14

fill-in with Klenow fragment



Α



В

Α





ChIP-CHIP

110382

13809

14373

15458

23957

18534

Α

R

P-CHIP		CGI genes		non-CGI genes			
Entrez ID	Symbol	Entrez ID	Symbol	Entrez ID	Symbol	Entrez ID	Symbol
HNF1A CGI		66665	5730528L13Rik	67432	0610010D20Rik	83379	Klb
14194	Fh1	77803	A930021C24Rik	71775	1300017J02Rik	259301	Leap2
56794	Hacl1	70317	Arl16	67119	2510048L02Rik	64898	Lpin2
16432	ltm2b	11899	Astn1	109280	9330176C04Rik	17288	Mep1b
HFN1A non-C	GI	71132	Cabyr	210373	A530095I07Rik	69826	Ms4a10
16790	Anpep	234388	Ccdc124	93732	Acox2	17700	Mstn
55938	Apom	27784	Commd8	11431	Acp1	17882	Myh2
15112	Hao1	352968	D830050J10Rik	233799	Acsm2	338370	Nalcn
15378	Hnf4a	13143	Dapk2	280662	Afm	18105	Nqo2
15486	Hsd17b2	13178	Dck	11576	Afp	22259	Nr1h3
16006	lgfbp1	13511	Dsg2	98870	AI182371	18171	Nr1i2
17777	Mttp	13518	Dst	11727	Ang	67528	Nudt7
100163	Pafah2	192193	Edem1	72074	Anks4b	258623	Olfr123
20704	Serpina1e	13839	Epha5	69787	Anxa13	259162	Olfr427
12401	Serpina6	13885	Esd	11807	Apoa2	18346	Olfr47
27219	Sgk2	14048	Eya1	231287	Atp10d	258731	Olfr491
72094	Ugt2a3	384009	Glipr2	26877	B3galt1	258307	Olfr493
CGI		216871	Gltpd2	545366	BC026782	259126	Olfr623
66540	3110001A13Rik	14732	Gpam	170752	Bcdo2	258934	Olfr802
11637	Ak2	14782	Gsr	228662	Btbd3	258812	Olfr923
224727	Bat3	15199	Hebp1	230558	C8a	628813	OTTMUSG0000000997
66205	Cd302	15473	Hrsp12	12352	Car5a	13180	Pcbd1
28126	D13Wsu177e	207683	Igsf11	30952	Cngb3	110385	Pde4c
76843	Dtl	16949	Loxl1	239447	Colec10	18815	Plg
27395	Mrpl15	116748	Lsm10	71791	Cpa4	67000	Prl3a1
17750	Mt2	212679	Mars2	70062	Ctaq2	56189	Prodh2
17966	Nbr1	108645	Mat2b	107869	Cth	30053	Reg3d
18510	Pax8	230125	Mcart1	15945	Cxcl10	192658	Rfpl4
215615	Rnpep	17434	Mocs2	107141	Cvp2c50	19733	, Ran
20019	Rpo1-4	270685	Mthfd1I	433247	Cyp2c68	12309	S100a
269831	Tspan12	23959	Nt5e	226105	Cyp2c70	20209	Saa2
217109	Utp18	18242	Oat	13909	EG13909	20700	Serpina1a
56490	Zbtb20	231602	P2rx2	240327	EG240327	20701	Serpina1b
on-CGI		75552	Pagr9	14060	F13b	20702	Serpina1c
11606	Aat	18769	Pkia	544763	F830116E18Rik	20703	Serpina1d
11699	Ambo	263406	Plekha3	14080	Fabp1	20388	Sftpb
11818	Apoh	22038	Plscr1	14161	Faa	20495	SIc12a1
242557	Ata4c	217734	Pomt2	110135	Fab	319848	SIc17a4
12263	C2	212627	Prnsan2	240894	Emo9	320718	SIc26a9

14317

14538

14618

66283

217674

107022

14823

226243

56185

54426

16171

16552

387510

11905	Serpinc1	
218103	Slc17a2	

C8b

Enpep

G0s2

Нрх

Nr0b2

Pck1

109108 Slc30a9 58246 Slc35b4 240726 Slco5a1 66616 Snx9 20660 Sorl1 234857 Spire2 52331 Stbd1 71954 Suds3 381085 Tbc1d22b 235300 Tmem136 56407 Trpc4ap 209584 Tyw3 56401 Varb	74011	Slc25a27	
58246 Slc35b4 240726 Slco5a1 66616 Snx9 20660 Sorl1 234857 Spire2 52331 Stbd1 71954 Suds3 381085 Tbc1d22b 235300 Tmem136 56407 Trpc4ap 209584 Tyw3 56401 Vanb	109108	Slc30a9	
240726 Slco5a1 66616 Snx9 20660 Sorl1 234857 Spire2 52331 Stbd1 71954 Suds3 381085 Tbc1d22b 235300 Tmem136 56407 Trpc4ap 209584 Tyw3 56401 Vanb	58246	Slc35b4	
66616 Snx9 20660 Sorl1 234857 Spire2 52331 Stbd1 71954 Suds3 381085 Tbc1d22b 235300 Tmem136 56407 Trpc4ap 209584 Tyw3 56401 Varb	240726	Slco5a1	
20660 Sorl1 234857 Spire2 52331 Stbd1 71954 Suds3 381085 Tbc1d22b 232078 Thnsl2 235300 Tmem136 56407 Trpc4ap 209584 Tyw3 56401 Vapb	66616	Snx9	
234857 Spire2 52331 Stbd1 71954 Suds3 381085 Tbc1d22b 232078 Thnsl2 235300 Tmem136 56407 Trpc4ap 209584 Tyw3 56401 Vapb	20660	Sorl1	
52331 Stbd1 71954 Suds3 381085 Tbc1d22b 232078 Thnsl2 235300 Tmem136 56407 Trpc4ap 209584 Tyw3 56401 Vanb	234857	Spire2	
71954 Suds3 381085 Tbc1d22b 232078 Thnsl2 235300 Tmem136 56407 Trpc4ap 209584 Tyw3 56401 Vapb	52331	Stbd1	
381085 Tbc1d22b 232078 Thnsl2 235300 Tmem136 56407 Trpc4ap 209584 Tyw3 56401 Vanb	71954	Suds3	
232078 Thnsl2 235300 Tmem136 56407 Trpc4ap 209584 Tyw3 56401 Vaph	381085	Tbc1d22b	
235300 Tmem136 56407 Trpc4ap 209584 Tyw3 56401 Vanb	232078	Thnsl2	
56407 Trpc4ap 209584 Tyw3 56401 Vanh	235300	Tmem136	
209584 <i>Tyw3</i>	56407	Trpc4ap	
56101 Vanh	209584	Tyw3	
50491 Vapu	56491	Vapb	
52357 Wwc2	52357	Wwc2	

Ptk2

Rnf121

Rragc

Sfrs1

Sfrs5

Shmt1

14083

75212

54170

110809

20384

20425

Grm8	
Habp2	
Hao3	
Hgfac	
lfnk	
ll17a	
Kif12	

Ftcd

Gcnt2

Gjb1

Gkn1

Gphb5

Gramd3

Tbxas1	
Tfpi2	
Tm4sf20	
Tmem106a	
Ttr	
Upp2	
Zranb1	

Slco1b2

Spink3

Spon2

Sucnr1

Sult1c2 Synpr

28253 20730

100689

84112

69083

72003

21391

21789

66261

22139

76654

360216

217203





Supplementary Table S1.

	probe	S	ENSEMBL trar	script Ids
	number	%	number	%
chr1	264,858	6.06	1,738	5.77
chr2	359,931	8.23	2,481	8.24
chr3	210,691	4.82	1,430	4.75
chr4	279,996	6.4	1,861	6.18
chr5	248,152	5.67	1,761	5.85
chr6	240,327	5.5	1,586	5.27
chr7	352,011	8.05	2,572	8.54
chr8	199,046	4.55	1,440	4.78
chr9	229,342	5.24	1,608	5.34
chr10	200,262	4.58	1,368	4.54
chr11	311,061	7.11	2,458	8.16
chr12	153,099	3.5	1,047	3.48
chr13	171,579	3.92	1,161	3.85
chr14	170,459	3.9	1,167	3.87
chr15	168,974	3.86	1,104	3.67
chr16	137,301	3.14	930	3.09
chr17	194,934	4.46	1,444	4.79
chr18	116,740	2.67	737	2.45
chr19	140,689	3.22	969	3.22
chrX	218,003	4.98	1,228	4.08
chrY	6,098	0	30	0.1
total	4,373,553		30,120	

Distribution of probes and ENSEMBL transcripts along mouse chromosomes

Probes were remapped to the mm8 mouse genome assembly by xMAN. The tiling probes were originally disigned to cover 33,559 ENSEMBL genes located on the mouse genome assembly of mm5.

p-values of	Num-	Percen-	χ square			
g:GOSt	ber of	tage of	test*			
analysis	genes	no-CGI		Ontology	Term	$\mathrm{DAVID}^{\#}$
			(p-values)	Type**		
3.06 x 10 ⁻⁹	94	62.80%	7.3 x 10 ⁻²	GO:BP	lipid metabolic process	NC
4.06 x 10 ⁻⁹	82	63.40%	7.3 x 10 ⁻²		cellular lipid metabolic process	NC
1.89 x 10 ⁻⁶	106	59.40%	2.2 x 10 ⁻¹		response to stress	
1.41 x 10 ⁻⁶	80	60.00%	2.4 x 10 ⁻¹		generation of precursor metabolites and	NC
					energy	
1.40 x 10 ⁻⁹	81	53.10%	9.7 x 10 ⁻¹		organic acid metabolic process	NC
2.91 x 10 ⁻⁹	80	53.80%	9.4 x 10 ⁻¹		carboxylic acid metabolic process	NC
4.98 x 10 ⁻⁶	38	68.40%	6.5 x 10 ⁻²		monocarboxylic acid metabolic process	
1.02 x 10 ⁻⁶	19	52.60%	9.5 x 10 ⁻¹	GO:MF	vitamin binding	NC
1.59 x 10 ⁻⁶	31	48.40%	5.9 x 10 ⁻¹		cofactor binding	NC
4.47 x 10 ⁻⁶	16	68.80%	2.2 x 10 ⁻¹		FAD binding	NC
5.97 x 10 ⁻⁸	28	71.40%	5.6. x 10 ⁻²	TF	M00790_1 (HNF-1)	n.d.
biased to no	CGI gen	ies				
4.85 x 10 ⁻⁶	69	81.20%	<u>5.2 x 10⁻⁶</u>	GO:BP	defense response	
1.96 x 10 ⁻¹⁰	64	84.40%	<u>9.4 x 10⁻⁷</u>		response to wounding	NC
1.18 x 10 ⁻⁸	48	83.30%	<u>3.8 x 10⁻⁵</u>		inflammatory response	
1.38 x 10 ⁻⁸	23	95.70%	<u>5.2 x 10⁻⁵</u>		acute inflammatory response	
9.46 x 10 ⁻⁹	253	66.00%	<u>1.4 x 10⁻⁴</u>	GO:CC	extracellular region	NC
2.39 x 10 ⁻⁸	234	65.80%	<u>3.0 x 10⁻⁴</u>		extracellular region part	
2.08 x 10 ⁻⁸	224	66.50%	<u>1.8 x 10⁻⁴</u>		extracellular space	NC
1.53 x 10 ⁻⁷	23	91.30%	<u>2.8 x 10⁻⁴</u>	KEGG	Complement and coagulation cascades	NC
2.61 x 10 ⁻⁶	83	69.90%	<u>3.1. x 10⁻³</u>	TF	M00790_2 (HNF-1)	n.d.
1.68 x 10 ⁻⁸	33	72.70%	2.7. x 10 ⁻²	TF	M00132_3 (HNF-1)	n.d.
8.18 x 10 ⁻¹²	32	75.00%	<u>1.5 x 10⁻²</u>	TF	M01011_1 (HNF1)	n.d.
biased to CG	a genes					
2.06 x 10 ⁻⁶	401	37.20%	<u>4.7 x 10⁻⁹</u>	GO:CC	cytoplasm	NC
8.65 x 10 ⁻⁸	313	38.30%	<u>9.8 x 10⁻⁸</u>		cytoplasmic part	NC
1.55 x 10 ⁻⁶	108	32.40%	2.4 x 10 ⁻⁵		mitochondrion	NC,CGI
5.03 x 10 ⁻⁶	58	32.80%	2.0 x 10 ⁻³		mitochondrial part	
7.34 x 10 ⁻¹⁰	526	46.80%	<u>8.1 x 10⁻³</u>	GO:MF	catalytic activity	NC

Supplementary Table S2. Annotation analysis of genes with liver T-DMRtags

*χ-square tests were applied to examine the difference in the proportions of CGI-containing and CGI-lacking genes for each criterion among all the genes containing T-DMRtags. Percentage of no CGI genes among 1817 genes carrying T-DMRtags is 53.6%. Statistically significant (less than 5%) are designated by underlined boldface.

**Ontology types of GO:BP, GO:CC, and GO:MF indicate biological process, cellular component, and molecular function in Gene Ontology criteria, respectively. KEGG and TF represent KEGG pathway database, and TRANSFAC database, respectively.

[#]The DAVID column indicates the over-representation of the terms in DAVID analysis among all genes classified into the same criterion according to the position of CGIs. NC: non CGI genes, CGI: CGI genes, n.d.: not determin

Supplementary Table S3. List of adaptors and primers

Name	Sequence	Genomic location of PCR *	Description
R24	AGCACTCTCCAGCCTCTCACCGCT		R-Adaptor pairs
R10	CGAGCGGTGA		D Adapter (Netl)
N24			R-Adaptor (Noti)
Ncq10	CGTTCCCTCG		N-Adaptor pairs
N18	GGCAACTGTGCTATCCGA		LM-PCR
R182	GCACTCTCCAGCCTCTCA		
Gnmt_F1	ACTACAACCCCAACCTTACTAAAAA	chr17:46196405-46196775	Fig. 2D Gnmt-1
Gnmt F2	AGGTAGTAAGTTTGGTTTTGGGTTT	chr17:46192018-46192480	Fig. 2D Gnmt-2
Gnmt R2	TCCCATACCCATACTACCCTAATAA		rig. 20 onini 2
Gnmt_F3	AATTGGGGTAAGTTTGTTTGTTTAG	chr17:46189976-46190462	Fig. 2D Gnmt-3
Gnmt_R3	TCCCAAAAACACATAAAAACTCATT		
Hnfla_F Hnfla_R		CNr5:115232432-115232905	Fig. 4A Hnf1a
Hnf4a F1	GTTTGTGATAGGGTTTGGGAATTAT	chr2:163230407-163230741	Fig. 4B Hnf4a-1
Hnf4a_R1	CTTTCTCATTTAAACAAACATTCCA		
Hnf4a_F2	GGTTTTTGGTGGTTTTTAGAGATTT	chr2:163231804-163232291	Fig. 4B Hnf4a-2
Hnf4a_ $K2$	GGGAGGGGTATGTATTGTGTGAGTA	chr2:163237981-163238371	Fig 4B Hnf4a-3
Hnf4a_R3	ACAAACACCCAACAAAACTAACATT		
Hnf4a_F4	AAAAATCAATCCTATCCAACATAACC	chr2:163239167-163239466	Fig. 4B Hnf4a-4
Hnf4a_R4	TGAAGTTGGGATATAAATTTAAAAAGG	abr2:162220647 162240004	Fig. 4D Hof4a 5
HIII4a_F5 Hnf4a_R5		0112.103239047-103240004	гіў. 46 піш4а-э
Nr1h3_F	TTAGGAAGAGATGTTTTTGTGGTTG	chr2:90994114-90994508	Fig. 4C Nr1h3
Nr1h3_R	CCACTACCCAACTAATACATCAAAA		-
Nr1i2_F	AGATTGGTTTTGTAGGTGGTTATTG	chr16:38212169-38212611	Fig. 4D Nr1i2
Nr112_R Rxra F1	TTGGATAGGTTTGGTATTTGTTTGT	chr2·27525521-27525845	Fig 4F Ryra-1
Rxra_R1	CACAAATCACTTCTTTAAAAAACACCA		
Rxra_F2	AAGTGTAGGATTGGAGGGAAGTATT	chr2:27527859-27528199	Fig. 4E Rxra-2
Rxra_R2	ATCTCCAAAATCACACATCCTTAAA	abre:104475717 104476066	
CII_FI C1r R1		CIII6.124475717-124476066	FIG. 55A C 11-1
C1r_F2	AAGGTTATTGTTAAGGGGAGATTGT	chr6:124477322-124477721	Fig. S3A C1r-2
C1r_R2	CATCTTTTCCTAAACATATAATCAACTC		
Proc_F1	ATTGTAAGATTGTGAAGGATTGTGG	chr18:32280721-32281125	Fig. S3A Proc-1
F2 F1	GATGGATTTTTGTAATTGTGTGTGA	chr2.91439647-91440086	Fig. S3A F2-1
F2_R1	AACACCATCCAACTCCTAACTTACA		
F2_F2	TGTTAGGGGTGGATATTTGTTTTA	chr2:91436998-91437368	Fig. S3A F2-2
F2_R2		abr2:02100760 02100144	Eig S2A Ego 1
Fga R1	CAACCAAAAATTCACACATTTAACA	CIII3.83100700-03109144	rig. SSAT ga-1
Fga_F2	GGAGGAATAAGGGGTTATATTTATTTT	chr3:83110480-83110781	Fig. S3A Fga-2
Fga_R2	CCAAATCTAAATCTCAAACAAACAA		
Fga_F3		chr3:83114472-83114909	Fig. S3A Fga-3
Fab F1	TTTGTTTGGGGTTATTAGATAATTT	chr3:83137743-83138091	Fia. S3A Fab-1
Fgb_R1	ACCAAACTTAAACAAATCCAACTCA		5 5
Fgb_F2	TGGTTTATGAGAAGTGATAAAAGAAAA	chr3:83136383-83136693	Fig. S3A Fgb-2
Fgb_R2		abr2:02124210 02124562	Eig S2A Eab 2
Fab R3	TTCATATTAAAACCATAATCTTCATCAA	CIII3.03134219-03134302	1 lg. 33A1 gb-3
F9_F1	GTAAGTTTTATTTAGTTTGTATTTTGGAA	chrX:56346175-56346555	Fig. S3A F9-1
F9_R1	ATAAAAATACACCACAAACCCTAT		F : 000 0 · 4 4
Serpina1e_F1		chr12:104357440-104357836	Fig. S3A Serpina1e-1
Serpina1e F2	TAGGTGTTTTTGGGGAGTTTTGAATA	chr12:104356571-104356882	Fig. S3A Serpina1e-2
Serpina1e_R2	CCAAACAAACTAAATCACATTCTCA		3 • • • • • •
Serpind1_F1	TGATTTTATGTAAGTTGGGTGAGTG	chr16:17247783-17248189	Fig. S3A Serpind1-1
Serpind1_R1		chr4:107421333-107421703	Fig. S3C Cpt2-1
Cpt2_R1	ACTAAAAACTCCCAAAACAAAC		1 ig. 000 0piz 1
Cpt2_F2	GGTTGTGAGTTTAAGGTTTGTTTTAT	chr4:107419415-107419712	Fig. S3C Cpt2-2
Cpt2_R2	CCTCAAAATTAAAACTCCTTACCAAAT	ab-10-00026020 00027040	
Gstz1_F1 Gstz1_R1	CATCTTTCCCCCCTTACCTTTAATAC	Chr12:88036830-88037318	FIG. 53C GStZ1-1
Gstz1_F2	TTTAAGTTATTGTTGGAATGGAGTTG	chr12:88037537-88037932	Fig. S3C Gstz1-2
Gstz1_R2	AAACACTAACAACAAACCACCTAAC		
Pygl_F1	GAGGTAATGGTTTTATTGGGAATTT	chr12:71146232-71146507	Fig. S3C Pygl-1
Pygi_Ki Pval F2	GTTGTATGATGGTTTTTGTGGAAAT	chr12:71144064-71144491	Fig. S3C Pval-2
Pygl_R2	CAACAAACCTATAAACCCTCTCAAC		J · JJ. =
Ahcy_F1	TATATGTGTTTGGGTGTTTTGTTTG	chr2:154763215-154763572	Fig. S3C Ahcy-1
ANCY K1	AUUUUAUTUUTATUTAUUAAUTATT		

Legends for Supplementary Figures

Figure S1

Selective amplification of DNA fragments by D-REAM. (**A**) Genomic DNA was digested with HpyCH4IV and subsequently ligated with the unphosphorylated R-adaptor pairs. The complementary strand of the ligated R24 were filled-in by using Klenow fragments of DNA polymerase I. The DNA fragments were then digested with TaqI and ligated with the unphosphorylated N-adaptor pairs (**Supplementary Table S2**). By complete fragment denaturation and hot-start Taq DNA polymerase, only strands obtained from fragments digested with HpyCH4IV can be used as templates for PCR with the N18 and R18 primers (indicated by the red dotted oval). (**B**) Monitoring selective amplification of HpyCH4IV digested fragments. The PCR mixture with different primer sets was sampled at different cycles of the thermal cycles (15, 20 and 25) and was subjected to agarose gel electrophoresis. (**C**) Selective amplification of NotI digested fragments. Genome DNA, which was digested by NotI instead of HpyCH4IV, was performed by D-REAM. The positions of the DNA molecular markers (M) of 0.5, 1, and 2 kbp are indicated along the sides of the figure. (**D**) The correlation of intensities of the probes between an experimental duplicated liver DNA samples of a mouse.

Figure S2

in silico analysis of mouse genome. (A) Steep peaks of HpaII (narrow blue), CpG dinucleotides (blue) and the GC percentage (gray) at TSS indicated that HpaII mainly localized in the CGI on promoter array, and agree the large quantity of tiny fragments. The frequencies of probes (green), HpyCH4IV (red) of the genomic regions, which were covered by the mouse promoter tiling array, are plotted. Dotted horizontal lines at CpG (obs/exp) 0.6 and GC percentage 50% represent the cutoff values for defining CpG islands. (B) The mouse genome (mm8, UCSC genome browser) was digested *in silico* with HpyCH4IV (upper panel) or HpaII (lower panel) by using the EMBOSS software package. The distribution of the length of fragments was plotted. The median and average sizes of HpyCH4IV and HpaII fragments were 907, 1468, 658 and 1620 bp, respectively. These plots

indicated that the range of distribution of HpyCH4IV fragments size is narrower than that of HpaII. The first quantile of sizes of HpaII fragments indicate the presence of large quantities of tiny fragment. On the other hands, the average sizes of them are larger than those of HpyCH4IV, indicating that HpyCH4IV is distributed throughout genome in more uniform manner than HpaII.

Figure S3

COBRA confirmed the methylation status of T-DMRtags. (**A**) The methylation status of T-DMRtags of the non-CGI genes involved in the coagulation and complement cascades are shown as plots displayed by the integrated genome browser (IGB) indicating T-DMRtags as arrowheads, and the chromosomal locations are indicated at the top of the panels. The regions indicated as yellow boxes were analyzed by restriction mapping. (**B**) The numbers at the top of the gel images correspond to those on the plots. L and C indicate liver and cerebral DNA, respectively. + and – indicate digestion with HpyCH4IV and no digestion, respectively. (**C**) The methylation status of T-DMRtags and CGIs in CGI genes. (**D**) Agarose electrophoresis gel images are displayed.

Figure S4

The list of candidate genes under the control of HNF1 and DNA methylation. (A) Fortythree of mouse orthologs of human genes that had been identified by ChIP-chip experiment of hepatocytes with anti-HNF1 antibody have T-DMRs. (B) Genes displayed in shadowed rectangle are classified to have HNF-1A motifs in 1-kb upstream regions of TSSs by MAPPER database. Those in list A are omitted from list B.

Figure S5

Tissue specificity of genes clustered by K-means clustering according to the methylation status of T-DMRs. (A) The expression levels of the genes in each cluster of Figure 5E are expressed in box-plot of the log-ratios (base = 2) of expression levels in tissues compared to those in the liver. The expression data were downloaded from SymAtlas web site. (B) The methylation status of T-

DMRs of the genes are shown as plots displayed in MATscores, which were obtained by MAT using cerebrum as control samples, by the integrated genome browser (IGB)..

Figure S6

Data flow of bioinformatics analysis in this study. Rectangles with right corners stand for public domain or raw data. Rhombuses and rectangles in round corners represent software and treated data, respectively.