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Supplementary methods

1. Expression, purification and functional evaluation of MutS fusion proteins

1.1 Construction of recombinant expression vectors for *Eco*MutS-CBM3-EGFP (eMutS) and CBM3-*Taq*MutS-EGFP (tMutS)

The *E. coli* MutS gene was amplified from pET32-muts (a gift from Dr. Tianyin Zhong) using the primers *Eco*MutS-Nhel-F and *Eco*MutS-SacI-R (Supplementary Table S1) and sub-cloned into the *Nhe*I and *Sac*I sites of the pET-21c vector to generate the p*Eco*MutS plasmid. Then, the fusion gene segments of CBM3 (GenBank: HF912725.1) and EGFP (GenBank:ACX42327.1), which were amplified using the primers CBM3-EGFP-SacI-F and CBM3-EGFP-NotI-R (Supplementary Table S1) and the pCG plasmid (46) as template, were inserted into the *Sac*I and *Not*I sites of the p*Eco*MutS vector to generate the p*Eco*MutS-CBM3-EGFP plasmid (Supplementary Figure S1a) for the expression of the eMutS fusion protein. The MutS gene from *T. aquaticus* was amplified from its genomic DNA using the primers *Taq*MutS-SalI-F and *Taq*MutS-NotI-R (Supplementary Table S1) and cloned into the *Sal*I and *Not*I sites of the pET-21c vector to generate the p*Taq*MutS plasmid. Then, the fusion gene segments of CBM3 and EGFP, which was amplified from the pCG plasmid using the primers CBM3-EGFP-NdeI-F and CBM3-EGFP-SalI-R, was inserted into the *Nde*I and *Sal*I sites of p*Taq*MutS to generate the pCBM3-EGFP-*Taq*MutS plasmid (Supplementary Figure S1b) for the expression of the tMutS fusion protein. Both MutS fusion proteins contained a 6-His tag, a cellulose-binding module (CBM3), a fluorescent EGFP marker and a DNA mismatch recognition protein (MutS).

1.2 Expression and purification of the eMutS and tMutS proteins

The constructed MutS fusion protein expression plasmids were independently transformed into *E. coli* BL21 star (DE3). The cells (200 ml) were cultivated until the mid-exponential phase (OD600 approx. 0.6), and MutS expression was induced using 1 mM isopropyl-D-thiogalactoside (IPTG). Then, the cells were cultivated at 16 °C for 12 h (for eMutS) or at 37°C for 4 h (for tMutS) in LB medium. After the cells were collected via centrifugation at 8000 × g for 10 min at 4 °C and washed with water, the cells were re-suspended in 20 ml of cell lysis buffer (20 mM Tris–HCl pH 7.6, 300 mM KCl and 5 mM imidazole). Then, the cells were lysed via ultra-sonication (Sonics...
Ultra-cell VCX 130, Sonics & Materials INC., CT, USA) using a 1 S pulse at 30% strength for 3 min in an ice bath. After centrifugation at 14000 × g for 15 min at 4 °C to remove the insoluble material, the proteins in the supernatant were purified using a Ni-NTA affinity column (Qiagen, Netherlands) according to the manufacturer’s protocol. The eMutS and tMutS proteins were eluted in lysis buffer (20 mM Tris-HCl pH 7.6, 300 mM KCl) containing 250 mM imidazole. Then, the proteins were dialyzed against binding buffer containing 20 mM Tris-HCl (pH 7.6), 100 mM KCl, 5 mM MgCl₂ and 1 mM DTT at 4 °C. The purified eMutS and tMutS proteins were verified via 4.8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Coomassie Brilliant Blue R-250 staining, and the gels were scanned using a Tanon 1600 Gel Imaging System (Tanon Science & Technology Co., Ltd., Shanghai, China). The purity of the purified proteins was analyzed using Tanon Gis v4.0 software. The protein concentrations were determined via the Bradford assay using bovine serum albumin as a standard.

1.3 Function evaluation of the MutS fusion proteins

Oligonucleotide design and re-annealing reactions

To evaluate the specific mismatched DNA binding properties of EcoMutS-CBM3-EGFP (eMutS) and CBM3-TaqMutS-EGFP (tMutS), 10 oligos were designed and obtained from Sangon Biotech Co (Shanghai, China) (Supplementary Table S2). These oligos (A1~A5 and B1~B5) can form all possible single-nucleotide mismatches when two oligos are dissociated and re-annealed to form heteroduplex DNA (Supplementary Table S3). Equimolar concentrations of the two complementary single-stranded oligos at a final concentration of 2 µM were dissolved in annealing buffer containing 10 mM Tris-HCl (pH 7.6), 50 mM NaCl and 1 mM EDTA. Then, the duplexes were re-annealed by slowly cooling from 100 °C to 25 °C in a water bath. Fourteen different types of duplexes were used in these studies, the sequences of which are presented in Supplementary Table S3.

MutS and DNA binding

To measure the nonspecific binding of MutS to perfectly matched DNA, various molar ratios of MutS (eMutS or tMutS) to DNA (58 bp homoduplexes and unpaired T) ranging from 0:1 to 40:1
were analyzed. The MutS-DNA binding reaction was performed using a 10-µl reaction mixture containing 5 mM MgCl₂, 100 mM KCl, 20 mM Tris-HCl (pH 7.6), 1 mM DTT, 0.1 µM re-annealed heteroduplex DNA (containing mismatch) or homoduplex DNA (perfectly matched) substrate and 0-4 µM of a specific purified MutS protein at room temperature (RT) for 10 min.

To determine the binding properties of eMutS and tMutS to different types of mismatches (Supplementary Table S3), binding reactions of eMutS or tMutS to various mismatches were performed using 10-µl reaction mixtures containing 5 mM MgCl₂, 100 mM KCl, 20 mM Tris-HCl (pH 7.6), 1 mM DTT, 0.1 µM re-annealed heteroduplex DNA (containing mismatches) or homoduplex DNA (perfectly matched) substrate and 1 µM or 2 µM of a specific purified MutS protein (eMutS: 1 µM; tMutS: 2 µM) at room temperature (RT) for 10 min. During this step, 58 bp perfectly matched homoduplex DNA (Supplementary Table S3, 58 bp homoduplex) (Supplementary Table S2, Seq A5 and B5) was set as the control.

After the reaction, 3 µl of 50% glycerol were added. Then, 10 µl of the mixtures were loaded for electrophoresis on a polyacrylamide gel (6% (w/v) acrylamide, 19:1 acrylamide:bisacrylamide, 10 mM MgCl₂ and 1x TBE) at room temperature for approximately 15 min. The gels were visualized via ethidium bromide (EB) staining according to the manufacturer’s recommendation (Molecular Probes) and were scanned using a Tanon 1600 Gel Imaging System. The results were analyzed using Tanon Gis v4.0 software. The concentration of the free DNA on the gel represented the binding properties of the MutS fusion proteins to the duplex DNA, as the MutS–DNA complex had a very low mobility in the gel. A higher concentration of free DNA indicated that less DNA was bound, demonstrating a weaker binding of the MutS protein to the DNA.
2. Evaluation of the binding properties of the MICCs to error-containing oligos

The binding ability of MICC to error-containing oligos was evaluated. An additional two oligos were synthesized by Sangon Biotech Co. (Shanghai, China) (Supplementary Table S2), which formed a 54 bp homoduplex (homoduplex-54) after a re-annealing reaction (Supplementary Table S3). This homoduplex was used to produce error-free duplex. A MICC (eMICC: immobilized with 600 pmol eMutS; tMICC: immobilized with 1200 pmol tMutS; etMICC: immobilized with 600 pmol eMutS and 600 pmol tMutS) was used to treat 60-pmol DNA samples containing 45 pmol of the 59 bp heteroduplexes (unpaired T: ‘+T’) and 15 pmol of the 54 bp homoduplex (Supplementary Table S3). Due to the size difference, the 59 bp heteroduplex and the 54 bp homoduplex could be separated via PAGE. After the DNA sample was loaded on the MICC, the eluate fractions were collected into 1.5-ml Eppendorf tube at 80 µl per fraction. Then, 8 µl of each fraction were analyzed via PAGE (6 % (w/v) acrylamide, 19:1 acrylamide:bisacrylamide, 10 mM MgCl₂ and 1x TBE). The gels were visualized via EB staining according to the manufacturer’s recommendation (Molecular Probes) and were scanned using a Tanon 1600 Gel Imaging System. The results were analyzed using Tanon Gis v4.0 software, and the quantity of the unbound 54 bp homoduplex was calculated using Tanon Gis v4.0 software using the 20 bp DNA ladder marker (TaKaRa) as a standard.
3. Microchip oligonucleotide design

3.1 EGFP oligonucleotide pool design

The design of the EGFP oligos is shown in Supplementary Figure S3. Briefly, the codon of EGFP gene (720 bp) was first optimized based on *E. coli* codon usage, and the restriction enzyme sites were removed using DNAWorks (54). Then, the optimized sequence was cut into three similar length fragments (~260 bp) containing approximately 30 bp of overlap between the neighboring fragments. Next, the fragments were further cut into a set of oligos using Gene2Oligo (55). Three of the EGFP fragments were cut to form an oligo pool containing 60 types of oligos. There was no gap between each consecutive oligo. Thus, these oligos could be assembled into fragments via either PCR-based assembly [e.g., polymerase cycling assembly (PCA)] or ligation-based assembly [e.g., ligase chain reaction (LCR)]. Then, six different pairs of primer sequences (Supplementary Table S5 and S6) were added at both ends of the oligos to divide them into six subpools. Each subpool (a1, a2, a3, c1, c2 or c3) was amplified using the corresponding pair of primers (Supplementary Table S6), and the primers sequences were removed via *Mly I* digestion prior to gene assembly. All of the oligo and fragment sequences are presented in the Supplementary Data of Sequences. The a1-3 subpools contained the sense strand of EGFP Fra 1, Fra 2 and Fra 3, respectively, and the c1-3 subpools contained the antisense strand of EGFP Fra 1, Fra 2 and Fra 3, respectively. Thus, the a1 and c1 subpools, the a2 and c2 subpools and the a3 and c3 subpools could be used to assemble EGFP Fra 1, Fra 2 and Fra 3, respectively (Supplementary Figure S3). These fragments, which contained approximately 30 bp overlap between the consecutive fragments, could be assembled into the full length EGFP gene via overlap extension PCR.

3.2 sMMO and Epo oligonucleotide pool design

The codons of the target genes were all optimized (sMMO gene cluster: *E. coli*; Epo A, B and C: *Streptomyces coelicolor*), and the unnecessary restriction endonuclease sites were removed using DNAWorks (54). All oligos were designed using the DNAWorks program with similar Tm values (70 °C) of the consecutive overlapping regions. The 253 and 640 oligos designed for the sMMO gene cluster and the Epo A, B and C genes, respectively, were divided into 12 and 45 separate amplification subpools by adding different pairs of 15-bp primers containing the *Mly I* restriction
site at the terminals of the oligos (Supplementary Table S4). Each subpool contained 11-32 distinct oligos ranging from 63 nt to 129 nt in length (Supplementary Table S4). The sequences of all of the oligos and fragments are presented in the Supplementary Data of Sequences. The synthesized oligos in one subpool were independently amplified, and the primer sequences were removed via \textit{Mly} I digestion prior to gene assembly (Supplementary Figures S5 and S6). The oligos of each subpool were ligated to form from one to three 286-456 bp length fragments or genes from the consecutive adjacent complementary pairs of oligos. There was no gap between the consecutive oligos; thus, they could be assembled into fragments via either ligation-mediated assembly or polymerization-mediated assembly (PCA, LCR or the combination of these two methods). These fragments, which contained approximately 30 bp overlaps between neighboring fragments, were assembled into the corresponding full-length genes. To simplify the subsequent processes, a pair of common primer sequences containing the \textit{Bbs} I restriction site were designed and added to the corresponding oligos during synthesis (Supplementary Figure S6).
4. Amplification and assembly of the oligonucleotide pools

The oligos were released from the microchip surface, forming an oligo pool that contained ~20 picomoles of total oligos as described previously (22). This oligo pool was used as the template for PCR without any additional purification. PCRs were performed using KOD Plus polymerase (TOYOBO, Osaka, Japan) using the corresponding primers (Supplementary Tables S5 and S6) according to the manufacturer’s instructions. The PCR parameters were set as follows: 94 °C for 5 min, 30 cycles of 15 s at 94 °C, 30 s at 50 °C and 20 s at 68 °C and a final extension step of 10 min at 68 °C. To remove the primer region of the oligos, the PCR products were cleaved using Mly I, followed by purification using the UNIQ-10 oligonucleotide purification kit (Sangon, Shanghai, China) according to the manufacturer’s instructions.

After removing the primer region of the oligos, PCA, LCR or the combination of these two methods (PCA-LCR) were performed to assemble the target fragments. For the PCA reaction, 10 pmol (500 ng) of purified primer-removed PCR products and 0.2 unit of KOD Plus DNA polymerase in 10 µl of KOD PCR buffer (20 mM Tris–HCl pH 7.5 at 25 °C, 2 mM MgCl₂, 0.5 mM dithiothreitol, 50 µg/ml BSA and 200 µM of each dNTP) were incubated at 94 °C for 2 min, followed by 15 cycles of 15 s at 94 °C and 2 min at 68 °C and a final elongation step at 68 °C for 5 min. For the LCR procedure, 10 pmol (500 ng) of purified digested PCR products and 40 units of Taq DNA ligase in 10 µl of Taq DNA ligase buffer (20 mM Tris–HCl, pH 7.6, 25 mM potassium acetate, 10 mM magnesium acetate, 10 mM dithiothreitol, 1 mM NAD⁺ and 0.1% Triton X-100) were incubated at 95 °C for 5 min, followed by 10 cycles of 15 s at 94 °C and 10 min at 55 °C. For the LCR-PCA method, first, 40 µl of the LCR reaction was performed as described above. Then, the reaction was purified, and these products were used as the template DNA for the PCA reaction as described above. One microliter of 10-fold diluted PCA or LCR reaction product was used as the template for the amplification of the assembled fragments using Pfu DNA polymerase. The reaction was performed using 50-µl PCR reaction mixtures containing 10 mM KCl, 16 mM (NH₄)₂SO₄, 2 mM MgSO₄, 20 mM Tris-HCl (pH 8.8), 0.1% Triton X-100, 0.1 mg/ml BSA, 2 mM dNTPs, 50 units/ml of Pfu DNA polymerase (Biocolor BioScience & Technology Company (BBST), Shanghai, China), 5 μM of each primer and 0.1 µl of the PCA or LCR reaction product. The PCR reaction was performed at 95 °C for 5 min, followed by 30 cycles
of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 50 s and a final step of 72 °C for 10 min. The PCR products were separated on a 3% (w/v) agarose gel and extracted using an Axygen gel extraction kit according to the manufacturer’s protocol (Axygen Biotechnology Hangzhou Ltd, Hongzhou, China).

Overlap extension PCR (OE-PCR) was used to fuse the assembled fragments into full-length genes. OE-PCR was performed using 50-µl reaction mixtures containing 100 mM Tris-HCl (pH 8.3), 10 mM KCl, 2 mM MgCl₂, 6 mM (NH₄)₂SO₄, 0.1% Triton X-100, 0.1 mg/ml BSA, 2 mM of each dNTP, 25 units/ml of PrimeStar DNA polymerase (TaKaRa, Dalian, China), 0.3 pmol (~50 ng) of each fragment and 2 µM of each primer. The PCR reaction was performed at 98 °C for 2 min, followed by 30 cycles of 98 °C for 10 s, 60 °C for 5 s and 72 °C for 1 min/kb and a final elongation step at 72 °C for 5 min. The PCR products were separated on a 1% (w/v) agarose gel and extracted using a Axygen gel extraction kit according to the manufacturer’s protocol (Axygen Biotechnology Hangzhou Ltd, Hangzhou, China).
Supplementary Figures:

Supplementary Figure S1. Circular diagram of the expression vectors of the MutS fusion proteins. (a) pEcoMutS-CBM3-EGFP (eMutS expression vector). (b) pCBM3-EGFP-TaqMutS (tMutS expression vector).
Supplementary Figure S2. MutS immobilization and the MICCs. (a) Immobilization of the MutS fusion protein. (i) Purified eMutS protein. (ii) Purified tMutS protein. (iii) Cellulose slurry (RAC). (iv) eMutS immobilized cellulose slurry (eMutS-cellulose slurry). (v) tMutS immobilized cellulose slurry (tMutS-cellulose slurry). (b) MICC. (i) tMutS immobilized cellulose column (tMICC). (ii) eMutS immobilized cellulose column (eMICC). (iii) e/tMutS immobilized cellulose column (e/tMICC). (iv) t/eMutS immobilized cellulose column (t/eMICC). (v) e+tMutS immobilized cellulose column (e+tMICC).
**Supplementary Figure S3.** Design of the EGFP gene oligonucleotides for synthesis on a microchip.
Supplementary Figure S4. Design of the sMMO gene cluster and the Epo A, B and C oligonucleotides for synthesis on a microchip (sMMO G oligonucleotides are described as an example).
Supplementary Figure S5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of the purified MutS fusion proteins. Ten microliters of the purified fusion protein were analyzed via 4.8% SDS-PAGE, stained with Coomassie Brilliant Blue R250 and scanned using a Tanon 1600 Gel Imaging System (Tanon Science & Technology Co., Ltd., Shanghai, China). Lane 1. eMutS (EcoMutS-CBM3-EGFP fusion protein, 147.8 kDa); Lane 2. tMutS (CBM3-TaqMutS-EGFP fusion protein, 140.3 kDa); Lane M. Protein Marker (PageRuler™ Prestained Protein Ladder, #SM0671 from Fermentas).
Supplementary Figure S6. Band-shift assay of the MutS fusion proteins bound to heteroduplexes containing a single-bp mismatch and perfectly matched homoduplexes. The 58 bp homoduplex (PM) (a, c) and the 59 bp heteroduplexes (un-paired T) (b, d) were incubated with eMutS (a, b) or tMutS (c, d) separately at various molar ratios (MutS:duplexes from 0:1 to 40:1) for 10 min at 25 °C. This band-shift assay was performed to analyze the nonspecific binding properties of the two homologs of MutS fusion proteins to mismatched DNA and to determine the optimal molar ratio of MutS to treated DNA. PM, perfectly matched homoduplex. Arrows indicate the unbound DNA.
Supplementary Figure S7. Band-shift assay of the MutS fusion proteins bound to various heteroduplexes containing a single-bp mismatch. The 59 bp heteroduplexes containing a single-bp mismatch and the 58 bp homoduplex (PM) were incubated in tMutS (a) or eMutS (b) for 10 min at 25 °C. The eMutS:DNA molar ratio was 10:1, and the tMutS:DNA molar ratio was 20:1. This band-shift assay was performed to examine the mismatch-binding properties of the two homologs of MutS fusion proteins to mismatched DNA. The binding properties of eMutS and tMutS to different types of mismatches (listed in Supplementary Tables S2 and S3) were determined. The 58 bp perfectly matched homoduplex DNA (58 bp homoduplex, Supplementary Tables S1 and S2 online) was set as the control. The labels of each lanes correspond to the respective mismatched base pair (G:T, G:T mismatch; +G, unpaired G); PM, perfectly matched homoduplex. Arrows indicate the unbound DNA.
Supplementary Figure S8. Error removal ability of MICCs for double-stranded oligonucleotides containing a single-bp mismatch. The cellulose column (CC) was not immobilized with MutS and was set as the control. The tMICC contained 1200 pmol of tMutS; the eMICC contained 600 pmol of eMutS; and the etMICC contained 600 pmol of eMutS and 600 pmol of tMutS. The column length was 2 cm. A 60 pmol DNA sample in which 3/4 of the oligonucleotides were +T 59 bp heteroduplexes and 1/4 of the molecules were 54 bp homoduplexes was eluted through the CC (a), the tMICC (b), the eMICC (c) or the etMICC (d), and the eluate was collected in fractions of 80 µl/tube and analyzed via PAGE. M: 20 bp DNA ladder Marker (TaKaRa), 1-5: Collected error-depleted DNA (Eluted fractions 1-5).
Supplementary Figure S9. Fragment (sMMO D) assembly. (a) Re-amplified error-depleted oligonucleotide subpool (72 bp to 109 bp). Lane 1, amplified oligo subpool; Lane M, 20 bp DNA ladder marker (TaKaRa). (b) Assembled fragment. The products were separated via PAGE on a 3% agarose gel. Lane 1, assembled fragment (309 bp); Lane M, 20 bp DNA ladder marker (TaKaRa).
Supplementary Figure S10. SDS-PAGE analysis of sMMO gene expression in *E. coli* BL21 (DE3). The synthetic sMMO genes were expressed in *E. coli* BL21 star (DE3) under the control of the T7/lac promoter. Lane M, protein marker (PageRuler™ Prestained Protein Ladder, #SM0671 from Fermentas); Lane 1, BL21 star (DE3) as a negative control; Lane 2, sMMO X (53 kDa); Lane 3, sMMO Y (45 kDa); Lane 4, sMMO Z (20 kDa); Lane 5, sMMO D (12 kDa); Lane 6, sMMO B (16 kDa); Lane 7, sMMO H (13 kDa); Lane 8, sMMO C (39 kDa); Lane 9, sMMO G (59 kDa). Arrows represent the target protein bands.
Supplementary Figure S11. Flow chart describing error removal of DNA pools using a MICC. One round of MICC error correction processing could be completed within 1.5 hours.
**Supplementary Tables:**

**Supplementary Table S1.** Primers used to construct the MutS expression vector.

| Name                        | Sequence                                                                 |
|-----------------------------|--------------------------------------------------------------------------|
| TaqMutS-Sall-F              | AAACGCGTGCACATGGAAGGCATGCTGAAGG                                         |
| TaqMutS-NotI-R              | AATAGAATTGCGGCCCCCCCTTCATGATCAAAG                                        |
| EcoMutS-SacI-R              | CGAGCTCACCAGGCCTTTAAGACG                                                |
| EcoMutS-NhelI-F             | CTAGCTAGCATTGCAATAGAAAATTTGC                                            |
| CBM3-GFP-SacI-F             | CGAGCTCATGCAGCTATCAGGCCAGCAATTTG                                       |
| CBM3-GFP-NotI-R             | ATAGAATTGCGGCCCTTGTACGCTCGTCATG                                         |
| CBM3-GFP-NdelI-F            | AAAAACATATGCGGTTTCATCGCTCGTCATG                                         |
| CBM3-GFP-Sall-R             | AAACGCGTGACCTTTGACAGCTCGTCATG                                           |

*a* Underline: the restriction enzyme site.
**Supplementary Table S2.** Oligonucleotides used to evaluate the DNA-binding properties of MutS

| Name | Nucleotide Sequences* | Length (mer) |
|------|-----------------------|--------------|
| **Top Strand (5’-3’)** | | |
| A1  | GCGGACTATTTAACACAGCTTTAGGCCTGGACGAGGTATCGATTAATCAGCGGCTTGTGCTCC | 59 |
| A2  | GCGGACTATTTAACACAGCTTTAGGCCTGGACGAGGTATCGATTAATCAGCGGCTTGTGCTCC | 59 |
| A3  | GCGGACTATTTAACACAGCTTTAGGCCTGGACGAGGTATCGATTAATCAGCGGCTTGTGCTCC | 59 |
| A4  | GCGGACTATTTAACACAGCTTTAGGCCTGGACGAGGTATCGATTAATCAGCGGCTTGTGCTCC | 59 |
| A5  | GCGGACTATTTAACACAGCTTTAGGCCTGGACGAGGTATCGATTAATCAGCGGCTTGTGCTCC | 59 |
| A6  | GCGGACTATTTAACACAGCTTTAGGCCTGGACGAGGTATCGATTAATCAGCGGCTTGTGCTCC | 59 |
| **Bottom strand (3’-5’)** | | |
| B1  | CGCCTGATAAAATTTGTCAGCAAATCCCGACATGCTCCATGATACCTAGCCCGAAGG | 59 |
| B2  | CGCCTGATAAAATTTGTCAGCAAATCCCGACATGCTCCATGATACCTAGCCCGAAGG | 59 |
| B3  | CGCCTGATAAAATTTGTCAGCAAATCCCGACATGCTCCATGATACCTAGCCCGAAGG | 59 |
| B4  | CGCCTGATAAAATTTGTCAGCAAATCCCGACATGCTCCATGATACCTAGCCCGAAGG | 59 |
| B5  | CGCCTGATAAAATTTGTCAGCAAATCCCGACATGCTCCATGATACCTAGCCCGAAGG | 59 |
| B6  | CGCCTGATAAAATTTGTCAGCAAATCCCGACATGCTCCATGATACCTAGCCCGAAGG | 59 |

*Underlined: mismatched nucleotides. The mismatch could be formed after re-hybridization of two ssDNA.
| Mismatch Type | Nucleotide Sequences | Seq. Name |
|---------------|----------------------|----------|
| G:A mismatch  | 5'-GCGGACTATTTAACACAGGCTTGGACGAGGTACTATGAATCGGCTTGGCTTCC-3' | A1       |
|              | 3'-CGCCTGATAAAATTGTGCAGAATCCCGAGCAATGCTCCATGATAATTAGGCGGAACGAGG-5' | B1       |
| C:A mismatch  | 5'-GCGGACTATTTAACACAGGCTTGGACGAGGTACTATGAATCGGCTTGGCTTCC-3' | A2       |
|              | 3'-CGCCTGATAAAATTGTGCAGAATCCCGAGCAATGCTCCATGATAATTAGGCGGAACGAGG-5' | B1       |
| C:C mismatch  | 5'-GCGGACTATTTAACACAGGCTTGGACGAGGTACTATGAATCGGCTTGGCTTCC-3' | A3       |
|              | 3'-CGCCTGATAAAATTGTGCAGAATCCCGAGCAATGCTCCATGATAATTAGGCGGAACGAGG-5' | B1       |
| T:T mismatch  | 5'-GCGGACTATTTAACACAGGCTTGGACGAGGTACTATGAATCGGCTTGGCTTCC-3' | A4       |
|              | 3'-CGCCTGATAAAATTGTGCAGAATCCCGAGCAATGCTCCATGATAATTAGGCGGAACGAGG-5' | B1       |
| Unpaired A   | 5'-GCGGACTATTTAACACAGGCTTGGACGAGGTACTATGAATCGGCTTGGCTTCC-3' | A5       |
| (+A)          | 3'-CGCCTGATAAAATTGTGCAGAATCCCGAGCAATGCTCCATGATAATTAGGCGGAACGAGG-5' | B1       |
| G:T mismatch  | 5'-GCGGACTATTTAACACAGGCTTGGACGAGGTACTATGAATCGGCTTGGCTTCC-3' | A1       |
| (G:T)         | 3'-CGCCTGATAAAATTGTGCAGAATCCCGAGCAATGCTCCATGATAATTAGGCGGAACGAGG-5' | B2       |
| Unpaired T   | 5'-GCGGACTATTTAACACAGGCTTGGACGAGGTACTATGAATCGGCTTGGCTTCC-3' | A5       |
| (+T)          | 3'-CGCCTGATAAAATTGTGCAGAATCCCGAGCAATGCTCCATGATAATTAGGCGGAACGAGG-5' | B2       |
| G:G mismatch  | 5'-GCGGACTATTTAACACAGGCTTGGACGAGGTACTATGAATCGGCTTGGCTTCC-3' | A1       |
| (G:G)         | 3'-CGCCTGATAAAATTGTGCAGAATCCCGAGCAATGCTCCATGATAATTAGGCGGAACGAGG-5' | B2       |
| T:C mismatch  | 5'-GCGGACTATTTAACACAGGCTTGGACGAGGTACTATGAATCGGCTTGGCTTCC-3' | A3       |
| (T:C)         | 3'-CGCCTGATAAAATTGTGCAGAATCCCGAGCAATGCTCCATGATAATTAGGCGGAACGAGG-5' | B3       |
To evaluate the binding specificities of eMutS and tMutS, 10 oligos were designed and then synthesis by Sangon Biotech Co. (Shanghai, China) (Supplementary Table S1). These oligos (A1 - A5 and B1 - B5) constitute all possible single-nucleotide mismatches when two sequences are re-annealed to form heteroduplexes. An equimolar ratio of the two complementary single-stranded oligos at a final concentration of 2 µM were dissolved in annealing buffer containing 10 mM Tris-HCl (pH 7.6), 50 mM NaCl and 1 mM EDTA. Then, the duplexes were re-annealed by slowly cooling from 100 °C to 25 °C in a water bath. Fourteen different types of duplexes were used in these studies, the sequences of which are presented in this Table.

*Bold and underlined: the nucleotides form the mismatches.*
**Supplementary Table S4.** Summary of the oligonucleotide designs for the EGFP gene, the sMMO gene cluster and the Epo A, B and C genes.

| Microchip | Genes a | Length (bp) | Oligos a | Oligo length (nt) | Subpool a | Oligos per subpool a | Fragments per subpool a | Fragment s a | Fragment length (bp) |
|-----------|---------|-------------|----------|------------------|-----------|---------------------|------------------------|---------------|---------------------|
| EGFP      | 1       | 720         | 60       | 69 ~ 118         | 6         | 10                  | 1                      | 3             | 258 ~ 260           |
| sMMO      | 8       | 7,029       | 253      | 70 ~ 129         | 12        | 11-32               | 1-3                    | 29            | 286 ~ 456           |
| Epo       | 3       | 13,989      | 640      | 63 ~ 111         | 45        | 11-28               | 1-2                    | 49            | 313 ~ 344           |

aThese datas represent the numbers of genes, oligos, oligo pools, subpools, MICCs and assembled fragments.
**Supplementary Table S5.** Primer sequences used for the EGFP gene, the sMMO gene cluster, and the Epo A, B and C genes.

| Primer ID | Seq.* |
|-----------|-------|
| MlyI-1F   | CACAGGA GTGTCCTCAC |
| MlyI-2F   | CCAAGGAGTCGCTAG |
| MlyI-3F   | CAGAGGA GTGTCCTGAG |
| MlyI-4F   | CCTACGAGTCGCAAC |
| MlyI-5F   | CATTCGAGTCGCTG |
| MlyI-6F   | CGTACGAGTCCTTC |
| MlyI-7F   | CTTACGAGTCGAT |
| MlyI-8F   | GGTACGAGTCGAT |
| MlyI-9F   | CTTACGAGTCGTC |
| MlyI-10F  | GGTACGAGTCGAT |
| MlyI-11F  | GTGTGGAGTCATATG |
| MlyI-12F  | GGTACGAGTCGTCAT |
| MlyI-13F  | GTTTTCAGTCATCC |
| MlyI-14F  | GCATGAGTCGAT |
| EGFP-1-DR | GGAAGGA GTGCTAG |
| EGFP-2-UR | CTTACGAGTCGTC |
| EGFP-2-DR | CTCAGGAGTCGTC |
| EGFP-3-UR | AGCAGGA GTGACCCGT |
| EGFP-3-DR | GTTGAGTCGTCAG |
| xMlyI-1F  | GTATGAGTCGTC |
| xMlyI-2F  | GTTCAGA GTGACG |
| xMlyI-3F  | GATAGAGTCGTCAG |
| xMlyI-4F  | GATGGA GTGACG |
| 6BbsI-1F  | CGAAGAACGAT |
| 6BbsI-2F (EGFP-1-UR) | ACGGAAGACCTTC |
| EGFP Fra-1-F | ATGTAGACGGAGGCGAG |
| EGFP Fra-1-R | GGACTTGAAGAAGCGT |
| EGFP Fra-2-F | CACATGACAGCAAC |
| EGFP Fra-2-R | CACCTTGAACGCGT |
| EGFP Fra-3-F | GCCGACAAGCAGAAG |
| EGFP Fra-3-R | TTAAGTACAGGT |

*Underlined: the restriction enzyme site*
**Supplementary Table S6.** Primers used for the EGFP gene, the sMMO gene cluster and the Epo A, B and C genes.

| Subpool Name (pool) | Oligos primer_F (5'->3') | Oligos primer_R (5'->3') | Fragments or genes primer_F (5'->3') | Fragments or genes primer_R (5'->3') |
|---------------------|--------------------------|--------------------------|--------------------------------------|--------------------------------------|
| EGFP-1-Upper        | MLY1_1F                  | EGFP-1-UR                | EGFP Fra-1-F                          | EGFP Fra-1-R                          |
| EGFP-1-Down         | MLY1_1F                  | EGFP-1-DR                | EGFP Fra-2-F                          | EGFP Fra-2-R                          |
| EGFP-2-Upper        | MLY1_1F                  | EGFP-2-UR                | EGFP Fra-3-F                          | EGFP Fra-3-R                          |
| EGFP-2-Down         | MLY1_1F                  | EGFP-2-DR                | EGFP Fra-3-F                          | EGFP Fra-3-R                          |
| EGFP-3-Upper        | MLY1_1F                  | EGFP-3-UR                | EGFP Fra-3-F                          | EGFP Fra-3-R                          |
| EGFP-3-Down         | MLY1_1F                  | EGFP-3-DR                | EGFP Fra-3-F                          | EGFP Fra-3-R                          |
| mmoX_1              | MLY1_1F                  | MLY1-3F                  | 6BBSI-1F                             | 6BBSI-2F                             |
| mmoX_2              | MLY1_1F                  | MLY1-3F                  | 6BBSI-1F                             | 6BBSI-2F                             |
| mmoX_4              | MLY1_1F                  | MLY1-4F                  | 6BBSI-1F                             | 6BBSI-2F                             |
| mmoX_5              | MLY1_1F                  | MLY1-4F                  | 6BBSI-1F                             | 6BBSI-2F                             |
| mmoY_1              | MLY1_1F                  | MLY1-5F                  | 6BBSI-1F                             | 6BBSI-2F                             |
| mmoY_2              | MLY1_1F                  | MLY1-5F                  | 6BBSI-1F                             | 6BBSI-2F                             |
| mmoY_3              | MLY1_1F                  | MLY1-6F                  | 6BBSI-1F                             | 6BBSI-2F                             |
| mmoY_4              | MLY1_1F                  | MLY1-6F                  | 6BBSI-1F                             | 6BBSI-2F                             |
| mmoG_1              | MLY1_1F                  | MLY1-11F                 | 6BBSI-1F                             | 6BBSI-2F                             |
| mmoG_2              | MLY1_1F                  | MLY1-12F                 | 6BBSI-1F                             | 6BBSI-2F                             |
| mmoG_3              | MLY1_1F                  | MLY1-11F                 | 6BBSI-1F                             | 6BBSI-2F                             |
| mmoG_4              | MLY1_1F                  | MLY1-12F                 | 6BBSI-1F                             | 6BBSI-2F                             |
| mmoG_5              | MLY1_1F                  | MLY1-12F                 | 6BBSI-1F                             | 6BBSI-2F                             |
| mmoG_6              | MLY1_1F                  | MLY1-12F                 | 6BBSI-1F                             | 6BBSI-2F                             |
| mmoB_1              | MLY1_1F                  | XLYI_2F                  | 6BBSI-1F                             | 6BBSI-2F                             |
| mmoZ_1              | MLY1_1F                  | MLY1-2F                  | 6BBSI-1F                             | 6BBSI-2F                             |
| mmoZ_2              | MLY1_1F                  | MLY1-2F                  | 6BBSI-1F                             | 6BBSI-2F                             |
| mmoD_1              | MLY1_1F                  | XLYI_1F                  | 6BBSI-1F                             | 6BBSI-2F                             |
| mmoC_1              | MLY1_1F                  | XLYI-3F                  | 6BBSI-1F                             | 6BBSI-2F                             |
| mmoC_2              | MLY1_1F                  | XLYI-3F                  | 6BBSI-1F                             | 6BBSI-2F                             |
| mmoC_3              | MLY1_1F                  | XLYI-4F                  | 6BBSI-1F                             | 6BBSI-2F                             |
| mmoC_4              | MLY1_1F                  | XLYI-4F                  | 6BBSI-1F                             | 6BBSI-2F                             |
| mmoH                | MLY1_1F                  | MLY1-9F                  | 6BBSI-1F                             | 6BBSI-2F                             |
| EPOA_1              | MLY1_1F                  | MLY1-3F                  | 6BBSI-1F                             | 6BBSI-2F                             |
| EPOA_2  | MLYI_1F | MLYI-4F  | 6BBSI-1F  | 6BBSI-2F  |
|---------|---------|----------|-----------|-----------|
| EPOA_3  | MLYI_1F | MLYI-5F  | 6BBSI-1F  | 6BBSI-2F  |
| EPOA_4  | MLYI_1F | MLYI-6F  | 6BBSI-1F  | 6BBSI-2F  |
| EPOA_5  | MLYI_1F | MLYI-7F  | 6BBSI-1F  | 6BBSI-2F  |
| EPOA_6  | MLYI_1F | MLYI-8F  | 6BBSI-1F  | 6BBSI-2F  |
| EPOA_7  | MLYI_1F | MLYI-9F  | 6BBSI-1F  | 6BBSI-2F  |
| EPOA_8  | MLYI_1F | MLYI-10F | 6BBSI-1F  | 6BBSI-2F  |
| EPOA_9  | MLYI_1F | MLYI-11F | 6BBSI-1F  | 6BBSI-2F  |
| EPOA_10 | MLYI_1F | MLYI-12F | 6BBSI-1F  | 6BBSI-2F  |
| EPOA_11 | MLYI_1F | MLYI-13F | 6BBSI-1F  | 6BBSI-2F  |
| EPOA_12 | MLYI_1F | MLYI-14F | 6BBSI-1F  | 6BBSI-2F  |
| EPOA_13 | MLYI_1F | XMLYI_2F | 6BBSI-1F  | 6BBSI-2F  |
| EPOA_14 | MLYI_1F | XMLYI-3F | 6BBSI-1F  | 6BBSI-2F  |
| EPOA_15 | MLYI_1F | XMLYI-4F | 6BBSI-1F  | 6BBSI-2F  |
| EPOB_1  | XMLYI_1F| MLYI-3F  | 6BBSI-1F  | 6BBSI-2F  |
| EpoB_2  | XMLYI_1F| MLYI-4F  | 6BBSI-1F  | 6BBSI-2F  |
| EpoB_3  | XMLYI_1F| MLYI-5F  | 6BBSI-1F  | 6BBSI-2F  |
| EpoB_4  | XMLYI_1F| MLYI-6F  | 6BBSI-1F  | 6BBSI-2F  |
| EpoB_5  | XMLYI_1F| MLYI-7F  | 6BBSI-1F  | 6BBSI-2F  |
| EpoB_6  | XMLYI_1F| MLYI-8F  | 6BBSI-1F  | 6BBSI-2F  |
| EpoB_7  | XMLYI_1F| MLYI-9F  | 6BBSI-1F  | 6BBSI-2F  |
| EpoB_8  | XMLYI_1F| MLYI-10F | 6BBSI-1F  | 6BBSI-2F  |
| EpoB_9  | XMLYI_1F| MLYI-11F | 6BBSI-1F  | 6BBSI-2F  |
| EpoB_10 | XMLYI_1F| MLYI-12F | 6BBSI-1F  | 6BBSI-2F  |
| EpoB_11 | XMLYI_1F| MLYI-13F | 6BBSI-1F  | 6BBSI-2F  |
| EpoB_12 | XMLYI_1F| MLYI-14F | 6BBSI-1F  | 6BBSI-2F  |
| EpoB_13 | XMLYI_1F| XMLYI_2F | 6BBSI-1F  | 6BBSI-2F  |
| EpoB_14 | XMLYI_1F| XMLYI-3F | 6BBSI-1F  | 6BBSI-2F  |
| EpoB_15 | XMLYI_1F| XMLYI-4F | 6BBSI-1F  | 6BBSI-2F  |
| EPOC_1  | MLYI_2F | MLYI-3F  | 6BBSI-1F  | 6BBSI-2F  |
| EpoC_2  | MLYI_2F | MLYI-3F  | 6BBSI-1F  | 6BBSI-2F  |
| EpoC_3  | MLYI_2F | MLYI-4F  | 6BBSI-1F  | 6BBSI-2F  |
| EpoC_4  | MLYI_2F | MLYI-4F  | 6BBSI-1F  | 6BBSI-2F  |
| EpoC_5  | MLYI_2F | MLYI-5F  | 6BBSI-1F  | 6BBSI-2F  |
| EpoC_6 | MLY1_2F | MLY1-5F | 6BBSI-1F | 6BBSI-2F |
|--------|---------|---------|----------|----------|
| EpoC_7 | MLY1_2F | MLY1-6F | 6BBSI-1F | 6BBSI-2F |
| EpoC_8 | MLY1_2F | MLY1-6F | 6BBSI-1F | 6BBSI-2F |
| EpoC_9 | MLY1_2F | MLY1-7F | 6BBSI-1F | 6BBSI-2F |
| EpoC_10| MLY1_2F | MLY1-8F | 6BBSI-1F | 6BBSI-2F |
| EpoC_11| MLY1_2F | MLY1-9F | 6BBSI-1F | 6BBSI-2F |
| EpoC_12| MLY1_2F | MLY1-10F| 6BBSI-1F | 6BBSI-2F |
| EpoC_13| MLY1_2F | MLY1-11F| 6BBSI-1F | 6BBSI-2F |
| EpoC_14| MLY1_2F | MLY1-12F| 6BBSI-1F | 6BBSI-2F |
| EpoC_15| MLY1_2F | MLY1-13F| 6BBSI-1F | 6BBSI-2F |
| EpoC_16| MLY1_2F | MLY1-14F| 6BBSI-1F | 6BBSI-2F |
| EpoC_17| MLY1_2F | XMLY1_2F| 6BBSI-1F | 6BBSI-2F |
| EpoC_18| MLY1_2F | XMLY1-3F| 6BBSI-1F | 6BBSI-2F |
| EpoC_19| MLY1_2F | XMLY1-4F| 6BBSI-1F | 6BBSI-2F |

The sequences of the primers are listed in Supplementary Table 5.
Supplementary Table S7. Effect of the combined MICCs using different packing modes on egfp gene synthesis

| Error Type     | e/tMICC | e+tMICC | t/eMICC |
|----------------|---------|---------|---------|
| Deletion       | 0       | 3       | 1       |
| Insertion      | 0       | 0       | 0       |
| Substitution   | 6       | 3       | 4       |
| Total errors   | 6       | 6       | 5       |
| Bases sequenced | 4,155   | 4,248   | 4,146   |
| Error frequency (error per kb) | 1.44 | 1.41 | 1.21 |
**Supplementary Table S8.** Effect of the combined MICCs using various eMutS/tMutS ratios on *egfp* gene synthesis

| Error Type         | eMutS : tMutS* | 1:0.5 | 1:1 | 1:2 | 1:3 |
|--------------------|----------------|-------|-----|-----|-----|
| Deletion           |                | 2     | 1   | 6   | 3   |
| Insertion          |                | 0     | 0   | 0   | 0   |
| Substitution       |                | 4     | 4   | 4   | 6   |
| Total errors       |                | 6     | 5   | 10  | 9   |
| Bases sequenced    |                | 2,599 | 4,146 | 5,193 | 5,199 |
| Error frequency (error per kb) |                | 2.31 | 1.21 | 1.93 | 1.73 |

*The molar ratio of eMutS to tMutS in the etMICCs; the molar ratio of eMutS to oligos were 10:1.*
**Supplementary Table S9.** Error analysis of the oligos comprising the sMMO gene cluster and the Epo A, B and C genes before and after etMICC error removal.

| Error type          | Untreated | etMICC |
|---------------------|-----------|--------|
| Multi-error<sup>a</sup> | 2         | 0      |
| Deletion            | 47        | 5      |
| Insertion           | 11        | 1      |
| Substitution        | 52        | 2      |
| Total errors        | 112       | 8      |
| Bases sequenced     | 9,151     | 9,083  |
| Error frequency (error per kb) | 12.24 | 0.88 |
| Error frequency (bases per error) | 81.71 | 1,135.38 |
| Correct oligo ratio | 35/109 (32.11%) | 107/115 (93.04%) |

<sup>a</sup>Error site located in a sequence that contained more than three adjacent consecutive nucleotide errors.
**Supplementary Table S10.** Error analysis of synthetic *egfp* gene sequences after one round of error removal at the fragment stage

| Error type     | eMICC<sup>a</sup> | tMICC<sup>a</sup> | etMICC<sup>a</sup> |
|----------------|--------------------|-------------------|--------------------|
| Multi-error<sup>b</sup> | 0                  | 1                 | 0                  |
| Deletion       | 14                 | 21                | 9                  |
| Insertion      | 0                  | 4                 | 1                  |
| Substitution   | 20                 | 29                | 21                 |
| Total errors   | 34                 | 55                | 31                 |
| Bases sequenced| 5,539              | 6,463             | 6,724              |
| Error frequency (errors per kb) | 6.14               | 8.51              | 4.61               |

<sup>a</sup>Random clones of the synthetic fragments and the gene sequences after one round of error removal using various MICCs at the fragment stage were sequenced. Then, the number of different types of errors was counted.

<sup>b</sup>Error site located in a sequence that contained more than three adjacent consecutive nucleotide errors.
Supplementary Table S11. The cost to prepare an etMICC.

| Compose            | Amount   | Cost   |
|--------------------|----------|--------|
| MutS protein       | 1.2 nmol | $0.013 |
| Empty Column       | 1        | $0.261 |
| Cellulose slurry   | 500 µl   | $0.100 |
| Total              |          | $0.374 |
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