

## **1. Supplementary of Fig2: details of construction of anticalin library for in vitro selection.**

**The process of amplification of BBP1:** 3min at 94°C, 30s at 94°C, 30s at 45°C, 1min at 72°C, 5cycles; 30s at 94°C, 30s at 60°C, 1min at 72°C, 25cycles; 5min at 72°C.

The PCR performed using PfuUltra™ II fusion HS DNA Polymerase and the primers Fbbp1 and Rbbp1.

**The process of amplification of BBP2:** 3min at 94°C, 30s at 94°C, 30s at 45°C, 1min at 72°C, 5cycles; 30s at 94°C, 30s at 60°C, 1min at 72°C, 25cycles; 5min at 72°C.

The PCR performed using PfuUltra™ II fusion HS DNA Polymerase and the primers F3437 and R5860.

**The process of amplification of BBP3:** 3min at 94°C, 30s at 94°C, 30s at 45°C, 1min at 72°C, 5cycles; 30s at 94°C, 30s at 60°C, 1min at 72°C, 25cycles; 5min at 72°C.

The PCR performed using PfuUltra™ II fusion HS DNA Polymerase and the primers F8797 and R8797.

**The process of amplification of BBP4:** 3min at 94°C, 30s at 94°C, 30s at 45°C, 1min at 72°C, 5cycles; 30s at 94°C, 30s at 60°C, 1min at 72°C, 25cycles; 5min at 72°C.

The PCR performed using PfuUltra™ II fusion HS DNA Polymerase and the primers F1416 and R2527.

**The process of amplification of BBP5:** 3min at 94°C, 30s at 94°C, 30s at 45°C, 1min at 72°C, 5cycles; 30s at 94°C, 30s at 60°C, 1min at 72°C, 25cycles; 5min at 72°C.

The PCR performed using PfuUltra™ II fusion HS DNA Polymerase and the primers Ftail and Rtail.

**BBP1 and BBP2 fragment were assembled.** In the SOE experiment, the reaction mixture (50ng BBP1 fragment, 50ng BBP2 fragment) was subjected to an initial denaturation step at 94°C for 3 min followed by 5 cycles of 94°C for 30 s, 40°C for 30 s, 72°C for 1 min and then followed by 25 cycles of 94°C for 30 s, 57.5°C for 30 s, 72°C for 1 min and a final extension step at 72°C for 5 min. To amplify the assembled fragment, 25pmol of each of the two primers Fbbp1 and R69 were added.

**BBP3 and BBP4 fragment were assembled.** In the SOE experiment, the reaction mixture (50ng BBP3 fragment, 50ng BBP4 fragment) was subjected to an initial denaturation step at 94°C for 3 min followed by 10 cycles of 94°C for 30 s, 53°C for 30 s, 72°C for 1 min and then followed by 25 cycles of 94°C for 30 s, 57.5°C for 30 s, 72°C for 1 min and a final extension step at 72°C for 5 min. To amplify the assembled fragment, 25pmol of each of the two primers F88971 and R25272 were added.

**BBP library were assembled.** In the SOE experiment, the reaction mixture (35ng BBP1+BBP2 fragment, 35ng BBP3+BBP4 fragment, 35ng BBP5 fragment) was subjected to an initial denaturation step at 94°C for 3 min followed by 5 cycles of 94°C for 30 s, 40°C for 30 s, 72°C for 1 min and then followed by 25 cycles of 94°C for 30 s, 57.5°C for 30 s, 72°C for 1 min and a final extension step at 72°C for 5 min. To amplify the assembled fragment, 25pmol of each of the two primers Fbbp1 and Rtail were added.

**The process of amplification of T fragment:**the PCR protocol consisted of an initial denaturation step at 94°C for 3 min followed by 15 cycles of 94°C for 30 s, 40°C for 30 s,

72°C for 1min and then followed by 20 cycles of 94°C for 30 s, 45°C for 30 s, 72°C for 1min a final extension step at 72°C for 5 min.

**The process of amplification of P fragment:**the PCR protocol consisted of an initial denaturation step at 94°C for 3 min followed by 35 cycles of 94°C for 30 s, 53°C for 30 s, 70°C for 1 min and a final extension step at 72°C for 5 min.

**T fragment and BBP library were assembled.** In the SOE experiment, the reaction mixture (50ng BBP library, 50ng T fragment) was subjected to an initial denaturation step at 94°C for 3 min followed by 15 cycles of 94°C for 30 s, 42°C for 30 s, 72°C for 1 min and followed by 8 cycles of 94°C for 30 s, 64°C for 30 s, 72°C for 1 min and then followed by 15 cycles of 94°C for 30 s, 45°C for 30 s, 72°C for 1 min a final extension step at 72°C for 5 min. To amplify the assembled fragment, 25pmol of each of the two primers FT7 and Rtail were added.

**Anticalin library were assembled.** In the SOE experiment, the reaction mixture (50ng T+BBP library, 50ng P fragment) was subjected to an initial denaturation step at 94°C for 3 min followed by 10 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min, 25pmol of each of the two primers FT7 and PRDR were added at cycle 11 and then followed by 25 cycles of 94°C for 30 s, 45°C for 30 s, 72°C for 1 min a final extension step at 72°C for 5 min. To amplify the assembled fragment.

## **2. Supplementary of sequences of 20 clones.**

After 5<sup>th</sup> and 8<sup>th</sup> cycle of selection, 20 BBP clones which is equal to the fifth and eighth round was sequenced and the result indicated the ratio of mutation to death for the BBP library of the fifth and eighth round were 30% and 40%, respectively(Fig

S1, Fig S2).

E2-5-1.seq	ATGAACTGCTACCCGAGCGTGCCTGTCCGAACTCAAA	40	E2-5-1.seq	GTACCACCGTGAATTTGTTTCAACGTTCTGTCCACCGA	320
E2-5-2.seq	ATGAACTGCTACCCGAGCGTGCCTGTCCGAACTCAAA	40	E2-5-2.seq	GTACCACCGTGAATTTGTTTCAACGTTCTGTCCACCGA	320
E2-5-3.seq	ATGAACTGCTACCCGAGCGTGCCTGTCCGAACTCAAA	40	E2-5-3.seq	GTACCACCGTGAATTTGTTTCAACGTTCTGTCCACCGA	320
E2-5-4.seq	ATGAACTGCTACCCGAGCGTGCCTGTCCGAACTCAAA	40	E2-5-4.seq	GTTATACCCGAGATGGTTTCAACGTTCTGTCCACCGA	317
E2-5-5.seq	ATGAACTGCTACCCGAGCGTGCCTGTCCGAACTCAAA	40	E2-5-5.seq	GTTATACCCGAGATGGTTTCAACGTTCTGTCCACCGA	317
E2-5-6.seq	ATGAACTGCTACCCGAGCGTGCCTGTCCGAACTCAAA	40	E2-5-6.seq	GTTATACCCGAGATGGTTTCAACGTTCTGTCCACCGA	317
E2-5-7.seq	ATGAACTGCTACCCGAGCGTGCCTGTCCGAACTCAAA	40	E2-5-7.seq	GTTATACCCGAGATGGTTTCAACGTTCTGTCCACCGA	317
E2-5-8.seq	ATGAACTGCTACCCGAGCGTGCCTGTCCGAACTCAAA	40	E2-5-8.seq	GTTATACCCGAGATGGTTTCAACGTTCTGTCCACCGA	317
E2-5-9.seq	ATGAACTGCTACCCGAGCGTGCCTGTCCGAACTCAAA	40	E2-5-9.seq	GTTATACCCGAGATGGTTTCAACGTTCTGTCCACCGA	317
E2-5-10.seq	ATGAACTGCTACCCGAGCGTGCCTGTCCGAACTCAAA	40	E2-5-10.seq	GTTATACCCGAGATGGTTTCAACGTTCTGTCCACCGA	317
Consensus	atgaactgctaccgagcgggtgcctgtccgaaactcaaa		Consensus	gt acc gaa gttttcaacgttctgtccaccga	
E2-5-1.seq	CGTGCAACAACCTTGGACGGTCTAACTACCCAGGCAAA	80	E2-5-1.seq	CAACAAAACCTACATCATCGGTTACTGCAAAATAGGAC	360
E2-5-2.seq	CGTGCAACAACCTTGGACGGTCTAACTACCCAGGCAAA	80	E2-5-2.seq	CAACAAAACCTACATCATCGGTTACTGCAAAATAGGAC	360
E2-5-3.seq	CGTGCAACAACCTTGGACGGTCTAACTACCCAGGCAAA	80	E2-5-3.seq	CAACAAAACCTACATCATCGGTTACTGCAAAATAGGAC	360
E2-5-4.seq	CGTGCAACAACCTTGGACGGTCTAACTACCCAGGCAAA	80	E2-5-4.seq	CAACAAAACCTACATCATCGGTTACTGCAAAATAGGAC	357
E2-5-5.seq	CGTGCAACAACCTTGGACGGTCTAACTACCCAGGCAAA	80	E2-5-5.seq	CAACAAAACCTACATCATCGGTTACTGCAAAATAGGAC	357
E2-5-6.seq	CGTGCAACAACCTTGGACGGTCTAACTACCCAGGCAAA	80	E2-5-6.seq	CAACAAAACCTACATCATCGGTTACTGCAAAATAGGAC	357
E2-5-7.seq	CGTGCAACAACCTTGGACGGTCTAACTACCCAGGCAAA	80	E2-5-7.seq	CAACAAAACCTACATCATCGGTTACTGCAAAATAGGAC	357
E2-5-8.seq	CGTGCAACAACCTTGGACGGTCTAACTACCCAGGCAAA	80	E2-5-8.seq	CAACAAAACCTACATCATCGGTTACTGCAAAATAGGAC	357
E2-5-9.seq	CGTGCAACAACCTTGGACGGTCTAACTACCCAGGCAAA	80	E2-5-9.seq	CAACAAAACCTACATCATCGGTTACTGCAAAATAGGAC	357
E2-5-10.seq	CGTGCAACAACCTTGGACGGTCTAACTACCCAGGCAAA	80	E2-5-10.seq	CAACAAAACCTACATCATCGGTTACTGCAAAATAGGAC	357
Consensus	cggtgcaacaacttggacgggtctaaactaccagggcaaatg		Consensus	caacaaaaactacatcatcggttactgcaaaataggac	
E2-5-1.seq	GTGGGAAGTCCGCAAAATACCCGTTTGGTGTCAAAATAC	120	E2-5-1.seq	GAAGCAAAAGGTCACAGCGAGACGTTCTGGTGTCTGT	400
E2-5-2.seq	GTGGGAAGTCCGCAAAATACCCGTTTGGTGTCAAAATAC	120	E2-5-2.seq	GAAGCAAAAGGTCACAGCGAGACGTTCTGGTGTCTGT	400
E2-5-3.seq	GTGGGAAGTCCGCAAAATACCCGTTTGGTGTCAAAATAC	120	E2-5-3.seq	GAAGCAAAAGGTCACAGCGAGACGTTCTGGTGTCTGT	400
E2-5-4.seq	GTGGGAAGTCCGCAAAATACCCGTTTGGTGTCAAAATAC	120	E2-5-4.seq	GAAGCAAAAGGTCACAGCGAGACGTTCTGGTGTCTGT	397
E2-5-5.seq	GTGGGAAGTCCGCAAAATACCCGTTTGGTGTCAAAATAC	120	E2-5-5.seq	GAAGCAAAAGGTCACAGCGAGACGTTCTGGTGTCTGT	397
E2-5-6.seq	GTGGGAAGTCCGCAAAATACCCGTTTGGTGTCAAAATAC	120	E2-5-6.seq	GAAGCAAAAGGTCACAGCGAGACGTTCTGGTGTCTGT	397
E2-5-7.seq	GTGGGAAGTCCGCAAAATACCCGTTTGGTGTCAAAATAC	120	E2-5-7.seq	GAAGCAAAAGGTCACAGCGAGACGTTCTGGTGTCTGT	397
E2-5-8.seq	GTGGGAAGTCCGCAAAATACCCGTTTGGTGTCAAAATAC	120	E2-5-8.seq	GAAGCAAAAGGTCACAGCGAGACGTTCTGGTGTCTGT	397
E2-5-9.seq	GTGGGAAGTCCGCAAAATACCCGTTTGGTGTCAAAATAC	120	E2-5-9.seq	GAAGCAAAAGGTCACAGCGAGACGTTCTGGTGTCTGT	397
E2-5-10.seq	GTGGGAAGTCCGCAAAATACCCGTTTGGTGTCAAAATAC	120	E2-5-10.seq	GAAGCAAAAGGTCACAGCGAGACGTTCTGGTGTCTGT	397
Consensus	gtgggaagtccgcaaaataccggtttggtgtcaaaatc		Consensus	gaagcaaaaaggctcacagcgagaccttctgggtctgt	
E2-5-1.seq	GGTAAATCGGTTGGGCTGAATACACCCCGGAAGGCAAA	160	E2-5-1.seq	CTCGTCCAAAGTCCCTGACCCGGTGAAGCCAAAACCGCTGT	440
E2-5-2.seq	GGTAAATCGGTTGGGCTGAATACACCCCGGAAGGCAAA	160	E2-5-2.seq	CTCGTCCAAAGTCCCTGACCCGGTGAAGCCAAAACCGCTGT	440
E2-5-3.seq	GGTAAATCGGTTGGGCTGAATACACCCCGGAAGGCAAA	160	E2-5-3.seq	CTCGTCCAAAGTCCCTGACCCGGTGAAGCCAAAACCGCTGT	440
E2-5-4.seq	GGTAAATCGGTTGGGCTGAATACACCCCGGAAGGCAAA	160	E2-5-4.seq	CTCGTCCAAAGTCCCTGACCCGGTGAAGCCAAAACCGCTGT	437
E2-5-5.seq	GGTAAATCGGTTGGGCTGAATACACCCCGGAAGGCAAA	160	E2-5-5.seq	CTCGTCCAAAGTCCCTGACCCGGTGAAGCCAAAACCGCTGT	437
E2-5-6.seq	GGTAAATCGGTTGGGCTGAATACACCCCGGAAGGCAAA	160	E2-5-6.seq	CTCGTCCAAAGTCCCTGACCCGGTGAAGCCAAAACCGCTGT	437
E2-5-7.seq	GGTAAATCGGTTGGGCTGAATACACCCCGGAAGGCAAA	160	E2-5-7.seq	CTCGTCCAAAGTCCCTGACCCGGTGAAGCCAAAACCGCTGT	437
E2-5-8.seq	GGTAAATCGGTTGGGCTGAATACACCCCGGAAGGCAAA	160	E2-5-8.seq	CTCGTCCAAAGTCCCTGACCCGGTGAAGCCAAAACCGCTGT	437
E2-5-9.seq	GGTAAATCGGTTGGGCTGAATACACCCCGGAAGGCAAA	160	E2-5-9.seq	CTCGTCCAAAGTCCCTGACCCGGTGAAGCCAAAACCGCTGT	437
E2-5-10.seq	GGTAAATCGGTTGGGCTGAATACACCCCGGAAGGCAAA	160	E2-5-10.seq	CTCGTCCAAAGTCCCTGACCCGGTGAAGCCAAAACCGCTGT	437
Consensus	ggtaaatcgggtgggctgaatacaccccggaaggcaaaa		Consensus	ctcgttccaaagtccctgaccgggtgaagccaaaaccgctgt	
E2-5-1.seq	CGCTCAAAAGTTTCGAAATACGTTTGTATCCACGGCAAGA	200	E2-5-1.seq	CGAAAACCTACCTGATCGCTCCCGGTTGCGACTCCCGAG	480
E2-5-2.seq	CGCTCAAAAGTTTCGAAATACGTTTGTATCCACGGCAAGA	200	E2-5-2.seq	CGAAAACCTACCTGATCGCTCCCGGTTGCGACTCCCGAG	480
E2-5-3.seq	CGCTCAAAAGTTTCGAAATACGTTTGTATCCACGGCAAGA	200	E2-5-3.seq	CGAAAACCTACCTGATCGCTCCCGGTTGCGACTCCCGAG	480
E2-5-4.seq	CGCTCAAAAGTTTCGAAATACGTTTGTATCCACGGCAAGA	200	E2-5-4.seq	CGAAAACCTACCTGATCGCTCCCGGTTGCGACTCCCGAG	477
E2-5-5.seq	CGCTCAAAAGTTTCGAAATACGTTTGTATCCACGGCAAGA	200	E2-5-5.seq	CGAAAACCTACCTGATCGCTCCCGGTTGCGACTCCCGAG	477
E2-5-6.seq	CGCTCAAAAGTTTCGAAATACGTTTGTATCCACGGCAAGA	200	E2-5-6.seq	CGAAAACCTACCTGATCGCTCCCGGTTGCGACTCCCGAG	477
E2-5-7.seq	CGCTCAAAAGTTTCGAAATACGTTTGTATCCACGGCAAGA	200	E2-5-7.seq	CGAAAACCTACCTGATCGCTCCCGGTTGCGACTCCCGAG	477
E2-5-8.seq	CGCTCAAAAGTTTCGAAATACGTTTGTATCCACGGCAAGA	200	E2-5-8.seq	CGAAAACCTACCTGATCGCTCCCGGTTGCGACTCCCGAG	477
E2-5-9.seq	CGCTCAAAAGTTTCGAAATACGTTTGTATCCACGGCAAGA	200	E2-5-9.seq	CGAAAACCTACCTGATCGCTCCCGGTTGCGACTCCCGAG	477
E2-5-10.seq	CGCTCAAAAGTTTCGAAATACGTTTGTATCCACGGCAAGA	200	E2-5-10.seq	CGAAAACCTACCTGATCGCTCCCGGTTGCGACTCCCGAG	477
Consensus	gc tcaaaagttttg tac gttatccacggcaaga		Consensus	cgaaaactacctgatcgctcccggttgcgactcccgag	
E2-5-1.seq	ATACTTTAATGAAAGTACCGCTACCGGTTGGTGTACTCC	240	E2-5-1.seq	AAACTGGTTTACAGCGACTTCTCTGAAGCGGCTGCAAAG	520
E2-5-2.seq	ATACTTTAATGAAAGTACCGCTACCGGTTGGTGTACTCC	240	E2-5-2.seq	AAACTGGTTTACAGCGACTTCTCTGAAGCGGCTGCAAAG	520
E2-5-3.seq	ATACTTTAATGAAAGTACCGCTACCGGTTGGTGTACTCC	240	E2-5-3.seq	AAACTGGTTTACAGCGACTTCTCTGAAGCGGCTGCAAAG	520
E2-5-4.seq	ATACTTTAATGAAAGTACCGCTACCGGTTGGTGTACTCC	240	E2-5-4.seq	AAACTGGTTTACAGCGACTTCTCTGAAGCGGCTGCAAAG	517
E2-5-5.seq	ATACTTTAATGAAAGTACCGCTACCGGTTGGTGTACTCC	240	E2-5-5.seq	AAACTGGTTTACAGCGACTTCTCTGAAGCGGCTGCAAAG	517
E2-5-6.seq	ATACTTTAATGAAAGTACCGCTACCGGTTGGTGTACTCC	240	E2-5-6.seq	AAACTGGTTTACAGCGACTTCTCTGAAGCGGCTGCAAAG	517
E2-5-7.seq	ATACTTTAATGAAAGTACCGCTACCGGTTGGTGTACTCC	240	E2-5-7.seq	AAACTGGTTTACAGCGACTTCTCTGAAGCGGCTGCAAAG	517
E2-5-8.seq	ATACTTTAATGAAAGTACCGCTACCGGTTGGTGTACTCC	240	E2-5-8.seq	AAACTGGTTTACAGCGACTTCTCTGAAGCGGCTGCAAAG	517
E2-5-9.seq	ATACTTTAATGAAAGTACCGCTACCGGTTGGTGTACTCC	240	E2-5-9.seq	AAACTGGTTTACAGCGACTTCTCTGAAGCGGCTGCAAAG	517
E2-5-10.seq	ATACTTTAATGAAAGTACCGCTACCGGTTGGTGTACTCC	240	E2-5-10.seq	AAACTGGTTTACAGCGACTTCTCTGAAGCGGCTGCAAAG	517
Consensus	atacttt aatgaaagtaccgctaccggttggtgtactcc		Consensus	aaactggtttacagcgacttctctgaagcggctgcaaa	
E2-5-1.seq	AAAATTTGTAATAATCTACCCAAATACCGTACCGGTTGGT	280	E2-5-1.seq	TCACAA	527
E2-5-2.seq	AAAATTTGTAATAATCTACCCAAATACCGTACCGGTTGGT	280	E2-5-2.seq	TCACAA	527
E2-5-3.seq	AAAATTTGTAATAATCTACCCAAATACCGTACCGGTTGGT	280	E2-5-3.seq	TCACAA	527
E2-5-4.seq	AAAATTTGTAATAATCTACCCAAATACCGTACCGGTTGGT	277	E2-5-4.seq	TCACAA	524
E2-5-5.seq	AAAATTTGTAATAATCTACCCAAATACCGTACCGGTTGGT	277	E2-5-5.seq	TCACAA	524
E2-5-6.seq	AAAATTTGTAATAATCTACCCAAATACCGTACCGGTTGGT	277	E2-5-6.seq	TCACAA	524
E2-5-7.seq	AAAATTTGTAATAATCTACCCAAATACCGTACCGGTTGGT	277	E2-5-7.seq	TCACAA	524
E2-5-8.seq	AAAATTTGTAATAATCTACCCAAATACCGTACCGGTTGGT	277	E2-5-8.seq	TCACAA	524
E2-5-9.seq	AAAATTTGTAATAATCTACCCAAATACCGTACCGGTTGGT	277	E2-5-9.seq	TCACAA	524
E2-5-10.seq	AAAATTTGTAATAATCTACCCAAATACCGTACCGGTTGGT	277	E2-5-10.seq	TCACAA	524
Consensus	aaaatttgtaaaatctacccaaa acc ggtg		Consensus	tcaaaa	

**Fig S1** Supplementary of sequences of 10 clones obtained from fifth round. (Color region represents the designed random mutation sites. Orange frame represents fatal mutations. The sequences of E<sub>2</sub>-5-1, E<sub>2</sub>-5-2 and E<sub>2</sub>-5-3 are fully identical and we named them E<sub>2</sub>-A. The sequences of E<sub>2</sub>-5-4 and E<sub>2</sub>-5-5 are fully identical and we named them E<sub>2</sub>-B.

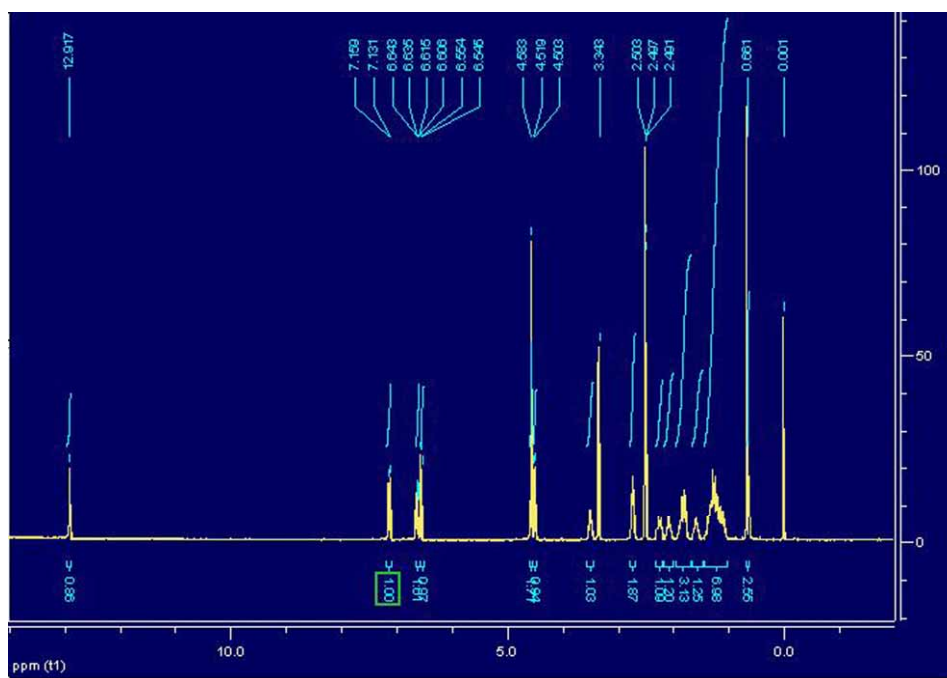
E2-8-1.SEQ	ATGAACGTGTACCACGACGGTGCCTGTCGGGAAGTCAAA	40	E2-8-1.SEQ	TGCTACCTTGGACTGTTTTCAACGTTCTGTCACCAGAC	318
E2-8-2.SEQ	ATGAACGTGTACCACGACGGTGCCTGTCGGGAAGTCAAA	40	E2-8-2.SEQ	TGCTACCTTGGACTGTTTTCAACGTTCTGTCACCAGAC	318
E2-8-3.SEQ	ATGAACGTGTACCACGACGGTGCCTGTCGGGAAGTCAAA	40	E2-8-3.SEQ	TGCTACCTTGGACTGTTTTCAACGTTCTGTCACCAGAC	318
E2-8-4.SEQ	ATGAACGTGTACCACGACGGTGCCTGTCGGGAAGTCAAA	40	E2-8-4.SEQ	TGCTACCTTGGACTGTTTTCAACGTTCTGTCACCAGAC	318
E2-8-5.SEQ	ATGAACGTGTACCACGACGGTGCCTGTCGGGAAGTCAAA	40	E2-8-5.SEQ	TGCTACCTTGGACTGTTTTCAACGTTCTGTCACCAGAC	318
E2-8-6.SEQ	ATGAACGTGTACCACGACGGTGCCTGTCGGGAAGTCAAA	40	E2-8-6.SEQ	TGCTACCTTGGACTGTTTTCAACGTTCTGTCACCAGAC	318
E2-8-7.SEQ	ATGAACGTGTACCACGACGGTGCCTGTCGGGAAGTCAAA	40	E2-8-7.SEQ	TGCTACCTTGGACTGTTTTCAACGTTCTGTCACCAGAC	318
E2-8-8.SEQ	ATGAACGTGTACCACGACGGTGCCTGTCGGGAAGTCAAA	40	E2-8-8.SEQ	TGCTACCTTGGACTGTTTTCAACGTTCTGTCACCAGAC	318
E2-8-9.SEQ	ATGAACGTGTACCACGACGGTGCCTGTCGGGAAGTCAAA	40	E2-8-9.SEQ	TGCTACCTTGGACTGTTTTCAACGTTCTGTCACCAGAC	318
E2-8-10.SEQ	ATGAACGTGTACCACGACGGTGCCTGTCGGGAAGTCAAA	40	E2-8-10.SEQ	TGCTACCTTGGACTGTTTTCAACGTTCTGTCACCAGAC	318
Consensus	atgaacgtgtaccacgacgggtgcctgtccggaagtcaaa		Consensus	t acc gaa gttttcaacgtt tctaccagac	
E2-8-1.SEQ	CGGTGCAAACTTCGACTGGTCTAACTACCAGGCAAAATG	80	E2-8-1.SEQ	AACAAAACTACATCATCGGTTACTTGGCTTACGAGC	358
E2-8-2.SEQ	CGGTGCAAACTTCGACTGGTCTAACTACCAGGCAAAATG	80	E2-8-2.SEQ	AACAAAACTACATCATCGGTTACTTGGCTTACGAGC	358
E2-8-3.SEQ	CGGTGCAAACTTCGACTGGTCTAACTACCAGGCAAAATG	80	E2-8-3.SEQ	AACAAAACTACATCATCGGTTACTTGGCTTACGAGC	358
E2-8-4.SEQ	CGGTGCAAACTTCGACTGGTCTAACTACCAGGCAAAATG	80	E2-8-4.SEQ	AACAAAACTACATCATCGGTTACTTGGCTTACGAGC	358
E2-8-5.SEQ	CGGTGCAAACTTCGACTGGTCTAACTACCAGGCAAAATG	80	E2-8-5.SEQ	AACAAAACTACATCATCGGTTACTTGGCTTACGAGC	358
E2-8-6.SEQ	CGGTGCAAACTTCGACTGGTCTAACTACCAGGCAAAATG	80	E2-8-6.SEQ	AACAAAACTACATCATCGGTTACTTGGCTTACGAGC	358
E2-8-7.SEQ	CGGTGCAAACTTCGACTGGTCTAACTACCAGGCAAAATG	80	E2-8-7.SEQ	AACAAAACTACATCATCGGTTACTTGGCTTACGAGC	358
E2-8-8.SEQ	CGGTGCAAACTTCGACTGGTCTAACTACCAGGCAAAATG	80	E2-8-8.SEQ	AACAAAACTACATCATCGGTTACTTGGCTTACGAGC	358
E2-8-9.SEQ	CGGTGCAAACTTCGACTGGTCTAACTACCAGGCAAAATG	80	E2-8-9.SEQ	AACAAAACTACATCATCGGTTACTTGGCTTACGAGC	358
E2-8-10.SEQ	CGGTGCAAACTTCGACTGGTCTAACTACCAGGCAAAATG	80	E2-8-10.SEQ	AACAAAACTACATCATCGGTTACTTGGCTTACGAGC	358
Consensus	cggtgcaaaacttcgactgggtcttaactaccagggcaaatg		Consensus	aacaaaaactacatcatcggttact tggcttacgagc	
E2-8-1.SEQ	GTGGGAAGTGCAGCAATACCCGGTGTGTTTGGTTAAATA	119	E2-8-1.SEQ	AAGACAAAAAGGTCACAGGACTCGGCTCGGGTGTGTC	398
E2-8-2.SEQ	GTGGGAAGTGCAGCAATACCCGGTGTGTTTGGTTAAATA	119	E2-8-2.SEQ	AAGACAAAAAGGTCACAGGACTCGGCTCGGGTGTGTC	398
E2-8-3.SEQ	GTGGGAAGTGCAGCAATACCCGGTGTGTTTGGTTAAATA	119	E2-8-3.SEQ	AAGACAAAAAGGTCACAGGACTCGGCTCGGGTGTGTC	398
E2-8-4.SEQ	GTGGGAAGTGCAGCAATACCCGGTGTGTTTGGTTAAATA	119	E2-8-4.SEQ	AAGACAAAAAGGTCACAGGACTCGGCTCGGGTGTGTC	398
E2-8-5.SEQ	GTGGGAAGTGCAGCAATACCCGGTGTGTTTGGTTAAATA	119	E2-8-5.SEQ	AAGACAAAAAGGTCACAGGACTCGGCTCGGGTGTGTC	398
E2-8-6.SEQ	GTGGGAAGTGCAGCAATACCCGGTGTGTTTGGTTAAATA	119	E2-8-6.SEQ	AAGACAAAAAGGTCACAGGACTCGGCTCGGGTGTGTC	398
E2-8-7.SEQ	GTGGGAAGTGCAGCAATACCCGGTGTGTTTGGTTAAATA	119	E2-8-7.SEQ	AAGACAAAAAGGTCACAGGACTCGGCTCGGGTGTGTC	398
E2-8-8.SEQ	GTGGGAAGTGCAGCAATACCCGGTGTGTTTGGTTAAATA	119	E2-8-8.SEQ	AAGACAAAAAGGTCACAGGACTCGGCTCGGGTGTGTC	398
E2-8-9.SEQ	GTGGGAAGTGCAGCAATACCCGGTGTGTTTGGTTAAATA	119	E2-8-9.SEQ	AAGACAAAAAGGTCACAGGACTCGGCTCGGGTGTGTC	398
E2-8-10.SEQ	GTGGGAAGTGCAGCAATACCCGGTGTGTTTGGTTAAATA	119	E2-8-10.SEQ	AAGACAAAAAGGTCACAGGACTCGGCTCGGGTGTGTC	398
Consensus	gtgggaagtgcagcaaataccgggtgtgtttggttaaata		Consensus	aagacaaaaaggctcac aggactcggctcgggtgtgttc	
E2-8-1.SEQ	CGGTAATGCGGTTGGGCTGAATCACCOCGGAAGCAAA	159	E2-8-1.SEQ	TCGTTCCAAAGTCTGACCGGTGAAGCCAAAACCGCTGTC	438
E2-8-2.SEQ	CGGTAATGCGGTTGGGCTGAATCACCOCGGAAGCAAA	159	E2-8-2.SEQ	TCGTTCCAAAGTCTGACCGGTGAAGCCAAAACCGCTGTC	438
E2-8-3.SEQ	CGGTAATGCGGTTGGGCTGAATCACCOCGGAAGCAAA	159	E2-8-3.SEQ	TCGTTCCAAAGTCTGACCGGTGAAGCCAAAACCGCTGTC	438
E2-8-4.SEQ	CGGTAATGCGGTTGGGCTGAATCACCOCGGAAGCAAA	159	E2-8-4.SEQ	TCGTTCCAAAGTCTGACCGGTGAAGCCAAAACCGCTGTC	438
E2-8-5.SEQ	CGGTAATGCGGTTGGGCTGAATCACCOCGGAAGCAAA	159	E2-8-5.SEQ	TCGTTCCAAAGTCTGACCGGTGAAGCCAAAACCGCTGTC	438
E2-8-6.SEQ	CGGTAATGCGGTTGGGCTGAATCACCOCGGAAGCAAA	159	E2-8-6.SEQ	TCGTTCCAAAGTCTGACCGGTGAAGCCAAAACCGCTGTC	438
E2-8-7.SEQ	CGGTAATGCGGTTGGGCTGAATCACCOCGGAAGCAAA	159	E2-8-7.SEQ	TCGTTCCAAAGTCTGACCGGTGAAGCCAAAACCGCTGTC	438
E2-8-8.SEQ	CGGTAATGCGGTTGGGCTGAATCACCOCGGAAGCAAA	159	E2-8-8.SEQ	TCGTTCCAAAGTCTGACCGGTGAAGCCAAAACCGCTGTC	438
E2-8-9.SEQ	CGGTAATGCGGTTGGGCTGAATCACCOCGGAAGCAAA	159	E2-8-9.SEQ	TCGTTCCAAAGTCTGACCGGTGAAGCCAAAACCGCTGTC	438
E2-8-10.SEQ	CGGTAATGCGGTTGGGCTGAATCACCOCGGAAGCAAA	159	E2-8-10.SEQ	TCGTTCCAAAGTCTGACCGGTGAAGCCAAAACCGCTGTC	438
Consensus	cggtaatgctgggtgggctgaatcaccocgggaagcaaa		Consensus	tctgtccaaagtctgac cgggtgaagccaaaaccgctgttc	
E2-8-1.SEQ	AGCGTCAAAGTTTCGAACTACAGTGTATCCACCGCAAA	198	E2-8-1.SEQ	GAAAACCTACCTGATCGGCTCCCGGTTGTGCATCCAGAG	478
E2-8-2.SEQ	AGCGTCAAAGTTTCGAACTACAGTGTATCCACCGCAAA	198	E2-8-2.SEQ	GAAAACCTACCTGATCGGCTCCCGGTTGTGCATCCAGAG	478
E2-8-3.SEQ	AGCGTCAAAGTTTCGAACTACAGTGTATCCACCGCAAA	198	E2-8-3.SEQ	GAAAACCTACCTGATCGGCTCCCGGTTGTGCATCCAGAG	478
E2-8-4.SEQ	AGCGTCAAAGTTTCGAACTACAGTGTATCCACCGCAAA	198	E2-8-4.SEQ	GAAAACCTACCTGATCGGCTCCCGGTTGTGCATCCAGAG	478
E2-8-5.SEQ	AGCGTCAAAGTTTCGAACTACAGTGTATCCACCGCAAA	198	E2-8-5.SEQ	GAAAACCTACCTGATCGGCTCCCGGTTGTGCATCCAGAG	478
E2-8-6.SEQ	AGCGTCAAAGTTTCGAACTACAGTGTATCCACCGCAAA	198	E2-8-6.SEQ	GAAAACCTACCTGATCGGCTCCCGGTTGTGCATCCAGAG	478
E2-8-7.SEQ	AGCGTCAAAGTTTCGAACTACAGTGTATCCACCGCAAA	198	E2-8-7.SEQ	GAAAACCTACCTGATCGGCTCCCGGTTGTGCATCCAGAG	478
E2-8-8.SEQ	AGCGTCAAAGTTTCGAACTACAGTGTATCCACCGCAAA	198	E2-8-8.SEQ	GAAAACCTACCTGATCGGCTCCCGGTTGTGCATCCAGAG	478
E2-8-9.SEQ	AGCGTCAAAGTTTCGAACTACAGTGTATCCACCGCAAA	198	E2-8-9.SEQ	GAAAACCTACCTGATCGGCTCCCGGTTGTGCATCCAGAG	478
E2-8-10.SEQ	AGCGTCAAAGTTTCGAACTACAGTGTATCCACCGCAAA	198	E2-8-10.SEQ	GAAAACCTACCTGATCGGCTCCCGGTTGTGCATCCAGAG	478
Consensus	agcgtcaaagtttcgaaactacagtgtatccaccgcaaa		Consensus	gaaaactacctgatcggctccccggttctgcactcccaga	
E2-8-1.SEQ	GAATACTTTCGGAAGGTACCGCTACCCGGTGGTGACT	238	E2-8-1.SEQ	ACTGGTTTACAGCGGACTTCTCTGAAGCCGCTGCAAAAGT	518
E2-8-2.SEQ	GAATACTTTCGGAAGGTACCGCTACCCGGTGGTGACT	238	E2-8-2.SEQ	ACTGGTTTACAGCGGACTTCTCTGAAGCCGCTGCAAAAGT	518
E2-8-3.SEQ	GAATACTTTCGGAAGGTACCGCTACCCGGTGGTGACT	238	E2-8-3.SEQ	ACTGGTTTACAGCGGACTTCTCTGAAGCCGCTGCAAAAGT	518
E2-8-4.SEQ	GAATACTTTCGGAAGGTACCGCTACCCGGTGGTGACT	238	E2-8-4.SEQ	ACTGGTTTACAGCGGACTTCTCTGAAGCCGCTGCAAAAGT	518
E2-8-5.SEQ	GAATACTTTCGGAAGGTACCGCTACCCGGTGGTGACT	238	E2-8-5.SEQ	ACTGGTTTACAGCGGACTTCTCTGAAGCCGCTGCAAAAGT	518
E2-8-6.SEQ	GAATACTTTCGGAAGGTACCGCTACCCGGTGGTGACT	238	E2-8-6.SEQ	ACTGGTTTACAGCGGACTTCTCTGAAGCCGCTGCAAAAGT	518
E2-8-7.SEQ	GAATACTTTCGGAAGGTACCGCTACCCGGTGGTGACT	238	E2-8-7.SEQ	ACTGGTTTACAGCGGACTTCTCTGAAGCCGCTGCAAAAGT	518
E2-8-8.SEQ	GAATACTTTCGGAAGGTACCGCTACCCGGTGGTGACT	238	E2-8-8.SEQ	ACTGGTTTACAGCGGACTTCTCTGAAGCCGCTGCAAAAGT	518
E2-8-9.SEQ	GAATACTTTCGGAAGGTACCGCTACCCGGTGGTGACT	238	E2-8-9.SEQ	ACTGGTTTACAGCGGACTTCTCTGAAGCCGCTGCAAAAGT	518
E2-8-10.SEQ	GAATACTTTCGGAAGGTACCGCTACCCGGTGGTGACT	238	E2-8-10.SEQ	ACTGGTTTACAGCGGACTTCTCTGAAGCCGCTGCAAAAGT	518
Consensus	gaatactttcgggaaggtaccgctaccgggtgggtgact		Consensus	aactggtttacagcgacttctctggaag ccgctgcaaaagt	
E2-8-1.SEQ	CCAAAAATGGTAAAATCTACCACAATTCACCTTGGGTGG	278	E2-8-1.SEQ	CAACAA	524
E2-8-2.SEQ	CCAAAAATGGTAAAATCTACCACAATTCACCTTGGGTGG	278	E2-8-2.SEQ	CAACAA	524
E2-8-3.SEQ	CCAAAAATGGTAAAATCTACCACAATTCACCTTGGGTGG	278	E2-8-3.SEQ	CAACAA	524
E2-8-4.SEQ	CCAAAAATGGTAAAATCTACCACAATTCACCTTGGGTGG	278	E2-8-4.SEQ	CAACAA	524
E2-8-5.SEQ	CCAAAAATGGTAAAATCTACCACAATTCACCTTGGGTGG	278	E2-8-5.SEQ	CAACAA	524
E2-8-6.SEQ	CCAAAAATGGTAAAATCTACCACAATTCACCTTGGGTGG	278	E2-8-6.SEQ	CAACAA	524
E2-8-7.SEQ	CCAAAAATGGTAAAATCTACCACAATTCACCTTGGGTGG	278	E2-8-7.SEQ	CAACAA	524
E2-8-8.SEQ	CCAAAAATGGTAAAATCTACCACAATTCACCTTGGGTGG	278	E2-8-8.SEQ	CAACAA	524
E2-8-9.SEQ	CCAAAAATGGTAAAATCTACCACAATTCACCTTGGGTGG	278	E2-8-9.SEQ	CAACAA	524
E2-8-10.SEQ	CCAAAAATGGTAAAATCTACCACAATTCACCTTGGGTGG	278	E2-8-10.SEQ	CAACAA	524
Consensus	ccaaaaatggtaaaatctaccacaattcaccttgggtgg		Consensus	caacaa	

**Fig S2** Supplementary sequences of 10 clones obtained from fifth round. (Color region represents the designed random mutation sites. Orange frame represents fatal mutations. The sequences of E<sub>2</sub>-8-1 and E<sub>2</sub>-8-2 are fully identical and we named them E<sub>2</sub>-C. The sequences of E<sub>2</sub>-8-3 and E<sub>2</sub>-8-4 are fully identical and we named them E<sub>2</sub>-D.

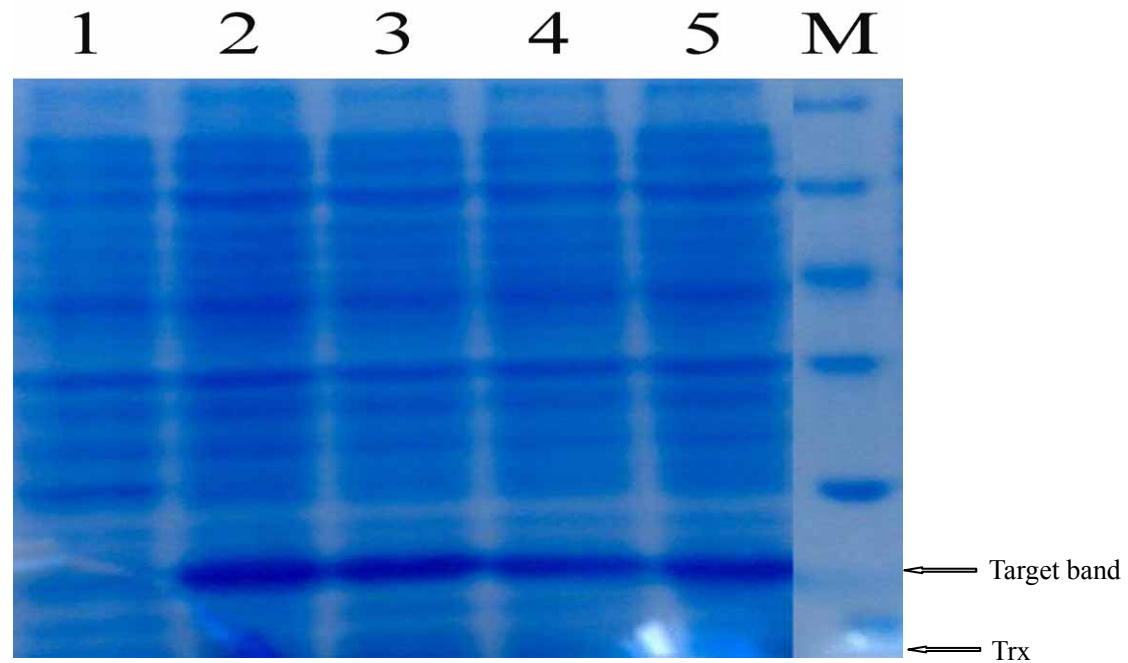
### 3. Supplementary of sequences of BBP template for designing 17 oligodeoxynucleotides (Table 1).

AACGTGTACCACGACGGTGCCTGTCCGGAAGTCAAACCGGTCGACAACTTCGACTGGTCT  
AACTACCACGGCAAATGGTGGGAAGTCGCCAAATACCCGAACAGCGTTGAAAAATACGG  
TAAATGCGGTTGGGCTGAATACACCCCGGAAGGCAAAGCGTCAAAGTTTCGAACTACCA  
CGTTATCCACGGCAAAGAATACTTTATTGAAGGTACCGCCTACCCGTTGGTGACTCCAAA  
ATTGGTAAAATCTACCACAACTGACCTACGGTGGTGTACCAAAGAAAACGTTTTCAAC  
GTTCTGTCCACCGACAACAAAACTACATCATCGGTTACTACTGCAAATACGACGAAGAC  
AAAAAAGGTCACCAGGACTTCGTCTGGGTGCTGTCTCGTTCCAAAGTCCTGACCGGTGAA  
GCCAAAACCGCTGTGCGAAAACCTACCTGATCGGCTCCCCGTTGTCGACTCCAGAACTG  
GTTTACAGCGACTTCTCTGAAGCCGCCTGCAAAGTCAACAAT

### 4. Supplementary of $^1\text{H}$ NMR of estradiol-3-carboxymethyl ether ( $\text{E}_2$ -3-CME).



**5. Supplementary of SDS-PAGE assay of four random selected soluble anti-E<sub>2</sub> (approximately 20kDa) (Fig. S3)**



Lane M: protein standards; lane 1: induced plasmid pTIG-trx; lane 2: the supernatant of cellular lysate of induced E<sub>2</sub>-A; lane 3: the supernatant of cellular lysate of induced E<sub>2</sub>-B; lane 4: the supernatant of cellular lysate of induced E<sub>2</sub>-C; lane 5: the supernatant of cellular lysate of induced E<sub>2</sub>-D;