Supplemental Data for

E2A-PBX1 functions as a coactivator for RUNX1 in acute lymphoblastic leukemia

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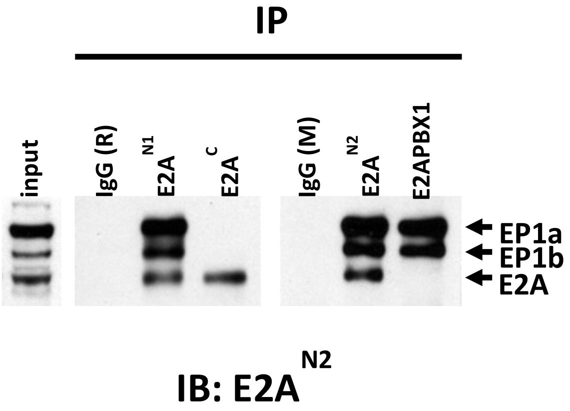
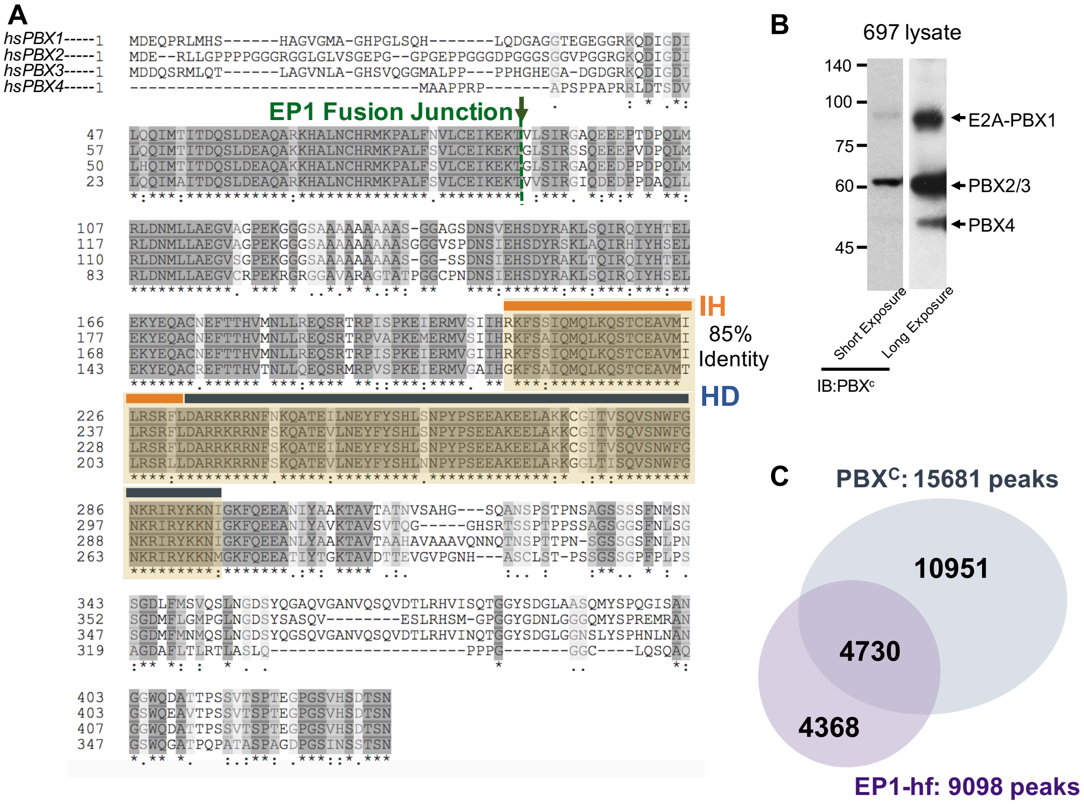
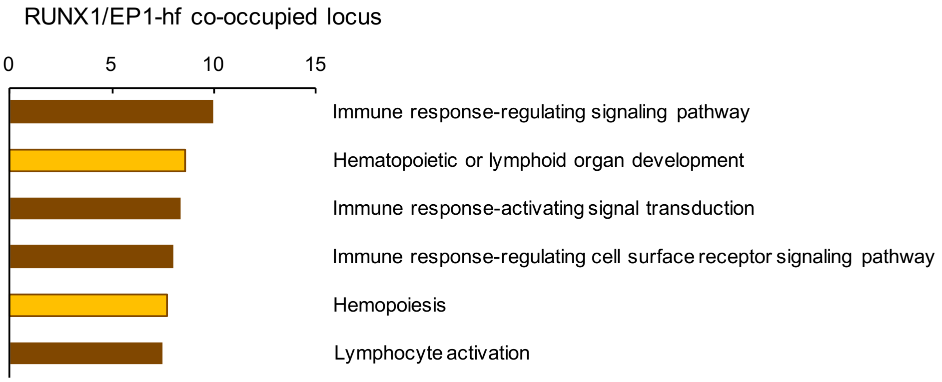


Supplemental Figure S1. E2A and E2A-PBX1 are separately enriched by the indicated antibodies. Immunoprecipitation (IP) assays with cell lysate from the E2A-PBX1+ 697 cell line were carried out with antibodies against the E2A N-terminus ($E2A^{N1}$ and $E2A^{N2}$), the E2A C-terminus ($E2A^{C}$) and the E2A-PBX1 fusion junction (E2APBX1). Immunoblotting with anti-$E2A^{N2}$ antibody validates the specificity of the antibodies. Normal IgGs from rabbit, IgG(R), and mouse, IgG(M), were used as IP control antibodies.

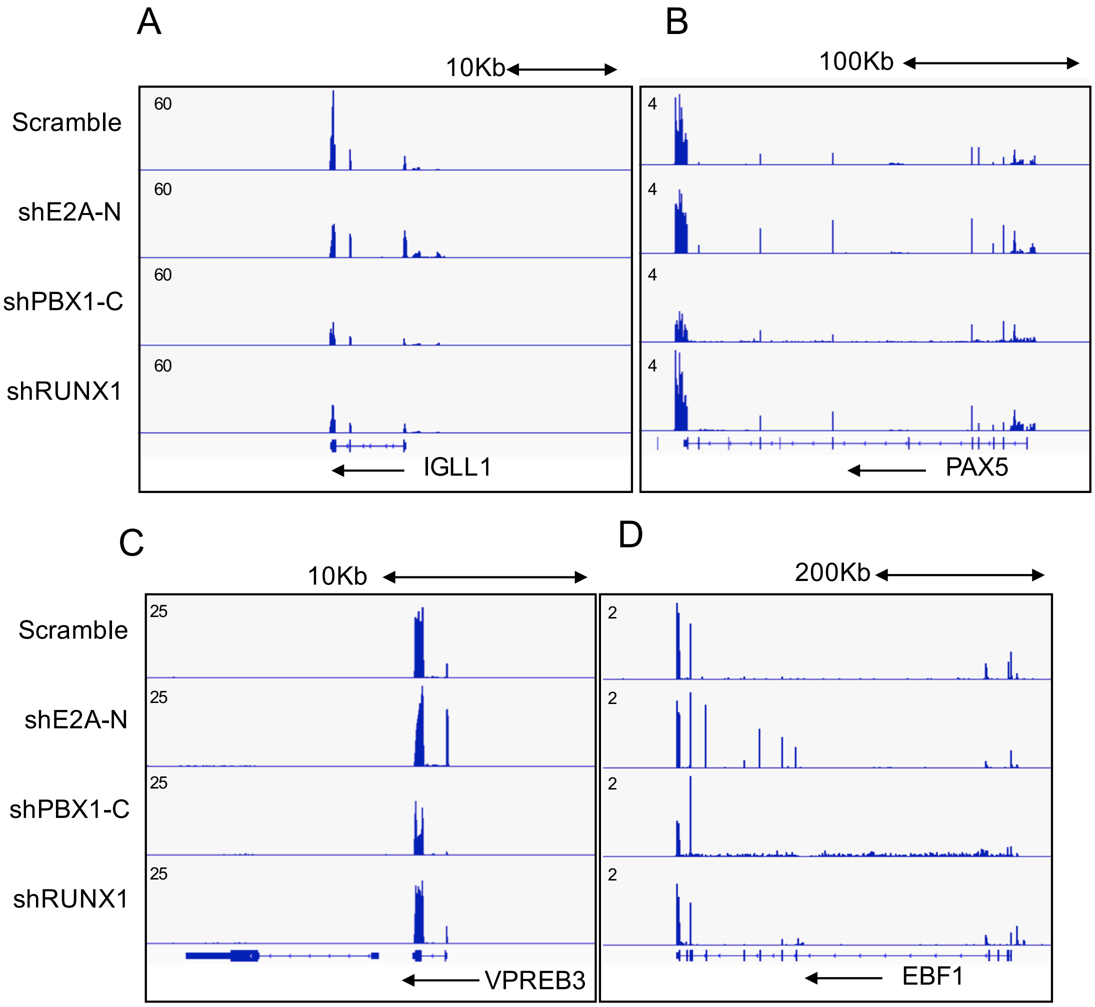


Supplemental Figure S2. The sequence alignment and protein levels of PBXs in 697 pre-B ALL**.** **(A)** The sequence alignment of human PBX proteins. The fusion junction at PBX1 for E2A-PBX1 (EP1) is indicated by green arrow. The inhibitory helix (IH) and homeodomain (HD) are marked. **(B)** The cell lysates from the E2A-PBX1+ 697 pre-B ALL cells were analyzed by immunoblotting with an anti-PBX^{C} antibody (sc-888) that were also used in ChIP-seq assay. **(C)** Venn diagram showing the overlap between PBX^{C} and EP1-hf ChIP-seq peaks.

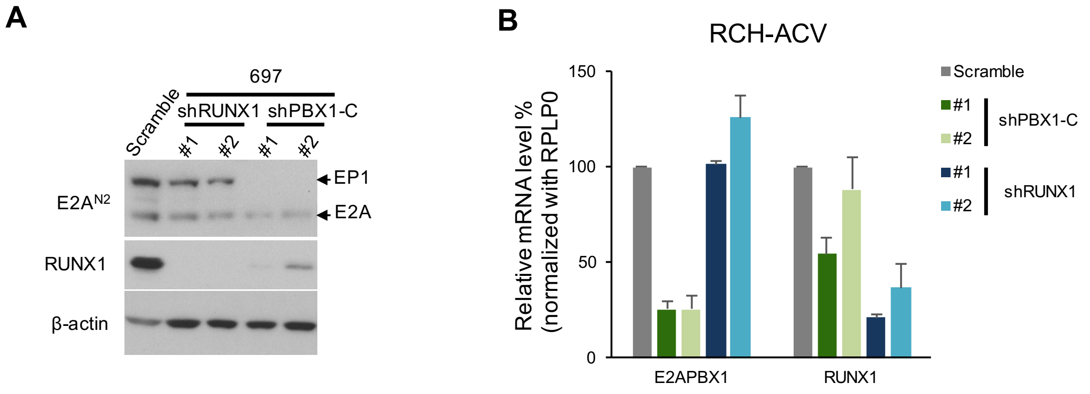


Supplemental Figure S3. Immune response signaling and hematopoietic pathways are enriched in RUNX1/EP1-hf co-occupied locus.

GREAT analysis identifies the indicated GO biological processes for the RUNX1/EP1-hf co-bound genomic loci.



Supplemental Figure S4. The expression levels of putative E2A targets are slightly decreased in E2A/E2A-PBX1 and RUNX1-depleted 697 lines. RNA-seq signals of *IGLL1* (A), *PAX5* (B), *VPREB3* (C), and *EBF1* (D) gene loci in 697 cell line treated with indicated shRNAs.



Supplemental Figure S5. RUNX1 and E2A-PBX1 are efficiently depleted by shRNA-treatment in 697 and RCH-ACV cell lines.

(A) Immunoblot of E2A, E2A-PBX1, and RUNX1 in 697 cell line treated with shRUNX1 and shPBX1-C. Actin as the gel loading control.

(B) RT-qPCR-determined mRNA levels of E2A-PBX1 and RUNX1 in RCH-ACV line transduced with indicated shRNAs. Data of three independent experiments are plotted as mean +/- sd after normalization to *RPLP0* levels and relative to those of shScr.

Supplemental Methods

Cell culture

HEK293 and HEK293T cell lines were maintained at 37°C in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin. Human pre-B leukemia lines 697 (DSMZ ACC 42), RCH-ACV (DSMZ AC-548) were grown at 37°C in RPMI 1640 supplemented with 10% FBS and 100 U/mL penicillin,100 µg/mL streptomycin. The lines with doxycycline-inducible stable expression of E2A-HA-FLAG, designated 697(E2A-hf), or E2A-PBX1-HA-FLAG, designated 697(EP1-hf), were established as previously described[1]. Positive clones were screening by immunoblotting with anti-FLAG antibody 24 hr post-induction with 1 µg/mL Doxycycline. Authentication of identities of parental and derived cell lines was ensured by the genetic signature profiling and fingerprinting analysis as recommended[2]. Every month, a routine examination of cell lines in culture for any possible mycoplasma contamination was performed using commercially available detection kits (Lonza). Cells with less than of 10 times of passages were used in the study.

Recombinant proteins and pull-down assays

Full length cDNAs for E2A-PBX1, E2A, PBX, RUNX1, and EBF1 were cloned in-frame to an N-terminal FLAG or HA tag into *pFastBac* vector (Thermo Scientific). Baculovirus-mediated protein expression was performed in High Five^{TM} cells according to manufacturer's instruction. Expressed proteins were immunopurified by anti-FLAG M2 or anti-HA beads and subjected to pull-down assay as previously described[3]. Briefly, 0.2 µg recombinant proteins (each) were incubated in 500 µL BC300 buffer supplemented with 0.2% Triton-X 100 and 0.1% BSA at 4°C for 2 hr followed by immunoprecipitation with indicated antibodies and protein A agarose. GST and GST-tagged PBX1^{C} [PBX1^{C} (484-852), FD (563-713), DM-IH (563-626), IH-HD (601-690), and HCM (691-713)] proteins were expressed in bacterial from pGEX6P1 vectors and purified using glutathione magnetic agarose beads (Pierce). For GST pull-down assays, GST proteins were immobilized on glutathione beads and followed by incubation with purified RUNX1 protein (0.2 μg) in BC100 buffer containing 0.2% Triton-X 100 and 0.1% BSA at 4°C for 2 hr. Precipitated protein complexes were analyzed by gel electrophoresis and stained with Coomassie brilliant blue or immunoblotting with indicated antibodies.

shRNA knockdown

Lentiviruses were prepared in HEK-293T cells according to a standard protocol from Addgene. For knockdown (KD), cells were infected with shRNA lentivirus plus 8 ug/mL polybrene in complete medium for 24 hr. After selection with 2 μg/mL puromycin for 48 hr, transduced cells were subjected to proliferation and colony -formation unit (CFU) assays or used for RNA extraction and cell lysate preparation. For cell proliferation assay, 1,000,000 cells/mL were plated in triplicate in 24-well plate at post-infection 96 hr. After incubation for 48 hr (post-infection 144 hr), cells were stained with trypan blue and viable cells were counted. For CFU assays of 697 line, 5,000 cells/mL cells were plated in triplicate in 12-well plate that contain a complete methylcellulose-based medium (MethoCult™ GF H4434; Stem Cell Technologies Inc.) supplemented with 20% FBS. After incubation at 37°C for 21 days, visible colonies (> 100 μm) were counted and imaged by a light microscope.

RT-qPCR

The quantitative RT-PCR was carried out as previously described[1]. In brief, total RNAs were isolated using a Quick-RNA™ MiniPrep Plus kit (Zymo Research) and cDNAs were synthesized with 1 μg of total RNA using RevertAid RT Reverse Transcription Kit (Thermo Scientific) according to the manufacturer's instructions. Quantitative PCR used SYBR green master mix (KAPA SYBR FAST qPCR Master Mix

ABI Prism, KM4102) was run in triplicate on an ABI StepOne Plus real time PCR system (ABI). The gene-specific primers used for RT-qPCR are listed in Supplemental Table S2.

Bone marrow transformation, Colony-formation unit (CFU) assay

E2A-PBX1 (WT or mutant) was cloned into MSCV-based retroviral vectors, and retroviruses was prepared according to manufacturer's instructions (Clontech Laboratories, Inc.). Murine bone marrow cells were isolated and subject to lineage-negative enrichment (Miltenyi Bio). The HSPC stimulation, retroviral infection and assays for out-growth and immortalization were performed as described before[4-6]. The CFU assays were conducted in as previously described[5,6]. In brief, 5,000 infected cells were plated in triplicate in 6-well plates containing the complete growth medium supplemented with 0.5% soft agar at bottom and layered with 0.3% soft agar and 1 mL of medium. Colonies were stained with iodonitritetrazolium chloride and directly counted under a light microscope.

Co-immunoprecipitation (CoIP) assay

HEK293T cells were co-transfected with pEF1α-E2A-PBX1 and pIRES-RUNX1 or pIRES-EBF1 vectors. At 24hr post-transfection, cell lysates were prepared in BC150 lysis buffer (50 mM Tris-HCl pH 7.9, 150 mM NaCl, 0.2% Triton-X 100) containing protease inhibitor cocktail (Roche). Proteins were immunoprecipitated by indicated resins and subjected to gel electrophoresis followed by immunoblotting assay.

ChIP assay

Briefly, 697 or doxycycline-treated stable 697 cells were cross-linked with 1% formaldehyde, quenched by 0.125 M glycine, and lysed in FA lysis buffer (50mM HEPES-KOH pH 7.5, NaCl, Triton-X 100, 0.1%SDS) supplemented with protease inhibitor cocktail. Chromatin was fragmented by a Covaris (S220) Sonicator and immunoprecipitated with indicated antibodies. Precipitated DNAs were purified by PCR purification kit (Qiagen). Quantitative PCR used QuantiNova Probe PCR kit (Qiagen 208052) in a StepOnePlus™ System (Applied Biosystems) with gene-specific primers (listed in Supplemental Table S2). The ΔCt method was used to quantify the signals and normalized to the ΔCt values of indicated house-keeping genes.

ChIP-seq and RNA-seq analysis

ChIP-seq and RNA-seq in 697 line were executed as previously described[1]. For data analysis, raw image data were converted into base calls and fastq files via the Illumina pipeline CASAVA version 1.8 with default parameters. For ChIP-seq analysis, 50-base reads were aligned to HG19 human genome using Bowtie software[7] with parameters "-n 2 -m 1 -l 36 --best". ChIP-seq peak calling was performed using MACS2 (1.4.0rc2) software[8] and identified peaks are listed in Supplemental Dataset_1. The bedtools intersect command[9] was used to identify overlapping peaks between indicated ChIP-seq profilings. For RNA-seq analysis, 50-base reads were mapped to HG19 human genome using TopHat[10] with the default parameters. The expression level for gene/transcript was quantified using the analyzeRepeats.pl command of Homer software[11] with default parameters (-strand both -count exons) and differential gene expression was analyzed using getDiffExpression.pl command. The quantified gene/transcripts level are represented as RPKM (reads per kilobase of transcripts per million fragments mapped) and listed in Supplemental Dataset_2.

Supplemental References

1. Shimada M, Chen WY, Nakadai T, et al. Gene-Specific H1 Eviction through a Transcriptional Activator-->p300-->NAP1-->H1 Pathway. *Mol Cell*. 2019;74:268-283 e265.
2. Yu M, Selvaraj SK, Liang-Chu MM, et al. A resource for cell line authentication, annotation and quality control. *Nature*. 2015;520(7547):307-311.
3. Chen WY, Zhang J, Geng H, Du Z, Nakadai T, Roeder RG. A TAF4 coactivator function for E proteins that involves enhanced TFIID binding. *Genes Dev*. 2013;27:1596-1609.
4. Wang GG, Song J, Wang Z, et al. Haematopoietic malignancies caused by dysregulation of a chromatin-binding PHD finger. *Nature*. 2009;459:847-851.
5. Xu B, On DM, Ma A, et al. Selective inhibition of EZH2 and EZH1 enzymatic activity by a small molecule suppresses MLL-rearranged leukemia. *Blood*. 2015;125:346-357.
6. Lu R, Wang P, Parton T, et al. Epigenetic Perturbations by Arg882-Mutated DNMT3A Potentiate Aberrant Stem Cell Gene-Expression Program and Acute Leukemia Development. *Cancer Cell*. 2016;30:92-107.
7. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods*. 2012;9:357-359.
8. Zhang Y, Liu T, Meyer CA, et al. Model-based analysis of ChIP-Seq (MACS). *Genome Biol*. 2008;9:R137.
9. Quinlan AR, Hall IM. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics*. 2010;26:841-842.
10. Trapnell C, Pachter L, Salzberg SL. TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics*. 2009;25:1105-1111.
11. Heinz S, Benner C, Spann N, et al. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol Cell*. 2010;38:576-589.

Supplemental Table S1. shRNA clone ID

Gene symbol	NM ID	Clone ID
TCF3/E2A	NM_003200	#1, TRCN0000017535
		#2, TRCN0000017534
PBX1	NM_002585	#1, TRCN0000274137
		#2, TRCN0000274085
RUNX1	NM_001754	#1, TRCN0000338490
		#2, TRCN0000338427
mRunx1	NM_009821	#1, TRCN0000084812
		#2, TRCN0000229574

Supplemental Table S2. RT-qPCR and ChIP-qPCR primers

RT-qPCR

	Forward	**Reverse**
EPHA3	GGACTACGAGGTCAAATACTATGAA	GTGCCTCTTGCCCTCAGAAT
MPP7	GCTGCCTCCTTGCCTCC	GCAGCCACGTTGTCTACCAA
KCNJ2	CTTACATGCCTCTGTACCCCC	CTGGGAGCCTTGTGGTTCTA
TRIM13	CACTAGCCGGAGTAGCCTCT	TTCTTCAAGCAGCTCCATCACA
CXorf21	AGCTTCTGGAGGATTGAAGAGT	GCACAACTCCAGTGGATGTCA
RUNX1	AAATGCTACCGCAGCCATGA	CTGTGGTAGGTGGCGACTTG
E2A-PBX1	CCAGCCTCATGCACAACCA	GGGCTCCTCGGATACTCAAAA
RPLP0	CACCATTGAAATCCTGAGTGATGT	TGACCAGCCCAAAGGAGAAG

ChIP-qPCR

	Forward	**Reverse**
EPHA3	TTAGCACAAAGCAGAGCCGT	TTTCCCTTTGCACCAACGAG
MPP7	GCTTGTGGTTCAGAACGCCT	AGGCGTAAAGTCTCCATGAGG
KCNJ2	GGGAGTTAACCTCCTTGGTCC	AGGGCTACAAGTAGAGCGAGG
TRIM13	CAGTTGTTGCCTCCAAACGG	TCGGGGTTTTGGTGACTCAG
CXorf21	TCAGGATGTCGTGGTCAAGTC	CCATGATTTTTCCGGTGCCC
RUNX1_IGR	CTCCTTAAGAGGTGGGCTGTC	AGGGCCGGTCTCATCACTA
RUNX1_EN	AGTCGAAGCGACTGTTGGTTT	AACAACTGTGCAATGGTTCTGA
RPL30	AACACCACAATCGCTACCGT	AAGAGACTGAACGGGCTTCG
GAPDH	TACTAGCGGTTTACGGGCG	TCGACAGGAGGAGCAGAGAGCGA
NCAPD2	ATGGTTGCCACTGGGGATCT	TGCCAAAGCCTAGGGGAAGA

Supplemental Table S3. Antibody list

anti-E2A (E2A^{N1})	this study
anti-E2A (E2A^{N2})	Santa Cruz, sc-416
anti-PBX1/2/3 (PBX^{C})	Santa Cruz, sc-888
anti-E2A-PBX1	BD Biosciences, 556021
anti-p300	Santa Cruz, sc-585
anti-RUNX1	Abcam, ab23980
anti-H3K27ac	Abcam, ab4729
anti-MED1	Bethyl, A300-793A
anti-FLAG	Sigma, F1804
anti-FLAG agarose	Sigma, A2220
anti-HA tag	Abcam, ab9110
anti-β-Actin	Sigma, A2228

Supplementary Table S4: Summary of number of reads and peaks detected in ChIP-seq experiments

ChIP-seq experiment	**Total reads**	**Uniquely aligned reads**	**Number of peaks detected**		**Mapping Efficiency (%)**
			P^{c}<=10^{-5}	**P<=10^{-9}**	
input 697[a]	38,341,191	21,436,723			56
input 697HF[b]	34,281,257	27,365,815			80
HA-Ctrl 697HF	34,890,188	28,349,401			81
E2A^{N1} 697	34,906,348	20,508,319	9,575	4,110	59
E2A^{C} 697	39,098,605	23,029,392	7,494	4,144	59
PBX^{C} 697	45,572,361	27,533,200	15,681[d]	8,800	60
E2A-hf 697HF	32,666,045	25,968,665	10,442[d]	5,748	79
EP1-hf 697HF	31,875,272	25,176,210	9,098[d]	4,818	79
RUNX1 697	36,956,521	30,315,686	45,475	31,232[d]	82
p300 697	47,386,452	29,982,714			63
H3K27ac 697	36,508,556	30,626,776			84
MED1 697	44,076,152	32,210,634			73

[a] input DNA of parental 697 line

[b] input DNA of stable HA-Ctrl 697HF lines

[d] p-value calculated by MACS peak calling program.

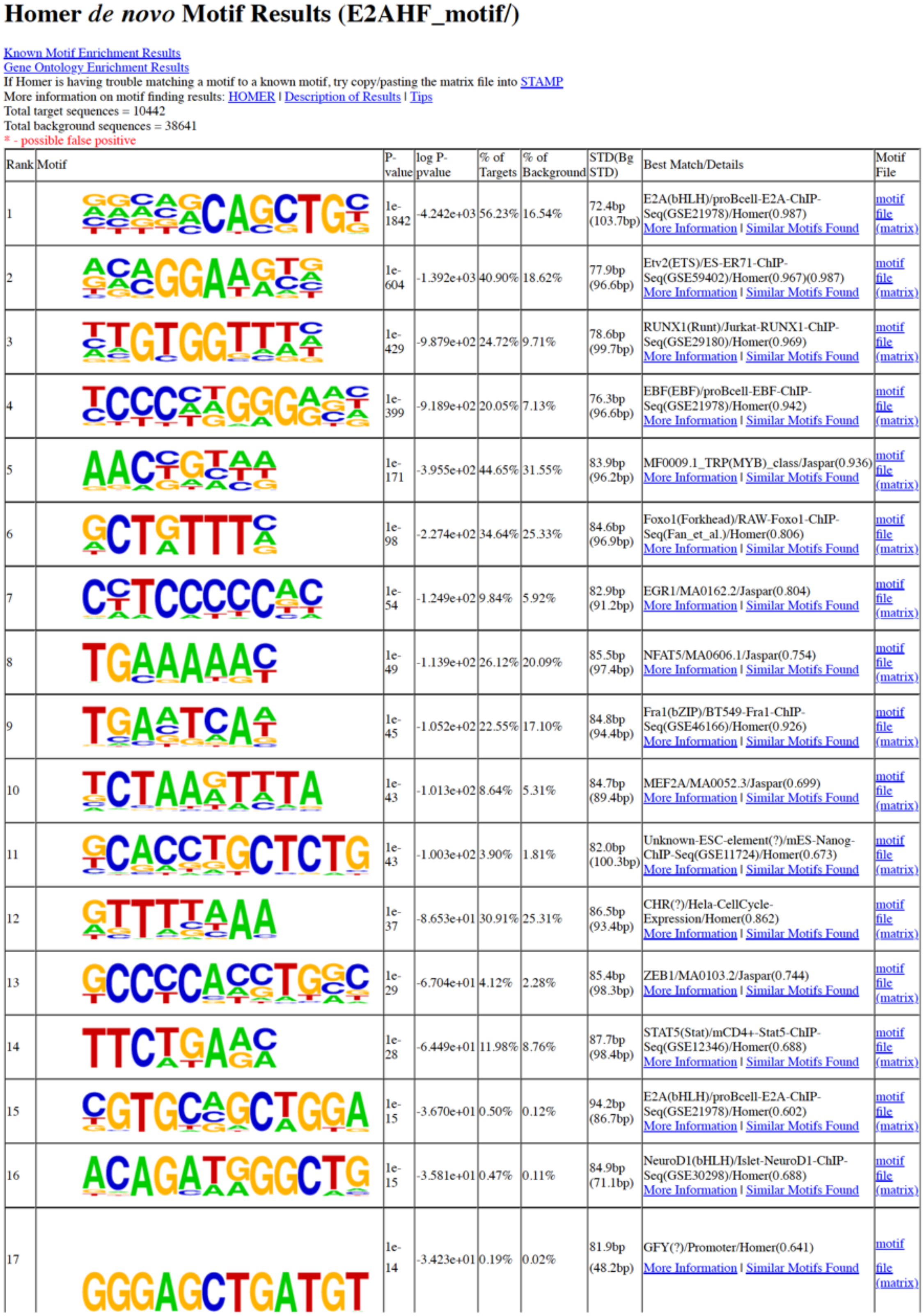
[d] peaks used for region overlapping analysis.

Supplementary Table S5: Summary of number of reads and alignment rate in RNA-seq experiments

RNA-seq experiment	Total reads	Uniquely aligned reads (%)^{a}	Aligned > 1 times (%)^{a}	Overall alignment rate (%)^{a}
697_Scr_#1	60,829,680	48,028,626 (78.96)	7,758,668 (12.75)	91.71
697_Scr_#2	65,157,731	47,994,771 (73.66)	12,233,741 (18.78)	92.42
697_shE2A_N#1	60,620,638	47,521,229 (78.39)	7,941,280 (13.1)	91.49
697_shE2A_N#2	68,465,992	50,530,075 (73.80)	11,757,059 (17.17)	90.98
697_shPBX1_C#1	36,913,154	24,213,055 (65.59)	11,722,164 (31.76)	97.35
697_shPBX1_C#2	41,784,716	32,876,419 (78.68)	7,678,863 (18.38)	97.06
697_shRUNX1_#1	69,995,952	52,517,289 (75.03)	11,846,971 (16.93)	91.95
697_shRUNX1_#2	38,234,911	30,156,425 (78.87)	6,977,994 (18.25)	97.12

[a] percent total reads

Supplemental Table S6. Complete output of Homer motif analysis.

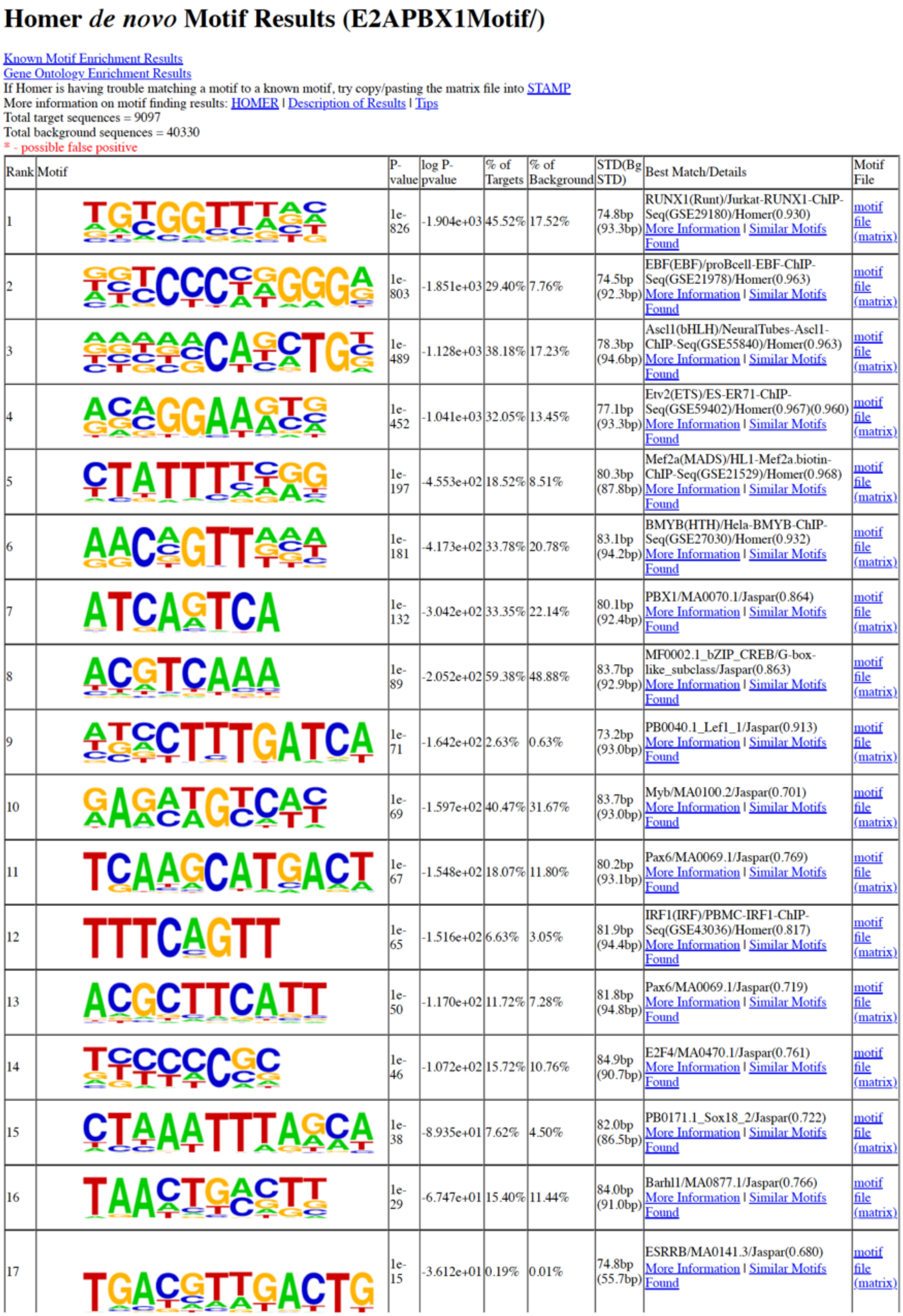


Homer *de novo* Motif Results (E2AHF_motif/)

Known Motif Enrichment Results
Gene Ontology Enrichment Results
If Homer is having trouble matching a motif to a known motif, try copy/pasting the matrix file into STAMP
More information on motif finding results: HOMER | Description of Results | Tips
Total target sequences = 10442
Total background sequences = 38641
* - possible false positive

Rank	Motif	P-value	log P-pvalue	% of Targets	% of Background	STD(Bg STD)	Best Match/Details	Motif File
1		1e-1842	-4.242e+03	56.23%	16.54%	72.4bp (103.7bp)	E2A(bHLH)/proBcell-E2A-ChIP-Seq(GSE21978)/Homer(0.987) More Information | Similar Motifs Found	motif file (matrix)
2		1e-604	-1.392e+03	40.90%	18.62%	77.9bp (96.6bp)	Etv2(ETS)/ES-ER71-ChIP-Seq(GSE59402)/Homer(0.967)(0.987) More Information | Similar Motifs Found	motif file (matrix)
3		1e-429	-9.879e+02	24.72%	9.71%	78.6bp (99.7bp)	RUNX1(Runt)/Jurkat-RUNX1-ChIP-Seq(GSE29180)/Homer(0.969) More Information | Similar Motifs Found	motif file (matrix)
4		1e-399	-9.189e+02	20.05%	7.13%	76.3bp (96.6bp)	EBF(EBF)/proBcell-EBF-ChIP-Seq(GSE21978)/Homer(0.942) More Information | Similar Motifs Found	motif file (matrix)
5		1e-171	-3.955e+02	44.65%	31.55%	83.9bp (96.2bp)	MF0009.1_TRP(MYB)_class/Jaspar(0.936) More Information | Similar Motifs Found	motif file (matrix)
6		1e-98	-2.274e+02	34.64%	25.33%	84.6bp (96.9bp)	Foxo1(Forkhead)/RAW-Foxo1-ChIP-Seq(Fan_et_al.)/Homer(0.806) More Information | Similar Motifs Found	motif file (matrix)
7		1e-54	-1.249e+02	9.84%	5.92%	82.9bp (91.2bp)	EGR1/MA0162.2/Jaspar(0.804) More Information | Similar Motifs Found	motif file (matrix)
8		1e-49	-1.139e+02	26.12%	20.09%	85.5bp (97.4bp)	NFAT5/MA0606.1/Jaspar(0.754) More Information | Similar Motifs Found	motif file (matrix)
9		1e-45	-1.052e+02	22.55%	17.10%	84.8bp (94.4bp)	Fra1(bZIP)/BT549-Fra1-ChIP-Seq(GSE46166)/Homer(0.926) More Information | Similar Motifs Found	motif file (matrix)
10		1e-43	-1.013e+02	8.64%	5.31%	84.7bp (89.4bp)	MEF2A/MA0052.3/Jaspar(0.699) More Information | Similar Motifs Found	motif file (matrix)
11		1e-43	-1.003e+02	3.90%	1.81%	82.0bp (100.3bp)	Unknown-ESC-element(?)/mES-Nanog-ChIP-Seq(GSE11724)/Homer(0.673) More Information | Similar Motifs Found	motif file (matrix)
12		1e-37	-8.653e+01	30.91%	25.31%	86.5bp (93.4bp)	CHR(?)/Hela-CellCycle-Expression/Homer(0.862) More Information | Similar Motifs Found	motif file (matrix)
13		1e-29	-6.704e+01	4.12%	2.28%	85.4bp (98.3bp)	ZEB1/MA0103.2/Jaspar(0.744) More Information | Similar Motifs Found	motif file (matrix)
14		1e-28	-6.449e+01	11.98%	8.76%	87.7bp (98.4bp)	STAT5(Stat)/mCD4+-Stat5-ChIP-Seq(GSE12346)/Homer(0.688) More Information | Similar Motifs Found	motif file (matrix)
15		1e-15	-3.670e+01	0.50%	0.12%	94.2bp (86.7bp)	E2A(bHLH)/proBcell-E2A-ChIP-Seq(GSE21978)/Homer(0.602) More Information | Similar Motifs Found	motif file (matrix)
16		1e-15	-3.581e+01	0.47%	0.11%	84.9bp (71.1bp)	NeuroD1(bHLH)/Islet-NeuroD1-ChIP-Seq(GSE30298)/Homer(0.688) More Information | Similar Motifs Found	motif file (matrix)
17	GGGAGCTGATGT	1e-14	-3.423e+01	0.19%	0.02%	81.9bp (48.2bp)	GFY(?)/Promoter/Homer(0.641) More Information | Similar Motifs Found	motif file (matrix)

18 *		1e-11	-2.688e+01	0.19%	0.02%	87.8bp (40.7bp)	MYBL2/MA0777.1/Jaspar(0.732) More Information | Similar Motifs Found	motif file (matrix)
19 *		1e-9	-2.126e+01	0.29%	0.07%	82.7bp (73.0bp)	EGR2/MA0472.2/Jaspar(0.651) More Information | Similar Motifs Found	motif file (matrix)
20 *		1e-8	-1.883e+01	0.09%	0.01%	85.7bp (68.2bp)	TFCP2/MA0145.3/Jaspar(0.555) More Information | Similar Motifs Found	motif file (matrix)
21 *		1e-7	-1.622e+01	1.43%	0.90%	82.1bp (85.7bp)	Maz(Zf)/HepG2-Maz-ChIP-Seq(GSE31477)/Homer(0.645) More Information | Similar Motifs Found	motif file (matrix)
22 *		1e-1	-4.390e+00	0.05%	0.01%	81.0bp (77.6bp)	TEAD(TEA)/Fibroblast-PU.1-ChIP-Seq(Unpublished)/Homer(0.730) More Information | Similar Motifs Found	motif file (matrix)



Homer *de novo* Motif Results (E2APBX1Motif/)

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Gene Ontology Enrichment Results
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More information on motif finding results: HOMER | Description of Results | Tips
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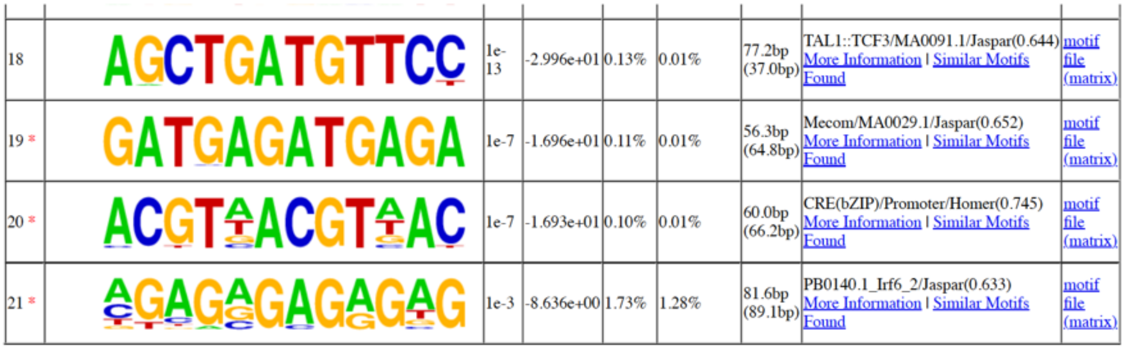
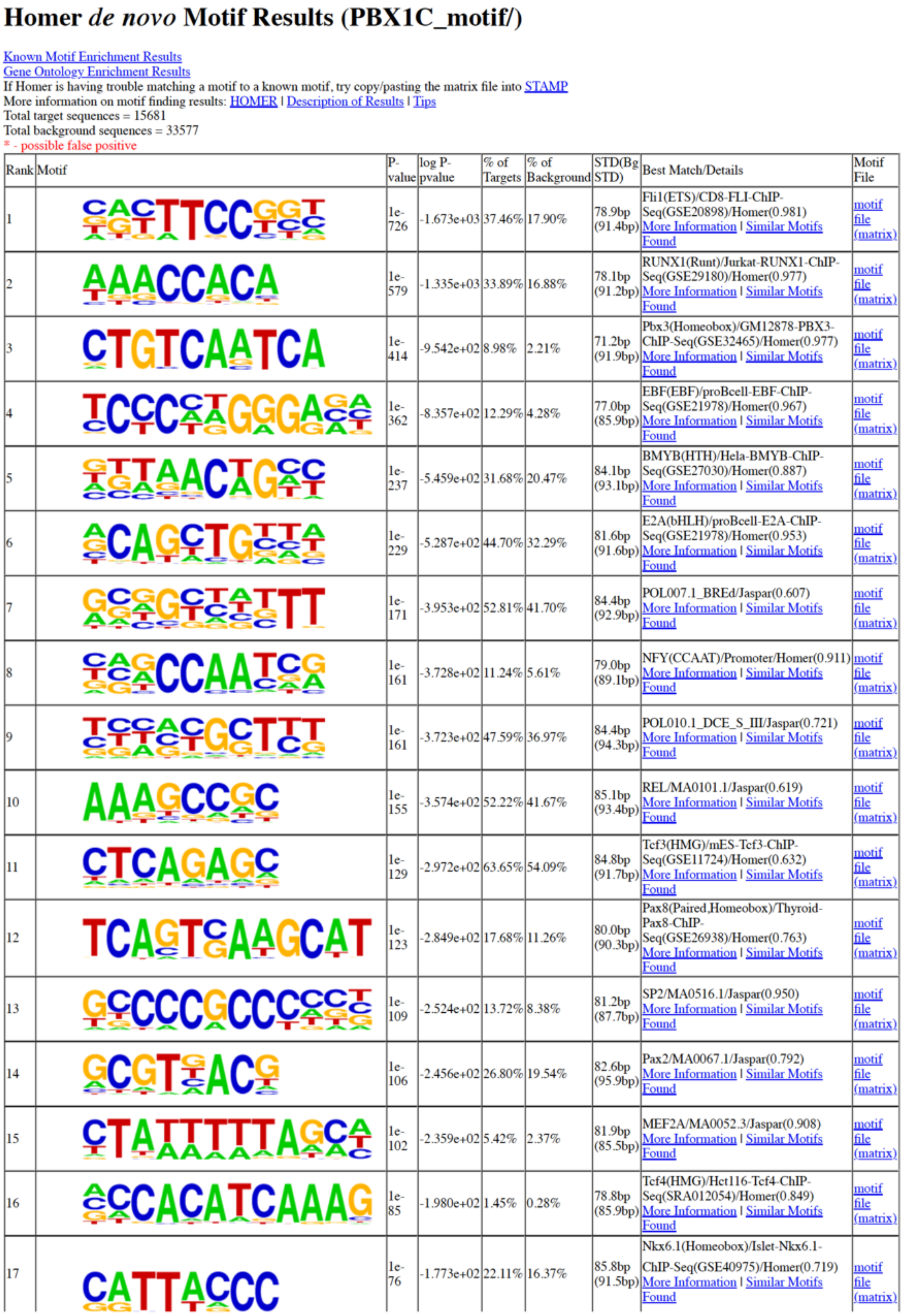
Total background sequences = 40330
* - possible false positive

Rank	Motif	P-value	log P-pvalue	% of Targets	% of Background	STD(Bg STD)	Best Match/Details	Motif File
1		1e-826	-1.904e+03	45.52%	17.52%	74.8bp (93.3bp)	RUNX1(Runt)/Jurkat-RUNX1-ChIP-Seq(GSE29180)/Homer(0.930) More Information | Similar Motifs Found	motif file (matrix)
2		1e-803	-1.851e+03	29.40%	7.76%	74.5bp (92.3bp)	EBF(EBF)/proBcell-EBF-ChIP-Seq(GSE21978)/Homer(0.963) More Information | Similar Motifs Found	motif file (matrix)
3		1e-489	-1.128e+03	38.18%	17.23%	78.3bp (94.6bp)	Ascl1(bHLH)/NeuralTubes-Ascl1-ChIP-Seq(GSE55840)/Homer(0.963) More Information | Similar Motifs Found	motif file (matrix)
4		1e-452	-1.041e+03	32.05%	13.45%	77.1bp (93.3bp)	Etv2(ETS)/ES-ER71-ChIP-Seq(GSE59402)/Homer(0.967)(0.960) More Information | Similar Motifs Found	motif file (matrix)
5		1e-197	-4.553e+02	18.52%	8.51%	80.3bp (87.8bp)	Mef2a(MADS)/HL1-Mef2a.biotin-ChIP-Seq(GSE21529)/Homer(0.968) More Information | Similar Motifs Found	motif file (matrix)
6		1e-181	-4.173e+02	33.78%	20.78%	83.1bp (94.2bp)	BMYB(HTH)/Hela-BMYB-ChIP-Seq(GSE27030)/Homer(0.932) More Information | Similar Motifs Found	motif file (matrix)
7		1e-132	-3.042e+02	33.35%	22.14%	80.1bp (92.4bp)	PBX1/MA0070.1/Jaspar(0.864) More Information | Similar Motifs Found	motif file (matrix)
8		1e-89	-2.052e+02	59.38%	48.88%	83.7bp (92.9bp)	MF0002.1_bZIP_CREB/G-box-like_subclass/Jaspar(0.863) More Information | Similar Motifs Found	motif file (matrix)
9		1e-71	-1.642e+02	2.63%	0.63%	73.2bp (93.0bp)	PB0040.1_Lef1_1/Jaspar(0.913) More Information | Similar Motifs Found	motif file (matrix)
10		1e-69	-1.597e+02	40.47%	31.67%	83.7bp (93.0bp)	Myb/MA0100.2/Jaspar(0.701) More Information | Similar Motifs Found	motif file (matrix)
11		1e-67	-1.548e+02	18.07%	11.80%	80.2bp (93.1bp)	Pax6/MA0069.1/Jaspar(0.769) More Information | Similar Motifs Found	motif file (matrix)
12		1e-65	-1.516e+02	6.63%	3.05%	81.9bp (94.4bp)	IRF1(IRF)/PBMC-IRF1-ChIP-Seq(GSE43036)/Homer(0.817) More Information | Similar Motifs Found	motif file (matrix)
13		1e-50	-1.170e+02	11.72%	7.28%	81.8bp (94.8bp)	Pax6/MA0069.1/Jaspar(0.719) More Information | Similar Motifs Found	motif file (matrix)
14		1e-46	-1.072e+02	15.72%	10.76%	84.9bp (90.7bp)	E2F4/MA0470.1/Jaspar(0.761) More Information | Similar Motifs Found	motif file (matrix)
15		1e-38	-8.935e+01	7.62%	4.50%	82.0bp (86.5bp)	PB0171.1_Sox18_2/Jaspar(0.722) More Information | Similar Motifs Found	motif file (matrix)
16		1e-29	-6.747e+01	15.40%	11.44%	84.0bp (91.0bp)	Barhl1/MA0877.1/Jaspar(0.766) More Information | Similar Motifs Found	motif file (matrix)
17		1e-15	-3.612e+01	0.19%	0.01%	74.8bp (55.7bp)	ESRRB/MA0141.3/Jaspar(0.680) More Information | Similar Motifs Found	motif file (matrix)



18	AGCTGATGTTCC	1e-13	-2.996e+01	0.13%	0.01%	77.2bp (37.0bp)	TAL1::TCF3/MA0091.1/Jaspar(0.644) More Information | Similar Motifs Found	motif file (matrix)
19 *	GATGAGATGAGA	1e-7	-1.696e+01	0.11%	0.01%	56.3bp (64.8bp)	Mecom/MA0029.1/Jaspar(0.652) More Information | Similar Motifs Found	motif file (matrix)
20 *	ACGTAACGTAAC	1e-7	-1.693e+01	0.10%	0.01%	60.0bp (66.2bp)	CRE(bZIP)/Promoter/Homer(0.745) More Information | Similar Motifs Found	motif file (matrix)
21 *	AGAGAGAGAGAG	1e-3	-8.636e+00	1.73%	1.28%	81.6bp (89.1bp)	PB0140.1_Irf6_2/Jaspar(0.633) More Information | Similar Motifs Found	motif file (matrix)



Homer *de novo* Motif Results (PBX1C_motif/)

Known Motif Enrichment Results
Gene Ontology Enrichment Results
If Homer is having trouble matching a motif to a known motif, try copy/pasting the matrix file into STAMP
More information on motif finding results: HOMER | Description of Results | Tips
Total target sequences = 15681
Total background sequences = 33577
* - possible false positive

Rank	Motif	P-value	log P-pvalue	% of Targets	% of Background	STD(Bg STD)	Best Match/Details	Motif File
1		1e-726	-1.673e+03	37.46%	17.90%	78.9bp (91.4bp)	Fli1(ETS)/CD8-FLI-ChIP-Seq(GSE20898)/Homer(0.981) More Information | Similar Motifs Found	motif file (matrix)
2		1e-579	-1.335e+03	33.89%	16.88%	78.1bp (91.2bp)	RUNX1(Runt)/Jurkat-RUNX1-ChIP-Seq(GSE29180)/Homer(0.977) More Information | Similar Motifs Found	motif file (matrix)
3		1e-414	-9.542e+02	8.98%	2.21%	71.2bp (91.9bp)	Pbx3(Homeobox)/GM12878-PBX3-ChIP-Seq(GSE32465)/Homer(0.977) More Information | Similar Motifs Found	motif file (matrix)
4		1e-362	-8.357e+02	12.29%	4.28%	77.0bp (85.9bp)	EBF(EBF)/proBcell-EBF-ChIP-Seq(GSE21978)/Homer(0.967) More Information | Similar Motifs Found	motif file (matrix)
5		1e-237	-5.459e+02	31.68%	20.47%	84.1bp (93.1bp)	BMYB(HTH)/Hela-BMYB-ChIP-Seq(GSE27030)/Homer(0.887) More Information | Similar Motifs Found	motif file (matrix)
6		1e-229	-5.287e+02	44.70%	32.29%	81.6bp (91.6bp)	E2A(bHLH)/proBcell-E2A-ChIP-Seq(GSE21978)/Homer(0.953) More Information | Similar Motifs Found	motif file (matrix)
7		1e-171	-3.953e+02	52.81%	41.70%	84.4bp (92.9bp)	POL007.1_BREd/Jaspar(0.607) More Information | Similar Motifs Found	motif file (matrix)
8		1e-161	-3.728e+02	11.24%	5.61%	79.0bp (89.1bp)	NFY(CCAAT)/Promoter/Homer(0.911) More Information | Similar Motifs Found	motif file (matrix)
9		1e-161	-3.723e+02	47.59%	36.97%	84.4bp (94.3bp)	POL010.1_DCE_S_III/Jaspar(0.721) More Information | Similar Motifs Found	motif file (matrix)
10		1e-155	-3.574e+02	52.22%	41.67%	85.1bp (93.4bp)	REL/MA0101.1/Jaspar(0.619) More Information | Similar Motifs Found	motif file (matrix)
11		1e-129	-2.972e+02	63.65%	54.09%	84.8bp (91.7bp)	Tcf3(HMG)/mES-Tcf3-ChIP-Seq(GSE11724)/Homer(0.632) More Information | Similar Motifs Found	motif file (matrix)
12		1e-123	-2.849e+02	17.68%	11.26%	80.0bp (90.3bp)	Pax8(Paired,Homeobox)/Thyroid-Pax8-ChIP-Seq(GSE26938)/Homer(0.763) More Information | Similar Motifs Found	motif file (matrix)
13		1e-109	-2.524e+02	13.72%	8.38%	81.2bp (87.7bp)	SP2/MA0516.1/Jaspar(0.950) More Information | Similar Motifs Found	motif file (matrix)
14		1e-106	-2.456e+02	26.80%	19.54%	82.6bp (95.9bp)	Pax2/MA0067.1/Jaspar(0.792) More Information | Similar Motifs Found	motif file (matrix)
15		1e-102	-2.359e+02	5.42%	2.37%	81.9bp (85.5bp)	MEF2A/MA0052.3/Jaspar(0.908) More Information | Similar Motifs Found	motif file (matrix)
16		1e-85	-1.980e+02	1.45%	0.28%	78.8bp (85.9bp)	Tcf4(HMG)/Hct116-Tcf4-ChIP-Seq(SRA012054)/Homer(0.849) More Information | Similar Motifs Found	motif file (matrix)
17		1e-76	-1.773e+02	22.11%	16.37%	85.8bp (91.5bp)	Nkx6.1(Homeobox)/Islet-Nkx6.1-ChIP-Seq(GSE40975)/Homer(0.719) More Information | Similar Motifs Found	motif file (matrix)

18		1e-71	-1.640e+02	25.76%	19.86%	84.7bp (91.7bp)	E2F(E2F)/Hela-CellCycle-Expression/Homer(0.751) More Information | Similar Motifs Found	motif file (matrix)
19		1e-56	-1.297e+02	3.72%	1.79%	80.6bp (88.4bp)	NRF(NRF)/Promoter/Homer(0.909) More Information | Similar Motifs Found	motif file (matrix)
20		1e-49	-1.129e+02	4.22%	2.26%	85.8bp (90.1bp)	FOS/MA0476.1/Jaspar(0.973) More Information | Similar Motifs Found	motif file (matrix)
21		1e-42	-9.733e+01	3.34%	1.73%	83.1bp (84.8bp)	PB0166.1_Sox12_2/Jaspar(0.714) More Information | Similar Motifs Found	motif file (matrix)
22		1e-32	-7.422e+01	1.27%	0.48%	78.9bp (85.6bp)	YY1(Zf)/Promoter/Homer(0.938) More Information | Similar Motifs Found	motif file (matrix)
23		1e-30	-7.073e+01	1.60%	0.70%	80.7bp (98.8bp)	GFX(?)/Promoter/Homer(0.920) More Information | Similar Motifs Found	motif file (matrix)
24		1e-21	-5.055e+01	0.11%	0.01%	60.3bp (75.2bp)	JUN/MA0488.1/Jaspar(0.691) More Information | Similar Motifs Found	motif file (matrix)
25		1e-21	-4.950e+01	0.17%	0.01%	54.6bp (63.6bp)	Sox3/MA0514.1/Jaspar(0.735) More Information | Similar Motifs Found	motif file (matrix)
26		1e-16	-3.906e+01	0.16%	0.02%	88.1bp (62.2bp)	PB0120.1_Foxj1_2/Jaspar(0.637) More Information | Similar Motifs Found	motif file (matrix)