CUTANA™ QUICK CLEANUP DNA PURIFICATION KIT

QUICK-START CARD

Scan for full manual before first use

CUT&RUN DNA PURIFICATION PROTOCOL (~30 MIN)

This protocol generates highly pure CUT&RUN DNA for downstream library prep.
The starting input is 85 µL CUT&RUN-enriched chromatin in 8-strip tubes.

1. For each CUT&RUN reaction, make 500 µL 85% Ethanol (EtOH) by combining 425 µL 100% EtOH and 75 µL molecular biology grade water. Prepare fresh, mix well, and store at room temperature (RT).
2. Vortex **SPRIselect** reagent (manufactured by Beckman Coulter, Inc.) thoroughly to resuspend beads. Slowly add 119 µL **SPRIselect** reagent to 85 µL CUT&RUN-enriched chromatin in 8-strip tubes.
3. Mix well by pipetting and/or vortexing to an even resuspension. Quick spin tubes to collect liquid. Incubate for 5 min at RT.
4. Place tubes on a magnetic rack for 2-5 min, until solution clears. Pipette to remove supernatant without disturbing beads.
5. Keep tubes on magnet. Add 180 µL **85% EtOH** directly to beads. Pipette to remove supernatant. Repeat one time.
6. Remove tubes from magnet. Quick spin with caps facing in, to avoid dislodging beads. Return to magnet and pipette to remove residual EtOH.
7. Remove tubes from magnet and air-dry, caps open, for 2-3 min at RT. Beads should appear damp matte brown (Figure 1). If beads are crackly/light brown, they are too dry.
8. Add 17 µL **0.1X TE Buffer** to elute DNA. Pipette and/or vortex to resuspend beads and incubate 2 min at RT.

FIGURE 1
Elute DNA before beads dry out.

9. Quick spin tubes and place on magnet for 2 min. Transfer 15 µL CUT&RUN DNA to new **8-strip tubes**.
10. Use 1 µL to quantify DNA with the Qubit™ fluorometer. Proceed to library prep using the CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001) or store DNA at -20°C.

CUT&TAG SEQUENCING LIBRARY PURIFICATION PROTOCOL (~30 MIN)

This protocol is designed for purification of CUT&Tag sequencing libraries after indexing PCR.
The starting input is 50 µL post-indexing PCR product in 8-strip tubes.

1. For each CUT&Tag reaction, make 500 µL 85% Ethanol (EtOH) by combining 425 µL 100% EtOH and 75 µL molecular biology grade water. Prepare fresh, mix well, and store at room temperature (RT).
2. Vortex **SPRIselect** reagent thoroughly to resuspend beads. Slowly add 65 µL **SPRIselect** reagent to 50 µL post-indexing PCR product in 8-strip tubes.
3. Mix well by pipetting and/or vortexing to an even resuspension. Quick spin tubes to collect liquid. Incubate for 5 min at RT.
4. Place tubes on a magnetic rack for 2-5 min, until solution clears. Pipette to remove supernatant without disturbing beads.
5. Keep tubes on magnet. Add 180 µL **85% EtOH**. Pipette to remove supernatant. Repeat one time.
6. Remove tubes from magnet and quick spin with caps facing in, to avoid dislodging beads. Return to magnet and pipette to remove residual EtOH.
7. Remove tubes from magnet and air-dry, caps open, for 2-3 min at RT. Beads should appear damp matte brown (Figure 1). If beads are crackly/light brown, they are too dry.
8. Add 17 µL **0.1X TE Buffer** to elute DNA. Pipette and/or vortex to resuspend beads and incubate 2 min at RT.
9. Quick spin tubes and place on magnet for 2 min. Transfer 15 µL CUT&Tag libraries to new **8-strip tubes**.
10. Use 1 µL to quantify sequencing libraries with the Qubit fluorometer. Examine fragment distribution on the Agilent TapeStation® or Bioanalyzer®. Proceed to sequencing or store at -20°C.

SEQUENCING LIBRARY CLEANUP (ADAPTER / PRIMER DIMER REMOVAL) (~30 MIN)

This protocol is designed for removal of small contaminating fragments from CUT&RUN and CUT&Tag sequencing libraries. In CUT&RUN libraries, small fragments represent adapter dimers (125-175 bp), while CUT&Tag libraries are prone to primer dimers (25-100 bp). This method removes all fragments <180 bp, making it suitable for CUT&RUN and CUT&Tag library cleanup.

The starting input for this protocol is a normalized library pool of 8 or more sequencing libraries, with a volume determined by the user. The protocol is designed for 8-strip tubes.

1. Prepare a normalized library pool in 8-strip tubes. Use your preferred sequencing buffer and molarity calculations from TapeStation/Bioanalyzer data (200-700 bp region):
   A. Dilute individual libraries to the same concentration, depending on final yields. 1-4 nM is ideal for NextSeq 2000 and NextSeq 500/550.
   B. Combine equimolar libraries into one tube. This is your normalized library pool for cleanup.
   C. Transfer 2 µL library pool to a new tube and set aside. Use this material to confirm small fragment removal after cleanup.
2. Make 1 mL 85% Ethanol (EtOH) by combining 850 µL 100% EtOH and 150 µL molecular biology grade water. Prepare fresh, mix well, and store at room temperature (RT).
3. Vortex **SPRIselect** reagent thoroughly to resuspend beads. Slowly add a 1X volume of **SPRIselect** reagent to library pool (e.g. add 40 µL beads to 40 µL library pool).
4. Mix well by pipetting and/or vortexing to an even resuspension. Quick spin tubes to collect liquid. Incubate for 5 min at RT.
5. Place tubes on a magnetic rack for 2-5 min, until solution clears. Pipette to remove supernatant without disturbing beads.

   (Optional): To ensure fragments of interest are not removed, transfer the supernatant to a new tube. Note the volume of the supernatant and add a 0.5X volume of **SPRIselect** reagent to the tube (e.g. add 19 µL beads to 38 µL supernatant). Mix well and proceed with DNA cleanup in parallel (Steps 6-11). This material can be recombined with the library pool if significant loss is observed.
6. Keep tubes on magnet. Add 180 µL **85% EtOH**. Pipette to remove supernatant. Repeat one time.
7. Remove tubes from magnet and quick spin with caps facing in, to avoid dislodging beads. Return to magnet and pipette to remove residual EtOH.
8. Remove tubes from magnet and air-dry, caps open, for 2 min at RT. Beads should appear damp matte brown (Figure 1). If beads are crackly/light brown, they are too dry.
9. Add 25 µL preferred sequencing buffer from Step 1 to elute the library pool. Larger elution volumes may be used, with the caveat that DNA concentration will be lower.
10. Pipette and/or vortex to resuspend beads and incubate 2 min at RT.
11. Quick spin tubes and place on magnet for 2 min. Transfer 24 µL eluted library pool to a new tube (8-strip tubes or 1.5 mL tube can be used).
12. Use 1 µL to quantify the library pool with the Qubit fluorometer. Confirm small fragment removal on the TapeStation or Bioanalyzer. Scan the QR code to see the **DNA Quick Cleanup DNA Purification Kit manual** for additional information and examples.
13. Record final library pool concentration. Proceed to sequencing or store at -20˚C.