CUTANA™ CUT&RUN Library Prep Kit

QUICK-START CARD

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Section I: End Repair (~75 min)

1. Transfer 5 ng of CUT&RUN enriched DNA to a new **8-strip PCR Tube**. Adjust volume to 25 µL with **0.1X TE Buffer**.
2. Prepare an End Repair Master Mix in a fresh 1.5 mL tube on ice by combining 4.2 µL **End Prep Buffer** and 1.8 µL **End Prep Enzyme** per reaction. Gently vortex (setting #7), quick spin, and return to ice.
3. Add 5 µL End Repair Master Mix to 25 µL DNA. Pipette up and down to clear tips, gently vortex to mix, and quick spin.
4. Place reactions in a thermocycler and run the following program with heated lid set to ≥75˚C:

STEP #	BLOCK TEMP	TIME	CYCLES	NOTES
1	20˚C	20 minutes	1	Reaction temperature
2	65˚C	30 minutes	1	Enzyme activation
3	4˚C	∞		Hold temperature

Section II: Adapter Ligation & U-Excision (~45 min)

5. Place 8-strip tubes directly on ice or in a pre-chilled aluminum block on ice.
6. Add 1.25 µL of 1.5 µM **Adapter for Illumina®** to each reaction on ice.
7. Prepare a Ligation Master Mix in a fresh 1.5 mL tube on ice by combining 16.5 µL **Ligation Mix** and 0.55 µL **Ligation Enhancer** per reaction. Gently vortex (setting #7), quick spin, and return to ice.
8. Add 15.5 µL Ligation Master Mix to each reaction on ice. Thoroughly vortex to mix (setting #7), quick spin, and return to ice.
9. Incubate tubes in thermocycler without a heated lid for 15 minutes at 20°C. Place tubes in a room temperature (RT) rack.
10. Add 1 µL **U-Excision Enzyme** to each reaction. Pipette to clear tips, gently vortex to mix, and quick spin.
11. Incubate in a thermocycler at 37°C for 15 minutes with a heated lid set to ≥47°C.
12. Remove tubes from thermocycler and quick spin. Continue to next step, or store tubes at -20˚C for future processing.

Section III: DNA Cleanup (~15 min)

***NOTE:** Multichannel pipetting is recommended for these steps.

13. Prepare 900 µL **FRESH** 85% Ethanol (EtOH) per reaction; if pausing protocol after **Section III**, make 450 µL per reaction.
14. Vortex **SPRIselect reagent** (manufactured by Beckman Coulter, Inc.*) thoroughly to ensure complete resuspension.
15. Slowly add 47.75 µL (1X reaction volume) **SPRIselect reagent** to each reaction.
16. Gently vortex 8-strip tubes and quick spin. Incubate at RT for 5 minutes.
17. Place 8-strip tubes on a magnet at RT for 2 minutes. Pipette to remove and discard supernatant.
18. Keeping tubes on magnet, add 180 µL 85% EtOH directly onto beads. Pipette to remove and discard supernatant.
19. Repeat previous step for a total of two washes, without removing tubes from magnet.
20. Remove tubes from the magnet, quick spin, and return tubes to magnet. Pipette to remove residual supernatant.
21. Remove tubes from magnet, leave caps open, and air dry the beads for 2 minutes.
22. Add 12 µL **0.1X TE Buffer** to each reaction to elute target DNA. Gently vortex to resuspend beads and quick spin.
23. Incubate at RT for 2 minutes. Place on magnetic rack for 2 minutes.
24. Transfer 10.5 µL eluted DNA to new **8-strip PCR Tubes**. Continue to next step or store tubes at -20˚C for future processing.

Section IV: Indexing PCR (~30 min)

***NOTE:** Before first use, transfer entire volume of **i5 Primers** to new **8-strip PCR Tubes**.

25. Following the primer selection instructions in **Appendix II**, assign a unique pair of **i5 & i7 Indexing Primers** to each reaction. Mark the combination as consumed in the **Primer Tracking Table** (below).

26. To each 10.5 µL reaction add the following reagents individually and in order. Visually inspect tips to confirm that the correct volume was aspirated and change tips between each addition to avoid cross-contamination.

    1 µL of the assigned **i7 Primer** (discard i7 Primer tube caps after using, and replace with fresh caps)
    1 µL of the assigned **i5 Primer**
    12.5 µL of **High Fidelity 2X PCR Master Mix** (mix well before using)

27. Vortex reaction to mix, then quick spin. Run PCR using the following parameters, with heated lid set to 105˚C:

STEP #	BLOCK TEMP	TIME	CYCLES	NOTES
1	98˚C	45 sec	1	Hot start activation
2	98˚C	15 sec	14	DNA melting
3	60˚C	10 sec		Hybrid annealing/extension
4	72˚C	60 sec	1	Final extension
5	4˚C	∞		Hold temperature

Section V: PCR Cleanup (~15 min)

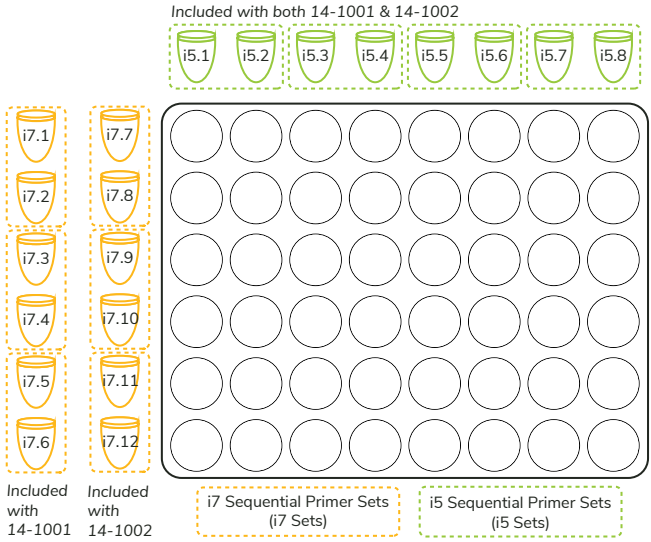
28. Follow **Section III: DNA Cleanup** protocol using 25 µL of **SPRIselect reagent** per reaction (1X reaction volume). Will result in 10.5 µL for each CUT&RUN library in fresh **8-strip PCR tubes**.

Section VI: Analysis of Library Fragment Size (~1 hr)

29. Use 1 µL to quantify CUT&RUN libraries using the Qubit™ fluorometer.

30. Prepare 5 µL of each library at 10 ng/µL, and load 1 µL on the Agilent BioAnalyzer® or Tapestation® for quality analysis. Obtain library concentration (200-700 bp range) and confirm mononucleosome fragment size distribution (~300 bp).

31. Store prepared CUT&RUN sequencing libraries at -20°C.



Primer Tracking Table

Mark consumed primer combinations on the provided table.

***IMPORTANT:** Each sequencing run must contain at least one i5 Sequential Primer Set (green dashed lines) AND one i7 Sequential Primer Set (orange dashed lines) to ensure proper index diversity for Illumina® systems. See **Appendix II** for a detailed Primer Selection Guide.