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 x 10^6 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.
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°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 µg of DNA at a minimum concentration of 75 ng/µl. 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 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.
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°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 µg of DNA at a minimum concentration of 5 ng/µl. 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 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.
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°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 ng/µl. 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.