EpiCypher® CUTANA™ CUT&RUN Genomic Core Instructions

For CUT&RUN Libraries, Library Prep, and Sequencing

If Performing CUT&RUN Library Prep for a Core Customer

1. Quantify CUT&RUN DNA using the Qubit fluorometer and 1X dsDNA HS Assay Kit (Invitrogen Q33230). Depending on the target, DNA yields can range from undetectable (by Qubit) to ≥10 ng.
    a. DO NOT examine fragment size analysis (e.g. TapeStation or Bioanalyzer) at this stage, as DNA quantity is below the sensitivity limit of these platforms and cannot be effectively resolved.
2. Ideally, 5ng should be used in library prep. If CUT&RUN DNA yields are less than 5 ng, use the entire DNA yield as input to library prep.
3. The preferred library prep workflow is the CUTANA™ CUT&RUN Library Prep Kit, as it is optimized for the small fragment sizes and yields generated by CUT&RUN.
4. To get quality CUT&RUN libraries from another library prep kit:
    a. After adapter ligation, perform DNA cleanup using a 1X CUTANA™ DNA Purification Beads (EpiCypher 21-1407) ratio (e.g. 50 µL beads to 50 µL PCR product). This will retain fragments >150 bp.
    b. PCR amplification cycles should be modified according to the below protocol
        i. 45 sec at 98°C (Hot start activation of DNA polymerase)
        ii. 15 sec at 98°C (DNA melting)
        iii. 10 sec at 60°C (Hybrid annealing/extension)
        iv. Repeat steps ii - iii for a total of 14X
        v. 1 min at 72°C (Final extension)
    c. Perform a final DNA clean-up with a 1X CUTANA™ DNA Purification Bead ratio.
5. Quantify purified CUT&RUN sequencing libraries using the Qubit fluorometer and 1X dsDNA HS Assay Kit (Invitrogen Q33230).
6. For each library, prepare 5 µL at 10 ng/µL. Examine diluted sequencing libraries on the Bioanalzyer (High Sensitivity Kit) or TapeStation (D1000 ScreenTape and Reagents) per manufacturer's instructions. You should see predominant enrichment of ~300 bp fragments.

If receiving prepared libraries from a Core customer

1. Quantify purified CUT&RUN sequencing libraries using the Qubit fluorometer and 1X dsDNA HS Assay Kit (Invitrogen Q33230).
2. For each library, prepare 5 µL at 10 ng/µL. Examine diluted sequencing libraries on the Bioanalzyer (High Sensitivity Kit) or TapeStation (D1000 ScreenTape and Reagents) per manufacturer's instructions. You should see predominant enrichment of ~300 bp fragments.
    a. If using a qPCR-based quantification method (e.g. KAPA Library Quantification Kits) note that these methods quantify all adapter-ligated fragments, not just the fragment sizes of interest.
    b. Sharp peaks at ~400 bp represent spike-ins (specific to EpiCypher CUT&RUN).

c. Peaks at ~150 bp represent adapter dimer. If adapter dimer comprises >5% of the library, it should be removed. We recommend pooling sequencing libraries and re-purifying the pool using a 1X ratio of CUTANA DNA Purification Beads. Do not re-purify individual sequencing libraries, as this risks losing ~300 bp library fragments.

Preparing CUT&RUN Libraries for Illumina Sequencing

1. Use the 200-700 bp concentration (from TapeStation or Bioanalyzer) to dilute each library to the desired ratio, typically 1-4 nM.
2. Pool libraries at equimolar ratios (e.g. dilute each library to 1 nM, then combine 5 µL of each library into one tube). We have found this method to be the best to ensure accurate pooling.
    a. Diluting and pooling can be done in a 96-well plate where each column/row/sample can be combined once diluted.
    b. We then recommend running this master pool on TapeStation or Bioanalyzer to ensure maximum flow cell loading. Again, if >5% of the pool is attributed to adapter dimers, we recommend re-purifying the entire pool using a 1X CUTANA DNA Purification Beads ratio.
3. CUT&RUN has a high signal-to-noise ratio, and it is recommended to sequence 5-10 million total reads per sample, so that 3-8 million unique reads are obtained.
    a. DO NOT sequence at ChIP-seq levels (>10 million reads). This will increase background and will make data analysis difficult.
4. We recommend 50 bp paired-end sequencing (2 x 50 bp reads). Longer reads may be used, but will require adapter trimming prior to bioinformatic analysis.