Native chromatin immunoprecipitation of histone marks to Identify enhancer status

M Madapura Pradeepa (pmadap@essex.ac.uk)
University of Essex
Yatendra Kumar
Institute of Genetics and Molecular Medicine, Edinburgh
Graeme Grimes
Institute of Genetics and Molecular Medicine, Edinburgh
Robert Illingworth
Institute of Genetics and Molecular Medicine, Edinburgh
Wendy Bickmore
Institute of Genetics and Molecular Medicine, Edinburgh

Method Article

Keywords: Enhancers, histone, acetylation, globular, poised, active enhancers

Posted Date: May 5th, 2016

DOI: https://doi.org/10.1038/protex.2016.033

Abstract

The comprehensive identification of functional enhancers in mammalian genomes remains a major challenge. Histone post-translational modifications like mono methylation histone H3 at Lysine 4 \(H3K4me1), in combination with acetylation at histone H3 lysine 27 \(H3K27ac) is widely used to distinguish active enhancers from inactive enhancers in a given cell type. Recently, a more complex picture of different histone acetylation marks at regulatory elements is emerging. Here we describe methods to comprehensively identify and functionally validate the status of enhancers in mammalian cells using histone H3 globular acetylation marks – H3K122ac and H3H64ac in combination with previously known enhancer marks. This protocol describes ChIP-sequencing method to classify enhancers based on histone marks in mammalian cell types.

Procedure

Day1 Chromatin preparation Important note • Shown are the volumes per IP for 10M cells. In the event that multiple IPs are carried out per chromatin source only one input need be taken per DNA preparation. • All cell processing steps up until the addition of RNase should be carried out with ice cold buffers and on ice where possible.

——- a. Harvest ~10M million cells and pellet at 500g for 3 minutes at 4 °C. b. Wash twice in 1000ul of ice cold PBS, centrifuging at 500g for 3 minutes at 4 °C. c. Resuspend cells in 1000 ul ice cold NBA Buffer. Add 1000 ul of NBB Buffer and incubate for 3 minutes on ice. Centrifuge for 3 minutes at 1000g, 4 °C. d. Resuspend nuclei in 1000 ul NBR buffer and centrifuge for 3 minutes at 2000g, 4 °C. Resuspend nuclear pellet in 600ul NBR* \(theoretically 100ng/ul). e. Add 10 µl RNaseA \(10 mg/ml) to your 600 µl and leave 5 minutes on the bench. f. Add 40U MNase \(Sigma, Boehringer units; 4U for 1M cells) and mix by pipetting. Incubate for 10 minutes at 20°C in a control temperature block. Mix by pipetting after 5 minutes \(MNase concentration, incubation temperature and incubation time mary vary based on titration). g. Stop by adding 600 µl \(equal volume) STOP Buffer. Then add appropriate volume of 1:1 \(NBR: STOP) buffer to a final volume sufficient for the number of IPs to be performed then incubate on ice over night. The Stop Buffer contains freshly added 2x Protease Inhibitors, 1mMDTT and 0.2mM PMSF. Day2 Check the Mnase digestion a. Spin down the chromatin at 12000 g for 10 minutes, take 20 micro litre of supernatant to new tube, b. Add1 ul of Proteinase K \(20 mg/ml) and incubate for 2 hours. c. Purify the DNA using qiagen Min-elute PCR purification kit according to manufacturer's protocol and elute DNA in 10 ul. d. Load in 1.5% agarose gel with DNA marker \(100 – 1000bp range) Preblock and binding of antibody to agarose beads N.B. type of Beads \(Protein A, Protein G, sheep anti-mouse IgG, sheep anti-rabbit IgG, etc.) depends on antibody being used, see table below. a. Collect enough ProteinA beads \(Dynabeads; Invitrogen) for 10ul bed volume \(20ul slurry) per IP. Remove the storage buffer and prewash the beads twice in 400ul Block solution \(supplemented with PMSF). Block solution can be stored in fridge for short periods. b. Transfer 5ul of washed beads to separate tubes \(1 per IP) and add 5 µg of antibody to each \(include IgG from same species as a negative control). Incubate for 2 hours on a

rotating wheel at 4°C. c. Wash antibody bound beads once in 1000 ul blocking solution \(supplemented with PMSF). Chromatin immunoprecipitation d. Centrifuge down chromatin for 5 minutes at max speed at 4 °C. Transfer supernatant to a new tube on ice, this is your released chromatin. Make sure you have no nuclear debris \(gives background) e. Keep 10% of chromatin as input and freeze at –20 °C. \(eg. 20 µl per 200ul ChIP chromatin) f. To antibody bound ProteinA or G beads, add chromatin \(minimum 200ul) and incubate for 3-4 hours on a rotating wheel at 4 °C. Washes Each wash in 1000ul – Rotate for 10 minutes at 4°C, gentle pulse spin down in a microcentrifuge, place tube in magnetic rack for 1 min at 4°C and withdraw the supernatant. g. Wash \(3x) with ChIP-W1\(1% NP40 and 1% Sodium deoxycholate) supplemented with 1x Protease Inhibitors, 1mM DTT and 0.2mM PMSF \(other buffers should be used if antibody is Sodium deoxycholate sensitive). N.B. The detergent and salt concentration may vary for different antibodies\! Hence, see also other suggested washes as well. Nadeoxycholate is a strong detergent, so if you have to reduce detergent go to 0.1%. h. Wash with 1x TE at RT \(not necessary to rotate), and remove was as above. i. Resuspend in 1x TE, transfer to a fresh tube and remove TE. Elution j. Add 100ul of Elution solution to the washed beads \(heated to 37°C). Vortex briefly and incubate for 15min on a 37C vibrating platform. DNA elution k. Adjust pH of elutions \(100ul total volume) by adding 7ul 2M TrisHCl pH 6.8 \(this changes the pH from 12 to approximately 8) and add 20ug of proteinase K and incubate ON at 55C. For 10% Input \(from step e) add 20ug of proteinase K \(You do not need to add Tris\!) and incubate ON at 55C. Day 3 a. Remove the dynabeads for the IPs using a magnetic rack. b. Purify inputs and IPs using Qiagen PCR purification columns according to manufacturer's protocol. c. Add 5x IP/Input volume of PB to IPs and Inputs and apply a maximum of 650 µl to each purification column and centrifuge max speed for 1 minute. Discard flow through, apply any remaining DNA and repeat procedure above. d. Wash once with 600 µl Buffer PE. e. After the wash remove the flow through and any residual wash buffer from the blue rim surrounding the filter with a pipette or by careful aspiration \(ensure to keep each sample separate and clean). Centrifuge the column assembly again for 1 minute in a fresh tube. f. Transfer columns to new-labelled tubes and elute with 10ul of Ambion \(or equivalent) water. Leave for 1 minute and centrifuge for 1 minute at max speed. Repeat procedure above with a further 10ul of milliQ water. g. Quantify the DNA accurately using Qubit quantification. PCR \(where appropriate – not if solexa libraries are to be made as PCR will be performed on the resulting library). Use 0.5-1ul of sample for quantitative real-time PCR. The input is used to generate a serial dilution to calculate a standard curve. Usually the input series includes 3 concentrations e.g.: no dilution / 1/3rd / 1/9th. Each sample should be replicated \(it is sufficient to have two replicates for inputs and three for unknown samples).

——- PCR set up DNA 1ul Primers \(F/R / 1mM) 5ul Sybr Green MM \(2x) 10ul Water up to 20ul

Figures

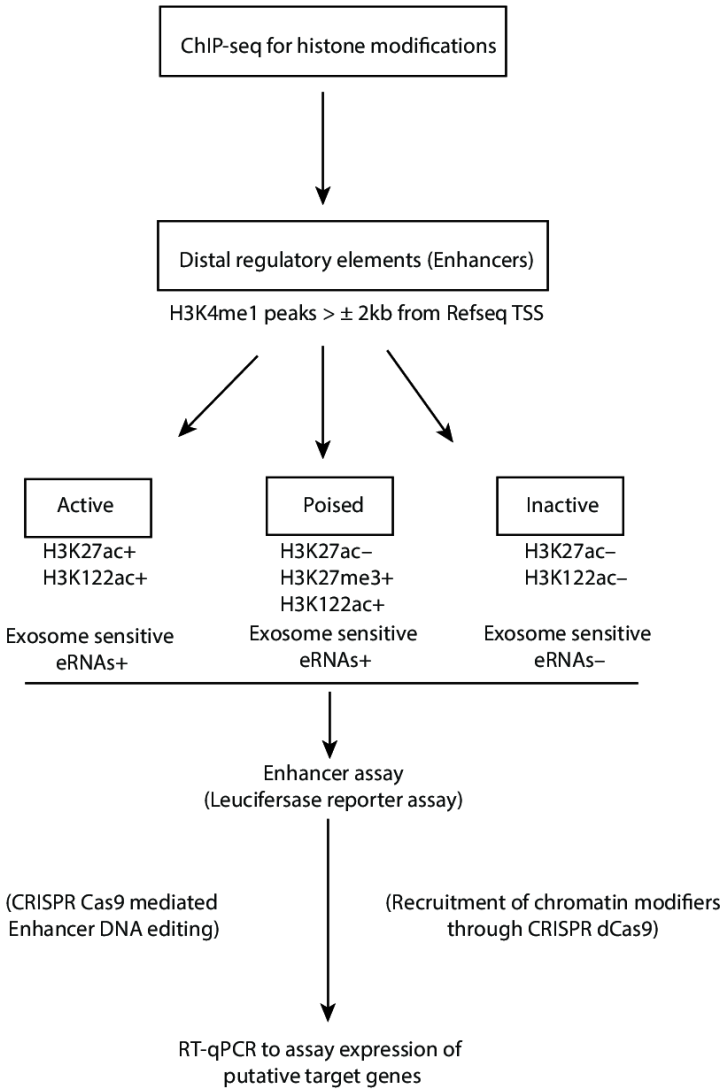

Figure 1

Figure 1

Overall design for identification of enhancer status using histone modifications

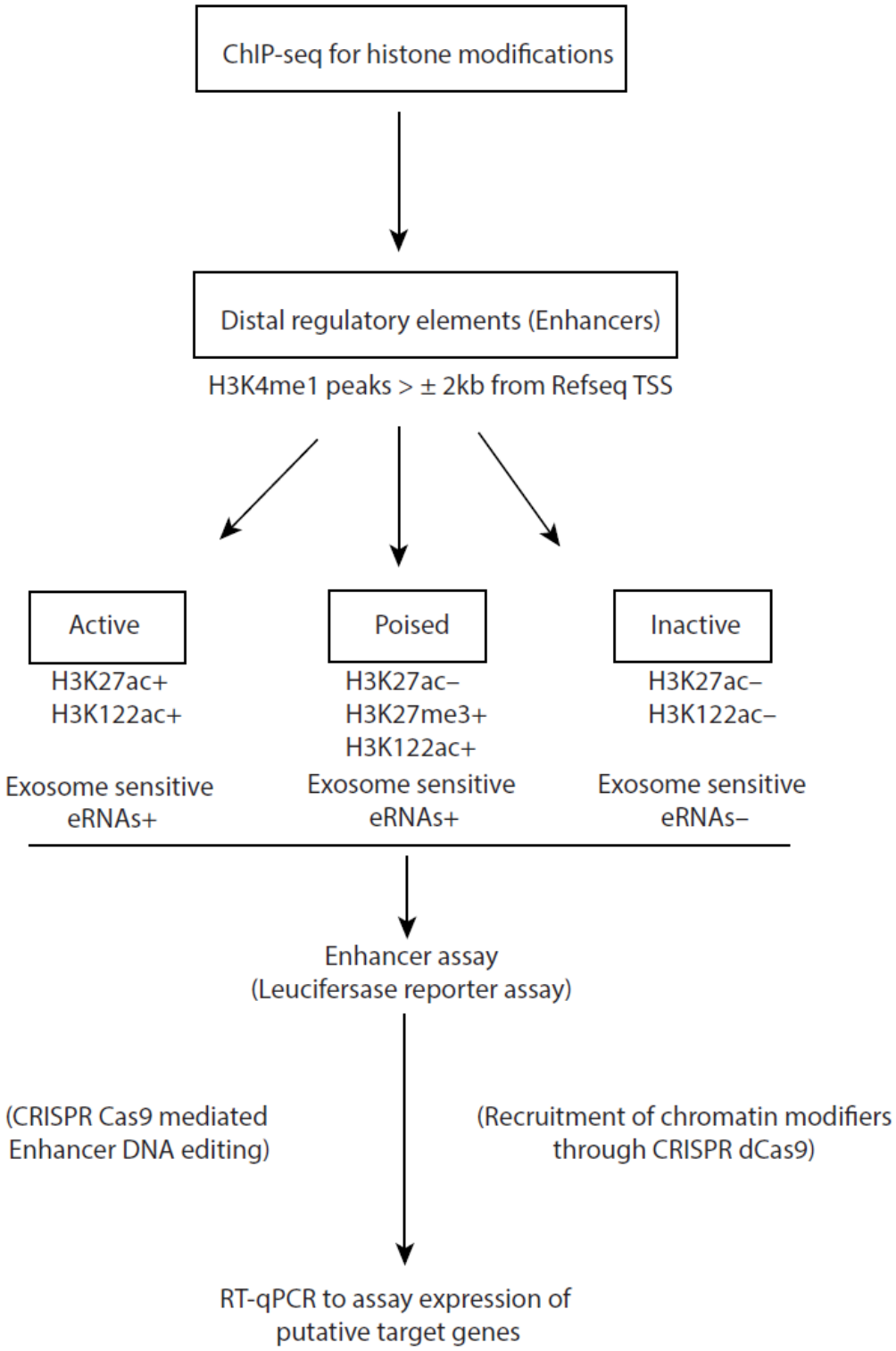

Figure 2

Figure 1 as an image file Overall design for identification of enhancer status using histone modifications