

## Sample Preparation for DNA Methylation Analysis

(Submit cells, tissues or DNA for Active Motif's MeDIP, RRBS & Bisulfite Sequencing Assays)

### MeDIP

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

#### I. Prepare frozen pellets from cell cultures

1. Grow  $2 \times 10^6$  to  $5 \times 10^6$  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  $800 \times g$  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  $800 \times g$  in a refrigerated centrifuge for 5 minutes to pellet the cells.
5. Decant PBS, freeze cell pellets on dry ice and store at  $-80^\circ\text{C}$ .
6. Ship on dry ice. Fill out Active Motif's [Sample Submission Form](#), include a hard copy with your samples and ship according to the instructions on the Sample Submission Form.

#### II. Freeze animal tissue

1. Remove an appropriate amount of tissue from the animal (100-200 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  $-80^\circ\text{C}$ .
3. Ship on dry ice. Fill out Active Motif's [Sample Submission Form](#), include a hard copy with your samples and ship according to the instructions on the Sample Submission Form.

#### 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 200 ng of DNA on a 1% agarose gel to show high molecular weight DNA.
4. Send 5 to 50  $\mu\text{g}$  of DNA at a minimum concentration of 75 ng/ $\mu\text{l}$ . Enclose a photo of the gel analysis with your sample.
4. Ship on dry ice or cold packs. Fill out Active Motif's [Sample Submission Form](#), include a hard copy with your samples and ship according to the instructions on the Sample Submission Form.

## RRBS

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

### I. Prepare frozen pellets from cell cultures

1. Grow  $5 \times 10^5$  to  $5 \times 10^6$  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  $800 \times g$  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  $800 \times g$  in a refrigerated centrifuge for 5 minutes to pellet the cells.
5. Decant PBS, freeze cell pellets on dry ice and store at  $-80^\circ\text{C}$ .
6. Ship on dry ice. Fill out Active Motif's [Sample Submission Form](#), include a hard copy with your samples and ship according to the instructions on the Sample Submission Form.

### II. Freeze animal tissue

1. Remove an appropriate amount of tissue from the animal (25-200 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  $-80^\circ\text{C}$ .
3. Ship on dry ice. Fill out Active Motif's [Sample Submission Form](#), include a hard copy with your samples and ship according to the instructions on the Sample Submission Form.

### 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 200 ng of DNA on a 1% agarose gel to show high molecular weight DNA.
4. Send 1 to 10  $\mu\text{g}$  of DNA at a minimum concentration of 5 ng/ $\mu\text{l}$ . Enclose a photo of the gel analysis with your sample.
5. Ship on dry ice or cold packs. Fill out Active Motif's [Sample Submission Form](#), include a hard copy with your samples and ship according to the instructions on the Sample Submission Form.

## Targeted Next-Gen Bisulfite Sequencing

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

### I. Prepare frozen pellets from cell cultures

1. Grow  $5 \times 10^5$  to  $5 \times 10^6$  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  $800 \times g$  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  $800 \times g$  in a refrigerated centrifuge for 5 minutes to pellet the cells.
5. Decant PBS, freeze cell pellets on dry ice and store at  $-80^\circ\text{C}$ .
6. Ship on dry ice. Fill out Active Motif's [Sample Submission Form](#), include a hard copy with your samples and ship according to the instructions on the Sample Submission Form.

### II. Freeze animal tissue

1. Remove an appropriate amount of tissue from the animal (25-200 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  $-80^\circ\text{C}$ .
3. Ship on dry ice. Fill out Active Motif's [Sample Submission Form](#), include a hard copy with your samples and ship according to the instructions on the Sample Submission Form.

### 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 200 ng of DNA on a 1% agarose gel to show high molecular weight DNA.
4. Send 500 ng to 5 ug of DNA at a minimum concentration of  $30 \text{ ng}/\mu\text{l}$ . Enclose a photo of the gel analysis with your sample.
5. Ship on dry ice or cold packs. Fill out Active Motif's [Sample Submission Form](#) completely and enclose it with your samples. Follow the Shipping Instructions on that document to send in your samples.