Oct 31, 2024

HiCAR for mouse HL-1 cells following enhancer perturbations

DOI
https://dx.doi.org/10.17504/protocols.io.36wgqn2yygk5/v1

Taylor Anglen[1], Irene M. Kaplow[1], Baekgyu Choi[2], Kevin Hagy[1], Duc Tran[1], Magan E. Ramaker[1], Svati Shah[1], Inkyung Jung[2], Ravi Karra[1], Yarui Diao[1], Charles A. Gersbach[1]

[1]Duke University; [2]Korea Advanced Institute of Science and Technology

Gersbach Lab

Taylor Anglen
Duke University

DOI: https://dx.doi.org/10.17504/protocols.io.36wgqn2yygk5/v1

Protocol Citation: Taylor Anglen, Irene M. Kaplow, Baekgyu Choi, Kevin Hagy, Duc Tran, Magan E. Ramaker, Svati Shah, Inkyung Jung, Ravi Karra, Yarui Diao, Charles A. Gersbach 2024. HiCAR for mouse HL-1 cells following enhancer perturbations. **protocols.io** https://dx.doi.org/10.17504/protocols.io.36wgqn2yygk5/v1

Protocol status: Working
We use this protocol and it's working

Created: October 20, 2024

Last Modified: October 31, 2024

Protocol Integer ID: 110366

Keywords: gsk3 inhibition, enhancer perturbations this protocol, following enhancer perturbation, seq methods in human ipsc, atac

Funders Acknowledgements:
NIH
Grant ID: HG012053

Abstract

This protocol describes ATAC-seq methods in human iPSC-CM following growth with or without GSK3 inhibition using CHIR99021.

Fibronectin + Gelatin coating tissue culture plates

1 5ug/ml fibronectin + 0.02% gelatin solution was added to tissue culture plates for 1 hour at 37C

Culture of HL-1 mouse atrial cardiomyocytes

2 HL-1 cells were culture per manufactures instructions in complete Claycomb medium (Claycomb basal medium + 10% FBS + Norepinephrine (0.1mM final concentration) + 1x Glutamax + 100 U ml−1 penicillin and 100 µg ml−1 streptomycin). (**1**)

Transfections for high-titer lentiviral production

3 Plate 1.2 × 106 or 7 × 106 HEK293T cells in a 6 well plate or 10 cm dish in the afternoon with 2 mL or 12 mL of complete opti-MEM (Opti-MEM‰ I Reduced Serum Medium supplemented with 1x Glutamax, 5% FBS, 1 mM Sodium Pyruvate, and 1x MEM Non-Essential Amino Acids). Based on previously published protocols. (**2**)

4 The next morning, transfect HEK293T cells with 0.5 µg pMD2.G, 1.5 µg psPAX2, and 0.5 µg transgene for 6 well plates or 3.25 µg pMD2.G, 9.75 µg psPAX2, and 4.3 µg transgene for 10 cm dishes using Lipofectamine 3000.

5 Exchanged media 6 hours after transfection and collect and pool lentiviral supernatant at 24 hours and 48 hours after transfection.

6 Centrifuged lentiviral supernatant at 600g for 10 min to remove cellular debris.

7 Concentrate lentivirus to 50–100× the initial concentration using Lenti-X Concentrator (Takara Bio). Resupsend viral pellet in dPBS

8 Snap freeze virus in liquid nitrogen and store at -80C

Determining viral titer

9 Plate 4×10^6 HEK293T cells per well in a 6-well plate in complete DMEM (DMEM supplemented with 1x Glutamax, 5% FBS, 1 mM Sodium Pyruvate, 1x MEM Non-Essential Amino Acids, and100 U/ml penicillin and 100 µg/ml streptomycin). Transduce individual wells add 0ul,0ul, 1ul, 5ul, 10ul, and 20ul of concentrated virus.

10 Change media 24 hours following transduction with complete DMEM.

11 Change media 24 hours following transduction with complete DMEM + blasticidin (8ug/ml final concentration). Add complete DMEM without blasticidin to one well that received no virus. This well will be the control well for MOI calculations. Change media every 48 hour until the well that received no virus but was supplemented with blasticidin has no remaining adherent cells, usually 96 hours.

12 Count surviving cells across all wells. Plot viral load (ul) vs % survival and generate a linear fit with the y-intercept = 0.

Transduce HL-1 cells

13 Transduce HL-1 with virus for a desired MOI of 0.3.

14 Change media with complete Claycomb medium 24 hours following transduction.

15 Change medium complete Claycomb medium + blasticidin (8ug/ml final concentration) 48 hours following transduction.

16 Change medium complete Claycomb medium + blasticidin (8ug/ml final concentration) every 48 hours for 96 hours total culture.

17 Change medium complete Claycomb medium every 48 hours following.

Remove stress response and fetal gene expression

18 10 days following transduction stress stimuli was removed from culture medium for HL-1 cells. Medium was changed on transduced HL-1 with Claycomb basal medium supplemented only with 1x Glutamax and 100 U ml−1 penicillin and 100 µg ml−1 streptomycin for 72 hours.

HiCAR

19 Culture of HL-1 has occurred for 13 days following transduction, and 72 hours following the removal of stress stimuli.

20 Add trypsin-EDTA (0.05%) to cells to disassociate (1ml for 6-well, 3ml for a 10-cm). Incubate at 37C for 5 minutes.

21 Add 1:1 Claycomb medium to quench the trypsin-EDTA (0.05%). Transfer cells to a conical tube and centrifuge at 300g for 5 minutes.

22 Proceed to HiCAR NGS library generation as described in the original publication. (**3**)

23 HiCAR libraries were then pooled at equal molarity. Targeted enrichment was performed for regions of interest, including the areas surrounding the *Myh6* transcription start site (TSS) and gene body, the *Myh7* TSS, and a distal regulatory element. Biotinylated probes and protocols from Twist Bioscience were used for the targeted enrichment. (**4**)

24 Enriched libraries were sequenced using an Illumina NextSeq 1000/2000 P2 kit on an Illumina NextSeq 2000.

25 Sequencing data was then processed using nf-core HiCAR pipeline v1.0.0 using the mm39 genome as the reference genome.

26 A contact matrix was created from cooler files generated by the HiCAR pipeline for all reads mapping between mm39 chr14:55110000-55260000.

27 Differential contact frequency within the region was determined using DESeq2. (**5**)

Protocol references

(**1**) Claycomb, W. C., Lanson, N. A., Stallworth, B. S., Egeland, D. B., Delcarpio, J. B., Bahinski, A., & Izzo, N. J. (1998). HL-1 cells: A cardiac muscle cell line that contracts and retains phenotypic characteristics of the adult cardiomyocyte (simian virus 40 T oncogenepotassium channelsdofetilide). In *Cell Biology* (Vol. 95). www.pnas.org.

(**2**) McCutcheon, S. R., Swartz, A. M., Brown, M. C., Barrera, A., McRoberts Amador, C., Siklenka, K., Humayun, L., ter Weele, M. A., Isaacs, J. M., Reddy, T. E., Allen, A. S., Nair, S. K., Antonia, S. J., & Gersbach, C. A. (2023). Transcriptional and epigenetic regulators of human CD8+ T cell function identified through orthogonal CRISPR screens. *Nature Genetics*, *55*(12), 2211–2223. https://doi.org/10.1038/s41588-023-01554-0

(**3**) Wei, X., Xiang, Y., Peters, D. T., Marius, C., Sun, T., Shan, R., Ou, J., Lin, X., Yue, F., Li, W., Southerland, K. W., & Diao, Y. (2022). HiCAR is a robust and sensitive method to analyze open-chromatin-associated genome organization. *Molecular Cell*, *82*(6), 1225-1238.e6. https://doi.org/10.1016/j.molcel.2022.01.023

(**4**) https://www.twistbioscience.com/sites/default/files/resources/2022-02/Protocol_NGS_TargetEnrichmentFastHybridizationProtocol_2FEB22_Rev4.0.pdf

(**5**) Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12). https://doi.org/10.1186/s13059-014-0550-8