CUTANA™ CUT&RUN Library Prep Kit

QUICK-START CARD

Scan for full manual - read before first use

Section I: End Repair (~75 min)

1. Thaw kit reagents stored at -20˚C and keep on ice during experiment. Mix **End Prep Buffer**; if a white precipitate is observed, bring to room temperature (RT), vortex to dissolve, and place on ice.
2. Transfer 5 ng CUT&RUN-enriched DNA to fresh **8-strip tubes**. Adjust to 25 µL with **0.1X TE Buffer**.
3. Prepare an End Repair Master Mix in a new 1.5 mL tube on ice. Per library prep reaction, combine 4.2 µL (•) **End Prep Buffer** and 1.8 µL (•) **End Prep Enzyme**. Gently vortex to mix, quick spin to collect liquid, and return to ice. This recipe includes excess volume to account for pipetting error.
4. Add 5 µL **End Repair Master Mix** to 25 µL CUT&RUN DNA in 8-strip tubes. Pipette up and down 5X to clear tips, gently vortex to mix, and quick spin.
5. Place reactions in a thermocycler with heated lid set to ≥75˚C. Run the program outlined in the table.
6. Quick spin tubes and place on ice. **Note:** It is critical to keep tubes on ice during adapter ligation (below).

STEP #	TEMP	TIME	CYCLES
1	20˚C	20 min	1
2	65˚C	30 min	1
3	4-12˚C	∞	1

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

7. Prepare a Ligation Master Mix in a new 1.5 mL tube on ice. Per reaction, combine 16.5 µL (•) **Ligation Mix** and 0.55 µL (•) **Ligation Enhancer**. Gently vortex, quick spin, and return to ice. This recipe includes excess volume to account for pipetting error.
8. Add 1.25 µL (•) **Adapter for Illumina®** and 15.5 µL **Ligation Master Mix** to each reaction, keeping tubes on ice. Vortex tubes thoroughly to mix, quick spin, and return to ice.
9. Place tubes in a thermocycler without a heated lid, with block set to 20˚C. Incubate 15 min.
10. Quick spin tubes and place in a RT rack. Add 1 µL/reaction (•) **U-Excision Enzyme**. Pipette up and down 3X to clear tips, gently vortex to mix, and quick spin.
11. Place tubes in a thermocycler with a heated lid set to ≥47ºC and block set to 37˚C. Incubate 15 min.
12. Quick spin tubes. Continue to next step or store at -20˚C for future processing.

Section III: DNA Cleanup (~15 min)

***NOTE:** Use of multi-channel pipettors is recommended

13. Prepare 1 mL 85% Ethanol (EtOH) per reaction by combining 850 µL 100% EtOH and 150 µL molecular biology grade water. Prepare fresh, mix well, and store at RT.
14. Vortex **SPRIselect** reagent (manufactured by Beckman Coulter, Inc.) to thoroughly resuspend beads. Slowly add 47.75 µL/reaction. Ensure pipette tip is free of extra bead droplets.
15. Mix well by pipetting and/or vortexing to resuspend. Quick spin to collect liquid. Incubate 5 min at RT.
16. Place tubes on magnet for 2-5 min. Pipette to remove supernatant without disturbing beads.
17. Keep tubes on magnet. Add 180 µL/reaction **85% EtOH**. Remove supernatant. Repeat one time.
18. Quick spin with caps facing in to avoid dislodging beads. Return to magnet and remove residual EtOH.
19. Remove tubes from magnet. Air-dry, caps open, 2-3 min at RT. Beads should appear damp matte brown (Figure 1). If beads are crackly/light brown, they are too dry.
20. Add 12 µL/reaction **0.1X TE Buffer** to elute DNA. Pipette/vortex to resuspend. Incubate 2 min, RT.
21. Place tubes on magnet for 2 min at RT. Transfer 10.5 µL eluted DNA to new **8-strip tubes**. Continue or store at -20˚C.

FIGURE 1
Elute DNA when "ideal."

EpiCypher®

Section IV: Indexing PCR (~30 min)

***NOTE:** Before first use, transfer entire volume of (ʊ) **i5 primers** to a new set of **8-strip tubes**.

22. Assign a unique pair of (ʊ) **i5 and** (•) **i7 primers** to each reaction, following the primer selection instructions in **Appendix 2**. Mark the combination as consumed in the **Primer Tracking Table** (below).

23. Mix the stock tubes containing **Hot Start 2X PCR Master Mix** and (ʊ) **i5 and** (•) **i7 primers** and quick spin. To each library prep reaction, add the following reagents individually and in order: 1 µL (•) **i7 primer**, 1 µL (ʊ) **i5 primer**, and 12.5 µL (•) **Hot Start 2X PCR Master Mix**.

    Change tips between each addition to avoid contamination. Visually inspect tips to confirm the correct volume was aspirated.

24. Mix well by vortexing tubes, avoid bubbles, and quick spin. Place tubes in a thermocycler with a heated lid set to 105°C. Perform PCR using parameters outlined in the table.

STEP #	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	∞	1	Hold temperature

Section V: PCR Cleanup (~15 min)

25. Vortex **SPRIselect** reagent to thoroughly resuspend beads. Slowly add 25 µL **SPRIselect** reagent to each indexing PCR reaction. Ensure pipette tip is free of extra bead droplets.

26. Proceed with library cleanup following steps 15-21 in **Section III**. The protocol generates 10.5 µL purified CUT&RUN sequencing libraries.

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

27. Quantify libraries using the Qubit™ fluorometer. Examine fragment size distribution on the Agilent TapeStation® or Bioanalyzer®. Proceed to sequencing or store at -20°C. Obtain library concentration (200-700 bp range) and confirm fragment size distribution (~300 bp).

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

Primer Tracking Table

Mark consumed primer combinations in the table.

***IMPORTANT:** Sequencing runs must contain libraries with a sufficient mixture of i5 and i7 indexes to ensure proper diversity for for Illumina® systems. At least one i5 Sequential Primer Set (grey dashed lines) AND one i7 Sequential Primer Set (red dashed lines) should be represented per sequencing run. See **Appendix 2** for guidance. For index sequences, visit epicypher.com/14-1001 and download the Excel spreadsheet under Documents and Resources.