RNA-Seq Sample Preparation

Customers may submit cells, animal tissues or purified total RNA for our RNA-Seq Service (Illumina-based sequencing).

I. Prepare frozen pellets from cell cultures

If cells are adherent it is best to add the lysis buffer directly to the culture plate. Use the RNA lysis buffer provided in your RNA isolation kit of choice and perform lysis and RNA isolation as described by the kit manufacturer. Complete the RNA isolation and provide total RNA to Active Motif as described in section III below. If you are unable to perform the RNA isolation, prepare cells as described below.

1. Grow cells in culture and harvest 2 x 10^6cells. A minimum amount of 2.5 x 10^5 is acceptable if necessary.
2. Transfer cell culture to a conical tube (If cells are adherent, scrape or trypsinize them from the culture surface prior to transferring to a 15 ml conical tube).
3. Centrifuge tubes at 800 x *g* in a refrigerated centrifuge for 5 minutes to pellet the cells and decant culture media.
4. Re-suspend cells in 10 ml chilled PBS per tube by pipetting up and down. Spin again at 800 x *g* in a refrigerated centrifuge for 5 minutes to pellet the cells.
5. Decant PBS and freeze cell pellets with liquid nitrogen or on dry ice.
6. Store at -80°C.

II. Freeze animal tissue

1. Remove the appropriate amount of tissue (approximately 50-200 mg for most tissues). Note the weight of each sample on the sample submission form.
2. Place tissue in a 1.5 ml microfuge tube or 15 ml conical and snap freeze on dry ice. Alternatively, wrap the tissue in aluminum foil and freeze with liquid nitrogen.
3. Store at -80°C.

III. Prepare total RNA

1. Prepare total RNA from cell culture or animal tissue using a column-based RNA purification kit such as the Qiagen RNeasy Mini/Midi kit or other suitable RNA purification kit.
2. Elute or re-suspend RNA in water and measure concentration/yield. We ask for 0.5 μg to 3 μg of total RNA at a concentration of 10-50 ng/ul.
3. Store at -80°C.