Electronic Supplementary Material (ESI) for Molecular BioSystems.

Supplementary Material

The effect of sequence context on the activity of cytosine DNA glycosylases.

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Sequences of oligonucleotides that were used to construct hUDG

5′-AGCTCAGTCATATGGGCGGCTTTGGAGAGAGCTGGAAGAA-3′
5′-CTTGATAAAATACGGTTTCCCGAACTCCCCGCTGAGGTGCTTCTTCCAGCTCTCTCCAAA-3′
5′-GGAAACCGTATTTTATCAAGCTAATGGGATTTGTTGCAGAAGAAAGAAAGCATTACACTG-3′
5′-ATCTGGGTCCAGGTGAAGACTTGGTGTGGGGGTGGATAAACAGTGTAATGCTTTCTTTCT-3′
5′-GTCTTCACCTGGACCCAGATGTGTGACATAAAAGATGTGAAGGTTGTCATCCTGGGACAG-3′
5′-GCAGAGCCCGTGAGCTTGATTAGGTCCATGATATGGATCCTGTCCCAGGATGACAACCT-3′
5′-TCAAGCTCACGGGCTCTGCTTTAGTGTTCAAAGGCCTGTTCCGCCTCCGCCCAGTTTGGA-3′
5′-AAAATCCTCTATGTCTGTAGACAACTCTTTATAAATGTTCTCCAAACTGGGCGGAGGCGG-3′
5′-CTACAGACATAGAGGATTTTGTTCATCCTGGCCATGGAGATTTATCTGGGTGGGCCAAGC-3′
5′-GCACGAACCGTGAGGACAGCGTTGAGAAGGAGAACACCTTGCTTGGCCCACCCAGATAAA-3′
5′-GCTGTCCTCACGGTTCGTGCCCATCAAGCCAACTCTCATAAGGAGCGAGGCTGGGAGCAG-3′
5′-TCGAGTTCTGATTTAGCCAGGACACAACTGCATCAGTGAACTGCTCCCAGCCTCGCTCCT-3′
5′-CTGGCTAAATCAGAACTCGAATGGCCTTGTTTTCTTGCTCTGGGGCTCTTATGCTCAGAA-3′
5′-TAGTACATGGTGCCGCTTCCTATCAATGGCACTGCCCTTCTTCTGAGCATAAGAGCCCCA-3′
5′-GGAAGCGGCACCATGTACTACAGACGGCTCATCCCTCCCCTTTGTCAGTGTATAGAGGGTTC-3′
5′-AGCAGCTCATTGGTCTTTGAAAAGTGTCTACATCCAAAGAACCCTCTATACACTGA-3′
5′-TCAAAGACCAATGAGCTGCTGCAGAAGTCTGGCAAGAAGCCCATTGACTGGAAGGAGCTG-3′
5′-TCCGAGTCGAATTCTCACAGCTCCTTCCAGTCAATGG-3′

hUDG was generated by complete gene synthesis from two rounds of PCR. The first round produced a mixed product of varying length that contained a small proportion of full length products. The product was subjected to further amplification using the terminal oligonucleotides to increase the amount of full length product. This product was further purified by PCR clean-up and cloned into pET28a. hUDG then underwent successful site directed mutagenesis to produce hCDG (N204D) and hCYDG (N204D:L272A), and expressed in *E. coli*.