Upload image

Jul 14, 2020

Performing CUT&RUN on adherent cells in a multi-well cell culture plate

Michi Miura[1]

[1]Department of Microbiology, The University of Hong Kong

1 Works for me dx.doi.org/10.17504/protocols.io.bijakcie

Michi Miura

ABSTRACT

This protocol describes a variant of the standard CUT&RUN procedure (Skene et al. *eLife* 2017; Skene et al. *Nature Protocols* 2018; Meers et al. *eLife* 2019). This streamlined protocol is immediately applicable to adherent cells maintained in a multi-well cell culture plate. Trypsinising cultured cells to harvest and attaching them onto Concanavalin A-beads are not required.

EXTERNAL LINK

https://www.biorxiv.org/content/10.1101/2020.07.07.191478v1

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

CUT&RUN reveals unique positioning of pre-initiated RNA polymerase II in the steady state of transcription
Michi Miura, Honglin Chen bioRxiv 2020.07.07.191478; doi: https://doi.org/10.1101/2020.07.07.191478

DOI

dx.doi.org/10.17504/protocols.io.bijakcie

PROTOCOL CITATION

Michi Miura 2020. Performing CUT&RUN on adherent cells in a multi-well cell culture plate. **protocols.io** dx.doi.org/10.17504/protocols.io.bijakcie

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

CUT&RUN reveals unique positioning of pre-initiated RNA polymerase II in the steady state of transcription
Michi Miura, Honglin Chen bioRxiv 2020.07.07.191478; doi: https://doi.org/10.1101/2020.07.07.191478

EXTERNAL LINK

https://www.biorxiv.org/content/10.1101/2020.07.07.191478v1

KEYWORDS

CUT&RUN

CREATED

Jul 14, 2020

LAST MODIFIED

Jul 14, 2020

PROTOCOL INTEGER ID

39234

GUIDELINES

In the manuscript *"CUT&RUN reveals unique positioning of pre-initiated RNA polymerase II in the steady state of transcription"*, this protocol was performed on the human lung carcinoma cell line A549 cultured in a 24-well polystyrene plate (https://www.biorxiv.org/content/10.1101/2020.07.07.191478v1). The protocol is expected to work for other adherent cells that are firmly attached to the plate.

Antibodies used in the above manuscript:
Millipore, 05-623 (mouse anti-RNA polymerase II, clone CTD4H8; 0.75 µg per well)
Abcam, ab5095 (rabbit anti-RNA polymerase II (phospho S2), polyclonal; 0.75 µg per well)
Abcam, ab5131 (rabbit anti-RNA polymerase II (phospho S5), polyclonal; 0.675 µg per well)

Recombinant pAG-MNase:
Purified recombinant pAG-MNase from two commercial venders were tested, of which only one from Cell Signaling (Product# 40366) worked well with this protocol.

MATERIALS TEXT

Recombinant pAG-MNase (Cell Signaling, 40366)

BEFORE STARTING

Perm buffer
20 mM HEPES-KOH pH 7.5
150 mM NaCl
0.5 mM Spermidine (Sigma, S2626)
0.1% Triton X-100
Proteinase inhibitor (Roche, 04 693 132 001)

4× STOP buffer
680 mM NaCl
40 mM EDTA (Sigma, E5134)
8 mM EGTA (AG Scientific, E-2491)
100 µg/ml RNase A (Invitrogen, 12091021)
0.1% Triton X-100

Other materials
Recombinant pAG-MNase (Cell Signaling, 40366)
Spike-in DNA (Cell Signaling, 40366) can be supplemented at step 2.6 if necessary (10 pg per well).

Cell seeding

1 Seed adherent cells in a standard 24-well cell culture plate

Seed the cells at an appropriate density so that the cells are 70 to 90% confluent when performing CUT&RUN.

CUT&RUN

2 Perform CUT&RUN

Before cell permeabilisation (step 2.2),
1. Remove the cell culture medium
2. Wash the cells with PBS

(Optional) Cells can be fixed prior to CUT&RUN (step 2.1)

2.1 (Optional) Cells can be fixed by one of the followings:

(1) Fix intact cells

Citation: Michi Miura (07/14/2020). Performing CUT&RUN on adherent cells in a multi-well cell culture plate. https://dx.doi.org/10.17504/protocols.io.bijakcie

1. Add 500 µl of 1.5% formaldehyde (diluted in PBS) to a well and incubate for 10 min
2. Wash cells ×3 with PBS
3. go to step 2.2 (Permeabilisation)

(2) Fix extracted cells
1. Add 500 µl Perm buffer to a well and incubate for 5 min
2. Wash cells ×2 with PBS
3. Add 500 µl of 1.5% formaldehyde (diluted in PBS) to a well and incubate for 10 min
4. Wash cells ×3 with PBS
5. go to step 2.2 (Permeabilisation)

2.2 Permeabilisation

1. Dispense 500 µl Perm buffer per well and incubate on the bench (i.e. at room temperature) for 15 min
2. Remove the buffer and wash the cells once with Perm buffer

2.3 Antibody incubation

1. Prepare antibody dilution* in Perm buffer (150 µl per well)
2. Dispense 150 µl of the diluted antibody to a well and incubate on the bench for an hour
3. Remove the antibody solution from the well and wash the cells ×2 with Perm buffer

*1:100 dilution for antibodies of 0.5 µg/µl stock concentration and 1:200 for antibodies of 1 µg/µl (https://www.biorxiv.org/content/10.1101/2020.07.07.191478v1).

2.4 pAG-MNase incubation

1. Prepare pAG-MNase dilution (Cell Signaling, 40366; 1:33 volume) in 150 µl Perm buffer per well
2. Dispense 150 µl of the diluted pAG-MNase into a well and incubate on the bench for an hour
3. Remove the pAG-MNase solution from the well and wash the cells ×2 with Perm buffer*

*At the second wash, place the cell culture plate on ice-cold water to get ready for the chromatin digestion (step 2.5).

2.5 Chromatin digestion

1. Prepare Perm buffer containing 5 mM $CaCl_2$ (add 1/20 volume of 100 mM $CaCl_2$ to Perm buffer) and chill it on ice
2. Make sure that the cells are chilled on ice-cold water
3. Dispense 150 µl cold Perm buffer containing 5 mM $CaCl_2$ and incubate the plate on ice-cold water for 30 min

2.6 Fragment release

1. Add 50 µl 4× STOP solution to a well (50 µl per well)
2. Gently rock the plate to mix the solution
3. Place the cell culture plate in an incubator at 37ºC for 30 min
4. Collect the supernatant (~200 µl)

2.7 Fragment purification

If the cells were not fixed, purify the DNA fragment using DNeasy Blood & Tissue Kit (Qiagen, 69504).

If fixed (step 2.1), add 10 µl of 20% SDS to the supernatant (1% in final concentration) and incubate overnight at 65ºC. Then add Proteinase K and incubate at 56ºC for an hour. Purify the DNA with

Citation: Michi Miura (07/14/2020). Performing CUT&RUN on adherent cells in a multi-well cell culture plate. https://dx.doi.org/10.17504/protocols.io.bijakcie

QIAquick PCR Purification Kit (Qiagen, 28104).

Citation: Michi Miura (07/14/2020). Performing CUT&RUN on adherent cells in a multi-well cell culture plate. https://dx.doi.org/10.17504/protocols.io.bijakcie