The ChIP-Seq project

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Overview

- Focus on technical aspects

  Description of applications (C programs)

  Where to find binaries, data files and src packages

  Release on SourceForge

  The Web Interface

- Large data sets have become available starting from the year 2007

  Barski et al. (2007): human CD4+ cell lines
  Histone modifications, POL II, CTCF (~2 millions tags per experiment)

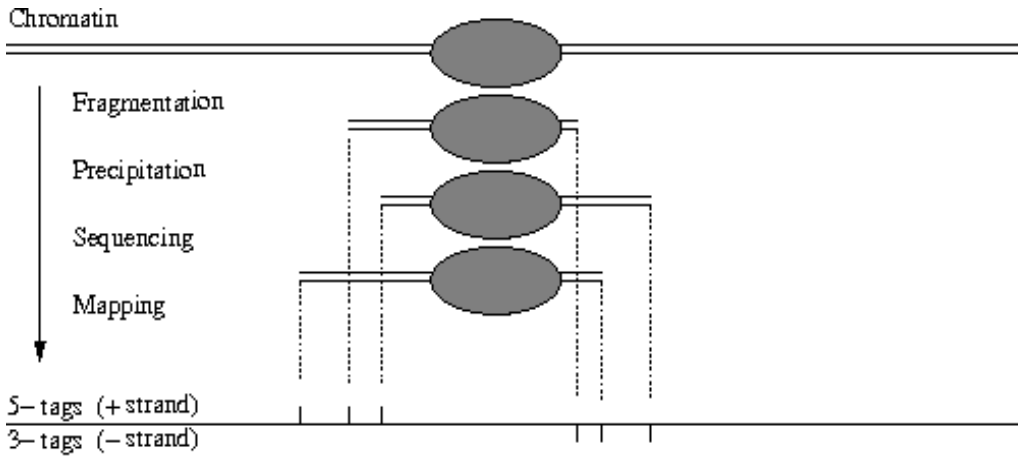
  Mikkelsen et al. (2007): four mouse cell lines
  Histone modifications (~2 millions tags per experiment)

  Robertson et al. (2007). INF-gamma stimulated HeLa cells
  STAT1 (>20 million tags per experiments)

- Very good data quality and reproducibility

- Choose to develop simple algorithms to achieve good results

ChIP-Seq Technique an Data



Our representation: SGA (Simple Genome Annotation) format

```
NC_000001.9     stim    559139  -       1
NC_000001.9     stim    559333  +       1
NC_000001.9     stim    559356  -       1
NC_000001.9     stim    559765  -       1
NC_000001.9     stim    559766  +       3
NC_000001.9     stim    559767  +       1
NC_000001.9     stim    559768  +       1
NC_000001.9     stim    559777  +       3
NC_000001.9     stim    559778  +       2
...
```

Fields of SGA format

1. Sequence ID
2. Feature
3. Position
4. Strand
5. Counts
6. Description

ChIP-Seq programs require SGA files to be sorted by chromosome name and position!

setenv LANG C; sort –s –k1,1 –k3,3 –k4,4

ChIP-seq data analysis: Biological questions

- ChIP-seq data for specific transcription factors
  - *Average length of an immuno-precipitated fragment (protected DNA regions)*
  - *The location of in vivo occupied sites*
  - *The strength of in vivo occupied sites*
  - *The in vivo binding specificity (consensus sequence, matrix)*
  - *Contextual features of in vivo occupied binding sites*
- ChIP-seq data for histone variants
  - *Which regions of the genome are enriched in a particular variants*
  - *Nucleosome phasing, position of individual nucleosomes*
  - *Epigenetic genome organization – definition of chromatin domains*
- Combined analysis
  - *Position of TF binding sites relative to nucleosomes*

ChipSeq Tools: Design principles and available tools

- Design principles

  - *Simple tools (easy to understand to non-specialists)*
  - *Fast algorithms*
  - *Generic methods if possible (not restricted to ChIP-seq data)*
  - *C-programs for basic programming tools*
  - *Auxiliary Perl tools to perform format conversion tasks and other useful tasks such as repeat masking and SGA fetching*

- Web interface (*http://ccg.vital-it.ch/chipseq*)

  - *Access and analysis of selected public data*
  - *Upload and analysis of private (user-owned) data*
  - *Combined analysis of private and public data*
  - *Interoperability with sequence analysis program (e.g. motif discovery)*
  - *Link to genome browser (preparation of customized WIG and BED files)*

ccg.vital-it.ch/chipseq

SIB Swiss Institute of Bioinformatics

ChIP-Seq

ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

Computational Cancer Genomics I ExPASy I EPFL

ChIP-Seq Analysis Server

The ChIP-Seq Server aims at providing tools for the analysis of ChIP-seq data and other types of MGA (mass genome annotation data).
The programs offered by this web server are listed below.

Access to ChIP-Seq tools	
ChIP-cor	Feature Correlation Tool
ChIP-center	Tag Centering Tool
ChIP-peak	Signal Peaks Detection Tool
ChIP-part	Partitioning Tool
WIG Files	WIG Files for public chIP-seq data

ChIP-Cor Application

❑ Input

- *Genomic tag count distributions for two features (reference, target)*
- *Features may be + and – strand tags from same experiments*
- *Applicable to other types of features, e.g. TSS positions*
- *Relative correlation distance, histogram step size and normalization*

❑ Output

- *A count correlation histogram (text file indicating the frequency of the target feature as a function of the relative distance to the reference feature)*

❑ Method

- *Consider reference positions which have at least one tag count.*
- *For each position, computes number of tag pairs that fall into a distance range.*
- *Different normalization options:*
  - *count density of target feature*
  - *global → relative target feature frequency (over-representation)*

❑ Purpose

- *Identification of average fragment size*
- *Reveals length distribution of enriched domains*
- *Provides clues for choosing parameters for peak and partitioning algorithms*
- *Positional relationship to other genomic features, e.g. transcription start sites*

❖ Output options on the Web

- *Histogram graph, feature extraction option*

Correlation plot: Example

Input data:

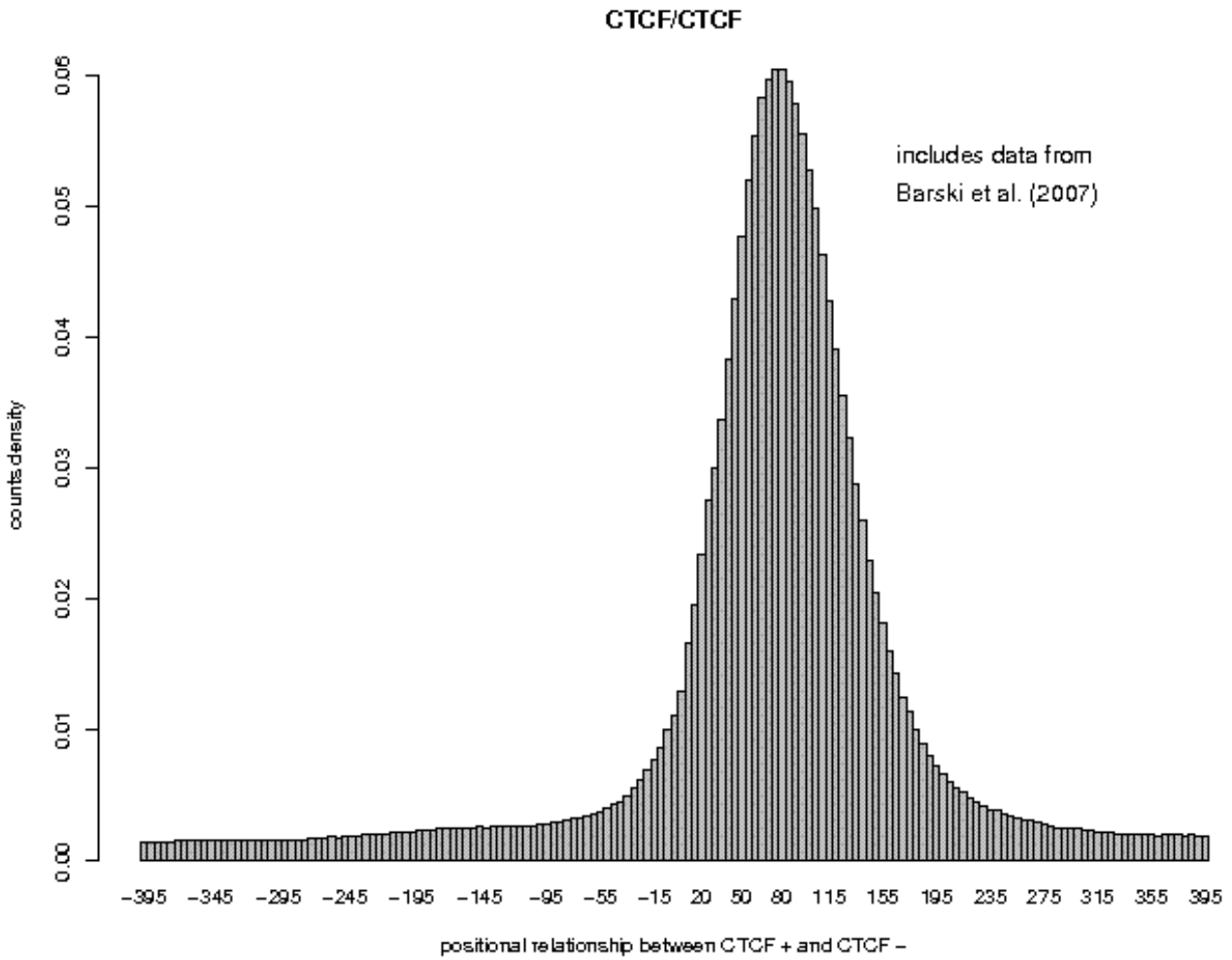
Ref: CTCF 5’ tags
Target: CTCF 3’ tags

Analysis parameters:

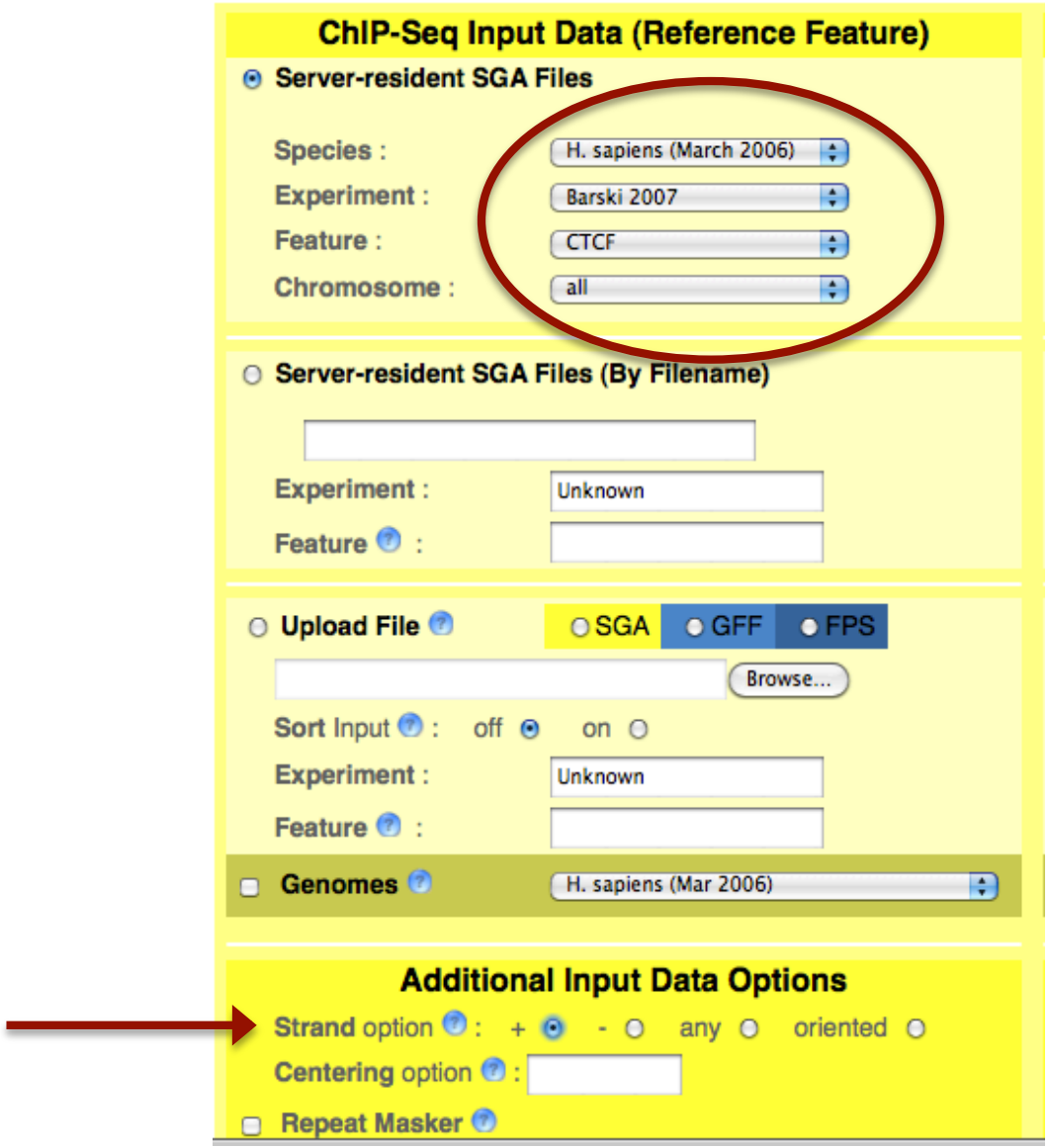
Range: –400,+400
Window width: 5
Count cut-off: 3
Y-axis: count-density

➢ Observations

➢Peak center: ~75
➢Peak count density: 0.06
➢Background: < 0.002



Correlation plot: input Data



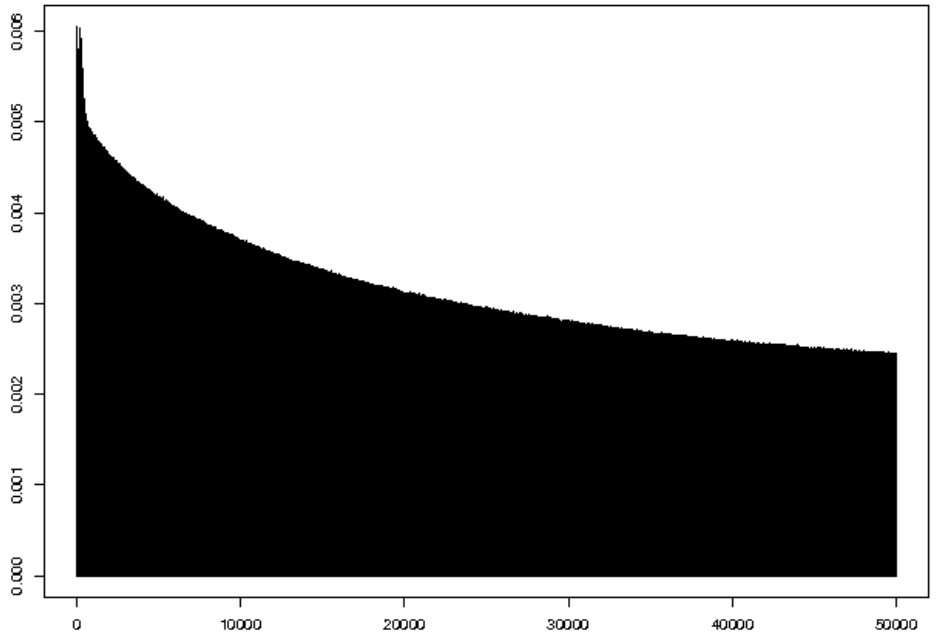
Auto-correlation plots for different histone modifications

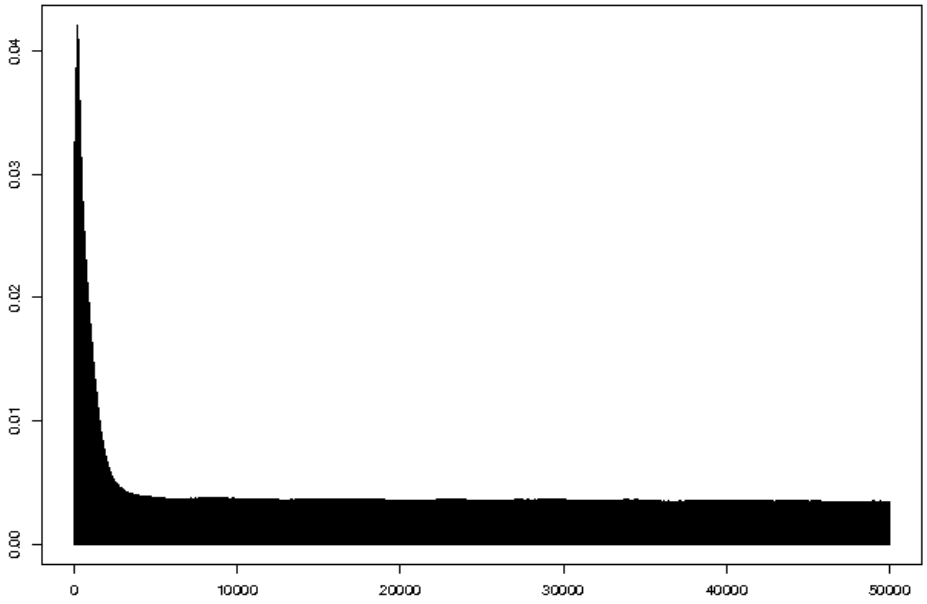
Top:

Auto-correlation plot of H3K36me3 in mouse ES cells

Bottom:

Auto-correlation plot of H3K4me3 in mouse ES cells





- Observations
  - H3K36me3 → long range correlation
  - H3K4me3 → short range correlation

ChIP-Score Application

❑ Input

- *Genomic tag count distributions for two features (reference, target)*
- *Count output threshold, distance range*

❑ Output

- *All reference sites that are enriched or depleted in target feature sites.*

❑ Method

- *Consider reference positions which have at least one tag count.*
- *For each position, computes cumulative target tag counts that fall into a distance range.*
- *Select those reference positions, which:*
  - *Have cumulative target tag count ≥ threshold (enriched feature)*
  - *Or have cumulative target tag count < threshold (depleted feature)*

❑ Purpose

- *Identification of enriched/depleted domains*
- *Further correlation to other genomic features, e.g. transcription start sites*

❖ Special ChIP-Cor Web server option

ChIP-Center Application

❑ Input

- *Oriented tag counts for a Chip-Seq feature*
- *Shift amount for centering tag positions*

❑ Output

- *Centered, un-oriented tag counts*

❑ Method

- *Moves by a given shift value tag positions to estimated center positions of DNA fragments*

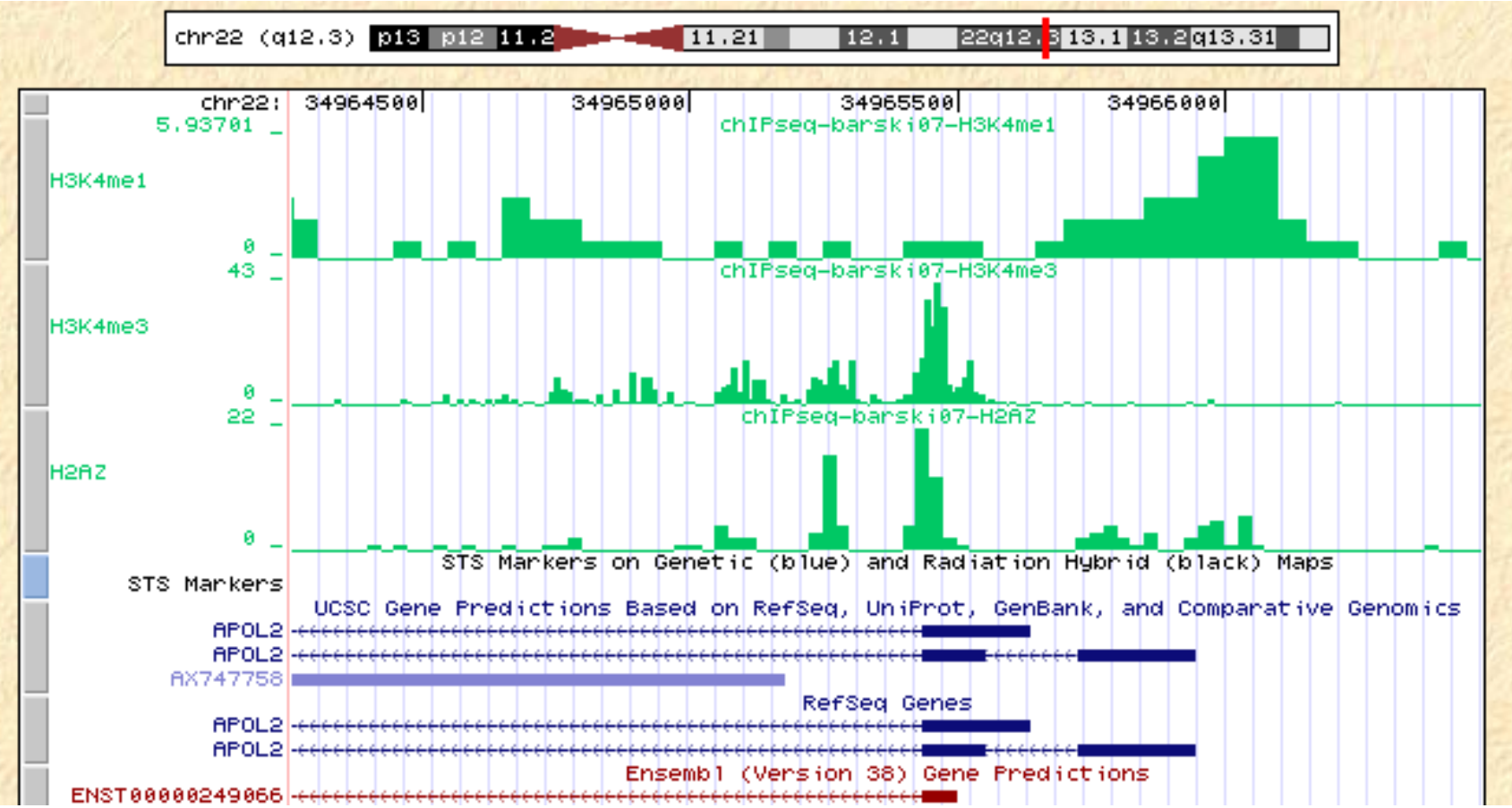
❑ Motivation

- *5' and 3' tag positions show relative displacement to each other*
- *best estimates for protein-binding site position:*
  5' end position + ½ fragment length
  or 3' end position – ½ fragment length
- *centered tag count distribution more useful as input for peak recognition and partitioning algorithm*

❖ Output options on the Web

- *WIG files for viewing data in a genome browser environment*

Example ChIP-center: Viewing customized WIG files in a UCSC browser environment



Based on data: from Barski et al. 2007, Cell 129, 823-837).
ChIP-Seq tags from both strands centered by 70 bp .
WIG file resolution: H3K4me1 50bp, H3K4me3 10 bp, H2A.Z 25 bp.

ChIP-peak Application

❑ Input

- *Centered tag counts*
- *Peak threshold (t), integration range of tag counts (w), Vicinity range (r)*

❑ Output

- *List of peak center positions (SGA or FPS format)*

❑ Method

- *Consider only positions which have at least one tag count.*
- *For each position, determines cumulative tag counts in windows of width w.*
- *select as peaks those positions, which*
  - *have cumulative tag count ≥ threshold t.*
  - *are local maximum with range ± r.*

❖ Special server options

- Download of sequences around peak center positions
- Provide several output formats: WIG, GFF, FPS

Example ChIP-Peak: Locating *in vivo* STAT1-binding sites

Input data

Robertson et al. (2007) Nature Methods 4, 651-657.
Cell material: Interferon γ-stimulated HeLa S3 cells.
About 15 million tags in total

Analysis parameters

Centering: 70bp, window 200bp, exclusion range 200 bp, threshold 100 counts

➢ Result: 4446 peaks

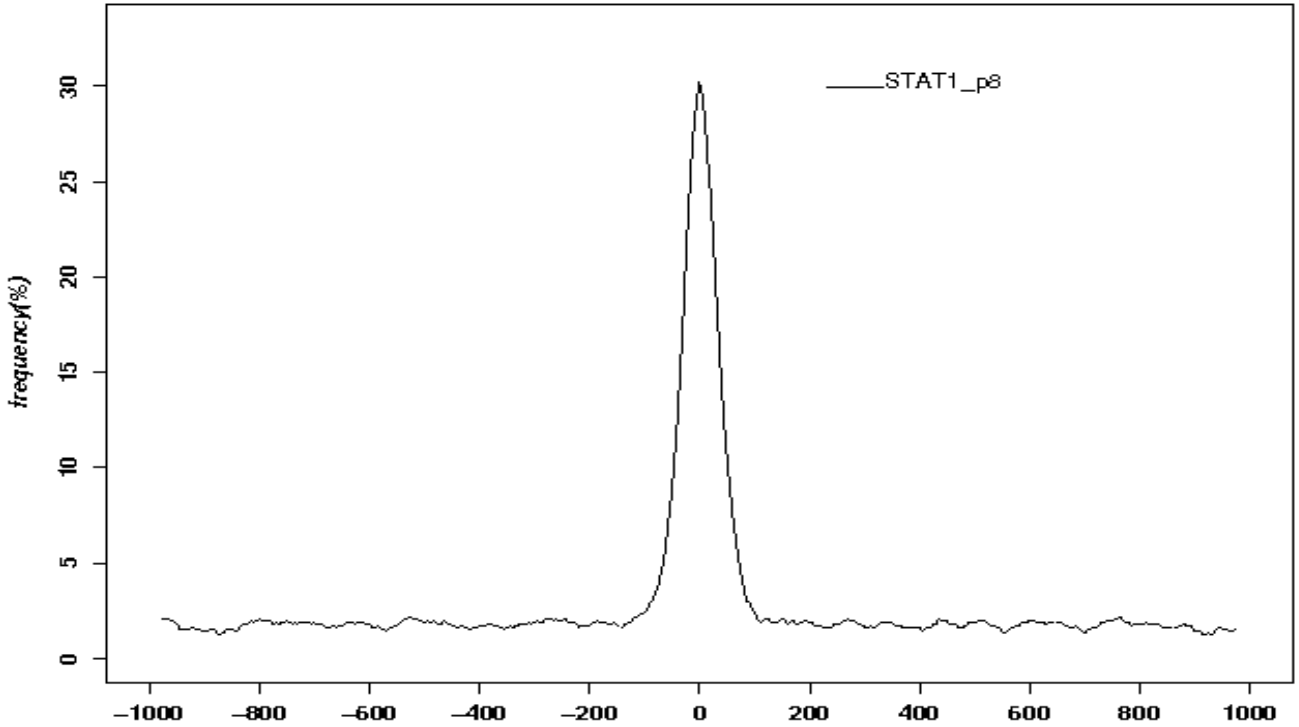
Sequence extraction range for downstream analysis: –1000, 1000

Donwstream sequence analysis

✧Distribution of TTCNNNGAA around STAT1 peak

✧Sliding window size 50

✧Figure produced with OPROF (SSA server)



STAT1 Sequence Motif Defined by ChIP-Seq data

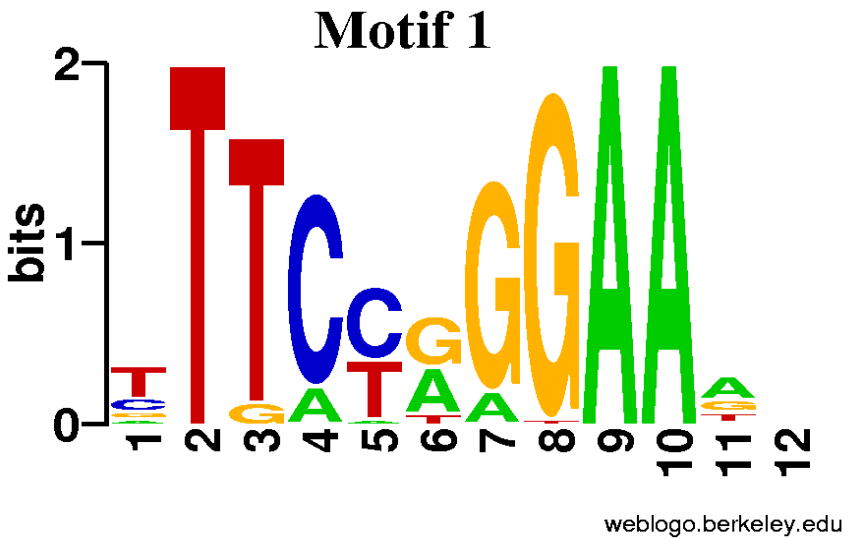
Input

4446 ChIP peak regions

200 bp

ab initio motif discovery

MEME (zoops)



Matrix from experimental *in vivo* sites

	-7	-6	-5	-4	-3	-2	-1	0
A	23	38	15	0	2	29	8	19
C	33	17	13	0	0	67	56	31
G	35	17	12	4	2	4	15	31
T	10	27	60	96	96	0	21	19

Matrix from SELEX

	-7	-6	-5	-4	-3	-2	-1	0
A	6	62	26	2	2	5	2	2
C	57	13	27	2	3	89	95	48
G	23	14	10	2	2	2	2	49
T	14	11	36	95	93	4	2	2

ChIP-partition Application

❑ Input

- Centered tag counts
- Count density threshold, transition penalty

❑ Output

- List of signal-enriched regions (beginning, end)

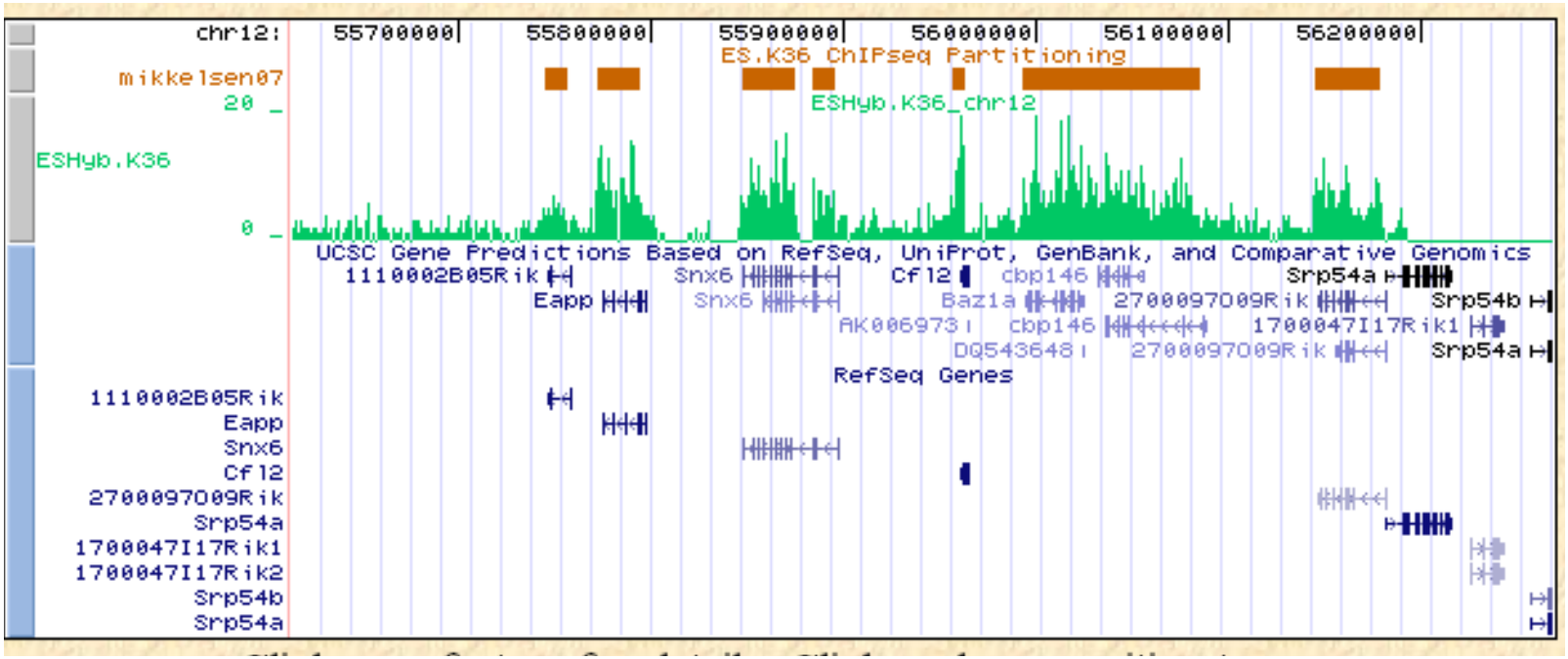
❑ Method

- *Optimization of a partition scoring function by a fast dynamic programming algorithm*
- *Two parameters: count density threshold, transition penalty*
- *Scoring functions for global results: sum of scores of*
  - *Transitions (penalty)*
  - *Signal-rich region: length × (local count-density – threshold)*
  - *Score for signal-poor region: length × (threshold – local count-density)*

❖ Output options on the Web

- *GFF, BED file for genome browser*

Viewing the results of the partitioning program in the genome browser



Custom tracks:

Mikkelsen07: results of ChIP-partition program (BED file)

ESHyb.K36: from: http://www.isrec.isb-sib.ch/WIG/HSM07_ESHyb.K36_m_chr12.wig

Where to find source and binary files

- On Vital-IT (at SIB – http://www.isb-sib.ch)

  /mnt/common/share/chip-seq-1.1.0

  /bin.em64t -> /mnt/local/bin

  chipcor chipscore chipcenter chippeak chippart

  /src

  chipcor.c chipscore.c chipcenter.c chippeak.c chippart.c

  /perl

  eland2sga.pl bed2sga.pl gff2sga.pl sga2.bed.pl sga2fps.pl counts_filters.pl fetch_sga.pl

- On SourceForge

  http://sourceforge.net/projects/chip-seq

  tarball file: chip-seq.1.1-0.tar.gz

  www : http://chip-seq.sourceforge.net

- Documentation

  Man pages, README files, C programs

  On going projects: ChIP-seq Web Tutorial, PDF User Manual, Reference Technical Manual

Where to find data files

- On all platforms (within the Vital-IT environment)

/db/chipseq

Experiments

Barski et al. (2007): human CD4+ cell lines
Histone modifications, POL II, CTCF (~2 millions tags per experiment).

Mikkelsen et al. (2007): four mouse cell lines
Histone modifications (~2 millions tags per experiment).

Robertson et al. (2007). INF-gamma stimulated HeLa cells
STAT1 (>20 million tags per experiments).

Boyle et al. (2008): CD4+ cell lines
Open chromatin studies (~10 millions tags per experiment).

Wang et al. (2008): human CD4+ cells lines
Histone acetylations and methylations (3,4 millions tags per experiments).

Schones et al. (2008): human CD4+ cells lines
Regulation of nucleosome positioning (100 millions tags per experiments).

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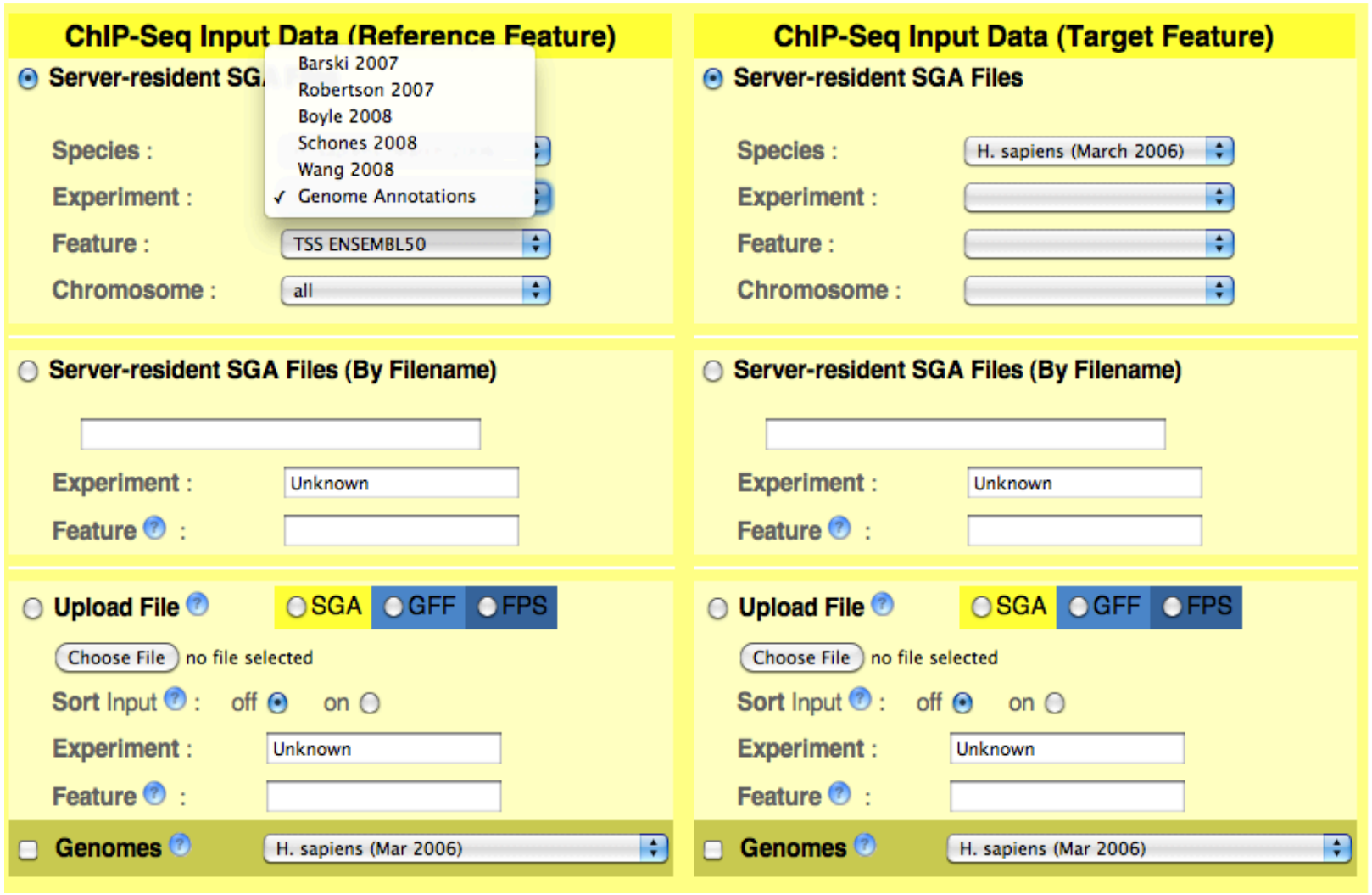
Genome Annotations

CAGE, ENSEMBL, DBTSS7 and EPD TSS
Repeat masks
Phastcons tracks from UCSC
ENSEMBL POLYA

Where to find data files (cont.)

- On the Web Server



Web Access Statistics

- Chip-Seq Web Server on ccg.vital-it.ch *Week: Jan 10 2010 - Jan 17 2010*

  - ✓ *Tot Number of Accesses : 828*
  - ✓ *Tot Number of IPs : 95*

  - ✓ *Number of Accesses from Switzerland: 316*
  - ✓ *Number of IPs from Switzerland: 10*

  - ✓ *Number of Accesses from Abroad: 512*
  - ✓ *Number of IPs from Abroad: 85*

  - ✓ *Number of Accesses from inside the UNIL: 4*
  - ✓ *Number of IPs from the UNIL: 3*
  - ✓ *Number of Accesses from the EPFL: 293*
  - ✓ *Number of IPs from the EPFL: 4*