Analysis of your ChIP-Seq data: Collaborating with the CCRIFX Core

Best practices to achieve meaningful results

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Acknowledgements

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What is the CCR Bioinformatics (CCRIFX) core?

- http://ccrifx.cancer.gov
- Commenced support efforts in January 2011
- Collaborative support for > 100 data projects
  - Design2Delivery
  - NGS data, microarray, metabolomics, data mining
- Education and training
- Workflow/pipeline development
- No additional cost to collaborate with the core

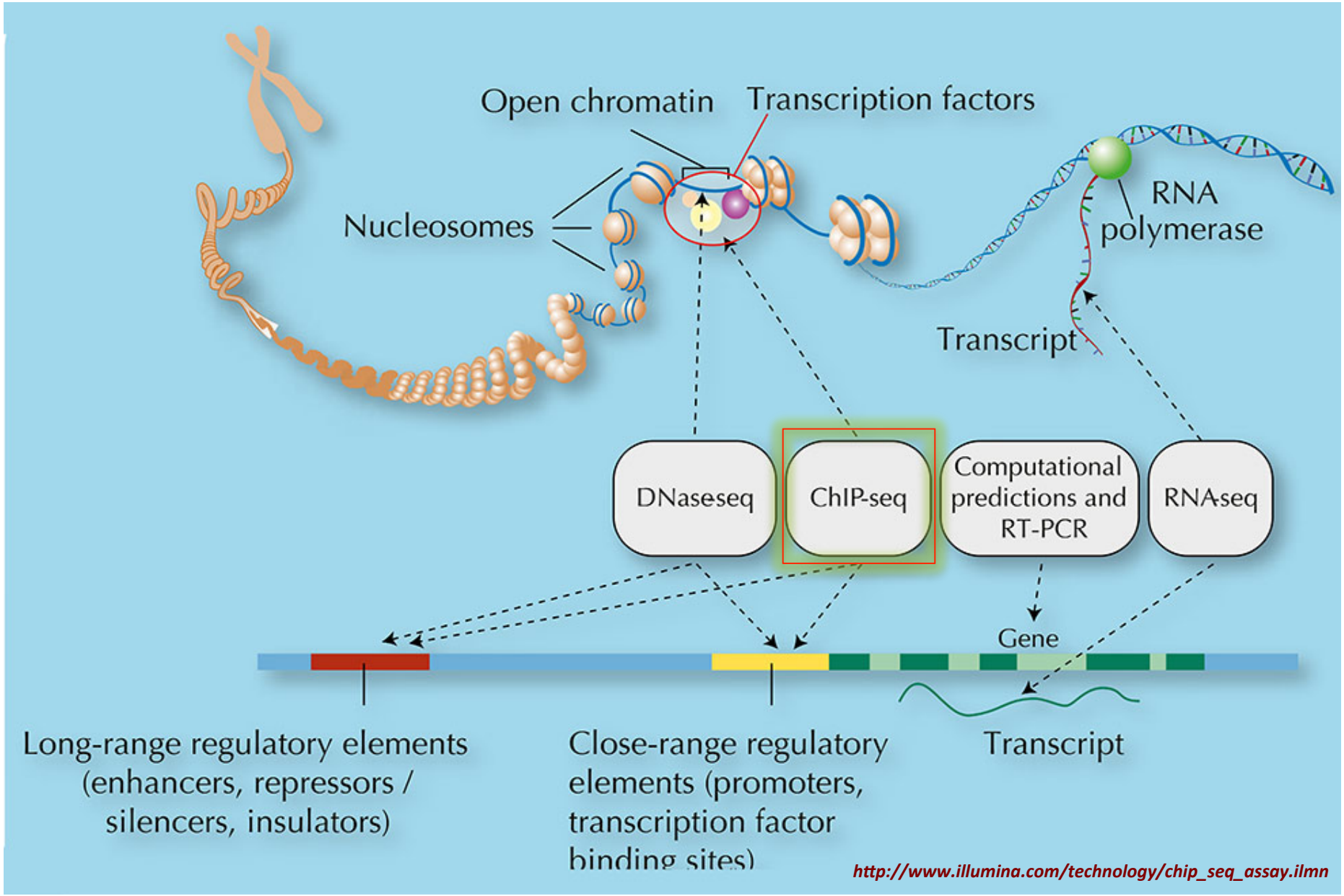
Outline

- **Introduction**
  - ✧ Challenges with ChIP-Seq
  - ✧ Snapshot of ENCODE recommendations

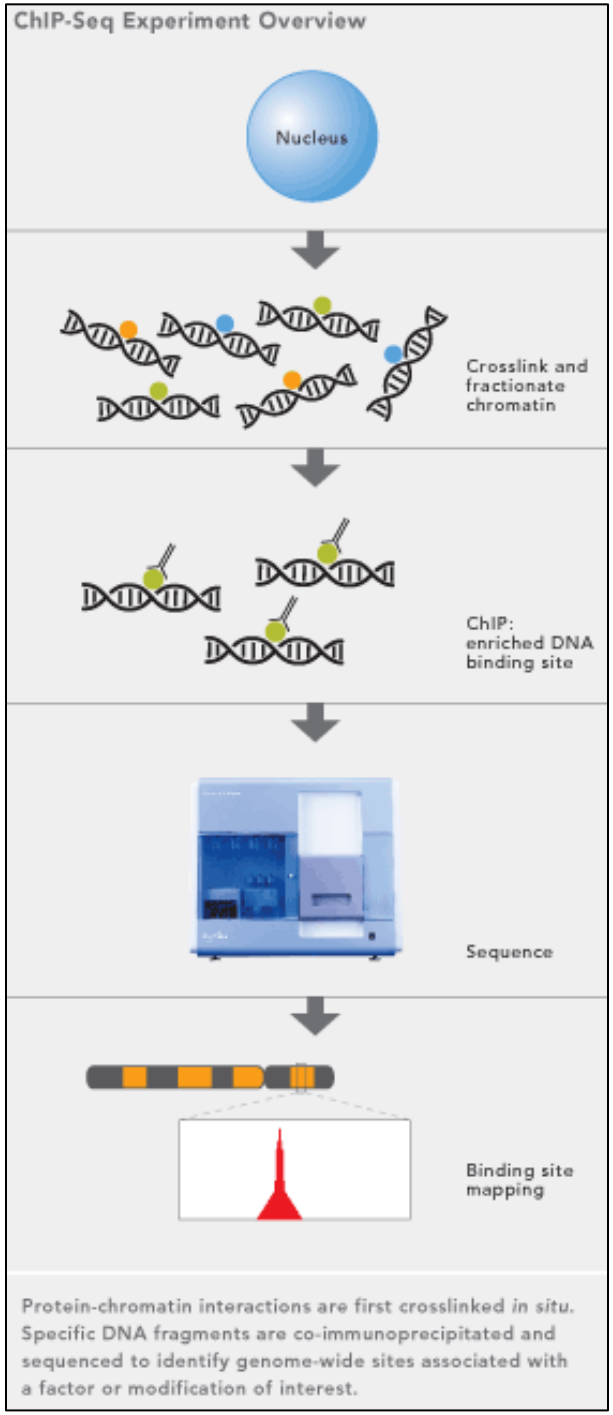
- **Consultations with the CCRIFX CORE**
  - ✧ Practical design considerations
  - ✧ Data/ metadata for the analysis

- **Examples of ChIP-Seq data** – QC metrics
- **Enhanced support** – enrichment, integration, methods
- **Summary of Proposed Guidelines for ChIP-Seq Assessment**

Targeting Different Elements in Biological Space using NGS



http://www.illumina.com/technology/chip_seq_assay.ilmn



Critical phases	Challenges across phases
Experimental Phase	▪ Variations in conditions ▪ Non-Specificity of antibodies ▪ Low IP efficiency ▪ Absence of Replicates/ Controls
Sequencing Phase	▪ Library construction ▪ Sequencing chemistry ▪ Instrumentation ▪ Depth of coverage
Analysis Phase	▪ Different protein classes require different analysis ▪ Parameter optimization ▪ Comparisons across experiments

Snapshot of ENCODE Recommendations

Really good antibody to start with!

EXPERIMENTAL DESIGN GUIDELINES

- At least 2 replicates
- Input Control for each condition
- Reproducibility
- Library complexity
- Adequate Sequencing depth to capture events across genome

DATA QUALITY ASSESSMENT

- Metrics at every stage possible to assess quality of experiment
- Cross-correlation for stranded reads
- Irreproducible Discovery Rate (IDR) for peak concordance in replicates

DATA REPORTING GUIDELINES

- Minimal Information for Chip-seq Experiment (MICE)
- Analysis Details
- High-throughput sequencing data

Important to Confirm that the Antibody Works!

BIOMEDICINE: NATURE NEWS | 1 AUGUST 2013 | VOL 500 | NATURE | 15

NIH mulls rules for validating key results

US biomedical agency could enlist independent labs for verification

"Having a third party validate every batch would be a fabulous thing," says Peter Park, a computational biologist at Harvard Medical School.

He notes that the consortium behind ENCODE — a project aimed at identifying all the functional elements in the human genome — tested more than 200 antibodies targeting modifications to proteins called histones and found that more than 25% failed to target the advertised modification.

Consultation with the CCRIFX Core

The Quest for a Successful ChIP-Seq –

- **The recommendation is to approach the CCRIFX Core prior to or during the ChIP-Seq experimental design process**
- **A practical goal is to maximize site discovery by**
  - Optimizing IP and
  - Sequencing deeply, within reasonable expense constraints
- **Goals of data quality assessment**
  - Identification of most suitable analytical approaches
  - Defining outcomes

Advantage of collaborating with CCRIFX

- Better understand research question
- Suggest viable experimental designs
- Guide on use of technologies
- Assist in data tracking and transfer of very large datasets
- Perform analysis and explain results
- Deliver figures and methods for manuscripts, presentations, posters
- Maximize the research impact of your invested effort

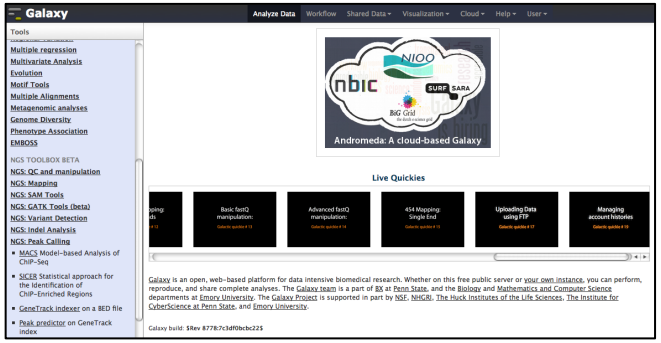
ChIP-SEQ DATA ANALYSIS

Most widely used data analysis tools

- MACS (**Model-based Analysis for ChIP-Seq**)
  - Widely used method for peak calling
- HOMER **(Hypergeometric Optimization of Motif EnRichment)**
  - Suite of tools for motif discovery and ChIP-Seq analysis
- SICER (**Statistical approach for the Identification of ChIP-Enriched Regions**
  - Clustering approach for identification of domains from histone modification
- CCRIFX Wiki - **https://ccrod.cancer.gov/confluence/display/ifx1/Differential+ChIP-seq+binding**

GUI tools/packages for ChIP-Seq analysis

Galaxy Server



Partek

- Genomatix NGS Suite (available through CCR OSTR)

Checklist for a ChIP-seq experiment

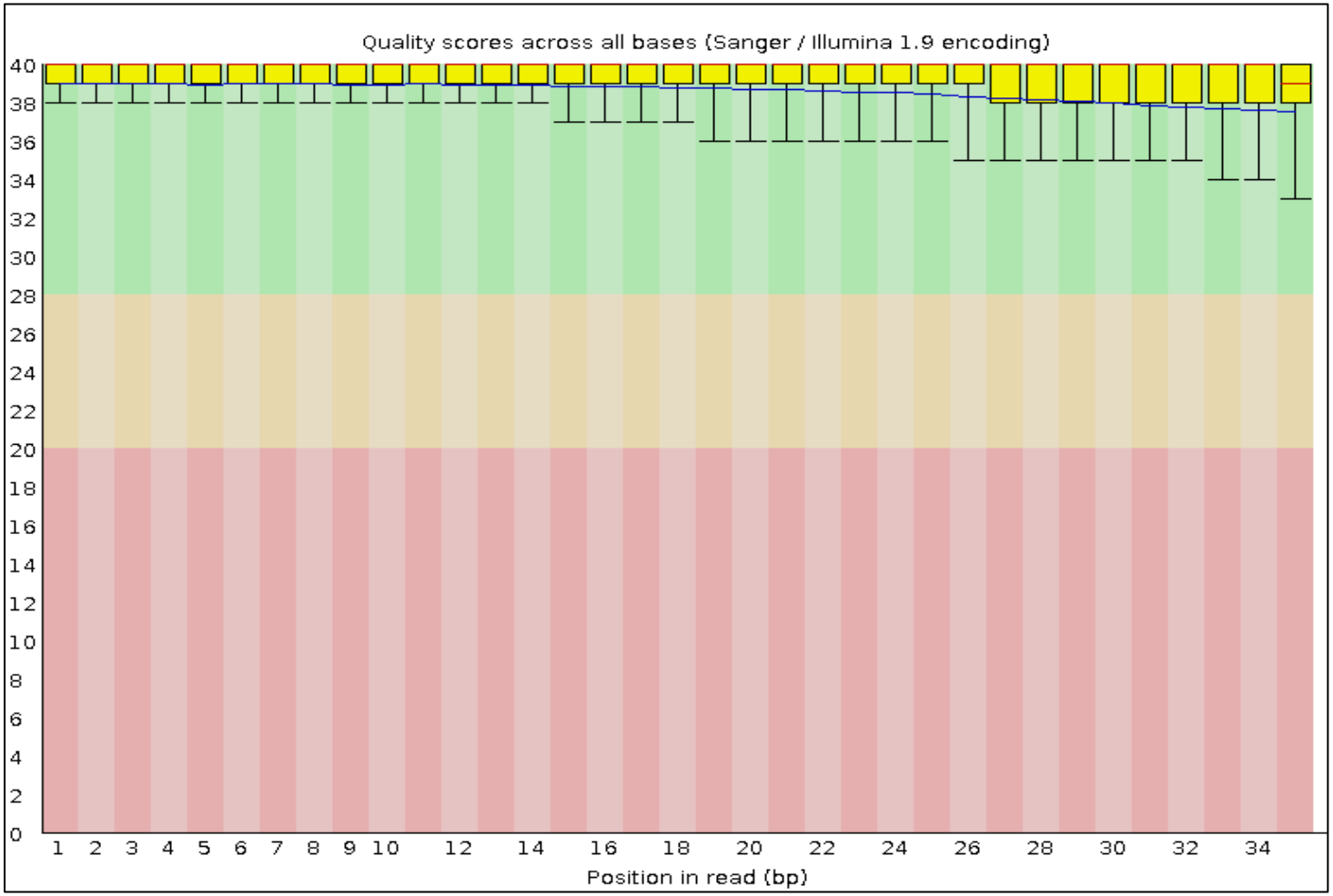
1. **Raw data** – format (.fastq, .bam); location (server, hard drive); SF CSAS#, if applicable
2. **Sample Metadata**
   a. Species and strains: human, mouse, etc.; Type: tissue, cell lines, etc.
   b. Total number of Samples; Experimental Groups; Replicates
3. **Native ChIP or cross-linking ChIP?**
   a. Antibody validation (immunoblots, IP assays, etc.)
   b. Good antibody specificity and IP efficiency? (Yes/No)
4. **Library Metadata**
   a. Sample prep/enrichment and Library construction protocols
   b. Sequencing platforms and instruments used
   c. Libraries: Type (PE/SE); Sequence read length
5. **Analysis-related specifications**
   a. Preferred reference genome (hg19, mm10, etc.), and annotation (Refseq, UCSC)
   b. Integrated analysis with other data types (microarray, RNA-Seq)

Illustrations of ChIP-Seq Analysis

Determining data suitability using QC plots

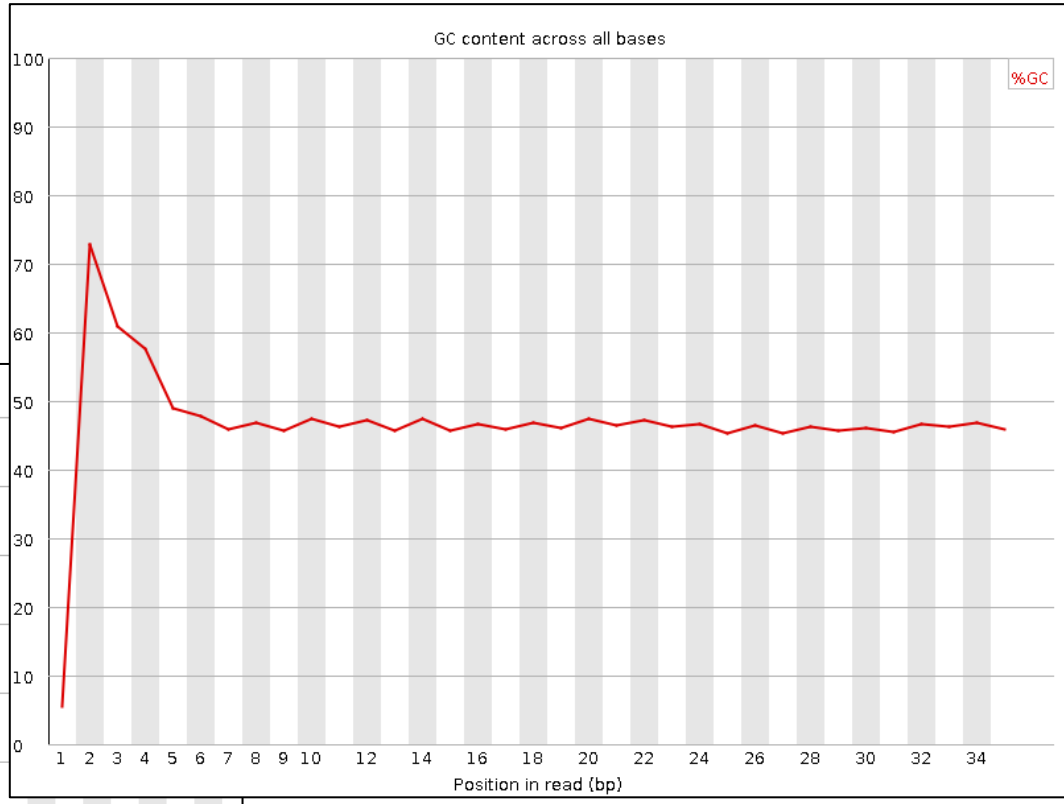
Features –

1. Qscore > 30 (green zone)
2. Less spread/bp

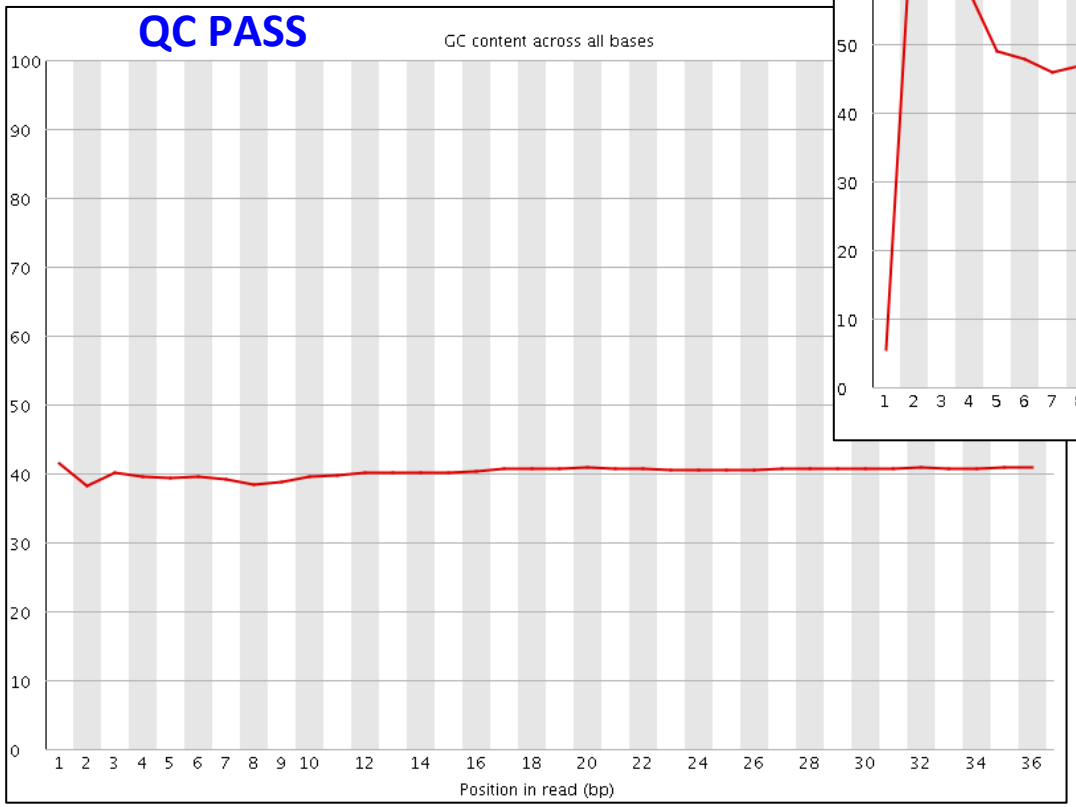


Per-base GC Content Distribution Plot

QC WARNING*



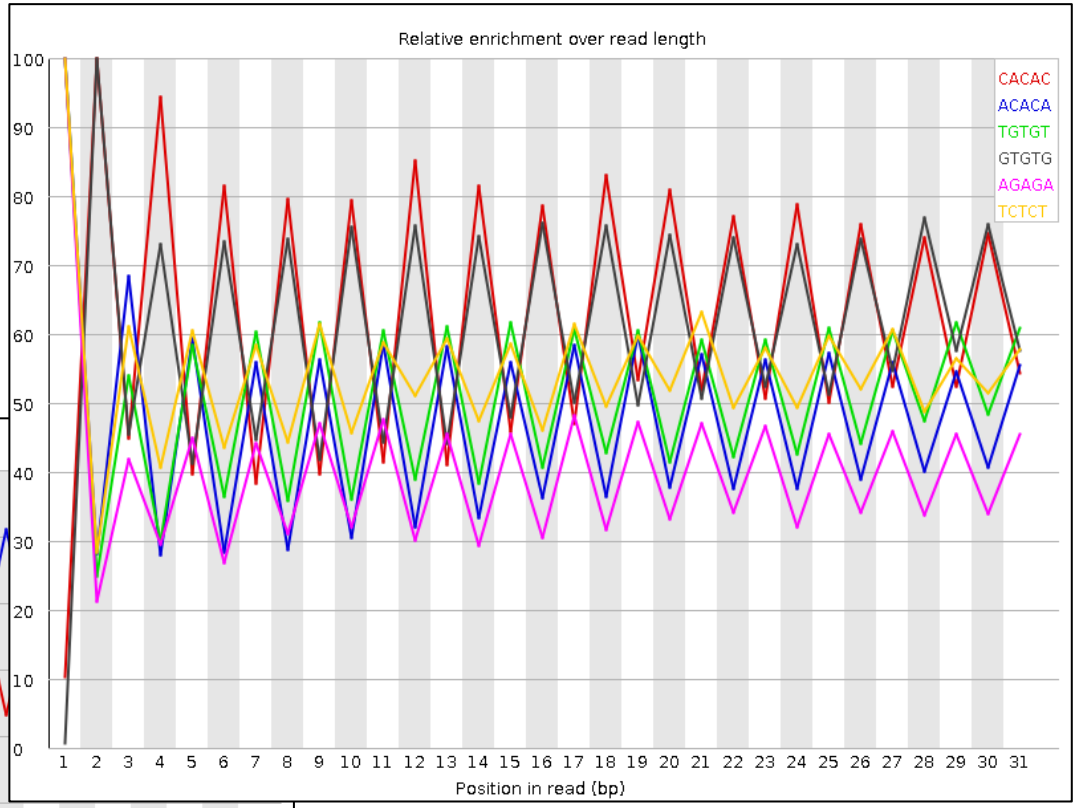
QC PASS



** Not a criteria to trim reads just based on this plot, but in combination with other QC parameters provides a basis to improve read quality*

K-mer (repeat) profile

QC WARNING*



Post-repeat removal

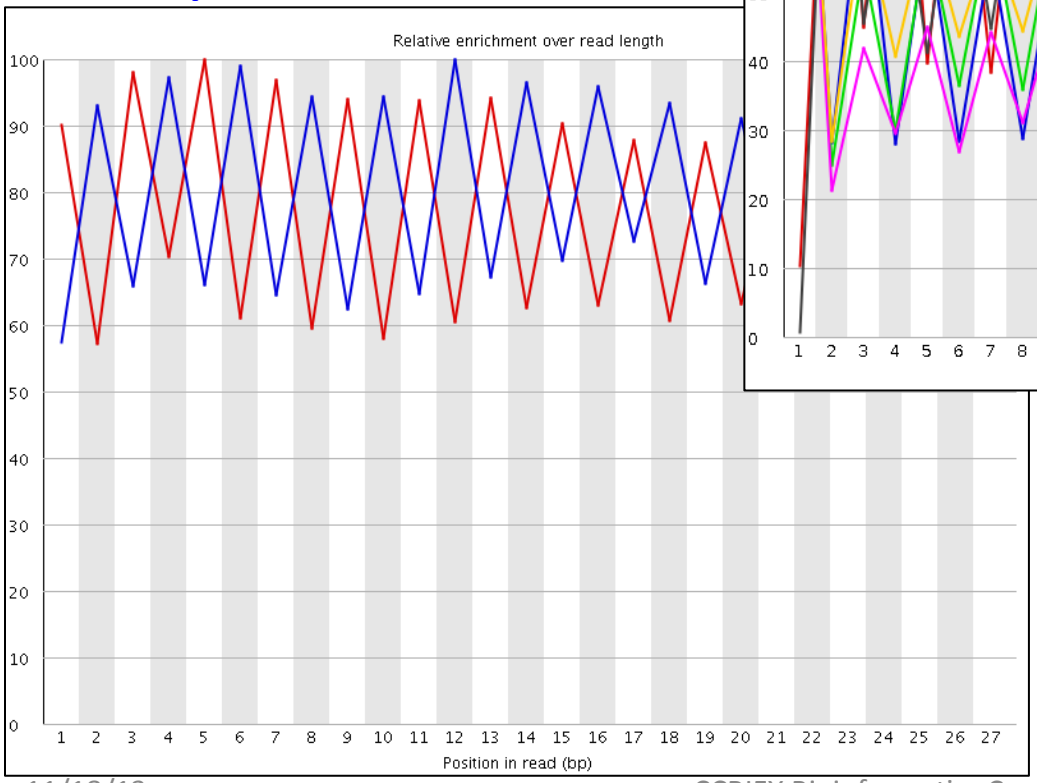
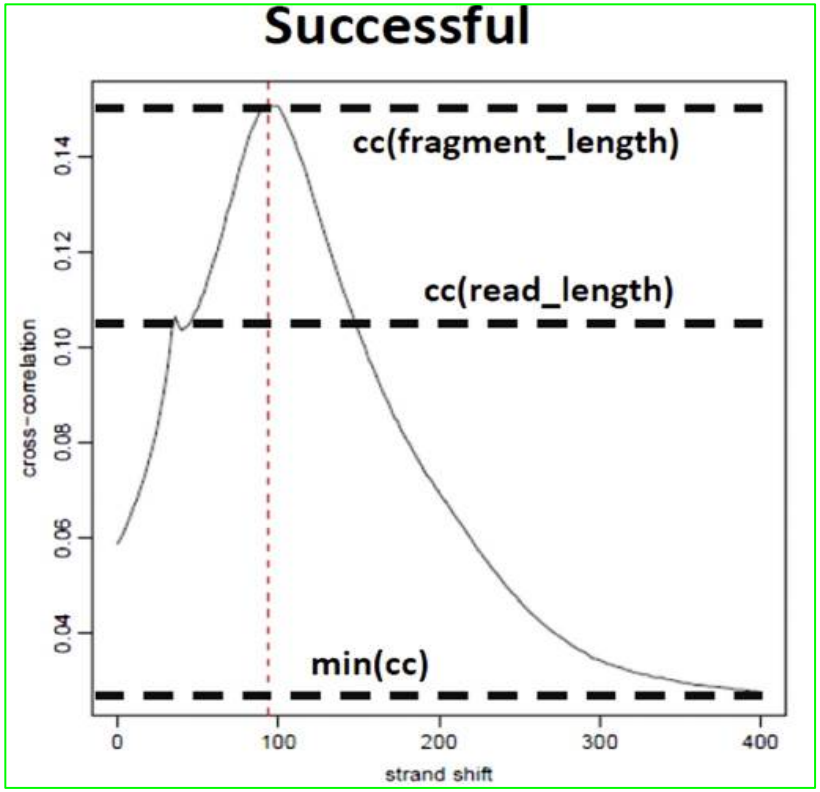
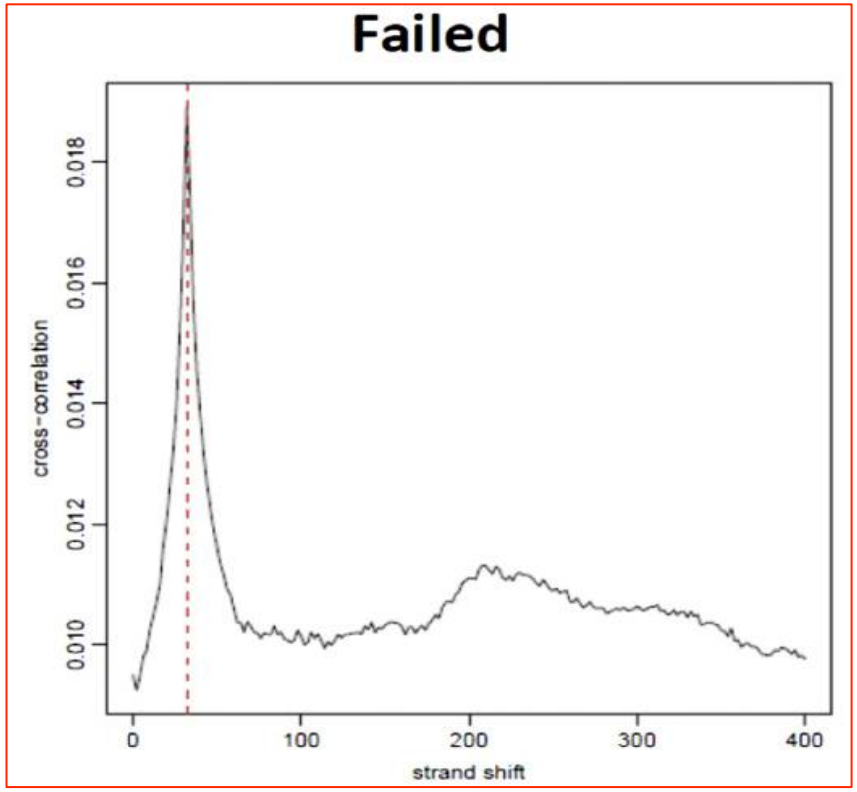


Figure from ENCODE publication on strand correlation





$$NSC = \frac{cc(fragment\ length)}{min(cc)}$$

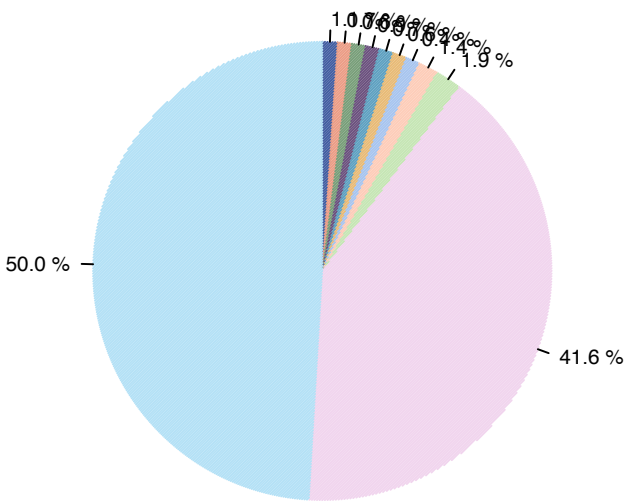
$$RSC = \frac{cc(fragment\ length) - min(cc)}{cc(read\ length) - min(cc)}$$

Stephen G. Landt, Georgi K. Marinov, Anshul Kundaje, et al. 2012 22: 1813-1831 Genome Res.; doi:10.1101/gr.136184.111

Pie chart with distribution of peaks across different genomic regions

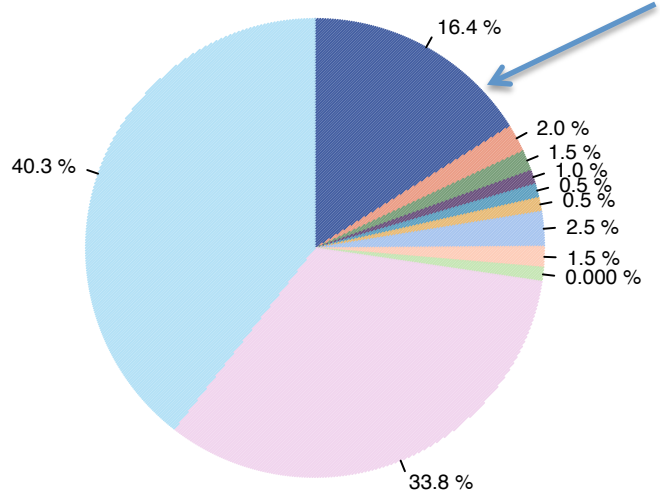
Output from CEAS (Cis-regulatory Element Annotation System)

Genome



- Promoter (<=1000 bp): 1.1 %
- Promoter (1000–2000 bp): 0.7 %
- Promoter (2000–3000 bp): 0.6 %
- Downstream (<=1000 bp): 0.9 %
- Downstream (1000–2000 bp): 0.7 %
- Downstream (2000–3000 bp): 0.6 %
- 5'UTR: 0.4 %
- 3'UTR: 1.4 %
- Coding exon: 1.9 %
- Intron: 41.6 %
- Distal intergenic: 50.0 %

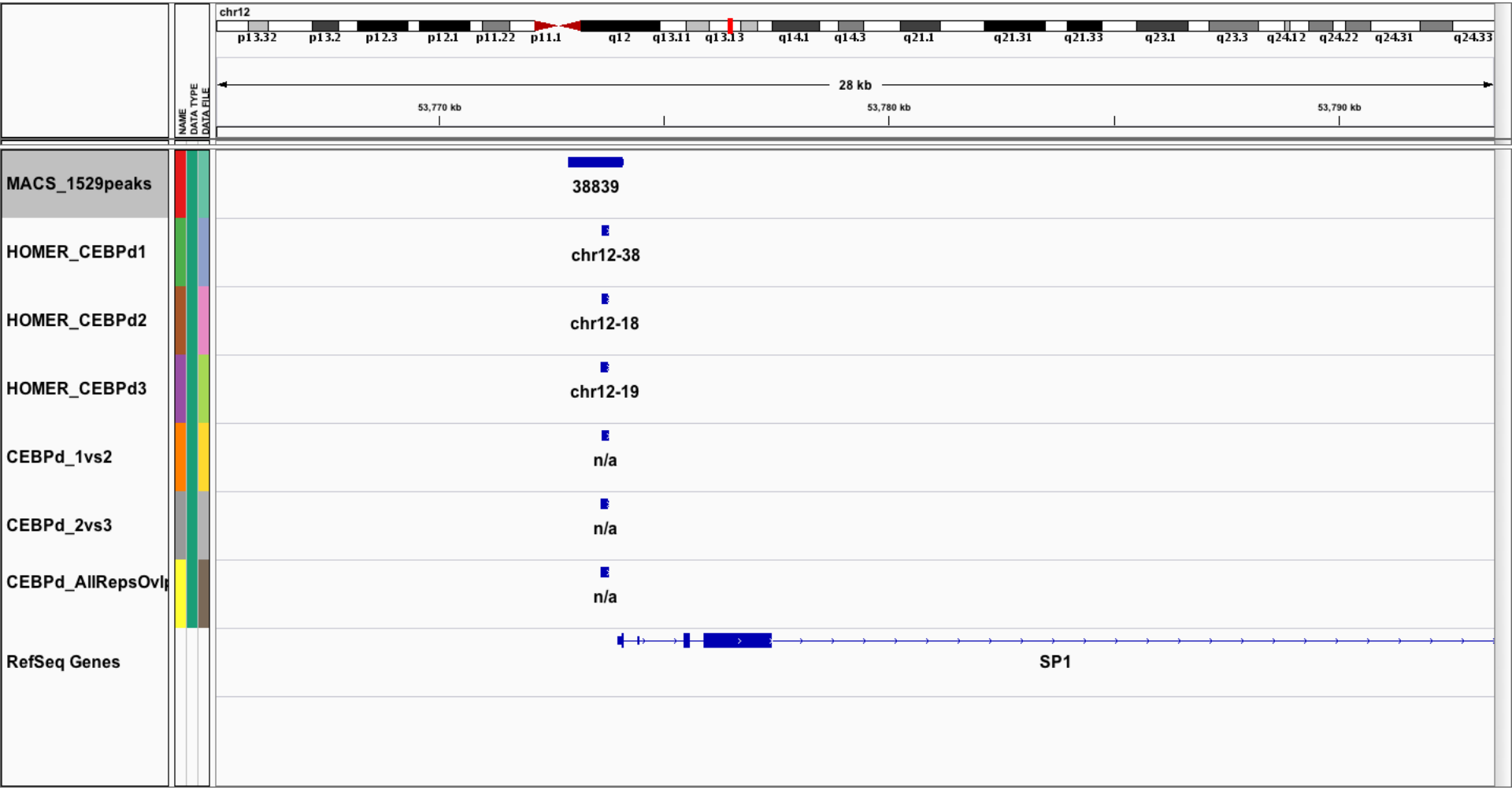
ChIP



- Promoter (<=1000 bp): 16.4 %
- Promoter (1000–2000 bp): 2.0 %
- Promoter (2000–3000 bp): 1.5 %
- Downstream (<=1000 bp): 1.0 %
- Downstream (1000–2000 bp): 0.5 %
- Downstream (2000–3000 bp): 0.5 %
- 5'UTR: 2.5 %
- 3'UTR: 1.5 %
- Coding exon: 0.000 %
- Intron: 33.8 %
- Distal intergenic: 40.3 %

Enrichment in promoter region

Visualization of BED files on IGV



SP1 Promoter Region

Proposed Guidelines for Assessing a ChIP-Seq Experiment

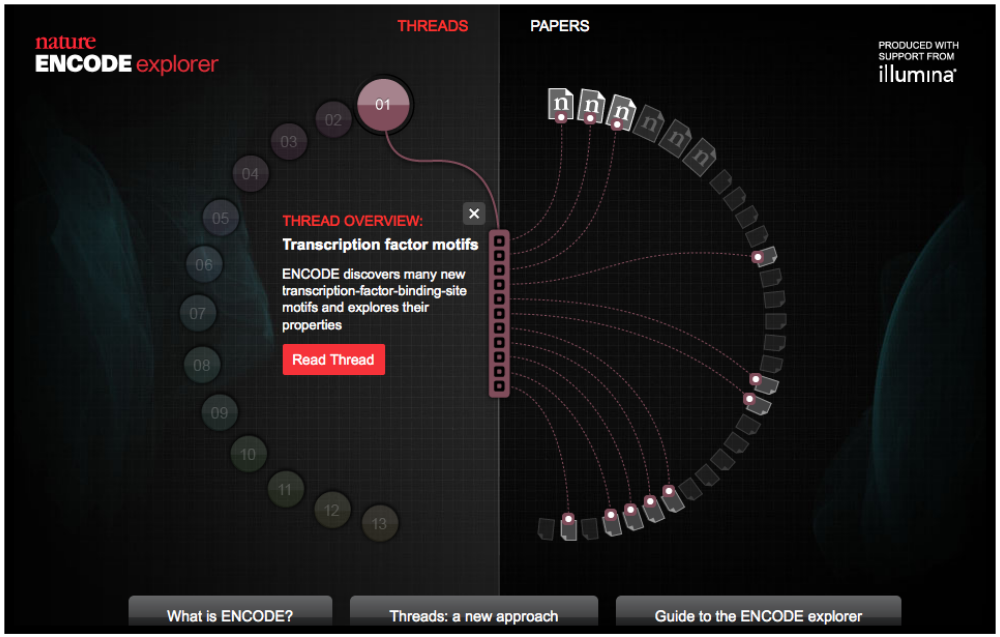
	Pre-ChIP-Seq Experimental Index		Post-ChIP-Seq Quality Index
1	Biological Replicates		Number of uniquely mapped reads
2	Documented ChIP-Valid Antibody		Significant statistics (FDR p-value; Fold-change)
3	Specific Sample Characteristics (Cell Line/Tissue; Culture Conditions; Comparisons; Multiple factors)		a. QC outputs b. Motif enrichment b. Browser Visualizations
4	Controls – Input/IgG or both		ENCODE Metric 1 - Non-Redundancy Fraction (NRF)
5	Available ChIP-PCR data/ Positive Controls		ENCODE Metric 2 – Strand cross-correlation coefficients (NSC/RSC)
6	Other corroborative evidence (e.g. publications)		ENCODE Metric 3 - Irreproducibility Discovery Rate (IDR)

Characteristics of a ChIP-Seq Experiment that is…

Good	Sub-optimal	Poor
• ChIP-validated Ab • Efficient IP • At least 2 experimental replicates • Input DNA control • Excellent sample quality and library complexity • Sufficient sequencing depth (>10x)	• Ab and IP issues • No replicates • Low quantity/quality of sample • QC Flags • Insufficient depth of sequencing	• Poor IP (Ab binding) • No Input • No replicates • Unbalanced experimental conditions (e.g. treatment, time)

Useful ENCODE Links

- Quality Metrics: http://encodeproject.org/encode/qualityMetrics.html#spreadsheets
- Data Coordination Center: http://encodeproject.org/encode/otherTerms.html
- Software Tools: http://genome.ucsc.edu/encode/encodeTools.html#metrics
- NATURE ENCODE Explorer: http://www.nature.com/encode/#/threads
- NPG Reporting Checklist: http://www.nature.com/authors/policies/checklist.pdf



Get in touch with us!

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