Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Biochip Based Epigenotyping Using a Methyl Binding Domain Protein

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Supplementary Fig. 1 Representation of the MBD protein structure. Amino acids 1-75 of the murine MBD1 protein are fused to a downstream enhanced green fluorescent protein (EGFP) tag. While not used directly for fluorescent detection, the EGFP tag significantly increased the soluble yield when expressed in *E. coli*. An N-terminal His_6 tag is used for immobilized metal affinity chromatographic (IMAC) purification of the protein from bacterial cells used to express it, and a C-terminal biotin acceptor peptide sequence (MAGGLNDIFEAQ**K**IEWHE) provides the recognition site for the BirA biotin ligase enzyme to covalently attach a biotin to the bolded lysine residue. 410 AA. Theoretical pI/MW: 5.98 / 46.3 kDa.

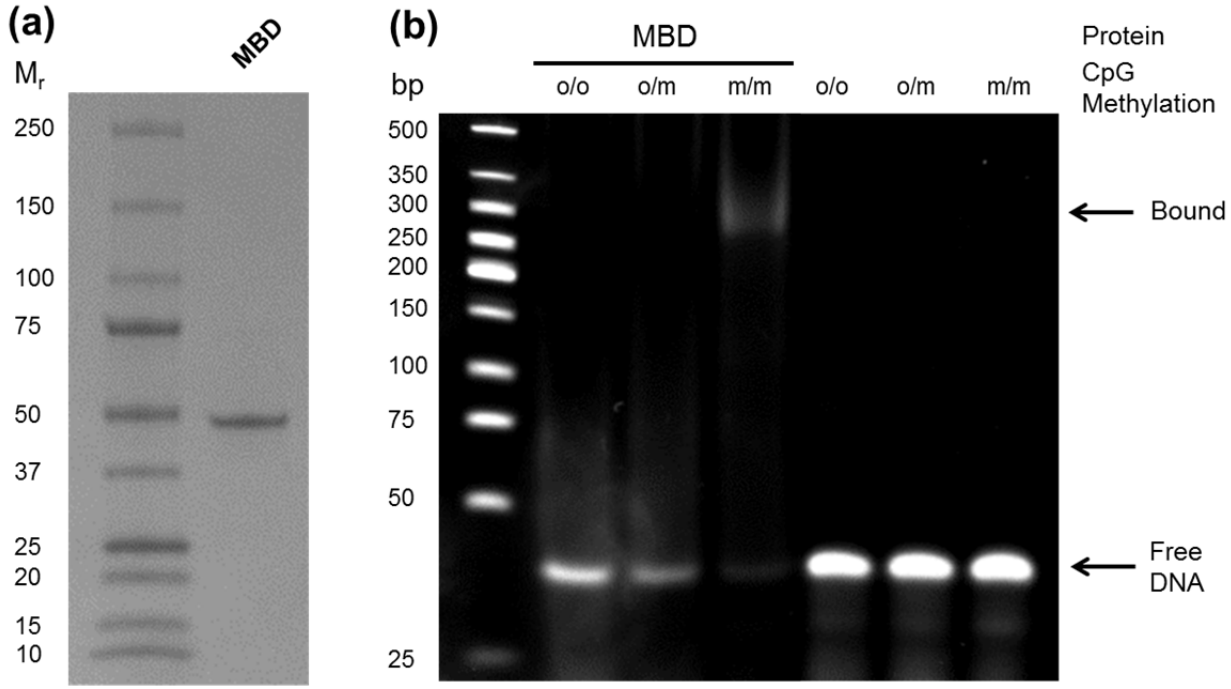
cDNA sequence:

ATGCACCATCATCATCATCATTCTTCTGGTCTGGTGCCACGCGGTTCTGGTATGAAAGAAACCGCTGCTGCTAAATTCGAACGCCAGCACATGGACAG
CCCAGATCTGGGTACCGACGACGACGACAAGGCCATGGCGATATCGGATCCGAATTCGATGCCAAAAAAGAAGAGAAAGGTAGATATCATGGCTGAGT
CCTGGCAGGACTGCCCAGCCCTGGGCCCTGGCTGGAAACGCCGAGAGTCCTTTCGAAAGTCAGGGGCCTCGTTTGGACGCTCAGACATCTATTACCAG
AGCCCCACAGGAGAAAAGATCCGAAGCAAAGTTGAGCTGACTCGGTACTTGGGCCCTGCATGTGATCTTACCCTGTTTGACTTCAGACAAGGCACCTT
ATGCCATCCCATTCCCAAGTACCCATACGACGTGCCAGACTACGCGTACCCATACGACGTCGCCACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCG
GGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTG
ACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCC
CGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGA
CCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAG
CTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGA
CGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCG
CCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCCCGGCATGGACGAGCTGTACAAG
ATGGCGGGCGGCCTGAACGATATTTTTGAAGCGCAGAAAATTGAATGGCATGAATAA

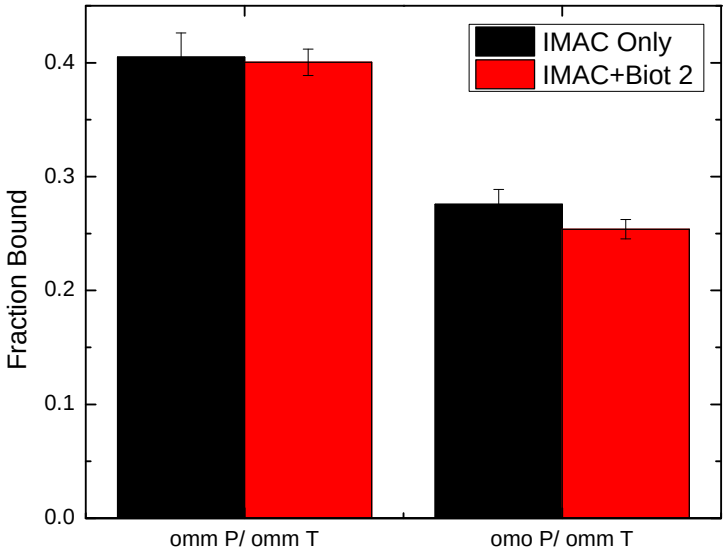
Primary amino acid sequence:

MHHHHHHSSG LVPRGSGMKE TAAAKFERQH MDSPDLGTDD DDKAMAISDP NSMPKKKRKV DIMAESWQDC PALGPGWKRR ESFRKSGASF
GRSDIYYQSP TGEKIRSKVE LTRYLGPACD LTLFDFRQGT LCHPIPKYPY DVPDYAYPYD VATMVSKGEE LFTGVVPILV ELDGDVNGHK
FSVSGEGEGD ATYGKLTLKF ICTTGKLPVP WPTLVTTLTY GVQCFSRYPD HMKQHDFFKS AMPEGYVQER TIFFKDDGNY KTRAEVKFEG
DTLVNRIELK GIDFKEDGNI LGHKLEYNYN SHNVYIMADK QKNGIKVNFK IRHNIEDGSV QLADHYQQNT PIGDGPVLLP DNHYLSTQSA
LSKDPNEKRD HMVLLEFVTA AGITPGMDEL YKMAGGLNDI FEAQKIEWHE

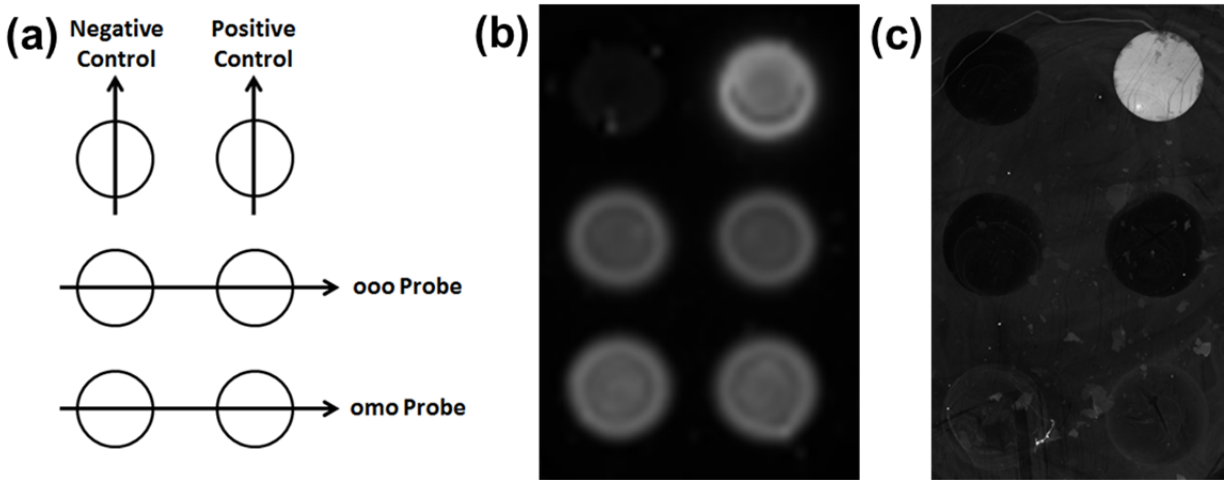
Supplementary Fig. 2 Characterization of the purified MBD protein. (a) Coomassie blue stained 4–15% SDS–PAGE gradient gel shows purified MBD protein (MW = 46.3 kDa). (b) Electromobility shift assay (EMSA) shows the capacity of purified MBD to bind methylated DNA. MBD protein was incubated with pre-hybridized dsDNA oligonucleotides with non- (o/o), hemi- (o/m) and singly, symmetrically-methylated (m/m) CpGs; concentration matched DNA samples were prepared without protein as controls. DNA with bound MBD protein migrates through the gel at a retarded rate thus appearing to have larger size when compared to the DNA ladder standard.



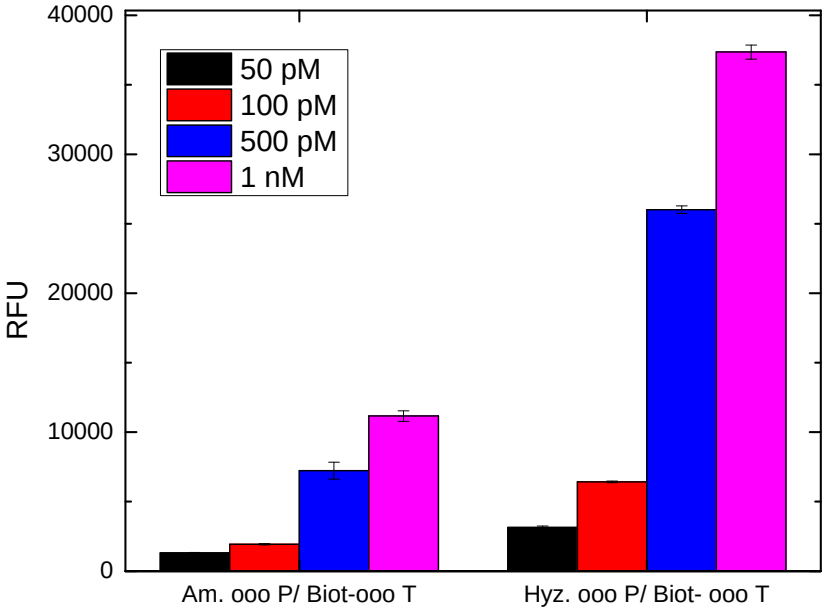
Supplementary Fig. 3 Comparison of the signal from doubly (omm) and singly (omo) methylated DNA using MBD purified using IMAC only or IMAC plus a streptavidin mutein affinity chromatography polishing step. The polishing step was not found to improve the fraction bound over the IMAC only case and was thus not routinely used.



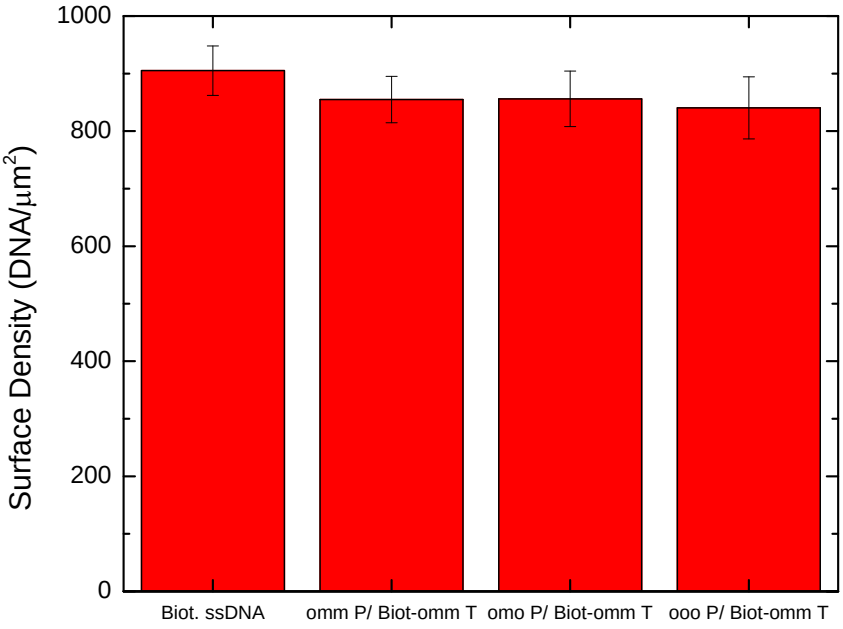
Supplementary Fig. 4 Biochip epigenotyping assay performance on a SCHOTT Nexterion Slide AL. (a) Biochip spotting layout for probe ssDNA. (b) Hybridization of biotinylated target ssDNA and streptavidin-Cy3 labeling shows that duplex DNA is being formed at each of the ooo and omo probe spots. (c) Hybridization of omo target ssDNA followed by 40 µg/mL MBD detection and streptavidin-Cy3 labeling. Fluorescence scanning shows high background signal from static MBD binding and omo probe/omo target signal not distinguishable from background on the self-assembled monolayer surface.



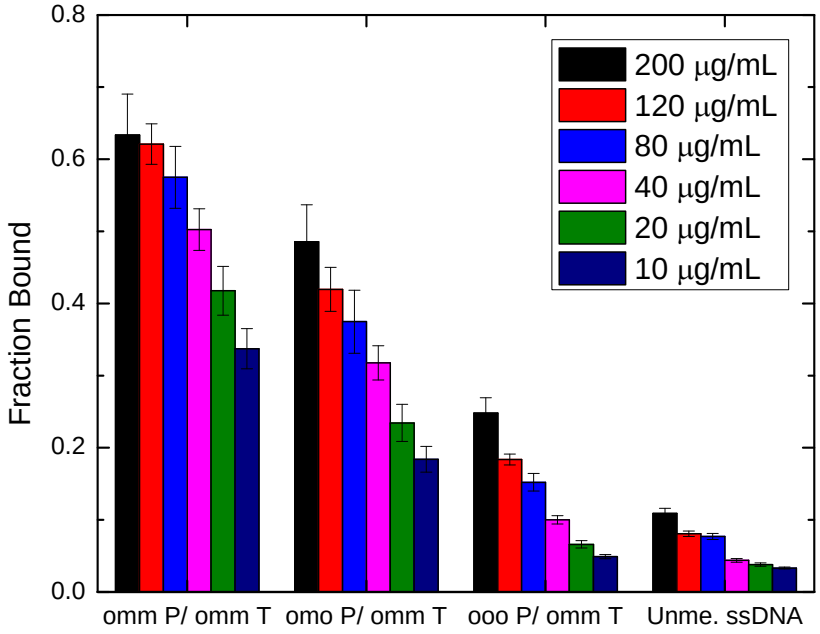
Supplementary Fig. 5 Comparison of capture performance of probe ssDNA oligos with either a primary amine (Am.) or hydrazide (Hyz.) modification to facilitate coupling to the aldehyde functional, agarose biochip. Sub-nanomolar concentrations of biotinylated target ssDNA were hybridized to each array and detected using streptavidin-Cy3 and fluorescence scanning. Spots printed using hydrazide functionalized probe produced a signal approximately threefold greater than those printed with the primary amine functionalized probe when incubated with target ssDNA at the same concentration.



Supplementary Fig. 6 The surface density of the biotinylated ssDNA control oligonucleotide and capture probes hybridized with 100 nM biotinylated target ssDNA were determined using streptavidin-Cy3 and fluorescence quantification. The 900±40 DNA/μm^2 surface density of the biotinylated ssDNA control was taken as the maximum for the hydrazide functionalized probes used in this study. Hybridization to each of the three (ooo, omo and omm) capture probes was equivalent within error; with 100 nM target, 94% of capture probes had bound target.



Supplementary Fig. 7 Comparison of fraction bound as a function of varying MBD concentration on arrays hybridized with 100 nM omm target ssDNA. The fraction of DNA probes with MBD bound decreases as a function of MBD concentration. While the highest MBD concentrations give larger fractional values, they have lower omm P:ooo P ratios indicating sub-optimal assay specificity. Using 40 µg/mL MBD decreases the fraction bound by as much as 13% but increases the omm P:ooo P ratio twofold relative to 200 µg/mL and has a non-specific binding level to unmethylated ssDNA comparable to the lowest MBD concentrations.



MBD Conc. (µg/mL)	ommP : oooP
200	2.6
120	3.4
80	3.8
40	5.0
20	6.3
10	6.9