Supplementary Information

TFIIH generates a five base-pair open complex during RNAP II transcription initiation and start-site scanning

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TATA-box dependence

While 88/992 (8.9%) traces showed activity with an intact TATA-box, 0/52 (0%) traces showed activity in the mutant (A → G, TGTGTGGTA) TATA-box that showed no transcription in a runoff transcription experiment (Fig. 1c, p = 0.008 for null hypothesis of no TATA-dependence).

pGOHis4TATA Sequence

The sequence of the PCR amplified region of pGOHIS4TATA used for making promoter DNA tether constructs is shown below:

GAGAGTACGCGTTCCTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAGGAGCGCCAGCAACGCGGCCTGGTGACG
GTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTAGTA
CCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAA
GAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCAGATCTGCAGCTGGCACGACAGGTTTCCCGA
CTGGAAAGCGGGCAGTGAGCGCAACGCGGTGAGTGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTGACACGTGA
TGCTCCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAAGTTCACACAGGAAACAGCTATGACCATGATTAC
GCCAAGCGCGCACTCGACCCTCACTAAAGGGAACAAAAGCTGGGTACCGCGATAAGCTTAGCAGCTTGTGAGGTCGA
AGCGCTTCTGGAGAAACTCAACGAGCTGGACGCGGATGAACAGGCAGACATCTGTGAATCGCTTCACGACCACGCTG
ATGAGCTTTACCGCAGCTGCCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACG
GTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCG
GGGCGCAGCCATGACCCAGTCACGTAGCGATAGCGGAGTGCCTGAACTGGATCAGTGCACAAGCTTGATATCGAATT
CCTGCAGCCCGGGGGATCGATCCGGGTGACAGCCCTCCGAATTCGAGCTCGGTACCCGGGGATCTGTCGACCTCGAG
AACAGTAGCACGCTGTGTATATAATAGCTAcGGAACGTTCGtTTCACCTCCGcTGcGTGTTGcACcTACAcAAATCC
TTAATACTCCATCCTTACTGTGTAATAGTAATACAATAGTTTACAACGTTTCTGTTCTGAATAATGACCGGATCCGG
AGCTTGGCTGTTGCCCGTCTCACTGGTGACGAGAACAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCCCGCG
CGTTGGCCGATTCAGATCTGCAGCTAGAGCGGCCGCCACCGCGGTGGAGCTCCAATTCGCCCTATAGTGAGTCGTAT
GACGCGCGCTCACTGGCCGTCGGTCGACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAGTCGCCTTGC
AGCACATCCCCCTTTCGCCAGCTGGCGTAACAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCC
TGAATGGCGAATGGGACGCGCCCTGTAGCGGCGCAGGACGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCT
ACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCG
TCAAGCTCGAGACCGGGGGCTCCCTTTAGGGTTCCGACTTAGTGCTTTACGGCACCTCGACCCCGAGAGACTTGATT
AGGGTGATGGTTCACGTAGAGGGCCATCGCCCTGATAGACGGTTCGTCGCCCTTTGACGTTGGAGTCCACGTTCTAG
ATCTGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTGAGAAGGGACTTT
GCCGATTTCGGCCTATTGACTAGAACAGGAGCTGATTCGACACGAAGGAACGCGAACTGAACACGATATCAACGCTT
ACAAGCTAGGTGGCACTTCTCGGGGAAATGTGCGCGGAACCCCTATGTGTGTACTTGTCTACGTACATTCAGGTATG
TATCCGCTCATGAGACAACTACCCTGACGACTGCTTCAGTCCTATTGAAACAGGAAGAGTATGAGTATTCAACATTT
CCGTGTCGCCCTGTATCCCTCCGTTGCGGCACATTGCCTTCCTGTTCTTGCTCACCCTAGGTCTCTC

The *S. cerevisiae HIS4* gene TATA sequence is shaded in red. The promoter was engineered to have a strong TSS using the sequence from the yeast U4 small RNA gene SNR14 (green, with start-site bold underlined). Flanking sequences were engineered to eliminate the presence of TATA-like sequences. Additional site-directed mutagenesis in the region between TATA and *SNR14* TSS was required to eliminate TATA-independent transcription, lower case bases. The primers used to amplify the region are shaded in grey (dark, forward primer; light, reverse primer complement). The restriction sites used for attaching 1 kb upstream digoxigenin-labeled DNA (MluI, ACGCGT) and 1 kb downstream biotin-labeled (StyI, CCTAGG) DNA are underlined.