Structural and biophysical study of chromatin and Its Regulation by Effector Protein

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УНИВЕРСИТЕТ МГУ-ППИ В ШЭНЬЧЖЭНЕ

1. Introduction

Chromatin refers to a mixture of DNA and proteins that form the chromosomes found in the cells of humans and other higher organisms. Histones package the massive amount of DNA in a genome into a highly compact form that can fit in the cell nucleus.145-147bp DNA binds to the octomer, which is composed of four histone proteins(H2A, H2B, H3, H4), to form the NCP. Linker DNA connects the NCP to form chromatin. Chromatin structure is important for regulating gene expression and for the proper condensation and segregation of chromosomes during cell division. Several human genetic diseases have been found to be due to mutations in genes producing proteins known or suspected to be involved in maintaining or modifying chromatin structure.

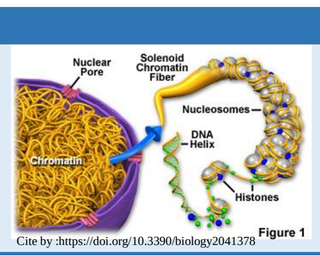


Figure 1

Cite by :https://doi.org/10.3390/biology2041378

2. In-vitro reconstruction

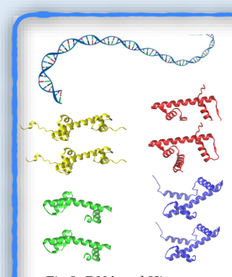
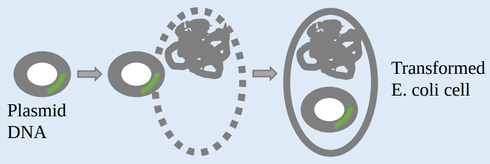


Fig 2 DNA and Histone

Histone preparation and purification (H2A H2B H3 H4)

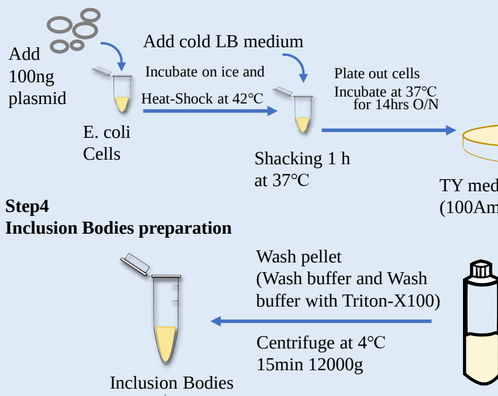
1.Transformation with histone gene
2.Histone Expression
3.Preparation of Inclusion bodies
4.Purification of histones by FPLC gel filtration
5.Purification of histones by FPLC ion exchange



Competent Cells
E. coli Rosetta 2 for H2B
E. coil BL21(DE3)pLysS

Fig 3 Paradigm of Transformation

Step1
Transformation with histone gene



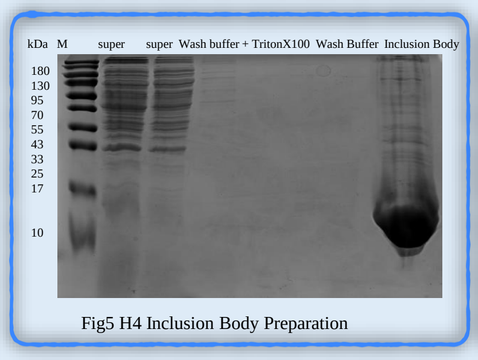
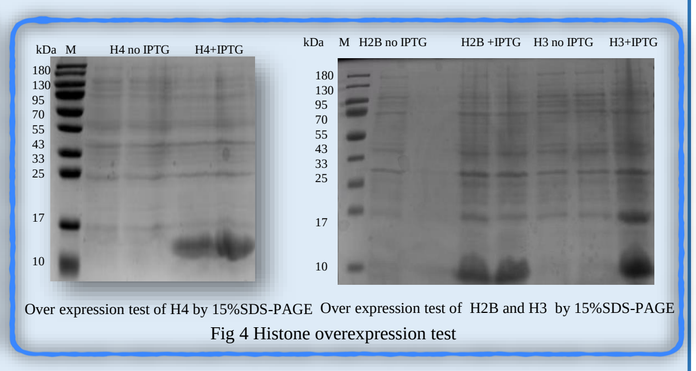


Fig5 H4 Inclusion Body Preparation



Over expression test of H4 by 15%SDS-PAGE Over expression test of H2B and H3 by 15%SDS-PAGE

Fig 4 Histone overexpression test

Step5

Purification protein by Superdex 200 pg

Unfolding protein than inject into system

Fig 6 FPLC Machine

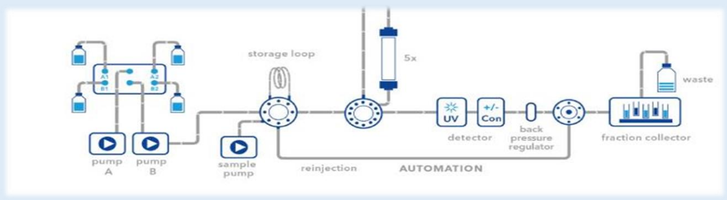


Fig 7 FPLC Systems.
Image quoted from product introduction(https://www.rubiconscience.com.au/fplc-systems/)

Histone purification by FPLC

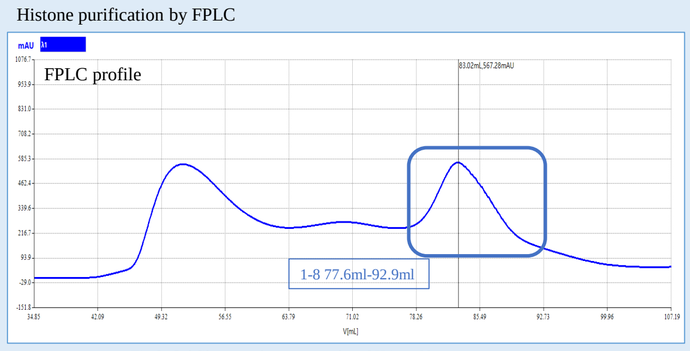


Fig 8 Gel filtration chromatography of H4 profile

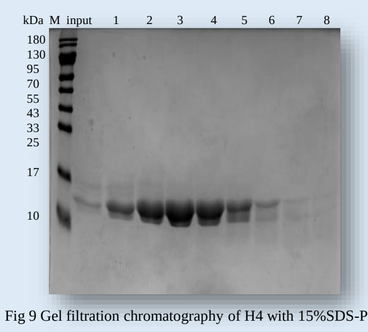


Fig 9 Gel filtration chromatography of H4 with 15%SDS-PAGE

Successfully separating the protein out and getting pure target protein.

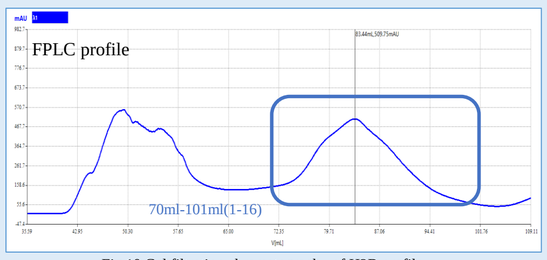


Fig 10 Gel filtration chromatography of H2B profile

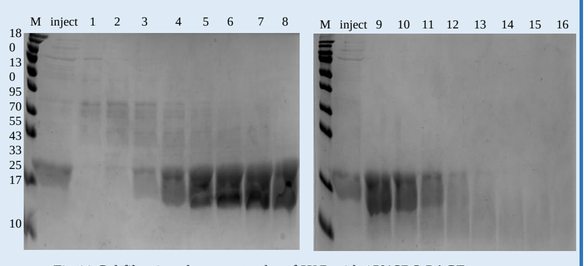


Fig 11 Gel filtration chromatography of H2B with 15%SDS-PAGE

Step 6 Dialysis

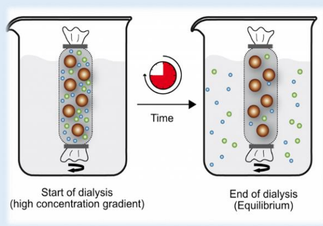


Fig 12 Paradigm of Dialysis
Cite from Dialysis Guide_scienova
https://www.blossombio.com/pdf/products/GDE_Sample%20Dialysis

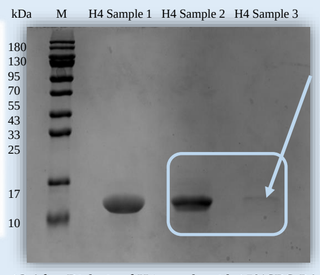


Fig 13 After Dialysis of H4 sample with 15%SDS-PAGE

High quality samples were obtained. There is almost no DNA contamination, and it can be used directly after dialysis and freeze-drying.

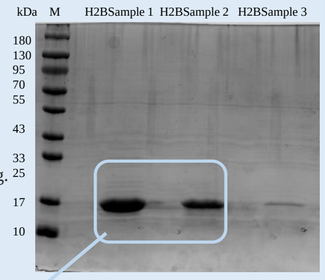


Fig 14 After Dialysis of H2B sample with 15%SDS-PAGE

A large number of protein samples were obtained, still
The sample $A_{260}/A_{280} > 0.7$ is still contaminated with DNA after dialysis, so ion exchange will be required.

Step 7
Recourse S

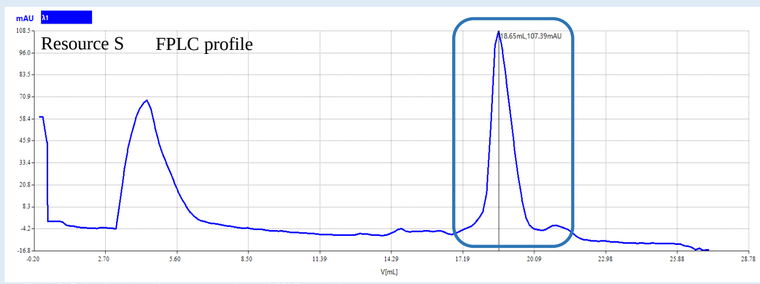


Fig 15 Gel filtration chromatography of H2B profile

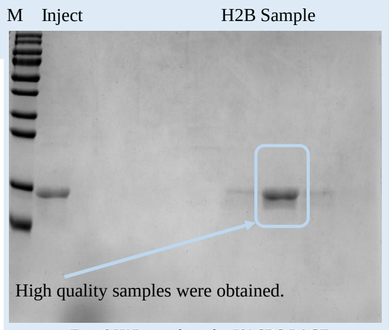


Fig 16 H2B sample with 15% SDS-PAGE

3. Preparation of DNA

DNA Production

1 Transformation

Transformation
with p 32-a8s-147 construct (in pUC19 vector) containing 32 sections (16 repeats each of 84 bp A and B half-sites)

2 Plasmid DNA overexpression

Pick colonies
Pick 5 of the largest, isolated colonies from the plate and inoculate 4 ml TB media

Cell culture
Incubate the tube at 37°C and shake at 220 rpm for 4 hr or until the media becomes turbid.

Colect and Cell culture
Transfer the culture to a 100 ml flask containing TB medium + appropriate antibiotic. Incubate in shaker at 37°C and Shake 220 rpm until it becomes turbid or 1-2 hrs

plasmid DNA over expression
Distribute the media evenly into twelve flasks containing TB medium (500 Ml) with antibiotic. Incubated in Shaker at 37°C for 19 hrs and shake at 220 rpm.

3 Plasmid Isolation

Washing pellet
Add only small amounts of alkaline lysis buffer I (25 mM Tris (8.0), 10 mM EDTA, 50 mM glucose)

Washing pellet
Centrifuge than add
lysis buffer II (1% SDS, 0.2 M NaOH),
lysis buffer III (4 M K-Acetate , 2 M Acetic acid)

Filter the supernatant
Filter the supernatant through sterile gauze (4 packs: fully unfolded, stacked, and refolded to give 8 layers). Add 0.52 volumes of isopropanol. Mix and allow it to stand for 30 minutes.

Resuspand
Add 10 ml of Te (10/50) and dissolved the pellet. Use 5 ml of TE to wash the entire centrifuge bottles. Bring the final volume to 20 ml in each tube.
Dissolve as completely as possible by putting in a small stire bar and vortexing.

Remove RNA
Add 120 µl RN ase A (10 mg/ ml) to each tube and incubated overnight in a 37 °C shaker.

Extract each 20 ml with 10 ml of phenol. Mix well by vortexing for a minute. Centrifuge at 27,000 g for 20 Min at 20 °C.

Purification
Extract the aqueous phase with 10 ml CIA (Chloroform- isoamyl alcohol, 20:1) using TEFLON tubes. Centrifuge at 12.000 g for 10 Min at 20 °C.

add 0.2 volume of 4 M NaCl and 0.4 volume of 40% PEG (make sure it gives final of 10 % PEG and 0.5 M NaCl)

dissolve the pellets in a total of 15 ml of TE (10, 0.1). Wipe tube to remove traces of PEG.

CIA to remove PEG. Centrifuge at 12.000g for 10 min at 20°C.

To get concentration and 260/280 ratio
Use a total of 10 ml TE 10/0.1 to dissolve the pellets. Measure the DNA concentration using UV Spec by diluting a sample 200X

Plasmid Isolation
Do a 100 µg EcoRV digestion test and run a 0.7% agarose gel to check material quality and digestibility

4 Eco RV digestion of plasmid DNA

Eco RV digestion of plasmid DNA
1 mg of p 32-a 8s-147 plasmid contains 9.82 nanomoles of Eco RV sites. We need 295.25 units of Eco RV to cut 1 mg of plasmid. In general, use 300 units to digest 1 mg of Plasmid DNA (300U/mg).

Protocol
1) The (final) EcoRV reaction setup: 2 mg/ml plasmid DNA 50mM Tris (7.6) 100mM NaCl 1mM MgCl2 1mM DTT Eco RV at 300 units per mg of DNA
2) Incubate reaction in a 50 ml PC tube (clear ones) in a shaking incubator at 37 °C for 24 hrs.
3) Check the samples on 10% (60: 1 acryl: bis) DNA PAGE to confirm total concentration of Eco RV cutting.
4) If cutting is not quite complete, add in another 100 U/mg enzyme and incubate overnight.

5 PEG Fraction ation of vector

PEG Fractionation of vector
Add 0.374 volume of 40% PEG 6000 and 0.200 volume of 4 M NaCl (this gives a final of 9.5% PEG and 0.5 M NaCl) Centrifuge at 27,000g for 20 minutes at 4 °C

Purification
Carefully remove the aqueous phase and add 1/10 volume of 4 M NaCl. Add 2.5 volume of 100% cold ethanol and incubate on ice for 30 min.

Centrifuge the tubes at 27,000g for 30 minutes at 4°C.

Dissolved the pellet in TE 10/0.1 to give a final DNA concentration of 5 mg/ml (should get 40% of original plasmid DNA mass)

Remove vector
Measures the DNA concentration by diluting a sample 100X. Make sure the UV spec is on for 20 minutes before taking the readings. Check all the samples on 10% (60:1 acryl: bis) DNA PAGE

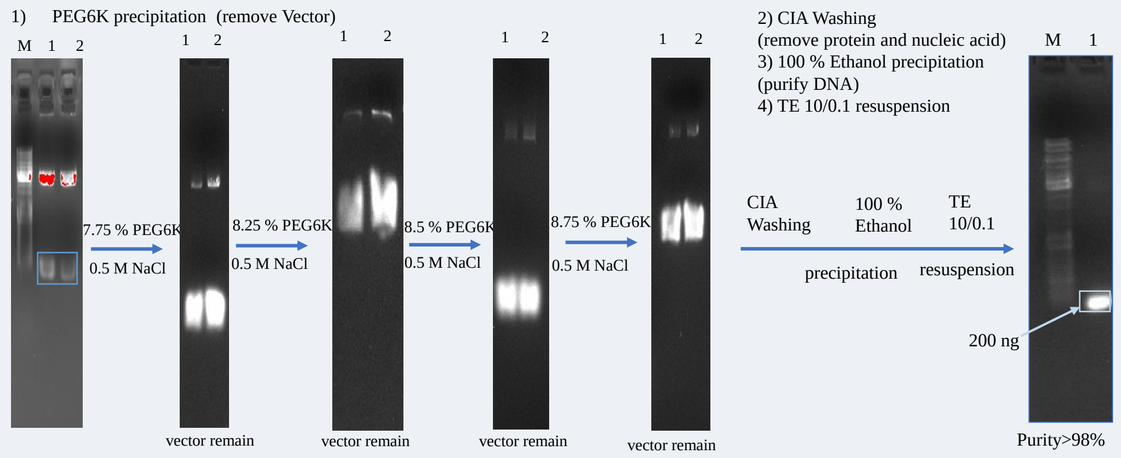


Fig 17 DNA preparation process, samples were checked by 1% agarose gel

4. NCP Reconstitution

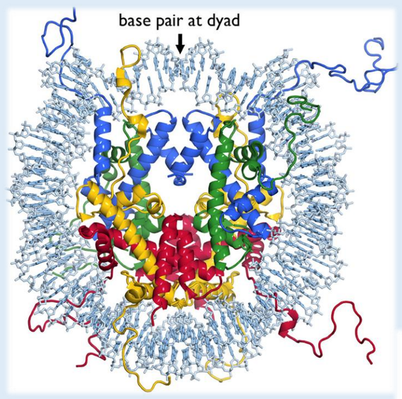


Fig 18 Diagram of NCP

Reconstitution:
The success of NCP reconstitution relies again on precise molar stoichiometry of HO to DNA ratio. So, we need accurate values for the HO and DNA concentrations beforehand.

1.Set up small-scale reconstitution
2.Use TCS buffer and peristaltic pump
3.After 12 h collect than centrifuge 10000g 5min

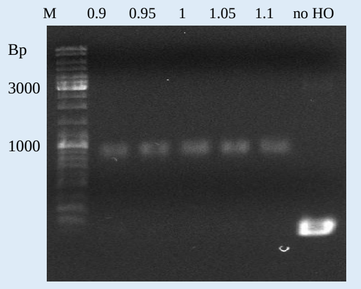


Fig 19 Reconstitution octomer and DNA

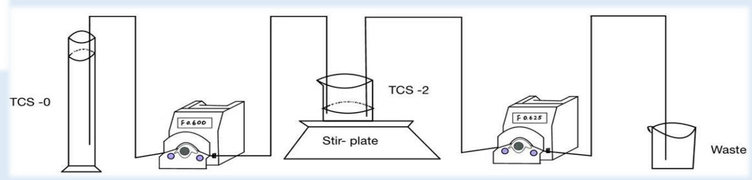


Fig 20 Reconstitution procedure

5. Effector protein

Fig 21 NMR Machine

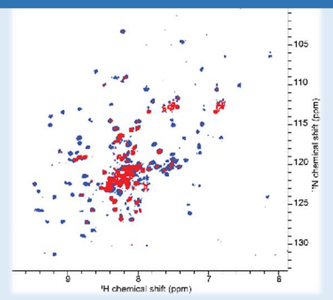


Fig 22 NMR spectrum

NMR-2D data showed interaction between chromatin and effector proteins.

Data processing is ongoing…

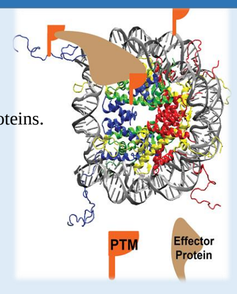


Fig 23 Effector protein interacted with chromatin

Acknowledgement

Guangdong University Students' Science and Technology Innovation Cultivation Special Fund（广东大学生科技创新培育专项资金）