## **Supporting information**

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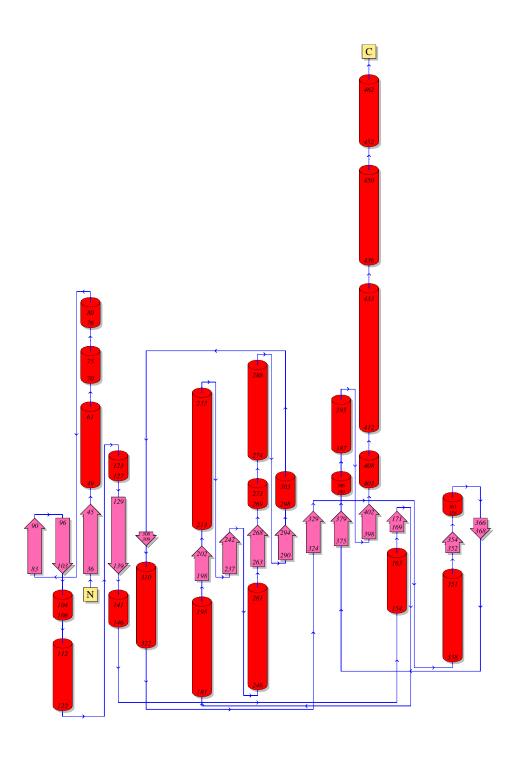
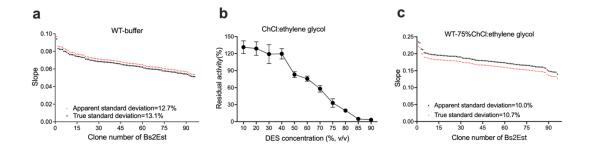
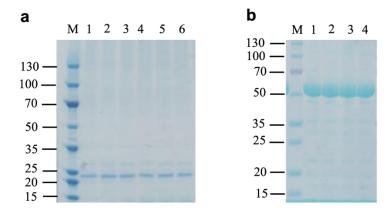


Figure S1. Secondary structure of Bs2Est



**Figure S2.** Screening system of Bs2Est in ChCl:ethylene glycol. (a) The slope of Bs2Est WT in the buffer. (b) Residual activity of Bs2Est WT at different ChCl:ethylene glycol concentrations. (c) Slope of Bs2Est WT in 75% ChCl:ethylene glycol (Concentration which can obtain residual activity of 30–40% of the BSLA WT was used in the incubation step for the Bs2Est activity assay). The standard deviation to evaluate the applicability of the 96-well MTP-based screening system for directed Bs2Est WT evolution. The apparent standard deviation was calculated without excluding the background, and the true stand deviation was calculated after deducting the background from the empty vector.



**Figure S3**. Identification and SDS-PAGE analysis of purified protein. A broad molecular weight standard protein (15-130 kDa) was used as a marker, and the remaining lanes were WT and variants. (a) From left to right was BSLA WT, T66H/G67D, K88E/N89K, M137D/N138D, M137D/N138H, Y161D/S162E/S163E. (b) From left to right was Bs2Est WT, K205H, E286H/E286D, E286K.

**Table S1.** Primers used for corner engineering of the BSLA gene in pet-22b.

Name	5'> 3' sequence
F1(5.6)- Forward Primer	TGAACACAAT <u>SRCSRC</u> GTTATGGTTC
F1(5.6)- Reverse Primer	GAACCATAAC <u>GYSGYS</u> ATTGTGTTCA
F1'(5.6)- Forward Primer	TGAACACAAT <u>RAARAA</u> GTTATGGTTC
F1'(5.6)- Reverse Primer	GAACCATAAC <u>TTYTTY</u> ATTGTGTTCA
F2(9.10)- Forward Primer	AGTCGTTATG <u>SRCSRC</u> GGTATTGGAG
F2(9.10)- Reverse Primer	CTCCAATACC <u>GYSGYS</u> CATAACGACT
F2'(9.10)- Forward Primer	AGTCGTTATG <u>RAARAA</u> GGTATTGGAG
F2'(9.10)- Reverse Primer	CTCCAATACC <u>TTYTTY</u> CATAACGACT
F3(15.16)- Forward Primer	GTATTGGAGGG <u>SRCSRC</u> TTCAATTTTGC
F3(15.16)- Reverse Primer	GCAAAATTGAA <u>GYSGYS</u> CCCTCCAATAC
F3'(15.16)-Forward Primer	GTATTGGAGGG <u>RAARAA</u> TTCAATTTTGC
F3'(15.16)- Reverse Primer	GCAAAATTGAA <u>TTYTTY</u> CCCTCCAATAC
F4(28.29)- Forward Primer	GCTATCTCGTA <u>SRCSRC</u> GGCTGGTCGCG
F4(28.29)- Reverse Primer	CGCGACCAGCC <u>GYSGYS</u> TACGAGATAGC
F4'(28.29)-Forward Primer	GCTATCTCGTA <u>RAARAA</u> GGCTGGTCGCG
F4'(28.29)- Reverse Primer	CGCGACCAGCC <u>TTYTTY</u> TACGAGATAGC
F5(32.33)- Forward Primer	TCTCAGGGCTGG <u>SRCSRC</u> GACAAGCTGTAT
F5(32.33)- Reverse Primer	ATACAGCTTGTCGYSGYSCCAGCCCTGAGA
F5'(32.33)-Forward Primer	TCTCAGGGCTGG <u>RAARAA</u> GACAAGCTGTAT
F5'(32.33)- Reverse Primer	ATACAGCTTGTC <u>TTYTTY</u> CCAGCCCTGAGA
F6(35.36)- Forward Primer	TGGTCGCGGGAC <u>SRCSRC</u> TATGCAGTTGAT
F6(35.36)- Reverse Primer	ATCAACTGCATAGYSGYSGTCCCGCGACCA
F6'(35.36)-Forward Primer	TGGTCGCGGGAC <u>RAARAA</u> TATGCAGTTGAT
F6'(35.36)- Reverse Primer	ATCAACTGCATA <u>TTYTTY</u> GTCCCGCGACCA
F7(38.39)- Forward Primer	GACAAGCTGTAT <u>SRCSRC</u> GATTTTTGGGAC
F7(38.39)- Reverse Primer	GTCCCAAAAATC <u>GYSGYS</u> ATACAGCTTGTC
F7'(38.39)-Forward Primer	GACAAGCTGTAT <u>RAARAA</u> GATTTTTGGGAC
F7'(38.39)- Reverse Primer	GTCCCAAAAATC <u>TTYTTY</u> SATACAGCTTGTC
F8(47.48)- Forward Primer	GACAAGACAGGC <u>SRCSRC</u> TATAACAATGGA
F8(47.48)- Reverse Primer	TCCATTGTTATAGYSGYSGCCTGTCTTGTC
F8'(47.48)-Forward Primer	GACAAGACAGGC <u>RAARAA</u> TATAACAATGGA
F8'(47.48)- Reverse Primer	TCCATTGTTATA <u>TTYTTY</u> GCCTGTCTTGTC
F9(66.67)-Forward Primer	GTTTTAGATGAA <u>SRCSRC</u> GCGAAAAAAGTG
F9(66.67)-Reverse Primer	CACTTTTTCGC <u>GYSGYS</u> TTCATCTAAAAC
F9'(66.67)-Forward Primer	GTTTTAGATGAA <u>RAARAA</u> GCGAAAAAAGTG
F9'(66.67)-Reverse Primer	CACTTTTTCGC <u>TTYTTY</u> TTCATCTAAAAC
F10 (70.71)- Forward Primer	ACGGGTGCGAAA <u>SRCSRC</u> GATATTGTCGCT
F10(70.71)- Reverse Primer	AGCGACAATATCGYSGYSTTTCGCACCCGT
F10' (70.71)- Forward Primer	ACGGGTGCGAAA <u>RAARAA</u> GATATTGTCGCT
F10' (70.71)- Reverse Primer	AGCGACAATATC <u>TTYTTY</u> TTTCGCACCCGT

F11(76.77.78)-Forward Primer ACTGGATATTGTCGCTSRCSRCGGGGGCGCGA ACACAC F11(76.77.78)- Reverse Primer F11(76.77.78)- Forward Primer ACTGGATATTGTCGCTCCCGYSGYSGYSAGCGACAATA TCCACT F11'(76.77.78)- Forward Primer F12(88.89)- Forward Primer F12(88.89)- Forward Primer F12(88.89)- Forward Primer F12'(88.89)- Reverse Primer F13(91.92)- Forward Primer F13(91.92)- Forward Primer F13(91.92)- Reverse Primer F14(102.103)- Forward Primer F14(102.103)- Reverse Primer F14(102.103)- Reverse Primer F15(105.106)- Forward Primer F15(105.106)- Forward Primer F15'(105.106)- Reverse Primer F16'(108.109)- Forward Primer F16'(108.109)- Reverse Primer F16'(108.109)		
F11(76.77.78)- Reverse Primer         GTGTGTTCGCGCCCCCGYSGYSGYSAGCGACAATA TCCACT           F11'(76.77.78)- Forward Primer         AGTGGATATTGTCGCTRAARAARAGGGGGCGCGA ACACAC           F11'(76.77.78)- Reverse Primer         GTGTGTTCGCGCCCCCTTYTTYTTYAGCGACAATAT CCACT           F12(88.89)- Forward Primer         CGAACACACTTTACTACATASRCSRCCTGGACGGCG GAAATAAAGT           F12(88.89)- Reverse Primer         ACTTTATTTCCGCCGTCCAGGYSGYSTATGTAGTAAA GTGTTCG           F12'(88.89)- Forward Primer         CGAACACACTTTACTACATARAARAACTGGACGGCG GAAATAAAGT           F12'(88.89)- Reverse Primer         ACTTTATTTCCGCCGTCCAGTTYTTYTATGTAGTAAA GTGTTCG           F13(91.92)- Forward Primer         ACTTTATTTCCGCCGTCCAGTTYTTYTATGTAGTAAA GTGTTTCG           F13(91.92)- Forward Primer         AACATTTATTTCCGCCGTCCAGTTYTTYTATGTAGTAAA GTGTTTCG           F13(91.92)- Forward Primer         AACTTTATTTCCGYSGYSCAGATTTTTTAT           F13(91.92)- Forward Primer         AACTTTATTTCCGYSGYSCAGATTTTTTAT           F13(91.92)- Forward Primer         ACTTTATTTCCTTYTTYCAGATTTTTAT           F13(91.92)- Forward Primer         GTCGTGACGSRCSRCGGCGCAAC           F13(91.92)- Forward Primer         GTCGTGACGSRCSRCGGCGCGAAC           F14(102.103)- Forward Primer         GTCGTGACGSRCSRCGCGCAAC           F14(102.103)- Forward Primer         GTCGTGACGCTTTYTTYCGTCACGAC           F15(105.106)- Forward Primer         TGCCTGTCGTCAAACGGYSGCCGCAACGTTGACGACAGGCAC <td< td=""><td>F11(76.77.78)-Forward Primer</td><td>AGTGGATATTGTCGCT<u>SRCSRCSRC</u>GGGGGCGCGA</td></td<>	F11(76.77.78)-Forward Primer	AGTGGATATTGTCGCT <u>SRCSRCSRC</u> GGGGGCGCGA
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CCACT		ACACAC
F12(88.89)- Forward Primer  CGAACACACTTTACTACATASRCSRCCTGGACGGCG GAAATAAAGT  F12(88.89)- Reverse Primer  ACTTTATTTCCGCCGTCCAGGYSGYSTATGTAGTAAA GTGTGTTCG  F12'(88.89)- Forward Primer  CGAACACACTTTACTACATARAARAACTGGACGGCG GAAATAAAGT  F12'(88.89)- Reverse Primer  F13(91.92)- Forward Primer  F13(91.92)- Forward Primer  F13(91.92)- Forward Primer  F13'(91.92)- Forward Primer  F13'(91.92)- Reverse Primer  F13'(91.92)- Reverse Primer  F14'(102.103)- Forward Primer  F14'(102.103)- Forward Primer  F14'(102.103)- Forward Primer  F15'(105.106)- Forward Primer  F15(105.106)- Forward Primer  F15'(105.106)- Reverse Primer  F15'(105.106)- Reverse Primer  F15'(105.106)- Forward Primer  CGTGACGCTTGGCGGCCGCAACGTTTGACGACA GGCA  F15'(105.106)- Forward Primer  CGTGACGCTTGGCGGCCRAARAACGTTTGACGACA GGCA  F15'(105.106)- Forward Primer  CGTGACGCTTGGCGGCCRAARAACGTTTGACGACAGGCA  F15'(105.106)- Forward Primer  CGTGACGCTTGGCGGCCRAARAACGTTTGACGACAGGCAC  F16'(108.109)- Forward Primer  CGGCGCGAACCGTTGCCGCCCAAGCGTC  ACG  F16'(108.109)- Forward Primer  CGGCGCGAACCGTRAARAACGTTTTTYCCGCCACCCGCCGCCGCCCCCCCCCCCCCC	F11'(76.77.78)- Reverse Primer	GTGTGTTCGCGCCCC <u>TTYTTYTTY</u> AGCGACAATAT
F12(88.89)- Reverse Primer  F12'(88.89)- Forward Primer  F12'(88.89)- Forward Primer  F12'(88.89)- Reverse Primer  F12'(88.89)- Reverse Primer  F12'(88.89)- Reverse Primer  F13(91.92)- Forward Primer  F13(91.92)- Forward Primer  F13(91.92)- Forward Primer  F13'(91.92)- Forward Primer  F13'(91.92)- Forward Primer  F13'(91.92)- Reverse Primer  F13'(91.92)- Reverse Primer  F14(102.103)- Forward Primer  F14(102.103)- Forward Primer  F14'(102.103)- Reverse Primer  F15'(105.106)- Forward Primer  F16(108.109)- Forward Primer  F16(108.109)- Forward Primer  F16'(108.109)- Forward Primer  F170'(108.109)- Forward Primer  F180'(108.109)- Forward Primer  F180'(108.109)- Forward Primer  F180'(108.109)- Forward Primer  F180'(108.109)- Forward Primer		CCACT
F12(88.89)- Reverse Primer  F12'(88.89)- Forward Primer  F12'(88.89)- Forward Primer  F12'(88.89)- Reverse Primer  F12'(88.89)- Reverse Primer  F13(91.92)- Forward Primer  F13(91.92)- Forward Primer  F13'(91.92)- Forward Primer  F13'(91.92)- Reverse Primer  F13'(91.92)- Reverse Primer  F14'(102.103)- Forward Primer  F14'(102.103)- Forward Primer  F14'(102.103)- Forward Primer  F15'(105.106)- Forward Primer  F15'(105.106)- Reverse Primer  F15'(105.106)- Reverse Primer  F16'(108.109)- Forward	F12(88.89)- Forward Primer	CGAACACACTTTACTACATA <u>SRCSRC</u> CTGGACGGCG
F12'(88.89)- Forward Primer  CGAACACACTTTACTACATARAARAACTGGACGGCG GAAATAAAGT  F12'(88.89)- Reverse Primer  ACTTTATTTCCGCCGTCCAGTTYTTYTATGTAGTAAA GTGTGTTCG  F13(91.92)- Forward Primer F13'(91.92)- Reverse Primer F13'(91.92)- Forward Primer F13'(91.92)- Reverse Primer AACTTTATTTCCGYSGYSCAGATTTTTAT F13'(91.92)- Reverse Primer F13'(91.92)- Reverse Primer F13'(91.92)- Reverse Primer F13'(91.92)- Reverse Primer GTCGTGACGSRCSRCGGCAAATAAAGTT AACTTTATTTCCTTYTTYCAGATTTTTAT GTCGTGACGSRCSRCGGCCGAAC GTT4'(102.103)- Reverse Primer GTCGTGACGSRCSRCGGCGCGAAC GT4'(102.103)- Forward Primer GTCGTGACGRAARAAGGCGCGAAC GT4'(102.103)- Reverse Primer GTCGCGCCTTYTTYCGTCACGAC GCA F15(105.106)- Forward Primer CGTGACGCTTGGCGGCSRCSRCCGTTTGACGACA GGCA F15'(105.106)- Reverse Primer TGCCTGTCGTCAAACGGYSGYSGCCCCAAGCGTC ACG F15'(105.106)- Reverse Primer CGTGACGCTTGGCGGCRAARAACGTTTTGACGACAG GCA F15'(105.106)- Forward Primer CGTGACGCTTGGCGGCRAARAACGTTTTTTTTGACGACAG GCA F15'(105.106)- Reverse Primer CGGCGCGAACCGTSRCSRCACAGGCAAGGCGC F16'(108.109)- Forward Primer CGGCGCGCAACCGTTGCTGTGTSTSCACAGGCAAGGCGC F16'(108.109)- Forward Primer CGGCGCGAACCGTRAARAACAGGCAAGGCGC F16'(108.109)- Forward Primer CGGCGCTTGCCTGTTTYTTYACGGTTCGCGCCG F16'(108.109)- Reverse Primer CGGCCCTTGCCTGTTTYTTTYACGGTTCGCGCCG F16'(108.109)- Reverse Primer CGGCCCTTGCCTGTTTYTTTYACGGTTCGCGCCG		GAAATAAAGT
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F12'(88.89)- Reverse Primer  F13'(91.92)- Forward Primer  F13(91.92)- Forward Primer  F13'(91.92)- Forward Primer  F14'(102.103)- Forward Primer  F14(102.103)- Forward Primer  F14'(102.103)- Forward Primer  F14'(102.103)- Forward Primer  F15'(105.106)- Forward Primer  F15'(105.106)- Reverse Primer  F15'(105.106)- Forward Primer  F15'(105.106)- Forward Primer  F16'(108.109)- Forward Primer  F16'(108.109)- Forward Primer  F16'(108.109)- Forward Primer  F16'(108.109)- Reverse Primer  F16'(108.109)- Reverse Primer  F16'(108.109)- Reverse Primer  F16'(108.109)- Forward Primer  F16'(108.109)- Forward Primer  F16'(108.109)- Reverse Primer		GTGTGTTCG
F12'(88.89)- Reverse Primer  F13(91.92)- Forward Primer  F13(91.92)- Reverse Primer  F13(91.92)- Forward Primer  F13'(91.92)- Forward Primer  F13'(91.92)- Forward Primer  F13'(91.92)- Reverse Primer  F14'(102.103)- Forward Primer  F14'(102.103)- Forward Primer  F14'(102.103)- Forward Primer  F14'(102.103)- Reverse Primer  F15'(105.106)- Forward Primer  F15'(105.106)- Reverse Primer  F15'(105.106)- Forward Primer  F15'(105.106)- Forward Primer  F16'(108.109)- Forward Primer  F16'(108.109)- Forward Primer  F16'(108.109)- Reverse Primer	F12'(88.89)- Forward Primer	CGAACACACTTTACTACATA <u>RAARAA</u> CTGGACGGCG
F13(91.92)- Forward Primer F13(91.92)- Reverse Primer F13(91.92)- Forward Primer F13'(91.92)- Forward Primer F13'(91.92)- Forward Primer F13'(91.92)- Reverse Primer F13'(91.92)- Reverse Primer F14'(102.103)- Forward Primer F14(102.103)- Forward Primer F14'(102.103)- Forward Primer F14'(102.103)- Reverse Primer GTCGTGACGSRCSRCGGCGCGAAC F14'(102.103)- Reverse Primer GTCGTGACGRAARAAGGCGCGAAC F14'(102.103)- Reverse Primer GTCGCGCCGYSGYSCGTCACGAC GTTCGCGCCGYSGYSCGTCACGAC GTTCGCGCCTTYTTYCGTCACGAC GTTCGCGCCTTYTTYCGTCACGAC GGCA F15(105.106)- Forward Primer GTCGTGACGCTTGGCGGCSRCSRCCGTTTGACGACA GGCA F15'(105.106)- Reverse Primer GTCGTGCGCTTGCTCAAACGGYSGYSGCCGCCAAGCGTC ACG F15'(105.106)- Forward Primer GCGTGACGCTTGGCGGCRAARAACGTTTTTCACGACAG GCA F15'(105.106)- Forward Primer GCGCGCGAACCGTSRCSRCACAGGCAAGCGTC ACG GCA F16'(108.109)- Forward Primer GCGCCCTTGCCTGTCAAACGTTYTTYGCCGCCCAAGCGTC GCGCCCTTGCCTGTCAAACGTTTTTCGCGCCCGCCG F16'(108.109)- Forward Primer GCGCCTTGCCTGTCAAACAGGCAAGGCGC GCGCGCGAACCGTRAARAACAGGCAAGGCGC GCGCCCTTGCCTGTCTTTTTTCACGGCCCCG F16'(108.109)- Forward Primer GCGCCCTTGCCTGTTTTTTTCACGGTTCGCGCCC GCGCGCAACCGTRAARAAACAGGCAAGGCGC GCGCCCTTGCCTGTTTTTTTTCACGGTTCGCGCCCG F16'(108.109)- Reverse Primer GCGCCCTTGCCTGTTTTTTTTCACGGTTCGCGCCCG GCGCCCTTGCCTGTTTTTTTTTT		GAAATAAAGT
F13(91.92)- Forward Primer F13(91.92)- Reverse Primer F13'(91.92)- Forward Primer F13'(91.92)- Forward Primer F13'(91.92)- Forward Primer F13'(91.92)- Reverse Primer F14'(102.103)- Forward Primer F14(102.103)- Forward Primer F14'(102.103)- Forward Primer F14'(102.103)- Forward Primer F14'(102.103)- Forward Primer F14'(102.103)- Reverse Primer F15(105.106)- Forward Primer F15(105.106)- Forward Primer F15(105.106)- Reverse Primer F15'(105.106)- Forward Primer F15'(105.106)- Forward Primer F16'(108.109)- Forward Primer F16'(108.109)- Forward Primer F16'(108.109)- Forward Primer F16'(108.109)- Reverse Primer	F12'(88.89)- Reverse Primer	ACTTTATTTCCGCCGTCCAG <u>TTYTTY</u> TATGTAGTAAA
F13(91.92)- Reverse Primer F13'(91.92)- Forward Primer F13'(91.92)- Reverse Primer F14(102.103)- Forward Primer F14(102.103)- Reverse Primer F14'(102.103)- Forward Primer F14'(102.103)- Forward Primer F14'(102.103)- Forward Primer GTCGTGACGSRCSRCGGCGCGAAC F14'(102.103)- Forward Primer GTCGTGACGRAARAAGGCGCGAAC F14'(102.103)- Reverse Primer GTCGTGACGRAARAAGGCGCGAAC F15(105.106)- Forward Primer GTCGTGACGRAARAAGGCGCGAAC GGCA F15(105.106)- Forward Primer GTCGTGACGCTTYTTYCGTCACGAC CGTGACGCTTYTTYCGTCACGAC GGCA F15'(105.106)- Reverse Primer GCCTGTCGTCAAACGGYSGYSGCCGCCAAGCGTC ACG F15'(105.106)- Forward Primer GCGTGACGCTTGGCGGCRAARAACGTTTGACGACAG GCA F15'(105.106)- Forward Primer GCGTGACGCTTGGCGGCRAARAACGTTTGACGACAG GCA F16'(108.109)- Forward Primer GCGCGCGAACCGTSRCSRCACAGGCAAGGCGC F16'(108.109)- Forward Primer GCGCCTTGCCTGTCGTCAAACGTTTCGCGCCG F16'(108.109)- Forward Primer GCGCCTTGCCTGTCGTCAAACGCTTCGCGCCG F16'(108.109)- Reverse Primer GCGCCTTGCCTGTCAAACAGCAAGGCAAGGCGC GCGCGCGAACCGTRAARAAACAGGCAAGGCGC GCGCGCGAACCGTRAARAAACAGGCAAGGCGC GCGCCCTTGCCTGTTYTTYACCGGTTCGCGCCG GCGCCCTTGCCTGTTYTTYACCGGTTCGCGCCG GCGCCCTTGCCTTGTTYTTYACCGGTTCGCGCCG		GTGTGTTCG
F13'(91.92)- Forward Primer F13'(91.92)- Reverse Primer F14(102.103)- Forward Primer F14(102.103)- Forward Primer F14'(102.103)- Forward Primer F14'(102.103)- Forward Primer F14'(102.103)- Forward Primer F14'(102.103)- Reverse Primer F14'(102.103)- Reverse Primer F15(105.106)- Forward Primer F15(105.106)- Forward Primer F15(105.106)- Forward Primer F15'(105.106)- Forward Primer F16'(108.109)- Reverse Primer	F13(91.92)- Forward Primer	ATAAAAAATCTG <u>SRCSRC</u> GGAAATAAAGTT
F13'(91.92)- Reverse Primer F14(102.103)- Forward Primer GTCGTGACGSRCSRCGGCGCGAAC F14'(102.103)- Reverse Primer GTCGTGACGRAARAAGGCGCGAAC F14'(102.103)- Forward Primer GTCGTGACGRAARAAGGCGCGAAC F14'(102.103)- Reverse Primer GTCGTGACGRAARAAGGCGCGAAC F15(105.106)- Forward Primer GTCGCGCCTTYTTYCGTCACGAC GGCA F15'(105.106)- Reverse Primer GCCTGTCGTCAAACGGYSGYSGCCGCCAAGCGTC ACG F15'(105.106)- Forward Primer GCGTGACGCTTGGCGGCRAARAACGTTTGACGACAG GCA F15'(105.106)- Reverse Primer GCGCGCTTGGCGGCRAARAACGTTTGACGACAG GCA F15'(105.106)- Reverse Primer GCGCTGTCGTCAAACGTTYTTYGCCGCCAAGCGTC ACG F16(108.109)- Forward Primer GCGCCTTGCCTGTGYSGYSACGGTTCGCGCCG F16'(108.109)- Reverse Primer GCGCCTTGCCTGTTTTTTYACGGTTCGCGCCG F16'(108.109)- Reverse Primer GCGCCTTGCCTGTTTTTTTYACGGTTCGCGCCG F16'(108.109)- Reverse Primer	F13(91.92)- Reverse Primer	AACTTTATTTCC <u>GYSGYS</u> CAGATTTTTTAT
F14(102.103)- Forward Primer F14(102.103)- Reverse Primer F14'(102.103)- Forward Primer F14'(102.103)- Forward Primer F14'(102.103)- Forward Primer F15(105.106)- Forward Primer F15(105.106)- Reverse Primer F15'(105.106)- Forward Primer F15'(105.106)- Reverse Primer F16'(108.109)- Forward Primer F16'(108.109)- Forward Primer F16'(108.109)- Forward Primer F16'(108.109)- Forward Primer F16'(108.109)- Reverse Primer	F13'(91.92)- Forward Primer	ATAAAAAATCTG <u>RAARAA</u> GGAAATAAAGTT
F14(102.103)- Reverse Primer GTTCGCGCCGYSGYSCGTCACGAC F14'(102.103)- Forward Primer GTCGTGACGRAARAAGGCGCGAAC F14'(102.103)- Reverse Primer GTTCGCGCCTTYTTYCGTCACGAC F15(105.106)- Forward Primer CGTGACGCTTGGCGGCSRCSRCCGTTTGACGACA GGCA F15'(105.106)- Reverse Primer CGTGACGCTTGGCGGCRAARAACGTTGACGACAGCGTCACG F15'(105.106)- Forward Primer CGTGACGCTTGGCGGCRAARAACGTTTGACGACAGGCA F15'(105.106)- Reverse Primer CGTGACGCTTGGCGGCRAARAACGTTTGACGACAGGCA F16'(108.109)- Forward Primer CGGCGCGAACCGTSRCSRCACAGGCAAGGCGC F16'(108.109)- Forward Primer GCGCCTTGCCTGTGYSGYSACGGTTCGCGCCG F16'(108.109)- Reverse Primer GCGCCTTGCCTGTTTYTTYACGGTTCGCGCCG F16'(108.109)- Reverse Primer GCGCCTTGCCTGTTTYTTYACGGTTCGCGCCG F16'(108.109)- Reverse Primer GCGCCTTGCCTGTTTYTTYACGGTTCGCGCCG	F13'(91.92)- Reverse Primer	AACTTTATTTCC <u>TTYTTY</u> CAGATTTTTTAT
F14'(102.103)- Forward Primer F14'(102.103)- Reverse Primer F15(105.106)- Forward Primer GCGCGCCTTYTTYCGTCACGAC F15(105.106)- Forward Primer GCGCGCCTTGGCGGCSRCSRCCGTTTGACGACA GGCA F15'(105.106)- Reverse Primer GCGTGACGCTTGGCGGCSRCSRCCGTTTGACGACA GGCA F15'(105.106)- Forward Primer CGTGACGCTTGGCGGCRAARAACGTTTGACGACAG GCA F15'(105.106)- Reverse Primer TGCCTGTCGTCAAACGTTYTTYGCCGCCAAGCGTC ACG F16'(108.109)- Forward Primer GCGCGCGAACCGTSRCSRCACAGGCAAGCGC F16'(108.109)- Forward Primer GCGCCTTGCCTGTCGYSGYSACGGTTCGCGCCG F16'(108.109)- Reverse Primer GCGCCTTGCCTGTTTTTTYACGGTTCGCGCCG F16'(108.109)- Reverse Primer GCGCCTTGCCTGTTTTTTTYACGGTTCGCGCCG GCGCCTTGCCTGTTTTTTTYACGGTTCGCGCCG	F14(102.103)- Forward Primer	GTCGTGACG <u>SRCSRC</u> GGCGCGAAC
F14'(102.103)- Reverse Primer F15(105.106)- Forward Primer  GCGCCTTYTTYCGTCACGAC  CGTGACGCTTGGCGGCSRCSRCCGTTTGACGACA  GGCA  F15(105.106)- Reverse Primer  TGCCTGTCGTCAAACGGYSGYSGCCGCCAAGCGTC  ACG  F15'(105.106)- Forward Primer  CGTGACGCTTGGCGGCRAARAACGTTTGACGACAG  GCA  F15'(105.106)- Reverse Primer  TGCCTGTCGTCAAACGTTYTTYGCCGCCAAGCGTC  ACG  F16(108.109)- Forward Primer  CGGCGCGAACCGTSRCSRCACAGGCAAGGCGC  F16'(108.109)- Forward Primer  CGGCGCGAACCGTRAARAACAGGCAAGGCGC  CGGCGCGAACCGTRAARAACAGGCAAGGCGC  CGGCGCGAACCGTRAARAAACAGGCAAGGCGC  CGGCGCGAACCGTRAARAAACAGGCAAGGCGC  GCGCCTTGCCTGTTTTTTYACCGGTTCGCGCCG  GCGCCTTGCCTGTTTTTTYACGGTTCGCGCCG  GCGCCTTGCCTGTTTTTTTYACGGTTCGCGCCG	F14(102.103)- Reverse Primer	GTTCGCGCC <u>GYSGYS</u> CGTCACGAC
F15(105.106)- Forward Primer CGTGACGCTTGGCGGCSRCSRCCGTTTGACGACA GGCA  F15(105.106)- Reverse Primer TGCCTGTCGTCAAACGGYSGYSGCCGCCAAGCGTC ACG  F15'(105.106)- Forward Primer CGTGACGCTTGGCGGCRAARAACGTTTGACGACAG GCA  F15'(105.106)- Reverse Primer TGCCTGTCGTCAAACGTTYTTYGCCGCCAAGCGTC ACG  F16(108.109)- Forward Primer CGGCGCGAACCGTSRCSRCACAGGCAAGGCGC  F16'(108.109)- Reverse Primer GCGCCTGTGYSGYSACGGTTCGCGCCG  F16'(108.109)- Reverse Primer GCGCCTTGCCTGTTGYSGYSACGGTTCGCGCCG  F16'(108.109)- Reverse Primer GCGCCTTGCCTGTTTYTTYACGGTTCGCGCCG  F16'(108.109)- Reverse Primer GCGCCTTGCCTGTTTYTTYACGGTTCGCGCCG	F14'(102.103)- Forward Primer	GTCGTGACG <u>RAARAA</u> GGCGCGAAC
GGCA  F15(105.106)- Reverse Primer  F15'(105.106)- Forward Primer  F15'(105.106)- Forward Primer  F15'(105.106)- Reverse Primer  F15'(105.106)- Reverse Primer  F16(108.109)- Forward Primer  F16(108.109)- Reverse Primer  F16'(108.109)- Forward Primer  F16'(108.109)- Forward Primer  F16'(108.109)- Reverse Primer  F16'(108.109)- Reverse Primer  GCGCCTTGCCTGTGYSGYSACGGTTCGCGCCG  F16'(108.109)- Reverse Primer  GCGCCTTGCCTGTTTTTTTYACGGTTCGCGCCG  GCGCCTTGCCTGTTTTTTTYACGGTTCGCGCCG	F14'(102.103)- Reverse Primer	GTTCGCGCC <u>TTYTTY</u> CGTCACGAC
F15(105.106)- Reverse Primer  F15'(105.106)- Forward Primer  CGTGACGCTTGGCGGCRAARAACGTTTGACGACAG GCA  F15'(105.106)- Reverse Primer  TGCCTGTCGTCAAACGTTYTTYGCCGCCAAGCGTC ACG  F16(108.109)- Forward Primer  F16'(108.109)- Reverse Primer  F16'(108.109)- Forward Primer  F16'(108.109)- Reverse Primer  F16'(108.109)- Reverse Primer  GCGCCGGAACCGTRAARAAACAGGCAAGGCGC  GCGCCGGAACCGTRAARAAACAGGCAAGGCGC  GCGCCCTTGCCTGTTTYTTYACGGTTCGCGCCG  GCGCCTTGCCTGTTTYTTYACGGTTCGCGCCG	F15(105.106)- Forward Primer	CGTGACGCTTGGCGGC <u>SRCSRC</u> CGTTTGACGACA
ACG  F15'(105.106)- Forward Primer  GCA  F15'(105.106)- Reverse Primer  F16'(108.109)- Forward Primer  F16'(108.109)- Forward Primer  F16'(108.109)- Forward Primer  F16'(108.109)- Forward Primer  F16'(108.109)- Reverse Primer  GCGCCTTGCCTGTGYSGYSACGGTTCGCGCCG  F16'(108.109)- Reverse Primer  GCGCCTTGCCTGTTTTTTYACGGTTCGCGCCG  GCGCCCTTGCCTGTTTTTTYACGGTTCGCCCCG		GGCA
F15'(105.106)- Forward Primer CGTGACGCTTGGCGGCRAARAACGTTTGACGACAG GCA  F15'(105.106)- Reverse Primer TGCCTGTCGTCAAACGTTYTTYGCCGCCAAGCGTC ACG  F16(108.109)- Forward Primer CGGCGCGAACCGTSRCSRCACAGGCAAGGCGC F16'(108.109)- Reverse Primer CGGCGCGAACCGTRAARAAACAGGCAAGGCGC F16'(108.109)- Reverse Primer CGGCGCGAACCGTRAARAAACAGGCAAGGCGC F16'(108.109)- Reverse Primer CGGCCGCGAACCGTRAARAAACAGGCAAGGCGC F16'(108.109)- Reverse Primer CGCCCTGTTTYTTYACGGTTCGCGCCG	F15(105.106)- Reverse Primer	TGCCTGTCGTCAAACG <u>GYSGYS</u> GCCGCCAAGCGTC
GCA  F15'(105.106)- Reverse Primer  TGCCTGTCGTCAAACG <u>TTYTTY</u> GCCGCCAAGCGTC ACG  F16(108.109)- Forward Primer  CGGCGCGAACCGT <u>SRCSRC</u> ACAGGCAAGGCGC  F16'(108.109)- Reverse Primer  GCGCCTTGCCTGT <u>GYSGYS</u> ACGGTTCGCGCCG  F16'(108.109)- Forward Primer  CGGCGCGAACCGT <u>RAARAA</u> ACAGGCAAGGCGC  F16'(108.109)- Reverse Primer  GCGCCTTGCCTGT <u>TTYTTY</u> ACGGTTCGCGCCG		ACG
F15'(105.106)- Reverse Primer  TGCCTGTCGTCAAACG <u>TTYTTY</u> GCCGCCAAGCGTC ACG  F16(108.109)- Forward Primer  F16'(108.109)- Reverse Primer  GCGCGCGAACCGT <u>SRCSRC</u> ACAGGCAAGGCGC  GCGCCTTGCCTGT <u>GYSGYS</u> ACGGTTCGCGCCG  CGGCGCGAACCGT <u>RAARAA</u> ACAGGCAAGGCGC  F16'(108.109)- Reverse Primer  GCGCCTTGCCTGT <u>TTYTTY</u> ACGGTTCGCGCCG	F15'(105.106)- Forward Primer	CGTGACGCTTGGCGGC <u>RAARAA</u> CGTTTGACGACAG
ACG F16(108.109)- Forward Primer CGGCGCGAACCGTSRCSRCACAGGCAAGGCGC F16(108.109)- Reverse Primer CGGCGCGAACCGTSRCSRCACAGGCAAGGCGC GCGCCTTGCCTGTGYSGYSACGGTTCGCGCCG CGGCGCGAACCGTRAARAAACAGGCAAGGCGC F16'(108.109)- Reverse Primer CGGCCCTTGCCTGTTTYTTYACGGTTCGCGCCG		GCA
F16(108.109)- Forward Primer CGGCGCGAACCGTSRCSRCACAGGCAAGGCGC F16(108.109)- Reverse Primer CGGCGCGAACCGTGCCTGTGYSGYSACGGTTCGCGCCG F16'(108.109)- Forward Primer CGGCGCGAACCGTRAARAAACAGGCAAGGCGC F16'(108.109)- Reverse Primer CGGCCCTTGCCTGTTTYTTYACGGTTCGCGCCG	F15'(105.106)- Reverse Primer	TGCCTGTCGTCAAACG <u>TTYTTY</u> GCCGCCAAGCGTC
F16(108.109)- Reverse Primer GCGCCTTGCCTGTGYSGYSACGGTTCGCGCCG F16'(108.109)- Forward Primer GCGCGCGAACCGTRAARAAACAGGCAAGGCGC F16'(108.109)- Reverse Primer GCGCCTTGCCTGTTTYTTYACGGTTCGCGCCG		ACG
F16'(108.109)- Forward Primer CGGCGCGAACCGT <u>RAARAA</u> ACAGGCAAGGCGC F16'(108.109)- Reverse Primer GCGCCTTGCCTGT <u>TTYTTY</u> ACGGTTCGCGCCG	F16(108.109)- Forward Primer	CGGCGCGAACCGT <u>SRCSRC</u> ACAGGCAAGGCGC
F16'(108.109)- Reverse Primer GCGCCTTGCCTGTTTYTTYACGGTTCGCGCCG	F16(108.109)- Reverse Primer	GCGCCTTGCCTGT <u>GYSGYS</u> ACGGTTCGCGCCG
• • •	F16'(108.109)- Forward Primer	CGGCGCGAACCGT <u>RAARAA</u> ACAGGCAAGGCGC
F16(123.124)- Forward Primer CCGGGAACAGATCCAAATCAAAAGSRCSRCTACAC	F16'(108.109)- Reverse Primer	GCGCCTTGCCTGT <u>TTYTTY</u> ACGGTTCGCGCCG
	F16(123.124)- Forward Primer	CCGGGAACAGATCCAAATCAAAAG <u>SRCSRC</u> TACAC
ATCCATTTACAGCAGTGCCG		ATCCATTTACAGCAGTGCCG
F17(123.124)- Reverse Primer CGGCACTGCTGTAAATGGATGTGTAGYSGYSCTTTT	F17(123.124)- Reverse Primer	CGGCACTGCTGTAAATGGATGTGTA <u>GYSGYS</u> CTTTT
GATTTGGATCTGTTCCCGG		GATTTGGATCTGTTCCCGG
F17'(123.124)- Forward Primer CCGGGAACAGATCCAAATCAAAAGRAARAATACACA	F17'(123.124)- Forward Primer	CCGGGAACAGATCCAAATCAAAAG <u>RAARAA</u> TACACA
TCCATTTACAGCAGTGCCG		TCCATTTACAGCAGTGCCG
F17'(123.124)- Reverse Primer CGGCACTGCTGTAAATGGATGTGTA <u>TTYTTY</u> CTTTTG	F17'(123.124)- Reverse Primer	CGGCACTGCTGTAAATGGATGTGTA <u>TTYTTY</u> CTTTTG
ATTTGGATCTGTTCCCGG		ATTTGGATCTGTTCCCGG

F18(130.131)- Forward Primer	TATACACATCCATTTACSRCSRCGCCGATATGATTGT
1 10(1001101) 1 olimaid 1 illioi	CAT
F18(130.131)- Reverse Primer	ATGACAATCATATCGGC <u>GYSGYS</u> GTAAATGGATGTG
	TATA
F18'(130.131)- Forward Primer	TATACACATCCATTTAC <u>RAARAA</u> GCCGATATGATTGT
	CAT
F18'(130.131)- Reverse Primer	ATGACAATCATATCGGC <u>TTYTTY</u> GTAAATGGATGTGT
	ATA
F19(137.138)- Forward Primer	GCAGTGCCGATATGATTGTC <u>SRCSRC</u> TACTTATCAA
	GATTAGATGG
F19(137.138)- Reverse Primer	CCATCTAATCTTGATAAGTA <u>GYSGYS</u> GACAATCATAT
E40//407 400\ E	CGGCACTGC
F19'(137.138)- Forward Primer	GCAGTGCCGATATGATTGTCRAARAATACTTATCAAG
F19'(137.138)- Reverse Primer	ATTAGATGG CCATCTAATCTTGATAAGTATTYTTYGACAATCATATC
F19 (137.136)- Reveise Filliei	GGCACTGC
F20(141.142)- Forward Primer	ATGAATTACTTA <u>SRCSRC</u> TTAGATGGTGCT
F20(141.142)- Reverse Primer	AGCACCATCTAAGYSGYSTAAGTAATTCAT
F20'(141.142)- Forward Primer	ATGAATTACTTA <u>RAARAA</u> TTAGATGGTGCT
F20'(141.142)- Reverse Primer	AGCACCATCTAA <u>TTYTTY</u> TAAGTAATTCAT
F21(146.147)- Forward Primer	ACTTATCAAGATTAGATGGT <u>SRCSRC</u> AACGTTCAAAT
( ,	CCATGGCGT
F21(146.147)- Reverse Primer	ACGCCATGGATTTGAACGTT <u>GYSGYS</u> ACCATCTAAT
	CTTGATAAGT
F21'(146.147)- Forward Primer	ACTTATCAAGATTAGATGGT <u>RAARAA</u> AACGTTCAAAT
	CCATGGCGT
F21'(146.147)- Reverse Primer	ACGCCATGGATTTGAACGTT <u>TTYTTY</u> ACCATCTAATC
	TTGATAAGT
F22(151.152)- Forward Primer	GGTGCTAGAAACGTTCAA <u>SRCSRC</u> GGCGTTGGACA
	CATCGGC
F22(151.152)- Reverse Primer	GCCGATGTGTCCAACGCC <u>GYSGYS</u> TTGAACGTTTC
F00//454 450\ F	TAGCACC
F22'(151.152)- Forward Primer	GGTGCTAGAAACGTTCAA <u>RAARAA</u> GGCGTTGGACA
F22'(151.152)- Reverse Primer	CATCGGC GCCGATGTGTCCAACGCC <u>TTYTTY</u> TTGAACGTTTCT
F22 (131.132)- Reveise Fillier	AGCACC
F23(157.158)- Forward Primer	CCATGGCGTTGGACAC <u>SRCSRC</u> CTTCTGTACAGCA
120(107:100) 1 GIWald 1 Illiloi	GCC
F23(157.158)- Reverse Primer	GGCTGCTGTACAGAAG <u>GYSGYS</u> GTGTCCAACGCCA
-(	TGG
F23'(157.158)- Forward Primer	CCATGGCGTTGGACAC <u>RAARAA</u> CTTCTGTACAGCA
•	GCC
F23'(157.158)- Reverse Primer	GGCTGCTGTACAGAAG <u>TTYTTY</u> GTGTCCAACGCCAT
	GG

GGACACATCGGCCTTCTG <u>SRCSRCSRC</u> CAAGTCAA
CAGCCTGATT
AATCAGGCTGTTGACTTG <u>GYSGYSGYS</u> CAGAAGGC
CGATGTCC
GGACACATCGGCCTTCTG <u>RAARAARAA</u> CAAGTCAA
CAGCCTGATT
AATCAGGCTGTTGACTTG <u>TTYTTYTTY</u> CAGAAGGCC
GATGTGTCC
CCTGATTAAAGAAGGG <u>SRCSRC</u> GGCGGGGGCCAG
AATACG
CGTATTCTGGCCCCCGCCGYSGYSCCCTTCTTTAAT
CAGG
CCTGATTAAAGAAGGG <u>RAARAA</u> GGCGGGGGCCAG
AATACG
CGTATTCTGGCCCCCGCC <u>TTYTTY</u> CCCTTCTTTAATC
AGG

**Table S2.** Primers used for corner engineering of the Bs2Est gene in pet-22b.

Name	5'> 3' sequence
F1(33.34)- Forward Primer	TATGCCAAGCCG <u>SRCSRC</u> GGACAATGGC
F1(33.34)- Reverse Primer	TATGCCAAGCCG <u>RAARAA</u> GGACAATGGC
F1'(33.34)- Forward Primer	GCCATTGTCC <u>GYSGYS</u> CGGCTTGGCATA
F1'(33.34)- Reverse Primer	GCCATTGTCC <u>TTYTTY</u> CGGCTTGGCATA
F2(38.39)- Forward Primer	TCGGACAATGG <u>SRCSRC</u> AAAGCACCTGAGC
F2(38.39)- Reverse Primer	TCGGACAATGG <u>RAARAA</u> AAAGCACCTGAGC
F2'(38.39)- Forward Primer	GCTCAGGTGCTTT <u>GYSGYS</u> CCATTGTCCGA
F2'(38.39)- Reverse Primer	GCTCAGGTGCTTT <u>TTYTTY</u> CCATTGTCCGA
F3(114.115)- Forward Primer	CTAGGAGCGGGC <u>SRCSRC</u> CCATTGTATGAC
F3(114.115)- Reverse Primer	CTAGGAGCGGGC <u>RAARAA</u> CCATTGTATGAC
F3'(114.115)- Forward Primer	GTCATACAATGG <u>GYSGYS</u> GCCCGCTCCTAG
F3'(114.115)- Reverse Primer	GTCATACAATGG <u>TTYTTY</u> GCCCGCTCCTAG
F4(119.120)- Forward Primer	GAGCCATTGTAT <u>SRCSRC</u> TCAAAACTTGCG
F4(119.120)- Reverse Primer	GAGCCATTGTAT <u>RAARAA</u> TCAAAACTTGCG
F4' (119.120)- Forward Primer	CGCAAGTTTTGA <u>GYSGYS</u> ATACAATGGCTC
F4' (119.120)- Reverse Primer	CGCAAGTTTTGA <u>TTYTTY</u> ATACAATGGCTC
F5(125.126)- Forward Primer	TCAAAACTTGCG <u>SRCSRC</u> GGAGAAGTCATT
F5(125.126)- Reverse Primer	TCAAAACTTGCG <u>RAARAA</u> GGAGAAGTCATT
F5' (125.126)- Forward Primer	AATGACTTCTCC <u>GYSGYS</u> CGCAAGTTTTGA
F5' (125.126)- Reverse Primer	AATGACTTCTCC <u>TTYTTY</u> CGCAAGTTTTGA
F6(128.129)- Forward Primer	GCGGCACAGGGA <u>SRCSRC</u> ATTGTCGTTACA
F6(128.129)- Reverse Primer	GCGGCACAGGGA <u>RAARAA</u> ATTGTCGTTACA
F6'(128.129)- Forward Primer	TGTAACGACAAT <u>GYSGYS</u> TCCCTGTGCCGC
F6'(128.129)- Reverse Primer	TGTAACGACAAT <u>TTYTTY</u> TCCCTGTGCCGC

F7(137.138)- Forward Primer F7(137.138)- Reverse Primer F7'(137.138)- Forward Primer F7'(137.138)- Reverse Primer F8(143.144)- Forward Primer F8(143.144)- Reverse Primer F8'(143.144)- Forward Primer F8'(143.144)- Reverse Primer F9(155.156)- Forward Primer F9(155.156)- Reverse Primer F9' (155.156)- Forward Primer F9' (155.156)- Reverse Primer F10(176.177)- Forward Primer F10(176.177)- Reverse Primer F10'(176.177)- Forward Primer F10'(176.177)- Reverse Primer F11(206.207)- Forward Primer F11(206.207)- Reverse Primer F11'(206.207)- Forward Primer F11'(206.207)- Reverse Primer F12(205.206)- Forward Primer F12(205.206)- Reverse Primer F12'(205.206)- Forward Primer F12'(205.206)- Reverse Primer F13(221.222)- Forward Primer F13(221.222)- Reverse Primer F13'(221.222)- Forward Primer F13'(221.222)- Reverse Primer F14(238.239)- Forward Primer F14(238.239)- Reverse Primer F14'(238.239)- Forward Primer F14'(238.239)- Reverse Primer F15(242.243)- Forward Primer F15(242.243)- Reverse Primer F15'(242.243)- Forward Primer F15'(242.243)- Reverse Primer F16(264.265)- Forward Primer F16(264.265)- Reverse Primer F16'(264.265)- Forward Primer F16'(264.265)- Reverse Primer F17(286.287)- Forward Primer F17(286.287)- Reverse Primer F17'(286.287)- Forward Primer F17'(286.287)- Reverse Primer

ACATTGAACTAT<u>SRCSRC</u>GGGCCGTTTGGC ACATTGAACTATRAARAAGGGCCGTTTGGC GCCAAACGCCCGYSGYSATAGTTCAATGT GCCAAACGGCCCTTYTTYATAGTTCAATGT GGGCCGTTTGGCSRCSRCCACTTGTCTTCA GGGCCGTTTGGCRAARAACACTTGTCTTCA TGAAGACAAGTGGYSGYSGCCAAACGGCCC TGAAGACAAGTG<u>TTYTTY</u>GCCAAACGGCCC GAGGCGTATTCTSRCSRCCTTGGGCTTTTA GAGGCGTATTCTRAARAACTTGGGCTTTTA TAAAAGCCCAAGGYSGYSAGAATACGCCTC TAAAAGCCCAAGTTYTTYAGAATACGCCTC AATATTTCAGCG<u>SRCSRC</u>GGTGATCCCGAT AATATTTCAGCGRAARAAGGTGATCCCGAT ATCGGGATCACCGYSGYSCGCTGAAATATT ATCGGGATCACCTTYTTYCGCTGAAATATT CCTGCGGCAAAASRCSRCTTCCAGAAAGCA CCTGCGGCAAAARAATTCCAGAAAGCA TGCTTTCTGGAAGYSGYSTTTTGCCGCAGG TGCTTTCTGGAATTYTTYTTTTTGCCGCAGG ATGCCTGCGGCASRCSRCCTGTTCCAGA ATGCCTGCGGCARAARAACTGTTCCAGA TCTGGAACAGGYSGYSTGCCGCAGGCAT ATGCCTGCGGCARAARAACTGTTCCAGA GCTTCTCGAACGSRCSRCAAAGAACAAGCG GCTTCTCGAACGRAARAAAAAAGAACAAGCG CGCTTGTTCTTTGYSGYSCGTTCGAGAAGC CGCTTGTTCTTTTTYTTYCGTTCGAGAAGC TTACAGGTCCTT<u>SRCSRC</u>AACGAGGGCCAA TTACAGGTCCTTRAARAAAACGAGGGCCAA TTGGCCCTCGTTGYSGYSAAGGACCTGTAA TTGGCCCTCGTTTTYTTYAAGGACCTGTAA GGGATTAACGAG<u>SRCSRC</u>CTGGATAAATTG GGGATTAACGAGRAARAACTGGATAAATTG CAATTTATCCAGGYSGYSCTCGTTAATCCC CAATTTATCCAG<u>TTYTTY</u>CTCGTTAATCCC GATCAGCTTCGGSRCSRCGAAAAAGAAA GATCAGCTTCGGRAARAAGAAAGAAA TTTCTTTTCGYSGYSCCGAAGCTGATC ATTTTCTTTTCTTYTTYCCGAAGCTGATC AAAACGCTGCCTSRCSRCCCAGAAAAAGCG AAAACGCTGCCTRAARAACCAGAAAAAGCG CGCTTTTTCTGG<u>GYSGYS</u>AGGCAGCGTTTT CGCTTTTTCTGGTTYTTYAGGCAGCGTTTT

F18(294.295)- Forward Primer F18(294.295)- Reverse Primer F18'(294.295)- Forward Primer F18'(294.295)- Reverse Primer F19(347.348)- Forward Primer F19(347.348)- Reverse Primer F19'(347.348)- Forward Primer F19'(347.348)- Reverse Primer F20(349.350)- Forward Primer F20(349.350)- Reverse Primer F20'(349.350)- Forward Primer F20'(349.350)- Reverse Primer F21(375.376)- Forward Primer F21(375.376)- Reverse Primer F21'(375.376)- Forward Primer F21'(375.376)- Reverse Primer F22(401.402)- Forward Primer F22(401.402)- Reverse Primer F22'(401.402)- Forward Primer F22'(401.402)- Reverse Primer F23(421.422)- Forward Primer F23(421.422)- Reverse Primer F23'(421.422)- Forward Primer F23'(421.422)- Reverse Primer F24(443.444)- Forward Primer F24(443.444)- Reverse Primer F24'(443.444)- Forward Primer F24'(443.444)- Reverse Primer

AAAGCGATCGCA<u>SRCSRC</u>GCTGCTTCCG AAAGCGATCGCARAARAAGCTGCTTCCG CGGAAGCAGCGYSGYSTGCGATCGCTTT CGGAAGCAGCTTYTTYTGCGATCGCTTT GTTGCCGATTTGSRCSRCCGTTCTCTGGAA GTTGCCGATTTGRAARAACGTTCTCTGGAA TTCCAGAGAACGGYSGYSCAAATCGGCAAC TTCCAGAGAACG<u>TTYTTY</u>CAAATCGGCAAC GATTTGTATCCGSRCSRCCTGGAAAGCCAA GATTTGTATCCGRAARAACTGGAAAGCCAA TTGGCTTTCCAGGYSGYSCGGATACAAATC TTGGCTTTCCAGTTYTTYCGGATACAAATC GCATCCGCACAG<u>SRCSRC</u>TACGCCCCTGTC GCATCCGCACAGRAARAATACGCCCCTGTC GACAGGGCGTAGYSGYSCTGTGCGGATGC GACAGGGGCGTATTYTTYCTGTGCGGATGC GCGTTTCACGCASRCSRCCTTCCTTTTGTC GACAAAAGGAAGGYSGYSTGCGTGAAACGC GCGTTTCACGCARAARAACTTCCTTTTGTC GACAAAGGAAG<u>TTYTTY</u>TGCGTGAAACGC GCAAAAGCGGAGSRCSRCGATGAGGTGAAA GCAAAAGCGGAGRAARAAGATGAGGTGAAA TTTCACCTCATCGYSGYSCTCCGCTTTTGC TTTCACCTCATC<u>TTYTTY</u>CTCCGCTTTTGC TTCGCCAAAACASRCSRCCCAAGCACCGAA TTCGCCAAAACARAARAACCAAGCACCGAA TTCGGTGCTTGGGYSGYSTGTTTTGGCGAA TTCGGTGCTTGGTTYTTYTGTTTTGGCGAA

**Table S3.** Kinetic characterization of the purified BSLA variants in buffer and DES.

Variant		Buffer		30 %ChCl:acetamide=1 :2		30 %TBPB:ethylene glycol =1:2			95% ChCl:ethylene glycol =1:2			
Variant												
						K <sub>cat</sub> /K			K <sub>cat</sub> /K			K <sub>cat</sub> /K
	<i>K<sub>M</sub></i> (mM)	K <sub>cat</sub> (min <sup>-</sup> 1)	$K_{cat}/K_M$ (min <sup>-1</sup> )	<i>K<sub>M</sub></i> (mM)	K <sub>cat</sub> (min⁻ ¹)	м (min <sup>-</sup> ¹mМ <sup>-</sup>	$K_M$ (mM)	$K_{cat}$ (min	м (min <sup>-</sup> ¹mМ <sup>-</sup>	K <sub>M</sub> (mM)	K <sub>cat</sub> (min <sup>-</sup>	<sup>M</sup> (min <sup>-</sup> <sup>1</sup> mM <sup>-</sup>
						1)			1)			<sup>1</sup> )
WT	0.004	0.18 9	44.704	0.145	0.188	1.297	0.365	0.396	1.085	0.088 6	0.208	2.348
T66H/G67D	0.025	0.60 6	24.240	0.073	0.128	1.753	0.383	0.299	0.781	0.157	0.223	1.420
K88E/N89K	0.013	0.18 9	14.538	0.140	1.434	10.243	0.217	2.110	9.724	0.226	2.174	9.619
Y161D/S162E/ S163E	0.017	0.23	13.706	0.026	0.039	1.500	0.530	0.246	0.464	0.065 4	0.089 9	1.375
M137D/N138H	0.0529	0.50 7	9.584	0.122	0.526	4.311	0.118	0.532	4.508	0.114	0.498	4.368
M137D/N138D	0.0812	0.56 3	6.933	0.124	0.560	4.516	0.150	0.590	3.933	0.116	0.518	4.466

Table S4. Kinetic characterization of the purified Bs2Est in buffer and DES.

Variant	Buffer			75% (v/v) ChCl:ethylene glycol				
	<i>K<sub>M</sub></i> (mM)	K <sub>cat</sub> (min <sup>-1</sup> )	$K_{cat}/K_M$ (min <sup>-1</sup> mM <sup>-1</sup> )	$K_M$ (mM)	<i>K<sub>cat</sub></i> (min⁻ ¹)	$K_{cat}/K_M$ (min <sup>-1</sup> mM <sup>-1</sup> )		
WT	0.0398	0.532	13.367	0.139	0.277	1.993		
205H	0.0079	0.090	11.416	0.042	0.105	2.500		
E286H/E286D	0.0187	0.203	10.856	0.248	0.239	0.964		
E286K	0.0214	0.123	5.747	0.048	0.111	2.313		