NATIONAL HUMAN GENOME RESEARCH INSTITUTE Division of Intramural Research

Current Topics in Genome Analysis Fall 2006

Week 5- Part I: Detection and Characterization of Noncoding Functional Elements

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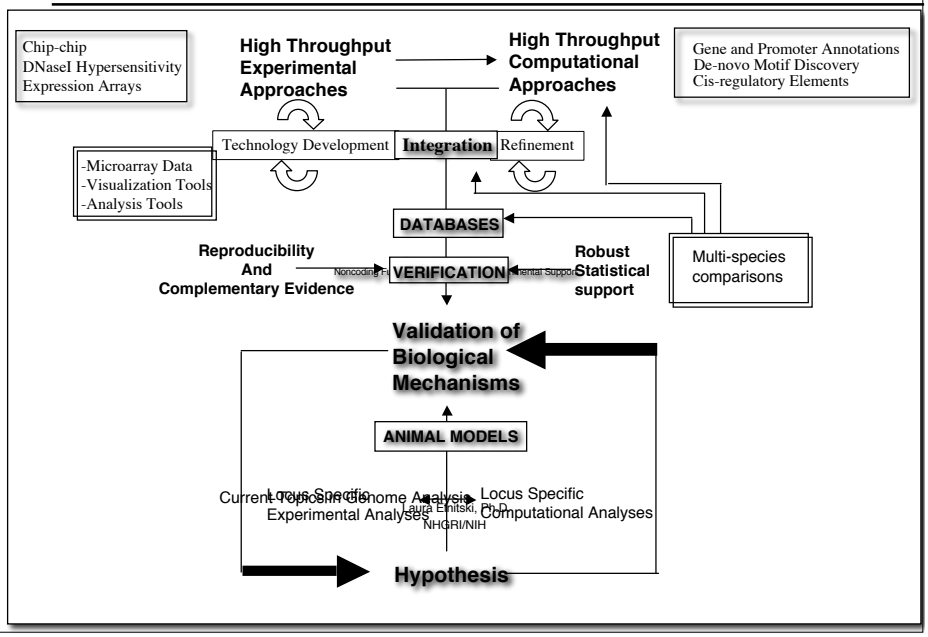
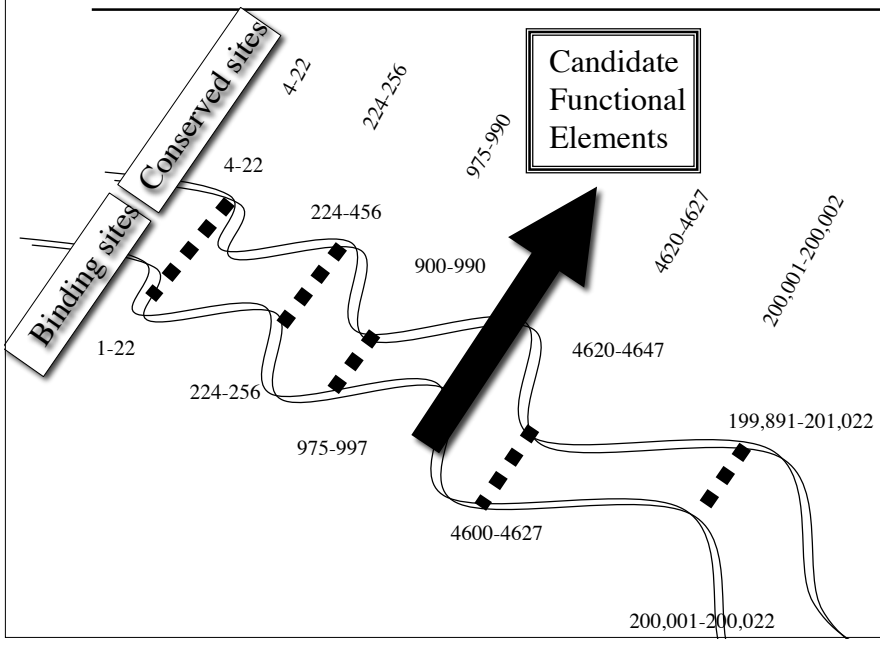
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES | NATIONAL INSTITUTES OF HEALTH | genome.gov/DIR

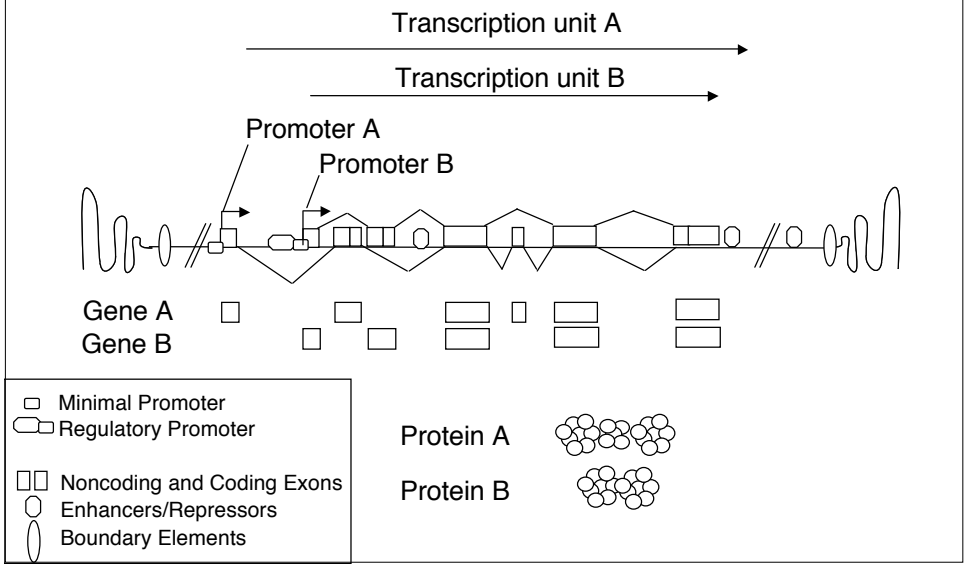
Fully Integrated Genomics Research



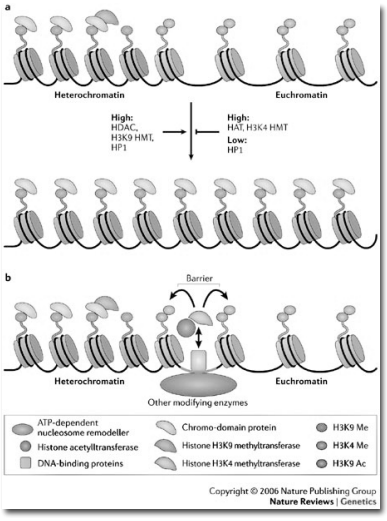
Intra- and Interspecies Sequence Comparisons

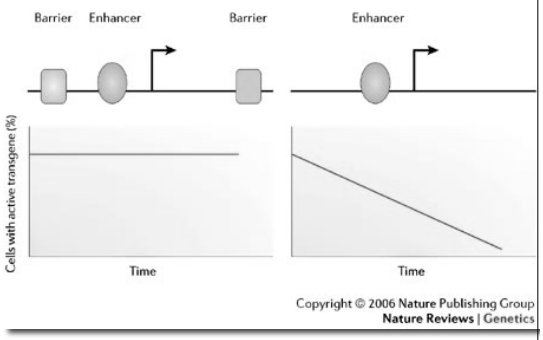


A Typical Genomic Locus



Boundary Elements

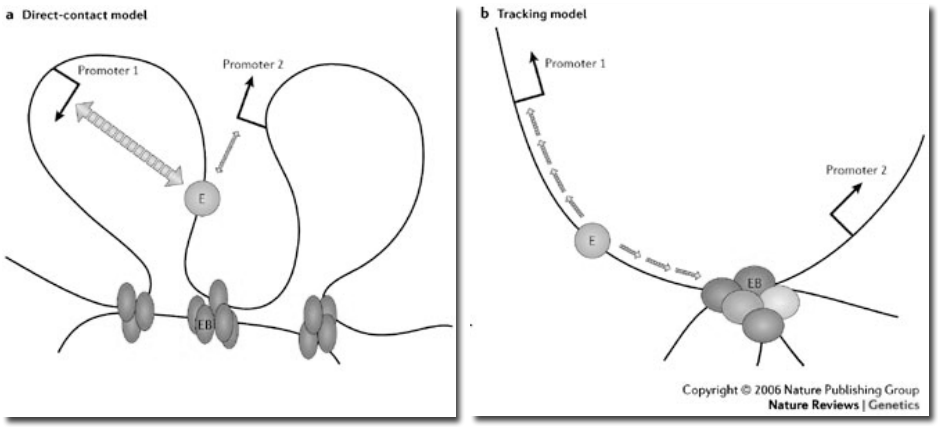




CTCF - 1st insulator
HS4 of the chicken beta-globin locus

Gaszner and Felsenfeld, 2006

Boundary Elements / Enhancer Blockers

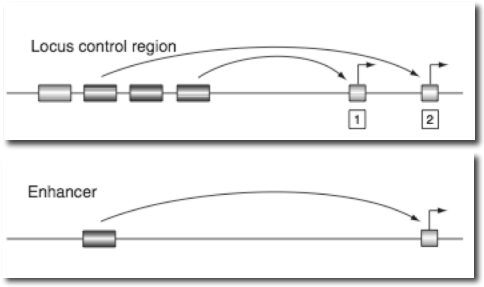


Can we find them through sequence specific searches?
Other indicators?

- Chromatin structure
- CTCF binding motifs
- CTCF occupancy

Enhancers

Distally located regulatory elements, 1kb - 1 Mb
Locus control regions *vs* single gene regulators

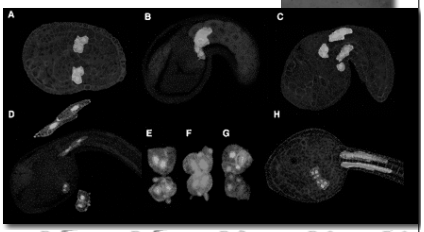


Enhancers

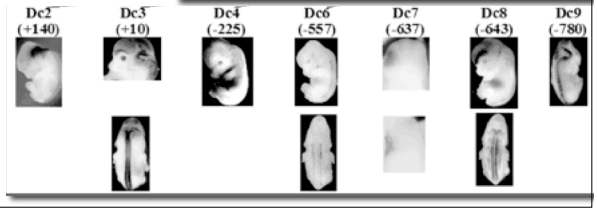
Many experimental systems using Luciferase or GFP assays:

Zebrafish
Drosophila
Chicken
Vertebrate cell lines

Ciona

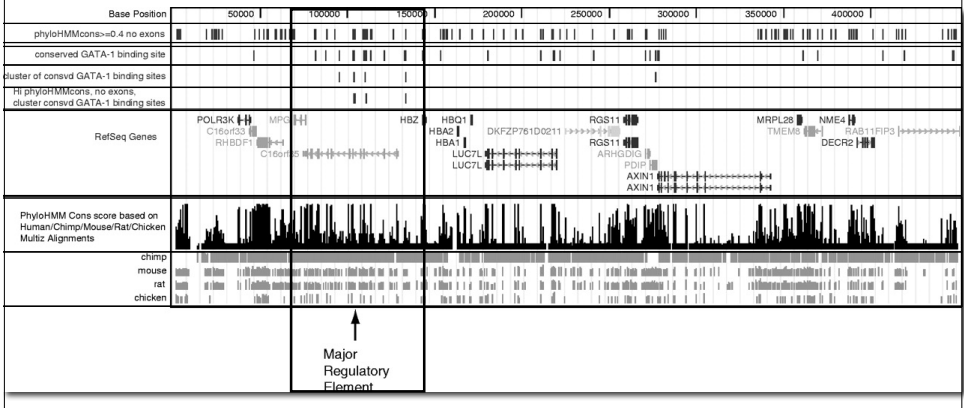


Xenopus



Transgenic mice

Computational Prediction of Enhancers



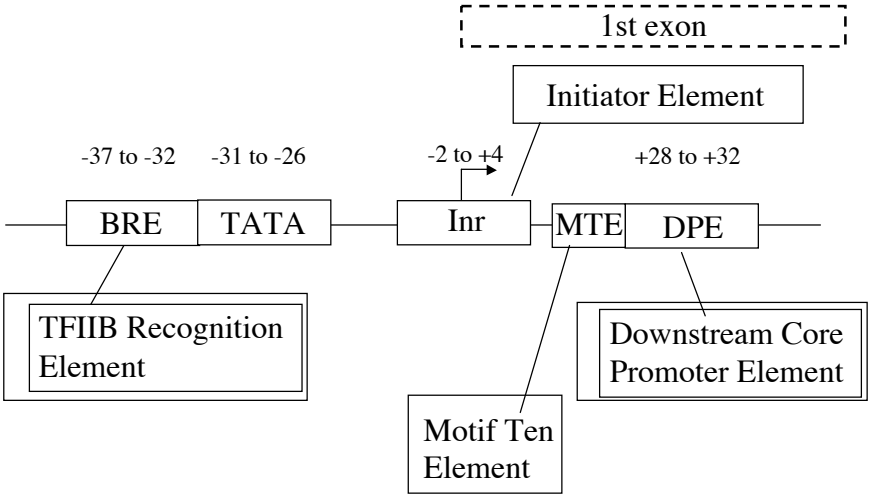
Core Promoters

- Proximal to transcription start site (+/- 35 bp)
- Determine timing of gene expression during development
- Interact with the pre-initiation complex

Riken CAGE Data — Cap Analysis of Gene Expression
http://fantom.gsc.riken.go.jp/

dbTSS — Database of Transcription Start Sites
http://dbtss.hgc.jp/

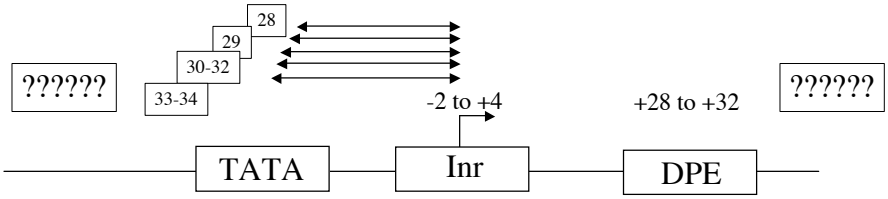
Core Promoters



Core Promoters

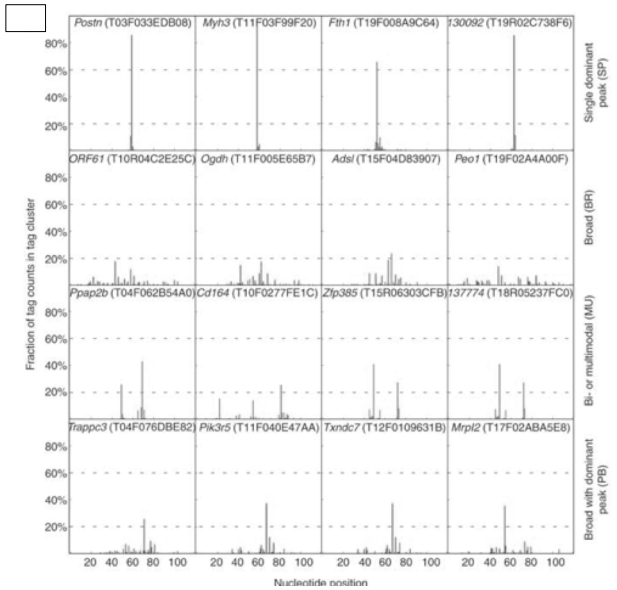
Transcriptional and structural impact of TATA-initiation site spacing in mammalian core promoters

Ponjavic, et al. 2006



Sequence searching
Stratification of datasets

Core Promoters



SP

BR

MU

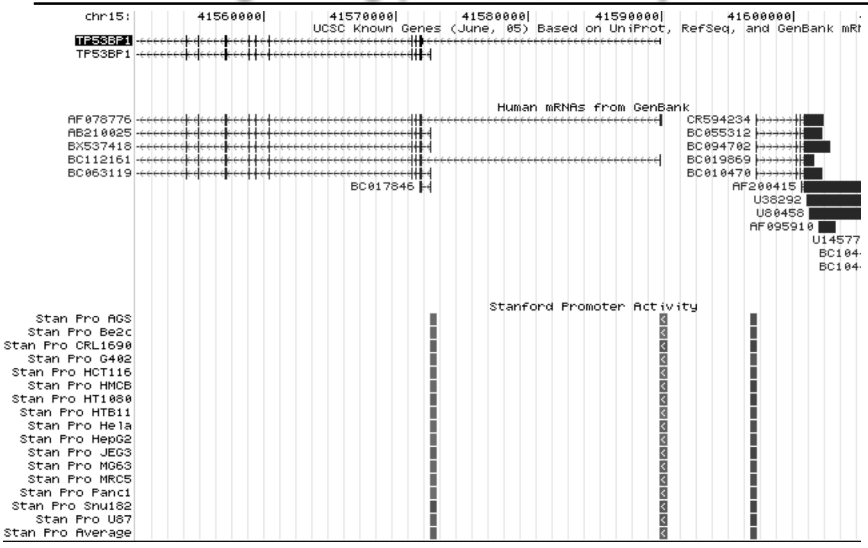
PB

Carninci et al. 2006

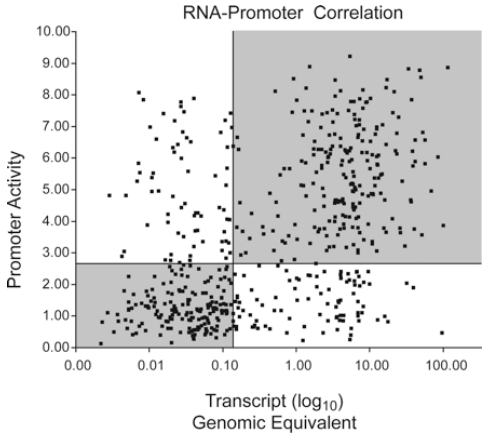
Core Promoters

- The transcriptional regulation and evolutionary plasticity of CpG island-associated promoters is also linked to epigenetic control of transcriptional activity. These promoters are rapidly evolving in mammals.
- The BR classes, most commonly based on CpG islands, represent the majority of mammalian promoters.
- Classical TATA-box promoter architecture represents a minority of the set of mammalian promoters in mouse and humans. This class is commonly associated with tissue-specific genes and high conservation across species

High Throughput Promoter Analyses



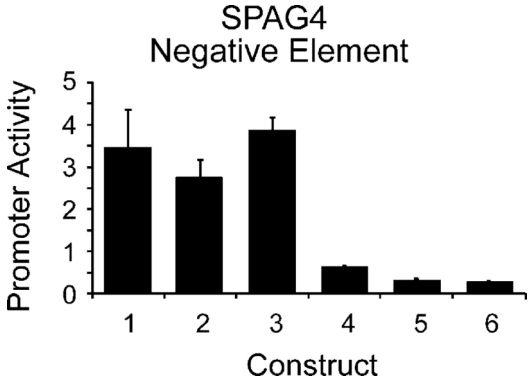
High Throughput Promoter Analyses



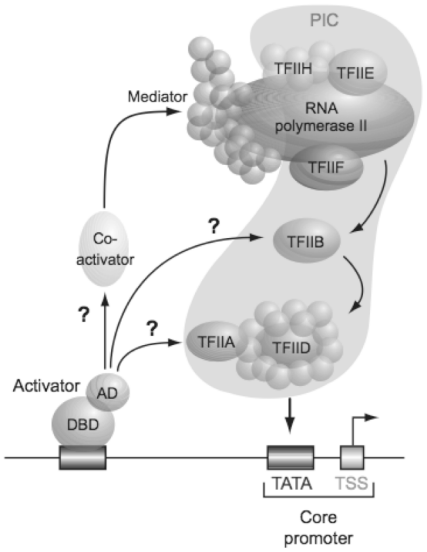
Cooper et al. Genome Res. 2006

Extended Promoters

- Upstream elements (~ 500-1 kb) that synergize with basal promoter
- Augment the level of expression or choice of tissue specificity
- Separation from enhancers? (negative elements- Cooper et al 2006)

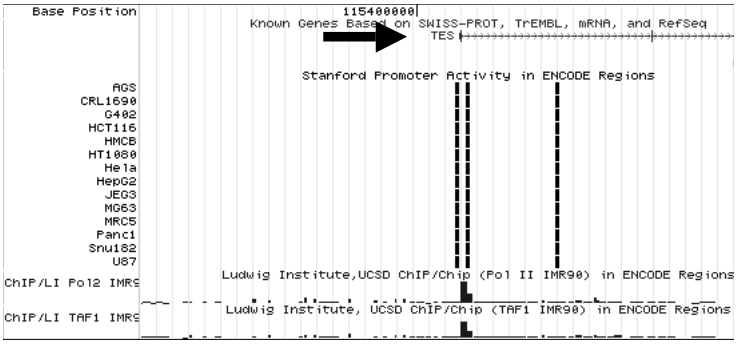


Promoter Classes



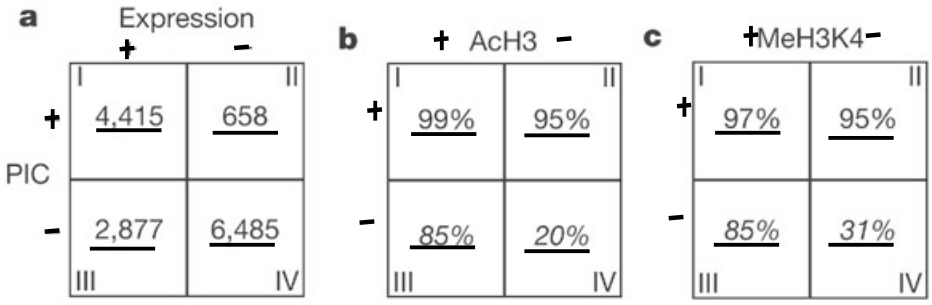
PIC = Pre-initiation complex

Promoter Features



UCSC Genome Browser

Promoter Classes



Kim et al., Nature 2005

Promoter Classes

As expected, formation of the PIC on promoters leads to transcription

➡ When PIC does not correlate with expression

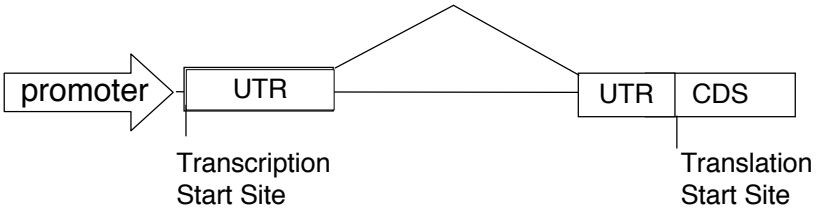
(a) PIC formation is not sufficient to initiate transcription or mRNAs are post-transcriptionally degraded.

(b) Transcription occurs, but the PIC assembles transiently, weakly or only during the early stage of fibroblast differentiation.

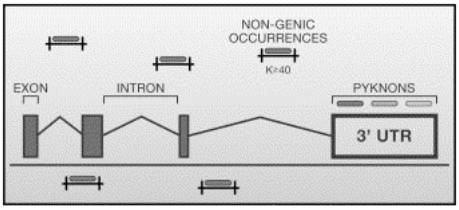
➡ Acetylation and methylation marks can correspond to both active and poised genes, defining the transcriptome capacity of the cell, requiring additional transcription regulators and machinery to collaborate with these epigenetic markers

5' UTRs

- Regulatory binding sites
- Small ORFs
- Spliced introns
- Secondary structure



3' UTRs- pyknons



Rigoutsos et al, Meynert and Birney 2006

- from the Greek word meaning "dense"
- typically 60-80 nucleotides long
- present in coding and noncoding DNA
- spacing of 18 to 22 nucleotides between copies

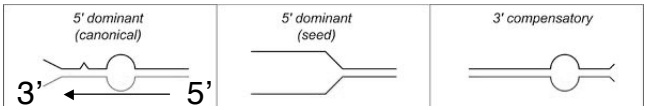
- 1/3 predicted to form double-stranded, energetically stable, hairpin-shaped RNA secondary structures with reverse complement copies.

- subsume approximately 40% of the known microRNA sequences, thus suggesting a possible link with posttranscriptional gene silencing and RNA interference.

3’ UTRs- microRNA target sites

- MicroRNAs (miRNAs) are small non-coding RNAs that serve as post-transcriptional regulators of gene expression in plants and animals.

MicroRNA target sites:
- 8-base length
- end with the nucleotide 'A’
- match through Watson–Crick pairing



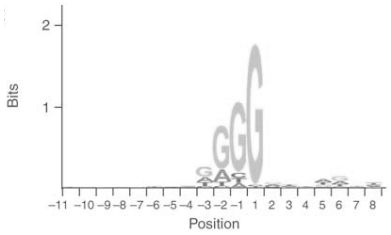
Brennecke et al. 2005

http://mirna.imbb.forth.gr/microinspector/

http://microrna.sanger.ac.uk/targets/v3/

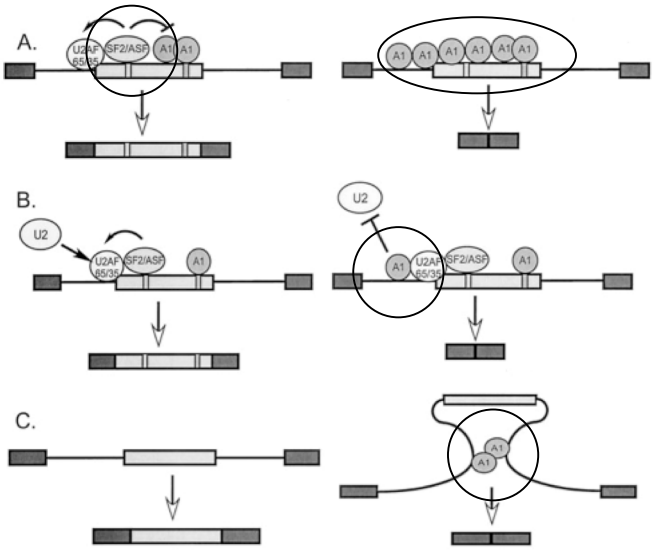
3’ UTRs

- Polyadenylation signals
- Various (A + T)-rich elements involved in controlling mRNA stability and degradation
- Binding sites for the Puf family of RNA-binding proteins (yeast) with possible human homologues
- Promoters



Carninci et al, 2006

Splicing Elements



Douglas L. Black 2003

Splicing Elements

- The same ESR sequence can function as an enhancer in one exon and a silencer in the other.
- Orthologous exons that are alternatively spliced in both human and mouse are more conserved than constitutively spliced ones
- This may be due to the presence of ESRs and is consistent with previous studies showing selective pressure against synonymous mutations in alternatively spliced exons

Goren et al. 2006

Splicing Enhancers

ESE FINDER http://rulai.cshl.edu/tools/ESE/

RESCUE-ESE http://genes.mit.edu/burgelab/rescue-ese/

Cis-acting elements bound by *trans*-acting factors

Sequence specific patterns recognized and bound by proteins

May mediate secondary interactions over long distances

May utilize proteins that
- do not directly bind DNA
- alter the DNA temporarily

Are impacted by the spatial positioning of nucleosomes

Predict them genome wide
Find mutations that affect them
Understand the biological role of proteins that bind there

Predicting transcription factor binding sites

Pre-mapped

- False positives
- False negatives

ab initio tools

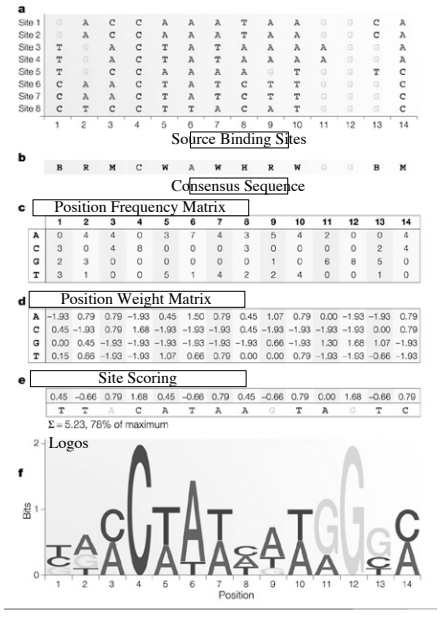
- See PDF table

Additional considerations

- Sequence conservation
- Evolutionary constraint
- Regulatory potential
- Novel Motifs

Clusters of interacting factors
Tissue specific factors

Binding Motif Notation



Resources for detecting regulatory sites

Service	Site
Whole genome binding site predictions	GALA Browser
	UCSC Genome Browser
	rVista
Position weight matrix repositories	JASPAR
	SELEXdb
	TRANFAC
Experimental data repositories	CAGE Basic Analysis Databases
	COMPEL
	EPD
	GenBank
	MPromDb
	OMGProm
	TRRD

Resources for detecting binding sites

Pattern matching	AlignACE
	ANN-Spec
	BayesianPhylogeneticFootprinter
	cisRED
	COmrad
	ConSite
	CompelPatternSearch
	DoOP
	Dragon ERE Finder
	ECR Browser
	Mapper
	MatInspector
	MDscan
	MotifVis
	P-Match
	PROMO
	PromoterPlot
	RSAT
	SeSiMCMC
	SiteSeer
	TESS
	TFBind
	TESEARCH
	Toucan
	TRED

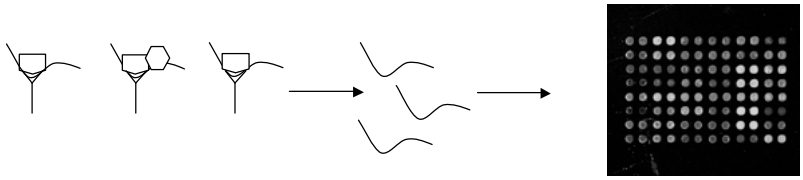
Resources for detecting binding sites

Pattern discovery	Consensus
	Decode.org
	Ensembl.org
	Eponine
	FootPrinter
	Gibbs Motif Sampler
	GLAM
	MITRA
	motifSampler
	MSCAN
	Oligo/Dyad-analysis
	oPOSSUM
	Weeder Web
	YMF
	Target Explorer

Resources for detecting binding sites

Cluster detection	Cluster Buster
	CREME
	Improbizer
	ModuleSearcher
	MEME
	Over-represented Transcription Factor Binding Site Prediction Tool (OTBS)
	TraFaC
	TFBInd
	TRANSCompel

ChIP-chip



- Detects Protein-DNA Interactions - Direct
- Protein-Protein Interactions - Indirect

ENCODE Consortium
31 ChIP datasets of 18 sequence specific factors were examined for enrichment in motifs.

Resources for detecting binding sites

ChIP-chip	Chip-chip antibodies
	Chip-chip protocols
	MPEAK
	PeakFinder
Transcripton factor information	oPOSSUM
PWM comparisons	T-Reg Comparator

Resources for ChIP-chip data

Resource	URL
ArrayExpress	www.ebi.ac.uk/arrayexpress
GALAXY2	www.bx.psu.edu
GEO	www.ncbi.nlm.nih.gov/geo
ENCODEdb	http://research.nhgri.nih.gov/ENCODEdb
Ensembl	www.ensembl.org
UCSC	www.genome.ucsc.edu

ChIP-chip Results

- The factors whose binding sites are most enriched at the 5' ends of genes include histone modifications, TAF1, RNA PolII with hypo-phosphorylated C terminal domain
- E2F2, a sequence-specific factor that regulates the expression of many genes at the G1 to S transition, is often tightly associated with TSSs.
- Repressive elements CTCF and SIRT1, and PU1 are underrepresented near the TSSs.
- Several factors had no enriched TRANSFAC motif nor could a motif be discovered using *ab initio* methods.

ENCODE 2006

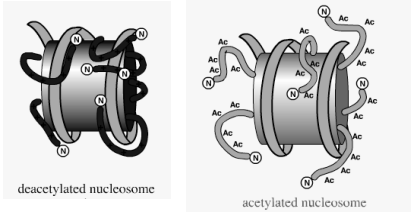
Detectable Histone Modifications

Gross rearrangements : movement or removal

Detected by DNAse I sensitivity

Reversible alterations: methylation, actylation, phosphorylation

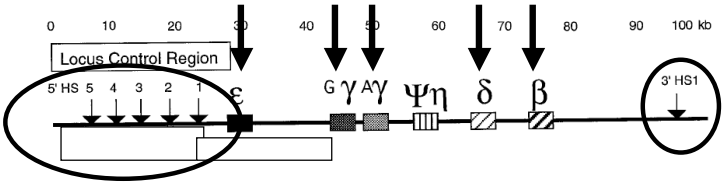
Detected by ChIP assays



http://www.broad.harvard.edu/chembio/lab_schreiber/anims/animations/hdac.html

Chromatin and Gene Expression

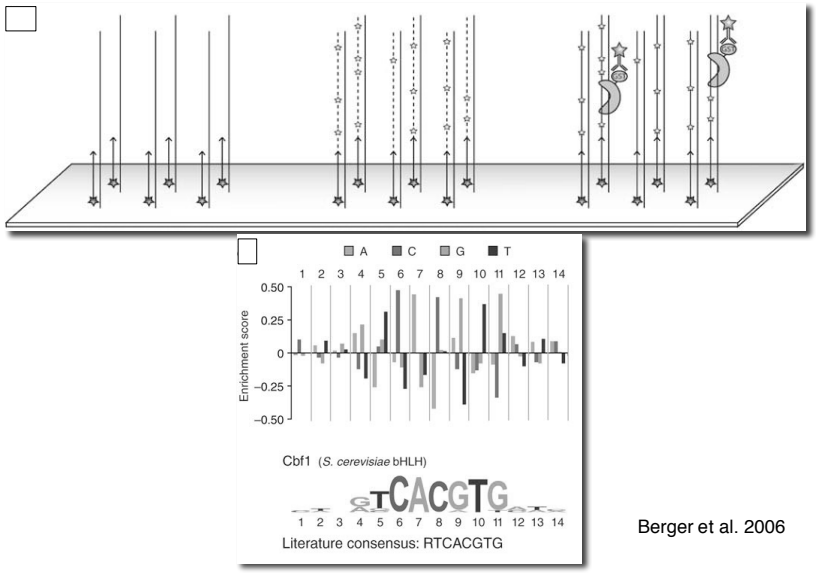
- Changes in chromatin conformation can be measured by sensitivity to DNase I
- DNase I *hypersensitivity* is an indicator of relaxed chromatin
- Often seen prior to detectable gene expression



Novel Motifs

- Certain TFs, such as Sp1 and C/EBP, bind to target sequences that vary widely.
- Evolutionary pressure to retain such binding sites is minimal owing to the high likelihood that alternative sites will be available within a regulatory region.
- This indicates that there might be two subtypes of TFBS: highly selected sites that rarely occur by chance and auxiliary sites that are available by convenience.
- Phylogenetic footprinting methods will be well suited for binding sites of TFs with greater binding specificity.

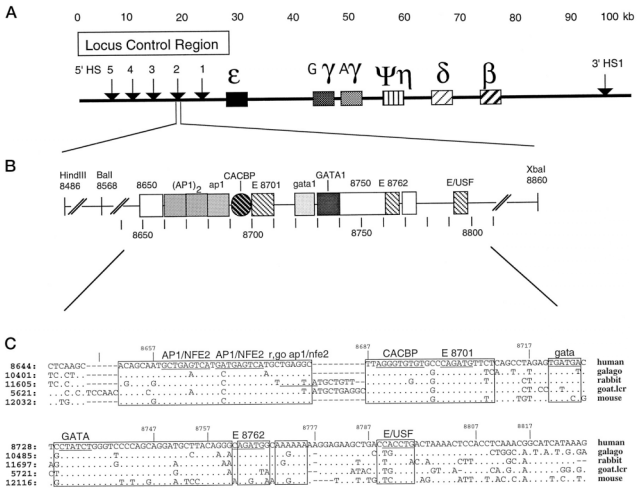
Protein Binding Arrays



Berger et al. 2006

Novel Motifs

Phylogenetic Footprinting
Shadowing



Characteristics Implicating Function

- Location in genome
- Conservation in a multiple sequence alignment
- Evolutionary Constraint
- Predictive tracks
- Clusters of transcription factor binding sites
- Experimental evidence
- Chromatin structure

Functional multiplicity

GALAXY2 Server

Enables Integrative Genomic Analysis

- Extracting Orthologous Sequences
- Phylogenetic Tree Construction
- Basic Statistics
- Operations
  - Complement
  - Restrict
  - Merge overlapping regions
  - Intersect
  - Union
  - Join Lists
  - Cluster
  - Proximity
  - Subtract

http://www.bx.psu.edu/

Questions / Follow-up

elnitski@mail.nih.gov Re: Current Topics

Supplementary Tables

Table 1(A). Web Servers for *in silico* predictions

Service	Site	URL
Whole genome binding site predictions	GALA Browser	www.bx.psu.edu
	UCSC Genome Browser	genome.ucsc.edu
	rVista	rvista.dcode.org
Position-weight matrix repositories	JASPAR	jaspar.cgb.ki.se
	SELEXdb	wwwmgs.bionet.nsc.ru/mgs/systems/selex/
	TRANFAC	www.gene-regulation.com
Experimental data repositories	CAGE Basic/Analysis Databases	fantom3.gsc.riken.jp
	COMPEL	compel.bionet.nsc.ru/new/compel/compel.html
	EPD	www.epd.isb-sib.ch
	GenBank	www.ncbi.nlm.nih.gov
	MPromDb	bioinformatics.med.ohio-state.edu/MPromD
	OMGProm	bioinformatics.med.ohio-state.edu/OMGProm
	TRRD	wwwmgs.bionet.nsc.ru/mgs/gnw/trrd/
Pattern matching	AliBaba2	www.gene-regulation.com/pub/programs/alibaba2/index.html
	AlignACE	Atlas.med.harvard.edu
	ANN-Spec	www.cbs.dtu.dk/services/DNAarray/ann-spec.php
	Bayesian Phylogenetic Footprint	bayesweb.wadsworth.org/cgi-bin/bayes_align12.pl
	cisRED	www.cisred.org
	COnreal	conreal.niob.knaw.nl
	ConSite	mordor.cgb.ki.se/cgi-bin/CONSITE/consite
	CompelPatternSearch	compel.bionet.nsc.ru/FunSite/CompelPatternSearch.html
	DoOP	doop.abc.hu
	Dragon ERE Finder	sdmc.lit.org.sg/ERE-V2/index
	ECR browser	ecrbrowser.dcode.org
	Mapper	bio.chip.org/mapper
	MatInspector	www.genomatix.de/products/MatInspector
	MDscan -	ai.stanford.edu/~xsliu/MDscan/
	MotifViz	biowulf.bu.edu/MotifViz
	P-Match	www.gene-regulation.com/cgi-bin/pub/programs/pmatch/bin/p-match.cgi
	PROMO	www.lsi.upc.es/~alggen
	PromoterPlot	promoterplot.fmi.ch
	RSAT	rsat.ulb.ac.be/rsat/
	SeSiMCMC	favorov.hole.ru/gibbslfm
	SiteSeer	rocky.bms.umist.ac.uk/SiteSeer/
	TESS	www.cbil.upenn.edu/tess
	TFbind	tfbind.ims.u-tokyo.ac.jp
	TFSEARCH	www.cbrc.jp/research/db/TFSEARCH.html
	Toucan	homes.esat.kuleuven.be/~saerts/software/toucan.php
	TRED	rulai.cshl.edu/TRED

Table 1(B). Web Servers for *in silico* predictions

Pattern discovery	Consensus	bifrost.wustl.edu/consensus
	Dcode.org	www.dcode.org
	Ensembl.org	www.ensembl.org
	Evoprinter	evoprinter.ninds.nih.gov
	FootPrinter	bio.cs.washington.edu/software.html
	Gibbs Motif Sampler	bayesweb.wadsworth.org/gibbs/gibbs.html
	GLAM	Zlab.bu.edu/glam
	MITRA	www1.cs.columbia.edu/compbio/mitra/
	motifSampler	www.esat.kuleuven.ac.be/~dna/Biol/Software.html
	MSCAN	mscan.cgb.ki.se/cgi-bin/MSCAN
	Oligo/Dyad-analysis	rsat.scmbb.ulb.ac.be/rsat/
	oPOSSUM	www.cisreg.ca
	Weeder Web	www.pesolelab.it/
	YMF	bio.cs.washington.edu/software.html
	Target Explorer	trantor.bioc.columbia.edu/Target_Explorer
Cluster detection	Cluster Buster	zlab.bu.edu/cluster-buster
	CRÈME	creme.dcode.org
	Improbizer	www.cse.ucsc.edu/~kent/improbizer/improbizer.html
	ModuleSearcher	homes.esat.kuleuven.be/~saerts/software/modulesearcher.html
	MEME	meme.sdsc.edu/meme/
	Over-represented Transcription Factor Binding Site Prediction Tool (OTFBS)	www.bioinfo.tsinghua.edu.cn/%7Ezhengjsh/OTFBS/index.html
	TraFaC	trafac.cchmc.org/trafac/index.jsp
	TFBind	tfbind.ims.u-tokyo.ac.jp
	TRANSCompel	www.gene-regulation.com/pub/databases.html#transcompel
ChIP-chip	Chip-chip antibodies	www.chiponchip.org/antibody.html
	Chip-chip protocols	genomecenter.ucdavis.edu/farnham/farnham/protocol.html
	MPEAK	www.stat.ucla.edu/~zmdl/mpeak/
	PeakFinder	research.stowers-institute.org/jeg/2004/cohesin/peakfinder
Transcripton factor information	ooTFD	www.ifti.org/ootfd
PWM comparisons	T-Reg Comparator	treg.molgen.mpg.de

* Note – tools or servers may belong to more than category in the table. Additional tools are frequently provided at each site. Web sites may occasionally be down due to server errors or discontinued altogether.

Table 2. Resources for Experimental protocols

Assay	Reference (Chapter)	
	Molecular Cloning (Sambrook and Russell 2001)	Current Protocols in Mol. Biol. (Ausubel et al. 2005)
DNase I Hypersensitivity	17.18	21
EMSA	17.13	12
DNase I Protection	17.4	21
ChIP*	N/A	21
Transfection	16	9

*Note: Additional information is available in (Allis and Wu, 2003).

Table 3. Web servers and repositories for publicly available high-throughput ChIP-chip binding data

Resource	URL
ArrayExpress	www.ebi.ac.uk/arrayexpress
GALAXY2	www.bx.psu.edu
GEO	www.ncbi.nlm.nih.gov/geo
ENCODEdb	http://research.nhgri.nih.gov/ENCODEdb
Ensembl	www.ensembl.org
UCSC	www.genome.ucsc.edu

Allis C.D. and C. Wu. 2003. *Chromatin and Chromatin Remodeling Enzymes, Part B*. Methods in Enzymology. Elsevier Inc.

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, K.A., Struhl, K. 2005. *Current Protocols In Molecular Biology*. John Wiley & Sons, Inc.

Sambrook, J. and Russell, D.W. 2001. *Molecular Cloning: A Laboratory Manual (3-Volume Set)*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.