CUTANA™ CUT&RUN Library Prep Kit

QUICK-START CARD

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Section I: End Repair (~75 min)

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

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

5. Quick spin 8-strip tubes and place directly on ice or in a pre-chilled aluminum block on ice.

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

6. Prepare a Ligation Master Mix in a 1.5 mL tube on ice. Per reaction, combine 16.5 µL (•) **Ligation Mix** and 0.55 µL (•) **Ligation Enhancer**. Gently vortex (setting #7), quick spin, and return to ice.
7. To 8-strip tubes on ice, add 1.25 µL/reaction (•) **Adapter for Illumina®** and 15.5 µL/reaction Ligation Master Mix. Vortex tubes thoroughly to mix, quick spin, and return to ice.
8. Incubate 15 min in a thermocycler set to 20˚C, without a heated lid.
9. Place tubes in a room temperature (**RT**) rack and add 1 µL/reaction (•) **U-Excision Enzyme**. Pipette up and down to clear tips, gently vortex to mix, and quick spin.
10. Incubate 15 min in a thermocycler set to 37ºC, with heated lid at ≥47ºC.
11. 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 from this point forward.

12. Make 900 µL **FRESH** 85% Ethanol (EtOH) per reaction; if pausing after **Section III**, make 450 µL/reaction.
13. Vortex **SPRIselect** reagent (manufactured by Beckman Coulter, Inc.*) thoroughly to resuspend.
14. Slowly add 47.75 µL/reaction **SPRIselect** reagent in 8-strip tubes.
15. Gently vortex tubes and quick spin. Incubate 5 min at RT.
16. Place 8-strip tubes on a magnet for 2 min at RT. Remove supernatant.
17. Keeping tubes on magnet, add 180 µL/reaction 85% EtOH. Remove supernatant.
18. Repeat the previous step one time.
19. Remove tubes from magnet, quick spin, and return to magnet. Remove residual supernatant.
20. Remove tubes from magnet, leave caps open, and air dry beads for 2 min.
21. Add 12 µL/reaction **0.1X TE Buffer** to elute target DNA.
22. Gently vortex to resuspend beads and quick spin. Incubate 2 min at RT.
23. 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 for future processing.

Section IV: Indexing PCR (~30 min)

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

24. Assign a unique pair of (○) **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).
25. 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 assigned (•) **i7 primer**
    1 µL assigned (○) **i5 primer**
    12.5 µL (•) **Hot Start 2X PCR Master Mix** (mix well before using)
26. Vortex 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)

27. Follow **Section III: DNA Cleanup** using 25 µL/reaction **SPRIselect** reagent. Results in 10.5 µL of each library.

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

28. Use 1 µL of each CUT&RUN library for quantification on the Qubit™ fluorometer.
29. Prepare 5 µL of each library at 10 ng/µL. Run 1 µL on the Agilent BioAnalyzer® or Tapestation® for quality analysis. Obtain library concentration (200-700 bp range) and confirm fragment size distribution (~300 bp).
30. Store prepared CUT&RUN sequencing libraries at -20ºC.

Primer Tracking Table

Mark consumed primer combinations in the table.

***IMPORTANT:** Each sequencing run must contain at least one i5 Sequential Primer Set (grey dashed lines) AND one i7 Sequential Primer Set (red dashed lines) to ensure proper index diversity for Illumina® systems. See **Appendix 2** for a Primer Selection Guide. For index sequences, visit epicypher.com/14-1001 and download the Excel spreadsheet under Documents and Resources.