-Alternative Splicing- Discovery of Splicing Regulatory Elements

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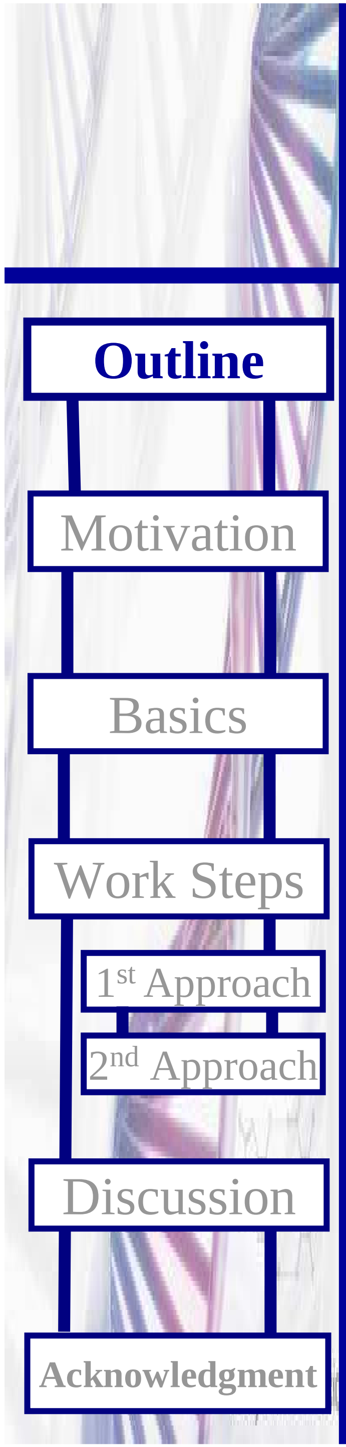
Outline

- Motivation
- Basics
- Work Steps
  - 1^{st} approach

    Inference of binding specificity of splicing regulatory factors from known binding sites
  - 2^{nd} approach

    Inference of regulatory elements using phylogeny-sensitive methods and comparative genomic data
- Discussion
- Acknowledgment



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Motivation

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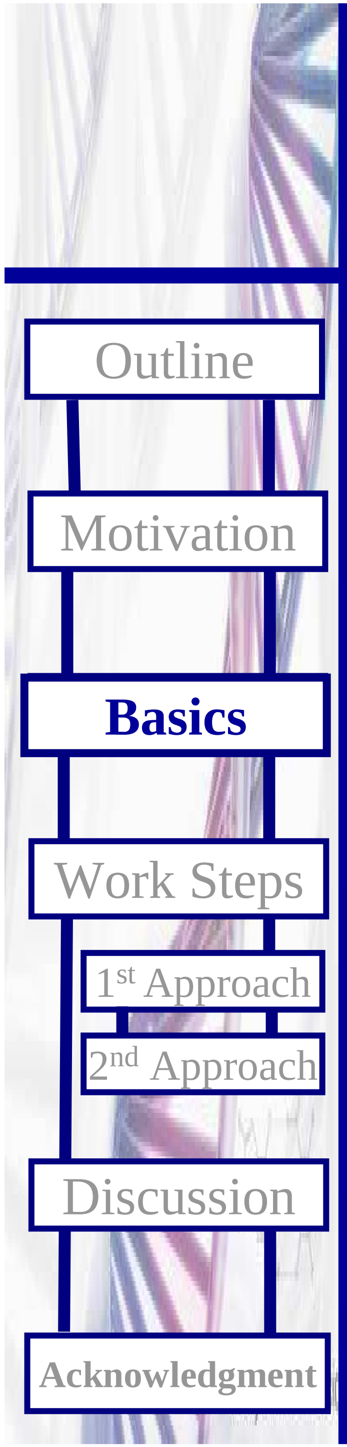
- What?
  - Molecular recognition is necessary for the regulation of biological processes

    e.g. specific recognition of sites for replication, initiation, termination of transcription by proteins

  →Find such regulating sequences to which proteins can bind

- Why?
  - Motifs correspond to changes in development or environment
  - Understand how complex processes are regulated in specific cellular context
  - Indicate relationships and ancestry between different species
  - Treatment of ailments (i.e. research in gene therapy)



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Basics

- Alternative Splicing
- Splice-regulating Sequences and corresponding RNA-binding Proteins
  - ESE (SRp)
  - ESS (hnRNP)

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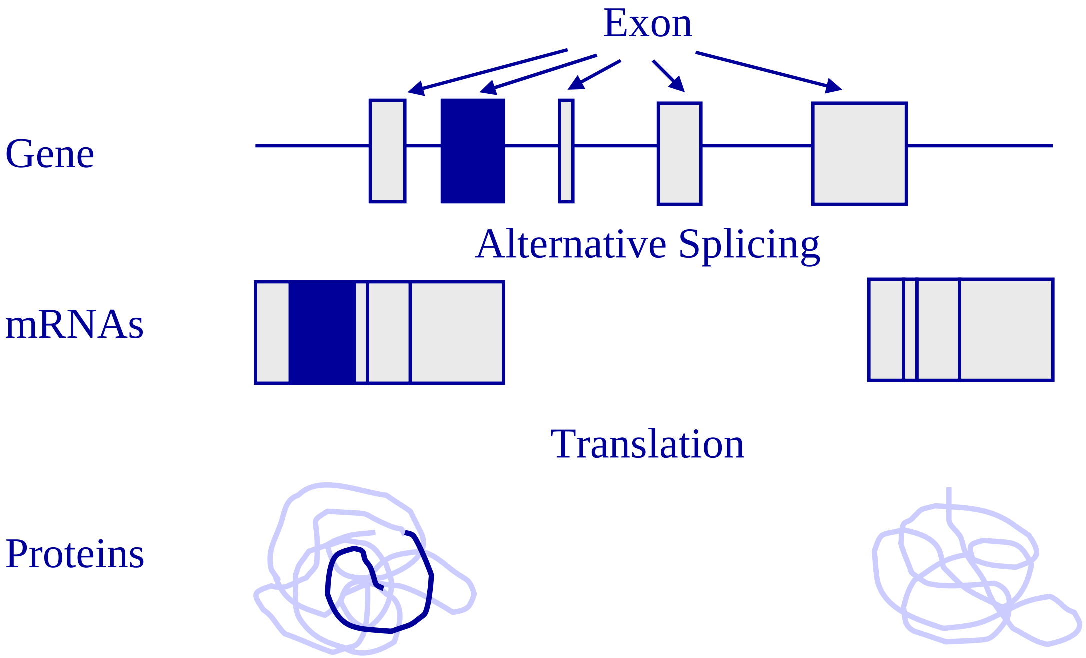
Basics

» Alternative Splicing

- Individual genes produce multiple protein isoforms
- Alternative use of exons or exon parts within pre-mRNA transcript
- Can be specific to a tissue, developmental stage or a condition
- ~40-60% of human genes are alternatively spliced



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Basics

» Splice-regulating sequences & RNA-binding Proteins

- Discrete and highly variable sequences within exons
- Important in defining constitutive and alternative exons
- Control splice site choice

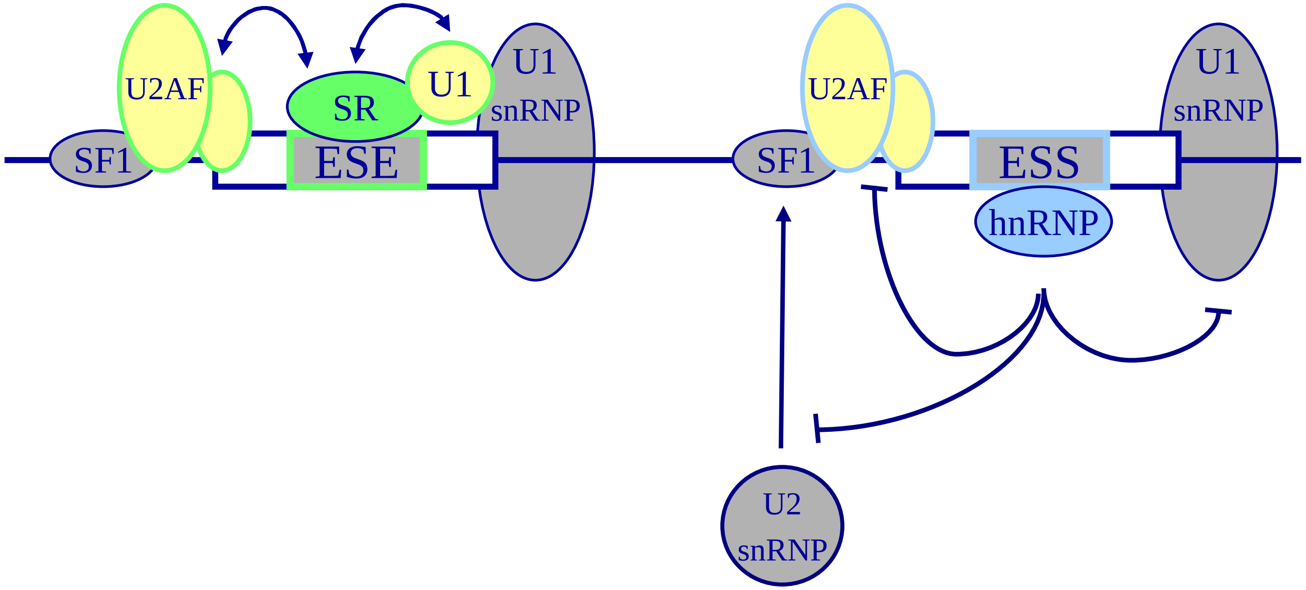
ESE (Exonic Splicing Enhancer)

- Activity involves their binding by members of a family of splicing regulators (often SRp - serine-arginine-rich proteins)
- Promote use of weak or regulated splice sites

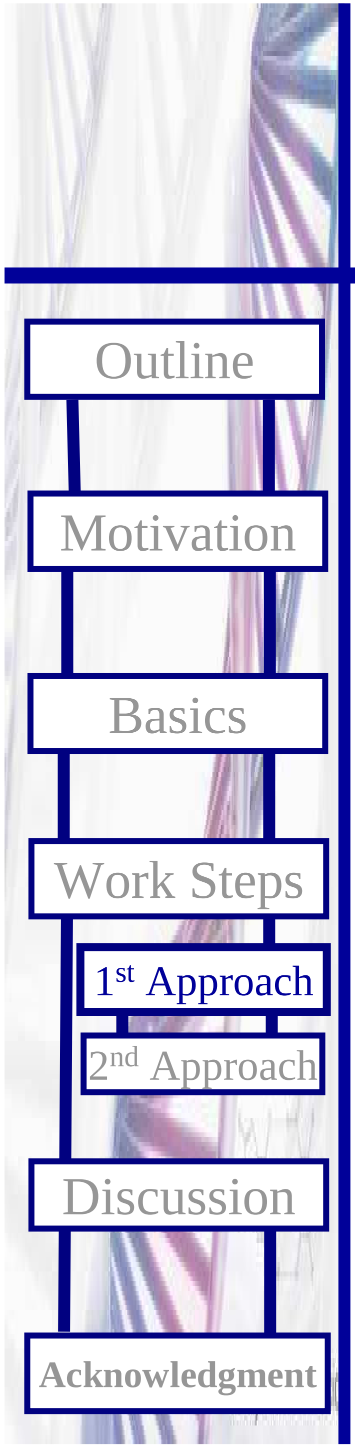
ESS (Exonic Splicing Silencer)

- Build complex with splice regulatory factors (often hnRNP - heterogeneous nuclear ribonucleoproteins)
- Repress use of splice sites

Basics



SRps and hnRNPs regulate the recognition of splice sites and the definition of intron and exon sequences



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Work Steps

► *First Approach*

Using known binding sites of splicing regulatory factors

- Second Approach

Work Steps

» First Approach (I)

- Inference of binding specificity of splicing regulatory factors from known binding sites (SELEX data)
- Extract sequences from [Singh & Valcárcel, 2005]

→ Preferred binding sites from splice regulatory factors

hnRNP	SRp
A1	9G8
C1/C2	ASF/SF2
E1/E2	SC35
H/H‘/F	SRp30c
I	SRp40c
K	SRp55
M	Tra2β
SXL	

Work Steps

» First Approach (II)

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- Cluster sequences of each protein with PROCSE

→ Weight matrices (WMs) of possible motifs with a length 6 up to 10 nt

- Extract most representative WMs

- Create profile with PROFILER
  - Background model using hg18
  - Random sequence (hg18, chr18, position 748411 to 763327)

  to calculate z-score along the sequence

- Run separately for WMs of hnRNP and SRp
  (window length of 3 at each site, 100 nt enhancer length)

Work Steps

» First Approach (III)

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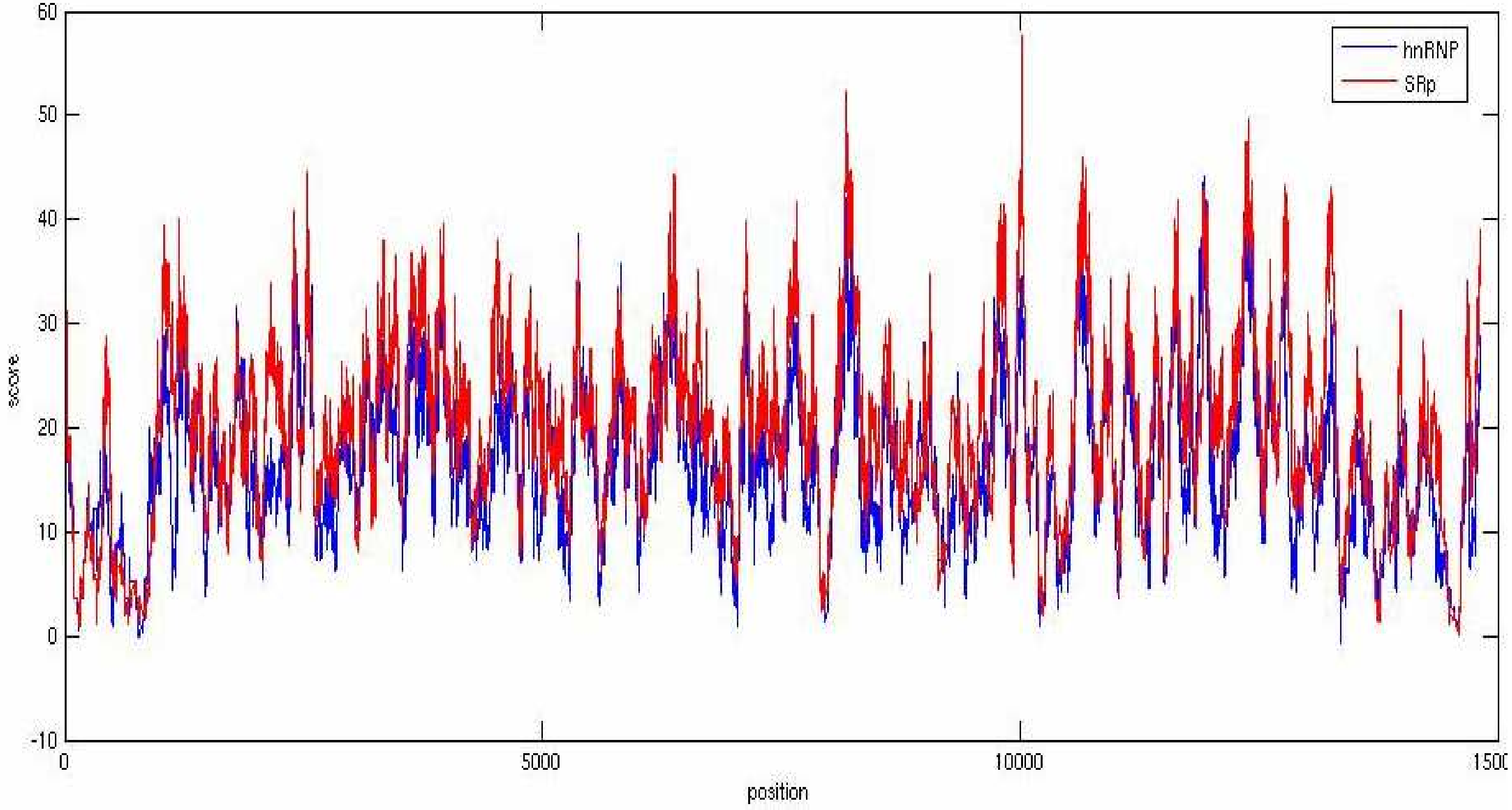

Work Steps

» First Approach (IV)

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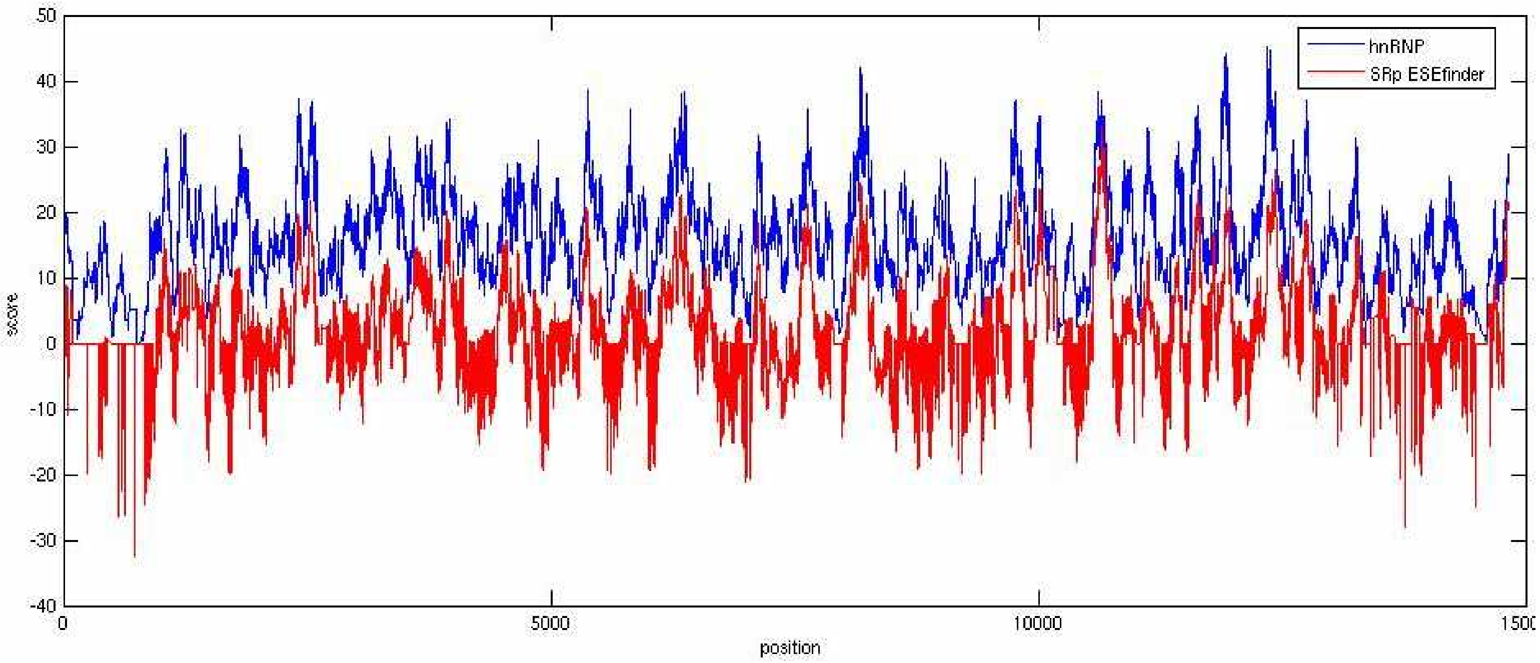
- Take WMs of ASF/SF2, SC35, SRp40, SRp55 from [Cartegni et al., 2003]
- Run PROFILER
- Compare output of hnRNP with output of these four WMs



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- First Approach

► *Second Approach*

Using comparative genomic data

Work Steps

» Second Approach (I)

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- Inference of regulatory elements using phylogeny-sensitive methods and comparative genomic data
- Get exons which are internal and non-coding using fantom3DB
- Get pairwise alignments of
  - rhesus (rhemac1)
  - chimp (pantro1)
  - cow (bostau2)
  - human (hg17)
  - rat (rn3)

  aligned to mouse (mm7) from UCSC Genome Browser

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» Second Approach (II)

- For each pairwise alignment:
  - MultiZSearch to get all sequences from mm7 and aligned ones which occur in the wanted exon regions
- Modify output for alignments on same exon
  - If overlapping
    - Remove alignments
  - If difference of boundaries > 10% difference of exon sites
    - Remove alignments

    Else concatenate alignments
- For each internal, non-coding exon create a FASTA file containing exon sequence and corresponding pairwise alignments, remove gaps
- Realign sequences for each FASTA with ClustalW

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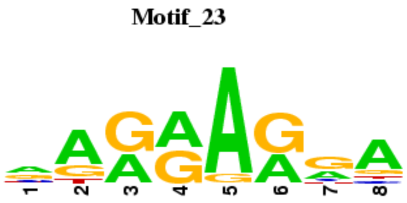
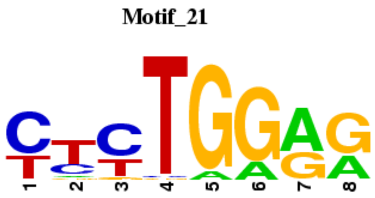
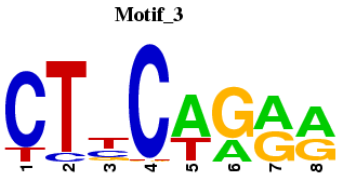
» Second Approach (III)

- Run PHYLOGIBBS
  - Comparative analysis of orthologues intergenic regions of related species

  → Identifies binding sites for regulatory proteins
  - Inputdata:
    - Aligned sequences splitted in 500 blocks per file
    - 10 motifs with length of 8 nt
  - Cluster WMs and create sequence logos with WEBLOGO





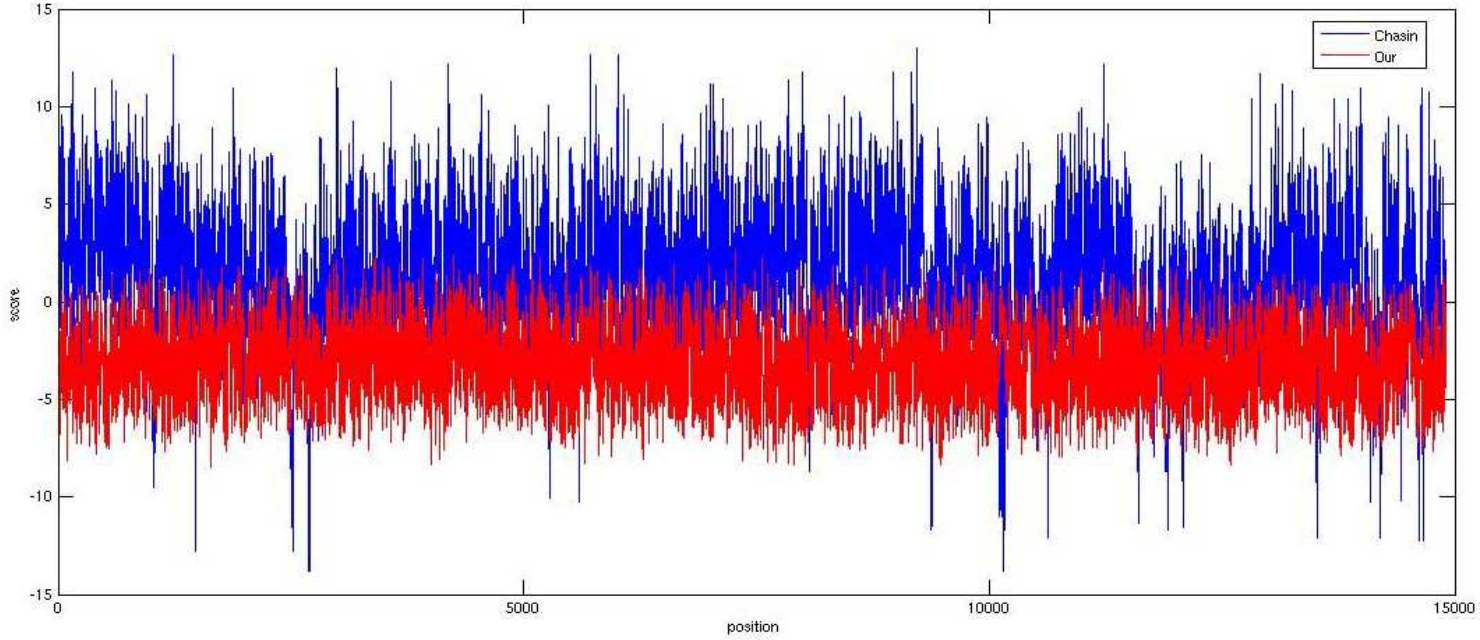


(out of 50 motifs)

Work Steps

» Second Approach (IV)

- Octamers with scores (if ES or EE) from [Chasin et al., 2004]
- Calculate max. log-likelihood over all WMs
- Make a profile for both scores with a random sequences (hg18, chr18, position 748411 to 763327)



Work Steps

» Second Approach (V)

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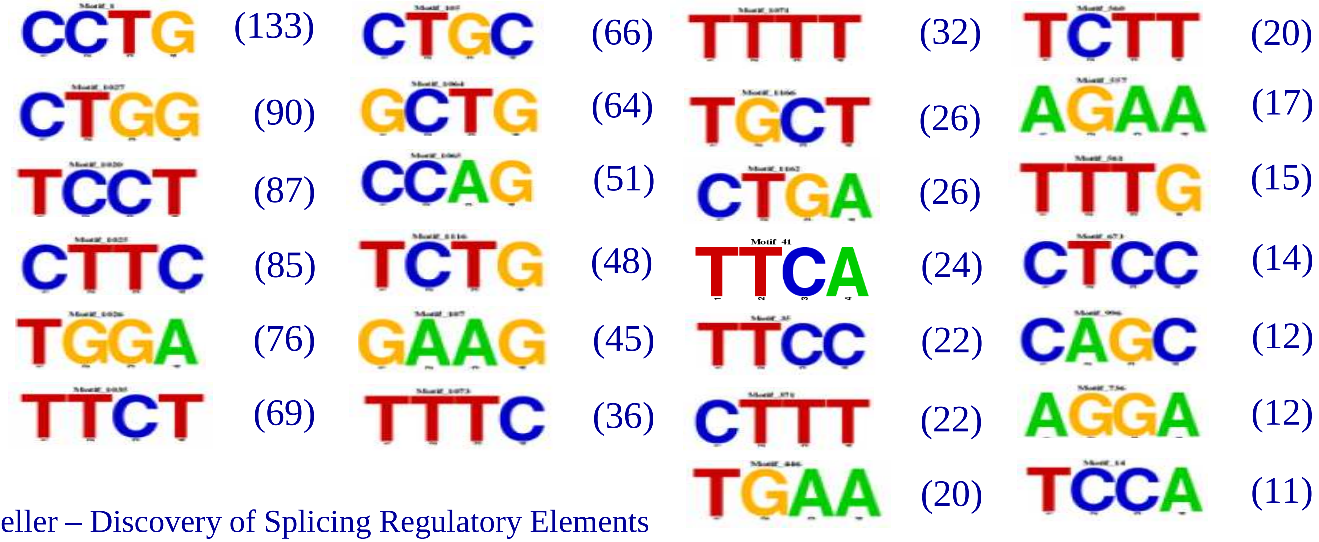
- Final input data for PHYLOGIBBS
  - 100 datasets of aligned sequences splitted randomly in 500 blocks; 5 motifs with length 4 nt
- Analyse output of PHYLOGIBBS
  - Sequence logos of all WMs to look for conserved motifs

  → Many motifs which occurred several times

  → Extract WMs of motifs with occurrence > 10 (26/1246)



Work Steps

» Second Approach (VI)

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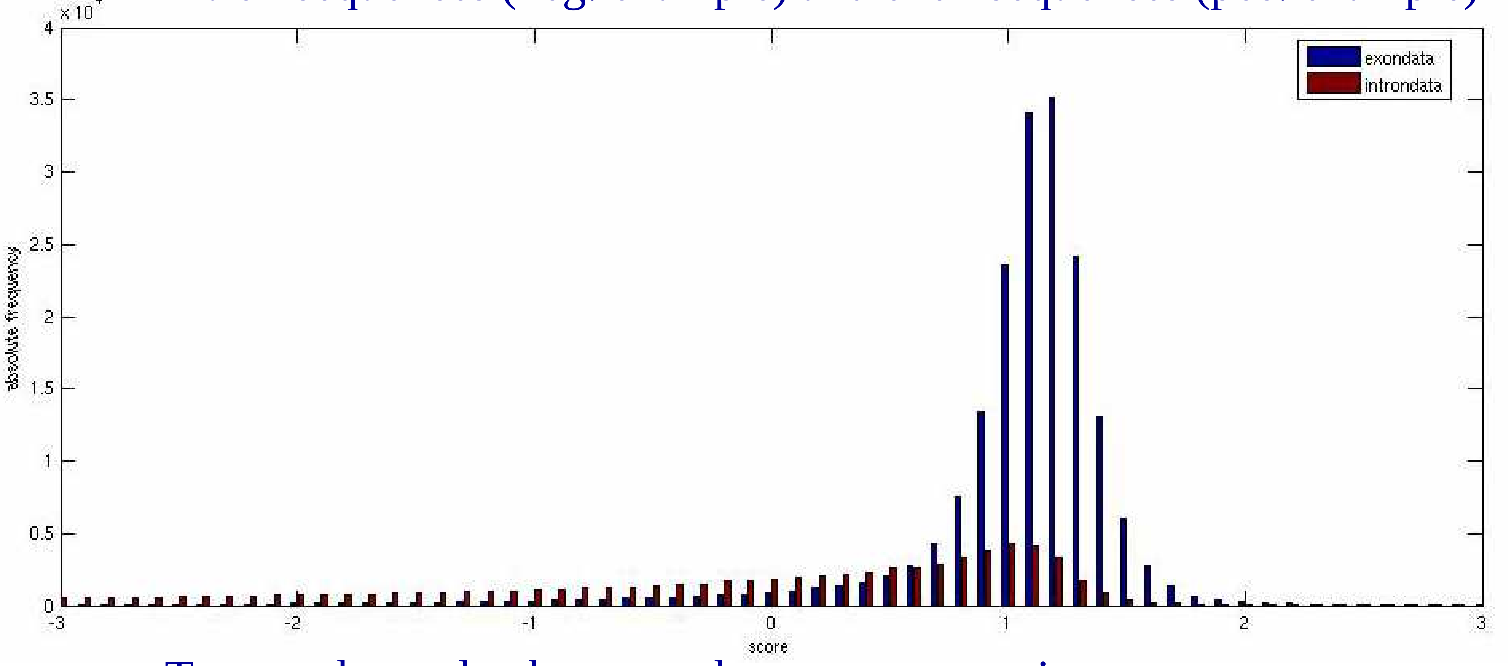
- Find out in which regions the motifs can be found
  - Appear as individuals
  - Appear in cluster
  - Predominantly in exon sequences
    - Whole sequences
    - Splice sites (10%)
  - Predominantly in intron sequences
    - Whole sequence
    - Flanking introns (size of exon sequence)

Work Steps

» Second Approach (VII)

- SVM^{light} from [Joachims, 2002] to decide with the motifs that have been found if a sequence is an exon or intron sequence
  - Create training sample for the known motifs
  - Intron sequences (neg. example) and exon sequences (pos. example)



  - Too much overlap between these two categories

    → Badly chosen motifs to define intron sequences ?

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Discussion

- Using SELEX data to find regulatory elements did not work out
- Using comparative genomic data with internal, non-coding exons
  - – Do the same for introns getting data from fantom3DB and compare the output of $\text{SVM}^{\text{light}}$ of exons
- Choose different motifs
- Use motifs with a different length
- Find other ways to calculate motifs

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Mihaela Zavolan and Group Members

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Thank you for your Attention!