ACTIVE MOTIF®

Sample Preparation for DNA Methylation Analysis

RRBS

(Use one of the following recommendations for sample submission.)

I. Prepare frozen pellets from cell cultures

1. Grow 1 x 10^5 to 5 x 10^6 cells in culture.
2. Transfer cell culture to a conical tube. (If cells are adherent, scrape them thoroughly from the culture surface prior to transferring to a conical tube).
3. Centrifuge tubes at 800 x *g* in a refrigerated centrifuge for 5 minutes to pellet the cells. Decant culture media.
4. Resuspend cells in 10 ml chilled PBS by pipetting up and down, then spin again at 800 x *g* in a refrigerated centrifuge for 5 minutes to pellet the cells.
5. Decant PBS, freeze cell pellets on dry ice and store at -80°C.

II. Freeze animal tissue

1. Remove an appropriate amount of tissue from the animal (20-200 mg for most tissues).
2. Place tissue in a 1.5 ml microfuge tube or 15 ml conical, freeze on dry ice and store at -80°C.

III. Prepare DNA

1. Prepare genomic DNA from cell culture or animal tissue using a Qiagen QIAmp DNA Mini Kit (cat # 51304) or comparable genomic DNA isolation method.
2. Elute or resuspend DNA in 10 mM Tris, pH 8.
3. Run 100-200 ng of DNA on a 1% agarose gel to show high molecular weight DNA.
4. Send 100 ng to 10 µg of DNA at a minimum concentration of 10 ng/µl.
   NOTE: Minimum concentration is required in order not to dilute reaction volume.