Microsynth

ChIP Sequencing Service For Studying Protein-DNA Interactions

Investigate genome-wide transcription regulation in a comparative setting
Investigate the active part of the chromatin

Introduction

Chromatin-immunoprecipitation (ChIP) followed by next generation sequencing of the immuno-precipitated DNA is a powerful tool for the investigation of protein-DNA interactions. ChIP-Seq studies are mainly performed to increase our understanding of transcription factor biology and histone modifications.

In the "ChIP" part, chromatin is isolated from cells or tissues and fragmented. Antibodies against chromatin-associated proteins are used to enrich for specific chromatin fragments. In the "Seq" part the isolated DNA is sequenced and aligned to a reference genome to determine specific DNA-protein binding loci at a nucleotide level.

Advanced bioinformatics analyses help to gain insights into common binding motifs as well as the protein function by analyzing affected gene regulatory networks. As a plethora of similar methods have emerged for functional experiments (e.g. DNase-Seq, Ribo-Seq, PAR-CLIP, etc.), downstream sequencing and analysis follow a similar pattern. Therefore, feel free to inquire about a sequencing and bioinformatics solution in case you plan any such project.

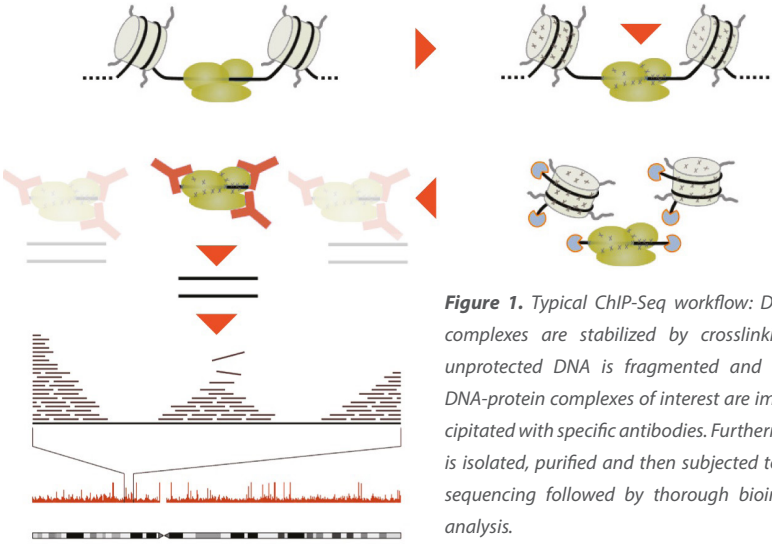

Figure 1. *Typical ChIP-Seq workflow: DNAprotein complexes are stabilized by crosslinking, while unprotected DNA is fragmented and remaining DNA-protein complexes of interest are immunoprecipitated with specific antibodies. Furthermore, DNA is isolated, purified and then subjected to NextGen sequencing followed by thorough bioinformatics analysis.*

Microsynth Competences and Services

Experimental Design

Please consider the importance of biological replicates as well as control/reference samples (e.g. by an unrelated IgG) along with your specific antibodies to allow an accurate and meaningful analysis – both are highly advised by Microsynth.

Sequencing

DNA purified by the customer is prepared following the Illumina TruSeq ChIP sample preparation protocol. The DNA libraries are then sequenced and the resulting reads are demultiplexed and trimmed of Illumina sequencing adapter residuals. The required sequencing depth

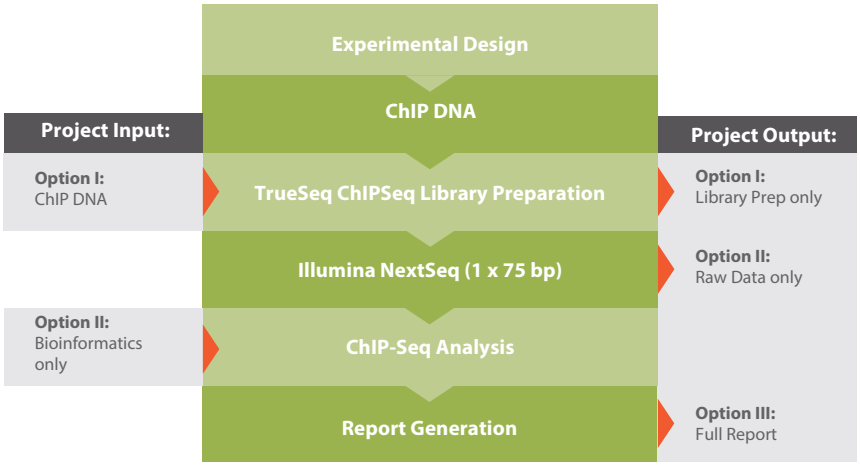

Figure 2. *Microsynth's workflow for ChIP-Seq projects. It lists all possible input and output points.*

Microsynth AG, Switzerland
Schützenstrasse 15 · P.O. Box · CH-9436 Balgach · Phone +41 71 722 83 33 · Fax +41 71 722 87 58 · info@microsynth.ch · www.microsynth.ch

depends strongly on the genome size, peak types, library complexity and aim of the study. As a rule of thumb, 10 million uniquely mapped reads per replicate should be aimed for human genomes given point-source peaks.

Bioinformatics Analysis

First, the sequenced reads are checked and filtered for their quality. Second, the sequence data is mapped to the reference genome (e.g. hg19, mm10, danRer7, etc.). Third, the analysis software HOMER is employed as a core module to detect DNA-binding sites ("peak-finding") and motifs suitable for your DNAbinding proteins (point-, broad-, or mixed sources such as transcription factors, certain chomatin marks or RNA polymerase II, respectively). An in-depth annotation (proximity to genes, gene ontology, etc.) is provided, in case a thoroughly annotated reference genome (e.g. human, mouse, zebrafish, etc.) is available.

Analysis Output

The most important results of the ChIP-Seq analysis are presented in form of an HTML document which allows user-friendly navigation through the assessment of the experiment, the annotation of the peaks, the binding motifs, their co-occurrence and the pathway analysis. Furthermore, numerous additional useful analysis results are provided alongwith the sequencing data in the FASTQ format. Some selected examples for further downstream analysis are shown in ***Figures 3-4*** and ***Table 1***.

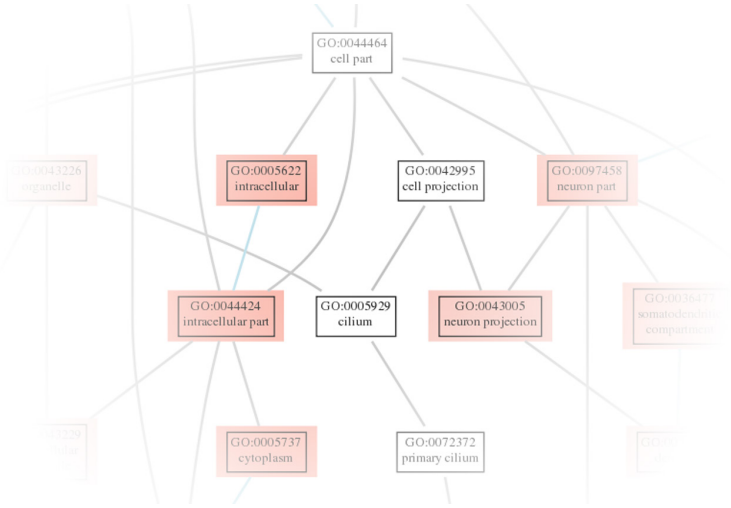

Figure 3. *For the pathway analysis, the three gene-ontology domains (cellular component, biological process and molecular function) are each displayed in a network graph as a preview for the most significant ontology terms. Here a detail of the "cellular component" graph is presented.*

Figure 4. *Among the enrichment analysis results, de novo motifs are detected and visualized. Significance is calculated and provided along with GO-terms and cross-links to further information (not shown).*

Table 1: *A selected excerpt of the comprehensive peak annotation is displayed. Additional information such as cross references, categorical data and motif patterns for each peak position is provided in the annotation table (as supported by the reference genome annotation).*

PeakID	Chr	Start	End	Strand	Peak Score	Focus Ration/ Region Size	Gene Name	Annota-tion	Distance to TSS	Nearest PromoterID	Gene Descripton
chr17-1	chr17	72303743	72303943	+	398.8	0.98	KIF19	intron	-18508	NM_153209	kinesin family
chr7-3	chr7	156860912	156861112	+	366.3	0.978	MNX1-AS1	intergenic	57461	NR_038835	MNX1 antisense RNA
chr19-5	chr19	23498870	23499070	+	244.2	0.968	IPOSP1	intergenic	-41917	NR_103742	importin 5 pseudogene 1
chr17-7	chr17	29942474	29942674	+	244.2	0.968	MIR365B	intergenic	40144	NR_029856	microRNA 365b

Related Topics

- microRNA Sequencing at Microsynth
- RNA Sequencing at Microsynth

Further Reading

1. *Langmead B, Salzberg S. Fast gapped-read alignment with Bowtie 2. Nature Methods. 2012, 9:357-359.*
2. *Heinz S, Benner C, Spann N, Bertolino E et al. Simple Combinations of Lineage-Determining Transcription Factors Prime cis-Regulatory Elements Required for Macrophage and B Cell Identities. Mol Cell. 2010, 38(4):576-589.*
3. *Landt S. G., Marinov Ge. K., Kundaje A. et al. ChIP-seq guidelines and practices of the ENCODE and modENCODE consortia. Genome Research. 2012. 22: 1813-1831.*
4. *Furey, Terrence S. ChIP-seq and beyond: new and improved methodologies to detect and characterize protein-DNA interactions. Nat Rev Genet. 2012. 13: 840-852*