**Additional Information for**

**The ChIP-exo Method: Identifying Protein-DNA Interactions with Near Base Pair Precision**

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**Oligonucleotide sequences (Related to Sections 2, 19, and 23)**

Note: /5Phos/ = 5’Phosphate, \* = phosphothiorate 3’ linkage, INDEX denotes 6 nucleotide barcode sequence

P7 Adapter

ExA2\_iX (PAGE purification)

5’/5Phos/CAAGCAGAAGACGGCATACGAGAT**INDEX**GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC\*T

ExA2-33 (PAGE purification)

5’GATCGGAAGAGCACACGTCTGAACTCCAGTCAC

P5 Adapter

ExA1-58 (PAGE purification)

5’AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATC\*T

ExA1-13 (standard desalting)

5’GATCGGAAGAGCG

P7 Primer (standard desalting)

5’ CTGGAGTTCAGACGT

PCR Primers P1.3 & 2.1

P1.3 (HPLC purification)

5’AATGATACGGCGACCACC

P2.1 (HPLC purification)

5’CAAGCAGAAGACGGCATACGA

**Quick Guide to Operating the BioRuptor (related to Section 5)**

1. Fill BioRuptor chamber halfway with ice to prechill for a few minutes. In the meantime, fill 2 buckets with ice (one to chill resonance adapter and spacers and the other to chill 15ml sample tubes). Fill 1L beaker with ice and fill with deionized water.
2. Remove ice from BioRuptor chamber by hand. Fill chamber with ice-cold water to the labeled line. Scoop out any large chunks of ice. It is critical that the water level is exact.
3. Insert chilled resonance adapter into 2x 15 mL polystyrene tubes and screw cap snuggly. The cap should not be so tight that the tip cannot be repositioned in the middle of the tube. Ensure that the metal tip is not touching the wall of the tube.
4. After closing lid, place 2 spacers in opposite positions, and then place 15 mL polystyrene tubes with inserted resonance adapter on top of spacers. Close door and turn on the sonicator after correct settings are verified. Set timer to 15 min. and keep track with independent timer.
5. Open door and remove 15 mL polystyrene tubes. Immediately remove resonance adapter and place 15 mL tubes on ice. Rinse resonance adapter with deionized water and place back on ice along with spacers.
6. Use a pump to aspirate the water from the BioRuptor chamber into a waste beaker. Refill chamber with ice-cold deionized water to fill line.
7. Repeat steps 2 to 6 as many times as necessary to sonicate all samples.

**Input Library Guide**

If input librariesare needed**,** collect 50ul sonicated nuclear lysate from Section 5.

1. To reverse crosslinks, add 50ul TE-RNaseA and 1ul of 20mg/mL Proteinase K to 50ul sample. Mix and incubate at 65°C overnight to reverse crosslinks.
2. Perform PCIAA extraction and ethanol precipitation of DNA from Section 18 and resuspend DNA pellet in 100ul of ddH2O.
3. Measure DNA concentration on Qubit Fluorometer 2.0 using 2ul of input sample. Total yield should be above 100ng for Illumina Library prep.

**Directions for ChIP Wash Cycles (related to Section 8)**

1. After aspirating previous wash or reaction while samples are on the magnet, immediately add the next wash buffer.
2. Invert tubes for 3 min at room temperature (RT) on a mini-tube rotator.
3. Spin tubes briefly.
4. Place tubes on the magnetic rack for 1 min, and then aspirate supernatant.
5. Immediately proceed to next wash.