High-Specificity Kilobase-Level Multiplex PCR Enabled by Graphene Oxide-Dendrimer-Encapsulated Gold Nanoparticles

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Table S1. Functional CYP450 loci selected as targets for multiplex PCR amplification.

Gene	SNP (rs ID)	Chromosome	Genomic position	CHB allele frequency	Allele	Drug / disease relevance
CYP2C8	rs11572103	chr10	95,058,349	1.00	T	Associated with paclitaxel metabolism[1-3]
CYP2A6	rs1801272	chr19	40,848,628	1.00	A	Associated with nicotine metabolism, smoking addiction and withdrawal response[4-6]
CYP2J2	rs890293	chr1	59,926,822	0.04 / 0.96	A / C	Associated with coronary heart disease, myocardial infarction and cardiovascular risk[7-9]
CYP1A2	rs2069514	chr15	74,745,879	0.72 / 0.28	G / A	Associated with myocardial infarction[10]
CYP2E1	rs2070676	chr10	133,537,633	0.19 / 0.81	G / C	Associated with alcohol metabolism and liver injury susceptibility[11-14]
CYP2B6	rs8192709	chr19	40,991,369	0.98 / 0.02	C / T	Associated with efavirenz metabolism[15, 16]
CYP2	rs1065	chr22	42,130,692	0.40 /	G /	Associated with the metabolism

D6	852			0.60	A	of codeine and β-blockers[17-20]
CYP2C19	rs4986893	chr10	94,780,653	0.04 / 0.96	A / G	Associated with clopidogrel metabolism[21]

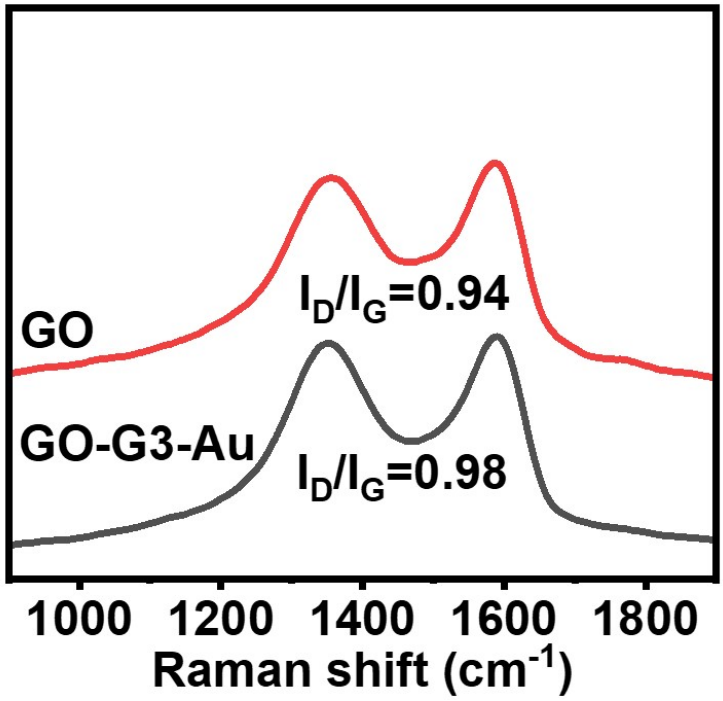

Figure S1. Raman spectra of graphene oxide (GO) and graphene oxide-dendrimer-encapsulated gold nanoparticles (GO-G3-Au).

Table S2. Comparative summary of PCR enhancement methods and the advantages of the GO-G3-Au system.

Method / Reference	Reported Enhancement	Limitation	Advantage of This Work
Au/GO[22]	1.5–2.4× yield increase of short amplicons	Mechanism speculative; lacking stability data	Achieved robust, high-yield and kb-level amplification with validated mechanism and material stability
AuNPs@GO[23]	Enhanced specificity; 90 min single-plex reaction	No decoupled AuNPs@GO analysis; insufficient mechanistic evidence	Applied to higher-plex PCR with high specificity, faster reaction and mechanistic insight

$(Au^0)_{300}$–G5.NH_2–mPEG^{24}	Specificity ratio improved to 0.45–0.53, around 20% higher intensity	Marginal improvement; unclear mechanism	Achieved high specificity (1.0) and high-yield amplification under challenging conditions
Molecular inversion probes[25]	Around 80% probe uniformity in non-model organisms	Limited by probe design and genomic interference	Superior multiplex uniformity, simplified operation, lower cost
GO-G3-Au (This work)	Greatly shortened kb-level multiplex PCR time (430→70 min) with improved specificity and multiplex uniformity ($\Delta Ct < 4$)	Scalability and tolerance require further study	Integrated electrostatic and thermal effects for highly specific, uniform, efficient amplification

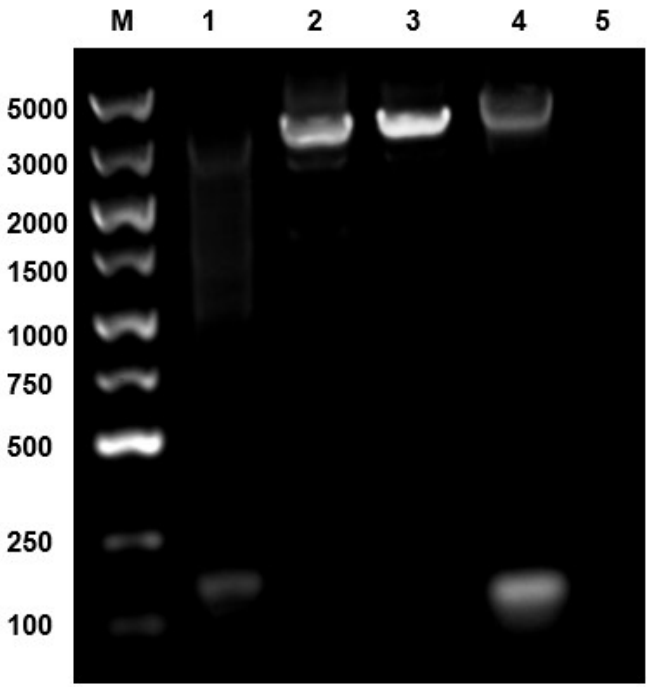

Figure S2. Electrophoresis results of semi-multiplex PCR performed using one forward primer and two reverse primers (matched and single-base mismatched) to evaluate the selective effect of GO-G3-Au on primer–template interactions. Lane 1 is the mismatched-primer-only group, lane 2 is the matched + mismatched primer group, lane 3 is the matched + mismatched primer group with GO-

G3-Au. Lane 4 is the matched-primer-only group. The final lane is the no-template control (NTC). M is the molecular weight marker, with units in bp. Data are representative of three independent experiments.

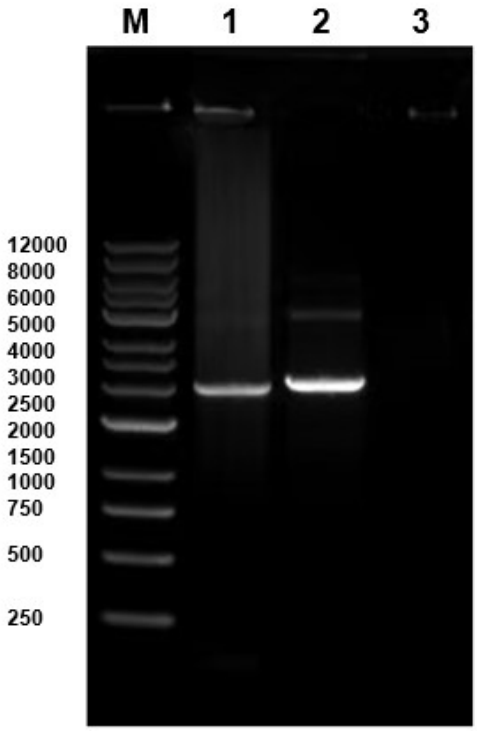

Figure S3. Electrophoresis results of a triplex mitochondrial PCR system with and without GO-G3-Au. Lane 1 is the PCR product without the nanomaterial, lane 2 is the PCR product with GO-G3-Au. Lane 3 is the NTC, and M is the molecular weight marker (bp). Data are representative of three independent experiments.

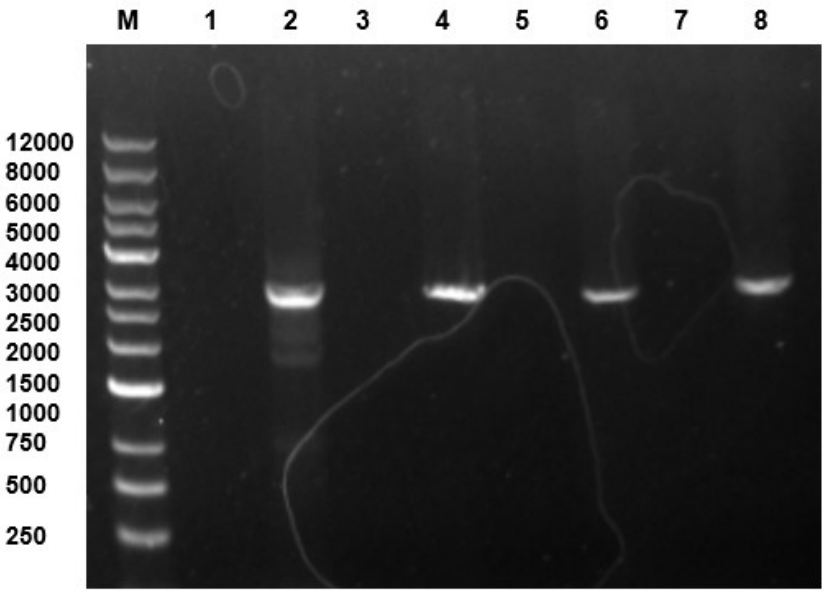

Figure S4. Evaluation of the long-term storage, freeze-thaw stability and experimental reproducibility of GO-G3-Au. Electrophoresis results of kb-level eight-plex PCR products amplified under different conditions: (Lanes 1 and 2) without nanomaterials, (Lanes 3 and 4) with freshly prepared GO-G3-Au, (Lanes 5 and 6) with GO-G3-Au stored at -20 °C for 5 months, and (Lanes 7 and 8) with GO-G3-Au subjected to three consecutive freeze-thaw cycles. The odd-numbered lanes (1, 3, 5, 7) represent the corresponding NTC for each reaction condition. Lane M: DNA marker (bp). These experiments were independently performed using different PCR instruments and by different operators, and comparable amplification patterns were obtained. Data are representative of three independent experiments.

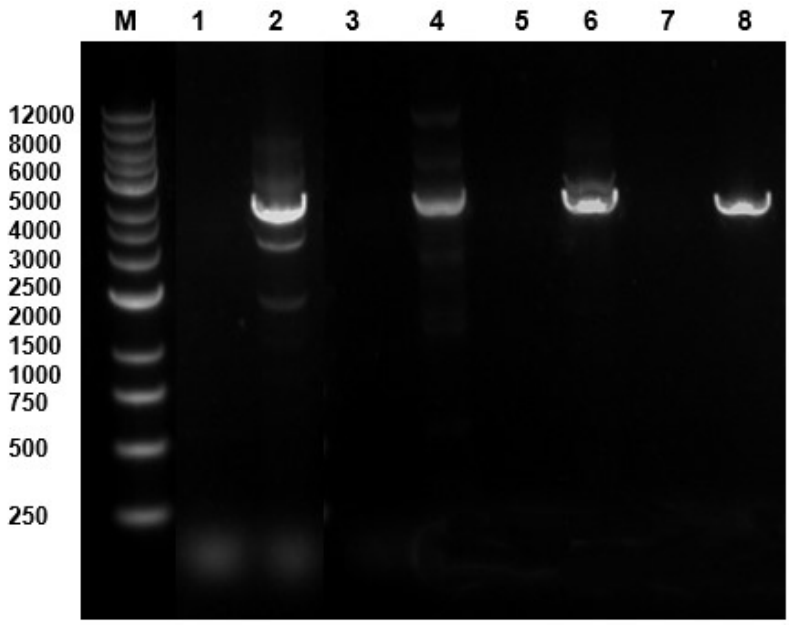

Figure S5. Electrophoresis results under different material conditions. Lanes 1, 3, 5 and 7: corresponding negative controls. Lane 2: PCR without nanomaterials. Lane 4: PCR with positively charged G3 dendrimer. Lane 6: PCR with thermally conductive AuNPs. Lane 8: PCR with GO-G3-Au composite. Lane M: DNA marker (bp). Data are representative of three independent experiments.

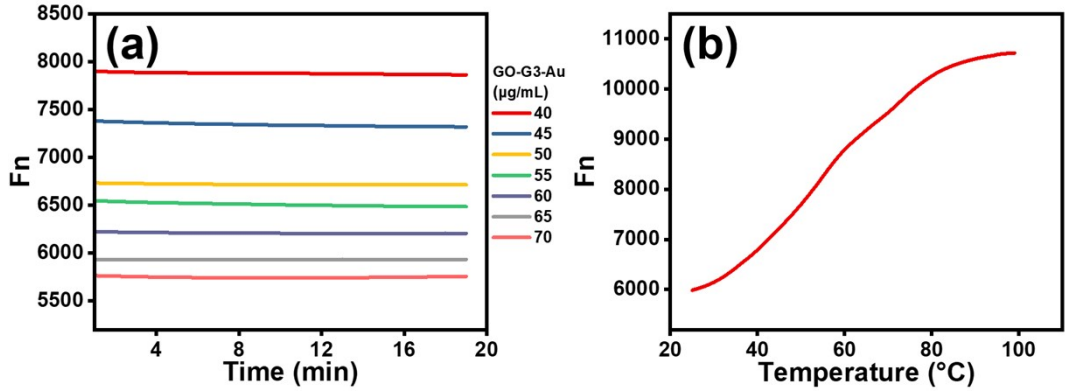

Figure S6. Fluorescence-based characterization of the interaction between FAM-labeled DNA and GO-G3-Au. (a) Time-dependent fluorescence quenching profiles of 0.4 μM FAM-labeled DNA in the presence of GO-G3-Au at different concentrations (40–70 μg/mL) recorded at 25 °C. (b) Temperature-dependent fluorescence response of 0.4 μM FAM-labeled DNA in the presence of GO-G3-Au (100 μg/mL) over the range of 25–100 °C.

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