Aug 25, 2025

CUT&RUN library generation

Nature Cancer

In 1 collection

DOI
https://dx.doi.org/10.17504/protocols.io.6qpvrw8dblmk/v1

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OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.6qpvrw8dblmk/v1

External link: https://doi.org/10.1038/s43018-025-01065-3

Protocol Citation: hanjingnk 2025. CUT&RUN library generation. **protocols.io** https://dx.doi.org/10.17504/protocols.io.6qpvrw8dblmk/v1

Manuscript citation:
Han, J., Lu, X., Guo, M. *et al.* Spatiotemporal control of SMARCA5 by a MAPK–RUNX1 axis distinguishes mutant KRAS-driven pancreatic malignancy from tissue regeneration. *Nat Cancer* (2025). https://doi.org/10.1038/s43018-025-01065-3

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

Created: August 23, 2025

Last Modified: August 25, 2025

Protocol Integer ID: 225310

Abstract

CUT&RUN library generation

Guidelines

30,000-60,000 GFP-positive cells were sorted by FACS and the CUT&RUN sequencing was performed as previously described[54] with minor modifications. 10% Kasumi-1 cells were added as spike-in. Concanavalin-A (ConA) beads were activated by washing twice using activation buffer (20 mM HEPES–KOH pH 7.9, 10 mM KCl, 1 mM CaCl2, 1 mM MnCl2). Sorted cells were resuspended in room temperature (RT) wash buffer (20 mM HEPES–KOH pH 7.5, 150 mM NaCl, 0.5 mM Spermidine, protease inhibitor cocktail) and added into ConA beads. After incubating for 10 min in RT, ConA-beads-bound cells were incubated overnight at 4 °C in antibody buffer (wash buffer supplemented with 0.01% digitonin and 2 mM EDTA) and antibody. The following primary antibodies were used: SMARCA5 (Abcam, ab72499, 2.5 µl and EpiCypher, 13-2007, 2.5 µl), RUNX1 (Abcam, ab229482, 2.5 µl) and CTCF (CST, 3418S, 1 µl). After antibody incubation, ConA-beads-bound nuclei were washed once with Digitonin buffer (wash buffer supplemented with 0.01% digitonin) then resuspended and incubated at 4 °C for 1 h in Digitonin buffer and 0.1 µl pAG-MNase (a gift from Dr. Wei Xie, Tsinghua University). ConA-beads-bound nuclei were then washed twice with Digitonin buffer and resuspended in 98 µl of Digitonin buffer and incubated on ice for 5 min. Then, 2 µl 100 mM CaCl2 was added and mixed gently to each 100 µl ConA-bound nuclei. The reaction was then incubated at 4 °C for 30 min. The reaction was stopped by addition of 100 µl 2× stop buffer (340 mM NaCl, 20 mM EDTA, 4 mM EGTA, 0.02% Digitonin and 50 µg/ml RNaseA, 50 µg/ml Glycogen) and incubated at 37 °C for 10 min. After incubation, ConA-bound nuclei were captured using a magnet and supernatant containing CUT&RUN DNA fragments were collected. The supernatant was incubated at 70 °C for 10 min with 2 µl 10% sodium dodecyl sulfate, 3 µL 10 mg/ml proteinase K and 2 µL 20 ng/µL carrier RNA (Qiagen, 1068337). DNA was purified using PCI reagent (Solarbio, P1012) and overnight ethanol precipitation with glycogen at −20 °C. DNA was resuspended in water and repaired to 5′ phosphorylated and 3′ dA-tailed DNA with NEBNext® Ultra™ II End Repair/dA-Tailing Module (NEB, E7546S). Barcodes (BIOO Scientific, 514153) were ligated with Quick Ligation kit (NEB, M2200). After size selection, 16 cycles of PCR were used to amplify the library. PCR-amplified libraries were purified using KAPA beads (Kapa Biosystems) and sent for sequencing.

Materials

- FACS-sorted GFP-positive cells (30,000–60,000)
- Kasumi-1 cells (10% spike-in)
- Concanavalin-A (ConA) beads
- Activation buffer: 20 mM HEPES–KOH pH 7.9, 10 mM KCl, 1 mM CaCl2, 1 mM MnCl2
- Room temperature (RT) wash buffer: 20 mM HEPES–KOH pH 7.5, 150 mM NaCl, 0.5 mM Spermidine, protease inhibitor cocktail
- Antibody buffer: wash buffer supplemented with 0.01% digitonin and 2 mM EDTA
- Digitonin (0.01%) and Digitonin buffer
- Primary antibodies: SMARCA5 (Abcam, ab72499 and EpiCypher, 13-2007), RUNX1 (Abcam, ab229482), CTCF (CST, 3418S)
- pAG-MNase (0.1 µl used; gift from Dr. Wei Xie, Tsinghua University)
- CaCl2 (100 mM)
- 2× stop buffer components: 340 mM NaCl, 20 mM EDTA, 4 mM EGTA, 0.02% Digitonin, 50 µg/ml RNaseA, 50 µg/ml Glycogen
- Sodium dodecyl sulfate (SDS), 10% (used 2 µl of 10%)
- Proteinase K (10 mg/ml)
- Carrier RNA (Qiagen, 1068337; 2 µl 20 ng/µl used per reaction)
- PCI reagent (Solarbio, P1012)
- Glycogen (for ethanol precipitation)
- Ethanol (for overnight precipitation at −20 °C)
- NEBNext® Ultra™ II End Repair/dA-Tailing Module (NEB, E7546S)
- Barcodes (BIOO Scientific, 514153)
- Quick Ligation kit (NEB, M2200)
- KAPA beads (Kapa Biosystems) for library purification
- PCR reagents (16 cycles indicated) and thermocycler
- Magnetic rack for bead capture
- General consumables: tubes, pipette tips, nuclease-free water

Before start

- Sort 30,000–60,000 GFP-positive cells by FACS.
- Prepare 10% Kasumi-1 cells to add as spike-in to samples.
- Activate ConA beads by washing twice with activation buffer (20 mM HEPES–KOH pH 7.9, 10 mM KCl, 1 mM CaCl2, 1 mM MnCl2).
- Resuspend sorted cells in RT wash buffer (20 mM HEPES–KOH pH 7.5, 150 mM NaCl, 0.5 mM Spermidine, protease inhibitor cocktail) before adding to ConA beads.
- Prepare antibody buffer (wash buffer + 0.01% digitonin + 2 mM EDTA) and pre-aliquot primary antibodies (SMARCA5, RUNX1, CTCF) at indicated volumes.

1 Sort 30,000–60,000 GFP-positive cells by FACS and add 10% Kasumi-1 cells as spike-in.

2 Activate Concanavalin-A (ConA) beads by washing twice with activation buffer (20 mM HEPES–KOH pH 7.9, 10 mM KCl, 1 mM CaCl2, 1 mM MnCl2).

3 Resuspend sorted cells in room temperature (RT) wash buffer (20 mM HEPES–KOH pH 7.5, 150 mM NaCl, 0.5 mM Spermidine, protease inhibitor cocktail) and add cells to the activated ConA beads; incubate for 10 min at RT.

4 Incubate ConA-beads-bound cells overnight at 4 °C in antibody buffer (wash buffer supplemented with 0.01% digitonin and 2 mM EDTA) with the appropriate primary antibody: SMARCA5 (Abcam, ab72499, 2.5 µl and EpiCypher, 13-2007, 2.5 µl), RUNX1 (Abcam, ab229482, 2.5 µl) or CTCF (CST, 3418S, 1 µl).

5 After antibody incubation, wash ConA-beads-bound nuclei once with Digitonin buffer (wash buffer supplemented with 0.01% digitonin).

6 Resuspend the beads and incubate at 4 °C for 1 h in Digitonin buffer containing 0.1 µl pAG-MNase (gift from Dr. Wei Xie, Tsinghua University).

7 Wash the ConA-beads-bound nuclei twice with Digitonin buffer, then resuspend in 98 µl Digitonin buffer and incubate on ice for 5 min.

8 Add 2 µl of 100 mM CaCl2 to each 100 µl ConA-bound nuclei, mix gently, and incubate the reaction at 4 °C for 30 min.

9 Stop the reaction by adding 100 µl 2× stop buffer (340 mM NaCl, 20 mM EDTA, 4 mM EGTA, 0.02% Digitonin, 50 µg/ml RNaseA, 50 µg/ml Glycogen) and incubate at 37 °C for 10 min.

10 Capture ConA-bound nuclei using a magnet and collect the supernatant containing CUT&RUN DNA fragments.

11 Incubate the collected supernatant at 70 °C for 10 min with 2 µl 10% sodium dodecyl sulfate (SDS), 3 µl 10 mg/ml Proteinase K and 2 µl 20 ng/µl carrier RNA (Qiagen, 1068337).

12 Purify DNA using PCI reagent (Solarbio, P1012) and perform overnight ethanol precipitation with glycogen at −20 °C.

13 Resuspend DNA in nuclease-free water and repair to 5′-phosphorylated and 3′-dA-tailed DNA using NEBNext® Ultra™ II End Repair/dA-Tailing Module (NEB, E7546S).

14 Ligate barcodes (BIOO Scientific, 514153) to repaired DNA using the Quick Ligation kit (NEB, M2200).

15 Perform size selection, then amplify the library by PCR for 16 cycles.

16 Purify PCR-amplified libraries using KAPA beads (Kapa Biosystems) and submit libraries for sequencing.

Protocol references

- CUT&RUN sequencing performed as previously described[54] (reference number 54 cited on this page).

Acknowledgements

- pAG-MNase was a gift from Dr. Wei Xie, Tsinghua University.