Supporting Information

Efficient Polymerase Chain Reaction assisted by Metal-Organic Frameworks

Chunli Sun [a], Yong Cheng,[b*] Yong pan[a], Juliang Yang [c], Xudong Wang [a, *] and Fan Xia[a,c*]

[a] *Hubei Key Laboratory of Bioinorganic Chemistry & Materia Medica, School of Chemistry and Chemical Engineering, Huazhong University of Science and Technology, 1037 Luoyu Road, Wuhan 430074, China*

[b] *Sate Key Laboratory of Material Processing and Die & Mould Technology, School of Materials Science and Engineering, Huazhong University of Science and Technology, 1037 Luoyu Road, Wuhan 430074, China*

[c] *Faculty of Materials Science and Chemistry, China University of Geosciences, Wuhan 430074, China*

** Correspondence to: Fan Xia: E-mail: xiafan@hust.edu.cn*

Yong Cheng: E-mail: chengy@hust.edu.cn

Experimental procedures

Synthesis of ZIF-8 and UIO-66

UiO-66 was synthesized as follow: after fully dissolving of $ZrCl_4$ (0.053 g, 0.227 mmol) and 1,4‑benzenedicarboxylic acid (H_2BDC) (0.034g, 0.227 mmol) in N,N`‑dimethylformamide (DMF) (24.9 g, 340 mmol) at room temperature, the mixture was sealed and placed in a pre-heated oven at 120 ˚C for 24 hours. Crystallization was carried out under static conditions. Then the resulting solid after cooling in air to room temperature was filtered, washed with DMF and dried at room temperature.

ZIF-8 was synthesized using 2-methylimidazole as a linker. Briefly, 6.56 g of 2-methylimidazole and 2.95 g of Zn $(NO_3)_2 \cdot 6H_2O$ were dissolved in 120 mL and 60 mL deionized water respectively, and adjust the pH of the former to 9.5 with TEA. Then, the two solutions were mixed under stirring for 20 min. After that, the reaction solution was filtered and washed with deionized water several times with air compressor. To accelerate the filtration rate of the entire process, the system was pressurized using nitrogen. Finally, the microcrystalline production was obtained. The excess 2-methylimidazole and pore-occluded water were completely removed by three sonification in deionized water for 2 h. Unless otherwise specified, all operations were performed at room temperature. Then, the clean, empty and stable ZIF-8 was heated to 120 °C under vacuum for 3 days to remove the solvents and other guest molecules in the pores. The final yield of ZIF-8 was 1.804 g.

Preparation of working solution

After weighing, 10 mg UiO-66 or ZIF-8 was dissolved in 1 ml deionized water. Then the solutions were ultrasonic until stable suspensions were formed. They were stored at 4 ^{0}C after autoclaving.

Characterization of ZIF-8 and UiO-66

Powder X-ray diffraction (PXRD) spectra were recorded with a Rigaku 2500 VBZ+/PC. The X-ray generated from a sealed Cu tube was monochromatic by a graphite crystal and collimated by a 0.5 mm MONOCAP (λ Cu-Kα = 1.54178 Å). The tube voltage and current were 40 kV and 40 mA, respectively.

Nitrogen physisorption isotherms were measured at 77 K, on a Quantachrome Autosorb Automated Gas Sorption instrument. Samples were off-gassed at 80 °C under nitrogen for approximately 12 h. Surface area measurements were calculated using the Brunauer-Emmett-Teller (BET) method, pore size distributions were calculated using density functional theory (DFT).

Malvern Zetasizer Nano ZS model ZS90 was used to measure Dynamic light-scattering (DLS) and Zeta potential of suspensions of UiO-66 or ZIF-8 diluted with 1×PCR buffer.

Scanning electron microscopy (SEM) images were obtained with a Hitachi SU8010 field emission scanning electron microscopy at an acceleration voltage of 10 kV after gold coating.

Primers of polymerase chain reaction (PCR):

A 283-bp DNA fragment will be amplified from λ-DNA (Takara Bio Inc.) using one pair of primers (Shanghai Sangon Biological Engineering and Technology and Service Co. Ltd.).

primer 1　5′- GGCTTCGGTCCCTTCTGT-3′;

primer 2　5′-CACCACCTGTTCAAACTCTGC-3′.

PCR system

PCR reagents were mixed in a final volume of 25 μL according to the following conditions: 10× reaction buffer (Shanghai Sangon Biological Engineering and Technology and Service Co. Ltd.), 0.2 μM primers, 0.25 mM each dNTP, and 0.025 U/μL rTaq DNA polymerase (Shanghai Sangon Biological Engineering and Technology and Service Co. Ltd.), 2.5 μL template. Unless otherwise specified, PCR parameters were set as follows: 3 min at 95 °C for predenaturation, followed by 35 cycles of 20 s at 94 °C, 30 s at 55 °C, and 30 s at 72 °C. The final extension was performed at 72 °C for 5 min. Amplifications were carried out in a S1000 Thermal Cycler (Gene-Rad Inc.).

Evaluation methods of PCR results

The PCR products (5 μL) with 1 μL 6×loading buffer (Shanghai Sangon Biological Engineering and Technology and Service Co. Ltd.) were analyzed by 1.0% (w/v)

agarose gel electorphoresis stained with Super Gel-Red and visualized and photographed on a Tanon-5200 Chemiluminescent Imaging System (Tanon Science and Technology, Shanghai, China). DL2000 marker (Takara Bio. Inc.) was employed to mark the size of the DNA. The gray value of bands was calculated by Image J software. The capability of the tested additives to enhance PCR were figured out by the intensity of target DNA band after amplification. The concentration of each additive that made PCR produce the maximal specificity and the brightest target band was identified to be the optimum concentration. Each reaction was performed at least three times.

Fluorescence measurements

All solutions were diluted with 1×PCR buffer. First, the fluorescence tetramethylrhodamine labeled ssDNA probes (FP, its sequence and its complementary probe sequence see Supporting Information Table S1) were mixed with UIO-66 (320 mg L-1), ZIF-8 (80 mg L-1) or 1×PCR buffer. After 3 hours incubation at room temperature, the mixtures were divided evenly into two groups, one group add equal volume buffer, and the other group add equal volume solutions with complementary probe (CP, 150 nM). After overnight at 4 ^{0}C, the samples were incubated at room temperature for 4 hours, then fluorescence measurements were carried out on the AFS5 spectrofluorometer (Edinburgh Instruments) with an excitation wavelength of 547 nm.

Supporting information figures

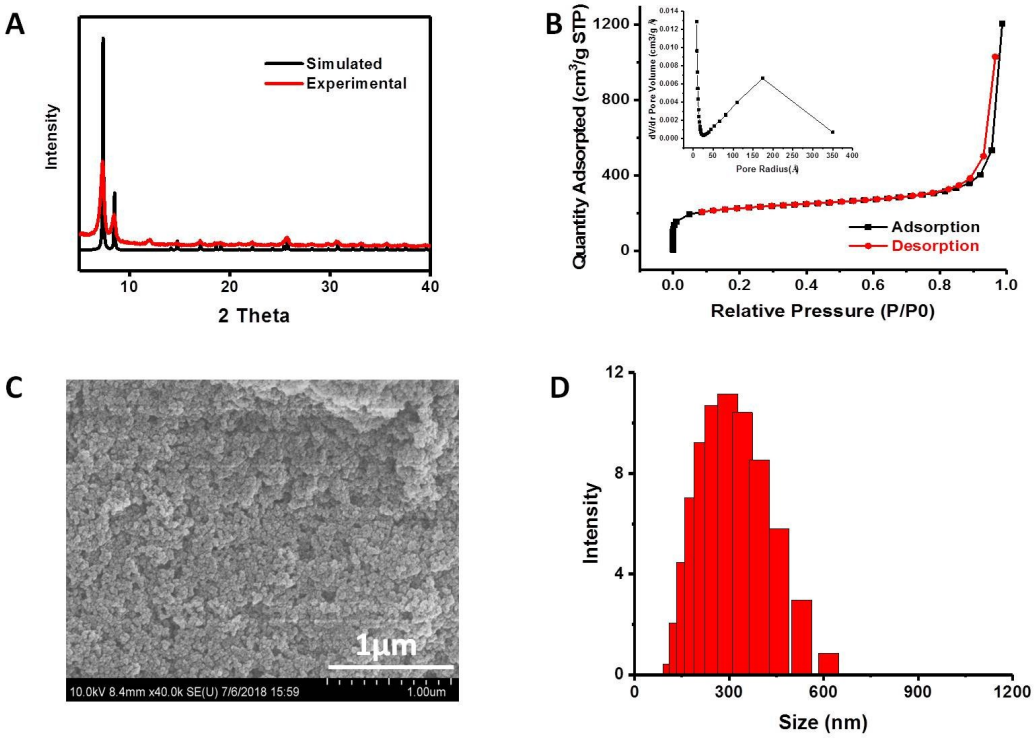


Figure S1 Characterization of the UiO-66.

A. PXRD patterns of UiO-66 showing agreement between the simulated and synthesized; B. Nitrogen gas adsorption isotherm at 77K for ZIF-8. P/P0 is the ratio of gas pressure (P) to saturation pressure (P0). Inset is the corresponding pore size distribution curves. C. SEM micrograph of UiO-66 particles; D. DLS spectrum showing the hydrodynamic diameter of UiO-66 particles in PCR buffer.

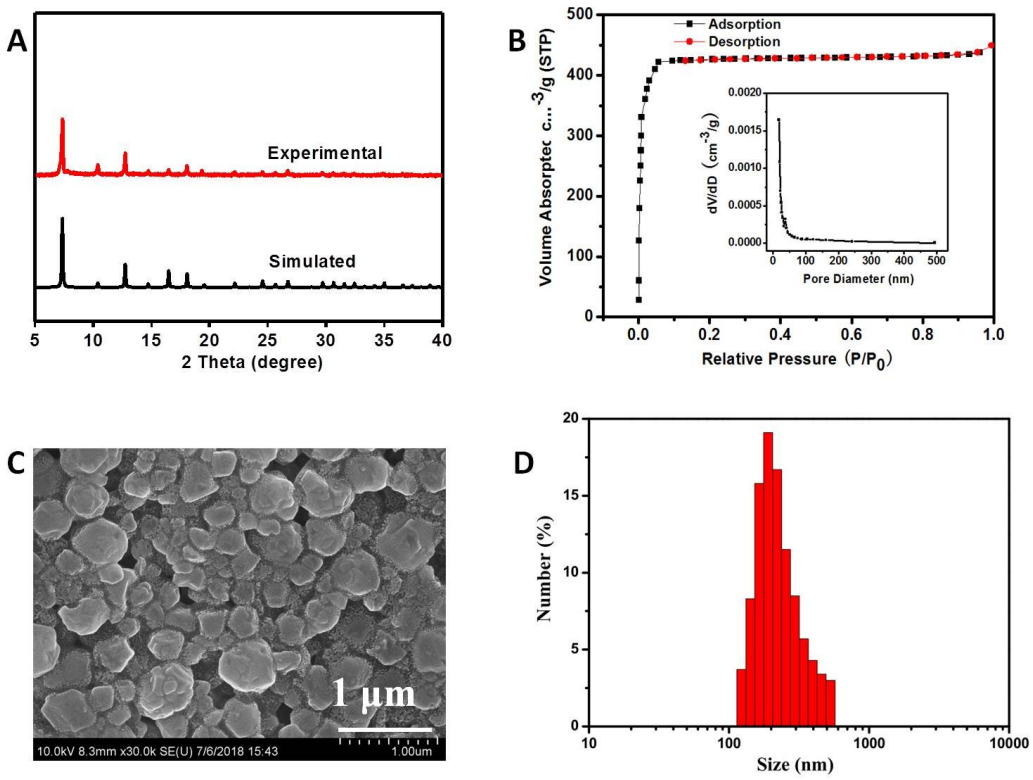


Figure S2 Characterization of the ZIF-8.

A. PXRD patterns of ZIF-8 showing agreement between the simulated and synthesized; B. Nitrogen gas adsorption isotherm at 77K for ZIF-8. P/P0 is the ratio of gas pressure (P) to saturation pressure (P0). Inset is the corresponding pore size distribution curves. C. SEM micrograph of ZIF-8 particles; D. DLS spectrum showing the hydrodynamic diameter of ZIF-8 particles in PCR buffer.

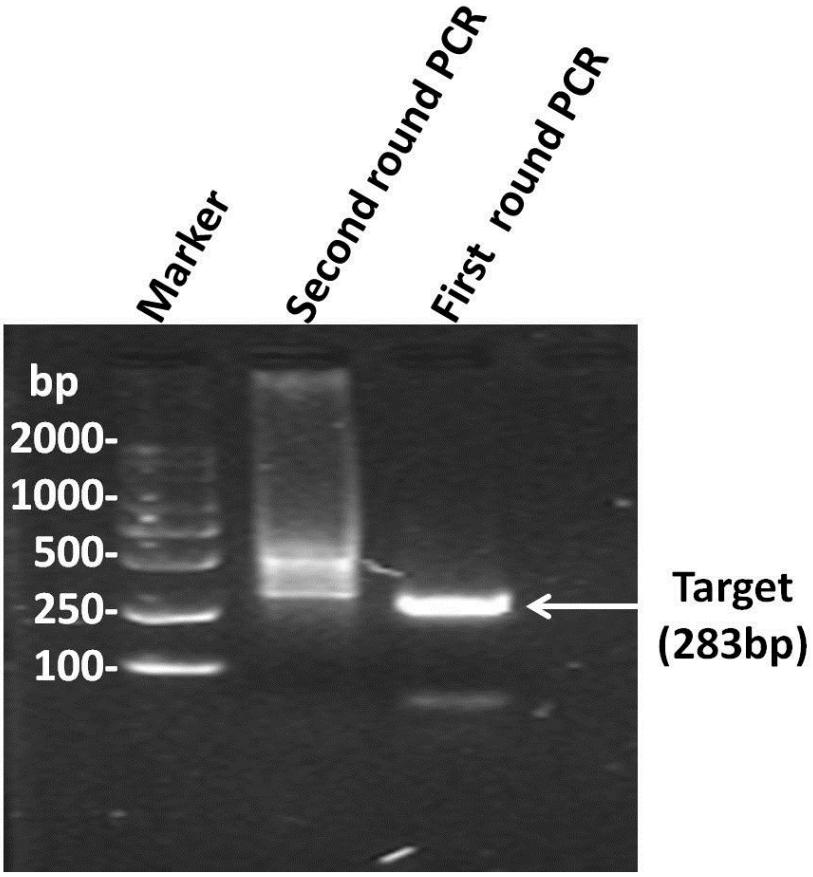


Figure S3. The results of the error-prone two round PCR. M, the DL2000 marker; Lane 1, the results of the second-round PCR; Lane 2, the results of the first round PCR.

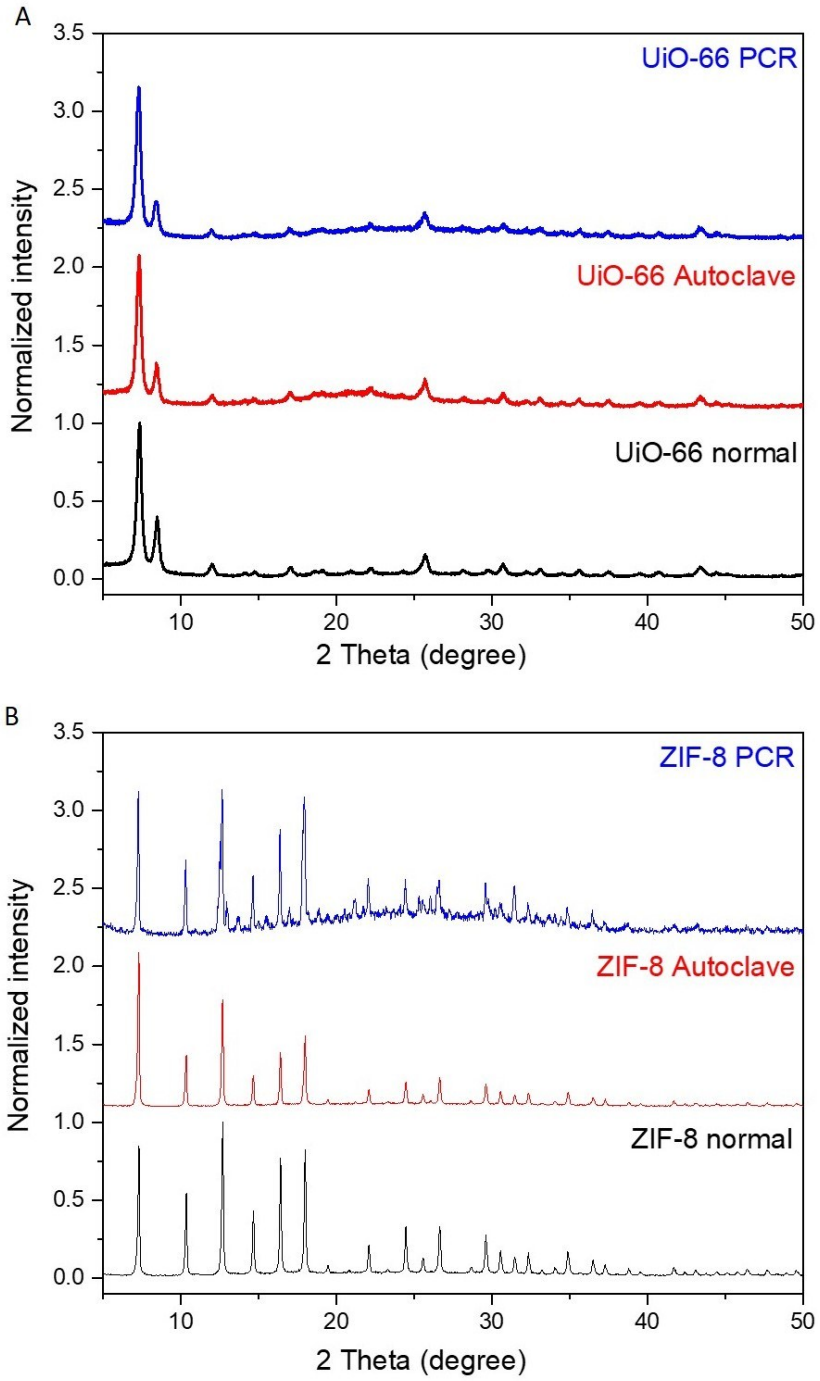


Figure S4. PXRD analysis of UiO-66 (A) and ZIF-8 (B) before and after PCR and autoclave.

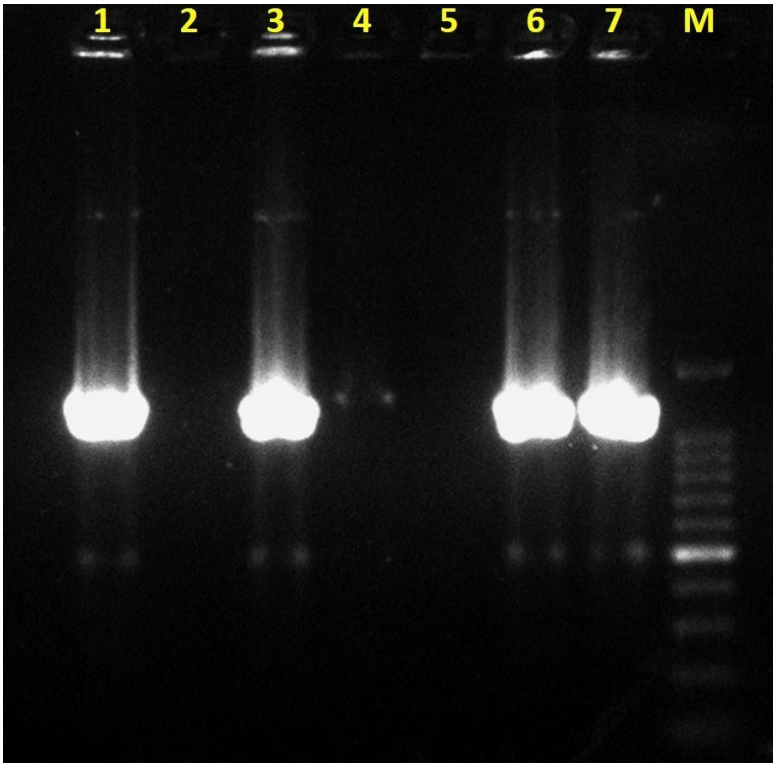


Figure S5. Gel electrophoresis of PCR production with different materials (Lanes 1-7, conventional PCR; Zn^{2+} in PCR; 2-methylimidazole in PCR; Zr^{4+} in PCR; 1,4-benzenedicarboxylic acid in PCR; ZIF-8 in PCR; UiO-66 in PCR).

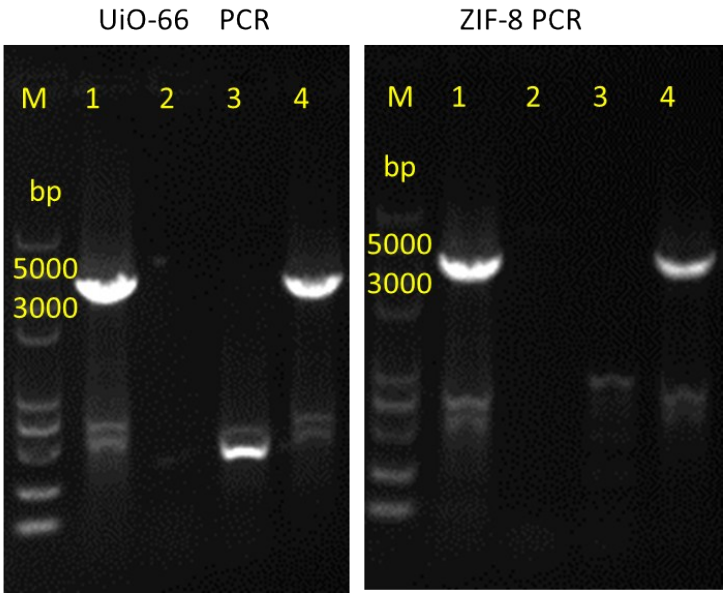


Figure S6. Gel electrophoresis of the effect of high concentrations of UiO-66 (left) and ZIF-8 (right) on PCR of 3530 bp amplified fragment. Target band: 3530bp; M: DNA marker; 1, conventional PCR; 2. PCR with 1000 mg·L^{-1} MOFs; 3, PCR with 100 mg·L^{-1} MOFs; 4. PCR with 50 mg·L^{-1} MOFs.

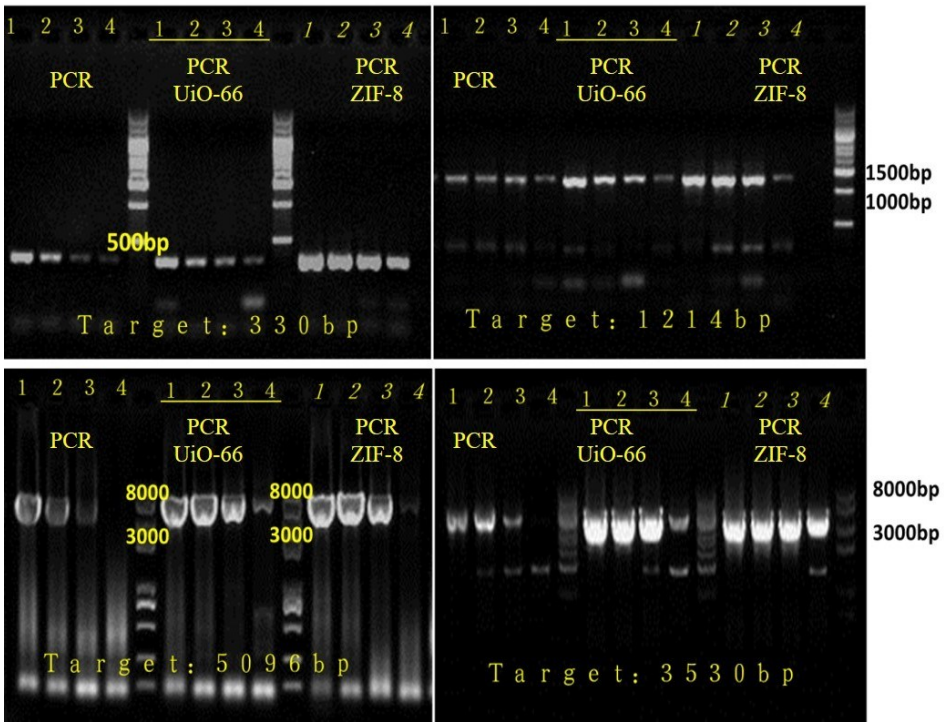


Figure S7. Gel electrophoresis of PCR production with the primer, product length, Tm value and extension time of the four different kinds of amplification sequence using plasmid as template.

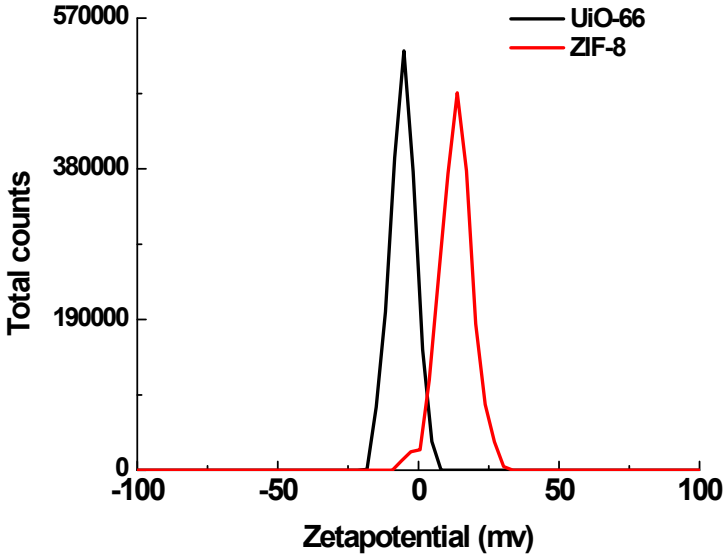


Figure S8. Zeta potential of UiO-66 and ZIF-8 particles in PCR buffer.

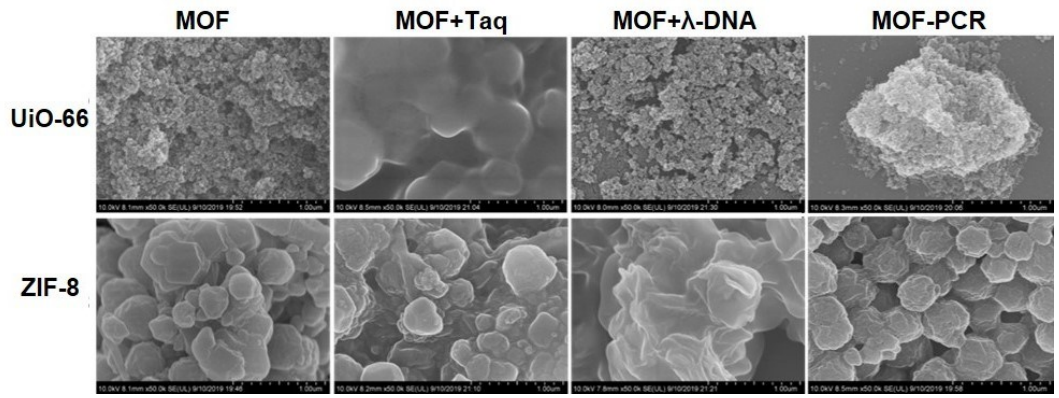
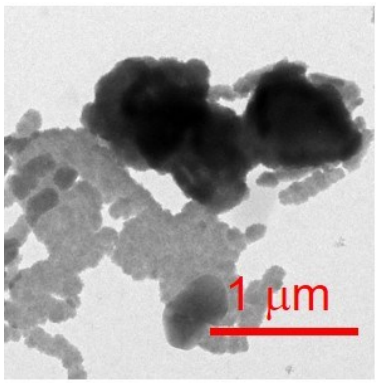
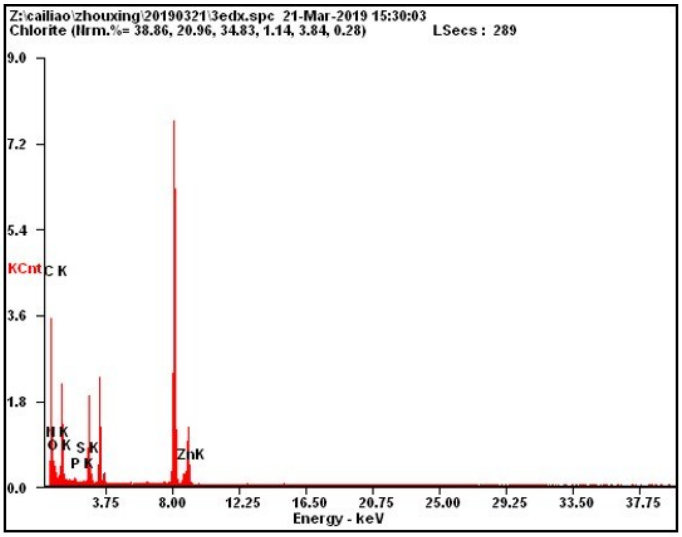
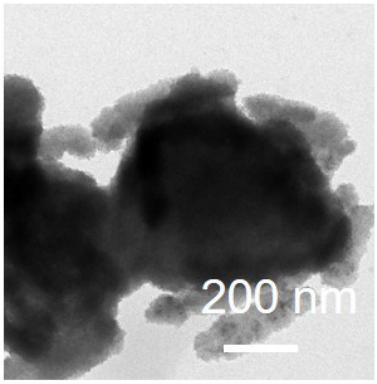


Figure S9. SEM images of MOFs particles: before and after the addition of Taq polymerase, λ-DNA and PCR.

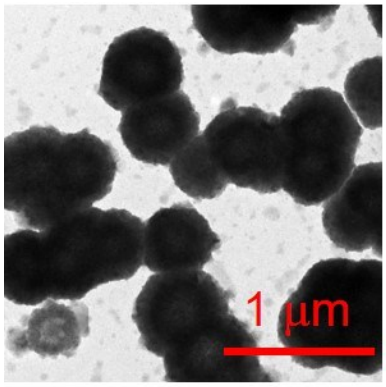


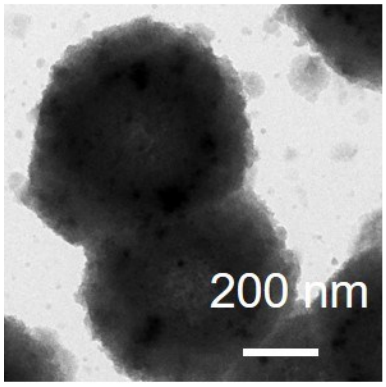


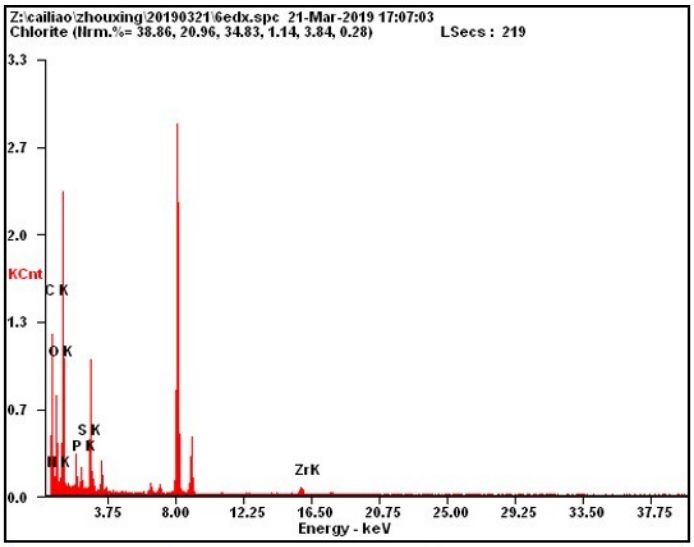


Elem	Weight %	Atomic %
C K	76.60	83.40
N K	12.50	11.70
O K	04.40	03.60
P K	00.00	00.00
S K	00.10	00.00
Zr K	06.40	01.30

Figure S10. TEM images and elemental analysis of UiO-66.

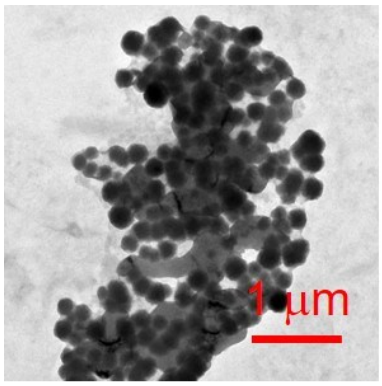


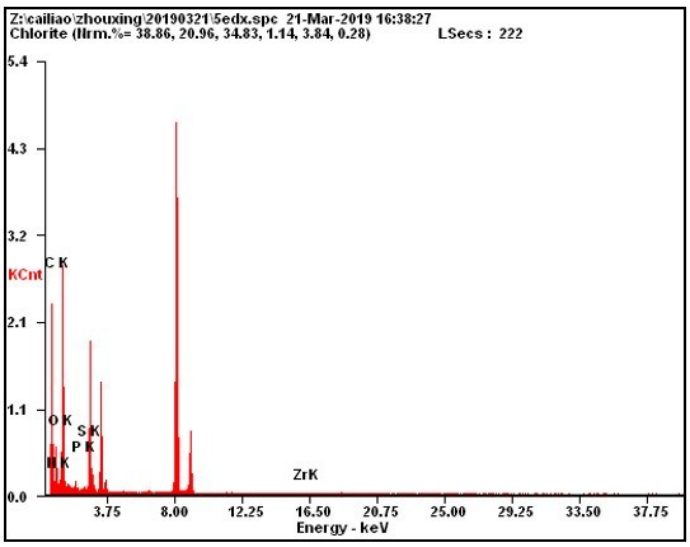


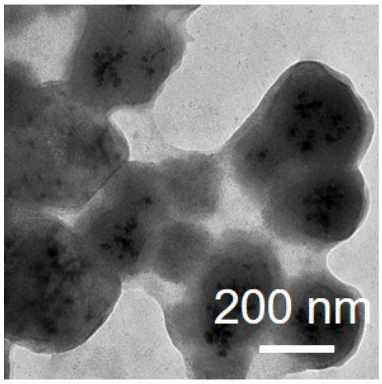


Elem	Weight %	Atomic %
C K	62.60	73.00
N K	03.80	03.80
O K	24.00	21.00
P K	02.10	01.00
S K	00.20	00.10
Zr K	07.30	01.10

Figure S11. TEM images and elemental analysis of UiO-66 after the addition of λ-DNA.

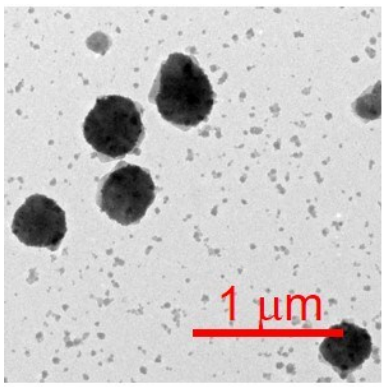


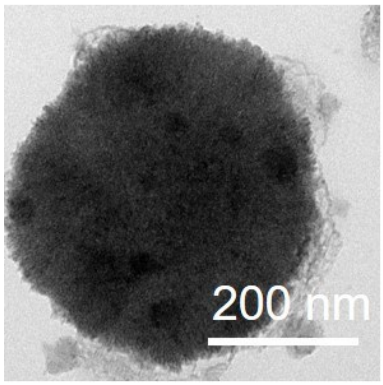


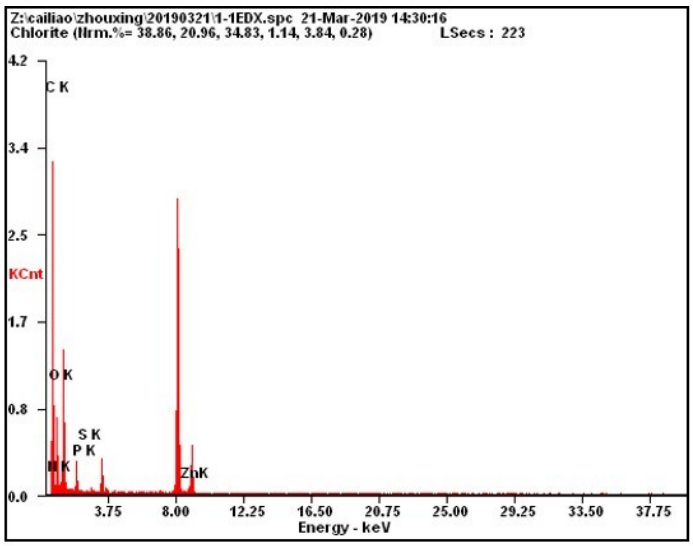


Elem	Weight %	Atomic %
C K	83.50	87.40
N K	03.20	02.90
O K	11.90	09.30
P K	00.10	00.00
S K	00.50	00.20
ZrK	00.80	00.10

Figure S12. TEM images and elemental analysis of UiO-66 after the addition of Taq polymerase.

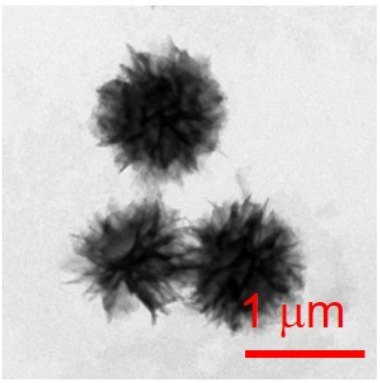


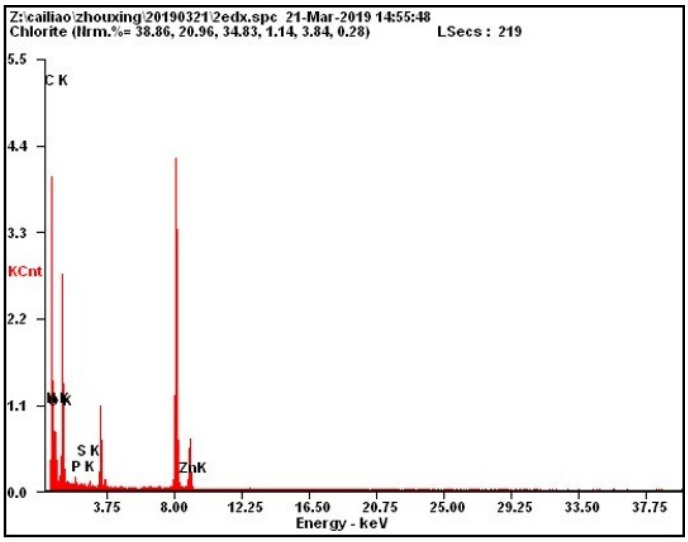


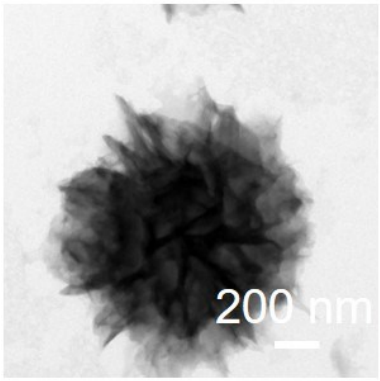


Elem	Weight %	Atomic %
C K	84.90	88.50
N K	01.80	01.60
O K	12.50	09.80
P K	00.00	00.00
S K	00.00	00.00
Zn K	00.90	00.20

Figure S13. TEM images and elemental analysis of ZIF-8.

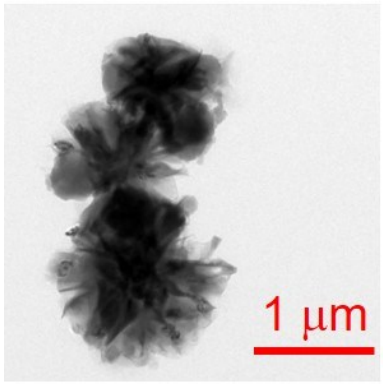


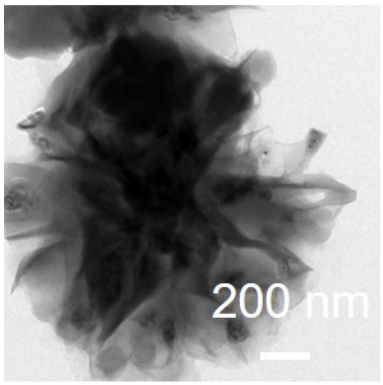


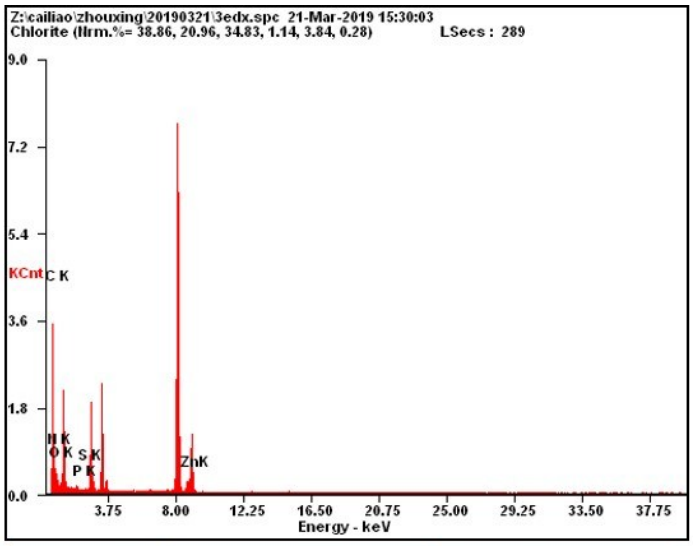


Elem	Weight %	Atomic %
C K	76.30	80.40
N K	14.30	13.00
O K	08.00	06.30
P K	00.10	00.00
S K	00.10	00.00
Zn K	01.10	00.20

Figure S14. TEM images and elemental analysis of ZIF-8 after the addition of λ-DNA.







Elem	Weight %	Atomic %
C K	76.60	83.40
N K	12.50	11.70
O K	04.40	03.60
P K	00.00	00.00
S K	00.10	00.00
Zn K	06.40	01.30

Figure S15. TEM images and elemental analysis of ZIF-8 after the addition of Taq polymerase.

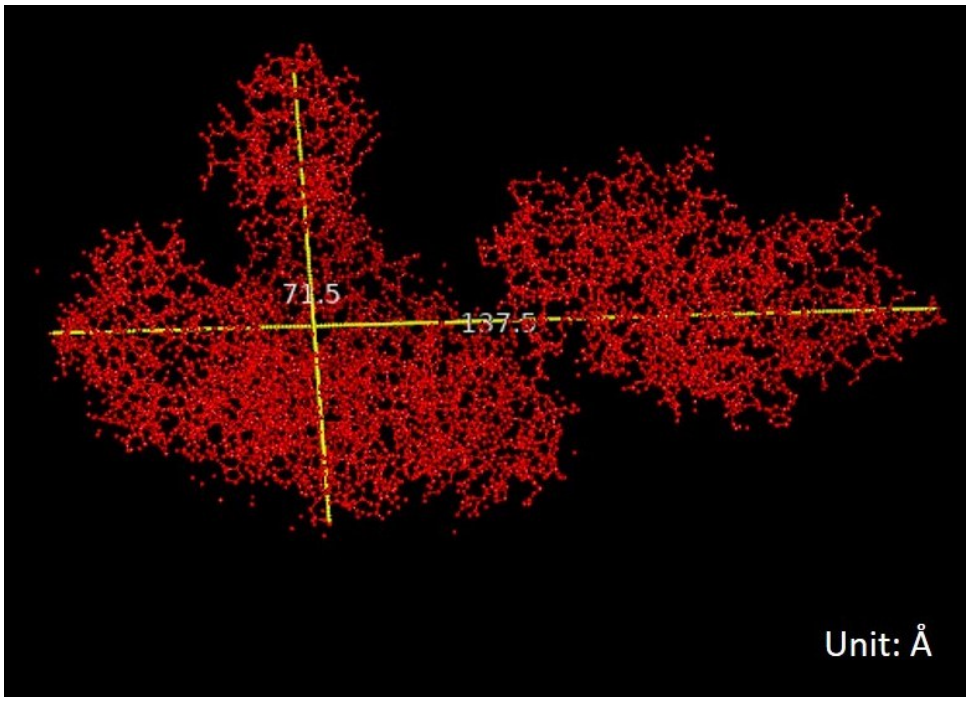


Figure S16. The crystal structure of the Taq enzyme (refer to http://www.rcsb.org/structure/1TAQ).

Supplementary Tables

Table S1 Some properties of ZIF-8 and UIO-66 samples for nitrogen adsorption experiments at 77 K

Sample	SBET[a](m^2/g)	Vp[b](cm^3/g)
ZIF-8	1408	0.6274
UIO-66	711	0.6878

[a] BET surface area. [b] Total pore volume.

Table S2. The sequence of CP and FP

Name	Sequence 5'→3'
FP	TGACATGGAGAGGATGAAGATG-**TAMRA**
CP	CATCTTCATCCTCTCCATGTCA-PC-TTTTT-Cholesterol

Table S3. The primer, product length, Tm value and extension time of the 4 different kinds of amplification sequence

Name	Primer	Sequence (5'-3')	Tm (℃)	Extention Time (s)	Product length (bp)
T1	1-F	GCAAGGGCGAGGAGCTGTTCACC	62	30	330
	1-R	CCTCGGCGCGGGTCTTGTAGTTG			
T2	2-F	GTCCAGGAGCGCACCATCTTCTTCA	63	60	1214
	2-R	TGCCGGGACTGGTGGTGGTGG			
T3	3-F	TCGACTTCAAGGAGGACGGCAACAT	61	60	3530
	3-R	GACAACGGGCCACAACTCCTCATAAAG			
T4	4-F	GCTGTGCCTTGGAATGCTAGTTGGAGT	61	90	5096
	4-R	GACGATGATTTCCCCGACAACACCA			