Cataolg No. 14-0052

CUTANA™ Quick Cleanup DNA Purification Kit V1

QUICK-START CARD

SCAN FOR MANUAL

Read latest manual before first experiment!

CUT&RUN DNA PURIFICATION PROTOCOL (~30 MIN)

* **The starting input is 84 µL CUT&RUN-enriched chromatin in 8-strip tubes.**

1. Prepare 500 µL 85% Ethanol (EtOH) per reaction by combining 425 µL 100% EtOH and 75 µL molecular biology grade water. Prepare fresh, mix well, and store at RT.
2. Vortex **SPRI beads** to thoroughly resuspend beads. Slowly add 118 µL/reaction (1.4X ratio). **Note:** If targets generate small fragments, a 1.8X bead ratio may help improve recovery.
3. Mix well by pipetting and/or vortexing. Quick spin in a mini-centrifuge to collect liquid. Incubate 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/reaction **85% EtOH**. Remove supernatant. Repeat one time.
6. Quick spin tubes with caps facing in, to avoid dislodging beads. Place on magnet and remove residual EtOH.

FIGURE 1
Elute DNA when "ideal."

7. Take tubes off magnet. Air-dry, caps open, 2-3 min at RT. Beads should be damp matte brown (Figure 1). If beads are crackly/light brown, they are too dry.
8. Add 17 µL/reaction **0.1X TE Buffer** to elute DNA. Pipette or vortex to resuspend and quick spin.
9. Incubate 2 min at RT. Quick spin tubes and place on magnet for 2 min.
10. Transfer 15 µL CUT&RUN-enriched DNA to new **8-strip Tubes**.
11. Quantify DNA with the Qubit fluorometer. Proceed to library prep or store DNA at -20ºC.

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

* **The starting input is 50 µL post-indexing PCR product in 8-strip tubes.**

1. Prepare 500 µL 85% Ethanol (EtOH) per reaction by combining 425 µL 100% EtOH and 75 µL molecular biology grade water. Prepare fresh, mix well, and store at RT.
2. Vortex **SPRI beads** to thoroughly resuspend beads. Slowly add 65 µL/reaction (1.3X ratio).
3. Mix well by pipetting and/or vortexing. Quick spin in a mini-centrifuge. Incubate 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**. Remove supernatant. Repeat one time.
6. Quick spin tubes with caps facing in to avoid dislodging beads. Place on magnet and remove residual EtOH.
7. Take tubes off magnet. Air-dry, caps open, 2-3 min at RT. Beads should be damp matte brown (Figure 1). If beads are crackly/light brown, they are too dry.
8. Add 17 µL/reaction **0.1X TE Buffer** to elute DNA. Pipette or vortex to resuspend and quick spin.
9. Incubate 2 min at RT. Quick spin tubes and place on magnet for 2 min.
10. Transfer 15 µL CUT&Tag sequencing libraries to new **8-strip Tubes**.
11. Quantify libraries with the Qubit fluorometer. Examine fragment distribution on the 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 (~150 bp), while CUT&Tag libraries are prone to primer dimers (25-100 bp).**
* **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 **SPRI beads** thoroughly to resuspend beads. Slowly add a 1X ratio of **SPRI beads** 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 - Purify short DNA fragments from removed fraction: To ensure library fragments are not lost, transfer the Step 5 supernatant to a new tube. Note the volume of the supernatant and add a 0.5X ratio of **SPRI beads** to the tube (e.g. add 19 µL beads to 38 µL supernatant). Mix well and proceed with DNA purification, starting at the 5 min incubation in Step 4. This material can be added back to the library pool at the end if significant loss is observed.

6. Keeping 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 a 1.5 mL tube can be used).
12. 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.