Duke/UNC/UT/EBI enhancer functional assay

To characterize whether DNase HS sites displayed enhancer activity, we cloned a number of DNase and/or FAIRE sites into a pGL3 vector just downstream of the Promega pGL3 promoter luciferase reporter gene **(Table 1)**. This pGL3 reporter construct contained a SV40 promoter. Each clone was cotransfected with a Renilla luciferase control plasmid into HeLa cells in quadruplicate. Transfection was performed using Lipofectamine 2000. Four hours after transfection, media was removed, replaced with fresh media, and incubated overnight for approximately 24 hours. Average firefly luciferase-to-Renilla luciferase ratios were determined (Promega Dual Glo). The overall enhancer value was determined by dividing this ratio by the baseline luciferase-to-Renilla ratio using the pGL3 promoter vector containing only the SV40 promoter. Therefore, an enhancer value of 1 displays no change relative to the promoter only vector. A positive control plasmid containing a potent SV40 enhancer displayed ~20-30 fold higher levels of basal level transcription **(Table 1)**. Enhancer values of cloned DNase/FAIRE regions ranged from 0.5-16 fold higher levels of basal level transcription. Some regions were cloned in the reverse orientation to confirm enhancer activity.

Table 1. Regions tested for enhancer activity by luciferase reporter assay. Coordinates (hg19) and primers used to amplify segments of DNA are indicated, and regions were cloned into the pGL3 promoter vector. Average signal over promoter indicates the relative enhancer activity. Enhancer_control contains the SV40 promoter and potent SV40 enhancer. Each sample was tested 4 times and standard deviation was calculated.

Name	Coords (hg19)	Forward	Reverse	Average signal over promoter	STDEV
Enhancer_control				34.36	15.96
Region_1	chrX:153361725-153362951	ACACATCTGCCTGACCATGA	ATGGCGTCCCTCCTCTCTAC	0.59	0.18
Region_2	chr11:64611307-64612444	CAGGCTGCAGTGGTCTTGTA	CCAGGTAAGTCTGGGTCTGG	0.61	0.16
Region_3	chr15:43802205-43803866	TGGGGGAGGCTAGACTAACA	AGTGGTTGGCTACTGGCATT	0.39	0.10
Region_4	chr19:54344845-54346662	GCAAATTGAGGCTTGGGTTA	GGCAATTTTGCCTCTTGTTT	1.28	0.37
Region_5	chr19:54382099-54383798	ATGATCACAGAACCCGCTATC	CTGGCTCTATGAATCTCTATGTCTC	0.58	0.16
Region_6	chr20:33891153-33892556	CCTCTTCCCTTTCTCCGTCT	GGCTTCTCTGGATGATGACC	0.78	0.19
Region_7	chr21:34754815-34755901	TTATCCAGCCAAAACCATCC	AAAACGCCAAAATGACATCC	0.94	0.13
Region_7_reverse	chr21:34754815-34755901	TTATCCAGCCAAAACCATCC	AAAACGCCAAAATGACATCC	1.34	0.46
Region_8	chr1:151482839-151484043	CCCAGCAACGTAAACGAAGT	CTGAAGGCTCGCTTTCCTCT	0.68	0.26
Region_9	chr21:35319596-35321303	CATGGCATATTTGCATGAGC	GAGAAAGGGTTTGGGTCCAG	3.46	2.63
Region_10	chr22:32026374-32027549	AGGAGACGGAGCTCTCAGC	CATGGGCTCTGCATCCTAGT	0.75	0.29
Region_11	chr5:131721718-131723070	GTGAGATCACCAGCCCAAGT	AACCCGTGGTGAAAAGAGTG	1.53	0.50
Region_11_Rep2	chr5:131721718-131723070	GTGAGATCACCAGCCCAAGT	AACCCGTGGTGAAAAGAGTG	0.68	0.14
Region_12	chr6:41690828-41691929	CTGCCCATGACCTTCTCACT	GGCTGAGGAACCACTGAACT	2.77	0.56
Region_12_reverse	chr6:41690828-41691929	CTGCCCATGACCTTCTCACT	GGCTGAGGAACCACTGAACT	2.27	0.51
Region_12_Rep2	chr6:41690828-41691929	CTGCCCATGACCTTCTCACT	GGCTGAGGAACCACTGAACT	1.67	0.32
Region_13	chr6:41701243-41702452	AAGCAGAGCATGGAGTCACC	AGTCCCTGGGCAGTCTCTTT	0.85	0.20
Region_14	chr9:131900863-131902480	ATCCCTCCTCAGTCCCTTTC	AGGGGAATCGTGTGAGTCAG	11.25	5.09
Region_14_reverse	chr9:131900863-131902480	ATCCCTCCTCAGTCCCTTTC	AGGGGAATCGTGTGAGTCAG	7.54	4.71
Region_14_Rep2	chr9:131900863-131902480	ATCCCTCCTCAGTCCCTTTC	AGGGGAATCGTGTGAGTCAG	9.47	4.17
Region_15	chr9:131901849-131903151	GCACACGTTGTGTTGGTTCT	ACCTCTGGGGAGCTTTGTTT	13.78	6.57
Region_15_Rep2	chr9:131901849-131903151	GCACACGTTGTGTTGGTTCT	ACCTCTGGGGAGCTTTGTTT	16.10	5.04
Region_16	chr9:132175942-132176979	AGGGGAAAACACAGCAAGTG	CAGAAGCCCAGACCTCTGAC	2.49	1.84
Region_16_Rep2	chr9:132175942-132176979	AGGGGAAAACACAGCAAGTG	CAGAAGCCCAGACCTCTGAC	7.16	5.08
Region_17	chr6:108323294-108324969	AACATGGGGGAAATTCAACA	GGTGCATGTGACCATTGACT	1.32	0.53
Region_18	chr18:25944790-25946297	TGCTCATATCTGGTATTCATCTTCA	CTCCTGTCTCCTGACCCATC	0.88	0.17
Region_19	chr7:114170868-114171982	TGCACAGTAAGAGTTAGGAATGATG	CAATGGAATATGTCAATGCTACG	0.66	0.18
Region_20	chr7:116416930-116418352	AACCAGTGACTAATGGGGAAA	ATTTTTCAGGCTGCTGCAAT	2.89	0.94
Region_21	chr7:116440376-116441997	AAGGGAAATGAGGCTTGGAG	ACGCAGGAGAATTGGACTTG	0.88	0.25
Region_22	chr7:114687466-114689140	AAACTTTCCACCCGACACAC	AATCGCCCTCTGAGGTTTTT	0.98	0.26
Region_23	chr21:33896270-33897669	ATTTCTGGCAGAGCCCTTTT	CCACTGCTTTTGCTTCTGG	0.94	0.26
Region_24	chr9:132004636-132006267	TACGGAGCTCCTGGACAGAT	CACAGCCCTAGGGTCCTACA	1.79	0.24
Region_25	chr1:151285166-151286681	GGGACAAAAGCCTTGACAGA	CCCACGTTTTTGATACAATGG	1.09	0.41
Region_26	chr5:56300124-56301703	GAGCTGACTGCCAAGGTCAT	AACCACACACCACTGTGTCAA	2.11	0.84
Region_27	chr20:33944777-33946386	GAGCAATTGAGGTTCAACAGG	GGAGGAGCAACTTGAGTTTGA	0.97	0.17
Region_28	chr7:116444398-116445819	TGCTTAAAAACCTATGCCCATT	CCAAAAAGGATGAAGAAAATCAA	0.86	0.20
Region_29	chr18:61307913-61309379	TCCTGTGATTTCCTCCTTGG	GGTAGACAGCGCTGCATCTT	1.40	0.19
Region_30	chr9:132178620-132179879	AAGGGTTGGGGTTCCTAACA	TGTGCACTTGGGCTTAGTTG	1.33	0.53
Region_31	chr21:33814023-33815530	GGGGGAAATTATGGGACTTG	GCTTCCATTGGTACAATGCTG	0.52	0.26
Region_32	chr18:61271228-61272884	TGAGGTAGGAGTCTGGACATGA	GATTCATCCCTTGGCACTGT	0.86	0.42
Region_33	chr22:33095840-33097265	CCTTGAGGCAAGGTCTATGA	GTGGGAGGGCAGTTTCTGTA	1.52	0.19
Region_34	chr7:114194483-114196031	GGCTAATGAAACTTGGTTCTGC	TGGTTCCTGACAGGTAGAGATG	0.78	0.10
Region_35	chr5:142059897-142061578	ACACAGGAGTCTGGGAATGG	TGCCACTGCTAGGGTAGCTT	0.76	0.24
Region_36	chr13:30983323-30984838	AGCGGGAAATCCTCTCTCAT	GCTTTTTCAATGAGGCCAAA	0.84	0.12
Region_37	chrX:153347580-153348829	CAAAACCCGGAAGTACACAGA	TTCCTGGCGTCATTCTAAGG	1.56	0.49
Region_38	chr20:34034654-34036016	CCAGAGTCCCTTCCCTTTTC	GAGTGGAGGAGTCCAGCAAG	1.27	0.41
Region_39	chr7:27227496-27229325	CAACTTCAGCTTGTGCCTGA	GCATTTAAAGGGCTGCATGT	1.00	0.45
Region_40	chr11:64496710-64498072	GCAGCTGCGTCATCAAAATA	TGCGATTTCTCCCAATCTCT	0.29	0.12
Region_41	chr12:40718658-40719810	ACATGGTGCATCCTTCCTCT	AACCCCTCAAATCACACCAC	1.02	0.45
Region_41_Rep2	chr12:40718658-40719810	ACATGGTGCATCCTTCCTCT	AACCCCTCAAATCACACCAC	0.96	0.30
Region_42	chrX:153942447-153943998	CAGAAACAGCCAATGAAAAGC	TTCCCATGATGCTGACACTC	0.66	0.23
Region_43	chr21:34752309-34754032	GACAGGGATTGCTCACCAAG	GAGGTGCCTTCCAGAAAGTG	5.62	2.19
Region_43_Rep2	chr21:34752309-34754032	GACAGGGATTGCTCACCAAG	GAGGTGCCTTCCAGAAAGTG	5.71	1.89
Region_44	chr1:151518747-151520479	ATGCCAAGTCTTTCCAGAGC	TTTTATGTGGCTGCCTACCC	0.84	0.25
Region_44_Rep2	chr1:151518747-151520479	ATGCCAAGTCTTTCCAGAGC	TTTTATGTGGCTGCCTACCC	5.37	0.97
Region_45	chr13:113365591-113367252	AGCAGGAAAATTGTCCCAAA	TCCAGCATGGCTGCTATAAA	2.52	0.77
Region_46	chr9:131928553-131930217	AAGTTATCTGGGCAGAGCTGA	CAGAAGGAACGGAAAAGGTG	1.11	0.32
Region_47	chr1:151520675-151522562	TCCGGCTCTGTTCTTTGAGT	AGTGAGCCCTGTGACACTCC	0.75	0.23
Region_48	chr11:64614022-64615656	AGGTTGAGGGAGGAGGTGTT	ATTGGAGCTGCAAAGGAATG	0.69	0.26
Region_49	chr5:56196280-56197558	GTGCCTGTGTCAGGAAGACA	GTGTGAGGGAAGGTGCAGAG	0.82	0.08
Region_50	chr8:119132495-119133711	GGCAGCGAGTCGAGTTTATT	TGGTCCATCTTATTCGTGTGC	2.52	0.84
Region_51	chr11:1792316-1794662	GGAGGAAAAAGGCAAAGAGG	GGAAGAGGACACAGCAGAGG	0.85	0.27
Region_52	chr21:35028032-35029703	AAGGGCCTTGAAACAAGACT	GGGCAAGGAAATAGCTGAAA	8.56	2.06
Region_53	chr1:151568770-151570269	TTGCTTACTCAGCAGCAATAGG	TATGTGAGTGACAGCCATTTCC	2.41	0.56
Region_54	chr13:30716151-30717784	GAAGCAGCTCCTGGACATTT	ATTGAGTCTGGGGTCAGGTG	0.83	0.58
Region_55	chr21:35054457-35056210	TGGCTACTCAGGACAACTATTGG	TTGCACACATTCCCAACCTA	1.22	0.17
Region_56	chrX:153754777-153756052	TTGCAATTCTGGGTTTCCTC	CCCTGGATTGGTGTCTCATC	0.96	0.29
Region_57	chrX:153617439-153618684	AGAGCCCAGCAAAGAGGAA	CACGACCTCTGGACGTTTCT	1.22	0.21
Region_57_Rep2	chrX:153617439-153618684	AGAGCCCAGCAAAGAGGAA	CACGACCTCTGGACGTTTCT	0.79	0.16
Region_58	chr13:30968702-30970733	GAGGGAGAAAACCAAACTTCC	TAGCAGGGCTTCTTCAGCAT	0.85	0.19
Region_58_Rep2	chr13:30968702-30970733	GAGGGAGAAAACCAAACTTCC	TAGCAGGGCTTCTTCAGCAT	0.81	0.24
Region_59	chr1:151223234-151224524	TTGCCCCAATAGGATGTGAT	TCAGACCAGATGACGATTTCC	0.87	0.39
Region_60	chr7:27228888-27229903	TCGGAATAAAGTGGGTCCAG	CCCTAAGCAATCCACCAAGA	0.74	0.37
Region_60_Rep2	chr7:27228888-27229903	TCGGAATAAAGTGGGTCCAG	CCCTAAGCAATCCACCAAGA	1.15	0.30