

Reduced Representation Bisulfite Sequencing (RRBS):

RRBS uses restriction enzymes and bisulfite conversion to enrich for CpG to assess genome wide methylation patterns. 1 ng-1 µg high molecular weight DNA is restriction enzyme digested (MspI-NEB, R0149S/ TaqI -NEB, R0106S) to yield various sized sticky end fragments. The 3' end is end repaired and A-tailing is performed on the 5' end of both strands (Kapa Hyper Prep Kit-Kapa, KK8501). Methylated sequencing adapters are ligated to the DNA fragments and the DNA fragments are purified (AMPureXP beads-Invitrogen, A63880). The purified DNA fragments undergo bisulfite conversion (EpiTect Bisulfite Conversion Kit-Qiagen, 59104) and are PCR amplified (HiFi Uracil+ Polymerase-KAPA, KK2801) using primers complementary to the sequencing adapters. The library is quantified via qPCR (KAPA qPCR Library Quantification Kit-KAPA, KK4844) and pooled at equimolar ratios. Libraries are sequenced using Illumina sequencing chemistry to yield 50M 75bp paired end reads per sample.

For detailed methods see: [Barwick et al. *Nature Immunology*. 2016.](#)