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 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.
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 (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.
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 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. 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.