Cancer Center
NCI-DESIGNATED COMPREHENSIVE CANCER CENTER

ChIP-seq/CUT&RUN/CUT&Tag Preparation and Best Practices

General considerations to increase the likelihood of high quality data

Consult Analyst
Engage with an analyst before beginning the experiment

Discuss goals available samples, batches, etc.

Define Experimental Variables
Define experimental question and design experiments to properly test while avoiding confounders

Validate your experimental groups (e.g. drug, genetic change) before launching a large scale experiment

Prevent Batch Effects
Do not introduce technical variables that cannot be controlled
(e.g. do not process your control and experimental groups on separate days or slides)

Randomize Sample Order
Minimize batch biases in large experiments by alternating processing of experimental and control group samples

Conduct Pilot Studies
Perform preliminary sequencing to confirm quality and positive/ negative controls

Pilot studies can save money!

Consult Analyst
Review raw data and results

Discuss data management plan

Quality Control
Conduct quality checks throughout data generation

Go/no-go decisions

Collect Metadata
Gather necessary sample information (e.g. sex, time point, batch)

Avoid Sample Pooling
Pooling can mask important variability

Ensure Sufficient Sample Size
Verify adequate sample numbers for analysis

Consult Data Generating Facility
Ensure compliance with sample input policies and understand the deliverables for the data generating facility
(e.g. buffers, concentrations)

Which method to choose?

Feature	ChIP-seq	CUT&RUN	CUT&Tag
Mechanism	Sonication	Micrococcal nuclease (MNase)	Transposase Tn5
Input cells	**High** (1-10M)	**Low** (50K-500K)	**Lowest** (1K-100K)
Sequencing depth	**High** (40-60M reads)	**Low** (10-20M reads)	**Low** (5-10M reads)
Background noise	High	Lower	Very low
Best for...	Transcription factors (TFs), cofactors, histone marks	TFs, cofactors, histone marks	Histone marks
Required optimizations	Cross-linking, cell lysis, sonication, antibody (if ChIP-grade not available)	Antibody, permeabilization	Antibody, permeabilization
Limitations	Cost, requires more optimizations	Lack of validated antibodies	Not ideal for TFs and cofactors
Advantages	Many validated ChIP-grade antibodies	Cheaper, versatile	Cheapest, low cell input

Best practices for these methods

Design & Controls
- ✓ Always use a **negative control** (IgG, Ct H3, input, or no antibody). Check IgG quality.
- ✓ Always use **a positive control** (H3K27ac for ChIP-seq or H3K4me3 for CUT&RUN/Tag) to validate protocol/kit.
- ✓ Run independent samples in **triplicates** (min. duplicates).
- ✓ **Spike-ins:** Use only if you expect global changes. If used, ensure identical cell counts.

Lab Fundamentals
- ✓ **Cell viability:** Ensure >90% live cells. Dead cells = high noise.
- ✓ **Never vortex** antibody solutions; mix by gentle pipetting or tube flicking.
- ✓ Use certified **DNAse-free water** if not using a kit.
- ✓ **Store aliquots** to avoid freeze-thaw cycles.
- ✓ **Never let magnetic beads dry out** during washes.

Validations
- ✓ Always run first a small sequencing pilot experiment to **validate the antibodies.**
- ✓ **TapeStation/BioAnalyzer:** Check fragment distribution after library creation.
- ✓ **Digitonin titration:** Assess cell permeabilization by using Trypan blue.
- ✓ **Sonication optimization:** Ensure a DNA fragment length range between 200-500 bp.

Contact the Biostatistics & Bioinformatics Shared Resource (BBSR) for cancer-related analysis

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Contact CUBE for all non-cancer-related projects