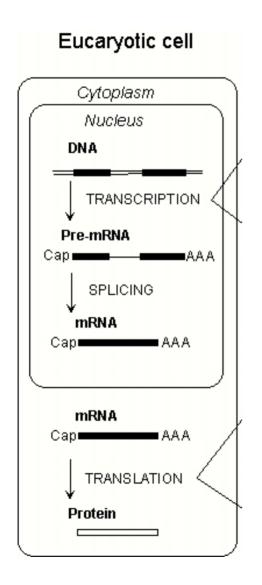
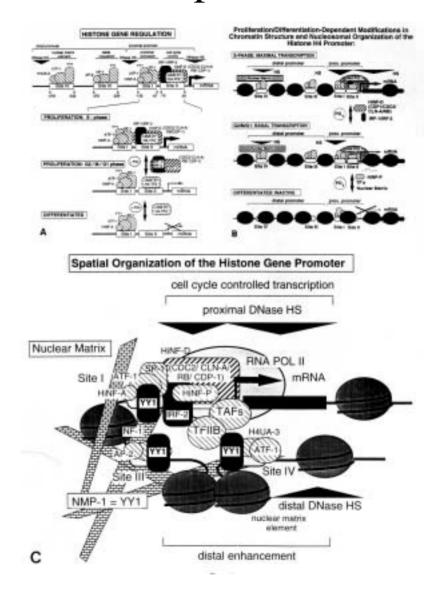
Promoter Analysis in silico Michael Q. Zhang

Cold Spring Harbor Laboratory

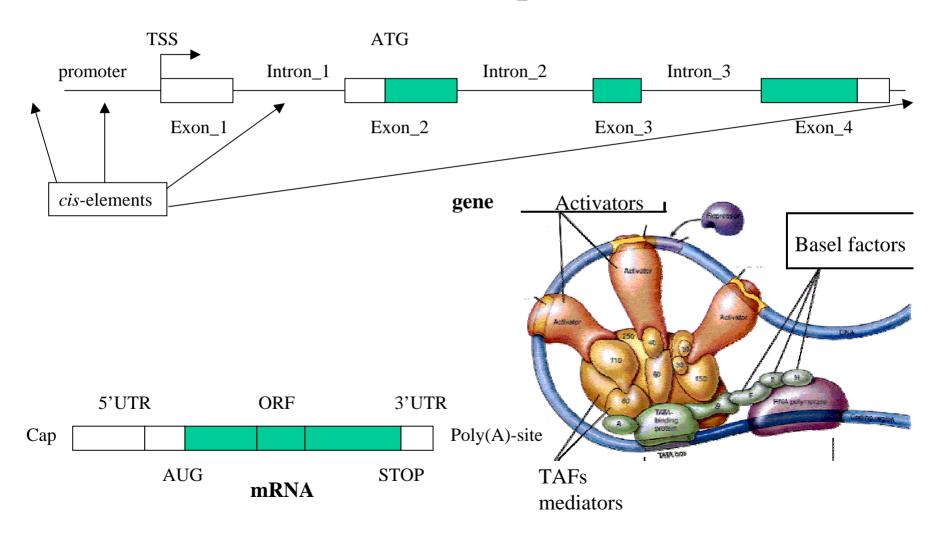
- Promoter: transcriptional control/regulation
- General promoter recognition problem
 - De novo prediction
 - Comparative analysis
- Specific promoter recognition problem
 - Database search and multiple alignment
 - Expression data analysis

Promoter and transcription

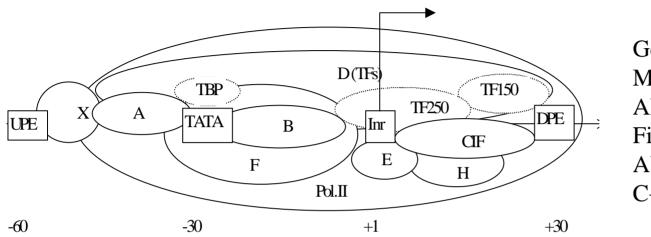




General promoter recognition Gene structure prediction



Core_Promoter (Zhang, Genome Res. 1998)



GenBank
M12523:1..1980
ALB gene=serum albumin
Firstexon=1737..1854
AUG=1776
C+G=0.33

Core_Promoter prediction:

	+1 .	TSS	Score
160 120 100 70	40 10 +20 +50 +80	1737	0.637
-160 -130 -100 -70	-40 -10 +20 +50 +80	1736	0.604
X_1 X_2 X_3 X	X_4 X_5 X_6 X_7 X_8	1727	0.588
X_{0} X_{10}	VVVV	1732	0.534
, , ,	X_{11} X_{12} X_{13} X_{13}	1731	0.531
-145 -100 -5:	55 -10 +35 +80	1728	0.498
C	DDA variables	1726	0.428

CpG_Promoter (Ioshikhes&Zhang Nature Genet. 2000)

CpG island: Length > 200 bp;C + G content > 50%;CpG ratio Obs/Exp > 0.6

- 135 genes
- 68 have CpG island around promoter
- 63 recognized
- SN = 0.47 (0.93)
- SP = 0.34 (1 Pos./26 kb; 1/36 kb is in fact)

•Promoter Scan gives

SN = 0.44

SP = 0.06 (1 Pos. / 4.7 kb)

GenBank	CpG_Promo	ter prediction:	Core_Promoter prediction:		
	CpG islands	Promoter-associated	TSS	Score	
D87675	88139319	+	8921	0.100	
>301kb	93289547	+	8923	0.094	
App gene encodes	976110203	+	8920	0.089	
Amyloid precursor protein	117256117511	-	8919	0.084	
• 1	176132176342	_	8922	0.078	
Firstexon=90019204	257735257942	_	8918	0.058	
AUG=9148	261475261750	-	8783	0.056	

First exon prediction (FirstEF)

(Daluvuri, Grosse & Zhang, submitted)

Performance statistics of FirstExonFinder based on cross validation

Exon Type	Sn	Sp	CC
CpG-related	0.92	0.97	0.94
Not CpG- related	0.74	0.6	0.65
All Exons	0.86	0.83	0.83

Promoter Prediction accuracy of FirstEF and PromoterInspector for Ch22

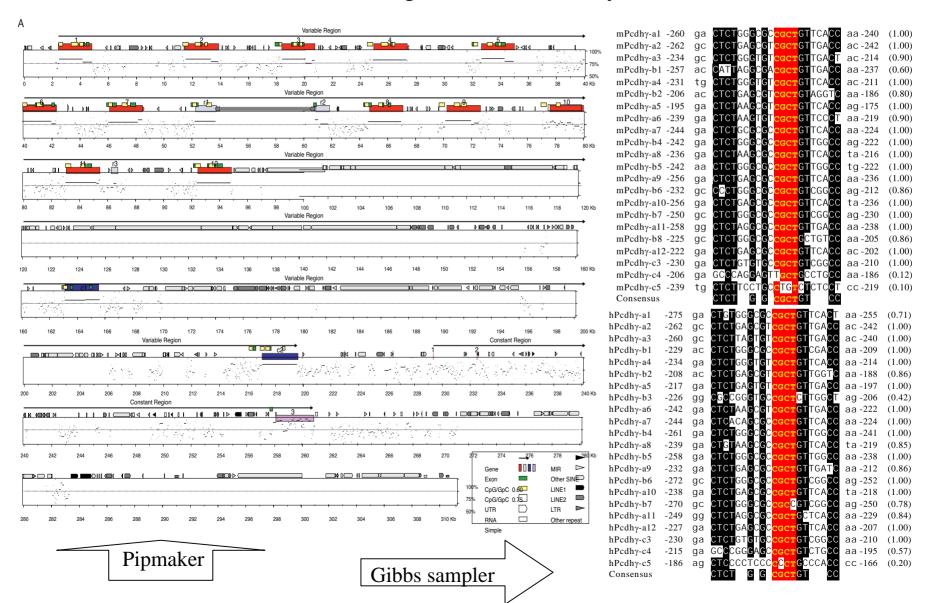
Program	TP	FP	Sn	Sp
FirstEF	46	40	79.30%	53.50%
PromoterInspector	28	37	48.30%	43.10%

Prediction Accuracy for Ch21&22 (Number of Real Promoters: 58)

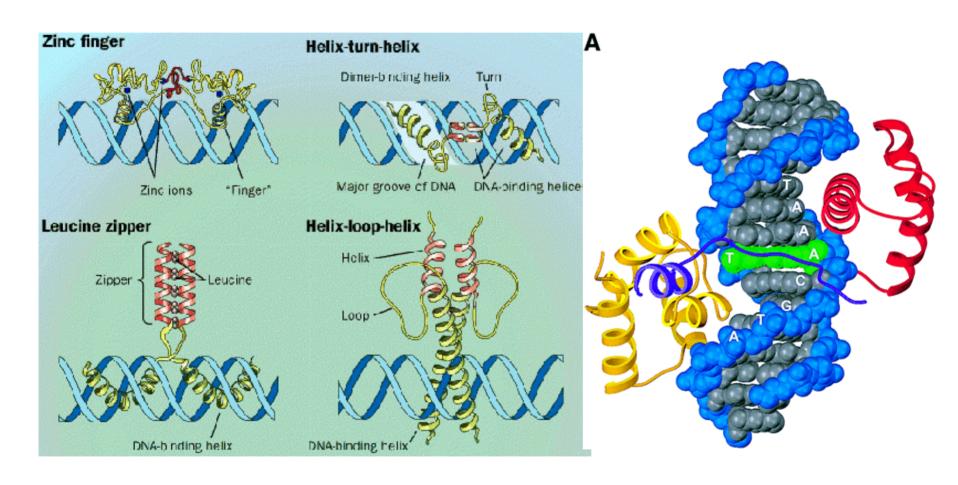
Chromo- some	Number of Exponentially mapped first exons	Number of correctly predicted first exons	Completely non-coding exons	Predicted non- coding exons
21	42	37 (88%)	14	10 (71%)
22	79	69 (87%)	28	23 (82%)
Total	121	106 (88%)	42	33 (79%)

Comparative genomics (Wu et al. Genome Res. 2001)

Maniatis lab (Harvard), Zhang lab (CSHL) & Myers lab (Stanford)



Specific promoter recognition (Gene expression prediction)



Liver-specific Promoter Database

(LSPD: Zhang&Zhang unpublished)



LSPD

The Liver Specific Gene Promoter Database

Liver Specific Genes

- List ~300 promoter regions responsible for liver specific transcriptions
- Collect ~400 experimentally verified regulatory regions and elements
- Provide information on transcription regulation of liver genes
- Compare transcription regulation of functionally or evolutionarily related genes
- Retrieve sequences of the promoter region

Regulatory Elements

- Provide information on transcription regulatory elements
- · Report the methods for verification of the elements
- Record binding affinity and regulatory function
- Summarize the site distribution and sequence consensus

Composite Sites

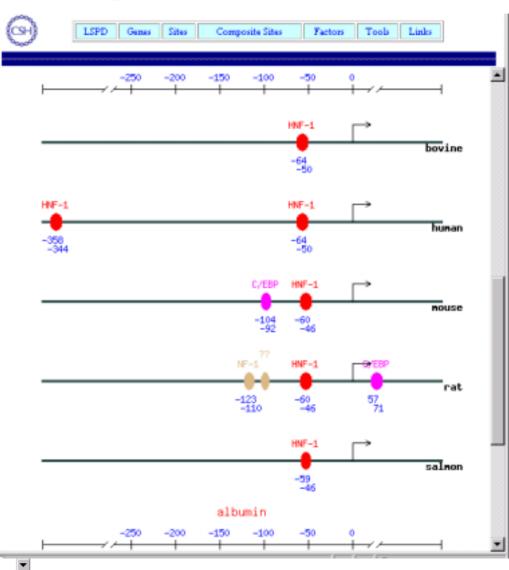
- Record interaction among transcription factors
- Explore space constraints and correlation of regulatory elements
- Characterize the condition for factors to interact

Transcription Factors

- o Provide alignment of all experimentally verified sites
- Construct matrix and consensus sequence
- . Summarize information on the factors and their roles in regulation

Analysis tools

- Retrieve promoter sequences
- Discover new motifs
- Search known motifs
- Build promoter models



LSPD (continued)



LSPD Genes Sites Com	posite Sites Factors Tools Links
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albumin

Brief_Name	albumin
Function	(Transport/Binding)
Cell	liver

Regulatory regions of ortholog genes:

	Accession #	TSS	ATG	# known sites	include?
bovine	X62448	241		1	
human	M92816	enhancer		1	
human	M12523	1737		2	
mouse	M63182	enhancer		5	
mouse	J04738	2043		2	
rat	M16825	153	4		
salmon	X79487	299		1	
xenopus laevis	Z26826	1518		1	
xenopus laevis	Z26825	1607		1	

- to 100 with respect to TSS 🔻 Go Retrieve regulatory sequences from |-500
- Multiple sequence alignment

CLUSTAL W (1.8) multiple sequence alignment Gene albumin

X.laevia_826825 X.laevia_826826 Mua_J04738	
Rat_616825 B.taucus_X62448 Human_612523	
S.9919c_X79487	

X.laevia_826825 X.laevia_826826 Mus J04738 Rat_816825 B.taucus X62448 Human 812523

S.9919c_X79487

CARCAGCARIAGGIAITIGACCIIARAAGIIGAIIG----ACAIIAGGAAATICCACA CARCAGCARTACATTATTIGACCITARAACITATTIG----ACTITAGGAA-CICCACA -AACATACGCAAGGGATTIAGTCAAACAACITTITGGCAAAGATGGTATGA--TITTGTA -GATITAGITAAACAACITITITI--TITCITITI TOCIAICA--AACATACACAAGGGATICAGICAARCAATITITIGGGAAGAATGCTATGAA-TITIGTA -ARTATACACAAGGERTITAGICAAACAATTITTIGGCAAGAATATTAIGAA-TITIGIA

AAGCTAAAACAACTGC--AAACAGAACAATTTGATAGGTTAATAATTTTCCAGATCTCTC ARGCTARARCARCIGG -- ARRCARCACATTITGRIAGGITARICATTITCCAGRICICIC ATODDETAGENACCANTGANATGCGRGE-TANGTATGETTANTGATCTACAGTTATTGGT V GODELAGEACCAATGAAATGAAAGE-TIAGTGTGTTTAATGATCTACAGTTATTGET ATCACTTATGRACCAATGRAATACARAGATGRGTCTAGTTAATAATCTACAATTATTGGC ATCOSTTOCCACCCAATGAAATACAAAGATGAGTCTAGTTAATAATCTACAATTATTOCT ATCOSTIGSTAGECARIGARIAE ROOM TO ACTIVATE AGEING AGE

HNF-1

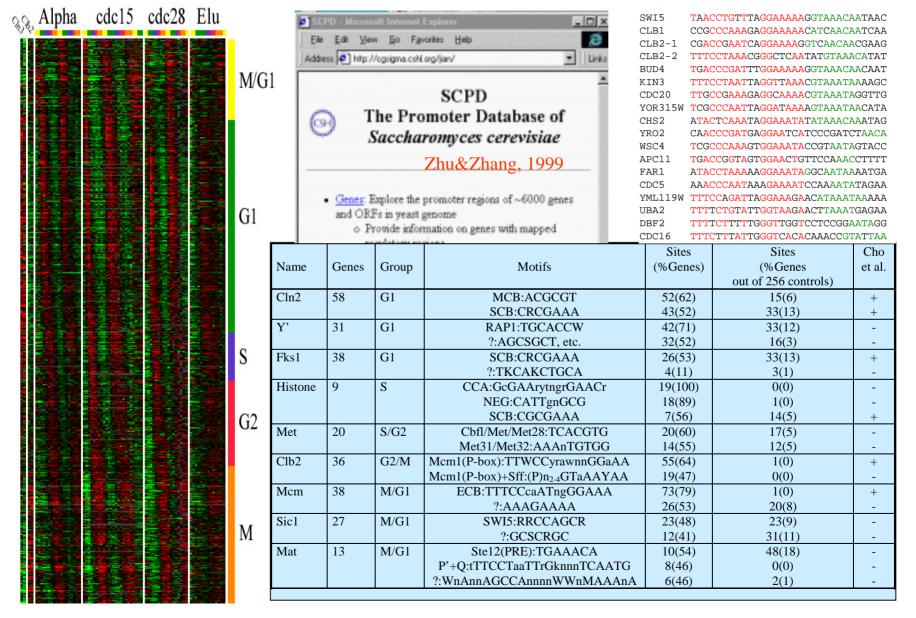
TFs	#exp. sites	motif
HNF1	47	(A/G)TTAAT(h) (IR(1))
HNF3	54	TRTT(G/T)RY (core)
HNF4	37	AGGTCA(h) (DR(1(2))
C/EBP	80	GCAAT(h) (palindrome)

•C/EBP frequency matrix

pos	3 1	2	3	4	5	6	7	8	9	10
A	29	6	1	21	18	3	15	68	68	13
C	16	6	3	61	7	10	46	10	6	16
G	20	6	20	34	20	58	0	2	0	14
T	15	62	56	19	25	9	19	0	6	37

Cell Cycle Regulation (Spellman et al. MBC,1998)

(Brown&Botstein labs – Futcher&Zhang labs)



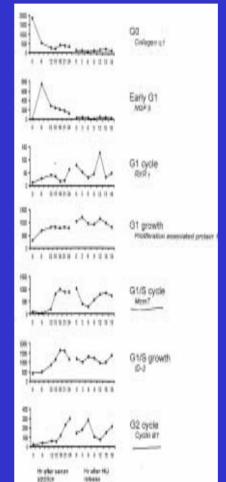
Cell Cycle Regulation (continued)

Computationally predicted E2F target genes confirmed by in vivo footprint (ChIP) (Kel et al. JMB 2001)

Gene	EMBL	Sequence of the potential sites	Position rel. start transcriptio n	Score, q	d(X)
c-fos , Homo sapiens	HSFOS	(-) gcCTTGGCGCGTGTcc (-) ggGGTGGCGCGCGGgc (+) ccTCTGGCGCCACCgt (-) acGGTGGCGCCAGAgg	-165176 -92103 -9079 -7889	0.915 0.836 0.878 0.830	2.92
JunB, Homo sapiens	HS207341	(+) gcTATCGCGCCAGAga (-) tcTCTGGCGCGATAgc (-) ggGCTGGCGCGGCgg	79 90 91 80 169 158	0.887 0.905 0.820	3.16
TGF-β1, Homo sapiens	HSTGFB1PR	(+) ctGTTTGCGGGCCga (+) ccCTTCGCGCCCTGgg (+) ctCTTGGCGCACGct (-) agCGTCGCGCCAAGag (+) ccTTTGCCGCCGGGga	-513502 -298287 28 39 40 29 85 96	0.804 0.912 0.928 0.830 0.854	2.03
ARF, Homo sapiens	AF082338	(-) acTTTCCCGCCCTGtg	-265276	0.859	
Mcm4 (Cdc21), Mus musculus	AB000629	(+) ggTTTCCCGCCAAAac (-) gtTTTGGCGGGAAAcc (+) gcAGTGGCGCCTTCcg (+) ccTTTGCCGCTGTGat (+) tgGGTGGCGCAGAAct (+) ttTGTCGCGCAGCAac	-443432 -431442 -329318 -297286 -127116 -2413	0.872 0.935 0.810 0.846 0.809 0.858	
MCM5 (P1- CDC46), Homo sapiens	HS286B10	(+) agTTTCGCGCCAAAtt (-) aaTTTGGCGCGAAAct (+) ttTTTCCCGCGAAAct (-) agTTTCGCGGGAAAaa	-187176 -175186 8 19 20 9	0.988 1.005 0.885 0.932	4.91 3.01 4.21
von Hippel- Lindau (VHL), Homo sapiens	AF010238	(+) aaGCTCGCGCCACTgc (-) gcAGTGGCGCGAGCtt (-) gtCTTCGCGCGCGCCtc	-270259 -258269 -2839	0.810 0.838 0.921	2.22
B-myb , Homo sapiens	HSBMYBDNA	(-) gtCCTGGCGCGCGGgc	-7283 -5342	0.831 0.866	5.50
Nucleolin, Homo sapiens Nucleolin,	HSNUCLEO CSNUCLEO	(-) ttTTTGGCGCCGGCtg (-) ccGTGGGCGCGCGgt (-) cgTTTGGCGCGCGCTtg	-297308 -256267 -296307	0.966 0.814 0.973	2.91 6.67
Cricetulus griseus					
Nucleolin, Mus musculus	MMNUCLEO	(-) agTTTGGCGCGGCTtg	-306317	0.973	1.76

Mammalian cell cycle regulation

(Ishida et al. MCB, 2001) (Nevins, Zhang labs, unpublished)



CDE......CHR

Cdc2 agcgcgtgagtttgaact
cdc25C ctggcggaaggtttgaattg
Cyc-A2 gtcgcggatacttgaactg
Cyc-B1 gcagtgcgggtttaaatct
Cyc-B2 gcggcggtatttgaatct
Ki-67 ggcgcgcggtatttgaatct
HABP tgggcgg-taggaact
H2A.X ccagagttggatattaaaa



	EZF
Cyc-E	gTTCCGCGCGCAGgg
P107	tTTTCGCGCGCTTtg
B-myb	aCTTGGCGGGAGAta
Mcm4	gTTTCCCGCCAAAac
Mcm5	gTTTCGCGCCAAAtt
	tTTTCCCGCGAAAct
DNApola	aCAGGGCGCCAAAcg
Rad51	gTTTGGCGGGAATtc

Sample Web Resources

Databases

- •TRANSFAC:transfac.gbf.de/TRANSFAC
- •TRRD/COMPEL:compel.bionet.nsc.ru/
- •EPD:www.epd.isb-sib.ch
- •MTIR:www.cbil.upenn.edu/MTIR/HomePage.html
- •LSPD (CSHL)
- •SCPD +Fly+Worm+Plant (CSHL)

- **Promoter finding**
- •CSHL:www.cshl.org/mzhanglab
- •PromoterInspector:www.gsf.de/biodv
- •PromoterScan:bimas.dcrt.nih.gov/molbio
- •Promoter2.0:www.cbs.dtu.dk/services/promoter
- •NNPP:www-hgc.lbl.gov/projects/promoter.html
- •TSSG/TSSW:dot.imgen.bcm.tmc.edu:9331/gene-finder/gf.html

Comparative genomics tools

- •PIPMaker: bio.cse.psu.edu/pipmaker
- •VISTA: www-gsd.lbl.gov/vistal

Motif finding

- •Gibbs sampler:bayesweb.wadsworth.org/gibbs/gibbs.html
- •MEME:meme.sdsc.edu/meme/website/
- •Consensus:bioweb.pasteur.fr/seqanal/interfaces/consensus-simple.html
- •ClustalW:www.ebi.ac.uk/clustalw/



Zhang Lab Members 2001

