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CutRun (Histone Modification) Library Preparation

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We use this protocol and it's working

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Abstract

This is a modified NEB library protocol for CutRun DNA. This protocol has been tested on H3K4Me3, H3K27Ac, H3K27Me3 CutRun. For the transcription factors CutRun, you may check: https://www.protocols.io/view/library-prep-for-cut-amp-run-with-nebnext-ultra-ii-kxygxm7pkl8j/v2?version_warning=no

Materials

CutRun assay was performed using Epicypher CutRun kit (CUTANA™ ChIC/CUT&RUN Kit**,** SKU: 14-1048) follow the instructions

Library preparation kit and indexed primers were purchased from NEB (NEBNext® Ultra™ II DNA Library Prep Kit for Illumina®, E7645S; Index, E7335S)

SPRIselect beads

Magnet stand for PCR tubes

End Repair (Green) 30m

1 Add the following components to a sterile nuclease-free tube: 20m

	A	B
	NEBNext Ultra II End Prep Enzyme Mix	3 µl
	NEBNext Ultra II End Prep Reaction Buffer	7 µl
	CutRun DNA (5-10 ng)	50 µl
	Total Volume	60 µl

2 Set a 100 µl or 200 µl pipette to 50 µl and then pipette the entire volume up and down at least 10 times to mix thoroughly. 10m
Perform a quick spin to collect all liquid from the sides of the tube

3 Place in a thermal cycler, with the heated lid set to ≥ 75°C, and run the following program: 1h
30 minutes @ 20°C
30 minutes @ 65°C
Hold at 4°C

Safe Stop Point.

Adaptor Ligation (Red) 30m

4 Adaptor dilution. Dilute the adaptors in dilution buffer (10mM pH7.5-8.0 Tris-HCl with 10 mM NaCl) at 25-fold (1:25). 7m
Dilution buffer: 10 uL 1M Tris-HCl, 2 uL 5M NaCl, 998 uL H2O

5 Add 2.5 uL adaptor to 60 uL End-Repair reaction mix from step3. Mix through by pipetting up and down 10 times. 8m
Add 30 uL Ultra II Ligation Mater Mix (viscous), add 1 iL Ligation Enhancer. Mix through by pipetting up and down 10 times, total volume is 93.5 uL. Quick spin down.

6 Incubate at 20°C for 15 minutes in a thermal cycler with the heated lid off 15m

Cleanup of Adaptor-ligated DNA without Size Selection

7 Vortex SPRIselect Beads to resuspend. 5m

Add 93.5 µl (1X) resuspended beads to the Adaptor Ligation reaction. Mix well by pipetting up and down at least 10 times. Be careful to expel all of the liquid out of the tip during the last mix. Vortexing for 3-5 seconds on high can also be used. If centrifuging samples after mixing, be sure to stop the centrifugation before the beads start to settle out.

8 Incubate samples on bench top for at least 5 minutes at room temperature 5m

9 Place the tube/plate on an appropriate magnetic stand to separate the beads from the supernatant. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing on the magnetic stand. 5m

10 After 5 minutes (or when the solution is clear), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets (Caution: do not discard beads). 3m

11 Add 200 µl of 80% freshly prepared ethanol to the tube/ plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets 5m

12 Repeat Step 11 once for a total of two washes. Be sure to remove all visible liquid after the second wash. If necessary, brieflyspin the tube/plate, place back on the magnet and remove traces of ethanol with a p10 pipette tip. 5m

13 Air dry the beads for up to 5 minutes while the tube/plate is on the magnetic stand with the lid open.
Caution: Do not over-dry the beads. This may result in lower recovery of DNA target. Elute the samples when the beads are still dark brown and glossy looking, but when all visible liquid has evaporated. When the beads turn lighter brown and start to crack, they are too dry. 5m

14 Remove the tube/plate from the magnetic stand. Elute the DNA target from the beads by adding 17 µl of 10 mM Tris-HCl or 0.1X TE. 1m

15 Mix well by pipetting up and down 10 times, or on a vortex mixer. Incubate for at least 2 minutes at room temperature. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing back on the magnetic stand. 3m

16 Place the tube/plate on the magnetic stand. After 5 minutes (or when the solution is clear), transfer 15 µl to a new PCR tube. 5m

Samples can be stored at –20°C.

PCR Enrichment of Adaptor-ligated DNA (Blue)

17 Add the following components to a sterile strip tube:
Adaptor ligated DNA fragment from step 16, 15 uL
NEBNext Ultra II Q5 Master Mix, 25 uL

i7 primer, 5 uL (** each sample should use a unique i7 primer if they will be sequenced together)
University PCR primer , 5 uL
Total volume is 50 uL.

18 Set a 100 µl or 200 µl pipette to 40 µl and then pipette the entire volume up and down at least 10 times to mix thoroughly.
Perform a quick spin to collect all liquid from the sides of the tube.

19 Place the tube on a thermal cycler and perform PCR amplification using the following PCR cycling conditions:
Step A: 98 C, 45s;
Step B: 98 C, 15s;
Step C: 60 C, 10s;
Go to Step B, 13 times;
Step D: 72 C, 60s;
Step E: 4 C, forever.

Clean Up of PCR Reaction

20 Vortex SPRIselect Beads to resuspend.
Add 50 µl (1X) resuspended beads to the Adaptor Ligation reaction. Mix well by pipetting up and down at least 10 times. Be careful to expel all of the liquid out of the tip during the last mix. Vortexing for 3-5 seconds on high can also be used. If centrifuging samples after mixing, be sure to stop the centrifugation before the beads start to settle out.

21 Incubate samples on bench top for at least 5 minutes at room temperature

22 Place the tube/plate on an appropriate magnetic stand to separate the beads from the supernatant. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing on the magnetic stand.

23 After 5 minutes (or when the solution is clear), carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets (Caution: do not discard beads).

24 Add 200 µl of 80% freshly prepared ethanol to the tube/ plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets

25 Repeat Step 24 once for a total of two washes. Be sure to remove all visible liquid after the second wash. If necessary, briefly spin the tube/plate, place back on the magnet and remove traces of ethanol with a p10 pipette tip.

26 Air dry the beads for up to 5 minutes while the tube/plate is on the magnetic stand with the lid open.

Caution: Do not over-dry the beads. This may result in lower recovery of DNA target. Elute the samples when the beads are still dark brown and glossy looking, but when all visible liquid has evaporated. When the beads turn lighter brown and start to crack, they are too dry.

27 Remove the tube/plate from the magnetic stand. Elute the DNA target from the beads by adding 33 µl of 10 mM Tris-HCl or 0.1X TE.

28 Mix well by pipetting up and down 10 times, or on a vortex mixer. Incubate for at least 2 minutes at room temperature. If necessary, quickly spin the sample to collect the liquid from the sides of the tube or plate wells before placing back on the magnetic stand.

29 Place the tube/plate on the magnetic stand. After 5 minutes (or when the solution is clear), transfer 30 µl to a new PCR tube.

Samples can be stored at −20°C.

Check Library Quality

30 Measure library concentration by using Qubit High sensitive dsDNA kit.

31 Check the library fragment size by TapeStation.
If primer-dimer exceed 5%, perform another round of clean up using 1X SPRIselect beads.

Protocol references

https://www.neb.com/en-us/-/media/nebus/files/manuals/manuale7103-e7645.pdf?rev=de09eaf8fcdf45e0ac8a66bf6fee75fb&hash=346DB0B0FD1203244DC01FBEAFA2D259