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Methylated or unmethylated dsDNA

Figure S1. Preparation of the full-length dsDNA. (A) Schematic illustrations of the preparation of full-length dsDNA. (B) Methylated and unmethylated dsDNA were electrophoresed in a 1% agarose gel. Lane M, 100 bp ladder used as size marker; lanes 1-2, methylated full-length dsDNA and unmethylated full-length dsDNA, respectively. (C) Schematic illustration of obtaining methylated or unmethylated full-length dsDNA from PCR products.



Figure S2. CD spectra of 260 bp of *DRD2* dsDNA. Temperature-dependent CD spectra of unmethylated (A) or methylated (B) dsDNA. (C) Comparison of CD spectra of unmethylated (blue) and methylated (red) *DRD2* dsDNA at 60 °C.

Unmethylated CpG site
Methylated CpG site

Du145 cell (71.9 %, n=8)

LNCaP cell (4.2 %, n=8)

CpG site No.								CpG site No.															
1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
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Figure S3. The methylation status by bisulfite sequencing provided by Funakoshi Co., Ltd. The percentages in the parentheses indicate the methylation levels. The circles correspond to each of the CpG sites. The closed circle indicates methylated CpG sites, and the open circle indicates unmethylated CpG sites. A set of horizontally aligned circles represents an individual clone. The vertical number represents individual CpG sites. The "n" refers to the number of sequenced genomes.

	Sequences (5' to 3')	DNA size (base)		
DRD2_	CCCCACCAAAGGAGCTGTACCTCCTCGGCGATCCCCGG	71		
target 1	CCTGGAACGGGTAGGAGGGGTTGGGGGATTCCG	·····		
DRD2_	Phosphate_CCATCCCTTGTTTTGAGGCGGGAACC	60		
target 2	CT <u>C</u> GAC <u>C</u> GCCCACTG <u>C</u> GCTCCCACCCACACCCAGAGTA	69		
	Phosphate_ATAAGCTGTGATTGCAGGCTGGGTCCTCACC			
DRD2_	GTCTGCTCGCCAGTCTTCTCCTTTGAGGACTCAGAAGCC	120		
target 3	AAGGGTTGCGGGAGGCACCACCCATCCAGCAAGTCAG	120		
	GCAAGGAGAGGAG			
	CTCCTCTCCTTGCCTGACTTGCTGGATGGGTGGTGCCTC			
complement 1	CCGCAACCCTTGGCTTCTGAGTCCTCAAAGGAGAAGAC	97		
	TGGCGAGCAGACGGTGAGGA			
DRD2	Phosphate_CCCAGCCTGCAATCACAGCTTATTACTCTGG			
complement 2	GTGTGGGTGGGAG <u>C</u> GCAGTGGG <u>C</u> GGT <u>C</u> GAGGGTTG <u>C</u> G	75		
	TTCC <u>C</u> GC			
DRD2	Phosphate_CTCAAAACAAGGGATGGCGGAATCCCCCAA			
complement 3	CCCCTCCTACCCGTTCCAGGCCGGGGATCGCCGAGGAG	88		
complement o	GTACAGCTCCTTTGGTGGGG			

Table S1 List of separated synthetic DNA sequences.

Bold and underline means unmethylated or methylated cytosine.

Table S2 The pairs of hybridized DNA strands for ligation in <i>DRD</i> 2.
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Fragments	Target	Complement
Fragment 1	DRD2_target 1	DRD2_complement 3
Fragment 2	DRD2_target 2 (Unmethylated or methylated)	DRD2_complement 2 (Unmethylated or methylated)
Fragment 3	DRD2_target 3	DRD2_complement 1

Table S3 The sequence of the *AR* region inserted in plasmid DNA and primers for PCR.

	Sequences (5' to 3')	DNA size (base)
AR region inserted in plasmid DNA	GGGCTAGAGCTAGCCTCTCCTGCCCTCGCCACG CTGCGCCAGCACTTGTTTCTCCAAAGCCACTAGG CAGGCGTTAGCGCGCGGTGAGGGGAGGG	283
PCR forward primer	GCTAGAGCTAGCCTCTCCTG	20
PCR reverse primer	CAGCTTGCTGGGAGAGC	17

Table S4 List of LAMP primer sequences.

Target	Sequences (5' to 3')	DNA size (base)						
LAMP primers for <i>AR</i>								
AR_FIP	GGCCTCCTTGCCTTCCCACGGGAGGGGGGAGAAAAGGAAAG	39						
AR_BIP	GGACCCGACTCGCAAACTGTTTGGCTCCCCGGGATCT	37						
AR_F3	GTTAGCGCGCGGTGAG	16						
AR_B3	TCCCGCTCTCCCAGCA	16						
LAMP primers	for DRD2							
DRD2_FIP	GCGTTCCCGCCTCAAAACAAGGCCTGGAACGGGTAGGA	38						
DRD2_BIP	TAAGCTGTGATTGCAGGCTGGGCAACCCTTGGCTTCTGAGT	41						
DRD2_F3	TGTACCTCCTCGGCGATC	18						
DRD2_B3	CTTGCTGGATGGGTGGTG	18						

LAMP, loop-mediated isothermal amplification

Table S5 List of PCR primer sequences for bisulfite sequencing.

Target	Forward	Reverse	Product size (bp)	Nucleotide coordinates (Human Jan. 2022)
Primers for PCR of AR	CCAAATTTGGTGAGTGCTGG	CCTTCGGCTCCTGTACAGC	438	chrX:65,973,549- 65,973,986
Primers for PCR of bisulfite treated genomic DNA of AR	ATTAAATTTGGTGAGTGTTG GTTTT	CAATCCTACCAAACACTTTC CTTAC	600	chrX:65,973,548- 65,974,147