance than 0.05% Tween-20. Let the cell pellets in scrapping buffer for 10 min is very necessary to make full contact. Additionally, the Simplified Method eliminates the homogenization step entirely. This reduction in steps not only saves a significant amount of time but also yields the similar result.

Homogenization method

Cells were washed twice with PBS and collected with a scraper. The cell mass was resuspended in 2 ml of PBS per 150-mm dish and centrifuged at 1500 rpm for 5 min. After removal of the supernatant, the cell pellets were either stored at −80°C or processed further.

Subsequent steps were performed at 4°C. The cell pellets were then suspended in five volumes of 4°C PBS and collected by centrifugation as described above. The cells were suspended in five packed cell pellet volumes of extract buffer (10 mM HEPES, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl and 0.5 mM DTT) and allowed to stand for 10 min. The cells were collected by centrifugation as described before, suspended in two packed cell pellet volumes of extract buffer and lysed by 10 strokes of a Kontes all-glass Dounce homogenizer (B-type pestle). The homogenate was checked by microscopy for cell lysis and centrifuged for 10 min at 2000 rpm to pellet the nuclei. The pellet obtained from the low-speed centrifugation of the homogenate was subjected to a second round of centrifugation for 20 min at 25 000×*g*, to remove residual cytoplasmic material, and this pellet was designated the nuclear sample.

Use of the NE-per nuclear and cytoplasmic extraction reagents kit

Cell nuclear extraction was performed according to the instructions of the kit (Thermo, 7833) manufacturer.

Western blotting

Whole-cell and nuclear pellets were lysed with NP-40 lysis buffer (Invitrogen, FNN0021), containing a 1% complete EDTA-free protease inhibitor mixture. Cell nuclear lysis required a sonication step. The samples were lysed on ice for 30 min and mixed by vortexing every 10 min, followed by sonication (five cycles of 2 s on and 8 s off). The supernatant was collected by centrifugation (12 000 rpm for 10 min). Protein quantification was performed with a BCA protein assay. Western blot experiments were performed according to we reported before [13]. Antibodies against the following were used for Western blotting: HIF-1α (R&D System, AF1935), HIF-1β (CST, 5537), calnexin (Abcam, ab133615), ATPB (Abcam, ab170947), GAPDH (ZSGB-BIO, TA-09), β-actin (ZSGB-BIO, TA-08) and histone H3 (Millipore, 05-1341).

Co-IP assays

The whole-cell sample and cell nuclear samples were lysed with the method described above. The samples were immunoprecipitated using IgG or HIF-1α antibody (R&D System, AF1935). Antibody-protein complexes were captured with protein G magnetic beads (Thermo 10003D Dynabeads). Co-IP assays were performed as we reported previously [16].

Ch-IP assays

The samples were incubated at 37°C for 15 min in 1% formaldehyde, and the reaction was terminated by addition of a glycine solution (0.125 M). The nuclei were lysed in nuclear lysis buffer (50 mM Tris, pH 8.0, 10 mM EDTA, 1% SDS) containing a 1% complete EDTA-free protease inhibitor mixture. Ch-IP assays were performed according to standard procedure [13]. The samples were immunoprecipitated using IgG or HIF-1α antibody (R&D System, AF1935). After Ch-IP assays, the enrichment of specific DNA fragments was examined by quantitative PCR and analyzed relative to the IgG level using the $2^{-\Delta\Delta CT}$ method.

Primers were designed according to the HRE site sequences as follows: forward, 5′-CCTCAGTTCCCTGGCAACATCTG-3′ and

reverse, 5′-GAAGAATTTGGCACCAAGTTTGT-3′ for the HRE site of *VEGF*; forward, 5′-GGCCGCTTCCCTGCACGTC-3′ and reverse, 5′-GCCGGGTTCTCCTTTGAAGGG-3′ for the HRE site of *BNIP3*.

Statistical analysis

At least three experiments were carried out, and the data from each experiment are expressed as the mean values ±SD (Student's *t* test, $^*P < .05$). A *P*-value < .05 indicated statistical significance.

Results

Comparison of the sucrose centrifugation method and the Simplified Method

First, we observed the nuclei obtained with both sucrose centrifugation method and the simplified method under a microscope. The morphology of the cell nuclei obtained by sucrose centrifugation method did not significantly differ with the use of different homogenization times. The nuclei isolated by the simplified method and those isolated by sucrose centrifugation exhibited the same morphology (Fig. 1A). To assess the purity of the nuclear fractions, Western blot analysis with antibodies against the following different organelle markers was performed: calnexin (an endoplasmic reticulum marker) [17], ATPB (a mitochondrial marker) [18], GAPDH (a cytoplasm marker) [13], and β-actin (an internal control). The nuclei isolated by these two methods contained a very small amount of cytoplasm and few organelles (Fig. 1B). The HIF-1α protein accumulated in the nucleus, and its concentration was higher than that in whole-cell samples from HUVECs that were treated with $CoCl_2$ [19]. In summary, sucrose centrifugation and the Simplified Method showed similar performances (Fig. 1B and C). However, the 80 min required for the Simplified Method was faster than the 160 min for the sucrose centrifugation method (Table 1).

Nuclear samples isolated by the Simplified Method were purer than those isolated by other isolation methods

We then compared our Simplified Method with two other commonly used methods, homogenization and the use of a kit. The nuclear samples isolated by homogenization still contained a large amount of calnexin and ATPB. Compared to the GAPDH level in the whole-cell proteins, the nuclear proteins isolated by homogenization contained less GAPDH. This indicated that the nuclear samples contained a high concentration of organelles. The nuclear samples isolated by the Simplified Method contained a small amount of ATPB, and calnexin and GAPDH were undetectable, indicating that the Simplified Method removed most of the cytoplasm and organelles. The nuclear samples isolated with a kit contained a small amount of ATPB and a large amount of GAPDH, and calnexin was undetectable. This indicated that the kit removed the organelles well, but a large amount of cytoplasm remained. Notably, the histone H3 protein levels in the nuclear samples isolated by homogenization and the Simplified Method were higher than that in the whole-cell sample (Fig. 2A and B). This is because nuclear proteins make up only a small fraction of the total protein in a whole cell.

The effect of nuclear samples isolated with different methods in Co-IP assays

HIF-1α dimerizes with HIF-1β to form HIF-1 during $CoCl_2$ treatment for processing [19]. A Co-IP assay using an anti-HIF-1α antibody was performed. We examined whether the HIF-1 complex was intact and the complex enrichment level by Western blot experiments with anti-HIF-1α and anti-HIF-1β antibodies. HIF-1α and HIF-1β were more enriched in samples obtained with the Simplified Method (labeled as Sucrose Centrifugation method in the Fig. 3) and the kit compared to samples obtained with homogenization and whole-cell samples (Fig. 3).

The effect of nuclear samples isolated by different methods in Ch-IP assays

Cells were exposed to $CoCl_2$ to increase HIF-1α accumulation. A Ch-IP assay was carried out with an anti-HIF-1α antibody to measure HIF-1-DNA binding in cells. Transcription factor and target gene HRE site enrichment were detected by ChIP-PCR [13]. BNIP3-HRE DNA fragments were more enriched in nuclear samples isolated by the Simplified Method with a kit. Compared with the whole-cell sample, the nuclear sample isolated by homogenization was enriched in BNIP3-HRE DNA fragments, but the level was lower than that with the two other isolation methods. The nuclear samples were more enriched in VEGF-HRE DNA fragments with the simplified method (Fig. 4).

Discussion

Determining the molecular mechanisms of nuclear proteins such as transcription factors has become increasingly important. In studies of nuclear proteins, the nuclear isolation process can effectively exclude disturbance from cytoplasmic proteins. Additionally, this process allows more functional nuclear proteins to be obtained. These advantages help protein–protein and protein–DNA studies of nuclear proteins. HIF-1, a transcription factor that regulates cellular responses to hypoxia, is composed of HIF-1α and HIF-1β. Under hypoxic conditions, HIF-1α accumulates in the cytosol and is translocated into the nucleus [2]. Then, the two subunits combine to form HIF-1, which binds HRE sites in target genes [3, 13, 14, 20]. BNIP3 and VEGF are two classic target genes of HIF-1 [15, 21–23]. Our goal is to evaluate the impact of different nuclear extraction methods on protein–protein interactions (CoIP) and protein–nucleic acid interactions (ChIP) through the assessment of HIF1 protein, ultimately selecting the optimal method for downstream analyses.

Sucrose centrifugation for human cell nuclear isolation was optimized. We named the new method the Simplified Method, which involves scrapping buffer containing Triton-X100, showed better performance in perforating the cell membrane. Under the conditions tested, the sucrose solution showed better extraction performance. The cell nuclear morphology did not show a significant difference with the use of different homogenization times when sucrose centrifugation was used. The Simplified Method removed the homogenate step, resulting in substantial time savings (Table 1) and a reduced the risk of protein degradation. Both the Simplified Method and the sucrose centrifugation method can isolate pure nuclei with a very small amount of endoplasmic reticulum, mitochondria and cytoplasm. Nuclear isolation by both methods yielded higher HIF-1α protein levels compared with whole-cell samples. This indicates that nuclear isolation could increase the levels of nuclear proteins for follow-up experiments.

Comparing the purity of nuclear samples isolated by homogenization, with the Simplified Method and with a kit, we found that the Simplified Method showed the best performance. We removed almost all of the endoplasmic reticulum and cytoplasm when the simplified method was applied. Very little mitochondrial residue remained in the isolated nuclear samples. This may

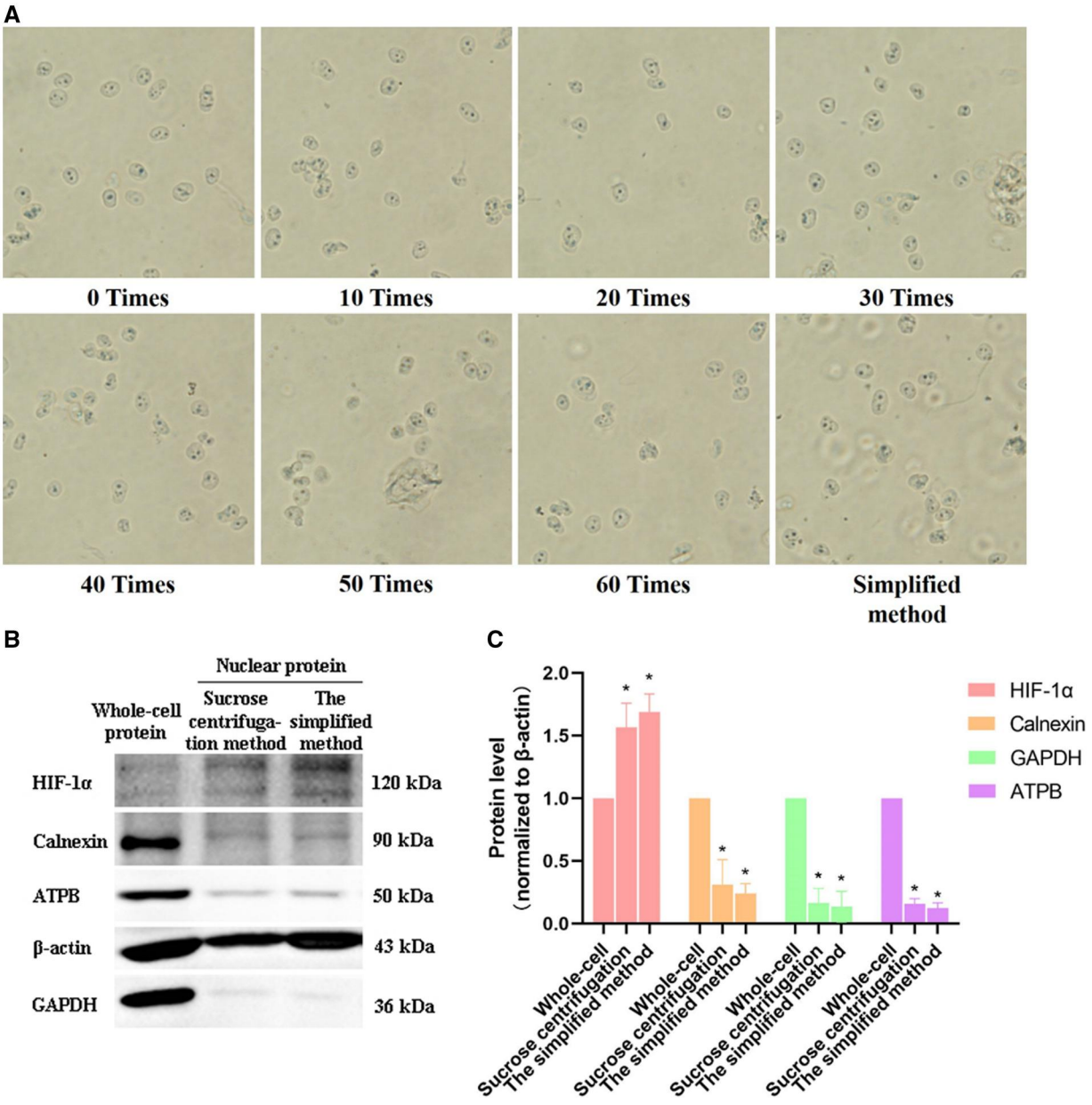

Figure 1. Comparison of the purity of nuclei isolated with sucrose centrifugation and the simplified method. (**A**) HUVECs were homogenized for different durations. The nuclear morphology was observed under a microscope. (**B**) HUVECs were treated with $CoCl_2$ at a final concentration of 100 μM for 22 h. The purity of the isolated nuclei was tested by immunobloting for calnexin, ATPB and GAPDH as endoplasmic reticulum, mitochondrial and cytoplasmic markers, respectively. β-Actin was used as a reference in the experiments. (**C**) Statistical analysis of the Western blotting results shown in (B). *Significantly different from the whole-cell sample ($P < .05$)

Table 1. Comparison of different nuclear isolation methods.

Method	Time (min)	Cytoplasm extraction capacity	The contamination in nucleus			Co-IP assay	Ch-IP assay
			Endoplasmic reticulum	Mitochondria	Cytoplasm		
Sucrose centrifugation method	160	No	-	+	-	/***	/***
Simplified method	80	No	-	+	-	**	*
Homogenate method	90	No	++	++	+	***	**
NE-PER Kit	120	Yes	-	++	++		

+ Protein detected.
++ More protein detected.
*Good effectiveness/performance.
**Better effectiveness/performance.
***Excellent effectiveness/performance.

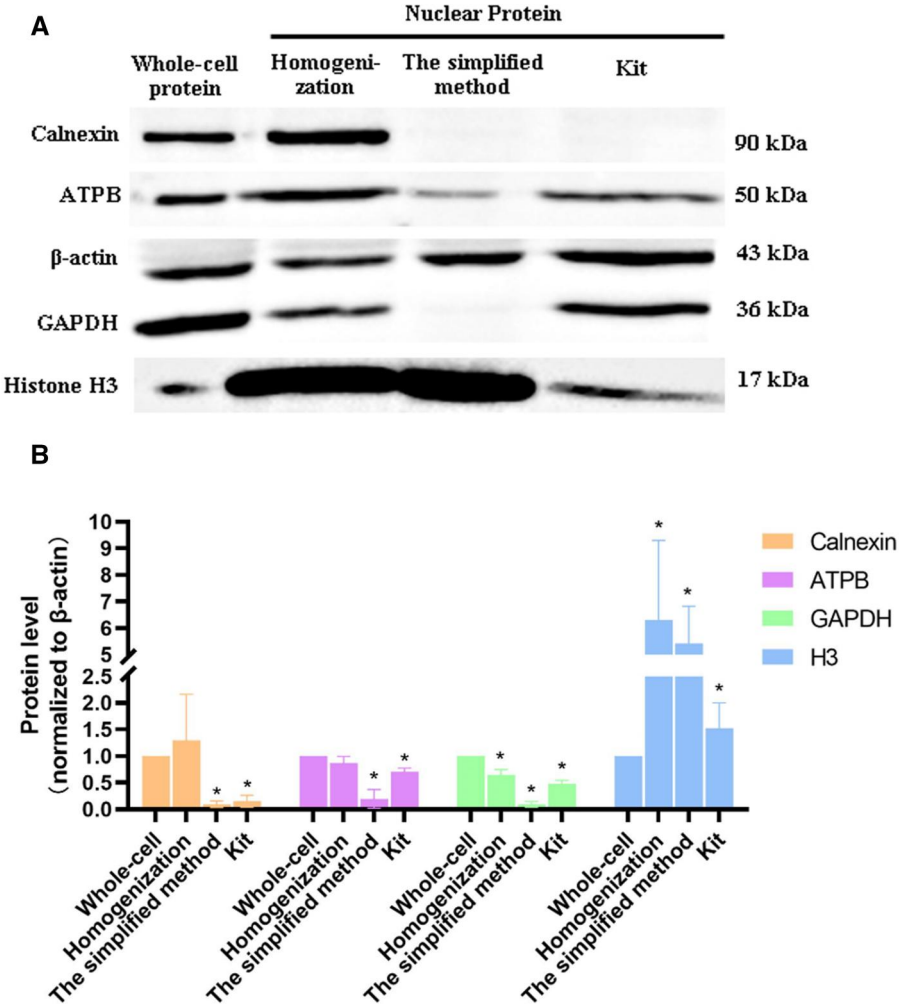

Figure 2. Nuclear samples isolated with diffident nuclear isolation methods. (**A**) The purity of the isolated nucleus was tested using calnexin, ATPB and GAPDH as endoplasmic reticulum, mitochondrial and cytoplasmic markers, respectively. Histone H3 is a nuclear marker. β-actin was used as a reference. (**B**) Statistical analysis of the Western blotting results shown in (B). *Significantly different from the whole-cell sample ($P < .05$)

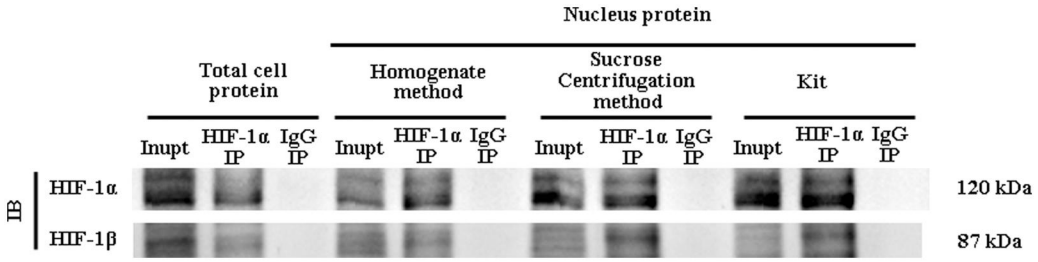

Figure 3. Co-IP assay with the use of different nuclear isolation methods. Whole-cell proteins and nuclear proteins were compared in a Co-IP assay. HUVECs were treated with $CoCl_2$ at a final concentration of 100 μM for 22 h. The nuclei were isolated by three diffident methods. The input was the cell lysate or nuclear lysate before immunoprecipitation. HIF-1α IP is the protein immunoprecipitated with an anti-HIF-1α antibody. IgG IP is the protein immunoprecipitated with goat IgG

be because the viscous sucrose buffer retained the mitochondria. The nuclei isolated by homogenization contained lots of endoplasmic reticulum and mitochondria compared with those in whole-cell samples from which the cytoplasm had been removed. However, the higher histone H3 protein level indicated that homogenization also increased the abundance of nuclear proteins. We obtained nuclear samples and cytoplasmic samples with an NE-PER nuclear and cytoplasmic extraction kit. However, the nuclear sample contained little contamination from mitochondria and cytoplasm. The histone H3 protein level in the whole-cell samples was lower than that in the nuclear samples because nuclear proteins constitute only a small percentage of the protein in a whole cell. Notably, every band in the Kit group is broader in Fig. 2A. This may be due to the higher salt concentration in the Kit's lysis buffer, which can sometimes lead to protein precipitation, impacting band quality [24].

In Co-IP assays, we detected higher levels of HIF-1β in nuclei isolated by the Simplified Method and with a kit. This result is

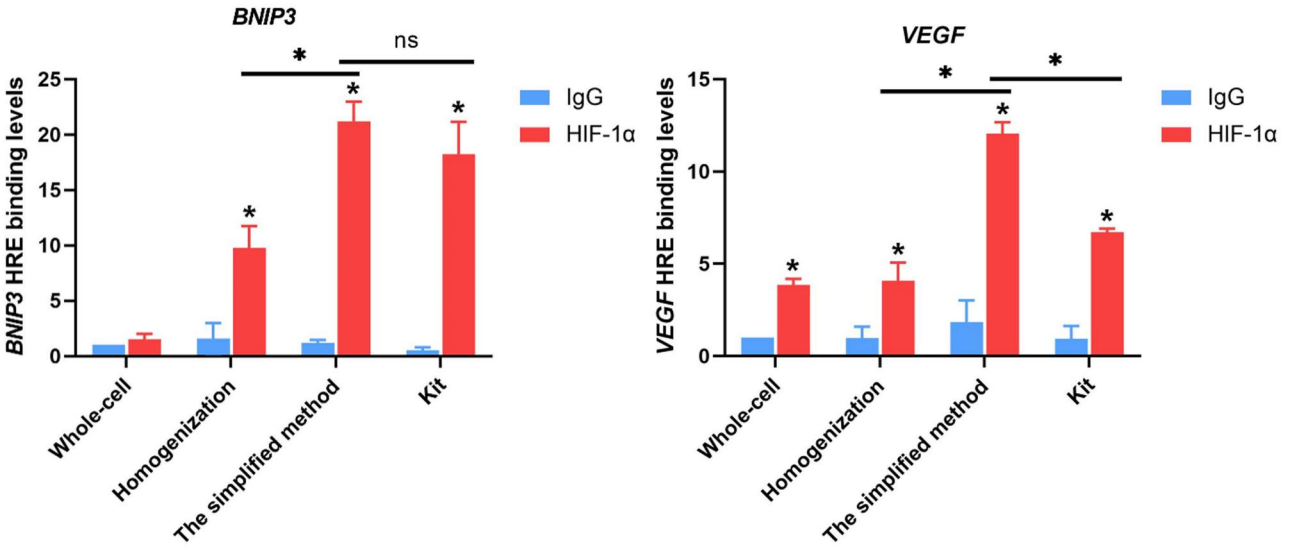

Figure 4. Ch-IP assay measuring the *BNIP3* and *VEGF* HRE regions with different nuclear isolation methods. HUVECs were treated with $CoCl_2$ for 22 h at a final concentration of 100 μM. The nuclei were isolated by three different methods. Anti-HIF-1α (R&D System, AF1935) antibodies and goat IgG were used for immunoprecipitation. *Significantly different from the IgG sample ($P < 0.05$)

consistent with the results of western blotting to assess nuclear sample purity. This is probably because HIF-1α molecules in the cytoplasm were not bound to HIF-1β. For Co-IP assays using antibodies against HIF-1α, the enrichment in the protein complex would be impacted with free HIF-1α in whole-cell sample and an "impure" nuclear sample. A similar result was also found in the Ch-IP assays. Target nucleic acids were better enriched in the Ch-IP assays with pure nuclear samples. The presence of organelles and cytoplasm may affect the efficiency of Ch-IP assays. Compared with the whole-cell sample, the nuclear samples isolated by each method showed better performance in terms of target nucleic acid enrichment. These results indicated that nuclear isolation is necessary for Ch-IP assays.

This research reports an improved nuclear isolation method that we have named the Simplified Method. This method offers substantial cost savings as well as a more efficient use of time, labor and resources. The nuclear samples isolated with the Simplified Method are highly pure and perform better in Co-IP and Ch-IP assays.

Author contributions

Pengfei Li (Formal analysis [equal], Methodology [equal]), Jingyao Zhang (Data curation [equal], Investigation [equal]), Xiaojuan Liu (Investigation [equal], Methodology [equal]), Zhijuan Wu (Investigation [equal], Methodology [equal]), Y. James Kang (Conceptualization [equal]), and Wenjing Zhang (Conceptualization [lead], Supervision [lead])

Conflict of interest statement. None declared.

Funding

This work was supported by grants from the Youth Foundation of Inner Mongolia Medical University (YKD2023QN036).

References

1. Stark GR, Darnell JE. Jr. The JAK-STAT pathway at twenty. *Immunity* 2012;**36**:503–14.
2. Ball AT, Mohammed S, Doigneaux C *et al.* Identification and development of cyclic peptide inhibitors of hypoxia inducible factors 1 and 2 that disrupt hypoxia-response signaling in cancer cells. *J Am Chem Soc* 2024;**146**:8877–86.
3. Semenza GL, Nejfelt MK, Chi SM, Antonarakis SE. Hypoxia-inducible nuclear factors bind to an enhancer element located 3' to the human erythropoietin gene. *Proc Natl Acad Sci USA* 1991;**88**:5680–4.
4. Wenger RH, Kvietikova I, Rolfs A *et al.* Hypoxia-inducible factor-1 alpha is regulated at the post-mRNA level. *Kidney Int* 1997;**51**:560–3.
5. Rani S, Roy S, Singh M, Kaithwas G. Regulation of transactivation at C-TAD domain of HIF-1alpha by factor-inhibiting HIF-1alpha (FIH-1): a potential target for therapeutic intervention in cancer. *Oxid Med Cell Longev* 2022;**2022**:2407223.
6. Mitchell S, Vargas J, Hoffmann A. Signaling via the NFκB system. *Wiley Interdiscip Rev Syst Biol Med* 2016;**8**:227–41.
7. Yu H, Lin L, Zhang Z *et al.* Targeting NF-κB pathway for the therapy of diseases: mechanism and cli nical study. *Signal Transduct Target Ther* 2020;**5**:209.
8. Mirzaei S, Saghari S, Bassiri F *et al.* NF-kappaB as a regulator of cancer metastasis and therapy response: A focus on epithelial-mesenchymal transition. *J Cell Physiol* 2022;**237**:2770–95.
9. Zhao L, Zou X, Deng J *et al.* hnRNPH1 maintains mitochondrial homeostasis by establishing NRF1/DRP1 retrograde signaling under mitochondrial stress. *Cell Death Differ* 2025;**32**:118–33.
10. Déjardin J, Kingston RE. Purification of proteins associated with specific genomic Loci. *Cell* 2009;**136**:175–86.
11. Deryabin A, Moraleva A, Dobrochaeva K *et al.* Human RPF1 and ESF1 in pre-rRNA processing and the assembly of pre-ribosomal particles: a functional study. *Cells* 2024;**13**:326.
12. Dignam JD, Lebovitz RM, Roeder RG. Accurate transcription initiation by RNA polymerase II in a soluble ex tract from isolated mammalian nuclei. *Nucleic Acids Res* 1983;**11**:1475–89.
13. Liu X, Zhang W, Wu Z *et al.* Copper levels affect targeting of hypoxia-inducible factor 1α to the p romoters of hypoxia-regulated genes. *J Biol Chem* 2018;**293**:14669–77.
14. Wu Z, Zhang WF, —Kang YJ, Kang YJ. Copper affects the binding of HIF-1α to the critical motifs of its target genes. *Metallomics* 2019;**11**:429–38.

15. Zhang Z, Qiu LF, —Lin C *et al.* Copper-dependent and -independent hypoxia-inducible factor-1 regulation of gene expression. *Metallomics* 2014;**6**:1889–93.
16. Li R, Bourcy K, Wang T *et al.* The involvement of vimentin in copper-induced regression of cardiomyocyte hypertrophy. *Metallomics* 2015;**7**:1331–7.
17. Lam STT, Lim CJ. Cancer biology of the endoplasmic reticulum lectin chaperones calretic ulin, calnexin and PDIA3/ERp57. *Prog Mol Subcell Biol* 2021;**59**:181–96.
18. Ha BG, Heo J-Y, Jang Y-J *et al.* Depletion of mitochondrial components from extracellular vesicles secreted from astrocytes in a mouse model of fragile X syndrome. *Int J Mol Sci* 2021;**22**:410.
19. Piret J-P, Mottet D, Raes M, Michiels C. $CoCl_2$, a chemical inducer of hypoxia-inducible factor-1, and hypoxia r educe apoptotic cell death in hepatoma cell line HepG2. *Ann N Y Acad Sci* 2002;**973**:443–7.
20. Ke Q, Costa M. Hypoxia-inducible factor-1 (HIF-1). *Mol Pharmacol* 2006;**70**:1469–80.
21. Zhang D, Lv FL, Gh W. Effects of HIF-1α on diabetic retinopathy angiogenesis and VEGF expres sion. *Eur Rev Med Pharmacol Sci* 2018;**22**:5071–6.
22. Lee H, Paik S-G. Regulation of BNIP3 in normal and cancer cells. *Mol Cells* 2006;**21**:1–6.
23. Liu Y, Cox SR, Morita T, Kourembanas S. Hypoxia regulates vascular endothelial growth factor gene expression in endothelial cells. Identification of a 5' enhancer. *Circ Res* 1995;**77**:638–43.
24. Mishra M, Tiwari S, Gomes AV. Protein purification and analysis: next generation Western blotting techniques. *Expert Rev Proteomics* 2017;**14**:1037–53.

Biology Methods and Protocols, 2025, 10, 1–7
https://doi.org/10.1093/biomethods/bpaf007
Methods Article