Supplementary Materials

Catalytic Synthesis of (S)-CHBE by Directional Coupling and Immobilization of Carbonyl Reductase and Glucose Dehydrogenase

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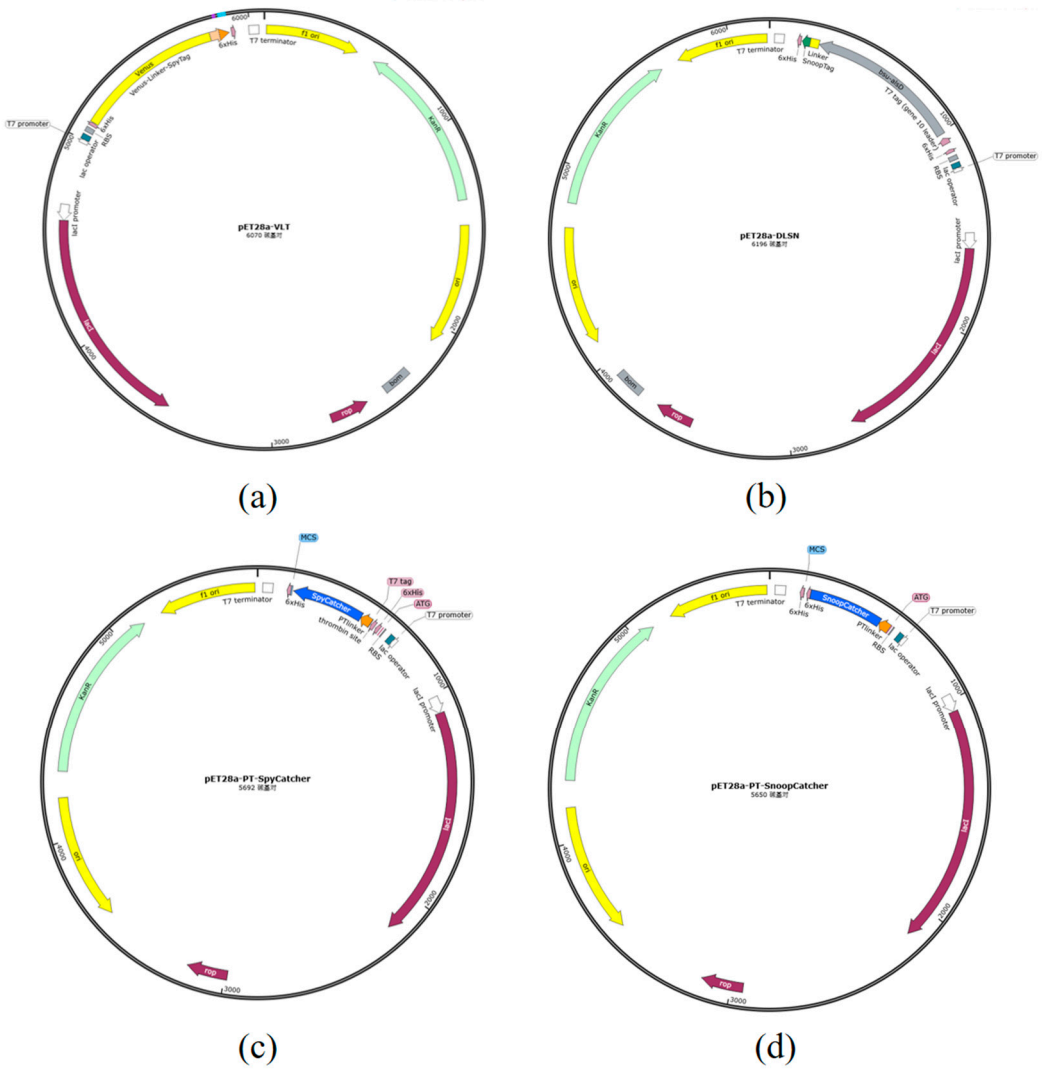

Figure S1. The plasmid profiles. (**a**) pET28a-VLT. (**b**) pET28a-DLSN. (**c**) pET28a-SpyCatcher. (**d**) pET28a-SnoopCatcher.

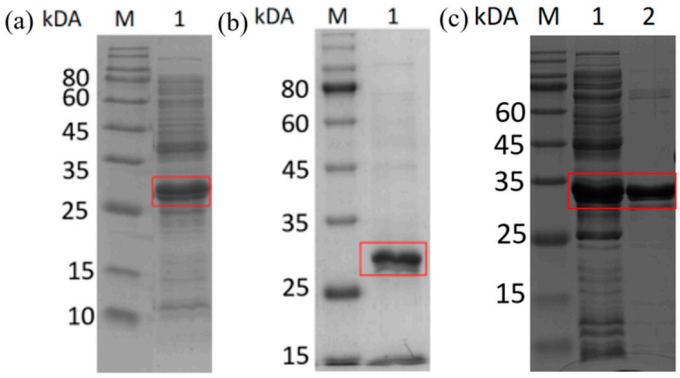

Figure S2. SDS-PAGE analysis of recombinant fusion protein expression in E. coli. (**a**) M: Protein Marker; lane 1: BsCR crude enzyme. (**b**) M: Protein Marker; lane 1: Purified BsCR enzyme solution. (**c**) M: Protein Marker; lane 1: BsGDH crude enzyme; lane 2: Purified BsGDH enzyme solution.

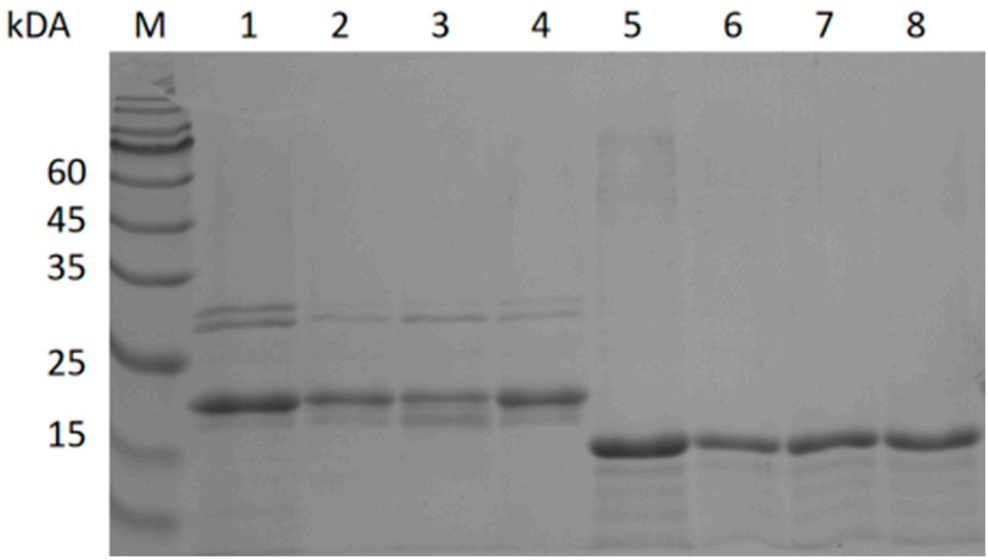

Figure S3. SDS-PAGE results of immobilization of purified SpyCatcher and SnoopCatcher ; M: Protein Marker; lane 1: Purified SpyCatcher; lane 2-4: The SpyCatcher supernatant occurred after fixation; lane 5: Purified SnoopCatcher; lane 6-8: SnoopCatcher After fixed supernatant.

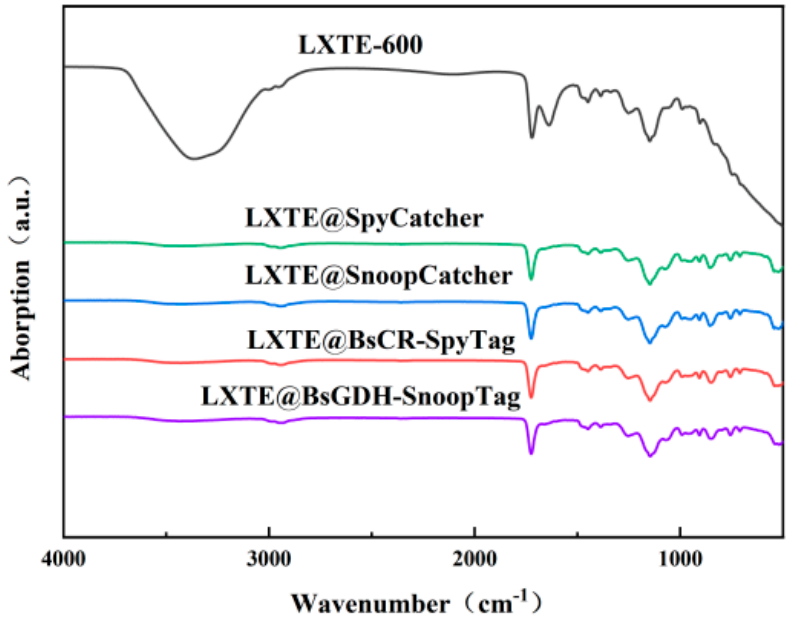

Figure S4. FT-IR spectra of the epoxy resin and the immobilized resin.

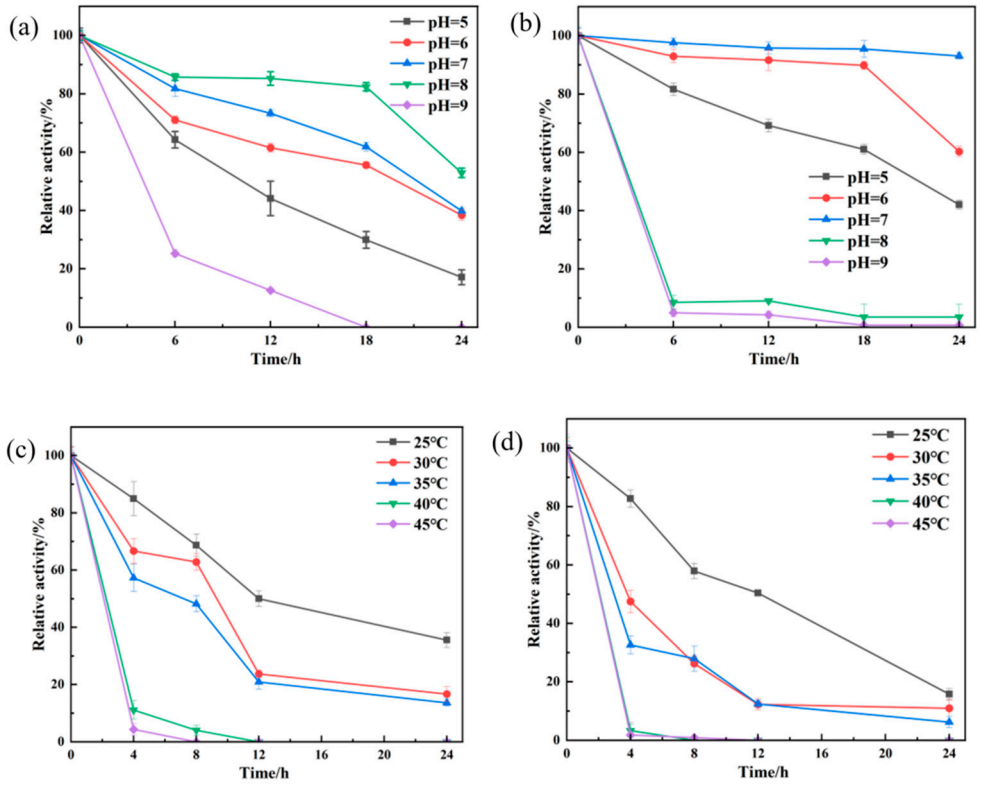

Figure S5. Effects of pH and temperature on BsCR-SpyTag and BsGDH-SnoopTag stability. (a) PH Stability of BsCR-SpyTag: various buffers of PH 5.0-9.0 for 24 hours at 4 ℃. (b) The pH stability of BsGDH-SnoopTag: various buffers of pH 5.0-9.0 for 24 hours at 4 ℃. (c) Temperature stability of BsCR-SpyTag: 25 ℃, 30 ℃, 35 ℃ ,40 ℃and 45 ℃, pH 7.0 , 24 hours. (d) Temperature stability of BsGDH-SnoopTag: 25 ℃, 30 ℃, 35 ℃ ,40 ℃and 45 ℃, pH 7.0 , 24 hours.

Table S1. Nucleic acid sequences of constructed fusion proteins:

Gene name	Nucleic acid sequences
Linker-SpyTag:	GGAGGCTCCGGATCCGCTGGCTCCGCTGCTGGTTCTGGCGAA TTCCGTGGCGTTCCGCACATCGTTATGGTTGATGCGTATAAAC GTTACAAA
Linker-SnoopTag:	GAATTCGCCAGAACCAGCAGCGGAGCCAGCGGATCCGGAGC CTCCAAGTTGGGTGACATCGAGTTCATCAAGGTCAACAAG
SpyCatcher:	GTTACCACCCTGAGCGGCCTGAGCGGTGAACAGGGCCCGAG CGGCGATATGACCACCGAAGAAGATAGCGCGACCCACATCA AATTCAGCAAACGTGATGAAGATGGCCGTGAACTGGCGGGC GCGACCATGGAACTGCGTGATAGCAGCGGTAAAACCATCAG CACCTGGATTAGTGACGGCCACGTGAAAGACTTTTATCTGTAT CCGGGTAAATATACCTTCGTTGAAACCGCTGCGCCGGATGGTT ACGAAGTTGCGACCCCGATCGAATTCACCGTTAACGAAGATG GTCAGGTTACCGTTGATGGTGAAGCGACCGAAGGTGATGCTC ACACC
SnoopCatcher:	AAACCGCTGCGTGGCGCGGTTTTCAGCCTGCAAAAACAGCA CCCGGATTACCCGGACATCTACGGCGCGATCGATCAGAACGG CACCTACCAGAACGTTCGTACCGGCGAAGATGGCAAACTGA

CCTTCAAAAACCTGAGCGATGGCAAATACCGTCTGTTCGAAA
ACAGCGAACCGGCGGGCTACAAACCGGTTCAGAACAAACCG
ATCGTTGCGTTCCAGATCGTTAACGGCGAAGTTCGTGATGTTA
CCAGCATCGTTCCGCAGGACATCCCGGCGACCTACGAATTTA
CCAACGGCAAACACTACATCACCAACGAACCGATCCCGCCG
AAA

yueD: ATGGAACTTTATATCATCACCGGAGCGTCAAAAGGGCTGGGT
CAAGCCATTGCATTACAGGCTTTAGAAAAGGGGCATGAAGTC
CATGCCTTATCCAGAACGAAAACAGATGTCTCTCACAAAAAA
CTAACGCAGCATCAAATAGACCTCATCAATCTCGAAGAAGCT
GAACAGCAATTTGAAACATTGCTCTCATCCATCGATTCAGATC
GTTATTCTGGTATTACCCTTATTAATAACGCCGGAATGGTAACG
CCGATCAAACGTGCCGGCGAAGCGTCTCTTGACGAGCTTCAG
CGCCATTATCAGCTGAACCTGACTGCGCCCGTGCTTTTGAGTC
AGCTGTTTACAAAACGGTTTGCTTCATACAGCGGCAAAAAGA
CGGTTGTCAACATTACTTCAGGAGCCGCCAAAAATCCATATA
AGGGATGGAGCGCGTATTGCAGTTCAAAAGCCGGGCTCGAC
ATGTTTACGAGGACATTCGGATTTGAACAGGAGGATGAAGAG
CTGCCGGTGAACATGATTTCGTTCTCACCTGGAGTGATGGAC
ACTGAGATGCAGGCCGTCATCCGTTCTTCATCGAAAAAGGAT
TTCCACCACATTGAACGATTCCGGAAATTAAATGAAACAGGA
AGCCTTCGCAGTCCGGACTTTATTGCCGGCACGCTGCTTTCTT
TACTAGAAAAAGGGACGGAAAACGGCCGCATTTATGATATTA
AAGAGTTTTTG

bsgdh: ATGTATCCGGATTTAAAAGGAAAAGTCGTCGCTATTACAGGA
GCTGCTTCAGGGCTCGGAAAGGCGATGGCCATTCGCTTCGGC
AAGGAGCAGGCAAAAGTGGTTATCAACTATTATAGTAATAAA
CAAGATCCGAACGAGGTAAAAGAAGAGGTCATCAAGGCGGG
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AGCGATTAAATATTTCGTAGAAAACGATATCAAGGGAAATGT
CATTAACATGTCCAGTGTGCACGAAGTGATTCCTTGGCCGTTA
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GTCAATAATATTGGGCCAGGTGCGATCAACACGCCAATCAAT
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AAGCATGATTCCAATGGGATATATCGGCGAACCGGAGGAGAT
CGCCGCAGTAGCAGCCTGGCTTGCTTCGAAGGAAGCCAGCTA
CGTCACAGGCATCACGTTATTCGCGGACGGCGGTATGACACA
ATATCCTTCATTCCAGGCAGGCCGCGGT

yueD-Linker-SpyTag: ATGGAACTTTATATCATCACCGGAGCGTCAAAAGGGCTGGGT

	CAAGCCATTGCATTACAGGCTTTAGAAAAGGGGCATGAAGTC
	CATGCCTTATCCAGAACGAAAACAGATGTCTCTCACAAAAAA
	CTAACGCAGCATCAAATAGACCTCATCAATCTCGAAGAAGCT
	GAACAGCAATTTGAAACATTGCTCTCATCCATCGATTCAGATC
	GTTATTCTGGTATTACCCTTATTAATAACGCCGGAATGGTAACG
	CCGATCAAACGTGCCGGCGAAGCGTCTCTTGACGAGCTTCAG
	CGCCATTATCAGCTGAACCTGACTGCGCCCGTGCTTTTGAGTC
	AGCTGTTTACAAAACGGTTTGCTTCATACAGCGGCAAAAAGA
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	ATGTTTACGAGGACATTCGGATTTGAACAGGAGGATGAAGAG
	CTGCCGGTGAACATGATTTCGTTCTCACCTGGAGTGATGGAC
	ACTGAGATGCAGGCCGTCATCCGTTCTTCATCGAAAAAGGAT
	TTCCACCACATTGAACGATTCCGGAAATTAAATGAAACAGGA
	AGCCTTCGCAGTCCGGACTTTATTGCCGGCACGCTGCTTTCTT
	TACTAGAAAAAGGGACGGAAAACGGCCGCATTTATGATATTA
	AAGAGTTTTTGGGAGGCTCCGGATCCGCTGGCTCCGCTGCTG
	GTTCTGGCGAATTCCGTGGCGTTCCGCACATCGTTATGGTTGA
	TGCGTATAAACGTTACAAA
bsgdh-Linker-SnoopTag:	ATGTATCCGGATTTAAAAGGAAAAGTCGTCGCTATTACAGGA
	GCTGCTTCAGGGCTCGGAAAGGCGATGGCCATTCGCTTCGGC
	AAGGAGCAGGCAAAAGTGGTTATCAACTATTATAGTAATAAA
	CAAGATCCGAACGAGGTAAAAGAAGAGGTCATCAAGGCGGG
	CGGTGAAGCTGTTGTCGTCCAAGGAGATGTCACGAAAGAGG
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	TGTGCCATCTCACGAAATGCCGCTCAAGGATTGGGATAAAGT
	CATCGGCACGAACTTAACGGGTGCCTTTTTAGGAAGCCGTGA
	AGCGATTAAATATTTCGTAGAAAACGATATCAAGGGAAATGT
	CATTAACATGTCCAGTGTGCACGAAGTGATTCCTTGGCCGTTA
	TTTGTCCACTATGCGGCAAGTAAAGGCGGGATAAAGCTGATG
	ACAGAAACATTAGCGTTGGAATACGCGCCGAAGGGCATTCGC
	GTCAATAATATTGGGCCAGGTGCGATCAACACGCCAATCAAT
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	AAGCATGATTCCAATGGGATATATCGGCGAACCGGAGGAGAT
	CGCCGCAGTAGCAGCCTGGCTTGCTTCGAAGGAAGCCAGCTA
	CGTCACAGGCATCACGTTATTCGCGGACGGCGGTATGACACA
	ATATCCTTCATTCCAGGCAGGCCGCGGTGAATTCGCCAGAAC
	CAGCAGCGGAGCCAGCGGATCCGGAGCCTCCAAGTTGGGTG
	ACATCGAGTTCATcAAGGTCAACAAG

Table S2. Amino acids sequences of constructed fusion proteins:

Fusion protein name	Amino acids sequences

Linker-SpyTag:	GGSGSAGSAAGSGEFRGVPHIVMVDAYKRYK
Linker-SnoopTag:	EFARTSSGASGSGASKLGDIEFIKVNK
SpyCatcher:	MGSSHHHHHHSSGLVPRGSHMPTPPTTPTPPTTPTPTPVTTLSGLS GEQGPSGDMTTEEDSATHIKFSKRDEDGRELAGATMELRDSSGKTI STWISDGHVKDFYLYPGKYTFVETAAPDGYEVATPIEFTVNEDGQV TVDGEATEGDAHT
SnoopCatcher:	PTPPTTPTPPTTPTPTPKPLRGAVFSLQKQHPDYPDIYGAIDQNGTY QNVRTGEDGKLTFKNLSDGKYRLFENSEPAGYKPVQNKPIVAFQI VNGEVRDVTSIVPQDIPATYEFTNGKHYITNEPIPPKHHHHHH
BsCR:	MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSMELYIITGAS KGLGQAIALQALEKGHEVHALSRTKTDVSHKKLTQHQIDLINLEE AEQQFETLLSSIDSDRYSGITLINNAGMVTPIKRAGEASLDELQRHY QLNLTAPVLLSQLFTKRFASYSGKKTVVNITSGAAKNPYKGWSAY CSSKAGLDMFTRTFGFEQEDEELPVNMISFSPGVMDTEMQAVIRSS SKKDFHHIERFRKLNETGSLRSPDFIAGTLLSLLEKGTENGRIYDIKE FL
BsGDH:	MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSMYPDLKGK VVAITGAASGLGKAMAIRFGKEQAKVVINYYSNKQDPNEVKEEVI KAGGEAVVVQGDVTKEEDVKNIVQTAIKEFGTLDIMINNAGLENP VPSHEMPLKDWDKVIGTNLTGAFLGSREAIKYFVENDIKGNVINM SSVHEVIPWPLFVHYAASKGGIKLMTETLALEYAPKGIRVNNIGPG AINTPINAEKFADPKQKADVESMIPMGYIGEPEEIAAVAAWLASKE ASYVTGITLFADGGMTQYPSFQAGRG
BsCR-SpyTag:	MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSMELYIITGAS KGLGQAIALQALEKGHEVHALSRTKTDVSHKKLTQHQIDLINLEE AEQQFETLLSSIDSDRYSGITLINNAGMVTPIKRAGEASLDELQRHY QLNLTAPVLLSQLFTKRFASYSGKKTVVNITSGAAKNPYKGWSAY CSSKAGLDMFTRTFGFEQEDEELPVNMISFSPGVMDTEMQAVIRSS SKKDFHHIERFRKLNETGSLRSPDFIAGTLLSLLEKGTENGRIYDIKE FLGGSGSAGSAAGSGEFRGVPHIVMVDAYKRYK
BsGDH-SnoopTag:	MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSMYPDLKGK VVAITGAASGLGKAMAIRFGKEQAKVVINYYSNKQDPNEVKEEVI KAGGEAVVVQGDVTKEEDVKNIVQTAIKEFGTLDIMINNAGLENP VPSHEMPLKDWDKVIGTNLTGAFLGSREAIKYFVENDIKGNVINM SSVHEVIPWPLFVHYAASKGGIKLMTETLALEYAPKGIRVNNIGPG AINTPINAEKFADPKQKADVESMIPMGYIGEPEEIAAVAAWLASKE ASYVTGITLFADGGMTQYPSFQAGRGEFARTSSGASGSGASKLGDI EFIKVNK

Table S3. Immobilization efficiency of the BsCR-SpyTag and BsGDH-SnoopTag

Immobilization efficiency of the BsCR-SpyTag	Immobilization efficiency of the BsGDH-SnoopTag
69.24%	69.96%