Supplementary Data

Myc-induced anchorage of the rDNA IGS region to nucleolar matrix modulates growth-stimulated changes in higher-order rDNA architecture

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Figure Legends

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Figure 1. The non-transcribed inter-genic spacer region (IGS) of the mouse rRNA gene has an enhanced propensity for nuclear matrix attachment.
The line graph shows the MAR-potential score in relation to nucleotide position for the rDNA repeat sequence from mouse. The lower panel shows a schematic depiction of the mouse rRNA repeat (GeneBank no. BK000964). Matrix attachment potential was predicted by MAR-Wiz software as implemented at http://genomecluster.secs.oakland.edu using a window size of 1000bp stepped in 100bp intervals. Scores in excess of 0.6 are generally considered as strong indicators of nuclear matrix binding potential.

Figure 2. The digestion efficiencies of restriction enzymes at sites throughout the mammalian rDNA repeat.
Nucleolar halos isolated from (A) growing TGR1 and (B) HeLa cells were digested with restriction enzymes as described in Materials and Methods. The upper panels depict rat and human rDNA repeats marked with restriction enzyme cutting sites and primer pairs designed for analysis of cutting efficiencies by qPCR. (C) Comparison restriction enzyme digestion of nucleolar halos from starved HeLa cells with and without serum addition and in the presence of c-Myc inhibitor, 10058-F4. (D) Analysis of cutting efficiencies of nucleolar halos from TGR1 cells in the presence or absence of treatment with Actinomycin D and c-Myc inhibitor, 10058-F4. The cutting efficiency for nuclear halos from HO15.19 is also shown. (E) Analysis of cutting efficiencies of nucleolar halos from Rat1MycER without or with induction of Myc-ER by 4-HT in the presence or absence of 10058F4. The calculation of cutting efficiency is based on the formula:

$$\left[1 - 2^{(CT_x - CT_{ic})_{Input} - (CT_x - CT_{ic})_{Ex}}\right] \times 100\%$$

"Input" represents DNA templates from nucleolar halos without restriction enzyme digestion; "Ex" indicates the DNA templates treated with restriction enzymes; CT is the threshold cycle of quantitative PCR amplification; "*x*" the indicated primer pairs spanning cutting sites of restriction enzymes (the sequences are shown in Supplementary Table 1-2); "*ic*"means internal control for DNA template loading (R0 for rat and H40 for human). The values are shown as the mean and standard deviation from three independent experiments.

Figure 3. The anchorage sites of nucleolar matrix on human rRNA genes.
(A) Preferential nucleolar matrix attachment on IGS of rDNA repeat. Top panel shows the scheme of rRNA repeat and amplicons representing locations throughout rDNA. The nucleolar halos isolated from growing HEK293, subjected to DNase I digestion, and separated into a non-matrix associated fraction and matrix associated fraction. (B) Myc-dependent regulation on nucleolar matrix attachment of rDNA IGS. MAR assays are performed on HeLa cells in the absence or presence of c-Myc inhibitor, 10058-F4, added prior to serum addition. The lower panel shows a statistically significant difference in pre-rRNA levels in cells un-treated or treated with c-Myc inhibitor.

Figure 4. The sequence of the rDNA non-transcribed spacer region is strongly divergent between mammalian species.
The upper panel shows mouse rDNA repeat unit derived from GeneBank no. BK000964, containing transcribed and non-transcribed regions. The middle panel shows human rDNA derived from GeneBank no. U13369. The lower panel shows rat rDNA, derived from accession numbers X04084, X03838, X61110, X00677, X16321, V01270 and X03695. Dotted sections of the diagram represent regions where rat sequence information is incomplete. Locations of E-boxes are marked with vertical lines. The percentage identity of mouse and rat sequences compared to human sequences is noted for each coding and non-coding region.

Figure 5. Occupancy of Myc and UBF on the human rDNA repeat.
Growing (A) HEK293 and (B) HeLa cells were cross-linked by 0.25% formaldehyde. Nucleoli were isolated and extracted chromatin DNA was sheared to 500 bp – 1, 000 bp according to the standard ChIP assay procedure. Quantitative real-time PCR analysis of Myc/UBF binding to the rRNA gene was calculated by the amplification signals of immunoprecipitated DNA by anti-Myc/anti-UBF antibody relative to signals from rabbit serum controls. The sequences of primer sets for PCR are shown in Supplementary Table 1. The values represented means and standard deviations deriving from three independent experiments.

Figure 1

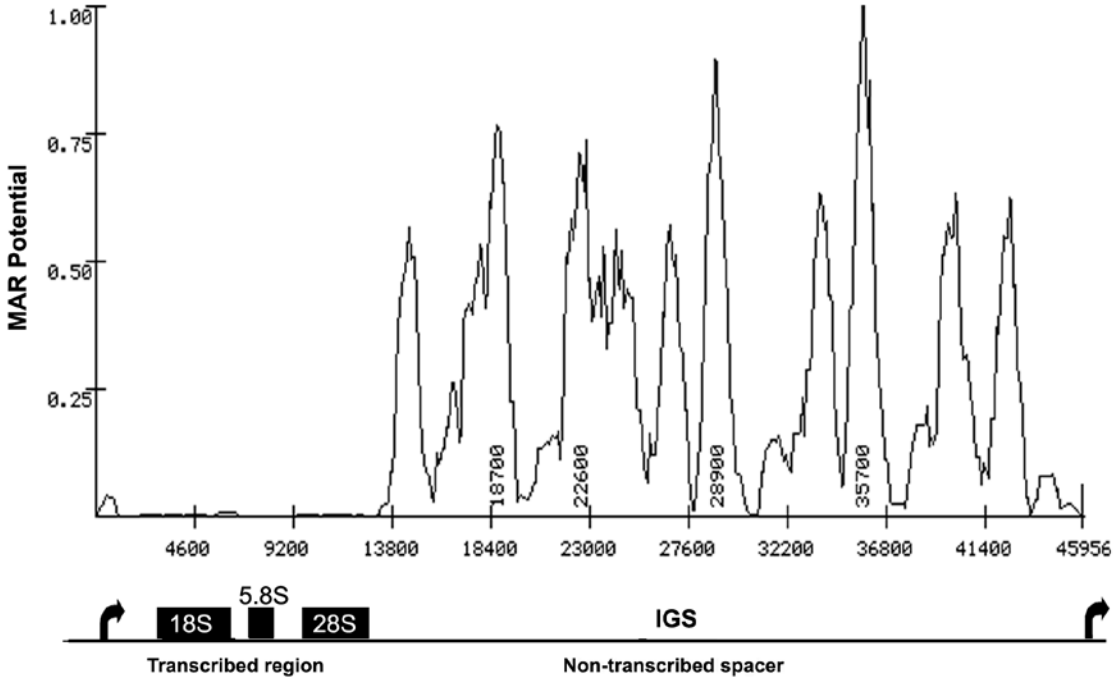
MAR Potential
1.00
0.75
0.50
0.25
18700
22600
28900
35700
4600
9200
13800
18400
23000
27600
32200
36800
41400
45956
5.8S
18S
28S
IGS
Transcribed region
Non-transcribed spacer

Figure 2

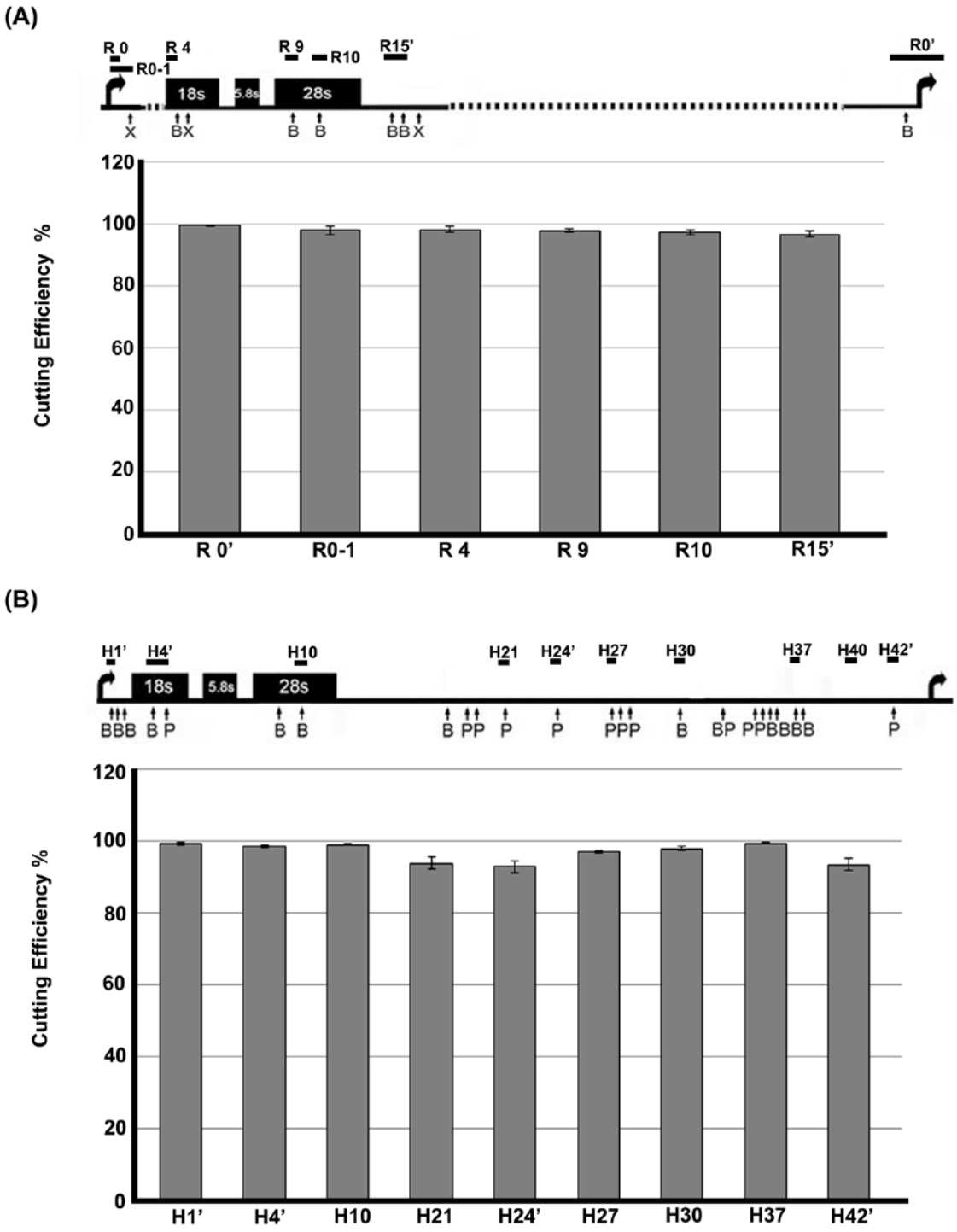
(A)
R 0
R0-1
R 4
R 9
R10
R15'
R0'
18s
5.8s
28s
X
BX
B B
BB X
B
Cutting Efficiency %
120
100
80
60
40
20
0
R 0'
R0-1
R 4
R 9
R10
R15'
(B)
H1'
H4'
H10
H21
H24'
H27
H30
H37
H40
H42'
18s
5.8s
28s
BBB B P
B B
B PP P
P
PPP
B
BP PPBBBB
P
Cutting Efficiency %
120
100
80
60
40
20
0
H1'
H4'
H10
H21
H24'
H27
H30
H37
H42'

Figure 2

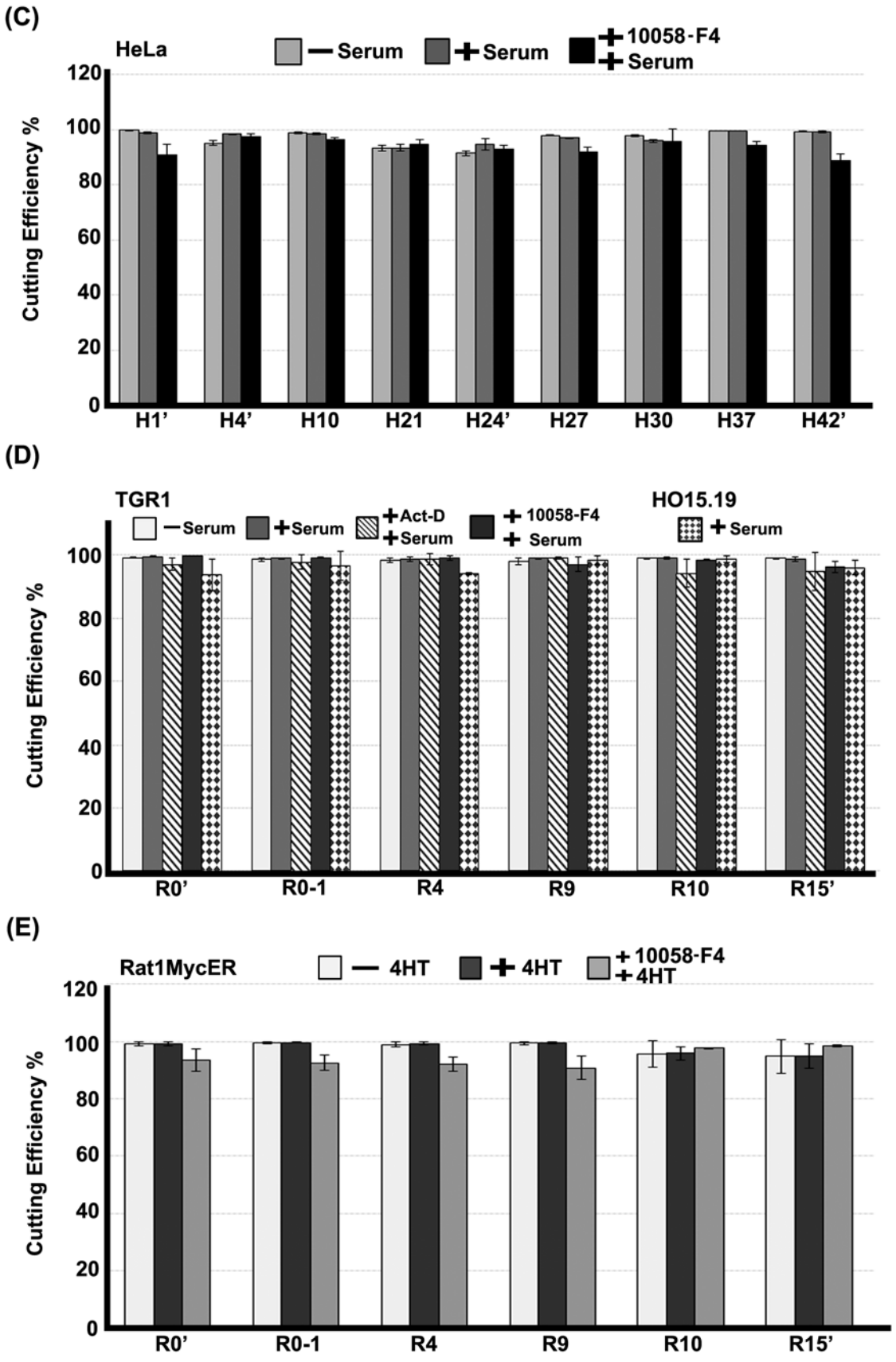
(C)
HeLa
— Serum
+ Serum
+ 10058-F4
+ Serum
Cutting Efficiency %
120
100
80
60
40
20
0
H1'
H4'
H10
H21
H24'
H27
H30
H37
H42'
(D)
TGR1
— Serum
+ Serum
+ Act-D
+ Serum
+ 10058-F4
+ Serum
HO15.19
+ Serum
Cutting Efficiency %
100
80
60
40
20
0
R0'
R0-1
R4
R9
R10
R15'
(E)
Rat1MycER
— 4HT
+ 4HT
+ 10058-F4
+ 4HT
Cutting Efficiency %
120
100
80
60
40
20
0
R0'
R0-1
R4
R9
R10
R15'

Figure 3

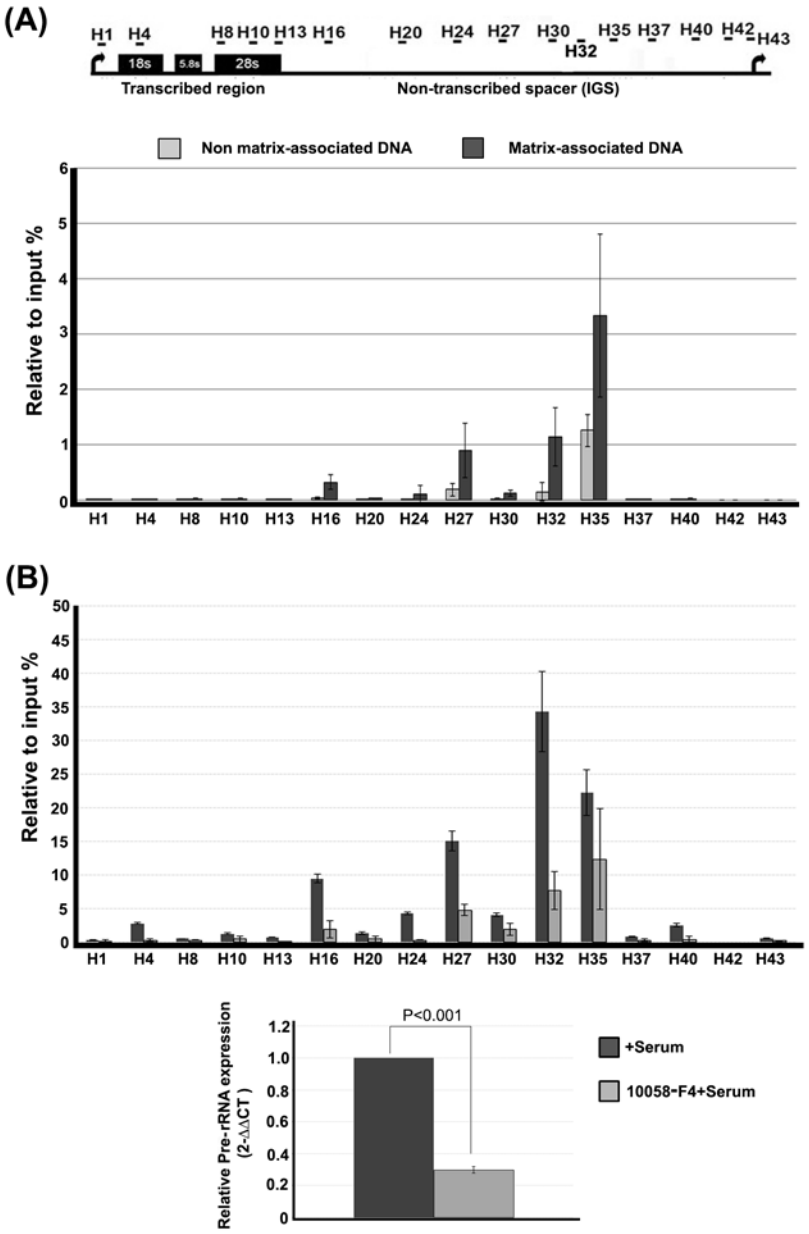
(A)
H1 H4 H8 H10 H13 H16 H20 H24 H27 H30 H32 H35 H37 H40 H42 H43
18s
5.8s
28s
Transcribed region
Non-transcribed spacer (IGS)
Non matrix-associated DNA
Matrix-associated DNA
Relative to input %
(B)
Relative to input %
Relative Pre-rRNA expression (2-ΔΔCT)
P<0.001
+Serum
10058-F4+Serum

Figure 4

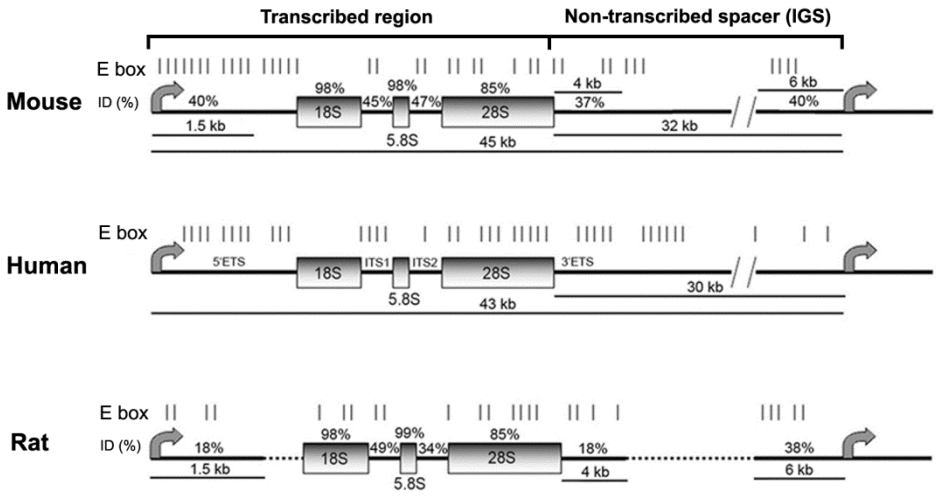
Transcribed region
Non-transcribed spacer (IGS)
E box
Mouse
ID (%)
40%
98%
45%
98%
47%
85%
4 kb
37%
6 kb
40%
18S
28S
1.5 kb
5.8S
45 kb
32 kb
E box
Human
5'ETS
18S
ITS1
ITS2
5.8S
28S
3'ETS
43 kb
30 kb
E box
Rat
ID (%)
18%
98%
49%
99%
34%
85%
18%
38%
18S
28S
1.5 kb
5.8S
4 kb
6 kb

Figure 5

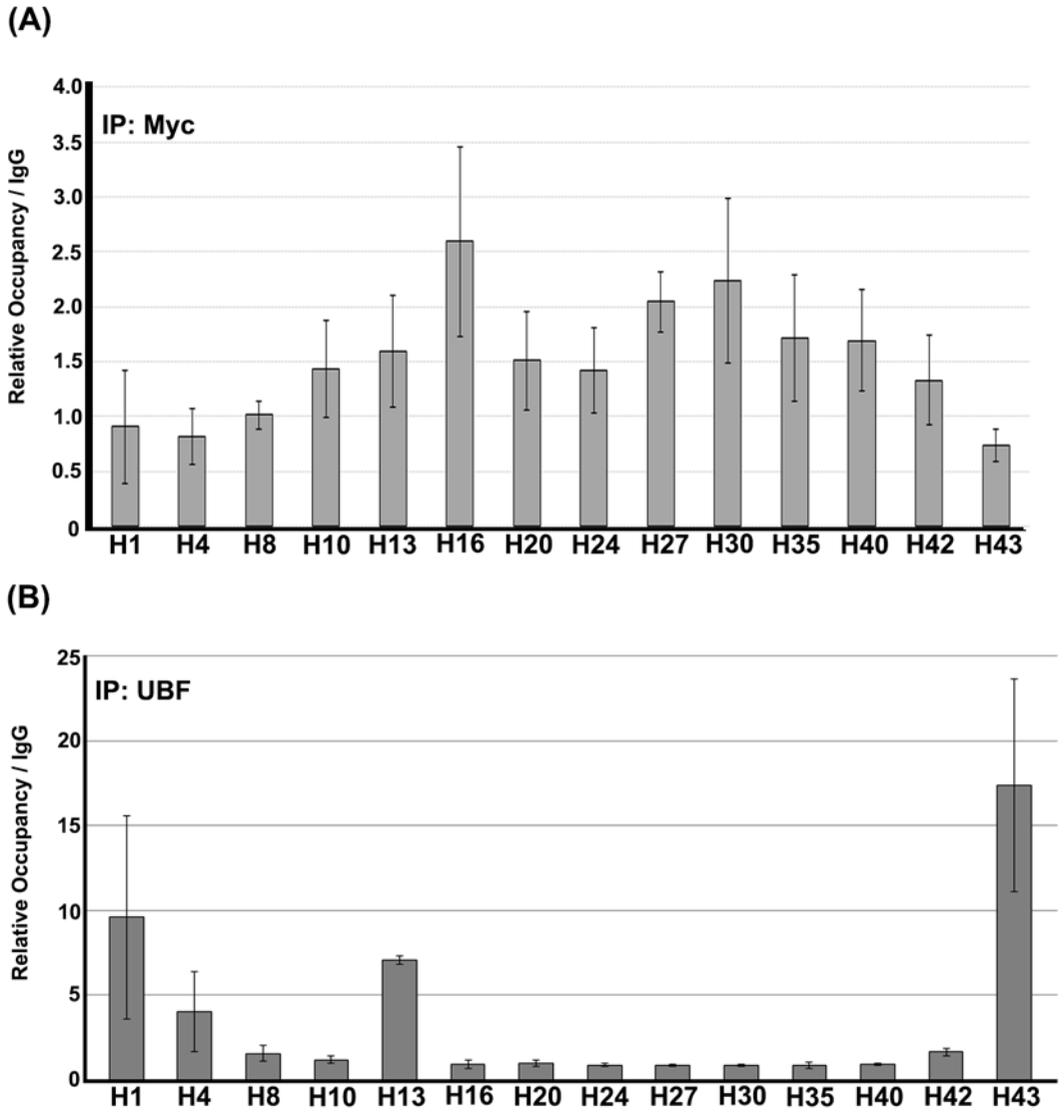
(A)
IP: Myc
Relative Occupancy / IgG
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
H1 H4 H8 H10 H13 H16 H20 H24 H27 H30 H35 H40 H42 H43
(B)
IP: UBF
Relative Occupancy / IgG
25
20
15
10
5
0
H1 H4 H8 H10 H13 H16 H20 H24 H27 H30 H35 H40 H42 H43

Table 1. Human ribosomal DNA primer pairs used to detect rRNA gene in MAR and ChIP assays

Region	Corresponding site # Forward	Reverse
47S*	TGTCAGGCGTTCTCGTCTC (307-325)	GACGTCACCACATCGATCAC (437-420)
H 1	CTCCGGGTTGTCCCTCAGGTG (821-842)	ACGTGCGCTCACCGAGAGCAG (1030-1010)
H 1'	AAAAGCCTTCTCTAGCGATCTGAG (1402-1425)	TACCATAACGGAGGCAGAGACAG (1489-1467)
H 4	CGACGACCCATTCGAACGTCT (3990-4010)	CTTGGATGTGGTAGCCGTTTCTC (4122-4100)
H 4'	AGAAACGGCTACCACATCCAAGG (4101-4123)	TCATGGCCTCAGTTCCGAAAACC (4572-4550)
H 8	GTAACGGCGAGTGAACAGGGAAG (8029-8041)	GCCGGTATTTAGCCTTAGATGGAG (8268-8245)
H10	TAACGCGACCGATCCCGGAGAA (10501-10522)	CCTTAGAGCCAATCCTTATCCCG (10818-10796)
H13	ACCTGGCGCTAAACCATTCGTAG (12855-12877)	GGACAAACCCTTGTGTCGAGG(12970-12959)
H16	ACACACACACACCCCGTAGTG (15816-15836)	ACGTGTATGTAAATGAAATGGG (15937-15916)
H20	TTATTCCCTTCCTGGAGTTGG (20241-20261)	CAGTTTTCAGCCCCAACACAC (20376-20356)
H21	AAGTGTGTGTTCCCGTGAGAGTG (21619-21641)	CACAGAGAGAAAGACAGAGAC (22120-22100)
H24	TTCACGTCTGTCATCCCGAGGTC (24151-24173)	CCTGACTCCATTTCGTATTTTC (24244-24223)
H24'	TTCACGTCTGTCATCCCGAGGTC (24151-24173)	AACAGACAGAGAGAAGGCCCTAG (24548-24526)
H27	AAATACAAAGCTGAGTCGGGAGC (27634-27656)	GTCGACTTTCAATAACAGTGGCC (27915-27893)
H29	GTAGGCGAATGCTGCTGCTGCTC (28981-29003)	AAGACAGACACAGTGAGAGAGAC (29321-29299)
H30	CTTGTGGAACCACTGGCTCTTTGA (30795-30818)	TGCAGAGGTATCCATTTGACCTC (30992-30970)
H32	TAACTACCACAGGGTTATGAC (32620-32640)	GTATAAAGATTAGCTGGGCGTGG (32862-32840)
H35	CTGGCTTTTCCCCCCTATTTCAC (35118-35140)	CAGCAAGCTTCGAGAAGGGGATC (35364-35342)
H37	TTCACCGTGTTGCCAAGGCTTG (37316-37338)	TCTGCCGTGAAACTGTCTGTC (37464-37444)
H40	GTTTCACTCTTGTTTCCACGGC (40739-40760)	ACACAAACATCAGCCAAGCCAG (40896-40875)
H42'	TTCGCCATCTGTCTCTTTTCCC (41711-41732)	TCTCTGGAGATGCCTCTCGGAAG (41901-41879)
H42	AGGCAGAGACGCGTTTTGGGCAC (41939-41961)	TGACTCACAAGCGACCGGCCA (42271-42251)
H43	GCTCCGTGTGTGGCTGCGATG (42839-42859)	GACGCGCGAGAGAACAGCAGG (152-132)

sequence derived from GeneBank no. U13369

★ primers for 47S in quantitative analysis of Pre-rRNA transcription

Table 2. Rat ribosomal DNA primer pairs used to detect rRNA gene in MAR assays

Region	Corresponding site	
	Forward	Reverse
R -4	AGTGCAAGGCCTTGGGTTCTGTC (129-151)[!]	AAAGCAGCCAGAAGCCTCAGACC (335-303)[!]
R -2	GGAGAGACGGAATGAGTGTGTG (2709-2730)[!]	CCCGCCTGCTTGCCTGTCAC (2905-2886)[!]
R 0'	GCGCCTCCGAGACTTTCTTTTTTC (1481-1504)[&]	CATGGACCTCTATATACGAC (262-243)[@]
R 0[★]	TATCTACCATGGCCTCCTCGG (43-64)[@]	CATGGACCTCTATATACGAC (262-243)[@]
R0-1	TATCTACCATGGCCTCCTCGG (43-64)[@]	GCACGGGAAGAAGTCGCTAAC (954-934)[@]
R 4	CCCTGTAATTGGAATGAGTCCAC (550-572)[#]	CCATTATTCCTAGCTGCGGTATC (872-850)[#]
R 5	CATGGCCGTTCTTAGTTGGTGG (1335-1356)[#]	ACGCTGAGCCAGTTCAGTGTAG (1554-1533)[#]
R 8	GGGAAATGTGGCGTACGGAAGAC (4005-4027)[#]	CTCGTGCCGGTATTTAGCCTTAG (4208-4187)[#]
R 9	ACGGACCAAGGAGTCTAACG (5086-5105)[#]	CCAGAGTTTCCTCTGCTTCG (5332-5313)[#]
R 10	TGTAGGTAAGGGAAGCGGCAAG (6559-6538)[#]	CCCTTAGAGCCAATCCTTATCCC (6598-6576)[#]
R13	CGGTACACCTGTCAAACGGTAAC (7781-7803)[#]	TAGAGGCGTTCAGTCATAATCCC (8282-8259)[#]
R14	TCAGACACACCAGAAGAAGGC (1861-1881)[¤]	AGAGAAAGGGGAATTGGATACTGG (2110-2087)[¤]
R 15'	GCGATCTGGGGAGTTGCTTTTC (2028-2049)[¤]	TGCACAACCATCATTGGAGGACG (2461-2439)[¤]
R15	AGGAGGATGAGGAGGAGGAAAG (2605-2626)[¤]	TTAAGTTGAGCAGAGCAGAGCAG (2842-2820)[¤]
R16	CTCTGGAGGAACACGGTCAGAAC (3651-3663)[¤]	GAATTCATCTGTGCGTCCCGCAG (4025-4003)[¤]

! sequence derived from GeneBank no. X04084
& sequence derived from GeneBank no. X61110
@ sequence derived from GeneBank no. X00677
# sequence derived from GeneBank no. V01270
¤ sequence derived from GeneBank no. X03695
★ primers for 45S in quantitative analysis of Pre-rRNA transcription

Table 3. Rat rDNA primer pair used for syntheses of control fragments in chromatin conformation capturing & MAR-loop assays

Primer	corresponding site	
	Forward	Reverse
r0	TTTCCAGGATCCTCCGGTCC (1601-1620) !	CATAAAGCTGCCCCAGAGAG (460-441) #
r4	AAACGGCTACCACATCCAAGG (449-469)&	CTGATCGTCTTCGAACCTCCG (1063-1043)&
r9	ACGGACCAAGGAGTCTAACG (5086-5105)&	CCAGAGTTTCCTCTGCTTCG (5332-5313)&
r10	ATCGAAAGGGAGTCGGGTTCAG (6209-6230)&	CCCTTAGAGCCAATCCTTATCCC (6598-6576)&
r14,r15,r16	TCAGACACACCAGAAGAAGGC (1861-1881)%	CAAGCTTTCCTCCTCCTCATCC (2630-2609)%

! sequence derived from GeneBank no. X61110
sequence derived from GeneBank no. X00677
& sequence derived from GeneBank no. V01270
% sequence derived from GeneBank no. X03695

Table 4. Rat rDNA primer used in MAR-loop assays

Primer	Corresponding site
r0	CATAAAGCTGCCCCAGAGAG (460-441)[&]
r4	CTGATCGTCTTCGAACCTCCG (1063-1043)[%]
r9	CCAGAGTTTCCTCTGCTTCG (5332-5313)[%]
r10	CCCTTAGAGCCAATCCTTATCCC (6598-6576)[%]
r14	GCGATCTGGGGAGTTGCTTTTC (2038-2049)[#]
r15	ACCACCGACTTGTAGGGATCC (2367-2387)[#]
r16	TGCACAACCATCATTGGAGGACG (2462-2448)[#]

! sequence derived from GeneBank no. X61110
sequence derived from GeneBank no. X03695
& sequence derived from GeneBank no. X00677
% sequence derived from GeneBank no. V01270

Table 5. Human rDNA primer pair used for syntheses of control fragments in chromatin conformation capturing & MAR-loop assays

Primer	Corresponding site #	
	Forward	Reverse
h1	AAAAGCCTTCTCTAGCGATCTGAG (1403-1425)	TACCATAACGGAGGCAGAGACAG (1489-1467)
h4	AGAAACGGCTACCACATCCAAGG (4101-4123)	TCATGGCCTCAGTTCCGAAAACC (4572-4550)
h9	TGAAACACGGACCAAGGAGT (9241-9260)	TGAGGGAAACTTCGGAGGGAACC (9600-9578)
h10	TAACGCGACCGATCCCGGAGAA (10501-10522)	CCTTAGAGCCAATCCTTATCCCG (10818-10796)
h18	TAGGAGAGGTATGGGAGAGGAC (17858-17879)	CTGGAGAAAGGGAAGAGAAGAGG (18311-18289)
h21	AAGTGTGTGTTCCCGTGAGAGTG (21619-21641)	CACAGAGAGAAAGACAGAGAC (22120-22100)
h24	TTCACGTCTGTCATCCCGAGGTC (24151-24173)	AACAGACAGAGAGAAGGCCCTAG (24548-24526)
h27	AAATACAAAGCTGAGTCGGGAGC (27634-27656)	GTCGACTTTCAATAACAGTGGCC (27915-27893)
h28	CACCGTTTTTGAAGATGGGGG (28381-28401)	GGCGGAGAAACTAAAACATCG (28594-28574)
h37	GTAGAGATGGGGTTTCACTGTGG (37110-37132)	AGAATGAGTTTAAGCACGCCAGCC (37494-37471)
h42	TTCGCCATCTGTCTCTTTTCCC (41711-41732)	TCTCTGGAGATGCCTCTCGGAAG (41901-41879)

sequence derived from GeneBank no. U13369

Table 6. Human rDNA primer used in chromatin conformation capturing & MAR-loop assays

Primer	Corresponding site #
h1	TACCATAACGGAGGCAGAGACAG (1489-1467)
h4	AGAAACGGCTACCACATCCAAGG (4101-4123)
h9	ATTTGCACGTCAGGACCGCTAC (9523-9502)
h10	TAACGCGACCGATCCCGGAGAA (10501-10522)
h18	AGTCCACCCTGTACGTCAACC (18174-18154)
h21	CACAGAGAGAAAGACAGAGAC (22120-22100)
h24	AACAGACAGAGAGAAGGCCCTAG (24548-24526)
h27	GTCGACTTTCAATAACAGTGGCC (27915-27893)
h28	GGCGGAGAAACTAAAACATCG (28594-28574)
h37	TCTGCCGTGAAACTGTCTGTC (37464-37444)
h42	TTCGCCATCTGTCTCTTTTCCC (41711-41732)

sequence derived from GeneBank no. U13369