Services Library Prep Submission Protocol for use with Active Motif Kits:

ATAC-Seq Assay Kit, CUT&Tag-IT® Assay Kit, CUT&Tag-IT® R-loop Assay Kit, ChIP-IT High Sensitivity® Kit, and CUT&RUN Assay Kit

There are two options for submitting your libraries. The first one is to ship your individual libraries to Active Motif and we will pool and balance libraries with full QC. The second option is to quantify and pre-pool the libraries before shipping to Active Motif and we will directly sequence the library.

I. Individual Libraries

If submitting individual libraries, Active Motif will conduct significant QC before performing full-scale sequencing. Each library will be checked for concentration and fragment distribution before performing shallow sequencing to check clustering on an iSeq. Libraries are then rebalanced and pooled before checking the concentration and fragment distribution once again before full-scale sequencing. However, we still recommend quantifying and checking the fragment distribution of each library before shipping to ensure that that sequencing will be successful.

1. The concentration of each library should >1 ng/µl as measured by Qubit® fluorometric quantification (ThermoFisher Scientific).
2. Library fragment distribution should be similar for all libraries to be pooled, with fragments appearing as described below when measured by TapeStation (Agilent) or gel electrophoresis:
   a. ATAC-Seq Assay Kit, nucleosomal pattern from 200-500 bp.
   b. CUT&Tag-IT® Assay Kit, nucleosomal pattern from 200-500 bp.
   c. CUT&Tag-IT® R-loop Assay Kit, nucleosomal pattern from 200-500 bp.
   d. CUT&RUN Assay Kit with AM DNA Library Prep Kit for Illumina, nucleosomal pattern from 200-500 bp.
   e. ChIP-IT High Sensitivity® Kit with AM DNA Library Prep Kit for Illumina, peak between 200 and 550 bp.
   f. mRNA-seq library, single peak around 500 bp.
3. Send the libraries in PCR strip tubes and wrap the lid with Parafilm to ensure that evaporation and loss does not occur.
4. Store at -20 °C.
5. Please provide i5 and i7 index barcodes for each sample when they are entered into the Sample Submission Portal. There should be no overlapping sample index barcodes.

II. Pre-Pooled Libraries

For customers sending in pre-pooled libraries we will run these directly on our sequencer. Please only pool libraries of the same type. Only experienced researchers should attempt this as we cannot guarantee that libraries are balanced and will produce good sequencing results if this option is chosen.

1. Final pooled concentration and volume should be at least 2 nM, 30 µl for CUT&Tag and CUT&RUN. For ATAC-Seq and ChIP-Seq we require at least 4 nM, 40 µl.
2. Send the libraries in PCR strip tubes and wrap the lid with Parafilm to ensure that evaporation and loss doesn't occur.
3. Store at -20 °C.
4. Please provide i5 and i7 index barcodes for each sample when they are submitted to the Sample Submission Portal. There should be no overlapping sample index barcodes.