**Supplementary material and methods**

Methyl-Seq was performed using Agilent’s SureSelectXT Methyl-Seq Target Enrichment System protocol version A.1 for Illumina multiplexed sequencing. Unless otherwise stated, product catalogues for all the reagents used in this assay can be found in protocol. Here, we detail sections of the protocol and also describe alterations made to the original protocol.

**DNA fragmentation**

3µg of DNA was diluted in low TE buffer to a volume of 130µl and was fragmented using E or S series of the Covaris sonicator. Sonication settings used were Duty Factor 10%, Peak Incident Power 175, cycles of Burst 200, treatment time 360 seconds, bath temperature was 4◦C – 8◦C.

**End-repair, A-tailing and adaptor ligation**

DNA was concentrated using a speed vac to a volume of 50µl followed by end-repair using 50µl of XT2 End-Repair Master Mix. The reaction was incubated on a thermal cycler for 30 min at 20◦C. DNA was subsequently purified using AMPure XP beads. Reaction was mixed with 180µl of AMPure XP beads and incubated for 10 min at room temperature. Beads were separated from solution using a magnetic separation device and washed twice using 200µl of 80% ethanol for each wash. Beads were incubated for 1 min for each ethanol wash. Beads were then dried using a thermal cycler at 37◦C for exactly 5 min. DNA was eluted with 22µl of nuclease-free water at 37◦C for 10 min. While beads were still present in DNA suspension, the mixture was mixed with 20µl of XT2 dA-Tailing Master Mix and incubated at 37◦C for 30 min. After incubation, 5µl of XT2 ligation master mix and 5µl of SureSelect Methyl-Seq methylated adaptor were added to the reaction and incubated at 20◦C for 15 min. To purify DNA from the reaction, AMPure XP beads were reactivated by mixing the reaction with equal volume of a buffer consisting of 20% polyethylene glycol/2.5M NaCl and incubated at room temperature for 10 min. Beads were then washed with 80% ethanol and eluted in 22µl of water as described above. Only the supernatant was retained for subsequent steps of the protocol. Using DNA 1000 bioanalyzer assay, 2µl of products after end-repair and 2µl of products after adaptor ligation were used to ensure successful enzymatic reactions. After end-repair, DNA fragments ranged between 140 – 180 bps. After adaptor ligation, fragments ranged between 170-230 bps.

**Hybridization**

Adaptor-ligated DNA was concentrated using a speed vac to a volume of 3.4µl. In the first PCR plate, capture library mix was made by mixing 5µl of SureSelect mouse Methyl-Seq Capture Library with 2µl of 25% RNase Block solution (0.5µl RNase Block and 1.5µl nuclease free water). In a second PCR plate, 3.4µl of adaptor-ligated DNA was mixed with 5.6µl of SureSelect Methyl-Seq block mix (2.5µl SureSelect Indexing block #1, 2.5µl SureSelect Indexing block #2 and 0.6µl 0SureSelect Indexing block #3) in the first row of the plate. Wells of the second PCR plate were sealed and incubated in the thermal cycler for 95◦C for 5 min followed by an incubation temperature of 65◦C. While at 65◦C, 40µl of hybridization buffer (25µl SureSelect Hyb #1, 1µl SureSelect Hyb #2, 10µl SureSelect Hyb #3, 13µl SureSelect Hyb #4) was added to the second row of the plate and incubated at 65◦C for 5 min. From the first PCR plate, 7µl of capture library mix was added to the third row of the second PCR plate and incubated at 65◦C for 2 min. Following this, 13µl of the hybridization buffer from the second row was transferred and mixed with the the capture library mix that is in the third row. 9µl of the adapter-ligated DNA and SureSelect Methyl-Seq block mix was then transferred and mixed with the reaction in the third row. The reaction was incubated at 65◦C for 24 hr.

**Isolation of DNA-RNA bait hybrids**

50µl of Dynabeads MyOne streptavidin T1 beads were washed in SureSelect Binding Buffer 3 times and resuspended in 200µl of SureSelect Binding Buffer. The hybridization reaction was transferred from the incubation plate at 65◦C to the streptavidin beads and incubated on a nutator for 30 min at room temperature. Beads were separated from solution using a magnetic separator and washed in 500µl of SureSelect Wash Buffer #1 by brief vortexing and incubation at room temperature for 15 min. Beads were then separated from solution by a magnetic separator and resuspended in 500µl of 65◦C-prewarmed SureSelect Wash Buffer #2 and vortexed briefly. They were incubated for 10 min at 65◦C in a water bath and separated from the supernatant using a magnetic separator. The supernatant was discarded. This wash step with SureSelect Wash Buffer #2 was performed 3 times in total. Beads were pelleted by centrifugation at maximum speed for 1 min and separated from residual supernatant by magnetization. After residual supernatant was removed, DNA was isolated from the beads by re-suspending the beads in 20µl of Qiagen pyromark denaturation buffer (Cat. No. 979007) and incubating this at room temperature for 20 min.

**Bisulfite conversion**

Bisulfite conversion was performed using Zymo research’s EZ DNA Methylation-Gold kit and was performed as described in the manufacturer’s protocol. Briefly, 20µl of isolated DNA was mixed with 130µl of CT conversion reagent and incubated in a thermal cycler at 64◦C for 2.5 hr. Sample was transferred to the Zymo-Spin IC column containing 600µl of M-binding buffer. The flow-through was discarded and the column was washed with 100µl of M-wash buffer. 200µl of M-desulphonation buffer was then added to the column and incubated for 20 min at room temperature. The column was then washed with 200µl of M-wash buffer and air dried at room temperature for 2 min. DNA was eluted in 10µl of M-elution buffer twice giving a total volume of 20µl.

Quantify bisulfite-treated DNA samples by qPCR

DNA was quantified using Agilent qPCR NGS library quantification kit (G4880A). Briefly, a standard curve was created from five 2-fold serial dilutions of the quantification standard included in the kit. The range covered was 10 pM, 5 pM, 2.5 pM, 1.25 pM, 0.625 pM and 0 pM. The standard was diluted in 1 x Dilution Buffer also included in the kit. Bisulfite-converted DNA was diluted 1/100 in 1 x Dilution Buffer. 2µl of the standard curve and bisulfite-converted DNA were mixed with 18µl of qPCR master mix (5.3 µl nuclease free water, 10 µl of 2 x Brilliant III Master Mix, 2 µl of 10 x GC Additive, 0.2 µl of Methyl-Seq PCR1 Primer F, 0.2 µl of Methyl-Seq PCR1 Primer R and 0.3 µl of Reference Dye that has been diluted 1/500 in nuclease free water). qPCR was performed using SYBR green instrument settings. The thermal cycling parameters used were 1 cycle of 95◦C for 2 min followed by 30 cycles of 95◦C for 30 seconds, 60◦C for 30 seconds and 72◦C for 30 seconds.

**PCR amplification of bisulfite converted libraries**

Amplification was performed using 18µl of bisulfite-converted DNA and 82µl of PCR reaction mix (30 µl nuclease free water, 50 µl SureSelect Methyl-Seq 2x Taq200 Master Mix, 1µl of Methyl-Seq PCR1 Primer F and 1µl Methyl-Seq PCR1 Primer R). Thermal cycling parameters were 1 cycle of 95◦C for 2 min, 7 - 10 cycles (\*see below for explanation) of 95◦C for 30 seconds, 60◦C for 30 seconds and 72◦C for 30 seconds, 1 cycle of 72◦C for 7 min, 1 cycle of holding temperature at 4◦C. Reactions were purified using AMPure XP beads as mentioned above and eluted in 26 µl free nuclease-free water. \*Where bisulfite-converted library concentration was > 800 pM, 7 cycles was used. If the library was between 400 pM - 800 pM, it was amplified by 8 cycles. If the library was between 200 pM – 400 pM, 9 amplification cycles were used. If the libraries were between 100 pM – 200 pM, 10 cycles were used.

**Indexing of libraries**

Libraries were indexed as described in the Methyl-Seq protocol. Briefly, 24 µl of amplified library was mixed with 26µl of indexing reaction mix (0.5µl f PCR Primer Index 1, 0.5 µl of SureSelect Methyl-Seq Common Indexing Primer and 25µl of SureSelect Methyl-Seq 2 x Taq2000 Master Mix). Thermal cycling conditions were 1 cycle of 95◦C for 2 min, 4 cycles of 95◦C for 30 seconds, 60◦C for 30 seconds and 72◦C for 30 seconds, 1 cycle of 72◦C for 7 min, 1 cycle of 1 cycle of holding temperature at 4◦C. Reactions were purified using AMPure XP beads as mentioned above and eluted in 24µl of nuclease-free water. On the bioanalyzer high sensitivity DNA assay, the indexed libraries should have a fragment size of 220 – 280 bps.